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Development of Heat Tolerant Breeding Lines and Varieties in Rice

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Rice cultivation is directly and significantly affected by climate change than other sectors of the economy. Agriculture is also a significant contributor to climate change and emission of nitrogenous oxide from rice field is one of the potent components causing global warming. Rice cultivation particularly in India is more affected by climate change and seasonal climate variability and its contribution is severe in yield reduction, crop failure and economic losses. The development of more sustainable, resilient agricultural systems could be achieved by identifying heat tolerant rice genotypes and development of new rice varieties for mitigating the yield losses under high temperature condition during summer and Kharif seasons. The chalky characteristic in rice grain is more prevalent with high temperatures that will bring quality and yield losses to rice growers. With a temperature rise of just 2 °C is sufficient to trigger the trait, researchers have noted that a 4 °C increase could ruin entire crop. The research focuses on identification of suitable rice genetic resources and development of heat tolerant rice varieties with high-quality grain and was carried out at Tamil Nadu Rice Research Institute, Aduthurai during 2007-08 and 2008-09. A total of 27 and 32 rice varieties from 7 countries, IRRI and WARDA were evaluated under International Rice Heat Tolerant Nursery (IRHTN, 2008 and 2009), the rice varieties viz., N22 and IDSA 77 were found to be high temperature tolerant. A total of 1669 F₄ breeding lines were screened for high temperature tolerance during June to September, 2009. A total of 154 breeding lines were advanced to F₅ generation and selection was carried out based on pollen sterility and spikelet sterility count (%), high-yielding potential with high grain quality above 35 °C under field condition and 38 °C under phytotron screening.

Key Words: Rice, Heat tolerance, Climate change, Chalky rice, Pollen sterility

Introduction

Rice is the staple food for 65% of the global population and forms the cheapest source of food, energy and protein. In India, rice is cultivated in 44 m ha by different methods under diverse environmental conditions. To meet the food demand of the growing population and to achieve food security in the country, the present production levels need to be increased by 2 million tonnes every year, which is possible through breeding rice varieties with sustainable production level against the effect of global warming. Atmospheric gases that absorb incoming energy and warm the lower atmosphere are known as greenhouse gases. About 75% of the natural greenhouse effect is due to water vapour. The next most significant greenhouse gas is carbon dioxide, followed by methane, nitrous oxide, ozone, synthetic halocarbons (such as chlorofluorocarbons and hydrofluorocarbons) and sulphur hexafluoride, all of which are influenced by human activities. Higher concentrations of greenhouse gases in the atmosphere will lead to increased trapping of infrared radiation. Scientists say the lower atmosphere is then likely to warm, changing the weather and climate.

The two greenhouse gases most linked to agricultural

activity (other than CO₂ from stubble burning) are methane and nitrous oxide. The main sources of methane are cattle, rice growing and leakages in natural gas production. Nitrous oxide results from fertilizer use and other agricultural activities, such as land clearing and cultivation. With 1.1 to 6.4 °C temperature increases in the next century rice areas of India and Pakistan may be endangered with temperature exceeding 40 °C. Breeding rice for high temperature has received little attention however; there is ample evidence in other crops that it is possible to breed varieties (cotton, tomato, peanuts and cowpea). Due to high temperature during flowering, rice produces chalkiness. Rice that breaks or powders during milling due to a defect called chalk affects growers, millers and consumers. In domestic markets, chalk can devalue the grain by up to 25% and in international markets by up to 30% or more (Collis and Braidotti, 2009).

Materials and Methods

Heat Tolerance Screening in the Field

A total of 1669 F₄ breeding lines were received from International Rice Research Institute, Los Baños, Laguna,

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Philippines during June 2009 and those accessions were screened for high temperature tolerance under an irrigated lowland field at Tamil Nadu Rice Research Institute, Aduthurai. The genotypes were seeded and seedling establishment was done in dry beds and transplanting was done 21 days after seeding. Each accession was transplanted in a 2 m length row. Row spacing was 30 x 20 cm and one seedling per hill was used. Recommended agronomic practices were followed. Pesticides and bird nets were used to protect the plants against pests. All other crop management practices were at the optimum level. Observations were recorded on 50% heading, peak anthesis, pollen sterility (%), grain sterility (%), plant height, panicle number per plant, lodging (%), phenotypic acceptability and grain yield.

Fifty percent heading was determined when the panicles are exerted in approximately 50% of the plants in the plot. Peak anthesis was recorded at the time of flowering in three consecutive days. Observation was done from 6.00 AM to 1.00 PM. Pollen sterility/fertility was determined by taking 10 spikelets each from the main panicle of the all plants from each accession (total of 100 spikelets/accession). Spikelets were sampled from top, middle and bottom portion of the panicles. Taking one anther each from the 100 spikelets, anthers were mixed, crushed, and stained with I₂KI in a glass slide. The slides were mounted on a microscope at 10x magnification and the fertile and sterile pollen were counted at 3 microscope fields. The spikelet fertility/sterility data was obtained at harvest by counting the no. of filled and unfilled grains. The plant showing highest pollen fertility was tagged on the day of 50% flowering and its spikelet fertility per cent record was measured during harvest. A plant from each accession showing more than 90% pollen fertility and spikelet fertility was selected and advanced to F₅ generation for confirming the high temperature tolerance during flowering later. One or two or three plants from each accession were selected and advanced to F₅ generation.

A total of 27 entries were evaluated under the Third International Rice Heat Tolerance Nursery and a total

of 32 entries were evaluated under the Fourth International Rice Heat Tolerance Nursery.

The plant height from 3 plants was recorded, number of panicle in 3 plants was recorded and lodging per cent was also noted during harvest. Phenotypic acceptability was measured (1=excellent, 3=good, 5=fair, 7=poor and 9=unacceptable). Grain yield was obtained from the bulk harvest of each row.

Results and Discussion

The genetic donors were evaluated in the Third and Fourth International Rice Heat Tolerance Nursery (2008 and 2009), the donors, viz., N22, Dular, IDSA 77, Giza 178 and Sadri were found to be high temperature tolerant during flowering (Table 1). The similar results were obtained earlier by Prasad *et al.* (2006). The time of day flowering (TDF) is the most important trait for rice high temperature tolerance, heat escape/avoidance, early flowering/short duration increase the pollen production, spikelet fertility and reduced the kernel damage.

A total of 105 families were advanced from F₄ generation to F₅ generation, those lines were showing early time of day flowering, less secondary branching in the panicle and higher pollen and spikelet fertility per cent. The higher pollen fertility and spikelet fertility was noticed despite exposure to >35 °C during the flowering time 1564 F₄ breeding lines were rejected due to higher pollen sterility/spikelet sterility. Sterility is due to poor anther dehiscence, low pollen production, and low numbers of germinating pollen grains on the stigma. Similar results were observed by Yoshida (1981). High temperature causes high percentages of spikelet sterility in dry season crops in many tropical and subtropical countries. For example, if the temperature exceeds 35 °C at anthesis and this state lasts for more than 1h, a high percentage of spikelet sterility occurs in a rice crops. Similar finding was noticed by Takeoka *et al.* (1991). The poor anther dehiscence was studied in the 1515 rejected lines. Anther dehiscence is due to rapid increase in pressure with swelling of pollen

Table 1. Genetic donors for high temperature tolerance (> 35 °C)

Cultivar	Origin	Ecotype/subspecies	Time of day flowering	Fertilization (%)
N22	India	<i>O. indica</i>	9.00 – 10.00AM	96
Dular	India	<i>O. indica</i>	9.00 – 9.45 AM	97
IDSA 77	Ivory Coast	<i>O. glaberrima</i>	9.00 – 10.30 AM	94
Giza 178	Egypt	<i>O. glaberrima</i>	9.00 – 10.00 AM	90
Sadri	IRRI	Tropical japonica	9.15 – 10.30 AM	92

grains. High temperature inhibits swelling of pollen grains and reduces anther dehiscence. Similar results were obtained by Goto *et al.* (2001).

The floral abnormality in spikelets, viz., stamenless, more than one ovary, decrease in the number of stamens in the spikelet and extra glume was noticed in more than 1200 rejected F₄ breeding lines. Those floral abnormality as a cause of spikelet sterility has played significant role in rice yield reduction. Similar results were revealed by Takeoka *et al.* (1991), Wang and Zhu (2000), Sanchez and Khush (2000), Li Rongbai (2002) and Yamaguchi *et al.* (2004). The floral abnormality happened in the susceptible breeding lines caused severe yield reduction due to poor pollen production and sterility of the plant. The high temperature affected the floral biology of the rice plant that causes significant yield reduction. Breeding for high temperature tolerant genotypes and varieties in rice may be the solution for sustainable agriculture production in the tropical and sub-tropical regions of India in the future, where the rice production, productivity and cropping season will be significantly decreasing due to high temperature (38 to 40 °C).

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Pot Culture Experiment on Screening for Tolerance to Drought in a Set of Wild *Hevea* Germplasm

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India received 4,548 wild germplasm accessions of *Hevea brasiliensis* as a result of the expedition conducted by International Rubber Research and Development Board in 1981 in the Amazon forests of Brazil, mainly concentrating in three states, namely Acre (AC), Rondonia (RO) and Mato Grosso (MT). This wild germplasm collection is conserved at Rubber Research Institute of India (RRII) and is now in various stages of evaluation. Wide variability observed in this large collection indicated a broad genetic base, potentially important in broadening the existing narrow genetic base of cultivated rubber. Being a likely repository of genes conferring tolerance to various biotic and abiotic stresses, this wild collection is useful in developing *Hevea* clones tolerant to stresses. For identifying the drought tolerance potential of this germplasm collection, a pot culture experiment was conducted at RRII using a set of 16 wild accessions along with the check clones RRII 105, RRII 208, RRIM 600 and Tjir 1. Data recording on growth and drought related morphological, physiological and anatomical parameters was done for assessing the clonal response of these accessions to drought stress. Plant height, girth, number of whorls, number of leaves, distance between whorls and petiole length were the growth parameters studied. As a physiological parameter, after noon leaf water potential (LWP) was recorded during the summer months after withholding irrigation and drought related anatomical parameters studied were xylem characteristics and intra-xylary phloem in twigs. Morphological characterization based on drought sensitive parameters such as leaf size, appearance of leaf surface, presence or absence of wax coating on leaf surface, leaf yellowing and senescence and vigor of plant was done and mortality rate among the accessions was assessed for identifying the potential drought tolerant accessions. It was found that among the 16 wild accessions studied, the accessions MT 1681 and MT 1623 were the potential ones for further detailed field evaluation towards developing a drought tolerant *Hevea* clone.

Key Words: *Hevea* germplasm, Drought tolerance, Characterization

India received 4,548 wild germplasm accessions of *Hevea brasiliensis* as a result of the expedition conducted by International Rubber Research and Development Board in 1981 in the Amazon forests of Brazil, mainly concentrating in three states, namely Acre (AC), Rondonia (RO) and Mato Grosso (MT). This wild germplasm collection is conserved at Rubber Research Institute of India (RRII) and is now in various stages of evaluation. Genetic diversity in this collection is closely related to the geographical provenance of the accessions (RRII, 2002) and considerable genetic variation among the accessions from Acre, Rondonia and Mato Grosso provenances has been reported by Varghese *et al.*, 2002. Wide variability observed in this large collection indicates that this collection has a broad genetic base, potentially important in broadening the existing narrow genetic base of cultivated rubber. Being a likely repository of genes conferring tolerance to various biotic and abiotic stresses, this wild collection is useful in developing *Hevea* clones tolerant to stresses.

Materials and Methods

A pot culture experiment was conducted at Rubber Research Institute of India, Kottayam using 16 wild

Hevea germplasm accessions of 1981 IRRDB expedition along with check clones RRII 105, RRII 208, RRIM 600 and Tjir 1 (Table 1) for identifying the potential drought tolerant accessions. The plants were grown in cement pots of size 2' height and 1' diameter in a Completely Randomized Design with five plants per accession, during 2003-2004. Data recording on morphological and physiological parameters related to drought tolerance was conducted during the pre- and post-summer periods and anatomical observations on primary xylem points and intra-xylary phloem were studied using twig samples. Twig samples of one year growth were collected from two trees per clone. Transverse section of twigs was prepared at 60-80 µm thickness. The sections were observed under a bright field microscope and stained in Toluidene Blue O. Number of primary xylem points (PXP) and number of intra-xylary phloem points (IXP) were quantified, and diameter of twigs were recorded and compared. Five sections per twig were observed for recording the characters.

Plant height, girth, number of whorls, number of leaves, internodal length and petiole length were the morphological parameters studied and afternoon leaf

Table 1. The list of wild accessions selected for the study along with the check clones

Accession	Accession	Accession	Check clones
MT 81	MT 4242	RO 1520	RRII 105
MT 200	MT 3714	RO 217	RRII 208
MT 1623	MT 60	AC 462	RRIM 600
MT 1681	MT 48	AC 441	Tjir 1
MT 1631	MT 1584		
MT 1710	MT 52		

water potential after withholding irrigation was used as a drought related physiological parameter. Morphological characterization based on drought sensitive parameters such as leaf size, appearance of leaf surface, presence or absence of wax coating on leaf surface, leaf yellowing and senescence and vigor of plant was done and mortality rate among the accessions was assessed for identifying the potential drought tolerant accessions. The clonal response of these accessions to drought stress was assessed to identify the potential drought tolerant wild accessions.

Results and Discussion

The list of wild accessions selected for the study is shown in Table 1. While assessing the variability among the accessions for morphological parameters during the pre stress period, wide variability was observed among the accessions for all the characters studied. MT 1623 showed superiority in growth and vigour during the pre stress period, and among the check clones, RRIM 600 and RRII 208 were the superior clones (Table 2). In the post stress period also (Table 3), MT 1623 showed

the maximum girth whereas the total foliage production was more in MT 200. Among the check clones RRIM 600 showed the superiority over the other clones. Analysis of data from the various field trials, and preliminary observations on sets of accessions from the nurseries, has revealed similar variability and significant differences among the accessions for most of the agro-morphologic traits, bark structural characters and juvenile yield indicating the scope for selection of accessions with desirable characters (Annamma *et al.*, 1989; Abraham *et al.*, 1992, 2000; Madhavan *et al.*, 1993; Mercy *et al.*, 1993, 1995, Rao *et al.*, 1996, 2008). The Shannon-Weaver Diversity indices (SDI) estimated for the 22 morphological qualitative traits during characterization of 195 accessions, also showed high levels of diversity for most traits (Suma *et al.*, 2006). In *Hevea*, growth and yield are affected under drought conditions and hence clones with good growth and vigor in the early growth stage are highly preferred for drought-affected areas. There are several earlier reports indicating the importance and usefulness of morphological characters like plant height, basal diameter, number of leaves, leaf area etc. in water stressed or drought condition (De Conceicao *et al.*, 1986). These characters give an indication of the general vigour of the genotype and hence studying these characters at the juvenile stage itself holds some importance.

Superiority in terms of girth increment and leaf water potential during the post stress period was high for MT

Table 2. Range and mean of growth characters in the pre stress period

Characters	Min.	Accession	Max.	Accession	G. Mean	Check clones				CD	VR
						RRII 105	RRIM 600	Tjir 1	RRII 208		
Height (cm)	83.8	MT1584	164.6	MT60	112.6	94.4	173.2	144.6	139.8	44.27	2.83**
Girth (cm)	4.5	MT1631	6.0	MT1623	5.24	4.5	5.8	5.6	5.30	—	NS
No. of whorls	1.0	MT1631	2.6	MT1623	1.89	1.5	2.6	1.8	2.75	0.88	3.16**
No. of leaves	8.0	MT 1631	27.0	MT200	19.27	18.0	34.6	27.4	27.5	10.8	3.59**
Inter nodal length (cm)	7.25	MT1584	28.38	MT1623	18.57	15.0	13.45	18.0	11.11	5.29	8.67**
Petiole length (cm)	8.88	MT60	23.38	MT3714	14.67	8.88	12.42	11.78	14.29	4.21	5.38**

** Significant at 1%

Table 3. Range and mean of growth characters in the post stress period

Characters	Min.	Accession	Max.	Accession	G. Mean	Check clones				CD	VR
						RRII 105	RRIM 600	Tjir 1	RRII 208		
Height (cm)	102.0	MT 1584	181.0	MT60	140.0	120.2	211.5	156.0	149.0	41.58	3.19**
Girth (cm)	4.38	MT1631	6.3	MT1623	5.35	4.6	6.3	5.6	5.3	0.94	3.33**
No. of leaves	11.4	MT 1710	38.25	MT200	21.86	23.0	40.75	29.6	26.25	12.14	3.0**

** Significant at 1%

accessions (Table 4). In an early evaluation of wild *Hevea* germplasm for drought tolerance based on growth and dry matter production, Mercy *et al.* (2006) has reported similar growth difference among the accessions. Check clones RRIM 208 and Tjir1 did not show any girth increment during this period, whereas it was highest for RRIM 600. With respect to afternoon LWP, some of the MT accessions showed higher LWP than the check clones and among the checks it was high for RRIM 600. An assessment on mortality rate among the 16 accessions indicated 100 per cent survival of two accessions viz., MT 1623 and MT 1681 (Table 5). Among the check clones, RRIM 208 and RRIM 600 were having less mortality, whereas in the clone RRIM 105 it was 100%. Following this, girth increment of accessions with less than 50% mortality was assessed over a summer period (Table 6) and was found that it was high for MT accessions, again showing their potentiality for growth in the drought condition. In a preliminary screening carried out in 100 wild accessions in the traditional region in Kerala based on various drought related morphological, anatomical, physiological and biochemical characters, Mercy (2001) has reported the superiority of Mato Grosso accessions. Among the check clones

Table 4. Superior accessions in terms of girth increment and AN Leaf water potential in the post stress period

Girth increment (%)	AN leaf water potential (-Mpa)
MT 3714 (8.16)	MT 3714 (-2.27)
MT 81 (6.00)	MT 1631 (-2.4)
MT 1623 (5.00)	MT 48 (-2..67)
MT 4242 (4.17)	MT 1681(-2.93)
MT 1584(4.08)	MT 1584 (-3.06)
RRIM 208 (0)	RRIM 208 (-3.07)
Tjir 1 (0)	Tjir 1 (-3.34)
RRIM 600 (8.62)	RRIM 600 (-2.8)
RRIM 105 (2.22)	RRIM 105 (dried)
VR= NS	VR=18.73**CD= 0.35

** Significant at 1%

Table 5. Assessment on mortality rate in the accessions

Accession	Mortality rate (%)	Accession	Mortality rate (%)
MT 1623	0	AC 462	60
MT 1681	0	MT 3714	60
MT 81	20	AC 441	80
MT 200	20	RO 1520	80
RO 217	20	MT 60	80
MT 52	40	MT 1631	80
MT 4242	40	RRIM 208	20
MT 48	40	RRIM 600	40
MT 1584	40	Tjir 1	60
MT 1710	60	RRIM 105	100

Table 6. Girth increment in accessions with less than 50% mortality over a summer period

Accession	Girth increment (%)
MT 81	60.38
MT 200	57.89
MT 1681	50
MT 4242	50
RO 217	44.74
MT 1623	41.27
MT 1584	21.57
MT 52	14.58
RRIM 208	34.53
Tjir 1	38.10
RRIM 600	79.84
RRIM 105	0
VR= 3.03**	CD=24.45

** Significant at 1%

RRIM 600 recorded the highest girth increment and the clone RRIM 105 was totally affected. Genetic level difference in response towards drought tolerance has been previously reported by Nair *et al.* (2005), while conducting laboratory screening in a set of 200 wild accessions for rapid screening of germplasm accessions for drought tolerance using the chlorophyll fluorescence technique. In an attempt to rapidly screen 3073 *Hevea* germplasm lines for intrinsic drought tolerance traits, using drought related morphological parameters during summer months, there was difference in response to drought tolerance (unpublished data). Previous studies using wild germplasm accessions have revealed genetic difference among the accessions for drought related biochemical parameters (Mercy *et al.*, 2005) and cell membrane stability (Mercy *et al.*, 2000). Similarly, genotypic difference among the wild accessions for drought related leaf and bark anatomical characters are also reported earlier (Mercy *et al.*, 2004).

In *Hevea*, intra-xylary phloem (IXP) appears in the peri central region of the stem as strands flanking the primary xylem points (PXP) groups (Fig. 1). The wild accessions showed wide variation with respect to PXP, IXP and diameter of twigs (Table 7). The number of primary xylem points varied from 29.20 (MT 4242) to 104.50 (MT 1623). With respect to IXP, the accession MT 1623 recorded significantly superior values compared to the check clone RRIM 600, which is a proven drought tolerant clone. Four accessions, viz., MT 1623, MT 200, MT 3714, and MT 48 showed better values than the general mean. Among the check clones, the highest IXP was recorded for RRIM 600 followed by RRIM 208 whereas, in Tjir 1 which is a drought susceptible clone it was relatively low. Three accessions, viz., MT 1623,

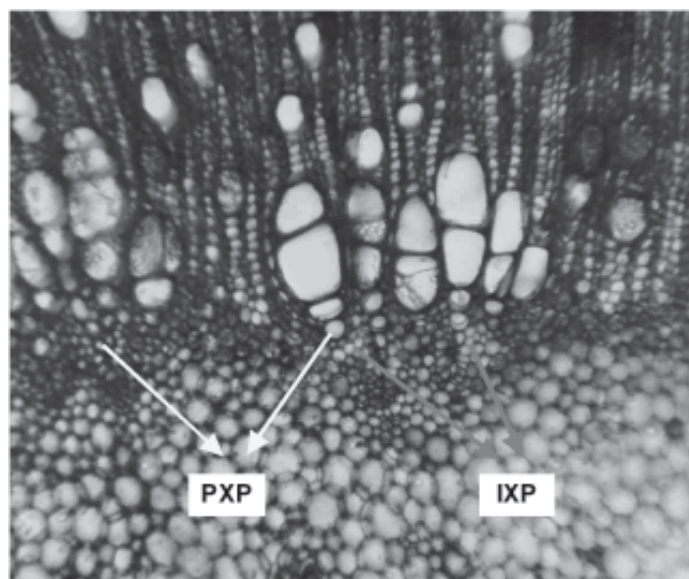


Fig. 1: Transverse section of a twig showing primary xylem and intraxylary phloem in *Hevea brasiliensis* (PXP–primary xylem points; IXP–intraxylary phloem)

Table 7. Potential accessions for structural traits along with twig diameter

Accession/clone	No. of proto xylem points (PXP)	No. of intraxylary phloem points (IXP)	Diameter of twig (mm)	IXP/ mm diameter of twig
MT 1623	104.50	77.00	15.60	4.94
MT 200	81.80	75.38	20.30	1.82
MT 3714	74.25	59.25	17.90	3.31
MT 48	54.67	38.79	12.80	3.03
Tjir 1	60.83	30.67	12.50	2.45
RRII 105	48.60	38.20	9.00	4.24
RRII 208	56.00	39.88	11.90	3.35
RRIM 600	57.48	49.15	7.70	6.38
V.R.	3.24**	3.71**	7.32**	
CD	30.15	30.40	3.88	
CV	26.53	42.04	15.08	

** Significant at 1%

MT 200 and MT 3714 recorded higher values for this trait as compared to RRIM 600, of which the former two accessions were significantly superior to RRII 208. The variability for IXP among the different accessions was higher than that of the variability for PXP, as indicated by the high CV for this trait. Diameter of twigs varied from 7.50 (MT 52) to 20.30 mm (MT 200). A relative comparison of the proportion of IXP per mm. diameter of twig also showed the superiority of the accession MT 1623.

Occurrence of an internal phloem (intraxylary) in the xylem tissue is reported in some species of Euphorbiaceae (Metcalfe and Chalk, 1950). In *Hevea*, the occurrence of adaxial (medullary) phloem associated

with protoxylem was noticed first, during the studies on periodicity of cambial activity by Premakumari *et al* (1985). Premakumari and Panikkar (1988) and Premakumari (1992) further observed that PXP and IXP are highly significant clonal characters and there is a positive correlation between the number of intra xylary phloem points and rate of girth increment under tapping. According to Zamski and Tsivion (1977) in tobacco, girdling activated the internal phloem to take on the functions of external phloem for transporting assimilates. Moreover, quantitative analysis of conductive tissues is observed to show a close correspondence of wood structure in relation to habitat (Carlquist and Debuhr, 2008). In the present study, the high variability for IXP

Table 8. Morphological characterization based on drought sensitive parameters

Accn.	Leaf size	Leaf surface	Wax coat	Yellowing (%)	Vigour	Senescence (%)
MT1631	Narrow	Flat	Nil	37.5	Low	100
MT 1710	Narrow	Curved	Nil	10	Medium	80
AC 462	Narrow	Flat	Nil	68.33	Medium	100
MT 3714	Narrow	Curved	Nil	100	Medium	84
AC 441	Narrow	Flat	Nil	100	Medium	100
MT 81	Broad	Flat	Nil	13.75	Medium	8.33
MT 200	Broad	Flat	Nil	61.25	High	45
RO 217	Narrow	Flat	Nil	40.0	High	34
RO 1520	Broad	Flat	Nil	88	Low	86
MT 1623	Narrow	Curved	Nil	7.5	High	0
MT 1681	Narrow	Curved	Nil	12.5	High	7.0
MT 52	Narrow	Flat	Nil	50.0	Low	54
MT 4242	Narrow	Flat	Nil	42.0	Medium	42
MT 60	Broad	Flat	Nil	76.25	Medium	80
MT 48	Broad	Flat	Nil	63.0	Medium	68
MT 1584	Broad	Flat	Nil	42.0	Medium	50
RRII 208	Broad	Flat	Nil	43.0	Medium	52
RRIM 600	Narrow/Small	Flat	Nil	48.0	High	47
Tjir 1	Narrow/small	Flat	Nil	61.25	Medium	55
RRII 105	Narrow/small	Flat	Yes	100.0	Medium	100

observed is in concurrence with earlier observations (Mary, 2004). The wild accession MT 1623, which showed superiority for most of the characters in this study, also exhibited significantly superior values for IXP, compared to the proven drought tolerant clones RRIM 600 and RRII 208 suggesting the use of this trait for selection towards potential drought tolerant accessions. The linear relationship observed for IXP tissues and growth as well as the possible activation of this tissue under conditions of stress assumes significance in this context.

Morphological characterization based on drought sensitive parameters such as leaf size, appearance of leaf surface, presence or absence of wax coating on leaf surface, leaf yellowing and senescence and vigor of plant was also done (Table 8) for identifying the potential drought tolerant accessions. It was found that among the 16 wild accessions studied, the accessions MT 1681 and MT 1623 were the potential ones for further detailed field evaluation towards developing a drought tolerant *Hevea* clone. Field level screening of the wild accessions conducted in the drought prone area in Dapchari, Maharashtra, also has proved genotypic difference among the accessions towards drought tolerance (Mercy *et al.*, 2009).

Natural rubber cultivation in India faces adverse effects of drought and cold stresses especially in the non-traditional rubber growing areas (Jacob *et al.*, 1999). As the genetically divergent germplasm accessions are

an excellent repository of various useful traits including stress tolerance, systematic screening of wild germplasm for drought and cold tolerance with the ultimate objective of developing location specific clones for non-traditional rubber growing areas holds much importance.

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Evaluation of Exotic Germplasm of Indian Mustard (*Brassica juncea*) for Economic Traits

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Genetic diversity in *Brassica* crop is limited as compared to the variability within the species and its relatives including exotic germplasm. The exotic germplasm lines have been found to be reservoir of genetic diversity, especially to several agro-morphological traits and disease resistance which causes severe yield losses in the country. Therefore, one hundred exotic accessions of Indian mustard were evaluated for various agro-morphological, quality traits and disease resistance against two major diseases of this crop, *i.e.*, Alternaria blight (*Alternaria brassicae*) and white rust (*Albugo candida*). Among the traits studied, the highest variability was observed for secondary branches per plant (CV 47.3%) followed by silique beak length (CV 43.6%) while the least variability was observed for oil content (CV 4.3%) followed by protein content (CV 6%). All the exotic accessions were small seeded. More than sixty percent of total accessions were late maturing. The accession EC511435 was identified as a useful donor for early flowering, more secondary branches per plant and high seed yield per plant as compared to best check. However, EC511407 and EC511435 were identified as donors for high oil content and high seed yield. Seed yield was positively and significantly correlated with main shoot length, siliques on main shoot, plant height and oil content. Seven accessions showed tolerance to Alternaria blight (percent disease severity or PDS < 10), while eleven accessions to white rust (PDS < 1). However, four accessions namely, EC511504, EC511507, EC511472 and EC511513 showed combined tolerance to both the diseases.

Key Words: Accessions, Donors, Evaluation, Variability

Introduction

Rapeseed-mustard is a group of seven cultivated oilseed crops of *Brassica* species. Indian mustard (*Brassica juncea* L. Czern & Coss) is an annual species belonging to family Brassicaceae and amongst the most important species of seven cultivated oilseeds of India. The family Brassicaceae contains about 3500 species and 350 genera and is placed among the 10 most economically important plant families (Warwick *et al.*, 2000). The crop *Brassica* display enormous diversity and are mainly used as sources of oil, vegetables, condiments, and fodder. A wider genetic base assumes priority in breeding research aimed at developing new varieties with desired traits. Germplasm play an important role in crop improvement programme of rapeseed-mustard (Kumar *et al.*, 2004; Kumar and Misra 2007; Misra 2008; Misra and Kumar, 2008). Genetic diversity within elite cultivars of a crop is limited compared to the variability within the species and its relatives including exotic germplasm. Exotic germplasm refers to crop varieties unadapted to a breeder's target environment, and is an underutilized resource for crop improvement (Holland, 2004). This exotic germplasm represent unique alleles for productivity that are absent

from elite crop gene pools, and may protect the crop against new biotic and abiotic stresses (Zamir, 2001). *Brassica* has narrow genetic base thus variability available in present germplasm of rapeseed-mustard is limited to few desired traits (Kumar *et al.*, 2000). There is no resistance source available for Alternaria blight and white rust, in available indigenous germplasm collection, however, few donors are available for some traits but they are location or race specific (Misra, 2004a). The exotic germplasm lines have been found to be reservoir of genetic diversity, especially to several agro-morphological traits and disease resistance which causes severe yield losses in the country. Estimated yield losses due to Alternaria blight varied between 10 to 70% in different species of these crops (Saharan and Chand, 1988; Kolte, 1991). It is evident that many of the exotic lines possess desirable traits which are otherwise not available in cultivated oilseed *Brassic*as. Thus, the exotic germplasm of crop plants are one of the important sources of some of the agronomical and disease resistance traits for the development of improved cultivars.

A number of rapeseed-mustard varieties have been developed by utilization of exotic germplasm (Misra,

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2004b). Some of the exotic lines were characterized and promising donors were also identified in *B. juncea* (Misra *et al.*, 2005a), *B. carinata* and *Eruca sativa* (Misra *et al.*, 2005b). There are limited reports on evaluation and utilization of exotic germplasm in rapeseed-mustard. Therefore, the present study was aimed to evaluate the diversity in exotic accessions of Indian mustard and their reaction to two major diseases of *Brassica* crop.

Materials and Methods

Seeds of one hundred exotic germplasm accessions of Indian mustard (*Brassica juncea* L. Czern & Coss) were evaluated for 15 agro-morphological, quality traits and disease resistance for two major diseases of the crop i.e. Alternaria blight (*Alternaria brassicae*) and white rust (*Albugo candida*). These exotic accessions of *B. juncea* were acquired from Canada through National Bureau of Plant Genetic Resources (Indian Council of Agricultural Research), New Delhi (India). The experiment was conducted during *rabi* season (post-rainy) of 2007-2008, under Augmented complete Block Design in 30 cm x 10 cm spacing, with three national checks (BIO 902, PCR 7 and RH 30) at the Directorate of Rapeseed-Mustard Research (formerly National Research Centre on Rapeseed-Mustard) Bharatpur, Rajasthan. Recommended standard agronomic package of practices were adopted as and when required to raise a healthy crop. The observations were recorded on randomly tagged five plants for different traits at appropriate growth stages for initiation of flowering, 50% flowering, maturity, plant height, primary branches and secondary branches per plant, main shoot length, siliquae on main shoot, siliqua length, siliqua beak length, seeds per siliqua and seed

yield per plant. 1000 seeds were counted by electronic seed counter (Contador, Germany) and weighed by electronic balance. Further, protein and oil content were analyzed by Near Infrared Reflectance Spectroscopy (Dickey- John, Instalab 600). While, Alternaria blight and white rust severity recorded on leaves and pods after 90 days sowing using standard pictorial scale of Conn *et al.* (1990). Range, mean, and coefficient of variation were computed using standard statistical methods (Gomez and Gomez 1984).

Results and Discussion

A range of variability observed in all the agro-morphological traits. Among the traits studied, the highest variability was observed (Table 1) for secondary branches per plant (CV 47.3%) followed by siliqua beak length (CV 43.6%) and the least variability was observed for oil content (CV 4.4%) followed by protein content (CV 6%). The over all mean value of initiation of flowering was 72.5 and ranged between 41 to 116 days, the accessions EC511435 was earliest in flowering followed by EC511615, EC537892 and EC537896. The mean value of days to 50% flowering was 89.2 and ranged 53-123 days, the accession EC511504 was earliest for this trait which followed by EC511640 and EC537894. The plant height ranged from 100 cm to 320 cm with mean value of 188.6 cm. Siliquae on main shoot ranged 8-64 and accession EC511663 had highest number of siliquae. Seed yield per plant ranged 0.39-58.8 and accession EC511435 gave highest seed yield. The accession EC511435 was identified as a useful donor for early flowering, more secondary branches per plant and high seed yield per plant as compared to the best check. There

Table 1. Range, mean and coefficient of variation (CV) for different traits in exotic accessions of Indian mustard

Traits	Range	Mean $\pm$ SEM	CV (%)	Mean values of checks		
				BIO 902	PCR 7	RH30
Initial flowering (days)	41-116	72.5 $\pm$ 1.6	23.3	59	52	51
50% flowering (days)	53-123	89.2 $\pm$ 1.3	15.3	65	64	58
Plant height (cm)	100-320	188.6 $\pm$ 3.3	21.3	169.3	167.5	223.5
Primary branches per plant	4-15	7.3 $\pm$ 0.2	30.5	7.6	6	7.5
Secondary branches per plant	2-22	8.5 $\pm$ 0.3	47.3	11.3	8	9
Main shoot length (cm)	10-65	34.8 $\pm$ 1.0	33.9	47.6	54.5	66.5
Siliquae on main shoot	8-64	31.9 $\pm$ 0.9	34.1	39.3	40	43
Maturity (days)	118-163	144.6 $\pm$ 0.8	6.3	136	126	130.5
Siliqua beak length (cm)	0.1-1.2	0.4 $\pm$ 0.02	43.6	0.94	0.98	0.96
Siliqua length (cm)	1.1-4.7	2.0 $\pm$ 0.1	39.5	3.7	4.65	4.65
Seed yield per plant (g)	1.4-38.8	7.4 $\pm$ 0.9	30.4	11.2	7.58	21
1000-seed weight (g)	1.1-4.2	2.3 $\pm$ 0.1	24.5	3.58	5.72	4.78
Seeds per siliqua	2.3-19	10.6 $\pm$ 0.3	28.7	13.95	17.28	12.48
Protein content (%)	19.2-26.2	23.1 $\pm$ 0.1	6.0	23.1	21.5	21.05
Oil content (%)	33.1-41.8	38.9 $\pm$ 0.2	4.3	37.6	39.1	39.08

has been a wide variability for seed yield contributing traits such as primary and secondary branches per plant, seed yield per plant, siliquae on main shoot and seeds per silique. EC511407 and EC511435 were identified as useful donors for high oil content and seed yield. Promising donors were identified (Table 2) for various economic traits which can be further used for future breeding programme. However, exotic lines were not found superior for some economic traits such as, 1000-seed weight, main shoot length, silique length and silique beak length among the tested accessions.

Accessions EC511472, EC511478, EC511504, EC511507, EC511513, EC511732, EC537897 and EC537898 showed tolerance to *Alternaria* blight (Per cent Disease Severity or PDS < 10), while accessions EC511472, EC511488, EC511489, EC511490, EC511504, EC511507, EC511513, EC511516, EC511517, EC511518 and EC511718 to white rust (PDS < 1). However, accessions EC511504, EC511507, EC511472 and EC511513 showed combined tolerance to both the diseases (Table 3). Tolerance to the two diseases has also been reported by earlier workers (Chattopadhyay and Séguin-Swartz, 2004; Misra *et al.*, 2005a).

Characterization and evaluation of germplasm is the primary step in any plant breeding programme. Earlier most of the studies were on indigenous accessions (Yadav *et al.*, 1997; Meena *et al.*, 2000; Misra *et al.*, 2004). In the present investigation, evaluation was done on exotic accessions for 15 agro-morphological, quality traits and disease resistance for two major diseases of the crop,

i.e., *Alternaria* blight (*Alternaria brassicae*) and white rust (*Albugo candida*) and several useful accessions were identified (Tables 2 and 3).

All the accessions were small seeded (light weight) and short silique. The exotic germplasm were very tall (>75%) and were late maturing (>60%). In general these had short main shoot length (>70%) with few siliquae (around 80%) and few seeds in each silique (>70%). In addition, these lines also had medium range of oil per cent. Therefore, the exotic germplasm might be a very useful source as one of the parent material for improvement of crop *per se*.

Correlation among different traits is one of the practical means in understanding the behaviour of the accessions and might be useful in selecting desirable clones in a breeding programme. Computation of correlation between seed yield contributing characters and yield are of considerable importance in plant selection. There is significant difference for the characters studied among the genotypes, which demonstrated adequate variability among them. Seed yield was positively and significantly correlated with main shoot length, siliquae on main shoot, plant height and oil content. Seed yield showed negative but significant correlation with initiation of flowering, 50% flowering and protein content. Some of the component characters also showed positive and significant correlation among themselves like 1000-seed weight with plant height, oil content and seed yield per plant, siliquae on main shoot with plant height and main shoot length (Table 4). This indicates that simultaneous

Table 2. List of promising genotypes identified

Traits	Name of genotypes
Initiation of flowering (days)	≤ 51 :EC511435, EC537896, EC537892, EC511615
50 % flowering (days)	≤ 58:EC511435, EC511615, EC537896
Plant height (cm)	≤ 167.5 :EC511504, EC537894, EC511640
Primary branches per plant	e" 7.6 :EC511734, EC511436, EC537892, EC537897
Secondary branches per plant	e" 11.3 :EC511436, EC537892, EC537899, EC511458, EC511435
Siliquae on main shoot	e" 43:EC511663, EC511419, EC511657
Seed yield per plant (g)	e"21:EC511435, EC511608, EC511604
Seeds per silique	e" 17.3 :EC511436, EC511632, EC537896
Days to maturity (days)	≤ 126 :EC537892, EC537896, EC511433
Protein content (%)	e" 23.1:EC511493, EC511440, EC511429
Oil content (%)	e" 39.1:EC511407, EC511679, EC511601

Table 3. Exotic accessions showing tolerance to various diseases

<i>Alternaria</i> blight tolerant genotypes	White rust tolerant genotypes
EC511472, EC511478 EC511504, EC511507, EC511513, EC511732, EC537897 and EC537898	EC511472, EC511488, EC511489, EC511490, EC511504, EC511507, EC511513, EC511516, EC511517, EC511518 and EC511718

Table 4. Correlation among different traits of exotic accessions

Traits	Initial flowering	50% flowering	Maturity	Plant height	Primary branches	Secondary branches	Main shoot length	Siliquae on main shoot	Protein content	Oil content	Seed yield/plant	1000 seed weight	Siliqua beak length	Siliqua length
50% Flowering	0.79*													
Primary branches	-0.07	-0.15	0.04	0.03										
Secondary branches	-0.08	-0.16	0.24*	0.26*	0.69*									
Main shoot length	-0.40*	-0.36*	-0.28*	0.07	0.01	0.10								
Siliquae on main shoot	-0.40*	-0.24*	-0.13	0.28*	-0.02	0.14	0.69*							
Protein content	0.20*	0.33*	0.28*	0.05	0.04	0.07	-0.22*	-0.15						
Oil content	0.02	0.12	-0.03	0.23*	-0.36*	-0.26*	0.04	0.14	-0.46*					
Seed yield/ plant	-0.39*	-0.47*	-0.03	0.19*	-0.04	-0.04	0.24*	0.22*	-0.41*	0.29*				
1000 seed weight	-0.19*	-0.28*	-0.05	0.18*	-0.22*	-0.03	0.17	0.16	-0.29*	0.19*	0.32*			
Siliqua beak length	-0.27*	-0.43*	-0.32*	-0.25*	0.19*	0.04	0.26*	0.17	-0.30*	-0.11	0.29*	0.37*		
Siliqua length	-0.28*	-0.42*	-0.25*	-0.18*	0.20*	0.09	0.23*	0.19*	-0.35*	-0.07	0.29*	0.38*	0.89*	
Seeds/ siliqua	0.02	0.05	0.12	0.14	0.03	0.16	0.13	0.14	0.08	0.03	0.00	0.11	0.04	0.11

* Significant at 5%

improvement for these components is possible in exotic lines of mustard. Similar trend for these traits have conformity with the some of the earlier reports in brassicas (Yadav *et al.*, 1997; Meena *et al.*, 2000; Misra *et al.*, 2004, 2005b).

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Correlation and Path Coefficient Analysis for Agronomical Traits in Forage Sorghum [*Sorghum bicolor* (L.) Moench]

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Forage sorghum germplasm field experiment was conducted at Sorghum Research Station, Deesa (Gujarat) during *kharif* 2008 by using 144 genotypes to study the variability parameters and genotypic and phenotypic correlations and path analysis among agronomic traits. Significant variation among all the genotypes was recorded for all the characters. Most of the characters had higher genotypic and phenotypic coefficient of variation which is indicative that character expression in this sorghum population was genetic and can be exploited in breeding programs. The genotypes also exhibited varying degrees of heritability and genetic advance. Characters such as plant height, stem girth and dry and green fodder yield responded positively to selection because of high broad sense heritability and high genetic advance. Plant height, stem girth and leaf length were positively and significantly associated with green fodder and dry fodder yield per plant. Leaf breath was positively and significantly associated with plant height, number of leaves per plant and leaf length. The path coefficient analysis indicated positive and significant correlation as well as high direct effect of dry fodder yield per plant, plant height, number of leaves per plant and stem girth on green fodder yield per plant.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) crop is genetically suited to hot and dry agro-ecologies with frequent drought, where it is difficult to grow other crops. In north Gujarat areas sorghum is mainly grown for dual and forage purposes. Therefore, it can play a vital role for the uplift of socio-economic status of the farmers of north Gujarat areas through development of high yielding varieties along with reasonable amount of green and dry fodder for the livestock.

The study of relationships among quantitative traits is important for assessing the feasibility of joint selection of two or more traits and hence for evaluating the effect of selection for secondary traits on genetic gain for the primary trait under consideration. A positive genetic correlation between two desirable traits makes the job of the plant breeder easy for improving both traits simultaneously. The path coefficient analysis allows partitioning of correlation coefficient into direct and indirect contributions (effects) of various traits towards dependent variable and thus helps in assessing the cause-effect relationship as well as effective selection. Hence, this study is aimed to analyze and determine the traits having greater interrelationship with yield utilizing the correlation and path analysis for different traits in forage sorghum.

Materials and Methods

One-hundred forty-four forage sorghum genotypes collected from Directorate of Sorghum Research (DSR), Hyderabad and local landraces of Gujarat were used for the present study. The trial was grown in augmented block design at Sorghum Research Station, Sardar Krushinagar Dantiwada Agricultural University, Deesa (Gujarat) during 2008 *kharif* season. Deesa is situated at latitude of 24.5° N and longitude 72° E and at an elevation of 136 m above the msl. The soil of the field was sandy in texture with pH value of 7.5 to 8.0 having good physical and chemical properties (Organic Carbon= 0.23, EC dsm⁻¹ = 0.232, K₂O= 259.9 kg/ha and P₂O₅= 46.2 kg/ha). The experimental unit was a single-row plot of 6.75 m long, spaced 0.45 m apart. NPK 120:40:00 fertilizers was applied as half basal dose of nitrogen and full dose of phosphorus at the time of sowing and half nitrogen applied after one month of sowing. Plots were thinned down after two weeks of crop emergence and plant-to-plant distance of 0.10 m was maintained. The total rainfall during the crop season was 495.4 mm. The other recommended agronomic practices were followed to raise a good crop. Data was taken on time to 50% flowering (days), plant height (cm), number of leaves per plant, leaf length (cm), leaf breath (cm), stem girth (cm), leaf stem ratio, green fodder yield per plant(g) and dry fodder yield per plant (g). Analysis of variance was done by method suggested by Panse

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and Sukhatme (1978). Genotypic and phenotypic coefficient of variability, heritability and genetic gain, genotypic and phenotypic correlation coefficient and path coefficient using standard method suggested by Burton (1952), Johnson, *et al.* (1955), Al-Jibouri, *et al.* (1958) and Dewey and Lu (1959), respectively.

Results and Discussion

Analysis of variance indicated significant differences among genotypes for all the traits studied, indicating thereby the presence of wide range of variability (Table 1). The genetic constants for the characters revealed that the magnitude of phenotypic coefficient of variation (PCV) was higher than the corresponding genotypic coefficient of variation (GCV) for all the traits denoting environmental factors influencing their expression to some degree or other. Wide differences between PCV and GCV implied their susceptibility to environmental fluctuation, whereas narrow differences between PCV and GCV suggested their relative resistance to environmental alteration. In the present study, the PCV and GCV were higher for plant height (cm), number of leaves per plant, leaf breadth (cm), stem girth (cm), leaf stem ratio, green fodder yield per plant (g) and dry fodder yield per plant (g) (Table 2). High amount of GCV and PCV suggested greater scope for selection of superior genotypes for these traits while the lower degree of GCV and PCV for leaf length and days to

50% flowering indicated that improvement for such traits may be achieved only up to some extent. Similar findings were also obtained from earlier reports (Warkad *et al.*, 2008).

The estimate of GCV and PCV alone is not much helpful in determining the heritable portion. The amount of advance to be expected from selection can be achieved by estimating heritability along with coefficient of variability. Burton also suggested that GCV and heritability estimate would give better information about the efficiency of selection (Burton, 1952). The heritability ranged from 63.56% for days to 50% flowering to 92.49% for stem girth. The high degree of heritability estimate for most of the traits suggested that the characters are under genotypic control. Similar result was also reported by Warkad *et al.* (2008). The high heritability doesn't necessarily mean high genetic gain and alone is not sufficient to make improvement through selection. The utility of heritability is increased when it is used to estimate genetic advance (Johnson *et al.*, 1955). Thus the genetic advance has an added edge over heritability as a guiding factor to breeders in selection programme. High heritability coupled with high genetic advance and GCV were noticed for green fodder yield/plant, dry fodder yield/plant and plant height indicating control of additive gene action. Simple selection model will be good enough to do needful and no additional gain is achieved by using sophisticated models.

Table 1. Analysis of variation for different traits in forage sorghum

Source	DF	Days to 50% flowering	Plant height (cm)	No. of leaves per plant	Leaf length (cm)	Leaf breadth (cm)	Stem girth (cm)	Leaf stem ratio	Green fodder yield per plant (g)	Dry fodder yield per plant (g)
Replication	6	10.90	917.90*	8.90	54.78	0.23	1.12	0.01	3232.59**	888.27**
Treatments	143	127.18**	1621.45**	10.71**	79.32	1.22**	2.35**	0.2**	4282.68**	891.03**
(a) Checks	3	1294.32**	2262.14**	24.79**	474.99**	1.18**	3.59**	0.1**	6852.63**	1737.00**
(b) Germplasm	140	102.17**	1607.72**	10.41**	70.84**	1.22**	2.32**	0.2**	4227.61**	872.90**
Error	18	16.79	286.59	3.46	71.52	0.23	0.17	0.01	771.47	185.91

Table 2. Variability parameters for different traits in forage sorghum

Characters	CV	SE	PCV	GCV	H ²	GA	GG
Days to 50% flowering	6.17	2.19	13.52	12.36	83.56	17.40	23.28
Plant height (cm)	8.12	9.05	29.95	36.90	92.17	67.87	87.16
No. of leaves per plant	19.63	0.99	36.60	29.91	66.79	4.44	50.36
Leaf length (cm)	13.05	4.52	13.88	9.00	90.00	0.17	0.27
Leaf breadth (cm)	8.88	0.26	24.06	21.69	81.24	1.85	40.26
Stem girth (cm)	12.51	0.22	49.67	47.77	92.49	2.90	94.64
Leaf stem ratio	30.89	0.04	43.29	35.99	69.09	0.21	61.62
Green fodder yield per plant (g)	23.33	14.85	50.73	45.87	81.75	109.50	85.43
Dry fodder yield per plant (g)	23.69	7.29	54.89	48.69	78.70	47.90	88.99

Genotypic and phenotypic correlation coefficients between the various traits, computed are presented in Table 3. Genotypic correlation coefficient provides measures of genetic association between traits and thus helps to identify the more important as well as less important traits to be considered in breeding programmes. For most of the association in this study, the genotypic correlation coefficients are higher than phenotypic correlation coefficients. Higher genotypic correlations of characters than phenotypic correlation coefficients had earlier been reported (Alhassan *et al.*, 2008). Expressions of higher genotypic than phenotypic correlations are indications of strong inherent relationships between these characters (Johnson, 1955). However, when phenotypic correlations are higher than genotypic correlations, environmental effect or non-additive effects are acting on the trait in the same direction. Plant height, number of leaves per plant, stem girth and leaf length

were positively and significantly associated with green fodder and dry fodder yield per plant. Leaf breadth was positively and significantly associated with plant height, number of leaves per plant and leaf length. However, number of leaves per plant was negatively and significantly correlated with leaf length. All other traits indicated no significant correlation among themselves. Green and dry fodder yield per plant had a strong association with leaf length, number of leaves per plant and plant height and stem girth. This indicated that when the number of leaves are many, there will be a greater surface area for photosynthesis; greater photosynthesis can translate into more photosynthates, ultimately resulting in increased fodder yield (Alhassan *et al.*, 2008). In maize also a positive association between photo synthetically active leaf number and grain and fodder yield had been indicated (Mohammed *et al.*, 2003). Path analysis provides an effective means of partitioning direct and indirect causes

Table 3. Correlation matrix for different traits in forage sorghum

Character	r	Plant height (cm)	No. of leaves per plant	Leaf length (cm)	Leaf breadth (cm)	Stem girth (cm)	Leaf stem ratio	Green fodder yield per plant (g)	Dry fodder yield per plant (g)
Days to 50% flowering	g p	0.111 0.079	0.213 0.081	-0.100 0.183	0.243 0.156	0.140 0.103	0.150 0.076	0.236 0.166	0.395* 0.333*
Plant height (cm)	g p		0.685** 0.550**	-0.220 0.330	0.769** 0.517**	-0.067 -0.022	-0.448* -0.284	0.796** 0.766**	0.538* 0.416*
No. of leaves per plant	g p			-0.491* -0.451*	0.635** 0.529**	-0.086 -0.031	-0.138 -0.142	0.833** 0.710**	0.276 0.399*
Leaf length (cm)	g p				0.600** 0.500*	0.200 0.195	-0.100 -0.246	0.723** 0.610**	0.390* -0.020
Leaf breadth (cm)	g p					-0.131 -0.111	-0.222 -0.256	0.207 0.233	-0.021 0.040
Stem girth (cm)	g p						-0.102 -0.106	0.630** 0.639**	0.499* 0.433*
Leaf stem ratio	g p							0.064 0.026	0.191 0.083
Green fodder yield per plant (g)	g p								0.873** 0.891**

Table 4. Path analysis for green fodder yield per plant (g) in forage sorghum

Character	Days to 50% flowering	Plant height (cm)	No. of leaves per plant	Leaf length (cm)	Leaf breadth (cm)	Stem girth (cm)	Leaf stem ratio	Dry fodder yield per plant (g)	r
Days to 50% flowering	-0.2623	-0.0828	-0.0571	-0.0245	0.2306	0.0183	-0.0273	0.5385	0.3334
Plant height (cm)	0.0292	0.5441	0.0841	0.012	0.0221	-0.0087	0.0819	0.0321	0.0796
No. of leaves per plant	-0.3857	-0.5098	0.868	-0.1322	0.6027	-0.0112	0.0252	0.3767	0.8337
Leaf length (cm)	0.3605	0.421	0.4188	-0.1222	-0.5456	-0.1744	0.6432	-0.2775	0.7238
Leaf breadth (cm)	-0.0637	-0.5719	-0.1706	-0.3451	0.9495	-0.0170	0.0406	-0.0293	-0.2075
Stem girth (cm)	-0.0368	0.0496	0.0231	-0.111	-0.1241	0.1305	0.0187	0.6804	0.6304
Leaf stem ratio	-0.0392	0.3337	0.0372	-0.1212	-0.2112	-0.0134	-0.1826	0.2612	0.0645
Dry fodder yield per plant (g)	-0.1035	-0.1772	-0.0742	-0.1452	-0.0204	0.0651	-0.0350	1.3640	0.8738

Res. Effect: 0.0774

of association while permitting a critical examination of the specific forces to produce a given correlation and measure the relative importance of each causal factor. The path coefficient analysis indicated positive and significant correlation as well as high direct effect of dry fodder yield per plant, plant height, number of leaves per plant and stem girth on green fodder yield per plant. A similar finding was also reported by Sankarapandian (2000). Results from the present study indicated that selection for plant height, leaf length, number of leaves per plant, stem girth will be the best indirect selection indices for increasing green and dry fodder yield.

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Characterization of Chow-Chow (*Sechium edule*) Germplasm of North-eastern Region of India for Economic Traits

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A total of thirty-eight accessions of Chow-chow were collected from Meghalaya (20), Mizoram (2) and Sikkim (16). These accessions were grown in augmented design at Barapani (Meghalaya) to study the economic traits both quantitative as well as qualitative. A range of fruit colour was observed such as dark green, light green, yellow, light yellow, cream and white. A total of 13 accessions were found without spine and remaining were having spine from soft to hard in nature. The fruit length varied from 5.7 cm (Sikkim-11) to 15.1 cm (Megha-21) with average length of 11.3 cm. The maximum fruit diameter was recorded 10.9 cm for Sikkim-16 followed by 10.3 for Megha-13 and 10.2 for Megha-14 while lowest 5.2 cm for Sikkim-3. The number of fruits per plant ranged between 2.5 (Sikkim-3) to 73.3 (Megha-20) with average fruit per plant of 28.2. The average fruit weight varied from 134 gm (Megha-9) to 406 gm (Megha-3). The highest fruit yield per plant was recorded 25.0 kg for Sikkim-17 while lowest 0.50 kg for Sikkim-3. The average fruit yield per plant was 7.6 kg. The accessions collected from Meghalaya were early maturing while the accessions collected from Sikkim late in maturity. Biochemical analysis of fruit revealed that higher TSS content observed for Megha-16 (6.2%), Sikkim-12 (6%) and Sikkim-10 (5.5%) while high level of ascorbic acid 24.7 mg/100gm in Sikkim-1 and followed by 23.4 mg/100gm in Sikkim-13.

Key Words: Chow-chow, Characterization, NEH region, Germplasm

Introduction

Chow-chow (*Sechium edule* Sw.), an underutilized cucurbitaceous vegetable, native to Central America and humid tropical region of Mexico (Singh, 1990), is monoecious, perennial vine with tuberous roots. Its vine produces large number of fruits that are single seeded and viviparous. In India, chow-chow is widely grown in Madurai and Nilgiri district of Tamil Nadu, Karnataka, West Bengal, Mandi district of Himachal Pradesh and entire North Eastern Hill (NEH) region. In NEH region, chow-chow is grown in kitchen gardens of every tribal, as an important component of their daily diet. Fruits of this crop are mainly used as vegetable and it is also used for making sweets and sauce. Though, it is a native of Mexico but considerable diversity is found in NEH region particularly, Meghalaya, Mizoram, and Sikkim (Rai *et al.*, 2006). Mizoram is the leading state with an estimated area of 845 ha and 10985 MT production (Sanwal, 2008) It is mainly propagated by means of seed (whole fruit with seed)/ sprouted fruits, which are main cause of variation existing in the region (Yadav *et al.*, 2005) and Rai *et al.* (2002). Therefore, an attempt has been made to collect and characterize chow-chow germplasm of this region.

Materials and Methods

A total of thirty eight accessions of chow-chow were collected from Meghalaya (20), Mizoram (2) and Sikkim (16) states of NEH region of India. The sprouted fruits of all the genotypes were planted at experimental farm of Division of Horticulture, ICAR Research Complex for NEH region, Umiam, Meghalaya during the rainy season of 2006-07 in Augmented Complete Block Design. The sprouted fruits of each genotype were planted in hill at spacing of 2.5m between row to row and 2 m within rows consisting 5 plants in each row, having five rows of each accession. The plants were trained on bamboo thatch. The observations were recorded for ten quantitative and qualitative traits, viz. fruit length, fruit diameter, number of fruits per plant, average fruit weight, fruit yield per plant, spine character, fruit colour, TSS, acidity and ascorbic acid. Mean data of quality and quantitative characters were subjected to statistical analysis.

Results and Discussion

The qualitative and quantitative traits are presented in Table 1. A range of fruit colour was observed such as dark green, light green, yellow, light yellow, cream

Table 1. Quantitative and qualitative characters of Chow-chow

Genotypes	Fruit length (cm)	Fruit diameter (cm)	No. of fruits/plant	Average fruit weight(gm)	Fruit yield/plant (kg)	Spine	Fruit colour	TSS %	Acidity %	Ascorbic acid (mg/100 g)
Megha-1	10.86	7.36	33.67	396	13.33	Less spine	Dark green	3.60	0.32	10.58
Megha-2	13.66	7.32	13.67	207	2.83	No spine	Light green	3.00	0.26	14.10
Megha-3	12.92	7.40	33.00	406	13.42	Hard, more spine	Dark green	3.40	0.38	14.10
Megha-4	9.12	5.70	13.67	137	1.88	No spine	Light yellow	2.80	0.19	14.10
Megha-5	13.52	7.44	33.67	248	8.33	Less spine	Yellow	3.00	0.32	10.58
Megha-6	12.00	6.92	39.00	256	10.00	Very hard	Light yellow	2.80	0.45	10.58
Megha-7	13.80	7.10	9.00	239	2.15	Less spine	Light green	3.00	0.38	17.63
Megha-8	10.94	6.70	27.33	159	4.33	No spine	Green	2.80	0.26	10.58
Megha-9	10.10	6.90	31.40	134	4.20	No spine	White	3.60	0.32	10.58
Megha-10	14.50	8.62	19.17	296	5.67	Less spine	White	2.60	0.26	17.63
Megha-12	12.80	8.90	37.50	326	12.25	Less spine	Light green	5.00	0.32	10.48
Megha-13	12.40	10.30	24.67	267	6.58	Less spine	Light green	4.80	0.32	10.40
Megha-14	12.20	10.20	32.67	220	7.20	Less spine	Light green	4.60	0.26	9.36
Megha-15	13.80	7.46	51.33	261	13.42	Less soft	Light green	5.00	0.38	9.88
Megha-16	12.20	7.70	50.33	215	10.83	Less spine	Light green	6.20	0.70	9.88
Megha-17	14.76	8.62	49.67	285	14.17	No spine	Light green	4.90	0.38	10.48
Megha-18	12.80	8.90	28.67	302	8.67	No spine	Light green	0.40	0.20	10.48
Megha-19	7.20	6.10	32.00	242	7.75	Soft spine	Light green	3.40	0.38	10.58
Megha-20	11.92	7.14	81.00	370	30.00	No spine	Light green	3.00	0.96	9.88
Megha-21	15.10	8.90	71.00	366	26.00	No spine	Light green	5.00	0.77	10.40
Sikkim-1	11.00	8.10	11.50	405	4.67	Less spine	Cream	3.00	0.32	24.68
Sikkim-2	7.50	5.40	13.33	375	5.00	Hard spine	Dark green	3.20	0.26	10.58
Sikkim-3	9.30	5.20	2.50	200	0.50	Less spine	Light green	3.00	0.26	14.10
Sikkim-4	12.10	6.30	18.00	267	4.80	No spine	Light green	2.40	0.38	17.63
Sikkim-5	12.70	7.50	5.33	354	1.89	No spine	Light green	3.00	0.32	10.58
Sikkim-6	10.20	7.50	10.33	350	2.58	Hard spine	Cream	4.00	0.70	14.10
Sikkim-8	9.50	7.80	9.50	149	1.42	Spine	Light green	5.80	1.15	15.28
Sikkim-9	11.10	7.30	11.00	200	2.22	Less spine	Dark green	3.60	0.32	10.58
Sikkim-10	11.10	8.90	35.00	190	6.67	No spine	Green	5.50	1.60	9.56
Sikkim-11	5.70	4.30	20.00	350	7.00	Less spine	Cream	5.00	1.66	10.48
Sikkim-12	12.80	10.00	17.00	250	4.25	Less spine	Cream	6.00	1.60	10.40
Sikkim-13	12.00	9.80	18.00	250	4.50	Hard spine	Light green	5.00	1.15	23.40
Sikkim-14	9.80	8.80	15.00	188	2.82	No spine	Light green	5.00	1.14	10.48
Sikkim-15	8.30	8.20	33.33	330	11.00	Less spine	Light green	4.00	1.60	13.00
Sikkim-16	12.80	10.90	23.33	271	6.33	Hard spine	Light green	5.00	0.90	10.48
Sikkim-17	6.60	6.40	79.00	405	32.00	Less spine	Cream	4.80	0.90	13.00
Mizoram-2	9.00	6.70	46.00	178	8.17	No spine	Dark green	4.80	0.45	17.63
Mizoram-3	12.30	9.30	22.40	295	6.60	Less spine	Dark green	4.80	0.06	10.40
Mean	11.29	7.71	28.16	270.65	7.60			4.33	0.60	12.59
Range	5.70-15.8	5.2 –10.9	2.5-73.33	134 –406	0.50-25.0			0.40-6.2	0.06-1.66	9.36-24.68
CD at 5%	2.06	1.54	5.86	19.45	3.36			0.42	0.12	1.14

and white. As far as the spine is concerned, 13 accessions were found without spine and remaining had spine from soft to hard. The fruit length varied from 5.7 cm (Sikkim-11) to 15.1 cm (Megha-21) with average length of 11.3 cm. The maximum fruit diameter was recorded 10.9 cm for Sikkim-16 followed by 10.3 for Megha-13 and 10.2 for Megha-14 while lowest 5.2 cm for Sikkim-3. The number of fruits per plant ranged between 2.5 (Sikkim-3) to 73.3 (Megha-20) with average fruit per plant of 28.2. The average fruit weight varied from 134 gm (Megha-9) to 406 gm (Megha-3). The highest fruit yield per plant was recorded 25.0 kg for Sikkim-17 while lowest 0.50 kg for Sikkim-3. The average fruit yield per plant was 7.6 kg. Biochemical analysis of fruit revealed

that higher TSS content observed for Megha-16 (6.2%), Sikkim-12 (6%) and Sikkim-10 (5.5%) while high level of ascorbic acid 24.7 mg/100gm in Sikkim-1 and followed by 23.4 mg/100gm in Sikkim-13.

The accessions collected were classified based on fruit maturity. Accessions from Meghalaya were early maturing (May–June) while the accessions Sikkim-11, 12, 14, 15 and 17 were late in maturity (mid October to January). The remaining accessions, Sikkim-2, 3, 4, 5, 6, 8, 9, 10 and 16, Mizoram-2 and 3 were medium (August to mid October) in maturity.

Considerable variation was found in respect of various fruit characteristics like number, size, shape, weight, diameter and colour as well as presence of spine

and other biochemical traits in present study. Similar variation is observed in some of the earlier reports on indigenous chow-chow collections (Singh *et al.*, 2002, Yadav *et al.*, 2005, Rai *et al.*, 2006, Sanwal *et al.*, 2008).

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Evaluation of Morphological Diversity in Chota Gokhru (*Tribulus terrestris* L.) and Bara Gokhru (*Pedalium murex* L.)

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An attempt has been made to study the morphological diversity of chota gokhru (*Tribulus terrestris* L.) and Bara gokhru (*Pedalium murex* L.), collected from different geographical and agro-climatic regions of Agra and adjoining areas. Both the herbs springing up soon after the first showers in the month of June. *T. terrestris* L. abundantly found in this region. However, *P. murex* L. was rarely observed on road side as well as protected areas. The great deal of morphological diversity collected from various parts of Agra. *Tribulus terrestris* is perennial prostrate herb with spreading branches up to 100 cm. However, at the bank of river Yamuna it showed great morphological diversity in plant height as well as leaf size. *P. murex* is erect herb, 2-3 feet in height and leaves are broad in size showed the variability. The fruit of *T. terrestris* are globose, hard and brown in colour, mature fruit split into 5-segmented nutlets (seeds). Seeds are hard and bear 2-3 sharp spines, 10 mm long and 4-6 mm broad point to point. The fruits of *P. murex* are as indehiscent capsules 1-2 cm x 0.5-1 cm, hard, pyramidal, 4-angles with a spreading. It exhibits variability in fruit and seed characters, shape, size and colour. The fruits and seeds are of high medicinal value as they are used for treatment of various diseases.

Key Words: Morphological diversity, *Tribulus terrestris*, *Pedalium murex*

Introduction

Tribulus terrestris L. commonly known as chota gokhru belongs to family Zygophyllaceae and is found in Asia, Africa, Europe, America and Australia (Kostova *et al.*, 2002; Topia *et al.*, 1994). It is an annual or perennial, hugging herbaceous plant that grows as a summer annual in colder climates. It is one of the major medicinal plant in India, and useful in several human diseases like, dysuria, renal, vesical calculi, anorexia, dyspepsia, helminthiasis, cough, asthma and rheumatic arthritis (Shivarajan and Balachandran, 1994; Warriar *et al.*, 1996).

Pedalium murex L. is commonly known as Bara gokhru of family Pedaliaceae, it is an erect or ascending annual medicinal herb. The various parts of the plant are used in human diseases. The leaves contain mucilage, the mucilaginous water derived from leaves is used for the treatment of kidney. The leaves are also used as a vegetable in various parts of the country (Saxena, 1979). Increasing demand of herbal plants at national and international level have resulted in the over exploitation and indiscriminate over harvesting of medicinal plants. The degree of disturbance to the species population and vulnerability to over exploitation effects demand and supply (Anonymous, 1979; Robbins, 2000).

The present investigation evaluates the morphological diversity of chota gokhru (*Tribulus terrestris* L.) and

bara gokhru (*Pedalium murex* L.) in the climatic conditions of Agra.

Materials and Methods

The present study was conducted at Keetham Jheel, Runkata (Reservoir) Agra, Tundla (Firozabad), and Chambal catchments area on the river bank of Yamuna and at Bateswar (Agra). The field data was recorded on five accessions of chota gokhru (*T. terrestris* L.) and Bara gokhru (*P. murex* L.) for various morphological traits viz. plant height (cm), leaf length (cm), leaf breadth (cm), petiole length (cm), leaf area (cm), flower length (cm), length of anther (cm), length of pistil (cm), length of fruit (cm), fruit breadth (cm), and seed length (cm), with help of range, mean, standard deviation and standard error (Khan, 2000).

Results and Discussion

Morphological data presented in Table 1 and 2 showed the considerable morphological diversity in all the characters but plant height, leaf size, shape, leaf area, fruit colour, shape, length and seed length in chota gokhru and bara gokhru *T. terrestris* L. is an important medicinal or perennial prostrate herb with pinnately compound leaf with leaflets. The colour and leaf area showed the maximum diversity on the Yamuna river bank at Keetham Jheel, Runkata (Agra) as well as Bateswar near Bah

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Table 1. Morphological variation in characters of *T. terrestris* L.

S.No	Characters	Mean	Range	Standard Deviation	Standard Error
1.	Plant height (cm)	90.00	20.0	8.94	4.00
2.	Leaf length (cm)	5.64	1.00	1.26	0.56
3.	Leaf width (cm)	3.56	0.70	7.96	3.56
4.	Petiole length (cm)	0.60	0.02	1.52	0.68
5.	Leaf area (cm)	16.20	20.80	2.23	16.30
6.	Flower length (cm)	2.50	0.10	5.50	2.50
7.	Length of anther (cm)	0.47	0.20	1.05	0.47
8.	Length of pistil (cm)	0.50	0.15	1.30	0.58
9.	Fruit length (cm)	0.72	0.50	1.60	0.70
10.	Fruit width (cm)	0.91	2.00	0.92	0.05
11.	Seed length (cm)	0.73	0.10	1.60	0.70

Table 2. Morphological variation in characters of *P. murex* L.

S.No.	Characters	Mean	Range	Standard Deviation	Standard Error
1.	Plant height (cm)	67.00	17.0	29.90	5.90
2.	Leaf length (cm)	5.40	0.9	2.40	0.48
3.	Leaf width (cm)	5.00	0.5	2.20	0.40
4.	Petiole length (cm)	1.50	0.4	3.40	0.69
5.	Leaf area (cm)	1.08	0.2	2.86	0.57
6.	Flower length (cm)	2.38	0.2	5.32	2.38
7.	Length of anther (cm)	1.24	0.1	2.77	1.24
8.	Length of pistil (cm)	1.48	0.1	3.30	1.40
9.	Fruit length (cm)	1.36	0.3	3.041	0.60
10.	Fruit width (cm)	1.06	0.1	2.30	1.06
11.	Seed length (cm)	0.68	0.3	1.50	0.68

(Agra). It produces flowers from May to October, the flower are hermaphroditic, small lemon yellow in colour and solitary. The fruit is woody with sharp, rigid spines that easily fall apart into four or five seeded nutlets. The nutlets are seeds which are hard and bear two or three sharp spines.

P. murex is a member of family Pedaliaceae, it is an erect tall herb, slightly succulent with much branching stem. The variability in height and thickness of the stem has been also observed in the various places of Agra. The stem is very smooth and hairy types have also been recorded on the river bank of Yamuna. However, in the plains, stem is very tough. The leaves are opposite simple, fleshy, base narrow, lower surface with small scales and about 1-5 cm long. The flowers are bisexual, solitary, regular, pentamerous and white-yellow in colour. The fruit an indehiscent capsule and pyramidal in shape with 0.5-1.00 cm in size. Seeds are hard with horizontal spines, there are three to twelve seeds in a fruit. Study of diversity of morphological characters based on various parameters revealed that maximum diversity in the leaf area, fruit and seed size and number of seeds per fruit have been recorded in chota gokhru and Bara gokhru at Agra and their adjoining area. A great deal of diversity occurs in fruits of *T. terrestris* and *P. murex* in this region, The leaf as well as fruit of both chota gokhru and bara

gokhru are medicinally important. The fresh leaves and stem of *P. murex* is a good source of mucilage. A single plant can produce 300-400 fruits and 600-1200 seeds in chota gokhru. However, *P. murex* produced 50-100 fruit plant. Such morphological diversity have been used significantly towards the adaptability, divergence of the species and selection of superior accessions leading to their use in plant breeding, conservation and commercial value.

Rengalakshmi, (2005) suggested that morphological characters, viz., plant height, hairiness, ear head shape and structure, seed colour, shape and lusture, and stem colour are used in the functional classification is a special purpose taxonomy that utilizes the practical knowledge on the landraces. Similarly, the morphological characters, such as stem juiciness, midrib colour, grain shape, plumpiness, covering and size, as well as glume colour and hairiness, are used to classify the sorghum landraces among the Ethiopian tribes (Teshome *et al.*, 1997). On the basis of present investigation it may be concluded that the morphological diversity of *T. terrestris* and *P. murex* shows rich diversity in fruits on the banks of river Yamuna and Chambal catchment area. The seeds also show great morphological diversity. Therefore, there is need of biochemical and molecular characterisation, evaluation and breeding programmes.

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Evaluation of Guava (*Psidium guajava* L.) Genotypes Based on Fruit Morphology, Physico-chemical Properties and Yield under Eastern Plateau Condition

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Thirty-two guava germplasm were evaluated on the basis of fruit morphology, physico-chemical properties and yield at ICAR Research Complex for Eastern Region, Research Center, Ranchi during 2007-08. High yielding cultivars were CHG-1, Allahabad Safeda, Mild Flesh, Sardar and White Flesh. The fruit of CHG-1 cultivar was round shaped with pink flesh and having smooth surface. On the other hand, the fruit of Allahabad Safeda cultivar was round shaped and had creamy pulp and smooth surface. The fruit of Sardar and White Flesh cultivars were oval shaped and had white pulp and rough surface. The characteristics of Mild Flesh cultivar was round fruited with white pulp and smooth surface. With regard to physico-chemical properties of the genotypes, the cultivar Spear Acid accounted for the maximum fruit weight (179.4 g) and ascorbic acid content (265.83 mg/100 gm pulp) whereas the cultivar CHG-5 had higher T.S.S. content of 10.26°B during rainy season. As winter crop, the cultivar Mild Flesh recorded the maximum fruit weight of 199.1 g whereas the cultivars Seed Drop and Kairala Seedling exhibited highest T.S.S. content of 12.86°B but later had the maximum ascorbic acid content of 358.33 mg/100 gm pulp. The only cultivar Barbados Superior did not produce fruit during winter. During summer season, six cultivars, viz. Mild Flesh, Seed Drop, Surkh Gudi, CHG-1, CHG-2 and Eskwala brought forth fruit. The cultivar Eskwala recorded the highest fruit weight of 139.8 g and the cultivar Seed Drop exhibited the maximum TSS content of 14.36°B and ascorbic acid content of 337.5 mg/100gm pulp. Hence, based on fruit morphology, quality and total yield CHG-1, Allahabad Safeda, Mild Flesh, Sardar, White Flesh, Spear Acid and Seed Drop were found promising for cultivation in the plateau region of Jharkhand.

Key Words: Guava, Evaluation, Fruit shape, Flesh colour, TSS, Ascorbic acid, Yield

Introduction

The guava (*Psidium guajava* L.) 'Apple of Tropics' is one of the major fruit crops grown in subtropical, sub-humid climate under eastern plateau and hill region. In the present investigation, thirty-two guava cultivars and seedlings which excelled over most other guava cultivars in term of productivity and physico-chemical characters were evaluated. Description of guava cultivars on the basis of botanical and pomological characters are most useful tools for systematic botanist and pomologist for further research and equally good for the people concerned with guava industry. Maintenance of superior genotypes in respect of fruit and other characters for further crop improvement programmes is essential. The soil and climatic conditions of eastern plateau and hill agro-climatic zone offer suitable conditions for successful cultivation of guava under irrigated and rain-fed conditions as well. The ease in cultivation and precociousness of guava (after two-years attains bearing stage) under these conditions makes it a suitable option for increasing the paddy equivalent yield of agriculture production system. Keeping the above point in view, the present investigation

was undertaken to evaluate guava genotypes on aspect of fruit morphology, nutritive quality and total yield under subtropical sub humid climate of Chotanagpur.

Materials and Methods

The experiment was conducted at ICAR Research Complex for Eastern Region, Research Center, Ranchi during 2007-08. This area is situated 620 m above mean sea level (msl) and at 23°25' N latitude and 85°20' East longitude experiencing an average annual rainfall of 110-140 cm. High humidity (78.14%-84.14%) and low evaporation rate (4.04 mm/day) is experienced after June which continues up to onset of winter (Singh, 1999). Soil is acidic and pH range from 5.0-6.5, which is ideal for guava cultivation. The important cultivars for evaluation were Kairala Seedling (T₁), Barkhana (T₂), Florida Flesh (T₃), Chittidar AC (T₄), Mild Flesh (T₅), Seed Drop (T₆), Barbados Superior (T₇), Surkh Gudi (T₈), Chittidar (T₉), Allahabad Collection (T₁₀), Sangam (T₁₁), Sardar (T₁₂), Allahabad Safeda (T₁₃), Mustafapur (T₁₄), Behat Coconut (T₁₅), Pear Shaped (T₁₆), Apple Colour (T₁₇), Harijha (T₁₈), Banarasi (T₁₉), Superior (T₂₀), White Flesh (T₂₁), Guatemala (T₂₂), CHG-1 (T₂₃), CHG-3 (T₂₄),

CHG-5 (T₂₅), CHG-2 (T₂₆), Spear Acid (T₂₇), Nasik (T₂₈), Eskwala (T₂₉), Sindh (T₃₀), Patiala (T₃₁) and Smooth Green (T₃₂). Cultivars were evaluated in rainy, winter and summer season. Each cultivar represented one treatment. The experiment was laid out in randomized block design with four replications. Fruit botanical descriptions were measured by the descriptor given by proceeding of the group workers meeting on subtropical fruits (AICRP on Sub-tropical fruits). Titratable acidity was estimated by titrating the fruit extract with 0.1 N NaOH using phenolphthalein as an indicator and expressed as percent citric acid equivalent. The estimation of vitamin C in fresh fruits was carried out using 2,4-6 dichlorophenol indophenol dye. Reducing and total sugar was estimated by Lane and Eynon method (Ranganna, 1977).

Results and Discussion

In our study, from yield point of view, five cultivars, viz., CHG-1 (41.12 kg/tree/year), Allahabad Safeda (37.76 kg/tree/year), Mild Flesh (36.99 kg/tree/year), Sardar

(36.03 kg/tree/year) and White Flesh (35.73 kg/tree/year) were high yielder. In a different experiment conducted at Sabour, Bihar, Singh *et al.* (2002) found that Allahabad Safeda exhibited superior yield of 72 kg and 42 kg during rainy and winter season, respectively as compared to the other germplasm. Kundu and Mitra (1994) reported that maximum yield of 5.8 kg and 44.6 kg during winter and rainy season, respectively, in the laterite tract of West Bengal. A close perusal of the Table 1 revealed that the fruit of CHG-1 cultivar was round shaped with pink flesh and having smooth surface and creamy white peel colour. On the other hand, the fruit of Allahabad Safeda cultivar was round shaped and had creamy pulp and creamy white peel colour with smooth surface. The fruit of Sardar and White Flesh cultivars were oval shaped and had white pulp and rough surface with light green peel colour. The only difference of these two cultivars was the former cultivar had broadly round fruit apex but later cultivar possessed flat fruit apex. The cultivar Mild Flesh was round shaped with white pulp and creamy

Table 1. Fruit morphological characters of guava cultivars assessed during 2007-2008

Cultivars	Fruit shape	apex	Base	Cavity	Surface	Peel colour	Peel thickness	Pulp colour
Kairala Seedling	Round	Broadly round	Tapering	Absent	Smooth	Light green	Thick	White
Barkhana	Round	Broadly round	Rounded	Absent	Smooth	Creamy white	Thick	White
Florida Flesh	Round	Broadly round	Rounded	Absent	Rough	Light green	Medium	White
Chittidar AC	Oval	Flat	Tapering	Absent	Rough	Light green	Thin	White
Mild Flesh	Round	Broadly round	Rounded	Absent	Smooth	Creamy white	Thin	White
Seed Drop	Round	Broadly round	Rounded	Shallow	Smooth	Yellowish reddish	Medium	White
Barbados Superior	Round	Broadly round	Rounded	Absent	Smooth	Light green	Thin	Cream
Surkh Gudi	Oval	Broadly round	Tapering	Shallow	Smooth	Light green	Thin	White
Chittidar	Oval	Flat	Tapering	Shallow	Smooth	Light green	Thin	White
Allahabad Collection	Round	Broadly round	Rounded	Absent	Rough	Yellowish reddish	Thin	Cream
Sangam	Oval	Broadly round	Tapering	Shallow	Smooth	Light green	Thin	Cream
Sardar	Oval	Broadly round	Tapering	Shallow	Rough	Light green	Medium	White
Allahabad Safeda	Round	Broadly round	Rounded	Shallow	Smooth	Creamy white	Thin	Cream
Mustafapur	Round	Broadly round	Rounded	Shallow	Smooth	Creamy white	Thin	White
Behat Coconut	Oval	Flat	Tapering	Shallow	Rough	Creamy Wwhite	Medium	Cream
Pear Shaped	Pyriform	Broadly round	Tapering	Absent	Smooth	Light green	Medium	White
Apple Colour	Round	Broadly round	Rounded	Absent	Rough	Yellowish reddish	Thin	White
Harijha	Oval	Broadly round	Rounded	Absent	Smooth	Light green	Thin	White
Banarasi	Oval	Broadly round	Tapering	Absent	Rough	Yellowish reddish	Thin	Cream
Superior	Round	Rounded obtuse	Tapering	Intermediate	Smooth	Light green	Thin	Cream
White Flesh	Oval	Flat	Tapering	Shallow	Rough	Light green	Thin	White
Guatemala	Oval	Broadly round	Tapering	Absent	Smooth	Light green	Thin	White
CHG-1	Round	Broadly round	Rounded	Absent	Smooth	Creamy white	Thin	Pinkish
CHG-3	Round	Broadly round	Rounded	Shallow	Rough	Light green	Medium	White
CHG-5	Round	Broadly round	Rounded	Absent	Smooth	Yellowish reddish	Medium	Cream
CHG-2	Round	Broadly round	Rounded	Absent	Rough	Yellowish reddish	Thin	Pinkish
Spear Acid	Oval	Flat	Tapering	Absent	Rough	Creamy white	Medium	White
Nasik	Pyriform	Broadly round	Tapering	Absent	Smooth	Creamy white	Thin	White
Eskwala	Round	Broadly round	Rounded	Absent	Smooth	Light green	Thick	White
Sindh	Round	Broadly round	Rounded	Shallow	Smooth	Creamy white	Thin	Cream
Patiala	Round	Broadly round	Rounded	Absent	Smooth	Creamy white	Medium	White
Smooth Green	Oval	Flat	Tapering	Shallow	Smooth	Light green	Medium	White

white peel colour. The cultivars Pear Shaped and Nasik were pyriform in shape with white pulp but peel colour of former cultivar was light green whereas later was creamy white.

In the present investigation, during rainy season, the maximum fruit weight was recorded in cultivar Spear Acid (179.41 g) (Table 2) and during winter season the cultivar Mild Flesh exhibited maximum fruit weight of 199.13 g (Table 3). This finding corroborates the results of Tandon *et al.* (1983) and Singh *et al.* (2002). The fruits of CHG-5 recorded the maximum TSS (10.26°B) in rainy season whereas in the winter season Kairala Seedling and Seed Drop recorded the maximum TSS of 12.86°B. These findings are in agreement with results reported by Kundu *et al.* (1995). In our present study, the maximum titratable acidity was observed in case of Chittidar AC (0.36%) during rainy season whereas;

Seed Drop recorded maximum acidity of (0.32%) during winter season followed by cultivar Kairala Seedling. In rainy season the maximum ascorbic acid content was found in case of cultivar Spear Acid (265 mg/100 g pulp) followed by Sooth Green (262 mg/100 g pulp). But in winter season, the maximum ascorbic acid was found in cultivars Kairala Seedling (358 mg/100 g pulp). In a different study, Tandon *et al.* (1983) found that Behat Coconut accounted for the maximum ascorbic acid of 295.7 mg/100gm in winter season. Singh *et al.* (1995) also reported that the maximum vitamin C was found in case of cultivar Superior in winter season. The highest total sugar was found in case of genotype CHG-3 (4.2%) during rainy season but the cultivar Florida Flesh accounted for the minimum total sugar of 4.37 per cent during winter season. Singh *et al.* (2002) reported that the maximum total sugar of 9.13% was found in case of

Table 2. Physico-chemical characters and yield of rainy season guava crop during 2008

Cultivars	Fruit size (cm ²)	Fruit weight (gm)	TSS (°B)	Acidity (%)	Reducing sugar (%)	Total sugar (%)	Ascorbic acid (mg/100 gm pulp)	Yield (kg/tree)
Kairala Seedling	36.30	112.41	8.80	0.24	2.55	2.81	158.33	14.40
Barkhana	39.18	113.00	9.36	0.22	2.05	3.34	243.33	11.90
Florida Flesh	37.17	117.83	8.43	0.18	1.92	2.57	246.66	17.60
Chittidar AC	36.77	113.58	8.63	0.36	2.21	2.55	185.00	22.13
Mild Flesh	29.32	98.00	10.00	0.31	2.70	3.44	171.66	19.93
Seed Drop	31.92	102.25	8.63	0.18	1.74	2.63	204.16	18.70
Barbados Superior	31.35	97.33	8.80	0.13	2.58	2.88	189.16	13.30
Surkh Gudi	38.22	138.66	9.73	0.15	1.92	3.00	208.33	12.53
Chittidar	47.81	159.75	8.73	0.14	2.13	2.94	227.50	19.90
Allahabad Collection	42.18	122.16	10.06	0.15	2.00	3.26	195.83	12.40
Sangam	31.48	108.16	9.00	0.16	2.20	3.42	187.50	13.50
Sardar	41.61	128.16	9.20	0.14	2.14	3.26	218.33	23.43
Allahabad Safeda	43.29	125.83	9.46	0.13	2.34	3.54	241.67	24.86
Mustafapur	28.71	78.83	9.93	0.18	2.11	3.57	244.16	17.90
Behat Coconut	33.35	102.75	9.46	0.16	2.05	2.90	241.67	18.90
Pear Shaped	29.28	96.50	9.33	0.19	1.90	3.39	242.50	18.56
Apple Colour	32.08	99.50	8.93	0.14	2.63	3.01	200.00	13.16
Harijha	39.01	154.16	10.06	0.16	1.97	3.41	183.33	17.80
Banarasi	32.10	87.25	9.53	0.16	2.28	3.57	173.33	15.70
Superior	34.38	100.33	9.53	0.19	2.16	3.08	181.66	16.40
White Flesh	41.05	128.16	8.80	0.13	2.17	2.73	231.66	24.20
Guatemala	41.38	127.08	9.40	0.14	2.02	3.38	141.66	17.93
CHG-1	34.20	102.41	10.06	0.14	2.12	3.61	210.83	23.93
CHG-3	39.05	128.75	9.20	0.18	2.68	4.20	185.83	19.56
CHG-5	36.69	105.83	10.26	0.15	2.14	3.23	201.66	22.26
CHG-2	32.67	104.58	8.20	0.17	2.32	2.69	194.16	18.46
Spear Acid	47.24	179.41	10.06	0.16	2.02	2.83	265.83	19.50
Nasik	32.48	78.25	9.59	0.18	2.68	3.30	210.83	18.53
Eskwala	34.33	130.16	9.0	0.27	2.46	3.44	192.50	20.16
Sindh	33.81	107.33	9.0	0.12	2.63	3.35	195.00	20.00
Patiala	30.19	92.33	8.06	0.13	1.88	3.14	203.33	20.53
Smooth Green	38.96	133.58	8.73	0.20	2.06	2.76	262.50	21.26
CD at 5 %	1.899	3.648	0.4138	0.062	0.237	0.285	15.237	1.702

Table 3. Physico-chemical characters and yield of winter season guava crop during 2008

Cultivars	Fruit size (cm ²)	Fruit weight (gm)	TSS (°B)	Acidity (%)	Reducing sugar (%)	Total sugar (%)	Ascorbic acid (mg/100 gm pulp)	Yield (kg/tree)
Kairala Seedling	38.43	131.73	12.86	0.30	3.13	3.53	358.33	9.56
Barkhana	31.58	103.66	11.66	0.23	2.73	3.07	250.00	8.26
Florida Flesh	38.33	154.00	11.86	0.20	2.79	4.37	250.00	9.20
Chittidar AC	33.63	145.80	10.06	0.25	3.07	3.37	229.16	11.20
Mild Flesh	46.37	199.13	9.26	0.16	2.74	3.01	216.66	12.13
Seed Drop	48.32	167.93	12.86	0.32	3.20	3.90	276.66	8.80
Barbadose Superior	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
Surkh Gudi	39.02	155.93	9.13	0.18	2.81	3.81	280.83	7.30
Chittidar	43.10	174.46	10.0	0.28	2.98	3.85	237.50	10.53
Allahabad Collection	35.73	139.53	9.26	0.28	2.70	3.40	212.50	7.33
Sangam	37.61	120.80	9.33	0.26	3.28	3.78	204.16	5.50
Sardar	48.43	130.66	11.66	0.17	2.47	4.12	279.16	12.6
Allahabad Safeda	43.16	129.60	12.33	0.15	2.95	4.25	300.00	12.9
Mustafapur	38.70	139.40	10.13	0.26	2.73	3.37	183.33	8.90
Behat Coconut	32.92	110.33	9.16	0.29	3.59	4.24	262.50	10.03
Pear Shaped	39.08	142.53	10.83	0.32	2.88	3.33	204.16	8.86
Apple Colour	40.08	173.06	9.83	0.19	2.89	3.59	191.66	6.76
Harijha	36.23	135.93	9.00	0.18	2.66	3.19	262.50	9.00
Banarasi	29.91	109.60	9.46	0.21	2.77	3.80	262.50	7.63
Superior	45.03	164.06	9.83	10.27	2.51	2.73	333.33	8.10
White Flesh	50.56	167.80	9.93	0.31	2.18	2.45	237.50	11.53
Guatemala	29.65	112.93	9.66	0.17	2.93	3.65	312.50	8.60
CHG-1	42.25	143.66	8.16	0.23	2.74	3.14	225.00	11.86
CHG-3	38.76	135.60	9.53	0.16	2.31	2.77	208.33	8.83
CHG-5	40.66	156.60	9.86	0.26	2.82	3.35	252.50	10.83
CHG-2	34.77	130.33	8.93	0.26	2.31	3.23	214.16	8.73
Spear Acid	37.34	139.33	9.76	0.23	2.79	3.55	207.50	9.56
Nasik	34.89	119.13	9.93	0.18	3.04	3.47	237.50	9.53
Eskwala	27.64	84.20	8.93	0.14	2.62	2.87	225.83	9.91
Sindh	37.97	134.80	9.73	0.20	2.73	3.11	205.83	10.00
Patiala	33.84	111.13	8.86	0.27	3.05	3.53	211.66	10.16
Smooth Green	33.81	119.66	8.06	0.21	2.98	3.41	250.00	9.66
CD at 5%	2.459	1.353	0.406	0.028	0.150	0.338	23.173	1.220

Table 4. Physico-chemical characters and yield of summer season guava crop during 2008

Cultivars	Fruit size (cm ²)	Fruit weight (gm)	TSS (°B)	Acidity (%)	Reducing sugar (%)	Total sugar (%)	Ascorbic acid (mg/100 gm pulp)	Yield (kg/tree)
Mild Flesh	29.57	90.15	13.12	0.295	2.59	5.24	285.62	4.93
Seed Drop	33.29	109.00	14.27	0.310	5.81	6.82	338.12	4.20
Surkh Gudi	33.94	110.72	12.07	0.297	2.40	5.33	289.37	3.73
CHG-1	24.92	67.57	11.67	0.277	2.10	4.81	212.62	5.33
CHG-2	34.59	96.07	11.25	0.265	2.79	4.64	235.87	4.10
Eskwala	35.69	138.70	12.22	0.270	2.95	5.44	315.37	3.76
CD at 5%	1.017	5.441	0.223	0.025	0.369	0.190	14.438	0.381

cultivar Allahabad Safeda during winter season at Sabour (Bihar) condition. The only cultivar Barbados Superior did not produce fruit during winter. During summer season, six cultivars, viz., Mild Flesh, Seed Drop, Surkh Gudi, CHG-1, CHG-2 and Eskwala brought forth fruit. In summer season, fruit size and weight was smaller than that of rainy and winter season crop. The cultivar Eskwala recorded the highest fruit weight of 139.8 g

and the cultivar Seed Drop exhibited the maximum TSS content of 14.36°B and ascorbic acid content of 337.5 mg/100 gm pulp (Table 4). Yield of summer season crop was also low due to cultivation under rainfed condition and higher temperature.

Hence, based on fruit morphology, fruit weight, TSS, Ascorbic acid content CHG-1, Allahabad Safeda, Mild Flesh, Sardar, White Flesh, Spear Acid, Seed Drop and

Kairala Seedling were found suitable for cultivation under rainfed condition in the plateau region of Jharkhand.

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Estimation of β -Carotene in Local Banana Cultivars from Kerala

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Bananas, a major staple food are the forth important food in the world. Bananas are nourishing fruits contains many minerals and vitamins. It is a rich source of energy low in protein and fat content and provides a balanced diet. At present a number of edible banana cultivars are spread throughout the world. India has more than 300 cultivars. Edible cultivars are derived from interspecific hybridization between two wild species *Musa accuminata* and *Musa balbisiana*. In Kerala cultivars like Palayamcodan (AAB), Red banana (AAA), Robusta (AAA), Nendran (AAB), Rasakadali (AB) and Chingan (AA) are available. In present study, biochemical analysis of 13 cultivars which belongs to AA, AB, AAA, AAB genomes, respectively was conducted. The analysis involved estimation of carbohydrates, starch, reducing sugars, proteins as well as β carotene. The cultivars which are studied are Kadali, Matty (AA), Annanvazha, Rasakadali (AB), Red banana, Robusta (AAA), Palayamcodan, Nendran, Poovan, Padatti (AAB). Among the cultivars Nendran has high carbohydrates, starch, reducing sugars as well as β carotene content and padatti has maximum proteins. β carotene content was quantified at differential stages of fruit development using HPLC.

Key Words: Banana, Musaceae, Scitaminae, *Musa accuminata*, *Musa balbisiana*, β -carotene, HPLC

Introduction

Vitamin A deficiency is considered as a global health problem (UNICEF, 1990). Food sources of Vitamin A include animal foods rich in Vitamin A and plant foods containing provitamin A carotenoids such as β carotene (Mc Laren, 2001). Carotenoids are widely distributed in the plant kingdom. These components contribute to the red, orange and yellow colours to fruits (Burns, 2003; Hughes, 2001). A number of carotenoids including β carotene, β carotene and β cryptoxanthin have provitamin A activity and they are converted to retinal by mammals (Burns, 2003) have twice provitamin A activity. There are several factors that restricts the potential of known food sources of Vitamin A. Animal foods as source of vitamin A are often too expensive for low income people, unacceptable for religious reasons or less available (Gibson, 2000). In many regions of world dark green leafy vegetable may not be given to young children, because of concern that it may not be digestible and Papaya is avoided in Southern India by pregnant woman because of a belief of miscarriage (Kuhnlein, 1997). Some people in India consider it as a food causes dysmenorrhea in women and impotency in men. Other vegetables like mangoes, pumpkins, squash, orange fleshed sweet potatoes have the problem of indigestion, taste and season dependent availability (Englberger, 2003).

Banana is a major food in the world, after rice, wheat and maize. These fruits are wholesome and fairly

well balanced source of nutrients containing various mineral salts, vitamins and high amount of carbohydrate with a little fat and protein (Simmonds, 1966; Ketiku, 1973; Ahenkaro *et al.*, 1997). These fruits are eaten raw as desert fruits or cooked as vegetable and available throughout the year. These are rich in antioxidant vitamins (Vitamin C, A and E), calcium, magnesium and potassium (Marisa, 2006). Bananas are not listed as a fruit rich in β Carotene since the Cavendish varieties available in US and Europe has little β Carotene content. It is observed that some banana cultivars from Micronesia and South East Asia have significant amount of β carotene, a precursor of Vitamin A (Abdon *et al.*, 1980; Puwastein *et al.*, 1999; Siong, 1985). Some cultivars of Micronesia like Karat and Uhten Yap have significant carotenoid contents (Stover, 1987) various cultivars from Africa also have high content of carotene (Daniells, 2001). However the nutritional analysis of many cultivars available in Kerala is not evaluated and present study reports the biochemical analysis of ten banana fruits locally available in Kerala.

Materials and Methods

Ten cultivars of banana fruits were purchased from the banana nursery, Peringmala, Kerala, South India and used in the present study. This includes Kadali, Matty (AA), Annanvazha, Rasakadali (AB), Red banana, Robusta (AAA), Palayamcodan, Nendran, Poovan, Padatti (AAB). The analyses of organic compounds, minerals as well as β -carotene were estimated from

ripened fruits. The β -carotene content at un-ripened, ripened and over-ripened fruits were estimated using HPLC only in the cv. Nendran.

Estimation of Carbohydrates

Carbohydrate estimation of the banana fruit was done as per the protocol defined by Hedge and Hofreiter (1962). Glucose was used as standard. UV spectrometer (Shimadzu 1700) was used for the estimation of carbohydrate present in the sample.

Estimation of Starch

The estimation of total starch content was done using the modified protocol by Thayumanavan and Sadasivam (1984). Glucose was used as standard. UV spectrometer (Shimadzu 1700) was used for the estimation of starch present in the sample

Estimation of Reducing Sugar

The reducing sugar was estimated by using Di nitro Salicylic acid with reference to the procedure Miller (1971). Amount of reducing sugar was calculated with the help of a standard graph using glucose. UV spectrometer (Shimadzu 1700) was used for the estimation of carbohydrate present in the sample

Estimation of Total Proteins

The estimation of total protein was done with the help of procedure by Lowry *et al.* (1951). The amount of protein present was calculated with the help of standard graph using BSA. UV spectrometer (Shimadzu 1700) was used for the estimation of protein present in the sample

Determination of Minerals

The mineral content of different banana samples was determined by procedure mentioned by Health Protection Branch Laboratories Bureau of Nutritional Science. Determinations of elements were done by the help of Flame Atomic absorption Spectrophotometer.

Spectrophotometric estimation of β -carotene

The extract was prepared according to the protocol. The carotene were extracted with ice cold acetone and it was filtered under vacuum. The extracts were then transferred to 100 ml petroleum ether and acetone was removed by washing with water. The colour developed was analyzed at 455 nm using spectrophotometer. Then the amount of β -carotene was estimated by the help of a standard graph. UV spectrometer was used for the estimation of β -carotene present in the sample.

HPLC Analysis of β -Carotene

The HPLC analysis was done with the help of procedure mentioned by Weissenberg *et al.* (1997).

Pigment Extraction

β -carotene was extracted from fruit paste using petroleum ether (bp 40-60°C) repeatedly in subdued light until colorless extracts were obtained. 100 ml of aliquots were evaporated in a stream of Nitrogen.

HPLC Determination

The extracts obtained were dissolved in mobile phase (1 ml), filtered through a 0.45 mm membrane disc and injected in to chromatograph. The column was regenerated by washing with 2-Propanol after analysis and then equilibrated with mobile phase.

Standard Solutions

Stock solution of β -carotene (Hi-Media, Bangalore) was prepared in petroleum ether. Aliquots (10-200 μ l) were evaporated in a nitrogen stream and the residues were dissolved in mobile phase (1 ml) and subjected to HPLC.

Results and Discussion

Estimation of organic compounds such as carbohydrates, starch, reducing sugar and protein showed that depending upon the cultivar, the nutritional components varied in the ripened fruits. Among different cultivars Padatty (AAB) have highest protein and Matty (AA) has the minimum protein. Nendran (AAB) has the higher content of total carbohydrate, starch as well as reducing sugar. From the analysis of different minerals, most bananas have a significant amount of minerals. Kadali (AA) have a higher content in Manganese and Magnesium but copper content is nil. Copper content is higher in Padatty (AAB), Iron in Rasakadali (AB), Potassium in Matty (AA), Calcium in Red banana (AAA) and Zinc in Robusta (AAA). β -carotene estimation showed that Nendran (AAB) has maximum content and Kadali (AA) has minimum content. The HPLC analysis of β -carotene in different stages of fruit ripening in Nendran showed that unripened fruit has higher β -carotene than ripened and over ripened fruits.

Studies in banana reported that protein content increases only the first 60-70 days but after that it remains constant (Peumans *et al.*, 2000). It is also observed that reducing sugar content is higher than that of starch in most cases. During ripening starch content decreased and the reducing sugar content increased (Henderson,

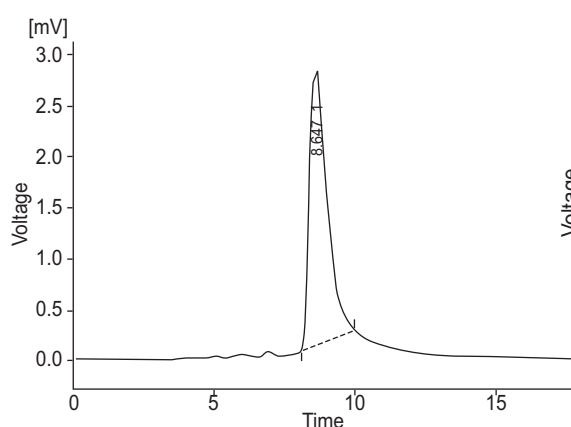
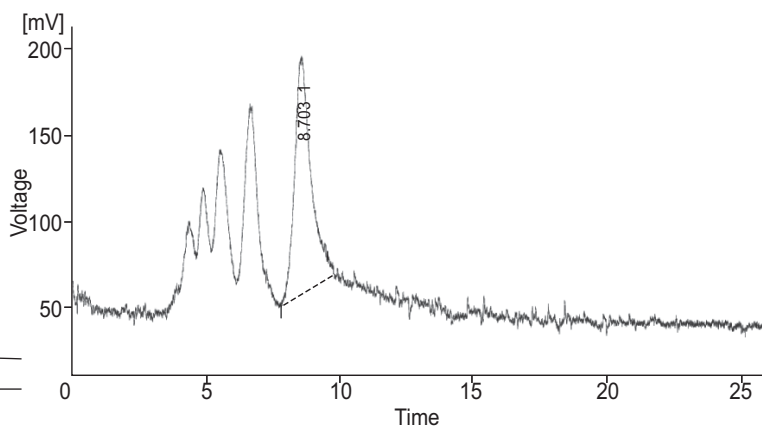
Fig. 1: HPLC chromatogram of β carotene standard

Fig. 2: HPLC chromatogram of pre-ripened Nendran

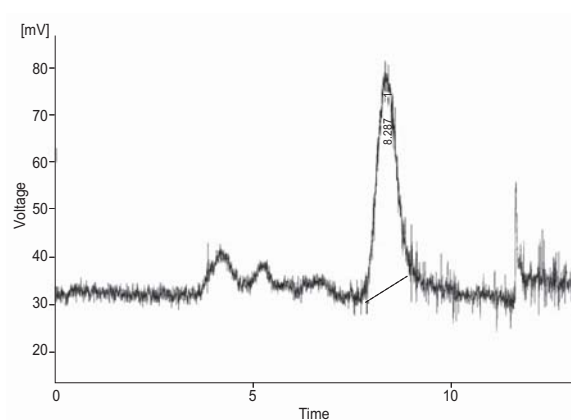


Fig. 3: HPLC chromatogram of ripened Nendran

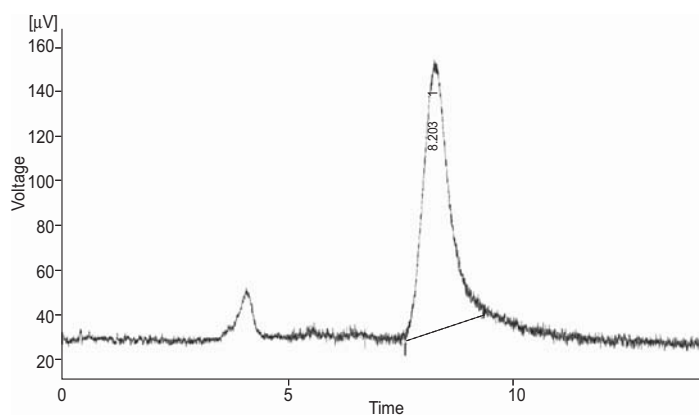


Fig. 4: HPLC chromatogram of over ripened Nendran

1956). Even though Zinc is higher in Robusta, Nendran (AAB) also has significant amount Zinc. This may indicate the high carotene content in Nendran because Zinc is an important component in carotenoids. The HPLC analysis of β -carotene was different from Spectrophotometric analysis. This variation may be due to the difference in procedure during extraction. Spectral analysis used acetone and HPLC analysis used Petroleum ether. In Nendran unripened fruits showed high β -carotene Giami and Alu (1994) also reported that total carotenoids in plantain almost halve during ripening

From this preliminary analysis the nutritional status of locally available banana fruits indicated variation among the cultivars. Also the cultivar Nendran showed potential for improvement in β -carotene content.

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Oil and Protein Quality of Black Seeded Soybean Germplasm

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Sixteen black seeded soybean lines locally known as *Kaley Bhatt*, collected from different parts of Uttarakhand area of India were grown in similar environmental and field conditions along with five creamish-white colored control lines. Nutritional quality of seeds in terms of oil-protein quantity, fatty acid profile of seed oil and amino acid profile of proteins were established and compared. *Kaley Bhatt* accessions produced small sized black seeds with average hundred seed weight of 7.62 g. In general, black seeds were more in protein and less in oil content in comparison to creamish white colored seeds. Fatty acid profiles of the collections were uniform irrespective of seed color and size. Significant and negative association exists between oleic-linoleic and oleic-linolenic acid. Some black seeded accessions were identified for their higher concentrations of linolenic acid (omega-3 fatty acid). Essential amino acid composition of *Kaley Bhatt* accessions varied with in range of 47.75 to 40.73 mol% of total amino acid. Maximum methionine concentration of 2.08 mol % was observed in IC548683.

Key Words: Soybean, Germplasm, Oil, Fatty acid profile, Protein

Introduction

Soybean serves as a major vegetable source of edible protein and oil (Smith *et al.*, 1987). Increased demand for these constituents has stimulated research efforts to develop soybean lines which can produce seed with higher concentration of oil and protein

Agronomically acceptable cultivars producing quality seed in terms of having combine oil and protein concentration of 62% can command significant market value over other related crops. However soybean oil is found to contain relatively high level of lipooxygenase enzyme, which react with linolenic acid (omega-3 fatty acid) present in seed oil and contribute to reduced oxidative stability and poor flavor quality (Mounts *et al.*, 1988). Presence of lesser levels of sulphur containing amino acid in seed proteins makes soybean less suited for human nutrition. Soybean oil also contains appreciable quantity of saturated fatty acids and in some cases; this value is about 16% (Mounts *et al.*, 1994). In modern time, the health issue involving saturated fats and blood serum cholesterol levels has received considerable public attention. Besides that, higher proportion of genetically modified *Brassica*, safflower, sunflower and peanut seed has been released for commercial production that gives high oil with desired quality (Fernandez-Martinez *et al.*, 1989; Mundel and Braun, 1999). Keeping all these developments in view it seems apparent that genetic modification research on soybean must have to be continued as per demand

of market, just to keep pace with the technological advances in other related crops.

In this direction, some good progress has been made in developing some genetic altered soybean lines (Burton *et al.*, 1989; Wilson *et al.*, 1976, 1981, 1988; Erickson *et al.*, 1988; Carver *et al.*, 1986), with one or other improved traits by exploiting germplasm resources. It is expected with the active involvement of germplasm collection and evaluation activities towards crop improvement programme will result future production of commercial soybean that will bear little resemblance to the soybean oil protein quality, available today. In continuation of these activities, the present work has been undertaken to study some black seeded soybean lines widely available in different pockets of Uttarakhand (India) for their nutritional quality.

In Uttarakhand region of India, the soybean is known as *Bhatt*. Based on colour, black seeded soybean is known as *Kaley Bhatt*. Similarly, yellowish white and brown seeded soybeans are known as *Safed Bhatt* and *Rata Bhatt*, respectively. Among *Kaley Bhatt* type some are very small in size, climber, tall and giant. Locally it is being grown in margins of agricultural field, mixed cropping with minor millets, i.e., foxtail, finger, barnyard and pseudo-cereals, i.e., amaranth. Others are rounded and medium type.

In the present experiment, sixteen black seeded soybean lines (*Kaley Bhatt*) and five yellowish white color, erect, small dwarf lines (*Safed Bhatt*) were taken for comparison of nutritional quality of the seeds.

Materials and Methods

Plant Materials

Sixteen black seeded soybean accessions collected from different parts of Uttarakhand (India) were grown along with five control lines. All control lines were rounded types and yellowish white seeded.

Accessions were grown in Randomized Block Design with three replications in June, 2007 and harvested after five months. Dried powdered seeds were taken for analysis.

The Soybean seeds were dried to 4-5% moisture level in oven set at 108°C for 16 to 18 h. The oil content of the seed samples were determined by non-destructive method using a Newport NMR analyzer (Model-4000) from Oxford Analytical Instruments Ltd. U.K, equipped with 40ml coil assembly. The instrument was kept in a room of constant temperature (23-25°C). Pure seed oil required to calibrate the instrument was extracted by solvent extraction method. The instrument was run by adjusting audio frequency (AF) gain of 400 and radio frequency (RF) current of 225 μ A with a gate width of 1.5 gauss. The NMR responses (signal/ mass) of the seed samples were compared to NMR response of pure oil (100%) for obtaining oil percentage of the soybean accessions.

Samples of soybean seeds were freshly grounded (Remi homogenizer) and weighed so that 40 mg oil are obtained when extracted with 10 ml solvent mixture consisting of chloroform:hexane:methanol (8:5:2 v/v/v). The extracts obtained were dried at 60°C in nitrogen gas for 30 min. Methyl esters of oil samples were prepared according to the method of Neff *et al.* (1994) with slight modifications as applied for Cruciferous species by Mandal *et al.* (2002). 1 μ l of the hexane extract was injected into a highly polar HP Innowax capillary column of 30 m length (inner diameter: 0.32 mm, film thickness: 0.5 μ m, split: 1:80). A Hewlett Packard gas chromatograph, model 6890 equipped with flame ionization detector (FID) was used. The injector and detector temperatures were 260°C and 275°C, respectively. Oven temperature was programmed from 150°C holding at 1 min to 210°C at the rate of 15°C/min, followed by 210°C to 250°C at the rate of 5°C/min for 12 min. Peaks of fatty acid

methyl esters were identified by comparing their retention time with that of the known standards, run under similar separation conditions, peak integration was performed applying HP3398A software.

Protein Content

Nitrogen content of the soybean seeds was determined by conventional Kjeldahl method with Kjeltec analyzer (Model-2300) from Foss Tecator, Sweden. Factor 6.25 was used to convert nitrogen to protein.

Amino Acid Profile of Total Seed Protein by High Performance Liquid Chromatography

HPLC based pre-column derivatization technique was used in the present study. Fluorescent active reagent, 6-aminoquinolyl-N-hydroxy succinimidyl carbamate of Millipore Corporation (Cohen and Michaud, 1993) was used for derivatization with protein hydrolysate amino acids.

Hydrolyzing Samples

5 mg powder samples were taken in a clean 6x50 mm sample tube. Tubes were placed in a reaction vial containing 200 μ l of constant boiling HCl (6 N) and a crystal of phenol, placed to the bottom of the reaction vial. Samples were hydrolyzed in an oven at 112°C to 116°C for 20 to 24 h. After hydrolysis is over, excess HCl was wiped off from tubes and dried under vacuum.

Derivatizing the Samples

Vacuum dried samples were dissolved in 750 μ l, 20 mM HCl solution. To 20 μ l of this protein hydrolysate amino acid solution, 20 μ l of AccQ-Fluor™ reagent (waters Part no. WAT052880) and 60 μ l of AccQ-Fluor™ Borate buffer (waters Part no. WAT052880) was added, vortex thoroughly and mixture was heated for 10 min at 55°C.

HPLC System for Separation of Amino Acid Derivatives

The HPLC system consisted of waters two pumps (Model no. 515), auto sampler and fluorescence detector (Waters 2475). Waters Empower software was used to control the operation and peak integration.

Derivatized sample was run in Waters AccQ-Tag™ column of length 3.9 x 150 mm (Part no. –WAT052885). Mobile phases A containing 10% solution of AccQ-Tag™ concentrate (Part no. WAT052890) and phase B consisting of 60% HPLC grade Acetonitrile were used as eluent in gradient conditions : initially A=100%, 2 min=98%,

15 min=93%, 19min=90%, 32 to 37min=67% followed by wash with 100% eluent B for 13 min. and re-equilibration for 10 min at 100% by eluent A.

Detection

Detection of individual amino acid derivative was made by fluorescence detector with excitation at 250 nm and emission at 395 nm with band width 18 nm. The polarity of the detector was positive with gain 10 and sampling rate 1.

Establishment of Factors to Convert Area Percentage of the Individual Amino Acid in Respective Concentration

From the area percentage of the individual amino acid of the standard mixtures (PIERCE Product no. NC10180/ vial No. 88122) run under similar condition, the factors were calculated to convert area percentage of the individual amino acid to respective concentration.

Results and Discussion

Sixteen black seeded soybean genotypes collected from different regions of Uttarakhand state of India were studied along with five normal colored control lines from same state, for major nutritive traits like oil, protein, fatty acid profile of seed oil and amino acid composition of protein, to make comparison between two different types of soybean samples.

Black seeded collections were comparatively very small in size. Hundred seed weight varied within the range of 10.29 to 5.77 g, with the mean value of 7.62 g, against 20.81 g mean seed weight recorded for control collections. The black seeded collections with average oil value of 18.65% was about 2.5% less than that of control line's oil value of 21.23%. But small seeded samples are more in protein content. Average protein value of 40.80% was about 2% more than the average protein value for the five control lines. IC548665, IC548683 and IC548669 were the three black seeded genotypes that contain more than 42% protein in their seeds. Among these, IC548665 and IC548683 showed combine oil plus protein concentration of about 62%. Out of five control lines, four were found having combine oil- protein percentage of about 60.

Fatty Acid Profile of Seed Oil

In general, average fatty acid profiles of the samples were uniform irrespective of seed color and seed size. Palmitic, stearic, oleic, linoleic, and linolenic acid components of the black seeded soybean oils varied

within the range of 10.78 to 8.17%, 5.23 to 2.24%, 25.95 to 16.23%, 60.52 to 51.89% and 14.70 to 7.66%, respectively. Values are very similar as mentioned earlier by different workers (Wilson, 1987).

Fatty acid profiles of the accessions along with other quality traits are shown in Table 1. IC548719 with total saturated fatty acid concentration of 11.87% was identified as low total saturated fatty acid containing line. IC419815 and IC548665 identified for their high oleic acid concentration of 25.95 and 22.86%, respectively

Poly unsaturated fatty acid (PUFA) content of the present accessions including control lines, varied within the range of 71 to 61%. Both the experimental and control lines gave average PUFA content of about 66%. Soybean oil is one of the few edible oils where appreciable amount of linolenic acid (omega-3 fatty acid) is present in PUFA components.

Higher level of lipoxigenase enzyme in soybean seed reacts with linolenic acid (omega-3 fatty acid) component of the oil and generates poor flavor if oil is not stored properly. Both linoleic and linolenic fatty acids are essential for human health (Pryde, 1980; Tinoco *et al.*, 1979). The linolenic acid acts as precursor for its conversion to long chain (LC) omega-3 PUFAs mainly eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA). Indu and Ghafoorunissa (1992) indicated that increasing dietary linolenic increases EPA concentrations in plasma phospholipids. The beneficial effects of PUFA are mainly due to EPA and DHA, the LC omega-3 PUFAs components. Dietary amounts of linoleic acid as well as the ratio of linoleic to linolenic acid in PUFA, appear to be important for the metabolism of linolenic to LC omega-3 PUFAs. Indu and Ghafoorunissa (1992) also showed that while keeping the amount of dietary linoleic acid constant, 3.7 g linolenic acid appears to have biological effects similar to those of 0.3 g LC omega-3 PUFA with conversion of 11 g linolenic acid to 1 g LC omega-3 PUFA. Thus ratio of 4 (15 g linoleic: 3.7 g linolenic) is appropriate for conversion. This ratio is also consistent with the Lyon Hear study (12). Simopoulos *et al.*, 2003 and Cleland, 1997 reported linoleic to linolenic acid ratio of 3:1 to 5:1 for exerting beneficial effects of PUFA. In the present study, omega-3 fatty acid concentration of *Kaley Bhatt* oils varied from 7.66 to 14.70% and 10.33% was the mean value against 9.56% mean value established for control lines. IC548669 and IC548612 were the two *Kaley Bhatt* collections selected for possessing high linolenic acid

Table 1. Value rich trait of soybean samples

S.No.	Accession No.	100 seed wt. (g)	Protein (%)	Total oil (%)	Palmitic	Stearic	Oleic	Linoleic	Linolenic	linoleic/linolenic
Percent of total oil										
1	IC548612	5.77	39.88	19.29	9.14	3.93	17.43	56.22	13.02	4.32
2	IC548623	6.62	39.45	18.34	9.01	3.23	19.09	57.43	11.23	5.11
3	IC548632	5.98	38.69	18.50	9.21	4.36	19.22	55.53	11.67	4.76
4	IC548636	5.83	38.92	18.73	8.74	4.96	20.23	56.67	9.39	6.04
5	IC548640	10.29	40.72	19.26	10.44	4.77	20.23	54.98	9.56	5.75
6	IC548643	9.12	39.50	20.13	10.32	4.41	17.03	58.13	10.11	5.75
7	IC548665	9.50	43.15	18.66	10.73	5.17	21.70	51.89	10.57	4.91
8	IC548669	7.00	42.47	16.30	9.41	3.30	16.23	57.25	14.70	3.89
9	IC548672	9.80	40.99	19.50	10.27	5.23	18.67	56.22	9.56	5.88
10	IC548676	9.26	41.31	19.38	9.59	3.89	19.08	57.75	9.69	5.96
11	IC548683	10.00	42.99	19.58	9.63	2.24	19.75	59.37	8.99	6.60
12	IC548719	6.22	40.33	18.87	8.17	4.25	16.78	60.52	10.25	5.90
13	IC548721	7.10	40.59	18.32						
14	IC548724	6.98	41.35	18.79	10.78	2.41	24.07	53.91	8.82	6.11
15	IC419815	5.92	40.72	17.60	9.35	3.42	25.95	53.61	7.66	7.00
16	IC524256	6.50	41.66	17.10	9.82	3.77	18.65	58.04	9.71	5.98
	Min.	5.77	38.69	16.30	8.17	2.24	16.23	51.89	7.66	3.89
	Max.	10.29	43.15	20.13	10.78	5.23	25.95	60.52	14.70	7.00
	Mean	7.62	40.80	18.65	9.64	3.96	19.61	56.50	10.33	5.60
	Std dev	1.70	1.34	0.98	0.75	0.92	2.65	2.27	1.75	0.84
	% Cv	22.35	3.29	5.258	7.82	23.25	13.53	4.02	16.99	15.01
17	Soybean-02	19.93	38.65	21.46	11.06	3.22	20.62	55.64	9.45	5.89
18	Soybean-21	21.53	36.01	21.95	10.54	4.00	17.45	57.22	10.56	5.42
19	Soybean-47	20.50	40.39	20.27	10.60	3.58	20.28	55.94	9.59	5.83
20	Soybean-54	21.20	38.60	21.61	10.10	4.21	21.35	55.65	8.69	6.40
21	Soybean-77	20.87	39.45	20.87	10.36	2.99	17.33	59.79	9.52	6.28
	Min.	19.93	36.01	20.27	10.10	2.99	17.33	55.64	8.69	5.42
	Max.	21.53	40.39	21.95	11.06	4.21	21.35	59.79	10.56	6.40
	Mean	20.81	38.62	21.23	10.53	3.60	19.41	56.85	9.56	5.96
	Std dev	0.621	1.631	0.665	0.35	0.51	1.88	1.77	0.67	0.39
	% Cv	2.99	4.22	3.13	3.36	14.23	9.69	3.11	6.96	6.57

value of 14.70% and 12.80%, respectively. Corresponding linoleic to linolenic fatty acid ratio were 3.89 and 4.32, respectively. The linoleic to linolenic fatty acid values for other *Kaley Bhatt* collections varied within the range of 3.89 to 7.00 against mean value of 5.96 recorded for five control lines.

Most of the common edible oils produced in modern days are rich in linoleic acid. These soybean oils with more than 10% linolenic acid stand a very good scope for use as an alternative for fish oil and other linolenic acid rich oil.

Amino Acid Composition of Seed Protein

In the present study, 6 aminoquinolyl-N-hydroxysuccinimidyl carbamate flour reagent of Waters (USA, Cat no. WAT 025890) was used for amino acid derivative formation. Afterwards the corresponding amino acid derivative gets separated in column and finally identified and quantified through fluorescence detector in HPLC system.

The Essential amino acid (EAA) composition of *Kaley Bhatt* collections varied with in range of 44.21 to 31.67 mol%, with mean value of 38.07 mol%. This mean value was about 7.36 mol% less when compared with the mean value of five control lines. Black seeded lines were found to contain more percentage of lysine and less percentage of tyrosine amino acid. Methionine content also was in higher side in control lines. More variation in the concentration of methionine was observed in *Kaley Bhatt* collections. IC548683 gave highest methionine concentration of 2.08 mol % against average 1.52 mol % recorded for *Kaley Bhatt* collections. Range and mean of the Essential Amino Acid composition, both for control and black seeded lines are shown in Table 2.

Variation in EAA compositions in black seeded lines in comparison to control lines was mainly due to the difference of lysine and tyrosine concentrations. Average tyrosine concentration of 2.61% was found in black seeded lines in comparison to 4.0% tyrosine found present

Table 2. Range and mean value of essential amino acid concentrations of soybean

EAA	Kaley Bhatt collections					Control lines				
	Min	Max.	Mean	Std dev	% Cv	Min	Max.	Mean	Std dev	% Cv
Arginine + Threonine	9.69	12.45	10.89	0.71	6.56	11.89	12.59	12.15	0.26	2.17
Half cystine	0.53	1.76	1.07	0.38	35.40	0.95	1.57	1.2	0.28	23.08
Tyrosine	1.03	3.76	2.61	0.89	34.24	3.51	4.48	4.046	0.49	12.16
Valine	4.97	5.78	5.31	0.25	4.77	5.15	5.4	5.254	0.11	2.15
Methionine	1.17	2.08	1.52	0.27	17.48	1.65	1.74	1.676	0.04	2.37
Lysine	4.53	6.48	5.39	0.57	10.64	4.04	5.26	4.54	0.66	14.48
Isoleucine	3.72	4.75	4.30	0.28	6.50	4	4.41	4.188	0.19	4.62
Leucine	6.57	8.24	7.48	0.50	6.62	6.78	7.41	7.08	0.30	4.22
Phenylalanine	3.62	6.68	5.10	0.89	17.47	4.46	6.13	5.304	0.79	14.84
Total EAA	40.73	47.75	43.68	1.75	4.00	43.88	46.75	45.438	1.46	3.21

control lines. On the other, lysine concentrations of black seeded lines varied within the range of 6.48% to 4.53%, 5.39% was the mean value. Average lysine value of 4.54% was observed in control lines. Maximum lysine concentration of 5.26% was observed in one of the creamish white coloured Control line. In this method, response of cystine amino acid was not satisfactory; as a result, good and clear cut cystine peaks were not detected, causing unreliable integration values. This might have contributed high CV% values for the samples. Except cystine, other essential amino acids got well separated and quantified with respect to standard amino acids, run under similar instrument programme. Comparative values rather than literature cited data were given more emphasis for drawing any conclusion about experimental results and for selecting superior line for possessing higher level of any essential amino acid. Kaley Bhatt collections were found to contain low tyrosine and high lysine residue in seed protein in comparison to present five normal colored control lines.

Essential amino acid components in seed protein of few selected accessions, which were identified for

their high and low protein, high methionine percentage in seed protein and high and low total essential amino acid composition are shown in Table 3 along with their protein values.

The present work was undertaken with the sole objective to quantify different nutritional parameters of Kaley Bhatt seeds and to identify any oil-protein related traits which might be considered specific for these black seeded accessions, if compared with any normal colored soybean line. Another objective has been to select value rich germplasm for use as resource material in development of a good soybean seed with improved nutritional quality.

Relationship between Different Quality Traits

Significant and direct associations exist between size and oil content of the seed. Associations between oil and protein content of the seed was also very strong but inverse. Among the fatty acid components, significant and negative association exists between oleic–linoleic and between oleic–linolenic acid as observed by earlier workers (Wolf, 1982).

Table 3. Lines showing high and low EAA, high protein and high methionine content

EAA	IC548665 Mol %	IC548724 Mol %	IC548683 Mol %	IC548632 Mol %	IC548669 Mol %
Arginine +Threonine	11.16	10.67	11.21	10.03	10.86
Half cystine	1.59	0.86	1.76	0.73	0.96
Tyrosine	2.56	2.98	3.76	1.31	3.03
Valine	5.31	5	5.28	5.6	5.42
Methionine	1.39	1.66	2.08	1.24	1.51
Lysine	5.81	5.87	4.53	4.87	5.34
Isoleucine	4.29	4.02	4.53	4.52	4.42
Leucine	7.42	7.02	7.92	8	7.31
Phenylalanine	4.68	4.26	6.68	6.01	4.9
EAA	44.21	31.67	36.54	42.31	43.75
Protein %	43.15	41.35	42.99	38.69	42.47

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Characterization of Mutant Lines of Mungbean [*Vigna radiata* (L) Wilczek]

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Mutation breeding is relatively quicker method for improvement of crops. The experiment comprised 32 (31 mutant lines + one parent line) lines of mungbean cv. Pusa 9072 in M₈ generation, which were isolated through appropriate selection procedure after mutagenic treatment. The analysis of variance showed significant differences for all eight morphological characters under study. The mutant AAIMM-10 recorded to be the best performer for seed yield per plant, while the mutant AAIMM-24 was taken 58 days to maturity. The higher magnitude of genotypic coefficient of variation and phenotypic coefficient of variation were recorded for seed yield per plant while moderate estimates for plant height and number of clusters per plant. The characters showed highest heritability for pod length and days to 50 per cent flowering. High genetic advance as per cent of mean was observed for seed yield per plant while moderate estimates for plant height and number of clusters per plant. It is concluded in present study that sufficient variability present in the mutant lines and there is an ample scope for genetic improvement through selection in mungbean.

Key Words: Mungbean, Mutant lines, GCV, PCV, Heritability, Genetic advance

Introduction

The pulses production of India is 15.9 million tones which is still deficit of the present consumption, i.e., 17.65 million tones (Ali and Kumar, 2008). The requirements of pulses is expected to rise further mainly due to ever-increasing population and preference for pulses as the cheapest source of dietary protein. Among the pulses, mungbean [*Vigna radiata* (L.) Wilczek syn. *Phaseolus aureus* Roxb; *P. radiatus* L.] is the most important crop. It is also known as greengram and is the most widely distributed among the six Asiatic *Vigna* species. Mungbean is rich in protein (25.9%), fat (1.0-1.5%), fibre (3.5-4.5%), ash (4.5-5.5%) and carbohydrate (59-65%) and provides 334-344 k cal energy (Srivastava and Ali, 2004).

Improvement of the cultivated plants largely depends on the extent of genetic variability available within the species. Mungbean is considered rather wild as it gives low seed yield (<1 t/ha), with uneven maturity. The major constraints in achieving higher yields of this crop are due to lack of genetic variability, absence of suitable ideotypes for different cropping systems, poor harvest index and susceptibility to diseases. Broad spectrum genetic variability is pre requisite for any successful breeding programme. Besides natural genetic variation available in mungbean germplasm collections, mutagenesis as a modern device has proven useful in obtaining novel traits and creating genetic variability (Sangsiri *et al.*, 2005). Therefore, an attempt has been made to create

additional genetic variability for quantitative traits in present study.

Materials and Methods

The experiment comprised 32 (31 mutant lines + one parent line) mutant lines of mungbean cv. Pusa 9072 in M₈ generation which were isolated through appropriate selection procedure after mutagenic treatment. The experiment was carried out at Allahabad Agricultural Institute-Deemed University, Allahabad during *zaid*, 2008 in randomized block design in three replications. Each genotype was grown in a plot size of 4 m² area with 30 x 10 cm spacing. The recommended cultural practices were adopted for the proper growth and stand of the crop. The observations were recorded on 10 randomly selected plants from each replication of each genotype for days to 50 per cent flowering, plant height (cm), number of clusters per plant, pod length (cm), number of seeds per pod, days to maturity, 100-seed weight (g) and seed yield per plant (g). The mean values were used for computation. Phenotypic coefficient of variation and genotypic coefficient of variation calculated after Burton (1952), heritability in broad sense (h²) after Burton and Devane (1953), and genetic advance, i.e., the expected genetic gain by using the procedure given by Johnson *et al.* (1955).

Results and Discussion

The analysis of variance (Table 1) showed significant differences for all eight morphological characters under

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Table 1. Analysis of variance for quantitative characters of mutants of mungbean

Characters	Mean sum squares		
	Replication (df=02)	Treatment (df=31)	Error (df=62)
Days to 50% flowering	26.84	23.41*	2.24
Plant height	27.30	196.60*	23.47
No. of cluster per plant	10.68	21.97**	4.42
Pod length	0.27	1.51*	0.13
Seeds per pod	4.61	3.75**	0.82
Days to maturity	4.13	19.53*	2.19
Seed yield per plant	5.26	105.77*	11.36
100-seed weight	0.26	0.55**	0.06

* **

study. All the characters under study indicated that there is ample scope for isolation of promising lines from present mutant lines for yield improvement. The data presented in Table 3 for mean performance of 31 mutant lines and the parent variety (Pusa-9072) for eight characters, i.e., days to 50% flowering, plant height, clusters per

plant, pod length, seeds per pod, days to maturity, seed yield/plant and 100-seed weight. The highest variability (Vp and Vg) was recorded for plant height (81.2 and 57.7) and seed yield per plant (42.8 and 31.5). While lowest values were observed for 100-seed weight (0.22 and 0.16), pod length (0.59 and 0.45), seeds per pod (1.79 and 0.97), days to maturity (7.97 and 5.77), and days to 50% flowering (9.29 and 7.05). The minimum variance between genotypic variance and phenotypic variance was estimated for 100-seed weight which revealed that this character is less influenced by environmental effect. Similar observations were reported by Idrees *et al* (2006).

In general estimates of phenotypic coefficient of variation (PCV) were higher than their corresponding genotypic coefficient of variation (GCV) however good correspondence was observed between GCV and PCV for all characters. A wide range of PCV was observed

Table 2. Mean performance of mutant lines derived from parent (Pusa 9072) for different characters

Accession No.	Days to 50% flowering	Plant height	No. of cluster/plant	Pod length	No. of seeds/pod	Days to maturity	100-seed weight (g)	Seed yield/plant(g)
AAIMM-1	39.33	30.05	7.65	4.05	9.13	62.50	2.80	7.52
AAIMM-2	38.33	53.40	11.68	6.66	10.98	60.00	3.67	10.25
AAIMM-3	37.33	49.30	13.38	5.48	11.10	65.50	3.23	19.63
AAIMM-4	35.00	46.73	18.20	7.08	10.70	64.00	3.70	16.83
AAIMM-5	39.67	40.10	16.60	6.55	10.70	63.00	3.88	14.83
AAIMM-6	34.33	63.20	14.76	6.62	10.50	64.50	3.72	25.57
AAIMM-7	34.33	60.53	13.38	7.39	12.10	63.00	3.38	20.14
AAIMM-8	38.00	49.09	19.33	7.69	12.00	59.50	4.40	12.33
AAIMM-9	34.67	57.37	13.80	6.97	12.30	62.00	3.65	26.12
AAIMM-10	41.00	54.60	12.90	7.02	10.00	60.00	4.21	33.08
AAIMM-11	39.33	60.22	19.20	7.91	10.90	66.50	3.50	20.33
AAIMM-12	39.67	54.00	20.94	7.71	12.10	66.00	5.25	19.25
AAIMM-13	35.00	47.73	15.33	7.19	10.60	65.00	3.76	25.10
AAIMM-14	38.00	45.97	16.15	7.24	12.90	62.50	3.63	19.64
AAIMM-15	33.00	33.40	11.10	6.20	8.40	68.00	3.78	13.44
AAIMM-16	36.33	40.93	12.17	7.53	10.95	65.50	3.35	16.50
AAIMM-17	36.67	46.43	12.18	6.72	9.85	69.50	3.46	14.43
AAIMM-18	33.33	43.73	13.20	6.84	10.20	65.00	3.61	18.71
AAIMM-19	35.00	38.13	15.38	6.62	9.70	63.50	3.84	15.12
AAIMM-20	36.33	42.90	13.60	6.46	10.50	65.50	3.25	25.84
AAIMM-21	34.33	44.15	17.20	7.21	11.70	64.00	3.95	17.95
AAIMM-22	33.33	37.45	12.70	6.23	10.00	63.00	3.34	23.08
AAIMM-23	34.33	35.05	15.69	6.69	9.65	62.00	3.90	12.15
AAIMM-24	31.67	39.20	12.80	7.00	10.00	58.50	4.00	20.52
AAIMM-25	34.00	42.42	15.53	6.81	12.80	69.00	3.55	25.35
AAIMM-26	32.67	43.27	15.35	6.82	10.70	64.00	3.70	23.26
AAIMM-27	31.33	47.57	12.70	6.46	9.10	63.00	3.40	16.84
AAIMM-28	32.67	49.53	16.80	6.35	11.90	66.00	3.42	30.33
AAIMM-29	32.00	37.37	11.98	6.25	9.25	65.00	3.78	20.23
AAIMM-30	33.00	44.80	13.70	6.36	9.80	65.00	3.16	26.09
AAIMM-31	32.00	41.83	14.00	6.58	10.90	64.50	3.24	25.00
Parental	31.67	53.40	14.10	7.15	11.50	66.50	3.68	26.52

Table 3. Estimates of variance and genetic parameters for quantitative characters of mutant lines of mungbean

Characters	σ^2_g	Variance σ^2_p	σ^2_e	GCV	PCV	h^2 (bs)%	GA	GG
Day to 50% Flowering	7.05	9.29	2.24	7.54	8.65	75.9	4.76	13.53
Plant height	57.71	81.18	23.47	16.49	19.56	71.1	13.19	28.64
No. of cluster/plant	5.84	10.27	4.42	16.69	22.13	56.9	3.75	25.95
Pod length	0.45	0.59	0.14	10.02	11.45	76.6	1.21	18.06
Seeds/pod	0.97	1.79	0.82	9.22	12.50	54.4	1.50	14.00
Days to maturity	5.77	7.97	2.19	3.75	4.40	72.5	4.21	6.57
100 seed weight	0.16	0.22	0.06	11.11	13.00	73.1	0.71	19.58
Seed yield/plant	31.46	42.83	11.36	28.13	32.81	73.5	9.90	49.66

* h^2 (bs) = Heritability broad sense, σ^2_g = Genotypic variance, σ
 σ

for traits ranging from 4.40 per cent for days to maturity to 32.81 per cent for seed yield per plant. Higher magnitude of PCV was recorded for seed yield per plant (32.81 %) and followed by number of clusters per plant (22.13%). While moderate estimates observed for plant height (19.56 %), and low estimates of 100-seed weight (13.00%), seeds per pod (12.50%), pod length (11.45%), days to 50% flowering (8.65%) and days to maturity (4.40%). GCV ranged from 3.75% (days to maturity) to 28.13 (seed yield per plant). Higher magnitude of GCV was recorded for seed yield per plant (28.13%) while moderate estimates for plant height (16.49 %) and number of clusters per plant (16.69 %). The low estimates of GCV values was observed in days to maturity (3.75 %), days to 50 per cent flowering (7.54%), seeds per pod (9.22%), pod length (10.02%) and 100-seed weight (11.11). Relatively low magnitudinal differences were observed between GCV and PCV for 100-seed weight, days to 50 per cent flowering, pod length, seeds per pod and days to maturity. This indicates less environmental influence in the expression of these attributes. Relatively high differences between GCV and PCV were observed for seed yield per plant, and number of clusters per plant. These findings suggested that greater influence of the environment in the expression of these traits. Rao *et al.* (2006) reported that the high magnitudinal differences between GCV and PCV for seed yield per plant and number of clusters per plant, whereas, environmental coefficient of variation contributed more in the expression of these characters.

High heritability was observed for traits viz. pod length (76.6%) and days to 50 per cent flowering (75.9%). According to Panse (1957) such characters governed predominantly by additive gene action and could be

improved through individual plant selection. Similar results were reported by Anwari and Sochendi (1999), Kumar *et al.* (2003), Idrees *et al.* (2006). The values of genetic advance as percent of mean were observed for plant height (13.19%), seed yield per plant (9.90%), days to 50 per cent flowering (4.76%), days to maturity (4.21%), Number of clusters per plant (3.75%), seeds per pod (1.50%), pod length (1.21%) and 100-seed weight (0.71%). All the characters studied showed the low genetic advance. A character exhibiting high heritability may not necessarily give high genetic advance. Johnson *et al.* (1955) reported that high heritability should be accompanied by high genetic advance to arrive at more reliable conclusion. Highest genetic advance as per cent of mean was observed for seed yield per plant (49.66) while moderate for plant height (28.64%) and number of clusters per plant (25.95%). The 100-seed weight, pod length, seeds per pod, days to 50 per cent flowering and days to maturity have low estimates of genetic advance as per cent of mean.

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Induced Genetic Variability through Physical and Chemical Mutagens in M_3 and M_4 Populations of Frenchbean (*Phaseolus vulgaris* L.)

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Rajmash (*Phaseolus vulgaris* L.), also known as rajma, French bean, kidney bean, common bean etc. is a popular pulse crop at hills and hill valleys but not so popular in the plains. Out of the two cultivars, 'Udai' and 'Malviya Rajmash 137' released for cultivation in plains, the cultivar 'Udai' is not widely accepted because of its shattering of pods at maturity, variegated seed colour and low yield as compared to Malviya Rajmash 137. The pure and healthy seeds of the variety 'Udai' were irradiated with gamma rays (5, 10, 15, 20 and 40 kR doses), ethyl methane sulphonate (10, 20, 40, 60 and 80 mM concentrations) and their combinations (5 kR + 10 mM, 10 kR + 20 mM, 15 kR + 40 mM, 20 kR + 60 mM and 40 kR + 80 mM doses/concentrations). In the present investigation, the dose of 15 kR was found to be most efficient among the different doses of gamma rays with few exceptions. In case of the EMS, the concentration of 20 mM was found to be the best. The most efficient treatment among the combination was found to be 10 kR gamma rays + 20 mM EMS.

Key Words: French bean, Genetic variability, Mutagens

Introduction

Pulses, also known as grain legumes, are an important group of crops, next to cereals. Legumes include the species of the family leguminosae which are consumed directly by human beings, mostly as mature dry seeds, but occasionally as immature green seeds or green pods with immature seeds enclosed. Traditionally pulses have been cultivated in rainfed conditions due to some wrong notion that they are meant for poor and marginal lands and can be grown without much care and input. Thus, these energy rich crops are being cultivated largely under energy starvation conditions. With the development of high yielding varieties of cereals and oilseeds, pulses are being pushed more and more towards marginal areas. The non-availability of high yielding varieties and lack of protection against biotic and abiotic stresses compounding the problem (Asthana, 1998; The Times of India, 2001). The 146 legume species available worldwide, about 80 species are consumed by human and only 14 of them are of major economic importance. Among the different pulse crops grown in India, the most important are chickpea and pigeon pea followed by urdbean, mungbean, lentil, pea, cowpea, lathyrus, horsegram and rajmash. Rajmash (*Phaseolus vulgaris* L.) also known as rajma, frenchbean, kidney bean, haricut bean, navy bean, common bean etc. is grown as legume crop, mainly at hilly tracts of India. The crop is not so popular in plains and grown during Rabi season as a minor crop.

Materials and Methods

Present investigation was carried out at the Crop Research Centre, Institute of Agricultural Sciences, Banaras Hindu University during 2001-03. Seeds of Frenchbean (Rajmash) cultivar 'Udai' was treated with two mutagens, viz., gamma rays and ethyl methane sulphonate (EMS) singly as well as in combination. The different doses/concentrations of gamma rays and ethyl methane sulphonate used for seed treatment are as five doses of gamma rays viz., 5 kR, 10 kR, 15 kR, 20 kR and 40kR, five concentrations of ethyl methane sulphonate viz. 10, 20, 40, 60 and 80 mM and also combination treatments of both the mutagens were used. The total sixteen treatments including control viz. T_1 -5 kR γ rays, T_2 -10 kR γ rays, T_3 -15 kR γ rays, T_4 -20 kR γ rays, T_5 -40 kR γ rays, T_6 -10 mM EMS, T_7 -20 mM EMS, T_8 -40 mM EMS, T_9 -60 mM EMS, T_{10} -80 mM EMS, T_{11} -5 kR γ rays + 10 mM EMS, T_{12} -10 kR γ rays + 20 mM EMS,

T_{13} -15 kR γ rays+40 mM EMS, T_{14} -20 kR γ rays + 60 mM EMS, T_{15} -40 kR γ rays + 80 mM EMS and T_{16} -Control. Seeds harvested in M_2 generation from the above mentioned treatments and also seeds of different macro-mutants selected in M_2 generation were used as a experimental materials for study in M_3 generation. The following viable macro-mutants were detected in M_2 generation are dwarf mutant, early maturing mutant, late maturing mutant, non-shattering mutant, high yielding mutant, light seed coat colour

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mutant and dark seed coat colour mutant. The M_2 seeds were space planted in three replications to raise M_3 generation. Seeds of viable macro-mutations were also space planted in three replications individually. The population was screened for macro-mutations in M_3 generation.

Fifty competitive normal looking plants from each replication of each treatment were taken randomly to record the observation. The observations were recorded on selected plants for the following parameters, viz. plant height, number of primary branches per plant, days to flowering, days to maturity, number of pods per plant, length of pod, number of seeds per plant, number of seeds per pod, yield per plant and 100-seed weight.

The M_3 seeds were space planted in three replications to raise M_4 generation and observations were recorded on selected plants for the same parameters as given in M_3 generation.

The statistical analysis for M_3 and M_4 generations was done for analysis of variance and other genetical parameter, viz., Genotypic co-efficient of variation (GCV), Phenotypic co-efficient of variation (PCV), Heritability (h^2) and Genetic Advance (GA).

Results and Discussion

Analysis of Variance (ANOVA)

The analysis of variance for the ten quantitative characters under study in M_3 and M_4 generation revealed that the treatments differed significantly for all the characters studied in both the generations. The analysis of variances has been presented for both the generations for micro mutations and macro (viable) mutants in Table 1 and 2, respectively.

It is obvious from the ANOVA that the treatments were found significant for all the quantitative traits under study in micro mutations in both the generation, i.e., M_3 and M_4 generations. It is also obvious from the ANOVA that the treatments were found significant for most of the quantitative traits under study in M_3 generation except average length of pod and in M_4 generation except average length of pod and 100-grain weight for macro mutants.

Performance of Viable Macro-mutants (Selected from M_2 Generation) in M_3 and M_4 Generations

A number of viable mutations for different morphological and yield traits were evaluated in M_3 and M_4 generations. These included mutations for plant height, earliness, non-shattering, high-yielding, lateness and seed coat colour. The mutations can be categorized into phenological and morphological mutants. Mutation affecting the time taken to flowering and maturity are grouped in to phenological while; those affecting the various plant parts constituted the morphological types (Table 3, 4).

Dwarf Mutant

The value of mean for plant height in control was 41.43 cm in M_3 generation. The plants with the height between 20-30 cm were grouped in to dwarf mutant category. The performance of dwarf mutant in M_3 generation for plant height was recorded with mean value of 27.73 cm which decreased significantly from the control and about same performance was found in M_4 generation whereas, mean value for plant height in control was recorded 43.17 cm and for dwarf mutant progeny it was recorded 27.60 cm. In M_3 this mutant showed a

Table 1. Analysis of variance for ten quantitative traits in Frenchbean cv. 'Udai' for micro-mutations in M_3 and M_4 generations

1	2	3	4									
Generation	Source	DF	Mean Sum of Squares (MSS)									
			Characters									
			Plant height	No. of primary branches per plant	No. of pods per plant	Average length of pods	No. of seeds per plant	No. of seeds per pod	Days to flowering	Days to maturity	100-grain weight	Grain yield per plant
M_3	Replication	2	0.0722	0.0764	0.2238	0.0087	1.251	0.0018	0.1171	0.3750	1.173	0.0505
	Treatments	15	16.81**	0.3141**	2.359**	0.8396**	27.42**	0.0428**	9.337**	10.36**	0.8289**	1.924**
	Error	30	0.2588	0.0191	0.0824	0.0344	0.6843	0.0071	0.2236	0.4138	0.1809	0.0484
M_4	Replication	2	0.0156	0.0308	0.0205	0.0173	0.4238	0.0012	0.8203	1.093	0.1230	0.0190
	Treatments	15	26.65**	0.3208**	2.842**	0.1314**	15.76**	0.241**	9.211**	10.95**	1.173**	1.20**
	Error	30	0.4789	0.0361	0.0575	0.0175	0.3853	0.0038	0.2554	0.2798	0.2164	0.0175

F Table Value at 1% = 4.31; ** Significant at 1%, level of significance

Table 2. Analysis of variance for 10 quantitative traits in Frenchbean cv. 'Udai' for macro-mutations in M₃ and M₄ generations

1	2	3	4									
Generation	Source	DF	Mean sum of squares (MSS)									
			Characters									
			Plant height	No. of primary branches per plant	No. of pods per plant	Average length of pods	No. of seeds per plant	No. of seeds per pod	Days to flowering	Days to maturity	100-grain weight	Grain yield per plant
M ₃	Replication	2	2.794	0.0162	0.1588	0.0920	2.026	0.0087	0.4609	1.984	0.4326	0.0649
	Treatments	7	50.92**	0.7561**	8.52**	0.1455	110.23**	0.073**	20.23**	21.82**	1.564**	7.157**
	Error	14	0.6493	0.0343	0.1501	0.0775	2.234	0.0065	0.7446	1.110	0.2639	0.1574
M ₄	Replication	2	1.444	0.0650	0.0112	0.1771	1.471	0.0133	0.8554	0.0312	0.2617	0.0426
	Treatment	7	60.04**	0.6580**	10.93**	0.0983	122.33**	0.0279*	17.82**	20.848**	1.446	8.834
	Error	14	0.4001	0.0407	0.1433	0.0815	2.383	0.0095	0.5522	0.3750	0.6820	0.0928

F Table value: At 1% level of significant=4.31; ** Significant at 1% level of significance; At 5% level of Significant =2.07; * Significant at 5% level of significance

Table 3. Mean performance of macro-mutants of Frenchbean cv. 'Udai' in M₃ generation

		Characters								
Control/macro-mutants	Plant height (cm)	No. of primary branches per plant	No. of pods per plant	Average length of pod (cm)	No. of seeds per plant	No. of seeds per pod	Days to flowering	Days to maturity	100-grain weight (g)	Grain yield per plant(g)
Control	41.43	4.20	1060	10.07	28.73	2.73	63.20	124.97	28.45	8.16
Dwarf mutants	27.73**	3.97	9.93*	9.63	25.27**	2.54**	63.93	126.43	28.76	7.27*
Early mutants	36.40**	4.00	9.93*	9.40**	25.57*	2.57*	57.53**	120.50**	26.98**	6.90**
Late mutants	38.80**	4.27	11.37*	9.73	28.73	2.45**	66.20**	130.27**	27.96	8.02
Non-shattering mutants	34.53**	3.60**	8.47**	9.47*	20.40**	2.41**	64.08	125.87	28.82	5.88**
High-yielding mutants	38.97**	5.23**	14.30**	9.43**	41.50**	2.90*	65.10**	125.57	26.99**	11.20**
Seed colour mutants (light)	37.47**	3.77**	10.23	9.57*	26.53	2.59*	64.20	125.10	28.14	7.47*
Seed colour mutants (dark)	37.77**	3.87**	10.37	9.50*	27.23	2.62	64.33	124.70	27.67	7.53
SE (d)	0.6569	0.1513	0.3163	0.2273	1.221	0.066	0.704	0.860	0.4194	0.324
CD (1%)	1.809	0.416	0.8698	0.625	3.355	0.1815	1.936	2.365	1.153	0.891
CD (5%)	1.343	0.3089	0.6458	0.4641	2.491	0.1347	1.437	1.756	0.8564	0.661

** Significant at 1% * Significant at 5%

Table 4. Mean performance of macro mutants of Frenchbean co-efficient 'Udai' in M₄ generation

		Characters								
Control/macro-mutants	Plant height (cm)	No. of primary branches per plant	No. of pods per plant	Average length of pod (cm)	No. of seeds per plant	No. of seeds per pod	Days to flowering	Days to maturity	100-grain weight (g)	Grain yield per plant(g)
Control	43.17	4.40	10.83	9.85	28.60	2.63	62.40	123.73	27.22	7.78
Dwarof mutants	27.60**	4.03*	9.80*	9.53	25.70*	2.62	63.97*	125.70*	28.70*	7.37
Early mutants	36.87**	4.23	10.07*	9.57	26.57	2.63	58.32**	120.30**	28.27	7.50
Late mutants	38.60**	4.57	11.50*	9.57	30.57	2.66	66.70**	129.10**	26.75	8.18
Non-shattering mutants	34.80**	3.77**	8.87**	9.32	23.13**	2.61	64.50**	127.00**	28.63**	6.62**
High-yielding mutants	39.43**	5.27**	15.17**	9.73	44.10**	2.90**	64.67**	127.03**	27.68	12.21**
Seed colour mutants (light)	36.57**	4.03*	10.07*	9.57	27.37	2.71	64.27	126.20**	27.48	7.51
Seed colour mutants (dark)	37.63**	4.00*	10.40	9.53	27.83	2.67	63.93*	124.87	27.45	7.64
SE (d)	0.5164	0.1647	0.3155	0.2331	1.26	0.0797	0.606	0.50	0.674	0.248
CD (1%)	1.420	0.4529	0.8676	0.6410	3.465	0.2191	1.666	1.3751	1.853	0.6842
CD (5%)	1.054	0.3363	0.6442	0.4759	2.5729	0.1627	1.237	1.021	1.376	0.508

** Significant at 1%; * Significant at 5%

significant decrease in yield per plant but in M_4 generation it was observed non significant from the control.

In present investigation, the dwarf mutants (detected in M_2 generation) raised in M_3 and M_4 generation to confirm their performance and it is evident from experimental findings that mean performance for plant height recorded in M_3 and M_4 generation in agreement with the hypothesis. Egiazaryan (1981) and Fadl (1988) also observed mutant for plant height after treatment of seeds of Frenchbean with gamma rays and ethyl methane sulphonate.

Early Maturing Mutant

The mean value for days to maturity was recorded 120.5 days in M_3 which significantly decreased from the mean value of control (124.97). In M_4 generation it was recorded (120.3 days) for the mutant progeny which also significantly low from control (123.73 days). But this mutant was significantly low for grain yield per plant in M_3 generation whereas, in M_4 it was non significant for the same.

Late Maturing Mutant

The mean performance for days to maturity were recorded with 130.27 days and 129.10 days in M_3 and M_4 generations for mutant progeny which were significantly higher than the mean of control 124.97 days and 123.73 days in M_3 and M_4 generations, respectively. There was an increase in mean values for grain yield per plant (8.18 g) from the control (7.78 g) in M_4 generation but increase was non-significant. Increase in yield was recorded due to significant increase in number of pods per plant (11.50) from the control (10.83) in M_4 generation. In M_3 generation mean value for grain yield per plant (8.02 g) was recorded which was no significantly low from the control (8.16 g). A number of workers have also reported about early maturing mutants in Frenchbean after treatment with mutagens (Kimani, 1988; Manrique *et al.*, 1988; Zagorcheva and Paryazov, 1983; Navale *et al.*, 1998; Tulmann, 1994).

In present study, it was obvious from the result that early maturing mutant progenies were found earlier but significantly low yielder than the control. Hence, these mutants were not advantageous whereas, late maturing mutant progenies were found late and non-significantly high yielder due to increase in number of pods per plant.

Non-Shattering Mutant

Non-shattering mutants raised in M_3 and M_4 generation

in replicated trials in the field which did not shatter even after fifteen to twenty days of maturity. Non-shattering mutants were found significantly low yielder (5.88 g and 6.62 g) as compared to be control (8.16 g and 7.78 g) in both the generations due to less number of branches and pods per plant. All plants of mutant progenies were found non-shattering in both the generations.

High Yielding Mutant

Mutants for higher yield raised in M_3 and M_4 generation in replicated trials and mean performance recorded for all quantitative traits in both the generations. These mutant progenies were found significantly high yielder in both the generations. The mean values for yield were recorded 11.20 g and 12.21 g in successive generations respectively which were found significantly in positive direction from the control. This increase in yield was due to significant increase in more number of primary branches per plant and number pods per plant from the control. Similar findings have been reported by Kandelaki (1981) and Nandi *et al.* (1995).

Light Seed Coat Colour Mutant

Mutants that were detected in M_2 generation for light seed coat colour raised in M_3 and M_4 generations in replicated trials in the field. The seed coat colour of this mutant (lighter colour) inherited to the M_3 and M_4 generations as in M_2 generation and for other quantitative traits were found either at par or decrease with the control.

Dark Seed Coat Colour Mutant

Mutant with dark seed coat colour raised in replicated trials in the field to see the performance for the seed coat colour in M_3 and M_4 generations. In case of dark mutant progenies white patches decreased leading to more dark red colour. None of the plants were obtained with change in colour in seeds obtained from all the pods and these mutants also showed same for dark seed coat colour in both the generations. This mutant also found either at par or decrease with the control for other quantitative traits.

Estimates of GCV, PCV, Heritability and Genetic Advance in M_3 and M_4 Generations for Micro Mutations

The estimates of phenotypic and genotypic co-efficient of variation (PCV and GCV), heritability in broad sense and genetic advance have been presented in Table 5.

Invariably, phenotypic co-efficient of variation values were higher than the corresponding genotypic co-efficient of variation in both the generations. Among all the parameters under study, maximum values for GCV and PCV were obtained for number of seeds per plant (11.65 and 12.09) followed by yield per plant (10.53 and 10.93) in M₃ generation and number of pods per plant (9.18 and 9.46) followed by number of seeds per plant (8.22 and 8.52) in M₄ generation. Lower values of GCV and PCV were obtained for days to maturity, 100-grain weight, and days to flowering in M₃ generation and in M₄ generation, it was obtained for days to maturity, 100-grain weight and average length of pod. Almost a similar finding was reported by Arya and Rana (1999) in French bean. Almost all the characters exhibited high values for heritability in both the generations. The highest heritability was recorded for plant height (0.955) followed by days to flowering (0.931), number of seeds per plant (0.929) and grain yield per plant (0.928) in M₃ generation and for grain yield per plant (0.957) followed by plant height (0.948), number of pods per plant (0.942) and

number of seeds per plant (0.930) in M₄ generation. The 100-grain weight exhibited the minimum value of heritability (0.544 and 0.596) in both the generations, respectively. The number of seeds per pod also exhibited low values of 0.624 and 0.638 of heritability in M₃ and M₄ generations, respectively. The highest value of genetic advance was scored for number of seeds per plant (5.93) followed by plant height (4.73) days to maturity (3.54) and days to flowering (3.47) in M₃ generation and in M₄ generation it was scored for plant height (5.92) followed by number of seeds per plant (4.5), days to maturity (3.74) and days to flowering (3.42). The minimum values (0.18 and 0.14) of genetic advance was recorded for the number of seeds per pod.

Several reports regarding heritability and genetic advance has been reported in Frenchbean. Singh *et al.* (1994) recorded high values of heritability and genetic advance for yield per plant. Somal *et al.* (1995) found maximum heritability for days to fifty per cent flowering, followed by 100-seed weight and plant height. Nandi *et al.* (1995) found that yield per plant had moderately

Table 5. Estimates of genotypic, phenotypic co-efficient of variation, heritability and genetic advance for micro-mutations of Frenchbean Cv. 'Udai' in M₃ and M₄ generations

Characters	M ₃ generations					M ₄ generation				
	GCV	PCV	h ²	GA	GA as percent of mean	GCV	PCV	h ²	GA	GA as percent of mean
Days to flowering	2.71	2.81	0.931	3.47	5.40	2.71	2.82	0.924	3.42	5.36
Days of maturity	1.45	1.54	0.889	3.54	2.83	1.51	1.57	0.927	3.74	2.99
Plant height	6.43	6.58	0.955	4.73	12.95	7.81	8.02	0.948	5.92	15.65
No. of primary branches	8.15	8.91	0.837	0.590	15.37	7.07	8.31	0.724	0.54	12.41
No. of pods per plant	9.03	9.50	0.902	1.70	17.62	9.18	9.46	0.942	1.93	18.40
Average length of pods	5.57	5.92	0.886	1.00	10.77	2.04	2.47	0.684	0.33	3.46
No. of seeds per plant	11.65	12.09	0.929	5.93	23.14	8.22	8.52	0.930	4.50	16.34
No. of seeds per pod	4.10	5.19	0.624	0.18	6.80	3.11	3.89	0.638	0.140	5.30
100-grain weight	1.58	2.14	0.544	0.71	2.41	1.99	2.58	0.596	0.90	3.17
Grain yield per plant	10.53	10.93	0.928	1.57	20.90	8.06	8.24	0.957	1.27	16.32

Table 6. Estimates of genotypic, phenotypic co-efficient of variation, heritability and genetic advance for macro-mutants of Frenchbean Cv. 'Udai' in M₃ and M₄ generations

Characters	M ₃ generations					M ₄ generation				
	GCV	PCV	h ²	GA	GA as percent of mean	GCV	PCV	h ²	GA	GA as percent of Mean
Days to flowering	4.01	4.23	0.897	4.97	7.82	3.77	3.95	0.912	4.72	7.42
Days to maturity	2.09	2.26	0.861	5.02	4.00	2.08	2.14	0.948	5.24	4.17
Plant height	11.17	11.39	0.963	8.27	22.57	12.11	12.23	0.980	9.09	24.68
No. of primary branches per plant	11.93	12.75	0.875	00.95	23.11	10.58	11.58	0.835	00.85	19.85
No. of pods per plant	15.69	16.10	0.949	03.35	31.45	17.50	17.86	0.960	03.83	35.36
Average length of pods	01.57	03.30	0.226	00.15	01.56	00.79	03.11	0.064	00.04	0.421
No. of seeds per plant	21.43	22.09	0.942	11.99	42.83	21.63	22.27	0.944	12.65	43.27
No. of seeds per pod	05.74	06.53	0.773	00.27	10.38	02.93	04.68	0.392	00.10	3.74
100-grain weight	02.35	02.99	0.622	01.07	03.82	01.82	03.49	0.272	00.54	1.94
Grain yield per plant	19.57	20.22	0.937	03.05	39.10	21.07	21.41	0.969	03.46	42.71

high heritability and genetic advance. High heritability values for days to flowering has also been reported by Somal *et al.* (1997). Jai Dev and Gupta (1997) found high heritability for most of the traits but genetic advance was moderate for different traits in M_2 generation. Similar finding has been reported by Nandi *et al.* (1988) and Dikshit *et al.* (1999). The highest values of heritability and genetic advance for days to flowering followed by days to maturity, yield per plant and 100-grain weight have been reported by Roy (2001). Singh *et al.* (2001) recorded highest estimates of heritability and genetic advance for number of pods per plant, number of seeds per pod and 100-seed weight in Mungbean.

Estimates of GCV, PCV, Heritability and Genetic Advance in M_3 and M_4 Generations for Macro-Mutants

The estimates of phenotypic and genotypic co-efficient of variation, heritability in broad sense and genetic advance have been presented in the Table 6.

Invariably, phenotypic co-efficient of variation values were higher than the corresponding genotypic co-efficient of variation. Among all the parameters under study, maximum values for GCV and PCV were obtained for number of seeds per plant (21.43 and 22.09) followed by grain yield per plant (19.57 and 20.22) in M_3 generation and in M_4 generation, it was found for the same characters but values were (21.63 and 22.27) followed by (21.07 and 21.41). Low values of GCV and PCV were obtained for average length of pods, 100-grain weight and days to maturity in both the generations. Almost all the characters exhibited high values for heritability in both the generations. The highest heritability was recorded for plant height (0.963) followed by number of pods per plant (0.949), number of seeds per plant (0.942) and grain yield per plant (0.937) in M_3 generation whereas, in M_4 generation, maximum heritability was exhibited by plant height (0.980) followed by grain yield per plant (0.969), number of pods per plant (0.960) and days to maturity (0.948). The average length of pods exhibited the minimum value of heritability (0.226 and 0.064) in M_3 and M_4 generations, respectively. The 100 grain weight also exhibited low values of 0.622 and 0.272 in M_3 and M_4 generations, respectively. The highest values of genetic advance were recorded for number of seeds per plant (11.99 and 12.65) and followed by plant height (8.27 and 9.09) in M_3 and M_4 generations, respectively. The minimum values (0.15 and 0.04) of

genetic advance were recorded for the average length of pods in M_3 and M_4 generation, respectively.

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Evolvable Features of Lignin Pathway in Wheat under Water Stress

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Recent advances in plant genomics have led to the identification of a vast number of potentially beneficial water-stress-related genes, plus technologies for gene over-expression or silencing. The characterization of key plant physiological mechanisms that restrain plant performance under drought, together with the associated regulatory genes, could therefore facilitate the development by breeders of improved crop varieties showing increased water-use efficiency and drought resistance. Plant growth is the result of daughter-cell production by meristematic cell divisions and subsequent massive expansion of the young cells. Cell expansion is in turn dependent on biophysical changes, which include a regulated loosening of primary cell walls and subsequent yielding to the hydrostatic (turgor) pressure generated by solute and water uptake into the cells. Lignin is a core component of plant cell wall and it is important for water transport, structural integrity, rigidity and pest resistance. Our light microscope and electronmicrographs data on three wheat (hexaploid) cultivars HD 2428 (drought susceptible), HD2285 (thermotolerant) and C306 (drought tolerant) displayed that weak linkage between lignin gene function and metabolic outcome may be advantageous, since it may enhance the tolerance, flexibility and robustness of metabolic regulation.

Key Words: Hexaploid wheat, Water stress, Vascular bundle

Introduction

In annual cropping systems, agricultural fitness is defined as the ability to produce economically harvestable yields of useful and commercially desirable plant products. Agricultural fitness is influenced by many metric factors including germination, pest resistance, survival, growth rate, lodging and yield. For example, glume and caryopses lignin content of the wild relative of sorghum (shattercane) is higher than that of cultivated sorghum [*Sorghum bicolor* (L.) Moench], and lignin content is positively correlated with survival over winter of shattercane seed (Fellows and Roeth, 1992). Agronomically favourable traits are not necessarily those that would promote survival of wild species in at least some environments. For example, crop life-spans are modified to accommodate growing seasons. However, in terms of agronomic fitness, reducing lignin may have positive, neutral, or negative effects. Lignin, a complex phenolic polymer, is integral to plant health, survival, and functions. Lignin provides strength and rigidity to plants and to plant structures such as xylem, which allows for the transport of water; and sclerenchyma and bundle sheath cells, which provide a natural barrier to microorganisms (Bird, 1988). Production of lignin has been demonstrated to be induced in plants in response to mechanical damage or infection by pathogens in numerous species including cucumber, carrot, radish, melon, potato, bean, and grasses.

Variable conclusions regarding the effect of reduced lignin on agricultural fitness are common and most

probably derive from interactions of specific genes (which may affect more than just lignin synthesis) and individual plant species, cultivars, and environments. Several studies have attempted to establish a relationship between lodging and lignin content in wheat. However, studies using growth regulators to elicit reduced lodging showed no relationship between lignin content and lodging in wheat (Clark and Fedak, 1977; Knapp *et al.*, 1987). An underlying assumption is that to remain agriculturally fit, plants with reduced lignin would need to remain morphologically similar to commercially desirable unmodified crops. Pincon *et al.* (2001) showed reduced plant size in tobacco transformed with COMT (caffeic acid 3-O-methyltransferase) and CCoAOMT (caffeoyl-CoA-O-methyltransferase) antisense sequences reducing lignin by 48%. Conversely, Zhong *et al.* (1998) demonstrated significantly reduced lignin (34–59%) in tobacco plants transformed with COMT and CCoAOMT antisense sequences compared with wild-type tobacco, but with normal morphological phenotype. Chabannes *et al.* (2001) reported a synergistic interaction of CAD (cinnamyl alcohol dehydrogenase) and CCR (cinnamyl CoA reductase) antisense transgenes leading to severe reduction in lignin content in transformants that had a normal morphological phenotype under controlled conditions, concluding that “expression of a specific transgene may have different impact depending on the genetic background.” Expected responses to significant biochemical or physiological modification of lignin content are reduced vigour and growth.

Here, the discussion is limited to expression of lignin under sudden imposition of water stress and gradual adaptation of wheat cultivars under high temperature in late sown conditions, and the associated changes in plant morphology, and implications.

Materials and Methods

Wheat cultivars C 306 (water stress tolerant), HD 2285 (heat tolerant during grain filling period) and HD 2428 (water stress susceptible) were planted under normal (15 November) and late sown (15 January) conditions. Sowing was done in earthen pots of 30 x 30 cm size filled with sandy loam soil and farmyard manure in 3:1 under natural environment. Each pot was fertilized corresponding to 120, 90 and 60 kg ha⁻¹ of N, P and K, respectively. Four seedlings were maintained in each pot. Plants were watered, as per requirement and stress was imposed by lack of water supply for 4 days. Plant protection measures were taken as required. The water stress treatment was imposed at ear emergence (1 cm length) and at grain filling stage (half grain). Seven days period was given for the revival of the plants from

water stress. For the estimation of relative water content (RWC) and all anatomical analysis using light and electron microscope, flag leaf of the mother shoot was selected. Each value of RWC, presented in figure is a mean of three replicates. For yield analysis five ears were selected at the time of harvest from the mother shoot. The flag leaf in January sowed plants develops at high temperature.

Yield components were assessed by analysis of complete factorial randomized design. Standard error was calculated for RWC and shown in graphs. Critical difference was calculated by using statistical package for agriculture (opstate).

Results and Discussion

The cells of plants grown under favorable environmental conditions were plasmolysed and cell volume is reduced due to water loss when subjected to water stress (Plate 1-A). The vascular bundle was lignified during revival from water stress in drought tolerant wheat cultivar (Plate 1-B). Therefore, this provided a clear example of a wall localized enzyme action *in vivo*. While in late sown

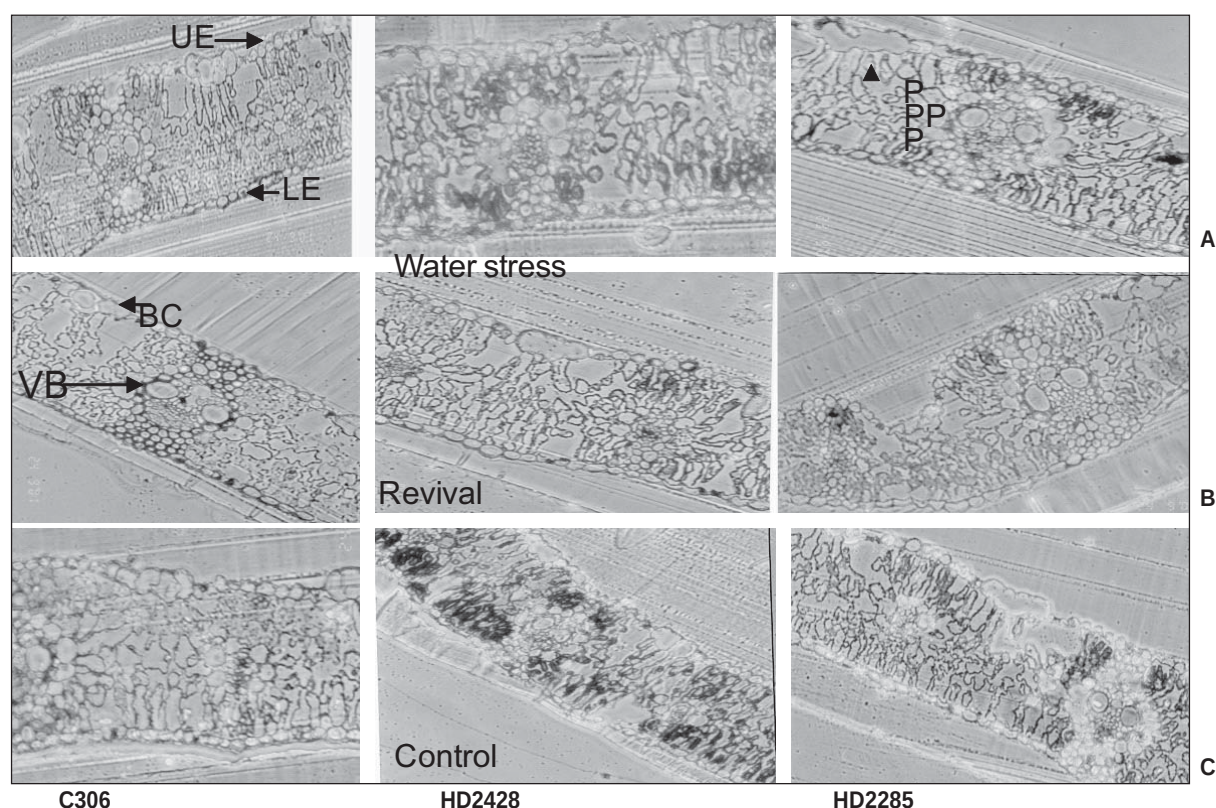


Plate 1: Transverse section of flag leaf of three cultivars of wheat under light microscope (20x) showing lignification of vascular bundle in C306 after revival of water stress. -10X; UE and LE (upper and lower epidermis), PP (palisade parenchyma), BC (bulliform cell) and VB (vascular bundle)

plant, cell volume is reduced due to the increased lignifications of cell wall (Plate 2). There was significant reduction in RWC under water stress in all cultivars. Genotype C 306 retained significantly higher RWC when submitted to water stress conditions under all sowing dates. HD 2285 exhibited intermediate value, while HD 2428 showed lowest value under water stress.

Presence of endoplasmic reticulum, ribosomes and Golgi apparatus indicate *de-novo* synthesis and modification of proteins in vascular bundle of C 306 during revival of stress (Plate 3). We have demonstrated that C306 is drought tolerant genotype because it synthesized lignin using reactive oxygen species induced Cu-Zn-SOD antioxidants (proposed to function in the apoplast in lignifications and that in the nucleus protects the cell against fatal mutations caused by O_2 molecules) in the vascular bundle during recovery from water stress.

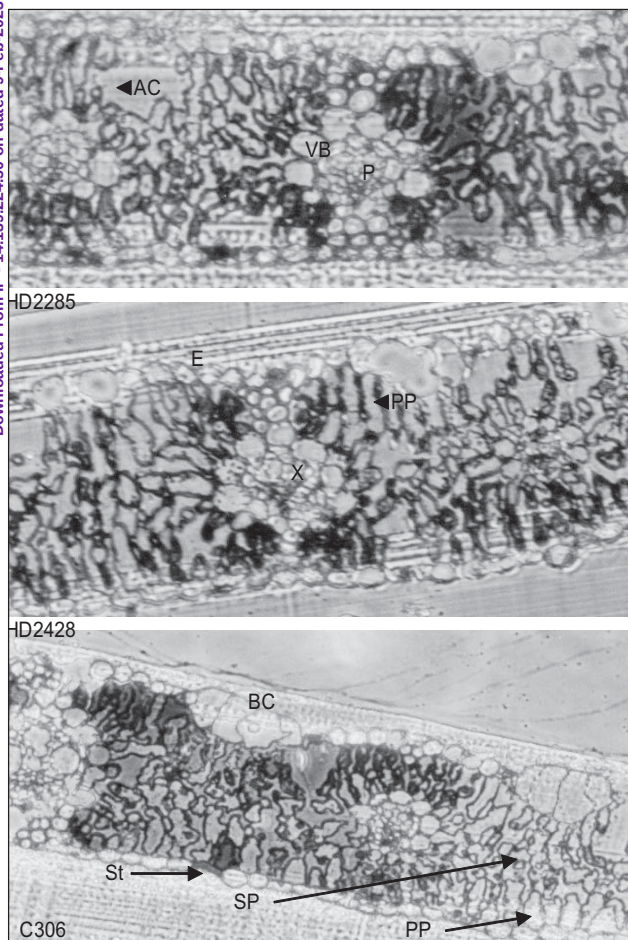


Plate 2: Transverse section of flag leaf of three cultivars of three cultivars sown in January under light microscope (20x) showing reduced cell size and lignification of all cells. E (Epidermal cells); X (Xylem); B (Bulliform cells); VB (Vascular bundle) and P (Phloem); PP (Palisade parenchyma); SP (Spongy parenchyma); St (Stoma)-10X

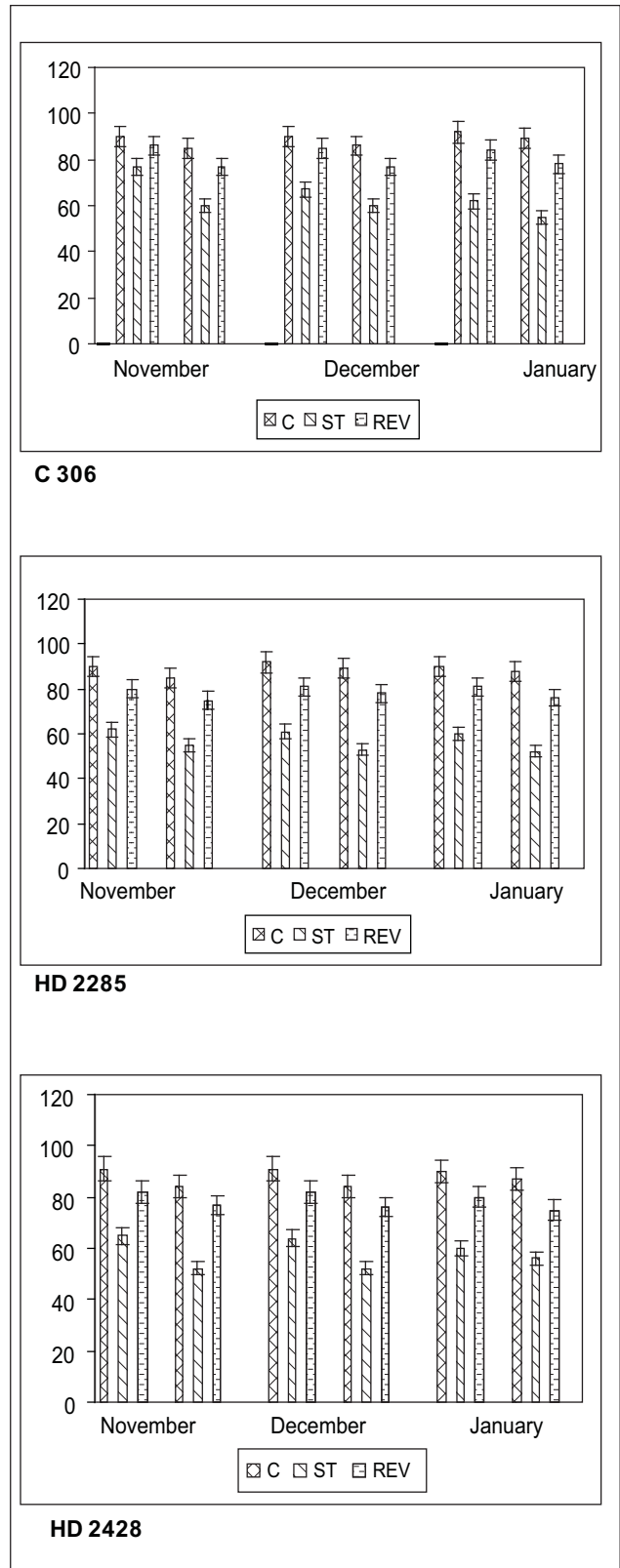


Fig. 1: Relative water contents (%) of flag leaf in C306, HD 2428 and HD 2285 at ear emergence and grain filling stages under water stress at different dates of sowing

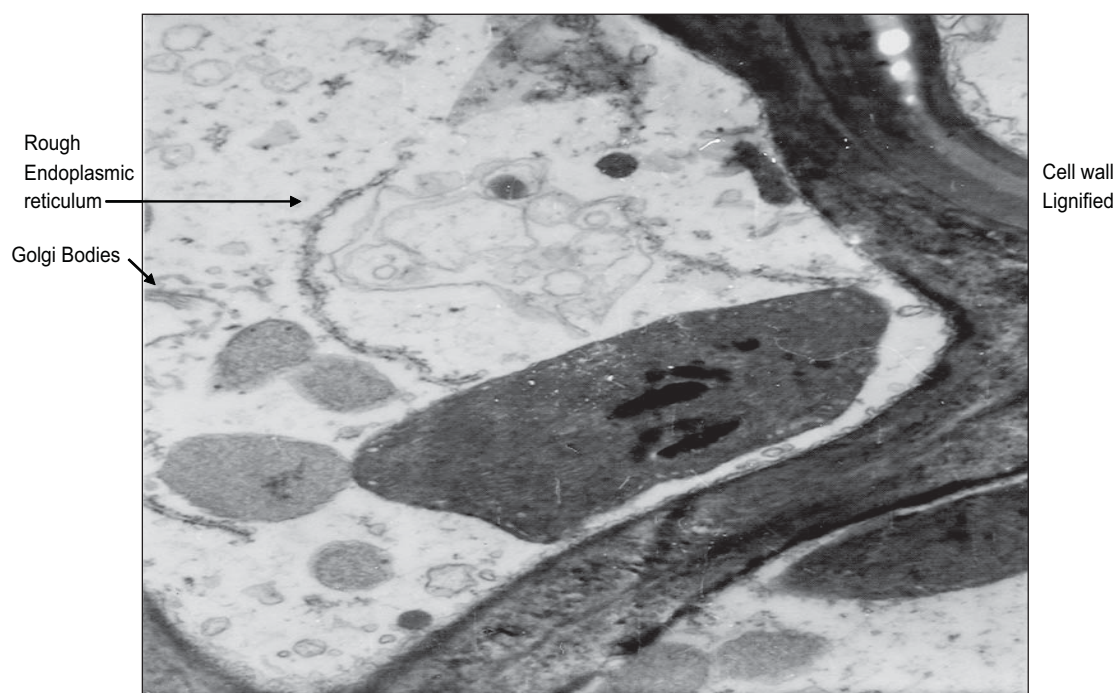
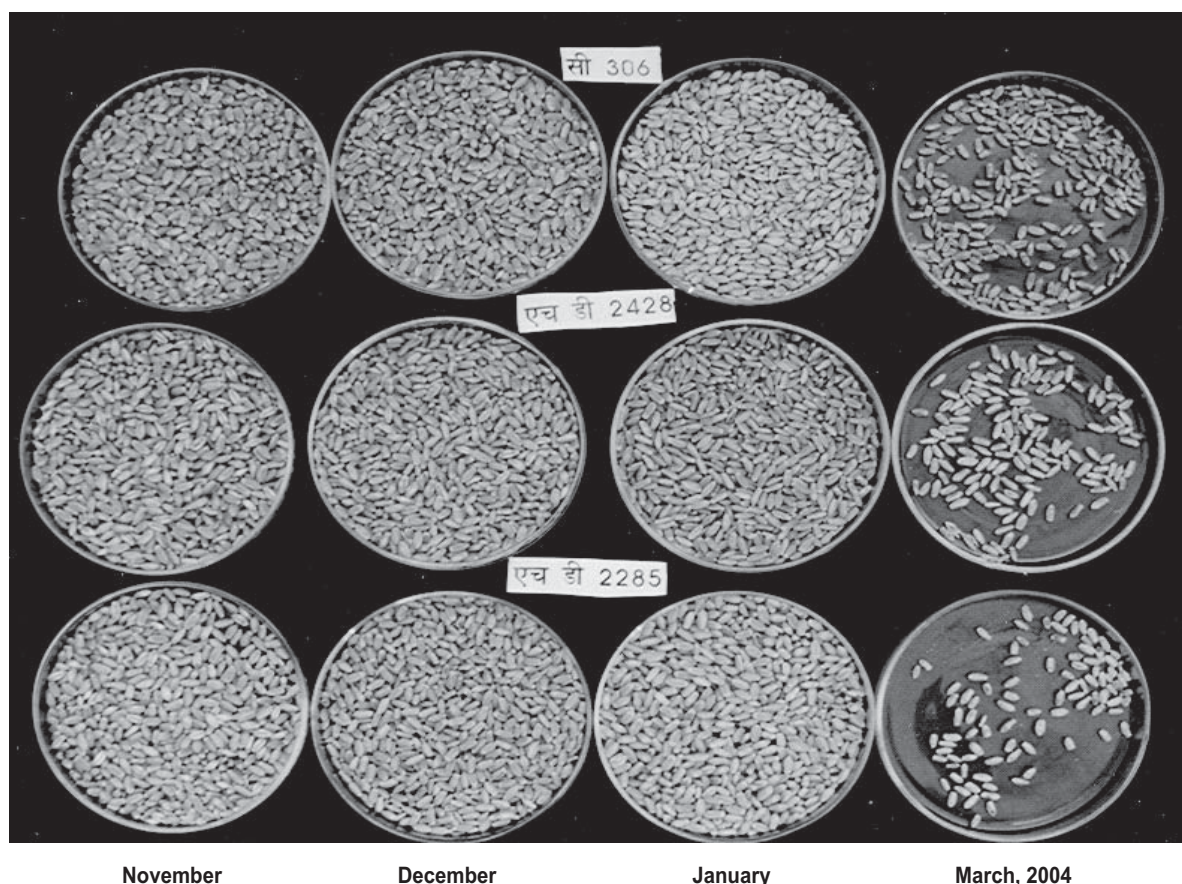


Plate 3: Golgi bodies and rough endoplasmic reticulum appear in vascular parenchyma cell during rehydration of tissue in response to water stress



November

December

January

March, 2004

Plate 4: Number and size of seeds in C306, HD2428 and HD2285 cultivars per plant seen at different dates

The cultivars can resist cavitations (breakage of water column) by lignification of vascular bundle under water stress conditions. The size of the xylem vessels were reduced due to secondary growth (lignin formation); reducing the efficiency of water flow in wheat at high temperatures. Furthermore, lignin is water insoluble and thus immobile; therefore, lignin must be made where it is found. In fact, lignin is initially formed in the middle lamella and primary cell wall (PCW) of cells such as xylem vessel elements and phloem fibers (Wardrop, (1971). The initial site of lignifications may thus be approximately 10 micron distant (the thickness of the secondary wall) from the nearest living protoplasm. Gene inactivation, in the *ref8 Arabidopsis thaliana* mutant, with a point mutation in the CYP98A3 gene, was associated with reduced cell expansion, altered sugar composition of cell walls, and decreased contents in crystalline cellulose and pectins (Nawroz *et al.*, 2006).

Cell sizes were reduced because of the increased rate of growth or rapid cell division of smaller size and different cell wall composition (indicated by vascular bundles and epidermal cells) under higher temperature. Secondary metabolism utilizes more energy in terms of synthesis of lignin. Therefore, photosynthates liberated by internode reduction are used in production of thick leaf (multiple layers of mesophyll tissue) and cell wall thickenings. Inhibition of cell expansion results in a slowing of leaf expansion early in the development of water deficits. Finally, it was expressed in reduced internode length, plant height, leaf area and duration of developmental phases in wheat under higher temperature in late sown conditions. Reduced cell expansion as a primary response to water deficit serves to reduce plant water use but lead to reduce plant productivity.

Reduction in 100-seed weight (grain weight and size) of wheat could have been by reduction in the endosperm cell expansion indicating involvement of abscisic acid accumulated in water deficit thus limiting the maximal storage capacity of the kernels by water stress at ear emergence stage. Narrow xylem vessels formation due to reduced cell size under the influence of abscisic acid, and early disconnection of grain could be another reason for smaller grain size under water stress particularly in late sowed crops (Plate 4). Our study with fresh tissue further showed that grain yield was reduced not only due to reduced leaf area and spike size but also by infertility of spikes and inability of pollen tube to reach the egg cell or ovule due to the

thickness of ovary wall in lignified tissue (observation under microscope). Several factors could be involved in driving lignin biosynthesis evolution, including environmental adaptation and development of new structures and greater plant stature. We cannot exclude any one of them. Drought tolerant cultivar synthesizes lignin around vascular bundle to avoid cavitations during rehydration under normal planting. While all the three contrasting cultivars lignified whole leaf tissue to survive under drought conditions created by high temperature in January sowed plants. The strong interactions among lignin reducing genes, the genetic background in which they are placed, and the environment in which the resulting plant lines will ultimately determine the agricultural fitness of reduced lignin cultivars and hybrids. Taken as a whole, it appears that reducing lignin content of crop plants can negatively impact their agricultural fitness. However, when evaluating individual events of reduced lignin, effect on agricultural fitness may be neutral or even positive. An example of such a positive reaction to reduced lignin content is provided in a study by Hu *et al.* (1999) that showed enhanced leaf, root, and stem growth in transformed poplar (*Populus tremuloides* Michx.) with a 45% reduction in lignin. It is essential that reduction in lignin content, whether resulting from mutations in lignin biosynthesis genes, from selection for traits associated with reduced lignin, or from transformation, be evaluated in diverse genetic backgrounds and gene combination, and in diverse environments to discover optimal combinations and a true measure of value to, and fitness in agricultural systems.

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Prediction of Plant Height in Okra [*Abelmoschus esculentus* (L.) Moench] through Seedling Markers

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Okra is especially valued for its tender and delicious fruits in different parts of the country. It has a great potential for earning foreign exchange and accounts for about 60% of the export of fresh vegetables excluding potato, onion and garlic. Plant height in okra affects the effective flowering branches and ultimately fruit yield. Wide range of plant height has been observed in okra and farmers fail to understand the plant habit on or before sowing of the crop. The present investigation was carried out with 25 okra cultivars at Indian Institute of Vegetable Research, Varanasi to find out seedling markers for plant height in okra. The observation were recorded on germination percentage, plumule length/shoot length, radical length, total seedling length, seedling fresh weight, seedling dry weight, dry matter percentage and plant height. The plant height ranged from 43.36 cm (No. 315) to 125.06 cm (IIVR-10). The maximum plumule length was found in HRB-55 (17.5 cm) and IIVR-10 (17.47) while minimum was recorded from No. 315 (10.60) and VRO-5 (11.60). Results indicated that varieties No. 315 and VRO-5 were found bushy in nature as well as with shortest plumule and radical length. None of the other characters had any direct relationship with plant height. Therefore, on the basis of plumule and radical length, the plant height in okra could be predicted.

Key Words: Okra, Variability, Correlation, Path analysis

Introduction

Okra (*Abelmoschus esculentus* L.) belongs to family *Malvaceae* known for its tender and delicious fruits in different parts of the country. It has a great potential for earning foreign exchange and accounts for about 60% of the export of fresh vegetables excluding potato, onion and garlic. Due to high iodine contents, the fruits are considered useful for control of goitre. Plant height in okra affects the effective flowering branches and ultimately fruit yield. Wide range of plant height has been observed in okra and farmers fails to understand the plant habit on or before sowing of the crop. So, present investigation was carried out with 25 okra cultivars at Indian Institute of Vegetable Research, Varanasi to find out seedling markers for plant height in okra.

Materials and Methods

Twenty-five okra genotypes selected for this study were diverse in plant height. A total of 75 seed of each genotype were grown in three replications in nursery bed. The observations were recorded on germination percentage, plumule length (cm), radical length (cm), total seedling length (cm), seedling fresh weight (g), seedling dry weight (g), and finally plant height (cm). For plant height, the seed were sown in the field and all the recommended package of practices were followed for raising a good crop.

Results and Discussion

The analysis of variance due to genotypes were found highly significant for all the characters studied. The germination percentage ranges from 43.33 to 93.33 (Table 1). The genotype EMS 8-1 showed the maximum (93.33 %) germination followed by NRO-4 (85.33%) and DVR-1 (84.66%) while Prabhani Kranti showed the minimum germination percentage (49.33%). Highest plumule length (shoot length) was found in HRB-55 (17.5 cm) followed by IIVR-10 (17.46 cm) and Larm-1 (17.03 cm) while lowest was recorded from No. 315 (10.6 cm) followed by VRO-5 (11.60 cm). Maximum radical length was found in VROH-2 (6.01 cm) followed by Arka Anamika (5.76 cm) and minimum radical length was recorded in no. 315 followed by VRO-5 (3.16 cm). The maximum total seedling length was found in HRB-55 (23.26 cm) followed by Arka Anamika (22.6 cm) and IIVR-10 (22.24 cm) whereas shortest seedling length was found in No. 315 (13.66 cm) followed by VRO-5 (13.66 cm).

The maximum seedlings fresh weight was recorded from cultivars P-7 (2.79 g) followed by DVR-3 (2.77 g) Larm-1 (2.77 g) and VRO-5 (2.76 g) whereas minimum was recorded in Arka Abhey (2.23 g) and VROH-4 (2.30 g). There is no significant difference for seedling dry weight among genotypes (Table 2). The maximum seedling dry matter per cent was found in

Table 1. Seedling characters of different genotypes of okra

Genotypes	Germination per cent	Plumule length (cm)	Radical length (cm)	Total length of seedlings (cm)
Arka Abhey	66.66	13.03	3.93	19.96
Arka Anamika	61.33	16.83	5.77	22.60
DVR-1	84.66	12.70	4.03	16.73
DVR-2	77.33	15.60	4.33	19.93
DVR-3	84.67	16.60	5.20	21.80
EMS-8-1	93.33	13.51	4.63	18.14
HRB-55	73.33	17.50	5.77	23.26
HRB-9-2	69.33	13.69	4.50	18.16
IIVR-10	76.66	17.47	4.77	22.24
IIVR-11	73.33	16.17	5.03	20.53
Larm-1	53.33	17.03	4.83	21.86
No. 315	70.66	10.6	3.07	13.66
P-7	70.66	16.50	5.30	21.86
Parbhani Kranti	49.33	15.80	4.13	19.93
Punjab Padmini	53.33	15.10	4.70	19.86
Pusa Makhamali	66.66	15.43	4.73	20.16
Pusa Sawani	50.66	15.10	4.33	19.73
VRO-5	80.00	11.60	3.17	14.76
VRO-6	84.00	14.60	4.07	18.66
VROH-1	80.00	14.33	5.07	19.40
VROH-2	80.00	16.63	6.01	21.91
VROH-3	70.66	14.07	4.53	18.60
VROH-4	85.33	12.63	3.60	16.23
VROH-5	74.66	14.43	4.23	18.66
VROH-6	74.66	16.03	5.77	21.80
CD at 5%	5.22	1.35	0.72	1.70

Table 2. Seedling characters of different genotypes of okra

Genotypes	Seedling fresh weight (g)	Seedling dry weight (g)	Dry matter (%)	Final plant height (cm)
No. 315	2.44	0.11	4.50	43.36
DVR-2	2.44	0.13	5.11	113.42
VROH-2	2.72	0.12	4.65	104.14
IIVR-11	2.53	0.11	4.34	125.06
DVR-1	2.65	0.13	5.18	95.16
VRO-5	2.76	0.12	4.47	61.03
Arka Anamika	2.67	0.13	4.42	99.70
VRO-6	2.40	0.11	4.70	121.2
Pusa Sawani	2.39	0.13	5.68	106.8
P-7	2.79	0.11	4.14	83.9
VROH-4	2.30	0.13	5.63	109.66
Punjab Padmini	2.66	0.13	5.21	96.76
VROH-3	2.55	0.14	5.48	98.36
EMS-8-1	2.48	0.11	4.29	65.36
HRB-9-2	2.51	0.13	5.31	77.03
VROH-5	2.66	0.13	4.99	123.60
HRB-55	2.59	0.11	4.24	71.1
Arka Abhey	2.23	0.12	5.00	96.03
Larm-1	2.77	0.11	4.08	106.0
Parbhani Kranti	2.69	0.12	4.55	93.83
Pusa Makhamali	2.57	0.12	4.67	75.47
DVR-3	2.77	0.13	4.79	97.97
IIVR-10	2.70	0.11	4.19	125.06
VROH-6	2.57	0.13	4.79	141.24
VROH-1	2.56	0.14	5.42	105.08
CD at 5%	0.38	0.03	0.74	12.42

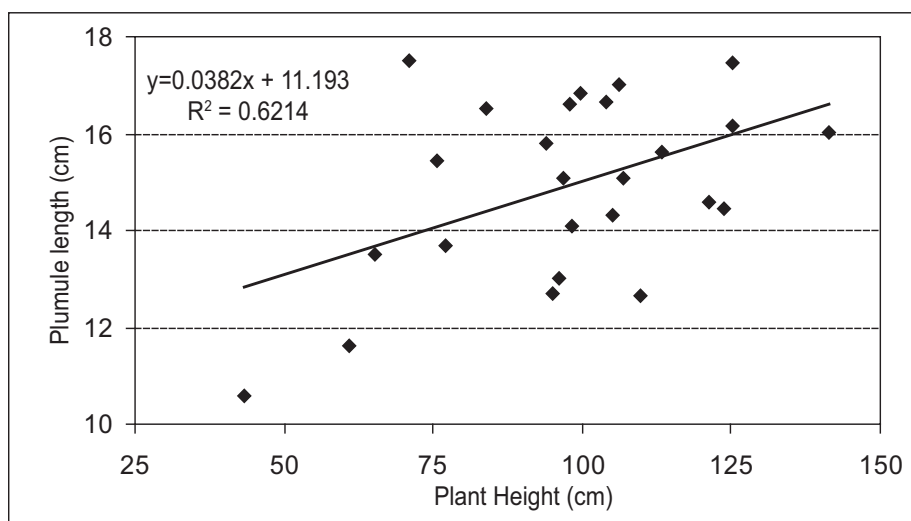


Fig. 1: Scatter plots illustrating the correlation between plant height and plumule length

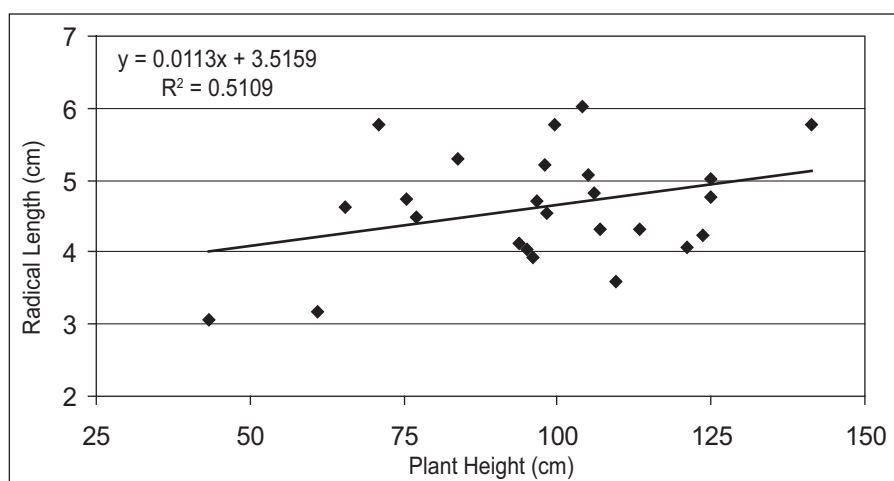


Fig. 2: Scatter plots illustrating the correlation between plant height and radical length

Pusa Sawani (5.68%) followed by VROH-4 (4.14%) while minimum was found in Larm-1 (4.08%) followed by P-7 (4.14%) and IIVR-10 (4.19%). The tallest genotype was VROH-6 (141.24 cm) followed by IIVR-10 (125.06) and IIVR-11 (125.06) while the smallest genotype was No. 315 (43.36 cm) followed by VRO-5 (61.03 cm).

Relationship between Plant Height and Plumule and Radical Length

There is a linear relationship between plant height and plumule length (Fig. 1) and also with radical length (Fig. 2). The genotypes which have higher plant height also have higher plumule and radical length. Martin and Rhode (1983) also found the linear relationship of plant height with radical length. The genotype No. 315 and VRO-5 were found bushy in nature as well as with shortest

plumule and radical length. With the increase in plant height, plumule and radical length also increased. All other characters did not have any clear cut relationship with plant height. These results are in close agreement with the finding of Berry *et al.* (1998). Thus, it is concluded that based on plumule and radical length, the nature of plant growth at seedling stage in okra can be predicted.

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Provenance Variation in Growth Characteristics of Wild *Hevea* Genotypes in Bastar Region of Central-eastern India

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A study to evaluate thirty wild genotypes of three provenances Acre, Matto Grosso and Rondonia along with six Wickham clones, was conducted for the screening of their existing nature and extent of variation under the drought prone conditions in Bastar region of central-eastern India. The region received rain for about four months (June-September) with only sixty-five rainy days and had more than seven months of dry period. In summer months ambient temperature during daytime crossed 38°C. The observation on variability in growth were recorded for girth, plant height, canopy height, canopy breadth, number of branches, branching height, branching angle and bark thickness at fourth year after field planting. Correlation, components of variance and heritability in broad sense were worked out. Wide inter and intra provenance variability was observed for all the characters studied. In general, Matto Grosso provenance showed comparatively high adaptability and superiority for all characters except branching height and branching angle; while in general Acre exhibited lowest growth performance. Several genotypes could be identified as superior to Wickham clones for certain important characters like bark thickness, plant height, canopy height and branching height. The existing wide variability would ultimately help to identify certain potential genotypes for future breeding and crop improvement programmes.

Key Words: *Hevea*, Provenance, Wild germplasm, Variability, Heritability

Introduction

The source of origin of almost all the present cultivated rubber (*Hevea brasiliensis*) has been evolved within the 'Wickham' genetic group, which represents a very small gene pool, since it was collected from a limited area (Schutles, 1977). The intensive directional selection over the years for yield alone has further narrowed the genetic base (Wycherley, 1969), and has further resulted in a slow down in genetic advances in breeding phases (Tan, 1981; Seguin *et al.*, 1995).

Considering the gravity of the situation, the International Rubber Research and Development Board (IRRDB), organized an expedition in vast region of the primary centre of origin of the crop, the Amazon basin, covering wide agro-climatic areas in the states, Acre, Matto Grosso and Rondonia in Brazil, for collection and conservation of the high variability. This resulted in collection of over 60,000 seeds and bud wood from 194 exceptionally good trees (Ong *et al.*, 1983). These genotypes were distributed to IRRDB member countries, and those received in India, were being conserved in conservation nurseries.

In India with non-availability of land in traditionally cultivated region (8° to 12° N), and to bridge the widening gap between production and consumption, steps were

taken to explore the possibilities of extending *Hevea* cultivation to less congenial areas. This necessitated evaluation of wild genotypes capable of withstanding stress conditions, for their further utilization in selection of stress/drought tolerant clones.

Earlier, no study has been made to assess the performance of wild *Hevea* germplasm under the drought prone conditions in Bastar region of central-eastern India. The present first ever study of its kind in this region was undertaken to identify the nature and extent of variability in wild population, which would ultimately help to identify genotypes for breeding programmes.

Materials and Methods

The study was conducted at the Regional Research Station at Sukma (19°5'N, 82°02'E, 202 m MSL) in the Bastar region of central-eastern India. The trial was located on plain land with uniform stand and soil status. The main soil types in Sukma are red and yellow soils (Khanna and Motiramani, 1972). The soil is acidic in nature (pH 5.3) and low in organic content (0.53%). Available P, K and Mg are 0.20, 4.25 and 4.62 mg per 100 gm soil, respectively. The soil exhibited considerable shrinkage and cracking on drying.

A field trial was laid out in 1996, to screen the wild *Hevea* germplasm. In total thirty wild genotypes of Acre,

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Matto Grosso and Rondonia provenances and six Wickham clones as controls were included in the trial (Table 1). The experiment was laid out in a simple lattice design with two replications. The spacing adopted was 4.9 m x 4.9 m with ten plants per plot. During drought period (March to May) the plants were given uniform life saving irrigation at fortnightly intervals, at the rate of 15 litres per plant during the first year (1996-97) and 30 liters during the further years of study period (1998-2000). The recommended cultural practices (Rubber Board, 1990) were followed.

Table 1. Genotypes planted in the field trial

Rondonia	Matto Grosso	Acre	Wickham clones
RO 2822, RO 2635	MT 44	AC 685	RRII 105
RO 2629, RO 3172	MT 196	AC 707	RRII 118
RO 2890, RO 5557	MT 2594	AC 607	RRII 208
RO 5369, RO 5348	MT 2217	AC 623	PB 260
RO 5463, RO 5408	MT 2229	AC 619	GT 1
RO 5553, RO 5554		AC 763	GL 1
RO 6135, RO 5329		AC 68	
RO 5363, RO 5430		AC 1950	
RO 5445			

The variability in growth, as girth of the stem at the height of 150 cm above the ground level, and observations on variations in other morphological characters, plant height, canopy height, canopy breadth, number of branches, branching height, branching angle and bark thickness were recorded during the fourth year after field planting for ten trees per genotypes.

The correlations between various growth parameters were computed following the methods of Panse and Sukhatme (1978). The genotypic co-efficient of variation (GCV), phenotypic co-efficient of variation (PCV) and broad sense heritability (h^2) were calculated according to Zoble and Talbert (1984).

Results and Discussion

The Bastar region of central-eastern India experiences a warm dry sub-humid climate. During the study period,

this region received a mean annual rainfall of 1530 mm with only 65 rainy days, almost confined to June to September, with July recording the highest rainfall. In general, during March to June, the mean maximum temperature was higher than 38°C (Table 2). In this region, soil moisture deficit and high summer temperature are the major environmental constraints.

The genotypes exhibited wide variation for the characters studied. The range of variation and mean values in wild *Hevea* germplasm are given in Table 3. In fourth year after field planting, the girth of the plants ranged from 16.98 cm (AC 707) to 27.3 cm (MT 2594), plant height from 474.65 cm (AC 685) to 802.30 cm (MT 196) and bark thickness from 1.85 mm (AC 685) to 3.21 mm (RO 2890). Similar wide variation in bark thickness was also reported among the three provenances by Azwar *et al.* (1995). Varghese (1992), Abraham *et al.* (1992, 2002), and Mercy *et al.* (1995) have also reported wide variability in wild germplasm in respect to certain growth morphological characters.

The wide variability was also recorded for other secondary characters, canopy height, canopy breadth and first branching height. The number of branches and

Table 2. Average temperature and rainfall during the experimental period (1996-2000)

Month	Temperature (°C)		Rainfall (mm)	No. of Rainy days
	Max.	Min.		
January	29.2	10.7	5.4	1
February	32.0	13.8	3.0	1
March	36.6	17.8	11.4	1
April	38.6	25.6	42.3	3
May	38.4	24.9	42.4	3
June	34.0	23.9	273.6	7
July	29.3	23.3	444.5	17
August	29.5	22.5	351.6	15
September	30.9	22.2	222.0	12
October	30.9	20.6	92.9	3
November	30.2	15.6	35.6	1
December	27.9	9.5	6.0	1
Total	—	—	1530.7	65
Mean	32.3	19.2	—	—

Table 3. Variability in growth characters of 4-year-old-trees

Characters	Wild genotypes			Wickham clones		
	Minimum	Maximum	General mean	Minimum	Maximum	General mean
Girth (cm)	16.98 (AC 707)	27.30 (MT 2594)	23.34	21.36 (GL 1)	29.26 (RRII 118)	26.77
Plant height (cm)	474.65 (AC 685)	802.30 (MT 196)	674.15	587.09 (RRII 105)	782.10 (RRII 118)	704.06
Canopy height (cm)	198.32 (AC 707)	494.00 (RO 3172)	356.17	292.50 (GL 1)	457.50 (RRII 118)	392.51
Canopy breadth (cm)	273.20 (AC 707)	467.50 (RO 5557)	403.41	380.00 (RRII 105)	512.00 (RRII 118)	440.86
Branching height (cm)	256.50 (AC 1950)	404.00 (RO 5430)	311.67	273.40 (RRII 105)	338.00 (GT 1)	302.11
No. of branches	7.66 (AC 707)	34.10 (RO 5553)	21.52	14.30 (RRII 105)	37.40 (GT 1)	24.95
Branching angle (°)	49.11 (AC 1950)	61.14 (AC 623)	55.51	55.26 (RRII 105)	61.51 (RRII 118)	58.17
Bark thickness (mm)	1.85 (AC 685)	3.21 (RO 2890)	2.43	2.91 (PB 260)	3.10 (RRII 208)	2.99

branching angle also widely varied. The branching height ranged from 256.50 cm (AC 1950) to highest as 404.00 cm (RO 5430) (Table 3), with a general mean value of 311.67 cm, which was higher than those of Wickham clones. The tendency of wild *Hevea* germplasm to branch at a higher than that of Wickham clones was also reported by Azwar *et al.* (1995) and Rao *et al.* (1999). Chapuset *et al.* (1995) also reported variation in branching behavior among the wild germplasm. Branching height related to early or late branching. Number of branches and canopy size have got considerable influence on the rate of growth, as stem and branching characters attributed to differences in dimensions of form of tree crown (Jankiewicz and Stecki, 1976), and has a direct effect on the spatial distribution of photosynthetic surface by forming the plant skeleton.

When the mean values of wild genotypes were compared with the Wickham clones, the general mean values of the wild germplasm for all the characters except the branching height were found to lower than those of Wickham clones. Though the wild genotypes in general showed lower general mean values than that of Wickham clones, the mean values of the wild genotypes particularly MT 196 for plant height, RO 3172 for canopy height, RO 5430 for branching height and RO 2830 for bark thickness, were found to show superiority over Wickham clones. The wide variation observed in the wild germplasm were in accordance with the general expectations that wild and primitive forms from the centre of origin exhibit more variability (Annamma *et al.* 1989).

A provenance wise comparison of the performance of wild genotypes is depicted in Table 4. The genotypes from Matto Grosso were found to be comparatively vigorous with highest provenance mean for girth, height, branching height, canopy breadth, and also exhibited the

almost highest mean values for canopy height and bark thickness. The general performance of Matto Grosso provenance was superior is in agreement with the general observations made by Chevallier (1988), Clement-Demange *et al.* (1990), Abraham *et al.* (1992), Mercy *et al.* (1995) and Rao *et al.* (1999). Though, the Matto Grosso provenance revealed the vigorous nature with higher mean values, the Rondonia and Acre provenances exhibited comparatively higher variability. Acre showed higher heterogeneity for the characters studied except for girth, branching height and bark thickness. In general, Rondonia provenance appeared to be intermediate in the mean values between Acre and Matto Grosso provenances. Chevallier (1988), Nicolas *et al.* (1988) and Rao *et al.* (1999) have reported similar results of intermediate position of the Rondonian genotypes.

Simple correlation coefficients between pairs of different characters are presented in Table 5. The girth is significantly correlated with plant height (0.840), branching height (0.557), canopy height (0.691), canopy breadth (0.792) and bark thickness (0.651). Plant height is also significantly correlated with the character except branching angle. Similarly, canopy height was positively correlated with canopy breadth. No significant correlation, however, could be detected between branching angle and any other trait. Significant high correlation between bark thickness and girth has been reported in wild *Hevea* genotypes by Madhavan *et al.* (1996) and Rao and Reghu (2000). Significant positive correlation of plant height with canopy height, indicates that the total height of the plant is influenced by the growth of the canopy (Nazeer *et al.*, 1992).

Components of variation in the population were estimated through the genotypic and phenotypic coefficients of variation, and heritability in the broad

Table 4. Provenance-wise comparison of 4-year-old trees

Characters	Provenance						Wickham clones	
	Acre		Matto Grosso		Rondonia		Mean	Range
	Mean	Range	Mean	Range	Mean	Range		
Girth (cm)	20.32	16.98 – 23.40	25.71	24.19 – 27.34	24.00	19.50 – 27.25	26.77	21.36 – 29.26
Plant height (cm)	591.68	474.65 – 717.54	723.97	635.00 – 802.30	706.80	592.60 – 749.58	704.06	587.09 – 782.10
Canopy height (cm)	288.60	198.32 – 377.00	389.48	276.20 – 429.70	390.43	318.70 – 494.00	392.51	292.50 – 457.50
Canopy breadth (cm)	355.86	273.20 – 441.50	428.36	344.90 – 455.20	426.01	373.20 – 467.50	440.86	380.00 – 512.00
Branching height (cm)	299.50	256.50 – 359.10	326.40	295.80 – 356.30	309.13	255.40 – 404.00	302.11	273.40 – 338.00
No. of branches	20.70	7.66 – 29.60	23.26	18.80 – 28.60	20.60	13.30 – 34.10	24.95	14.30 – 37.40
Branching angle (°)	56.44	49.11 – 61.14	55.47	55.44 – 56.52	54.64	52.78 – 60.72	58.17	55.26 – 61.51
Bark thickness (mm)	2.21	1.85 – 3.04	2.51	2.00 – 3.09	2.57	2.01 – 3.21	2.99	2.91 – 3.10

Table 5. Correlation coefficients among growth characters of wild genotypes

Characters	Characters						
	Plant height	Branching height	No. of branches	Canopy height	Canopy breadth	Branching angle	Bark thickness
Girth	0.840**	0.557**	0.110	0.691**	– 0.792**	– 0.269	0.651**
Plant height		0.538**	0.652**	0.902**	0.876**	– 0.314	0.550**
Branching height			– 0.103	0.134	0.314	– 0.087	0.257
No. of branches				0.131	0.257	– 0.356	0.252
Canopy height					0.873**	– 0.319	0.496
Canopy breadth						– 0.239	0.618**
Branching angle							– 0.110

* Significant at P<0.05

** Significant at P<0.01

sense (Table 6). A high phenotypic coefficient of variation (PCV) was observed for number of branches (40.83), canopy height (27.77) and bark thickness (23.43), whereas it was lowest for branching angle (5.83). The highest estimate of genotypic coefficient of variation (GCV) was for number of branches (24.36) followed by canopy height and bark thickness. For all the characters studied, the PCV was higher than the GCV ; and the existence of high magnitude of genetic variability was evidenced by medium to high estimates of PCV and GCV for majority of the characters. Earlier, almost similar moderate estimates of coefficients of variation was reported by Abraham *et al.* (1992, 2002) and Rao *et al.* (1999) for certain morphological growth characters in wild *Hevea* germplasm.

Heritability in the broad sense (h^2) was found to be medium to high for the morphological characters. Higher h^2 estimates were recorded for plant height (0.58), girth (0.53) and bark thickness (0.46). Rao *et al.* (1999) and Abraham *et al.* (2002) have reported moderate to high h^2 in the wild germplasm with respect to certain growth parameters like height, girth, branching height and bark thickness. In the present study moderate to high heritability for most of the characters suggests the predominance of additive gene action in the inheritance of these traits and hence indicates the possible good response of these characters to selection.

Table 6. Phenotypic and genotypic coefficients of variation and heritability in growth characters of wild germplasm

Characters	PCV	GCV	Heritability (H^2)
Girth	16.28	11.89	0.53
Plant height	15.42	11.82	0.58
Canopy height	27.77	18.58	0.45
Canopy breadth	20.00	11.59	0.34
Branching height	19.68	9.40	0.23
No. of branches	40.83	24.36	0.36
Branch angle	5.83	5.19	0.79
Bark thickness	23.43	15.94	0.46

The present study showed a general superiority of Matto Grosso provenance for growth characters and adaptability. The wide variability observed for the growth characters, therefore can be utilized for broadening the genetic base, for further use in breeding and improvement of cultivated rubber.

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In vitro Multiplication and Conservation of *Saccharum* Germplasm

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Sugarcane germplasm have been maintained in the world germplasm repository at Sugarcane Breeding Institute Research Centre in the field gene bank. Field gene bank is always at the risk of biotic and abiotic factors and complimentary strategies need to be worked out to support the field gene bank. *In vitro* techniques offer different methods of conservation, and slow growth preservation is being successfully employed in many vegetative propagated crops. In this study, attempt was made to standardize optimal *in vitro* multiplication of different species of *Saccharum* and also studied to delay the sub-culturing period by retarding the growth of the *in vitro*-maintained material. It was observed that MS medium supplemented with 1 mg/l Kn and 6 mg/l BAP was more effective in inducing multiple shoots from the shoot apex in most of the species studied and 2% mannitol was found effective in controlling the growth.

Key Words: *Saccharum*, Sugarcane, *In vitro* multiplication, Micropropagation, *In vitro* conservation, Field gene bank

Introduction

Saccharum germplasm includes five species, viz., *S. officinarum*, *S. spontaneum*, *S. barberi*, *S. sinense* and *S. robustum*. *S. officinarum* is the thick cane, high sugared tropical sugarcane which is also known as the 'noble' cane. *S. barberi* and *S. sinense* are the north Indian and Chinese forms, respectively, which were under cultivation in the sub-tropical areas before the advent of man made hybrid varieties in the early part of twentieth century. At present, these varieties are not commercially cultivated for sugar and are mainly confined to collections in research stations only. The related genera of *Saccharum* such as *Erianthus*, *Miscanthus*, *Narenga*, *Sclerostachya*, *Sorghum*, *Imperata*, etc. and the commercial hybrids developed in India and foreign countries were also the part of sugarcane germplasm which are also maintained in field gene bank at Kannur. The germplasm assembly at SBI Research Centre, Kannur now consists of 3368 accessions. These include *S. officinarum* (759), *S. barberi* (42), *S. sinense* (30), *S. robustum* (145), *S. spontaneum* (79), Indian hybrids (1027), Foreign hybrids (611), IND collections (393), IA clones (130) and allied genera (152). The germplasm accessions are clonally propagated every year and are maintained in the field. The field gene bank is exposed to various diseases and pests and other natural calamities like drought, flood, etc. Genetic resources being the safe deposit for the present and future crop improvement programmes, it is imperative to maintain the germplasm through whatever mode of conservation available to safeguard it from any kind of loss during conservation. The viability of the *in vitro* technique in micropropagation and for crop improvement have been studied by Sreenivasan and Sreenivasan, 1984 and 1992. Potential of *in vitro*

meristem culture technique in sugarcane storage of germplasm was also reported by Sreenivasan and Srinivasan, 1985. Hence to adopt a complementary strategy to support field gene bank *in vitro* multiplication and slow growth conservation studies were conducted in the sugarcane germplasm.

Materials and Methods

Two clones each from all five species were studied for *in vitro* multiplication in MS media supplemented with Kinetin 1mg/l and BAP at 0, 2, 4, 6, 8 mg/l. The shoot apex were taken from the field and surface sterilized by wiping with 95% ethyl alcohol. The outer leaf sheaths were removed and the shoot apex of 1-2 cm length with two whorls of leaf sheath were inoculated in the liquid media in three culture tubes each in three replication. Explants were collected from two clones each from five species viz., *S. officinarum* (White transparent and NC 25), *S. barberi* (Lalri, Matnashaj), *S. sinense* (Uba reunion, Uba seedling), *S. robustum* (NG77-213, NG 77-90) and *S. spontaneum* (IND 81-318, PLAG 84-8). The cultures were transferred to same media after one week to avoid the excess phenolics exudated to the medium. After three weeks of inoculation the response were observed and sub cultured in the same media for three weeks. The number shoots induced were recorded. For rooting, fully matured 60 shoots were inoculated in MS solid medium supplemented with 1 mg/l IBA; and for hardening, 50 rooted plantlets were transplanted to plastic cups with 1:1 sand and soil and kept in high humidity condition under shade for two weeks. For prolonging the sub culturing period by growth retardation mannitol were supplemented to the basal MS medium at 0%, 1%, 2% and 4% level. Ten fully grown plantlets with roots were

inoculated in the media in three replications on a filter paper bridge in liquid media and another set in media solidified with 0.75% Agar. Initial shoot length and fresh weight were recorded. The fresh shoot weight and shoot length were measure at one month intervals after culling out two tubes per replication. The observations were recorded till the shoots started drying.

Results and Discussion

Shoot elongation was recorded in all the clones in the treatment T1 where only one or two side shoots were observed (Table 1). All clones except *S. spontaneum* clones showed maximum shoot induction in media supplemented with Kn 1mg/l + BAP 6 mg/l. *S. spontaneum* showed maximum shoot production in media supplemented with 1 mg/l Kn + 2 mg/l BAP. The maximum multiplication rate of 1:11 was observed in NC 25. In BAP, concentration above 6 mg/l did not have any advantages and in *S. spontaneum* browning of explants was noticed. The result indicated that BAP at 6 mg/l was better for induction of multiple shoots from the shoot apex for most of the clones and for *S. spontaneum* lower concentration was desirable. The multiplication rate of 1:10 was desirable in view of maintenance of germplasm where faster multiplication rate is discouraged to reduce the chance of somaclonal variation. Hundred per cent rooting was observed in MS medium supplemented with 1 mg/l IBA with in three weeks. The hardened plants under shade and high humid condition were transplanted after two weeks to pots and 90% of the plants could be established.

One of the difficulty that arises during maintenance of germplasm clones under *in vitro* conservation is that it continues to grow in the culture medium and difficult to accommodate in the culture vessel and need trimming and constant replacement of culture media (Sreenivasan and Srinivasan, 1985). Slow growth preservation by using osmotic regulators (mannitol) and growth retarding substances like ABA in culture medium have been employed successfully in other crops (Ashmore, 1997; Chandran *et al.*, 2001). In this study, the treatment with 2% mannitol in liquid medium was found effective in retarding the growth in good health condition up to 120 days in culture. After 120 days the medium get completely exhausted and required to be replenished or sub culturing had to be done. Compared to solid medium the liquid medium was found better for *in vitro* maintenance. This may be due to the exudated phenols get dispersed in the liquid medium whereas in solid medium it accumulate on the surface where the explant is in contact with the medium resulting death of cells at the cut end and affect the proper absorption of the nutrients. The increase in fresh weight was more in liquid medium as the absorption rate of the nutrient is faster in liquid medium compared to solid medium. A slow and linear increase was observed in medium supplemented with 1% and 2% mannitol (Table 2). In solid medium supplemented with 2% mannitol, the increase in fresh weight was not observed after 90 days in culture; this may be due to the drying of shoots. In medium supplemented with 4% mannitol, the shoots were completely dried within 90 days.

Table 1. Shoot induction in 5 different species of *Saccharum*

Genotype	No of shoot tip cultured	Average no of shoots induced/explant				
		T1	T2	T3	T1	T5
		1 mg/l Kn	1 mg/l Kn+ BAP 2 mg/l	1 mg/l Kn+ BAP 4 mg/l	1 mg/l Kn+ BAP 6 mg/l	1 mg/l Kn+ BAP 8 mg/l
<i>S. officinarum</i>						
White transparent	45	1.3	2.0	2.8	9.2	5.9
NC 25	45	1.0	2.1	2.7	11.0	4.0
<i>S. robustum</i>						
NG 77-213	45	2.4	3.0	3.2	9.1	5.3
NG 77-90	45	2.0	2.7	3.3	8.0	3.7
<i>S. barberi</i>						
Lalri	45	1.7	3.3	3.1	10.7	3.1
Matnashaj	45	1.0	2.6	2.8	6.0	3.5
<i>S. sinense</i>						
Uba reunion	45	1.6	2.5	3.3	6.3	5.0
Uba seedling	45	1.7	3.7	2.2	8.2	4.7
<i>S. spontaneum</i>						
IND 81-318	45	2.0	6.0	2.1	2.3	2.0
PLAG 84-8	45	1.9	8.3	2.0	2.7	1.7

Table 2. Fresh weight increase in different treatment of mannitol in culture medium

		Initial fresh weight of shoot (mg)	Fresh wt after 30 days (mg)	Fresh wt after 60 days (mg)	Fresh wt after 90 days (mg)	Fresh wt after 120 days (mg)
T1	Liquid medium + 0% Mannitol	104.0	338	902	702	1200
T2	Solid medium + 0% Mannitol	104.6	228	493	353	508
T3	Liquid medium + 1% Mannitol	104.8	155	724	550	800
T4	Solid medium + 1% Mannitol	104.6	240	910	721	942
T5	Liquid medium + 2% Mannitol	90.0	200	215	525	610
T6	Solid medium + 2% Mannitol	105.0	150	425	425	0
T7	Liquid medium + 4% Mannitol	90.0	125	348	100	0
T8	Solid medium + 4% Mannitol	105.0	120	350	0	0

Shoot elongation was found maximum in medium without any addition of mannitol where it reaches up to 26 cm within 120 days and in treatment the maximum retardation was observed in liquid medium supplemented with 2% mannitol and in solid medium with the same composition and in the medium supplemented with 4% mannitol the shoots were dried with in 120 days of culture (Fig. 1). Hence, it is inferred that 2% mannitol can be supplemented to the liquid culture medium to retard the growth of the shoots under *in vitro* condition and can be used in *in vitro* germplasm maintenance without affecting the viability and vigour of the material. The *in vitro* maintenance of germplasm also facilitate the easy exchange of germplasm with minimal quarantine procedures and the supply of the material can also be ensured round the year irrespective of crop season.

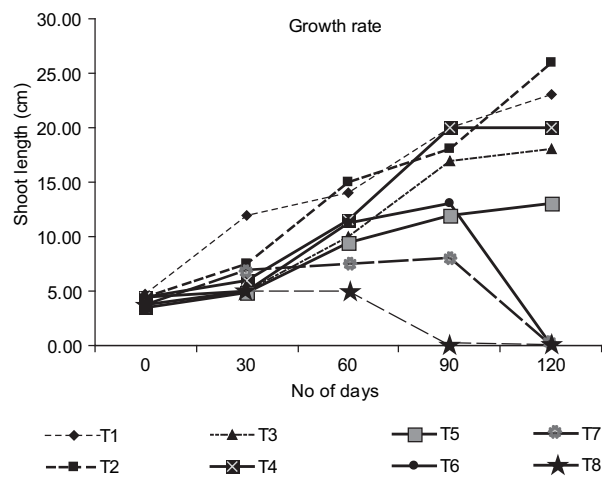
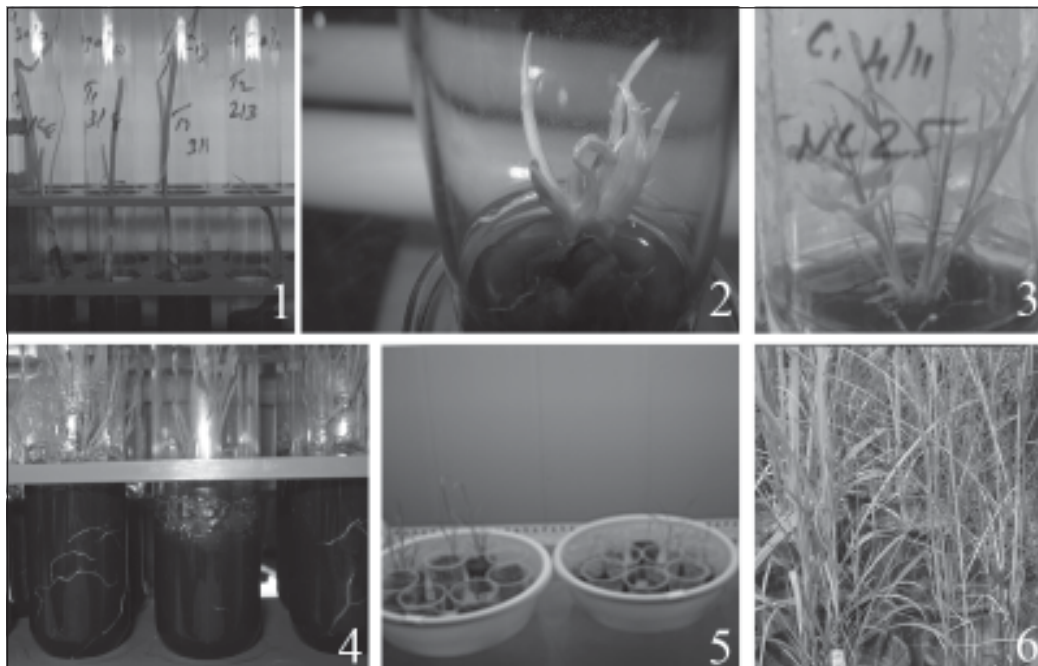
Fig. 1: Growth rate of *in vitro* maintained shoots in different treatment

Fig. 2: Various stages of development of shoot in germplasm clones; 1. Elongation of single shoots from explant, 2. Multiple shoot induction, 3. Multiple shoots derived from NC 25, 4. Rooting in solid medium, 5. Hardening in plastic cups, 6. Transplanted plants in pots

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Somatic Embryogenesis and Plant Regeneration in Diploid Banana Cultivars (*Musa acuminata* cv. Chingan and *Musa acuminata* cv. njalipoovan) from Kerala

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Plant regeneration by somatic embryogenesis was attempted with diploid banana cultivars *Musa acuminata* cv. Chingan (AA) and *Musa acuminata* cv. Njalipoovan (AB). *In vitro* plants grown on solid MS medium supplemented with BA (4.4-17.6 μ M) and TDZ (0.45-9 μ M) were used as explant source. The basal meristem including 3-4 leaf primordial and excised apices were inoculated on MS medium supplemented with 2,4-D (0.225-9 μ M) and BA (0.044-8.8 μ M) for callus induction. Pale yellow embryogenic callus initiated after three weeks of inoculation and subsequent subculture were done in the same medium to enhance callus proliferation. Maximum callus proliferation was observed in 2,4-D (4.5 μ M) along with BA (2.2 μ M). Embryogenic calli were isolated and transferred to 100 ml erlenmeyer flask with 20 ml of liquid MS medium with zeatin (0.46-4.56 μ M) and malt extract (100 mg/l) or malt extract and ascorbic acid (0.01-0.5 mg/l). The culture medium was refreshed every third week, embryogenic complexes and compact structures were removed. Small embryo-like structures and embryogenic calli were transferred to fresh medium. After 4-5 subculture the embryos were isolated and inoculated on petridish containing MS basal medium. Leaf and root initials were developed after 2-3 weeks. These were then transferred to same medium in culture bottles. After 3 weeks, plants of 4-5 cm height were transferred to vermiculate with 100 per cent survival rate.

Key Words: Somatic embryogenesis, *Musa acuminata* cv. Chingan, *Musa acuminata* cv. Njalipoovan

Introduction

Edible bananas (*Musa* spp.) are the major staple food for rural and urban consumers in the tropical and sub tropical countries and an important source of rural income. However, in the banana production system, the non-availability of disease free, true-to-type planting material, low fertility due to triploidy, slow propagation and long time span from one generation to the next are the major constraints. Local or indigenous cultivars are generally more useful and tolerant to local conditions and have lesser growth requirements to obtain satisfactory yield. Most of cultivars in Kerala, South India are adapted to domestic cultivation and have unique morphological features as well as fruit qualities. South India is well-known for the presence numerous diploid banana cultivars. Diploid banana varieties are a well known source of genetic resistance to many biotic stresses that have limited banana cultivation. Somatic embryogenesis offers an ideal system for the production of somatic embryos on a large scale for use in the preparation of synthetic seeds, propagation and genetic transformation. Somatic embryogenesis technique in the genus *Musa* developed new, high performance micropropagation techniques and that of cell regeneration system useful for genetic improvement. The production of synthetic seeds by encapsulating somatic embryos and vegetative propagules

became an applied technique with potential for mass propagation of elite plant species. The advantages of using artificial seeds include ease of handling, transportation and potential storage, higher scale-up potential and low cost of production and subsequent propagation (Redenbaugh *et al.*, 1986). Synthetic seed technology will have a significant impact on crop production, in both vegetatively propagated and seed propagated crops. For the vegetatively propagated plants, synthetic seeds would allow direct planting material of clonal varieties and may provide a means for maintenance of elite germplasm. The objective of this work was to establish a protocol of cell suspension culture and plant regeneration via somatic embryogenesis of two diploid banana cultivars (*Musa acuminata* cv. Chingan (AA) and *Musa acuminata* cv. Njalipoovan (AB)).

Materials and Methods

In vitro grown plants were established from inflorescence explants obtained from field grown plants at Department of Botany, University of Kerala, Kerala, India. The *in vitro* plants were obtained on MS medium supplemented 7.6 μ M BA. The shoot buds were obtained on MS medium supplemented with 4.5 μ M TDZ. The cultures were maintained at a temperature of 25 $\pm$ 1 $^{\circ}$ C, with 50-70% relative humidity and 16 h photoperiod. Thin longitudinal sections of approximately 1cm square pieces from leaf

base (basal meristem) were excised from *in vitro* grown plants, after the removal of leaves and roots from the plantlets. Shoot apices of approximately 1 cm square pieces were excised from *in vitro* grown shoot buds maintained on MS medium supplemented with TDZ. Approximately 6-8 sections were cut from the shoot apex and 2-4 sections were dissected from basal leaf meristem. The explants were inoculated on MS medium supplemented with 2,4-D (0.225-9 μM) and BA (0.44-8.8 μM) for callus initiation. These calli were subcultured at an interval of 3 weeks in the same medium for callus proliferation.

In 3-month-old embryogenic calli derived from both the explants were used to establish the suspension cultures. Approximately 100 mg of callus transferred to 100 ml Erlenmeyer flask containing 20 ml of liquid MS medium supplemented with zeatin (0.46-4.6 μM) or ascorbic acid (0.01-0.5 mg/l) along with malt extract (100 mg/l) in a gyratory shakers at 80 rpm. Subcultures were done once every 21 days by adding 10 ml of fresh medium.

During subculturing compact structures were removed, small embryo like structures were isolated and transferred to fresh medium. After 4-5 subculture, these embryos were transferred to petri dish containing MS basal medium. After the development of shoot and root initials these were transferred to same medium in culture bottles. After 3 weeks, plants of 4-5 cm height were transferred to vermiculate with 100 per cent survival rate.

Results and Discussion

The shoot apices and basal meristem showed callus development in the presence of 2,4-D (0.225-9 μM) and BA (0.44-8.8 μM) in various combinations. High frequency of embryogenic callus proliferation was obtained in presence of 2, 4-D (4.5 μM) along with BA (2.2 μM). Initially the callus morphology was pale yellow or white compact and later milky white globular structures developed along with white callus. Embryogenic tissues developed within 1-2 months after callus initiation (Fig. 1). The embryogenic tissues were subcultured and maintained on the same hormonal combination for 12 weeks for maximum proliferation. These embryogenic calli were transferred to liquid MS medium with zeatin (0.46-4.6 μM) or ascorbic acid (0.01-0.5 mg/l) along with malt extract (100 mg/l). The suspension showed presence of free cells and cell aggregates in the liquid medium. Embryogenic cell aggregates were obtained after 3rd or 4th subculture. Both the shoot apices and leaf sheath derived calli from each cultivar showed maximum embryogenic potential in the presence of zeatin (1.84 μM) along with malt extract. In both the cultivar presence of ascorbic acid (0.01-0.5 mg/l) along with malt extract (100 mg/l) showed development of embryogenic cell aggregates which later grown to plants/shoots. However, only a little embryo development was observed in this medium.

The embryos developed in the liquid medium were isolated and transferred to petri dish containing basal MS medium (Fig. 2). Root and shoot initials were

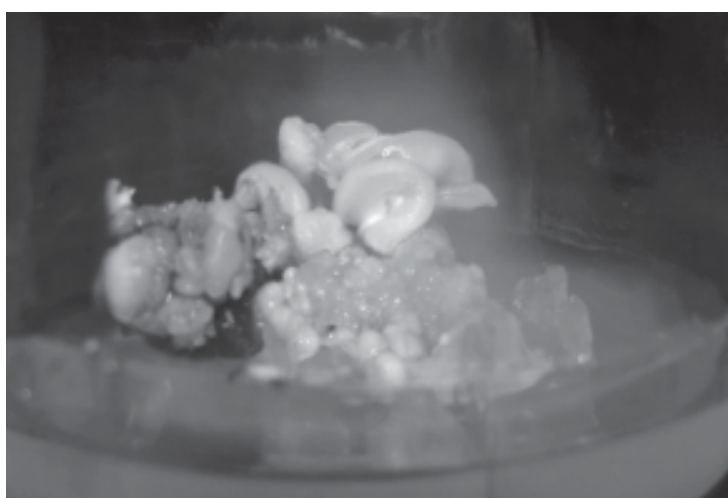


Fig. 1: White embryogenic callus

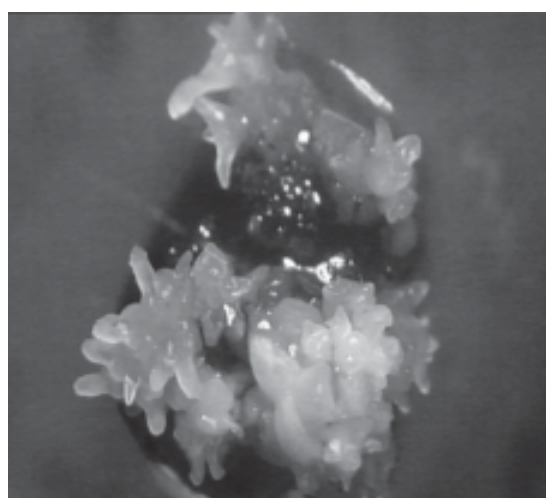


Fig. 2: Initiation of shoot primordial on MS basal medium

Table1. Induction of somatic embryo from the diploid banana cv. Njalipoovan and cv. Chingan

MS Medium		Average no. of embryos from shoot apices		Average no. of embryos from leaf sheath	
Zeatin (μ M)	Ascorbic acid (mg/l)	Chingan	Njalipoovan	Chingan	Njalipoovan
0.4	—	33.5000 $\pm$ 0.73c	10.8750 $\pm$ 0.44d	23.5000 $\pm$ 3.11cd	16.6250 $\pm$ 0.18c
0.92	—	34.0000 $\pm$ 0.53c	14.5000 $\pm$ 1.95c	30.7500 $\pm$ 0.36b	18.5000 $\pm$ 0.18b
1.84	—	42.3750 $\pm$ 0.49a	27.5000 $\pm$ 0.18a	35.2500 $\pm$ 0.31a	21.5000 $\pm$ 0.18a
2.76	—	37.3700 $\pm$ 0.18b	18.2500 $\pm$ 0.16b	26.3750 $\pm$ 0.77c	18.2500 $\pm$ 0.6b
3.68	—	31.6200 $\pm$ 0.41d	15.1250 $\pm$ 0.35c	23.8750 $\pm$ 0.61cd	16.5000 $\pm$ 0.18c
4.6	—	28.0000 $\pm$ 0.26e	14.6250 $\pm$ 0.83c	21.3750 $\pm$ 0.18d	13.8750 $\pm$ 1.24d
—	0.01	16.5000 $\pm$ 0.37d	8.0000 $\pm$ 0.00e	7.5000 $\pm$ 0.32de	9.8750 $\pm$ 0.22d
—	0.02	13.2500 $\pm$ 0.64e	8.8750 $\pm$ 0.12d	13.1300 $\pm$ 0.35c	12.0000 $\pm$ 0.18b
—	0.04	13.3500 $\pm$ 0.18e	10.6250 $\pm$ 0.46c	14.7800 $\pm$ 0.32b	11.2500 $\pm$ 0.16c
—	0.06	11.8750 $\pm$ 0.12f	9.7500 $\pm$ 0.36d	17.4300 $\pm$ 0.36a	12.2500 $\pm$ 0.25b
—	0.08	18.6250 $\pm$ 0.18c	11.2500 $\pm$ 0.36bc	15.6300 $\pm$ 0.18b	13.5000 $\pm$ 0.18a
—	0.1	25.2500 $\pm$ 0.36a	15.5000 $\pm$ 0.32a	9.1300 $\pm$ 0.12d	12.3750 $\pm$ 0.26b
—	0.3	23.1250 $\pm$ 0.39b	11.7500 $\pm$ 0.16b	7.6300 $\pm$ 0.41de	12.3750 $\pm$ 0.18b
—	0.5	15.8750 $\pm$ 0.29d	9.6250 $\pm$ 0.26d	6.1300 $\pm$ 0.29f	9.2500 $\pm$ 0.16e

Significance was determined by ANOVA: significant at $P < 0.05$
SE=Standard Error; Data represent the mean of eight replications

developed within 2-3 weeks. These plantlets were transferred to basal medium in culture bottles. After 3 weeks, plants of 4-5 cm height were transferred to vermiculate with 100 % survival rate (Fig. 3).

Plant regeneration of banana has been reported from various explant sources and from a variety of cultivars. In cultivar Grand Nain, which has a genomic constitution of AAA, Novak *et al.* (1989) used bases of leaf sheath and corm sections from *in vitro* grown plants for embryogenesis. For the cultivar Bluggoe, which has an ABB genomic composition, Dheda *et al.* (1991) cultured thin sections from highly proliferate shoot tip cultures to produce embryogenic cell suspension cultures. Male flowers are used as the starting material for initiating embryogenic cultures in cv. Grand Naine (Escalant

et al., 1994; Cote *et al.*, 1996; Navarro *et al.*, 1997; Becker *et al.*, 2000).

Developing embryogenic culture systems with reliable regeneration efficiency from important varieties of banana is a pre requisite for realizing the potential of cellular and molecular tools of crop improvement (Smith *et al.*, 2005). Toward this goal, studies were made to develop protocols for somatic embryogenesis and plant regeneration from different banana cultivars. According to Dheda *et al.* (1991) elongated and highly vacuolated cells were apparently originated from the non-meristematic tissue. In the present study, elongated, thin, free cells appeared in the cell suspension was non meristematic, but cell aggregates showed embryogenic potential.

Somatic embryogenesis in the presence of malt extract was reported from different triploid banana cultivars (Khalil *et al.*, 2002; Cote *et al.*, 1996; Ganapathi *et al.*, 2001). Similar type of observation was reported by Navarro *et al.* (1997) in a diploid and triploid Cavendish banana. Addition of ascorbic acid (0.01-0.5 mg/l) reduced blackening of medium. Only cell aggregate developed into embryos. Similar cell aggregates were described in suspension cultures of corn (Emons and Kieft, 1992) and wheat (Tabaeizadeh and Campeau, 1992). These cell clusters were called pro-embryogenic masses (Georget *et al.*, 2000) and indicated the embryogenic potential of suspension culture. In the present study, the cvc. Chingan (AA) showed maximum embryo development in the medium supplemented with zeatin from both explants. The differential response of

**Fig. 3: Plantlet in vermiculite**

two cultivars with respect to embryogenesis suggested that genetic factors affect the rate of embryogenic potential. The literature also revealed that somatic embryogenesis is genotype dependent phenomena (Assani *et al.*, 2002; Gahan and George, 2008). However the embryogenic frequency in banana was not only dependent on the genome group, but it also varied with the variety with in the genome groups and even one experiment to another (Strosse *et al.*, 2006).

In this procedure, the establishment of plant regeneration system for *Musa* spp. via somatic embryogenesis was achieved in a limited time period of 120-150 Days. Strosse *et al.* (2006) reported plant regeneration system for *Musa* spp. via somatic embryogenesis within a period of 14-42 months. Somatic embryogenesis was reported as time consuming, labour intensive and rather in efficient method for raising regenerable suspension cultures in case of banana. Several commercial and elite clones have been induced in to embryogenesis. A large number of banana genotype still need to be explored for embryogenic potential for use in propagation and genetic improvement. The protocol developed in this work is suitable for large scale propagation of diploid banana cultivars such as *Musa acuminata* cv. Chingan and *Musa acuminata* cv. Njalipoovan.

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A Comparative Study on the Nutritional and Anti-Nutritional Characters of Species of *Amaranthus* L. (Amaranthaceae)

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Amaranthus belongs to the family Amaranthaceae and is widely distributed in tropical countries. The genus comprises over 70 species of which 15 are consumed as vegetables. The present study concerns with the estimation of nutritional and anti nutritional components of four *Amaranthus* species, namely, *A. cruentus*, *A. spinosus*, *A. tricolor* and *A. viridis* collected from Thiruvananthapuram district, is the southern part of Kerala. The evaluated parameters were protein, carbohydrate, ascorbic acid, phenol, flavanoid, moisture and oxalate. The protein content ranged between 4.8-9.7 mg/g. High protein content was detected in *A. spinosus*. *A. cruentus* was characterized by presence of rich carbohydrate (23.6 mg/g). The carbohydrate and phenol content of genus ranged between 5.2-23.6 mg/g and 5.1-11 mg/g respectively. Highest flavanoid content was observed in *A. spinosus* and it ranged between 0.0029-0.005 mg/g. Ascorbic acid content and moisture was high in *A. viridis* and it ranged between 14-25 mg/g and 40-65%, respectively. The anti nutritional factor oxalate was found to be highest in *A. cruentus* and *A. tricolor* (15-14.09 mg/g) whereas it was comparatively very low in *A. spinosus* and *A. viridis*. The nutritional factors namely, carbohydrate, protein and ascorbic acid were markedly high as per the Recommended Dietary Allowances (1974). This study suggested the cultivation and consumption of *A. spinosus* and *A. viridis* which are not commonly used.

Key Words: *Amaranthus*, Nutrients, Anti-nutrients

Introduction

Many of the local vegetables are under-exploited because of inadequate scientific knowledge of their nutritional potentials. Vegetables are the indispensable constituents of human diets they supply the body minerals, vitamins and hormone precursors in addition to protein and energy.

The genus *Amaranthus* is a leafy vegetable and also a grain type of the family Amaranthaceae distributed widely in the tropics and sub tropics and it comprised of 60-75 species (Allen, 1964) out of which 15 occur in Indian subcontinent. *Amaranthus* is very palatable and well suited for human nutritional needs. *Amaranthus* is a tender annual growing to a height of 2-8 feet. The leaves are oval or oblong and some species are having purple colored lamina. The flowers are unisexual and developed on branched flowering clusters.

Though various researchers reported the compositional evaluation and functional properties of edible species of wild species of *Amaranthus*, studies related with the nutritional properties of wild species are scanty. Present investigation was to assess many of the potentials of nutritional and anti-nutritional properties of four *Amaranthus* species viz, *A. cruentus*, *A. spinosus*, *A. tricolor* and *A. viridis*. Among these *A. cruentus* is grain type *A. tricolor* is the common vegetable and *A. spinosus* and *A. viridis* are weeds. The evaluated

parameters in this study are protein, carbohydrate, ascorbic acid, phenol, flavanoid, moisture and oxalate.

Materials and Methods

The leaves were collected from Thiruvananthapuram. Estimation of protein, ascorbic acid, moisture and oxalate were done immediately after collection. Leaves were then dried and ground. The leaf powder was analyzed for carbohydrate content. The powder subjected to extraction (10 mg: 150 ml) with methanol using soxhlet apparatus. Estimation of phenol and flavanoid were made from the dried extracts.

Protein estimation of the leaves was done by the method of Lowry *et al.*, (1951). The method suggested by Hedge and Holfreiter (1962) was used for the estimation of carbohydrate. Ascorbic acid content of the fresh leaves were determined by the titration with 2, 6 dichloro phenol indophenols. (Harris and Ray, 1935). Moisture percentage was estimated by drying in a hot air oven and calculation was done by the formula $W-D/DX100$ (AOAC, 1984). Total phenol was estimated by Folin Ciocalteu assay (Singleton *et al.*, 1999). Flavanoid extraction and estimation was done according to Zhishen *et al.* (1999) Extraction and estimation of total oxalate was done by the procedure described by AOAC (1984). The proximate analysis of the above

Table 1.

Parameters	<i>A. cruentus</i>	<i>A. spinosus</i>	<i>A. tricolor</i>	<i>A. viridis</i>
Protein (mg/g)	6.70	9.74	5.00	4.80
Carbohydrate (mg/g)	23.6	5.22	7.25	8.16
Ascorbic Acid (mg/g)	15.00	24.95	18.40	14.32
Phenol (mg/g)	9.25	5.1	9.50	11.00
Flavanoid (mg/g)	0.43	0.50	0.31	0.29
Moisture (%)	60	79	76	85
Oxalate (mg/g)	15.01	8.05	14.75	5.72

mentioned results were summarized in Table1.

Result and Discussion

It is evident that all the four species were rich in ascorbic acid. Ascorbic acid is necessary for healthy teeth, gums and bones and is essential for the proper functioning of adrenal and thyroid glands. It has also antioxidant properties. Ascorbic acid in the leaves varied from 14.32-24.95 mg/100g. *A. spinosus* is a weedy species possessed high amount of ascorbic acid (24.95) compared to the cultivated species, *A. tricolor* (18.4). According to the food and nutrition board (1974), the Recommended Dietary Allowances of ascorbic acid (vitamin C) for infants, children and adults are 40, 45 and 60 mg per day. Akubugwo *et al.* (2007) reported that ascorbic acid content in the *A. hybridus* leaves as 25.40 mg/100 mg. Shukla *et al.* (2003) reported variable quantity of ascorbic acid from foliage cuttings vegetable amaranth.

Phenol content in the leaves show a wide range of 5-11 mg/100 g. Phenolics are well known for their

diverse physiological properties, anti-carcinogenic, anti-inflammatory and antioxidant activities. (Rice Evans *et al.*, 1997) the presence of phenol content in *A. viridis* leaves is much higher than the other three species (11 mg/100 g). Phenol content was lowest in the weedy species *A. spinosus*. Presence of high phenol content in *A. viridis* points out the significance in the use of carcinogenic properties.

Flavanoid content of the four species ranged between 0.29-0.50 mg/100 g dry weight. Highest content of flavanoid was observed in *A. spinosus* (0.50 mg) where as the cultivated species *A. tricolor* possessed only 0.31 mg and the least value 0.29 mg was detected in *A. viridis*. Akubugwo *et al.* (2008) estimated the flavanoid content in *A. hybridus* as 0.83 mg/100 g. According to Michelle *et al.* (2008) *Urtica dioica* leaves (wild) possess 0.89 mg/100 g as quercetin equivalents.

The protein content was highest in the leaves of *A. spinosus* (9.74 mg/g) and the lowest in *A. viridis* (4.8 mg/g). *A. cruentus* and *A. tricolor* were 6.7 and 5 mg/gm respectively. Protein content in the present stud was lower than that reported by Prakash and Pal (1991), Mnkeni *et al.* (2007) and Akubugwo *et al.*, (2007). Shukla *et al.* (2003) reported the protein content in the cultivars of *A. tricolor* as 1.95-3.06 g/100 g. Low-level protein content observed in the present study due to the differences and variation in agro-climatic changes. Singh *et al.* (2000) suggested this in their study on the nutritional composition of leafy vegetables and herbs.

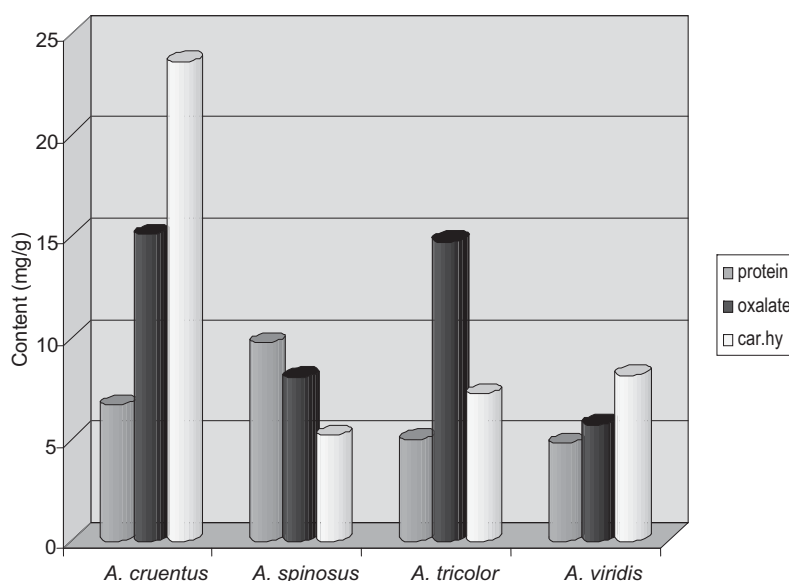


Fig. 1: Representation of carbohydrate, protein and oxalate

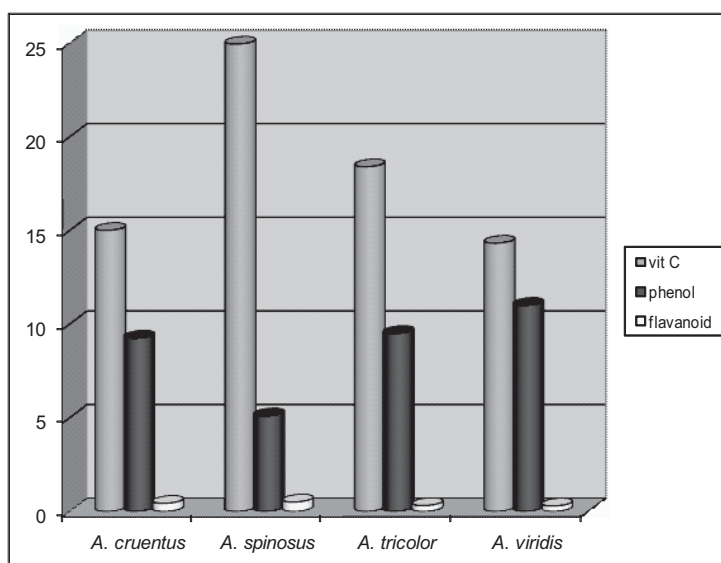


Fig. 2: Representation of vitamin C, phenol and flavanoid (mg/100 g)

A wide range of variation (95.22-23.6 mg/g) was observed in the quantity of carbohydrate. High quantity was found out in the *A. cruentus* the grain type amaranth. The leafy vegetable *A. tricolor* had 7.25 mg. The weedy species *A. spinosus* and *A. viridis* possessed 5.22 and 8.16 respectively.

Moisture content in the leaves ranged between 60-85%. Maximum moisture was observed in *A. viridis* (85%). *A. cruentus* contains less water content (60%) than others, *A. spinosus* (79%) and *A. tricolor* (76%). A similar value of moisture content was reported by Singh *et al.* (2000) in *A. tricolor*, Akubugwo *et al.* (2007) in *A. hybridus* and Marcone (2000) in *A. hypochondriacus*.

Oxalate is a common constituent of a plants with dicarboxylic acid anion, is considered to be anti nutritional factor as well as toxin. It can render some mineral nutrients unavailable by binding them to form insoluble salts that are not absorbed by the intestine (Libert and Franeschi, 1987). The four species contain prominent percentage of oxalate. *A. cruentus* contains high value (15.1 mg/g) and the lowest value was observed in *A. viridis*. *A. tricolor* and *A. spinosus* possessed 14.75 and 8.05 mg/g.

From the foregoing discussion, it may be concluded that *A. spinosus* had the best combination of high ascorbic acid, moderate amount of carbohydrate, phenol, flavanoid and protein and low amount of oxalate. The study suggest that the applicability of utilizing the weedy relatives for the improvement of cultivated species *A. tricolor* by transferring the traits related to increase the ascorbic

acid, protein and flavanoid content and the anti nutritional factor oxalate.

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Bulk Line Analysis in Coconut (*Cocos nucifera* L.) for Inferring Relationship between Talls, Dwarfs and Niu Leka Dwarf Forms

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Coconut is an important oil crop of India. Coconut (*Cocos nucifera* L.) is botanically classified in to two major groups based on its stature as Tall palms and as Dwarf palms. The Talls can also be referred to as var. Typica (Nav) and the Dwarfs as var. Nana (Griff). Further, intermediate forms of coconut referred to as Aurantiaca are also available. The technique Bulk Line analysis is employed for the identification of tall / dwarf bulk specific RAPD marker. Thirty random primers were used to amplify the DNA bulks of talls, dwarfs and intermediate coconut types. Out of 30 primers 22 primers showed polymorphism between the DNA bulks. The primers OPM 02, OPM 06, OPC 13 produced specific markers. Out of 40 polymorphic markers five markers were common to tall bulk and dwarf bulk; on the other hand 29 markers were common between the tall bulk and Niu Leka Dwarf. To reveal the overall relationship between the bulked samples presence or absence of RAPD marker data were analysed with the NTSYS software. Jaccard's similarity coefficient was highest between talls and Niu Leka Dwarf. The resultant dendrogram showed clustering of talls and Niu Leka types together whereas, dwarf types were separated out.

Key Words: Coconut, Bulk Line Analysis, RAPD, Marker

Introduction

The coconut palm, *Cocos nucifera* Linn. is a perennial oil yielding tree crop of tropics. It belongs to the family Arecaceae under the class monocotyledons. With its tall, slender and uniformly thick stem and massive crown with large number of leaves, bearing bunches of nuts in their axils is one of the most beautiful and useful trees in the world. It perhaps yields more products of use to mankind than any other tree. Coconut has been given different names such as Kalpa Vriksha (tree of Heaven), the consols of East, Mankind's Greatest Provider in the Tropics, Tree of Life, Tree of abundance and Tree of plenty etc.

Each and every part of the coconut palm is useful to man in one way or another. The most important products are tendernut, copra, oil, oil cake, desiccated coconut and fibre. The genus *Cocos*, formerly included besides *Cocos nucifera* Linn. over thirty species mostly confined to Central and South America. It is, however, now usual to regard one of them which is cosmopolitan in the tropics as monotypic genus containing *Cocos nucifera* L. the coconut, other species having been assigned to several other new genera. Coconut has a pantropic distribution mainly in coastal regions at 20° either side of the equator. Grown in 11.6 million hectares in 86 countries of tropics including India. The area and production of coconut in India (2002-03) are

1.82 M ha and 9.5 M nuts respectively (FAO STAT, 2004).

Coconuts are generally classified into tall palms and dwarf palms. Tall palms are widely planted and they can grow upto 20-30 m height. They are normally cross pollinating and therefore considered to be heterozygous. Talls constitute the polymorphic populations (Perera *et al.*, 2000). They are slow to mature, flowering 6-10 years after planting and have economic life of 65-75 years. On the other hand, Dwarf palms grow to a height of 10-15 m, flowering 3-4 years after planting and they are self pollinating and considered to be homozygous and has economic life of 30-40 years. The dwarfs are presumed to have originated from talls either by mutation (Menon and Pandalai, 1958) or by inbreeding (Swaminathan and Nambiar, 1961; Lebrun *et al.*, 1998). Harries (1978) recognized three basic dwarf types in coconut. They are nana, javanica and Niu Leka types. The Niu Leka dwarf coconut is characterized by its short internodes that result in a stout trunk, dense canopy and broad leaves. Harries (1978) notes that in all respects other than height, Niu Leka Dwarf resembles tall: swollen trunk base, out crossing reproductive behavior, lack of bright red and yellow fruited phenotypes and large fruit size. He suggested that Niu Leka Dwarf represented distinct domesticated types relative to other dwarf classes. Unlike the dwarf types, which are highly autogamous

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and thus homozygous, the Niu Leka Dwarf despite its name, is primarily out crossing like that Tall types (Whitehead, 1976; Harries 1978) and this is more heterozygous (Teulat *et al.*, 2000; Meerow *et al.*, 2003).

Coconut palms are identified as tall / Dwarf based on its stature. Genetically it was observed that loss of alleles and (Perera *et al.*, 2003) genetic diversity in dwarfs. But no specific markers have been identified for dwarfs / talls. Hence in the present study, the Bulk Line Analysis was employed for the identification of tall / dwarf specific markers. Earlier, Bulk Line Analysis (Tan *et al.*, 1998) was used to map the rice Rf gene. Identification of the marker for specific trait can be carried out by using various genetic stocks, such as near isogenetic lines (NILs, Muehlbauer *et al.*, 1988), bulked segregant analysis (BSA, Michelmore *et al.*, 1991) and segregating progenies. However, all these genetic stocks are restricted to advancing generations through specific crosses. This disadvantage appears more significant for a crop having a long period for advancing a new generation like coconut. It will take 7-10 years for advancing one generation in coconut. But Bulk Line Analysis method is not based on the segregating population. The informative markers can be identified is based on the polymorphism detected by the primer between a pair of bulks having same phenotype, not between the pair of parents.

Materials and Methods

Two gram leaf samples were taken from 116 talls types, 32 dwarf types and 12 Niu Leka types. (Table 1).

DNA Extraction

DNA was extracted from sprouting leaflets (pale yellow color). Two gram of leaf material was frozen in liquid nitrogen and ground to powder in a pestle and mortar. 0.50 g of Poly Vinyl Poly Pyrrolidone (PVPP, MW 40000) was added to the ground powder. Then the DNA extraction was done using Plant DNA extraction kit (Nucleon Phyt PURE) DNA pellet was air dried and resuspended in 100 µl TE. DNA concentration was measured in a spectrophotometer and the intactness was checked in 0.8% agarose gel. DNA was diluted to 15 ng per µl.

Bulking of DNA

Equal amounts (50 ng) of DNA samples from the 116 tall individuals were pooled to constitute the Tall Bulk. DNA samples from 32 dwarf individuals were pooled to constitute the Dwarf Bulk. DNA samples from 12

Table 1. Coconut accessions used for bulking the DNA

S.No.	Accession	Type	No. of samples
1	Kong Thienyong Tall	Tall	4
2	Straight Settlement Green Tall	Tall	4
3	Straight Settlement Apricot Tall	Tall	4
4	Philippines Kalambahim Tall	Tall	4
5	Laguna Tall	Tall	4
6	Philippines Palawan Tall	Tall	4
7	Philippines Dalig Tall	Tall	4
8	San Roman Tall	Tall	4
9	Markham Valley Tall	Tall	4
10	Nufella Tall	Tall	4
11	Nugili Tall	Tall	4
12	Nuwallis Tall	Tall	4
13	Nu Quamen Tall	Tall	4
14	Kupien Tall	Tall	4
15	Nuwehnug Tall	Tall	4
16	Lifou Tall	Tall	4
17	British Solomon Island Tall	Tall	4
18	Jamaica Tall	Tall	4
19	Saint Vincent Tall	Tall	4
20	Panama Tall	Tall	4
21	Nigerian Tall	Tall	4
22	Kaithathali Tall	Tall	4
23	Indian Spicata	Tall	4
24	Indian East Coast Tall	Tall	4
25	Verrikobbari Tall	Tall	4
26	Nadora Tall	Tall	4
27	Nicobar Tall	Tall	4
28	Hazari Tall	Tall	4
29	Navassi Tall	Tall	4
30	Niuleka Dwarf	Intermediate	12
31	Chowghat Green Dwarf	Dwarf	4
32	Laccadive Dwarf	Dwarf	4
33	Chowghat Orange Dwarf	Dwarf	8
34	Gangabondam Green Dwarf	Dwarf	8
35	Gangabondam Brown Dwarf	Dwarf	4
36	Malayan Orange Dwarf	Dwarf	4

individuals of the intermediate accession (Niu Leka Dwarf) were pooled to constitute the Intermediate Bulk (Table 1). Thirty random primers were used to amplify the bulked samples

RAPD Analysis

Amplification reactions were carried out in 10 µl volume containing 30 ng of template DNA, 200 mM of each dNTPs, 0.45 U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., India) and 25 pmol of random primer (Operon technologies, USA). Amplifications were performed in a Thermo Cycler (MJ Research Inc., USA) programmed for an initial denaturation at 94 °C for 2 min., 40 cycles of 1 min. denaturation at 94 °C, 1 min. annealing at 37 °C and 2 min. extension at 72 °C and a final extension of 7 min. at 72 °C. The amplification products were subjected to electrophoresis through a 1.80 % agarose gel using 1X TBE buffer at 90 volts for 2 h in Bio-Rad submarine electrophoresis

unit. The ethidium bromide stained gels were documented using the Alpha ImagerTM 1200 – Documentation and Analysis system (Alpha Innotech Corporation, USA). Amplification reaction was done twice for reproducible bands.

Data Analysis

Only the clear, unambiguous and reproducible bands were considered for scoring. Each band was considered to be a single locus. Data were scored as “1” for the presence and “0” for the absence of a DNA band of each accession. DNA band size was estimated by comparing the DNA bands with a 1 Kb DNA ladder or lambda DNA *Eco* RI / *Hind* III double digest (MBI Fermentas, India).

The binary data matrices were entered into the NTSYS pc package (Exeter Software, USA). The data were analysed using Qualitative routine to generate Jaccard's similarity coefficient. Similarity coefficients were used to construct a dendrogram using UPGMA (Unweighted Pair Group Method with arithmetic Average) and SHAN (Sequential Hierarchical and nested clustering) routine.

Results

Bulked DNA samples of talls, dwarfs and intermediate (Niu Leka Dwarf) types were amplified with 30 RAPD primers. Out of which 22 primers produced specific markers for bulked samples (Table 2 and 3). A total of three markers were observed for tall bulk, eight markers for dwarf bulk and two markers for intermediate type. The primer OPM 02 produced one tall specific marker (OPM 02₁₄₂₅) and one dwarf specific marker (OPM 02₄₂₀) and three markers common to tall bulk and intermediate bulk. The primer OPM 06 produced one dwarf specific marker (OPM 06₁₆₇₃), two markers common to talls and dwarfs and four markers common to tall bulk and intermediate bulk. The primer OPC 13 produced Niu Leka Dwarf specific marker (OPC 13₁₃₂₀). There were only five markers that were common to tall bulk and dwarf bulk; on the other hand 29 markers were common between the tall bulk and Niu Leka Dwarf. There were two markers OPBE 08₁₆₇₂ and OPE 02₉₀₉ common to dwarfs and Niu Leka Dwarf. RAPD marker profiles of the bulked DNA samples with different primers are shown in Plate1.

Table 2. List of RAPD markers specific to bulked DNA samples of talls, dwarfs and intermediates

Primer	No of specific markers (bp)					
	Tallbulk	Dwarfbulk	Intermediate(NLAD)	Tall bulk and dwarf bulk	Tall bulk and intermediate	Dwarf bulk and intermediate
OPBE 02	0	0	0	1	3	0
OPBE 03	0	0	1	0	1	0
OPBE 06	1	0	0	0	1	0
OPBE 08	0	1	0	0	2	1
OPBE 14	0	0	0	0	2	0
OPBE 16	0	0	0	0	1	0
OPC 04	0	0	0	0	1	0
OPC 10	0	0	0	0	1	0
OPC 12	0	0	0	0	1	0
OPC 13	0	0	1	0	0	0
OPC 15	0	1	0	0	2	0
OPE 02	1	0	0	0	0	1
OPE 12	0	0	0	1	1	0
OPE 16	0	1	0	1	0	0
OPE 18	0	0	0	0	1	0
OPG 08	0	1	0	0	1	0
OPM 02	1	1	0	0	3	0
OPM 06	0	1	0	2	4	0
OPM 07	0	0	0	0	1	0
OPM 09	0	0	0	0	2	0
OPM 10	0	2	0	0	0	0
OPM 20	0	0	0	0	1	0
Total	3	8	2	5	29	2

Table 3. RAPD markers detected in the tall bulk, dwarf bulk Niu Leka Dwarf, markers common to tall and dwarf, tall and intermediate and dwarf and intermediate

Tallbulk	Dwarfbulk	Intermediate(NLAD)	Tall bulk and dwarf bulk	Tall bulk and intermediate	Dwarf bulk and intermediate
OPBE 06 ₅₇₈	OPBE 08 ₉₂₀	OPBE 03 ₁₃₂₀	OPBE 02 ₁₂₃₃	OPBE 02 ₁₄₅₁	OPBE 08 ₁₆₇₂
OPE 02 ₆₁₈	OPC ₁₅₆₃₈	OPC 13 ₁₃₂₀	OPE 12 ₈₅₄	OPBE 02 ₃₂₀	OPE 02 ₉₀₉
OPM 02 ₁₄₂₅	OPE 16 ₁₄₈₀		OPE 16 ₁₃₁₂	OPBE 02 ₂₁₈	
	OPG 08 ₈₂₄		OPM 06 ₁₁₂₁	OPBE 03 ₂₅₃	
	OPM 02 ₄₂₀		OPM 06 ₁₀₄₅	OPBE 06 ₁₆₆₁	
	OPM 06 ₁₆₇₃			OPBE 08 ₁₀₄₂	
	OPM 10 ₁₇₅₈			OPBE 08 ₈₁₃	
	OPM 10 ₁₄₄₀			OPBE 14 ₁₂₀₂	
				OPBE 14 ₉₈₇	
				OPBE 16 ₁₄₁₂	
				OPC 04 ₄₄₀	
				OPC 10 ₄₇₂	
				OPC 12 ₅₅₈	
				OPC 15 ₆₁₇ OPC 15 ₆₀₂	
				OPE 12 ₄₄₂	
				OPE 18 ₅₈₃	
				OPG 08 ₄₇₈	
				OPM 02 ₁₆₂₃	
				OPM 02 ₆₇₂	
				OPM 02 ₆₄₆	
				OPM 06 ₁₇₄₁	
				OPM 06 ₁₄₅₄	
				OPM 06 ₉₀₈	
				OPM 06 ₆₀₂	
				OPM 07 ₅₉₂	
				OPM 09 ₁₀₇₇	
				OPM 09 ₈₈₉	
				OPM 20 ₄₉₃	

To reveal the overall relationship between the bulked samples, the presence / absence of RAPD marker data were analysed using the software NTSYS pc. 30 primers produced a total of 291 markers. Out of which, 40 markers were polymorphic. Jaccard's similarity coefficient between the tall bulk and the dwarf bulk was 0.819, between the tall bulk and Niu Leka Dwarf was 0.935, between the dwarfs and Niu Leka Dwarf was 0.810. The dendrogram grouped the tall bulk and Niu Leka Dwarf together and the dwarf bulk was separated out (Fig. 1).

Discussion

The utility of bulk line analysis (Tan *et al.*, 1998) was tested in coconut for the identification of dwarfs / tall palms. BLA method is not based on the segregating population and the informative markers identified is based on the polymorphism detected by the primer between a pair of bulks, not between the pair of parents.

Based on the method of BLA, RAPD markers were identified that were specific to tall and dwarfs. Eight

RAPD markers were identified for dwarfs. The interesting observations was, most of tall bulk markers were shared by the intermediate type, Niu Leka Dwarf. This again confirms the Niu Leka Dwarf is not a true dwarf type and it is intermediate between tall and dwarfs and more close to tall. Highest similarity found between tall and Niu Leka Dwarf reveals the closeness between them. Hence, our data suggest that, Niu Leka Dwarf can be placed towards the tall. Dendrogram constructed revealed the overall relationship among tall, dwarf and Niu Leka Dwarf. Meerow *et al.* (2003) reported Niu Leka Dwarf had the closest genetic identity with the Panama Tall and Spicata Tall and had largest number of unique SSR alleles. Our data revealed that the Niu Leka Dwarf shared most of the markers to the tall and clustered with the tall.

The DNA markers identified based on the bulked samples has immense tool for juvenile selection of tall / dwarf at early stages among hybrids of tall x dwarf or dwarf x tall. There was no such standard molecular

level guidelines available so far for the identification of seedlings of talls and dwarfs at nursery. But, some available discriminators viz., time taken for sprouting, rate of leaf production, length and width of leaves and time to first leaf splitting, needs six months to one year to observe these characters. Moreover, all traits are influenced by environment and require proper field experiments. Fernando *et al.* (1993) used seedling characteristics to identify the hybrids and they reported 20 per cent chance of misclassification. But, when DNA markers are available seedling can be identified unambiguously. Microsatellite markers would be better a tool for bulk line analysis also. The effectiveness of these markers needs to be tested in the field conditions.

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Nutritional and Technological Advancements in the Promotion of Ethnic and Novel Foods Using the Genetic Diversity of Minor Millets in India

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An appropriate food processing technology stimulates agricultural production, ensures availability of quality products, value addition and helps in creating jobs and thus plays a crucial role in the economic progress and industrial development in the rural areas of India. The research on small or minor millet species was undertaken to explore the inherent technological opportunities for better utilization of resources in designing value added and sustainable foods in different sectors of food industries under the project “Value addition to nutritious millets to enhance food security and income of the rural poor” supported by International Fund for Agricultural Development (IFAD), Rome, Italy. The traditional fermented breakfast food ‘*paddu*’ made with little millet has light and fluffy textural quality of cooked starch and is highly acceptable for taste and texture as compared to that made with rice. Small seeded little millet showed an advantage over rice due to several technological features like less water uptake, less soaking, grinding, and baking time and high product yield. Introduction of *paddu* in restaurants was acceptable to the consumers and was found sustainable in hotels. Indigenous *papads* such as ‘*nere happala*’ and ‘*mudde happala*’ prepared from the gelatinized mass of three days soaked millets, viz., *ragi* (finger millet), little millet and foxtail millet without addition of any chemical additive like ‘*happla khara*’ enhanced the characteristic aroma and sour taste with good expansion of fried *papad*. *Papads* were highly acceptable for all the sensory attributes. This natural fermented food which is harmless to health has cost effective preparation and, requires less laborious technology. When taken as a home based food industry by women entrepreneurs of Jekinakatti village of Haveri district in Karnataka, it was found profitable as compared to black gram *dhal papad*. Incorporation of 50% whole grain foxtail millet or *ragi* flour to standard recipe with change in proportion of fat yielded acceptable cookies of different types with good spreading quality, crispy texture and sweet taste. Muffins of foxtail millet and *ragi* were light, puffy and highly acceptable for taste. Incorporation of millet flour in cookies and muffins increased the protein, fibre, and micronutrient contents. Millet based products showed good potential to enter bakery industry especially in rural sector as a nutritious snack item for school children. Thus, ethnic millet *papads*, fermented breakfast food ‘*paddu*’ and bakery products have good scope for marketing and income generation through rural women entrepreneurs. Value addition to millets is a highly strategic intervention in the popularization of nutritionally rich crops. As this project has demonstrated, the success and sustainability of efforts in this domain is closely dependant upon an effective synergy among highly complementary disciplines, including conservation of plant genetic resources, crop improvement, agronomy and marketing.

Key Words: Small millets, Food technology, Value addition, Ethnic and novel foods, Women empowerment

Introduction

Neglected and underutilized crop genetic resources are very vital for sustainable agriculture (Eyzaguirre *et al.*, 1999, Bhag Mal, 2007) and minor millets belong to this important group of crops (Dutta *et al.*, 2007). Plant genetic resources play a critical role in enhancing adaptation and resilience of agricultural production systems (Jarvis *et al.*, 2007). The important minor millets cultivated in India are finger millet (*Eleusine coracana* (L.) Gaertner), kodo millet (*Paspalum scrobiculatum* L.), foxtail millet (*Setaria italica* (L.) Pal), little millet (*Panicum sumatrense* Roth ex Roemer & Schultes), proso millet (*Panicum miliaceum* L.) and barnyard millet (*Echinochloa colona* (L.) Link (Riley *et al.*, 1993). These millets have remained the food

for the people of the lower economic strata and traditional consumers, because of their coarse texture, characteristic flavour, intensely coloured seed coat and cultural attachments (Yenagi, 2004). However, the non-availability of processed products similar to rice or wheat is the primary reason for their consumption being confined to traditional consumers. Culture has preserved the significance of millets through the promulgation of their use in the preparation of traditional ethnic dishes during special occasions and rituals (Yenagi *et al.*, 2004). Ethnic foods made out of small millets have excellent taste, crispy texture, light and fluffy characteristics and superior textural quality of cooked starch. They are also highly acceptable for taste and texture by both rural and urban

consumers (Yenagi, 2005). The promotion of indigenous and value added products through different communication techniques enhanced the consumers knowledge and readiness to incorporate millets in their daily diet. An appropriate food processing technology stimulates agriculture production, ensures availability of quality products, value addition and helps in creating jobs. Such an approach is thus strategic in supporting both the economic progress and industrial development in rural areas of India (Yenagi, 2007). Because women are the main actors behind the knowledge, production and consumption of genetic resources of small millets, the enhanced use of these crops also provides an opportunity of empowerment of this marginalized sector of the Indian society. It is under this background that the IFAD funded research project on small millets is being pursued to explore the inherent technological opportunities for better utilization of these resources in designing value added and sustainable foods in different sectors of food industries in India.

Material and Methods

Selection of ethnic and novel foods

Products, accepted by both rural and urban consumers across Karnataka State and which have entered the entrepreneurial activities of women Self Help Groups (SHGs) as home based food processing activity, were selected for evaluation of nutritional and technological opportunities for better utilization in the market as compared to other products already existing in the market. Ethnic foods like *mudde happala* and fermented breakfast food - *paddu* and novel foods such as biscuits, *chakali* and *laddoos* were selected.

Evaluation of Ethnic and Novel Foods of Millets for Technological and Nutritional Qualities

Technological properties: The observations were recorded on weight of the dough, handling properties of the dough, weight of the end product, number of pieces obtained, weight of individual unit, time taken for cooking, yield obtained, spread ratio, expansion ratio, per cent increase in volume for the control recipe and millet based recipe as per the standard procedure.

Objective and subjective quality characteristics of products: Products prepared from the women entrepreneurs were procured and evaluated for sensory quality characteristics subjectively and objectively.

Sensory evaluation of ethnic and novel foods: Products were evaluated on nine point hedonic scale (1-very poor,

9-excellent) by a panel of ten trained members at Food Laboratory, Department of Food Science and Nutrition, College of Rural Home Science, University of Agricultural Sciences, Dharwad (Srilakshmi, 2007).

Nutrient composition of ethnic and novel foods: Selected products were assessed for nutrient composition by computation method using 'Nutritive value of Indian foods', and compared between control and millet recipes (Gopalan *et al.*, 2004).

Results and Discussion

Technological features of ethnic and novel foods of millets in comparison with standard foods are presented in Table 1 and Table 2. Significant variation was observed for most of the technological features of different millet products, viz., total weight of the end product, number of pieces obtained, spread ratio of biscuits, expansion ratio of *papads*, per cent increase in the volume of baked *paddu*, baking/cooking/frying time, making time/grinding time of the product as compared to the respectable standard products. It is evident from Table 2 that technological features of ethnic and novel foods of millets differed significantly during different stages of processing. Time taken for grinding, baking of *paddu*, preparation of *papad*, roasting of flour, making of *laddu* and frying of *chakali* of millet was significantly lower than the standard recipes. This may be due to the smaller size of millet grains which take lesser time for hydration and cooking (Malleshi and Hadimani, 1993). The expansion ratio of fried *papad* and spread ratio of biscuits were significantly lower than the standard recipes whereas the per cent increase in the volume of *paddu* was significantly higher than standard recipes. These unique technological features of millet products showed the possibility of their use as time and fuel saving grains. These technological features can replace or serve as an alternative to rice or wheat or any pulse flour in conventional or novel food preparations such as fermented foods, fried foods, and convenient foods or in bakery industries.

The evaluation of ethnic and novel foods of millets as compared to standard ones revealed that the sensory scores of all the quality parameters were higher for standard recipes as compared to those made with millets (Table 3). These differences were however non-significant for most of the traits. Furthermore, it is also interesting to note that though sensory scores were low for millet products for most characters, their rating scored 7 and above, which indicated that these products were under the category of "very good" for acceptability. The results

of the study show that millet products have a potential to be widely acceptable and popularized in the national market. The price of the main ingredient is one of the important factors for assessing the cost benefit ratio of the end product. The cost of Bengal gram *dhal* is Rs. 50/kg, which is far higher than that of millets (Rs.12-14/kg). The cost of pulse based recipe-*laddoo* in the market is around Rs.160-180/kg. Therefore, replacement of Bengal gram flour with that of millet yielded therefore a cheaper product with on par acceptability for all the sensory quality parameters (Table 3). Similarly, the per cent increase in the volume of the *paddu* is one of the important quality and economic character which adds additional profit to millet products. The number of biscuits that can be prepared with a unit quantity of millet flour is also higher as compared to that produced with the flour of other grains and this gain in productivity is certainly another interesting feature that can be used for enhancing the marketing of millet-based products.

From a nutritional point of view, the ethnic and novel products of millets were found to be comparatively richer in protein and mineral content than those made out of rice or wheat (Table 4). Although, no significant difference was observed for the sensory quality characters and technological features of standard biscuits and millet biscuits, the latter were found to bear greater amounts of proteins and calcium. Such a fact represents a highly strategic element in our attempt to enhance the nutritional

values of bakery products in India and deserve greater attention of policy makers and industrial sector. Indeed, since substitution of less refined millet flour to standard recipes, has improved the nutritional quality of the products by increasing macro as well as micronutrients, recipes based with small millets need to be widely popularized for combating hidden nutritional deficiencies, particularly high among school going children.

Besides their nutritional contribution, small millets also play a strategic role as instrument of empowerment of women. In the framework of the IFAD-NUS project, hundreds of women were trained on preparing value-added products, of great acceptability and demand at the local level. As a result of their acquired skills, the trained women gained greater self-confidence in their food production activities at both household and market level, with positive impact on their own family and communities. Furthermore, complementary training efforts in marketing food products made some of these women, associated with SHGs promoted by the project, also successful entrepreneurs.

Conclusion

The ethnic millet *papads*, *chakali*, fermented breakfast food '*paddu*', novel foods like biscuits, *laddu* all prepared with small millets proved to have a good scope for enhancing nutrition security, marketing and income generation of community members, particularly rural

Table 1. Technological features of processed ethnic and novel foods of millets in comparison to standard foods

Type of food		Main ingredient	Qty. of main ingredient (g)	Total weight of the dough (g)	Total weight of the cooked food (g)	Weight of one piece (g)	Total number of pieces
<i>Paddu</i>	Rice	Rice	200	—	147.00**	7.00*	21
	Millet	Little millet	200	—	136.50	6.50	21
t-value	—	—	—	—	24.37	1.36	NS
<i>Papad</i>	Rice	Rice	250	753.66*	370.00**	5.00	74.00**
	Millet	Little millet	250	702.33	345.00	5.00	69.00
t-value	—	—	—	2.70	16.27	NS	17.10
Biscuit	<i>Maida</i>	<i>Maida</i>	200	—	461.66**	11.26**	43.00
	Millet	<i>Maida + Ragi flour</i>	100+100	—	422.66	9.93	45.00**
t-value	—	—	—	—	12.78	29.49	35.01
Ladoo	<i>Besan</i>	<i>Besan</i>	250	545.66	545.66	30.46	17.00
	Millet	<i>Besan flour + Foxtail millet</i>	125+125	551.0	551.00	30.23	18.00**
t-value	—	—	—	1.67 ns	1.38 ns	0.49 NS	2.47
<i>Chakali</i>	Rice	Rice	250	599.00	510.00	—	—
	Millet	Little millet	250	598.00	538.66**	—	—
t-value	—	—	—	0.5 ns	14.76	—	—

* Significant ** Highly significant NS-Non Significant

Table 2. Technological features of processed ethnic and novel foods of millet during different stages of processing

Food	Type of processing	Technological features observed	t-value
Preparation of fermented breakfast food - <i>paddu</i>	Grinding time	Time taken to grind soaked rice was more (18.5** sec) than little millet (4.5 sec).	175.14
	Baking time	Time taken for baking of one set of rice <i>paddu</i> was more (9.19 min**) than little millet (6.30 min)	118.43
	Per cent increase in the volume of the baked product	Per cent increase in the volume of baked product of one set of rice <i>paddu</i> was lower (21.8%) than little millet <i>paddu</i> (25.45%**)	16.55
<i>Papad</i> preparation	Rolling of <i>papad</i>	<i>Papad</i> pressing was very easy with the dough of little millet as compared to rice	—
	Time taken to roll	The time taken for rolling of one rice <i>papad</i> was higher (25 sec**) than the little millet (22 sec)	29.86
	Frying quality	The expansion ratio of fried rice <i>papad</i> was higher (2.23**) than little millet (1.92)	14.96
Biscuit making	Spreading quality	Spreading ratio of standard biscuits was higher (4.00 cm**) than <i>ragi</i> biscuits (3.17)	19.21
<i>Ladoo</i> making	Time taken for roasting	Time taken for roasting of Bengal gram flour to attain golden brown colour was more (40 min**) as compared to foxtail millet flour (35 min)	8.34
	Time taken for <i>ladoo</i> making	Foxtail millet dough was very easy to handle during preparation of <i>ladoo</i> and the time taken was lesser (20min) than standard recipe (30min**)	13.08
<i>Chakli</i> preparation	Pressing of <i>chakli</i>	Pressing of little millet dough was very easy as compared to rice	—
	Frying time	The frying time of one set of rice <i>chakli</i> was more (6.5 min**) compared to millet (5.0min)	1.22

* Significant ** Highly significant

Table 3. Mean sensory evaluation scores of ethnic and novel foods of millets in comparison to standard recipes

	Colour and appearance	Texture	Taste	Aroma	Overall acceptability
A. Ethnic foods					
<i>Paddu</i>					
Standard	8.3**	8.0*	8.2	7.8**	8.0
Millet	7.5	7.6	7.9	7.1	7.8
t-value	3.53	2.44	N.S	3.13	N.S
<i>Papad</i>					
Standard	7.8**	8.1**	8.1	7.8	8.0
Millet	7.1	7.0	7.4	7.5	7.6
t-value	2.27	3.97	N.S	N.S	N.S
B. Novel foods					
<i>Biscuit</i>					
Standard	7.6**	6.4	6.5	6.5	7.0
Millet	6.2	6.9	6.5	6.7	7.1
t-value	3.32	N.S	N.S	N.S	N.S
<i>Ladoo</i>					
Standard	7.5	6.8	7.2	6.9	7.3
Millet	7.6	7.7	7.7	7.3	7.6
t-value	N.S	N.S	N.S	N.S	N.S
<i>Chakli</i>					
Standard	7.9*	8.0**	8.2**	7.8**	7.9**
Millet	7.2	7.0	7.3	7.1	7.0
t-value	2.27	3.0	2.74	2.27	3.25

* Significant ** Highly significant NS-Non Significant

women. Value addition also showed to be a highly strategic intervention in the popularization of nutritionally and technologically rich local crops which are currently largely neglected and underutilized. As this project has demonstrated, the success and sustainability of efforts in this domain are, however, largely dependent upon an effective synergy among highly complementary disciplines, including crop improvement, nutrition, food technology, marketing, education, public awareness and policies.

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Table 4. Nutritive value of ethnic and novel foods

Parameters	Rice	Millet
Paddu-100g		
Carbohydrates(g)	123.02	111.63
Protein(g)	19.03	20.85
Fat(g)	10.96	12.58
Ash(g)	2.02	4.75
Crude fibre(g)	0.75	12.42
Calcium (mg)	68.86	94.08
Energy(kcal)	666.84	643.18
Calcium (mg)	68.86	94.08
Energy(kcal)	666.84	643.18
Papad-100 g		
CHO(g)	81.82	78.51
Protein(g)	5.79	7.90
Fat(g)	3.06	4.06
Ash(g)	0.52	2.48
Crude fibre(g)	0.66	3.74
Energy(kcal)	377.98	382.18
Calcium (mg)	14.56	34.60
Biscuit-100g		
CHO(g)	57.80	58.69
Protein(g)	4.78	4.35
Fat(g)	26.36	27.88
Ash(g)	0.28	4.55
Crude fibre(g)	0.12	0.92
Energy(Kcal)	487.79	503.07
Calcium (mg)	60.40	90.16
Ladoo-100 g		
CHO(g)	64.46	63.97
Protein(g)	9.58	7.54
Fat(g)	18.91	18.39
Ash(g)	1.27	1.40
Crude Fibre(g)	0.55	2.08
Energy(kcal)	500.00	483.34
Calcium (mg)	28.86	24.81
Chakli-100 g		
CHO(g)	45.48	39.13
Protein(g)	6.92	7.49
Fat(g)	32.33	32.12
Ash(g)	1.16	1.88
Crude fibre(g)	1.04	5.05
Energy(kcal)	501.33	475.58
Calcium (mg)	65.27	72.00

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SSR and ISSR Markers based Population Genetic Structure of Coconut (*Cocos nucifera* L.) Germplasm Accessions

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The coconut palm (*Cocos nucifera* L.) is one of the major perennial oil crops of tropics. Population genetic structure was assessed among 33 accessions (4 individuals per accession) with ISSR and SSR markers. The molecular marker data were analyzed with POPGENE and ARLEQUIN software. The parameters derived were, Shannon's index, differentiation indices (Fst and Gst) and molecular variances. The diversity was partitioned into 'within population' (59.82% and 68.56% based on ISSR and SSR markers, respectively) and 'between populations' (40.18% and 31.44% based on ISSR and SSR markers, respectively). Relatively high 'between populations' diversity was present in the accessions belonging to South Pacific region reflecting higher population differentiation. Dwarfs and intermediate coconut types also maintained high 'between populations' diversity due its autogamous behaviour. There was overall reduction in the number of markers (ISSR and SSR) among the dwarfs and intermediate populations. The study provided useful information regarding the genetic makeup of the coconut germplasm accessions and their utilization in breeding.

Key Words: Coconut, Diversity, ISSR, Population structure, SSR

Introduction

Coconut (*Cocos nucifera* Linn.) is named as Tree of Abundance, Tree of Life, the Consols of East etc., is an important perennial oil yielding plantation crop of the tropics. *C. nucifera* ($2n = 2 \times = 32$) is a member of monocotyledonous family Arecaceae (Palmaceae). It is the only species of the genus *Cocos* belonging to the subfamily Cocoideae. Almost every part of the tree is used for its food and industrial products. It has been grown in 86 countries including India. Presently, the crop is now facing relative decline in cultivation in many countries, largely due to the impact of diseases on the yields and low farmer productivity. Hence, an urgent need is to evolve high yielding coconut palms either through hybridization/through selection in well collected and maintained germplasm. Assessment of population genetic structure will help in germplasm collecting, conservation and utilization processes.

Various molecular marker techniques like RFLPs (Lebrun *et al.*, 1998), RAPD (Ashburner *et al.*, 1997; Everard, 1999; Upadhyay *et al.*, 2004), AFLP (Perera *et al.*, 1998; Teulat *et al.*, 2000), ISTR (Rohde *et al.*, 1995), SSR (Perera *et al.*, 1999; Rivera *et al.*, 1999; Meerow *et al.*, 2003) and ISSR have been reported in coconut.

In the present study, population genetic structure among world wide collections of coconut germplasm

accessions was assessed using DNA markers, viz., ISSR and SSR.

Materials and Methods

One hundred and thirty two individual palms from 33 coconut germplasm accessions (4 palms per accession referred as population) were used. The accessions belong to a collection maintained at International Coconut Gene Bank for South Asia. These are conserved *ex situ* at Central Plantation Crops Research Institute, Kasaragod, India. These accessions belong to different geographic origin. The details on the plant stature (type), origin (place of collection) and geographic location are available Manimekalai and Nagarajan (2006). The accessions were grouped according to the geographic region as accessions belonging to South East Asia, South Pacific, South Asia, Atlantic and America and Africa.

DNA Extraction

DNA was extracted using Plant DNA extraction kit (Invitrogen) as per the manufacturer's instructions.

ISSR Analysis

Primers targeting the SSR were obtained from University of British Columbia (Canada). Amplification reactions were carried out in 10 ml volume containing 30 ng of template DNA, 200 mM of each dNTPs, 0.45 U of Taq polymerase (Bangalore Genei Pvt. Ltd. India)

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and 0.8 mM of primer. The PCR products were subjected to electrophoresis through a 1.80% agarose gel using 1X TBE buffer at 90 volts for 3 h in Bio-Rad submarine electrophoresis unit. The ethidium bromide stained gels were documented using the Alpha Imager™ 1200 Documentation and Analysis system (Alpha Innotech Corporation, USA).

SSR Analysis

SSR analysis was carried out as described by Perera *et al.* (2000). Primer sequences were obtained from CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement), Montpellier, France).

Data Analysis

ISSR markers

Only the clear, unambiguous and reproducible bands were considered for scoring. Each band was considered to be a single locus. Data were scored as “1” for the presence and “0” for the absence of a DNA band of

each accession. DNA band size was estimated by comparing the DNA bands with a 1 Kb DNA ladder. The binary data matrix was entered into the software POPGENE version 1.32 (Yeh and Boyle, 1999). The population genetic diversity parameters estimated were, observed for number of alleles per locus, effective number of alleles per locus, Nei's (1973) gene diversity, Shannon's information index and G statistics. For describing coconut population based on their geographic region and plant type, 6 groups have been identified. Group I consisted of tall populations of South East Asia; Group II comprised of South Pacific populations; Group III consisted of Atlantic and American populations; Group IV has populations belonging to Africa; Group V consisted of South Asian populations. The dwarfs and intermediate types are put under Group VI.

SSR markers

Microsatellite loci were scored individually and the different alleles were recorded for each population. Sizing of alleles was done by comparing with 30 bp ladder.

Table 1. Details of coconut germplasm populations, place of collection and geographic region

No.	Accession	Type	Country of origin	Geographic region
1	Kong Thienyong Tall	Tall	Borneo	Southeast Asia
2	Straight Settlement Green Tall	Tall	Malaysia	Southeast Asia
3	Straight Settlement Apricot Tall	Tall	Malaysia	Southeast Asia
4	Philippines Kalambahim Tall	Tall	Philippines	Southeast Asia
5	Laguna Tall	Tall	Philippines	Southeast Asia
6	Philippines Palawan Tall	Tall	Philippines	Southeast Asia
7	Philippines Dalig Tall	Tall	Philippines	Southeast Asia
8	San Roman Tall	Tall	Philippines	Southeast Asia
9	Markham Valley Tall	Tall	Papua New Guinea	South Pacific
10	Nufella Tall	Tall	New Caledonia	South Pacific
11	Nugili Tall	Tall	New Caledonia	South Pacific
12	Nuwallis Tall	Tall	New Caledonia	South Pacific
13	Nu Quamen Tall	Tall	New Caledonia	South Pacific
14	Kupien Tall	Tall	New Caledonia	South Pacific
15	Nuwehnug Tall	Tall	New Caledonia	South Pacific
16	Lifou Tall	Tall	Guam Islands	South Pacific
17	British Solomon Island Tall	Tall	Solomon Islands	South Pacific
18	Jamaica Tall	Tall	Jamaica	Atlantic
19	Saint Vincent Tall	Tall	Trinidad	Atlantic
20	Panama Tall	Tall	Panama	America
21	Nigerian Tall	Tall	Nigeria	Africa
22	Kaithathali Tall	Tall	India	South Asia
23	Indian Spicata	Tall	India	South Asia
24	Indian East Coast Tall	Tall	India	South Asia
25	Verrikobbari Tall	Tall	India	South Asia
26	Nadora Tall	Tall	India	South Asia
27	Nicobar Tall	Tall	India	South Asia
28	Hazari Tall	Tall	India	South Asia
29	Navassi Tall	Tall	India	South Asia
30	Niuleka Dwarf	Intermediate	Fiji	South Pacific
31	King coconut	Intermediate	Sri Lanka	South Asia
32	Laccadive Dwarf	Dwarf	India	South Asia
33	Chowghat Orange Dwarf	Dwarf	India	South Asia

(Ratnambal *et al.*, 1995; 2000)

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The allele size data were analyzed using the software ARLEQUIN version 2.0 (Schneider *et al.*, 2000). For each population the genetic diversity parameters estimated were number of polymorphic sites and gene diversity. The genetic structure of population was investigated by Analysis of Molecular Variance (AMOVA) (Excoffier *et al.*, 1992).

Results

Based on ISSR Markers

Variability and Level of Polymorphism

ISSR primers detected a total of 120 markers across 33 coconut populations, out of which 104 were polymorphic (86.6 %). The number of markers for each primer varied from nine (UBC 854) to 17 (UBC 854), with a mean of 12 markers per primer. The number of polymorphic markers for each primer varied from seven (UBC 835) to 15 (UBC 854 and UBC 855) with a mean of 10.4. The product size ranged from 206 bp to 2618 bp (Table 2).

The number of observed alleles among populations varied from 1.1667 (King coconut) to 1.7833 (Nicobar tall). The number of effective alleles ranged from 1.1404 (King coconut) to 1.6833 (Nicobar tall). Gene diversity for each population varied from 0.0746 (King coconut) to 0.3555 (Nicobar Tall). Among 33 coconut populations, Nicobar tall produced the highest number of polymorphic markers (94), while King coconut had the least (20).

Shannon's Information Index

Shannon's index provided information regarding within population diversity. The Shannon's index for individual population is given in Table 3. The population, Nadora tall had the highest index (0.5032). While, King coconut

had the lowest index (0.1061) followed by Philippines Palawan tall (0.1615), Nuwallis tall (0.1702) and Straight Settlement Apricot tall (0.1760). The mean Shannon's index was 0.2687. The mean Shannon's index among the tall populations was higher (0.2823) when compared to the dwarf and intermediate populations (0.2323).

Multiple Population Analysis

Partitioning of genetic diversity in to 'within population' and 'between populations' was calculated for each group of populations using G_{st}. The total diversity (H_t) was the highest (0.3652) for the populations belonging to South Asia (Group V) and the 'within population' diversity was also high for that group (0.2256). The proportion of total diversity present 'within population' was high for the Atlantic and American accessions (Group III) (0.6443).

Table 3. Shannon's index based on ISSR markers for the individual coconut population

Population	Shannon's index
Kong Thienyong Tall	0.2673
Straight Settlement Green Tall	0.2439
Straight Settlement Apricot Tall	0.1760
Philippines Kalambahim Tall	0.2342
Laguna Tall	0.2350
Philippines Palawan Tall	0.1615
Philippines Dalig Tall	0.2990
San Roman Tall	0.1890
Markham Valley Tall	0.2241
Nufella Tall	0.2306
Nugili Tall	0.3948
Nuwallis Tall	0.1702
Nu Quamen Tall	0.2428
Kupien Tall	0.3821
Nuwehnug Tall	0.2294
Lifou Tall	0.3255
British Solomon Island Tall	0.3648
Jamaica Tall	0.2626
Saint Vincent Tall	0.3005
Panama Tall	0.2699
Nigerian Tall	0.4336
Kaithathali Tall	0.2259
Indian Spicata	0.2850
Indian East Coast Tall	0.4501
Verrikobbari Tall	0.2392
Nadora Tall	0.5032
Nicobar Tall	0.2329
Hazari Tall	0.2372
Navassi Tall	0.2169
Niuleka Dwarf	0.2420
King coconut	0.1061
Laccadive Dwarf	0.2146
Chowghat Orange Dwarf	0.2757
Mean	0.2687
Mean among the talls	0.2823
Mean among the dwarfs	0.2323

Table 2. Details of ISSR markers produced among coconut populations

Primer	Total markers (No.)	Polymorphic markers (No.)	Polymorphism (%)	Product size (bp)
UBC815	13	13	100.0	2618-554
UBC834	12	10	83.3	1252-206
UBC841	10	8	80.0	2316-698
UBC810	9	8	88.8	2443-879
UBC824	10	9	90.0	2455-506
UBC835	10	7	70.0	2375-514
UBC854	17	15	88.2	1545-299
UBC855	15	15	100.0	2459-606
UBC889	14	10	71.4	2069-290
UBC823	10	9	90.0	2287-877
Total	120	104	86.6	
Mean	12.0	10.4	86.6	

Highest 'within population' diversity was present among Atlantic and American accessions (Group III, $G_{st} = 35.57\%$) followed by South Asian accessions (Group V, $G_{st} = 38.21\%$). Relatively high 'between population' diversity was present among South Pacific accessions (Group II, $G_{st} = 42.98\%$) and Intermediate and dwarf types (Group VI, 42.92%). On an average 'within population' diversity was higher (59.82%) than 'between population' diversity (40.18%) (Table 4).

Based on SSR Markers

Variability and Level of Polymorphism

Seven highly polymorphic SSR loci detected a total of 82 alleles, and all were polymorphic. The total number of alleles for each locus varied from two (CnCirE12) to 17 (CnCirE2) with an average of 11.71 alleles per locus. The allele size varied from 115 bp (CnCirE2) to 278 bp (CnCirE10) (Table 5). The number of polymorphic sites (Polymorphic alleles) ranged from 0 to 7. The intermediate population, King coconut had no polymorphic sites. The dwarf population, Chowghat Orange Dwarf had only 2 polymorphic sites. The intermediate population Niuleka Dwarf, had more polymorphic sites (6) when compared to other intermediate population King coconut (0). Among tall populations, Navassi Tall had the least number of polymorphic sites (3). The average number of polymorphic sites was calculated and found to be more among the tall populations (6.45) compared to dwarf populations (3.50). Total number of alleles was more among the tall populations (82) when compared to dwarfs (17). Gene diversity among 33 coconut populations varied from 0.734 (Nugili tall) to 0.000 (King coconut).

Population Genetic Structure Inferred by Analysis of Molecular Variance (AMOVA)

The partitioning of diversity into 'among groups' of coconut population, 'between populations' and 'within population' is shown in Table 6. The diversity present

'among the groups' was highly significant (13.10 %) reflecting a moderate differentiation among the geographical groups of coconut populations. The diversity present 'between population' was 18.38 per cent, which was highly significant reflecting great differentiation among populations. The component 'within population' accounted 68.56 per cent of diversity and found to be highly significant. The population differentiation was also reflected by fixation indices. The F_{st} statistic was 0.31437, which was found to be highly significant reflecting great level of diversity present 'between populations' and 'between groups'. Accordingly the within population diversity accounted for 68.56% of observed diversity in the coconut germplasm used in this study. On an average, the total diversity was partitioned 'within population' (68.56%) rather than between populations (31.44%).

Discussion

Based on ISSR Markers

ISSR primers have exhibited high per cent (86.6) of polymorphism reflecting its high informativeness. In the present study, it was found there was reduction in number of alleles and effective alleles in dwarfs and intermediate populations (King coconut, Laccadive dwarf and Chowghat orange dwarf). This result was in agreement with report of Perera *et al.* (2003).

Based on Shannon's index, Nadora tall showed the highest genetic diversity within it. The least genetic diversity was shown by King coconut. The mean Shannon index among tall was found to be higher (0.2823) when compared to the dwarfs and intermediates (0.2323). Among the tall, Straight settlement apricot tall, Philippines palawan tall, San ramon tall and Nuwallis tall were found to have less index and consequently less 'within population' diversity. The diversity was for the South asian population was contributed by Nicobar tall, Verrikobbari tall and Kaithathali tall. Previously, Upadhyay *et al.* (2004) reported higher diversity in the Philippine

Table 4. Partitioning of genetic diversity between populations and within population based on ISSR markers

Group	Individuals (No.)	Ht	Hs	Hs / Ht	Gst	Nm
I	32	0.2645	0.1554	0.5874	0.4126	0.7119
II	36	0.3159	0.1801	0.5702	0.4298	0.6633
III	12	0.3373	0.2173	0.6443	0.3557	0.9058
V	32	0.3652	0.2256	0.6179	0.3821	0.8085
VI	16	0.2546	0.1453	0.5708	0.4292	0.6650
Mean				0.5982	0.4018	

Group IV excluded from the analysis because of single population; Ht - Total genetic diversity; Hs - Within population genetic diversity; Hs/Ht- Proportion of total genetic diversity "within population"; Gst - Proportion of total genetic diversity "between population"; Nm- Gene flow

Table 5. Total alleles and product size of the SSR markers produced across coconut populations

Locus	Total alleles (No.)	Product size (bp)
CnCirA3	9	210-248
CnCirB12	15	135-183
CnCirC3	18	188-272
CnCirC12	10	120-190
CnCirE2	17	115-185
CnCirE10	11	226-278
CnCirE12	2	164-174
Total	82	
Mean	11.71	

Table 6. Analysis of molecular variance based on average of seven SSR loci

Source of variation	Sum of squares	Variance components	Diversity (%)
Among groups	109.151	0.37215 Va	13.10**
Between populations	165.857	0.52043 Vb	18.33**
Within population	453.575	1.94667 Vc	68.56**
Total	728.583	2.83926	

Fst : 0.31437; va- variance components among groups; vb-variance components between population; vc-variance components within population

Ordinary Talls based on Shannon's index. Shanon's index reflect the within population diversity (Ashburner *et al.*, 1997). Dwarfs and intermediate populations showed reduced number of polymorphic alleles and 'within population' diversity. Reduction in 'within population' diversity in dwarfs and intermediate types are also reported (Perera *et al.*, 1998; Upadhyay *et al.*, 2004).

Based on ISSR markers, genetic diversity was partitioned more in 'within population' (59.82%) rather than 'between population' (40.18%). This observation was in accordance with the earlier studies using RAPD (Upadhyay *et al.*, 2004), SSRs (Perera *et al.*, 2001). G_{st} values which reflect the population differentiation was least for the Atlantic and American populations which revealed high 'within population' diversity. Earlier Ashburner *et al.* (1997) analyzed a set of South Pacific coconut populations and reported more of 'within population' diversity (60%) and proportion of the diversity found between populations was less (40%). Among the five different geographic groups, relatively higher 'between population' diversity was present in the populations of South Pacific region (G_{st} = 0.4298). It was suggested that relatively high 'between population' diversity in the coconut populations of the South Pacific region has probably arisen because of the establishment of populations by few individuals (Ashburner *et al.*, 1997). The low differentiation of populations of South Asia and Atlantic

and America may be attributed to the gene flow between populations. High gene flow between populations will reflect low levels of population differentiation. However very few individuals were used for the present study and few for Atlantic and American accessions.

Based on SSR Markers

Seven SSR loci detected 100% polymorphism among 33 coconut populations. One of the dwarf populations namely, Chowghat orange found to have 71% of homozygous loci. The intermediate populations, King coconut showed homozygous at all 7 loci. Niu Leka dwarf, known to be a cross-pollinator among the dwarfs was homozygous for only one locus out of 7 loci tested. Since it is known to be a cross-pollinator. In general, dwarf populations showed a reduction in diversity and in number of total alleles (17) when compared to talls (82). This result agrees with Perera *et al.* (2003) who suggested that the dwarf coconuts are subset of tall coconuts and was directly evolved from tall coconut variety as a result of an event of domestication.

The hierarchical analysis of molecular variance (AMOVA) partitioned most of the diversity (68.56%) 'within population'. This result was comparable with the result obtained by ISSR markers in the present study. AMOVA suggested moderate level of differentiation between groups based on geographic region and plant stature. Higher 'within population' diversity obtained in the present study agrees with the general observation that woody perennial out breeding species like coconut and other crops maintain most of their variation within population (Hamrick and Godt, 1989; Bartish *et al.*, 2000; Perera *et al.*, 2001; Oraguzie *et al.*, 2001; Archak *et al.*, 2003; Belaj *et al.*, 2003).

The results obtained based on ISSR and SSR markers were comparable, however, SSR markers were more efficient in describing homozygous/heterozygous nature of the population. The data obtained suggest the importance of prior knowledge on the amount and distribution of genetic diversity among population for appropriate collection and conservation strategies. Diverse accessions from different geographic regions could be utilized in heterosis breeding.

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Efficiency of DNA Marker Systems in Discriminating *Cajanus cajan* (L.) Millsp. and its Wild Relatives

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Two multilocus markers, viz., RAPD (25 primers), ISSR (11 primers) and one single locus marker, viz., SSR (10 primer pairs) were used to assess the genetic diversity among 32 genotypes of pigeonpea and to find the efficient marker system to discriminate the genotypes at molecular level. Diversity analysis was done with the help of statistics such as marker index, mean marker index, polymorphism information content, principal component analysis and dendrogram. The random primers, viz., OPG06, OPG15, OPG03 and the ISSR primers, viz., UBC842, UBC876, and UBC857 are considered to be efficient in discriminating the genotypes. The RAPD, ISSR and SSR markers clustered the genotypes into 10, 12 and 7 clusters, respectively. Among the three marker systems used for the analysis, ISSR markers revealed a higher amount of divergence among the genotypes by producing 215 markers, 99.53 per cent polymorphism, and an average of 19.54 markers than SSR and RAPD. The present study clearly elucidated the superiority of ISSR markers for genetic diversity studies over RAPD and SSR markers.

Key Words: Pigeonpea, Polymorphism, Wild relatives

Introduction

Evaluation of germplasm in terms of diversity and relatedness is an important component of plant genetic resource management. Also the estimation of genetic diversity between different genotypes is the first and foremost process in any plant breeding program. Lynch (1988) proposed that the technique of DNA fingerprinting could be employed for the estimation of relatedness. Earlier, morphological and isozyme markers were used to assess the relatedness between the cultivated and their wild relatives, but these markers had the environmental influences.

The recently developed molecular marker technology has opened up new vistas for the assessment of genetic relatedness among genotypes at molecular level. DNA based markers have the obvious advantage of sampling the genome directly. PCR-based markers system like RAPD can be used for DNA fingerprinting of genotypes. Fingerprinting genomes using arbitrary primers was first reported by Welsh and McClelland (1990). RAPD markers have been successfully used by several earlier workers in various leguminous crops to determine genetic diversity, viz., soybean (Abdelnoor *et al.*, 1995), *Pisum* (Hoey *et al.*, 1996), pigeonpea (Ratnaparkhe *et al.*, 1995). RAPD and RFLP analyses have been used to arrive at the phylogenetic relationship among the cultivated and wild species of pigeonpea (Ratnaparkhe *et al.*, 1995).

Microsatellites are recently gaining importance over RFLP, RAPD, since they display considerable polymorphism due to variation in number of repeat units. This technique has been used for DNA fingerprinting and variety identification in many crops like rice (Yang *et al.*, 1994; Olufowote *et al.*, 1997), wheat (Roder *et al.*, 1995; Gupta and Varshney, 2000), pearl millet (Chowdari *et al.*, 1998) and pigeonpea (Burns *et al.*, 2001). ISSR markers have been successfully used to estimate the extent of genetic diversity at inter- and intra-specific level in a wide range of crop species, which include rice (Joshi *et al.*, 2000), wheat (Nagaoka and Ogiwara, 1997), *Vigna* (Ajibade *et al.*, 2000). DNA polymorphism in blackgram mutants was identified with ISSR markers (Souframanien *et al.*, 2002). With the above background two multilocus markers, viz., RAPD (25 primers), ISSR (11 primers) and one single locus marker, viz., SSR (10 primer pairs) were used in the present study to assess the genetic diversity among 32 genotypes of pigeonpea and its wild relatives and to find the efficiency of the different marker systems to discriminate the genotypes at molecular level.

Materials and Methods

The experiments were conducted at Centre for Plant Molecular Biology, Tamil Nadu Agricultural University during 2001-2003. The seeds were obtained from the Pulses Breeding Station, Centre for Plant Breeding and

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Genetics, and raised in transgenic greenhouse, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, India.

Plant Material and DNA Extraction

Thirty two pigeonpea genotypes, which included 23 cultivated species and nine wild relatives, were subjected to diversity analysis. The seeds of all cultivated genotypes were obtained from Pulse Breeding Station, TNAU, Coimbatore (Table 1) and the wild relatives were obtained from International Crops Research Institute for the Semi-Arid Tropics, (ICRISAT), Patancheru, India. DNA from the thirty two genotypes was extracted following Gawal and Jarret (1991) with slight modifications.

RAPD Analysis

DNA from the 32 genotypes was amplified using a set of 25 arbitrary oligonucleotide decamer primers obtained from Operon Technologies, USA. Amplification reaction were in volume of 15 ml containing 25-50 ng of genomic DNA, 0.5 mM of primer, 10 mM of dNTPs (2.5 mM each), 50 mM KCl, 10 mM Tris Hcl, (pH8.3), 2.5 mM MgCl₂, 0.01% gelatin, 0.15 units of *Taq* polymerase (Bangalore Genei Pvt. Ltd., Bangalore). Amplification were performed in PTC thermal cycler (MJ Research Inc., USA) programmed for an initial denaturation of 94°C for 4 min, 45 cycles of 1 min. denaturation at 94°C, 1 min annealing at 37°C, and extension at 72°C for 2 min, followed by final extension for 5 min at 72°C and then at 4°C for storage. PCR amplified products (10 ml) were subject to electrophoresis in a 1.2% agarose gel in 1xTBE buffer at 50 V for 5 h using Broviga™ standard submarine gel electrophoresis unit.

ISSR Analysis

A total of 11 primers were used for the study obtained from University of British Columbia (UBC) was used. PCR amplification was carried out in 15 ml reaction containing 25 ng of genomic DNA, 1 mM of primer, 10 mM of dNTPs (2.5 mM each), 50 mM KCl, 10 mM Tris Hcl (pH8.3), 2.5 mM MgCl₂, 0.01% gelatin, 0.15 units of *Taq* polymerase (Bangalore Genei Pvt. Ltd., Bangalore). Amplification was done using a PTC thermal cycler (MJ Research Inc., USA) programmed for initial denaturation at 94°C for 5 min, 45 cycles of 1 min denaturation at 94°C, 1 min annealing at 37°C, 2 min extension at 72°C, final extension of 5 min at 72°C and then at 4°C for storage. The PCR products (5 ml) were run in a 4% denaturing polyacrylamide gel electrophoresis (PAGE) at a constant current of 80

Table 1. Level of polymorphism detected by RAPD, ISSR and SSR analysis across 32 *Cajanus cajan* and its wild relatives

Parameters	Type of marker		
	RAPD	ISSR	SSR
Genotypes screened	32	32	32
Number of primers used	25	11	10
Total number of markers	249	215	95
Range of markers across primers	6-17	8-26	4-13
Average number of markers	9.96	19.54	9.50
Number of monomorphic markers	1	1	0
Number of polymorphic markers	248	214	95

watts for 3 hours and 45 minutes and resolved by silver staining (Panaud *et al.*, 1996).

SSR Analysis

A total of 10 pigeonpea specific SSR primer pairs reported by Burns *et al.* (2001) were synthesized by Sigma-Genosys, USA and used for the present the study. PCR amplification was carried out in 15 ml reaction containing 25 ng of genomic DNA, 0.4 mM of primer, dNTPs (2.5 mM each), 50 mM KCl, 10 mM Tris Hcl, (pH 8.3), 2.5 mM MgCl₂, 0.01% gelatin, 0.15 units of *Taq* polymerase (Bangalore Genei Pvt. Ltd., Bangalore). Amplification was done using a PTC thermal cycler (MJ Research Inc., USA) programmed for initial denaturation at 94°C for 3 min, 27 cycles of 30 sec denaturation at 94°C, followed by annealing for 30 sec (temperature vary according to the primer), extension at 72°C for 1 min, final extension for 3 min. at 72°C and then at 4°C for storage. The PCR products (5 ml) were run in a 4% denaturing polyacrylamide gel electrophoresis (PAGE) at a constant current of 60 watts for 1 h and 15 minutes and resolved by silver staining procedure (Panaud *et al.*, 1996).

DATA Analysis

Clearly resolved, unambiguous polymorphic band were scored visually for their presence or absence with each primer. The scores were obtained in the form of a matrix with '1' and '0', which indicate the presence and absence of bands in each variety, respectively. Polymorphism information content (PIC) was calculated for the SSR primer pairs based on the formula $PIC = 1 - \sum f_i^2$, where f_i is the frequency of the i^{th} allele (Smith *et al.*, 1997). Marker index was calculated for RAPD and ISSR markers (multilocus), in order to characterize the capacity of each primer to reveal or detect polymorphic loci among the genotypes. It was calculated based on the formula $MI = 1 - \sum p_i^2$, where p_i is the frequency of the i^{th} allele, (Ghislain *et al.*, 2002). Mean marker index was calculated

for RAPD and ISSR markers (multilocus markers), based on the formula $MMI = (1/N) (1-Sp_i^2)$, where p_i is the frequency of the i^{th} allele and N is the total number of markers that a primer produced.

The data obtained by scoring the RAPD, ISSR and SSR profiles of different primers were subject to cluster analysis. Similarity matrix was constructed using Jaccard's coefficient and the similarity values were used for sequential agglomerative hierarchical non-overlapping (SAHN) clustering based on unweighted pair group method with arithmetic averages (UPGMA) using NTSYSpc version 2.0 (Rohlf, 1998).

Results and Discussion

Several studies were made to test the efficiency of these markers in terms of informativeness in exploring the genetic variability among the genotypes. Comparison between RFLP and PCR based methods in pea (Lu *et al.*, 1996), among RFLP, RAPD, AFLP and SSR markers in soybean (Powell *et al.*, 1996), between RAPD and RFLP markers in *Theobroma cacao* (Lerceteau *et al.*, 1997), among ISSR, RFLP and RAPD markers in wheat (Nagaoka and Ogihara, 1997), between RAPD and SSR markers in kiwifruit (Palombi and Damiano, 2002), between RAPD and ISSR in blackgram (Souframanien *et al.*, 2002), between RAPD and SSR markers in rice (Ravi *et al.*, 2003) have been reported so far. In the present investigation, two multilocus marker systems, viz., RAPD and ISSR and one single locus marker system, viz., SSR were evaluated for their efficiency in revealing the genetic diversity among the genotypes studied.

The major advantage of the PCR based marker systems is the generation of fragments from both single and multilocus. In the present study, 25 RAPD, 11 ISSR and 10 SSR primers were used to assess the genetic diversity and relatedness among a set of 32 genotypes in pigeonpea. All the primers produced high level of polymorphism (~100 per cent). This is because of highly divergent genotypes (cultivars and wild relatives) were included in the study. Maciel *et al.* (2001) also reported such a high level of polymorphism among population consisting of both cultivated and wild relatives.

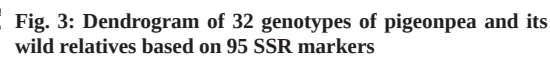
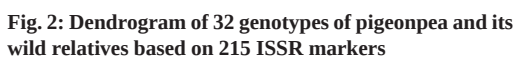
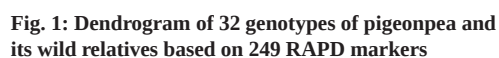
Level of polymorphism detected by RAPD, ISSR and SSR analysis across 32 pigeonpea genotypes is presented in Table 1. The average number of fragments amplified by RAPD primers among the genotypes was 9.96 with a range of 6 to 17. Ratnaparkhe *et al.* (1995)

reported an average of eight markers per primer. However, Maciel *et al.* (2001) reported the generation of RAPD fragments ranging from 7 to 31 in common beans. Such a high variation in the number of fragments produced by these arbitrary primers may be attributed to the differences in the binding sites through out genome of the genotypes included. ISSR primers generated 8 to 26 markers with average of 19.54 markers and SSR primer pairs generated 4 to 13 alleles and average of 9.5 alleles. Earlier, generation of 4 to 12 markers in Vigna (Ajibade *et al.*, 2000) and 8 markers in *Phaseolus vulgaris* (Galvan *et al.*, 2003) by ISSR primers and generation of 2-5 alleles with SSR primers in pigeonpea (Burns *et al.*, 2001) and 5.3 alleles in common bean (Metais *et al.*, 2002) were reported. Generation of higher number of markers or alleles in the present study can be attributed to the higher amount of genetic diversity among cultivars and wild relatives of pigeonpea.

Marker index (MI) reveals the amount of information that can be obtained from a particular primer. In the present study, marker index for RAPD and ISSR was in the range of 3.701 to 11.671 and 5.300 to 31.722, respectively. The higher MI value indicates the more informativeness of the primer. Hence, the random primers, viz., OPG06, OPG15, OPG03 and the ISSR primers, viz., UBC842, UBC876, and UBC857 are considered to be worth in future studies in the field of taxonomical and genetic resource management. Among these two multilocus marker system, ISSR primers had higher index values compared to RAPD suggesting their superiority in generating polymorphism among the genotypes than RAPD primers as reported by Galvan *et al.* (2003). PIC also provides the estimate of discriminatory power of a locus in the case of SSR marker system by taking into account not only the number of alleles expressed, but also the relative frequency of those alleles. The PIC values of the 10 SSR ranged from 0.844 to 0.982 with a mean of 0.921 whereas a wide range of PIC values (0.12 to 0.72) with a mean of 0.44 was reported by Metais *et al.* (2002) in common beans. This contradictory situation of obtaining a higher mean and range in the present study may be attributed to the wide diversity among the cultivars and their relatives.

Genetic Relationships between Pigeonpea Cultivars and Wild Relatives

Cluster analysis was used to group the genotypes to construct dendrogram based on RAPD (Fig. 1), ISSR (Fig. 2) and SSR (Fig. 3) data. The three marker systems



clearly distinguished the cultivars and wild forms into different clusters as expected since they were morphologically and genetically well differentiated. This is in accordance with studies in lima bean (Fofana *et al.*, 1997). It is interesting to note that these markers were able to further subdivide the 23 cultivars into five, seven and three clusters by RAPD, ISSR and SSR markers, respectively, which revealed the existence of sufficient amount of genetic variability available among these cultivars that could be exploited further. Archak S *et al.* (2002) reported that the cultivars derived from a common parent always tend to cluster together. Villand *et al.* (1998) observed the grouping of both wild and cultivated tomatoes in a single cluster and concluded that this might be due to the introgression of genes from the wild relatives into cultivated genotypes. In the present investigation, based on RAPD markers, ICPW28, a wild accession belongs to *Cajanus cajanifolius* and a cultivated line, ICPL86020 formed a single cluster due to their genetic relatedness. This type of relatedness was established only by the RAPD marker system, because RAPD scans the whole genome, whereas SSR and ISSR (to some extent) search for specific sites in the genome. Such information on the relatedness between the cultivated line and wild relatives would enable the breeders to introgress the favourable genes from the wild relatives through conventional breeding without any cytological barrier.

The wild relatives taken for the study belong to three different genera, viz., *Cajanus*, *Rhynchosia* and *Flemingia*. The accessions belonging to the genus *Cajanus* were grouped together which is in accordance with the results of Sivaramakrishnan *et al.* (2002). In the present study, larger amount of genetic diversity was revealed among wild relatives by all the three marker systems and the reduced genetic variability among the cultivars might be due to genetic erosion over a long period of breeding (Brown, 1978).

Among the nine wild accessions studied, five belong to secondary, two belong to tertiary and two belong to unknown origin of gene pool (Van der Maeson, 1990).

These wild accessions belonging to the same gene pool were discriminated into 3-4 clusters by different marker systems revealing sufficient amount of genetic diversity among them. The clustering among the wild relatives was according to their gene pool origin classified based on crossability, evolution of form and chemotaxonomy. All the clusters had different species belonging to the same genera except in one cluster where included two different genera, i.e., *Cajanus platycarpus* and *Rhynchosia aurea* which belong to tertiary gene pool were grouped together by ISSR marker system. Galvan *et al.* (2003) also reported that the ISSR markers had the ability to differentiate the common bean genotypes based on gene pool origin, i.e., Mesoamerican origin and Andean origin. Among the wild species, both accessions belonging to Australian origin and Indian origin clustered together suggesting the movement gene pool form the centre of origin to other regions.

For overall comparison of all the three marker systems used in the present study, the different parameters, viz., mean number of markers generated, level of polymorphism, marker index, PIC values, clustering pattern and the mean intercluster distances are summarised hereunder:

From Table 2 it is evident that among the two multilocus markers, viz., RAPD and ISSR, ISSR was the most efficient based on all the parameters studied. Even though the mean similarity index was slightly higher in RAPD than ISSR, ISSR was able to generate more markers with less number of primers than RAPD.

Ajibade *et al.* (2000) and Galvan *et al.* (2003) also concluded that ISSR would be a better tool than RAPD for phylogenetic studies. When comparing the SSR with RAPD and ISSR based on the information on the level of polymorphism and the PIC value, SSR was more efficient in generation of polymorphism among the genotypes and high informativeness (PIC), but they were less efficient in discriminating closely related genotypes/cultivars. Hence it can be stated that even though SSR markers are highly polymorphic, for genetic diversity

Table 2. Comparison of different parameters among the three markers

Marker system	Mean number of markers	Level of polymorphism %	Mean similarity index	Mean marker index (PIC)	Number of clusters	Average intercluster distance
RAPD (25)	9.96	99.60	0.535	0.655	10	0.489
ISSR (11)	19.54	99.53	0.478	0.751	12	0.582
SSR (10)	9.50	100.00	0.458	0.921	7	0.598

(Figures in the parenthesis are number of primers used)

studies generation of more number of markers is of prime importance. Since SSR are more loci specific, they can be used for mapping studies (Wu and Tanksley, 1993). ISSR markers are multilocus markers and produce a higher percentage of polymorphism (Blair *et al.*, 1999; Ajibade *et al.*, 2000; Martin and Sanchez-Yelamo, 2000; Galvan *et al.*, 2003) and reveal relationship between genotypes both at intra-specific and inter-specific levels (Huang and Sun, 2000).

The present study, clearly established the utility of molecular markers like RAPD and ISSR in assessing the genetic diversity among the cultivars and their wild relatives to identify the best parental lines for further breeding programmes and for effective management of genetic resources.

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CMSD: A Database of Microsatellite Markers Genotyped in Indigenous Coconut Accessions

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The increased use of molecular markers in varietal identification has led to the need for an appropriate storage place and this has paved way for the development of a marker database containing allelic data of coconut accessions as revealed by microsatellite markers. The main features of the information system are a SSR database, SSR analysis tool, variety identification tool, data submission form and brief information about microsatellites. The database contains details of 11200 allelic data of 400 palms comprising 62 indigenous coconut accessions analyzed using 14 microsatellite primer pairs. Variety identification tool contains a display of 14 pairs of allelic entry corresponding to the 14 microsatellite primers. The result pages display the information about the query sample matches to a reference sample in the database and the score values they attain. Based upon the maximum score value, it can be concluded that the unknown sample is genetically related to the particular reference sample. The SSR allelic size data have been used for creating a DNA barcode system too, providing a more convenient handling of numerical data. This identification system with data warehouse can be accessed from the home page of Bioinformatics Centre, Central Plantation Crops Research Institute (ICAR), Kasaragod (<http://www.bioinfpcperi.org/cmsd/home.php>).

Key Words: Microsatellites, Coconut, Database

Introduction

Morphological and physiological markers are frequently used to determine distinctness, uniformity and stability (DUS) for registration and granting of Plant Breeder's Rights to new varieties of crops. New varieties have to be distinct from all existing varieties by at least one character. Additionally, they have to meet established standards with respect to uniformity and stability of the characteristics used to demonstrate distinctness. Morphological characters used for registration purposes can also be used for varietal identification. However, many of the morphological descriptors have limitations—they are multi-genic, quantitative or continuous characters and their expression can be altered by environmental factors. Furthermore, the number of registered varieties increases over time because of which, it is impossible for any testing authority to check efficiently each newly submitted variety against all existing varieties in common knowledge. Hence, to circumvent these problems, it has been suggested that modern methods should be evaluated for variety identification and related uses (Cooke, 1999).

Polymorphisms existing in DNA molecule of plant genomes can be exploited for genotyping of cultivars. DNA-based markers have many advantages for plant variety identification over the more traditionally used morphological characters because of their independence from environmental influences, their generally high

level of polymorphism and their almost unlimited availability. There are several approaches to DNA profiling, of which microsatellites have proven to be efficient tools for characterizing coconut cultivars. Microsatellites or simple sequence repeat (SSR) loci are short tandem repeats of 1-6 bp, which are ubiquitous in both prokaryotes and eukaryotes both in protein-coding and non-coding regions (Toth *et al.*, 2000). Presently, they serve as the most widely used markers in plants. They have also been used widely in coconut in a variety of applications including genetic distance measurements, phylogeny reconstructions, population genetics, genetic mapping and elucidating evolutionary history (Rivera *et al.*, 1999; Perera *et al.*, 1999; Merrow *et al.*, 2003; Devakumar *et al.*, 2006; Rajesh *et al.*, 2008). Databases of DNA profiles of major coconut accessions are essential to the application of DNA marker-based variety identification.

The main objective of this present study was to build a database containing allelic data of indigenous coconut accessions as revealed by microsatellite markers.

Materials and Methods

Four hundred palms representing 62 coconut accessions (3-4 palms per accession) native to India were subjected to diversity analysis. DNA was extracted from spindle leaves of the palms using the standardized protocol (Upadhyay *et al.*, 1999) with slight modifications. A

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total of 14 highly polymorphic SSR primer pairs from the coconut microsatellite kit (Baudouin and Lebrun, 2002) were used in the present study. PCR reaction was conducted in volumes of 20 µl containing 35 ng genomic DNA, 0.2 µM each of forward and reverse primers, 50 µM of each dNTPs (M/s Bangalore Genei Pvt. Ltd., Bangalore), 1X buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂) and 0.3 Unit of Taq DNA polymerase (M/s Bangalore Genei Pvt. Ltd., Bangalore). PCR amplifications were performed on an Eppendorf gradient thermal cycler with a PCR profile of 94°C for 5 min followed by 30 cycles of 1 min at 94°C, 2 min at the different annealing temperatures standardized for the individual SSR locus, and 2 min at 72°C with a final extension for 5 min at 72°C. After amplification, a volume of 8 µl of loading buffer (98 per cent formamide, 10 mM EDTA, 0.005 per cent each of xylene cyanol and bromophenol blue as tracking dyes) was added to each of the amplified product and denatured at 94°C for 5 min, snap cooled using ice and separated on 5 per cent denaturing polyacrylamide gels containing 7 M urea at a constant power of 100 W. The patterns of amplified products across the samples were resolved by silver staining. The alleles were scored individually based on comparison with the molecular ladder and also the control samples.

The user friendly web interface for querying the database was done in PHP, a server side scripting language. The back end of the database was developed with MySQL (www.mysql.com). 11,200 allelic data were stored in a single table and there are 31 fields.

Results and Discussion

The Coconut Microsatellite Database (CMSD) is committed to provide the scientific community with

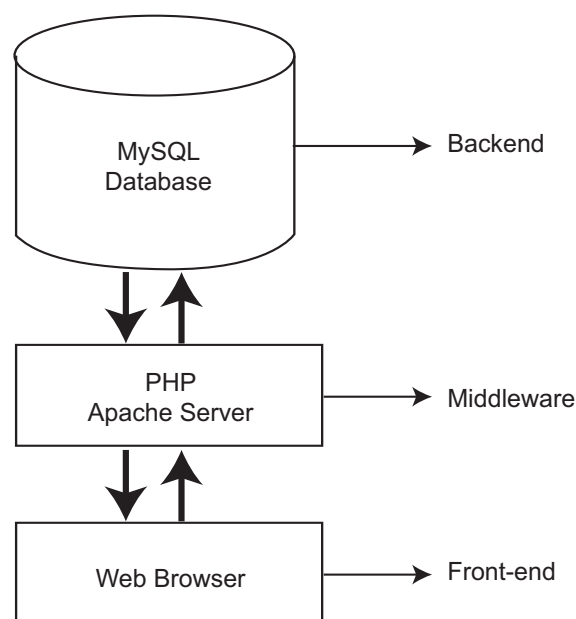


Fig. 1: An illustration of three level architecture used in the development of CMSD

comprehensive information on microsatellites occurring in indigenous coconut accessions. Three level schema architecture and flow of data has been shown in Figure 1. The screenshot of the CMSD homepage has been shown in the Figure 2. Currently CMSD contains 11200 allelic information of 400 coconut palms comprising 62 accessions extracted using 14 microsatellite markers. There is provision for updating any number of newly derived microsatellite data and the updation process is fully automated.

The CMSD interface provides mainly search and variety identification tool and SSR analyzer tool. In 'search tool', users can obtain the details of microsatellite markers by selecting either the microsatellite primer or accession-wise option (Fig. 3). If the primer option is

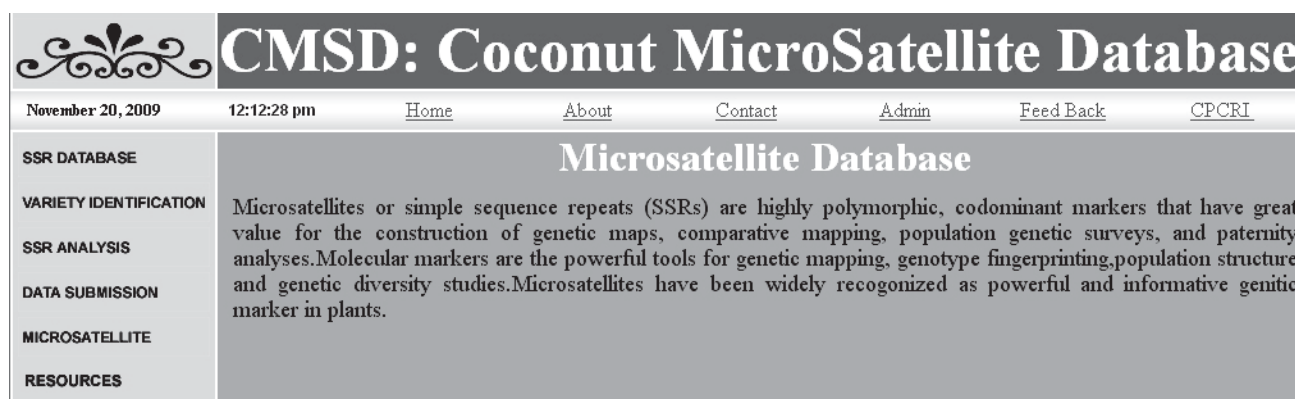


Fig. 2: A screen shot of CMSD home page

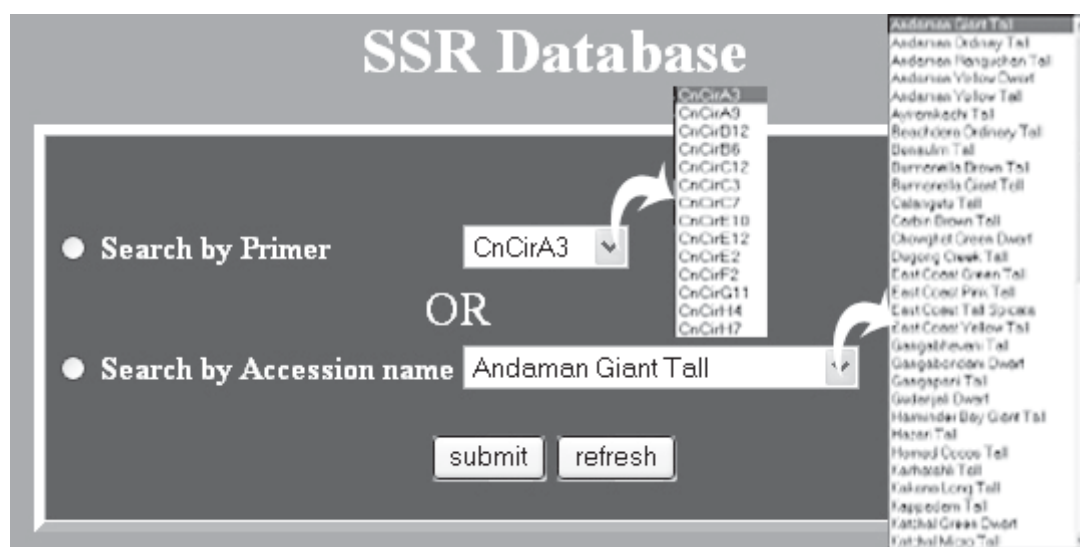


Fig. 3: Search tool with options for selecting either by the microsatellite primer or by accession

chosen, the allelic information of the 400 samples with primer sequence, annealing temperature, number of alleles, product sizes etc. are displayed. The accession-wise selection displays allelic size of the 14 microsatellite primers of the accession selected by the user. The 'variety

identification tool' displays the highly identical sample population to a query with their obtained score, percent identity, percent similarity and matched alleles (Fig. 4). This tool is highly beneficial to the researchers aiming to identify an unknown coconut population from a mixed

Sl.No.	Population	Sample	Score	Similarity	Identity	Match Primers
1	West Coast Tall	RT1	28	100	100	CnCir A3 Allele 1, CnCir A3 Allele 2, CnCir A9 Allele 1, CnCir A9 Allele 2, CnCir B6 Allele 1, CnCir B6 Allele 2, CnCir B12 Allele 1, CnCir B12 Allele 2, CnCir C3 Allele 1, CnCir C3 Allele 2, CnCir C7 Allele 1, CnCir C7 Allele 2, CnCir C12 Allele 1, CnCir C12 Allele 2, CnCir E2 Allele 1, CnCir E2 Allele 2, CnCir E10 Allele 1, CnCir E10 Allele 2, CnCir E12 Allele 1, CnCir E12 Allele 2, CnCir F2 Allele 1, CnCir F2 Allele 2, CnCir G11 Allele 1, CnCir G11 Allele 2, CnCir H4 Allele 1, CnCir H4 Allele 2, CnCir H7 Allele 1, CnCir H7 Allele 2
2	West Coast Tall	RT6	18	64.29	64.29	CnCir A3 Allele 1, CnCir A3 Allele 2, CnCir A9 Allele 1, CnCir A9 Allele 2, CnCir B6 Allele 1, CnCir B6 Allele 2, CnCir C3 Allele 1, CnCir C12 Allele 1, CnCir C12 Allele 2, CnCir E10 Allele 1, CnCir E10 Allele 2, CnCir F2 Allele 1, CnCir F2 Allele 2, CnCir G11 Allele 1, CnCir G11 Allele 2, CnCir H4 Allele 1, CnCir H4 Allele 2, CnCir H7 Allele 2
3	West Coast Tall	WCT8	17	60.71	60.71	CnCir A3 Allele 1, CnCir A3 Allele 2, CnCir A9 Allele 1, CnCir A9 Allele 2, CnCir B6 Allele 1, CnCir B6 Allele 2, CnCir C7 Allele 1, CnCir C7 Allele 2, CnCir E10 Allele 1, CnCir E10 Allele 2, CnCir E12 Allele 1, CnCir E12 Allele 2, CnCir F2 Allele 1, CnCir G11 Allele 2, CnCir H4 Allele 1, CnCir H4 Allele 2, CnCir H7 Allele 2
4	Ayirankachi Tall	AYRT8	15	53.57	53.57	CnCir A3 Allele 1, CnCir A3 Allele 2, CnCir B6 Allele 2, CnCir C3 Allele 1, CnCir C7 Allele 2, CnCir E10 Allele 1, CnCir E10 Allele 2, CnCir E12 Allele 2, CnCir F2 Allele 1, CnCir F2 Allele 2, CnCir G11 Allele 1, CnCir G11 Allele 2, CnCir H4 Allele 1, CnCir H4 Allele 2, CnCir H7 Allele 2
5	Ayirankachi Tall	AYRT6	15	53.57	53.57	CnCir A3 Allele 1, CnCir A3 Allele 2, CnCir A9 Allele 1, CnCir A9 Allele 2, CnCir B6 Allele 1, CnCir B6 Allele 2, CnCir C7 Allele 2, CnCir E10 Allele 2, CnCir F2 Allele 1, CnCir F2 Allele 2, CnCir G11 Allele 1, CnCir G11 Allele 2, CnCir H4 Allele 1, CnCir H4 Allele 2, CnCir H7 Allele 2
6	Katchal Micro Tall	KM4	15	53.57	53.57	CnCir A3 Allele 1, CnCir B6 Allele 1, CnCir B6 Allele 2, CnCir C7 Allele 1, CnCir C7 Allele 2, CnCir E10 Allele 2, CnCir E12 Allele 1, CnCir E12 Allele 2, CnCir F2 Allele 1, CnCir F2 Allele 2, CnCir G11 Allele 1, CnCir G11 Allele 2, CnCir H4 Allele 1, CnCir H4 Allele 2, CnCir H7 Allele 2
7	Benaulim Tall	BENT7	15	53.57	53.57	CnCir A3 Allele 1, CnCir A3 Allele 2, CnCir B6 Allele 2, CnCir C3 Allele 1, CnCir C7 Allele 1, CnCir C7 Allele 2, CnCir E10 Allele 2, CnCir E12 Allele 1, CnCir E12 Allele 2, CnCir F2 Allele 1, CnCir F2 Allele 2, CnCir G11 Allele 1, CnCir H4 Allele 1, CnCir H4 Allele 2, CnCir H7 Allele 2
8	Tiptur Tall	TPT2	15	53.57	53.57	CnCir A3 Allele 1, CnCir A3 Allele 2, CnCir B6 Allele 2, CnCir B12 Allele 1, CnCir C3 Allele 1, CnCir C3 Allele 2, CnCir C7 Allele 1, CnCir E10 Allele 2, CnCir E12 Allele 1, CnCir E12 Allele 2, CnCir F2 Allele 1, CnCir F2 Allele 2, CnCir H4 Allele 1, CnCir H4 Allele 2, CnCir H7 Allele 2
9	Nadora Tall	NAD89	15	53.57	53.57	CnCir A3 Allele 1, CnCir A3 Allele 2, CnCir A9 Allele 1, CnCir A9 Allele 2, CnCir B6 Allele 1, CnCir B6 Allele 2, CnCir B12 Allele 2, CnCir C12 Allele 1, CnCir C12 Allele 2, CnCir E10 Allele 2, CnCir E12 Allele 1, CnCir E12 Allele 2, CnCir F2 Allele 1, CnCir F2 Allele 2, CnCir H7 Allele 2

Fig. 4: The 'variety identification tool' displaying the highly identical sample population to a query with their obtained scores, percent identity, percent similarity and matched alleles

population. CMSD also provides an interface to a 'SSR analyzer tool', a programme designed to predict mono- to deca- microsatellite repeats from any given nucleotide sequence. The data submission form helps in easy submission of newly derived data from anywhere in the world. This facility is very useful to maintain the database with current information which is not publicly accessible. The 'resources page' provides basic information about microsatellites and protocols for microsatellite marker genotyping. The individual SSR allelic size values were applied for creating barcodes making it possible to easily identify the cultivars at DNA level at first sight and thus providing a more convenient handling of numerical data. User comments and feedback are always welcome through the 'feedback page' provided. CMSD is hosted by the Bioinformatics Centre, CPCRI and is accessible via the World Wide Web interface at <http://www.bioinfpcpri.org/cmsd/home.php>.

We have demonstrated that the selection of microsatellite markers and construction of a consensus database as carried out in this work constitute a suitable system for use in network activities in which a common, centrally held database is continually fed with data from different laboratories. The existence of such DNA databases would facilitate the testing of new varieties of crops against all existing ones, reducing the need for individual laboratories or testing centres to maintain their own large reference collections (Donini *et al.*, 2000).

The ability of identifying unknown samples with such a database was examined by genotyping 62 populations and their samples subsequent comparison to known reference varieties. As well as being useful in its own right and serving as a tool for variety identification, the constructed database of more than might be of interest for breeding companies and variety registration agencies for additional purposes such as description of the gene pool represented in indigenous coconut accessions. To maximise the value of the current database, however, it would be necessary to include additional characters, such as pest and disease resistance information. Furthermore, such a database only retains its value if it is permanently updated with new data. There are some important issues that arise from this methodology, such as the need for a careful selection of markers (rejecting any that cause difficulties in any laboratory, for instance), duplication of analyses in at

least two laboratories, and having information about the possible heterogeneity of varieties for identification and related purposes. Last but not least, to be of any practical value, there must be information on a sufficiently large number of accessions. Given attention to these factors, it is clearly possible to construct consensus databases that have a wide range of practical applications in the variety and seeds area and for genetic resource analysis generally.

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Comparative Analysis of Morphometric and Molecular Diversity in Cashew (*Anacardium occidentale* L.) Genotypes

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It is noted that seed propagation resorted to, in cashew from the time of its introduction and allogamous nature of the crop have led to the inadvertent spread of non-descript genetic material throughout the state of Goa and the adjoining region, however, with enormous natural variability in it. This cashew seedling progeny in the Konkan region is considered to be the vital source of potent natural recombinants possessing desired commercial traits. Understanding the genetic divergence in cashew was hitherto based on morphological traits and yield components. Keeping in view the comparative analysis of morphometric data with that of molecular data, studies were taken up in 57 cashew genotypes. RAPD analysis differentiated all the cashew genotypes into two broad groups. Grouping of genotypes into six morphometric clusters and two molecular based clusters substantiated the fact that morphometric variables could be always under the influence of environment and thus often would mislead in understanding the actual divergence, where lies, the significance of diversity at molecular level. On the other hand, it would be a great deal of task to actually interpret the molecular data in the total absence of morphometric data. As regards to phenotypic inventory in the background of molecular diversity, the comparative analysis of both molecular results and clustering based on morphometric data reflected rather varied picture about the grouping pattern of genotypes. The studies aptly facilitated the correct selection of parental combination in crop breeding programmes with suitable objectives.

Key Words: Cashew genotypes, Genetic variability, Molecular diversity, Comparative analysis

Introduction

Cashew (*Anacardium occidentale* L.) has gained special status in the international scenario as a plantation crop of considerable foreign exchange earnings. It is cultivated in more than 28 countries of Latin America, Asia, Africa and Australia for its delightful nutritious kernels and apples. This crop was introduced in India on the coasts of Goa in 1570 AD by Portuguese for afforestation and soil conservation. Seed propagation resorted to, till recently, coupled with allogamous nature of the crop has led to the enormous natural variability. The seedling progeny in Goa, thus, is the vital source of potent natural recombinants possessing desired commercial traits, besides having the potential genotypes for specific characters such as resistance or tolerance to biotic/abiotic stress (Mathew and Nagabhushanam, 1988; Desai and Dhandar, 1998). Understanding the genetic divergence in cashew was hitherto based on morphological traits and yield attributes. However, application of molecular markers has emerged as the precise complementary approach to study the genetic diversity (Staub and Serquen, 1996; Lee, 1999). Earlier studies indicated the use of DNA markers to reveal the genetic diversity in cashew (Neto *et al.*, 1995; Karihaloo and Archak, 2000; Mneney *et al.*, 2001; Dhanaraj *et al.*,

2002; Rout *et al.*, 2002; Archak *et al.*, 2003; Samal *et al.*, 2003). However, information about the phenotypic inventory in the background of molecular diversity is very meager in cashew. Keeping this in view, importance of interpreting the molecular diversity trends in the light of morphological diversity trends in local cashew genotypes is emphasized in the study.

Material and Methods

Fifty seven genotypes of cashew (Table 1) planted in RBD at 6 m × 6 m spacing in the clonal germplasm bank (of softwood grafts) at ICAR Research Complex for Goa, Old Goa, formed the experimental material for the study. All the trees received the uniform management practices. At the start of this study, cashew trees were of six years age (planted during 1997-98). Observations on 20 morphometric parameters were recorded as per the standard descriptors of cashew (IBPGR, 1986; Swamy *et al.*, 1998), consecutively for two seasons 2004-05 and 2005-06).

Morphometric data of 20 growth and yield related attributes was subjected to principal component analysis as suggested by Iezzoni and Pritts (1991). Further, first 14 principal components accounting for 96.5 per cent

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Table 1. Local cashew genotypes and their geographical sources

Sl. no.	Genotype	Accession no.	District	Zone	Village
1	Dhave-1	GCC-94/6	North Goa	Sattari	Dhave
2	Dhave-2	GCC-94/7	North Goa	Sattari	Dhave
3	Dhave-3	GCC-94/8	North Goa	Sattari	Dhave
4	Tiswadi-3	G-CS-4-5	North Goa	Tiswadi	Old Goa
5	Tiswadi-4	G-CS-4-6	North Goa	Tiswadi	Old Goa
6	Tiswadi-5	G-CS-4-7	North Goa	Tiswadi	Old Goa
7	Karapur-1	GCC-94/4	North Goa	Ponda	Karapur
8	Karapur-2	GCC-94/5	North Goa	Ponda	Karapur
9	Ganje-1	GCC-94/1	North Goa	Ponda	Ganjam
10	Ganje-1-2	GCC-94/2	North Goa	Ponda	Ganjam
11	Ganje-2	GCC-94/3	North Goa	Ponda	Ganjam
12	Red Local	GC-CS-10	North Goa	Tiswadi	Old Goa
13	Sweet Round	GC-CS-11	North Goa	Tiswadi	Old Goa
14	Balli-1	GC-94/10	South Goa	Quepem	Balli
15	Goa-1 (Local check)	GC-94/11	South Goa	Quepem	Balli
16	Vengurla-4 (Introduced check)		A high yielding variety introduced from RFRS, Vengurla		
17	Kuddi-1	G-CS-7-12	South Goa	Quepem	Kuddi
18	Kuddi-2	G-CS-7-13	South Goa	Quepem	Kuddi
19	Bardez-6	GC-CS-1-6	North Goa	Bardez	Painwado
20	Bardez-9	G-CS-1-9	North Goa	Bardez	Porvorim
21	Pernem-4	G-CS-10-4	North Goa	Pernem	Torse
22	Zorinth-1	G-CS-6-11	South Goa	Salcette	Sancoale
23	Zorinth-2	G-CS-6-12	South Goa	Salcette	Sancoale
24	Loutolim-1	G-CS-6-2	South Goa	Salcette	Loutolim
25	Loutolim-3	G-CS-6-4	South Goa	Salcette	Loutolim
26	Loutolim-4	G-CS-6-5	South Goa	Salcette	Loutolim
27	Sarvan-1	G-CS-2-1	North Goa	Bicholim	Sarvan
28	SB-2	G-CS-5-3	South Goa	Canacona	Painguinim
29	Tiswadi-2	G-CS-4-4	North Goa	Tiswadi	Ela
30	Tiswadi-7	G-CS-4-9	North Goa	Tiswadi	Old Goa
31	Agonda-1	G-CS-5-6	South Goa	Canacona	Agonda
32	Agonda-2	G-CS-5-7	South Goa	Canacona	Agonda
33	Agonda-5	G-CS-5-12	South Goa	Canacona	Agonda
34	KN 2/98	G-CS-7-7	South Goa	Quepem	Suntegal
35	Sattari-1/00	G-CS-8-8	North Goa	Sattari	Keri
36	Sattari-3/00	G-CS-8-10	North Goa	Sattari	Keri
37	Sattari-4/00	G-CS-8-11	North Goa	Sattari	Keri
38	Sattari Dwarf	G-CS-8-9	North Goa	Sattari	Br. Karmali
39	Sattari-5/00	G-CS-8-12	North Goa	Sattari	Br. Karmali
40	Sattari-30/4	G-CS-30-4	North Goa	Sattari	Br. Karmali
41	Ashley-1	G-CS-6-13	South Goa	Salcette	Raia
42	Silva-1/55	G-CS-	North Goa	Salcette	Varca
43	Valpoi-1	G-CS-8-2	North Goa	Sattari	Valpoi (Dhave)
44	Valpoi-2	G-CS-8-2	North Goa	Sattari	Dhave
45	Valpoi-3	G-CS-8-3	North Goa	Sattari	Polekar Dhave
46	Valpoi-4	G-CS-8-4	North Goa	Sattari	Valpoi (Charvane)
47	Valpoi-5	G-CS-8-5	North Goa	Sattari	Valpoi (Charvane)
48	Valpoi-7	G-CS-8-7	North Goa	Sattari	Valpoi (Charvane)
49	Tudal-1	G-CS-5-15	South Goa	Canacona	Tudal
50	Tudal-3	G-CS-5-17	South Goa	Canacona	Tudal
51	Tudal-4	G-CS-5-24	South Goa	Canacona	Tudal
52	Tudal-5	G-CS-5-14	South Goa	Canacona	Tudal
53	Kholla-2	G-CS-5-9	South Goa	Canacona	Kholla
54	Kholla-3	G-CS-5-20	South Goa	Canacona	kholla
55	Kholker-1	G-CS-7-1	South Goa	Canacona	Cuncolim
56	Mayem-1	G-CS-2-5	North Goa	Bicholim	Mayem
57	Sanguem-1	G-CS-9-3	South Goa	Sanguem	Kirlpal (Dhabal)

variation, were identified and their scores were used for non-hierarchical Euclidean cluster analysis. The analysis was done by using SPAR 1 statistical package.

Molecular diversity studies were also carried out for the above 57 cashew genotypes using RAPD marker for its simplicity and cost effectiveness.

Total DNA of each cashew genotype was isolated using tender leaves of new flush by following Cetyl Trimethyl Ammonium Bromide (CTAB) protocol of Murray and Thompson (1980), with minor modifications. Leaf sample (2-3 g) of each genotype ground in liquid nitrogen was extracted in warm CTAB extraction buffer containing 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% (w/v) CTAB, 1% (w/v) PVP and 0.2% (v/v) mercaptoethanol. The pellet of DNA was suspended in $T_{10} E_1$ for purification by treating with RNase (5 μ l of 10 mg/ml) followed by deproteinization, using equal volume of phenol: chloroform: isoamyl alcohol (25:24:1). The solution was centrifuged at 10,000 rpm for 10 min at room temperature. The aqueous upper layer was collected in a fresh tube and treated with equal volume of chloroform: isoamylalcohol (24:1) and centrifuged at 10,000 rpm for 10 minutes. The upper aqueous layer was taken into a fresh tube and 1/10th volume of 3 M sodium acetate and two volumes of chilled absolute ethanol were added and incubated at 4°C for 1 hr. The DNA was pelleted by centrifugation at 10,000 rpm for 10 minutes, washed with 70 per cent ethanol, air dried and dissolved in 50 μ l of $T_{10} E_1$ buffer for further use. Concentration of DNA was diluted as described in Sambrook and Russel (2001) to working concentration of 100 ng/ μ l for further use in Polymerase Chain Reaction.

Screening of Primers and PCR Amplification

From seventy four deca-mer RAPD primers (Operon, USA) screened initially using template DNA of cashew genotypes, eventually, a set of 37 primers was selected based on their ability to produce distinct, clearly resolved and reproducible amplicon profiles.

Polymerase chain reactions were carried out in a final volume of 20 μ l consisting of 40 ng of Genomic DNA, Assay buffer (1X 10 mM Tris-HCl pH-9.0), 10mM dNTPmix (2.5 mM each), 5pM random primer, 1 U Taq DNA polymerase (Bangalore Genei) in thin walled PCR tubes. Amplification was carried out in Thermal Master Gradient Cyler, (Eppendorf, Germany) with the programme involving a pre-denaturation step at 94.0°C for 3 minutes, 40 cycles each of initial denaturation for 1.0 minute at 94.0°C, primer annealing at 38.0°C for 1.2 minutes and primer extension at 72.0°C for 2.0 minutes with a final extension at 72.0°C for 20 minutes.

The amplified products were resolved by electrophoresis on 1.5 per cent agarose gels stained with ethidium bromide (0.5 μ g ml⁻¹ of agarose) run at 60 volts in Tris Acetate EDTA buffer (1.0X TAE) for 4 h.

PCR amplicons resolved on agarose gel were scored to generate binary data by giving score 1 and 0 for the presence and absence of amplicon bands, respectively, at different levels of molecular weight, for all the genotypes. Binary data generated for all the primers was subjected to DARwin 5.0.128 programme for interpretation of the results. The dissimilarity matrix of 57 genotypes was generated based on band sharing data and weighted neighbour-joining tree was constructed.

Clustering patterns of genotypes based on morphometric data and molecular data were compared for diversity inventory of cashew genotypes.

Results and Discussion

Principal component analysis based on 20 growth and yield related characters of the selected cashew genotypes revealed that only first 14 principal components qualified, accounting for about 96.52 per cent of the total variation and scores of these 14 principal components grouped all the genotypes into six clusters (Table 2). In cluster-III, a maximum of 15 genotypes grouped together indicating the genetic nearness among them, while 13 genotypes came together in cluster-I. The genotypes having higher nut weight, higher kernel weight and bigger apple with cluster means of 9.52 g, 2.8 g and 102 g, respectively, were grouped together in Cluster-III. In this cluster, the nut weight of the genotypes Tiswadi-4 (No.5), Tiswadi-5 (No.5), Sweet Round (No.13), Kuddi-1 (No.17), Kuddi-2 (No.18), Sattari-5 (No.39), Valpoi-1 (No.43), Valpoi-3 (No.45), Valpoi-5 (No.47), Tudal-3 (No.50), Tudal-5 (No.52) and Mayem-1 (No.56) clustered together in cluster-I (Table 2). These genotypes have such traits as small-medium nut size, higher shelling percentage, shorter flowering duration, lesser N-S canopy spread and lower tree height in common. In the same way, the genotypes, Red local, Goa-1, Vengurla-4 and Sattari Dwarf with higher number of shoots per m² canopy, number of leaves per twig, number of flowering shoots per square meter canopy, flowering intensity, sex ratio, nuts per panicle, and shelling per cent, but with smaller-medium nut weight and kernel weight came together in cluster-VI, which was completely distinct from the cluster-III. On the contrary, cluster-IV had those genotypes with higher nut weight, apple weight, kernel weight and distinctly higher yielding ones, with higher sex ratio. Tiswadi-3 (No.4), Balli-1 (No.14), Bandez-9 (No.20), Agonda-1 (No.31), KN-2/98 (No.34) and Valpoi-7 (No.48) were the conspicuously distinct genotypes compared to others. The clustering pattern of the genotypes probably reflected the genetic history of the genotypes. This pattern of clustering on

Table 2. Distribution of cashew genotypes in different morphometric clusters and important characteristics

Cluster No.	Total No. genotypes	Genotypes (*Sl.No.)	Main characters and cluster means
I	13	Tiswadi-4 (*5), Tiswadi-5 (6), Sweet Round (13), Kuddi-1 (17), Kuddi-2 (18), Sattari-5 (39), Valpoi-1 (43), Valpoi-3 (45), Valpoi-5 (47), Tudal-3 (50), Tudal-5 (52), Kholker-1 (55), Mayem-1 (56) (Med. growth and dwarf types)	Tree height (m) : 3.1 Can. sprd-NS (m): 5.0 Can. sprd-EW(m): 5.2 No. shoots/m ² can: 17.7 No. leaves/twig : 13.9 Leaf area/twig (cm ²): 1230 Shelling percent : 30.7
II	9	Dhave-1 (1), Dhave-2 (2), Dhave-3 (3), Karapur-1 (7), Karapur-2 (8), Ganje-1 (9), Ganje-1-2 (10), Ganje-2 (11), Ashley-1 (41) (Vigorous growth habit)	Tree height (m) : 5.3 Can. sprd-NS (m) : 6.5 Can. sprd-EW (m): 6.8 Leaf area/twig (cm ²): 1809 Nut weight (g) : 7.9 Apple weight (g) : 93.0 Nut yield (kg/tree) : 5.4
III	15	Loutolim-3 (25), Loutolim-4 (26), Tiswadi-2 (29), Tiswadi-7 (30), Agonda-2 (32), Agonda-5 (33), Sattari-1 (35), Sattari-3 (36), Sattari-4 (37), Valpoi-2 (44), Valpoi-4 (46), Tudal-1 (49), Tudal-4 (51), Kholla-2 (53), Kholla-3 (54) (Bold nut and bigger apple)	Nut weight (g) : 9.52 Kernel weight (g): 2.8 Apple weight (g) : 102
IV	6	Tiswadi-3 (4), Balli-1 (14), Bardez-9 (20), Agonda-1 (31), KN 2/98 (34), Valpoi-7 (48) (Bold nuts, high yield : yield attributes)	Sex ratio: 0.33 Nuts/panicle: 2.5 Nut weight (g): 10.6 Kernel weight (g): 3.1 Apple weight (g): 107.0 Nut yield (kg/tree): 7.2
V	10	Bardez-6 (19), Pernem-4 (21), Zorinth-1 (22), Zorinth-2 (23), Loutolim-1 (24), Sarvan-1 (27), SB-2 (28), Sattari-30/4 (40), Silva-1/55 (42), Sanguem-1 (57) (Bold nut and bigger apple)	Nut weight (g): 7.9 Apple weight (g): 82.5
VI	4	Red Local (12), Goa-1 (15), Vengurla-4 (16), Sattari Dwarf (38) (Yield attributes and yield)	No. shoots/m ² can.: 22.0 No. leaves/twig: 16.2 Fl. shoots/m ² can.: 16.5 Sex ratio: 0.3 Nuts/panicle: 3.9 Shelling percent: 31.9 Nut yield (kg/tree): 6.2

the basis of previous breeding or genetic history, is similar to the report of Swamy *et al.* (2002) and Aliyu and Awopetu (2007) on cashew. Thus, in the present study, the distinct genotypes with potential genetic history for desired traits have been identified, which could be either useful in planning subsequent breeding programmes or could themselves be the evolved potential new varieties for commercial cultivation. In this regard, the short listing of redundant collections and the importance of core collection concept is emphasized by Noirot *et al.* (1996).

Molecular Diversity

The weighted neighbour-joining diversity tree (Fig. 1), constructed based on the RAPD data precisely differentiated all the 57 cashew genotypes in to two broad clusters. Cluster-1, the broad group comprised of 35 genotypes, which in turn indicated eight sub-clusters. While the cluster-II had 22 genotypes with only three sub-clusters.

Phenotypic Inventory in the Background of Molecular Diversity

It is noted that non-hierarchical Euclidean cluster analysis based on morphometric variables grouped all the 57

genotypes into six clusters, while the molecular data based on weighted neighbour-joining diversity tree grouped genotypes into two major clusters. This substantiates the

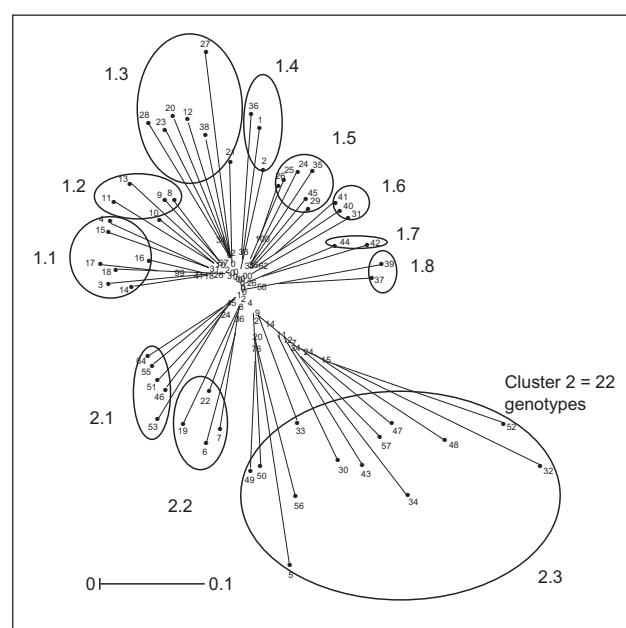


Fig. 1: Weighted neighbour joining tree of 57 cashew genotypes based on RAPD data

fact that morphometric variables are always under the influence of environment and thus often mislead in understanding the actual divergence, where lies, the significance of diversity at molecular level. In general, the morphometric clustering pattern in turn is refined by the molecular diversity pattern, which revealed that actual genetic variability in the germplasm accessions under the study is a population driven mainly by two sources.

Comparison of both diversity approaches, by and large, showed the partial consensus (Table 3) which clearly relates to the necessity of precise understanding of genetic diversity. For instance, the cashew genotypes, viz., Red Local (No. 12), Goa-1 (No. 15), Vengurla-4 (No. 16) and Sattari Dwarf (No. 38) of cluster-VI in morphometric grouping were also separately clustered in major cluster-1 of the molecular diversity tree, however scattered in sub-cluster-1.1 and 1.3 (Table 2). Similarly, the genotypes Tiswadi-5 (No. 6), Tudal 3 (No. 50), Tudal 4 (No. 5), Kholker-1 (No. 55), Mayem-1 (No. 56), Valpoi-1 (No. 43), Valpoi-5 (No. 47) and Tudal-5 (No. 52) of morphometric cluster-I were separately grouped in cluster-2 of weighted neighbour-joining diversity tree (Fig. 1 and Table 2), which were in turn scattered in sub-clusters 2.1 to 2.3. This kind of partial agreement of diversity studies are also reported by Samal *et al.* (2003). It is indeed imperative to precisely understand the genetic make up for the desired traits. In fact, it would be a great deal of task to actually interpret the molecular data in the total absence of morphometric data. Although, classical phenotype features are extremely useful, the efficiency of selection may be reduced by age, developmental stage and by environmental effects on measured traits (Mneny

et al., 2001). Any breeding programme will require more reliable information about level of genetic diversity by both approaches for practical significance. In this respect, present studies identified the genotypes with bold nut phenotypic trait scattered across both the major molecular clusters-1 and 2, which indicated that bold nut trait is derived originally from two different genetic back grounds. For instance, the bold nut genotypes such as Tiswadi-3 (S.No.4), Balli-1 (S.No. 14), Bardez-9 (S.No. 20) and Agonda-1 (S.No.31) and Valpo-2 (S.No.44) are scattered in different sub-clusters of the major molecular cluster-1 derived from one genetic background while bold nut genotypes such as Tudal-1 (S.No.48) and Valpoi-7 (S.No.49), derived from the other genetic background are clustered in sub-cluster-2.3 of the major molecular cluster-2. On the contrary morphometric clustering grouped Valpoi-2 and Tudal-1 in cluster-III and Agonda-1 and Valpoi-7 in cluster-IV although their molecular backgrounds were from two different sources. This information facilitates the breeder in selecting the correct parents truthful to desired traits in crop improvement programmes, which the morphometric diversity studies alone will not provide the breeder with. It is therefore felt that consideration of molecular data supplemented duly by morphological background would be precise practical solution in making the meaningful breeding programmes, since the QTLs (Quantitative Trait Loci) accounted ultimately are through morphological expressions.

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Table 3. Phenotypic inventory in the background of molecular diversity

Weighted neighbour joining tree clusters	Morphometric clusters					
	I	II	III	IV	V	VI
Cluster-1						
Sub-cluster 1.1	*17, 18	3		4, 14,		15, 16
Sub-cluster 1.2	13	8, 9, 10, 11				
Sub-cluster 1.3				20	21, 23, 27, 28	12, 38
Sub-cluster 1.4		1, 2	36			
Sub-cluster 1.5	45		25, 26, 29, 35		24	
Sub-cluster 1.6		41		31	40	
Sub-cluster 1.7			44		42	
Sub-cluster 1.8	39		37			
Cluster-2						
Sub-cluster 2.1	55		46, 51, 53, 54			
Sub-cluster 2.2	6	7			19, 22	
Sub-cluster 2.3	5, 50, 52, 43, 47, 56		30, 32, 33, 49	34, 48	57	

* Serial numbers of genotypes

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Mobilizing Neglected and Underutilized Crops to Strengthen Food Security and Alleviate Poverty in India

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The M.S. Swaminathan Research Foundation, Chennai in collaboration with Bioversity International, Rome has been implementing a project on neglected and underutilized crops with the support of the International Fund for Agricultural Development, Rome. The neglected and underutilized crops chosen for this study included finger millet (*Eleusine coracana*), Italian or foxtail millet (*Setaria italica*) and little millet (*Panicum sumatrense*). Two locations where these crops have been traditionally under cultivation and intrinsically linked with food security, livelihood and cultural identity of local tribal communities, were chosen as target sites. These crops by virtue of their extremely high resilience to the harsh agro-climatic conditions, particularly in marginal soils, hilly terrain and under aberrant rainfall, are well adapted to offer a dependable level of food security to the poor. They are also valued for their better nutritious profile of the grains and high quality straw. The grains have high bio-available minerals, particularly calcium and iron, vitamins, dietary fibre, and other phyto-chemicals. Their unique physico-chemical composition renders them slow digestible and with low glycemic index, which is a valued nutraceutical property in food grains. The long shelf life of these grains is also an attribute preferred by the communities living in regions characterized by poor communication.

A unique aspect of this study was that major activities were carried out in farmer participatory mode with a location specific blend of traditional and scientific know how. Major activity components and their outcome in enhancing food and nutritional security and income generation of the communities discussed in the paper are participatory seed selection, shift from marginal to modern crop management, seed selection and quality seed production and sharing, promotion of local landraces, value addition of grain and value chain development to achieve enhanced income, particularly to farm women, multifaceted capacity building, strengthening of common property local infrastructure and creation of community institutions for building sustainability to the change. The paper also discusses the increasing relevance of these crop species in the context of advancing climate change.

Key Words: Food security, Underutilized crops, Millets, Value addition, Participatory variety selection, Gene-seed-grain bank, Self help groups, Landraces

Introduction

The agriculture of biodiversity rich India is known for cultivation of few hundreds of species where in the process over historic period had evolved variable wealth of genetic diversity in all these species. While about 7,000 plant species are found useful in agriculture, only about 150 species among them are largely used and less than 30 plant species meet about 90 per cent of world's food requirement. The more recent intensification of agricultural research, production and associated policy support at the national and global levels had been narrowing the species base with emphasis only on a few of them belonging to cereal and other crop groups, while many species are left out of priority. Such shrinking species content in the food basket is a matter of major concern (Prescott and Prescott, 1990; Frison *et al.*, 2006; Swaminathan 2005). On the food front, currently about 60% of calories and 50% proteins are derived only from three major cereals, rice, wheat and maize (FAO, 1995). This process has resulted in the marginalization of a large group of locally important

crops, appreciated by communities because of their adaptability to marginal farming conditions, relevance to local food culture and diverse nutritional values and nutraceutical advantages. These crops belonging to categories such as cereals and pseudocereals, legumes, vegetables, oilseeds, roots and tubers, aromatic and medicinal plants, fruits and nuts, have earned collective names such as 'neglected and underutilized' or 'forgotten', 'orphan', 'minor' crops (Padulosi *et al.*, 2004, 2008). Many of these species are relevant to the poor people in many parts of the world. As important component of local crop diversity, they exert significant influence on the cultural traditions and diversity and contribute to the self-identity, self-esteem and visibility of local communities (Thies, 2000; Williams and Haq, 2002). The extraordinary hardiness of many of these species and their ability to cope with adverse growing and climatic conditions offer great promise in the era of climate change (Bala Ravi *et al.*, 2006). In this context, they are the 'crops of the future'.

The production systems followed in the cultivation of these crops are usually very marginal, largely based on local landraces conserved by farmers, with least or no external inputs or often under default organic farming. Their continued neglect, in spite of their resilience and strength in providing a favourable harvest under very marginal production situations of arid, hilly and mountainous regions, is leading to the rapid loss of their competitiveness, genetic resources as well as the associated traditional knowledge on production, processing and utilization. Their decreasing cultivation is being confined to fragile agro-ecological regions, which are largely inhabited by tribal or other socio-economically poor farming communities trapped below the poverty line (Bala Ravi, 2004). These crops are predominantly grown as mixed or inter crop along with fodder yielding cereals like maize or sorghum, grain legumes like chick pea or pigeon pea, and oil seeds like mustard or niger. The lack of technology for processing their produce and hence the continued dependence on traditional processing methods of high drudgery is also eroding their competitiveness. These are the motivations behind the farmer participatory studies undertaken by M.S. Swaminathan Research Foundation in collaboration with Bioversity International with the support of the International Fund for Agricultural Development. The results and conclusions reported here are based on the efforts of such investigations carried out during 2007 and 2008 seasons and involving 654 farm families in Tamil Nadu and Orissa States.

Materials and Methods

Three minor millets, finger millet (*Eleusine coracana*), little millet (*Panicum sumatrense*) and foxtail millet (*Setaria italica*) were targeted by the work, focusing on eleven villages in Kolli Hills (Namakkal District, Tamil Nadu) and Kundura (Koraput District, Orissa), areas where such crops used to be or continue to be grown as traditional food. The four major objectives of the study are: (1) Promote conservation of genetic diversity of underutilized and neglected crops by encouraging their cultivation, consumption and commercialization; (2) Enhance the food security and income generation potential of target species through better use of genetic diversity, improved agronomic practices and marketing; (3) Build and strengthen the capacity of women and men farmers through need based trainings and infra-structure support. The study was initiated with a bench mark survey of socio-economic-technological status of the community in the domains of project intervention. This was done with

a structured questionnaire and using samples of 105 and 148 households in Kundura and Kolli Hills, respectively. All local landraces of target species were initially identified and taken to on-farm conservation under the community managed Village Gene-Seed-Grain Bank approach developed and advocated by the MSSRF (2001). While practicing such on-farm conservation of all local landraces, farmers in each village were encouraged in participatory variety selection from scientifically laid out replicated variety trials comprising all land races and locally appropriate improved varieties. Introduction of improved varieties enlarged the genetic diversity accessible to farmers.

Participatory variety selection (PVS) in finger millet was conducted by using 26 varieties, including five local landraces and remaining improved varieties. Three trials were conducted in three villages with each variety grown in 3 x 3 m sub-plots and each trial replicated twice. At different stages of crop growth variety performance including their response to major disease (blast) was systematically recorded (in 1-5 scale) by the project staff. At physiological maturity stage, each farmer was requested to identify the best three visually determined varieties (in order of preference) from each replication and at each village location. The selection involved actively 116 farmers, including 65 women farmers. Care was taken that the decision of one farmer on selection is not prejudicing that of another. Focus Group Discussions at the trial sites revealed that farmers selected varieties using attributes like maturity duration, grain and straw yield, grain size, colour, appearance, taste and disease resistance, in their own way. The ranks given by the farmers to varieties selected by them were compiled using a scoring pattern of 5 points for first superior variety, 3 points for second superior and 1 point for the third superior variety. This ranking was assessed with data on average plot yield and disease response to determine the final variety selection.

Improved agronomic intervention had the following components: (1) Use of quality seeds of participatory selected varieties, including traditional varieties; (2) Promotion of density regulated row planting instead of the traditional broadcasting with high seed rate; (3) Structured intercropping of millet and non-millet traditional crops instead of their broadcasting as multi-species seed mix; (4) Promotion of use of farmyard manure and fertilizer with option to the farmer to choose either or both; (5) normalization of plant density by

thinning/transplanting and promoting weeding, inter-cultivation and often with a top dress, and (promotion of homestead vermin-compost production to replace farmyard manure and fertilizer. Several farmer participatory demonstrations on the said improved method were conducted in all villages together with traditional method in equal area. Data on cost-benefit aspects of both methods were collected. Field days were organized at a few of these demonstrations to sensitize other farmers on the merit of the advocated production system. The number of such demonstrations in Kolli Hills and Kundura were 4 and 5, respectively in 2007, and 7 and 15, respectively in 2008. While the main crop was finger millet in both Kolli Hills and Kundura, the intercrops in Kolli Hills were pigeonpea, little millet, mustard, *Dolichos* and maize and in Kundura pigeonpea, black gram, maize and niger. Family labour was accounted in calculating the cost of production.

During the last 30 years or so, small millets, which were the principal crops in Kolli Hills were substantially replaced by tapioca (Finnis, 2007), as the latter offers more than double profit. The local farmers who prefer millets for consumption are retaining very little area under millets as well as about 21 landraces of four millet species. However, shrinking area of cultivation is a growing challenge to the conservation of local agrobiodiversity. On the other end, surveys indicated that local communities retained an interest in consuming these grains. Participatory demonstrations were thus organized to promote finger millet cultivation as an intercrop with tapioca using a shorter duration variety and taking advantage of the slow early growth of tapioca. Seven such participatory demonstrations were conducted during 2007 and 2008. Data on crop competition and economics of production were also collected.

Value addition of millet grains is another strategic approach followed to enhance economic competitiveness of these crops. Intervention in this domain was designed as to leverage the specific nutritional and nutraceutical advantages of these grains (Hulse *et al.*, 1980; Swaminathan, 1995; Chetan and Malleshi, 2007). As the farming community knew only the traditional processing methods, which is tedious, and traditional cuisine from these grains, training programmes on commercially attractive products were organized together with the Rural Home Sciences Departments of the University of Agricultural Sciences, Bangalore and Dharwad. Selected women farmers of several self help groups (SHGs) and

farmers' clubs (FCs) organized in the project villages were deputed to these Home Science Departments to receive extensive practical training on development of different value added products, both traditional and novel and maintaining product quality and hygiene. Market orientation to the production of locally appropriate products was promoted by providing infrastructure such as flour mill, grain de-hulling unit and other facilities for processing and product packaging and labeling and training at group level with focus on women farmers.

Results and Discussion

Bench Mark Survey

The benchmark survey revealed that no farmer was aware of either improved varieties of these millets or their improved cultivation. The traditional practices of broadcasting millets as pure or mixed crop using high seed rate and no plant density regulation or intercultivation provided a very low yield, which on average was 317 kg/ha in Kundura and 857 kg/ha in Kolli Hills. These gains, on an average, contributed to 58% of the daily grain intake and 100 per cent of households cultivated and consumed millets in Kundura, while in Kolli Hills millets constituted only 7% of the food basket and 39% of the households cultivated and consumed these grains. Cultivation of millets contributed only 2.8% to the household income in Kolli Hills, and 14.6% in Kundura. Prior to this project intervention, none of the farm households in Kundura and Kolli Hills knew about post harvest processing, value added product development and commercialization potential of such products. Average annual household income in Kolli Hills was Rs. 17,407, while the same in Kundura was Rs. 3,861. The household income from millet cultivation was 4% of total income, while that in Kolli Hills was 3.2%. According to the poverty level defined by the Indian Planning Commission (GOI, 2007) these income levels, all the households in Kundura and 65.5% of those in Kolli Hills were below the poverty line.

Participatory Variety Selection (PVS)

The advantage of participatory crop variety selection is well established (Witcombe and Virk, 2001; Witcombe and Joshi, 1996). Participation of farmers in seed selection with their strong traditional knowledge and the skill adds to their involvement and ownership to the technology. The results of farmer participatory selection conducted under the present study are presented in Table 1. Farmers' ranking showed the highest preference to Indaf 9, a variety

with showy head and long fingers, which was followed by GPU 48, GPU 45, GPU 28, and Subhra (a rare white grain variety). Analysis of variance across location data showed significant differences in grain yield among varieties. The relationship between the ranking of varieties on the basis of average yield (as seen in Table 1), and farmer selection index was fairly good, particularly among the top ten varieties. The CD value showed that the yield differences between these ten varieties were not significant and the top four were significantly superior to the best local variety Badomandia. The top five varieties, Subra and all local varieties were chosen for propagation and seed conservation through the Village Gene-Seed-Grain Bank. All these improved varieties were found to be fairly tolerant to blast disease.

Yield Enhancement

Productivity improvement was planned with use of quality seeds of farmer selected varieties and deployment of improved production technology. The farmer participatory productivity enhancement demonstrations with options left to farmers on choice of intercrops and fertilizer application clearly brought out the comparative advantage of the improved practices over the traditional ones. The results from 31 trials conducted across two seasons at 11 villages in Kundura and Kolli Hills, provided clear trend on the impact of quality seeds and improved practices in increasing productivity and income from millets and millet based intercropping systems. The millet yield in Kundura, on an average, was two to three-folds higher than the traditional practice (Fig. 1). Here, millet farming is usually done with family labour and its exchange. On reckoning this labour in cost of production, it was observed that traditional millet cultivation resulted in a loss to

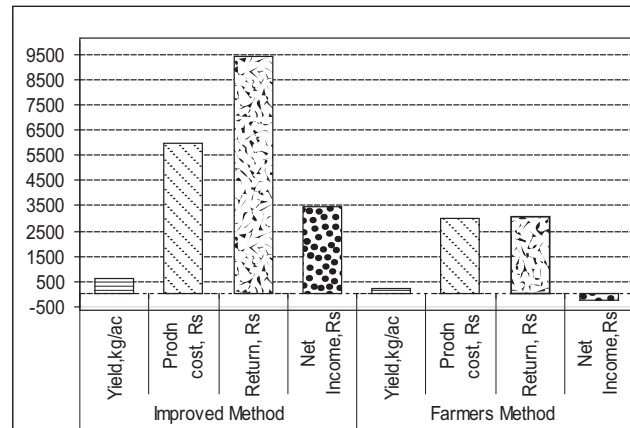


Fig. 1: Yield and income increase with improved method at Kundura

several farmers due to low yield. As estimated in the benchmark survey, the grain yields and income from traditional millet farming practices were higher in Kolli Hills in comparison with the same in Kundura. Therefore, the average gain achieved by PVS seeds and improved practices was comparatively lesser here. The yield increase

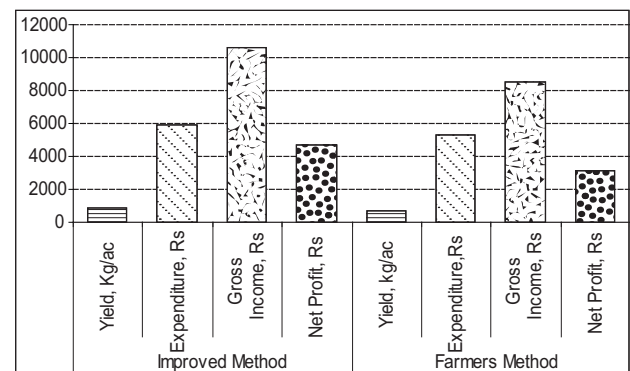


Fig. 2: Yield and income increase with improved method at Kolli hills

Table 1. Over all PVS score and grain yield of varieties studied at Kundura

Sl. No.	Variety	Av. yield (g/9m ²)	Farmers' ranking	Sl. No.	Variety	Av. yield (g/9m ²)	Farmers' ranking
1	GPU 28	2655.3	4	14	VL 149	1851.7	11
2	GPU 26	2644.3	10	15	Neelanchal	1790.5	14
3	PR 202	2514.8	9	16	RAU 8**	1780.5	15
4	GPU 45	2510.3	3	17	MR 33*	1763.5	17
5	GPU 48	2237.7	2	18	VR 708**	1692.5	6
6	L 5	2116.0	19	19	HR 91*	1685.8	23
7	Indaf 9*	2105.2	1	20	Badomandia	1677.5	25
8	PES 110**	2154.3	7	21	Birimandia*	1613.0	20
9	Indaf 5	2050.8	8	22	BM 91*	1577.8	13
10	Subhra	2031.0	5	23	Janhamandia**	1460.7	24
11	HR 374	1928.5	21	24	Dasahramandia*	1459.5	26
12	IE 3090	1855.5	18	25	Telugumandia	1396.2	16
13	OEB 10	1853.2	12	26	Dibyanshu**	1386.0	22
Mean		1915.1		CD@5%		761.0	

Note: *, ** means varieties affected by leaf blast or leaf and finger blast, respectively.

from improved practices, however, was about 60% higher than that of traditional practices (Fig. 2). In terms of increased income due to improved practices, the gain was Rs. 7,536/ha in Kundura and Rs. 4,342/ha in Kolli Hills. When these millets were intercropped with the traditional intercrops either under improved or farmers' practices, the net income increased by 30% and 27%, respectively.

Finger Millet Intercrop in Tapioca

The purpose of this participatory trial was to demonstrate that a quick crop of finger millet could be raised from the very same land where tapioca is planted and thus the land could be profitably used to meet both food and cash needs of farm family. The short duration finger millet variety, GPU 48, matures in 90-95 days when tapioca plants reach the growth stage with 15-20 leaves and canopy width of about 40 cm, leaving sizeable inter-row space free. The growth of tapioca was monitored in both areas with intercropped and without intercrop. These results showed that finger millet intercropping with tapioca caused noticeable growth reduction to the latter, although this was compensated during rest of the growing period with no significant drop in tuber yield. The average finger millet grain yield achieved from four trails during 2008 was 1080 kg/ha, which fetched a net income of rupees 10,006/ha. The finger millet intercrop with tapioca in other words offered 50% more income than that from tapioca (Table 2). Wider application of this intercrop would mitigate the decline in millet production due to the conversion of millet grown land to tapioca and to generate additional income from same unit of land. This would also remove the threat of tapioca causing to the loss of local millet genetic diversity.

Value Addition

Enhanced capacity of the community members, particularly farm women organized under SHGs, was built through in-campus training in Home Science Colleges, village level training on good practices in product making, maintaining product quality, packaging, and

marketing. SHGs were provided hand holding support by the project staff during initial period of product development and its standardization in quality, hygiene, packaging and marketing as well as building market linkages. Supply of small grain processing units functional at the project village level made a huge difference to the drudgery that these women were facing in processing these grains for domestic consumption. Financial resource for enterprise building was leveraged through creation of thrift fund by the SHGs and linkage with local banks, where the SHGs maintain their savings account.

SHGs in Kolli Hills identified millet-based malt, *chakkli* (*murukku*) and *laddu*, while those in Kundura selected malt and *nipputtu* (a sweetened ready to eat powder) for commercialization. The following example on the production and marketing of little millet malt is useful to highlight the underexploited potentials of minor millets. Little millet is the second best cereal grain suited to malting after barley¹⁸. This malt is a traditional weaning solid food for children from 6 month age onwards in view of its high digestibility and nourishment. Malting enhances the energy value of the grain, renders its protein, rich calcium, iron more bio-available and enlarges its vitamins such as niacin and folic acid (Malleshi and Desikachar, 1986). Little millet malt has blended components such as legume, which enhances the protein content and achieves better essential amino acid balance to the product. Thus, little millet malt is an excellent health food and beverage for all age groups. During 2007 and 2008, the SHGs at Kolli Hills and Kundura had produced and marketed more than one tonne of finger millet malt. The cost-benefit details of malt produced from 100 kg raw material and delivered at market end from both the locations are presented in Figure 3. While finger millet grain fetched Rs. 8-12 at the farm gate, depending on the location and time of sale, its value addition and marketing by farmers fetched them three-fold increase in income. In addition, value addition generated additional employment in the villages, value added the spare time

Table 2. Yield and income from pure and inter-cropped tapioca

Location	Tapioca (sole crop)			Tapioca with finger millet intercrop			
	Yield (kg/ha)	Cost of production (Rs.)	Net income/ ha (Rs.)	Yield (kg/ha)		Cost of production (Rs.)	Net income/ ha (Rs.)
				Finger. millet	Tapioca		
Village 1	9,518	12,825	19,725	1,215	8,910	16,033	29,020
Village 2	7,155	10,463	14,008	945	6,839	13,163	21,568
Mean	8,337	11,644	16,866	1,080	7,875	14,598	25,294

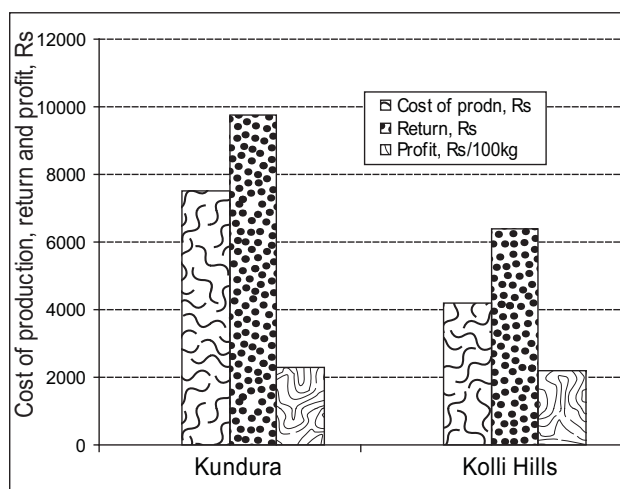


Fig. 3: Cost-benefit aspects of finger millet malt (costing of 100 kg raw material)

of the farm women and enhanced their social status and self-esteem.

A trademark was established for all millet products produced and commercialized from Kolli Hills and the malt is packaged in designer carton with international standards being followed for product labeling (Fig. 4). Introduction of similar pattern for the millet products developed and commercialized from Kundura is also under way.

This study demonstrates that the advantages offered by the underutilized and neglected crops, which enjoy high adaptive advantages under marginal agro-ecological and edaphic situations, in enhancing the food and nutritional security of local communities and enhancing their income. Importance of these crops in local food security and cultural diversity is well appreciated (Swaminathan, 2005;



Fig. 4: Packed Finger millet malt

Thies 2000; Thrupp, 1998). Some of these crops are also amenable to be grown along with competing cash crops and thus supplement the local food production. The unique strength of certain underutilized and neglected crops in their rich and favourable nutritional composition, nutraceutical value and product development offers uncommon opportunities for income generation to the farmers, in particular the farm women. This study also showcases the over all impact of such interventions on these groups of crops to the socio-economics of the communities conserving and cultivating them, enhancing their food and nutritional security and strengthening of their traditional food culture. This study confirmed earlier work carried out in previous years also in India (Padulosi, 2003). These crops and their genetic resources, which are being threatened by their status of neglect, have promising potential in the era of climate change in view of their unique adaptive strength. Time has come to review and reassess the importance of these crops in the future agriculture of India and elsewhere in the world.

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Participatory Varietal Selection: A Case Study on Small Millets in Karnataka

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Modern plant breeding has catered more to the needs of rich farmers who could afford high management under irrigated situations. In contrast, subsistence farmers growing millets in unfavourable environments use low levels of inputs and have not been benefited by high yielding variety (HYV) technology. In the present study, the usefulness of the participatory approach for identifying cultivars for harsh environments and acceptable to resource poor farmers has been demonstrated. In little millet five varieties, viz., CO 2, PRC 3, OLM 20, Sukshema and TNAU 98 were selected for testing on farmers fields. Trials was conducted at Jekinakatti village of Savanur taluk and Chandapur Tanda of Ranebennur Taluk during 2002; Harabagonda village of Byadagi Taluk during 2003 in Haveri district. Simillary, for foxtail millet study was carried out in Metriki, Balukundi (during 2002) and Janekunte village (during 2003) of Bellary District using five varieties, namely, HMT 100-1, RS 118, TNAU 173, Krishnadevaraya and Narashimharaya and for finger millet, varieties were GPU 26, GPU 28, MR 1, L 5 and Indaf 9. Trials was conducted at Koda (Hirekerur Taluk) and Bisanahalli (Byadagi Taluk) of Haveri District; Badeladaku (Kudligi Taluk) and Hirekolach (Hivinahadagali Taluk) of Bellary district Kharif 2002. In Pre- and post harvest Focus Group Discussions (FGDs) revealed that the variety “Sukshema” in little millet “HMT 100-1” in foxtail millet and “GPU 28” in finger millet meet the criteria of farmer’s requirement. Farmers also opined that the new variety has better grain and fodder yield potential and lodging resistance and they would adopt them in future.

Key Words: Little millet, Foxtail millet, Finger millet, Participatory selection

Introduction

Among rainfed crops, small millets as a group figure prominently. India is largest producer of small millets, which are often referred to as coarse cereals. Realisizing the nutritional superiority of these grains they are now considered as nutri-cereals (Nutritious grains). Small millets grown in India are finger millet, kodo millet, foxtail millet, little millet, proso millet and barnyard millet, which account for about 2.7 million ha and their cultivation extending from sea level in coastal Andhra Pradesh to 8,000 feet above sea level in hills of Uttarakhand and North-Eastern states. These crops are grown in diverse soils, varying rainfall regimes and in areas widely differing in thermo- and photo-periods. The resilience exhibited by these crops is helpful in their adjustment to different kinds of ecological niches and have made them quite indispensable to rainfed, tribal and hill agriculture where crop substitution is difficult. That is why it is important to enhance production and productivity of these crops to ensure food and nutritional security.

In Karnataka, these grains are mainly used as food for human consumption. The straw is a precious fodder for bovines. The grain is processed and consumed in traditional way and almost the entire produce is utilized at the farm/ village level. In spite of superior nutritional

value of grains, their use is confirmed more to rural areas and very little finds its way to urban markets. The promotion of these crops can lead to efficient natural resource management and holistic approach in sustaining precious agro-biodiversity. Karnataka state accounts 50 per cent of the area; 54 per cent of total finger millet production of the country. Finger millet is being grown widely in southern part of the state while little millet and foxtail millet are important for Northern Karnataka.

Although, many varieties have been released for cultivation in these crops their adoption by farmers is minimal. In vast dry land areas where these crops are grown, situations are differing from the areas prevailing as the research station are encountered. As a result, improved varieties found superior well in research stations may not perform upto the expectation in farmers field (Thiele *et al.*, 1997; Baidu-Forsen, 1997; Sthapit *et al.*, 1996). This situation has lead to farmers not showing preference to new varieties leading to negligible coverage by them in farmers field. Hence, farmers continue to cultivate local varieties having lower genetic potential as a consequence the grain yield productivity is low.

Keeping these things in view, the study was planned by providing cultivars acceptable to farmers through

participatory varietal selection. This gives closer farmer involvement from the initial stages of varietal evaluation and selection and help in identification of better varieties suitable for a given situation. This paper discusses the results of a case study on farmer participatory varietal selection in little millet, foxtail millet and finger millet in Northern Karnataka.

Materials and Methods

The project area consisted of Haveri and Bellary districts of Northern Karnataka. The details of the villages where the experiments were initiated are given in Table 1.

PRA (Participatory Rural Appraisal) was part of the study to understand the needs and preference of farmers on varietal choice. This was done by individual farmer survey using proforma developed for this purpose. Survey was conducted in project villages during summer 2002. Participatory Rural Appraisal showed that for all small millet growing farmers' grain and fodder yield were the main consideration. But, in finger millet, they also wanted blast resistance varieties.

Based on the farmers requirement from the basket of varieties available, five varieties in each crop were selected for testing with farmers from those released for Karnataka but not adopted and from those that were

promising in state as well as All India Coordinated Trials (Table 2).

In each village, one variety along with the local was grown in an area of 1 acre by the individual farmer adopting the prevailing cultivation practices. In the same village each variety was given to 3 farmers to serve as replications. Five test entries were tested by 15 farmers at each village. Also, two farmers were given all the five varieties to gauge the comparative performance of all the five varieties.

After sowing, regular visits of the scientists during the crop growth period were made to keep up the continuous interaction with the farmers. This enabled proper execution of the trials as well as in the gathering farmers perception on the testing material.

Results and Discussion

The performances of the varieties in each FAMPAR (Farmers Managed Participatory Rural) trials in each village were judged visually as well as quantitatively by a group of 20 farmers specially formed for this purpose, so that the final judgment and ranking of varieties were solely made by the farmers themselves. The group visited all the trial plots. Scientists, extension officers and key officials also accompanied the group.

Table 1. Study areas for participating variety selection in millets

Crop	Year	Project area
Little millet	2002	Jekinakatti, Savanur Tq; Haveri Distt. Chandapur Tanda, Ranebennur Tq; Haveri
	2003	Harabagonda, Byadgi Tq; Haveri
Foxtail millet	2002	Metriki, Sandur Tq; Bellary Distt. Balakundi, Bellary Tq and Dist
	2003	Janakunte, Bellary Tq and Distt.
Finger millet	2002	Koda, Hirekerur Tq; Haveri Bisalahalli, Ranebennur Tq; Haveri Hirekolachi, Huvinahadagali Tq, Bellary Badeladaku, Kudligi Tq; Bellary Distt.

Table 2. Varieties selected for the study

Crop	Name of the variety	Special features
Little millet	i) Sukshema	Released for Northern Karnataka in 2002
	ii) Co 2	All India release
	iii) PRC 3	All India release
	iv) OLM 20	All India release
	v) TNAU 98	Promising variety in Co-ordinated trial
Foxtail millet	i) HMT 100-1	Promising variety at station trial
	ii) RS-118	State released variety
	iii) TNAU-173	Promising variety in station trials
	iv) Krishnadevaraya	All India release
	v) Narashimharaya	All India release
Finger millet	i) GPU 26	Blast resistance released for Karnataka in 1999
	ii) GPU 28	Blast resistance released for Karnataka 1998
	iii) MR 1	High yielding
	iv) L 5	High yielding
	v) Indaf 9	High grain and fodder yield

The input provided by the group was used for formulating a pre-harvest ranking of varieties along with local check, by making important characters, namely, crop duration, panicle type and size, disease resistance, drought tolerance, grain density and estimated yield potential. After harvest, grain and fodder yield data were collected from all trial plots for a more critical comparison.

In FAMPAR, little millet trials mean grain yield data indicated that the cultivar Sukshema was superior to all the five entries in all the three locations (Table 3). Variety Sukshema recorded an overall mean yield of 10.30 q/ ha with the yield increase of 74% over local. The next performing variety was TNAU 98 which ranked second with a overall mean yield of 8.88 q/ ha, accounting to the tune of 50% increased yield over local. The varieties OLM 20, PRC 3 and CO 2 recorded 8.32 q/ ha, 8.18 q/ ha and 7.58 q/ ha, respectively.

In foxtail millet, grain yield data indicated that HMT 100-1 was superior to all the five entries over the locations. HMT 100-1 recorded an overall mean yield

of 15.35 q/ ha with a yield increase of 36% over local (Table 4). The next best performing variety was Krishnadevaraya which ranked second with an overall mean yield of 13.73 q/ ha with increase yield of 22% over local. The varieties TNAU 173, Narasimharaya and RS 118 recorded mean yield of 13.50 q/ ha, 13.15 q/ ha and 12.46 q/ ha, respectively.

In finger millet among five cultivars tested GPU 28 was significantly superior in mean yield data over all the locations. Variety GPU 28 recorded 20.52 q/ha which was followed by L 5 which recorded 19.90 q/ ha. Where as, GPU 26, MR 1, Indaf 9 recorded 16.42 q/ ha, 15.22 q/ ha and 14.30 q/ ha, respectively (Table 5). The best performing variety GPU 28 showed 50% increase over local which was followed by L 5 (45%).

Participatory rural appraisal showed that the little millet growing farmers, grain and fodder yield were the main considerations. Based on yield, the variety “Sukshema” was found significantly superior to other

Table 3. Mean performance of five FAMPAR little millet varieties over three locations

S. No.	Variety	Grain yield q/ ha			Mean	Increase over local check (%)
		Jekinakatti	Channapura Tanda	Harabagonda		
1	Sukshema	14.66	10.08	6.16	10.30	73.69
2	TNAU 98	12.46	9.16	5.03	8.88	49.74
3	OLM 20	12.56	8.25	4.16	8.32	40.30
4	PRC 3	12.56	8.00	4.00	8.18	37.94
5	Co 2	11.33	7.91	3.50	7.58	27.82
6	Local	8.59	5.44	3.76	5.93	—

Table 4. Mean performance of five FAMPAR-foxtail millet varieties over three locations

S. No.	Variety	Grain yield q/ ha			Mean	Increase over local check (%)
		Halakundi	Metriki	Janakunte		
1	HMT 100-1	14.90	15.06	16.10	15.35	36.44
2	Krishnadevaraya	13.10	11.90	16.20	13.73	22.04
3	TNAU 173	14.00	13.40	13.10	13.50	20.00
4	Narasimharaya	12.50	12.65	14.30	13.15	16.88
5	RS 118	12.85	11.85	12.70	12.46	10.75
6	Local	11.14	11.12	11.50	11.25	—

Table 5. Mean performance of five FAMPAR-finger millet varieties over four locations

S. No.	Varieties	Grain yield q/ ha				Mean	Increase over local check (%)
		Haveri		Bellary			
		Koda	Bisanalli	Hirekolachi	Bedaladauku		
1	GPU 28	19.60	18.50	25.00	19.00	20.52	49.56
2	GPU 26	16.40	14.4	20.6	14.3	16.42	19.67
3	L 5	19.90	18.10	23.60	18.00	19.90	45.04
4	MR 1	13.80	13.10	20.50	13.50	15.22	10.93
5	Indaf 9	12.50	14.00	18.60	12.10	14.30	4.22
6	Local	13.00	11.80	18.30	11.80	13.72	—

entries. This variety was preferred by the farmers because of early maturity (85 days), high tillering, non-lodging and drought tolerant.

In foxtail millet, the farmers accepted “HMT 100-1” which matured in 85 days with moderate tillers, thin stems and healthy foliage even at harvest and superior to TNAU 173 and Narashimharaya in yielding ability.

In finger millet, the farmers selected “GPU 28” for normal planting in the 2nd fortnight of June to 1st fortnight of July. GPU 28 followed by L 5, GPU 26 and MR 1 were found superior for yield. Indaf 9 was not preferred by the farmers due to low yields and susceptibility to blast.

Pre- and post-harvest Focus Group Discussions (FGDs) by considering some of the important characters, namely, crop duration, ear head type and size, disease resistance, drought tolerance and grain and fodder yield revealed that the varieties “Sukshema” in little millet, “HMT 100-1” in foxtail millet and “GPU-28” in finger millet came very near to the farmers requirements. The farmers of the village had been satisfied with the performance of the these varieties and were keen to plant more areas with these varieties in the ensuing season.

Seed production programme had been taken at Agricultural Research Station, Hanumanamatti, for production of quality seeds of little millet variety “Sukshema”, and foxtail millet variety “HMT 100-1” and finger millet variety “GPU 28”. The quantity of seeds produced for distribution to farmers of the project village is presented in Table 6.

The spread of high yielding varieties has been limited in small millet. Hence, large scale demonstrations had been conducted to demonstrate the full potential of selected varieties identified from participatory varietal selection vis-a-vis local cultivars. The cluster of villages that were involved in conducting FAMPAR trails during 2002 and 2003 and areas where these crops were dominant were selected for demonstrations. The total number of demonstrations conducted during 2003 and 2004 in Haveri and Bellary districts are presented in the Table 7.

In each demonstration, farmers were given 4 kg seeds of improved variety and asked to grow selected variety along with local in area of 0.8 ha (one acre each of improved variety and local) by adopting prevailing cultivation practices. Regular visit by the scientists during the crop growth period were made to keep up the continuous interaction with the farmers. This enabled proper execution of demonstrations as well as gathering farmers perception on improved varieties. The crop-wise breakup of the details of beneficiaries in large scale demonstrations are given in Table 8.

In little millet the mean grain yield under improved variety (Sukshema) was 8.00 q/ ha in comparison with 5.72 q/ ha of local variety. The per cent increase in productivity of the improved variety over local was 40%. The fodder yield recorded by Sukshema was 4.60 t/ ha whereas of 3.43 t/ ha under local genotype. The productivity of finger millet in large-scale demonstrations registered an increase in grain yield to the extent of 63% with improved variety GPU 28 over

Table 6. Seed produced and distributed under the project during 2003-04

S. No.	Crop	Variety	2003 (Quantity)	2004 (Quantity)	Total (Quantity)
1	Little millet	Sukshema	10 q	22 q	32 q
2	Foxtail millet	HMT 100-1	10 q	10 q	20 q
3	Finger millet	GPU 28	4 q	4 q	8 q

Table 7. Number of demonstrations conducted

District	Crop	Year		Total
		2003	2004	
Haveri	Little millet	35	50	85
	Finger millet	35	9	44
	Foxtail millet	15	9	24
Bellary	Foxtail millet	10	35	45

Table 8. The mean productivity of grain and fodder yield increased through frontline demonstrations conducted

Sl. No.	Crop	Grain yield (q/ ha)		Increase over local (%)	Fodder yield (t/ ha)		Increase over local (%)
		Demonstration	Traditional		Demonstration	Traditional	
1.	Little millet	8.00	5.72	39.8	4.60	5.43	34.1
2.	Finger millet	11.74	7.21	62.8	3.61	2.45	47.3
3.	Foxtail millet	11.52	7.99	44.1	4.29	3.11	37.9

local. Similarly, in foxtail millet, the increase in grain and fodder yield of improved variety “HMT 100-1” over local was 44% and 38%, respectively. The mean grain and fodder yield of HMT 100-1 was 11.52 q/ ha and 4.29 t/ ha, respectively, compared to 7.99 q/ ha and 3.40 t/ ha, respectively, of local variety.

The superior variety bred and released for large scale cultivation needs to be maintained for its genetic and physical purity in order to exploit its full genetic potential. The improved varieties are prone for genetic deterioration as they have a very carefully built up genetic constellation/ gene combination for higher productivity, regional adaption and inbuilt genetic resistance for biotic and abiotic stresses. In a well managed crop improvement programme maintenance of released variety becomes very important in order to prolong the consistency of

performance. In this context training was given for production and supply of good quality seeds. The farmers who have been involved in conducting Frontline Demonstrations were given training for adopting proper procedure for maintaining the genetic architecture of the variety.

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On-farm Conservation—Initiatives by NBPGR in the Western Himalayan Region

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Introduction

Large number of plant genetic resources have been collected and conserved *ex situ* in the gene banks, the world over. These genetic resources have been effectively used for breeding new crop varieties and enhancing food production. While doing so, the very important form of conservation *in situ* (on-farm), which has resulted into large *ex situ* collections, received less attention and lots of valuable materials were lost from farmer's field. On-farm conservation has been recognized as a participatory and dynamic approach involving farmers and their long established skills and knowledge on genetic resources. Being dynamic, it makes continuum in the evolutionary processes, thereby create useful genetic variability within traditional agricultural systems (Brush, 1999; Bisht *et al.*, 2006). It also supports large number of rare alleles and genotypes evolved spatially and temporally than the accessions conserved in the gene banks (Jarvis *et al.*, 2008). These rare combinations of new alleles are capable to cope with wide variety of stresses and have been reviewed in detail (Oldfield and Alcorn 1987; Iwanaga 1995; Maxted *et al.*, 1997a,b; Brush and Meng 1998; Sthapit *et al.*, 2008). Germplasm specialists must therefore, explore genetic variations to identify germplasm with traits for developing crops cultivars adapted to these stresses from materials conserved and cultivated on-farm as they have withstood the competition with their improved allies over the years and is testimony to their resilience to frequently changing environments. Despite these facts, changing cropping patterns, increasing interest towards cash crops, large scale migration leaving agricultural field abandoned and couple of other factors discussed later in the text are posing challenge for promoting on-farm conservation (Maikhuri *et al.*, 1996; Rana *et al.*, 2001) not only in cash crops dominating regions but also in remote and marginal hill areas which by and large practice traditional farming systems. The present study was, therefore, designed to understand the dynamics of crops diversity and farmers' innovation, initiative, opportunities and limitations in the on-farm conservation of PGR.

Materials and Methods

The selection of site is a very important process to achieve the objectives of on-farm conservation and therefore, it should have ecological, socio-economic and institutional consideration (Sthapit and Jarvis, 2005). The Western Himalayan region is characterized by a complex mosaic of distinct micro-environments housing variety of climate, soil, geological, vegetation and crop growing features (Rao and Saxena 1994; Maikhuri *et al.*, 2001; Rana *et al.*, 2008) and therefore, provide unique opportunity for undertaking on-farm conservation. The important indicators that we used for selecting the site were (i) number of house holds/ population structure, (ii) targeted crops, (iii) ecological, crop species and varietal diversity, (iv) cropping and land use pattern, (v) farming communities enthusiasm/ interest, (vi) potential to undertake on-farm conservation activities, (vii) imminent threat of genetic erosion, (viii) level of awareness among farmers/ farmer groups, (ix) level of technological interventions, (x) livelihood strategies/ dependence of agriculture, and access to market. In totality, we surveyed 24 sites and finally selected three which were meeting the criteria and objective laid to start on-farm conservation programme on two rice landraces, viz., *Chuhatu* and *Jattu* and two crops, viz., grain amaranth and finger millet (Fig. 1). Each site is a cluster of villages with overlapping boundaries and agricultural fields.

The programme mainly focused on participatory, dynamic, and farmer-based approach for the conservation, enhancement and utilization of crops and varieties of local importance. We carried out survey and collection work in 2006 and 2007 involving farmers, scientists and local extension specialists at 24 sites. After doing critical analysis three sites as mentioned above were selected to undertake the work more focused and systematic. Women farmers, in particular, were encouraged to participate because they provided more information and had greater role in seed conservation. As a first step, we collected the germplasm, evaluated, purified and given back to farmers. Germplasm comprising grain amaranth (284 accessions) and finger millet (126

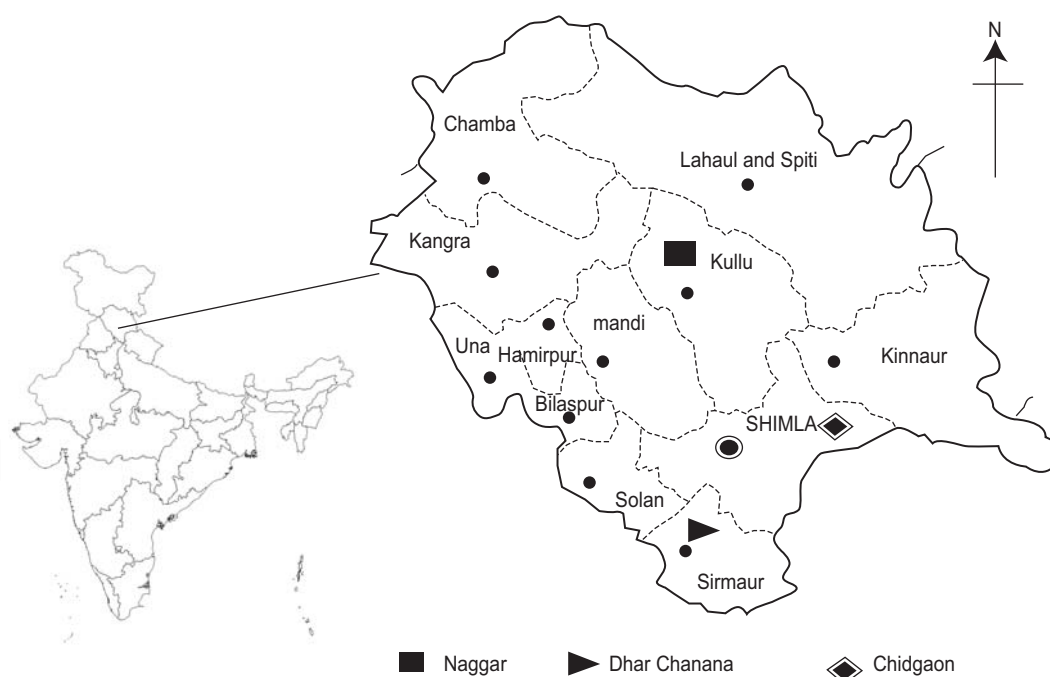


Fig. 1: Sites for on-farm conservation in Himachal Pradesh

accessions) were collected from different sites in the year 2006 and 2007. These were evaluated at NBPGR Regional Station, Shimla and elite types selected involving farmers were given back to farmers. In case of rice, 48 landraces including the two, i.e., *Chuhatu* and *Jattu* were collected in the year 2008. The material is being evaluated at NBPGR Regional Station, Bhowali and Himachal Pradesh Agricultural University, Regional Station, Malan.

Results and Discussion

(i) Naggar: Geographically, It is located at an average elevation of 1875 amsl, $32^{\circ}12'49''\text{N}$ and $77^{\circ}12'02''\text{E}$ in Kullu district and selected for promoting cultivation of *Jattu* landrace—a cold tolerant red grained rice. It occupies around 600-800 ha area spread in Chhaki, Halanseri, Rajhan, Sarsai and Naggar villages. The landholdings varied from 0.21-6.45 ha/family while area under *Jattu* varied from 0.04-2.20 ha/family.

(ii) Chidgaon: It is located in Shimla district at an average elevation of 1750 amsl and $31^{\circ}13'12''\text{N}$ and $77^{\circ}48'25''\text{E}$. It was selected for promoting a cold tolerant medicinal red grain landrace, viz. *Chuhatu* which is grown in ~1000 ha in Masli, Dadholi, Jannadee, Badhiara, Jagla, Todsa and Thana villages. The average area under *chuhatu* vary from 0.08-1.6 ha/family as compared while total landholdings were 0.24–4.40 ha/family.

(iii) Dhar Chanana: It is located in Sirmour district at an average elevation 1550 amsl and $30^{\circ}52'51''\text{N}$ and $77^{\circ}42'18''\text{E}$. This site was primarily selected for grain amaranth and finger millet Two landraces of rice, viz., *Bhaoj* and *Katan* which are grown since many years and now facing threat from cash crops, has been included. The area under grain amaranth and finger millet vary from 150-200 ha and 25-50 ha, respectively, in Khadah, Deuthi, Anog, Chanana, Chindi, Dhatauli Kothi villages. The area under grain amaranth varied from 0.01-0.4 ha/family while it was 0.009-0.08 ha/family for finger millet.

The amount of genetic diversity in an agricultural system is perhaps one of the important characteristic for its stability and long-term sustainability. Wide range of crops and varieties are grown in the sites both as pure and inter-cropped. Farmers in general practice low input agriculture and conserving significant amount of crop diversity which is more in Dhar Chanana than Chidgaon and Naggar. The major crops grown in *kharif* season include rice, maize, mash, rajmash, horse gram, buckwheat, amaranth, finger millet, foxtail millet, potato, tomato, *Capsicum*, apple, pear, and many other seasonal vegetables, fruits and spices while these were wheat, barley, lentil, pea, garlic and mustard in *rabi* season. The varietal composition includes landraces, locally adapted obsolete varieties (for cereals, pulses,

pseudocereals and small millets) and newly released varieties and hybrids (fruits and vegetables). The agriculture is primarily rainfed but rice is grown along the riverside/ valleys under irrigated condition for which water is obtained either from natural springs/ snow melt.

The analysis of the socio-economic surveys revealed very low level of awareness (varied from 3-18%) on the genetic resources and their management not only among farmers but also in local extension workers. Therefore, as a second step, we organized brainstorming sessions involving all the stakeholders at Shimla, Palampur and Kullu. They were sensitized on the importance of agro-biodiversity, medicinal and nutritional value of traditional crops and varieties, role of genetic resources in the land use management, against various stresses and ensuring food security. As a result, it was noticed that there was significant improvement in the awareness and attitude of farmers towards traditional crops and varieties and we could successfully promote and reintroduce these crops particularly amaranth and finger millet even in those areas from where they had disappeared, for instance, areas dominated with commercial or cash crops. They were also taken to sites of germplasm evaluation to have their inputs to identify elite types.

Studies are underway to document and build on the existing knowledge and practices relating to landraces production and management on selected sites. The data on ethnobotany and population biology, focusing on the population structure and dynamics of the traditional crops and landraces for more effective planning and management of on-farm conservation strategy is being generated. Another aspect of the programme would be to restore some selected landraces particularly of rice in areas where farmers had once planted them extensively, but now those regions are dominated by cash crops or improved varieties. We came across around 24 farmers who showed willingness to grow some of their old landraces, if seed material is made available to them. We also planned to expand on-farm conservation programme including creation of community gene banks in other crops like barley, maize and French bean and also to identify and establish strategic on-farm pockets as models.

Despite the fact that traditional crops and varieties are finding new market particularly in the emerging regimes of new tourism policies such as homestead tourism, and have better scope over their improved allies in the context of climate change and organic farming. Nevertheless, there are some concerns and/or operational

difficulties which we come across during field work. For instance, gradual reduction in area of traditional crops and farmers preferences for other introduced (cash) crops is induced by the economic and socio-cultural factors. In Chidgaon and Naggar, farmers particularly the young farmers were found more interested to take up apple and off-season vegetables than rice cultivation. The reason behind was not only the economic consideration, but the amount of labour being put in for growing rice and grain amaranth. About 64% of the farmers including young were of the view that they can get more money by growing apple which needs onetime planting and less afterwards care. While talking on food security issue, they were less worried and probably owing to public distribution system where food grains were sold at much cheaper rate. It was observed that dependence of farmers for food grains on ration shop and open market was very high, i.e., 40-100% in Chidgaon and Naggar than Dhar Chanana where it was 10-55% because farmers were growing more number of crops. Moreover, 58% of the farmers in Dhar Chanana had reserve stocks of amaranth and finger millet ranging from 3.0 to 25 q/ family, thus this site was found more food secured than others.

There has been a sea change in the food habits. A shift has occurred from coarse grains (maize, amaranth, buckwheat and small millets) to fine grains (wheat and rice) as the latter being considered "symbol of progress" and easy to digest. By and large, the younger generation is not keen to adoption of traditional technologies and food recipes as better substitutes are available (the views were recorded during surveys). Moreover, there is no guaranteed buyer or assured buy back system for products of on-farm conservation, be it landraces or crops. Further, there is no in-built mechanism to incentivise the farmers who are willing to adopt on-farm conservation activities. As a result, despite being aware on the loss of genetic resources, farmers are shifting to better options. Besides, there are many other associated factors leading to operational difficulties such as opting agriculture as profession is least priority, subject to natural vagaries, attack of wild animals, rural to urban migration, fixed targets to enhance seed replacement rate by the department of agriculture and agriculture universities, selling seeds of modern varieties at subsidized cost and lack of valuation/ value addition of the traditional crops and varieties.

Conclusion and Perspectives

The present programme has been systematically initiated only two years ago; it will be too early to conclude our findings. Nevertheless, on-farm conservation has potential to sustain the evolutionary systems that are responsible for generating variability and provide therefore, a valuable option for conserving crop diversity. The ability of traditional varieties and crops to survive under stress is conditioned by a broad genetic base than the more uniform new cultivars which have narrow genetic base and poor adaptability to micro-climatic niches of mountains. Though on-farm conservation has prospects, but at the same time it has challenges emerging from the changing agricultural scenario. Therefore, the complexities of on-farm conservation are required to be understood in the background of these challenges including population growth and increasing demand for food. Nevertheless, conserving biodiversity in its dynamic form should be identified as top priority in any conservation strategy.

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On-farm Management of Rice Varieties in Kumaon Himalayas of Uttarakhand

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In heterogenous agro-ecosystems, local farming communities used to grow different cultivars adaptable in different eco-niches. They are the managers of crop diversity as well as custodians of the important genetic wealth. The Himalayan highlands are important centre of crop diversity due to high ecological heterogeneity and socio-cultural integrations. Rice crop genetic diversity managed by the local farm households in Kumaon Himalaya of Uttarakhand state has been documented in the present study. Farmers management and on-farm conservation of existing rice varieties were studied in detail. Farmers variety selection criteria, seed flow, exchange and traditional storage techniques, which are the important processes involved in on-farm management were also studied in greater detail. This study examines and discusses the processes of seed and variety selection, adaptation in different agro-ecosystem in respect of management of crop diversity for subsistence needs.

Key Words: On-farm management of rice, Uttarakhand, Primitive cultivars, Kumaon Himalaya

Introduction

Rice (*Oryza sativa* L.) is one of the major crop of kharif season in Uttarakhand. It is the single most important food crop and 90% of it is grown and consumed in Asia (Khush and Brar, 2005). It is well known that rice emerged as staple food in South-East Asia that proliferated and subsequently dominated the world food bowl along with other food crops (Paroda, 2004). Rice contributes 26% in the world's food production (FAO, 1996). In Kumaon Himalayas it is grown since time immemorial. Before green revolution era, the total cultivation of rice was dependent on traditional local cultivars. The old traditional rice cultivars were very well known in the Gazetteer of Himalayan provinces (Atkinson, 1882). After green revolution, many rice varieties were developed by various regional, national and international organizations, but these high yielding varieties (HYVs) were suitable to irrigated areas only. In Kumaon Himalaya only 15% cultivable land is irrigated. In rainfed cultivation, mostly traditional cultivars are grown by the farm households. These local cultivars are very much suitable to the agro-climatic conditions and eco-niches. Despite the spread of many improved varieties, several local named varieties are still grown in the region because of their well adaptability (Bisht *et al.*, 2006). Farming communities in the Kumaon Himalaya are completely dependent on local cultivars and seed system, which are suitable for their environmental, socio-economic and ethnic requirements. These local varieties are managed by the local farmers in different agro eco-niches. Farmers management of diversity refers to the cultivation of a diverse set of more or less

specialized crop population. These population are named and recognized as units by the farmers. These are known as farmers varieties as opposed to the improved varieties.

Today there is a world wide concern over the loss of crop diversity. A particular concern is the substitution of a diverse set of genetically variable crop landraces by a few genetically uniform modern varieties (Brush, 1991; Harlan, 1992; Hawkes, 1983; National Research Council, 1983; Plucknett *et al.*, 1987). The need to conserve the wide array of diversity has been recognized as very important for many decades. It has resulted in to the creation of gene banks around the world *i.e.*, *ex-situ* conservation (Hawkes, 1983; Plucknett *et al.*, 1987). Lately, on-farm (*in-situ*) conservation and management has been advocated as a complementary method to *ex-situ* conservation (Altieri and Merrick, 1987; Brush, 1991; IPGRI, 1993; Old Field and Alcorn, 1987).

On-farm conservation of plant genetic resources can be defined as the continued cultivation and management of a diverse set of crop populations by farmers in the agro-ecosystem, where a crop has evolved. It is a dynamic and is aimed at maintaining the evolutionary processes that continue to shape the diversity.

In Kumaon Himalayas, farmers have developed and nurtured crop diversity and this process is still continue amongst the farmers, in spite of socio-economic and technological changes. For conservation and management of diversity, the process of seed selection, flow of seed material and local storage methods are the major components involved. The present study aimed at documentation of the local rice cultivars grown by the

farm households in Kumaon Himalaya is undertaken. The results are described in the light of farmers management of rice varieties for sustainable livelihood in this Himalayan region.

Materials and Methods

Data on rice genetic resources were collected from primary sources with the help of planned structural and unstructured interview schedules/questionnaires at individual household levels during 2007 and 2008 cropping seasons. Sample households were randomly selected from all the 33 development blocks of five districts of Kumaon Himalaya of Uttarakhand state. Three to four villages were selected from each development block representing the distinct agro ecological niches. In each selected village 5% households were randomly selected for interview. For randomization, lottery system was adopted. Thus a total of 363 farm households were interviewed for documentation of rice varieties grown by them. The district wise details of development blocks and number of villages surveyed and respondent farmers are presented in Table 1.

During the survey of the region, a non-participant observation method was also used while recording the information of rice cultivars.

Using participatory rural appraisal (PRA), information was obtained on the erosion and shift in cultivar diversity and changes. The information for current status was validated by taking observations in the fields of the farmers for the cultivars diversity grown by them. Respondent households were also asked to fill a questionnaire for extracting their knowledge on rice cultivars and diversity available under on-farm management. Local seed exchange systems, selection methods and storage techniques were also studied in greater details. All possible care was also taken to avoid the duplicacy of varieties by comparing information provided by different farm households and social groups.

Table 1. Districts, number of development blocks, villages and households surveyed for collection of data of rice diversity

District	Number of development blocks	Number of villages surveyed for the study	Number of households sampled
Almora	11	40	112
Bageshwar	4	16	58
Champawat	4	17	39
Nainital	7	21	63
Pithoragarh	8	29	91
Total	33	123	363

Information obtained was authenticated from knowledgeable elderly farmers particularly from women folks, who are considered as the manager of crop diversity in the area surveyed.

Results and Discussion

The cropping system in Kumaon Himalaya is built around two major seasons, *Kharif* (April to October) and *Rabi* (October to April). Rice is the major crop of *Kharif* season. It is grown in both conditions irrigated as well as rainfed. In the hills, practice of mixed cropping is very common, however rice is grown as mono crop. There are three different agro-ecosystems-rainfed locally known as *uprao*, irrigated locally known as *talao* or *sera* and marshy land locally called *simar* or *gazar*. Rice is grown in all the three agro-ecosystems in the entire hill region. A detailed inventory of local farmer named rice cultivars grown in different agro-ecosystem of Kumaon Himalaya is presented in Table 2. A total of 131 traditional and improved /HYVs have been documented in the present study, of which 73 are categorized as rainfed, 29 as irrigated and 29 as common. Common cultivars are also suitable for marshy areas (*simar*). Most of the cultivars 73 are grown under rainfed conditions. None of the improved varieties are cultivated in rainfed areas in the entire Kumaon region. In irrigated agro-ecosystem, there are 29 traditional cultivars grown by the farmers as against 10 improved cultivars. In earlier studies a total of 117 cultivars have been documented, of which 74 were categorized as rare, and 43 as common. Most of the cultivars were recorded in rainfed agro-ecosystems (Bisht *et al.*, 2007). In an ancient document the Gazetteer of North-Western provinces, a total of 48 traditional cultivars of rice were mentioned in Kumaon Himalayas (Atkinson, 1882). The present study reveals larger number of cultivars under cultivation in the region. It is mention worthy that the cultivars mentioned more than 100 years back are still under cultivation in the region. The study revealed that the rainfed (*uprao*) cultivation is a reservoir of traditional rice cultivars. Farmers have identified and selected the cultivars for different eco-niches, which atleast produce some yield in spite of adverse climatic conditions. That's why a large number of cultivars grown in the rainfed ecosystem. Local farming communities have maintained and conserved them for centuries. They have also acquired a good traditional knowledge in respect of these different varieties.

In irrigated agro-eco system 29 traditional local named varieties are grown as against 10 improved

Table 2. Rice cultivars grown and managed by the local farmers of Kumaon Himalaya in various agro-ecosystems

Agro-ecosystem(s)	Traditional cultivars	Improved cultivars
Rainfed agro-eco system (upraon cultivation)	<i>Dhurbasmati, Boran, Paktauli, Akadi, Kirmity, Banpas, Roti, Sontu, Jhumaria, Musmad, Hat, Makhur, Jawan, Masur, Telhat, Syont, Jungledhan, Jhadua, Nanbasmati, Thulbasmati, Ud, Parange, Jhakui, Bakul, Pingau kaludhan, Dangazai, Ram-Sita, Bakudhan, Dan naulia, Nauldudh, Giddha, Bamkua, Phamkua, Timilia Goldhan, Maildhan, Batasu, Gadai, Choria, Thai, Ram manua, Roti, Tawali, Sirmodia, Uskal, Lumadia, Ghesu, Timasia, Gadalu, Bhadgar, Sudia, Bhuria, Janoli, Madgar, Hayal Nalu, Jhusia, Matiya, Chamar, Lahangi, Jungloi, Dotiyali, Dhank, Rokhiyal, Khardudh, Tilasi, Pokhiya, Lal jaria Jhini, Bagesaria</i>	—
Irrigated agro-ecosystem (Talaon or Sera cultivation)	<i>Jiruli, Rajmati, Kapkoti, Dal badal, Rajula, Lali, Chhoti, bakul, Jama, Jorhat, kalyuri, katisal, Anjan, Siyud Kalajamali, Kashmiri, Jamali, Askotia Jyoli, Binduli, Parvati, Ramgarhi, Lohin, Sunkhar, Rajdhan, Sita, Musia, Lumadia, Pandudha</i>	Govind, China-4, Saket, Taichun, Native-8, IR-8, Indrasan, IR-24, VL-206, Pant-11
Common (grown in both rainfed and irrigated agro-eco-system) (suitable for Simar or Gazar cultivation)	<i>Thapachini, Dudh, Joliya, Gazai, Laldhan, Nandhani, Simanjari, Suntola, Sanwdhan, Ratdhan, Silkdhan, Patauli, Gazai, Kavdhan, Chhotia, Khazia, Chimbhuri, Basmati, Kalparia, Kalthuni, Sal Gadalu, Janoli, Pyolia, Ghini, Kalajaria, Jhusia kala, Jhusia safed, Ponthia</i>	Anjani, Mangraj

Table 3. Occupancy of rice cultivation area by different types of cultivars in different agro-ecosystems

Agro-ecosystem	Occupancy of area under improved cultivars (HYVs) %	Occupancy of area under
traditional cultivars (%)		
Rainfed agro-ecosystem	100.00	0.00
Irrigated agro-ecosystem	31.00	69.00
Marshy agro-ecosystem	84.00	16.00
Average	71.67	28.33

particularly in rice, based on certain characteristic features (Mehta *et al.*, 2008). Farmers varieties including crop populations, which farmers have identified and named as units (Lando and Mak, 1994). The large diversity of rice is managed by farmers as an adaptable strategy to cope with heterogeneous and uncertain ecological, socio-economic environment including different soil types (Das and Das, 2004).

In Kumaon Himalayas, farm holdings are marginal to small. These small pieces of fields are scattered over the entire village land and different eco-niches, which compels farmers to grow different cultivars for different type of eco-niches and soil types. As a strategy to produce atleast for their subsistence needs, farmers grow more number of varieties. The present study reveals that at different altitude levels, the number of varieties also differ. Rice is grown up to 2,200 msl in the hills. In the lower altitudinal areas, mostly irrigated land is used to grown improved varieties and in the higher reaches (above 2,000 msl) a limited number of varieties, which are adaptable in the cold climate are grown. Middle hills (between 1,001-2,000 msl), where both types of land (irrigated and rainfed) is found available for cultivation is found as the reservoir of a large number of varieties. Maximum an average of 19 varieties were found between 1,500-2,000 msl altitude followed by an average of 18.40 varieties between 1,001-1,500 msl. The largest number of varieties *i.e.*, 53 are grown in the villages situated between 1,501-2,000 msl in district Pithoragarh and followed by 45 varieties in the villages of district Almora situated between 1,001-1,550 msl (Table 4).

The study also reveals that the individual farm households grow and maintain atleast one or more rice landraces ranging between 1-9. Individual farm households grow and maintain an average of 3.08 different cultivars in Kumaon Himalayas. The highest average number of rice cultivars are maintained per household in district

varieties. Although 15% of the cultivable land under irrigation in Kumaon Himalaya and rest is under rainfed (Bisht *et al.*, 2007). Approximately 69% of the cultivable irrigated area is occupied by 12 high yielding varieties (Table 3).

Local cultivars, which are categorized as common are found very widely adapted in the region as they are suitable for both conditions rainfed as well as irrigated. An average 71.67% rice cultivation is occupied by traditional cultivars as against 28.33% by HYVs in the entire Kumaon Himalaya (Table 3).

Rainfed agro-ecosystem is completely dominated by traditional cultivars, whereas 84% of rice cultivation in marshy areas is dominated by these local cultivars. It is interesting to note that all the traditional cultivars under cultivation in the region are farmer named,

Bageshwar (4.19) followed by Champawat (3.28), Pithoragarh (3.12), Almora (2.59) and Nainital (2.25) Table 5.

Since the topography, geographical situations, altitudes, micro-eco-niches, availability of irrigation water vary from district to district, which attributes the variation in the number of cultivars under cultivation. In the region, heterogeneity of topography, altitude, water regime, environment, temperature, soil types etc. were observed to be very high. This heterogeneity has created a large number of micro-eco niches, which in turn are responsible for creation of a large number of local landraces/primitive cultivars to suit varied environments (Mehta *et al.*, 2008). As the HYVs are adaptable to a wide environment, they do not cater to the needs of the various micro-eco-niches prevailing in the region. Perhaps that is why a large number of farm households prefer to grow traditional cultivars inspite of introduction of HYVs.

Selection of varieties is also an important task of the farmers. Farmers use multiple criteria to select the varieties of their choice. The selection criteria is influenced by different socio-economic, geographical and technological factors, viz., performance of the variety with respect to agro-ecological conditions, uses of the output and input requirements. In hill farming system, use of input is very limited, seeds and farm yard manures are used as inputs. Assured yield, good straw quality, drought tolerance, quality of rice, etc. are the major selection criteria adopted by the farmers in this region. Farmers have identified and selected the rice varieties for different agro-ecosystems on the basis of their trials and observation. The varieties grown in rainfed areas are considered as drought tolerant (Table 2). Like wise the cultivars *Thapachini*, *Joliya* and *Chotia* are found good yielding. However, *Jamai*, *Thapachini* and *Bakua* are considered good in taste. Hill agriculture is interlinked with animal husbandry, hence fodder is also an important byproduct of crop yield. *Thapachini* and *Uskar* are good straw yielding varieties (Mehta *et al.*, 2008). Generally

good straw yielding cultivars are selected by the farming communities for cultivation in their fields. These varieties are under cultivation in Uttarakhand since time immemorial. Thus, the fact is that farmers have multiple criteria to select what varieties to plant as well as where, when and how to do it has been well established and reflect their concerns (Bellon, 1991; Brush *et al.*, 1981; Brush, 1992; Lando and Mak, 1994; Lambert, 1985; Sperling *et al.*, 1993).

Seed Flow and Exchange

The exchange of seed material is common historical pattern all over the world that currently continues, particularly with the introduction of modern varieties. Several studies have documented the flow of seed of different varieties among small farmers (Cromwell, 1990; Dennis, 1987; Loutte, 1994; Sperling *et al.*, 1993). These flows can happen within a village, a region, a country or even countries. It is evident from the survey that there was no formal system of seed exchange in the region, which was the limiting factor for continuous survival and on-farm management of local cultivars especially those grown by marginal farmers. However, the seed flow and exchange of varieties by using barter system is practiced in the region. Exchange of seed material within a village is observed very larger in comparison to outside of villages. Farmers preserve the seed material from their own harvest to be sown in the next cropping season. In Kumaon Himalayas, 79% of the seed material is arranged by the local farming communities from their own harvest, 16.2% seed material is collected from the neighbouring farmers, relative and friends. Only 4.8% seed material is procured from government or non-government seed agencies. It includes improved varieties developed by different research and development organizations (Table 5).

It was also observed in the study area that sometimes a married daughter carried the seed material from parental house to her-in-laws house and vice-versa. Similarly some service personnel like military men collect the

Table 4. Number of rice varieties grown by the farmers in different altitudinal gradients in Kumaon Himalaya

S.No.	Altitudinal gradients msl	Districts					Average number of varieties
		Almora	Bageshwar	Champawat	Nainital	Pithoragarh	
1	Up to 500	—	—	—	9	—	9.00
2	501-1000	28	—	—	11	03	14.00
3	1001-1500	45	16	10	9	12	18.40
4	1501-2000	11	10	18	03	53	19.00
5	Above 2001	—	17	04	—	08	9.67

Table 5. Average rice varieties grown and maintained per households in different districts of Kumaon Himalayas

S.No.	District	Range	Average rice varieties grown and maintained by per household
1	Almora	1-9	2.59
2	Bageshwar	1-6	4.19
3	Champawat	1-6	3.28
4	Nainital	1-4	2.25
5	Pithoragarh	1-9	3.12
Average varieties/household		3.08	

seed material from very far-flung areas. These varieties were known after the place or state of their collection. *Punjabi*, *Kashmiri*, *Kapkoti*, *Askoti*, *Ramgarhi*, *Jorhat* etc. are the cultivars introduced in the region and are performing well here since long. Introduction of these cultivars had enriched the rice diversity of the region and are flourishing very well in this Himalayan highland.

Seed Storage and Conservation System

Farmers, on the basis of performance and preference select the seed material of various varieties to be stored for the next cropping season. Generally each and every cultivar were selected for seed purposes. Selection of panicles for seed were done by old and experienced women folk from healthy crops fields. In order to ensure good germination only healthy and shining seeds were selected and harvested separately. The seed material was sun dried very carefully. Before storing, the moisture content of the seeds were tested by crushing them with teeth. By virtue of their age old knowledge of the viability of grains, healthy grains were selected and stored every season, thereby enhancing the genetic potential of the crop to withstand biotic and abiotic stresses (Ravishankar and Selvam 1998). To protect the seed material from insects, cow dung or oak wood ashes are mixed with seed material before putting them in the storage bins. For safe and secure storage, the leaves and rhizomes of Bach (*Acorus calamus*), Timur (*Zanthoxylum armatum*),

Bakayan (*Melia azadirachta*), Turmeric (*Curcuma longa*) were added in the dried seed material. These plant materials emit, a pungent smell, which act as repellent to insects. Storage of each cultivar was done separately to facilitate their identification and collection from specific fields. Generally seeds were stored in the containers, wooden bins and bamboo/*ringal* made bins. Bamboo/*ringal* made bins were plastered with mud, cow dung, mustard cake, and cow urine. Before putting the seeds in the storage containers or bins, they were sun dried carefully. After putting the seed material in the storage containers/bins, they were made airtight by covering the mouth with straw and cloth plastered with cow dung, mud and cow urine. Such storage systems have served farmers as their own seed banks from centuries. An urgent need was felt to strengthen at the village level for *in-situ* (on-farm) conservation of crop diversity (Swaminathan, 1998). Traditional seed storage system and repositories conserve the genes, which are very conveniently adaptable in various agro-eco-niches of this Himalayan region.

The present study is an evidence from the detailed documentation of rice cultivars in Kumaon Himalaya. This information collected from the house hold levels or farmers plots may not be an appropriate measurement to analyze the diversity, but indicates a vast wealth of rice diversity available in the region. In this Himalayan region, it is observed that out side forces influencing cropping system and diversity are negligible. Local agro-eco-systems, micro-eco-niches, climatic conditions, soil types etc. are the various attributes, which compels farmer to adopt more and more different cultivars adaptable to different situations. Agriculture for subsistence is completely dependent on local cultivars. These cultivars are managed by local farming communities inhabiting in various agro-eco niches. Such types of agro-eco-niches are suitable for on-farm conservation of crop varieties. The region as a whole may acts as a reservoir of rice cultivars possesses various characteristic features.

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Table 5. Sources of seed material of local farmers of Kumaon Himalaya

S.No.	Source of material	Percentage of seed material (%)
1	Harvest from their own fields	79.00
2	Exchange of seed material from neighbours, relatives and friends	16.20
3	Procurement of seed material from government and non-government agencies	4.80

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Empowerment of Women Farmers through Value Addition on Minor Millets Genetic Resources: A Case Study in Karnataka

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Finger millet is an important dryland crop valued for food grain and straw. One of the striking features of finger millet is its resilience and ability to adjust to marginal agro-climatic conditions in terms of soil fertility, rainfall and other weather parameters. The grain is nutritious with balanced protein, higher calcium and iron and dietary fibre. This offers ample opportunities for developing and promoting nutritious utility products from finger millet. In view of this, a study was undertaken to enhance the income of finger millet growing farmers by increasing productivity and strengthening the capacity of farming community for value addition and marketing of value added products. The process involved selection of two improved varieties by farmer participatory approach, organizing several field enhancement trials in farmers' fields for three years, empowering farmers in quality seed production and supply conservation using village seed bank approach and leveraging Self Help Groups (SHGs) of farm women for value addition of grain and its marketing. The crop management, also involving the developmental departments, resulted in 30-35 per cent increase in yield. On-farm intercrop trials of finger millet with field bean or pigeonpea during three years indicated higher monetary returns in comparison with the farmers' practice.

The rural mobilization under this study focused on SHGs constituted by small and landless farmers of villages in Doddabomanahalli and B.R. Hills of southern Karnataka. The nutritional status of women of these villages revealed 60-70% nutrient adequacy with low Hb levels in 50% of them. The pattern of group savings and lending showed that most of the credit was used for improving the sustainability of their agriculture and subsidiary activities. Empowerment of women was undertaken through trainings based on the need assessment carried out in a participatory mode. The training was imparted for preparation of value added products of finger millet (malt and energy dense mix) quality control, labeling, marketing of the produce with attractive cost benefit ratio. Empowerment of SHG women through training and nutrition education had positive impact on leadership qualities, enhanced self confidence, skills to undertake value added products and their marketing, domestic consumption, income generation and all converging to improved nutrition and economic status.

Key Words: Women empowerment, Small millets, Genetic resources, Value addition, Self help groups, Nutrient intake, Dietary pattern, Socio-demographic profile

Introduction

The social and economic status of women is a reliable indicator of their empowerment in a society. There is an exhaustive list of goals to be achieved through self-help programme which empower women with respect to saving habits, credit for production and commercial purposes, opportunities for entrepreneurship, functional literacy, developing leadership qualities, gender sensitivity and awareness about the socio-political, economic and cultural issues. The empowerment of women through self-help groups (SHGs), a non formal cooperative organization would benefit not only the individual women but also the family and community as a whole through collective action for development (Holvoet 2005, Tesoriero 2006).

Empowering women needs a holistic approach to participate in decision making in the household, community

and local domestic sector and prepare women to take up leadership position in agricultural activities. SHGs in rural India are bringing a silent revolution not only in terms of providing access to micro credit to communities but also in contributing towards a greater sustainability in agriculture in various ways, including a better use of marginalized local agrobiodiversity. In this backdrop, the present work aimed at empowering SHGs through improved processing and value addition of finger millet, a species of high nutritional value but suffering from a status of neglect for research and development. The specific objectives of this study were i) to understand the social and economic empowerment of the women members of SHGs, ii) to assess the dietary pattern with reference to small millets and iii) to empower the women through interventions in training and value addition.

Materials and Methods

The methodology involved quantitative as well as qualitative assessment conducted in two villages of Kolar District in Karnataka State, namely, Doddabommanahalli and Seegenahalli from Chintamani Taluk and the work was carried out during 2008-2009 in the framework of a global project funded by the International Fund for Agriculture Development (IFAD) on neglected and underutilized species (Padulosi 2007). The work encompassed 8 SHGs, each group comprising of 19-20 women. The quantitative data was collected with the help of semi-structured interviews. The interviews' format included questions relating to the family income, savings and loan schemes available to SHG members. Secondary information was collected from books, ledger and registers maintained by SHGs. The data on socioeconomic status included the age, education level of family, family size, occupation, land holdings and family income. Dietary pattern was assessed by 24 hour recall method for 7 days. Sets of pre-standard vessels were used to obtain estimates of raw and cooked foods consumed by the subjects. Subsequently, the individual consumption of nutrients like energy, protein, fat, iron, calcium, thiamine, riboflavin and niacin were calculated using food consumption table and compared with the recommended dietary allowance (Gopalan *et al.*, 1996) and the adequacy of nutrients was calculated as per the method suggested by Thimmayamma (1987).

The training programmes of the project concentrated on processing and value addition methods for finger millet. Altogether, 120 SHG members were trained of which 40 were selected for detailed skill development in processing of value added products. Secondly, intensive value added product trials both on and off the campus were conducted to select the suitable products having commercial potential for income generation activities and to facilitate enterprise building by the SHG women. Further training was also imparted to these women on nutrition education, importance of value addition in food products, Hazard Analysis and Critical Control Point (HACCP), handling of milling unit, labeling and marketing of the product. The impact of the training programme on the empowerment of women was also assessed.

Results and Discussion

Socio-demographic Profile of Women Members of SHGs

The socio-demographic profile of the members of SHGs is presented in Table 1. The age profile indicated that the SHG members belonging to the age group of 26-35 years

formed the largest class (60%) followed by that of 36-40 years (26%). With regard to literacy, 24% of members were illiterate, 60% had primary school level literacy and only 2% had high school education. The occupation pattern indicated that majority of them were labours, largely involved in dairy and sericulture activities. Majority of the rural women were having two children (70%) and the family size was 2-4 members in 90% of the samples. The results also showed that 90% of families were nuclear.

Table 1. Socio-demographic profile of women members of SHGs

Variables	Category	Respondents	
		Number	Percentage
Age	21-25 Years	7	14
	26-35 Years	30	60
	36-40 Years	13	26
Education	Illiterate	12	24
	Primary	30	60
	Secondary	7	14
	High School	1	2
Occupation	Housewife	2	4
	Laborer	48	96
Type of family	Nuclear	45	90
	Joint	5	10
Family size	2-4 members	45	90
	5-7 members	5	10
Number of children	One	10	20
	Two	35	70
	Three and above	5	10

Dietary Pattern of SHG Families

The assessment of dietary pattern of SHG members showed that all the families were non vegetarian by habit. The food from animal sources was consumed twice in a week by 45.3% of the families. The common meal of the family was cereal for breakfast and finger millet dumpling with *dhal* (pulse) and vegetables for lunch and dinner. The commonly consumed fruits were banana, papaya and guava. Milk and milk product were used only for tea and coffee and not for consumption as such by children / pregnant/lactating women as they sell the most part of milk to dairy cooperatives.

Nutrient Intake

The mean intake of nutrients by SHG women, viz. energy, protein, fat, iron, thiamine, riboflavin and niacin, were below the recommended dietary allowances (RDA), except for calcium (Table 2). The statistical analysis of the difference in mean values of RDA and actual intake by t-test showed that the inadequacy was highly significant (1% level of probability). The inadequacy of micronutrients is attributed to inadequate intake of protective foods. The

adequacy of calcium is attributed to consumption of finger millet which has high calcium intake. The share of millets in the dietary energy and protein was 50%.

Table 2. Mean dietary pattern of SHG women

Nutrients	RDA	Nutrient intake Mean	SD	% Adequacy	't' value
Energy (kcal)	2225	1600	219	72	32.72**
Protein (g)	50	34	7.7	68	21.02**
Fat (g)	20	10.8	8.4	54	7.58**
Iron (mg)	30	19	4.2	63	17.23**
Thiamine (mg)	1.1	0.77	0.3	70	9.39**
Riboflavin (mg)	1.3	0.88	0.4	68	12.86**
Niacin (mg)	14	9.3	3.3	65	17.44**
Vitamin A (mg)	600	378	343	63	7.93**

** Highly significant

Empowerment of SHG Members through Training

After analyzing the socioeconomic and dietary intake of SHG members, interventions were undertaken by the team of the University of Agricultural Sciences (UAS), Dharwad aimed at empowering these groups through enhancement of their capacities in value addition and nutrition. The different training methods employed in these efforts and their degree of preference by SHGs are presented in Table 3.

Table 3. Training methods preferred by SHG women

Training method	Percentage of preference
Demonstration	98
Lecture with projected aids	91
Video film	90
Exhibition	80
Field visit	75

The training activities organized through the project included lecture-cum-demonstration related to value added products of finger millet involving also fruits and vegetables processing for home consumption. Finger millet is a dominant grain crop in the target areas. Major portion of the grain harvested is marketed without value addition and the rest is kept for domestic consumption. The training on value addition of finger millet in the village was aimed at enhancing the income from the marketed grain through value addition. The different SHGs identified finger millet malt and energy dense mix (*Hurihittu*) as the products for value addition and marketing. The nutrient content of the products which were promoted through these interventions ranged from 327-364 kcal of energy, 4-13 g protein, 1-12 g fat, 65-300 mg calcium and 1-5 mg iron, values that are on par with several products available in the market but costing about three times more.

Sale of the Value-added Products

The value added products, were first commercialized by the SHGs during the *Krishi Mela* (farmers' fair) festival held on 16-19 November, 2008 at the UAS Campus, Bangalore and then at Chintamani, where an event organized on 4 December 2008, by the University witnessed a large participation of both rural and urban people. As a result of increasing demand of the products, these are now sold in retail outlets of Chintamani, general stores and health care centers under the brand name established by the SHG (Shrinidhi Balaji and Seekal).

Economics of Value Addition in Finger Millet

The SHGs were trained in processing of finger millet, cleaning, milling, packaging and labeling. The University team provided the necessary technical and certification support as well as the market linkages. These products are being marketed at Rs.100 per kg. At this price, on an average, the net income generated by the SHG is substantial as shown in Table 4 for finger millet malt and Table 5 for finger millet based '*Hurihittu*'. The calculation of cost of production and income was made for 2400 kg final products in both the cases. This model was found scalable provided market demand is expanded with consistency in quality, timely delivery and suitable market promotion.

Table 4. Economics of ragi malt production

Particulars	Amount (Rs.)
Variable cost (raw material+ fuel+ labour + 10% interest)	126,000
Fixed cost (depreciation of equipment+interest)	1,846
Space rental (500/month)	6,000
Total cost of production 2400 kg/annum	133,846
	134,000 (rounded amount)
Gross income = $100 \times 2400 =$	Rs. 240,000
Net income = Gross income – total expenditure = Rs. 240,000 – 135,000 =	100,000
B/C ratio (benefit/cost) = $240,000/135,000 =$	1.7 (benefit of one rupee 70 paise for every rupee spent)

From these data, it is evident that as a result of the interventions, the skills, self confidence and leadership enhanced through the training courses played an important role in boosting the income generating activities pursued by their women members.

Table 5. Economics of ragi energy mix production

Particulars	Amount (Rs.)
Variable cost (raw material+ fuel+ labour + 10%interest)	138,000
Fixed cost (depreciation of equipment+interest	1,850
Space (500/month)	6,000
Total cost of production 2400kg/annum	145,850
	1,46,000 (rounded amount)
Gross income = $120 \times 2400 =$	Rs. 288,000
Net income = Gross income – total expenditure = Rs. 288,000 – 146,000 =	142,000
B/C ratio (benefit/cost) = $240,000/140,000 =$	1.97 (benefit of one rupee 97 paisa for every rupee spent)

Conclusions

The study shed more light on the role played by SHGs in promoting socio-economic empowerment of women in India through a more effective use of local biodiversity and its products. The analyses has confirmed the strategic role that such types of interventions can play in rural and urban situations where women are too often marginalized in income generation activities and decision making processes. As a result of capacity building interventions to enhance local skills in value addition, SHG women were able to generate substantial income and use this towards their own family welfare developments. The training interventions by the University played a strategic role by increasing self confidence of SHGs in undertaking small scale food processing of value added products of finger millet at the village and city level.

The ultimate empowerment of target groups, was the result of a larger multi-stakeholder effort which involved several players, such as Bioversity International, the M.S. Swaminathan Research Foundation and the University of Agricultural Sciences of Bangalore in participatory variety selection, provision of high quality seed, dissemination of best cultivating practices, supplying of processing/milling units, training and education courses on nutrition and food technology, public awareness campaigns which altogether contributed to substantial benefits by SHG women members in pilot areas. The other important message of these results was that it is indeed possible to improve peoples' livelihood using local crops, such as small millets, which have the potential to contribute to enhance both incomes as well as nutrition security of people, particularly in poor and marginal areas where they have recognized advantages in terms of higher adaptation and resilience.

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Weed Seeds Intercepted in *Trifolium* spp. Germplasm Imported from USA and Egypt

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A total of eighty three seed samples of red clover (*Trifolium pretense* L.) and berseem clover (*T. alexandrinum* L.) germplasm were imported from USA and Egypt, respectively through National Bureau of Plant Genetic Resources, New Delhi during 2007. While processing the consignment for quarantine clearance, a total of 17 species of weeds were intercepted, of which nine species are not yet reported from India, whereas, eight species are known to occur in different parts of the country. Five exotic weed species had a Weed Risk Assessment (WRA) score of more than 12 indicating their potential to become serious weed and fifteen weed species were found viable even after two and half years of storage at room temperature. The observations indicated that import of red clover and berseem clover seeds from USA and Egypt has the risk of introduction and dissemination of exotic weeds into India. The weed species intercepted, their weed potential and seriousness using Weed Risk Assessment (WRA) system are discussed.

Key Words: *Trifolium*, Plant Quarantine, Exotic Weeds, Weed Risk Assessment

Introduction

Plant Genetic Resources in the form of seeds or vegetative propagules etc. are regularly imported for research and crop improvement programmes in our country. However, such imports also lead to the introduction of weeds and other pests and thus, pose quarantine risk. Increasing trade and globalization coupled with liberalized policies further increase the risk of introduction of exotic weeds through bulk imports. This could lead to decrease in native biodiversity, reduced productivity of different ecosystems, reduced input-use efficiency and increased production cost. It is estimated that one-third of oilseeds, half of the food grains and an equal amount of pulses produced currently are lost due to weeds. The potential yield losses due to weeds could be as high as 65 per cent depending upon the crop, type of weed species, degree of weed infestation, and management practices (Yaduraju *et al.*, 2006). Some of the major alien invasive weeds that have entered into the country along with the movement of plant material etc. include *Chromolaena odorata* L., *Eichhornia crassipes* (Mart.) Solms, *Lantana camara* L., *Mikania micrantha* H.B.K., *Mimosa* spp., *Parthenium hysterophorus* L., *Salvinia molesta* D.S. Mitchell, etc. (Yaduraju *et al.*, 2003). The environmental and socio-economic impacts of harmful invasive alien plant species are evident in case of alien aquatic weeds like water hyacinth (*E. crassipes*), water lettuce (*Pistia stratiotes* L.) and terrestrial weeds such as *L. camara*, *P. hysterophorus* and *Phalaris minor* Retz. (Rana *et al.*, 2003). The Central Government had imported nearly 63 lakh metric tones of wheat during 2006-07 from different countries in which 25 weed species were intercepted. The

National Invasive Weed Surveillance (NIWS) team has traced five invasive weeds which came to India through this wheat import (Joseph John, 2009).

National Bureau of Plant Genetic Resources (NBPGR), New Delhi is the nodal agency that facilitates exchange of plant germplasm meant for research between India and different countries. It has the power vested by the Plant Protection Adviser to the Government of India, under the Plant Quarantine (Regulation of Import into India) Order 2003, of the Destructive Insects and Pests Act (1914) to carry out quarantine of the plant germplasm including transgenic imported for research purpose. In order to ensure effective implementation of Plant Quarantine (Regulation of Import into India) Order 2003, it is essential that all imported seed samples are free from weeds of quarantine importance. Therefore, all samples of red clover and berseem clover were examined for the presence of weed seeds especially to determine the presence of quarantine weeds. The present study highlights the interception of weeds from imported germplasm of red clover and berseem clover from USA and Egypt and assessment of their potential risk through Weed Risk Assessment (WRA) system.

Materials and Methods

A total of 83 samples of red clover (77) from USA and berseem clover (6) from Egypt were processed at NBPGR for quarantine clearance during 2007. All seed samples were visually examined under higher magnification for detection of weed seeds. Each sample was spread in a thin uniform layer on a clean white drawing sheet and examined under illuminated magnifier (IMG50 with

magnification 6X) for separating the weed seeds. They were segregated into different types on the basis of their shape, size, colour, texture, etc. and observed under stereoscopic binocular (Wild (Heerbrugg) M5-86173, magnification 10X). Identification of weed seeds was done upto species level based on their morphological characters using Identification Keys (Anonymous, 1998) and with the help of Weed Seed Identification Kit developed by Academy of Grain Technology, Australia (Anonymous, 1997). Three weed species namely *Amaranthus retroflexus* L., *Chenopodium album* L. and *Panicum texanum* Buckl. were subjected to grow out test in glass house in isolation and identified on the basis of their vegetative characters (Nayar and Pandey, 2009) and subsequently plants were incinerated. All weed seeds were tested for their viability by wet blotter method in *Petri* dishes under strict quarantine conditions in incubation room at 21°C temperature and germination was counted on eighth day. The weed species were also assessed through Weed Risk Assessment (WRA) system for their weed potential and seriousness (Moolchand *et al.*, 2001). Weed Risk Assessment is a question based scoring system, containing 49 questions about the species. The questions include details of the plant's climatic preferences, biological attributes, reproduction and dispersal methods (Table 2). A minimum number of

questions must be answered before an assessment is made. The WRA uses responses to the questions to generate a numerical score that is positively correlated with weediness. The plants which have score between 0-6 are non weeds, 7-11 are common weeds and those having >12 score, are serious weeds (Groves *et al.*, 2001).

Results and Discussion

The results indicated that out of 77 samples of *T. pretense* imported from USA, 26 samples were found to be contaminated with 12 weed species. Similarly out of six samples of *T. alexandrinum* imported from Egypt, all samples were found to be contaminated with five weed species. Weed species intercepted from USA included *Amaranthus retroflexus* L., *Carrichtera annua* L., *Chenopodium album* L., *Ipomoea hederacea* L. (Jacq.), *Medicago sativa* L., *Panicum texanum* Buckl., *Phalaris paradoxa* L., *Polygonum convolvulus* L., *P. hydropiper* L., *P. persicaria* L., *Trifolium microcephalum* L. and *T. variegatum* L. while those from Egypt included *Cichorium intybus* L., *C. spinosum* L., *Rumex crispus* L., *R. pulcher* L. and *R. solicifolius* Weinm. (Fig. 1). Among 17 weed species intercepted, nine species such as *C. annua*, *P. paradoxa*, *P. persicaria*, *T. microcephalum*, *T. variegatum*, *C. spinosum*, *R. crispus*, *R. pulcher* and

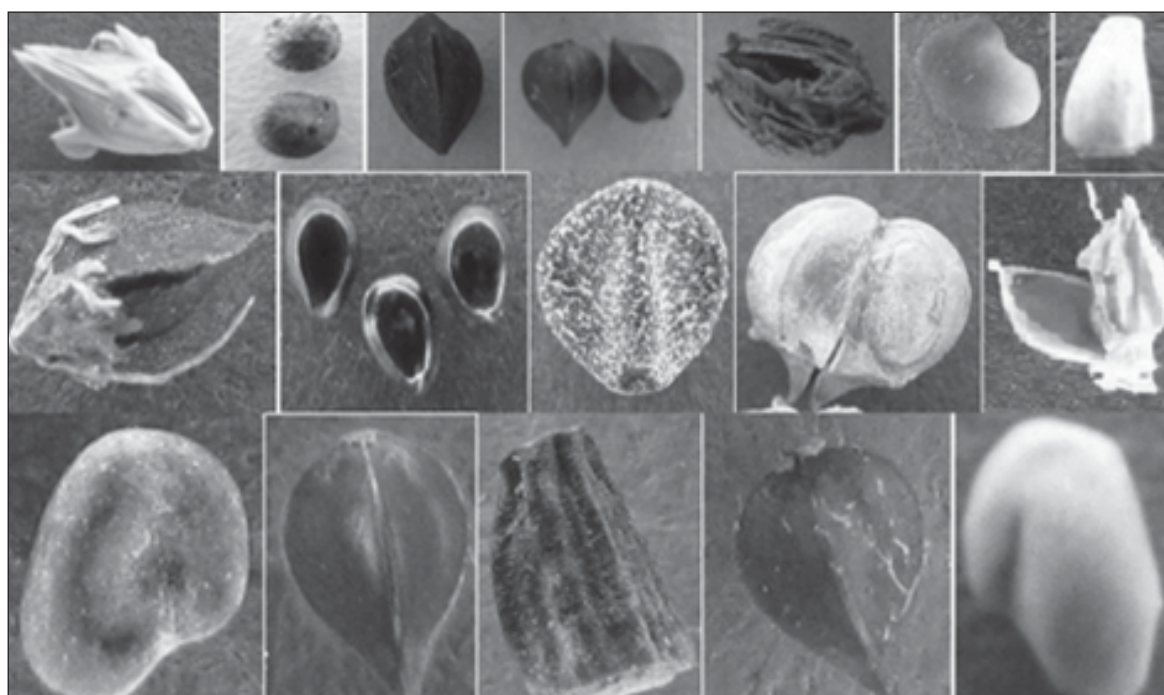


Fig. 1: Weed species intercepted: (First row from left to right) *Phalaris paradoxa*, *Chenopodium album*, *Polygonum persicaria*, *Rumex pulcher*, *Polygonum convolvulus*, *Trifolium microcephalum*, *Cichorium intybus* (second row from left to right) *Polygonum hydropiper*, *Amaranthus retroflexus*, *Ipomoea hederacea*, *Carrichtera annua*, *Panicum texanum*, and (third row from left to right) *Trifolium variegatum*, *Rumex crispus*, *Cichorium spinosum*, *Rumex solicifolius* and *Medicago sativa*.

R. solicifolius are not yet reported from India whereas, eight species occur in different parts of the country (Holm *et al.*, 1979). Fifteen weed species were found viable even after long storage of seeds. Five of these had a WRA score of >12 indicating their high potential to become serious weed in agriculture (Groves *et al.*, 2001). Out of 12 weed species intercepted in red clover seeds from USA, six species namely *A. retroflexus*, *C. album*, *P. paradoxa*, *P. convolvulus*, *P. hydropiper* and *P. persicaria* have developed multiple resistance against herbicides in USA (Moss, 2002). If these weeds are introduced into India, they may cause havoc to Indian agriculture. Our farmers are already struggling with *Phalaris minor* weed in wheat crop as it has developed resistance against isoproturon herbicide (Malik and Singh, 1995). Gradual decrease in the efficiency with the development of resistance have turned into deeper problem resulting in crop failure at many farms (Malik, 1995). Bhan and Williams (2006) reported that grain yield of wheat was reduced by 14.8% with *P. minor* and 17.4% in competition with *P. paradoxa*. This indicates that *P. paradoxa* is a more aggressive competitor with wheat than *P. minor*. Weed seeds identified in the imported germplasm, their viability and WRA score are given in Table 1.

The observations indicated that import of *T. pratense* and *T. alexandrinum* seeds could be source of introduction of exotic weeds into India. The WRA scores reveal that 14 species have potential to become serious weed in agriculture. It is evident that seeds of fifteen weed species were viable even after two and half years of storage at normal temperature indicating their ability to grow and spread under field conditions. All the weed seeds were removed from *T. pratense* and *T. alexandrinum* seeds by mechanical cleaning and only weed free samples were released and made available to the importer.

In order to prevent introduction of weeds, particularly the ones that are problematic in related countries need to be subjected to Weed Risk Assessment. Weeds seeds in imported samples could be serious threat to the country if not detected. Relatively unknown potential of these spreading to larger areas may turn to noxious and warrant study of their effect on the ecosystems besides effect on human and animal health. Critical quarantine examination of all the imported seeds is necessary to check the introduction of new weeds into the country. There is also an urgent need to design safeguards and strengthening of quarantine regulations to lower the risk of their entry.

Table 1. Weed seeds intercepted, number and %age of contaminated samples, germination (%) and Weed Risk Assessment (WRA) score

Weed species intercepted	Common name	Family	Average contamination % (Number basis)	No. of contaminated samples	% of contaminated samples	Germination (%)	WRA score
Red clover seeds from USA							
<i>Amaranthus retroflexus</i>	Pigweed	Amaranthaceae	02	11	14.28	45	07
<i>Carrichtera annua</i> *	Wards weed	Brassicaceae	03	06	07.79	62	08
<i>Chenopodium album</i>	Lambsquarters	Chenopodiaceae	04	14	18.18	00	06
<i>Ipomoea hederacea</i>	Ivyleaf morning-glory	Convolvulaceae	01	05	06.49	40	13
<i>Medicago sativa</i>	Alfalfa	Papilionaceae	02	03	03.89	72	00
<i>Panicum texanum</i>	Texas millet	Poaceae	01	07	09.10	82	13
<i>Phalaris paradoxa</i> *	Hood canarygrass	Poaceae	01	10	12.98	32	14
<i>Polygonum convolvulus</i>	Black bindweed	Polygonaceae	02	08	10.38	26	10
<i>P. hydropiper</i>	Common smartweed	Polygonaceae	02	12	15.58	28	09
<i>P. persicaria</i> *	Ladysthumb	Polygonaceae	01	04	05.19	30	08
<i>Trifolium microcephalum</i> *	Big-flowered clover	Papilionaceae	01	03	03.89	68	06
<i>T. variegatum</i> *	Whitetip clover	Papilionaceae	01	05	06.49	59	08
Berseem clover seeds from Egypt							
<i>Cichorium intybus</i>	Chicory	Asteraceae	06	06	100.00	80	07
<i>C. spinosum</i> *	Spiny chicory	Asteraceae	03	06	100.00	74	15
<i>Rumex crispus</i> *	Curly dock	Polygonaceae	02	06	100.00	00	14
<i>R. pulcher</i> *	Fiddleleaf dock	Polygonaceae	03	05	83.33	20	08
<i>R. solicifolius</i> *	Willow-leaved dock	Polygonaceae	02	04	66.66	32	09

* Weeds not yet reported from India

Table 2. Specimen of Weed Risk Assessment (WRA) question sheet

Answer yes (y) or no (n), or don't know (leave blank or ?), unless otherwise indicated

		Botanical name: Common name: Family name		Outcome: Score: Your name:
				History/Biogeography
A	1	<i>Domestication/ cultivation</i>	1.01	Is the species highly domesticated? If answer is 'no' go to question 2.01
C			1.02	Has the species become naturalized where grown
C			1.03	Does the species have weedy races
	2	<i>Climate and distribution</i>	2.01	Species suited to Indian climates (0-low; 1-intermediate; 2-high)
			2.02	Quality of climate match data (0-low; 1-intermediate; 2-high)
C			2.03	Broad climate suitability
C			2.04	Native or naturalized in regions with extended dry periods
			2.05	Does the species have a history of repeated introductions outside its natural range
C	3	<i>Weed elsewhere</i>	3.01	Naturalized beyond native range
E			3.02	Garden/amenity/disturbance weed
A			3.03	Weed of agriculture/horticulture/forestry
E			3.04	Environmental weed
			3.05	Congeneric weed
				Biology/Ecology
A	4	<i>Undesirable traits</i>	4.01	Produces spines, thorns or burrs
C			4.02	Allelopathic
C			4.03	Parasitic
A			4.04	Unpalatable to grazing animals
C			4.05	Toxic to animals
C			4.06	Host for recognized pests and pathogens
C			4.07	Causes allergies or is otherwise toxic to humans
E			4.08	Creates a fire hazard in natural ecosystems
E			4.09	Is a shade tolerant plant at some stage of its life cycle
E			4.10	Grows on infertile soils
E			4.11	Climbing or smothering growth habit
E			4.12	Forms dense thickets
E	5	<i>Plant type</i>	5.01	Aquatic
C			5.02	Grass
E			5.03	Nitrogen fixing plant
C			5.04	Geophyte
C	6	<i>Reproduction</i>	6.01	Evidence of substantial reproductive failure in native habitat
C			6.02	Produces viable seed
C			6.03	Hybridises naturally
C			6.04	Self-fertilisation
C			6.05	Requires specialist pollinators
C			6.06	Reproduction by vegetative propagation
C			6.07	Minimum generative time (years)
A	7	<i>Dispersal mechanisms</i>	7.01	Propagules likely to be dispersed unintentionally
C			7.02	Propagules dispersed intentionally by people
A			7.03	Propagules likely to disperse as a produce contaminant
C			7.04	Propagules adapted to wind dispersal
E			7.05	Propagules have dormancy
E			7.06	Propagules bird dispersed
C			7.07	Propagules dispersed by other animals (externally)
C			7.08	Propagules dispersed by other animals (internally)
C	8	<i>Persistence attributes</i>	8.01	Prolific seed production
A			8.02	Evidence that a persistent propagule bank is formed (>1 yr)
A			8.03	Well controlled by herbicides
C			8.04	Tolerates or benefits from mutilation, cultivation or fire
E			8.05	Effective natural enemies present in India

A= agricultural, E = environmental, C= combined

Source: Modified from Australian WRA Model (Groves *et al.*, 2001)

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Dr R.K. Arora: Obituary

(1932-2010)

Dr. Rajeshwar Kumar Arora, an eminent scientist in the field of plant genetic resources (PGR) and former Head of Exploration Division and Officiating Director, National Bureau of Plant Genetic Resources (NBPGR) passed away on 3 March 2010. Dr. R.K. Arora made significant contributions in the field of germplasm collection, use and management of PGR and systematics, taxonomy, ecology, phytogeography and ethnobotany of crop plants, their wild relatives and underutilized species native to the Indian region. He was awarded the Dr. Harbhajan Singh Memorial Award in 1998 instituted by the Indian Society of Plant Genetic Resources, New Delhi for his contributions towards the collection and study of plant genetic resources of India over a period of four decades.



heteroclita, dual purpose grasses *Digitaria cruciata*, rice bean and wild *Vigna* from north-eastern region. He also participated in exploratory trips for collection of vetiver with desirable oil quality from Rajasthan and Uttar Pradesh and the Chota Nagpur plateau and Western and Eastern Ghats for search and collection of progenitor wild species of native legumes such as green gram, black gram and pigeon pea. Dr. Arora also undertook collection missions in Africa and travelled widely in his capacity as expert in plant genetic resources to USSR, Indonesia, New Zealand, etc. He subsequently became the Officiating Director of NBPGR

(1987-88) before leaving he took a lead in coordinating PGR programmes for South Asian region and joined as Associate Coordinator (1989-1991)/ Coordinator (1992-1998) at the Regional Office for South Asia, New Delhi of the International Board of Plant Genetic Resources (IBPGR) later designated as the Bioversity International Sub-regional Office for South Asia, New Delhi (erstwhile IBPGR/ IPGRI). Here he was associated with promoting conservation and use and coordinating diverse activities and projects on of tropical fruit species in Asia. After retirement he continued to work as Honorary Fellow in the Bioversity International Office up to June 2009.

Dr. Arora was born in Kamalia, Lyallpur (presently Faisalabad in Pakistan) on 14 December 1932. He completed his graduation and post-graduation from Panjab University, Chandigarh and began his career as Assistant Ecologist (1960-1964) and Systematic Botanist (1964-1967) at the Botanical Survey of India, Calcutta after completing his Ph.D. in 1961 on the flora of Western Ghats. He joined the Indian Council of Agricultural Research as Senior Scientist in the Division of Plant Introduction, Indian Agricultural Research Institute (IARI), New Delhi in 1968, where besides survey and collection of germplasm he was also involved in teaching systematic botany and economy botany (1968-76). Dr. Arora headed the Plant Exploration Division (1976-1982; 1986-1987) of the NBPGR (erstwhile Plant Introduction Division, IARI) and undertook over 30 exploration and collection trips to remote areas with high diversity viz. cold-tolerant wheat and barley land races from the Pangri Valley in Himachal Pradesh, fodder grasses and wild legumes from Zaskar and Kargil in Jammu and Kashmir, and *Coix lacryma-jobi*, *Moghania heterophylla*, *Hodgsonia*

Dr R.K. Arora was a prolific writer, and in addition to over 160 publications in national and international journals, he authored/ co-authored/ edited nearly twenty books on PGR of the Indian region among which are the Wild Edible Plants of India in 1978 (revision with emphasis on conservation and utilization in 1996), Wild Relatives of Crop Plants in India in 1984 and Genetic Resources of Less-known Cultivated Food Plants published in 1985. Dr. Arora was the founder Editor-in-Chief (1987-88) of the Indian Journal of Plant Genetic Resources (IJPGR) published by the Indian Society of Plant Genetic Resources (ISPGR), and also headed the team involved in the

publication of the Annual Report, Research Highlights, Newsletter and other institute publications of the NBPGR (1978-1985). Dr. Arora also instituted the best paper award under the aegis of ISPGR in December 2009.

His scientific contributions led to his election as Fellow of several scientific bodies such as the National Academy of Agricultural Sciences (NAAS), India, Indian Ethnobotanical Society and the Indian Botanical Society. He was also on the Board of Trustees of TAAS (Trust for Advancement of Agricultural Sciences), New Delhi. He was awarded with Dr. J. W. Harshberger Medal for 1996 instituted by the Society of Ethnobotany of India, Lucknow for his significant contributions in field of ethnobotany.

Dr. Arora, in spite of his vast experience and expertise in various spheres of activities relating to PGR, was an unassuming person. He was able to execute and complete a large number of assignments and academic works by literally 'burning the midnight oil'. When working on projects of a collaborative nature he contributed a 'lion's share'. He made his collaborators and co-workers aware of their strengths and qualities and provided a lesson and mutual benefits. His demise is a major loss to plant science in general, and PGR in particular, as he was undoubtedly a doyen in this field.

Drs. E. Roshini Nayar and Anjula Pandey
(Principal Scientists, Division of Plant Exploration
and Germplasm Collection, NBPGR, New Delhi)

A Tribute to Dr. R.K. Arora

In the sad demise of Dr. R. K. Arora, destiny has snatched from India, rather South-East Asia one of the ablest and devoted explorers of plant genetic diversity. He botanised from hot dry deserts of Rajasthan to dense evergreen forests of north-east and east India, and from cold & dry regions of Leh-Ladakh to humid evergreen forests far south in pennisular India. Dr. Arora's hard and scholarly work on collection study and analysis of land races and wild relatives of crop plants contributed very largely to our knowledge of India's biodiversity, conservation of valuable germplasm and significant improvement in many economic crops in India and other countries. His scientific conclusions have remained undisputed.

My association with Dr. Arora dates back to middle of last century when he worked as Research Scholar in the Botanical Survey of India (BSI), at Pune; he accompanied me in several field trips in North and South Canara in the Western Ghats and coastal Maharashtra. Later, he joined the BSI and our association became closer when we lived in different floors in the same building at Calcutta. We spent long evenings discussing plans of field work, seminars, conferences and publications. Even after he moved to ICAR we regularly met. As a member of the Quarterly Review Team of the NBPGR during 1980s it pleased us greatly to see the outstanding work, organisation and guidance which he provided to his

colleagues as a senior scientist and later Head of NBPGR. Dr. Arora had excellent communication skills, and his lectures and writing received wide acclaim. He was a perfect gentleman, always cool with a pleasant smile. He shunned publicity and advertisement; but his excellent work attracted attention of international bodies and the FAO and IBPGR invited him for several consulting assignments and later made him the head of the Regional South-East Asia office of IBPGR at Delhi. He served with the IBPGR till his failing eyesight and other health problems intervened.

This tribute will not be complete without a few words of sincere admiration for his very supportive wife, Smt Krishna Arora, who happily allowed his frequent long absences for field work or assignments as Consultant; she not only single-handedly managed the house but also ably organised the education and development of their two loving children. Hundreds of friends share the grief of the bereaved family. Dr. R.K. Arora still lives in the immortal work which he did for the study and conservation of plant genetic resources.

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