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# Studies on *Vigna umbellata* Land Races from NE India for Nutritional Quality and Characterization through Seed Protein Profiling

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Thirty land races of the seed legume *Vigna umbellata*, collected from the hilly state of Nagaland, North-East India were screened for nutritive value. Crude protein content varied from 14.66% to 26.88%. Total carbohydrate varied from 44.3% to 57.6%; ash content from 3.6% to 6.32%. Lipid content varied from 1.0% to 2.04% and crude fibre varied from 3.8% to 4.4%. Calorific values were in the range of 271.04 kcal/100 gm to 316.04 kcal/100 gm. SDS-PAGE analysis of seed protein revealed a total of 35 protein bands. Dendrogram revealed the presence of four major clusters. Diversity was evident from the fact that while RB-27, 28, 29 and 30 had 21 protein bands each, RB-11 had only 6 protein bands. Our study indicates that rice bean is a promising pulse crop for future because of its wide adaptability and high nutritive values.

**Key Words: Rice bean, *Vigna umbellata*, Nutritive value, SDS-PAGE, Seed protein**

## Introduction

Search for new pulse crops is gaining momentum and getting priority because conventional pulses are stagnating in productivity and not in a position to meet the ever growing demand. Land races represent the natural gene pool for a crop species from which superior or desired cultivars are sorted out for use in genetic improvement programs. In India, the hilly states of North-East, particularly Nagaland, is the home for many little known, underutilized but promising seed legumes, the foremost among which is *Vigna umbellata*, referred to as rice bean with large number of land races which are yet to be assessed properly. In Nagaland the villagers have been cultivating rice bean for centuries as single crop or as mixed crop with maize. Most villages are isolated and land locked which help in perpetuation of a large number of land races over centuries. Grain legumes or pulses are the primary sources of plant protein for millions of people in tropical and sub-tropical countries and in countries like India and pulses are integral part of diet for rich poor alike. In view of the growing concern for nutritional security study of non-conventional grain legumes deserve priority since it is a foregone conclusion that animal protein can not cater to the needs of growing population in underdeveloped and developing countries. In a country like India which is the biggest producer as well as biggest importer of pulses, production of pulses has been stagnating for the last half a century (Gadgil *et al.*, 1999). On the other hand since population is steadily increasing, per capita grain availability has been dropping alarmingly. Considering a recommended requirement of 40 gm pulses per day/person, India's projected requirement for 2010 is 22.61 m tonnes and for 2020 the requirement is 26.76 m tonnes. But even in the initial years of 21st century India's pulses production have been about 13 m tonnes or below except in 2003-04 when it was 15.23 m tonnes. Two main reasons are cited for the stagnation of pulses production. Most pulse crops are recalcitrant and have limited genetic variability which make genetic improvement difficult. Secondly, pulse crops require specific agro-climate and soil type for which further expansion of pulse growing area is not possible (Asthana, 2000).

In this context *Vigna umbellata* (Thunb) Ohwi and Ohashi assumes great importance for its nutritional quality, high grain yield and multipurpose utility as feed, fodder, cover crop and green manure. Moreover, unlike most conventional pulses it can be grown under wide range of agro-climatic condition and soil type (Chandel, 1980). The present study was undertaken to collect and evaluate the land races of Nagaland with respect to major nutritive values and their characterization through seed protein profiling in an effort to short list the promising land races for future and further works.

## Materials and Methods

Seeds of different land races of rice bean were collected through visit to various villages of the hills of Nagaland of North-East India. The altitudes of collection areas were in the ranges of 300 msl to 1700 msl. Under field condition of Nagaland rice bean mature and become ready for harvest in the month of November. The land races were

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primarily based on seed colour and morphology. Collections were made from farmers field as well household granaries and catalogued as RB–1, RB–2 etc. A total of 30 land races were collected and grown in the experimental garden of the School of Agricultural Sciences and Rural Development, Medziphema (altitude 305 msl), where climate is sub- tropical, mostly humid with moderate temperature and medium to high rainfall. The land races were grown in randomized blocks with three replications. All the land races were climbers and bamboo poles were erected to give mechanical support. The mature seeds were harvested in the month of November.

Chemical analysis for nutritional components was done on dry weight basis. For this the sample was finely grounded and dried in an oven at 60°C till constant weight was recorded. Crude protein was estimated using microkjeldahl method. Total carbohydrate of the grains was determined using the method described by Clegg (1956). Lipid content was estimated by extracting the sample with petroleum ether in a Soxhlet Apparatus for 8 hours. Subsequently the solvent was evaporated away to get the lipid as residue. Crude fibre of the grains was determined as per the method of Sadasivam and Manickam (1996). For ash content the sample was ashed in a muffle furnace at 600°C for four hours and the difference in weight was recorded from which ash content was calculated. Three replications were made for each sample. Calorific values were computed as per the formula given by Sherman (1952).

Seed protein profile for the cultivars were analysed by SDS-PAGE technique as outlined by Laemmli (1970). The seeds were washed with distilled water and then blotted dry. 200 mg of seed sample were grounded in a pre-chilled morter with ice cold 0.5 M Tris buffer, pH 6.5. The extract was centrifuged in a refrigerated centrifuge at 8000 rpm for 10 minutes at 4°C. Sample weight to extract volume was adjusted to 1:5 ratio and the seed protein was resolved in 15% polyacrylamide gel. From the protein profile similarity indices were generated using Nei and Li co-efficient (Nei and Li, 1979). The similarity matrix was used to generate the dendrogram by UPGMA using NTSYS pc.v.2.02j.

**Results and Discussion**

Considerable variability were observed with respect to protein content, which was evident from the fact that protein content varied from 14.66% in RB-14 to 26.88% in RB-16 (Table 1). Frequency distribution analysis showed that 7 land races had 17% protein; 5 land races had 21% while 4 land races had 18% protein (Fig. 1). Thus the variation among the land races has been found to be highly significant. Among the major nutritional components, total carbohydrate constituted the major fraction and like protein, carbohydrate content also exhibited considerable variation. This is evident from the fact that it varied from 44.3% in RB-16 to 57.6% in RB-17. However, 23 land races out of 30 in the present study had 50% or more total carbohydrate. Among the major nutritional components of the grain, lipid occurred in the lowest quantity. Unlike crude protein and carbohydrate, lipid content did not exhibit appreciable variation. The range of variation was from 1.0% in RB-7, 13, 18 and 30 to 2.04 in RB-5 with the exception of RB-16 with 0.8%. Total minerals in the form of ash content exhibited appreciable variation. RB-12 with 3.6% recorded lowest while RB-16 with 6.32% recorded highest ash content. Among all the nutritional components, crude fibre exhibited least variation as evident from the range of variation which was 3.8% to 4.4%. Total calorific value exhibited significant variation and varied in the range of 271.04 kcal/100 gm in RB-14 to 316.04 kcal/100 gm in RB-29.

Seed protein profile exhibited remarkable variability. Total 35 protein bands were detected ranging in size from 109.0 to 13.5 Kd. Proteins with size range 109.0 to 59.5 Kd were considered as High Molecular Weight (HMW) proteins; those in the size range of 50.4 to 23.0 Kd were considered as Medium Molecular Weight (MMW) proteins and the remaining as Low Molecular Weight (LMW) proteins. Highest number of protein bands were observed for RB-27, 28, 29 and 30; each with 21 bands. Second highest number were recorded for RB-18, 22 and 23; each with 19 protein bands. Lowest number of protein bands were observed in RB-11 with 6 bands which include no low molecular weight protein. Proteins with medium molecular weight were comparatively more abundant. The protein with size 29 Kd exhibited highest frequency, being present in 25 land races. Proteins with size 33.6 and 26.2 Kd exhibited second highest frequency, being present in 20 land races. Lowest frequency was observed for the protein with 50.4 Kd which was detected in only 3 land races. Among the HMW group, the protein with size 91.8 Kd was the most frequent as it was present in 20 land races, followed by the protein 83.0 Kd which was present in 17 land races.

On the other hand, the protein with highest molecular weight of 109.0 Kd was found in only 4 land races.

**Table 1. Chemical composition of the grains of rice bean with respect to major chemical constituents. The values are in terms of gm/ 100 gm dry weight**

| Cultivars | Crude Protein | SD ± | Total Carbohydrate | SD ± | Lipid | SD ± | Ash | SD ± | Crude fibre | SD ± | Calorific Value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RB-1 | 21.69 | 0.743 | 48.00 | 1.212 | 1.33 | 0.030 | 5.46 | 0.039 | 4.04 | 0.091 | 290.73 |
| RB-2 | 20.22 | 0.590 | 47.46 | 1.103 | 1.73 | 0.027 | 4.02 | 0.045 | 3.90 | 0.100 | 286.05 |
| RB-3 | 17.02 | 0.094 | 54.86 | 0.928 | 1.50 | 0.000 | 3.90 | 0.036 | 4.10 | 0.075 | 300.78 |
| RB-4 | 17.17 | 0.392 | 50.03 | 1.049 | 1.70 | 0.062 | 4.00 | 0.0 | 4.33 | 0.065 | 283.98 |
| RB-5 | 19.43 | 0.478 | 48.83 | 0.987 | 2.04 | 0.057 | 5.11 | 0.040 | 4.38 | 0.120 | 291.28 |
| RB-6 | 17.11 | 0.346 | 54.00 | 1.154 | 1.04 | 0.038 | 4.03 | 0.0 | 4.05 | 0.083 | 293.44 |
| RB-7 | 16.41 | 0.297 | 57.20 | 1.016 | 1.00 | 0.033 | 3.85 | 0.050 | 4.28 | 0.0 | 303.44 |
| RB-8 | 15.57 | 0.438 | 57.60 | 0.930 | 1.60 | 0.025 | 3.76 | 0.047 | 4.30 | 0.100 | 307.08 |
| RB-9 | 18.07 | 0.363 | 55.06 | 1.396 | 1.31 | 0.018 | 3.90 | 0.040 | 4.00 | 0.062 | 303.98 |
| RB-10 | 17.17 | 0.124 | 53.66 | 1.262 | 1.42 | 0.021 | 3.90 | 0.036 | 4.13 | 0.071 | 295.68 |
| RB-11 | 21.33 | 0.453 | 53.04 | 0.887 | 1.14 | 0.0 | 5.20 | 0.057 | 3.90 | 0.083 | 307.22 |
| RB-12 | 17.86 | 0.392 | 53.25 | 1.016 | 1.60 | 0.064 | 3.60 | 0.0 | 4.06 | 0.111 | 298.64 |
| RB-13 | 19.85 | 0.407 | 53.87 | 1.622 | 1.02 | 0.053 | 4.04 | 0.0 | 4.22 | 0.080 | 303.60 |
| RB-14 | 14.66 | 0.085 | 50.40 | 1.098 | 1.24 | 0.018 | 3.88 | 0.039 | 4.31 | 0.076 | 271.04 |
| RB-15 | 16.74 | 0.127 | 56.41 | 1.452 | 1.66 | 0.042 | 4.26 | 0.047 | 4.14 | 0.0 | 306.96 |
| RB-16 | 26.88 | 0.381 | 44.30 | 1.085 | 0.80 | 0.0 | 6.33 | 0.053 | 3.81 | 0.068 | 291.92 |
| RB-17 | 14.73 | 0.356 | 57.60 | 0.864 | 1.66 | 0.045 | 4.00 | 0.0 | 4.42 | 0.070 | 303.72 |
| RB-18 | 19.64 | 0.524 | 57.00 | 1.272 | 1.03 | 0.042 | 4.41 | 0.054 | 4.31 | 0.096 | 315.56 |
| RB-19 | 18.98 | 0.247 | 52.63 | 1.201 | 1.54 | 0.025 | 3.86 | 0.046 | 4.27 | 0.102 | 299.82 |
| RB-20 | 18.56 | 0.103 | 56.28 | 1.621 | 1.36 | 0.027 | 4.39 | 0.0 | 3.93 | 0.069 | 310.74 |
| RB-21 | 20.23 | 0.624 | 46.80 | 0.990 | 1.30 | 0.023 | 4.93 | 0.040 | 4.00 | 0.110 | 279.82 |
| RB-22 | 23.74 | 0.235 | 48.22 | 0.959 | 1.43 | 0.040 | 5.60 | 0.043 | 4.03 | 0.091 | 300.71 |
| RB-23 | 21.06 | 0.367 | 46.81 | 1.047 | 1.63 | 0.025 | 5.40 | 0.066 | 4.11 | 0.080 | 285.84 |
| RB-24 | 23.58 | 0.478 | 50.60 | 0.827 | 1.58 | 0.035 | 3.81 | 0.042 | 4.00 | 0.078 | 310.22 |
| RB-25 | 22.30 | 0.416 | 53.26 | 1.048 | 1.33 | 0.020 | 4.04 | 0.060 | 3.84 | 0.097 | 313.70 |
| RB-26 | 17.01 | 0.609 | 54.80 | 1.567 | 1.40 | 0.0 | 4.10 | 0.0 | 4.04 | 0.073 | 299.84 |
| RB-27 | 21.77 | 0.556 | 52.80 | 0.927 | 1.77 | 0.047 | 5.12 | 0.063 | 3.92 | 0.062 | 313.58 |
| RB-28 | 21.70 | 0.117 | 45.02 | 0.990 | 2.02 | 0.0 | 5.30 | 0.050 | 4.21 | 0.0 | 284.80 |
| RB-29 | 18.56 | 0.103 | 56.41 | 1.101 | 1.81 | 0.023 | 4.13 | 0.0 | 4.03 | 0.116 | 316.04 |
| RB-30 | 17.32 | 0.107 | 57.88 | 0.991 | 1.00 | 0.0 | 4.30 | 0.044 | 3.81 | 0.060 | 309.48 |
| CD 5% | 1.19 | | 1.94 | | 0.25 | | 1.20 | | 1.12 | | 2.048 |
| CD 1% | 1.46 | | 2.59 | | 0.34 | | 1.60 | | 1.49 | | 2.759 |

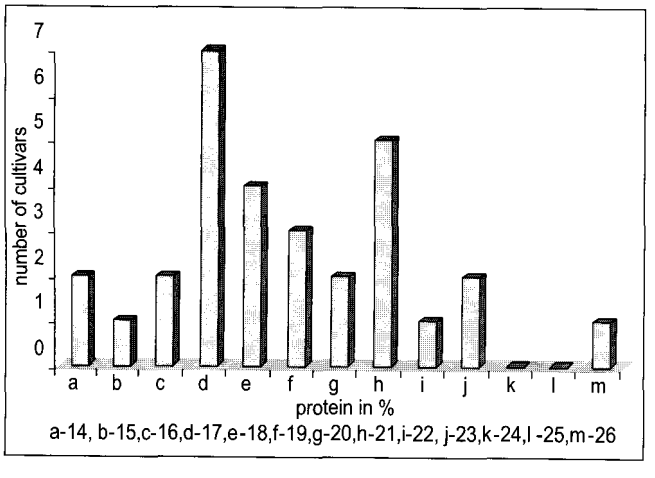


**Fig. 1: Frequency distribution for protein content among the 30 cultivars of rice bean**

Proteins with low molecular weight were less abundant in distribution. The most prominent among LMW proteins were the ones with molecular weight 22.4, 18.5 and 14.3 Kd; each of which were present in 11 land races. A protein band of 55.0 Kd was found in RB-28 which was not detected in others. Likewise another protein band of 15.5 Kd was found to be unique for RB-2.

Grain legumes are considered as a major source of plant protein and hence nutritional quality of a pulse crop is mainly judged on the basis of its protein content. In India the most commonly used pulses which are part of every day diet for majority population are lentil, mung bean, black gram, pigeon pea and chick pea and their average protein content are – 25.1, 24.5, 24.0% (Gopalan *et al.*, 1989); 22.9 and 22% (Chaturvedi and Ali, 2002),

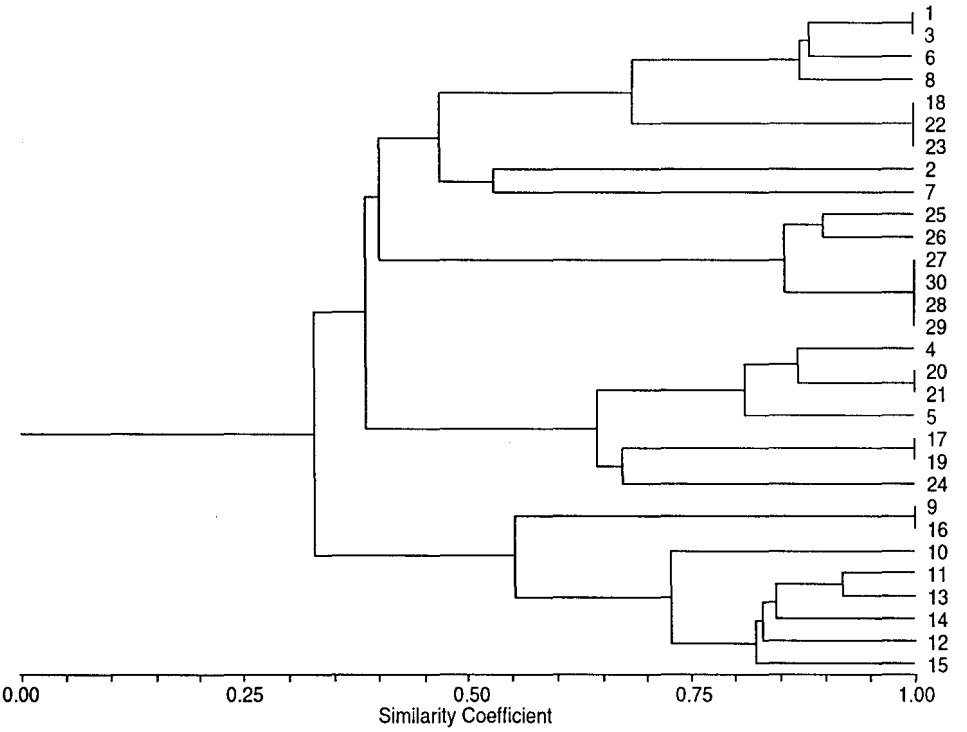


**Fig. 2: Dendrogram of the thirty rice bean cultivars based on seed protein profile**

respectively. The present study showed that while some land races were inferior, some were at par and at least one was superior to the conventional pulses in matter of protein content. Like protein wide range of variation was observed for total carbohydrate also which varied from 44% to 57%. In major pulses, carbohydrate content varies as – lentil 57.6%, black gram 46.3%, green gram 51.7% and chick pea 54.6% (Belitz and Grosch, 1999). In the present study, 5 land races had 57%; another 5 land races had 53% while only one land race had 44% carbohydrate. Therefore, most land races of rice bean are at par with the major pulses in matter of carbohydrate content. For major pulses total mineral in the form of ash content varies from 3.0% in Chick pea to 3.8% in Green gram (Belitz and Grosch, 1999). As against this ash content in the present study varied from 3.6% to 5.6% and as many as 8 land races were found to have over 5% ash content. It is therefore, clear that rice bean land races are superior to most conventional pulses with regard to ash content. Crude fibre for major pulses exhibit wide variation form 0.7% in Lentil to 4.1% in green gram and 5.3% in chick pea whereas the same varied from 3.8% to 4.4% among the rice bean land races. Lipid content among the land races were comparable to that of the major pulses and constituted the smallest and hence less significant nutritional component.

Seed protein profiling by SDS-PAGE has emerged as a reliable and powerful technique for resolving taxanomical and evolutionary problems; species and cultivar identification that are of great importance in many breeding studies (Naik and Kole, 2002). It has been demonstrated that composition of seed protein is highly constant and not influenced by environmental conditions, seasonal fluctuation and age of mature seeds (Ladizinsky and Hymowitz, 1979). In the present study, the land race RB-11 was characterized by only 6 protein bands while RB-27, 28, 29 and 30 were characterized by 21 protein bands, which reflect the degree of diversity among the land races studied. Dendrogram analysis revealed the presence of 4 major clusters and showed that while some land races were closely related, some others were distantly related which indicated the diversity prevalent (Fig. 2). On the other hand some land races viz. RB-27, 28, 29 and 30 were found to be same at molecular level in terms of seed protein profile although they appear different at morphological level. Seed protein profile has been successfully used by a number of workers for diverse purposes like characterization of cultivars, elucidating phylogenetic relationship and to develop molecular marker for specific trait and for identification of hybrid. Gomathinayagam and Ramaswamy (1994) used seed protein profile to find out interspecific and intraspecific

relationship in cow pea and its related species. Chandran *et al.* (2002) worked out the intraspecific relationship among 70 cultivars of ground nut by the same process. Panigrahi (2001) identified species specific seed protein for pigeon pea and its related species *Cajanus cajanifolius*. Chand and Kole (2002) identified a seed protein as molecular marker for genotype resistant to Cercospora leaf spot in Mungbean while Pattnaik and Kole (2002) identified similar molecular marker for identifying mungbean susceptible to Mungbean Yellow Mosaic Virus. Seed proteins are known to be controlled by multi gene family (De Lumen, 1990) and their Mendelian monogenic inheritance has been established (Panella *et al.*, 1993; Naik and Kole, 2002). The polymorphism in seed protein profile observed in the present study therefore indicate that high degree of genetic diversity exists among the land races of rice bean. The floral morphology of rice bean is akin to most other *Vigna* species where self pollination is the rule. In India's North Eastern state of Nagaland, rice bean has been cultivated by villagers in isolated villages in hilly terrain for centuries as part of subsistence agriculture. Under such condition selection pressure is less which may be a reason for wide genetic diversity. Because of its ability to grow in diverse geographical and climatic condition, the genetic diversity as well as diversity in nutritional parameters can be exploited to breed superior cultivars to make it a promising pulse crop for future.

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# Assessment of Genetic Diversity among Wild Accessions of *Abrus precatorius* L. using RAPD and ISSR Markers

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Genetic diversity was estimated among eight wild accessions of *Abrus precatorious* L. collected from different areas of Maharashtra, Orissa and West Bengal using Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) markers. Out of 40 random primers used, 28 were polymorphic, generating a total of 175 amplification products with an average of 6.25 products per polymorphic primer. Out of 35 ISSR primers, 20 were found polymorphic, generating a total of 81 amplification products with an average of 4.01 bands per polymorphic primer. Mean polymorphic information content values were 0.77 and 0.67 for RAPD and ISSR, respectively. Pair wise Jaccard's similarity coefficient values ranged from 0.357 to 0.740 for RAPD markers and 0.245 to 0.829 for ISSR markers, indicating high genetic variability among these genotypes. The accessions IC418115 from Thane and IC392840 from Mayurbhanj displayed the maximum genetic similarity. The cluster analysis discriminated all the accessions from Maharashtra in one group. The degree of genetic variation detected using RAPD and ISSR analysis among eight accessions suggests that these markers can be employed for genetic diversity studies in *Abrus precatorius*.

**Key Words: *Abrus precatorius* L., Genetic diversity, ISSR, Medicinal plant, RAPD**

## Introduction

The genus *Abrus* is a small pantropic genus (Isely, 1990) of the tribe Viceae of Papilionoidae-Leguminosae (Hutchinson and Dalziel, 1958). It grows in tropical climates such as India, Sri Lanka, Thailand, the Philippine Islands, South China, tropical Africa and the West Indies. *Abrus precatorius* L. is a wild plant that grows best in fairly dry regions at low elevations. *Abrus precatorius* is an important medicinal plant of India that holds a reputed position in Ayurvedic System of Medicine. The leaves of *Abrus* contain glycyrrhizin, triterpenol saponins, flavonol glycosides, isoflavoquinones, abrine etc; (Windholz, 1983). The seeds of *Abrus* have high protein content, *i.e.*, 17-20 mg/ml (Gupta *et al.*, 2006a), carbohydrates, lipids, saponins, and sterols; seeds are also abortifacient, anodyne, aphrodisiac, antimicrobial, diuretic, emetic, expectorant, febrifuge, hemostat, laxative, purgative, refrigerant and sedative. Vermifuge paste of seeds is applied locally in sciatica, stiffness of shoulder joints and paralysis. Roots are used for gonorrhoea, jaundice and haemoglobinuric bile (http://www.motherherbs.com/abrus-precatorius.html). The oil extracted from seeds promotes the growth of human hair. The seeds of *Abrus* under the name of *Rati*, weighing about 1 carat each, have been used in India since ancient times for the purpose of weighing gold (http://www.botanical.com).

There is an urgent need to look for new sources of varieties to widen the genetic base of the high yielding varieties to make them more adaptable to the local environment. Wild populations found within the same agro climatic conditions of the cultivated varieties offer the advantage of being utilized more easily for the breeding purpose than those found in far away places. So to conserve the precious genetic resources of wild populations for their proper utilization, it is imperative to assess the genetic variability present among wild populations along with their genetic inter-relationships with the cultivated relatives (De Bustos *et al.*, 1998). Both natural and man-made factors have been responsible for limiting the distribution and genetic diversity of medicinal plant species and are causing them to become rare or even extinct. Prior to breeding programmes for increasing the pharmacological alkaloid content, it is imperative to assess the range of chemical and genetic variability in natural populations of *Abrus precatorius* and to establish the possible concordance/ discordance relationship between the genetic and chemical profiles. Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP), the Polymerase Chain Reaction (PCR)-based markers are most widely used genetic tools for assessment of the genetic variation in the medicinal plants (Singh *et al.*, 1999; Bejaj *et al.*, 2002;

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Deshwal *et al.*, 2005; Padmesh *et al.*, 2006). RAPD markers are generated by using short, decamer oligonucleotides of random sequence (Williams *et al.*, 1990). These markers are generally dominant and detect variation in both coding as well as non-coding regions of the genome. RAPD analysis is technically simple and suitable for large-scale germplasm characterization and can be performed even in a moderately equipped laboratory (Rafalski and Tingey, 1993).

The ISSRs are semiarbitrary markers amplified by PCR in the presence of one primer complementary to a target microsatellite (Zietkiewicz *et al.*, 1994). ISSRs have been proven to be a rapid, simple, and inexpensive way to assess genetic diversity in plants (Kantety *et al.*, 1995; Tsumura *et al.*, 1996; Tanyo-lac, 2003), to identify closely related cultivars (Fang *et al.*, 1997, 1998; Eiadthong *et al.*, 1999; Martins *et al.*, 2003), and to study evolutionary processes, such as reproductive systems (Liston *et al.*, 2003) and gene flow (Wolfe *et al.*, 1998). ISSRs have also been recently utilized as markers to assess genetic diversity in medicinal plants (Fracaro and Echeverrigaray, 2006; Apte *et al.*, 2006).

So far, no report is available on the molecular characterization and determination of genetic relationship among the accessions except that of Gupta *et al.* (2006b), in which genotypic variations were analyzed in three *Abrus precatorius* accessions using three isozymes, *viz.*, esterase, acid phosphatases and peroxidase.

In the present study, DNA profiling of eight accessions of *Abrus precatorius* L. has been carried out using RAPD and ISSR markers with an objective of evaluating genetic relationships among the accessions collected from different areas of Maharashtra, Orissa and West Bengal.

## Materials and Methods

### *Plant Materials*

The experimental material comprised the seed samples of eight wild accessions of *Abrus precatorius* L. that were collected from three states of India, *viz.*, Maharashtra, Orissa and West Bengal under National Agricultural Technology Programme (NATP) and conserved in the National Gene Bank, NBPGR, New Delhi. Seed characteristics such as seed colour, seed weight, seed size and shape were analysed. The detailed description of the *Abrus* accessions along with their seed characteristics used in the present study is given in Table 1.

### *Genomic DNA Extraction*

The collected seeds of *Abrus* were germinated as per ISTA rules (2003) after scarification using BP between towel papers method at 25°C. Total genomic DNA was isolated from young leaves from the 2-3 weeks old seedlings of

**Table 1. Details of *Abrus precatorius* L. accessions used**

| Accession no. | Collection no. | Seed weight (g) | Seed size (mm) | Seed shape | Seed colour | Source (village/state) |
|---|---|---|---|---|---|---|
| IC418096 | BBJ-606 | 2.56 | 0.8 | Ovoid | Red with black eye | Ramwadi, Thane (Maharashtra) |
| IC418097 | BBJ-607 | 2.11 | 0.7 | Ovoid-subglobose | Dull white with brown eye | Jambhulpada, Thane (Maharashtra) |
| IC418103 | BBJ-613 | 2.64 | 0.8 | Subglobose | White | Bebgi, Thane (Maharashtra) |
| IC418115 | BBJ-625 | 2.57 | 0.8 | Ovoid | White | Dabosa, Thane (Maharashtra) |
| IC418120 | BBJ-630 | 2.07 | 0.7 | Ovoid-subglobose | Red with black eye | Vaki, Thane (Maharashtra) |
| IC392840 | SARC/DP-10 | 2.55 | 0.9 | Ovoid | Red with black eye | Aonlabadi, Mayurbhanj (Orissa) |
| IC391930 | SARC/AS-48 | 2.61 | 0.9 | Ovoid | Red with black eye | Jaladia, Keonjhar (Orissa) |
| IC427646 | SBC-2/50 | 2.52 | 0.5 | Subglobose | Dull white with brown eye | Satjele Emblibadi, 24 Paragana (West Bengal) |

*Abrus* accessions using modified SDS (sodium dodecyl sulphate) protocol described by Dellaporta *et al.* (1983) along with the modifications. Different percentages of Poly Vinyl Pyrrolidone (MW 40,000) were used in the extraction buffer and 0.2% was found satisfactory to tackle the problem of phenolics. The isolated DNA was quantified by VersaFluor™ Fluorometer and its quality was checked on 0.8% agarose gels by horizontal electrophoresis.

***RAPD and ISSR Analyses***

RAPD analysis was performed using 40 random sequence decamer primers (Operon Technologies Inc., Alameda, USA) to screen polymorphism among *Abrus* accessions. Amplification reactions were carried out in 25µl reaction mixture containing 25 ng of genomic DNA, 1x*Taq* DNA polymerase buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3), 1.5 mM $MgCl_2$, 200 µM dNTPs (Promega, USA), 10 pmoles of random primer and 1 unit of *Taq* DNA polymerase (Bangalore Genei, India). PCR amplification of the isolated DNA was done in a PTC-200 Thermal Cycler (MJ Research Inc, USA). The sequential amplification steps involved: 1 cycle at 94°C for 5 min (initial denaturation); followed by 40 cycles of 1 min at 94°C (denaturation), 1 min at 37°C (primer annealing) and 2 min at 72°C (primer extension). The last cycle was followed by 7 min final extension at 72°C. Thirty five ISSR primers (The University of British Columbia, Canada) were used for ISSR analysis. ISSR amplifications were performed in a total volume of 25 µl with 25 ng of genomic DNA, 1x *Taq* DNA polymerase buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3), 1.0 mM $MgCl_2$, 200 µM dNTPs (Promega, USA), 10 pmoles of primer and 1 unit of *Taq* DNA polymerase (Invitrogen, USA). The amplification reactions were carried out on PTC-200 Thermal Cycler (MJ Research Inc, USA) with the following thermal profile: one cycle of 94°C for 4 min (initial denaturation) followed by 35 cycles of 45 sec at 94°C (denaturation), 45 sec at 39°C (primer annealing), and 2 min at 72°C (primer extension), and finally, one cycle of 7 min at 72°C.

The amplified PCR products were size fractionated on 1.6% agarose gel in 1x Tris-acetate EDTA (TAE) buffer stained with ethidium bromide. The electrophoretic patterns were visualized under UV light and photographed using Gel Documentation System (Alpha Innotech Corporation, USA). The reproducibility of the amplification was confirmed by repeating each experiment twice.

**Data Analysis**

Allele sizes (in nucleotide base pair) were determined on the basis of its migration relative to standard size marker, *i.e.*, 1Kb DNA ladder (MBI Fermentas). RAPD and ISSR bands were scored for a binary data matrix, coded by 1 and 0, respectively for their presence and absence across all the *Abrus* accessions, for each primer. The pair wise genetic similarities among all the pairs of samples were estimated with Jaccard's similarity coefficient (Jaccard, 1908). The statistical analysis was performed using NTSYS-pc software (version 2.11S). UPGMA (Unweighted Pair Group Method of Arithmetic Means) was employed to construct the dendrogram using SAHN program to group accessions into discrete clusters based on RAPD and ISSR data.

Polymorphism information content (PIC) was also determined for each SSR primer set as described by Smith *et al.* (1997).

$$PIC = 1-\Sigma f_i^2$$

where $f_i$ is the frequency of $i^{th}$ allele.

**Results and Discussion**

***Genetic Diversity among Abrus accessions using RAPD and ISSR Markers***

Eight accessions of *Abrus precatorius* L. used in the present study were collected from different areas of Maharashtra, Orissa and West Bengal and were characterized morphologically with respect to seed weight, seed size, seed shape and seed colour to find the relationship with molecular markers. Forty RAPD primers were screened for polymorphism in *Abrus precatorius* L. accessions, of which 28 primers (70.0%) generated polymorphic patterns (Table 2). A total of 175 bands were produced with an average of 6.25 bands per polymorphic primer. Among these, 152 (86.85%) were polymorphic ranging between 2 to 12; the maximum number of bands was detected with OPC 18 (Fig. 1). The average PIC was 0.77, ranging from 0.44 to 0.89. The lowest and highest PIC values were recorded for primer OPX 04 and OPC 19, respectively. In ISSR analysis, 35 ISSR primers were used to study polymorphism and 20 of them (57.15%) showed polymorphism (Table 3). Polymorphic primers generated a total of 81 fragments with an average of 4.05 products per polymorphic primer. Out of these, 73 (90.12%) were polymorphic. The number of products amplified by the polymorphic primers varied from 1 to 8 (Fig. 2), the maximum number of products were detected with the primers UBC 811 and UBC 834. The average

**Table 2. List of polymorphic primers and their sequence used for RAPD analysis**

| Primer Code | Sequence (5′-3′) | Bands scored | | Amplicon size range (in bp) | PIC |
|---|---|---|---|---|---|
| | | Total | Polymorphic | | |
| OPC 02 | GTGAGGCGTC | 6 | 1 | 550-2200 | 0.83 |
| OPC 03 | GGGGGTCTTT | 5 | 5 | 400-1300 | 0.76 |
| OPC 04 | CCGCATCTAC | 8 | 7 | 500-1900 | 0.84 |
| OPC 05 | GATGACCGCC | 7 | 2 | 280-2000 | 0.84 |
| OPC 06 | GAACGGACTC | 6 | 2 | 450-2000 | 0.80 |
| OPC 07 | GTCCCGACGA | 4 | 4 | 500-1300 | 0.77 |
| OPC 09 | CTCACCGTCC | 3 | 3 | 200-850 | 0.62 |
| OPC 10 | TGTCTGGGTG | 5 | 3 | 350-1200 | 0.74 |
| OPC 11 | AAAGCTGCGG | 9 | 9 | 400-1500 | 0.86 |
| OPC 15 | GACGGATCAG | 4 | 3 | 700-1550 | 0.74 |
| OPC 16 | CACACTCCAG | 8 | 8 | 400-1800 | 0.85 |
| OPC 17 | TTCCCCCCAG | 3 | 3 | 900-1500 | 0.62 |
| OPC 18 | TGAGTGGGTG | 12 | 12 | 200-1900 | 0.88 |
| OPC 19 | GTTGCCAGCC | 10 | 10 | 200-1750 | 0.89 |
| OPF 17 | AACCCGGGAA | 4 | 4 | 500-1200 | 0.74 |
| OPG 08 | TCACGTCCAC | 7 | 7 | 450-1500 | 0.84 |
| OPG 20 | TCTCCCTCAG | 9 | 9 | 250-2000 | 0.85 |
| OPK 20 | GTGTCGCGAG | 6 | 6 | 500-1800 | 0.79 |
| OPM 14 | AGGGTCGTTC | 5 | 5 | 800-1500 | 0.76 |
| OPP 19 | GGGAAGGACA | 10 | 10 | 600-2000 | 0.88 |
| OPS 07 | TCCGATGCTG | 6 | 6 | 400-1800 | 0.82 |
| OPX 04 | CCGCTACCGA | 2 | 2 | 1200-1500 | 0.44 |
| OPX 05 | CCTTTCCCTC | 7 | 7 | 750-2000 | 0.83 |
| OPX 07 | GAGCGAGGCT | 2 | 2 | 1500-1900 | 0.50 |
| OPY 17 | GACGTGGTGA | 10 | 6 | 250-1400 | 0.88 |
| OPY 20 | AGCCGTGGAA | 6 | 6 | 600-1500 | 0.80 |
| OPZ 19 | GTGCGAGCAA | 2 | 2 | 750-1000 | 0.50 |
| OPZ 20 | ACTTTGGCGG | 9 | 8 | 500-2000 | 0.87 |

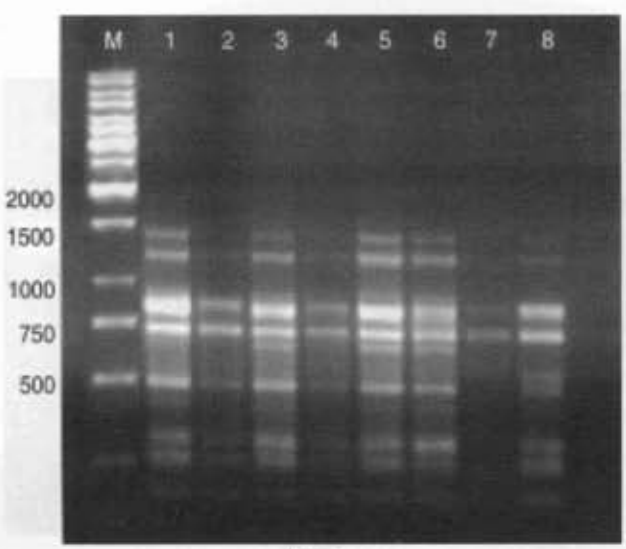


Fig. 1a

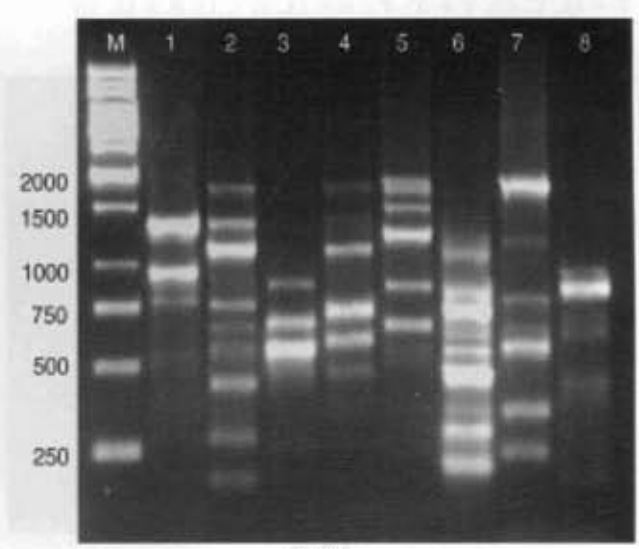


Fig. 1b

**Fig. 1: RAPD profile of 8 *Abrus precatorius* L. accessions amplified by primers OPC 04 (1a) and OPC 18 (1b)**
**Lane-1:IC418096, Lane-2:IC418097, Lane-3:IC418103, Lane-4:IC418115, Lane-5:IC418120, Lane 6:1C392840, Lane-7:IC427646 and Lane-8:IC391930, Lane M-1kb DNA ladder**

PIC was 0.67, and the lowest and highest PIC values were 0.32 (UBC 815) and 0.85 (UBC 811), respectively.

### Genetic Similarity Matrix and Cluster Analysis

RAPD and ISSR data were used to make pair wise comparisons of the accessions based on shared and unique amplification products to generate a similarity matrix. Similarity coefficient values based on Jaccard's coefficient ranged from 0.357 to 0.740 with RAPD and from 0.245 to 0.829 with ISSR markers, reflecting broad range of genetic similarity (Tables 4, 5). The accessions IC418115 from Thane (Maharashtra) and IC392840 from

**Table 3. List of polymorphic primers and their sequence used for ISSR analysis**

| Primer Code | Sequence (5'-3') | Bands scored | | Amplicon size range (in bp) | PIC |
|---|---|---|---|---|---|
| | | Total | Polymorphic | | |
| UBC 801 | ATA TAT ATA TAT ATA TT | 4 | 2 | 800-2000 | 0.75 |
| UBC 805 | TAT ATA TAT ATA TAT AC | 3 | 3 | 1200-2500 | 0.60 |
| UBC 809 | AGA GAG AGA GAG AGA GG | 6 | 4 | 900-1400 | 0.80 |
| UBC 811 | GAG AGA GAG AGA GAG AC | 8 | 8 | 450-2000 | 0.85 |
| UBC 812 | GAG AGA GAG AGA GAG AA | 7 | 7 | 600-1800 | 0.84 |
| UBC 813 | CTC TCT CTC TCT CTC TT | 5 | 5 | 500-1200 | 0.75 |
| UBC 814 | CTC TCT CTC TCT CTC TA | 2 | 2 | 1000-1500 | 0.41 |
| UBC 815 | CTC TCT CTC TCT CTC TG | 2 | 1 | 800-1500 | 0.32 |
| UBC 817 | CAC ACA CAC ACA CAC AA | 3 | 2 | 550-1000 | 0.54 |
| UBC 820 | GTG TGT GTG TGT GTG TC | 2 | 1 | 1500-1800 | 0.50 |
| UBC 827 | ACA CAC ACA CAC ACA CG | 4 | 4 | 1000-1500 | 0.74 |
| UBC 828 | TGT GTG TGT GTG TGT GA | 4 | 4 | 850-1200 | 0.72 |
| UBC 829 | TGT GTG TGT GTG TGT GC | 2 | 2 | 450-1000 | 0.50 |
| UBC 833 | ATA TAT ATA TAT ATA TYG | 2 | 1 | 1250-1500 | 0.84 |
| UBC 834 | AGA GAG AGA GAG AGA GYT | 8 | 7 | 500-2200 | 0.84 |
| UBC 836 | AGA GAG AGA GAG AGA GYA | 7 | 6 | 800-2000 | 0.84 |
| UBC 840 | GAG AGA GAG AGA GAG AYT | 6 | 6 | 400-1600 | 0.83 |
| UBC 843 | CTC TCT CTC TCT CTC TRA | 2 | 1 | 900-1500 | 0.50 |
| UBC 845 | CTC TCT CTC TCT CTC TRG | 2 | 2 | 1000-1400 | 0.50 |
| UBC 886 | VDV CTC TCT CTC TCT CT | 5 | 5 | 750-2000 | 0.78 |

V = (A, C, G); Y = (C, T); N = (A, G, C,T); R = (A, G)

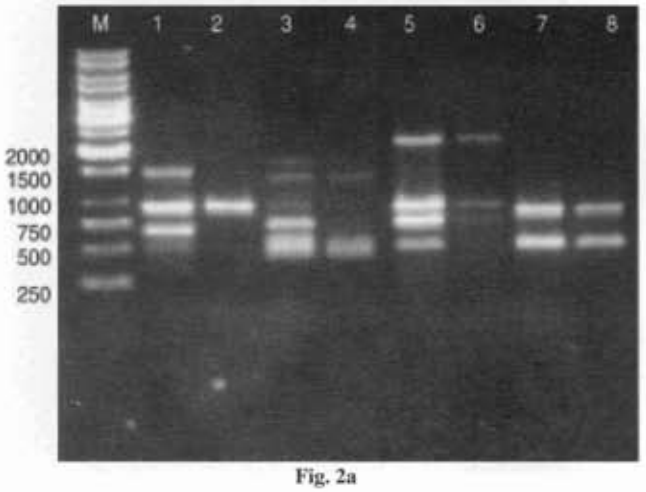


Fig. 2a

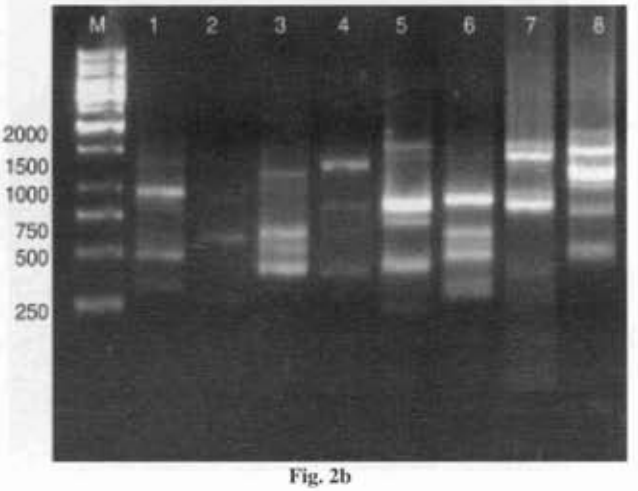


Fig. 2b

**Fig. 2: ISSR profile of 8 *Abrus precatorius* L. accessions amplified using ISSR primers UBC 813 (2a) and UBC 840 (2b)**
**Lane-1: IC418096, Lane-2: IC418097, Lane-3: IC418103, Lane-4: -IC418115, Lane-5: IC418120, Lane-6: IC392840, Lane-7: IC427646 and Lane-8: IC391930, Lane M: 1Kb DNA ladder**

Mayurbhanj (Orissa) displayed the maximum genetic similarity where as IC418096 from Thane (Maharashtra) and IC391930 from Keonjhar (Orissa) showed the minimum genetic similarity as revealed by RAPD and ISSR.

In RAPD dendrogram, the accessions of *Abrus* clustered in two major groups (I and II) with subgrouping in cluster I (Fig. 3). The first cluster was divided into two sub groups a and b, one comprising of IC418096, IC418097 and IC427646 and other having IC418103, IC418120, IC418115 and IC392840. All the five accessions from Maharashtra were grouped in to two sub groups of the same cluster. The second cluster comprised of single accession belonging to Orissa, *viz.*, IC391930. In ISSR dendrogram, the two major clusters I and II were formed (Fig. 4), one consisting of six accessions IC418096, IC418103, IC418115, IC392840, IC418120 and IC418097 and other comprising IC427646 and IC391930. All the five accessions from Maharashtra were grouped in the same cluster. The cluster analysis based on RAPD and ISSR data revealed that the accessions IC418115 collected from Thane and IC392840 from Mayurbhanj shared the maximum similarity indicating that they are closely related where as IC418096 from Thane and IC391930 from Keonjhar were found far apart among the eight accessions. The cluster analysis discriminated all the accessions collected from Maharashtra in two sub-groups of single cluster although they showed morphological variations with respect to the seed characters. The accessions IC418115 collected from Thane (Maharashtra) and IC392840 from Mayurbhanj (Orissa) displayed the maximum genetic similarity where as IC418096 from Thane (Maharashtra) and IC391930 from Keonjhar (Orissa) showed the minimum genetic similarity as revealed by RAPD and ISSR data. The accessions IC392840 and IC391930 from Orissa having the similar seed characteristics were grouped into different clusters based on RAPD and ISSR data.

So far, there is no report available for molecular characterization and determination of genetic diversity in *Abrus precatorius* except that of Gupta *et al.* (2006b) study where the genotypic variation was studied in three *Abrus precatorius* accessions, *i.e.*, pink, red and white morphotypes using three isozyme systems, *viz.*, esterase, acid phosphatases and peroxidase. Thirty-two polypeptide bands ranging from Rm value 0.06 to 0.71 were detected. The similarity index analysis indicated that the pink genotype as possibly the natural hybrid of the white and red genotypes.

In the present study, eight accessions of *Abrus precatorius* L. collected from different areas of Maharashtra, Orissa and West Bengal were characterized morphologically with respect to seed weight, seed size, seed shape and seed colour and were used to find the

**Table 4. Similarity matrix of *Abrus precatorius* L. accessions analyzed using RAPD data based on Jaccard's coefficient**

| | IC418096 | IC418097 | IC418103 | IC418120 | IC418115 | IC392840 | IC427646 | IC391930 |
|---|---|---|---|---|---|---|---|---|
| IC418096 | 1.000 | | | | | | | |
| IC418097 | 0.655 | 1.000 | | | | | | |
| IC418103 | 0.583 | 0.452 | 1.000 | | | | | |
| IC418120 | 0.594 | 0.472 | 0.722 | 1.000 | | | | |
| IC418115 | 0.516 | 0.420 | 0.665 | 0.686 | 1.000 | | | |
| IC392840 | 0.506 | 0.390 | 0.644 | 0.719 | **0.740** | 1.000 | | |
| IC427646 | 0.561 | 0.586 | 0.409 | 0.466 | 0.415 | 0.396 | 1.000 | |
| IC391930 | **0.357** | 0.476 | 0.248 | 0.302 | 0.265 | 0.313 | 0.416 | 1.000 |

**Table 5. Similarity matrix of *Abrus precatorius* L. accessions analyzed using ISSR data based on Jaccard's coefficient**

| | IC418096 | IC418097 | IC418103 | IC418120 | IC418115 | IC392840 | IC427646 | IC391930 |
|---|---|---|---|---|---|---|---|---|
| IC418096 | 1.000 | | | | | | | |
| IC418097 | 0.544 | 1.000 | | | | | | |
| IC418103 | 0.653 | 0.423 | 1.000 | | | | | |
| IC418120 | 0.589 | 0.441 | 0.792 | 1.000 | | | | |
| IC418115 | 0.608 | 0.443 | 0.808 | 0.767 | 1.000 | | | |
| IC392840 | 0.533 | 0.426 | 0.803 | 0.761 | **0.829** | 1.000 | | |
| IC427646 | 0.357 | 0.415 | 0.290 | 0.265 | 0.290 | 0.288 | 1.000 | |
| IC391930 | **0.245** | 0.371 | 0.197 | 0.169 | 0.197 | 0.210 | 0.440 | 1.000 |

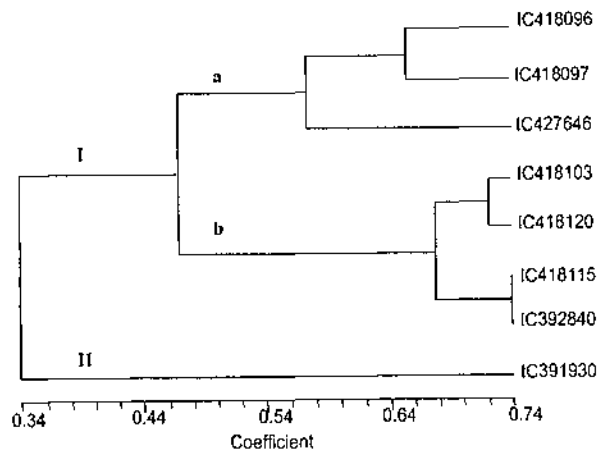


Fig. 3: Dendrogram generated by unweighted pair group method with arithmetic means based on RAPD data

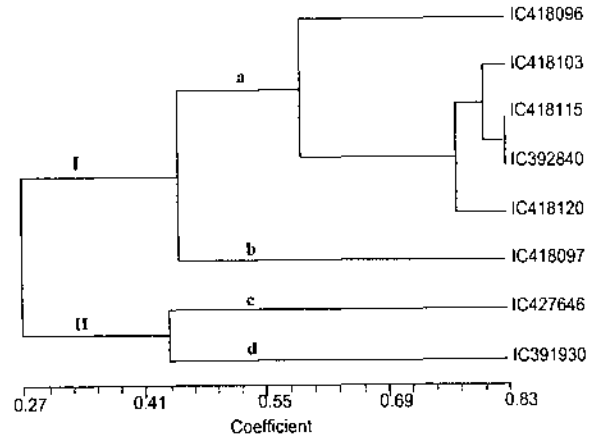


Fig. 4: Dendrogram generated by unweighted pair group method with arithmetic means based on ISSR data

relationship with molecular markers using RAPD and ISSR analyses. RAPD and ISSR analyses revealed an average of 86.85% and 90.12% polymorphism, respectively across all the eight *Abrus* accessions. In some of the recent reports, such high percentage polymorphisms using RAPD and ISSR markers have also been reported for other medicinal plants such as *Trigonella* (Dangi *et al.*, 2004) and *Mucuna pruriens* (Padmesh *et al.*, 2006).

The high levels of polymorphism detected in the accessions of *Abrus precatorius* may be attributed to the broad genetic base of the species that in the process of speciation might have acquired novel gene combinations for better adaptability in the changing environmental conditions. The high number of polymorphic products generated by certain RAPD primers may be due to the fact that in RAPD even small divergence between two accessions can result in distinct patterns as polymorphism may be due to single nucleotide change within the primer binding site, insertion or deletion with the amplified region so that part of the primer binding site in one of the strand is missing, complete absence of complementary sites, and the region between the binding sites on opposite strands is beyond the normal amplifiable length (Padmesh *et al.*, 2006).

Polymorphic information content (PIC) provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles (Smith *et al.*, 1997). In the present study, a high level of polymorphism was observed among the eight *Abrus* accessions with both the marker systems, and average PIC values were 0.77 and 0.67 for RAPD and ISSR, respectively. The values of genetic similarity based on Jaccard's coefficient for all the eight accessions, ranged from 0.357 to 0.740 for RAPD markers and from 0.245 to 0.829 for ISSR markers, respectively, reflecting broad range of genetic dissimilarity (Tables 4, 5).

The cluster analysis discriminated all the accessions collected from Maharashtra in two sub-groups of single cluster although they showed morphological variations with respect to the seed characters. The accessions IC418115 collected from Thane (Maharashtra) and IC392840 from Mayurbhanj (Orissa) displayed the maximum genetic similarity where as IC418096 from Thane (Maharashtra) and IC391930 from Keonjhar (Orissa) showed the minimum genetic similarity as revealed by RAPD and ISSR data. Based on RAPD and ISSR data, the accessions IC392840 and IC391930 from Orissa exhibiting the similar seed characteristics were grouped into different clusters.

The present study indicates the utility of RAPD and ISSR markers for measuring genetic diversity and for DNA fingerprinting that would prove valuable for the breeding programme of *Abrus precatorius* L. These results are important for future genetic improvement, identification, and conservation of *Abrus precatorius* germplasm.

**Acknowledgements**

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# Selection of Elite Maintainers with *eui* Gene using RAPD Analysis for Development of New CMS Lines in Hybrid Rice Breeding

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Random Amplified Polymorphic DNA (RAPD) analysis was employed for genetic diversity study over 32 rice genotypes including 29 agronomically superior maintainer lines with *eui* gene for good panicle exertion and 3 restorers. A total of 102 amplification products were detected with 12 polymorphic RAPD primers of which 69 (67.64%) were polymorphic. Polymorphic primer showed 50 to 80 amplicon polymorphism. The genetic similarity ranging between 54.2% ($L_{19}$-$L_5$) to 92% ($L_{11}$-$L_2$) suggested that the maintainers and restorers under study are divergent. Cluster generated through UPGMA analysis revealed two main clusters. Cluster I comprised two genotypes only, both derived from the same cross. Genotypes in cluster II were redistributed into a number of subclusters at different similarity coefficient levels indicating different levels of genetic relationships. The maintainer lines $L_2$, $L_{14}$, $L_{17}$, $L_{20}$ and $L_{29}$ used in the study are suggested to be exploited for development of CMS lines to realize higher level of heterosis. The hybrids involving parents with maximum dissimilarity coefficients displayed non-significant estimates of heterosis for grain yield per plant.

**Key Words: Rice, RAPD, WA-CMS, Heterosis**

## Introduction

Rice is grown in 114 countries across the world, on 150 million hectare area that constitutes nearly 11% of the world cultivated land (Rai, 2006). Exploitation of heterosis is the most pragmatic approach to increase the rice yield potential. However, the success is determined not only by the performance of the parents *per se* but also by the divergence between them (Yuan *et al.*, 1994; Gopal and Minocha, 1997). Molecular markers based on polymerase chain reaction (PCR) method offer several advantages over the conventional morphological markers. Random Amplified Polymorphic DNA (RAPD) uses arbitrary 10-base primers to amplify the random portions of the genome (Williams *et al.*, 1990; Naghia *et al.*, 2002). The fragments produced are easily visualized on ethidium bromide stained gel and polymorphism can be detected between the amplification products of different individuals. The level of correlation between molecular marker based distances and hybrid performance is dependent on the germplasm (Saghai-Maroof *et al.*, 1997). The RAPD analysis (Williams *et al.*, 1990) being relatively simple and based on the use of commercially available random primers provides useful information about the molecular distance within and among varieties.

Since most of the three-line hybrids either already released or in the advanced trials since start of the programme in 1994 utilize IR58025A as the sole CMS line with wild abortive (WA) cytoplasm, therefore, the extent of exploitable heterosis is limited to a point. Correlation of molecular diversity with heterosis reflects usefulness of RAPD in predicting heterosis based on RAPD marker polymorphism (Xiao *et al.*, 1996). We report here the genetic divergence among the maintainer lines specifically bred for improved plant type, duration, yield potential and grain quality at the GB Pant University of Agriculture and Technology, Pantnagar and three adapted varieties/ restorers by employing RAPD markers. This will aid in finding new vistas to develop CMS lines in diverse genetic backgrounds for the greater exploitation of heterosis in hybrid rice improvement programme.

## Materials and Methods

### *Plant Material*

Plant material used for this study consisted of 32 rice genotypes (Table 1) including 29 improved maintainer lines with good yielding ability and other agronomic characters that were bred specifically for the development of superior cytoplasmic male sterile lines. The elite lines were also selected for better panicle exertion as one of the parents in the cross involved '*eui*', the single recessive gene for elongated uppermost internode. The material also included three widely adapted superior genotypes being used as restorers in our hybrid breeding programme *viz.*,

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**Table 1. Parentage of the elite maintainer lines and restorer genotypes**

| S.No. | Designation | Code | Parentage |
|---|---|---|---|
| 1. | UPR 2899-78-4-2B | $L_1$ | UPRI 95-140/IR Basmati |
| 2. | UPR 2899-78-4-3B | $L_2$ | -do- |
| 3. | UPR 2899-78-5-1B | $L_3$ | -do- |
| 4. | UPR 2899-78-6-2B | $L_4$ | -do- |
| 5. | UPR 2900-80-1-1B | $L_5$ | Haryana Basmati 1/UPRI 95-139 |
| 6. | UPR 2906-254-2-6-5B | $L_6$ | Govind/UPRI 95-139 |
| 7. | UPR 2906-257-2-8-1B | $L_7$ | Haryana Basmati 1/UPRI 95-139 |
| 8. | UPR 2911-271-2-4B | $L_8$ | UPRI 95-139/IR 58025B |
| 9. | UPR 2911-271-2-6B | $L_9$ | -do- |
| 10. | UPR 2911-271-3-2B | $L_{10}$ | -do- |
| 11. | UPR 2911-271-7-1B | $L_{11}$ | -do- |
| 12. | UPR 2911-271-8-15B | $L_{12}$ | -do- |
| 13. | UPR 2911-271-8-3B | $L_{13}$ | -do- |
| 14. | UPR 2911-271-8-4B | $L_{14}$ | -do- |
| 15. | UPR 2911-271-8-9B | $L_{15}$ | -do- |
| 16. | UPR 2911-271-12-2B | $L_{16}$ | -do- |
| 17 | UPR 2912-273-8-1B | $L_{17}$ | UPRI 95-141/IRBB 21 |
| 18. | UPR 2900-80-1-4B | $L_{18}$ | Haryana Basmati 1/UPRI 95-139 |
| 19. | UPR 2900-80-9-2-1B | $L_{19}$ | -do- |
| 20. | UPR 2900-80-9-2-2B | $L_{20}$ | -do- |
| 21. | UPR 2903-87-1-1B | $L_{21}$ | Pusa Basmati 1/IR 58025B |
| 22. | UPR 2903-87-1-2B | $L_{22}$ | -do- |
| 23. | UPR 2904-207-1-3B | $L_{23}$ | BG 1321/UPRI 95-141 |
| 24. | UPR 2906-254-2-6-2B | $L_{24}$ | Govind/UPRI 95-139 |
| 25. | UPR 2911-271-2-1B | $L_{25}$ | UPRI 95-139/IR 58025B |
| 26. | UPR 2911-271-2-5B | $L_{26}$ | -do- |
| 27. | UPR 2911-271-3-3B | $L_{27}$ | -do- |
| 28. | UPR 2911-271-8-7B | $L_{28}$ | -do- |
| 29. | UPR 2911-271-12-3B | $L_{29}$ | -do- |
| 30. | UPRI 93-287 | $T_1$ | MRC 19340 |
| 31. | Pant Dhan 4 | $T_2$ | IR 262/Remajda |
| 32. | Ajaya | $T_3$ | IET 4141/CR 98-7216 |

Pant Dhan 4, Ajaya and UPRI 93-287, which have shown fertility restoration capability to wild abortive (WA) based cytoplasmic male sterility.

During 1998-1999 off season, all the 29 maintainers were crossed to UPR 93-287 and Pant Dhan 4 in line x tester fashion. To understand the effect of diversity on the extent of heterosis in crosses, the complete set of hybrids and parents were evaluated in the randomized complete block design (RCBD) using two replications and under two different environments based on fertility. The environments were created using two fertilizer doses *viz.*, optimum (120N: 40P: 40K kg/ha) and high (180N: 80P: 40K kg/ha) fertility, referred as $E_1$ and $E_2$, respectively. Data on yield related characters were obtained to calculate heterosis.

### *DNA Extraction*

For each genotype, 100 mg of fresh leaves from four-week old seedlings were weighed for DNA extraction using CTAB miniprep DNA extraction protocol (Saghai-Maroof *et al.*, 1984). DNA was quantified in a TKO 100 Fluorometer (Hoefer, San Fransisco, CA).

### *RAPD Analysis*

A total of 70 RAPD primers (A, B, C, D and E series) were initially taken (Operon Technology Alameda, California, USA) for this study. Amplifications were carried out with 1x PCR buffer (10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, 2% formamide), 1.5 mM $MgCl_2$, 200 $\mu$M dNTPs, 200 nM primer, 1 unit of *Taq* DNA polymerase (Bangalore Genei, India) and 25 ng of genomic DNA. Amplifications were performed using a 96-well thermal cycler (Perkin-Elmer, USA) programmed for an initial step of 5 min at 94°C followed by 45 cycles of 1 min at 94°C, 1 min at 37°C and 2 min at 72°C. A final extension step at 72°C was programmed for 7 minutes. Amplification products were separated on 1.4% agarose gels prepared in 1x TAE buffer (40 mM Tris-acetate and 1 mM EDTA pH 8.0) at 60 V for 3 h using a horizontal gel electrophoresis system (Bio-rad, Hercules, CA, USA). Gels were stained with ethidium bromide. A 100 bp DNA ladder plus (MBI, Fermentas, Burlington, Ontario, Canada) was run alongside the amplified products to determine their approximate size. Gel was photographed using Gel-Documentation system (Gel Doc Mega, Biosystematica, UK).

### *Data Analysis*

Reproducible amplified fragments of RAPD were scored manually. Weak bands of negligible intensity and smeared bands were excluded from the final data analysis. Band profiles were scored with 1 indicating the presence, and 0 indicating the absence of a band to construct a binary qualitative data matrix. Pair-wise comparisons of genotypes were employed to calculate Jaccard's similarity coefficient (GS): a / (n-d), where a = number of positive matches; d = number of negative matches and n = total sample size (Jaccard, 1908).

A dendrogram was constructed using the unweighted pair group method with arithmetic averages (UPGMA) and computation for multivariate analysis was done using the computer programme NTSYS-pc Version 1.8 (Rholf, 1993).

Statistical analysis was carried out to assess the heterosis as per Fonseca and Patterson (1968). Pant Dhan 4, a widely cultivated variety was taken as check to calculate standard heterosis.

## Results and Discussion

### *RAPD Analysis*

In the present study, band profiling for the genotypes was done with replicated PCR amplification to check the

reproducibility of the technique. Out of 70 RAPD primers, only 12 primers with clear and reproducible band pattern were chosen for further study. Details of the polymorphic RAPD primers and the level of polymorphism are presented in Table 2.

Twelve polymorphic primers (Table 2) generated 102 fragments (8.5 bands per primer) among all the 32 genotypes, of which 67.64% (69 bands) were polymorphic for one or more genotypes. Similar level of polymorphism (69.4%) using RAPD markers have been reported by He *et al.* (2004) based on the genetic diversity study on allelopathic rice germplasm. The number of polymorphic fragments ranged from 4 to 10 (5.75 average). None of the primers revealed 100% polymorphism; however, the primers OPA-19, OPB-17 and OPE-09 detected 75% or higher polymorphism. The distinct banding patterns as produced by the primer OPE-09 (Figs. 1a and b) are adequate enough to differentiate all the 32 genotypes at molecular level. These results reflect that RAPD is technically simpler, quicker, relatively inexpensive and non-radioactive as also observed by Samec (1993). The results suggest that RAPD is suitable marker system for DNA fingerprinting and diversity analysis at whole genome level with minimum inputs, in contrast to the most popular STMS markers which is single locus, therefore needed in more number to generate information about rice fingerprint. RAPDs have been widely used to detect polymorphism in germplasm characterization and genetic distance studies (Naghia *et al.*, 2002; He *et al.*, 2004) and population differentiation (Yu *et al.*, 2005).

***Genetic Diversity Based on RAPD Markers***

Analysis of the relationship based on 102 amplified products (RAPD markers) through UPGMA analysis revealed that the genetic relationship based on Jaccard's coefficients ranged between 54.2% ($L_{19}$-$L_5$) to 92% ($L_{11}$-$L_2$). The dendrogram (Fig. 2) revealed 2 major clusters. Cluster I comprised of lines $L_{19}$ and $L_{20}$ (81.1% genetically similar), whereas, Cluster-II contained all the remaining 30 genotypes, including three improved cultivars *viz.*, $T_1$, $T_2$ and $T_3$. Both of the lines ($L_{19}$ and $L_{20}$) in cluster-I were derived from a single cross Haryana Basmati1 × UPRI 95-139. All other lines derived from this cross grouped in different sub clusters of cluster II at different genetic similarity levels. The cluster-II was sub grouped into 5 sub clusters (IIA-IIE). Sub-clusters IIA and IIB were comprised of two genotypes each whereas, sub-cluster IIC comprised of four genotypes. In sub-cluster IID highest number of genotypes i.e. 14 genotypes were grouping together whereas, in sub-cluster IIE six genotypes were grouping together. The genotypes L7 and L18 were ungrouped in the sub-clusters. Amongst the restorers, $T_1$ (UPRI93-287) was closer to most of the elite maintainer lines in comparison to Pant Dhan 4 ($T_2$) and Ajaya ($T_3$). The lines $T_2$ and $T_3$ were 79.4% similar. It suggests the presence of high level of genetic divergence in the plant material under study and therefore, enhanced prospects of obtaining higher standard heterosis by crossing restorers to elite maintainer converted into cytoplasmic male sterile lines. Two different lines $L_2$ (UPRI 95-140 / IR Basmati) and $L_{11}$ (UPRI 95-139 / IR

**Table 2. RAPD Primers along with their sequences and level of polymorphism detected**

| Primer Designation | Sequence (5′ - 3′) | Total number of bands | Polymorphic loci | |
|---|---|---|---|---|
| | | | Frequency | (%) |
| OPA-04 | AATCGGGCTG | 8 | 5 | 62.50 |
| OPA-08 | GTGACGTAGG | 5 | 4 | 80.00 |
| OPA-19 | CAAACGTCGG | 9 | 7 | 77.77 |
| OPB-10 | CTGCTGGGAG | 8 | 5 | 62.50 |
| OPB-17 | AGGGAACGAG | 4 | 3 | 75.00 |
| OPD-03 | GTCGCCGTCA | 10 | 7 | 70.00 |
| OPD-08 | GTGTGCCCCA | 8 | 4 | 50.00 |
| OPE-03 | CCAGATGCAC | 6 | 4 | 66.66 |
| OPE-07 | AGATGCAGCC | 6 | 3 | 50.00 |
| OPE-04 | TGCGGCTGAG | 11 | 8 | 72.72 |
| OPE-09 | ACGGCGTATG | 13 | 10 | 76.92 |
| OPE-03 | GGCTGCAGAA | 14 | 9 | 64.28 |
| | Total | 102 | 69 | – |
| | Average | | 5.75 | 67.64 |

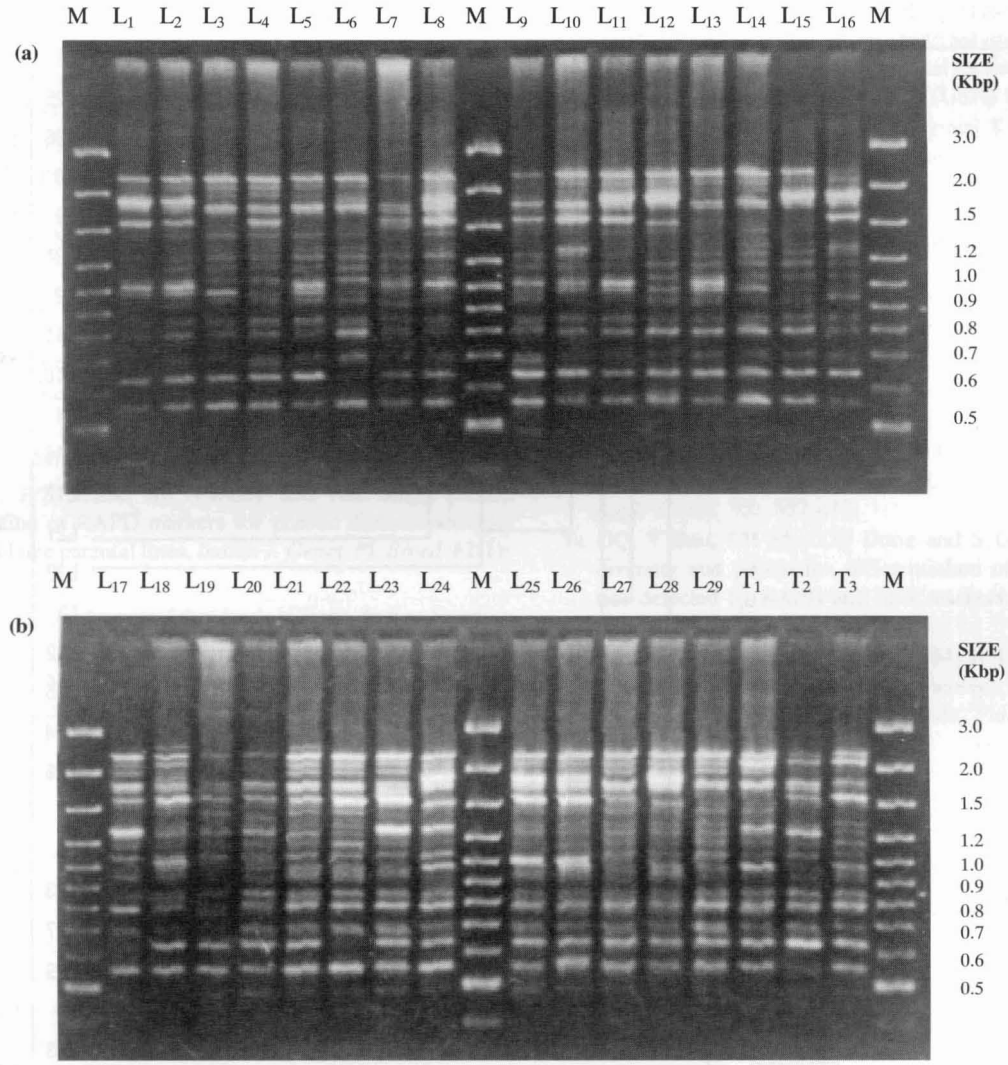


**Fig. 1: RAPD profile of 29 maintainer lines ($L_1$-$L_{29}$) and three improved cultivars of rice with primer OPE-09, Lane M: DNA size marker "100 bp DNA ladder plus"**

58025 B), although of different origin were close to each other with the genetic distance of 0.08 (92% similarity). Interestingly, the study also revealed the intermingling of the lines originating from different crosses into the same cluster and vice-versa. This could be due to the fact that some of the lines had one of the parents common in these crosses. For example, 12 of the lines originating from two crosses *viz.*, $L_8$ to $L_{16}$ and $L_{25}$ to $L_{29}$ (UPRI 95-139 × IR 58025B) and $L_{21}$-$L_{22}$ (Pusa Basmati 1 × IR58025 B) involved IR58025B as common parent. Similarly, the lines $L_5$, $L_7$ and $L_{18}$ to $L_{20}$, derived from the cross Haryana Basmati 1 × UPRI 95-139 and the lines $L_8$ to $L_{16}$ and $L_{25}$ to $L_{29}$ derived from the cross UPRI 95-139 × IR 58025B, UPRI 95-139 is common. Within the cluster- II, the lines derived from UPRI 95-139 × IR 58025B ($L_8$ to $L_{16}$) and $L_{25}$ to $L_{29}$ were grouped into five different sub-clusters, based on their relative genetic similarity. Such a variation could be attributed to the selection of diverse plant characteristics related to plant type, duration, grain and yield attributes during segregating generations ($F_2$-$F_4$) leading to divergence among these lines.

The present study showed higher level of heterosis for yield and related attributes with $L_2$, $L_{14}$, $L_{17}$, $L_{20}$ and $L_{29}$ crossed with $T_1$/$T_2$ (Table 1). The result suggested use of these maintainer lines for CMS line development to realize higher heterosis in hybrid rice development. Linear relationship between heterosis of specific crosses and divergence of combining parents in this study could not be established as also reported by Dudley *et al.* (1991), since the primers used were random and limited, so may

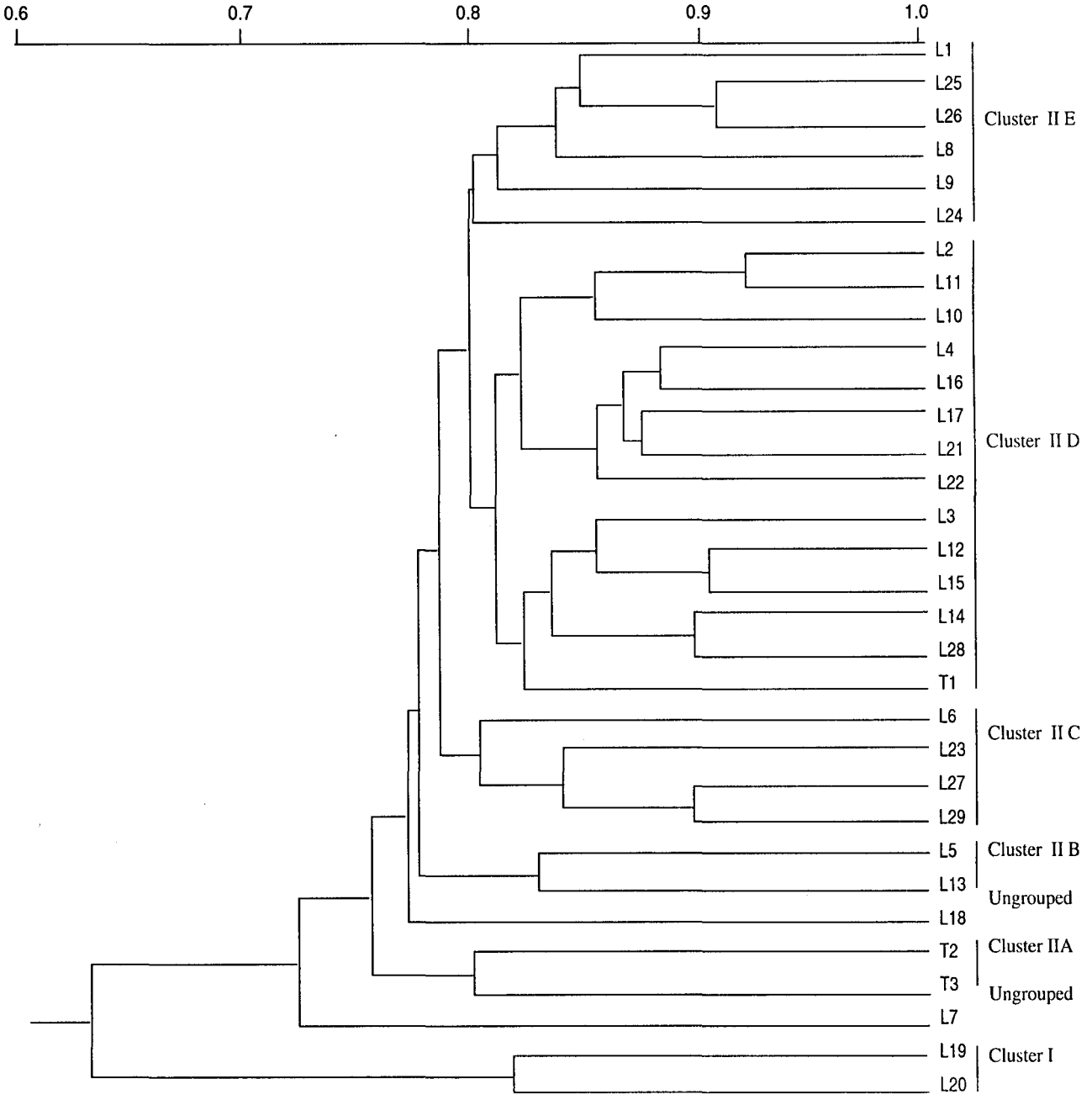


**Fig. 2: Dendrogram generated based on RAPD primers for 29 maintainer lines and three improved cultivars of rice**

not be able to cover the complete genome. The results however indicated top heterotic crosses for grain yield per plant and other characters revealing low level of genetic diversity between parents. On the other hand, the hybrids involving parents with maximum dissimilarity coefficients ($L_{19}$ x $T_2$) with genetic similarity of 62.1%, displayed non-significant estimates of heterosis (-19.89) for grain yield per plant. It suggested the prospects of higher level of heterosis with parents having optimum level of diversity.

In the present study, the RAPD markers had revealed the genetic diversity among lines and grouped them into distinct clusters and subclusters. The findings will have immediate practical implication in the hybrid breeding programme for the development of CMS lines of varying duration, plant height and productivity traits with optimum genetic diversity, which can exhibit enhanced level of heterosis. The present study emphasizes the use molecular markers in parental improvement and development of heterotic rice hybrids.

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# Carpological and Palynological Characterization of Germplasm of Oregano (*Origanum syriacum* L.) in Lebanon

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The morphobiometric characters of the pollens and mericarps of 74 accessions of *Origanum syriacum* L. collected in the Damour River area of Mount Lebanon, Lebanon, were studied for the first time. The detailed analysis of the morphobiometric descriptors and the further elaboration of these by means of cluster analysis distinguished two principal groups. These groups seem to correspond to the two varieties of *O. syriacum* described by Ietswaart (1980), *viz.*, *O. syriacum* L. var. *syriacum* and *O. syriacum* L. var. *bevanii* (Holmes) Ietswaart. On the other hand, with regard to their pollen characteristics, the studied accessions could be considered as ecoform variants. The greater uniformity detected in pollen traits could be ascribed to the enthomogamic pollination mechanisms present in *Origanum syriacum* and the related gene flow taking place among the populations under study. It can be hypothesized that such mechanisms might have in fact contributed to the natural selection of these populations, favoring greater uniformity in pollen traits rather than in those related to the mericarps. The diversity detected by this study in mericarps and pollen grains represents a useful diacritic element for the better understanding of *Origanum syriacum*, suggesting at the same time the presence of genetic variation that could be exploited in crop improvement programmes targeting this species.

**Key Words:** ***Origanum syriacum*, Morphology, Mericarps, Pollen grains, Lebanon**

## Introduction

The genus *Origanum* L. (Family Lamiaceae) is characterized by annual, biennial or perennial herbs distributed predominantly in the eastern Mediterranean (75%) and only a few species in the Western Mediterranean region. The genus consists of 42 species and a total of 49 taxa including species, subspecies, and varieties (Ietswaart, 1980; Calström, 1984; Danin, 1990; Danin and Künne, 1996).

Oregano has been always playing an important role in the daily life of many populations around the world. The cultivation of oregano in its natural habitat is particularly significant in Morocco, Turkey, Lebanon, Albania, which are major world exporting countries of this spice. In spite of its high social and economic value, oregano can be considered a neglected crop from the stand point of its genetic resources (Padulosi, 1997). Very little research has been carried out with regard to the better understanding of its genetic diversity, gene pool, conservation and sustainable use. Oregano species are also valuable for their widespread use in gardening and in medicine due to the presence of essential oils with antimicrobic, cytotoxic and antioxidant properties (Lagouri *et al.*, 1993; Sivropoulou *et al.*, 1996). The possibility of inter-specific crosses within the *Origanum* genus (Ietswaart, 1980) is also an opportunity for crop improvement programmes, including those aiming at enhancement of agronomic performance (resistance to biotic and abiotic stresses) and improvement of its values as condiment and medicinal plant.

In order to shed more light on the genetic diversity of *Origanum* and its sustainable use, a study was carried out in Lebanon during 2003-2004. The study focused on the palynological and carpological characterization of wild accessions of *Origanum syriacum* collected in Lebanon.

## Materials and Methods

Seventy four wild populations of *O. syriacum* were sampled from a study area of 100 km² in the watershed of the Damour River in Mount Lebanon, a region characterized by a Mediterranean ecosystem (Fig. 1, 2). The collected mericarps were planted in nearby fields. Table 1 provides a complete list of the material investigated. The characterization and the determination of the morphobiometric parameters of the accessions relative to the mericarps were carried out with a stereomicroscope Jena Citoval 2 provided with magnification lens up to 16x.

For the shape and colour of the mericarps, the number codes as suggested by Berggren (1969) were used. Table 2 and Figs. 3 and 4 report biometric and carpological characters considered in the study.

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**Fig. 1: Harvesting of *Origanum syriacum* plants**

**Fig. 2: A view of the flora of the Mount Lebanon**

**Table 1. Codes of 74 accessions of *Origanum syriacum* present in the villages of Mount Lebanon**

| Codes | Accessions | Villages | Codes | Accessions | Villages |
|---|---|---|---|---|---|
| 1 | A Da 1 | Ain Dara | 38 | K Nab 18 | Kfar Nabrakh |
| 2 | A Da 4 | Ain Dara | 39 | K Nab 21 | Kfar Nabrakh |
| 3 | Agh 3 | Aghmid | 40 | K Nab 25 | Kfar Nabrakh |
| 4 | Agh 5 | Aghmid | 41 | K Nab 26 | Kfar Nabrakh |
| 5 | Agh 8 | Aghmid | 42 | K Nab 29 | Kfar Nabrakh |
| 6 | Agh 13 | Aghmid | 43 | K Nab 31 | Kfar Nabrakh |
| 7 | Agh 14 | Aghmid | 44 | K Nab 32 | Kfar Nabrakh |
| 8 | Agh 15 | Aghmid | 45 | K Nab 35 | Kfar Nabrakh |
| 9 | Agh 17 | Aghmid | 46 | K Nab 36 | Kfar Nabrakh |
| 10 | A Ha 3a | Ain El Halazoun | 47 | M Ba 1 | Majdel Baana |
| 11 | A Ha 4 | Ain El Halazoun | 48 | M Ba 2ab | Majdel Baana |
| 12 | Amm 1 | Ammiq | 49 | M Me 3 | Majdel Meoush |
| 13 | Amm 6 | Ammiq | 50 | M Me 4 | Majdel Meoush |
| 14 | Amm 11 | Ammiq | 51 | M Me 8 | Majdel Meoush |
| 15 | Amm 12 | Ammiq | 52 | M Me 9 | Majdel Meoush |
| 16 | Amm 15 | Ammiq | 53 | M Me 11 | Majdel Meoush |
| 17 | Amm 17 | Ammiq | 54 | M Me 16 | Majdel Meoush |
| 18 | A Tr 1 | Ain Trez | 55 | M Me 17 | Majdel Meoush |
| 19 | A Tr 3 | Ain Trez | 56 | M Me 19 | Majdel Meoush |
| 20 | Bir 6 | Bireh | 57 | M Me 20 | Majdel Meoush |
| 21 | Bir 7 | Bireh | 58 | M Me 26 | Majdel Meoush |
| 22 | Bir 9 | Bireh | 59 | M Me 27 | Majdel Meoush |
| 23 | Bir 13 | Bireh | 60 | M Me 28 | Majdel Meoush |
| 24 | Bmh 2 | Bmehray | 61 | M Me 31 | Majdel Meoush |
| 25 | Bmh 3 | Bmehray | 62 | Ram 2A | Ramlieh |
| 26 | Bmh 4 | Bmehray | 63 | Ram 4 | Ramlieh |
| 27 | Bmh 6 | Bmehray | 64 | Ram 6 | Ramlieh |
| 28 | Gea 2 | Geael | 65 | Ram 8 | Ramlieh |
| 29 | Gea 4 | Geael | 66 | R Naa 2a | Rouaisset En Naamane |
| 30 | K Nab 4 | Kfar Nabrakh | 67 | R Naa 3 | Rouaisset En Naamane |
| 31 | K Nab 7 | Kfar Nabrakh | 68 | R Naa 7 | Rouaisset En Naamane |
| 32 | K Nab 8 | Kfar Nabrakh | 69 | R Naa 8 | Rouaisset En Naamane |
| 33 | K Nab 10 | Kfar Nabrakh | 70 | R Naa 9 | Rouaisset En Naamane |
| 34 | K Nab 12 | Kfar Nabrakh | 71 | War 1 | Warhanieh |
| 35 | K Nab 14 | Kfar Nabrakh | 72 | War 4 | Warhanieh |
| 36 | K Nab 15 | Kfar Nabrakh | 73 | War 9 | Warhanieh |
| 37 | K Nab 15a | Kfar Nabrakh | 74 | War 10 | Warhanieh |

For the characterization and the determination of the morphobiometric parameters of pollen material, the technique of Erdtman (1960) was deployed and the data are presented in Table 3. For the measurements of the parameters studied, an optical microscope Zeiss with a magnification of x100 was used. For the SEM observations (Jeol 35), pollen grains were placed on stubs and covered by gold dust to a thickness of 100Å (Fig. 5a, 5b). For the palynological terminology, the work of De Leonardis *et al.* (1986) was considered as a reference.

**Table 2. Mean values of the carpological parameters of 74 accessions of *Origanum syriacum* (mm)**

| Codes | Le | Wi | Th | A | B | C | D | E | F | Colour | Shape |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.00 | 0.61 | 0.38 | 0.24 | 0.13 | 0.14 | 0.37 | 0.23 | 0..22 | 15,23,28,29,31 | 3,4,39 |
| 2 | 0.99 | 0.62 | 0.31 | 0.19 | 0.19 | 0.13 | 0.32 | 0.18 | 0.18 | 22,23,29 | 3,4 |
| 3 | 1.10 | 0.50 | 0.32 | 0.21 | 0.16 | 0.17 | 0.37 | 0.20 | 0.22 | 16,22,23,29,30,31 | 2,3,4,38,46,47 |
| 4 | 1.00 | 0.65 | 0.36 | 0.22 | 0.18 | 0.17 | 0.38 | 0.19 | 0.20 | 23,28,29,30,31,35 | 2,3,46,47,48 |
| 5 | 0.96 | 0.62 | 0.36 | 0.20 | 0.16 | 0.15 | 0.36 | 0.21 | 0.19 | 22,23,28,29,30 | 2,3,4,37,47 |
| 6 | 1.00 | 0.66 | 0.33 | 0.23 | 0.17 | 0.18 | 0.40 | 0.20 | 0.20 | 21,29,30 | 3,4,39,47 |
| 7 | 1.00 | 0.66 | 0.33 | 0.19 | 0.19 | 0.19 | 0.40 | 0.18 | 0.19 | 22,23,26,27,28,29 | 3,4,37,38,47,48 |
| 8 | 1.00 | 0.69 | 0.38 | 0.20 | 0.12 | 0.18 | 0.38 | 0.18 | 0.20 | 21,22,23,29,30 | 2,3,4,47,48 |
| 9 | 1.07 | 0.66 | 0.38 | 0.20 | 0.19 | 0.18 | 0.40 | 0.20 | 0.22 | 21,22,29,30 | 2,3,37,46,47 |
| 10 | 0.90 | 0.54 | 0.36 | 0.23 | 0.20 | 0.18 | 0.40 | 0.20 | 0.22 | 22,23,29,30,32 | 2,3,37,38,46,47,48 |
| 11 | 1.00 | 0.65 | 0.32 | 0.19 | 0.17 | 0.16 | 0.30 | 0.14 | 0.19 | 22,23,29,30,31 | 3,46,47,48 |
| 12 | 1.00 | 0.65 | 0.33 | 0.20 | 0.16 | 0.17 | 0.37 | 0.17 | 0.19 | 22,23,29 | 3,46,47,48 |
| 13 | 0.99 | 0.67 | 0.34 | 0.22 | 0.15 | 0.20 | 0.45 | 0.19 | 0.19 | 22,23,28,29,30 | 3,46,47,48 |
| 14 | 0.90 | 0.67 | 0.34 | 0.21 | 0.14 | 0.16 | 0.38 | 0.19 | 0.18 | 22,28,29,30 | 3,4,46,47,49 |
| 15 | 0.90 | 0.63 | 0.33 | 0.19 | 0.15 | 0.16 | 0.30 | 0.17 | 0.17 | 26,27,28,29,30 | 3,4,46,47,48 |
| 16 | 1.00 | 0.67 | 0.37 | 0.20 | 0.15 | 0.17 | 0.40 | 0.20 | 0.20 | 21,23,28,29 | 2,3,37,46,47 |
| 17 | 0.88 | 0.56 | 0.31 | 0.23 | 0.15 | 0.12 | 0.34 | 0.25 | 0.23 | 22,23,28,29,30 | 3,4,6,47,48 |
| 18 | 0.90 | 0.55 | 0.30 | 0.18 | 0.15 | 0.12 | 0.30 | 0.18 | 0.20 | 22,23,28,29,30 | 2,3,46,47 |
| 19 | 0.80 | 0.55 | 0.33 | 0.20 | 0.17 | 0.14 | 0.34 | 0.19 | 0.20 | 23,28,29,30,31 | 3,4,46,47,48 |
| 20 | 0.90 | 0.66 | 0.34 | 0.20 | 0.13 | 0.16 | 0.30 | 0.19 | 0.22 | 23,29,30,31 | 4,47,48 |
| 21 | 1.10 | 0.66 | 0.38 | 0.23 | 0.18 | 0.18 | 0..38 | 0.23 | 0.21 | 22,23,29,30,40 | 3,4,46,47,48 |
| 22 | 0.98 | 0.66 | 0.36 | 0.23 | 0.17 | 0.16 | 0.30 | 0.23 | 0.19 | 23,26,29,30 | 4,46,47,48,49 |
| 23 | 1.20 | 0.68 | 0.38 | 0.26 | 0.20 | 0.24 | 0.45 | 0.24 | 0.22 | 23,29,30 | 4,37,38,39,48 |
| 24 | 1.10 | 0.70 | 0.41 | 0.22 | 0.17 | 0.17 | 0.39 | 0.23 | 0.21 | 22,23,29,30 | 3,4,37,39,47,48 |
| 25 | 1.10 | 0.65 | 0.39 | 0.22 | 0.18 | 0.16 | 0.30 | 0.20 | 0.22 | 22,29,30 | 4,37,38,39,47,48 |
| 26 | 1.10 | 0.75 | 0.36 | 0.21 | 0.19 | 0.16 | 0.40 | 0.21 | 0.21 | 29,30 | 38,46,47,48 |
| 27 | 1.00 | 0.65 | 0.37 | 0.21 | 0.15 | 0.15 | 0.35 | 0.23 | 0.23 | 23,29,30,31 | 4,38,39,47,48 |
| 28 | 1.00 | 0.69 | 0.35 | 0.24 | 0.14 | 0.18 | 0.38 | 0.21 | 0.22 | 29,30 | 38,39,47,48 |
| 29 | 1.00 | 0.71 | 0.33 | 0.22 | 0.22 | 0.14 | 0.33 | 0.21 | 0.23 | 23,29,30 | 47,48 |
| 30 | 1.00 | 0.67 | 0.37 | 0.22 | 0.15 | 0.13 | 0.28 | 0.20 | 0.22 | 23,29,30,31 | 38,47,48 |
| 31 | 1.10 | 0.69 | 0.33 | 0.20 | 0.12 | 0.16 | 0.33 | 0.20 | 0.23 | 23,29,30 | 37,39,47,48 |
| 32 | 1.00 | 0.68 | 0.36 | 0.23 | 0.14 | 0.16 | 0.33 | 0.23 | 0.20 | 23,29,30,31 | 37,38,47,48 |
| 33 | 0.90 | 0.66 | 0.33 | 0.23 | 0.13 | 0.14 | 0.30 | 0.20 | 0.23 | 29,30,31 | 47,48 |
| 34 | 0.90 | 0.69 | 0.37 | 0.22 | 0.16 | 0.14 | 0.33 | 0.19 | 0.20 | 23,29,30 | 38,39,47,48 |
| 35 | 1.00 | 0.68 | 0.37 | 0.21 | 0.12 | 0.16 | 0.30 | 0.19 | 0.19 | 23,27,29,30,31 | 38,47,48 |
| 36 | 0.90 | 0.61 | 0.30 | 0.20 | 0.14 | 0.16 | 0.30 | 0.20 | 0.20 | 29,30,32 | 2,3,4,5,47 |
| 37 | 0.90 | 0.65 | 0.30 | 0.20 | 0.14 | 0.16 | 0.30 | 0.20 | 0.20 | 29,30,32 | 2,3,4,5,47 |
| 38 | 0.90 | 0.69 | 0.38 | 0.19 | 0.14 | 0.13 | 0.30 | 0.16 | 0.20 | 29,30,31 | 47,48,49 |
| 39 | 1.05 | 0.67 | 0.38 | 0.21 | 0.14 | 0.13 | 0.30 | 0.16 | 0.23 | 22,23,28,29,30,31 | 38,47,48,49 |
| 40 | 1.00 | 0.69 | 0.35 | 0.20 | 0.15 | 0.12 | 0.30 | 0.16 | 0.18 | 29,30,31 | 38,47,48,49 |
| 41 | 1.09 | 0.66 | 0.35 | 0.24 | 0.14 | 0.15 | 0.30 | 0.17 | 0.17 | 23,27,29,30,31 | 47,48 |
| 42 | 1.05 | 0.64 | 0.37 | 0.22 | 0.15 | 0.14 | 0.30 | 0.17 | 0.17 | 28,29,30,31 | 38,39,47,48,49 |
| 43 | 0.90 | 0.71 | 0.35 | 0.22 | 0.13 | 0.14 | 0.30 | 0.17 | 0.16 | 23,27,29,30 | 38,47,48,49 |
| 44 | 1.02 | 0.68 | 0.40 | 0.22 | 0.16 | 0.17 | 0.40 | 0.19 | 0.22 | 23,29,30 | 39,47,48 |
| 45 | 0.99 | 0.69 | 0.36 | 0.20 | 0.15 | 0.16 | 0.36 | 0.17 | 0.18 | 29,30 | 38,39,47,48 |
| 46 | 1.00 | 0.65 | 0.37 | 0.21 | 0.14 | 0.12 | 0.32 | 0.17 | 0.18 | 23,29,30 | 38,39,47,48 |
| 47 | 1.00 | 0.64 | 0.37 | 0.20 | 0.13 | 0.16 | 0.30 | 0.18 | 0.19 | 23,29,30 | 46,47,48 |
| 48 | 1.04 | 0.66 | 0.38 | 0.20 | 0.16 | 0.12 | 0.30 | 0.17 | 0.19 | 23,29,30 | 38,47,48 |
| 49 | 0.98 | 0.62 | 0.37 | 0.20 | 0.13 | 0.14 | 0.30 | 0.18 | 0.18 | 23,29,30 | 28,46,47 |
| 50 | 1.01 | 0.52 | 0.37 | 0.20 | 0.14 | 0.17 | 0.36 | 0.17 | 0.19 | 23,29,30 | 38,47,48 |
| 51 | 0.90 | 0.62 | 0.36 | 0.19 | 0.14 | 0.14 | 0.30 | 0.15 | 0.16 | 23,29,30 | 46,47 |
| 52 | 0.90 | 0.63 | 0.37 | 0.20 | 0.16 | 0.16 | 0.30 | 0.17 | 0.19 | 23,27,29,30 | 38,47,48 |
| 53 | 1.07 | 0.75 | 0.39 | 0.23 | 0.16 | 0.16 | 0.38 | 0.18 | 0.18 | 23,29,30 | 38,39,47,48 |
| 54 | 1.10 | 0.69 | 0.36 | 0.20 | 0.17 | 0.16 | 0.37 | 0.18 | 0.18 | 23,28,29,30 | 47,48 |
| 55 | 1.03 | 0.63 | 0.39 | 0.20 | 0.19 | 0.16 | 0.41 | 0.19 | 0.19 | 23,29,30 | 38,39,47,48 |
| 56 | 1.03 | 0.67 | 0.35 | 0.20 | 0.18 | 0.17 | 0.40 | 0.18 | 0.19 | 23,29,30 | 47,48 |
| 57 | 1.02 | 0.62 | 0.40 | 0.20 | 0.16 | 0.14 | 0.40 | 0.18 | 0.17 | 23,29,30 | 38,47,48 |
| 58 | 1.07 | 0.68 | 0.37 | 0.21 | 0.14 | 0.16 | 0.37 | 0.18 | 0.17 | 23,28,29,30,31 | 38,47,48 |
| 59 | 1.04 | 0.77 | 0.37 | 0.21 | 0.15 | 0.12 | 0.35 | 0.18 | 0.19 | 23,29,30,31 | 38,47,48,49 |
| 60 | 1.04 | 0.66 | 0.37 | 0.21 | 0.15 | 0.16 | 0.38 | 0.19 | 0.19 | 28,29,30 | 47,48 |
| 61 | 1.08 | 0.69 | 0.34 | 0.25 | 0.16 | 0.14 | 0.35 | 0.18 | 0.19 | 23,29,30 | 47,48 |
| 62 | 0.96 | 0.65 | 0.32 | 0.23 | 0.17 | 0.12 | 0.35 | 0.17 | 0.17 | 23,29,30 | 38,47,48 |
| 63 | 1.00 | 0.65 | 0.33 | 0.21 | 0.15 | 0.14 | 0.34 | 0.17 | 0.18 | 22,23 | 37,38,39,48 |
| 64 | 1.00 | 0.66 | 0.32 | 0.19 | 0.15 | 0.13 | 0.30 | 0.15 | 0.17 | 22,23,29,30 | 38,46,47,48 |
| 65 | 0.99 | 0.62 | 0.33 | 0.19 | 0.11 | 0.16 | 0.30 | 0.16 | 0.17 | 23,29,30 | 38,47,48 |
| 66 | 1.00 | 0.65 | 0.33 | 0.20 | 0.14 | 0.14 | 0.33 | 0.17 | 0.18 | 23,28,29,30 | 38,39,47,48 |
| 67 | 1.03 | 0.67 | 0.32 | 0.21 | 0.18 | 0.14 | 0.36 | 0.16 | 0.17 | 22,23,29,30 | 38,47,48,49 |
| 68 | 1.01 | 0.69 | 0.39 | 0.19 | 0.13 | 0.14 | 0.30 | 0.16 | 0.17 | 23,28,29,30 | 38,46,47,48 |
| 69 | 0.90 | 0.66 | 0.33 | 0.20 | 0.15 | 0.13 | 0.30 | 0.16 | 0.17 | 23,28,29,30 | 38,46,47,48,49 |
| 70 | 0.90 | 0.66 | 0.35 | 0.23 | 0.13 | 0.14 | 0.3 | 0.16 | 0.17 | 22,23,28,29,30 | 38,39,47,48 |
| 71 | 1.01 | 0.67 | 0.37 | 0.19 | 0.15 | 0.14 | 0.3 | 0.15 | 0.16 | 23,29,30,40 | 38,39,47,48 |
| 72 | 1.16 | 0.70 | 0.32 | 0.23 | 0.15 | 0.17 | 0.37 | 0.19 | 0.19 | 29,30 | 38,47,48,49 |
| 73 | 1.03 | 0.67 | 0.38 | 0.21 | 0.14 | 0.13 | 0.3 | 0.15 | 0.19 | 23,29,30 | 38,47,48 |
| 74 | 1.18 | 0.77 | 0.37 | 0.20 | 0.18 | 0.15 | 0.38 | 0.18 | 0.18 | 23,29,30 | 38,47,48 |

Le: length of mericarps; Wi: width of mericarps; Th: thickness of mericarps; A: length of areola; B: width of left areola cavity; C: width of right areola cavity; D: width of areola; E: length of areola seen laterally; F: width of areola seen laterally

**Table 3. Mean values of palynological parameters of 74 accessions of *Origanum syriacum* (µm)**

| Codes | P. | E. | P.ex | E.ex | Mes | E.lum | P.lum |
|---|---|---|---|---|---|---|---|
| 1 | 40 | 38 | 3.3 | 2.4 | 14 | 1.4 | 1.2 |
| 2 | 42 | 37 | 3.5 | 2.4 | 12 | 1.4 | 1.2 |
| 3 | 37 | 37 | 3.4 | 2.4 | 12 | 1.5 | 1.2 |
| 4 | 40 | 37 | 3.5 | 2.4 | 13 | 1.5 | 1.2 |
| 5 | 40 | 37 | 3.5 | 2.4 | 12 | 1.5 | 1.3 |
| 6 | 41 | 39 | 3.5 | 2.4 | 12 | 1.9 | 1.5 |
| 7 | 39 | 31 | 2.8 | 2.1 | 9 | 0.6 | 0.6 |
| 8 | 38 | 34 | 2.7 | 2.0 | 11 | 0.6 | 0.6 |
| 9 | 41 | 37 | 3.2 | 2.4 | 13 | 1.3 | 0.6 |
| 10 | 38 | 36 | 2.9 | 2.4 | 12 | 1.3 | 0.6 |
| 11 | 38 | 34 | 2.9 | 2.4 | 12 | 1.2 | 0.6 |
| 12 | 39 | 35 | 3.2 | 2.5 | 12 | 1.2 | 1.2 |
| 13 | 42 | 37 | 3.3 | 2.6 | 13 | 1.3 | 1.2 |
| 14 | 39 | 37 | 2.5 | 2.4 | 12 | 0.6 | 0.6 |
| 15 | 41 | 39 | 3.2 | 2.7 | 13 | 1.5 | 1.2 |
| 16 | 31 | 33 | 2.6 | 2.0 | 11 | 1.1 | 1.0 |
| 17 | 33 | 30 | 2.7 | 2.0 | 12 | 1.2 | 1.1 |
| 18 | 41 | 33 | 3.3 | 2.6 | 13 | 1.2 | 1.2 |
| 19 | 29 | 31 | 2.5 | 2.0 | 12 | 1.0 | 1.0 |
| 20 | 34 | 28 | 2.7 | 2.0 | 10 | 1.2 | 1.0 |
| 21 | 33 | 30 | 2.8 | 2.0 | 11 | 1.2 | 1.0 |
| 22 | 31 | 30 | 2.8 | 2.0 | 12 | 1.5 | 1.0 |
| 23 | 31 | 33 | 2.9 | 2.0 | 11 | 1.1 | 1.0 |
| 24 | 32 | 30 | 3.0 | 2.0 | 11 | 1.2 | 1.0 |
| 25 | 32 | 29 | 2.9 | 2.0 | 11 | 1.5 | 1.0 |
| 26 | 32 | 32 | 2.9 | 2.0 | 11 | 1.2 | 1.0 |
| 27 | 30 | 31 | 3.0 | 2.0 | 12 | 1.5 | 1.0 |
| 28 | 37 | 36 | 3.2 | 2.0 | 14 | 1.3 | 1.2 |
| 29 | 32 | 29 | 3.0 | 2.0 | 11 | 1.1 | 1.1 |
| 30 | 29 | 31 | 2.6 | 1.8 | 12 | 1.2 | 1.0 |
| 31 | 30 | 33 | 2.8 | 1.9 | 12 | 1.2 | 1.0 |
| 32 | 28 | 31 | 2.7 | 1.9 | 11 | 1.3 | 1.0 |
| 33 | 29 | 32 | 2.9 | 2.2 | 12 | 1.3 | 1.0 |
| 34 | 31 | 32 | 2.5 | 1.7 | 12 | 1.5 | 1.0 |
| 35 | 29 | 30 | 3.0 | 2.1 | 11 | 1.2 | 1.0 |
| 36 | 32 | 34 | 3.0 | 2.0 | 13 | 1.5 | 1.0 |
| 37 | 28 | 32 | 2.7 | 2.0 | 12 | 1.3 | 1.0 |
| 38 | 32 | 29 | 2.6 | 2.0 | 11 | 1.4 | 1.0 |
| 39 | 32 | 30 | 2.6 | 2.0 | 12 | 1.3 | 1.0 |
| 40 | 41 | 37 | 2.6 | 2.4 | 13 | 1.4 | 1.2 |
| 41 | 30 | 32 | 2.7 | 2.0 | 12 | 1.1 | 1.0 |
| 42 | 31 | 30 | 2.8 | 2.0 | 12 | 1.1 | 1.0 |
| 43 | 29 | 32 | 2.8 | 2.0 | 12 | 1.5 | 1.0 |
| 44 | 30 | 30 | 2.7 | 2.0 | 11 | 1.1 | 1.0 |
| 45 | 33 | 30 | 2.8 | 2.0 | 11 | 1.2 | 1.0 |
| 46 | 29 | 31 | 2.7 | 2.0 | 11 | 1.5 | 1.0 |
| 47 | 30 | 30 | 2.7 | 2.0 | 11 | 1.6 | 1.0 |
| 48 | 26 | 28 | 2.8 | 2.4 | 13 | 1.2 | 1.2 |
| 49 | 30 | 29 | 2.6 | 2.0 | 10 | 1.3 | 1.0 |
| 50 | 29 | 27 | 2.6 | 2.0 | 11 | 1.2 | 1.0 |
| 51 | 30 | 31 | 2.7 | 2.0 | 10 | 1.5 | 1.1 |
| 52 | 37 | 37 | 2.7 | 2.1 | 11 | 1.3 | 1.0 |
| 53 | 30 | 29 | 2.7 | 2.0 | 11 | 1.2 | 1.1 |
| 54 | 29 | 32 | 3.0 | 2.0 | 11 | 1.3 | 1.0 |
| 55 | 28 | 31 | 2.8 | 2.0 | 11 | 1.2 | 1.0 |
| 56 | 31 | 28 | 3.0 | 2.0 | 10 | 1.5 | 1.2 |
| 57 | 30 | 31 | 2.8 | 2.0 | 12 | 1.5 | 1.0 |
| 58 | 32 | 30 | 2.5 | 2.5 | 12 | 1.1 | 1.0 |
| 59 | 34 | 32 | 3.0 | 2.0 | 12 | 1.6 | 1.3 |
| 60 | 31 | 32 | 3.0 | 2.0 | 12 | 1.7 | 1.3 |
| 61 | 31 | 30 | 2.7 | 2.1 | 10 | 1.4 | 1.1 |
| 62 | 37 | 39 | 2.8 | 2.2 | 14 | 1.2 | 1.2 |
| 63 | 37 | 35 | 2.9 | 2.4 | 13 | 1.2 | 1.2 |
| 64 | 39 | 37 | 3.0 | 2.4 | 14 | 1.3 | 1.2 |
| 65 | 32 | 29 | 2.4 | 2.1 | 10 | 1.2 | 1.2 |
| 66 | 29 | 27 | 2.5 | 2.0 | 10 | 1.1 | 1.0 |
| 67 | 25 | 27 | 2.5 | 2.0 | 10 | 1.1 | 1.0 |
| 68 | 30 | 29 | 2.5 | 2.0 | 12 | 1.3 | 1.0 |
| 69 | 30 | 29 | 2.8 | 2.0 | 11 | 1.2 | 1.1 |
| 70 | 29 | 29 | 2.7 | 2.0 | 12 | 1.2 | 1.0 |
| 71 | 32 | 29 | 2.5 | 2.0 | 11 | 1.2 | 1.0 |
| 72 | 29 | 31 | 2.5 | 2.0 | 12 | 1.1 | 1.0 |
| 73 | 31 | 29 | 2.5 | 2.0 | 10 | 1.1 | 1.0 |
| 74 | 29 | 30 | 2.3 | 1.8 | 11 | 1.2 | 1.0 |

P.: polar axis; E.: equatorial axis; P.ex: exine thickness in polar view; E.ex: exine thickness in equatorial view; Mes: mesocolpium; E.lum: length of the lumina in equatorial view; P.lum: length of the lumina in polar view.

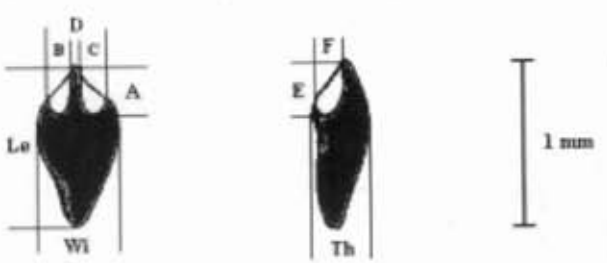


**Fig. 3: Frontal and lateral views of *Origanum syriacum* mericarp**


**Fig. 4: a, b, c Different shapes in *Origanum syriacum* mericarp**

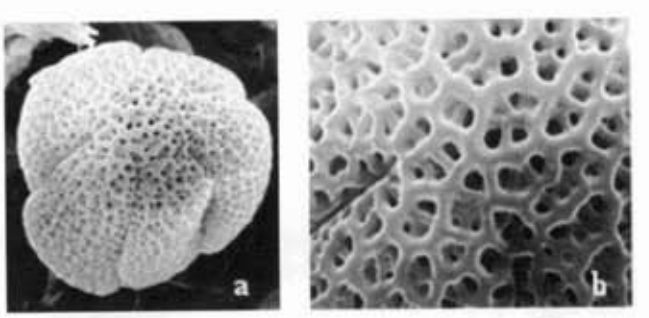


**Fig. 5: SEM micrographs of *Origanum syriacum* pollen: a) pollen with six colpi (x3.000), b) reticulate ornamentation with lumina of the exinic wall (x10.000)**

The measurements were recorded on 30 mericarps and 30 pollen grains for each accession.

Based on cluster analyses relative to the mericarps and pollen grains using the Euclidean distance of the morphobiometric characters (length, width, polar axis,

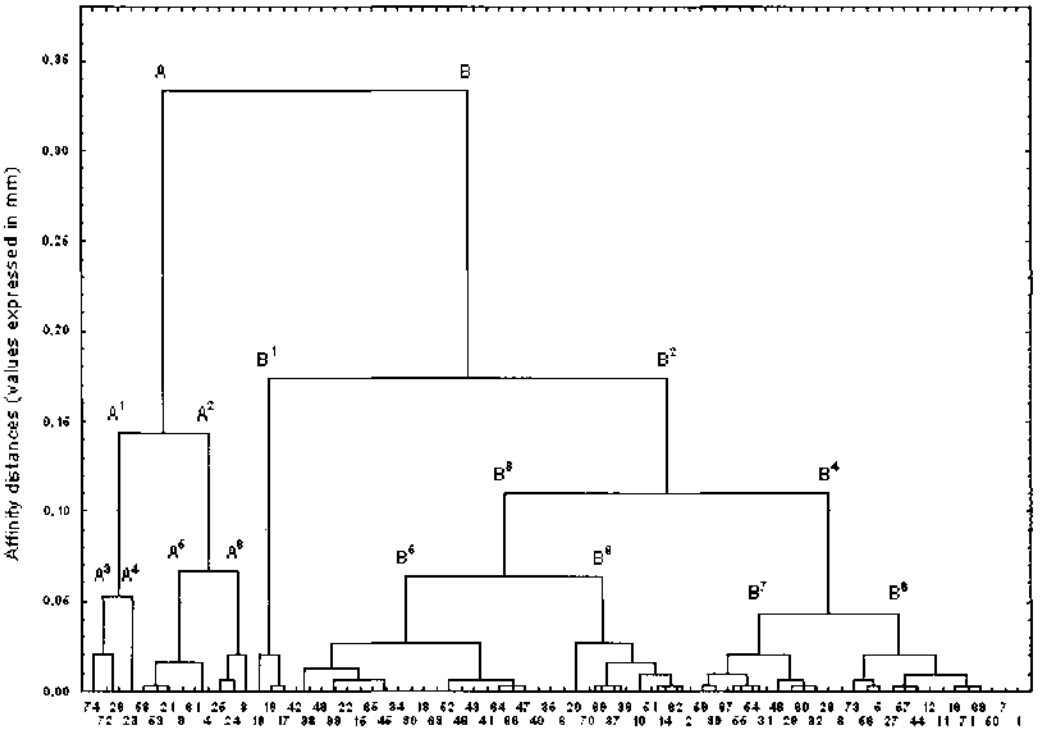


**Fig. 6: Average linkage cluster analysis of 74 *Origanum syriacum* accessions based on length (Le) of the mericarps**

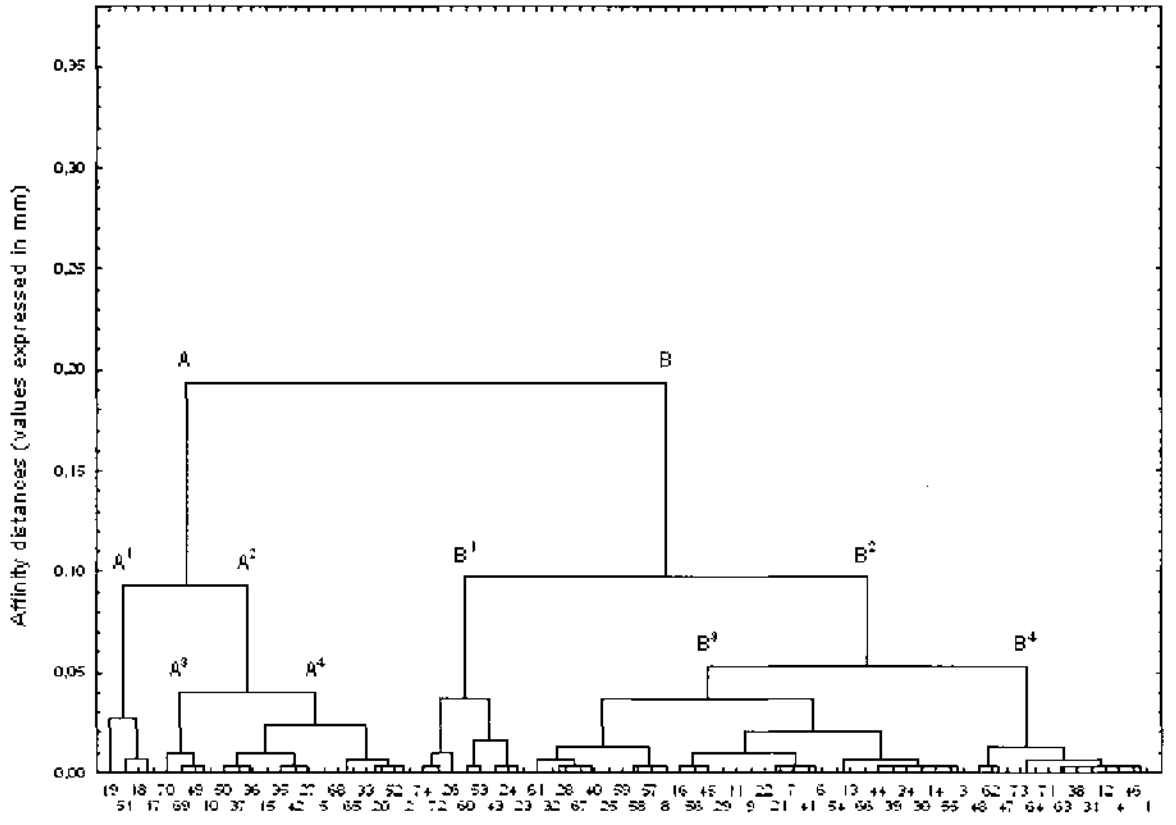


**Fig. 7: Average linkage cluster analysis of 74 *Origanum syriacum* accessions based on width (Wi) of the mericarps**

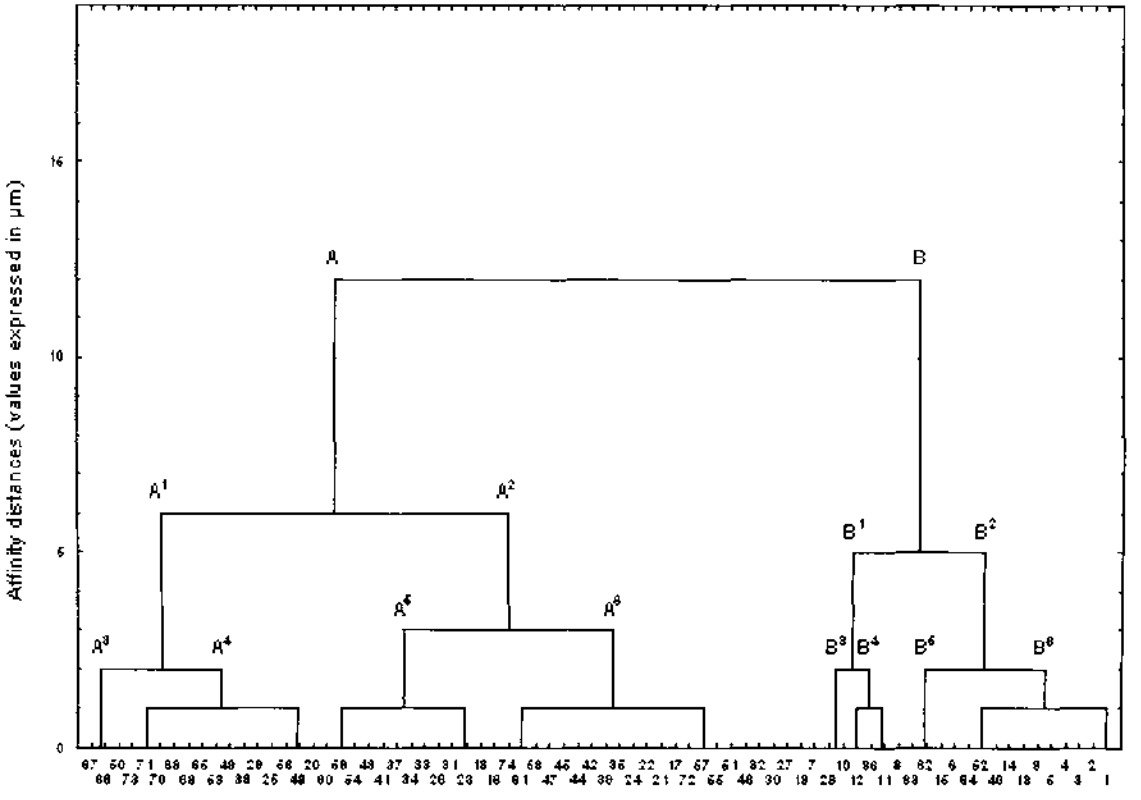


**Fig. 8: Average linkage cluster analysis of 74 *Origanum syriacum* accessions based on equatorial axis (E.) of the pollen grains**

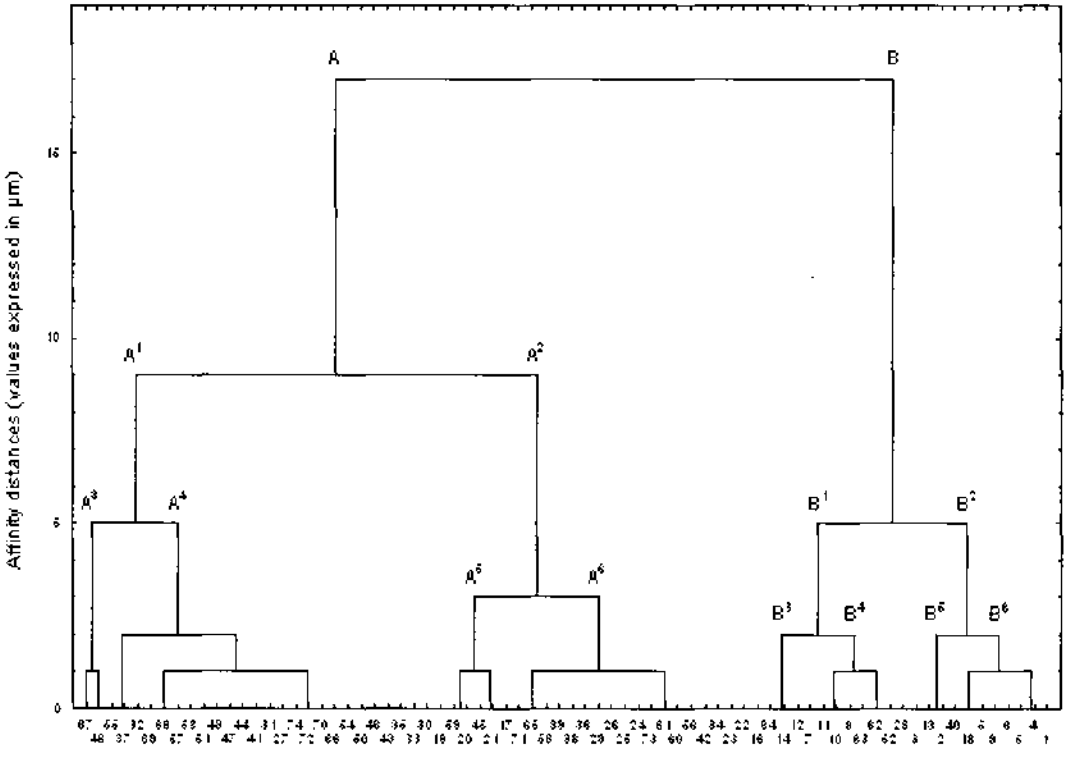


**Fig. 9: Average linkage cluster analysis of 74 *Origanum syriacum* accessions based on polar axis (P.) of the pollen grains**

equatorial axis), the 74 accessions of *O. syriacum* used in the study were grouped in four clusters (Fig. 6, 7, 8, 9).

**Results and Discussion**

The study revealed that the mericarps had a colour that varied from egg-yellow to brown-purple, but the most frequent colours were orange-brown, red-brown and nut-brown (Table 2). The shape varied from restricted-elliptical to wide-ovoidal, but the most frequent were ovoidal and tight-ovoidal (Fig. 4a, 4b, 4c). The mean values of different carpological parameters (Table 2) indicated that the mericarp length (Le) varied from 0.70 mm in the accessions Amm 12, A Tr 3, R Naa 8 to 1.40 mm in M Me 31; the width (Wi) from 0.4 mm in Agh 3, A Tr 1, A Tr 3 to 0.9 mm in Agh 17, War 10; the thickness (Th) from 0.2 mm in A Tr 1, K Nab 15, K Nab 15a to 0.5 mm in Agh 5,13,15, Amm 15, Bmh 3, K Nab 18, 21,32, War 4; length of areola (A) from 0.18 mm in A Tr 1 to 0.26 mm in Bir 13; width of left areola cavity (B) from 0.12 mm in Agh 15, Knab 7, K Nab 14 to 0.22 mm in Gea 4; width of right areola cavity (C) from 0.12 mm in Amm 17, A Tr 1, K Nab 25, K Nab 36, M Ba 2ab, M Me 27, Ram 2a to 0.24 mm in Bir 13; width of areola (D) from 0.28 mm in K Nab 4 to 0.45 mm in Amm 6, Bir 13; length of areola seen laterally (E) from 0.14 mm in A Ha 4 to 0.25 mm in Amm 17; and the width of areola seen laterally (F) from 0.16 mm in K Nab 31, M Me 8 to 0.23 mm in Amm 17, Bmh 6, Gea 4, K Nab 7, K Nab 10, K Nab 21.

The pollen grains are characterized by monads, isopolar and radiosymmetrical shapes. The openings are formed by 6 longitudinal colpi running in parallel or slightly nearer in the central part. The tectum has a primary reticulum with wide lumina and a secondary reticulum with a smaller lumina (Fig. 5a, 5b). The mean values of different palynological parameters (Table 3) indicated that the polar axes (P.) varied from 24 μm in R Naa 3 to 46 μm in Agh 17; equatorial axes (E.) from 25 μm in Bir 6, Ram 8, R Naa 2a,3,7,8 to 44 μm in A Da 1, Agh 13; the thickness of the exine in polar view (P.ex) from 2.0 μm in War 10 to 3.5 μm in A Da 1,4, Agh 3,5,8,13,14,15,17, Amm 1,6,12, A Tr 1, Gea 2; thickness of the exine in equatorial view (E.ex) from 1.5 μm in K Nab 4,7,8,12, War 10 to 2.9 μm in Amm 1,6,12, A Tr 1; distance between the two colpi in equatorial view (Mes) from 7 μm in Agh 14, Bir 6 to 24 μm in Agh 5; length of the lumina in equatorial view (E.lum) from 0.6 μm in Agh 14,15, Amm 11 to 2.4 μm in A Da 1,4 Agh 8,13; length of the lumina in polar view (P.lum) from 0.6 μm in Agh 14,15,17, A Ha 3a,4 to 2.4 μm in Agh 13.

The characteristics of the fruits, the presence of endosperm and the shape of the corolla are some of the useful characters in the phylogeny of the Lamiaceae and have been used as important elements in recent classifications (Cantino and Sanders, 1986; Cantino, 1992; Cantino *et al.*, 1992). There also exists an extensive literature on the study of the pericarp, which indicates the usefulness of this character for the classification of this family (Hedge, 1970; Marin *et al.*, 1994; Rejdali, 1990; Ryding, 1992, 1994, 1995; Campion-Bourget, 1993). The variation in chemical constituents in *O. syriacum* had earlier been reported (Fleisher and Fleisher, 1991; Dudai *et al.*, 1992). Interesting results on the taxonomy, evolution, conservation and utilization of the genus *Origanum* are also available in literature (Padulosi 1997). However, little or no research at all had ever been carried out on the morphobiometry of mericarps of *Origanum* species. More generally, in *O. vulgare* L. and *O. majorana* L., Fabre and Nicoli (1965) observed mericarps with a dimension of 0.5-1.0 mm, ovoid in shape, smooth walls, not hairy and areola with basal position and conical shape. *O. onites* L. (present in the sect. Majorana (Miller) Bentham together with *O. syriacum*) has a colour from orange-brown to brown and a shape from elliptic to widely obovate. From the comparison of the mericarps of these two species, it is apparent that there is a greater thickness of mericarp in *O. onites* both in frontal and lateral view, while the length of the areola and its concavity are smaller.

From the palynological point of view, the family Lamiaceae has been well studied especially with regard to its important melisso-palynological contribution (Maurizio and Louveaux, 1962; Ubera, 1981; De Leonardis *et al.*, 1982, 1984, 1992; Negrin Sosa and La-Serna Ramos, 1985; Zizza *et al.*, 1985; León-Arencibia and La-Serna Ramos, 1992). Pla Dalmau (1961) examined the pollen grains of *O. vulgare* L. defining the surface of the exine as being a rough claveate type. Moore and Webb (1978) included the pollen of the genus *Origanum* in the type *Mentha* (with *Mentha*, *Lycopus*, *Thymus* and *Salvia*), characterized by a perforated eureticolate tectum and distinct from the *Prunella* type (with *Prunella*, *Nepeta* and *Glechoma*) that has a suprareticulate tectum. Luque and Candau (1987) ascribed *O. compactum* Bentham and *O. virens* Hoffmanns. & Link to the type *Salvia verbenaca* characterized by pollen with a exinic wall and double

reticulum. It is possible to palynologically include the genus *Origanum* in one of the melissotypes described by Persano Oddo and Ricciardelli D'Albore (1989). Furthermore, morphobiometric characters in both palynological and carpological studies, always played an important role to distinguish wild and/or cultivated varieties (Vaughan and Whitehouse, 1971; Roselli, 1979; Recupero-Reforgiato *et al.*, 1988; De Leonardis *et al.*, 1993, 1996a, 1996b, 1998, 2002, 2004; De Leonardis and Fichera, 1995).

In the present study, interestingly the results obtained from the measurements carried out on mericarps and pollen grains of the populations of *O. syriacum* revealed that the length and the width of the mericarps, the size of the polar and equatorial axes of the pollens did not have comparable values within the populations observed. As a matter of fact, it can be concluded from the results (Table 2, 3) that parameters such as length, width, polar axis and equatorial axis did not lead to similar groupings in the dendrograms. Consequently, there was no correspondence among the populations presented in the dendrograms.

Such a condition does not prejudice, however the validity of the dendrograms if they are taken into consideration only in relation to single parameters (Fig. 6, 7, 8, 9). In the dendrograms related to the length and the width of the mericarps (Fig. 6, 7) it was in fact possible to discriminate two groups (A and B) in their turn subdivided in further subgroups, as it can be inferred in the dendrograms elaborated on the basis of the values polar axes and equatorial axes of the pollen (Fig. 8, 9). In the mericarps, however, the presence of numerous subgroups highlighted a greater diversity manifested also through a significant diversification of shapes within the same populations. In the pollen grains, on the contrary, the observed fewer number of subgroups indicated a greater affinity among populations. Many gametophytic characteristics were reported to be related to sporophytic traits (Ottaviano and Mulcahy, 1986). These correlations can be explained in physiological terms considering that the growth parameters in both sporophyte and gametophyte depend on the same metabolic activities (Ottaviano *et al.*, 1980). In this study, the palynological uniformity (observed in reticulate exine, exacolpic, radiosymmetry etc.) did not reflect the evolutionary tendencies observed by Punt (1967, 1971 and 1976) and Walker and Doyle (1975) in many other genera. But at the same time, the discrepancy between the pollen uniformity and mericaps diversity observed here could be explained with the enthomogamous mechanism of pollination of the Lamiaceae characterized by intense gene flow among populations which impact mainly pollen grains making them more uniform than mericarps.

In conclusion, the two groups (A and B) identified in the study, could either correspond to the two varieties of *O. syriacum* described by Ietswaart (1980) (*O. syriacum* L. var. *syriacum* and *O. syriacum* var. *bevanii* (Holmes) Ietswaart), or simply be considered as ecoforms related to the specific climatic, edaphic and altitudinal conditions in which the sampled specimens had been growing. The results from this work also indicated the occurrence of diverse populations within *O. syriacum* in Lebanon, an area particularly promising as source of resistant genes to abiotic stresses (e.g. resistant to drought) in view of very dry conditions where the populations were sampled, making these genetic resources an interesting material for breeding aiming at improving agronomic and other economically useful traits in the species.

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# Characterization of Some Macromutations Induced by Single and Combination Treatments of Gamma Rays, EMS and SA in Urdbean (*Vigna mungo* L. Hepper)

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Two cultivars of urdbean (*Vigna mungo* L. Hepper), namely, PDU1 and T9 were treated with single and combination doses/concentrations of gamma rays, ethyl methane sulphonate (EMS) and sodium azide (SA). Number of various types of morphological macromutations were induced in $M_2$ generation. Of these, thirteen mutants from PDU1 and twelve from T9 were identified as true breeding for plant morphology, pod and seed characters and early maturity in $M_3$ generation. Many macromutants showed significant improvement in yield and other yield components as compared to their parents.

**Key Words: Urdbean ((*Vigna mungo*), Macromutations, Gamma rays, Ethyl methane sulphonate (EMS), Sodiumazide (SA)**

## Introduction

Urdbean (*Vigna mungo* L. Hepper) is an important pulse crop of India grown on an area of about 3.25 million hectares with a production of 1.45 million tonnes with an average yield of 4.48 q/ha (Anonymous, 1999-2000). Since urdbean is a highly self-pollinated crop, the natural variability available is far less to make selections for its improvement. Induced mutagenesis has immense potential in creating genetic variability among naturally exhausted population which is utilized in selection programme for obtaining desirable improvement. The present study was conducted to induce and isolate useful macromutants which could be utilized for genetic improvement of this crop.

## Materials and Methods

Two cultivars of urdbean (*Vigna mungo* L. Hepper), namely, PDU1 and T9 were used as experimental materials. Three hundred pure, healthy and dry seeds of each cultivar were used for each treatment. The seeds were irradiated with $^{60}$Co gamma rays at 15, 30, 45 and 60 kR doses at IARI, New Delhi. For chemical mutagenic treatments, the seeds were presoaked in distilled water for 6 hr and then treated with ethyl methane sulphonate (0.02, 0.04, 0.06 and 0.08 M concentrations) in freshly prepared phosphate buffer (pH 7.0) or sodium azide (2, 4, 6 and 8 mM concentrations) in phosphate buffer (pH 3.0). For combination treatment, the gamma irradiated seeds at 15-60 kR doses were presoaked in distilled water for 6 hr and then treated with 0.02 M EMS or 4 mM SA in the manner described earlier. The mutagen treated seeds were thoroughly washed in running water for an hour to avoid traces of the chemicals, if any.

The mutagen treated seeds along with the untreated control seeds were sown in five rows each measuring 5 m with 30 x 10 cm spacing to raise $M_1$ generation in *kharif*, 1996. The surviving plants were harvested separately and individual plant progenies were raised in $M_2$ generation in Summer, 1997. A number of macromutations were induced in various treatments in $M_2$ generation. The macromutants having sufficient seeds were grown in $M_3$ generation in RBD with three replications in *kharif*, 1997 at Research Farm, Institute of Agricultural Sciences, BHU, Varanasi and they were characterized and also confirmed for their breeding behaviour. The observations were recorded on ten competitive plants from each replication for various yield and yield components in $M_3$ generation.

## Results and Discussion

Several types of macromutations were induced by different mutagenic treatment in $M_2$ generation with respect to growth habit, leaf structure, maturity period and flower-, pod- and seed characteristics in both the cultivars, *viz.*, PDU1 and T9. A dose dependent increase in the frequency and spectrum of macromutations was recorded in $M_2$ generation. A total of 31 and 34 types of macromutants were induced in cvs. PDU1 and T9,

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respectively. The probable causes of these macromutations may be chromosomal changes and most probably point or gene mutations. Most of these macromutations are reported to be polygenic in nature (Yadav and Singh, 1988; Saini and Mahana, 1989; Thakur and Sethi, 1993).

Thirteen and twelve true breeding macromutants for plant morphology, pod- and seed-characters and early maturity derived from cultivars PDU1 and T9, respectively were isolated in $M_3$ generation. These macromutants showed distinct morphological features as compared to their parents (Tables 1 and 2). Ten macromutants derived from both cultivars showed significantly higher seed yield than their parents.

Dwarf, tall, bushy, spreading, tendriller and long peduncled mutants were isolated in $M_3$ generation. The *dwarf* mutants were characterized by condensed nodes, shorter internodes and low yield as compared to the control. The plants exceeding the height of the respective controls by at least 10 cm were placed in tall category.

**Table 1. Mean values of yield and yield traits of macromutants in cultivar PDU1 in $M_3$ generation**

| S.No. | Macromutant | Mutagen treatment | Plant height (cm) | Days to maturity | Number of branches plant | Number of pods/ plant | Pod length (cm) | Number of seeds/ pod | Seed yield/ plant (g) | 100-seed weight (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Tall | 30 kR + 4mM SA | 52.50** | 79.20 | 5.36** | 36.40** | 4.25 | 5.85 | 7.50** | 4.50 |
| 2. | Bushy | 45 kR | 42.82 | 81.50 | 6.90** | 34.86** | 4.36 | 5.60 | 7.30** | 4.25 |
| 3. | Spreading | 30 kR + 4mM SA | 40.24 | 85.50** | 7.58** | 27.60** | 4.35 | 5.85 | 7.10** | 4.40 |
| 4. | Dwarf | 30 kR + 4mM SA | 31.10** | 75.60** | 3.15* | 20.15** | 4.10 | 5.58 | 6.15 | 4.25 |
| 5. | Broad leaved | 0.08 M EMS | 38.63* | 85.25** | 4.50 | 20.58** | 3.90** | 5.15** | 5.90 | 4.05 |
| 6. | Hairy | 45 kR + 0.02 M EMS | 38.36** | 82.37 | 4.52 | 18.22** | 4.40 | 5.86 | 5.35** | 3.90** |
| 7. | Thick podded | 0.02 M EMS | 39.52 | 82.50 | 3.92 | 32.86** | 5.05** | 6.80** | 7.32** | 4.82** |
| 8. | Short podded | 0.08 M EMS | 44.50 | 81.85 | 4.35 | 35.68** | 3.25** | 4.90** | 6.10 | 3.85** |
| 9. | Large seeded | 8 mM SA | 45.50** | 80.68 | 4.55 | 32.87** | 4.75** | 6.25** | 7.25** | 5.45** |
| 10. | Small seeded | 45 kR | 42.56 | 82.86 | 4.55 | 30.50** | 3.90** | 4.80** | 4.85** | 3.05** |
| 11. | Round seeded | 15 kR +0.02 M EMS | 41.96 | 84.85** | 3.90 | 30.80** | 3.95** | 4.95** | 5.05** | 3.50** |
| 12. | Brown seeded | 4 mM SA | 46.80** | 82.90 | 4.35 | 32.80** | 4.35 | 5.70 | 6.90** | 4.50 |
| 13. | Early maturing | 15 kR + 0.02 M EMS | 35.50** | 65.00 | 4.05 | 20.15** | 4.15 | 5.56 | 5.15** | 4.15 |
| | | Control (PDU1) | 42.00 | 80.50 | 4.25 | 25.50 | 4.30 | 5.75 | 6.25 | 4.30 |
| | | SE ± | 1.29 | 1.30 | 0.296 | 1.64 | 0.105 | 0.136 | 0.237 | 0.147 |

* Significant at 5 per cent level
** Significant at 1 per cent level

**Table 2. Mean values of yield and yield traits of macromutants in cultivar T9 in $M_3$ generation**

| S.No. | Macromutant | Mutagen treatment | Plant height (cm) | Days to maturity | Number of branches plant | Number of pods/ plant | Pod length (cm) | Number of seeds/ pod | Seed yield/ plant (g) | 100-seed weight (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Tall and erect | 15 kR + 4mM SA | 44.28** | 80.55** | 5.15** | 28.62** | 4.29 | 5.75 | 6.75** | 4.35 |
| 2. | Tendriller | 60 kR | 30.65** | 82.86 | 2.05** | 15.70** | 4.05 | 4.95** | 4.58** | 4.50** |
| 3. | Bushy | 0.08 M EMS | 37.85* | 83.07 | 5.90** | 32.86** | 4.25 | 5.80 | 6.50** | 4.15 |
| 4. | Narrow leaved | 4 mM SA | 37.26** | 82.80 | 4.25 | 18.75** | 4.26 | 5.26 | 4.75** | 4.08 |
| 5. | Dwarf | 45 kR + 4mM SA | 25.60** | 78.65** | 2.75** | 19.80 | 4.10 | 5.16** | 5.16 | 4.36 |
| 6. | Early maturing | 60 kR | 32.82* | 70.56** | 3.65 | 20.85 | 4.25 | 5.75 | 4.80** | 4.46** |
| 7. | Hairy | 30 kR | 32.46** | 85.26 | 3.70 | 18.26** | 4.25 | 5.65 | 4.62** | 4.26 |
| 8. | Thick and long podded | 15 kR + 0.02 M EMS | 40.81** | 84.67 | 4.50* | 30.65** | 5.25** | 6.95** | 6.90** | 4.55** |
| 9. | Long peduncled | 30 kR + 4 mM SA | 38.52** | 86.80** | 4.95** | 20.26 | 4.58** | 6.05** | 5.10 | 4.10 |
| 10. | Large seeded | 0.08 M EMS | 36.75 | 80.37** | 4.25 | 27.55** | 4.75** | 6.50** | 6.60** | 5.35** |
| 11. | Chocolate seeded | 60 kR + 4 mM SA | 30.56** | 86.82** | 4.50* | 25.86* | 4.25 | 5.62 | 6.05* | 4.35 |
| 12. | Short podded | 0.08 M EMS | 38.20* | 82.80 | 4.15 | 30.86** | 3.05** | 4.30** | 4.25** | 3.90** |
| | | Control (T9) | 35.25 | 83.50 | 3.90 | 22.50 | 4.15 | 5.50 | 5.50 | 4.20 |
| | | SE ± | 1.31 | 1.09 | 0.215 | 1.53 | 0.127 | 0.127 | 0.246 | 0.090 |

* Significant at 5 per cent level
** Significant at 1 per cent level

The bushy mutants were also induced in both the cultivars which were characterized by shorter internodes, compact stature and with higher number of pods. The spreading mutant exhibited branching parallel to the ground and covered more area than the normal plants. In the tendriller mutant, the leaflets were modified into tendrils. The long peduncled mutant had the characteristic of increased peduncle length joining the pods. Similar mutants have been reported by Pande and Raghuvanshi (1988), Singh and Yadav (1991), Sansiri *et al.* (2005) and Singh (2007) in greengram and Thakur and Sethi (1993) and Gautam and Mittal (1998) in blackgram. Two true breeding leaf mutants, namely, narrow leaved from T9 and broad leaved from PDU1 were isolated in $M_3$ generation. The broad leaved mutant exhibited increased leaf area and with significantly reduced plant height and number of pods per plant. The narrow leaved mutant showed reduced leaf area with decreased seed yield as compared to the parents. Many types of leaf mutants have been reported following mutagenesis in urdbean (Singh and Raghuvanshi, 1985; Thakur and Sethi, 1993; and Gautam and Mittal, 1998) and mungbean (Tickoo, 1987; Sansiri *et al.*, 2005; Singh, 2007).

Early maturing mutants were isolated from both the cultivars in $M_3$ generation. These mutants are 10 to 15 days earlier to the parents. The early maturing mutants have been reported in urd (Gautam and Mittal, 1998) and mung (Singh and Yadav, 1991).

Short, thick and long podded mutants were induced by different mutagenic treatments in both the cultivars in $M_3$ generation. The short podded mutants had relatively less number of seeds per pod with lower yield than the control. Such mutants have been reported by Thakur and Sethi (1993) and Gautam and Mittal (1998) in urdbean. The thick and long podded mutants had significantly increased pod length, greater number of seeds per pod and more seed yield over their parents. Gautam and Mittal (1998) observed such mutants in urdbean. Brown and chocolate testa colour mutants were isolated in $M_3$ generation from the black testa colour of the parents. Both the mutants exhibited a significant increase in the seed yield over the respective parents. The large seeded mutants were induced in both the cultivars which showed significantly increased pod length, number of seeds per pod, 100 seed weight and seed yield as compared to the controls. The small and round seeded mutants were also induced which showed significantly lower yield than the parents. The seed mutants for varied testa colours, shapes and sizes have been reported by Ali Khan and Veeraswamy (1974) in redgram and Singh *et al.* (1982) in greengram.

Gamma rays, EMS and SA have been demonstrated to induce mutations by different mechanisms. The combination of these mutagens in appropriate sequences has been shown to cause a synergistic increase in mutation frequency as observed during the present study. Similar results were also reported by Cheng (1987) and Cheng and Gao (1988). The combined treatments induced a higher mutation frequency, resulting in a higher mutation efficiency and an expansion of the spectrum of morphological mutations, thus offering more opportunities for selection in mutation breeding practice.

Many mutants, such as tall, bushy, early maturing, long- and thick-podded, large seeded, hairy, etc. induced during the present study, show the desirable characteristics from the breeders' point of view and hold promise for isolation of improved types from their progenies in the later generations.

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# *In vitro* Callogenesis and Plant Regeneration from Anther Culture in Groundnut (*Arachis hypogaea* L.)

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Anther or microspore culture is one of the efficient techniques for *in vitro* plant regeneration and has been exploited for haploid breeding. GG 2, a cultivated genotype of species *Arachis hypogaea* L. sub sp. *fastigiata* var. *vulgaris* was tested for *in vitro* callogenesis from anther and regeneration of anther derived calli. Callogenesis from anther starts four to five days after culturing. Callogenesis ranged from 31.5% to 91.2% in MS media with different combinations of growth regulators. Maximum callogenesis observed in medium containing MS salts with vitamins of B5+12.36 µM NAA+2.22 µM BAP+87.64 µM sucrose and 0.8 % agar (m/v). Somatic embryos were induced from anther callus in medium containing half strength MS salts with vitamins of B5+2.47 µM NAA+6.66 µM BAP+87.64 µM sucrose and 0.8 % agar. Regenerated plants were confirmed as diploid (2n=4x=40) by root tip analysis indicating that plants developed either from sporophytic tissue or diploidization.

**Key Words: Anther culture, Callogenesis, Groundnut, *In vitro*, Plant regeneration**

## Introduction

Groundnut is the principal oil seed crop of India. It is annually grown in about 7.6 million ha with a total production of 7.8 million tonnes of nuts-in-shell. Although India is the largest producer of groundnut in the world, its share in the world is not proportionate as the crop is grown mainly as rainfed crop with high level of fluctuation in the production due to different abiotic and biotic stresses. Development of tolerant/resistant varieties at a regular pace is needed in the present breeding programmes involving both traditional and nontraditional methods.

Anther or microspore culture is one of the efficient techniques for *in vitro* plant regeneration and has been exploited in haploid breeding (Croughan *et al.*, 1987; Veilleux, 1994; Martinez *et al.*, 1996; Yang, 1997; Chen *et al.*, 1997). However, the success hampered by the difficulty of inducing callus from anther and inducing morphogenesis for different crops and cultivars (Keshari *et al.*, 1992;Yan *et al.*, 1996; Raina and Zapata, 1997). In groundnut, Bajaj *et al.* (1980) first reported induction of pollen embryos and pollen callus in anther cultures of *A. hypogaea* and *A. glabrata*. Bajaj *et al.* (1981) later reported regeneration of genetically variable plants from the anther derived callus of *A. hypogaea* and *A. villosa*. However, regeneration of plants from anther in groundnut remains unexplored due to low frequency of callogenesis and somatic embryogenesis. Several other workers (Martin and Rahechault, 1976; Sudhakar and Moss, 1990; Willcox *et al.*, 1991; Yeh and Tseng, 1999; Tseng and Yeh, 2000; Yeh and Liaw, 1998; Morginski and Fernandez, 1979; Morginski and Fernandez,1980; Still *et al.*,1987; Lee and Yeh, 2001; Pongsupasamit *et al.*, 2001; Sastri *et al.*, 1981; Pitman, 1981) though had reported anther culture, responsive stages of microspore, media for callus induction and differentiation but techniques failed to provide substantial progress in repeatability; high frequency callogenesis and plant regeneration from anther culture. The objective of this study was to develop an efficient protocol for induction of anther calli and regeneration of plants in high-frequency.

## Materials and Methods

Healthy immature (2-3 mm in length) flower buds, suitable to bloom in next day morning were collected in tap water from field grown plants during 9-10 AM. Flower buds were then surface sterilized by agitating in 70% ethanol and freshly prepared 0.1% mercuric chloride solution for 10 minutes each and washed thoroughly in sterilized distilled water for three to four times. Sterilized flower buds then dissected longitudinally with sterilized needle inside the laminar flow and anthers of 0.5-1.0 mm in length were used as explants.

Anthers were inoculated in solidified medium consisting MS (Murashige and Skoog, 1962) basal salts, vitamins of B5 (Gamborg *et al.*, 1968), different doses of α-Naphthalene acetic acid (NAA) (2.47, 4.94, 7.41, 9.89, 12.36 and 14.83 µM), 6-Benzyl adenine (BAP) (2.22, 4.44 and 6.66 µM), 87.64 µM sucrose and 0.6 % agar (m/v). The medium was adjusted to pH 5.8 before autoclaving

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at 121° C for 15 minutes and cooled before use. Single anther was inoculated on the surface of the solidified medium in 25 x 150 mm test tubes (Borosil, Mumbai, India) containing 10 $cm^3$ solidified media and then cultured at an irradiation of 30 $\mu molm^{-2}s^{-1}$ with 16-h photoperiod and 25±2°C temperature. All compounds and plant growth regulators used in the experiments were from Himedia laboratories Pvt. Ltd., Mumbai, India. Experiments were carried out with 3 replicates with 50 explants per replication and each treatment was repeated three times. Colour, physical properties of anther derived callus were recorded after 30 days of culture by visual observations. Callus was classified into green (G), brown (B), dark brown (D) and creamy (Cr). Physical nature of callus recorded based on compactness of callus and shape of individual growing cell of callus. Callus was grouped into compact (C) and friable (F) based on compactness of callus. Callus was further grouped into nodular (N) and normal/round (R) based on shape of growing cells. Data were statistically analyzed using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1976).

**Results and Discussion**

Callogenesis started four to five days after culturing in media with all combinations of NAA and BAP used in the experiment. However, maximum (91.2%) callogenesis recorded in hormonal combination of 12.36 μM NAA and 2.22 μM BAP (Table 1) and callus was green, nodular and compact. However, colour and structure of the callus varied with the combinations of growth hormones (Table 1). This indicates that combination of 12.36 μM NAA and 2.22 μM BAP is comparatively more effective with greater repeatability for induction of anther callus in groundnut. Lee and Yeh (2001) also reported 91.9% callogenesis. Occasional root development from callus after 25 to 30 days in same medium indicating that callus tended to be differentiated with times in the same medium. Tseng and Yeh (2000) reported similar results of root formation from anther-derived callus. Two weeks old anther calli were sub-cultured in medium consisting MS basal salts, vitamins of B5, 2.68 μM NAA, different doses of BAP (2.22, 4.44, 6.66, 8.88, 11.11, 13.33, 15.55, 17.77, 20.00, 22.22 μM), 2.89 μM $GA_3$, 87.64 μM sucrose and 0.6 % agar (m/v). The calli did not differentiate in any combinations of growth regulators till three weeks of culture. But cultured callus was comparatively more green and supportive in medium supplemented with 4.44 μM, 6.66 μM and 8.88 μM BAP. The calli cultured in medium with 6.66 μM BAP and 2.68 μM NAA and 2.89 μM $GA_3$

**Table 1. Effect of NAA and BAP on anther callogenosis from field grown plants of *A. hypogaea* L. cv. GG2. Mean with different letters are significantly different according to DMRT (P<0.05)**

| NAA μM$^{-1}$ | BAP μM$^{-1}$ | Callus induction (%) | Type of Callus |
|---|---|---|---|
| 2.47 | 2.22 | 56.4±0.45efg | GRF |
| 2.47 | 4.44 | 31.5±0.82j | DRF |
| 2.47 | 6.66 | 33.6±0.32j | CrRC |
| 4.94 | 2.22 | 54.1±0.10g | GRF |
| 4.94 | 4.44 | 64.8±0.37cd | GRC |
| 4.94 | 6.66 | 43.1±0.78 hi | CrRF |
| 7.41 | 2.22 | 48.4±0.54gh | GRF |
| 7.41 | 4.44 | 54.5±0.48g | DRC |
| 7.41 | 6.66 | 37.4±0.77ij | DRF |
| 9.89 | 2.22 | 62.7±0.29def | BRF |
| 9.89 | 4.44 | 62.9±0.20def | BRF |
| 9.89 | 6.66 | 72.1±0.46bc | CrRF |
| 12.36 | 2.22 | 91.2±0.17a | GNC |
| 12.36 | 4.44 | 77.3±0.74b | DRC |
| 12.36 | 6.66 | 68.3±0.64cd | GRC |
| 14.83 | 2.22 | 55.5±0.17fg | BRF |
| 14.83 | 4.44 | 64.0±0.92dc | GRC |
| 14.83 | 6.66 | 64.4±0.73d | GRF |

differentiated after 35 days, which gradually produced multiple shoots after 55 days (Fig. 1). In general, delayed differentiation (between 35-45 days after culturing) of callus was noticed in groundnut and it requires one or 2 sub-culturing. The combination of 6.66 μM BAP and 2.68 μM NAA and 2.89 μM $GA_3$ was further repeated 7 times and confirmed delayed regeneration (between 35-45 days after culturing) and at least one sub-culturing after 15 days. Ninety four percent shoot induction observed in calli cultured in MS medium with 6.66 μM BAP, 2.68 μM NAA and 2.89 μM $GA_3$ (Table 2) and produced 1.5% shoots per callus (Table 3). The repeatability and high frequency regeneration are very much essential for further experimentation which is missing in earlier report (Yeh and Tseng, 1999; Tseng and Yeh, 2000; Yeh and Liaw, 1998; Lee and Yeh, 2001; Pongsupasamit *et al.*, 2001). Young generated shoot tips (1-2 cm) were shifted to growth medium (MS salts, vitamins of B5, 13.33 μM BAP, 5.37 μM NAA, 2.89 μM $GA_3$, 87.64 μM sucrose and 0.8 % agar (m/v) already standardized in the authors' laboratory. Later, growing shoots (4-5 cm) were shifted to rooting medium (MS salts, vitamins of B5, 5.37 μM NAA, 87.64 μM sucrose and 0.6% agar (m/v) already standardized in authors' laboratory. Hardening of plants carried out in test tubes containing liquid Hogland's solution under *in vitro* condition. Plants were kept initially covered with polythene sheet/packets to prevent excess transpiration and exposed to natural condition inside the culture room after 2 weeks. Healthy plants were shifted to earthen pots containing 50:50 mixtures (v/v) of soil

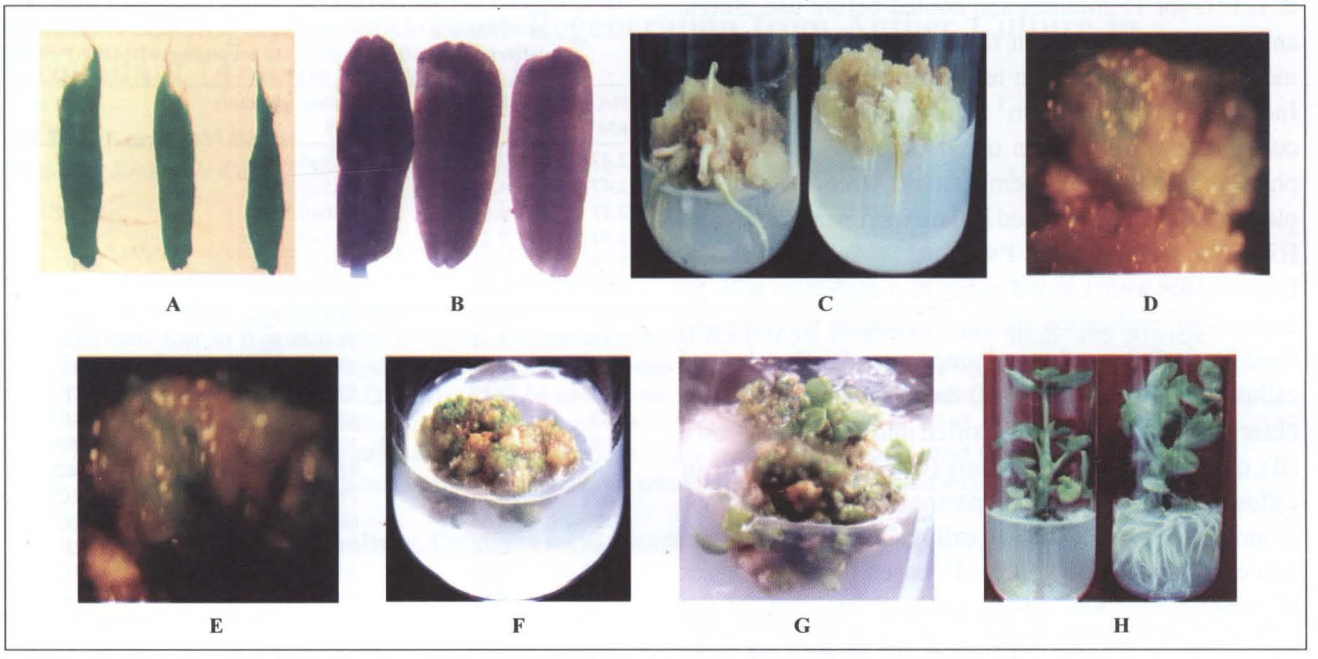


**Fig. 1: Regeneration of plantlets from anther derived callus of *Arachis hypogaea* L.**
**A-Immature flower buds, B-Anthers used as explants, C-Induced callus with root formation, D & E-Somatic embryo, F & G-Organogenesis from somatic embryo, H-Growing shoots and plantlets**

and sand. Root tip analysis of 26 generated plants confirmed 2n=40 chromosomes. It seems that plants have been regenerated from the anther tissue or from microspore mother cell. It is unlikely that regenerated plants could be spontaneous doubled haploids reported in other crops (Anderson, 2003) and callus takes more time (35-45 days) for regeneration. Further experiments may be required for confirmation of the origin and status of regenerated plants.

**Table 2. Effect of NAA and BAP in regeneration of anther callus of *A. hypogaea* L. cv. GG2**

| NAA µM/l | BAP µM/l | Regeneration (%) | Type of callus |
|---|---|---|---|
| 2.47 | 2.22 | 0 | BNC |
| 2.47 | 4.44 | 0 | DRF |
| 2.47 | 6.66 | 100 (50) | GRF |
| 2.47 | 8.88 | 0 | DRF |
| 2.47 | 11.10 | 0 | BRF |
| 2.47 | 13.32 | 0 | BRF |
| 2.47 | 15.54 | 0 | CrNC |
| 2.47 | 17.76 | 0 | BRF |
| 2.47 | 19.98 | 0 | GRC |
| 2.47 | 22.20 | 0 | GNC |
| 4.94 | 2.22 | 0 | BRF |
| 4.94 | 4.44 | 0 | GCF |
| 4.94 | 6.66 | 0 | DRF |
| 4.94 | 8.88 | 0 | DRC |
| 4.94 | 11.10 | 0 | BRF |
| 4.94 | 13.32 | 0 | GRC |
| 4.94 | 15.54 | 0 | CrRF |
| 4.94 | 17.76 | 0 | BRF |
| 4.94 | 19.98 | 0 | BRF |
| 4.94 | 22.20 | 0 | DRF |

**Table 3. Percent regeneration of anther callus of cv. GG 2 and number of shoot induction per callus in MS medium with hormonal combinations of 6.66 µM BAP, 2.68 µM NAA and 2.89 µM $GA_3$**

| No. of callus plated | Callus regenerated (%) | Number of shoot/callus |
|---|---|---|
| 50 | 100 | 1.28 |
| 50 | 84.0 | 1.50 |
| 50 | 96.0 | 2.00 |
| 50 | 92.0 | 1.30 |
| 50 | 100.0 | 1.50 |
| 50 | 96.0 | 1.50 |
| 50 | 92.0 | 2.00 |
| Mean | 94.28 | 1.58 |

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# Genetic Diversity and Variability in Mungbean [*Vigna radiata* (L.) Wilczek]

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Fifty two genotypes of mungbean (*Vigna radiata* L.) were evaluated in randomized block design during rainy season of 2005 at Agricultural Research Station, Mandor, Jodhpur for eight characters to estimate genetic diversity, variability, heritability and genetic advance as a percentage of mean. Characters like seed yield, pod length, pods/ plant, primary branches/ plant, 100-seed weight and pod length exhibited high variability and heritability coupled with genetic advance. The hierarchical cluster analysis indicated the presence of considerable genetic divergence among the genotypes. The genotypes were grouped into eight clusters using Ward's minimum variance method. The inter-cluster Euclidean$^2$ distance was maximum between cluster V and VIII (54.3) followed by cluster V and VII (41.7) and cluster II and III (40.0). Cluster V showed by the maximum cluster means for plant height, primary branches/ plant, pods/ plant and days to 50% flowering, cluster II for pod length and seeds/pod, cluster VII for seed yield and cluster VIII for 100-seed weight. Contribution of different characters towards total genetic divergence revealed maximum contribution of pod length (24.5%) followed by seed yield (19.5%) and 100-seed weight (17.7%).

**Key Words: Mungbean, Euclidean$^2$ distance analysis, Genetic divergence, Genetic variability**

## Introduction

Mungbean (*Vigna radiata* L.) is one of the important pulse crop grown in India. Assessment of genetic diversity in germplasm collection can facilitate classification and identification of diverse genotypes with possible utility for specific utilization in breeding programme. The hybridization between diverse lines produces a greater heterosis than those between closely related strains (Hallaur and Miranda, 1988). The utility of classifying germplasm to select diverse parents for hybridization had been appreciated (Bhatt, 1970). To increase the productivity of any crops, breeders have aimed to develop high yielding and better quality cultivars and normally it is achieved by selecting the desirable segregants from the segregating generation following hybridization. Therefore, an attempt has been made in present study to estimate genetic variability, heritability, genetic advance and genetic diversity among the genotypes of mungbean.

## Materials and Methods

Fifty two genotypes of mungbean [*Vigna radiata* (L.) Wilczek] were grown in randomized block design with three replications during the rainy season of 2005 at Agricultural Research Station, Mandor, Jodhpur. Each entry was sown in a four rows of 5 m length following crop geometry of 30 x 10 cm. The crop was raised under rainfed condition and all cultural operations were done as and when necessary. The data were recorded on five competitive plants taken from each replication for plant height, primary branches/ plant, pod length (cm), pods/ plant, seeds/ pod and 100-seed weight (g) whereas seed yield, days to 50% flowering and days to maturity were recorded considering whole plot in each replication. The data were subjected to analysis of variance following standard method. Clustering was performed by procedure of Ward's minimum variance method (Ward, 1963) and the other genetic parameters such as variability, heritability and expected genetic advance as percent of mean were estimated using INDOSTAT software (Indostat Services, Hyderabad).

**Table 1. Estimate of genetic parameters of seed yield and its component traits in mungbean**

| Characters | Mean | Range | *GCV | PCV | $h^2$(%) | GA |
|---|---|---|---|---|---|---|
| Seed yield (kg/ha) | 857.0 | 347–1209 | 20.1 | 23.4 | 74 | 35.7 |
| Days to 50% flowering | 42.0 | 39–48 | 4.0 | 5.2 | 59 | 6.4 |
| Days to maturity | 66.0 | 63–69 | 2.1 | 2.9 | 54 | 3.2 |
| Plant height (cm) | 59.9 | 50–72 | 7.9 | 10.8 | 53 | 11.9 |
| Primary branches/ plant | 3.6 | 2.6–4.9 | 12.5 | 17.2 | 53 | 18.6 |
| Pod length (cm) | 8.1 | 6.8–10.2 | 9.4 | 10.9 | 75 | 16.8 |
| No. of pods/plant | 36.4 | 25–56 | 16.3 | 19.9 | 67 | 27.5 |
| Seeds/pod | 11.2 | 9.8–13.2 | 4.5 | 7.2 | 39 | 5.8 |
| 100 seed weight (g) | 4.1 | 2.9–5.5 | 12.9 | 14.5 | 79 | 23.7 |

*GCV = Genetic coefficient of variation
PCV = Phenotypic coefficient of variation
$h^2$= Heritability, GA = Genetic Advance as percentage of mean

## Results and Discussion

The analysis of variance revealed significant differences among the genotypes for all the characters. The range,

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mean, coefficient of variability, heritability in broad sense and genetic advance for yield and its component characters are presented in Table 1. High magnitudes of phenotypic as well as genotypic coefficient of variation observed for seed yield, number of pods per plant, 100-seed weight and primary branches/ plant showed ample amount of variation for these characters. The heritability was high (range 53-79%) for all the characters except seeds/ pod (39%). Traits like 100-seed weight, pod length, seed yield and pod length had high estimate of heritability along with high genetic advance as percent of mean, suggesting the probability of a greater amount of additive gene action for these characters. Thus, selection of these traits would be effective for crop improvement. These results are in agreement with those reported earlier (Lal and Mishra, 2006; Sirohi and Kumar, 2006; Samad and Lavanya, 2005; Khairnar *et al.*, 2003; Reddy *et al.*, 2003; Yadav *et al.*, 2001).

A hierarchical cluster analysis of Ward's minimum variance method produced a dendrogram showing successive fusion of individuals which clearly partitioned the genotypes into eight clusters (Fig. 1). The genotypes within each cluster were closer to each other than the genotypes grouped in to different clusters. Maximum number of genotypes (14) were included in cluster I followed by 10 in cluster VII, seven each in cluster VI

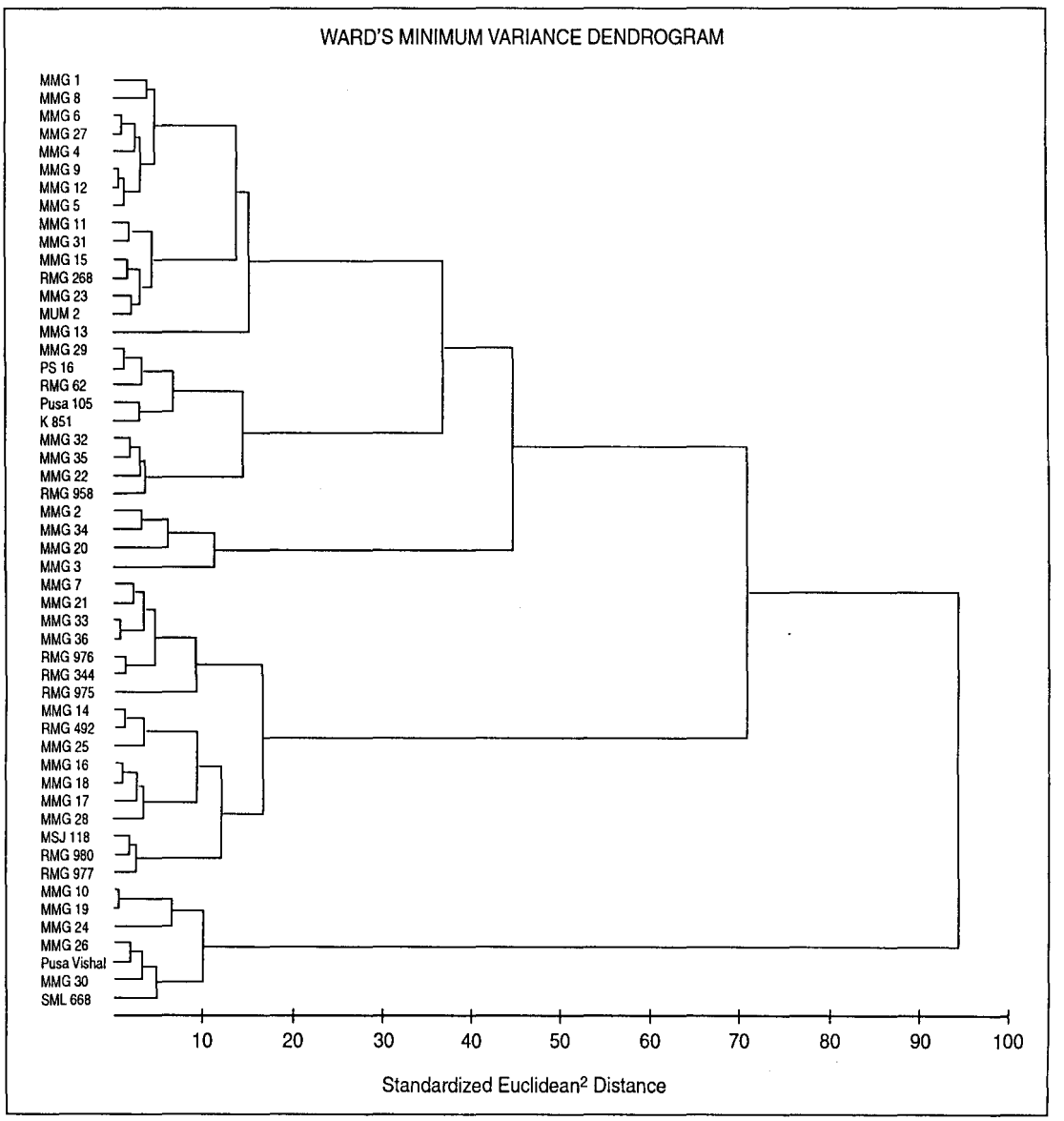


**Fig. 1: Dendrogram showing different clusters of mungbean**

**Table 2. Average intra- and inter- cluster values among eight clusters in mungbean**

| Cluster | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V | Cluster VI | Cluster VII | Cluster VIII |
|---|---|---|---|---|---|---|---|---|
| Cluster I | 6.7 | 18.8 | 14.2 | 10.7 | 22.5 | 10.8 | 13.8 | 18.4 |
| Cluster II | | 0.0 | 40.0 | 31.3 | 33.3 | 30.9 | 37.1 | 29.4 |
| Cluster III | | | 6.9 | 11.2 | 28.3 | 20.1 | 17.8 | 28.4 |
| Cluster IV | | | | 5.3 | 17.8 | 11.2 | 15.7 | 35.1 |
| Cluster V | | | | | 13.5 | 27.2 | 41.7 | 54.3 |
| Cluster VI | | | | | | 7.0 | 10.6 | 28.8 |
| Cluster VII | | | | | | | 8.1 | 22.5 |
| Cluster VIII | | | | | | | | 9.0 |

**Table 3. Cluster means and percent contribution of different characters in mungbean**

| Characters | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V | Cluster VI | Cluster VII | Cluster VIII | Contribution to diversity (%) |
|---|---|---|---|---|---|---|---|---|---|
| Seed yield (kg/ha) | 849.0 | 583.0 | 677.0 | 823.0 | 674.0 | 993.0 | 994.0 | 832.0 | 19.5 |
| Days to 50% flowering | 42.7 | 42.7 | 44.3 | 44.2 | 45.4 | 40.9 | 41.1 | 41.3 | 7.2 |
| Days to maturity | 65.8 | 65.0 | 67.5 | 67.8 | 68.1 | 65.5 | 65.0 | 64.0 | 6.1 |
| Plant height (cm) | 61.3 | 52.1 | 62.3 | 62.7 | 69.9 | 61.8 | 56.2 | 52.9 | 3.1 |
| Primary branches/plant | 3.8 | 4.5 | 3.5 | 3.6 | 4.8 | 3.8 | 3.2 | 3.2 | 4.8 |
| Pod length (cm) | 8.3 | 9.4 | 7.7 | 7.2 | 8.2 | 7.5 | 7.6 | 9.4 | 24.5 |
| Pods/plant | 36.0 | 39.2 | 28.4 | 40.6 | 45.7 | 41.8 | 35.6 | 30.4 | 13.5 |
| Seeds/pod | 11.6 | 13.2 | 10.6 | 11.3 | 11.5 | 11.2 | 10.8 | 11.1 | 3.0 |
| 100 seed weight (g) | 4.3 | 4.1 | 3.98 | 3.69 | 3.92 | 3.74 | 3.73 | 5.2 | 17.7 |

and cluster VIII, five in cluster III, four each in cluster IV and cluster V whereas cluster II was having only a single genotype.

Average intra- and inter-cluster Euclidean$^2$ distances are sown in Table 2. Maximum intra-cluster distance was observed in cluster V (13.52) followed by cluster VIII (8.99) indicating wide genetic variability within the genotypes of these two clusters. The highest inter-cluster distance was observed between clusters V and VIII (54.3) followed by clusters V and VII (41.7), and clusters II and III (40.0), suggesting wide diversity between genotypes of these clusters. Therefore, genotype belonging to these clusters may be used in hybridization programme for improvement of mungbean. The least inter-cluster distance was observed between clusters I and IV (10.7) indicating close relationship between the genotypes of these two clusters.

The diversity was also supported by the appreciable amount of variation among the cluster means for different characters (Table 3). Cluster V showed by the maximum cluster means for plant height, primary branches/ plant, pods/ plant and days to 50% flowering; cluster II for pod length and seeds/ pod; cluster VII for seed yield (kg/ha) and cluster VIII for 100-seed weight (g). These results showed that different clusters were superior for different characters. Amongst the characters, pod length contributed maximum towards genetic divergence (24.5%) followed by seed yield (19.5%) and 100-seed weight (17.7%). These results are in conformity with those reported by Sandhu and Brar (2002).

In the present study, the maximum inter-cluster distance observed between clusters V (genotypes MMG 2, MMG 34, MMG 20 and MMG 3) and VIII (genotypes MMG 10, MMG 19, MMG 24, MMG 26, Pusa Vishal, MMG 30 and SML 668) would be resulted into transgressive segregation. As seed yield, pod length and 100-seed weight contributed maximum towards the divergence, direct selection of these traits help in crop improvement.

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# Genetic Variability, Correlation and Path Analysis in Pea (*Pisum sativum* L.)

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Thirty-five genotypes of indigenous and exotic origin were grown over eight environments to study the genetic variability, correlation and path coefficients among thirteen quantitative characters in pea (*Pisum sativum* L.). Wide range of variability was observed for most of the characters under study. Significant differences in the magnitude of PCV and corresponding GCV were observed for branches per plant, pods per plant, seed yield and harvest index suggesting greater role of environment in the expression of these traits. Plant height, grain yield per plant, dry matter yield, 100-seed weight and number of pods per plant had high phenotypic and genotypic coefficient of variations coupled with moderate to high estimates of heritability and expected genetic advances. Seed yield showed positive and significant association with number of pods per node. Seed yield was negatively associated with days to 50% flowering, days to green pod picking and days to maturity. Path coefficient analysis revealed that yield per plant is directly affected by number of pods per plant, dry matter yield, pod length, harvest index and number of seeds per pod and thus it may be treated as selection criteria for isolating higher yielding genotypes in pea.

**Key Words: *Pisum sativum*, Genetic variability, Heritability, Genetic advances, Correlation, Path analysis**

## Introduction

Development of high yielding cultivars requires a thorough knowledge of the available genetic variation and also the extent of association among the traits of economic importance. It is well established fact that the differences among the plants, are the result of genetic difference among the plants, the environment in which they are growing and genotype environment interaction. Information on the relative magnitude of genotypic and phenotypic variances are important in judging the potential of germplasm and help in selection of parents for their use in breeding programmes.

Correlation studies provide an opportunity to study the magnitude and direction of association of one character with another, while path coefficient analysis gives the direct and indirect contribution of independent variables on dependent variable. Path analysis is a standardized partial regression coefficient, which splits the correlation coefficient into the measures of direct and indirect effects. It is important for a plant breeder to find out which of the characters are correlated with yield to bring about genetic improvement in crop plants. Hence, an experiment was conducted to study the genetic variability, correlation and path coefficients among yield and yield component traits in pea.

## Materials and Methods

Thirty-five genotypes of pea of indigenous (27) and exotic origin (8) were evaluated in a randomized block design. Three replications each in eight environments were created by manipulating the dates of sowing and fertilizer dosages with N:P kg/ha 20:40 and 10:40 dates of sowing as 10-15 November and 25-30 December at experimental farm, Kisan Post Graduate College, Simbhaoli, Ghaziabad, CCS University, Meerut during *rabi* 1996-97, 1997-98. Each plot consisted of 3 m long row spaced at 45 cm apart and the plant-to-plant distance was maintained at 15 cm.

Observations were recorded on five randomly selected plants per genotype in each replication for the characters like days to flowering, days to green pod picking, days to maturity, pods/ node, dry matter yield (g), number of seeds/ pod, seed yield/ plant (g), harvest index and 100-seed weight (g). The data for eight environments was pooled and analysed. Standard statistical procedures were followed for estimating various genetic parameters. Phenotypic and genotypic coefficient of variations, heritability and genetic gain were calculated following the Burton and De Vane (1953). The genotypic and phenotypic correlation coefficients were calculated following Dewey and Lu (1959) and path analysis following the method of Fisher (1954).

## Results and Discussion

Significant differences were observed among the genotypes for all the characters studied indicating presence of adequate genetic variability in the experimental material. The magnitude of genotypic

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coefficients of variation (GCV) and phenotypic coefficients of variation (PCV) were comparatively higher for seed yield per plant, dry matter yield, plant height and number of pods per plant as compared to other characters (Table 1). These findings are supported by earlier workers (Dhobal 1996; Vikas Singh *et al.,* 1996; Kumar *et al.,* 1998; Tyagi *et al.,* 1997). The lowest magnitude of GCV and PCV were observed for days to maturity. Marked differences between PCV and corresponding GCV were observed for harvest index, number of pods, number of branches, seed yield and dry matter yield per plants, confirming the predominance of GXE interaction and greater role of environment on the expression of these characters. Narrow differences observed between PCV and GCV values for 100-seed weight, days to maturity, days to flowering, pod length and plant height, indicated less environmental influence on the expression of these traits and these characters are rather stable. Therefore, selection based on these parameters may be comparatively more effective

Broad sense heritability was high (>70%) for 100-seed weight, plant height, days to flowering, days to maturity and pod length but observed low for number of pods per plant, harvest index, seed yield and number of branches per plant (Table 1). Similar results have also been reported by Singh (1995), Dhobal (1996) and Gupta *et al.* (1998). High heritability coupled with high genetic advance over the mean was observed for plant height, dry matter yield, 100-seed weight and grain yield per plant indicating the preponderance of additive gene effect and desired improvement in these characters can be brought through direct selection of these component traits. Mohanta *et al.* (2001) also observed high heritability coupled with high genetic advance for seed yield per plant, pods per plant, plant height, seeds per pod and 100-seed weight, suggesting additive gene effects. The findings also get supported by Kumar *et al.* (1998). Pod length and number of seeds per pod exhibited high heritability with low genetic advance indicating non-additive genetic effects. Low heritability estimates with low genetic advance were observed for branches per plant, number of pods per plant and seed yield per plant indicating high degree of non-heritable genetic effects for these characters in pea.

Grain yield is the result of interaction of a number of interrelated characters with positive and negative effects on yield per se. Therefore, selection should be based on those component characters which have positive correlation with grain yield. Character association reveals the mutual relationship between two characters and it is very important parameter for deciding the nature of selection to be followed by improvement in the crop under investigation. In general, the genotypic correlations were greater in magnitude than the corresponding phenotypic correlations. In the present study, the seed yield per plant was positively associated with number of pods per plant, dry matter yield per plant, pod length, number of seeds per pod and number of pods per node (Table 2). Kaloo *et al.* (1976) observed positive association of seed yield with number of pods per plant and number of pods per

**Table 1. Estimation of genetic variability in pea (*Pisum sativum* L)**

| Characters | Mean | PCV (%) | GCV (%) | H (%) | G.A. | G.A. Over Mean (%) |
|---|---|---|---|---|---|---|
| Days to flowering | 75.79 | 4.34 | 3.89 | 80.10 | 5.43 | 7.16 |
| Days to green pod picking | 99.89 | 4.23 | 3.05 | 52.10 | 4.53 | 4.54 |
| Days to maturity | 118.19 | 2.61 | 2.26 | 75.10 | 4.77 | 4.05 |
| Plant height (cm) | 89.25 | 32.46 | 29.16 | 80.70 | 48.16 | 53.96 |
| No. of branches/plant | 2.75 | 18.89 | 10.96 | 33.60 | 0.36 | 13.09 |
| No. of pods/plant | 11.53 | 31.45 | 15.03 | 22.80 | 1.71 | 14.83 |
| Pod length (cm) | 5.61 | 12.66 | 10.79 | 72.70 | 1.06 | 18.89 |
| No. of pods/node | 1.63 | 19.13 | 13.74 | 51.60 | 0.33 | 20.24 |
| Dry matter yield (g) | 27.87 | 35.59 | 24.36 | 46.80 | 9.57 | 34.34 |
| No. of seeds/pod | 4.61 | 14.57 | 10.66 | 53.80 | 0.74 | 16.05 |
| Seed yield/plant (g) | 9.07 | 41.30 | 24.40 | 35.00 | 2.69 | 29.66 |
| Harvest index | 31.50 | 21.23 | 10.23 | 23.20 | 3.20 | 10.16 |
| 100 seed weight (g) | 16.04 | 16.11 | 15.82 | 96.50 | 5.13 | 31.98 |

PCV = Phenotypic Coefficient of Variation
GCV = Genotypic Coefficient of Variation
H = Heritability
GA = Genetic Advance

**Table 2. Estimates of phenotypic and genotypic correlation coefficients among different thirteen characters**

| Characters | | Days to flowering | Days to green pod picking | Days to maturity | Plant height (cm) | No. of branches/ plant | No. of pods/ plant | Pod length (cm) | No. of pods/ node | Dry matter yield (g) | No. of seeds/ pod | Seed yield/ plant (g) | Harvest index | 100-seed weight (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Days to flowering | P | 1.00 | 0.65** | 0.77** | 0.09 | 0.13 | -0.16 | -0.10 | 0.13 | 0.14 | -0.32 | -0.12 | -0.35 | 0.23 |
| | G | 1.00 | 0.89** | 0.91** | 0.09 | 0.22 | -0.33 | -0.12 | 0.22 | 0.15 | -0.50 | -0.20 | -0.72 | 0.26 |
| Days to green pod picking | P | | 1.00 | 0.69 | 0.05 | 0.05 | 0.06 | -0.03 | 0.70 | 0.14 | -0.21 | -0.07 | -0.23 | 0.22 |
| | G | | 1.00 | 0.97 | 0.07 | 0.19 | -0.21 | -0.04 | 0.23 | 0.24 | -0.36 | -0.12 | -0.58 | 0.31 |
| Days to maturity | P | | | 1.00 | 0.22 | 0.10 | 0.03 | 0.02 | 0.10 | 0.31 | -0.28 | -0.07 | -0.22 | 0.41* |
| | G | | | 1.00 | 0.07 | 0.27 | 0.09 | 0.03 | 0.21 | 0.45** | -0.41 | 0.15 | -0.45 | 0.48** |
| Plant height (cm) | P | | | | 1.00 | 0.34** | 0.26 | -0.13 | -0.44 | 0.55** | -0.04 | 0.20 | -0.28 | 0.18 |
| | G | | | | 1.00 | 0.63** | 0.43* | -0.19 | -0.62 | 0.68** | -0.21 | 0.23 | -0.56 | 0.20 |
| No. of branches/ plant | P | | | | | 1.00 | 0.32 | -0.16 | -0.10 | 0.40** | 0.06 | 0.22 | -0.09 | -0.04 |
| | G | | | | | 1.00 | 0.44* | -0.34 | -0.43 | 0.73** | -0.07 | 0.16 | -0.68 | -0.09 |
| No. of pods/plant | P | | | | | | 1.00 | 0.07 | 0.21 | 0.67** | 0.06 | 0.83** | 0.37* | 0.09 |
| | G | | | | | | 1.00 | 0.06 | 0.14 | 0.82** | 0.13 | 0.79** | 0.20* | 0.20 |
| Pod length (cm) | P | | | | | | | 1.00 | 0.52** | 0.18 | 0.50** | 0.35** | 0.35 | 0.39** |
| | G | | | | | | | 1.00 | 0.76** | 0.23 | 0.60** | 0.57** | 0.75** | 0.46** |
| No. of pods/node | P | | | | | | | | 1.00 | 0.11 | 0.25 | 0.36** | 0.45** | 0.30 |
| | G | | | | | | | | 1.00 | 0.09 | 0.29 | 0.57** | 0.81** | 0.42* |
| Dry matter yield (g) | P | | | | | | | | | 1.00 | 0.09 | 0.69** | -0.01 | 0.36 |
| | G | | | | | | | | | 1.00 | 0.10 | 0.80** | 0.05 | 0.52** |
| No. of seeds/pod | P | | | | | | | | | | 1.00 | 0.30 | 0.40* | -0.16 |
| | G | | | | | | | | | | 1.00 | 0.41 | 0.58** | -0.23 |
| Seed yield/ plant (g) | P | | | | | | | | | | | 1.00 | 0.55** | 0.36* |
| | G | | | | | | | | | | | 1.00 | 0.65** | 0.61 |
| Harvest index (%) | P | | | | | | | | | | | | | 0.13 |
| | G | | | | | | | | | | | | | 0.28 |
| 100 seed weight (g) | P | | | | | | | | | | | | | 1.00 |
| | G | | | | | | | | | | | | | 1.00 |

*, ** Significant at 1% and 5% level

node. Teotia *et. al.* (1983) also observed that seed yield was positively associated with number of seeds per pod, length of pod and number of pods per plant. Seed yield was negatively associated with days to 50% flowering, days to green pod harvesting and days to maturity. This kind of association puts a significant limitation in breeding high yielding and early maturing varieties. Number of pods per plant had significant and positive association with dry matter yield per plant at both phenotypic and genotypic levels (Table 2).

Path-coefficient analysis helps in understanding the magnitude of direct and indirect contribution of each character on the dependent character, like grain yield in the present study. Partitioning of correlation coefficient into direct and indirect effects provide the information about the nature and magnitude of effects of other characters on seed yield. In present investigation, the path coefficient analysis (Table 3) revealed that direct and indirect effects at genotypic level were higher than corresponding phenotypic effects. Highest positive direct effect on seed yield per plant was exhibited by number of pods per plant (0.61), 100-seeds weight (0.26 g) and dry matter yield per plant (0.24 g). Highest indirect positive effect was observed via number of pods per plant (0.61), dry matter yield (0.16 g) and harvest index (0.23). Number of pods per plant, pod length, number of pods per node, dry matter yield per plant, number of seeds per pod, harvest index and 100-seed weight showed significantly positive correlation with seed yield per plant. It was observed that seed yield per plant is directly affected by number of pods per plant, harvest index and number of pods per plant while it is indirectly affected via number of pods per node, 100-seed weight, plant height and number of branches per plant. Thus, it appears that number of pods per plant, number of pods per node, number of seeds per pod, pod length, dry matter yield, harvest index

**Table 3. Path coefficient analysis of phenotypic (P) and genotypic (G) correlation coefficient to determine the direct and indirect effects of different triats on yield in pea**

| Characters | | Days to flowering | Days to green pod picking | Days to maturity | Plant height (cm) | No. of branches/ plant | No. of pods/ plant | Pod length (cm) | No. of pods/ node | Dry matter yield (g) | No. of seeds/ pod | Seed yield/ plant (g) | Harvest index | 100-seed weight (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Days to flowering | P | 0.14 | -0.04 | -0.03 | -0.01 | -0.01 | -0.10 | -0.001 | -0.01 | 0.03 | -0.07 | -0.08 | 0.06 | -0.01 |
| | G | 0.44 | -0.09 | -0.36 | 0.01 | -0.08 | -0.10 | 0.06 | 0.07 | 0.10 | -0.26 | -0.14 | 0.11 | -0.20 |
| Days to green pod picking | P | 0.09 | -0.06 | -0.03 | -0.01 | 0.00 | -0.04 | 0.00 | -0.01 | 0.03 | -0.04 | -0.05 | 0.06 | -0.07 |
| | G | 0.38 | -0.10 | -0.35 | 0.01 | 0.07 | -0.16 | 0.02 | 0.07 | 0.16 | -0.18 | -0.11 | 0.12 | -0.12 |
| Days to maturity | P | 0.11 | -0.04 | -0.04 | -0.02 | 0.00 | 0.02 | 0.00 | -0.01 | 0.08 | -0.06 | -0.09 | 0.11 | -0.07 |
| | G | 0.40 | -0.10 | -0.36 | 0.03 | -0.10 | 0.03 | -0.02 | 0.06 | 0.31 | -0.21 | -0.09 | 0.19 | 0.15 |
| Plant height (cm) | P | 0.01 | 0.00 | -0.01 | -0.09 | -0.01 | 0.16 | 0.00 | 0.04 | 0.13 | -0.01 | -0.06 | 0.05 | 0.20 |
| | G | 0.04 | -0.01 | -0.09 | 0.12 | -0.23 | 0.12 | 0.10 | -0.19 | 0.46 | -0.06 | -0.11 | 0.08 | 0.23 |
| No. of branches/ plant | P | 0.02 | -0.003 | -0.01 | -0.03 | -0.04 | 0.19 | 0.00 | 0.01 | 0.10 | 0.01 | -0.02 | -0.01 | 0.22 |
| | G | 0.10 | -0.02 | -0.10 | 0.07 | -0.36 | 0.13 | 0.18 | -0.13 | 0.50 | -0.03 | -0.13 | -0.04 | 0.16 |
| No. of pods/ plant | P | -0.02 | 0.00 | -0.001 | -0.02 | -0.01 | 0.61 | 0.00 | -0.02 | 0.16 | 0.01 | 0.09 | 0.02 | 0.83** |
| | G | -0.14 | 0.02 | -0.03 | 0.05 | -0.16 | 0.29 | -0.03 | 0.04 | 0.56 | 0.07 | 0.04 | 0.08 | 0.79** |
| Pod length (cm) | P | -0.01 | 0.002 | -0.001 | 0.01 | 0.01 | 0.04 | 0.01 | -0.04 | 0.04 | 0.11 | 0.08 | 0.10 | 0.35** |
| | G | -0.05 | 0.004 | -0.01 | -0.02 | 0.12 | 0.02 | -0.52 | 0.23 | 0.15 | 0.31 | 0.15 | 0.19 | 0.57** |
| No. of pods/ node | P | 0.02 | -0.004 | 0.00 | 0.04 | 0.004 | 0.13 | 0.01 | -0.08 | 0.03 | 0.05 | 0.11 | 0.08 | 0.36** |
| | G | 0.10 | -0.02 | -0.07 | -0.07 | 0.15 | 0.04 | -0.39 | 0.30 | 0.06 | 0.15 | 0.16 | 0.17 | 0.57** |
| Dry matter yield (g) | P | 0.02 | -0.01 | -0.01 | -0.05 | -0.02 | 0.41 | 0.00 | -0.01 | 0.24 | 0.02 | 0.00 | 0.09 | 0.69** |
| | G | 0.07 | -0.02 | -0.16 | 0.08 | -0.26 | 0.24 | -0.12 | 0.03 | 0.68 | 0.05 | 0.01 | 0.21 | 0.80** |
| No. of seeds/pod | P | -0.04 | 0.01 | -0.01 | 0.00 | 0.00 | 0.04 | 0.01 | -0.02 | 0.02 | 0.22 | 0.09 | -0.04 | 0.30 |
| | G | -0.22 | 0.04 | 0.15 | -0.01 | 0.02 | 0.04 | -0.31 | 0.09 | 0.07 | 0.52 | 0.11 | -0.09 | 0.41* |
| Harvest index (%) | P | -0.05 | 0.01 | 0.01 | 0.02 | 0.00 | 0.23 | 0.00 | -0.04 | 0.00 | 0.09 | 0.23 | 0.03 | 0.55** |
| | G | -0.31 | 0.06 | 0.16 | -0.07 | 0.25 | 0.06 | -0.39 | 0.24 | 0.04 | 0.30 | 0.20 | 0.11 | 0.65** |
| 100 seed weight (g) | P | 0.03 | -0.01 | -0.02 | -0.02 | 0.00 | 0.06 | 0.0.004 | -0.03 | 0.09 | -0.04 | 0.03 | 0.26 | 0.36* |
| | G | 0.11 | -0.03 | -0.17 | 0.02 | 0.03 | 0.06 | -0.24 | 0.13 | 0.35 | -0.12 | 0.06 | 0.40 | 0.61** |

and 100-seed weight can be considered as selection criteria for yield improvement in peas.

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# Shoot Multiplication in *Dioscorea* Species

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Standardized protocols for shoot induction and multiplication through axillary shoot proliferation from single node explants of nine species of *Dioscorea* namely, *Dioscorea bulbifera, D. pentaphylla, D. hispida, D. intermedia, D. hamiltonii, D. wightii, D. belophylla, D. spicata* and *D. oppositifolia* of the Southern Western Ghats. Cytokinins were effective in promoting shoot induction and multiplication in these species. Of the different cytokinins used, optimum results were obtained with BA (2-4 mg/l) followed by TDZ and Kinetin (Kn) in all the species. In *D. pentaphylla,* 12 shoots were obtained on MS medium supplemented with BA (4 mg/l) and Kn (2 mg/l) whereas in *D. oppositifolia* up to 8 shoots were produced in BA (2 mg/l) and Kn (1 mg/l). BA (2 mg/l) along with NAA (0.5 mg/l) produced 8-10 shoots in *D. pentaphylla* and *D. intermedia.*

**Key Words: *Dioscorea*, Nodal segment culture, Shoot multiplication**

## Introduction

The genus *Dioscorea* L., commonly called true yams, has about 620 species, which are both agriculturally and pharmaceutically important (Purseglove 1972; Coursey 1967). Of these, 23 species occur in the Indian subcontinent and 44% of these are endemic. Many wild species are of medicinal and pharmacological importance as they are the source of steroidal sapogenins especially diosgenin, a precursor for commercially synthesized sex hormone and corticosteroids. Moreover, they have industrial, aesthetic and ritualistic improtance. Yams are vegetatively propagated due to heterozygosity/shy flowering/non-synchronization in flowering/non-flowering or due to failure of seed set. These, therefore, need biotechnological interventions for their clonal multiplication as well as conservation. Reports on the *in vitro* propagation of species of *Dioscorea* using meristem tip or nodal explants of vines or tuber sprouts offer the prospects of germplasm storage by means of minimal-growth or cryopreservation, will be of application in the national and international exchange of germplasm.

Micropropagation is popular due to large-scale multiplication of high quality clonally propagated plants and it provides a means of germplasm storage for maintenance of disease-free stocks (Withers 1989). Ammirato (1984) reported that plants which are produced by nodal or meristem culture show a high degree of uniformity. The present experiments were carried out with the objective of formulating micropropagation protocols through nodal segment culture in *Dioscorea* species for their large-scale multiplication, conservation and exchange.

The *in vitro* propagation of cultivated species of *Dioscorea* using meristem tip or nodal explants of vines or tuber sprouts has been attempted by various workers (Mantell *et al.*, 1978; Ammirato, 1982; Forsyth and van Staden, 1982; Malaurie *et al.*, 1988, 1993; Nair and Chandrababu, 1996). Clonal propagation using nodal segments or meristems has also been reported in wild species like *D. deltoidea* (Grewal *et al.*, 1977), *D. floribunda* (Chaturvedi 1975; Sita *et al.*, 1976), *D. composita* (Ammirato 1982; Alizadeh *et al.*, 1998), *D. abyssinica* and *D. mangenotiana* (Lauzer *et al.*, 1992).

## Materials and Methods

One accession each from 12 species of *Dioscorea* (Table 1) having distribution in Southern Western Ghats, procured from NBPGR Regional Station, Thrissur and grown in the green house of the Department of Botany, University of Kerala, Thiruvananthapuram served as the source of explants for the experiments.

Single node segments from vines of healthy mother plants of 4-months were used. The explants were initially washed in running tap water for 30-60 minutes, then in aqueous 10% Labolene for 15-20 minutes and again in tap water and in sterile distilled water to remove the adhering surface contaminants. They were then treated with 0.1% $HgCl_2$ for 5-20 minutes followed by rinsing with sterile double distilled water 4-5 times inside the laminar airflow. The explants were then trimmed to approximate size (1-2 cm) by removing off cut ends after blotting in sterile filter paper before inoculation.

For shoot multiplication, surface sterilized single node segments (1-2 cm) were inoculated onto culture

**Table 1. Materials used**

| # | Species name | IC number | Locality of collection |
|---|---|---|---|
| 1 | *Dioscorea bulbifera* L. | 202349 | Peechi, Thrissur, Kerala |
| 2 | *Dioscorea pentaphylla* L. | 202367 | Peechi, Thrissur, Kerala |
| 3 | *Dioscorea hispida* Dennst | 202370 | Nilambur, Malappuram, Kerala |
| 4 | *Dioscorea tomentosa* Heyne | 202379 | South Canara, Karnataka |
| 5 | *Dioscorea pubera* Bl. | 202382 | Begur, Wynad, Kerala |
| 6 | *Dioscorea intermedia* Thw. | 202384 | Santhanpara, Idukki, Kerala |
| 7 | *Dioscorea spicata* Roth. | 202383 | Thirthahally, Coorg, Karnataka |
| 8 | *Dioscorea oppositifolia* L. | 202386 | Valpara, Palghat, Kerala |
| 9 | *Dioscorea wightii* Hook. f. | 214855 | Mundanthurai, Tamil Nadu |
| 10 | *Dioscorea belophylla* Voight | 248181 | Devakolly, Coorg, Karnataka |
| 11 | *Dioscorea wallichii* Hk. f. | 202312 | Courtallum, Tirunelveli, Tamil Nadu |
| 12 | *Dioscorea hamiltonii* Hk. | 202328 | Vadakkancherry, Thrissur, Kerala |

tubes containing 15 ml of MS basal medium (Murashige and Skoog, 1962) augmented with cytokinins like BA, Kn, TDZ (0.5-5.0 mg/l) alone or in combination with auxins like IAA, IBA or NAA (0.5-2.0 mg/l). Combinations of BA and Kn were also tried (0.5-5.0 mg/l). The cultures were then kept in the culture room maintained under 12 h photoperiod at a light intensity of 3000 lux at a temperature of 25±2°C and at 70-80% RH. The responding explants were transferred to fresh media either of the same composition or to lower concentrations for further proliferation and elongation. The shoot induction was recorded for seven replicates per treatment, each repeated three times.

## Results and Discussion

### *Effect of Cytokinins Alone*

*Dioscorea* species, belonging to the family Dioscoreaceae of the order Dioscoreales, were micropropagated through shoot multiplication. Single node segments showed various responses in accordance with the plant growth regulators supplied in the medium. About 80-90% contaminant free cultures were obtained following treatment of nodal segments with 0.1% $HgCl_2$ for 12 or 15 minutes.

Following 10 days of inoculation, sprouting of the axillary bud was observed. Elongation and multiplication of the shoot was observed after 30 days of culture. Response of different species has been presented below.

Multiple shoots were induced by cytokinins when used alone. The cytokinins BA, Kn and TDZ induced axillary bud proliferation at various concentrations (0.5–5 mg/l). It was observed that lower and higher levels of cytokinins, (less than 1 mg/l and above 5 mg/l) failed to induce shoot bud proliferation in all the species of *Dioscorea*. BA at 4 mg/l gave the best result in inducing 4 shoots in *D. pentaphylla* (Fig. 1) while Kn (3 mg/l) induced 3 shoots (Fig. 2). In *D. hispida*, TDZ at 3 mg/l produced 3 shoots (Fig. 3), while a single shoot was developed in BA at 1 mg/l (Fig. 4).

In *D. bulbifera*, BA (2 mg/l) initiated 3 shoots after 30 days of culture (Fig. 5; Table 2). When sub-cultured


**Fig. 1-5: Effects of cytokinins in shoot multiplication in different Dioscorea species**

**Table 2. Effect of cytokinins alone on shoot multiplication**

| Species | BA (mg/l) | Kn (mg/l) | TDZ (mg/l) | No. of shoots*(30 days) |
|---|---|---|---|---|
| *D. bulbifera* | 2.0 | – | – | 3 ± 0.22 |
| | 1.0 | – | – | 2 ± 0.18 |
| | – | 2.0 | – | 2 ± 0.13 |
| | – | 3.0 | – | 2 ± 0.18 |
| *D. pentaphylla* | 1.0 | – | – | 1 ± 0.18 |
| | 2.0 | – | – | 3 ± 0.13 |
| | 4.0 | – | – | 4 ± 0.29 |
| | – | 3.0 | – | 3 ± 0.13 |
| *D. hispida* | – | – | 3.0 | 3 ± 0.20 |
| | – | 1.0 | – | 1 ± 0.10 |
| *D. pentaphylla* | – | – | 1.0 | 3 ± 0.22 |
| | – | – | 2.0 | 2 ± 0.18 |
| | – | – | 3.0 | 4 ± 0.29 |
| *D. intermedia* | 1.0 | – | – | 3 ± 0.18 |
| | 2.0 | – | – | 5 ± 0.29 |
| *D. hamiltonii* | 3.0 | – | – | 3 ± 0.22 |
| *D. wightii* | 4.0 | – | – | 2 ± 0.13 |
| | 5.0 | – | – | 2 ± 0.22 |
| | – | 2.0 | – | 1 ± 0.18 |
| | – | 3.0 | – | 3 ± 0.22 |
| | – | 4.0 | – | 1 ± 0.18 |
| *D. wightii* | – | – | 0.5 | 2 ± 0.12 |
| | – | – | 1.0 | 5 ± 0.25 |
| *D. belophylla* | – | – | 1.0 | 22 ± 1.25 |
| | – | – | 2.0 | 3 ± 0.22 |
| *D. spicata* | – | – | 3.0 | 3 ± 0.18 |
| | – | – | 4.0 | 3 ± 0.22 |
| | – | – | 5.0 | 3 ± 0.22 |

* Values represent mean of seven replicates ± SE

on to the same medium, multiplication occurred at the same rate. The shoots were further subjected to elongation in basal MS medium.

A maximum of 5 shoot buds were obtained in *D. intermedia* in BA at 2 mg/l (Fig. 6), while at 1 and 3 mg/l, 3 shoots got developed in *D. hamiltonii* (Fig. 7). At BA (4-5 mg/l), 2 shoots got differentiated in *D. wightii* (Fig. 8). The shoots produced in 5 mg/l were suppressed and did not elongate in *D. oppositifolia* (Fig. 9). When Kn (0.5-5 mg/l) was used instead of BA, the shoot development was inhibited. At 3 mg/l, a maximum of 3 stunted shoots was formed in *D. wightii* (Fig. 10). In this species, when TDZ was used at different concentrations (0.5-5 mg/l), multiple shoot development was noticed. A maximum of 5 shoots were initiated in *D. wightii* (Fig. 11), whereas, 22 shoots were produced in

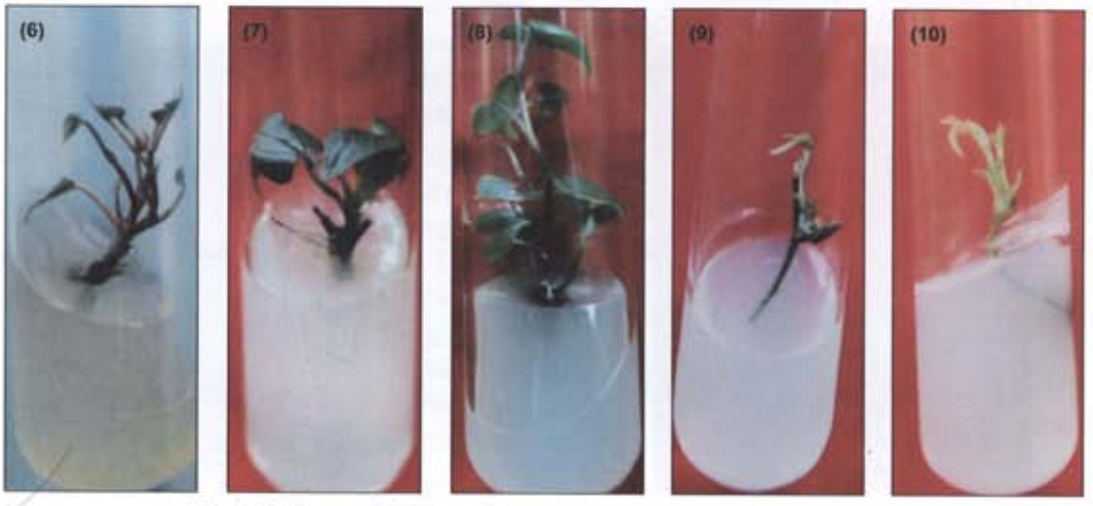


**Fig. 6-10: Rsponse of different Dioscorea species to shoot multiplication media**

*D. belophylla* in TDZ at 1 mg/l, (Fig. 12; Table 2). At higher concentrations of TDZ (>3 mg/l), 3 shoots with lush dark green leaves were produced in *D. spicata* (Fig. 13), while, at lower concentration (0.5 mg/l), 2 shoots having branches were initiated in *D. belophylla* (Fig. 14).

### *Effect of Cytokinins in Combination*

BA and Kn when used in combination resulted in better shoot multiplication compared to that when used singularly in *D. pentaphylla* and *D. oppositifolia*. Synergistic effect of BA (4 mg/l) and Kn (2 mg/l) resulted in 12 shoots in *D. pentaphylla* (Fig.15; Table 3), while, an increase in the Kn concentration to 4 mg/l reduced the number of shoots to 4 (Fig.16; Table 3). BA (2 mg/l) and Kn (1 mg/l) induced 8 shoots in *D. oppositifolia* (Fig.17; Table 3).

### *Effect of Cytokinins in Combination with Auxins*

Cytokinins *viz.*, BA/Kn (0.5–5.0 mg/l) in conjunction with NAA/IAA/IBA (0.5-2.0 mg/l) induced axillary bud proliferation in some species. In *D. bulbifera*, BA at 2-3 mg/l along with NAA/IAA (0.5 mg/l) resulted in single shoot formation (Fig. 18), with basal callusing. While in presence of IBA (0.5 mg/l), 2 shoots were induced (Fig. 19). The shoots produced in BA+IAA containing medium were long having 7-10 culturable nodes after 30 days of culture. When BA was replaced with Kn, in all combinations, a single shoot was induced (Table 4).

Addition of NAA (0.5 mg/l) along with BA (2 mg/l) induced a maximum of 8-10 shoots per node in *D. pentaphylla* (Fig. 20). Five shoots were obtained in 1 and 4 mg/l BA (Fig. 21) and 3 in 3 mg/l BA along with 0.5 mg/l NAA (Fig. 22). On the other hand, IBA (0.5 mg/l) when used in place of NAA, in conjunction with BA (2-3 mg/l), single shoot formation was noticed in *D. pentaphylla* (Table 4). Combinations of BA with IAA and Kn with IAA/IBA/NAA resulted in single shoot formation only (Table 4). In *D. hispida*, Kn (2 mg/l) along with NAA (0.5 mg/l) resulted in the formation of a single healthy shoot (Fig. 23).

**Table 3. Synergistic effect of cytokinins (BA + Kn) on shoot multiplication**

| Species | BA (mg/l) | Kn (mg/l) | No. of shoots (30 days)* |
|---|---|---|---|
| *D. bulbifera* | 2.0 | 1.0 | 2 ± 0.26 |
| | 2.0 | 1.0 | 1 ± 0.18 |
| *D. pentaphylla* | 4.0 | 2.0 | 12 ± 0.25 |
| | 4.0 | 4.0 | 4 ± 0.20 |
| | 2.0 | 2.0 | 3 ± 0.22 |
| *D. oppositifolia* | 2.0 | 1.0 | 8 ± 0.25 |
| | 4.0 | 4.0 | 2 ± 0.13 |
| | 2.0 | 2.0 | 1 ± 0.18 |
| | 4.0 | 2.0 | 1 ± 0.18 |

* Values represent mean of seven replicates ± SE

**Table 4. Effect of cytokinins along with auxins on shoot multiplication**

| Species | BA (mg/l) | Kn (mg/l) | NAA (mg/l) | IBA (mg/l) | IAA (mg/l) | No. of shoots (30 days) |
|---|---|---|---|---|---|---|
| *D. bulbifera* | 2.0 | – | 0.5 | – | – | 1 ± 0.18 |
| | 3.0 | – | 0.5 | – | – | 1 ± 0.18 |
| | 2.0 | – | – | 0.5 | – | 2 ± 0.13 |
| | 3.0 | – | – | 0.5 | – | 2 ± 0.13 |
| | | 2.0 | 0.5 | – | – | 1 ± 0.22 |
| | | 3.0 | – | 0.5 | – | 1 ± 0.18 |
| *D. pentaphylla* | 2.0 | – | – | 0.5 | – | 1 ± 0.18 |
| | 2.0 | – | 0.5 | – | – | 8 ± 0.25 |
| | 1.0 | – | 0.5 | – | – | 5 ± 0.25 |
| | 4.0 | – | 0.5 | – | – | 5 ± 0.25 |
| | 3.0 | – | 0.5 | – | – | 3 ± 0.22 |
| *D. hispida* | – | 2.0 | 0.5 | – | – | 1 ± 0.18 |
| *D. intermedia* | 2.0 | – | 0.5 | – | – | 10 ± 0.32 |
| | 3.0 | – | 0.5 | – | – | 5 ± 0.25 |
| | 3.0 | – | – | 0.5 | – | 5 ± 0.25 |
| | – | 2.0 | 0.5 | – | – | 1 ± 0.18 |
| | 3.0 | – | – | – | 0.5 | 1 ± 0.18 |
| | – | 3.0 | – | – | 0.5 | 1 ± 0.18 |

* Values represent mean of seven replicates ± SE

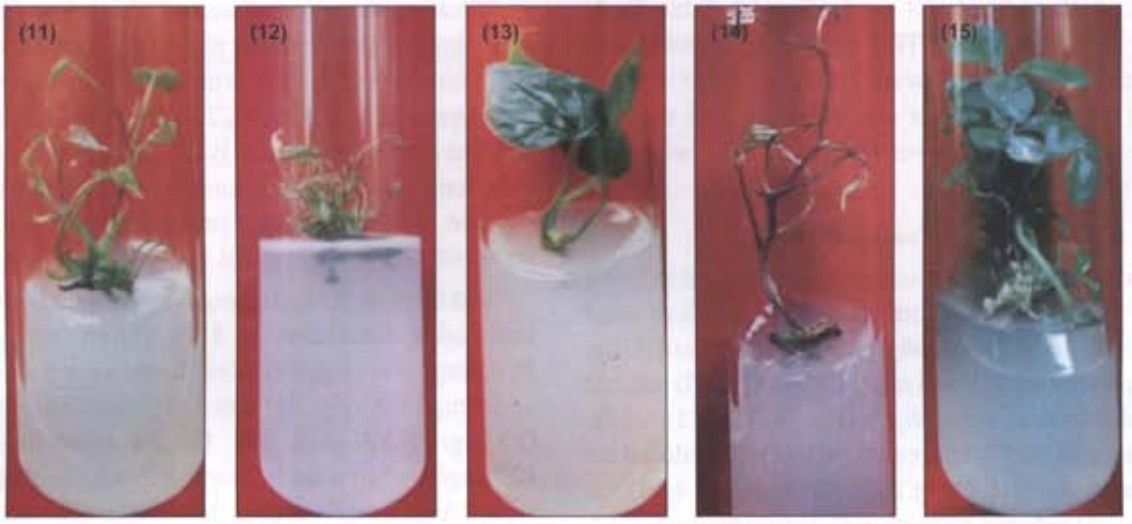


**Fig. 11-15: Effect of varying TDZ concentrations in Dioscorea species**

NAA (0.5 mg/l) along with BA (2-3 mg/l) induced 10 and 5 shoots, respectively in *D. intermedia* (Fig. 24, 25) as compared to 5-6 shoots at BA (3 mg/l) and IBA (0.5 mg/l) (Fig. 26; Table 4). However, BA along with IAA as well as all the combinations of Kn along with NAA/IAA/IBA resulted in single shoot development.

Selection of appropriate nutrient medium forms the initial and essential step for the success and fulfillment of all experimental systems of plant tissue culture. In the present study, micropropagation through shoot tip and nodal segment cultures was attempted in MS.

Of the different methods of *in vitro* propagation, multiple shoot induction is the most frequently used multiplication technique in plant micropropagation systems. The production of plants from axillary shoots has proved to be the most applicable and reliable method of *in vitro* propagation. According to George and Sherrington (1984), shoot tip/nodal segment culture depends on stimulating axillary shoot growth by overcoming the dominance of shoot apical meristem by the incorporation of growth regulators in to the medium.

The use of nodal segments as initial explants for the *in vitro* propagation of different *Dioscorea* species has been reported in *D. floribunda* (Chaturvedi, 1975; Sinha and Chaturvedi, 1979; Sita *et al.*, 1976), *D. bulbifera* (Forsyth and van Staden, 1982), *D. composita* (Datta *et al.*, 1982) and in *D. alata* and *D. rotundata* (Mantell *et al.*, 1978; Nair and Chandrababu, 1996).

In the present study, shoot multiplication was achieved in different species of *Dioscorea* by culturing single node segments in MS medium supplemented with different concentrations and combinations of cytokinins *viz.* BA/Kn/TDZ alone or in combination with auxins IAA/IBA/NAA. The number of shoots produced in different species varied in these hormonal regimes. Of the different cytokinins used for shoot multiplication, BA (2-4 mg/l) was found to be the best followed by TDZ and Kn in all the species of *Dioscorea.* The superiority of BA over other cytokinins for shoot proliferation has been established for plants such as *D. alata* (Mantell *et al.*, 1980), *D. bulbifera* (Forsyth and van Staden, 1982), *D. opposita* (Kobayashi, 1991) and *D. cayenensis* and *D. trifida* (Mitchell *et al.*, 1995b). However, in *D. belophylla*, 22 shoots were obtained in TDZ at 1 mg/l.

Cytokinins in combination showed better response than cytokinin alone for shoot multiplication in *D. pentaphylla*. In the present investigation, on BA+Kn, there was higher number of shoots in some species. A maximum of 12 shoots were produced in *D. pentaphylla* in presence of BA (4 mg/l) and Kn (2 mg/l), while up to 8 shoots in *D. oppositifolia* in presence of BA (2 mg/l) and Kn (1 mg/l). George and Sherrington (1984) were of the opinion that adding more than one cytokinin to the medium results in improved production of shoot or shoots of better quality. Mitchell *et al.* (1995a) also affirmed the potentiality of using more than one cytokinin in the medium for better shoot multiplication in *D. trifida*.

Addition of auxins along with cytokinins had a positive impact on shoot multiplication in some *Dioscorea* species. Of the various auxins used along with cytokinins, NAA/IAA/IBA, NAA at 0.5 mg/l gave the optimum results. BA at 2 mg/l along with NAA (0.5 mg/l) produced


**Fig. 16-26: Effect of cytokinin concentrations in Dioscorea species**

8-10 shoots in *D. pentaphylla* and 10 shoots in *D. intermedia*. Similar results have been reported in various *Dioscorea* species such as *D. floribunda* (Sita *et al.*, 1976), *D. bulbifera* (Forsyth and van Staden, 1982), *D. abyssinica* and *D. mangenotiana* (Lauzer *et al.*, 1992) and *D. alata, D. esculenta* and *D. rotundata* (Nair and Chandrababu, 1996).

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# Comparative Study of Wild Mangoes of Andamans (India) for Nutritional Composition and Rootstock Potential

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Out of reported five species of mango, *M. andamanica, M. griffithi* and *M. comptosperma* are reported from Andaman and Nicobar Islands, India. Detailed explorations and studies were carried out in Andamans for their habitat, botanical description, and nutritional composition of fruits, rootstock studies and grafting success of *M. indica* on these species. Nutritionally, *M. andamanica* and *M. griffithi* were found rich in vitamin C(ascorbic acid) (99.5 mg/100 g and 37.9 mg/100 g, respectively), total sugars (17.80 and 9.09%, respectively), which is comparable with traditional varieties of *M. indica*. Seedlings *M. comptosperma* showed more vigour, success and survival after grafting of *M. indica*. *Ex situ* conservation of these wild species and *M. andamanica* in particular was carried out by collecting of stones and raising of seedlings in bulk for further improvement and exploitation.

**Key Words: Wild Mango, Andaman, Botany, Nutrition, Grafting, Rootstock**

## Introduction

India holds rich genetic diversity of both cultivated and wild relatives of several horticultural crops including mango, which is decidedly the most important fruit for million of people. Perhaps, mango, originated in the Indo-Burma region and before domestication was growing wild in the forests of India, especially in the hilly areas in the northeast. Many indigenous genotypes are lost in recent past due to intervention of man and urbanization. Nevertheless, a great genetic diversity still exists owing to the large geographical area, diverse climatic conditions and allopolyploid nature of its origin.

Andaman and Nicobar archipelago (India) unique to India, have a vast variety and diversity in many tropical indigenous and unexploited fruit tree species (Parkinson, 1932; Rao, 1986 and Singh *et al.*, 1996).

Genus *Mangifera* belongs to the dicotyledonous family Anacardiaceae and almost all commercial cultivars of mango are included in a single species i.e. *M. indica*. Out of 41 recognized *Mangifera* species, valid number stands up to 39 (Mukherjee, 1985). Five species *viz., M. indica, M. khasiana, M. sylvatica, M. comptosperma* and *M. andamanica* were reported from India (Mukherjee *et al.*, 1983; Sreekumar *et al.*, 1996). Out of reported species 3 species *viz., M. andamanica, M. griffithi and M. comptosperma* are reported from the small stretch of Andaman and Nicobar islands.

In recent years, man and its associated anthropic factors have been radically changing the ecosystems, causing continuous loss of forestland. Consequently, many valuable and endemic species become rare and extinct. *M. andamanica* has fallen in the list of endangered species (Mukherjee, 1985).

Except few of the botanical studies no efforts were made to study the wild mangoes of Andaman to evaluate their nutritional value, their exploitation as rootstock multiplication and conservation of these rare and endangered species. These wild species can be a good stock for crop improvement and further utilization for processing and making value added products.

These explorations and detailed study were therefore conducted to gather maximum stones of different species in a region that is menaced by the genetic erosion as a result of the development of the agriculture and industrialization, for their botanical description, habitat, nutritional composition of fruits, rootstock studies and grafting success of these species and mass multiplication and conservation of rare and endangered species.

## Materials and Methods

Series of explorations during different seasons of vegetative growth and fruiting were conducted in several Andaman and Nicobar islands; situated of the Eastern coast of India, in the Bay of Bengal (10° 31′ and 13° 42′ N latitude and 92° 14′ and 90 ° 16′ E longitude) having a typical tropical and humid climate; annual average precipitation of 3086 mm with 82% relative humidity. Vegetative, flowering and fruiting characteristics of different mango species of wild were studied during different seasons. Flowering and fruiting of different species of mango was recorded from December to May during 1996-1998. The ripe fruits were collected and analyzed for different quality parameters (size and weight

of fruit, thickness of peel, weight of stone, TSS, acidity, vitamin C (ascorbic acid) and total sugar). TSS of the fruit was determined using hand refractometer and expressed in Brix. Titratable acidity and ascorbic acid were calculated using methods described by Rangana (1986). Titratable acidity was determined a 10 g aliquot of the homogenates made up to 100 ml. Titratable acidity was expressed in gm citric acid per 100 g fresh weight. Vitamin C was calculated as ascorbic acid mg/100 g fresh weight using 2,6-dichloro indophenol dye.

The healthy and bold stones of these species were raised for rootstock study. Grafting of Neelum variety of *M. indica* by soft wood grafting techniques (Singh and Suryanarayana, 1996) was done on 2 months old rootstock of different wild mangoes of these islands. 25 grafts were made for each species, which were replicated thrice.

Success of grafting and growth studies was carried out in the nursery at Central Agricultural Research Institute, Port Blair, India. The stones of these species and that of *M. andamanica* in particular were collected graded and raised in bulk for *in situ* conservation of rare and extinct species.

## Results and Discussion

### *Taxonomical Distribution/Botanical Description*

These wild species of mango i.e. *M. andamanica, M. griffithi and M. comptosperma* were studied for their morpho-taxonomical distribution (Table 1).

**I. *M. andamanica:*** Rare and endemic, was collected from Chidyatapu and Choudhari (South Andaman), 30-40 m high of upright growth. Flowers were 1/4$^{th}$ inch in diameter. Fruit was very small yellow orange in colour.

**II. *M. griffithi:*** First time reported wild from Mount Harriet of South Andaman. Tree was small 6-8 m high, having a characteristic thin leaf stalk (±3 mm) and prominent secondary veins, along lower surface with characteristic small fruits. Flowers were small 1/4$^{th}$ inch in diameter, white in colour.

**III. *M. comptosperma*:** It is not found common, usually found in middle Andaman; long island: South Andaman (Rutland). Tree was large 50-100 m high with greyish bark, leaves 5-10 inch long. Flowers 1/4$^{th}$ inch in diameter, white in colour. Fruits were compact in shape.

### *Fruit Characteristics/ Nutritional Composition*

These species were found in flowering and fruiting during January to April. The fruits of *M. andamanica* and *M. griffithi* were small and oblong in shape while that of *M. comptosperma* were comparatively larger and compressed in shape. Single fruit weight varied from 15.5g (*M. griffithi*) to 50.5 g (*M. comptosperma*). Maximum length (7.0 cm) of fruit was found in *M. comptosperma* and minimum (3.7 cm) in *M. andamanica*. Minimum weight of stone (23.6 g) was recorded in *M. andamanica*. Minimum weight of peel (19.35%) and maximum pulp percentage (52.25%) was recorded in *M. griffithi*, which was followed by *M. andamanica* (10.0%). Minimum thickness of fruit peel (0.15 cm) was recorded in *M. griffithi* and *M. andamanica* (0.18 cm). Maximum total soluble solids were recorded in fruits of *M. griffithi* (18.4°B) and *M. andamanica* (10.0°B) in *M. andamanica*. Minimum acidity was found in *M. griffithi* (3.0%) and *M. andamanica* (3.8%). Vitamin C content of the fruits was maximum in *M. andamanica* (99.5 mg/100 g) followed by *M. griffithi* (37.9 mg/100g). Total sugars were found maximum (17.80 and 9.09%) in *M. griffithi* and *M. andamanica,* respectively. Overall

**Table 1. Habit and habitat, vegetative growth and flowering of wild mangoes of Andaman and Nicobar Islands (India)**

| Species | Distribution | Tree characteristics | Flowering/Fruiting |
|---|---|---|---|
| *M. andamanica* | Endemic and rare: distributed in Andaman forests, Chidyatapu, Chouldhari (South Andaman) | The trees are of upright growth (30-40 m). Leaves 3-5 inch long obovate to broadly ablanceolate or eliptic | Flowers 1/4$^{th}$ inch in diameter, fruit very small, yellow orange in colour. Flowering and fruiting in Feb-April. Fruit yellowish orange |
| *M. griffithi* | South Andaman Island (Mount Harriet) | The trees are small (6-8 m high) with greyish brown bark and a dense canopy of green elliptic or obovate leaves. Allied to *M. longinifera* Griff by having a thin leaf stalk (±3 mm) and prominent secondary veins, along the lower surface with characteristic small fruits | Flowers small <1/4$^{th}$ inch in diameter, light yellow in colour. flowering and fruiting in Feb-April. Fruits are yellow with crimson tinge. |
| *M. camptosperma* | Not common, usually found in middle Andaman; long island: South Andaman, Rutland | The trees are large (50-100 m high) with greyish bark cut streaked dark reddish brown with little milky juice. Leaves 5-10 inch long, lanceolate, shortly acuminate in 16-24 pairs | Flowers 1/4$^{th}$ inch in diameter, white in colour in ample terminal panicle. Flowering and fruiting Feb.-April. Fruits are yellowish green. |

physical compositions of fruits indicate that *M. andamanica* and *M. griffithi* are having nutritional composition comparable with table varieties of *M. indica* (Table 2).

### *Rootstock and Grafting*

Maximum germination of stones was found in *M. griffithi* (76%) and *M. andamanica* (70%). Two months after sowing of the stones, the rootstock seedlings of maximum height (40.0 cm) were found in *M. comptosperma*. The seedlings of the *M.comptosperma* were found to be vigorous and stout. After grafting (soft wood) of *M. indica* scion, on these seedlings, maximum survival of 72% was found in *M. comptosperma, w*hile survival (8.0%) was least in *M. andamanica* and *M. griffithi*. After 60 days of grafting, none of the grafts survived on *M. andamanica* and *M. griffithi* whereas 50% of grafts survived in *M. comptosperma*. Number of leaves after 30 days of grafts were recorded in *M. comptosperma* (7.0%). In this rootstock it took least days for first sprouting of foliage i.e. 15 days while in others it took 18 days each. Performance of seedlings of these species for rootstock and grafting revealed that *M. comptosperma* showed more success and survival. Failure of *M. andamanica* and *M. griffithi* may be due to less vigorous growth of their seedlings and their stem, which was not found suitable for grafting. As for as nutritional aspect is concerned, *M. andamanica* (TSS 10.0°B and Vitamin C 99.5 mg/ 100 g) and *M. griffithi* (TSS 18.4°B and Vitamin C 37.5 mg/100 g) were found good in nutritional value (Table 2). These species need proper and sustainable exploitation for using in post harvest industry, which in turn will improve the socio economic aspect of local tribal community and local farmers living in interiors.

For *ex situ* conservation of these species, 1000 stones of each species were collected and raised seedlings were conserved in arboretum of the Central Agricultural Research Institute, Port Blair, Andaman and Nicobar Islands (India) for further detailed investigation.

**Table 2. Fruit physico-chemical characteristics of wild mango species**

| Species | Weight of fruit (g) | Length of fruit (cm) | Breadth of fruit (cm) | Weight of stone (gm) | Length of stone (cm) | Breadth of stone (cm) | Weight of peel (gm) | Weight of pulp (gm) | Thickness of peel (cm) | TSS (°B) | Acidity (%) | Vit. C (Ascorbic acid mg/ 100 g) | Total sugar (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *M. andamanica* | 20.0 | 3.7 | 2.9 | 4.32 (23.6%) | 3.8 | 1.8 | 8.82 (48.2%) | 5.5 (28.09) | 0.18 | 10.0 | 3.8 | 99.5 | 9.09 |
| *M.griffithi* | 15.5 | 4.0 | 2.10 | 3.98 (25.6%) | 3.7 | 1.8 | 3.00 (19.35%) | 8.1 (52.25) | 0.15 | 18.4 | 3.0 | 37.9 | 17.80 |
| *M. comptosperma* | 50.5 | 7.0 | 3.0 | 28.05 (55.54%) | 5.8 | 2.1 | 12.0 (23.8%) | 10.68 (22.0) | 0.22 | 6.0 | 6.0 | 22.0 | 4.80 |

Mean of 10 fruits/treatment

**Table 3. Grafting of *M. indica* Var. Neelum on wild mango species**

***Table 3.1. Stone germination and vegetative growth of seedlings of wild mango species***

| Species | Stone germination % | Vegetative growth of rootstock seedlings (2 months after sowing of stones) | | | Colour of Bark |
|---|---|---|---|---|---|
| | | Height (cm) | No. of leaves | Diameter (cm) | |
| *M. andamanica* | 70.0 | 22.0 | 5 | 0.40 | Greenish Brown |
| *M. griffithi* | 76.0 | 24.0 | 6 | 0.35 | Brownish Grey |
| *M. comptosperma* | 30.0 | 40.0 | 7 | 0.75 | Dark Brown |

Mean of 10 fruits/treatment

***Table 3.2. Growth performance of mango grafts on rootstock of wild mango species***

| Species | Survival (%) | | Days taken for | No. of leaves | |
|---|---|---|---|---|---|
| | After 30 days | After 60 days | first foliage | After 30 days | After 60 days |
| *M. andamanica* | 8 | 0 | 18 | 2 | 0 |
| *M. griffithi* | 8 | 0 | 18 | 2 | 0 |
| *M. comptosperma* | 72 | 50 | 15 | 3 | 7 |

Mean of 10 fruits/treatment

## Conclusion

The wild mango species lying scattered in wild and semi wild conditions in Andaman and Nicobar Islands (India) are found quite rich in nutritional composition and have great potential to be used as rootstock for grafting of different cultivars of *M. indica*. Nutritionally *M. andamanica* and *M. griffithi* are found rich in vitamin C and sugars and can be exploited for making value added products. Whereas, *M. comptosperma* was found to perform better as rootstock for grafting success of *M. indica*.

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# Evaluation Studies on Plum Germplasm under Temperate Region of Himachal Pradesh

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Seventeen plum accessions were evaluated for important qualitative and quantitative characters under Shimla conditions with two checks, 'Burbank Grand Prize' and 'Methley'. Qualitative characters expressed large variation for various character states proving the variability in the germplasm. Highest Coefficient of Variation was observed for fruit weight (53.87%) followed by percentage of stone in fruit (50.73%) and stone weight (37.84%) indicating that there is enough potential for exploiting the variability present in these characters. Like the check 'Burbank Grand Prize', EC034052 (Tarrol), EC552694 (Blackamber) and EC552684 (Fortune) had high productivity along with acceptable fruit size and weight and minimum percentage of stone in the fruit (<3.0). Meanwhile they ripen 1-3 weeks before the above mentioned checks, bestowed to attract early market. None of the accessions studied could surpass the check 'Methley', which had the rare blend of fruit quality traits and productivity.

**Key Words: Plum, *Prunus*, Evaluation, Germplasm**

## Introduction

The most important commercial species of plum belong to only two groups *viz.*, the European and Japanese types. The European plums are *Prunus domestica* L. and related forms have hexaploid chromosome number. Within *Prunus domestica* L., several groups of cultivars are recognized, such as Green Gage and Prunes. Its *insititia* subspecies include bullaces, damsons, mirabelles and St. Julien types. The term 'Japanese plum' covers not only *Prunus salicina* Lindl., but a wide range of plums developed by intercrossing various diploid species. Plum ranks fourth in importance and production and is widely cultivated in the hilly terrains lying between 1500 to 2200 m msl in India. In Himachal Pradesh, plums occupy a significant place both in respect to acreage and production next to apple (Sharma, 2005). Chanana (2001) suggested that plum can be grown in a wide range of subtropical and warm temperate climate. Plum species and cultivars are quite diverse in fruit characters such as fruit size, shape, color, texture, aroma and quality. On the other hand, plums exhibit limited capacity for adaptation, and as a result, in each area only specific cultivars are grown with an exception to few major cultivars. Evaluation studies in mid hills and subtropical plains of Northern India were done by few workers (Lal and Mishra, 1980; Tripathi *et al.*, 1984; Dhatt *et al.*, 1992; Singh *et al.*, 2002). In high hills like Shimla, similar work was lacking at least in the last two decades. Hence, the present study is an endeavor for the characterization and evaluation of plum genetic resources, thereby utilization either directly or for plum improvement work.

## Materials and Methods

Present study was carried out at field gene bank of NBPGR Regional Station, Phagli, Shimla (31° 05' 924" N latitude, 77° 09' 580" E longitude; 1924 msl). Seventeen plum accessions comprising 14 exotic (from four countries) and three indigenous were taken into account. Well known and recommended varieties for high hills such as 'Methley' (Japanese type) and 'Burbank Grand Prize' (European type) were considered as checks. All the plants of the accessions were of 8-10 years age. Out of 17 accessions studied, nine belong to Japanese type, two to European type and six of unknown origin (Table 1). The plants were grafted on wild *Prunus armeniaca* (*chuli*) rootstock. Experimental plants were grown under similar soil and cultural conditions. Observations were recorded on date of full bloom (when 80% flowers open) and fruit harvest (eating-ripe stage), days to fruit harvest (from date of full bloom), fruit length (mm), fruit width (mm), fruit weight (g), fruit shape, fruit color, fruit bloom intensity, pulp texture, fruit juiciness, fruit taste, stone adherence to pulp, total soluble solids (TSS) (using Digital Hand-held 'Pocket' Refractometer PAL-1), stone weight, percentage of stone in the fruit, and productivity status. Descriptor states for qualitative characters were followed from the descriptor developed by NBPGR (Mahajan *et al.*, 2002). Fruit data were recorded by randomly selecting ten fruits from each accession. Mean values of three years (2002-2004) data

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were taken to draw the conclusion. Variability parameters were also worked out.

**Results and Discussion**

Qualitative characters on 17 accessions studied are mentioned in Table 1 along with their alternate identity. Their diverse nature was represented by most of the descriptor states as mentioned by Mahajan *et al.* (2002). Out of 17 accessions studied, six exhibited dark-violet/ violet fruit color while nine had fruits with fine-textured pulp. As like the checks, there was no completely free-stone accession available in the germplasm. Partially clinging nature of stone with pulp was displayed by four accessions. About half the accessions recorded high/ medium productivity.

Date of full bloom ranged from 3rd March to 19th March while a difference of 26 days between the earliest (EC393740 and EC552689) and latest (EC552682 and EC552692) maturing accessions was noticed. The welcome situation is that the high yielding cultivars were ripening at a week interval satisfying the need for a long and continuous supply of fruits for market. All the four accessions showing high productivity status (EC552684, EC034052, EC552694 and EC552689) ripened 1-3 weeks before the check 'Burbank Grand Prize'. Tripathi *et al.* (1984) reported that the cultivar 'Settler' is early in ripening under Chaubattia (Uttaranchal) conditions. The present study reveals that out of 17 accessions 10 were earlier than both the checks studied, which indicates the scope for genetic improvement for earliness. A vast range of variation was exhibited by the characters 'fruit weight' (6.41-69.03 g) and 'percentage of stone in fruit' (1.58-11.06%). Bal (2003) also reported a wide range of variability of 8.36 to 28.5 g for fruit weight in 20 indigenous collections of plum. Coefficient of Variation (CV) was highest for fruit weight (53.87%) followed by percentage of stone in fruit (50.73%) and stone weight (37.84%) indicating that there is enough potential for exploiting the variability present in these characters (Table 2). Variability, both in intra- and inter-specific level might have contributed to the above diversity.

In plum, deep red/violet color with large size fruits (>40 mm), sweet pulp, small and free-stone are desirable characters. All these characters along with high productivity and earliness impart huge demand and command premium price. It is quite impossible to get all the desirable attributes in single cultivar. Like the check 'Burbank Grand Prize', EC034052 (Tarrol), EC552694 (Blackamber) and EC552684 (Fortune) had high productivity along with acceptable fruit size and weight and minimum percentage of stone in the fruit (<3.0). Later two had fine textured pulp. Even though, EC552689 (Red Plum) registered high productivity and earliest ripening, its unacceptable fruit size and weight may hinder the commercial aspect (Table 2). Fruit thinning experiments may help in increasing fruit size in this cultivar.

**Table 1. Important qualitative characters on plum accessions studied**

| S.No. | Accession | Alternate identity | Fruit shape | Fruit color | Fruit bloom intensity | Pulp texture | Fruit taste | Fruit juiciness | Stone adherence to pulp | Product-ivity status |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | EC552684 | Fortune[b] | Ovate | Dark violet | Low | Fine | Acidic | Less juicy | Cling | High |
| 2 | EC552682 | Plum Black[c] | Elliptic | Violet | Low | Fine | Sub acidic | Less juicy | Cling | Low |
| 3 | EC034048 | Settler[c] | Round | Greenish violet | Medium | Coarse | Sub acidic | Less juicy | Partially-cling | Low |
| 4 | EC552695 | Japan Plum[b] | Cordate | Yellow | Low | Intermediate | Medium sweet | juicy | Cling | Low |
| 5 | EC552688 | Yellow Plum[b] | Ovate | Reddish yellow | Medium | Fine | Medium sweet | juicy | Cling | Low |
| 6 | EC034052 | Tarrol[b] | Oblong | Dark violet | Low | Intermediate | Medium sweet | Less juicy | Cling | High |
| 7 | EC552692 | Green Gage[a] | Elliptic | Red | Medium | Intermediate | Sub acidic | juicy | Cling | Low |
| 8 | EC552694 | Blackamber[b] | Round | Dark violet | Low | Fine | Acidic | Less juicy | Cling | High |
| 9 | EC382626 | Au-Rosa[b] | Ovate | Red | Low | Fine | Sub acidic | Less juicy | Cling | Medium |
| 10 | EC393740 | Azar Shah[a] | Round | Yellow | Medium | Fine | Acidic | juicy | Cling | Low |
| 11 | EC552687 | Santa Rosa[b] | Elliptic | Dark violet | Low | Coarse | Sub acidic | juicy | Cling | Medium |
| 12 | EC552689 | Red Plum[c] | Elliptic | Violet | Low | Coarse | Sweet | juicy | Partially-cling | High |
| 13 | EC382624 | Au-Cherry[b] | Elliptic | Red violet | Low | Fine | Acidic | Less juicy | Cling | Medium |
| 14 | EC552685 | Kubo[c] | Round | Red violet | Low | Intermediate | Medium sweet | juicy | Partially-cling | Low |
| 15 | IC020827 | BDJ-228[c] | Round | Red violet | Low | Fine | Sweet | juicy | Partially-cling | Low |
| 16 | IC020085[c] | — | Round | Red | Low | Intermediate | Sweet | juicy | Cling | Medium |
| 17 | Kohinoor[b] | — | Round | Red violet | Low | Fine | Sweet | juicy | Cling | Low |
| Burbank Grand Prize[a] (Check 1) | | | Round | Red | Low | Fine | Sweet | Less juicy | Cling | High |
| Methley[b] (Check 2) | | | Round | Red violet | Low | Fine | Sweet | juicy | Partially-cling | Medium |

[a]European type [b]Japanese type [c]Unknown

**Table 2. Pooled mean values of quantitative characters in plum accessions studied**

| S.No | Accession | Date of full bloom | Date of fruit harvest | Days to fruit harvest | Fruit length (mm) | Fruit width (mm) | Fruit weight (g) | TSS (%) | Stone weight (g) | Percentage of stone in fruit |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | EC552684 | 11/3 | 21/6 | 102 | 40.23 | 38.74 | 41.62 | 11.80 | 0.99 | 2.38 |
| 2 | EC552682 | 18/3 | 4/7 | 108 | 31.80 | 31.96 | 25.81 | 18.17 | 1.65 | 6.39 |
| 3 | EC034048 | 13/3 | 22/6 | 101 | 26.94 | 25.17 | 16.75 | 19.47 | 0.61 | 3.64 |
| 4 | EC552695 | 19/3 | 12/6 | 85 | 34.41 | 32.52 | 23.51 | 15.27 | 0.80 | 3.40 |
| 5 | EC552688 | 9/3 | 22/6 | 105 | 43.94 | 43.16 | 46.17 | 14.27 | 1.33 | 2.88 |
| 6 | EC034052 | 10/3 | 14/6 | 96 | 45.80 | 45.43 | 69.03 | 15.63 | 1.09 | 1.58 |
| 7 | EC552692 | 14/3 | 4/7 | 112 | 30.36 | 30.97 | 25.93 | 15.07 | 1.18 | 4.55 |
| 8 | EC552694 | 12/3 | 14/6 | 94 | 36.77 | 39.76 | 31.74 | 12.07 | 0.82 | 2.58 |
| 9 | EC382626 | 18/3 | 22/6 | 96 | 40.48 | 38.59 | 31.74 | 14.73 | 1.58 | 4.98 |
| 10 | EC393740 | 9/3 | 8/6 | 91 | 20.40 | 22.01 | 6.41 | 11.70 | 0.61 | 9.52 |
| 11 | EC552687 | 8/3 | 13/6 | 97 | 32.91 | 33.33 | 24.61 | 17.97 | 1.50 | 6.10 |
| 12 | EC552689 | 3/3 | 8/6 | 97 | 29.42 | 31.72 | 21.41 | 16.60 | 1.29 | 6.03 |
| 13 | EC382624 | 12/3 | 23/6 | 103 | 42.50 | 40.95 | 34.77 | 12.53 | 1.59 | 4.57 |
| 14 | EC552685 | 9/3 | 12/6 | 95 | 31.01 | 29.65 | 18.55 | 15.90 | 0.67 | 3.61 |
| 15 | IC020827 | 10/3 | 13/6 | 95 | 33.89 | 31.74 | 20.81 | 16.97 | 1.16 | 5.57 |
| 16 | IC020085 | 12/3 | 15/6 | 95 | 31.75 | 30.49 | 17.09 | 11.23 | 1.89 | 11.06 |
| 17 | Kohinoor | 8/3 | 9/6 | 93 | 26.26 | 27.88 | 10.81 | 20.13 | 0.44 | 4.07 |
| Burbank Grand Prize (Check 1) | | 27/3 | 29/6 | 94 | 42.80 | 45.36 | 57.13 | 13.30 | 1.01 | 1.77 |
| Methley (Check 2) | | 15/3 | 20/6 | 97 | 34.42 | 33.92 | 22.44 | 16.13 | 1.18 | 5.26 |
| Mean | | | | 97.94 | 34.05 | 33.77 | 27.46 | 15.27 | 1.13 | 4.88 |
| Maximum value | | | | 112.00 | 45.80 | 45.43 | 69.03 | 20.13 | 1.89 | 11.06 |
| Minimum value | | | | 85.00 | 20.40 | 22.01 | 6.41 | 11.23 | 0.44 | 1.58 |
| S.E | | | | 1.59 | 1.66 | 1.55 | 3.59 | 0.67 | 0.10 | 0.60 |
| CV (%) | | | | 6.71 | 20.08 | 18.95 | 53.87 | 18.11 | 37.84 | 50.73 |

None of the accessions studied could surpass the check 'Methley', which had the rare blend of fruit quality traits and productivity. Barring the productivity, IC020827 (BDJ-228) and 'Kohinoor' showed their distinction for most of the fruit quality characters studied. They were also early maturing with high TSS under the present study. Hence they can be used as breeding material for genetic improvement.

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# Preliminary Observations on Fruit Ripening, Predation and Seed Dispersal in the Wild *Momordica* Species

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The present paper presents observations on fruit ripening and dispersal, predation and seed dispersal in the wild *Momordica* species. Fruit morphology of the genus *Momordica* L. is highly adapted for bird dispersal. Association of specific species of birds in seed dispersal and ant predation in seed germination was observed. An understanding of the biotic agents involved in the perpetuation of biological processes is a prerequisite for devising a holistic conservation strategy for the wild *Momordica* genepool.

**Key Words: *Momordica*, Phenology, Fruit ripening, Seed predation and Dispersal**

## Introduction

The genus *Momordica* L. (Family Cucurbitaceae) assumes significance for conservation of genetic diversity in India by virtue of being the wild relatives of cultivated bitter gourd, besides their direct utility as nutritious vegetables and multipurpose medicinal plants. Six species occur in India and among them, *M. dioica* Roxb. and *M. charantia* var. *muricata* (Willd.) Chakrav. have wider distribution scattered over the entire geographical territory (Joseph, 2005; Joseph *et al.*, 2006; Joseph and Antony, 2007). *M. sahyadrica* Joseph & Antony is endemic to the Western Ghats (Joseph and Antony, 2007) and *M. balsamina* L. is restricted to the arid belt of Rajasthan and Gujarat (Joseph, 2005). *M. cochinchinensis* (Lour.) Spreng., commonly known as 'sweet gourd', occurs in a wild state in Andaman Islands and North Eastern States (Bharathi *et al.*, 2006). *M. subangulata* ssp. *renigera* (G.Don) deWilde (teasle gourd), a native of northeast India, is now cultivated in the whole North East, West Bengal and Andaman Islands (Joseph *et al.*, 2006)). *M. charantia* and *M. balsamina* are monoecious annuals whereas the rest of the species dealt here are dioecious perennials with tuberous roots. All are essentially seed propagated though vegetative propagation through adventitious tubers are observed in *M. subangulata* ssp. *renigera*.

However, a perusal of its status in *ex situ* holdings and genebanks indicate its poor representation and neglect in the existing biodiversity conservation programmes. For devising a sound conservation strategy for the wild genepool of *Momordica*, taking into account its present conservation status and perceived threat of genetic erosion, it is mandatory to have a good understanding of the phenology and reproductive biology of the genus.

Phenology involves different stages in the lifecycle of the plant/species such as sprouting of dormant tubers and seed germination, flowering, fruit ripening and senescence. Fruit ripening is a genetically determined and regulated event that prepares the fruit or seed for dispersal. However, the control mechanism in ripening varies because of the species diversity and variation in morphology of the fruit (Brady, 1987). Ripening is regarded primarily as a manifestation of senescence of the fruit in which intercellular organization begins to break down (Sucher, 1973). The work on ripening of fruits has been restricted to horticultural crops especially to temperate pome and stone fruits, and citrus, avocado and mango to some extent (Hulme, 1971). The most readily apparent phenomena in the ripening process are colour changes which involve chlorophyll loss resulting in subsequent synthesis of new pigments, alterations in flavour, changes in acidity, astringency, sweetness and softness of the fleshy tissues (Rhodes, 1970).

## Materials and Methods

In the germplasm characterization plot, ten plants each of *M. charantia* var. *muricata*, *M. balsamina*, *M. subangulata* spp. *renigera*, *M. sahyadrica* and *M. dioica* were observed regularly during the Kharif season during 2002, 2003 and 2004. Fruits of all the five taxa were observed from female flower opening to bursting of orange ripe fruits through colour breaker, mature ripe and red ripe stages. Ten fruits in each species were tagged the day after pollination. Days taken to reach bursting of fruits were calculated and major morphological stages associated with ripeness recorded. As stage of

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harvest for vegetable is also important, the time up to which the seeds remain tender and soft, while attaining maximum size and weight was determined in another set of ten fruits in each species by passing a sharp knife through the fruit from $10^{th}$ to $18^{th}$ day of fruit development.

In the germplasm characterization plots, observations were recorded on fruit presentation, seed predation and dispersal. Bird activity in and around *Momordica* vines were observed at close quarters from a hidden place. Vernacular names of common birds were used to record the bird visits and then their zoological identity later confirmed with the ornithologists of Kerala Forest Research Institute, Peechi, Kerala. Polythene sheets were kept spread below the bearing vines and fallen seeds (de-pulped) if any, were counted. Similarly, seed predation was calculated by placing 100 seeds each with both washed and red fleshy aril intact on a newspaper sheet and observing their predation by various species of ants.

**Results and Discussion**

Ripeness in fruits is determined using several methods and these methods vary depending upon the fruit type and specific requirement. The visual assessment of appearance is the simplest method. The changes in colour from green to yellow or orange is effective in many crops. Fruit ripening and loosening from the plant are considered as indicators of maturity in a variety of fruits (Jackson, 1986). There are several approaches to quantify the ripening process. The number of days required to reach fruit maturity from full bloom is a reliable indicator (Jackson, 1986).

*M. balsamina* has the shortest and *M. subangulata* ssp. *renigera* has the longest ripening span (Table 1). Build up of red tinge in spine tips do not indicate seed coat hardening in *M. subangulata* ssp. *renigera*, whereas, full size and turgid appearance are a reliable indicator of fruit maturity. Physiologically mature fruits soften within 24-48 hours from harvesting in *M. sahyadrica* and *M. dioica*, whereas, *M. subangulata* ssp. *renigera* fruits remain green and fresh for longer periods. Thus, *M. subangulata* ssp. *renigera* has better keeping quality and shelf life than others.

Mature fruits in all species attain full size, become bright in colour (green or whitish green or off white), fruit surface is turgid, with bumps and tubercle bases spread out. When the fruits begin to ripen, the exterior colour changes to orange yellow and the pulp (seed aril and placenta) becomes gelatinous and scarlet red. Coinciding with colour changes, the fruit pulp loses bitterness and become sweet. Fruits start developing yellow colour and then change to orange colour gradually and uniformly in *M. subangulata* ssp. *renigera* whereas in all other taxa, fruits change colour suddenly and is localized and spreads within a day or two. Thus in *M. subangulata* ssp. *renigera,* redness cannot be taken as indicator of ripeness. The fruits remain intact until bursting open in to three or more pieces from base, usually in a star like configuration. Ripe fruits do not abscise, as the pendant fruit stalks remain strong and split fruit wall dry up and shrivel or wither and crumble due to rain and sun.

Species of frugivorous birds and ants feeding on seeds are given in Table 2. *Megalima lineata* (barbet) is often a pest of coffee estates, feeding on coffee berries. It is interesting to note that highest population of *M. sahyadrica* is found in coffee estates, hence adding credence to bird dispersal. Although crows, koyal, crow-phesant ('uppan') and sparrows were found as infrequent visitors, seed feeding was not observed. Even though, seeds per fruit varied from 15.0 to 30.0 (up to 50.0 in *M. sahyadrica*), no depulped seed was found on the floor (the fleshy fruit wall is not eaten by any of these birds), giving credence to the inference that while feeding on the fleshy aril, the seeds are gulped. All the *Momordica* species have very thick seed coat, enabling their passage through bird gut undamaged, but slightly reduced in size as it is partially digested. Strong pendulous fruit stalks, attractive orange red colour, fruits splitting open from base and rolling back and expanding in a star like

**Table 1. Observations on various stages of fruit maturity**

| Species | No. of days to full fruit size | No. of days to colour breaker | No. of days to red stage | No. of days to bursting stage | No. of days seeds remain tender |
|---|---|---|---|---|---|
| *M. charantia* var. *muricata* | 12 | 25 | 26-27 | 29-30 | 8 |
| *M. balsamina* | 9 | 22 | 24 | 25 | 8 |
| *M. dioica* | 15 | 28 | 29 | 30-31 | 12 |
| *M. sahyadrica* | 15 | 30 | 31 | 32-33 | 12 |
| *M. subangulata* ssp. *renigera* | 18 | 20 | 35 | 38 | 15 |

**Table 2. Seed dispersal and predation in various *Momordica* species**

| Species | Biotic agent | Zoological name | Common/vernacular name | Activity |
|---|---|---|---|---|
| *M. charantia* var. *muricata* | Ant (Family Formicidae) | *Myrmicaria* Saeeuders sp.<br>*Odontomachus* Latrelli sp.<br>*Monomorium* Mayx sp. | koonan<br>katturumbu<br>shavam theeni urumbu* | Feeding on red sweet aril<br>Carrying dry seeds and storing it in ant hives |
| | Bird | *Rufus treepie*<br>*Megalima viridis*<br>*Pycnonotus* sp. | South Indian Tree Pie<br>Small green barbet<br>Red whiskered bulbul | Feeding fleshy aril, gulping seeds |
| *M. dioica* | Ant (Family Formicidae) | *Odontomachus* Latrelli sp.<br>*Myrmicaria* Saeeuders sp.<br>*Monomorium* Mayx sp.<br>*Solenopsis* Westw. sp. | katturumbu<br>koonan<br>shavam theeni urumbu<br>neyyurumbu* | Feeding on red sweet aril<br>Carrying dry seeds and storing it in ant hives |
| | Bird | *Pycnonotus* sp.<br>*Megalima viridis* | Red whiskered Bulbul Barbet | Feeding fleshy aril, gulping seeds |
| *M. sahyadrica* | Ant (Family Formicidae) | *Odontomachus* Latrelli sp.<br>*Myrmicaria* Saeeuders sp.<br>*Monomorium* Mayx sp.<br>*Crematogaster* Leud sp. | katturumbu<br>koonan<br>shavam theeni urumbu<br>small black ants | Feeding on red sweet aril<br>Carrying dry seeds and storing it in ant hives |
| | Bird | *Megalima viridis*<br>*Rufus treepie*<br>*Pycnonotus* sp. | Small green barbet<br>Tree Pie<br>Bulbul | Feeding fleshy aril, gulping seeds |
| *M. subangulata* ssp. *renigera* (Family Formicidae) | Ant | *Odontomachus* Latrelli sp.<br>*Myrmicaria* Sauceders sp.<br>*Monomorium* Mayx sp. | katturumbu<br>koonan<br>shavam theeni urumbu | Feeding on red sweet aril<br>Carrying dry seeds and storing it in ant hives |
| | Bird | *Megalima lineata*<br>*Rufus treepie*<br>*Pycnonotus* sp. | Lineated barbet<br>Tree Pie<br>Bulbul | Feeding fleshy aril, gulping seeds |

configuration exhibiting rows of scarlet red aril (covered seeds), and small round or flat dented seeds are adaptations for bird dispersal. Ethnobotanical interviews with the tribal inhabitants and spotting of *M. dioica* seedlings rooted in crevices of stonewalls add further credence to the involvement of birds in seed dispersal (Joseph, 2005).

Ripe fruits of all species were found densely covered with ants and they were predating on red fleshy sweet aril. Many species of ants were found carrying dry seeds of *M. dioica*, *M. sahyadrica* and *M. subangulata* ssp. *renigera* and were recovered from their hives. Besides, carrying to small distance, ant feeding may help to scarify the seeds by releasing formic acid. *M. dioica* seeds are reported to contain up to 39 per cent oil (Dubey and Gaur, 1989). The ant genera falling under Formicidae family in general has been reported as primary seed dispersing ants and *Crematogaster* species has been found as an important dispersal agent in tropical forests (Beattie, 1985).

The unit of dispersal (diaspore) is seed in the case of *Momordica*. A two-pronged strategy for dispersal is evident; colourful arils as food reward for birds and oiliferous seeds for dispersal by ants. The fleshy aril (sarcotesta) is of placental origin and it turns to scarlet crimson colour on ripening. The sweet and slimy aril attracts a host of birds, snails and ants. Seed coat is hard enough and passes unharmed through the guts and bird dispersal is the rule in the genera. The fallen seeds on the floor are carried to a small distance (even up to 8 m) by ants.

The place of production of seeds does not have the carrying capacity to grow and sustain all of them. An unhealthy competition is avoided by dispersing seeds even at the danger of some casualities. Dependence on animals for seed transport means that the plants are susceptible to dispersal failure when their seed vectors become rare or extinct. Disruption of this mutualism can have serious consequences for the maintenance of plant/species populations. This again highlights the need for devising a holistic approach for conservation of *Momordica* genepool involving the biotic agents as well.

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# Morphological Descriptors for Ethiopian Wild *Coffea arabica* L. Genetic Resources Conserved in India

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Eighteen accessions of wild *Coffea arabica* from different geographic locations of Ethiopia available in the gene bank of Central Coffee Research Institute; Balehonnur, Karnataka, India, were evaluated for vegetative, inflorescence and flowering traits. Three cultivated varieties of Ethiopian Arabica coffee *viz.*, Agaro, Cioccie and Tafarikela were considered as standards in the present study. Morphological descriptor of International Plant Genetic Resource Institute was used as the basic format for the characterization. Vegetative characters showing large variation were identified to be young leaf colour and angle of insertion of primary branches. Among the reproductive traits studied, flower number per axil was found to manifest large variation. These traits offer scope for selection and thus, this information is expected to be useful for conservation of genotypes in the Gene Bank and selection and utilization in the breeding programmes.

**Key Words: *Coffea*, Descriptors, Characterization, Germplasm**

## Introduction

Coffee is one of the world's most valuable commodities, contributing largely to the economy of more than 50 countries of Asia, Latin America and Oceania. Of approximately 100 taxa of the genus *Coffea* (Family Rubiaceae), only two species are economically important *i.e., Coffea arabica* L. and *Coffea canephora* Pierre ex Froehner, accounting respectively for about 70 per cent and 30 per cent of the world coffee production. In India, the area under coffee cultivation is around 3,48,995 ha, of which arabica and robusta account for 48 per cent and 52 per cent, respectively. The annual average production is around 2,80,000 metric tons, a share of 4.54 per cent of the world production. Arabica coffee is believed to have originated in the Southwestern highlands of Ethiopia (erstwhile Abyssinia) (Sylvain, 1955; Meyer, 1965). Literature on coffee provides a vivid description of the spread of *C. arabica* from Arabia Felix (present Yemen) to other countries (Chevalier, 1929; Cramer, 1957; Meyer, 1965; Smith, 1985; Wrigley, 1988). Morphological traits were the earliest markers used in germplasm collection, management and utilization in breeding (Smith and Smith, 1992). Study of morphological and agronomic traits is the best approach to understand the genetic constitution of the coffee plant and helped in various breeding programmes for the improvement of yield, quality, disease resistance and other desirable characters. Morphological characters such as vegetative development, angle of insertion of primary branches, young leaf colour, leaf length, leaf width, number of days from rainfall to flowering, inflorescence position, inflorescence on old wood, number of flowers per axil, number of flowers per fascicle, number of fascicles per node, inflorescence stalk length, corolla tube length, anther insertion and number of stamens per flower have considerable importance in the coffee breeding programme (Vishveshwara and Ahmed, 1975; Srinivasan and Vishveshwara, 1973; Srinivasan, 1969, 1980, 1985; Selvakumar and Sreenivasan, 1986; Montagnon and Bouharmont, 1996).

Wild relatives and progenitors of cultivated plants represent a strategic component of germplasm collections. Characterization of the accessions maintained in the gene bank and related documentation is imperative for the optimal conservation of genetic resources and effective use in breeding programmes (Ferreira, 2005). As the genetic base and variability of agricultural crops are relatively narrow, introgression of genes from wild sources can substantially influence the breeding progress. Morphological descriptors are reliable, easy to study and relatively less expensive to evaluate with very few limitations. These descriptors enable correct taxonomic ranging and precise characterization of genetic resources and define infraspecific variation. As wild species constitute the base of genetic resources for coffee breeding, interspecific crosses have been realised to test breeding possibilities by introgression (Louarn, 1992). Despite the importance of morphological data in breeding, very little work has been done on the characterization and evaluation

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of coffee germplasm available at the Central Coffee Research Institute (CCRI), India. The specific objective of this study is to generate descriptors of the various accessions from different geographical locations of Ethiopia, maintained in CCRI Gene Bank to understand the organization of existing morphological diversity in the wild coffee germplasm in a universally acceptable format (IPGRI) for current and future use in genetic improvement of the crop.

## Materials and Methods

Fifteen different coffee germplasm accessions enlisted as S. 2440, S. 2441, S. 2600, S. 2601, S. 2602, S. 2615, S. 2604, S. 2608, S. 2642, S. 2644, S. 2649, S. 2650, S. 2707, S. 2709, S. 2708 in the Gene Bank planting register of Botany Division (CCRI), and growing in the Germplasm blocks of CCRI, constitute the experimental material for studying variations in the morphological characters such as, vegetative, inflorescence and floral morphology. The materials S. 2440 and S. 2441 were collected from Abyssinia during 1955 and the rest were from different geographical locations of Ethiopia collected in the wild during an FAO sponsored expedition to Ethiopia in 1964 for exploration of coffee germplasm and the establishment of World Coffee Collections (Narasimhaswamy, 1965). Accessions S. 2600 and S. 2601 represent Harrar province; S. 2602 and S. 2615 represent Shoa province; S. 2604 and S. 2608 represent Sidamo province; S. 2642 and S. 2644 represent Kaffa province; S. 2649 and S. 2650 represent Illubabor province; S. 2707 and S. 2709 represent Gojjam province and S. 2708 represents Eritrea province. The study was conducted during the peak periods of vegetative and reproductive growth. For all vegetative characters, average of 10 plant observations was taken; for inflorescence and floral characters average of 10 inflorescence and 10 flower observations were taken, as recommended by the International Plant Genetic Resource Institute (IPGRI, 1996). Descriptors and numerical weightages used for genetic diversity are presented in Tables 1 and 2. The three cultivated types of Ethiopian arabica coffee *viz.*, Agaro, Cioccie and Tafarikela were included as standards.

**Table 1. Plant morphological descriptors of *Coffea* spp. used for diversity analysis of vegetative characters**

| Characters | Weightage |
|---|---|
| Plant habit | 1 Bush (<5 m – without distinct trunk)<br>2 Shrub or small tree (<5 m - one or more trunks)<br>3 Tree (>5 m - single trunk) |
| Plant height | 1 Very short; 3 Short; 7 Tall; 9 Very tall |
| Overall appearance | 1 Elongated conical; 2 Pyramidal; 3 Bushy |
| Vegetative development | 1 Monopodial; 2 Sympodial |
| Branching habit | 1 Very few branches (primary)<br>2 Many branches (primary) with few secondary branches<br>3 Many branches (primary) with many secondary branches<br>4 Many branches (primary) with many secondary and tertiary branches |
| Angle of insertion of primary branches | 1 Drooping; 2 Horizontal or spreading; 3 Semi-erect |
| Stipule shape | 1 Round; 2 Ovate; 3 Triangular; 4 Deltate (equilaterally triangular); 5 Trapeziform; 6 Other |
| Stipule arista length | Measured in mm |
| Young leaf colour | 1 Greenish; 2 Green; 3 Brownish 4 Reddish brown; 5 Bronze; 6 Other |
| Leaf shape | 1 Obovate; 2 Ovate; 3 Elliptic; 4 Lanceolate; 5 Other |
| Leaf apex shape | 1 Round; 2 Obtuse; 3 Acute; 4 Acuminate; 5 Apiculate; 6 Spatulate; 7 Other |
| Leaf length | Measured in mm (Average of ten mature leaves, measured from petiole end to apex) |
| Leaf width | Measured in mm (Average of ten mature leaves, measured at the widest part) |
| Leaf petiole length | Measured in mm (Average of ten one-year leaves, measured from the base to the insertion with the blade) |
| Leaf petiole colour | 1 Green; 2 Dark brown; 3 Other |
| Young shoot colour | 1 Green; 2 Dark brown; 3 Other |
| Mature leaf colour | 1 Green; 2 Dark brown; 3 Other |
| Bud wax colour | 1 Yellowish; 2 Other |

**Table 2. Plant morphological descriptors of *Coffea* spp. used for diversity analysis of inflorescence and flower characters**

| Characters | Weightage |
|---|---|
| Number of days from rainfall to flowering | Number of days (d) |
| Inflorescence position | 1 Axillary |
| | 2 Terminal |
| Inflorescence on old wood | 0 Absent<br>1 Present |
| Number of flowers per axil | Measured in numbers (Average of 10 axils, randomly selected from different nodes) |
| Number of flowers per fascicle | Measured in numbers (Average of 10 fascicles, randomly selected from different nodes) |
| Number of fascicles per node | Measured in numbers (Average of 10 nodes, randomly selected from different branches) |
| Inflorescence stalk length | Measured in mm (Average of ten inflorescences, randomly selected from different nodes) |
| Corolla tube length | Measured in mm (Average of ten flowers, randomly selected from different nodes) |
| Number of petals per flower | Average of 10 flowers, randomly selected from different nodes |
| Anther insertion | 1 Excluded<br>2 Included |
| Number of stamens per flower | Average of 10 flowers, randomly selected from different nodes |

## Results and Discussion

Morphological description of cultivated coffee is available; however the characterization of wild *Coffea arabica* accessions in gene banks is far from complete. The germplasm showed a wide range of variations in vegetative, inflorescence and floral characters. Mean performance of morphological characters studied and weightage used for indexing genetic diversity are presented in Tables 3 and 4. Angle of insertion of primaries in S. 2440 and S. 2441 varied from drooping to semi-erect type, while rest of the accessions is with semi-erect type. Stipule arista length was found to be maximum in the germplasm accession S. 2600 (5.3 mm) and the lowest was observed in S. 2708 (3.3 mm). More variation was recorded in young leaf colour. Among the accessions S. 2440, S. 2441, S. 2604, S. 2608, S. 2649, S. 2650 and S. 2708 this varied from greenish to brownish colour; while S. 2600, S. 2601 and S. 2615 manifested brownish colour; whereas S. 2602, S. 2642, S. 2644, S. 2707 and S. 2709 showed only greenish colour. Leaf shape was found to be ovate in all except S. 2644 which was varying from ovate to lanceolate. Highest leaf length was recorded in S. 2644 (153.3 mm) and lowest in S. 2708 (110.5 mm). Leaf width was maximum in S. 2649 (64.3 mm) and minimum in S. 2441 (48.2 mm). Maximum leaf petiole length was recorded in S. 2600 (9.7 mm) and minimum in S. 2604 (5.7 mm). No differences were recorded in plant habit, plant height, overall appearance, vegetative development, branch habit, stipule shape, leaf apex shape, petiole colour; young shoot colour, leaf colour and bud wax colour characters.

Among the vegetative characters, young leaf colour showed the highest range of variation indicating its value in selection exercises. This is closely followed by the angle of insertion of primary branches that has the potential to determine the plant habit that is also a very valuable agronomic trait.

Highest number of flowers per axil was recorded in S. 2649 (9.1) and lowest number in S. 2604 (3.8). Number of flowers per fascicle was found to be maximum in S. 2649 (3.9) and minimum in S. 2604 (2.5). Number of fascicles per node was highest in S. 2615 (4.5) and lowest in S. 2707 (3.0). Inflorescence stalk length was maximum in S. 2615 (6.5 mm) and minimum in S. 2440 (3.8 mm). Maximum length for corolla tube was recorded in S. 2709 (13.6 mm) and lowest in S. 2601 (10.0 mm). Number of petals and stamens per flower was varying from 5 to 6 in all except S. 2644 in which it was only 5. No differences were found in the number of days from rainfall to flowering, inflorescence position, inflorescence on old wood and anther insertion, characters.

Among the reproductive characters, flower number per axil offered the largest variation implying scope for selection. This is a very important agronomic trait that translates into yield.

From the experimental findings it was observed that for the vegetative characters studied all the germplasm were closer to Cioccie, except S. 2642 and S. 2649 that share some characters with Agaro also (Table 5). For five variable inflorescence and floral characters, only germplasm S. 2649 and S. 2708 were closer to Tafarikela

**Table 3. Mean performance of 10 plant characters and weightage on vegetative parts of plants of different accessions of germplasm of coffee on the basis of IPGRI descriptors**

| Germplasm | Plant habit | Plant height | Overall appea-rance | Vegetative develop-ment | Branch habit | Angle of Insertion | Stipule shape | Stipule arista length | Young leaf colour | Leaf shape | Leaf apex shape | Leaf length | Leaf width | Leaf petiole length | Petiole colour | Young shoot colour | Leaf colour (mature) | Bud wax colour |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S. 2440 | 2 | 7 | 1 | 2 | 4 | 3 to 1 | 2 | 5.1 | 1 to 3 | 2 | 4 | 140.2 | 51.9 | 6.4 | 1 | 1 | 1 | 1 |
| S. 2441 | 2 | 7 | 1 | 2 | 4 | 3 to 1 | 2 | 4.4 | 1 to 3 | 2 | 4 | 137.0 | 48.2 | 6.7 | 1 | 1 | 1 | 1 |
| S. 2600 | 2 | 7 | 1 | 2 | 4 | 3 | 2 | 5.3 | 3 | 2 | 4 | 136.4 | 61.4 | 9.7 | 1 | 1 | 1 | 1 |
| S. 2601 | 2 | 7 | 1 | 2 | 4 | 3 | 2 | 4.5 | 3 | 2 | 4 | 128.9 | 55.6 | 7.9 | 1 | 1 | 1 | 1 |
| S. 2602 | 2 | 7 | 1 | 2 | 4 | 3 | 2 | 4.0 | 1 | 2 | 4 | 136.2 | 59.7 | 6.7 | 1 | 1 | 1 | 1 |
| S. 2615 | 2 | 7 | 1 | 2 | 4 | 3 | 2 | 3.4 | 3 | 2 | 4 | 142.3 | 62.2 | 6.5 | 1 | 1 | 1 | 1 |
| S. 2604 | 2 | 7 | 1 | 2 | 4 | 3 | 2 | 4.2 | 1 to 3 | 2 | 4 | 139.5 | 58.4 | 5.7 | 1 | 1 | 1 | 1 |
| S. 2608 | 2 | 7 | 1 | 2 | 4 | 3 | 2 | 4.6 | 1 to 3 | 2 | 4 | 140.2 | 56.8 | 6.1 | 1 | 1 | 1 | 1 |
| S. 2642 | 2 | 7 | 1 | 2 | 4 | 3 | 2 | 4.2 | 1 | 2 | 4 | 147.5 | 56.5 | 6.3 | 1 | 1 | 1 | 1 |
| S. 2644 | 2 | 7 | 1 | 2 | 4 | 3 | 2 | 4.4 | 1 | 2 to 4 | 4 | 153.3 | 56.1 | 5.9 | 1 | 1 | 1 | 1 |
| S. 2649 | 2 | 7 | 1 | 2 | 4 | 3 | 2 | 3.8 | 1 to 3 | 2 | 4 | 150.6 | 64.3 | 7.1 | 1 | 1 | 1 | 1 |
| S. 2650 | 2 | 7 | 1 | 2 | 4 | 3 | 2 | 4.4 | 1 to 3 | 2 | 4 | 144.0 | 61.1 | 9.0 | 1 | 1 | 1 | 1 |
| S. 2707 | 2 | 7 | 1 | 2 | 4 | 3 | 2 | 4.3 | 1 | 2 | 4 | 124.0 | 48.4 | 8.1 | 1 | 1 | 1 | 1 |
| S. 2709 | 2 | 7 | 1 | 2 | 4 | 3 | 2 | 4.7 | 1 | 2 | 4 | 138.7 | 56.2 | 9.0 | 1 | 1 | 1 | 1 |
| S. 2708 | 2 | 7 | 1 | 2 | 4 | 3 | 2 | 3.3 | 1 to 3 | 2 | 4 | 110.5 | 51.7 | 6.2 | 1 | 1 | 1 | 1 |
| Agaro | 2 | 7 | 1 | 2 | 4 | 3 | 3 | 4.0 | 2 | 4 | 4 | 160.0 | 72.5 | 8.5 | 1 | 1 | 1 | 1 |
| Cioccie | 2 | 7 | 1 | 2 | 4 | 2 to 1 | 3 | 4.5 | 2 | 4 | 4 | 142.5 | 59.5 | 7.5 | 1 | 1 | 1 | 1 |
| Tafarikela | 2 | 7 | 1 | 2 | 4 | 3 to 1 | 3 | 4.5 | 2 | 4 | 4 | 145.0 | 64.0 | 8.5 | 1 | 1 | 1 | 1 |
| Standard deviation | | | | | | | | 0.53 | | | | 11.08 | 6.00 | 1.23 | | | | |

**Table 4. Mean performance of 10 flower characters and weightage on flower parts of different accessions of germplasm of coffee on the basis of IPGRI descriptors**

| Germplasm | Number of days from rainfall to flowering | Inflorescence position | Inflorescence on old wood | Number of flowers per axil | Number of flowers per fascicle | Number of fascicles per node | Inflorescence stalk length | Corolla tube length | Number of petals per flower | Anther insertion | Number of stamens per flower |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S. 2440 | 8 | 1 | 1 | 6.8 | 3.5 | 3.9 | 3.8 | 12.0 | 5 to 6 | 1 | 5 to 6 |
| S. 2441 | 8 | 1 | 1 | 5.2 | 3.0 | 3.9 | 4.8 | 11.6 | 5 to 6 | 1 | 5 to 6 |
| S. 2600 | 8 | 1 | 1 | 6.9 | 3.2 | 4.2 | 5.6 | 11.4 | 5 to 6 | 1 | 5 to 6 |
| S. 2601 | 8 | 1 | 1 | 4.6 | 2.8 | 3.7 | 5.0 | 10.0 | 5 to 6 | 1 | 5 to 6 |
| S. 2602 | 8 | 1 | 1 | 7.4 | 3.3 | 3.8 | 5.7 | 11.8 | 5 to 6 | 1 | 5 to 6 |
| S. 2615 | 8 | 1 | 1 | 8.1 | 3.1 | 4.5 | 6.5 | 11.2 | 4 to 6 | 1 | 4 to 6 |
| S. 2604 | 8 | 1 | 1 | 3.8 | 2.5 | 3.2 | 5.3 | 11.1 | 5 to 6 | 1 | 5 to 6 |
| S. 2608 | 8 | 1 | 1 | 5.9 | 3.5 | 3.9 | 5.4 | 12.8 | 5 to 6 | 1 | 5 to 6 |
| S. 2642 | 8 | 1 | 1 | 6.8 | 3.6 | 3.6 | 5.5 | 12.7 | 5 to 6 | 1 | 5 to 6 |
| S. 2644 | 8 | 1 | 1 | 9.1 | 3.8 | 4.0 | 6.1 | 12.3 | 5 | 1 | 5 |
| S. 2649 | 8 | 1 | 1 | 6.9 | 3.9 | 3.2 | 6.0 | 11.3 | 5 to 6 | 1 | 5 to 6 |
| S. 2650 | 8 | 1 | 1 | 6.6 | 3.0 | 3.6 | 5.9 | 13.5 | 5 to 6 | 1 | 5 to 6 |
| S. 2707 | 8 | 1 | 1 | 7.1 | 3.3 | 3.6 | 6.0 | 13.0 | 5 to 6 | 1 | 5 to 6 |
| S. 2709 | 8 | 1 | 1 | 7.6 | 3.4 | 3.6 | 5.8 | 13.6 | 5 to 6 | 1 | 5 to 6 |
| S. 2708 | 8 | 1 | 1 | 5.9 | 3.1 | 3.0 | 6.2 | 10.6 | 5 to 6 | 1 | 5 to 6 |
| Agaro | 8 | 1 | 1 | 5.5 | 2.8 | 3.8 | 3.5 | 8.2 | 5 | 1 | 5 |
| Cioccie | 8 | 1 | 1 | 4.3 | 2.0 | 4.1 | 3.6 | 6.5 | 5 | 1 | 5 |
| Tafarikela | 8 | 1 | 1 | 4.5 | 3.7 | 3.2 | 3.4 | 10.7 | 5 | 1 | 5 |
| Standard deviation | | | | 1.42 | 0.48 | 0.39 | 1.00 | 1.79 | | | |

and rest of the studied germplasm shared characters with all three standards, e.g. S. 2440, S. 2604, S. 2608 and S. 2644 have more of the characters of Cioccie and Tafarikela; S. 2441, S. 2600, S. 2615 and S. 2642 have more of the characters of Agaro and Cioccie; S. 2601, S. 2602, S. 2650, S. 2707 and S. 2709 shared more of the characters with Agaro and Tafarikela (Table 6).

All consistently observed characters are possible manifestations of adaptation that has a genetic basis. Many recent studies of interspecific/intraspecific interactions have emphasized variations in traits and interaction outcomes within populations, among populations, and across geographic ranges because such variation contributes to the evolution and co-evolution of these interactions that lead to the manifested adaptation (Thompson, 1988). The characters like plant habit, height, phyllotaxy, angle of insertion and leaf shape is an adaptation for good realization of sunlight, which influences higher rate of photosynthesis and other biosynthetic processes. Petiole colour, young shoot colour and leaf colour are probably an adaptation to protect against insect damage and pathogens. Characters like stipule shape bud wax colour are of taxonomic value and the characters like number of days from rainfall to flowering, inflorescence position, inflorescence on old wood, number of flower per axil, per fascicle, inflorescence stalk length, and corolla tube length are of agronomic value. Sylvain (1958) states that the variability of Ethiopian germplasm of coffee is an indicator of its richness and the existing genetic diversity is greater than that indicated only by morphological differences. Recent studies on molecular markers confirmed this (Lashermes *et al.*, 1995, 1996a,b, 1999; Anthony *et al.*, 2001). Genetic basis of the above discussed characters can be

**Table 5. Comparison of wild *Coffea arabica* germplasm with the standard types for five vegetative characters**

| Germplasm | Angle of Insertion | Stipule arista length | Leaf length | Leaf width | Leaf petiole length | No. of characters resembling | | | Germplasm closer to standard (T) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Agaro | Cioccie (A) | Tafarikela (C) | |
| S. 2440 | T | T | C | C | C | nil | 3 | 2 | Cioccie |
| S. 2441 | T | C | C | C | C | nil | 4 | 1 | Cioccie |
| S. 2600 | A | T | C | C | A/T | 1 | 2 | 1 | Cioccie |
| S. 2601 | A | C | C | C | C | 1 | 4 | nil | Cioccie |
| S. 2602 | A | A | C | C | C | 2 | 3 | nil | Cioccie |
| S. 2615 | A | A | C | C | C | 2 | 3 | nil | Cioccie |
| S. 2604 | A | A | C | C | C | 2 | 3 | nil | Cioccie |
| S. 2608 | A | C | C | C | C | 1 | 4 | nil | Cioccie |
| S. 2642 | A | A | T | C | C | 2 | 2 | 1 | Agaro / Cioccie |
| S. 2644 | A | C | A | C | C | 2 | 3 | nil | Cioccie |
| S. 2649 | A | A | T | T | C | 2 | 1 | 2 | Agaro/Tafarikela |
| S. 2650 | A | C | T | C | A/T | 1 | 2 | 1 | Cioccie |
| S. 2707 | A | C | C | C | A/T | 1 | 3 | nil | Cioccie |
| S. 2709 | A | C | C | C | A/T | 1 | 3 | nil | Cioccie |
| S. 2708 | A | A | C | C | C | 2 | 3 | nil | Cioccie |

**Table 6. Comparison of wild *Coffea arabica* germplasm with the standard types for five inflorescence and floral characters**

| Germplasm | Number of flowers per axil | Number of flowers per fascicle | Number of fascicles per node | Inflorescence stalk length | Corolla tube length | No. of characters resembling | | | Germplasm closer to standard |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Agaro | Cioccie | Tafarikela | |
| S. 2440 | A | T | C | C | T | 1 | 2 | 2 | Cioccie / Tafarikela |
| S. 2441 | A | A | C | C | T | 2 | 2 | 1 | Agaro / Cioccie |
| S. 2600 | A | A | C | C | T | 2 | 2 | 1 | Agaro / Cioccie |
| S. 2601 | T | A | A | C | T | 2 | 1 | 2 | Agaro / Tafarikela |
| S. 2602 | A | T | A | C | T | 2 | 1 | 2 | Agaro / Tafarikela |
| S. 2615 | A | A | C | C | T | 2 | 2 | 1 | Agaro / Cioccie |
| S. 2604 | C | A | T | C | T | 1 | 2 | 2 | Cioccie / Tafarikela |
| S. 2608 | A | T | C | C | T | 1 | 2 | 2 | Cioccie / Tafarikela |
| S. 2642 | A | T | A | C | T | 2 | 1 | 2 | Agaro / Tafarikela |
| S. 2644 | A | T | C | C | T | 1 | 2 | 2 | Cioccie / Tafarikela |
| S. 2649 | A | T | T | C | T | 1 | 1 | 3 | Tafarikela |
| S. 2650 | A | T | A | C | T | 2 | 1 | 2 | Agaro / Tafarikela |
| S. 2707 | A | T | A | C | T | 2 | 1 | 2 | Agaro / Tafarikela |
| S. 2709 | A | T | A | C | T | 2 | 1 | 2 | Agaro / Tafarikela |
| S. 2708 | A | T | T | C | T | 1 | 1 | 3 | Tafarikela |

substantiated and validated by research on biochemical and molecular aspects that is presently undertaken.

## Conclusion

Vegetative characters of the studied genetic resources are nearer to Cioccie type and for inflorescence and floral characters most of the genetic resources are in an intermediate condition between Cioccie and Tafarikela; Agaro and Cioccie; Agaro and Tafarikela. Natural out crossing and autogamous nature of wild arabica germplasm might have led the transfer and fixation of genes conditioning these character complexes in the wild populations in the course of their evolution. Subsequent selection of types for cultivation from the wild types fixed the characters in Cioccie, Agaro and Tafarikela that are included as standards in the present study. Such germplasm, thus, can be considered for utilization in further breeding programme and should be conserved in the gene bank.

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# Classification for Core Collection of Rapeseed-Mustard (*Brassica juncea* L.) Germplasm

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Based on passport data of maturity (early, medium and late), stratified random sampling procedure was adopted to select 385 accessions of Indian mustard (*Brassica juncea* L.) maintained at NRCRM, Bharatpur, India, which was taken for this study. Observations of 17 characters were taken into consideration for the classification. All the accessions were classified in 9 clusters. Core collections of different cluster were selected with the help of following method, which is the main objective of the project. Mean ± SD of each character of different clusters were calculated and taken into consideration for selecting the core accessions of each cluster. All the characters in different clusters were studied and the character that has more than Mean ± SD was marked rank first. All the accession that ranked first in a minimum of four characters, were selected as core collection of that group. This way, out of 385 accessions only 116 accessions were selected as core collection, which is 30.13%. Now, it will be easy to maintain and easier for the breeder to select for breeding purposes. Viewing the Euclidean square distance, different accession of *Brassica* can be selected for breeding purposes as per the requirements of the breeder.

**Key Words: Rapeseed-Mustard, Agro-morphological characters, Variability, Cluster analysis, Core collection, Euclidean square distance, DAS (days after sowing)**

## Introduction

Among the oilseeds, *Brassica* group of crop is a highly diverse group of crop with great economic value. After groundnut oilseed *Brassica* are the second most important source of edible oil production in India. Productivity of *Brassica* in our country has not increased despite selections and intensive breeding programme, reason being most of the parents selected in hybridization programme were selected for seed yield, which is almost homogeneous in nature. Hence, pedigree method could not provide the desirable improvements in the yield. The selection of pedigree by the breeder from the huge germplasm for hybridization programme depends mainly on specific character or yield, which is not the proper way of selection by which we are lagging in improving the productivity in comparison to world scenario. Maintenance and selection of pedigree from the huge *Brassica juncea* L. germplasm, which is maintained at National Research Center on Rapeseed-Mustard, Bharatpur, India, is not only a cumbersome task but also quite difficult for the breeder to select the pedigree for breeding programme. Hence, this study was carried out in *Brassica* germplasm, considering principal component analysis as a tool, in which all the 17 characters were considered together to calculate principal component score to classify the entire population into different groups. A methodology was given to select core collection which have distinctly variable, agro-morphological characters useful for the breeder for selecting them for their breeding programme to attain maximum productivity, viewing the crop productivity level in rest of the world (Mishra *et al.*, 1991).

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## Materials and Methods

Based on passport data of maturity (early, medium and late), stratified random sampling procedure was adopted to select three hundred eighty three germplasm accessions of *Brassica juncea* L. along with two check varieties, Bio-902 and PCR-7, were grown for characterization, under irrigated condition in augmented design at National Research Centre on Rapeseed-Mustard, Sewar Bharatpur (India) during *rabi* 2002-03-04. Each accession was grown in two-row; a plot of three-meter length and plant and row distance was maintained at the spacing of 30 cm×10 cm, respectively. Standard agronomic and plant protection measures were followed to raise a healthy crop.

The observations were recorded on randomly tagged five plants for different traits such as initiation of flowering (DAS); 50% flowering (DAS); maturity duration (days); plants height (cm); primary branches per plant (no); secondary branches per plant (no); main shoot length (cm); siliquae on main shoot (no); siliqua length (cm); siliqua beak length (cm); seed per siliqua (no); total yield (g); seed yield per plant (g); 1000-seed weight (g); harvest index (%); oil content (%) and protein content (%) at appropriate growth stages of the crop. 1000-seed was counted by the electronic seed counter (the old mill

company, USA) and weighed by electronic balance. Further, oil and protein content were analyzed by NIR (Dickey-Johan, USA) method. All the observed data were tabulated in Microsoft Excel programme and considered for cluster analysis.

**Results and Discussion**

A total of 385 accessions of Indian mustard germplasm including two check varieties of Bio-902 and PCR-7 were taken for the study. The 17 characters, which are presented in Table 1, along with Characters' mean, C.V. and Percentage contribution to divergence were considered to be the base for the classification. Using Principal component analysis 385 accessions were classified into 9 groups/clusters. These 9 groups were taken, considering F-Statistics (Rao, 1952), in the process of statistical analysis, which is higher in the case of cluster # 9. The analysis revealed that the most variable cluster is cluster # 8 followed by cluster # 4, cluster # 6, cluster # 7, cluster # 5, cluster # 9 etc viewing within mean sum of square (Table 2). It also revealed that most variable character are Seed weight/plant (CV 38.65) followed by Secondary branches (CV 36.04), Total weight (CV 32.46), Siliqua beak length (CV 21.27), Seed/Siliqua (CV 21.16) etc. (Table 1). Arranging percentage contribution of the characters to genetic divergence in descending order (Table 1) it reveals, 10 characters namely, Siliqua beak length (SBL); Siliqua length (SL); Seed/siliqua (SS); Day to maturity (DM); Protein content (PC); 1000-seed weight (1000WS); Initial flowering (IF); Harvest index (HI); Plant height (PH); Secondary branches (SB) explained 67.54%, where as 5 principal component explained 65.06% and 10 principal component explained 88.07% towards genetic divergence (Hepziba *et al.*, 1995; Mishra *et al.*, 1998). Hence, classification done through principal component analysis is justified. Initial flowering is highly correlated with 50% flowering (r=0.859) followed by seed weight/plant, total weight (r=0.811); secondary branches, primary branches (r=0.652); total weight, secondary branches (r=0.548); plant height, 50% flowering (r=0.518); harvest index, seed weight/plant (r=0.484); siliqua on main shoot, plant height (r=0.482); day to maturity, plant height (r=0.445); protein content, 50% flowering (r=0444); day to maturity, 50% flowering (r=0.434); primary branches, plant height (r=0.431); total weight, primary branches (r=0.430) etc. (Table 4). Considering Table 4, description of quantitative characters for each cluster were classified as, >Over all mean + Standard deviation = VL; > Over all mean = L; < Over all mean = M; < Over all mean – Standard deviation = S (Table 5). Descriptions of quantitative characters (Table 5) were tested with Chi-square. Calculated Chi-square = 22.11. Tabulated Chi-square value for 8 d.f. at 1% level = 20.09 (Mishra *et al.*, 1998).

Hence, Chi-square is significant at 1% level, which justifies the classification of accession in 9 clusters with different characteristics.

**Table 1. Characters, their means, coefficient of variance (CV) and percentage contribution to divergence**

| S.No. | Character Name | Mean | CV | Percentage contribution to divergence |
|---|---|---|---|---|
| 1. | Initial flowering (IF) | 52.89 | 17.01 | 6.55 |
| 2. | 50% flowering (50%F) | 65.12 | 18.79 | 4.50 |
| 3. | Plant height (PH) | 148.00 | 13.65 | 6.11 |
| 4. | Primary branches (PB) | 6.11 | 17.96 | 5.20 |
| 5. | Secondary branches (SB) | 9.67 | 36.04 | 5.29 |
| 6. | Main shoot length (MSL) | 49.49 | 15.38 | 5.07 |
| 7. | Day to maturity (DM) | 141.93 | 2.67 | 6.99 |
| 8. | Siliqua beak length (SBL) | 0.72 | 21.27 | 8.15 |
| 9. | Siliqua length (SL) | 3.60 | 13.16 | 7.51 |
| 10 | Siliqua on main shoot (SMS) | 36.48 | 16.22 | 5.23 |
| 11. | Seed weight/plant (SW/P) | 7.50 | 38.65 | 4.96 |
| 12. | Total weight (TW) | 33.61 | 32.46 | 4.55 |
| 13. | 1000 seed weight (1000SW) | 3.74 | 19.54 | 6.58 |
| 14. | Harvest index (HI) | 22.42 | 21.15 | 6.43 |
| 15. | Seed/siliqua (SS) | 14.09 | 21.16 | 7.16 |
| 16. | Protein content (PC) | 22.16 | 3.23 | 6.77 |
| 17. | Oil content (OC) | 39.25 | 5.01 | 295 |

**Table: 2. Classified #9 clusters**

| Cluster | N | Within MSS | Cluster members |
|---|---|---|---|
| 1 | 52 | 9.31 | UPII-81, UPII-132, HP-1, UPI-82, UPII-79, UPII-100, P-32, UPII-87, UPII-33, NCRQR-6-91, UPII-56, UPII-58, KLM 227, UPII-86, RK-2-4, RN 573, P-98, JGM 9005, P-15, KLM-12A, P-72, UPII-80, P-44, P-11, UPII-14,P-50,UPII-9,UPII-123,P-69,UPII-35,NDR200202,UPII-60,UPII-84, UPII-22, UPII-110, UPII-10, P-80, UPI-74, P-48,UPII-78, UPII-76, UPII-44, P-68, P-71, P-58, P-65, P-20, P-45, P-24, JGM-01-04, P-74, P-23. |
| 2 | 59 | 8.56 | NPJ90, UPII-25, UPII-124, UPII-37, UPII-51, UPI-55, UPII-47, UPI-29, UPII-92, UPI-39, UPII-105, UPI-22, UPI-38, RH-114, TKG-18-64, UPII-29, UPII-48, UPI-78, P-61, UPI-69, UPII-125, RM11, UPI-53, P-25, UPI-72, PR 2001-87, UPII-46, RK02-5, UPII-22, UPII-122, UPI-77, UPI-31, UPII-61, P-33, RH115, P-37, RH118, P-92, PRQ2005-1, NDRE200216, UPII-118, TKG-180-9, UPI-24, 330RKP-2, UPI-51, UPII-26, UPI-76, UPI-63, UP-I-68(BJ), UPI-73UPI-2, UPI-36, NPJ-82, RK-01-3, RGN-74, UPII-50, UPI-14, SKM-158, UPI-26. |
| 3 | 70 | 7.41 | UPII-40, P-28, UPII-54, UPI-90, UPII-39, PR-2001-84, P-63, PR-2001,UPI-70, P-93, UPII-127, UPII-93, UPII-53, HUJM-01-02, UPI-44, UPI-71, UPII-128, UPI-23, UPII-64, UPI-52, UPI-50, UPI-43, UPI-56, UPII-112, SKM-149, UPII-94, UPII02, JGM2-1, UPII-69, P-42, UPII-75, RK-02-6, UPI-42, RS-2, RH-27, UPII-8,UPI-67,YET22,5084,SKM-9927, UPII-90,UPI-48, UPI-42, UPII-96, P-30, RM-51, UPII-1, UPII-49, UPI-6, UPII-52,UPI-20, UPI-75,NUDHYS-2,UPI-28,UPI-41, UPI-45, UPII-97, P-6, UPII-3, UPI-85, UPII-82, UPII-12, P-60, UPI-81, NDR200203, RK02-2, PR404, SKM2026, UPII-91, UPI-19, |
| 4 | 2 | 13.70 | RL9927, UPI-61. |
| 5 | 46 | 10.30 | UPII-38, UPII-41,P-62, UPII-101, UPII-99, UPII-77,UPII-95, UPII-88, UPII-34,RGN-79, UPII-62, JMWR-2-40,UPII-28, Bio-Q-108, UPII-65, RGN-89,UPII-130,UPII-129, UPII-13,UPII-68,UPII-45,UPII-15,UPII-36, P-77, UPII-103, UPII-120, UPII-20, UPI-62, UPII-57, UPII-19, P-53, UPII-43, P-56, P-10, P-103, P-95, NUDH-YJ-1, UPII-73, P-8, UPII-18, RH(OE)-0103, P-106, P-16, P-19, P-82, UPII-71. |
| 6 | 51 | 10.63 | UPII-85,NDRS-2002,UPII-6,UPII-131,P-31,HUJM-101,P-51,RK02-1, **PCR7,**HUJM105,UPI-1,PR2005,UPI-9UPII-89,P-29,RN510,P-52, DHR9701,JMM927,UPI-57,RM-49,UPI-12,RK-02-3,UPI-49,P-35, UPI-13,UPI-60,UPII-121,RH9615,NDRS2001,UPI-5,YET-17, NDRS-2003,UPI-3,YRN-6,ORM(M)-25-1,UPI-46,UPI-85,UPI-11, PR2001-42, P-38, SKM9816, UPI-89, UPII-4,UPII-115,SAL-9,RH-116,UPI-4,HP-2,NPJ90,UPI-21. |
| 7 | 33 | 10.58 | RK-2-4, UPI-66, RH(OE)1-1, RH-28, RK2002, UPI-54, UPI-30, SKM9819, UPI-37, UPII-98, NDR-8209, UPII-111, 614-41-4, UPI-34, HUJM-106, 611-1-3-6, P-40, RS-7, UPI-125, RGN-55, UPI-7,UPI-8, UPI-40, UPI-10, RP-2006, PR-2001-78, P-36, UPI-16, RH-0117, **Bio902**, PR2001-65, JMWR-13. |
| 8 | 31 | 14.60 | UPII-83, UPII-24, P-9, UPII-30, UPII-17, P-26, P-22, P-105, P-99, P-104, P-47, P-7, P-84, P-1, UPII-16, P-102, P-18, P-78, P-83, P-107, P-41, P-13, P-96, P-57, P-5, P-4, P-49, P-14, P-66, P-54, P-79. |
| 9 | 41 | 9.74 | UPII-67, UPII-63, UPII-66, UPII-42, UPII-32, RN554, P-12, P-27, UPII-126, UPI-58, UPI-33, UPI-33S, SKM9827, UPII-27,P-44, UP11-5, UPI-59 UPII-119UPII-31, NPJ-92, UPI-86, BAUSMM-11, UPII-110, Krishna-2-1, UPI-88, Bio-772R, PRO-2103, UPI-35, UPI-17, Bio-36-99, UPII-23, UPII-11, SALE3, UPI-79, RW-02-01UPI-18, UPI-27, UPI-15, Krishna Shorty, UPI-32, NDRE-200201,RNT-1. |

**Table 3. Correlations of different characters**

| Character | IF | 50%F | PH | PB | SB | MSL | DM | SBL | SL | SMS | SW/P | TW | 1000SW | HI | SS | PC | OC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IF | | 0.859 | 0.427 | 0.279 | 0.239 | -0.398 | 0.384 | -0.144 | -0.105 | 0.138 | -0.101 | 0.023 | -0.310 | -0.224 | 0.097 | 0.386 | 0.206 |
| 50%F | | | 0.518 | 0.399 | 0.315 | -0.442 | 0.434 | -0.174 | -0.178 | 0.168 | -0.085 | 0.076 | -0.350 | -0.284 | 0.101 | 0.444 | 0.280 |
| PH | | | | 0.431 | 0.220 | -0.040 | 0.445 | -0.081 | -0.024 | 0.482 | 0.249 | 0.426 | -0.179 | -0.232 | 0.074 | 0.206 | 0.332 |
| PB | | | | | 0.652 | -0.312 | 0.114 | -0.182 | -0.233 | 0.153 | 0.222 | 0.430 | -0.302 | -0.281 | -0.017 | 0.264 | 0.117 |
| SB | | | | | | -0.030 | 0.027 | -0.174 | -0.179 | 0.287 | 0.352 | 0.548 | -0.250 | -0.214 | 0.065 | 0.174 | 0.079 |
| MSL | | | | | | | -0.103 | 0.173 | 0.191 | 0.397 | 0.342 | 0.283 | 0.360 | 0.177 | -0.032 | -0.403 | -0.007 |
| DM | | | | | | | | -0.075 | -0.001 | 0.155 | 0.048 | 0.122 | -0.017 | -0.110 | 0.038 | 0.062 | 0.220 |
| SBL | | | | | | | | | 0.358 | -0.067 | 0.085 | 0.026 | 0.187 | 0.140 | 0.006 | -0.186 | -0.128 |
| SL | | | | | | | | | | -0.003 | 0.076 | 0.035 | 0.215 | 0.075 | 0.158 | -0.165 | 0.032 |
| SMS | | | | | | | | | | | 0.269 | 0.361 | -0.086 | -0.091 | 0.083 | 0.034 | 0.205 |
| SW/P | | | | | | | | | | | | 0.811 | 0.223 | 0.484 | 0.040 | -0.186 | 0.056 |
| TW | | | | | | | | | | | | | 0.087 | -0.083 | 0.013 | -0.073 | 0.088 |
| 1000SW | | | | | | | | | | | | | | 0.274 | 0.018 | -0.390 | -0.056 |
| HI | | | | | | | | | | | | | | | 0.037 | -0.212 | -0.048 |
| SS | | | | | | | | | | | | | | | | 0.017 | 0.207 |
| PC | | | | | | | | | | | | | | | | | -0.002 |
| OC | | | | | | | | | | | | | | | | | |

**Table 4. Cluster wise mean of different characters along with over all mean of all the characters**

| Cluster name | IF | 50%F | PH | PB | SB | MSL | DM | SBL | SL | SMS | SW/P | TW | 1000SW | HI | SS | PC | OC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 57.06 | 76.37 | 165.27 | 7.13 | 12.45 | 45.39 | 144.31 | 0.67 | 3.41 | 38.25 | 9.02 | 39.92 | 3.38 | 22.84 | 15.16 | 22.52 | 40.43 |
| 2 | 48.76 | 58.97 | 144.12 | 6.28 | 10.74 | 52.69 | 138.58 | 0.79 | 3.85 | 36.96 | 7.88 | 37.20 | 3.69 | 21.23 | 14.08 | 22.04 | 38.82 |
| 3 | 48.26 | 57.71 | 138.20 | 5.57 | 8.33 | 53.74 | 141.96 | 0.66 | 3.38 | 35.95 | 7.09 | 29.25 | 4.01 | 24.31 | 12.39 | 21.80 | 38.89 |
| 4 | 53.00 | 61.00 | 151.90 | 6.60 | 9.90 | 48.60 | 146.00 | 0.73 | 3.41 | 36.20 | 8.00 | 36.50 | 3.62 | 21.71 | 10.78 | 21.63 | 20.71 |
| 5 | 54.74 | 70.74 | 157.71 | 6.48 | 8.75 | 44.20 | 142.67 | 0.63 | 3.33 | 37.17 | 4.93 | 28.13 | 3.20 | 17.81 | 14.03 | 22.69 | 39.80 |
| 6 | 50.12 | 60.08 | 147.21 | 5.35 | 7.06 | 53.18 | 143.41 | 0.86 | 4.09 | 35.63 | 7.29 | 30.22 | 4.50 | 24.23 | 15.70 | 21.65 | 39.82 |
| 7 | 50.64 | 61.55 | 160.73 | 6.41 | 11.46 | 57.07 | 143.09 | 0.74 | 3.61 | 41.96 | 13.42 | 54.47 | 4.08 | 24.95 | 14.07 | 21.74 | 39.33 |
| 8 | 75.52 | 91.10 | 165.85 | 6.98 | 12.90 | 41.51 | 144.81 | 0.67 | 3.51 | 38.42 | 5.84 | 32.77 | 3.05 | 18.42 | 14.29 | 22.96 | 40.04 |
| 9 | 47.51 | 56.00 | 115.24 | 5.18 | 7.15 | 44.09 | 137.73 | 0.74 | 3.59 | 28.99 | 5.27 | 21.96 | 3.67 | 24.18 | 13.81 | 22.27 | 38.01 |
| Over all mean | 52.89 | 65.12 | 148.00 | 6.11 | 9.67 | 49.49 | 141.93 | 0.72 | 3.60 | 36.48 | 7.50 | 33.61 | 3.74 | 22.42 | 14.09 | 22.16 | 39.25 |

**Table 5. Description of quantitative characters for each cluster**

| Cluster name | IF | 50%F | PH | PB | SB | MSL | DM | SBL | SL | SMS | SW/P | TW | 1000SW | HI | SS | PC | OC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | L | L | L | L | L | M | L | M | M | L | L | L | M | L | L | L | L |
| 2 | M | M | M | L | L | L | M | L | L | L | L | L | M | M | M | M | M |
| 3 | M | M | M | M | M | L | L | M | M | M | M | M | L | L | M | M | M |
| 4 | L | M | L | L | L | M | VL | L | M | M | L | L | M | M | S | M | S |
| 5 | L | L | L | L | M | M | L | M | M | L | M | M | M | M | M | L | L |
| 6 | M | M | M | M | M | L | L | L | VL | M | M | M | VL | L | L | M | L |
| 7 | M | M | L | L | L | L | L | L | L | L | VL | VL | L | L | M | M | L |
| 8 | VL | VL | L | L | L | S | L | M | M | L | M | M | M | M | L | L | L |
| 9 | M | M | S | M | M | M | S | L | M | S | M | S | M | L | M | L | M |

> Over all mean =L; < Over all mean = M; <Overall mean - SD = S; > Over all mean + SD = VL

**(a) *Characteristics of different clusters***

Cluster # 1 has maximum plant height, a high total plant weight and has the highest oil content amongst all clusters but has a low harvest index. Cluster # 2 is early maturing and is comparable to cluster # 1 in oil content but again has a low harvest index. Cluster # 3 is similar to cluster # 2 but has a lower total weight but an improved harvest index. Cluster # 4 has the lowest oil content while cluster # 5 has the lowest harvest index. Cluster # 6 & cluster # 8 are much better than the above-mentioned clusters but have greater plant height and a high total plant weight. Cluster # 7 has the maximum total plant weight and rest of the traits comparable to cluster # 6 and 8. Cluster # 9 is the best cluster when all traits are compared as it has the lowest plant height, earliest maturing, has lowest total plant weight, high harvest index and high oil content (Mishra *et al.*, 1991).

**(b) *Methodology to select core collections from different classified groups***

Core accessions of different clusters were selected with the following description. Mean ± SD of each character of different clusters were calculated and taken into consideration for selecting the core accessions of each cluster. All the characters in different cluster were studied and the character, which is more than Mean ± SD were marked rank first. All the accessions that ranked first in a minimum of four characters were selected as core accession. This way, out of 385 accessions only 116 accessions were selected as core one, which is 30.13 % (Table 6) (Mishra *et al.*, 1991). Selection of core collection of the germplasm was adopted with the help of discriminant function analysis as well as with the help of Mean and S.D. It was observed that core collection selected in either of the above methods is identical. Hence, in present study Mean and S.D. were considered appropriate to select representative/core accession to maintain the germplasm, because discriminant function analysis was little difficult and need specific software for the purpose. In the present study 30.13% accessions of germplasm were selected as core collection to retain the same correlation structure of the characters as found in the base collections. In the present study, when only 10 % optimum sample size was considered to retain as core collection, it vacillated correlation structure between the characters of core and base collections.

**Table 6. Representative varieties of different clusters along with (%) of total no. of Accessions in each cluster**

| Cluster no. | Total no. of accessions in a cluster | Representative varieties | Total no. of representative accessions | (%) of total no. of accessions in a cluster |
|---|---|---|---|---|
| 1 | 52 | UP II-100, P-15, P-72, UP II-14, UP II-9, UP II-84, UP II-10, UP I-74, P-48, P-68, P-71, P-65, P-20, JGM-01-04, P-74, P-23 | 16 | 30.77 |
| 2 | 59 | RNS-72, UP II-92, UP II-105, UP I-69, UP II-21, P-II-12 UP I-31, RH 115, P-37, RH 118, UP I-24, 330-2KP 2, UP-I-68 (BJ), UP I-73, NPJ-82, RK-01-3, RGN-74 | 17 | 28.81 |
| 3 | 70 | UP II-39, P-63, PR-2001, UP I-70, UP II-93, HUJM-01-02, UP I-71, UP I-23, UP I-50, P-42, RH-27, 508 4, SKM-9927, UP I-48, UP I-42, RM-51, UP II-49, UP I-20, UP I-28, UP II-3, UP II-118, P-60, UP I-81, UP II-91 | 24 | 34.29 |
| 4 | 2 | Nil | Nil | Nil |
| 5 | 46 | UP II-38, P-62, UP II-99, UP II-95, UP II-88, RGN-79, RGN-89, UP II-129, UP II-20, P-53, UP II-43, P-56, P-10, NUDH-YJ-I, UP II-18, P-106, P-16, P-82 | 18 | 39.13 |
| 6 | 51 | UP II-85, UP II-131, HUJM-101, PR-2005, UP II-89, RN 510, DHR-9701, UP I-13, UP II-121, RH 9615, NDRS-2001, YET 17, UP I-3, YRN-6, UP-I-11, P-38, SKM-9816, HP-2 | 18 | 35.29 |
| 7 | 33 | NDR-8209, UP I-34, RGN 55, UP I-8, P-36, RH –0117, Bio-169-96, Bio 902, PR 2001-65, JMWR-13 | 10 | 30.30 |
| 8 | 31 | P-99, UP II-16, P-102, P-107, P-41, P-57, P-49, P-54 | 8 | 25.81 |
| 9 | 41 | UP II-67, UP II-31, Bio -772 R, UP I-27, RNT-1 | 5 | 12.20 |
| Over all | 385 | | 116 | 30.13 |

### (c) *Method for selection of accession for breeding programme*

Germplasm accession between the groups from core collection can be selected for hybridization programme, considering the Euclidean square distance (Table 7). It reveals that breeding can be performed after selecting the specific characters suitable to the breeder as per requirement of the breeding programme from the clusters which have maximum Euclidean square distance such as cluster # 9 and # 6 having maximum Euclidean square distance (122.84) followed by cluster # 9 and # 3 (117.41); # 9 and #1 (114.38); # 9 and # 7(113.43); # 9 and # 8 (107.91); # 9 and #4 (104.03); #9 and # 2(88.72); # 9 and #5 (82.78); # 8 and #2 (40.76); # 2 and # 1 (38.09); # 8 and # 3 (34.64), respectively (Mishra *et al.*, 1998; Reddy *et al.*, 2003).

### (d) *Future aspects for improving production and productivity to sustain yield*

In all the clusters except in cluster # 9 the photosynthates that are being produced by the green parts of the plant are being mobilized to different plant sinks where they are being utilized to increase plant height and total plant weight. But the harvestable sink (seeds) remains deprived of those essential photosynthates, which form the major constituents that sustain the quality and yield of the crop.

Hence, there is a need that all the five representative varieties of cluster # 9 be screened thoroughly for those genes that are responsible to block the unnecessary translocation of the valuable photosynthates to the non harvestable sinks, so that they can be partitioned unidirectionally towards the harvestable seeds and thus, improve the harvest index and the oil content .

**Table 7. Euclidean square distance matrix for 9 clusters**

| Clusters | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 38.09 | 28.32 | 20.71 | 22.39 | 12.71 | 22.30 | 10.40 | 114.38 |
| 2 | | 0.00 | 11.65 | 20.10 | 11.39 | 29.64 | 14.98 | 40.76 | 88.72 |
| 3 | | | 0.00 | 12.42 | 10.40 | 15.74 | 8.61 | 34.64 | 117.41 |
| 4 | | | | 0.00 | 4.61 | 10.85 | 7.63 | 20.55 | 104.03 |
| 5 | | | | | 0.00 | 13.33 | 8.71 | 18.11 | 82.78 |
| 6 | | | | | | 0.00 | 10.54 | 16.98 | 122.84 |
| 7 | | | | | | | 0.00 | 24.37 | 113.43 |
| 8 | | | | | | | | 0.00 | 107.91 |
| 9 | | | | | | | | | 0.00 |

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# Evaluation of Exotic Germplasm of Kabuli Chickpea

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The objective of the present study was to identify high yielding, large (>40g/100 seeds) and extra-large (>50g/100 seeds) germplasm accessions of Kabuli chickpea with resistance/tolerance against Fusarium wilt. Consequently, during *rabi* 2002-03,150 exotic germplasm accessions belonging to diverse geographical regions were raised in augmented design and studied for phenological and agro-morphological traits. The results indicated high variability for traits, *viz.*, yield/plant, pods/plant and 100 seed weight, respectively. While moderate variability was detected for plant height. 13 accessions found desirable for seed size were further studied during *rabi* 2003-04, for plant type, yield (kg/ha), 100 seed weight and seed coat characteristics. Four accessions, namely, ICC12033, ICC14199, ICC14197 and ICC14203 demonstrated high yield potential of >18q/ha. Their respective 100 seed weight was recorded as 60.2, 54.73, 58.31 and 46.16. Based on screening in wilt sick nursery, an accession ICC14199 displayed complete resistance whereas ICC14203 showed moderate resistance against *Fusarium* wilt (race 2). Efforts are underway to utilize the desirable accessions in Kabuli improvement.

**Key Words: Kabuli chickpea, exotic accessions, yield potential, seed size, *Fusarium* wilt**

Chickpea is among the major pulse crops in Indian subcontinent. India leads the chickpea producing countries with 75% and 73% as its share in area and production, respectively (FAO, 2005). However, most of the varieties of kabuli types have low yield potential, small to medium seed size and eventually succumb to various biotic and abiotic stresses. Narrow genetic base is attributed as one of the reasons of non-performance of these varieties. In countries like Turkey, Spain, Canada etc., varieties with 50-60g/100-seed weight are cultivated as these fetch premium price in the market. Hence, exotic material with wider genetic variation for yield and yield contributing traits, resistance/tolerance to *Fusarium* wilt, *Ascochyta* blight, drought and cold and plant type which is amenable to mechanical harvesting as well as suitable for different environmental niches is urgently sought. This investigation is an attempt to evaluate the phenological and yield and its component traits in exotic kabuli accessions as well as screen their reaction against wilt (*Fusarium oxysporum* f. sp. *ciceri*) race 2 so as to identify the promising entries to be utilized in augmenting breeding programmes.

## Material and Methods

The investigation was carried out at Kanpur, which falls in Northern part of India; its latitude and longitude being 20°27'N and 80°14'E, respectively. A total of 150 kabuli germplasm accessions (98 from NBPGR, New Delhi and 52 from ICRISAT Hyderabad), originating from diverse geographical regions was selected. The accessions were sown on November 2, 2002 and evaluated by a Petersen's augmented design (Petersen,1985) with three commercial varieties, namely, KAK2, L550 and BG1003 as checks. Therefore, five blocks were formed each one having 30 different genotypes and a replication of the checks. The experimental plots consisted of two rows, 3 m long, each with row to row and plant to plant distance of 30 cm and 10 cm, respectively. Cultural operations, weed control and fertilizations were made according to standard practices. Observations were recorded on days to flower (50%), days to pod (1st), days to maturity (95%), %wilt incidence and plant height (cm) upon maturity. On the basis of five competitive plants, information was collected on number of primary branches/plant, secondary branches/plant, pods/plant, and seed/pod and yield/plant (g). Plot yields (g) were converted into kg/ha using the formula (plot yield (g)/plot size x 10). 27 accessions were designated as promising entries on the basis of their overall performance. However, 13 entries were identified as large to extra-large seeded with low to moderate wilt incidence. These entries along with two checks namely KAK2 and L550 were raised in RBD with two replications during *rabi* 2003-04. The entries were raised on a plot size of 2.7 sqm with row to row and plant to plant spacings of 30 cm, and 10 cm, respectively. The cultural practices, etc as followed during previous year were resorted to. Information was gathered on various traits as done previously. Recorded data were analysed using the INDOSTAT programme.

Seven most promising lines displaying low wilt incidence under natural conditions were screened for scoring their reaction against *Fusarium* wilt (race 2).

During rabi 2004-05, the accessions along with three commercial varieties, *viz.,* KAK2 and L550 were sown in a wilt sick plot (WSP) – a 15-year old diseased nursery wherein pathogen inoculum was continuously added and followed by growing highly susceptible varieties/strains every year since 1989-90.The accessions were planted in 4.5 m long rows with row-row and plant-to-plant spacing of 30 cm and 10 cm, respectively. The susceptible check entry, JG62–a twin-podded desi was sown after every test entry. Two rows of each entry alternated with one row of infector JG62 enabling the development of heavy pathogen inoculum and maintenance of high disease pressure in the nursery. The extent of disease incidence in the susceptible infector rows served as a standard check for susceptibility of the test material. The crop was raised as per the recommended cultural practices for the chickpea pathological trials. The wilt incidence in each accession was scored and the lines characterized as per the guidelines of All India Co-ordinated Research Project on improvement of chickpea (Anonymous, 2004). Accordingly, plant count upon emergence was noted and the entries were regularly observed for wilt incidence. A count of wilted plant/s per accession was made and final data was recorded 2-weeks before maturity. The information on plant count upon emergence and maturity and the number of dead plants because of infection were compiled and % FW due to mortality was calculated .

**Results and Discussion**

Chickpea is quantitatively a long day plant (van der Maesen, 1972) . Its sown during October-November and matures during last week of March-mid April. Under North Indian conditions the crop thus encounters decreasing temperature and decreasing photoperiod until flowering begins, and increasing temperature as well as day length during pod development and maturity (Khanna-Chopra and Sinha, 1987).

During our investigation, 20 accessions showed first flush of flowering within 50-60 DAS (date of sowing being 02/11/2002). This period was an exceptional phase characterized by high day as well as night temperatures which started falling from last week of December with next 30 days being exceptionally cold, humid and foggy, with mean day temperature below 5$^{o}$C at the time of pod establishment (weather bureau, IIPR, data not given). The flowers /pods degenerated and the lines eventually re-flowered during 103-110 DAS. Such lines produced only a few pods by 117-120 DAS. These lines also displayed 100 % wilting at podding stage. Eventually shrivelled seeds were harvested. No further studies could be conducted on these accessions.

The mean values, range and CV (%) for the ten traits recorded, are given in Table 1. All the accessions displayed 50% flowering between 100-126 DAS with the mean of 108.68 DAS. An average of 115.81 DAS were taken for pod initiation. The accessions were found to mature by 152.01 DAS. A low coefficient of variation (cv) for these three parameters was discernible (Table 1). The plant height ranged from 27.3 cm to 68.3cm. Furthermore, only two accessions namely, ICC9593 and ICC16463 possessed 2 seeds/pod. Traits, *viz.,* yield/plant, number of pods/plant and 100 seed weight exhibited higher variability, % cv being 83.87, 74.26, and 64.27, respectively, followed by number of primary (39.94) and secondary (31.22) branches. On the other hand, moderate variability (20.18%) was found for plant height. These figures indicated the scope of exercising selection for direct improvement in yield and indirectly through the number of primary and secondary branches. Raju *et al.* (1978) and Sood and Kumari (2000) have also reported similar observations in kabuli chickpea.

Based on yield and its contributing traits, 27 accessions were found promising. In kabuli types, grain quality determines its desirability for consumption purposes and hence acts as a yard stick for price determination. In recent times, the demand for large (>40g/100 seeds) and extra - large (>50g/100 seeds) kabuli varieties is on the rise. Currently, this demand is met through import. Hence, there is a strongly felt need to boost production of l ırge and extra large varieties so as to be self sufficient nc ı only in meeting the domestic need but also having surplus for export by evolving a suitable strategy. In other words, donors possessing target trait/s need to be identified and exploited in developing the target varieties. This implies the intense screening of exotic germplasm from diverse world regions as one of the first steps for varietal development. During this investigation, out of 27 promising kabuli accessions, 13 were found to be large to extra large seeded and were therefore studied further. These entries were analyzed thoroughly for plant type, grain yield (kg/ha), 100-seed weight (g) and seed coat characteristics. This information is compiled in Table 2. All the entries were found to be taller than both the local checks. Furthermore, seven entries, *viz.,* ICC12033, 12034, 14197, 14199, 14203, 14204 and 14204 were found to be erect with semi-compact growth habit. Six accessions, namely, ICC7344, 14214, 14194, 14195 and

**Table 1. Phenological and agro-morphological variability in exotic germplasm accessions of kabuli chickpea**

| Trait | Mean | Range | CV (%) | Extreme accession/s |
|---|---|---|---|---|
| DF( 50%) | 108.68 | 100-126 | 4.31 | EC381867, EC382436 |
| DP(Ist) | 115.81 | 102-130 | 4.57 | EC381867, EC382352 |
| DM(95%) | 152.01 | 150-165 | 1.39 | EC381909, EC381616/EC381617 |
| Plant height(cm) | 48.39 | 27.3-68.3 | 20.18 | ICC1969, EC 387190 |
| Primary branches/plant | 4.62 | 2.0-9.0 | 39.94 | ICC16552, ICC4753 |
| Secondary branches/plant | 7.92 | 2.3-18.0 | 31.22 | EC382383, ICC16673 |
| Pods per plant | 33.72 | 1.0-103.0 | 74.26 | EC382379, ICC4753 |
| Seed/pod | 1.01 | 1.0-2.0 | 9.95 | ICC9593, ICC16463 |
| Yield/plant | 6.46 | 0.2-36.12 | 83.87 | EC382379, ICC12033 |
| 100 seed weight (g) | 24.79 | 2.5-61.4 | 64.27 | EC381893, ICC12034 |

**Table 2. Yield (kg/ha), 100-seed weight (g), plant types and testa characteristics of highly promising exotic kabuli germplasm accessions**

| Accession # | Source of origin | Yield kg/ha | G/100 seeds | Plant type | Testa characteristics |
|---|---|---|---|---|---|
| ICC14214 | Mexico | 250 | 55.90 | Tall spreading, open canopy | Milky white,bursting type |
| ICC14194 | „ | 561 | 50.01 | Tall, semi-spreading, open canopy | „ |
| ICC14195 | „ | 945 | 45.34 | „ | Beige, non-bursting type |
| ICC14196 | „ | 800 | 53.80 | Tall, spreading open canopy | Milky white, non-bursting |
| ICC7344 | „ | 1160 | 51.80 | Tall, semi-spreading, open canopy | Milky white non-bursting |
| ICC12034 | „ | 1078 | 61.0 | Tall, erect, semi-open canopy | Beige, bursting |
| ICC12033 | „ | 2060 | 60.20 | „ | Beige, bursting+non-bursting |
| ICC14197 | „ | 1877 | 58.31 | „ | Milky white non-bursting |
| ICC14199 | „ | 2020 | 54.73 | „ | Beige, non-bursting |
| ICC14205 | „ | 360 | 52.79 | | Milky white non-bursting |
| ICC14204 | „ | 880 | 53.53 | „ | Beige, non-bursting |
| ICC14203 | „ | 1840 | 46.16 | „ | Milky white non-bursting |
| EC381882 | Turkey | 501 | 51.91 | Tall, spreading type, open canopy | Milky white, bursting+ non-bursting |
| KAK2 (local check) | | 981 | 38.0 | Medium height, semi-spreading, open canopy | Beige, non-bursting |
| L550 (local check) | | 1230 | 18.0 | Medium height, erect, semi-open canopy | „ |
| | | CV(%) 13.04 CD @5% level 310.43 | CV(%) 5.636 CD @5% level 6.07 | | |

14196, and EC381882, displayed open canopy. Three accessions each displayed spreading and semi-spreading canopies, respectively. The study further established four accessions, namely, ICC12033, ICC14199, ICC14197 and ICC14203 as high yielding entries. The yield potential of these accessions was found above 18q/ha. The 13 test entries were also studied for their seed size and testa characteristics (Table 2). Entries namely ICC12033 &12034 displayed 100 seed weight of >60g while nine others viz. ICC14204, 14214, 14194, 14196, 14197, 14199, 7344, 42051 and EC381882 showed in the range of 50-60 g.Two entries *viz.*, ICC14195 and14203 displayed 100 seed weight of around 45 g. The best check KAK2 showed 100-seed weight of 38 g. Additionally, eight accessions displayed milky white testa while rest exhibited beige. However, it was found to be intact (non-bursting type) in seven of these entries, *viz.*, ICC14195, 14196, 14197, 14199, 14203, 14204 and 14205, respectively . Four accessions exhibited bursting type seed coat, whereas, two namely, ICC12033 and EC 381882 produced both the types.

**Table 3. Reactions of highly promising exotic kabuli germplasm accessions against FOC race 2 under WSP conditions**

| Accession # | % FW | Reaction type* |
|---|---|---|
| ICC14199 | 5.6 | R |
| ICC14203 | 18.7 | MR |
| ICC14195 | 36.2 | S |
| ICC14196 | 48.1 | S |
| ICC7344 | 39.2 | S |
| ICC14197 | 22.4 | S |
| ICC14205 | 56.4 | S |
| KAK2 (ch) | 23.7 | S |
| L550 (ch) | 80.6 | S |
| JG62 (susceptible ch) | 100.0 | Highly susceptible |

* R: resistant, MR: moderately resistant, S: susceptible

**Table 4. Highly desirable accessions of exotic kabuli chickpea germplasm and their useful trait/s**

| Useful trait/s | Desirable accession/s |
|---|---|
| Primary branches/plant | ICC4753, ICC16541 |
| Secondary branches/plant | ICC5099, ICC16673 |
| Plant height (cm), 100-seed weight (g) | IC accessions given in Table 2 |
| Pods/plant | ICC 4753, ICC9361, ICC11255 |
| Yield (kg/ha) | ICC12033, ICC14199, ICC14197, ICC14203 |
| Resistance against *Fusarium* wilt | ICC14199, ICC14203 |

Wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (FOC) is a major biotic factor capable of reducing chickpea yield by 10 to 15 per cent with 100 % loss under specific situations (Singh and Dahiya, 1973; Haware and Nene, 1980). Of the four physiological races reported from India (Haware and Nene, 1982) Kanpur isolate (race 2) is known to be the most aggressive (Gurha and Mishra, 1982). Hence, one of the major breeding objectives is to develop cultivar/s with resistance to FOC race 2. In the present investigation, an attempt has been made to screen exotic kabuli accessions against *Fusarium* wilt both under natural as well as sick plot conditions. The results on wilt incidence under natural conditions indicated low (<5%) to high including 100% in exotic material. 20 accessions were found to wilt completely before the on-set of maturity. However, 27 accessions showed upto 40% mortality due to wilt. Based on their low wilting (0-20%), 13 large to extra-large seeded accessions were earmarked for screening under sick plot conditions. However, six of these entries exhibited bursting seed coat (Table 2) and could not be studied further. Therefore, seven entries were grown in sick nursery (*Rabi* 2004-05) and reactions against wilt were scored as per the guidelines of AICRP on chickpea improvement (Anonymous, 2004). The results are presented in Table 3. One entry ICC14199 showed 5.6% mortality (i.e. % FW) and was thus designated as resistant (R) on account of wilting of plants below 10%. Likewise, an accession ICC14204 exhibited wilt incidence in the range of 10-20% and was thus classified as moderately resistant (MR), its % FW being 18.7. Rest of the four entries were found susceptible, % FW being in the range of 20.1 to 100.0 (Table 3).

Limited genetic variation has been a major constraint in attaining improvement in different pulse crops including chickpea. Use of exotic material is necessary when desired gene/s are not available in local cultivars. All available kabuli varieties besides being low yielders are susceptible to a number of biotic and abiotic stresses and have small to medium sized seeds. A clear understanding of genetic variability and interrelationship between yield and yield-related traits would complement breeders' efforts in evolving superior varieties. During this investigation some accessions of the exotic germplasm of kabuli chickpea displayed useful variations for important traits especially wilt, plant height, primary and secondary branches, pods/plant, yield potential,100 seed weight (Table 4).These accessions especially ICC14199, 14203, 14197 and 12033 offer possibilities in broadening the genetic base of the local kabuli cultivars as well as providing a better chance to recover transgressive segregants. Efforts are being made to utilize these accessions in kabuli improvement.

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# Mechanisms for Operationalization of Access to Germplasm in India in the New Regime

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## Introduction

The diversity in life forms is the basis of continuous evolution of life on earth. The dependence of human beings for food, fodder, shelter and other needs on various components of biodiversity, especially the plant genetic resources (PGR), is also undoubted. Sustainable use of PGR for food and agriculture is, therefore, of critical importance. Since most countries depend largely on PGR that have originated elsewhere, it is essential and extremely important to develop mechanisms for the access and sharing of these resources among and within the nations. It is, more so, after the realization of owners rights, breeders' rights (1970s) and Intellectual Property Rights (IPRs), and owing to the advances in biotechnology during 1990s.

Access to genetic resources under different regimes is governed by: (i) "Sovereign rights of Nations" under the legally binding Convention on Biological Diversity (CBD), 1992. The Convention provides for appropriate access to genetic resources and transfer of relevant technologies on mutually agreed terms and subject to prior informed consent and, (ii) "Facilitated access" to plant genetic resources for food and agriculture under the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), 2001 which has a provision of "Multilateral System" of exchange under mutually agreed terms.

Of these, the CBD (1992) had obligated all parties (Convention Countries) to provide for conservation of biological diversity, sustainable use of its components and equitable sharing of the benefits arising out of the use of biological resources. This also includes provisions for regulation of access to biological resources reaffirming the sovereign rights of nations over their biological resources. The Biological Diversity Act (BDA) of India was formulated after India became signatory to the CBD, and it provides for all the objectives of CBD as mentioned above (Brahmi *et al.*, 2004a).

## Established Institutional Mechanism under the BDA

For effective implementation of the Act it is envisaged to have National Biodiversity Authority (NBA), State Biodiversity Boards (SBBs) and Biodiversity Management Committees (BMCs).

The NBA deals with matters relating to requests for access by foreign individuals, institutions or companies, and those relating to transfer of results of research to any foreigner. Imposition of terms and conditions to secure fair and equitable sharing of benefits arising out of utilization of biological resources and approvals for seeking any form of Intellectual Property Rights (IPR) in or outside India for an invention based on research or information pertaining to a biological resource or knowledge associated thereto obtained from India, are also dealt by NBA.

The SBBs are being constituted for every state in India to deal with matters relating to access by Indians for commercial purposes and restrict any activity which violates the objectives of conservation, sustainable use and equitable sharing of benefits.

Institutions of self-government in their respective areas would constitute a BMC for conservation, sustainable use, and documentation of biodiversity and chronicling of knowledge relating to biodiversity. BMC shall be consulted by the NBA and SBB on matters related to use of biological resources and associated knowledge within their jurisdiction.

## Provisions for Regulation of Access to Biological Diversity

Chapter II of the BDA deals with regulation of access to biological diversity (Sections 3-7). Section 3 of the Act restricts non-Indians defined in the Act, not to undertake biodiversity related activities, without approval of National Biodiversity Authority.

Section 4 provides for conditions of transfer of results of research related to biodiversity with the approval of the NBA. Such transfer includes the results of any research relating to any biological resources occurring or obtained from India for monetary consideration or otherwise to any person who is not a citizen of India or a body corporate or organization which is not registered or incorporated in India.

However, 'transfer' does not include publication of research papers or dissemination of knowledge in any

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seminar or workshop, if such publication is as per the guidelines issued by the Government of India.

Exemption to provisions of section 3 and 4 has been provided in Section 5. Sub section (1), for collaborative research projects involving transfer or exchange of biological resources or information relating thereto, including Government sponsored institutions of India, and such institutions in other countries. Such collaborative research projects should conform to the policy guidelines issued by the Central Government in this regard and be approved by the Government of India. The Ministry of Environment and Forests (MoEF) has issued such guidelines vide notification no. S.O. 1911(E) dated 8 November, 2006. These guidelines cover exchange of germplasm and information under collaborative research projects including bilateral and multilateral agreement, memorandum of understanding, work plans, under any international collaborative research projects.

Section 6 deals with the application for intellectual property rights. The section states that "No person shall apply for any intellectual property right by whatever name called in or outside India for any invention based on any research or information on a biological resource obtained from India, without obtaining the pervious approval of the NBA before making such application". This permission, however, may be obtained after the acceptance of the patent but before sealing of the patent by the patent authority concerned. NBA may, while granting the approval under this section, impose benefit sharing fee or royalty or both or impose conditions including the sharing of financial benefits arising out of the commercial utilization of such rights.

**Access to PGR with Reference to Protection of Plant Varieties and Farmers Rights (PPVFR) Acts, 2001**

The researchers have been given exemptions under the PPVFR Act, to use a protected variety for research, including development of new varieties, except in case of commercialization of the newly developed variety or in case of repeated use of a variety as a parental line (Brahmi *et al.*, 2004 b).

**Registration of New Varieties**

The applications for registration of new variety under the PPVFR Act need to be accompanied with complete passport data of parental lines used for breeding, including geographical location in India from where the material was obtained. Secondly, proof of lawful acquisition of the material used for breeding is also required to be submitted.

Therefore, the breeders are required to maintain full passport data of material used and documentary evidence of its acquisition. This can be a Material Transfer Agreement (MTA) or written certified document showing consent of the owner of the material. These could be farming communities, public sector institutions or private organizations (Dhillon *et al.*, 2006).

**Access to PGRFA under the Multilateral System of ITPGRFA**

Access under this system is to be facilitated to all member countries, for the crops mentioned in Annex 1 of ITPGRFA and solely for utilization and conservation for research, breeding and training. This does not include chemical, pharmaceutical and/or other non-food/ feed or industrial uses (Dua *et al.*, 2004). The access is to be accorded free of charge or if charged, the fee should not exceed the minimal cost involved. The governing body of the Treaty has ratified a Standard Material Transfer Agreement (SMTA) for use among member countries. Salient provision of the SMTA is that the material received under the multilateral system of the treaty shall be freely available to others for use in research, breeding and training. The third and subsequent parties accessing the material shall be bound by the same conditions of the SMTA. The recipients can not claim any IPRs on the material in the form received and if any commercial utilization is done, the benefits would be returned to a trust fund of the Treaty. This sharing of benefits would be to the tune of 1.1% of the total share of profits.

The Consultative Group on International Agricultural Research (CGIAR) institutions have also agreed to be part of the multilateral system and access to germplasm of all their mandate crops would be under the condition of SMTA.

**Existing Access Regulations in India**

Access to PGR for research purpose from outside India has been regulated by quarantine regulatory provisions under Plant Quarantine Order, 2003 (Regulation of Import into India) of the Destructive Insects and Pests (DIP) Act of 1914, known as PQ Order, 2003. Material imported into India is to be accompanied with an import permit issued by the Director, National Bureau of Plant Genetic Resources (NBPGR) and Phytosanitary Certificate (PSC) issued from the country of origin. Under the PQ order, freedom of import commodities from pests of quarantine importance has been incorporated. "Additional/ Special Declarations on the basis of standardized Pest Risk

Analysis (PRA) particularly for seed/planting material have been taken care of. Four categories of agricultural imports have been included in the revised order. These are: (i) Prohibited plant species (Schedule IV), (ii) Restricted species where import permitted only by authorized institutions (Schedule V), (iii) Restricted species permitted only with additional declarations of freedom from quarantine/ regulated pests and subject to specified treatment certifications (Schedule VI), and (iv) Plant material imported for consumption/industrial processing permitted with normal phytosanitary certificate (Schedule VII).

For import of transgenic germplasm, a statuary clearance (Fig. 1) from Review Committee on Genetic Manipulation (RCGM) of the Department of Biotechnology, Govt. of India is required to be obtained before applying for import permit (Singh *et al.*, 2001).

The responsibility for import and export of plant genetic resources for research purposes, whether under

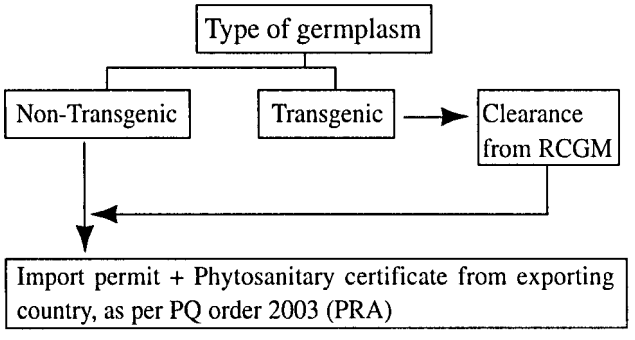


**Fig. 1: Clearance procedure for import of germplasm**

memoranda of understanding (MoUs) for collaborative research projects developed among countries for research purposes or by for individual public or private organizations, has been delegated by the Ministry of Agriculture to the Indian Council of Agricultural Research (ICAR)/ Department of Agricultural Research and Education (DARE). NBPGR is the nodal agency recognized for PGR exchange within the country as well as outside the country. After enactment of the Biological Diversity Act, and Protection of Plant Varieties and Farmers' Rights Act and ratification of International Treaty on Plant Genetic Resources for Food and Agriculture by India, NBPGR has to follow new rules for exchange of plant genetic resources for food and agriculture for which the following checklist needs to be kept in view:

1. Status of indentor or the requesting party is to be considered (Fig. 2). The public sector research organizations may address the request to the holder of germplasm or route the request through Director NBPGR. The germplasm will be sent as per the provisions under MTA. The MTAs lists the mutually agreed conditions for the transfer done between two parties, and has some essential clauses, viz. name(s) and addresses of both parties, name(s) identity and other passport details of the materials to be exchanged, purpose of transfer, cost involved, treatment of derivatives after further research IPR(s) generated, acknowledgements and arbitration conditions or dispute settlement etc. (Tiwari, 2006).
2. For Indian private seed companies with all Indian collaborators and full Indian funding, the material may be sent after confirming the R&D status of the company (Govt./State Govt. approved/notified) and under the conditions of Material Transfer Agreement (MTA).
3. For Indian private seed companies with foreign collaborators or foreign funding, the request shall be directed to NBA. The same will be applicable to multinational seed companies with centres in India.
4. Exchange of germplasm with CGIAR organization based in India or with Centres in India would be based on the bilateral MoUs signed between ICAR and these institutions from time to time and shall be restricted only to their mandate crops.
5. Export of germplasm under bilateral agreements and other MoUs/Work Plans between India and other countries shall be for the specific crops mentioned is submitted to DARE after recommendations from NBPGR and to NBA for final approval (Fig. 3).
6. For exchange of PGRFA under the multilateral system of the ITPGRFA, only those crops that are mentioned in Annex I to the Treaty shall be considered. Presently, Annex I covers 35 food crops and 29 forage genera based on world food security and interdependence of countries. This exchange shall be only among the contracting countries (countries signatory to the Treaty) and under the conditions of SMTA, as discussed above, approved by the Governing Body of the Treaty.

## Conclusion

Access to germplasm and information under the new regime in India has to taken into account the established

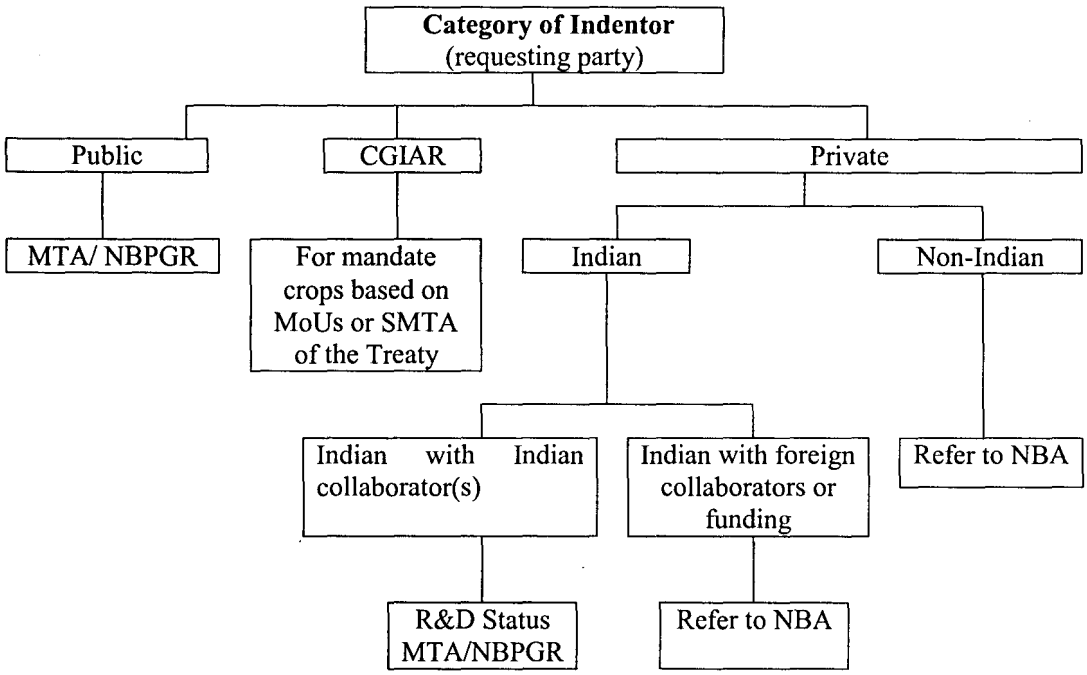


**Fig. 2: Procedure for handling cases for request of germplasm**

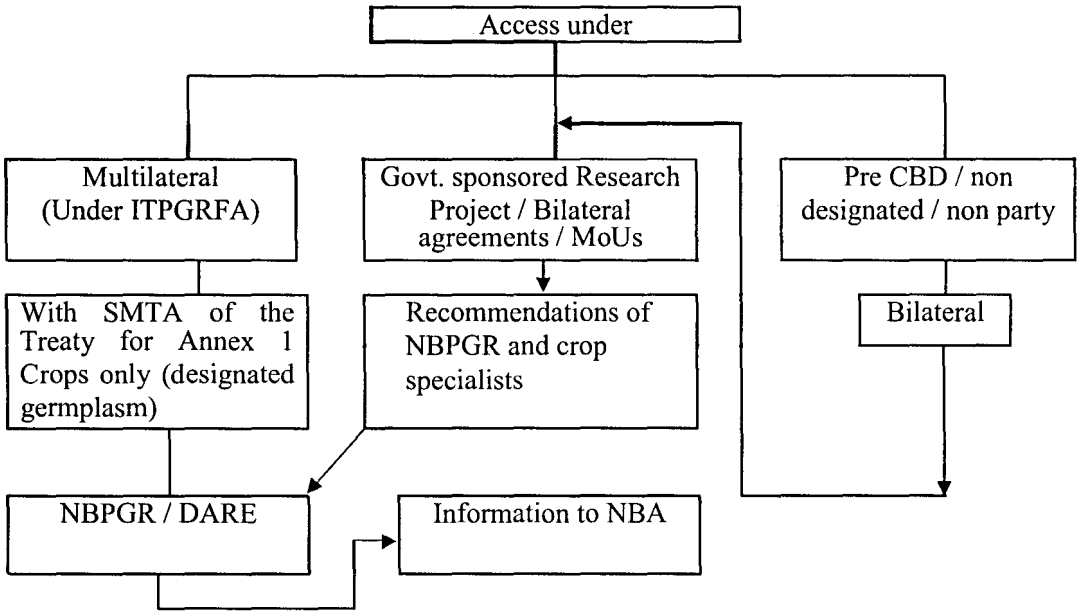


**Fig. 3: Conditions for access under different conditions**

institutional mechanism and various acts in force relating to agrobiodiversity. The requests of indentors are to be dealt with depending on the status of requesting party and the conditions for access under different categories. NBPGR/DARE, the nodal agency for the purpose, has to regulate the access based on projects approved by Govt. Department, State or Central Government, or MoU/ Workplan and other relevant agreements, key investigators/ institute involved, details of the biological resources/ associated knowledge to be exchanged (exported), special status of material if any as per any international agreement (e.g. FAO designated, INGER Nurseries, etc.), intended use for research only and quantity for exchange to be limited to experimental

purpose only. Conditions of MTA also need to be strictly followed and monitored.

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# Collection and Characterization of Wheat Germplasm from North-West Himalaya

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One hundred and fifty five germplasm accessions of wheat collected from North-Western Himalaya were characterized for qualitative and quantitative traits. The germplasm have shown wide range of variability for all the characters studied. Maximum accessions were found to possess spreading type of growth, late maturity, white ear, awned, amber, semi- hard oblong bold grain, grain size (38-50 g) and medium brush hair length. Results for quantitative traits also reveal a wide range for ear length (6.0-13.8 cm) and thousand grain weight (25-66 g). Seven genotypes were found to possess seedling resistance against 21R55 pathotype of leaf rust whereas only two genotypes have shown resistant reaction against 121R63-1 pathotypes of leaf rust. The data on yield contributing traits reveals the potential of some of the germplasm accessions *viz.*, WLG43, WLG44 and WLG171 for their utilization in wheat improvement programme.

**Key Words: Wheat, Germplasm, Variability, Characterization**

Among high yielding varieties of wheat (*Triticum aestivum*), the most popular present day varieties have been derived only from six broad groups (Rao, 1988). This narrow genetic base largely due to development and adoption of High Yielding Varieties (HYVs) points towards collection, evaluation and conservation of wheat germplasm. The North-Western hills of the country are one of the rich repositories of germplasm of winter wheat and rye (Partap *et al.*, 2001). However, these genetic resources have considerably eroded from the traditional wheat growing areas but still some of the landraces/ farmers' varieties are in cultivation in high hills and tribal areas of Jammu & Kashmir and Himachal Pradesh. Locally, the wheat in this region is known by various vernacular names such as *Kanak, Gehun, Ghoon, Gandham, Tor* and *Toe*. Although, such varieties and landraces have low yielding capacity besides disease susceptibility but they do possess yield stability, which is important for subsistence farming in the tribal areas.

Moreover, they are good sources of genes for traits such as drought tolerance (as most of them are grown in rainfed conditions) and for nutrition and dough quality (selected by the farmers with very long experience and local preferences) by virtue of which they are still existing and or competing with their superior counterparts. The impact of high yielding varieties (HYVs) is clearly visible in this region also, but it is more in irrigated valley areas than rain fed, remote and tribal areas. About 55% loss of wheat genetic resources has been recorded in this region over the last 30 years (Rana *et al.*, 2000). In view of above, a systematic germplasm collection programme was initiated in the genetic diversity rich areas especially the *changar* and *kandi* areas (highly rainfed) and tribal belt of North-Western Himalaya for strengthening our wheat germplasm programme. The objectives of the present study are to (i) collect local germplasm of wheat, (ii) locate out the sites having maximum genetic variability and (iii) characterize the germplasm to identify trait specific promising genetic stocks.

## Materials and Methods

The study area was confined to the two states as mentioned above. Three explorations specific to wheat were undertaken from 2000 to 2003. Wheat accessions were collected while conducting multi-crop explorations. Germplasm explorations were conducted in April to May for main season crop and in August-September for off-season crop (cold arid region). In all about 40 sites spreading over 16 districts located at different elevations ranging from 800-2600 msl were explored and 155 germplasm accessions were collected. Since the collections were constituted of random samples, the single ear selection was performed during *rabi* 2003-2004 at Tutikandi reseach farm of Indian Agricultural Research Institute, Regional Station, Shimla to purify the germplasm. Single ear head progenies of 155 accessions were evaluated in detail for various morpho-physiological traits during the year 2004-05. The observations were recorded on Growth class, Coleoptile pigmentation,

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Maturity class, Ear colour, Ear length, Awn type, Grain colour, Grain texture, Grain shape, Grain size and Brush hair length. Another set of germplasm accessions along with susceptible check *Agra local* was planted in glass house for recording their response against predominant pathotypes of brown rust according to the method described by Stakman *et al.* (1962).

## Results and Discussion

### *Characteristic features of the sites visited and landraces collected*

The agro-climatic conditions of the areas chosen for field study were highly varied for rainfall (500-2000 mm), topography (valleys, sub-mountainous to mountainous) and mean monthly temperature (–0.10-35°C). Wheat is sown in October-November in warm areas (subtropical) and from March-May (once the snow melting starts) in cold arid region of Pangi, Spiti, Upper Kinnaur and Ladakh. However, winter wheat in this region is sown in November-December (before the snowfall). Improved varieties of wheat are occupying 90-95% in plains and valleys of Jammu, Kangra, Mandi, Sirmour and Una, whereas it was 40-50% in rain-fed hilly terrains (Rana *et al.*, 2000; Sharma and Rana, 2005). While undertaking explorations the sites having maximum genetic variability for traits of economic importance and those sites which have high number of known landraces were considered as areas with rich genetic diversity (Fig. 1). The important traits for which genetic variability was observed were drought tolerance, cold tolerance, growth habit and adaptability to poor environments.

In general, there was not much variability within a landrace but while critically examining the fields; mechanical mixture was found to be very common. About

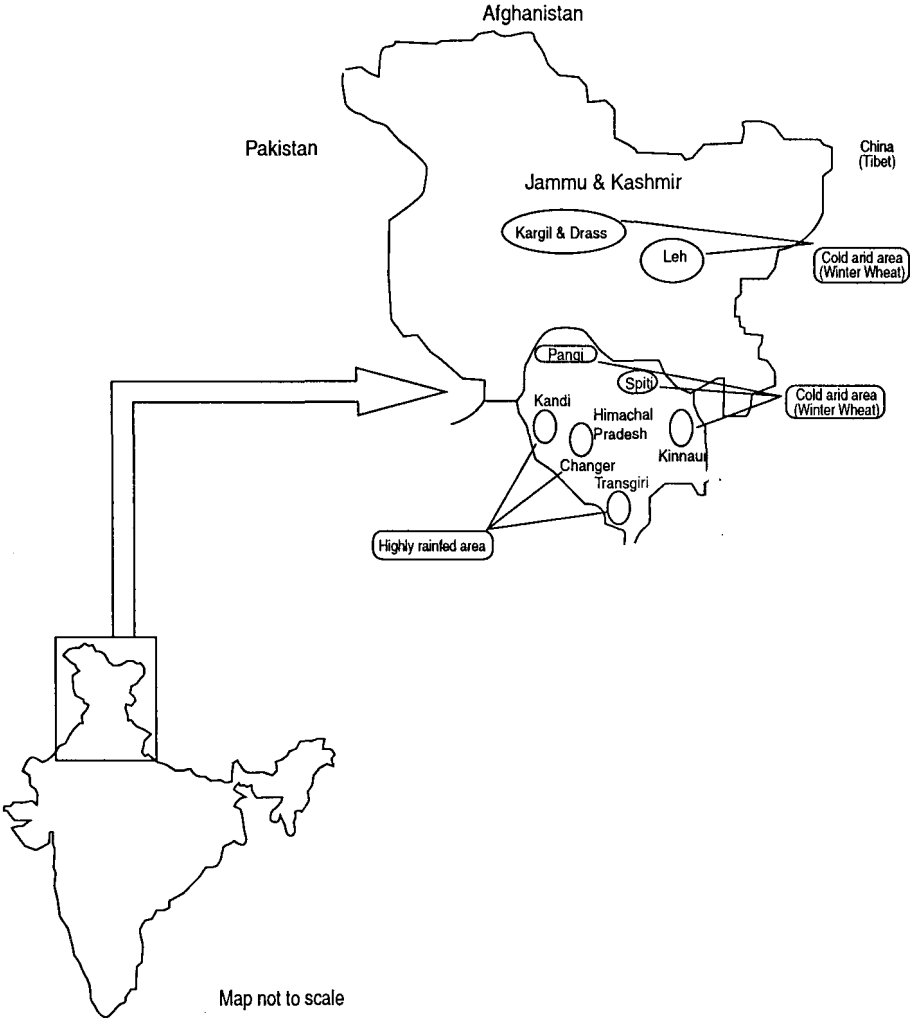


**Fig. 1: Areas rich in wheat genetic variability**

70% of the local wheat growing in the hills has named varieties and 30% were found without local names and generally called "*desi kanak*". The sites, where maximum variability was observed were located in Chamba, Sirmour, Kangra, Mandi, Hamirpur, Kullu, Lahaul & Spiti districts of Himachal Pradesh and Kargil, Drass, Leh, Poonch, Rajouri of Jammu & Kashmir. One hundred and fifty five germplasm accessions collected represent different agro-climatic areas *viz.*, Chamba (20), Sirmour (14), Kangra (15), Mandi (14), Hamirpur (9), Lahaul & Spiti (9) and others (16) in Himachal Pradesh and Kargil (16), Drass (23), Leh (12), Poonch (4) and others (3) in Jammu and Kashmir.

Genetic variability including farmers' varieties/ landraces was observed more in remote. It was observed that folk varieties/landraces have been named by the farmers based on characteristics they possess. For instances, *brad kanak* which means spreading type and have more number of tillers; *lalpuri* – red grains; *Misri* – sweet, soft and white, suitable for making bread. Adopting the same pattern about 35 named landraces *viz.*, *Bhangru, Bharadoo, Chawera, Cheeuni, Chiti Kanak, Daru, Darmori, Daron, Desi Mundal, Gazaria, Jael, Joth, Jhuladi, Kasieun, Kothek, Kothi, Kankoo, Kiawali, Lal kanak, Lalpuri, Latar, Kalee bauri, Mundra, Mandaleu, Marodum, Misri, Mundu, Paluwa, Ralieun, Rigaliya, Rundan, Shruin, Trimudi,* were observed in Himachal Pradesh only. The characteristic features of some important landraces as described by the farmers given in Table 1. As it is mentioned that wheat genetic resources are depleting at very fast rate as compared to other crops, thus concerted efforts are required to capture this gene pool. Although enough germplasm has been preserved under *ex situ* conditions but there are hardly any efforts to conserve the germplasm on farm which is perhaps very important for sustaining the wheat breeding programmes in the long run.

**Characterization of the Germplasm at the Experimental Farm**

The data recorded has been on one hundred and fifty five local wheat germplasm accessions for qualitative and quantitative traits. Considerable phenotypic variability was observed for coleoptile colour (green, light purple, purple, dark purple), growth habit (spreading, semi-spreading, semi-erect, erect), awn type (awned, scur, awnless), ear colour (brown, white), grain texture (hard, semi-hard, soft), grain colour (medium red, light red, amber, amber-white, white), grain shape (elliptical, oblong, ovate, round), grain size (bold, medium, small), brush hair length (long, medium, small). Results for quantitative traits also reveal a wide range for ear length (6.0-13.8 cm) and thousand grain weight (25-66 g) in the germplasm.

Maximum accessions were found to possess spreading type of growth, late maturity, white ear, medium spike length (9-12 cm), awned, amber grain colour, semi-hard grain, oblong grain shape, bold grain size (43-50 g) and medium brush hair length (Table 2). Germplasm accessions (Table 3), WLG41, WLG72, WLG73, WLG75, WLG76,WLG77, WLG89, WLG96, WLG101, WLG171 were found to possess long ear (>12cm) and

**Table 1. Characteristic features of the landraces as described by farmers**

| Name of the landrace | Characteristic feature |
|---|---|
| Rundal | Awnless, tall, thin stem, makes good bread, good yield |
| Jhuldi | Awned, long ears, bold grain size |
| Sirohun | Awned, gives good yield in rotation with paddy but less with maize |
| Kathuan | Pink pigmentation in the ear, poor yielder, very good quality |
| Kishal | Awned, good bread making quality, average yielder |
| Kankoo | Good plant vigour, awnless, tall, average yield, non-shattering, bread tasty and does not dry quickly, flour white |
| Dharmouri | Red grained, high tillering ability, drought tolerant |
| Desi mundal | Awnless, bread tasty, disease resistant, non-shattering type |
| Brad kanak | Awned, more straw, drought resistant, flour brown but bread tasty, very high tillering, shattering type, lodging resistant |
| Bharadoo | Late maturing, more tillers, good dough quality |
| Ralieun | Easy threshable, flour white, high yield, bread tasty |
| Mundu, Misri | Cold tolerant, amber colour, soft bread |
| Shruin | Awnless, tall, small grain, good dough quality |

**Table 2. Frequency distribution of 155 local germplasm for morphological characters**

| Characteristic feature | Frequency distribution for different descriptors |
|---|---|
| Growth class | Spreading (64); Semi-spreading (23); Semi-erect (49); Erect (15); $ (04) |
| Coleoptile pigmentation | Green (102); Light purple (12); Purple (26); Dark purple (9); $ (6) |
| Ear colour | White (115); Brown (37); $ (3) |
| Ear length | Short <9.0 cm (60); Medium 9-12 cm (82); Long >12.0 cm (11); $ (02) |
| Awn type | Awned (124); Scurs (02); Awnless (23); $ (6) |
| Maturity class | Medium early (29); Medium late (53); Late (64); Very late (05); $(04) |
| Grain colour | Red (01; Medium Red (20); Light Red (57); Amber (67); Amber - White (09); $ (01) |
| Grain texure | Soft (65); Semi-Hard (82); Hard (07); $ (01) |
| Grain shape | Elliptical (39); Oblong (96); Ovate (18); Round (01); $ (01) |
| Grain size | Small < 32g (27); Medium 32–42g (47); Bold 43- 50g (54; Extra Bold>50g (27) |
| Brush hair length | Short or lacking (01); Medium (132); Long (22) |

$ Not recorded

**Table 3. Promising accessions of local wheat germplasm for yield contributing traits and seedling leaf rust resistance**

| Grain yield component/leaf rust pathotype | Promising accessions |
|---|---|
| Ear length (>12 cm) | WLG41, WLG72, WLG73, WLG75, WLG76, WLG77, WLG89, WLG96, WLG101, WLG171 |
| 1000-Grain weight (e"60g) | WLG43, WLG44, WLG54, WLG119, WLG122, WLG132, WLG135, WLG137, WLG163, WLG167, WLG169, WLG171, WLG174 |
| Pathotype 21R55 | WLG14, WLG17, WLG23, WLG32, WLG36, WLG70, WLG71 |
| Pathotype 121R63-1 | WLG23, WLG88 |

WLG43, WLG44, WLG54, WLG119, WLG122, WLG132, WLG135, WLG137, WLG163, WLG167, WLG169, WLG171, WLG174 had extra bold grains (e"60 g thousand grain weight). The data on yield contributing traits reveals the potential of some of the germplasm accessions *viz.*, WLG43, WLG44 and WLG171 for their utilization in wheat improvement programme. Seven genotypes were found to possess resistance against 21R55 whereas only two genotypes had shown resistant reaction against 121R63-1 pathotypes of leaf rust at seedling stage (Table 3). However, there may be chances of disease escape; therefore more confirmation of resistant lines at adult plant stage is required for validating them as gene sources against above pathotypes of brown rust.

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