Seed Morphobiometry of Wild and Cultivated Taxa of *Phaseolus* L. (Fabaceae)

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A morphobiometric analysis of seeds of *Phaseolus* L. from 50 populations belonging to 31 wild and cultivated taxa was carried out. Based on the outcome of the study an identification key was developed comprising 25 morphotypes of which 23 related to individual taxa [*P. angustissimus* Gray, *P. filifomis* Benth., *P. acutifolius* Gray var. *latifolius* Freeman, *P. lunatus* L. var. *sylvester* Baudet, *P. polyanthus* Greenm., *P. lunatus* L. var. *lunatus* cv.-gr. "Sieva", *P. lunatus* L. var. *lunatus* cv.-gr. "Big Lima", *P. lunatus* L. var. *lunatus* cv.-gr. "Potato", *P. maculatus* Scheele, *P. lunatus* L., *P. microcarpus* Mart., *P. leptostachyus* Benth. var. *leptostachyus*, *P. grayanus* Woot. & Standl., *P. vulgaris* L. var. *aborigeneus* (Burk.) Baudet, *P. marechalii* Delgado, *P. vulgaris* L. var. *vulgaris*, *P. ritensis* Jones, *P. polystachios* (L.) Britt., Sterns & Pogg. var. *polystachios*, *P. hintonii* Delgado, *P. pauciflorus* Don, *P. parvulus* Greene, *P. pluriflorus* Maréchal, Mascherpa & Stainer, *P. micranthus* Hook. & Arn.) and two that refer to several taxa (*P. vulgaris* morphotype with *P. vulgaris* L., *P. coccineus* L., *P. glabellus* Piper, *P. oligospermus* Piper and *P. acutifolius* var. *tenuifolius* morphotype with *P. acutifolius* Gray var. *tenuifolius* (Woot. & Standl.) Gray, *P. acutifolius* Gray var. *acutifolius*, *P. xanthotrychus* Piper, *P. zimapanensis* (Delgado) Jaaska). The different patterns of seminal tegument allowed 31 taxa to cluster into three groups: (1) *Phaseolus angustissimus* Gray group (wrinkled seed coat) with two morphotypes, (2) *Phaseolus lunatus* L. group (smooth tegument with striae) with ten morphotypes and (3) *Phaseolus vulgaris* L. group (smooth tegument without striae) with 13 morphotypes. All the taxa exhibited uniformity in size and variability in tegument colour of seeds irrespective of the source of population and the type of habitat. Characterization of taxa into definite morphotypes and the groups could be useful for biosystematic investigations and the marker-based genetic selection approaches in this important leguminous crop.

Key Words: Biometry, Morphology, *Phaseolus*, Seeds

The bean, cultivated since classical times, is the second most important legume in the world for human consumption after soybean. The change in dietary habits and the globalization of the agrifood sector has led to the spread of bean varieties particularly selected for the canning industry, thus reducing the genetic diversity of *Phaseolus* species grown worldwide. This has had a strong impact on access by people to the large number of its species, varieties and ecotypes, suited to a certain specific environment where they have been selected by generations of farmers. In addition, the loss of this diversity is also accompanied by the loss of agronomic, organoleptic and sensory traits which can no longer be used by people for meeting their livelihood needs. Moreover, the possibility of crosses between different species of *Phaseolus* L., may involve not only an improvement in the production of fruits and seeds but also ensure genetic diversity of all those properties that characterize this genus (Maréchal *et al*., 1978; Mok *et al*., 1978; Leonard *et al*., 1987; Hoc *et al*., 2003; Kusova and Myers, 2005).

There is a great deal of scientific articles on *Phaseolus* that have mainly focused on chemotaxonomy, phytogeography, cultivation practices, *ex situ* conservation, physiological and agronomic characteristics, resistance to abiotic and biotic stresses, etc. (Brücher, 1953; Berglund-Brücher, 1969; Berglund-Brücher *et al*., 1969; Castiñeiras *et al*., 1994a, 1994b; Guerra *et al*., 1994, Hernández *et al*. 1994; Miháliková and Benková, 1995; Freytag and Debouck, 2002; Gutiérrez *et al*., 2004; Delgado-Salinas *et al*., 2006). Less attention has been paid to investigate the morphological variability in seed and in particular of its shape, size, hilum, chalaza or tegument sculpture. These characters although may appear less significant than others, are in fact very useful for the identification of morphotypes and assist to illustrate evolutionary trends of taxa at the generic level (Benson, 1982; De Leonardis *et al*., 2002). The purpose of this study was to add more light on the morphobiometric variability of *Phaseolus* seeds in order to search for possible morphological markers that can be successfully used

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by breeders as in other studies of Fabaceae (Gunn, 1970; Ladizinsky and Adler, 1976a, 1976b; Auckland and Singh, 1977; Kupiha, 1977; Ahmad and Godward, 1980; Lersten and Gunn, 1981; De Leonardis *et al*., 1993, 1995, 1996).

Materials and Methods

Seeds originating from 50 populations of 31 taxa of *Phaseolus* were used for this study. The samples were kindly provided by the National Botanic Garden of Belgium from Wild Phaseolinae Collection.

Morphometric data was gathered form a sample of 30 seeds for each taxon and performed under a stereo-microscope (Jeva Citoval) supplied with 16x eye piece and 1x objective lenses (Table 1). Measurements on large-size (≥ 4,0 mm) seeds were performed with a vernier caliper. Each seed was measured for length (Le), width (Wi), thickness (Th), distance between the micropyle and higher end of the seed (D1), distance between micropyle and the center of the chalaza (D2), distance between the center of the chalaza and the lower end of the seed (D3), distance between higher end of the seed and hilar collar near the chalaza (D4), distance between lower end of the seed and hilar collar near the chalaza (D5), hilum length (Le.hi), hilum width (Wi. hi), chalaza shape (Fig. 1). The map of sketch shapes reported in Fig. 2 has been taken from the descriptive terminology edited by Systematics Association (1962). The seed colour, numeric codes were given following a methodology described by Berggren (1969).

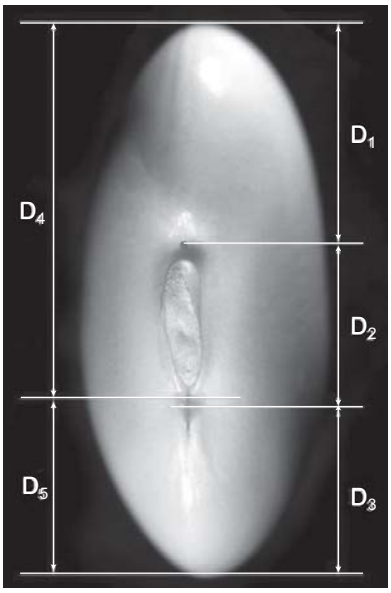

Fig. 1. Measures of some characters made on *Phaseolus* seeds (see abbreviations in Materials and Methods)

Results and Discussion

Table 1 and Fig. 1 show size (in mm) and dimension of seed, respectively. As for length, width and thickness of the seeds of greater size belong to *P. lunatus* (NI 776), while the seeds of smaller size are those belonging to *P. pauciflorus* (Le 1.9). The distance between the micropyle and higher end of the seed (D1) is in the range 0.5 (*P. pauciflorus*) and 6.6 (*P. lunatus* var. *lunatus* NI 426). The distance between micropyle and the center of the chalaza (D2) varies between 0.6 (*P. pauciflorus*) and 5.4 (*P. polyanthus* NI 519). The distance between the center of the chalaza and the lower end of the seed (D3) is in the range 0.9 (*P. acutifolius* var. *tenuifolius* NI 705, *P. micranthus, P. pauciflorus*) and 5.8 (*P. lunatus* var. *lunatus* NI 426). Distance between higher end of the seed and hilar collar near the chalaza (D4) varies between 0.8 (*Ph. pauciflorus*) and 1.30 (*P. polyanthus* NI 519). Distance between lower end of the seed and hilar collar near the chalaza (D5) varies between 1.1 (*P. acutifolius* var. *tenuifolius* NI 705, *P. micranthus*, *P. pauciflorus*) and 6.2 (*P. lunatus* var. *lunatus* NI 426). The hilar length and width vary respectively, between 0.5 (*P. pauciflorus*) and 4.9 (*P. polyanthus* NI 519) and between 0.5 (*P. pauciflorus*) and 0.30 (*P. polyanthus* NI 519). The surface is generally smooth with or without striae, except *P. angustissimus*. and *P. filiformis* having a rugulose surface. The colour varies from white to beige, red to dark brown often with the presence of marbling. In a lateral view the seed range from strictly elliptic to broadly elliptic (rarely oval or rhomboid). The chalaza is heart-shaped, sometimes barely visible

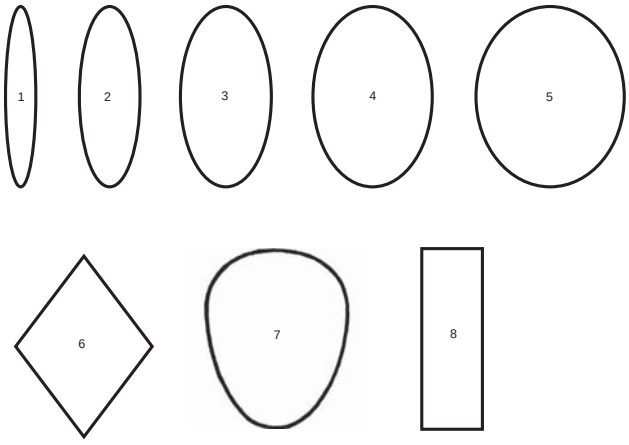

Fig. 2. The map of sketch shapes: 1-2) narrowly elliptic, 3-4) elliptic, 5) broadly elliptic, 6) rhombic, 7) broadly obovate, 8) narrowly oblong

Table 1. Mean values (expressed in mm) of the seed parameters and morphological characters of 50 populations of *Phaseolus*

Codes	Taxa	Le	Wi	Th	D1	D2	D3	D4	D5	Lateral shape	Transversal	Le.hi	Wi.hi	Chalaza shape	Seed coat	Colour	Countries
1	*P. acutifolius* Gray var. *acutifolius* NI 576	4.3	3.1	2.1	1.5	1.3	1.5	2.4	1.7	4,5	10	1.1	0.9	H-s	S	19,39 M 33	Mexico
2	*P. acutifolius* Gray var. *acutifolius* NI 851	4.1	3.4	2.2	1.8	1.0	1.3	2.5	1.6	4,5	7,8,9,10,11	0.9	0.7	H-s C	S	18 M 37; 20 M 28; 29 M 33	Mexico
3	*P. acutifolius* Gray var. *latifolius* Freeman NI 901	7.5	5.2	3.8	2.7	1.8	3.1	3.5	3.2	2,3	8	1.8	1.3	H-s C	Ss	10	El Salvador
4	*P. acutifolius* Gray var. *latifolius* Freeman NI 588	8.1	5.4	3.5	3.1	1.6	3.4	3.9	3.6	4	2,10	1.4	1.1	H-s C	S	4	Mexico
5	*P. acutifolius* Gray var. *tenuifolius* (Woot. & Standl.) Gray NI 705	3.3	2.7	1.5	1.3	1.1	0.9	2.2	1.1	5	9,10,11	0.9	0.7	H-s C	S	20 M 36; 33 M 20	Mexico
6	*P. acutifolius* Gray var. *tenuifolius* (Woot. & Standl.) Gray NI 1272	3.3	2.7	1.8	1.1	1.0	1.2	1.8	1.4	4	9	0.8	0.7	H-s C	S	20 M 14,36; 30 m 36	Mexico
7	*P. angustissimus* Gray NI 788	4.0	3.2	2.4	1.3	0.9	1.8	1.8	2.2	4	9	0.8	0.7	H-s	R	28,32,33 M 37	Arizona
8	*P. angustissimus* Gray NI 1303	5.1	3.9	2.7	2.0	1.2	2.1	2.7	2.4	5	9	1.1	0.8	H-sfv C	R	19 M 37	Mexico
9	*P. coccineus* L. NI 722	7.0	5.7	3.8	2.5	2.8	2.3	4.2	2.8	4,5	9	1.9	1.2	H-sfv C	S	33 M 29; 33 M 20	Mexico
10	*P. coccineus* L. NI 819	5.5	4.8	2.6	1.6	2.3	1.7	3.4	2.0	5	10	1.9	1.4	H-s	S	33 M 20	Mexico
11	*P. coccineus* L. NI 1430	9.2	8.3	6.6	2.5	3.6	2.0	6.2	3.1	28	8	3.1	2.0	H-s	S	36	Mexico
12	*P. filiformis* Benth. NI 689	3.2	2.6	1.6	1.3	0.7	1.1	1.8	1.3	4	9	0.6	0.6	H-s	R	19 M 37	Arizona
13	*P. filiformis* Benth. NI 859	3.1	2.9	1.6	1.3	0.9	1.1	1.8	1.3	5,28	9,10	0.7	0.7	H-s	R	19 M 33	Mexico
14	*P. glabellus* Piper NI 1304	4.9	4.7	3.2	1.5	2.2	1.3	3.5	1.4	5	8	1.9	1.3	H-sfv C	S	20 M 33	Mexico
15	*P. grayanus* Woot. & Standl. NI 724	5.4	4.1	3.1	1.4	1.5	2.6	2.5	2.9	4,40	8,9	1.5	1.5	H-s	S	19, 30 M 36	Mexico
16	*P. hintonii* Delgado NI 716	3.8	4.0	1.9	1.2	0.9	1.6	1.9	1.8	28	9,10	0.7	0.7	H-s	S	36	Mexico
17	*P. leptostachyus* Benth. var. *leptostachyus* NI 714	3.6	3.5	2.3	1.1	0.9	1.5	1.8	1.8	5	9	0.7	0.7	H-s	S	20 M 33,39; 36	Mexico
18	*P. leptostachyus* Benth. var. *leptostachyus* NI 1036	3.6	3.2	2.2	1.2	1.1	1.4	2.0	1.6	5	9	0.9	0.7	H-sfv C	S	40 M 37; 21 M 31; 23 M 37	Mexico
19	*P. lunatus* L. NI 776	18.1	8.6	4.1	4.3	3.7	3.8	7.3	4.7	5	9,10	3.4	1.8	H-s C	Ss	10; 36 M 21,22	Mexico
20	*P. lunatus* L. NI 1414 A	8.5	6.6	3.6	4.0	3.1	2.2	5.6	3.0	4	10	1.8	1.5	H-s	S	36	Mexico
21	*P. lunatus* L. var. *lunatus* cv.-gr. “Lima”NI 426	15.9	13.2	6.7	6.6	4.6	5.8	9.0	6.2	4	9,10	4.2	2.2	H-s C	Ss	17	Florida
22	*P. lunatus* L. var. *lunatus* cv-gr. “Potato” NI 783	9.9	8.4	5.8	3.6	3.0	3.4	4.9	3.6	4,5	8,9	2.7	2.0	H-s C	Ss	23,29 M 30; 30 M 32; 31 M 30	Brazil
23	*P. lunatus* L. var. *lunatus* cv-gr. “Sieva” NI 549	12.2	8.9	4.1	4.2	3.6	4.5	7.3	4.9	4	9,10	3.3	1.6	H-s	Ss	31	California
24	*P. lunatus* L. var. *silvester* Baudet NI 745	9.0	7.2	4.1	2.8	2.8	3.8	5.1	4.4	4	9,10	2.4	1.6	H-s C	S	19,20,29,31 M 37	Mexico
25	*P. lunatus* L. var. *silvester* Baudet NI 1605	7.8	6.0	3.1	2.4	3.0	4.5	4.5	3.4	5	10,11	2.2	1.7	H-s	S	19,33 M 36	Costa Rica
26	*P. maculatus* Scheele NI 1237	7.9	6.5	3.8	1.9	2.0	3.7	3.8	4.1	4,5	37,38,39	2.1	1.5	H-sfv C	R	18,19,22,23,40	Mexico
27	*P. marechalii* Delgado NI 402	5.6	4.5	3.4	1.7	2.2	1.7	3.4	2.2	5	8	1.9	1.5	H-s	S	21,29,30 M 36	Mexico
28	*P. micranthus* Hook. & Arn. NI 1590	2.6	2.3	1.4	1.1	0.8	0.9	1.5	1.1	5	9,10	0.7	0.7	H-sfv C	S	20,29 M 36; 36	Mexico
29	*P. microcarpus* Mart. NI 809	4.1	2.8	1.9	1.6	0.9	1.6	2.3	1.8	28	32,44	0.7	0.8	H-s	S	19,22 M 36	Mexico
30	*P. oligospermus* Piper NI 1116	5.6	5.2	3.1	1.8	1.9	1.4	3.4	1.7	4,5	9	2.1	1.8	H-s	S	20,22,23 M 36	Guatemala

Codes	Taxa	Le	Wi	Th	D1	D2	D3	D4	D5	Lateral shape	Transversal	Le.hi	Wi.hi	Chalaza shape	Seed coat	Colour	Countries
31	*P. parvulus* Greene NI 800	3.3	2.2	2.1	1.1	1.1	1.1	2.0	1.3	2	8	0.9	0.7	H-sfv C	S	19,40 M 36	Arizona
32	*P. pauciflorus* Don NI 711	1.9	1.8	1.4	0.5	0.6	0.9	0.8	1.1	4,5	8	0.5	0.5	H-sfv C	S	22,29 M 36	Mexico
33	*P. pluriflorus* Maréchal, Mascherpa & Stainier NI 869	3.0	2.3	2.1	0.9	1.0	1.1	1.5	1.5	2,4,6	9	0.9	0.6	H-sfv C	S	30 M 36	Mexico
34	*P. polyanthus* Greenm.NI 519	15.4	11.7	6.0	4.8	5.4	5.2	9.7	5.8	5	9	4.9	2.8	H-s	Ss	29	Mexico
35	*P. polyanthus* Greenm.NI 1011	12.4	10.1	5.5	4.0	4.5	3.8	8.3	4.2	4	9	4.2	2.1	H-s	Ss	10	Colombia
36	*P. polystachyos* (L.) Britt., Sterns & Pogg. var. *polystachous* NI 563	6.1	4.5	3.2	1.9	2.7	1.5	4.2	1.9	4,5	8,9,10	2.6	1.5	H-s C	S	33	Florida
37	*P. ritensis* Jones NI 795	6.8	5.4	2.8	2.4	1.6	2.7	3.6	3.0	5,40	9	1.4	1.1	H-s	S	14 M 20; 18 M 36; 30 M 23	Arizona
38	*P. ritensis* Jones NI 797A	6.0	5.1	3.2	2.4	1.7	2.3	3.4	2.7	4,28	9	1.6	1.1	H-sfv C	S	29 M 33	Arizona
39	*P. vulgaris* L. NI 575	6.4	4.4	2.7	2.4	1.7	2.4	3.7	2.7	4	9	1.5	1.2	H-s	S	20 M 33; 30 M 36	Mexico
40	*P. vulgaris* L. NI 613	6.9	4.9	3.5	2.4	2.4	2.1	4.3	2.7	4	9	2.3	1.4	H-s	S	22,23,40	Mexico
41	*P. vulgaris* L. NI 695	7.6	4.5	3.1	2.6	2.2	2.1	4.5	2.6	4	9	2.1	1.3	H-s	S	22,23,33,40	Mexico
42	*P. vulgaris* L. NI 928	6.1	4.2	2.6	2.0	2.0	2.1	3.7	2.4	4	9	2.0	1.6	H-s C	S	20,30 M 36	Colombia
43	*P. vulgaris* L. var. *aborigeneus* (Burk.) Baudet NI 573	7.9	5.8	2.8	2.9	2.1	2.9	4.7	3.3	4	9	2.0	1.5	H-s	S	28,30 M 36	Brazil
44	*P. vulgaris* L. var. *aborigeneus* (Burk.) Baudet NI 621	7.0	4.9	2.8	2.4	1.9	2.7	4.2	2.9	4	9	1.6	1.2	H-s	S	19,20 M 33	Argentina
45	*P. vulgaris* L. var. *vulgaris* NI 637	8.0	5.8	4.4	3.6	2.5	1.9	5.7	2.3	4	9	2.5	1.7	H-s	S	29	Brazil
46	*P. vulgaris* L. var. *vulgaris* NI 659	9.9	5.9	4.0	3.8	3.1	3.0	6.5	3.4	4	9	2.9	1.6	H-s	S	36	Colombia
47	*P. xanthotrichus* Piper NI 1332	3.4	3.5	1.7	1.0	1.1	1.4	1.8	1.6	5	9,10	0.8	0.7	H-s C	S	19,20,29 M 36	Guatemala
48	*P. xanthotrichus* Piper NI 1558	2.7	2.3	1.4	0.9	0.7	1.3	1.4	1.5	4,14	9,10,31	0.8	0.6	H-s	S	21,23 M 36	Mexico
49	*P. zimapanensis* (Delgado) Jaaska NI 1239	2.5	2.4	1.6	0.8	0.8	1.0	1.4	1.2	5	8,9	0.8	0.7	H-s C	S	20 M 36	Mexico
50	*P. zimapanensis* (Delgado) Jaaska NI 1600	3.4	3.2	1.6	1.1	1.1	1.3	1.9	1.6	5	9,10	0.9	0.7	H-s C	S	20,23 M 36	Mexico

Le: length seed; Wi: width seed; Th: thickness seed; Le.hi: length hilum; Wi.hi: width hilum; D1, D2, D3, D4, D5: see in Materials and Methods; H-s: heart-shaped; H-s C: heart-shaped cracked; H-sfv C: heart-shaped few visible and cracked; S: smooth; Ss: smooth with striae; R: rugulose; M: marbling.

with stereo-microscope, with or without fissure. Fig. 3 shows the direct proportionality between length, width and thicknes of the seeds belonging to fifty *Phaseolus* populations.

An analysis of the morphobiometric data allowed to develop the following identification key for 25 morphotypes (Fig. 4) selected in the study.

1	Rugulose seed coat	**2**
1	Smooth seed coat with striae	**3**
1	Smooth seed coat without striae	**11**
2	Seed Thickness 2.2-2.6	*P. angustissimus* type
2	Seed Thickness < = 2.1	*P. filiformis* type
3	Seed Length > 6.0	**4**
3	Seed Length < 6.0	**10**
4	Bright hilum edge	*P. acutifolius* var. *latifolius* type
4	Dark hilum edge	5
5	Narrowly elliptic hilum shape	**6**
5	Elliptic hilum shape	**7**
5	Obovate hilum shape	*P. lunatus* var. *silvester* type
6	Seed Thickness > 4.1	*P. polyanthus* type
6	Seed Thickness < 4.1	*P. lunatus* var. *lunatus* cv.-gr. "Sieva" type
7	Seed Thickness > 4.9	**9**
7	Seed Thickness < 4.9	**8**
8	Distance between the micropyle and higher end of the seed > 3.0	*P. lunatus* type
8	Distance between the micropyle and higher end of the seed < 3.0	*P. maculatus*
9	Seed Width > 10	*P. lunatus* var. *lunatus* cv.gr. "Big Lima"type
9	Seed Width < 10	*P. lunatus* var. *lunatus* cv.gr."Potato" type
10	Obovate hilum	*P. microcarpus* type
10	Subcircular hilum	*P. leptostachyus* var. *leptostachyus* type
11	Hilum Length > 1.1	**12**
11	Hilum Length < 1.1	**17**
12	Elliptic hilum	*P. vulgaris** type
12	Obovate hilum	**13**
12	Narrowly elliptic hilum	**15**
13	Distance between higher end of the seed and hilar collar near the chalaza ≤ 2.9	*P. grayanus* type
13	Distance between higher end of the seed and hilar collar near the chalaza > 2.9	**14**
14	Seed shape elliptic in lateral view	*P. vulgaris* var. *aborigineus* type
14	Seed shape broadly in lateral view	*P. marechalii* type
15	Distance between higher end of the seed and hilar collar near the chalaza > 5.3	*P. vulgaris* var. *vulgaris* type
15	Distance between higher end of the seed and hilar collar near the chalaza < 5.3	**16**
16	Hilum Length < 2.0	*P. ritensis* type
16	Hilum Length > 2.0	*P. polystachios* var. *polystachios* type
17	Circular hilum	*P. hintonii* type
17	Subcircular hilum	*P. acutifolius* var. *tenuifolius** type
17	Obovate hilum	**18**
17	Elliptic hilum	**19**
18	Seed Length< 2.1	*P. pauciflorus* type
18	Seed Length > 2.1	*P. parvulus* type
19	Seed Thickness > 1.6	*P. pluriflorus* type
19	Seed Thickness < 1.6	*P. micranthus* type

Only two morphotypes include several taxa

*P. vulgaris** morphotype: *P. vulgaris* NI 575, NI613, NI 695, NI 928; *P. coccineus* NI 722, NI 819, NI 1430; *P. glabellus*, *P. oligospermus.*

P. acutifolius var. *tenuifolius** morphotype: *P. acutifolius* var. *tenuifolius* NI 705, NI 1272; *P. acutifolius* var. *acutifolius*, NI 576, NI 851; *P. xanthotrychus* NI 1332, NI 1558; *P. zimapanensis* NI 1239, NI 1600.

The International Biotechnology and Plant Genetic Resources Institute had carried out an international collaboration in 1982 and in 2001 to develop a list of

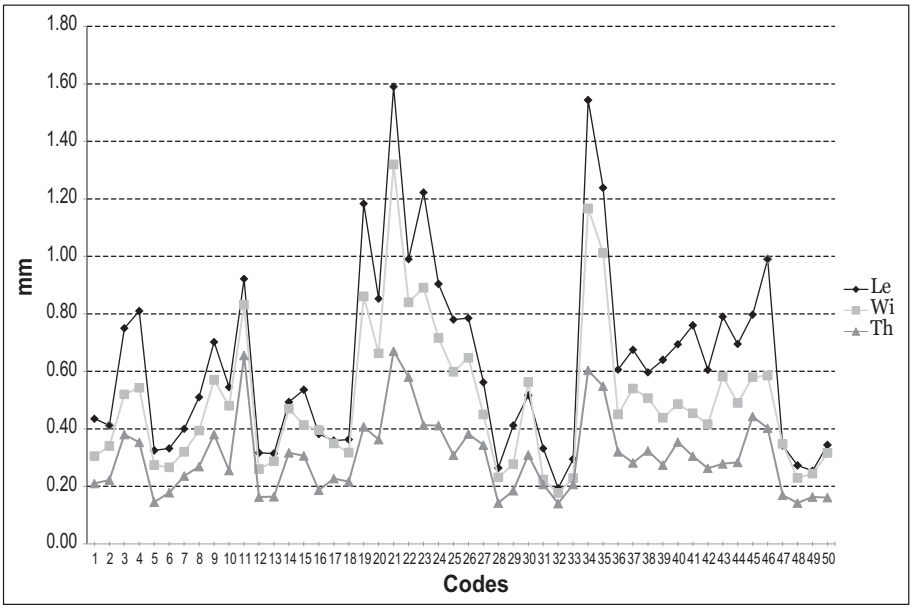

Fig. 3. Proportional dimensions between length, width and thickness of the seeds belonging to 50 *Phaseolus* populations

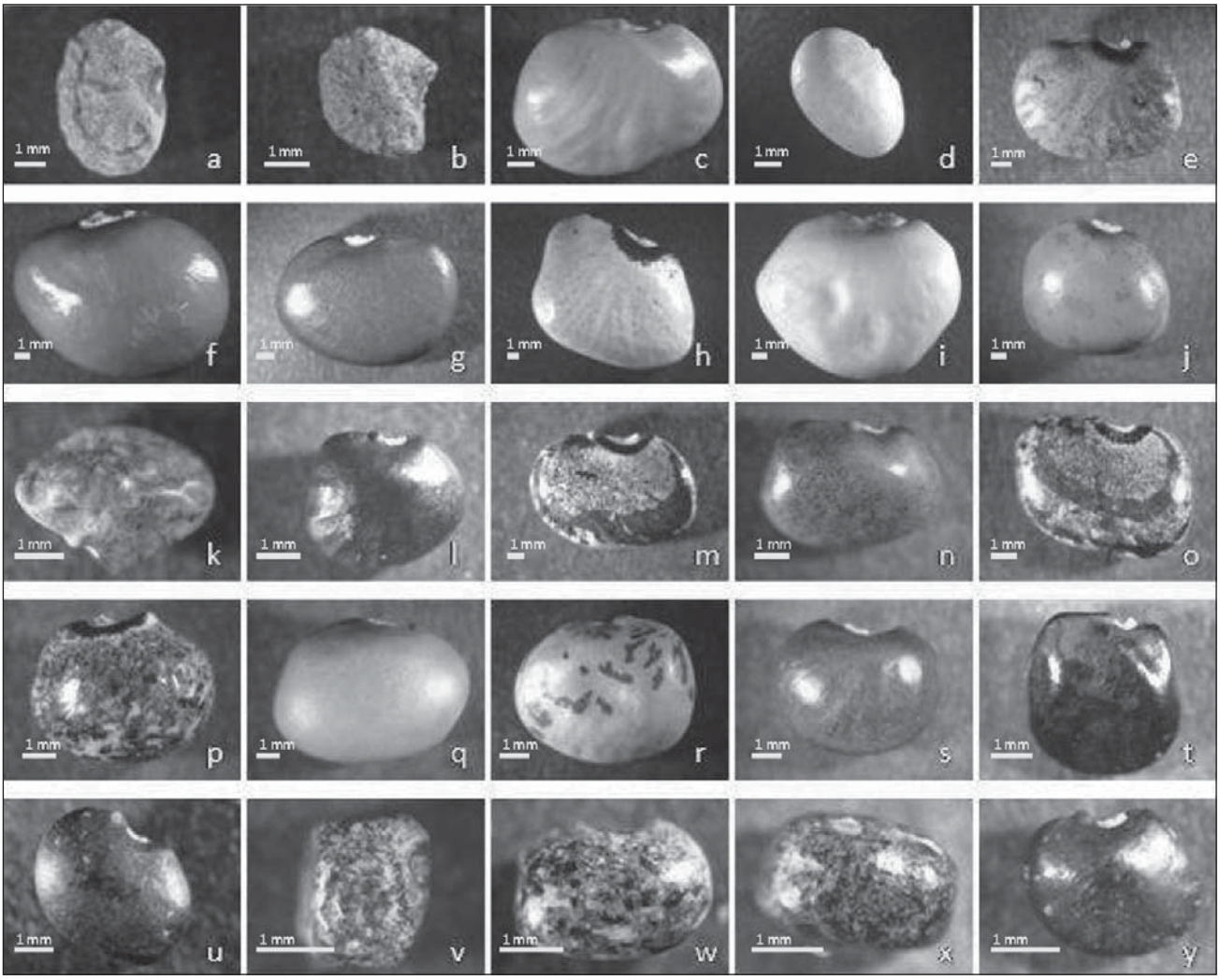

Fig. 4. Stereomicroscope micrographs of *Phaseolus* taxa: a) *P. angustissimus,* b) *P. filiformis,* c) *P. maculatus,* d) *P. acutifolius* var. *latifolius,* e) *P. lunatus* var. *silvester,* f) *P. polyanthus,* g) *P. lunatus* v. *lunatus* cv.gr. "Sieva", h) *P. lunatus,* i) *P. lunatus* var. *lunatus* cv.gr. "Big Lima", j) *P. lunatus* var. *lunatus* cv.gr. "Potato", k) *P. microcarpus,* l) *P. leptostachyus* var. *leptostachyus,* m) *P. vulgaris,* n) *P. grayanus,* o) *P. vulgaris* var. *aborigineus,* p) *P. marechalii,* q) *P. vulgaris* var. *vulgaris,* r) *P. ritensis,* s) *P. polystachios* var. *polystachios,* t) *P. hintonii,* u) *P. acutifolius* var. *tenuifolius,* v) *P. pauciflorus,* w) *P. parvulus,* x) *P. pluriflorus,* y) *P. micranthus*

descriptors for *P. vulgaris* (IBPGR, 1982) and *P. lunatus* (IBPGR, 2001) in order to (i) better understand the genetic variability of germplasm accessions maintained in gene banks, (ii) facilitate the sharing of information among experts, and (iii) promote a more effective use of the genetic diversity of *Phaseolus* around the world.

In a research on the phenotypical characters of *P. vulgaris* ecotypes, McClean *et al*. (2002) have shown that the variability in the colour of the seeds belonging to same or different populations, seems to be regulated by epistatic genes. Casini (2003) reported variable sizes of seeds within *P. vulgaris* from different geographical areas and opined that ecological conditions such as extreme humidity, high temperatures, low sunlight, unbalanced supply of nutrients and poor soil fertility play an important role in causing infra-specific variability. On the basis of form, colour and size of the seeds of *P. vulgaris* and *P. lunatus* Gutiérrez *et al*. (2004) have distinguished six groups in populations present in Andean region of Venezuela.

In this study, we did not detect variability in seed size within the same populations. However, varietal differences were conspicuous in some of the taxa (*e.g. P. acutifolius* var. *acutifolius*, var. *latifolius*, var. *tenuifolius*). With regard to colour, we confirmed the variability within the same population or in distinct populations of the same taxon; whereas the form of seeds was found to be generally elliptical both in the

same population and in distinct populations of the same taxon (three types of elliptic conditions refer to hilum: see number 12 in the key). More rarely, we encountered rhombic shapes (*P. coccineus* NI 1430, NI 859; *P. filiformis*, *P. hintonii* NI 716, NI 809; *P microcarpus*, *P. ritensis* NI 797A), broadly obovate shapes (*P. grayanus* NI 724, *P. ritensis* NI 795) and narrowly oblong shapes (*P. xanthotrichus* NI 1528). These findings are consistent with the work on *P. vulgaris* by Anderberg (1994) who described this species as being characterized by "reniform-ellipsoid shape, spotted, mottled and marbled colour, lustrous, glossy and smooth surface".

Unlike in *Origanum syriacum* L., a non leguminous species (De Leonardis *et al*., 2007), the traits related to the size of the seed (length, width, thickness) were directly proportional to the dimensions of all the other features investigated such as hilum and chalaza (Fig. 3). Seed size in cultivated crops is known to be usually greater than their wild taxa (De Leonardis *et al*., 1993; 1996). Our data also confirm the expected increased seed size of cultivated taxa (*P. acutifloius*, *P. coccineus*, *P. lunatus*, *P. polyanthus*, *P. vulgaris*) compared with wild taxa (Table 1).

The study allowed the development of an identification key composed of 23 morphotypes which are ascribable to specific taxa along with other two morphotypes that were ascribable to more taxa. If we consider the surface tegument of the seeds (result of genetic expression) the study confirms the uniformity in the expression of this trait in populations of the same taxon even when present in different habitats. These findings led us to cluster the taxa examined in three groups: 1) *P. angustissimus* group (rugulose seed coat) that includes two morphotypes, 2) *P. lunatus* group (tegument smooth with striae) which brings together 10 morphotypes; 3) *P. vulgaris* group (smooth seed coat without striae) that includes 13 morphotypes.

Based on our seed coat data, *P. lunatus* (smooth with striae) and *P. polystachios* (smooth without striae) are found in distinct taxonomic groups, in accordance with the work of Delgado-Salinas *et al*. (2006), but in disagreement with that of Freytag and Debouck (2002). Our observations demonstrate that *P. maculatus* and *P. ritensis* are morphologically distinguishable entities According to Freytag and Debouck (2002) only *P. maculatus* can be separated from the group containing *P. polystachios* species because of its seminal tegument.

P. vulgaris and *P. lunatus* appear to be taxonomically distant entities, findings which are not in agreement with what reported earlier in literature (Degreef & Baudoin, 1996; Duran *et al*., 2005; Delgado-Salinas *et al*., 2006; Sammour *et al*., 2007). The taxonomic affinities among these species reported by Marechal *et al*. (1978), based on a vast morphological study of the *Phaseolus-Vigna* complex, are partially validated in this study. Their study has shown that (i) the similarity between *P. vulgaris*, *P. coccineus* and *P. glabellus*, (ii) the taxonomic distance of the *Phaseolus-Vigna* complex from *P. lunatus*, (iii) the affinities between *P. angustissimus* and *P. filiformis* (both with rugulose seed coat) and (iv) the intermediate position of *P. acutifolius* with the complex *P. vulgaris-P. coccineus* and *P. lunatus*. However, our study demonstrates a greater affinity between *P. vulgaris*, *P. polystachios* var. *polystachios* and *P. ritensis* and between *P. lunatus* and *P. polyanthus*. The elaboration of the identification key based on morphobiometric characters and/or grouping into three affinity groups based on type of sculpture of tegument have allowed the identification of morphotypes that could be useful in biosystematic studies and for assisting breeders in marker-guided genetic selection of *Phaseolus*.

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Meiotic Chromosome Studies in the Diversity Diagnostics of *Cyamopsis tetragonoloba* Accessions – an Important Gum-Yielding Plant of Indian Thar Desert

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Cyamopsis tetragonoloba (Linn.) Taub (Fabaceae) is an important commercial crop of India. It is a multipurpose including gum-yielding, drought tolerant, kharif pulse crop grown on light textured soils of arid and semi arid regions. *C. tetragonoloba* is cytogenetically poorly understood. The 15 accessions originating from various regions of India, were investigated to ascertain the nature and extent of genotype differentiation, if any, in the meiotic details at diplotene, diakinesis, metaphase I, anaphase I and anaphase II. The accessions did show suitable variations with respect to meiotic pairing properties and recombination index. Eight accessions, for example, were well differentiated from the remaining seven accessions by the presence of quadrivalent(s) in otherwise diploid species and so on. The present input will have an important role in the improvement programme of the crop.

Key Words: Accessions, Chaisma frequency, Chromosome associations, *Cyamopsis tetragonoloba*, Variation

Introduction

Cyamopsis (Fabaceae) comprises four species. *C. tetragonoloba*, commonly known as cluster bean or *'guar'*, is an important grain legume known for its diverse uses. The demand for the produce and products of this crop species is increasing by leaps and bounds (Bewal *et al*., 2009). *Guar* is chiefly used for vegetable, animal feed, food additive, medicinal, gum/resin and other diverse purposes. It is also used as thickener and stabilizer in cheese and in cold- meat processing, and in salad dressings, ice-creams, lollipops, bakery products, meat and sausages. *Guar* Gum when used in oil well drilling muds gives a better colloid thereby reducing water losses. It regulates the viscosity of mud solution, stabilizes and regulates the flow properties of the drilling muds. The commercial quality *guar* gum can also be modified into derivatives of industrial importance (Sharma *et al.*, 2003). Despite the multipurpose importance of *Cyamopsis*, there is still paucity of information with regard to cytogenetic approach, such as meiotic behavior of germplasm resources. The details about degree of pairing and types of associations, recombination index and disjunction of chromosome/chromatids at anaphase I/II in various accessions can provide information on subtle differences within species, if any (Kumar *et al*., 2002; Kumar and Rao, 2002; Kumar and Rao, 2003). It is universally known that various stages of meiosis are genetically controlled (Zickler and Kleckner, 1998; 1999). Disturbance in the synaptic process due to various kinds of mutation, has been a subject of vast studies in many plant species (Bennett, 1984; John, 1990; Maguire and Reiss, 1996; Dave, 1998). The present work is concerned with species cytogenetics particularly with regard to the insight into extent and nature of genotype differentiation on the basis of meiotic behavior properties.

Materials and Methods

The seeds of different accessions (Table 1) of *Cyamopsis tetragonoloba* were obtained from the Division of Plant Improvement and Biotechnology, Central Arid Zone Research Institute, Jodhpur, Rajasthan. Flower buds of appropriate size were collected from mature plants. The buds were fixed on the spot in freshly prepared 1:3 glacial acetic acid: 95% ethanol mixture for at least 24 h at room temperature. These were subsequently transferred to 70% ethanol and stored at 10°C.

Anthers were squashed in 1% acetocarmine. On an average, 20-25 pollen mother cells (PMCs) were analyzed at diplotene/diakineses and metaphase I. During compilation of the meiotic data, it was observed that only minor differences existed between diakinesis

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Table 1. Mean number and range of chromosome associations at diplotene/diakinesis/metaphase I in *Cyamopsis tetragonoloba* accessions

Accession number	No. of cells analyzed	2n	Chromosome associations												Univalents		
			Quadrivalents			Bivalents											
						Total			Ring			Rod					
			No	Mean	Range	No	Mean	Range	No	Mean	Range	No	Mean	Range	No	Mean	Range
IC-370468	25	14	1	0.04±0.04	0-1	158	6.32 ±0.6	6-7	148	5.92±1.03	5-7	10	0.4±0.64	0-2	26.00	1.04±1.09	0-2
IC-373467	25	14	6	0.24±0.43	0-1	133	5.32±1.28	3-7	122	4.88±0.89	3-7	11.00	0.44±0.50	0-1	30.00	1.2±086	0-2
IC-40039	25	14	1	0.04±0.2	0-1	167	6.68±0.47	0-7	156	6.24±0.92	3-7	10.00	0.4±0.5	0-1	16.00	0.64±0.94	0-2
IC-40045	25	14	–	–	–	171	6.84±0.37	6-7	167	6-68±0.62	6-7	4.00	0.16±0.3	0-1	8.00	0.32±0.7	0-2
IC-368961	25	14	–	–	–	175	7.00 ± 0.31	–	171	6.84± 0.46	5-7	4.00	0.16± 0.46	0-2	0.00	0.00	0-0
SRV10JT14		14	–	–	–	172	6.88± 0.32	6-7	164	6.56± 0.57	5-7	8.00	0.32± 0.46	0-1	6.00	0.24± 0.6	0-2
SRV11JT15		14	–	–	–	172	6.88± 0.32	6-7	170	6.80± 0.4	6-7	2.00	0.80± 0.27	0-1	6.00	0.24± 0.64	0-2
SRV13JT17		14	6	0.24±0.43	0-1	133	5.32±1.28	3-7	122	4.88±0.89	3-7	11.00	0.44±0.50	0-1	30.00	1.2±086	0-2
SRV28JT32		14	6	0.24±0.59	0-2	147	6.12±1.05	4-7	137	5.48±1.63	2-7	12.00	0.48±077	0-2	36.00	1.401±2.04	0-6
SRV29JT32		14	8	0.32±0.47	0-1	133	5.32±1.78	2-7	129	5.16±1.15	3-7	12.00	0.48±0.71	0-2	34.00	1.36±1.60	0-4
SRV31JT35		14	–	–	–	175	7.00± 0.00	–	170	6.80± 0.4	6-7	5.00	0.20± 0.4	0-2	0	0.00	0-0
PLG-520		14	1	0.04±0.2	0-1	167	6.68±0.47	5-7	156	6.24±0.92	3-7	10.00	0.4±0.5	0-1	16	0.64±0.94	0-2
PLG-600		14	6	0.24±0.43	0-1	171	6.84±0.37	6-7	167	6-68±0.62	6-7	4.00	0.16±0.3	0-1	8.00	0.32±0.7	0-2
PLG-747		14	–	–	–	175	7.00± 0.00	–	157	6.28± 0.72	4-7	18.00	0.72± 0.72	0-3	0	0.00	0-0
PLG-752		14	–	–	–	133	5.32±1.78	5-7	129	5.16±1.15	3-7	12.00	0.48±0.71	0-2	34.00	1.36±1.60	0-4

and metaphase I stages with respect to associations and chiasma frequency. Therefore, the authors have analyzed the data for both these meiotic stages together, taking approximately equal number of cells from both the stages. About 15-20 PMCs were analyzed at anaphase I and/or II. For percentage pollen stainability, the pollen grains were stained in 1:1 glycerine: acetocarmine mixture. On an average, 10 slides were scored for stainable pollen. Photomicrographs of chromosome preparations were taken from temporary slides with Trinocular Research Microscope (Olympus, model BX60F).

Results and Discussion

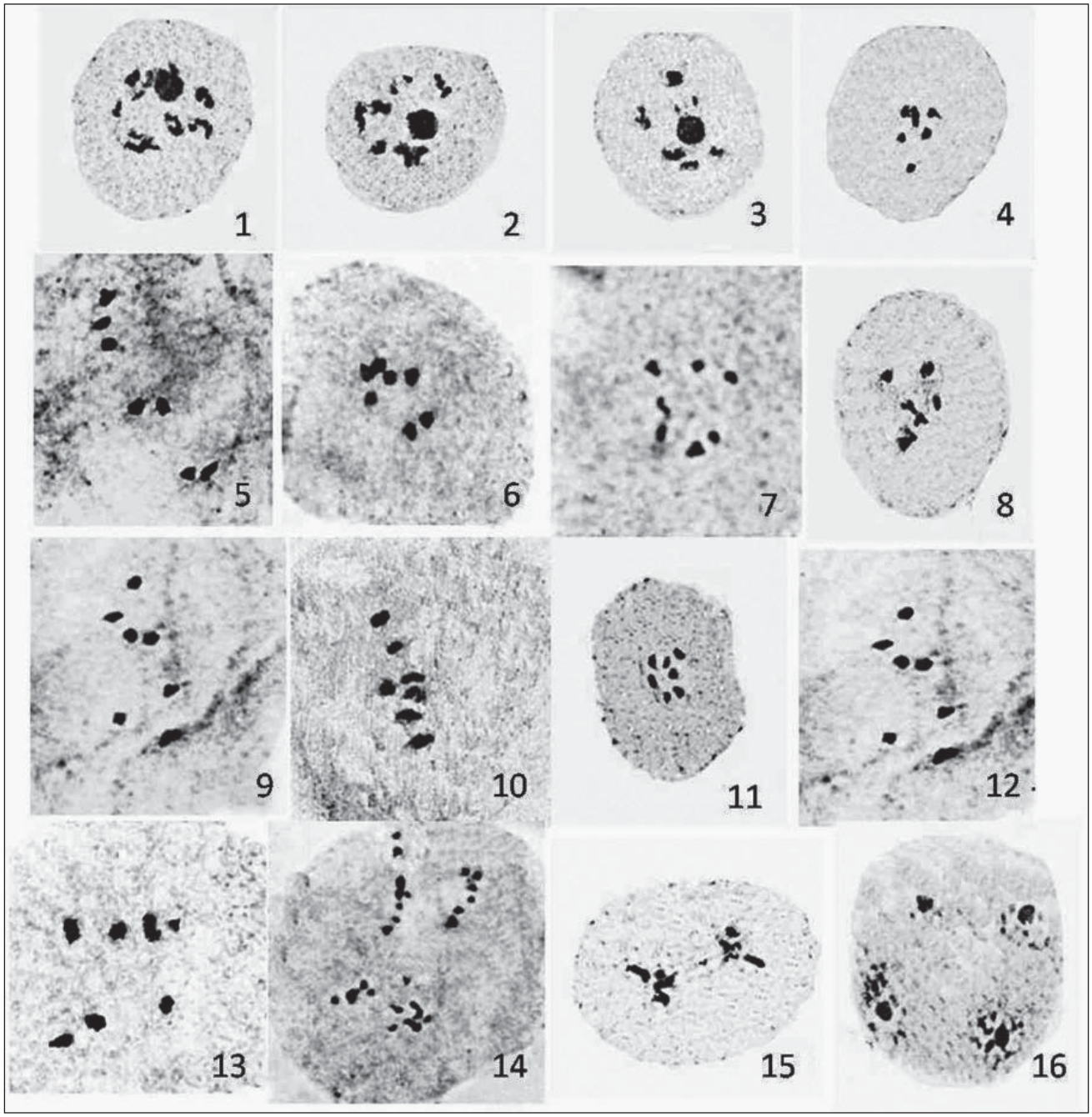
The details with regard to chromosome associations at diplotene/diakinesis/metaphase I, recombination index and distribution of chromosomes at respective poles and pollen stainability of the analyzed accessions are given in Tables 1-3 and illustrated in Figs 1-28.

From Table 1, it is amply clear that all the accessions presently investigated were characterized by gametic number of 7 (n=7). No deviation with respect to this number (n=7, 2n=14) has been encountered in any of the 375 PMCs analyzed for 15 accessions. In ascertaining the true basic number of the genus, it is essential to know the chromosome number of all the species. The perusal of literature indicates that the chromosome number of only two (*C. tetragonoloba*, 2n=14 and *C. psoroloides*, 2n=14) out of the four species is known. Based on the chromosome number reported for the two species and the meiotic behavior of the presently investigated species, it can be stated that 7 could be the true basic number of the genus.

Barring three accessions (*C. tetragonoloba* IC-368961, SRV31JT35 and PLG-747), all the remaining accessions had a mixture of bivalents and univalents at diplotene/ diakinesis/ metaphase I. The three accessions have 7 bivalents in all PMCs analysed. The least (5.32) number of bivalents per PMC (76%) was observed in the four accessions, *viz.* IC-373467, SRV13JT17, SRV29JT32 and PLG-752. The highest number (1.40) of univalents per PMC was recorded in *C. tetragonoloba* SRV28JT32 followed by 1.361 in SRV29JT32 and PLG-752. Such variation of chromosome association across various accessions is not uncommon in the plant species from Thar desert (Kumar *et al*., 2002; Rao and Kumar, 2003; Kumar and Rao, 2003; Rawat *et al.*, 2006, Rawat *et al*., 2007). The presence of univalents in various PMCs, by and large, did not influence the distributional pattern of chromosomes at anaphase I in, 13 out of the 15 accessions, presently

Table 2. Mean number, range of chiasmata and terminalization coefficient and pollen stainability

Accession	No. of cell analysed	2n	Chaismata									Terminilized coefficient	Pollen stainability percentage
			Total			Terminalised			Unterminalised				
			No.	Mean	Range	No.	Mean	Range	No.	Mean	Range		
IC-370468	25	14	317	12.68±1.5	0 – 14	231	9.24± 1.3	0 – 14	86	3.2± 1.7	7-1	0.73	94
IC-373467	25	14	311	12.44± 1.40	11 – 14	246	9.84±3.17	4– 13	65	2.6± 1.80	0-7	0.79	92
IC-40039	25	14	324	12.96± 1.05	11 – 14	253	10.00± 2.26	5 – 13	71	2.91± 1.90	0-6	0.78	88
IC-40045	25	14	338	13.52± 0.90	12 – 17	265	7.84± 2.09	4 – 11	160	6.40± 1.89	3-9	0.78	90
IC-368961	25	14	356	14.24± 0.90	12 – 17	196	7.84± 2.09	4 – 11	160	6.40± 1.89	3-9	0.81	92
SRV10JT14	25	14	343	13.72± 0.87	11 – 15	201	8.04± 2.39	4 – 12	142	5.68± 2.34	1-11	0.70	94
SRV11JT15	25	14	354	14.16± 1.40	12 – 16	200	8.00±2.17	3 – 11	154	6.16± 1.80	3-11	0.77	88
SRV13JT17	25	14	317	12.68±1.5	0 – 14	231	9.24± 1.3	0 – 14	86	3.2± 1.7	7-1	0.72	93
SRV28JT32	25	14	300	12.00± 2.46	7 – 14	217	8.68± 2.41	3 – 11	83	3.32± 1.84	1-7	0.73	96
SRV29JT32	25	14	312	12.48±200	0 8-14	229	9.16 ±2.46	7-13	83	3.2±1.84	1-6	0.90	94
SRV31JT35	25	14	324	12.96± 1.05	11 –14	253	10.00± 2.26	5 – 13	71	2.91± 1.90	0-6	0.74	90
PLG-520	25	14	343	13.72± 0.87	11 – 15	201	8.04± 2.39	4 – 12	142	5.68± 2.34	1-11	0.70	92
PLG-600	25	14	351	14.04±0.87	13 – 15	184	7.36± 1.80	3 – 11	167	6.68± 2.07	2-11	0.90	89
PLG-747	25	14	342	13.68± 0.88	12 – 15	146	5.84± 2.37	1 – 9	146	5.84± 2.32	1-9	0.74	98
PLG-752	25	14	356	14.24± 0.90	12 – 17	196	7.84± 2.09	4 – 11	160	6.40± 1.89	3-9	0.81	96



Figs. 1-28: Male meiosis in *Cyamopsis tetragonoloba* accessions
Figs. 1-3: Diakinesis 7II (IC-373467); Fig. 4: Metaphase I 7II (IC-373467); Fig. 5: Metaphase I 7II (IC-370468); Figs. 6-7: Metaphase I 7II (IC-40039); Fig. 8: Metaphase I 7II (SRV29JT32) ; Figs. 9 and 12: Metaphase I 7II (SRV10JT14); Fig. 10: Metaphase I 7II (SRV11JT15); Figs. 11 and 1: Metaphase I 7II (SRV28JT32); Fig. 13: Metaphase I 7II (SRV31JT35); Fig. 14: Anaphase II (SRV31JT35); Fig. 15: Anaphase I (SRV28JT32); Fig. 16: Anaphase II (SRV13JT32).

analyzed. However, in two accessions the distribution was affected leading to occurrence of lagging univalent/ bivalents in a few PMCs. Precocious separation of rod bivalents or early separation of synapsed homologues is generally the reason for regular occurrence of univalents in many of the tree species (Kumar, 2000; Singh, 1993). However, the present observations in *Cyamopsis tetragonoloba* indicate that the probability of existence of heterozygosity among few bivalents might be the reason for formation of univalents. In five accessions (IC 373467,

Table 3. Anaphase I distribution

Accession number	2n	No.of cell analysed	Chromosome distribution	No. of cells	Percentage
IC-370468	14	15	7:7	15	100
IC-373467	14	15	7:7	14	93.3
			7:1U:6	1	6.6
IC-40039	14	15	7:7	15	100
IC-40045	14	15	7:7	15	100
IC-368961	14	15	7:7	15	100
SRV10JT14	14	15	7:7	15	100
SRV11JT15	14	15	7:7	15	100
SRV13JT17	14	15	7:7	15	100
SRV28JT32	14	15	7:7	15	100
SRV29JT32	14	15	7:7	15	100
SRV31JT35	14	15	7:7	15	100
PLG-520	14	15	7:7	15	100
PLG-600	14	15	7:7	13	100
			6:1U:7	1	
			7:1U:6	1	
PLG-747	14	15	7:7	15	100
PLG-752	14	15	7:7	15	100

IC 373467, SRV29 JT32 and SRV28JT32), 1-2 bivalents at diplotene were closely associated with the nucleolus thereby indicating that 1-2 pairs of nucleolar chromosomes exist in the species.

The highest and lowest recombination index was recorded in four and one accessions, respectively. In general, two chiasma per bivalent was the rule. In most of the cases, the chiasmata was found to be located at distal ends. Interstitial chiasma were generally absent even at diplotene. Due to the fact that no bivalent had more than two chiasma makes it difficult to assume random distribution of chiasmata. Distal chiasmata prevents some chromosome segments in bivalents from undergoing recombinations resulting in preservation of specific gene combinations intact (John and Lewis, 1965; Sybenga, 1972; Singh, 1993; Gupta, 1995). Occurrence of distally localized chiasmata has been attributed to gene control, nature of chromosome pairing and/or interference pattern or even the availability of short segment for crossing over (Kumar, 2000; Stace, 2000; Kumar and Rao, 2002).

All but two accessions were characterized by the equal (7:7) distribution of chromosomes at anaphase I. Subsequent course of meiosis was normal resulting in good pollen stainability. Two accessions showed unequal (7:6) distribution of chromosomes at anaphase I in one cell each. The existence of univalent(s) and (or) multivalents is considered to be main reason for abnormal distribution of chromosomes at anaphase I (Stebbins, 1971; Sybenga, 1972; Singh, 1993; Gupta, 1995; Rao and Chandel, 1991; Kesavacharyulu, 1988; Arya *et al.*, 1987; Rao and Kumar, 2003; Kumar *et al*., 2002; Rawat *et al.*, 2007).

Three accessions were isolated from the remaining accessions by the presence of one quadrivalent in a few cells. The reason(s) for the formation of quadrivalents in the diploid cytotype is not clear.

Bewal *et al*. (2009) have induced colchitetraploidy in few accessions of *Cyamopsis tetragonoloba* and reported widespread occurrence of quadrivalent formation in PMCs analyzed. However their putative diploids were characteristic in not showing the presence of quadrivalents in them. Therefore, the sporadic occurrence of quadrivalent in diploids, may be attributed to plausible partial homology between otherwise non homologous chromosomes arising out of structural rearrangements (Stebbins, 1971).

Thus, it is concluded that chromosome structural changes rather than numerical changes have resulted in intra-specific diversity in *Cyamopsis tetragonoloba*.

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Characterization of Exotic Commercial Hybrid Clones of Sugarcane

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Fifteen exotic commercial hybrids were characterized for their morphological, agronomic and quality traits. The study showed wide morphological variation among the clones, though they are commercial canes developed for improved traits, including yield. For the agronomic data, the difference was significant only for the number of millable canes, HR brix, cane length and yield. Five clones had HR Brix value at 7th month (> 21%) indicating it's potential as a source for early high sucrose accumulation. One clone showed sucrose above 20% (SP 83-5073) at maturity and it may be a potential parent for improving the juice quality. Only one clone (SP 80-1816) was found yield on par with the best check Co 62175 for CCS yield/plot, indicating that most of the clones were not adapted to the local environment.

Key Words: Characterization, Exotic commercial hybrids, Germplasm, Sugarcane

Introduction

Sugarcane Breeding Institute, Research Centre, Kannur (Cannanore) has the mandate of maintaining and evaluating the germplasm of sugarcane, including commercial hybrids. Several catalogues were brought out as a result of elaborate characterization and evaluation of the world collection of sugarcane germplasm (Kandasami *et al*., 1983; Ramana Rao *et al*., 1985; Sreenivasan and Nair 1991; Nair and Sreenivasan, 1995; Nair *et al*., 1997; Sreenivasan *et al*., 2001a, 2001b; Balasundaram *et al*., 2005). Since1962, the world collection of sugarcane germplasm is maintained at this Centre. Apart from enhancing the indigenous germplasm collections made from within the country, the commercial hybrid germplasm was also enhanced by introducing more hybrid clones from other countries. During 2000-2001, 15 new clones were added to the collection *viz.*, eight SP hybrids from Brazil, six CP hybrids from USA and one hybrid from China. For understanding the adaptability and for effective utilization of the germplasm, extensive characterization of the newly introduced hybrid clones was carried out.

Materials and Methods

Fifteen commercial hybrid clones (Table 1) were grown in an augmented block design of 6 ft rows with an inter-row spacing of 90 cm. The test clones were plated in three blocks with two checks (Co 62175 and Co 8231) in each block. The crop was raised for three consecutive crop seasons, starting from 2004. The crop was planted in February and harvested in the month of January. The soil is sandy loam with a pH of 5.7 and the temperature ranges between 18º C and 36º C. The annual rainfall ranged from 2,852 to 3,695 mm during the period and the crop was properly irrigated with good quality water during summer months. Recommended packages and practices were followed for maintenance. Morphological observations were recorded during the 9th month in 2004-05 based on the descriptors by Artschwager and Brandes, 1958 (Table 2). Pollen fertility was studied by acetocarmine staining. The yield data were recorded from five canes each in all the three years (2005-07) during 11th month and the average was computed over the years. ANOVA is calculated using the online software for Augmented block analysis.

Results and Discussion

The clones characterized showed wide variation despite the fact that these clones are commercial hybrids selected from the population for desirable traits. Probably the

Table 1. Passport information of the hybrid clones

Clone	Source	Parents
CP 57-614	Canal Point, USA	CL 47-143xCP 53-17
CP 70-1133	-do-	CP 56-63 (poly cross)
CP 72-2086	-do-	CP 62-374xCp 63-588
CP 78-2114	-do-	Unknown
CP 79-318	-do-	CP 65-357X L65-69
CP 80-1827	-do-	CP 70-1133x CP 73-1311
Yuetang 85-177	China	Not available
SP 79-2233	Sao Paulo, Brazil	H 56-2954 (poly cross)
SP 80-185	-do-	BO 17 (poly cross)
SP 80-1816	-do-	SP 71-1088xH57-5028
SP 80-1842	-do-	SP 71-1088xH57-5028
SP 80-3280	-do-	SP 71-1088xH57-5028
SP 81-1763	-do-	Not available
SP 81-3250	-do-	CP 70-1547x SP 71-1279
SP 83-5073	-do-	SP 71-1406x SP 71-1088

Table 2. Descriptors and descriptor states for morphological characters

Descriptor(s)	Descriptor state(s)
Plant height	0-9 scale
Tillering	0-9 scale
Rind stripe	ST- striped, NS- Non striped
Rind colour (exposed and freshly exposed)	BP- Brownish purple, P- Purple, LP- Light purple, DP-Dark purple, YG- Yellowish green, GB- Greenish brown, GY- Greenish Yellow, LG- Light green, YB- yellowish brown, LG- Light green, GP Greenish purple, CRY-Creamy yellow, Y-Yellow, PG- Purplish green
Root band colour (exposed & freshly exposed)	-do-
Internode wax	NP- Not prominent, P-Prominent, VP- Very Prominent
Growth crack	A- Absent, FS- Few Shallow, FD- Few Deep
Internode shape (major & minor)	CYL- Cylindrical, OBC- Obconical, BOB- Bobbin shaped, CON- Conical, CUR- Curved
Internode alignment	ST- Straight, ZIG- Zig-zag
Internode cross section	R- Round, OV- Oval
Piping of stem	S- Solid, SLH- Slightly hollow, H- Hollow
Pithiness of stem	NP- No pith, SLP- Slightly pithy, PITH- Pithy
Wax band shape	FU- Funnel shaped, CON- Conical
Bud groove	A - Absent, IND- indicated, SS- Short shallow, DL- Deep long
Node swelling, root band swelling	NS- Not swollen SSW- Slightly swollen, SW- Swollen,
Root band shape	TUM- Tumultant, CYL- Cylindrical, OBC, Obconical,
Root eye alignment	IR-Irregular, R-Regular
Bud shape	OVT- Ovate, SQPE- Squarish pentagonal, RC- Round with central pore, RW- Round with wings
Bud size	MS- Medium small, MB- Medium Bold, SB- small bold, LB- Large Bold, MF- Medium flat
Bud extension	B-Below growth ring, T-Touching the growth ring
Bud germ pore	SA-Sub apical, C- Central
Canopy	OSD- Open semi droopy, OE-Open erect, OTD- Open tip droopy, FTD- Fans shaped tip droopy, CTD Compact tip droopy, FE- Fan erect,
Leaf sheath clasping	M- Medium, L- Loose
leaf sheath colour	G- Green, GPT- Green with purple tinge,
leaf sheath wax	M- Medium. L- Low, H- High, VH- Very high
leaf sheath scaries, wax band width	N- Narrow, M- Medium, W- Wide
leaf colour	G- Green DG- Dark green
Wax band, Root band, Corky cracks, Corky patches, Bud cushion, Hair group	P- Present, A- Absent

diverse environmental factors, diverse parents and different breeding conditions might have contributed to the inheritance of diverse traits into their progenies resulting in variation for morphological characters. All the clones showed good agronomical traits also as a result of selection for better yield. In general clones were medium tall or tall types with moderate to high tillering. Internode thickness ranged from 2.1 cm (SP 83-5073) to 3.2 cm (Yuetang 85-177) and nodal thickness from, 2.2 cm to 3.0 cm. All clones except CP 57-614 were having non striped rind. The exposed rind colour was ranging from yellowish green to dark purple and freshly exposed rind colour was yellowish green to light purple. Though rind colour is a quantitative character that is generally influenced by the environment, it is widely used in sugarcane for identifying and describing the commercial varieties. The rind wax was very prominent in eight clones. Growth cracks are one of the undesirable characters for commercial canes. CP 72-2086, CP 78-2114, SP 80-1816, SP 80-1842, SP 80-3280 and SP 81-3250 had few deep cracks and in CP 80-1827 few shallow cracks were observed. In all other clones, growth cracks were absent.

The predominant internode shape recorded was cylindrical, while in CP 72-2086 and CP 79-318 it was bobbin shaped, and in SP 79-2233, SP 80-185, SP 80-185 and SP 81-3250 it was conical. The stem of Yuetang 85-177 and CP 80-1827 did had pith, and in others the stem was having slight pith or had broad pith as in CP

72-2086, CP 78-2114 and SP 81-3250. The internode cross section of all CP varieties was round except in CP 79-318 where it was oval. Most of the SP clones showed oval cross section, except SP 79-2233, SP 81-3250 and Yuetang 85-177 where in it was round.

Prominent wax band was present in all the clones. In most of the clones it was funnel shaped while in SP 80-185, SP 80-1816 and SP 80-1842, it was conical. Root band colour was ranging from yellow to purple and root band width ranged from 4.3 cm (SP 81-1763) to 8.0 cm in CP 57-614. The number of root eyes was irregularly arranged in two rows in most of the cases except in SP 81-3250, CP 78-2114, CP 72-2086 and CP 57-614 in which it was in three rows. Bud size was medium small (CP 57-614) to large bold as in CP 79-318 with either ovate, squarish pentagonal or rhomboid in shape. The other bud characters *viz.*, bud groove, bud cushion, bud extension, bud germ pore, bud hair group 10 and 26 also showed variation among the clones (Table 3). The blade carriage determines the look of the variety in the field. The clones showed blade carriage ranging from compact erect (SP 81-1763) to open semi-droopy (CP 57-614).

Most of the clones had green or greenish purple leaf sheath or green sheath with purple tinge. Sheath wax was another character that was very prominent in most of the clones. High leaf sheath wax was observed in SP 80-1842 and SP 83-5073. Leaf sheath scaries was narrow in CP 78-2114, SP 80-185, SP 80-1842 and SP 81-1763 and in others it was wide. Leaf colour was green on all the clones, except Yuetang 85-117 where it was dark green. Hairiness of leaf blades and sheath can be used effectively for characterizing sugarcane clones. In sugarcane though leaf blade marginal hair group 53 is always present, the dorsal patch of hair on leaf sheath (hair Group 57) generally distinguishes clones on the basis of it extent (Moore,1987). HG 57 was dense in SP 80-1816, CP 80-1827 and CP 70-1133 and in other it was absent or slightly present whereas HG 56 was absent in all the clones. Flowering is an essential trait required for utilizing the clones for conventional breeding programme. In the germplasm collection of exotic hybrids, on an average 50% of the clones only flowers regularly. Of the 15 clones evaluated, 3 clones (SP 79-2233, SP 80-1842, and SP 81-1763) did not flower and other clones flowered in the month of November, from first week to fourth week. Pollen fertility ranged from 10-94% among the flowering clones. The detailed morphological traits are given in Table 3.

The yield evaluation of the clones revealed the adaptability of the introduced clones to the new environment. The variation for yield data was significant for number of millable canes, HR brix, cane length and yield (Table 4). The adjusted mean of yield data from augmented block design analysis are given in table 5. The number of millable canes was highest in SP 81-3250 and followed by SP 80-3280. Five clones CP 70-1133, CP 79-318, CP 80-1827, SP 80-1842 and SP 83-5073 had HR Brix value at 7^{th} month (> 21) indicating its potential as a source for early high sucrose accumulation. Among the early high brix clones, CP 70-1133 and CP 79-318 were having pollen fertility above 60 per cent which can be used as male parent and SP 83-5073 with low pollen fertility (16%) as female parent. However, intervention of artificial induction of flowering may be required in the case of SP 80-1842 for utilization in breeding programme as it did not flowering under normal condition. As the heavy rainfall coincides with the formative phase of the sugarcane at the evaluation site, the cane thickness is generally low and it ranged from 1.91 (CP 78-2114) to 2.65 cm in CP 80-1827. Single cane weight ranged from 0.58 kg (SP 79-2233) to 1.08 kg in SP 80-1816. At maturity the brix value ranged from 17.8 to 22.0 (SP 83-5073). Only one clone showed sucrose above 20% (SP 83-5073) at maturity and it may be a potential parent for improving the juice quality. Only one clone (SP 80-1816) was found yield on par with the best check Co 62175 for CCS yield/plot, indicating poor adaptability of the clones to the local environment with high rainfall and excess soil moisture condition.

Extensive evaluation is the first step for utilization for the germplasm. This study has revealed the potential of the introduced clones for different morphological, yield and quality traits which will be very useful for the crop improvement programme in sugarcane. The morphological characterization of the clones not only help to distinguish the clones and describe, but also enable the breeder to identify and select for morphological trait associated with more complex heritable processes such as stress resistance, disease resistance and yield (Rosario *et al.*, 1978; Mc David and Midmore, 1980).

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Table 3. Morphological traits of 15 exotic hybrids

		CP 57-614	CP 70-1133	CP 72-2086	CP 78-2114	CP 79-318	CP 80-1827	Yuetang 85-177	SP 79-2233	SP 80-185	SP 80-1816	SP 80-1842	SP 80-3280	SP 81-1763	SP 81-3250	SP 83-5073
1	Plant height (0-9 scale)	7	6	5	7	5	5	6	7	7	8	7	7	7	6	7
2	Tillering (0-9 scale)	5	6	3	7	5	5	4	6	5	7	6	7	7	7	8
3	Internode thickness (cm)	2.6	2.6	2.7	2.6	2.6	3.1	3.2	2.2	2.5	2.3	2.2	2.5	2.5	2.4	2.1
4	Node thickness (cm)	2.6	2.6	2.7	2.6	2.9	2.7	3.0	2.7	2.4	2.4	2.2	2.3	2.5	2.6	2.2
5	Rind stripe	ST	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
6	Rind colour exposed	BP	P	YG	BP	PG	LP	LP	BP	P	DP	LP	DP	YG	GB	DP
7	Rind colour freshly exp.	GY	LP	LG	CRY	LP	YG	YB	LG	GP	LP	LG	LP	GY	LG	YG
8	Internode wax	VP	VP	NP	P	VP	P	P	P	VP	VP	VP	VP	NP	VP	VP
9	Root band	P	P	P	P	P	P	P	P	P	P	P	P	P	P	A
10	Corcky cracks	P	P	P	P	A	P	A	A	A	A	A	A	P	P	A
11	Corky patches	P	P	P	P	P	P	P	A	P	P	A	P	P	P	P
12	Growth crack	FD	A	FD	FD	A	FS	A	A	A	FD	FD	FD	A	FD	A
13	Internode shape major	OBC	CYL	BOB	CUR	BOB	CYL	CON	CON	CON	CYL	CYL	CYL	CYL	CON	CYL
14	Internode shape minor	CYL	CYL	CYL	CON	CYL	CYL	CON	CYL	CYL	CYL	CYL	CYL	BOB	CYL	BOB
15	Internode alignment	ST	ST	ZIG	SZIG	ST	ZIG	ST	SZIG	ST	ST	ST	ST	ST	ST	ST
16	Internode cross section	R	R	R	R	OV	R	R	R	OV	OV	OV	OV	OV	R	OV
17	Piping of stem	S	S	H	SLH	H	SLH	SLH	SLH	SLH	S	SLH	SLH	SLH	H	S
18	Pithiness of stem	SLP	SLP	PITH	PITH	SLP	NP	NP	SLP	SLP	SLP	SLP	SLP	SLP	PITH	SLP
19	Wax band	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
20	Wax band shape	FU	FU	FU	FU	FU	FU	FU	FU	CON	CON	CON	FU	FU	FU	FU
21	Wax band width	M	W	W	W	W	W	W	W	W	W	W	W	W	W	W
22	Bud groove	A	SS	IND	A	DL	IND	DL	A	A	IND	IND	IND	A	A	A
23	Node swelling	SSW	SW	NS	SW	SW	SW	SW	SW	SSW	SSW	NS	SSW	SSW	SW	SSW
24	Root band colour exposed	GY	G	YG	GB	YG	G	G	G	P	P	G	P	Y	G	P
25	Root band col. fresh. expo	CRY	CRY	LG	CRY	CRY	CRY	CRY	YG	CRY	YG	LG	LG	Y	LG	CRY
26	Root band width (cm)	8.0	6.7	6.3	6.0	5.0	6.3	6.7	6.0	7.7	7.3	6.3	7.3	4.3	8.3	5.3
27	Root band swelling	SW	SSW	NS	SW	SSW	NS	SW	SSW	SSW	SSW	SSW	SSW	SSW	SSW	NS
28	Root band shape	OBC	CYL	CYL	TUM	OBC	OBC	TUM	CYL	CYL	OBC	CYL	OBC	OBC	OBC	CYL
29	Number of root eyes	3	2	3	3	2	2	2	2	2	2	2	2	2	3	2
30	Root eye alignment	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	R	IR	IR	R	RR
31	Bud shape	OVT	SQPE	RC	OVT	RC	SQPE	OVT	SQPE	SQPE	OVT	OVT	SQPE	OVT	RW	OVT
32	Bud size	MS	MB	MB	MS	LB	MS	MB	MB	MB	MF	SB	MB	MB	MB	MS
33	Bud cushion	A	A	P	P	A	A	P	P	P	P	P	P	A	P	P
34	Bud Extension	B	B	B	B	T	B	T	B	T	T	B	T	B	B	B
35	Bud germpore	SA	C	C	SA	C	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA
36	HG10 (bud)	A	A	A	A	A	A	A	A	A	A	A	A	P	A	A
37	HG26 (bud)	A	A	A	A	A	A	P	A	A	P	A	A	P	A	A
38	Canopy	OSD	OE	FTD	OTD	OE	FE	FTD	FTD	OE	CTD	FE	FE	CE	CTD	FE
39	Leaf sheath clasping	M	M	L	M	L	M	L	L	M	M	L	L	L	L	L
40	leaf sheath colour	G	GPT	G	G	G	GPT	G	GPT	GPT	GPT	GPT	GPT	GPT	GPT	GPT
41	leaf sheath wax	M	M	M	M	L	H	M	H	M	H	VH	H	H	H	VH
42	leaf sheath scaries	N	M	W	N	W	W	M	M	N	M	N	M	N	M	M
43	leaf colour	G	G	G	G	G	G	DG	G	G	G	G	G	G	G	G
44	leaf length (cm)	155	146	155	160	154	150	136	193	141	166	157	152	163	186	150
45	leaf width (cm)	3.4	4	4.5	3.9	4.5	5.7	5.5	4.5	4.3	4.8	3.6	4	4.6	4.7	4.5
46	Hair group 53	A	A	P	A	A	P	A	A	P	A	A	A	P	P	P
47	Hair group 57	A	D	P	A	P	D	D	P	A	D	P	P	P	P	P
48	Hair group 60	A	P	A	A	A	A	P	A	A	P	A	A	A	A	A
49	Hair group 56	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
50	Flowering (week/month)	1/11	3/11	2/11	2/11	2/11	1/11	1/11	NF	1/11	2/11	NF	1/11	NF	3/11	4/11
51	Pollen fertility (%)	54	64	69	78	63	94	10	–	73	80	–	62	–	55	16

Table 4. ANOVA for 10 yield traits

Traits	Treatment Adjusted		Block adjusted		
	Df	MS	Df	MS	F
No. of millable canes	16	25.0547**	2	0.92	8.25
Hand refractometer brix	16	2.5416*	2	0.14	1.95
Cane thickness	16	0.0770	2	0.00	0.05
Cane length	16	897.6734**	2	27.13	4.25
Single cane weight	16	0.0588	2	0.00	1.44
Brix (%)	16	1.2764	2	0.07	0.79
Sucrose (%)	16	1.6939	2	0.04	0.36
Purity (%)	16	6.2232	2	1.05	0.95
Yield	16	11.9565*	2	1.70	13.51
Ccs/p (sugar yield/plot)	16	0.1331	2	0.03	1.34

Table 5. Adjusted mean of yield and quality traits

	Clone	NMC	HRBM	ctk	Cl	scwt	Brix (%)	Suc (%)	Purity (%)	Yield kg/p	ccs/p
1	CP 57-614	18.52	18.61	1.94	262.22	0.78	17.83	15.78	86.51	15.31	1.86
2	CP 70-1133	18.22	21.49	2.33	230.13	0.78	18.54	17.10	92.45	16.69	2.14
3	CP 72-2086	11.52	19.93	2.58	208.91	0.94	18.86	17.20	91.15	8.82	1.23
4	CP 78-2114	19.92	18.84	1.91	229.93	0.66	19.00	17.66	93.12	12.96	1.82
5	CP 79-318	15.92	22.41	2.24	227.44	0.82	18.90	16.81	89.42	13.74	1.71
6	CP 80-1827	14.72	21.81	2.65	213.86	0.92	19.02	17.39	90.83	13.68	1.60
7	Yuetang 85-177	11.32	19.93	2.57	220.95	0.92	18.15	15.42	84.88	12.21	1.09
8	SP 79-2233	21.05	18.73	2.05	188.00	0.58	18.22	16.77	91.71	11.86	1.26
9	SP 80-185	16.72	19.72	2.25	258.15	0.93	17.85	15.91	88.98	17.21	1.76
10	SP 80-1816	21.32	20.41	2.24	247.66	1.08	19.06	17.98	93.91	18.30	2.19
11	SP 80-1842	18.67	21.94	2.23	289.34	0.88	20.10	18.06	90.07	13.67	1.93
12	SP 80-3280	21.87	20.51	2.22	257.82	0.86	18.52	16.48	89.42	16.42	2.09
13	SP 81-1763	20.37	20.13	2.11	246.14	0.84	20.40	18.98	93.31	13.48	2.02
14	SP 81-3250	21.97	18.89	2.19	220.60	0.72	19.39	16.90	87.52	15.86	2.01
15	SP 83-5073	19.67	21.67	2.10	242.62	0.73	22.20	20.44	92.19	12.79	2.09
16	Co 62175	17.23	17.90	2.40	225.50	1.18	17.90	16.10	90.11	20.55	2.19
17	Co 8231	27.70	21.10	1.80	184.44	0.48	20.27	18.50	91.56	13.41	1.64
CD5%		**2.49**	**1.47**	**0.28**	**14.03**	**0.14**	**1.63**	**1.79**	**7.80**	**2.64**	**1.05**
CV		**1.71**	**1.31**	**5.5**	**1.11**	**2.94**	**1.54**	**1.86**	**1.10**	**2.30**	**7.60**

(NMC = Number of millable canes, HRB = Hand refractometer brix at middle of the cane at 7th month, Ctk = Cane thickness, cl = Cane length, brix = Brix %, Suc = Sucrose %, Pur = Purity%, Yld/P = Cane yield/plot, CCS/P (2 meter row) = Sugar yield/plot (kg)

block design analysis in the IASRI website and for guiding in the analysis of data.

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Preliminary Screening of Indigenous *Curcuma* Germplasm against Taphrina Leaf Blotch and Colletotrichum Leaf Spot

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A total of 237 accessions of various species of the genus *Curcuma* such as *Curcuma brog* Val. (2), *C. amarissima* Rosc. (5), *C. aromatica* Salisb. (34), C. *caesia* Roxb. (5), *C. comosa* Roxb. (2), C. *ferruginea* Roxb. (2), *C. haritha* Mangaly & Sabu (15), *C. latifolia* Rosc. (2), *C. leucorrhiza* Roxb. (6), *C. aeruginosa* Roxb. (38), *C. montana* Roxb. (1), *C. amada* Roxb. (49), C. *soloensis* Val. (1), *C. zanthorrhiza* Roxb. (45), *C. longa* var. *vanaharidra* Vela *et al*. (1), C. *amada* var. *glabra* Vela *et al*. (7) and *C. raktakanta* Mangaly & Sabu (8) and two unidentified entities (14 accessions) collected from different ecogeographical regions of India and maintained at Vellanikkara were subjected to observation on incidence of Taphrina leaf blotch and Colletotrichum leaf spot diseases under natural epiphytotic conditions at Vellanikkara which is a hot spot for these diseases. The results indicated a complete absence of Taphrina leaf blotch symptom in ten entities containing 97 accessions. Four entities with 14 accessions showed complete absence of symptoms of both the diseases over the years. With respect to Colletotrichum leaf spot disease variation among the species and within the species over the years was noticed.

Key Words: Colletotrichum leaf spot, *Curcuma,* Germplasm, Taphrina leaf blotch

Introduction

The genus *Curcuma* consists of an important condiment such as turmeric and several medicinally useful as well as edible wild and cultivated species. The genus includes around 120 species (Skornickova and Sabu, 2002) distributed mainly in tropical and sub-tropical Asia extending to Australia. India has largest area under turmeric cultivation. Sporadic cultivation of mango ginger (*C. amada*) and yellow zedoar (*C. zanthorrhiza)* is also noticed. Apart from this, a large number of wild species numbering 29 also occur (Karthikeyan *et al.*, 1989). However, according to Velayudhan *et al.* (1999) the number of Indian species may be around 40. Several distinct entities of the genus have been collected and maintained at NBPGR Regional station, Thrissur without proper identity in some accessions due to lack of flowering, large number of species numbering 19 happens to be from southern region along the Western Ghats (Sabu, 2006). As a part of the exploration and collection of genetic resources of turmeric and its wild relatives in India, NBPGR had amassed a huge wealth leading to their characterization, classification and conservation. A total of 248 accessions belonging to 16 species of *Curcuma* such as *C. aeruginosa, C. amada, C. amada* var. *glabra, C. amarissima, C. aromatica, C. brog, C. caesia, C. comosa, C. ferruginea, C. haritha, C. latifolia, C. leucorrhiza,* (Velayudhan *et al.,* 2009a), *C. longa* var. *vanaharidra* (Velayudhan *et al.,* 2009b), *C. Montana, C. raktakanta, C. soloensis,* and *C. zanthorrhiza* along with some unidentified entities have been conserved at NBPGR, Vellanikkara, Thrissur. Turmeric (*C. longa* L.) being an important crop of both ethnic and commercial importance in India (Velayudhan *et al.*, 1999), two diseases such as leaf blotch disease caused by *Taphrina maculans* Butl. and leaf spot caused by *Colletotrichum capscici* (Syd.) Butl. commonly occur in all turmeric-growing areas in India. *T. maculans* was first described by Butler (1911) from Gujarat and Uttar Pradesh in India and also from Pakistan. Reddy *et al.* (1963) and Upadhyay and Tyagi (1974) reported varietal reaction to leaf blotch in turmeric. Recently, Prasadji *et al.* (2005) reported variation in turmeric with respect to incidence of these diseases. However, information on this aspect in case of various tuber bearing species of the genus *Curcuma* could not be traced in literature. Hence as a part of the preliminary characterization and evaluation, the surviving accessions of various species of *Curcuma* have been subjected to observation on the incidence of the above two diseases under natural field conditions. The results of the study are dealt in the paper.

Materials and Methods

A total of 236 accessions of various *Curcuma* species (Table 1) such as *C. brog* Val.(2), *C. amarissima* Rosc. (5), *C. aromatica* Salisb. (34), C. *caesia* Roxb. (5), *C. comosa* Roxb. (2), C. *ferruginea* Roxb. (2), *C. haritha* Mangaly & Sabu (15), *C. latifolia* Rosc. (2), *C. leucorrhiza* Roxb.

(6), *C. aeruginosa* Roxb. (38), *C. montana* Roxb. (1), *C. amada* Roxb. (49), *C. soloensis* Val. (1), *C. zanthorrhiza* Roxb. (45), C. *raktakanta* Mangaly & Sabu (8), *C. longa* var. *vanaharidra* (1) and *C. amada* var. *glabra* (7), and two unidentified entities *Curcuma* sp. 1 (6), and *Curcuma* sp. 2 (8) were planted in 1994 on permanent raised beds in partially shaded and rain fed conditions at Vellanikkara (10º 50' N latitude and 76º 20' E longitude and 20 m altitude) with 3,631 mm average annual rainfall and laterite soil. Three plants per collection were planted in lines at line-to-line and plant-to-plant distance of 1x 0.3 m. The plants were allowed to regenerate and perpetuate themselves there itself for facilitating observation on flowering. The beds were weeded out once during June and FYM along with dry decayed mulch were applied with one earthing up. These plants exhibited symptoms of Taphrina leaf blotch and Colletotrichum leaf spot disease every year at the late vegetative crop growth period during October-November. Since these diseases have been recurring at the site every year since 1978, the year of start of *Curcuma* conservation and the place is undoubtedly a hot spot for the diseases. Plants kept on permanent beds favoured the natural inoculum build up and spread during the main rainy season every year. During 2004 and 2006 plants grew vigorously and got infection at later stage and were observed for incidence of both the diseases. Observation was made on three plants subjectively and was scored on 0-9 scale based on magnitude of disease expression on all the leaves of all the suckers of each plant as done in the case of turmeric by Velayudhan and Liji (2003) and infestation severity was calculated as per the formula given by Misra and Chowdhury (1997). In case of *C. aeruginosa*, 36 accessions were observed during 2004 and 38 during 2006. In *C. amada* 47 accessions were observed in 2004 and 49 during 2006. In *Curcuma* sp. 27 accessions could be observed during 2004 and six during 2006. Out of nine classes observed from 0-8, class 0 indicated the absence of any symptom, classes 1-3 low, classes 4-5 medium and classes 6-8 high incidence of each disease separately. The data were analyzed as per standard statistical procedures. Plant pathologists from Department of Pathology, KAU, Vellanikkara, have helped in identification of diseases.

Formula for calculation of dicease severity:

$$\text{Infection severity (\%)} = \frac{\text{Sum of all infection ratings}}{\text{Total number of ratings} \times \text{Maximum score}} \times 100$$

Table 1. Materials used for the study

Species	Origin	Years	
		2004	2006
Curcuma sp. 1	Kerala	6	6
Curcuma sp. 2	Odisha	8	8
C. aerugenosa Roxb.	Kerala, Karnataka	36	38
C. amada Roxb.	Kerala, Tamil Nadu, NE	47	49
C. amada var. *glabra* Vela *et al.*	Kerala	7	6
C. amarissima Roxb.	North-east (NE)	5	5
C. aromatica Salisb.	South	34	34
C. brog Val.	North-east (NE)	2	2
C. caesia Roxb.	North-east (NE)	5	5
C. comosa Roxb.	North-east (NE)	2	2
C. ferrugenia Roxb.	North-east (NE)	2	2
C. haritha Mangaly & Sabu	Kerala	15	15
C. latifolia Rosc.	North-east (NE)	2	2
C. leucorrhiza Roxb.	Odisha	6	6
C. longa var. *vanaharidra* Vela *et al.*	Andhra Pradesh	1	1
C. montana Roxb.	Odisha	1	1
C. raktakanta Mangaly & Sabu	Kerala, Karnataka	8	8
C. soloensis Val.	North-east (NE)	1	1
C. *zanthorrhiiza* Roxb.	Kerala, Taminadu	45	45
		236	**236**

Results and Discussion

Frequency distribution of the diseases for each year is given in Table 2 along with disease severity percentage of diseases for each species. Range, mean, SD for both diseases in each species after pooling the data for two years is furnished in Table 3. Accessions, which were completely free of symptoms in both the years, are listed separately for each disease in Table 4.

The results indicated that more variation is existing in the case of Colletorichum leaf spot disease as compared to that for Taphrina leaf blotch. Among various species observed, *C. caesia* (5) *C. ferruginea* (2), *C. comosa* (2), *C. montana* (1) and *Curcuma* sp. 3 (5) as depicted in Table 4 did not express any disease symptoms. Seed setting is absent in all the entities except in the case of *C. montana* probably pointing to triploid nature of most of these species. *C . montana* with seed set probably appears to be an entity that may be exploited in conventional breeding programme for disease resistance in turmeric if proved resistant after screening under controlled conditions. Taphrina leaf blotch disease incidence was observed in different accessions of *C. brog* (2), *C. amarissima* (5), *C. aromatica* (34), *C. latifolia* (2), *C. leucorrhiza* (6), *C. aeruginosa* (36), *C. amada* (47 during 2004 and 48 during 2006), *C. harita* (15 during 2004 and 14 during 2006), *C. raktakanta* (8 during 2004), *C. zanthorrhiza* (45 during 2004 and 43 during 2006), *C. amada* var. *glabra* (seven during 2004 and six during 2006) and *Curcuma* sp. 2 (eight during

Table 2. Frequency class distribution of Taphrina leaf blotch and Coletotrichum leaf spot disease for 2004 and 2006

Species	Year	Disease	Score									Disease severity (%)
			0	1	2	3	4	5	6	7	8	
Curcuma sp. 1	04	*Taphrina*	6									0
	06	*Taphrina*	6									
	04	*Colletotrichum*	6									0
	06	*Colletotrichum*	6									
Curcuma spp. 2	04	*Taphrina*	8									12.5
	06	*Taphrina*	7	1								
	04	*Colletotrichum*	5	3								16.67
	06	*Colletotrichum*	3	2	1	1			1			
C. aeruginosa	04	*Taphrina*	36									0
	06	*Taphrina*	38									
	04	*Colletotrichum*	1		1	4	5	16	6	3		63.13
	06	*Colletotrichum*	1		2	4	16	14	1			
C. amada	04	*Taphrina*	47									0.52
	06	*Taphrina*	48	1								
	04	*Colletotrichum*	2	17	20	5	2	1				35.94
	06	*Colletotrichum*	48		1							
C. amada var. *glabra*	04	*Taphrina*	7									0
	06	*Taphrina*	6									
	04	*Colletotrichum*	5		1		1					21.43
	06	*Colletotrichum*	2	2	1	1						
C. amarissima	04	*Taphrina*	5									0
	06	*Taphrina*	5									
	04	*Colletotrichum*	4		1							15
	06	*Colletotrichum*	4	1								
C. aromatica	04	*Taphrina*	34									0
	06	*Taphrina*	34									
	04	*Colletotrichum*		15	8	6	1	4				39.64
	06	*Colletotrichum*			3	4	14	6	5	1	1	
C. brog	04	*Taphrina*	2									0
	06	*Taphrina*	2									
	04	*Colletotrichum*		1	1							62.5
	06	*Colletotrichum*		2								
C. caesia	04	*Taphrina*	5									0
	06	*Taphrina*	5									
	04	*Colletotrichum*	5									0
	06	*Colletotrichum*	5									
C. comosa	04	*Taphrina*	2									0
	06	*Taphrina*	2									
	04	*Colletotrichum*	2									0
	06	*Colletotrichum*	2									
C. ferruginea	04	*Taphrina*	2									0
	06	*Taphrina*	2									
	04	*Coletotrichum*			2							50
	06	*Coletotrichum*				2						
C. harita	04	*Taphrina*	15									23.3
	06	*Taphrina*	1	6	2	3	1	1	1			
	04	*Colletotrichum*	11	3	1							6.1
	06	*Colletotrichum*	14						1			
C. latifolia	04	*Taphrina*	2									0
	06	*Taphrina*	2									
	04	*Colletotrichum*			2							50
	06	*Colletotrichum*	2									
C. leucorrhiza	04	*Taphrina*	6									0
	06	*Taphrina*	6									
	04	*Colletotrichum*	4	2								25
	06	*Colletotrichum*	5	1								

Species	Year	Disease	Score									Disease severity (%)
			0	1	2	3	4	5	6	7	8	
C. longa var. *vanaharidra*	04	*Taphrina*	1									0
	06	*Taphrina*	1									
	04	*Colletotrichum*	1									0
	06	*Colletotrichum*	1									
C. montana	04	*Taphrina*	1									0
	06	*Taphrina*	1									
	04	*Colletotrichum*	1									0
	06	*Colletotrichum*	1									
C. raktakanta	04	*Taphrina*	8									34.36
	06	*Taphrina*		5	3							
	04	*Colletotrichum*	3	4	1							25
	06	*Colletotrichum*	6	2								
C. soloensis	04	*Taphrina*	1									50
	06	*Taphrina*		1								
	04	*Colletotrichum*		1								100
	06	*Colletotrichum*		1								
C. zanthorhiza	04	*Taphrina*	45									4.4
	06	*Taphrina*	43	2								
	04	*Colletotrichum*	44	1								1.11
	06	*Colletotrichum*	45									

Table 3. Species-wise minimum and maximum, mean, Standard deviation for *Taphrina* leaf spot disease and *Colletotrichum* leaf blotch disease

Species	Parameters	Taphrina leaf spot	Colletotrichum leaf blotch	Number of collections
Curcuma sp. 1	Range	0	0	5
	Mean	0	0	
	SD	0	0	
Curcuma sp. 2	Range	0-1	0-6	8
	Mean	0.063	1	
	SD	0.25	1.59	
C. aerugenosa	Range	0	0-7	38
	Mean	0	4.49	
	SD	0	1.20	
C. amada	Range	0-2	0-5	48
	Mean	0.01	0.91	
	SD	0.10	1.14	
C. amada var. *glabra*	Range	0-2	0-5	7
	Mean	0.01	0.91	
	SD	0.10	1.14	
C. amarissima	Range	0	0-1	5
	Mean	0	0.25	
	SD	0	0.5	
C. aromatica	Range	0	1-8	35
	Mean	0	3.26	
	SD	0	1.76	
C. brog	Range	0	1-2	2
	Mean	0	1.25	
	SD	0	0.5	
C. caesia	Range	0	0	5
	Mean	0	0	
	SD	0	0	
C. comosa	Range	0	0	2
	Mean	0	0	
	SD	0	0	
C. ferruginea	Range	0	0	2
	Mean	0	0	
	SD	0	0	
C. harita	Range	0-6	0-6	15
	Mean	1.67	0.36	
	SD	1.67	1.18	
C. latifolia	Range	0	0-2	2
	Mean	0	1	
	SD	0	1.155	
C. leucorrhoea	Range	0	0-1	6
	Mean	0	0.29	
	SD	0	0.47	
C. longa var. *vanaharidra*	Range	0-1	0	1
	Mean	0.5	0	
	SD	0.5	0	
C. montana	Range	0	0	1
	Mean	0	0	
	SD	0	0	
C. raktakanta	Range	0-2	0-2	8
	Mean	0.69	0.5	
	SD	0.79	0.79	
C. soloensis	Range	0-1	1-1	1
	Mean	0.5	1	
	SD	696.36	0	
C. zanthorrhiza	Range	0-1	0-3	45
	Mean	0.02	0.23	
	SD	0.15	0.23	

Table 4. Species showing absence of disease symptoms

Species	Number of collections	Collection identity
Species and collections showing absence of symptoms of both diseases		
Curcuma sp. 3	5	IC266596, 349879B, 210466, 349850A and 349879
C. caesia	5	IC313064, 313085, 266511, 266608 and 331735
C. comosa	2	IC313097 and 313092
C. ferrugenia	2	IC 266513 and 313098
C. latifolia	2	IC266595 and 313093
C. montana	1	IC406441
Species and collections showing absence of *Colletotrichum* only		
C. longa var. *vanaharidra*	1	IC360212
Species and collections showing absence of *Taphrina* only		
C. aeruginosa	33	IC 88593, 266532, 88842, 88843, 88844, 88845, 88846, 70009, 70013, 70015, 70061, 70072, 88848, 70110b, 88849, 88888, 88917, 88914, 88912, 313083, 266538, 88941, 266546, 266550, 266551, 266553, 266561, 266564, 266566, 266585, 266588, 266591, 266524, 406438, 406439 and 349989
C. amarissima	5	266531, 29968, 266511-B, 210290 and 137087
C. aromatica	32	IC 88850, 88852, 88929, 88853, 88856, 88857, 88858, 88859, 88860, 88861, 313087, 266537, 88938, 88957, 266543, 266544, 313090, 406440, 266569, 266572, 266577, 266579, 266580, 266581, 266582, 266583, 266590, 360204, 360205, 210361 A, 266605, 262716, 34974, 33215 and 349878
C. brog	2	IC 29881 and 313086
C. latifolia	2	IC 266595 and 313093
C. leucorrhiza	5	IC 210263, 210267, 210284, 210325 and 210328

2004 and seven during 2006) showed complete absence of symptoms. On the contrary, Colletotrichum leaf spot disease in the observed species showed good amount of variation between the species, accessions and years as presented in Tables 2 and 3. *C. brog* (2), *C. comosa* (2) and *C. ferrugenia* (2) had mild infection of *Colletotrichum* during both the years. *C. aromatica* showed maximum variation (0-5 scales during 2004 and 0-8 scales during 2006) followed by *C. aeruginosa* with 0-7 during 2004 and 0-6 during 2006 with one free during each year). However, *C. amada* exhibited a wide range of variation (0-6 scale during 2004) and 45 accessions were found to be free from disease. In *C. raktakanta* three got infected in 0-2 scale during 2004 and all were free from infection during 2006. Accessions belonging to *C. harita, C. leucorrhiza, C. zanthorrhiza, C. soloensis* and *C. amada* var. *glabra* and species 2 had mild infection during both the years. Thus**,** more number of species and accessions showing almost a vertical resistance to *Taphrina* and a horizontal resistance to *Colletotrichum* may be probably indicative of the inherent heterogeneity in the genus right from its origin through dibasic amphidiploidy from two unknown progenitors into entities with 2n=42 (Ramachandran, 1961) and later on diversification by triplodization into triploid entities with 2n = 63 chromosomes and palmately branched rhizomes (sessile tubers). Further, among other species, only *C. longa* var. *vanaharidra* with a single collection was free of *Colletotrichum* on one side and susceptible to *Taphrina* on the other side. This entity, though wild is very much related to cultivated turmeric (*C. longa* L.) in almost all characters including those of rhizome quality such as colour and aroma except for its thin fragile leaves with purple brown tint along the midrib. Among the species observed, C*urcuma* sp., *C. amada, C. haritha, C. raktakanta, C. soloensis* and *C. zanthorrhiza* were susceptible to both the diseases at varying intensities. In the case of *C. amada* (Table 2) 47 accessions were free of Taphrina leaf blotch during 2004 where as, the newly added one accession from Orissa with central spike was susceptible during 2006 indicating some heterogeneity in the species with respect to its flowering behaviour. With respect to *Colletotrichum* spot incidence, it was present in all the collections in 2004 and absent in 2006 except in one newly added accession. This probably indicates the influence of climatic and other uncontrollable factors influencing the disease infection on the species. The most susceptible species to Colletotrichum leaf spot appeared to be *C. aromatica*. Under the wild situation, also the disease is rampant in all the localities in Western Ghats from where collections of this have been amassed. Overall results indicated that majority of the species and collections were free from Taphrina leaf blotch and a fair amount of

expression of Colletotrichum leaf spot disease symptom in the genus. With respect to Taphrina leaf blotch disease, severity percentage was 0 in 12 identified entities and in one unidentified species as given in Table 2. Severity percentage for Colletotrichum leaf spot disease varied from 0% in *C. montana*, *C. caesia* and *C. amada* to 100% in *C. soloensis*. Further screening of the accessions without disease symptoms in the present studies is needed under controlled conditions. This can prove to be a very important criteria in the utilization of these germplasm in turmeric improvement. The results may also be useful in tracing the origin and evolution of various species in relation to these diseases. Both vertical and horizontal resistance is suspected in the genus for the diseases.

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Diversity for Resistance to Stem and Leaf Rusts in Indian Wheat Germplasm

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In India, three wheat species –*Triticum aestivum* L. emend. Fiori & Paol (bread wheat), *T. durum* Desf. (durum wheat) and *T. dicoccum* Schrank (khapli wheat) are under cultivation, which are attacked by stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn.) and leaf rust (*P. triticina* Eriks). Out of 120 genotypes each of durum and bread wheats tested including released varieties, advanced generation lines, genetic stocks and landraces, 67 bread wheat and 71 durum genotypes showed resistance to stem rust; and 45 bread wheat and 50 durum genetic stocks showed resistance to leaf rust, based on field scores of five successive crop seasons (2004-2009) under rust epiphytotic conditions. On the basis of patterns of their seedling responses to 24 pathotypes of stem rust and 40 of leaf rust, at least 17 diverse groups among bread wheats and 18 among durums could be recognised for resistance to stem rust, while 12 groups among bread wheats and nine among durums could be identified for leaf rust resistance, indicating wider resistance base of Indian wheat germplasm. In all, 31 bread wheat and 36 durum genotypes showed resistance to both the rusts, which can serve as donors in wheat crop improvement.

Key Words: Diversity, Donors, Leaf rust, Stem rust, Wheat

Introduction

Rust diseases could potentially be most serious destabilizing factors in wheat production. Of the three rust diseases attacking wheat crop, leaf rust occurs throughout the country, while stem rust is more common in the warmer central and peninsular parts of India. Like other biotic and abiotic stresses, monoculture practice might lead to vulnerability against rust pathogens, and thus, diversity is the only safeguard to it. Search for diverse sources of rust resistance is, therefore, a prerequisite for any wheat improvement programme. Hence, a comprehensive study involving a large number of durum and bread wheat genotypes representing a cross section of the Indian wheat germplasm was conducted to: 1) identify genotypes showing high levels of stable resistance under heavy rust inoculum pressure in the field and; 2) explore diversity for resistance to stem and leaf rusts through glasshouse seedling tests of these genotypes with the most of the variability of stem rust and leaf rust populations in India. The present communication reports the results of this study, which can be useful to wheat workers in the country for selecting effective and diverse rust resistant donors.

Materials and Methods

A total of 240 genotypes (120 each of durum and bread wheats) were used in the study. These were selected carefully to represent a cross section of the Indian wheat germplasm and included recent and old released cultivars, advanced generation lines, indigenous as well as commonly used exotic genetic stocks, and land races. To ensure the genetic purity, seeds of most of these genotypes were procured from the respective wheat breeders/originating centres. The test genotypes are listed in Table 1 and 2 along with their respective seed source.

Screening of Wheat Genotypes for Field Resistance to Stem and Leaf Rusts

Seeds of the test genotypes were hand-sown in each crop season during November 2nd fortnight, with ~2 cm seed to seed spacing in 1.0 m rows, spaced 30 cm apart. Recommended agronomic practices were followed for raising the crop. Rust spreader rows consisting of mixtures of susceptible wheat varieties were planted all around the experimental plot, and after every 20th test entry. These were inoculated using hypodermic syringes and sprays with aqueous suspension of uredospores of most of the important pathotypes of stem and leaf rusts, freshly collected from the glasshouse. The spore suspension was sprayed onto the test rows as well, but no syringe inoculations were made on them. Disease scores were recorded, combining rust severity as per the modified Cobb's scale (Peterson *et al.*, 1948) and field response. Disease severity was multiplied by respective field response value to obtain co-efficient of infection (Nayar *et al.*, 1997) for each line. Averages of the co-efficient of infection values of the five crop seasons were computed to determine the Average

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Table 1. List of bread wheat genotypes tested along with their seed source

Bread wheat genotypes	Seed source
AKW 381, AKW 1071, AKW 2951, AKW 3294	PKV-Akola
CSP 44, Frontana, IWP 72, Nainari 60, Pavon 76, PBW 175, PBW 343, PBW 498, WG 138, WL 410, WL 711	PAU-Ludhiana
DWR 16, DWR 162, DWR 195	UAS-Dharwad
GW 120, GW 173, GW 190, GW 273, GW 322, GW 326, J 405	SDAU-Vijapur
CPAN 1235, CPAN 1676, CPAN 1796, CPAN 1842, CPAN 3004, DL 153-2, DL 803-3, HD 1941, HD 1949, HD 1982, HD 2009, HD 2135, HD 2189, HD 2270, HD 2278, HD 2285, HD 2307, HD 2329, HD 2380, HD 2402, HD 2428, HD 2501, HD 2687, HD 2733, HDR 77, Kalyan Sona, Sonalika	IARI-New Delhi
HI 385, HI 617, HI 784, HI 1077, HI 1123, HI 1418, HI 1434, HI 1479, NP 4	IARI-Indore
HP 1209, HP 1731, HP 1744, HP 1761	IARI-Pusa
HPW 155	HPKVV-Palampur
HS 86, HS 295, HS 365, HS 375, HS 420, HS 1097-17, HS 1138-6-1	IARI-Shimla
HUW 37, HUW 206, HUW 234, HUW 468	BHU-Varanasi
HW 517	IARI-Wellington
JOB 666	RAU-Jobner
K 68, K 7410, K 7827, K 8020, K 8027, K 8962, K 9006, K 9107, K 9465, K 9644	CSAUA&T-Kanpur
KRL 1-4	CSSRI-Karnal
Lok 1	Lok Bharti-Sanosara
MACS 2496	ARI-Pune
MP 1048, MP 1109	JNKVV-Powarkheda
NI 5439, NI 5643, NI 9406, NIAW 34	MPKV-Niphad
Raj 1482, Raj 1972, Raj 3077, Raj 3765, Raj 3777	RAU-Durgapura
UP 262, UP 301, UP 2003, UP 2113, UP 2338, UP 2390, UP 2425, UP 2473	GBPUA&T-Pantnagar
VL 404, VL 421, VL 804, VL 832	VPKAS-Almora
WH 147, WH 283, WH 416, WH 542, WH 712	CCSHAU-Hisar

Coefficient of Infection (ACI) values. Genotypes having ACI value of <5.1, and highest disease score upto 50R / 40RMR / 30MR / 20MRMS / 20X / 15MSS / 10S, were classified as 'resistant'.

Glasshouse Evaluation of Wheat Genotypes for Seedling Resistance to Rust Pathotypes

Seedling tests were conducted at 22°C±3°C in an air-conditioned glasshouse during November-February months, using standard glasshouse procedures (Nayar *et al*., 1997). Wheat genotypes were tested with 24 pathotypes of stem rust, and 40 of leaf rust as given below. These included all the pathotypes of the two rusts being currently maintained and supplied by the Directorate of Wheat Research, Regional Station, Flowerdale, Shimla.

Stem Rust Pathotypes

11 (79G31), 11A (203G15), 14 (16G2), 21-1 (24G5), 21A-2 (75G5), 24 (18G3), 24A (5G19), 34-1 (10G13), 40A (62G29), 40-1 (62G29-1), 42 (19G35), 42B (7G35), 117 (37G3), 117-1 (166G2), 117-2 (33G3), 117-3 (167G3), 117-4 (166G3), 117-5 (166G2-2), 117-6 (37G19), 117A (38G2), 117A-1 (38G18), 122 (7G11), 184 (53G1), and 295 (7G43).

Leaf Rust Pathotypes

10 (13R19), 11 (0R8), 12 (5R5), 12-1 (5R37), 12-2 (1R5), 12-3 (49R37), 12-4 (69R13), 12-5 (29R45), 12-6 (5R45), 12A (5R13), 17 (61R24), 20 (5R27), 63 (0R8-1), 77 (45R31), 77-1 (109R63), 77-2 (109R31-1), 77-3 (125R55), 77-4 (125R23-1), 77-5 (121R63-1), 77-6 (121R55-1), 77-7 (121R127), 77-8 (253R31), 77A (109R31), 77A-1 (109R23), 104 (17R23), 104-1 (21R31-1), 104-2 (21R55), 104-3 (21R63), 104A (21R31), 104B (29R23), 106 (0R9), 107 (45R3), 107-1 (45R35), 108 (13R27), 108-1 (57R27), 162 (93R7), 162-1 (93R47), 162-2 (93R39), 162-3 (29R7), and 162A (93R15).

Table 2. List of durum wheat genotypes tested along with their seed source

Durum wheat genotypes	Seed source
AKDW 2997-16, AKDW 3347, and AKDW 4256	PKV-Akola
DW 1001	DWR-Karnal
A 9-30-1, A 28, GW 1, GW 12, GW 1114, GW 1128, GW 1139, GW 1158, GW 1163, GW 1170, GW 1172, GW 1175, GW 1182, GW 1189, GW 1207, GW 1209, and VD 97-15	SDAU-Vijapur
B 662, Baxi 288-18, Bijaga Red, Castel Porziano, DBP 02-08, ED 2398-A, Guji `S', HD 4502, HD 4530, HD 4672, HD 4676, HD 4687, HD 4692, HD 4694, HD 4696, HD 4701, HG 110, HI 7483, HI 7747, HI 8381, HI 8498, HI 8540, HI 8550, HI 8591, HI 8592, HI 8620, HI 8622, HI 8630, HI 8651, HI 8653, IWP 5019, Line 1172, Malvi Local, Motia, NP 404, Sarangpur Local, Trinakaria and Yuk	IARI-Indore
Bijaga Yellow, DWR 1006, KDW 137, Local Red, and UAS 2021	UAS-Dharwad
MACS 9, MACS 1967, MACS 2778, MACS 2788, MACS 2846, MACS 2884, MACS 3061, MACS 3063, MACS 3125, MACS 3208, MACS 3453, and MACS 3503	ARI-Pune
JNK 4W-184, MPO 215, MPO 615, and MPO 1106	JNKVV-Powarkheda
NI 146, NI 5749, NIDW 9, NIDW 15, NIDW 70, and NIDW 295	MPKV-Niphad
DWL 5023, PBW 34, PDW 215, PDW 233, PDW 245, PDW 251, PDW 254, PDW 269, PDW 271, PDW 272, PDW 273, PDW 274, PDW 275, PDW 277, PDW 278, PDW 283, PDW 287, PDW 289, and WG 7143	PAU-Ludhiana
Raj 911, Raj 1555, Raj 6516, Raj 6560, Raj 6562, Raj 6566, and RKD 97	MPUA&T-Kota
RD 930, and RS 749	IARI-New Delhi
UPD 45	GBPUA&T-Pantnagar
P 6046, P 7073, WH 896, WH 913, and WH 929	CCSHAU-Hisar

Infection types (ITs) on the seedlings were recorded 12-15 days after inoculation, on a 0-4 scale. Differential sets 'A' and 'B', and the desired lines from set `0' in respect of stem rust and leaf rust (Nayar *et al*., 2001) were also tested simultaneously for ascertaining the purity of the test pathotypes. The Infection Types (ITs) 3, 3^+, 34 and 4 produced by a pathotype on a host line indicated latter's susceptibility to that pathotype, whereas lower ITs ('0', '1', '2' and 'X') indicated resistance (Nayar *et al*., 1997).

Results and Discussion

In all, 67 bread wheat and 71 durum genotypes showed resistance to stem rust; and 45 bread wheats and 50 durums were classified as resistant to leaf rust, based on the above mentioned ACI and highest disease score limits. On the basis of their patterns of seedling reaction to 24 pathotypes of stem rust, and 40 of leaf rust, at least 17 diverse groups among bread wheats (Table 3) and 18 among durums (Table 5) could be recognised for stem rust resistanc, while a minimum of 12 groups among bread wheats (Table 4) and nine among durums (Table 6) could be identified for leaf rust resistance. Responses to rust pathotypes found 'virulent' on the two wheat species in the present and earlier study (Mishra *et al*., 2001) formed the basis of delineation of resistance groups. Some of the groups can be further classified, based on magnified resolution of differences in resistance to various pathotypes of a race group.

In all, 31 bread wheats (Table 7) and 36 durum genotypes (Table 8) showed resistance to both stem and leaf rusts. Many of these being released varieties, can serve as donors for resistance and other desired traits. The bread wheat genotypes belonged to nine diverse groups for stem rust resistance and to 11 for leaf rust resistance (Table 7). The durum genotypes belonged to 15 groups for stem rust resistance, and to eight for leaf rust resistance (Table 8). However, these groups can be further classified, based on differences in highest disease score. For example, three distinct sub-groups can be recognized within group A-2 for stem rust resistance among bread wheats (Table 7).

Among more than 40 designated genes for stem rust resistance, the genes *Sr2*, *Sr11* and *Sr31* are most common among Indian bread wheat genotypes, while *Sr2*, *Sr7b*, *Sr9e*, *Sr11* and *Sr12* have been commonly postulated in durum genotypes (Nayar *et al*., 2001). Of these, only *Sr31* is effective against all Indian stem rust pathotypes, *Sr2* provides a moderate level of adult-plant resistance, while others are ineffective. Genotypes carrying *Sr31* are listed in group A-1 of Table 3, and those having *Sr2* in Tables 3, 5, 7 and 8. *Sr2* has been the most important gene for stem

Table 3. Diverse groups for stem rust resistance in bread wheat (at least 17 groups)

A **Resistant to all stem rust pathotypes**

A-1 **Genotypes carrying stem rust resistance gene *Sr31***
AKW 1071, CPAN 3004*, DL 803-3, DWR 162*, DWR 195*, GW 190*, HD 2687*, HD 2733, HP 1761, HS 365, HS 375*, HUW 206*, MACS 2496*, PBW 343*, PBW 498, UP 2338*, UP 2425*, VL 804, and WH 542.

A-2 **Genotypes not carrying stem rust resistance gene *Sr31***
AKW 2951, DWR 16, HD 2380*, HD 2402*, HI 1418*, HI 1434*, HP 1731, HS 295*, HS 420*, NI 9406*, Raj 3765*, and UP 2390.

B **Resistant to all stem rust pathotypes except pathotypes 40A/40-1**
CPAN 1235, CPAN 1676, CPAN 1796, GW 273, HD 2009, HD 2135*, HD 2270, HD 2278*, HI 1479*, HW 517*, J 405, K 9107*, K 9465, NIAW 34, Raj 3777, UP 301, UP 2473*, and VL 421.

C **Resistant to all stem rust pathotypes except 40A / 40-1 + 117-complex pathotypes**
GW 173* (Susceptible to pathotypes 40-1 and 117-1)
HD 2501* (Susceptible to 40-1 and 117-4)
HD 2285 (Susceptible to 40A, 117-1, 117-4, and 117-5)

D **Resistant to all stem rust pathotypes except 40A / 40-1 + 295 + 117-complex pathotypes**
AKW 3294* (Susceptible to pathotypes 40-1, 295, 117-1, and 117-4)
HI 1077* (Susceptible to pathotypes 40A, 40-1, 295, 117-3, and 117-4)

E **Resistant to most stem rust pathotypes except 40A / 40-1 + others**
E-1 HI 784 and UP 2003 (Susceptible to pathotypes 40-1 and 11)
E-2 K 9006* (Susceptible to 40A, 40-1 and 184)
E-3 WH 712 (Susceptible to 40A, 40-1, 11, 184, and 295)
E-4 Pavon 76* (Susceptible to 40A, 40-1, 11, 24, 117-3, and 117-4)
E-5 HI 1123 (Susceptible to 40-1, 11, 24, 117-5, and 295)
E-6 CPAN 1842 (Susceptible to 40A, 40-1, 11, 11A, 24, 24A, 42B, and 117-6)

F **Resistant to stem rust pathotypes 40A and 40-1, but susceptible to others**
F-1 HD 2189* (Susceptible to pathotype 11)
F-2 GW 322* (Susceptible to 117-1)
F-3 K 8020* (Susceptible to 11, and 42B)
F-4 K 7827 (Susceptible to 11, 11A, and 24)
F-5 GW 326* (Susceptible to 117-1, 117-4, 117-5, and 295)
F-6 HD 2307 (Susceptible to11, 24, 42B, 117-1, 117-4, 117-5, and 295)

*Stem rust resistance gene *Sr2* postulated (after Nayar *et al*., 2001; Bhardwaj *et al*., 2003)

Table 4. Diverse groups for leaf rust resistance in bread wheat (at least 12 groups)

A **Resistant to all leaf rust pathotypes (pts)**
HP 1761, HS 365, HUW 468, and PBW 498

B **Resistant to all leaf rust pts except 77-complex (77-c) pt(s)**

B-1. Resistant to 77-5, but susceptible to other 77-c pt(s)
GW 326 and K 7827 (Susceptible to pathotype 77-6)
Raj 3765 and UP 1109 (Susceptible to 77-6 and 77-7)
VL 832# (Susceptible to 77-6 and 77A-1)

B-2 Susceptible to 77-5 and other 77-c pt(s)
UP 2473 (Susceptible to pathotypes 77-5 and 77-6)
HP 1209# and Raj 3777 (Susceptible to 77-2, 77-5, and 77-6)
HI 1418 and UP 2390 (Susceptible to 77-5, 77-6 and 77-7)
GW 322, HD 2380, HD 2402, and HI 1434 (Susceptible to 77-2, 77-5, 77-6 and 77-7)
Raj 1972 (Susceptible to 77-2, 77-5, 77-6, 77-7, and 77-8)
CPAN 1235 (Susceptible to 77-4, 77-5, 77-6, and 77-7)
CPAN 1796 (Susceptible to 77, 77A-1, 77-5, and 77-6)
Frontana# (Susceptible to 77A-1, 77-1, 77-3, 77-5, 77-6, and 77-8)
UP 301 (Susceptible to 77A, 77-1, 77-2, 77-3, 77-4, 77-5, 77-6, and 77-8)
HI 784 (Susceptible to 77, 77A-1, 77-1, 77-3, 77-4, 77-5, 77-6, and 77-8)

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C **Resistant to all leaf rust pts except 77-c and 104-c pts**
HS 295# (Susceptible to 77-1, 77-3, 77-4, 77-5, 77-6, 77-7, 77-8, 104B, and 104-2)
KRL 1-4 (Susceptible to 77, 77A-1, 77-1, 77-3, 77-4, 77-5, 77-6, 77-7, 77-8, 104, and 104A)
UP 2003 (Susceptible to 77, 77A, 77A-1, 77-1, 77-3, 77-4, 77-5, 77-6, 77-8, 104A, 104B, and 104-2)

D **Resistant to all leaf rust pts except 12-c, 77-c and 104-c pt(s)**
HD 2135 (Susceptible to 12A, 12-2, 77-2, 77-5, 77-6, 77-7, and 104-2)
HD 2501# (Susceptible to 12A, 77-2, 77-5, 77-6, 77-7, 104B, and 104-2)
Nainari 60# (Susceptible to 12-4, 77-1, 77-2, 77-3, 77-4, 77-5, 77-6, and 104-3)

E **Resistant to all leaf rust pts except 12-c, 77-c, 104-c, and 162-c pt(s)**
HD 2733 (Susceptible to 12-3, 12-5, 77-1, 77-5, 77-6, 104-2, and 162-2)
HP 1731# (Susceptible to 12-5, 77-1, 77-5, 77-6, 77-7, 104-2, and 162-2)
VL 804 (Susceptible to 12-1, 77-1, 77-5, 77-6, 77-7, 104-2, and 162-2)
HPW 155 (Susceptible to 12A, 12-5, 77A-1, 77-3, 77-4, 77-6, 77-8, 104B, 162-2, and 162-3)
K 9107# (Susceptible to 12-1, 12-4, 77, 77A-1, 77-1, 77-2, 77-3, 77-5, 77-6, 77-8, 104-3, and 162-3)

F **Resistant to various leaf rust pathotypes**
F-1 WG 138 (Susceptible to pathotypes 20, 77-1, 77-5, and 77-6)
F-2 HI 1479 (Susceptible to 77, 77A-1, 77-1, 77-3, 77-4, 77-5, 77-6, 77-8, and 107)
F-3 CPAN 1842 (Susceptible to 77, 77A-1, 77-1, 77-2, 77-3, 77-4, 77-5, 77-6, 77-8, 162-2, and 162-3)
F-4 WH 542# (Susceptible to 12-1, 12-5, 12-6, 77-1, 77-5, 77-6, 77-7, and 162-2)
F-5 Similar resistance patterns
NIAW 34# (Susceptible to 12-1, 12-5, 77A-1, 77-1, 77-3, 77-5, 77-6, 104B, 108, and 162A)
HP 1744# (Susceptible to 12-2, 12-5, 20, 77A-1, 77-2, 77-5, 104-2, 104-3, 108, 162, and 162A)
VL 404# (Susceptible to 12, 12A, 12-2, 12-4, 77A-1, 77-1, 77-3, 77-5, 77-6, 104A, 104B, 104-2, 104-3, 108, 108-1, 162, 162-2, and 162-3)
F-6 Similar resistance patterns
WH 283 (Susceptible to 10, 20, 77, 77A-1, 77-1, 77-2, 77-3, 77-5, 77-6, 104A, 108, and 108-1)
JOB 666 (Susceptible to 10, 12-5, 77, 77A, 77A-1, 77-1, 77-2, 77-3, 77-5, 77-6, 77-8, 107, and 162-2)
K 9465 (Susceptible to 10, 12, 12A, 12-1, 12-2, 12-3, 17, 77A-1, 77-3, 77-5, 77-6, 77-7, 104A, 104-3, 107-1, 108, 162A, 162-2, and 162-3)

#Leaf rust resistance gene *Lr34* postulated (after Nayar *et al*., 2001)

Table 5. Diverse groups for stem rust resistance in durum wheat (at least 18 groups)

A. **Resistant to all stem rust pathotypes**
AKDW 3347, B 662, GW 1114, GW 1139*, GW 1182, HD 4672, JNK 4W-184, MACS 2788*, MACS 2846, MACS 2884*, MPO 615, NIDW 9, P 6046, PDW 289, Raj 911, Raj 1555, Raj 6562, RS 749, UPD 45, and Yuk.

B. **Resistant to all stem rust pathotypes except 117-complex pathotypes**
B-1 **Resistant to all stem rust pathotypes except the pathotype 117-6**
GW 1128, GW 1163, HD 4530, HI 8550, MACS 2778, MACS 3208, and MACS 3453
B-2 **Resistant to all stem rust pts except 117-6 and other 117-c pt(s)**
HD 4696 and HI 8498* (Susceptible to pathotypes 117-6 and 117A)
HG 110, Line 1172, and MACS 1967 (Susceptible to 117-6 and 117A-1)
DBP 02-08 (Susceptible to 117-6 and 117-3)
HI 8651* and HI 8653 (Susceptible to 117-6, 117A, and 117-1)
NIDW 70 (Susceptible to 117-6, 117A, and 117-4)
MACS 3061* (Susceptible to 117-6, 117-1, and 117-2)
PDW 245 (Susceptible to 117-6, 117A, 117-2, and 117-3)
AKDW 4256 (Susceptible to 117-6, 117A, 117A-1, 117-1, and 117-3)
GW 1170 (Susceptible to 117-6, 117A, 117A-1, 117-1, 117-2, and 117-3)
MACS 3063* (Susceptible to 117-6, 117, 117A, 117-2, 117-3, and 117-5)
GW 1209 (Susceptible to 117-6, 117A, 117-1, 117-2, 117-3, and 117-5)
PDW 283 (Susceptible to 117-6, 117, 117A, 117-2, 117-4 and 117-5)
PDW 215 (Susceptible to 117-6, 117A, 117A-1, 117-1, 117-3, 117-4, and 117-5)
HI 8591* (Susceptible to 117-6, 117, 117A, 117-1, 117-3, 117-4, and 117-5)
B-3 **Resistant to all stem rust pts except pt(s) of 117-c other than 117-6**
GW 1207, NIDW 15*, and RKD 97 (Susceptible to pathotype 117)
HI 8592, Raj 6516*, RD 930, and UAS 2021 (Susceptible to 117A)
GW 1172 (Susceptible to 117A-1)
MACS 3125* (Susceptible to 117 and 117A)
PBW 34 (Susceptible to 117-1, 117-3 and 117A-1)
HI 8540 (Susceptible to 117, 117A, 117-1, 117-2, 117-3 and 117-4)
Resistant to different pts except one or more of 117-c + other selected pt(s)

C-1 Trinakaria (Susceptible to pathotypes 11 and 117-6)
C-2 ED 2398-A (Susceptible to 21-1, 24, and 117-1)
C-3 HD 4676 (Susceptible to 24, 117, and 117A)
C-4 WG 7143 (Susceptible to 24, 42B, 122, 117A, 117-3, and 117-6)
C-5 PDW 277 (Susceptible to 42B, 117, 117A, 117A-1, 117-1, and 117-6)
C-6 IWP 5019 (Susceptible to 184, 117A, 117A-1, 117-1, 117-4, 117-5, and 117-6)
C-7 HI 8381* (Susceptible to 40A, 117, 117A, 117-1, 117-2, 117-3, 117-5, and 117-6)
C-8 NI 5749 (Susceptible to 11A, 40A, 122, 295, 117, 117A-1, 117-1, 117-3, 117-4, and 117-5)
C-9 HD 4502 (Susceptible to 11, 11A, 14, 21-1, 21A-2, 24, 24A, 34-1, 42, 42B, 184, 117A, 117A-1, 117-1, 117-3, 117-4, 117-5, and 117-6)

D Resistant to 117-complex and most of the other stem rust pathotypes
D-1 Guji 'S' and VD 97-15 (Susceptible to pathotype 122)
D-2 Castel Porziano(Susceptible to 21-1)
D-3 Bijaga Red (Susceptible to 40A)
D-4 NIDW 295* (Susceptible to 184)
D-5 HI 7747 (Malavraj) (Susceptible to 14 and 122)

*Stem rust resistance gene *Sr2* postulated (after Nayar *et al*. 2001)

Table 6. Diverse groups for leaf rust resistance in durum wheat (at least nine groups)

A Resistant to all leaf rust pathotypes
AKDW 3347, Bijaga Red, DWR 1006, ED 2398-A, GW 1114, GW 1189, HD 4502, HD 4530, HD 4672, HD 4676, HD 4692, HD 4696, HI 8592, HI 8653, IWP 5019, MACS 2778, MACS 3063, MPO 615, MPO 1106, NIDW 9, NIDW 295, PDW 251, PDW 278, PDW 287, PDW 289, Raj 6562, Raj 6566, RD 930, RS 749, Trinakria, UAS 2021, WG 7143, and Yuk.

B Resistant to most of the leaf rust pathotypes
B-1 HD 4701 and HI 8630 (Susceptible to pathotype 12-5)
B-2 Castel Porziano, Guji 'S', HI 8498, and Raj 6560 (Susceptible to pathotype162-2)
B-3 GW 1209 and HI 8381 (Susceptible to pathotype 11)
B-4 DBP 02-08 (Susceptible to pathotype 10)
B-5 AKDW 4256 and WH 929 (Susceptible to 12-5 and 162-2)
B-6 UPD 45 (Susceptible to 162-2 and 162-3)
B-7 Similar resistance patterns
VD 97-15 (Susceptible to 11, 12-2, 12-5, 106, and 162-2)
MACS 3061 (Susceptible to 11, 12-5, 104-3, 106, and 162-2)
GW 1170 (Susceptible to 11, 12-2, 12-5, 104-3, 106, 162-2, and 162-3)
B-8 Similar resistance patterns
MACS 3503 (Susceptible to 12-2, 104-2, 104-3, and 162-2)
Raj 6516 (Susceptible to 12-5, 104-3, 162-2, and 162-3)

[a]Number of genotypes included in each group are given in parentheses

rust resistance and one of the most commonly deployed ones in modern plant breeding (McIntosh *et al*., 1995). A stem rust race 'Ug99', carrying virulence to several resistance genes including *Sr31*, is a potential threat to wheat crop in many countries including India. "Rebuilding the *Sr2*-complex", that is pyramiding diverse resistance genes in combination with *Sr2* has been recommended as the long term strategy for achieving durable resistance to stem rust including Ug99 (Joshi *et al*., 2008). Five genotypes *viz*., GW 273, GW 322, HD 4672, HI 8498, and MACS 2846, found resistant to stem rust, are among the nine Indian genotypes that showed resistance to Ug99 at Kenya (Joshi *et al*., 2008). It may be noted that these five genotypes belonged to four diverse groups; and GW 322 and HI 8498 carried the gene *Sr2* (Tables 3 and 5), and hence, need to be encouraged in cultivation and utilization in wheat improvement.

Out of more than 60 named genes for leaf rust resistance, *Lr13*, *Lr23*, *Lr26*, and *Lr34* are more common in Indian bread wheat genotypes (Nayar *et al*., 2001). With the exception of the partially effective adult-plant resistance gene *Lr34*, others are not effective against the recent pathotypes of leaf rust races 12, 77 and 104 (Nayar *et al*., 2001). The gene *Lr34* has been postulated in 12 of

the 45 leaf rust resistant bread wheat genotypes (Tables 4 and 7). Among bread wheat genotypes resistant to both stem and leaf rusts, HD 2501, HS 295 and K 9107 carried both *Sr2* and *Lr34* (Table 7). The gene *Lr23*, most commonly postulated in Indian durum genotypes, is ineffective against recent leaf rust pathotypes (Nayar *et al*., 2001). However, several studies (see Mishra *et al*., 2002) indicated that durum wheat carries unique leaf rust resistance genes, which can contribute to our resistance gene pool.

Diversity for rust resistance in Indian wheat germplasm was reported in earlier studies, based on seedling/adult-plant tests, generally involving a limited number of host genotypes and rust pathotypes. Seven patterns for stem rust resistance were observed among 97 bread wheats resistant to all the stem rust pathotypes (Bhardwaj *et al*., 2003). Sixteen bread wheat genotypes could be classified into three groups based on their seedling and adult-plant reactions to leaf rust pathotypes 77-2, 77-5 and 104-2 (Singh *et al*., 2000). In another study, 37 bread wheat genotypes were placed in five groups on the basis of seedling infection types and field responses to three leaf rust pathotypes 77-1, 77-2 and 104B (Bahadur, 2000). On the basis of infection types to leaf rust pathotypes 77, 77A, 77-1 and 77-2, at seedling and adult plant stages, 22 groups were recognized among 37 bread wheats (Shiwani

Table 7. Bread wheat genotypes resistant to both stem and leaf rusts

S. No.	Genotype	Stem rust			Leaf rust		
		Highest disease score[x]	ACI	Group (Table3)	Highest disease score	ACI	Group (Table 4)
1	CPAN 1235	10MR	1.90	B	5R	0.28	B-2
2	CPAN 1796 (R)	40RMR	5.04	B	20MRMS-TS	5.08	B-2
3	CPAN 1842	TS	0.36	E-6	10MRMS	2.04	F-3
4	GW 322[*] (R)	20RMR	2.48	F-2	10MSS	3.94	B-2
5	GW 326[*]	10RMR-TS	1.78	F-5	10MRMS	2.36	B-1
6	HD 2135[*] (R)	15MR-TS	3.08	B	5MSS	3.10	D
7	HD 2380[*] (R)	20MR	5.00	A-2	5MSS	3.50	B-2
8	HD 2402 (R)	20MR	4.64	A-2	5MRMS-TS	1.34	B-2
9	HD 2501[*#] (R)	5MR	2.20	C	10MSS	5.08	D
10	HD 2733 (R)	20R-TMR	1.48	A-1	20MRMS-TS	4.24	E
11	HI 784 (R)	30RMR-TS	4.20	E-1	5MSS	1.98	B-2
12	HI 1418[*] (R)	30RMR	3.04	A-2	5MRMS	0.92	B-2
13	HI 1434[*]	10R	0.52	A-2	10S	4.04	B-2
14	HI 1479[*] (R)	10R	1.24	B	10R-TMS	0.72	F-2
15	HP 1731[#] (R)	20R-5MR	1.74	A-2	10S	5.00	E
16	HP 1761 (R)	10R-TMR	0.76	A-1	TMR	0.12	A
17	HS 295[*#] (R)	20MRMS	4.16	A-2	10MRMS	1.81	C
18	HS 365 (R)	15R-TMR	0.92	A-1	TR	0.04	A
19	K 7827	20MRMS	4.64	F-4	5S	1.66	B-1
20	K 9107[*#] (R)	20RMR	3.64	B	5MRMS-TS	1.32	E
21	K 9465 (R)	40RMR-TMS	3.16	B	TMS	0.32	F-6
22	NIAW 34[#] (R)	30RMR-TMS	3.24	B	5MSS	4.66	F-5
23	PBW 498	20RMR	3.60	A-1	TMR	0.20	A
24	Raj 3765[*] (R)	20MRMS	4.84	A-2	10MSS	2.76	B-1
25	Raj 3777 (R)	20MR	3.28	B	20MRMS-TS	3.22	B-2
26	UP 301 (R)	20RMR	2.56	B	10S	3.66	B-2
27	UP 2003 (R)	20RMR	2.76	E-1	10S	4.40	C
28	UP 2390	20RMR	3.28	A-2	5S	2.32	B-2
29	UP 2473[*]	15MR	3.04	B	5MRMS	0.84	B-2
30	VL 804 (R)	20MR-5MRMS	4.84	A-1	5MSS	2.82	E
31	WH 542[#] (R)	5MR	1.58	A-1	10MSS	4.08	F-4

(R) = Released variety; [*]Stem rust resistance gene *Sr2* postulated (after Nayar *et al*., 2001; Bhardwaj *et al*., 2003); [#]Leaf rust resistance gene *Lr34* postulated (after Nayar *et al*., 2001); [x] Rust severity: T = trace infection, 5, 10, 15, 20, 30 etc. indicate per cent host tissue infected; Host response: R=Resistant, MR=Moderately Resistant, MS=Moderately Susceptible, S=Susceptible

and Saini, 1993). Seven groups were recognized among 61 bread wheat cultivars, based on field tests with leaf rust pathotypes 12-2, 77-1, 77-2 and 104-2 (Sawhney *et al*., 1992).

On the basis of evaluation with leaf rust race 77 in glasshouse and under field conditions, 362 durum wheat accessions were classified into three groups (Honrao and Rao, 1996). Seedling tests with five to ten leaf rust cultures revealed 23 reaction patterns among 43 durum wheat accessions (Singh *et al*., 1992). In another study, 36 durum wheat stocks could be classified into nine groups based on responses to eight leaf rust cultures (Sharma *et al*., 1986).

Table 8. Durum wheat genotypes resistant to both stem and leaf rusts

S. No.	Genotype	Stem rust			Leaf rust		
		Highest disease score*	ACI	Group (Table 5)	Highest disease score	ACI	Group (Table 6)
1	AKDW 3347	5R	0.64	A	20RMR-TMS	4.88	A
2	AKDW 4256	10MSS	3.10	B-2	20MR-TS	5.00	B-5
3	Bijaga Red (R)	10MSS	4.60	D-3	10MRMS-TS	1.82	A
4	Castel Porziano	5R-TMR	0.36	D-2	10MSS	4.70	**B-2**
5	DBP 02-08	5MR-TS	0.95	B-2	10MSS	4.87	**B-4**
6	ED 2398A	TR	0.08	C-2	5MRMS-TS	0.96	**A**
7	Guji `S'	TMR	0.16	D-1	TMS	0.44	**B-2**
8	GW 1114	10RMR-TMS	0.92	A	5MRMS	2.20	**A**
9	GW 1170	5MSS	2.02	B-2	10MRMS-TS	4.56	**B-7**
10	GW 1209	5MSS	1.70	B-2	10MRMS	5.00	**B-3**
11	HD 4502 (R)	5S	2.10	C-9	5MSS	2.88	**A**
12	HD 4530 (R)	10MSS	4.10	B-1	5MRMS-TS	0.96	**A**
13	HD 4672 (R)	5MSS	3.30	A	10MRMS	4.80	**A**
14	HD 4676	10S	4.60	C-3	10MRMS	2.32	**A**
15	HD 4696	10MRMS-TS	2.80	B-2	15RMR-TMS	1.62	**A**
16	HI 8381* (R)	10MSS	4.72	C-7	10MSS	4.40	**B-3**
17	HI 8498* (R)	10MSS	3.10	B-2	10MSS	4.82	**B-2**
18	HI 8592	10MR-TS	1.56	B-3	20MRMS	3.68	**A**
19	HI 8653	20MRMS-TS	3.36	B-2	10MRMS-TS	2.12	**A**
20	IWP 5019	10MRMS	2.24	C-6	5R	0.20	**A**
21	MACS 3061*	5S	1.72	B-2	10MRMS-TS	3.80	**B-7**
22	MACS 3063*	10MSS	3.00	B-2	20MRMS	4.80	**A**
23	MPO 615	20MR-TMS	2.24	A	TR	0.20	**A**
24	NIDW 9	10RMR-TS	1.60	A	5S	2.36	**A**
25	NIDW 295* (R)	20MRMS-TS	4.16	D-4	10MRMS	4.80	**A**
26	PDW 289	10MSS	4.90	A	20MRMS-TS	4.60	**A**
27	Raj 6516*	5MS	2.6	B-3	10MSS	5.06	**B-8**
28	Raj 6562	5MR-TMS	0.72	A	10MRMS-TS	2.70	**A**
29	RD 930	10S	4.84	B-3	5MSS	4.10	**A**
30	RS 749	30MR-TMS	3.16	A	10MRMS	4.50	**A**
31	Trinakaria	5MR-TS	0.68	C-1	10MRMS	2.28	**A**
32	UAS 2021	10MRMS-TS	2.32	B-3	20MR-10X	4.96	**A**
33	UPD 45	10S	3.40	A	5MSS	3.24	**B-6**
34	VD 97-15	20R	0.92	D-1	10MRMS-TS	3.0	**B-7**
35	WG 7143	5S	2.44	C-4	5MSS	4.96	**A**
36	Yuk	5S	2.16	A	20MRMS-TS	4.90	A

(R) = Released variety

* Stem rust resistance gene *Sr2* postulated (after Nayar *et al*. 2001); ᵡ Rust severity: T = trace infection, 5, 10, 15, 20, 30 etc. indicate per cent host tissue infected; Host response: R=Resistant, MR=Moderately Resistant, MS=Moderately Susceptible; S=Susceptible, X=Mesothetic response (different types of pustules on the same leaf

However, the present study adopted a more comprehensive approach, as seedling responses to most of our stem and leaf rust variability in general, and to the 'virulent' pathotypes in particular, formed the basis for assessing diversity for resistance in both durum and bread wheat genotypes with proven field resistance. Hence, wheat workers in the country can choose effective and diverse rust resistance donors from the ones under report.

The genotypes listed below, resistant to both stem and leaf rusts, showed resistance to all the three rusts in multilocation tests conducted during 1997-2003 (Singh *et al*., 2004).

Bread wheat: HI 1434, HI 1479, HP 1761, HS 295, HS 365, K 9107, NIAW 34, PBW 498, UP 2473, and VL 804.

Durum wheat: GW 1170, HD 4672, HD 4696, HI 8498, MACS 3061, MACS 3063, NIDW 9, PDW 289, Raj 6516, and UAS 2021.

Hence, these genotypes can be utilized as sources of multiple rust resistance throughout the country.

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Collecting Genetic Resources of Wild *Moringa oleifera* Lam. from Western Himalayas

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The present communication deals with survey, exploration and germplasm collection of wild *Moringa oleifera* Lam. in foothills of western Himalayas. A total of 23 accessions including one cultivated type were collected and conserved at National Bureau of Plant Genetic Resources, New Delhi. Fairly rich variability was observed with respect to several economic traits including cluster bearing, bitter content in fruits, prolific yield, etc. in wild/semi-wild moringa germplasm. Decline in population of young plants and seedlings in their native range was observed. Brief observations on botany of wild types collected, ethnobotany and conservation are also highlighted.

Key Words: Collection, *Moringa oleifera***, Vegetable, Western Himalayas, Wild**

Introduction

The genus *Moringa* Adans. (family Moringaceae) has 13 species (Olson, 2003), with centre of diversity in the Horn of Africa (National Research Council, 2006). Two species *viz*. *M. oleifera* Lam. (syn. *M. pterygosperma* Gaertn.) and its closest relative *M. concanensis* Nimmo occur in India. *M. oleifera* (the drumstick tree, horse radish tree, West Indian Ben, *murungai*, *sainjna*) is a medium sized fast-growing and extremely adaptable tree, native to India and Pakistan and occurs wild in the foothills of western Himalayas from North West Frontier Province (now Khyber Pakhtunkhwa), Pakistan in the west (Anwar and Rashid, 2007) to Uttarakhand up to 700 m above mean sea level (msl), and also northern parts of Uttar Pradesh (Kanjilal, 1933). It is widely cultivated as multipurpose tree species and naturalized in the tropical and sub-tropical regions of the world. Plants are principally utilized in India for fruit and leaves as vegetable especially in peninsular region, to some extent for edible flowers at local level and seed oil having industrial value and also as medicinal plant. Since ages in south India, perennial types had been cultivated as single tree in homesteads, cattle sheds, field boundaries, fences and as groups of trees on village waste lands, and rarely as intercrop/alley crop. In the last two decades improved annual cultivars (seed-propagated ones) have replaced around 60% of perennial moringa (stem-propagated) cultivating areas in south India (Rajangam *et al*., 2001).

Despite the rich nutritional content in fruit, leaf and its potential in industry, moringa has been denied attention for improvement and commercial utilization particularly in northern India. Because of high heterozygosity, genotypes existing in varied geographical areas provide scope for crop improvement programmes (Sundarajan *et al*., 1970) through broadening the genetic base. It is probable that moringa germplasm collected from centre of origin can show greater genetic diversity and also superior in having high functional property (Asian Vegetable Research and Development Centre, 2002; Bosch, 2004). Moreover germplasm with specific traits such as cluster-bearing habit, low tree canopy, tolerance to biotic and abiotic stress (Rajangam *et al*., 2001) and high seed oil are the desirable traits in moringa improvement programmes. The national and regional collections mainly represent variability in cultivated gene pool. Hence an exploration was undertaken to survey and collect the wild *M. oleifera* germplasm in parts of Punjab, Himachal Pradesh and Haryana states of India.

Materials and Methods

An exploration was undertaken in the foothills of western Himalayas (30° 75’ to 32° 26’ N latitude; 75° 40’ to 76° 90’ E longitude; altitude ranging from 230 to 700 m above msl) covering the eleven districts *viz*. Ludhiana, Hoshiarpur, Pathankot, Gurdaspur, Ropar (Punjab), Kangra, Una, Bilaspur, Hamirpur, Solan (Himachal Pradesh) and Panchkula (Haryana) of India during May 2010 (Fig. 1). General observation on occurrence under

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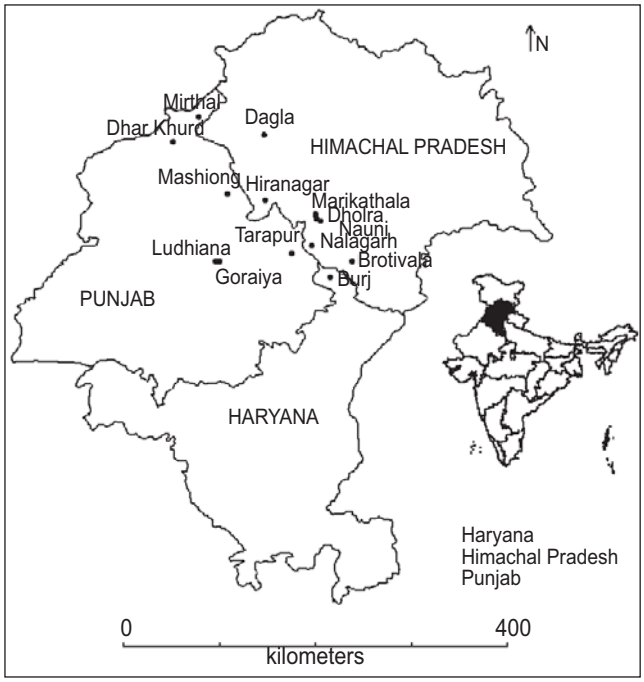

Fig. 1. Sites of *Moringa oleifera* germplasm collected in western Himalayas

natural conditions, indigenous knowledge was validated through local people. Vouchers of herbarium specimens and seed samples (HS20281, HS20282, HS20285; SS2893-2902) were deposited in National Herbarium of Cultivated Plants, NBPGR, New Delhi.

Sampling and Data Recording

Collections were made following random sampling from a population of 10 to 20 plants except for selected traits in which biased sampling on individual trees was made (trees were also marked for future bud-wood collection). Passport data on place of collection, latitude, longitude, altitude, vernacular name, frequency of occurrence, sample type, sampling method, biological status (*i.e.* wild, semi-wild, cultivated), associated vegetation and habitat/ecology were recorded at each site along with distinct traits like bitterness in fruit (as per respondents), fruit-bearing nature, productivity status, etc. Each collection was assigned with a unique collector number. Tree-dried (matured) fruits were harvested and ten representative fruits were selected for recording the data on fruit length, girth and colour and seeds per fruit. Seed samples were randomly taken for recording 100-seed weight while 20 representative seeds were selected for studying seed colour, prominence of wings and ease of seed coat detachability (recorded using arbitrary means), kernel per cent, size and shape.

Seedling Establishment Studies

A total of 50 seeds of each accession were put in for germination tests using paper towel method. The germinated seeds were shifted at two-leaf stage (15 days after planting) in pots containing sand, soil and farmyard manure in equal proportion. Seedling growth was recorded (10 seedlings/accession) after 15, 30 and 45 days. Notes on seedling establishment, variation in seedling morphology, etc. were also recorded. One set of germplasm of collected material was conserved in genebank at NBPGR, New Delhi.

Results and Discussion

General and Habitat Particulars

Moringa oleifera is known by different names in areas explored *viz*. *Sunna* in Una district, *Rasunna* in Bilaspur district, *Sunana* in Kangra district and *Swejna*/*Saijhna* in other areas. In most cases, trees were of 35-100 year old, juvenile plants were seen only sparingly in wild and semi-wild localities. Most of the populations were seen near human habitations and field boundaries (hereafter called as semi-wild), in such localities, all the respondents (approx.100) informed that all trees were self-sown. Good population stand was noticed in places namely Dhar Khurd (20 plants) in Gurdaspur district, Dagla (50) and Pind Padhya (20) in Kangra district, Gandawal (100) in Una district, Bilaspur (100), Nauni (50) and Kandrour-Bilaspur (100) in Bilaspur district, Chandimandir-Burj (300) in Panchkula district, Anandpur Sahib-Kiratpur Sahib (50) in Ropar district and Mirthal-Pathankot (100) in Pathankot district.

In few areas, plants were seen truly wild, occurring in stands as a component of the mixed broad-leaved/conifer forest or *khair* [*Acacia catechu* (L.f.) Willd.] forest with associated species like *Cassia fistula* L., *Syzygium cumini* (L.) Skeels, *Bombax ceiba* L., *Holoptelea integrifolia* (Roxb.) Planch., *Dendrocalamus strictus* (Roxb.) Nees, etc. The soils were well drained with often low organic matter. The wild trees were not distinguishable from the semi-wild ones on any consistent character. Places where truly wild population noticed were Batauli in Bilaspur district (31° 34’ N; 76° 80’ E; 547 m; 20 plants), Bhojpur-Jakhbar in Kangra district (32° 14’ N; 75° 71’ E; 347 m; 30), Rohin in Bilaspur district (31° 34’ N; 76° 75’ E; 600 m; 20) and Baduhi in Una district (31° 57’ N; 76° 19’ E; 350 m; 20) while single trees of landraces/cultivars introduced from peninsular India were observed under cultivation in kitchen garden for fruit purpose in few places.

Morphological Observations

Pollarded trees with young flush were noticed commonly in Himachal Pradesh and Panchkula district of Haryana but sparingly in Punjab. In non-pollarded trees, fruits at dehiscing stage were seen without any leaves or with new leaves. Gregarious bearing and fruit bearing in huge bunches were occasionally noticed in Punjab. In many places, the plants were noticed with slender as well as normal-sized fruits in adjacent branches, indicating the limitation of source preventing sink to develop to normal size. It signifies that better agronomic practices may boost the productivity. In general, irrespective of altitude, wild (hereafter used in the context for both wild and semi-wild populations) plants were abundant fruit-producers comparing to the cultivated types. Sometimes dehiscing fruits were noticed with reddish tinge. Although a cross-pollinated species, self pollination might be operating in wild plants in significant manner since a single plant sets fruits abundantly without having nearby plants for at least one kilometre, supporting the earlier statements on moringa pollination system by Jyothi *et al*. (1990) and Muluvi *et al*. (2004). Botanical studies of cultivated and wild types revealed some distinction (Table 1) which is hardly ever mentioned in botanical description of this species, since the latter is mainly based on the cultivated type.

Extent of Variability and Potential Value

The knowledge on the extent of variability in the morphological characters serves as a base for germplasm utilization. Out of 23 accessions collected from diverse habitats, 22 were taken for present analysis; one being collected in the form of vegetative material. Fruits in the wild types were bitter/semi-bitter and even free from bitterness as informed by local people. Three wild plants of 50-100 year old (including KP/PKS/RC/10-15) yielding bitter-free fruits were found in Batauli and Palthi (in Bilaspur district) and were occasionally used in culinary purpose like in South India. Variation was also noticed in leaflet size, dry pod colour (reddish brown/light brown/dark brown), bearing nature, prominence of seed wings, strength of seed coat, kernel shape (round/ovate/spindle) and seed colour (Table 2, Fig. 2). KP/PKS/RC/10-3, 4, 8 and 15 bore fruits in huge clusters. The seed of all the wild accessions possessed white seed coat colour except one (KP/PKS/RC/10-4) having ivory-white colour. Anwar and Rashid (2007) also reported pale-yellow to whitish seed coat colour in wild *M. oleifera* collected from Pakistan. Ovate kernel shape was exhibited by 80 % of the accessions. All the accessions had prominent wings (measuring 0.5-0.9 cm) except KP/PKS/RC/10-5, which had rudimentary or less developed wings. In line with the latter sample, a cultivated sample with wingless seeds was reported in China (Lianli and Olson, 2001). Unlike the thick and hard seed coat in cultivated sample (KP/PKS/RC/10-23), wild materials had relatively fragile and papery seed coat which could be broken with thumb pressure.

In addition to varying levels of fruit yield in individual plants sampled, good range of variation was noticed in quantitative characters studied such as fruit length (22.67-60.44 cm), fruit girth (2.20-3.44 cm), 100-seed weight (6.40-14.70 g), percentage of kernel to seed (67.80-82.21), kernel length (6.27-10.95 mm) and kernel diameter (3.36-8.52 mm) except one *i.e.* seeds/fruit which exhibited a narrow range of 12.10 to 18.70. High amount of variability (as expressed by CV) was exhibited in the traits like fruit length, 100-seed weight and kernel diameter. Raja and Bagle (2008) reported that fruit length and girth have positive direct effect on yield per plant in annual moringa. In this regard, KP/PKS/RC/10-20 was noteworthy to mention in having appreciable fruit length (60.44 cm) and girth (3.13 cm).

Table 1. Morphological comparison of wild and cultivated types of *Moringa oleifera*

Particulars	Wild†	Cultivated*
Leaf	Petiole strictly pale green in colour	Petiole also violet-purple
Flower	White or pink in colour	White or yellowish white, rarely pink
Fruit	Size 15-50(-60) x 1.0-1.5 cm, thin, slender, less fleshy, bitter; 15-20 seeds/fruit	(15-)20-120(-150) x 1.3-2.5(-3.3) cm, thick walled, fleshy, bitter-free; up to 26 seeds/fruit
Seed	Size (with wings) 15-30(-32) x (6-)8-12 mm, seed coat white, mealy, fragile; wings soft, membranous, caducous	25-32(-40) x 10-26(-30) mm; seed coat black to brown at least in the ¾ area from centre, hard; wings smaller, persistent
Phenology	Deciduous (during winter); one time flowering (February-March) followed by fruiting (March-May); leaf emergence (February-May)	Evergreen (occasionally semi-deciduous during winter in north India); flowering almost throughout the year with less pace in winter

*References: Resmi *et al*. (2005); Parrotta (2009) and Radovich (2009)
†Based on observations recorded on approx. 220 trees in area of exploration

Table 2. Fruit and seed characters of *Moringa oleifera* germplasm collected during exploration

S.N.*	Fruit length (cm)	Fruit girth (cm)	Seeds per fruit	100-seed weight (g)	Per cent kernel	Kernel length (mm)	Kernel diameter (mm)	Fruit color (dry)	Kernel shape	Wing prominence	Seed-coat detachability	Remarks
1	33.34	3.44	15.40	14.70	69.68	10.32	6.41	LB	Ovate	++	†	–
2	40.27	2.85	18.70	12.08	76.45	10.19	7.26	RB	Ovate	++	†	Gregarious bearing
3	44.38	3.06	15.30	11.24	72.95	9.18	6.11	DB	Ovate	++	†	Gregarious bearing
4	28.31	2.53	16.60	10.22	79.35	8.91	5.88	LB	Ovate	+++	†	Gregarious bearing
5	35.48	2.92	14.10	8.64	67.81	7.41	3.36	RB	Ovate	+	††	Big-sized leaflet
6	22.67	2.34	13.50	10.32	82.21	6.76	5.05	RB	Ovate to round	++	†	Big-sized leaflet
7	37.40	2.83	14.20	9.38	76.57	8.47	5.07	LB	Ovate	++	††	–
8	44.55	2.79	14.30	9.04	76.96	9.23	6.72	RB	Ovate	+++	†	Gregarious bearing
9	45.19	2.97	16.10	10.04	73.71	8.04	4.18	RB	Ovate	+++	†	Gregarious bearing
10	35.34	2.47	15.30	9.98	81.17	10.28	6.85	LB	Ovate	+++	†	Less-bitter fruits; big-sized leaflet
11	47.18	2.96	14.20	13.78	76.92	10.39	8.52	LB	Ovate	++	††	–
12	25.34	2.20	13.20	12.29	68.93	10.95	5.88	LB	Ovate		††	–
13	50.64	2.66	13.60	7.42	72.78	6.33	4.65	LB	Ovate	++	†	–
14	40.32	2.46	13.70	11.68	80.90	10.12	6.52	LB	Ovate	++	†	–
15	45.21	2.75	14.90	12.22	77.33	9.55	6.84	DB	Ovate	+++	†	Fruits bitter-free
16	47.89	2.57	13.90	7.89	79.05	8.78	5.54	LB	Ovate		††	–
17	30.77	2.75	12.30	6.40	72.88	6.27	4.02	LB	Ovate to round	+++	†	–
18	37.38	2.81	12.10	10.24	70.31	8.86	5.13	LB	Ovate	+++	†	Big-sized leaflet
19	57.34	2.86	17.80	8.37	71.88	9.31	5.76	RB	Ovate	+++	††	–
20	60.44	3.13	16.40	8.44	72.04	10.11	6.07	LB	Spindle	++	†	–
21	42.53	2.68	13.50	11.34	67.80	9.50	5.33	LB	Spindle	++	†	–
22	30.56	5.62	15.30	16.78	76.98	10.27	16.46	DB	Round	+++	†††	Fruits fleshy
Mean ± S.E	**40.57±2.11**	**2.76±0.06**	**14.72±0.37**	**10.27±0.46**	**74.65±0.98**	**8.97±0.30**	**5.79±0.26**					
Range	**22.67-60.44**	**2.20-3.44**	**12.10-18.70**	**6.40-14.70**	**67.80-82.21**	**6.27-10.95**	**3.36-8.52**					
CV (%)	**23.88**	**10.23**	**11.45**	**20.43**	**5.99**	**15.20**	**20.62**					

*Serial nos. 1-22 indicates collector nos. KP/PKS/RC-10-1 to KP/RKS/RC-10-22 respectively, last one is the cultivated type which is not considered for mean, range and CV; LB - Light brown, RB - Reddish brown, DB - Dark brown; † - Easy, †† - Slightly difficult, ††† - Difficult

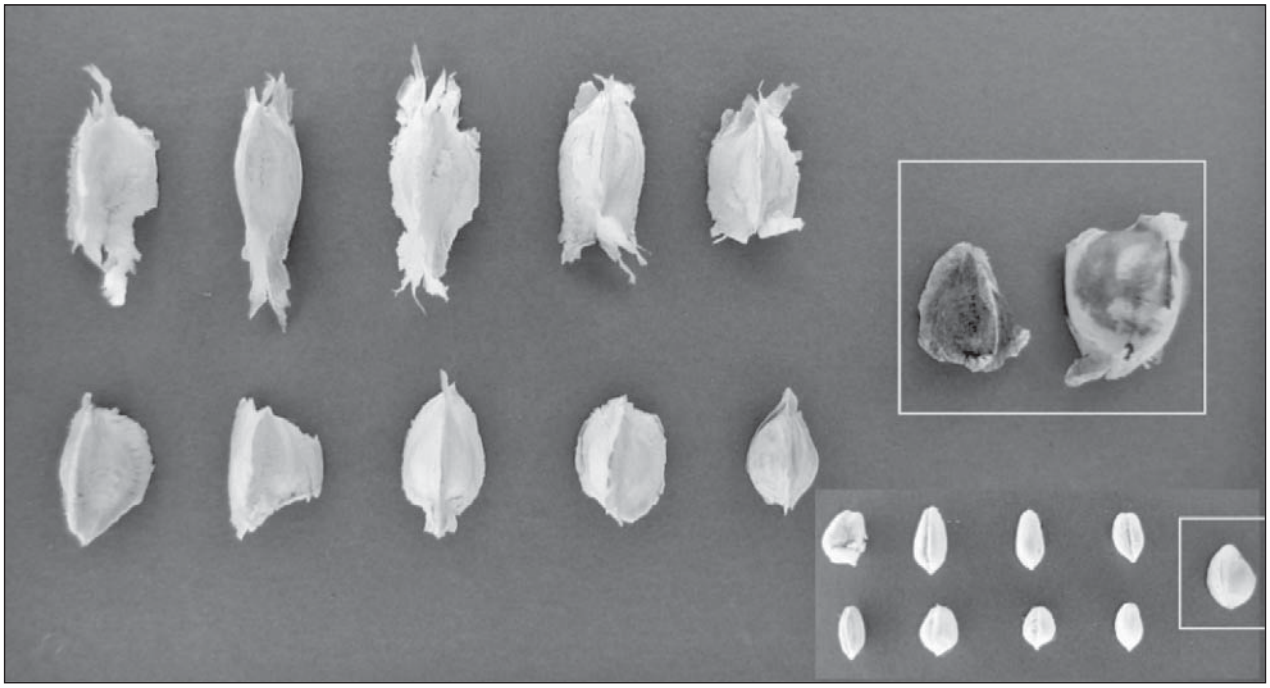

Fig. 2. Variability in seed and kernel (inset) characters of *Moringa oleifera* germplasm collected (cultivated sample in box)

The traits namely number of seeds/plant, 100-seed weight, kernel weight and size will have significance in identification of the oil-yielding types. The oil analysis of seeds of wild germplasm ranged from 20.90 to 36.80% while the cultivated sample yielded 39.20% (Dr. J Radhamani, NBPGR, pers. comm.). The present study confirms the findings of earlier reports such as Anwar and Rashid (2007) and Parrotta (2009) with respect to lower values of oil% (data not presented), fruit length, number of seeds/fruit, 100-seed weight and kernel size in wild germplasm in comparison with cultivated ones. Seed oil of wild *M. oleifera* from native range in Pakistan was found to have quality attributes identical with the cultivated moringa oils reported in literatures, hence, could be employed for edible and commercial applications (Anwar and Rashid, 2007).

Ethnobotany

Immature fruits of wild types were generally bitter, hence not generally used for vegetable purpose. In most areas surveyed, young fruits (10 day after anthesis; 10-15 cm long) were occasionally employed for making pickle. Flowers were less commonly used in *raita* (a dish prepared from boiled flowers and curd) preparation in Gandawal in Una district. At Burj village (Panchkula district), contractors were routinely collecting the flowers from wild trees (by destructive harvesting) in jute bag (moist) for sale in big markets (₹ 50/kg). Arora and Pandey (1996) also reported that young flowers (both pink and white forms) in packets are commonly sold during February-March in city markets of northern India. Also people seldom cooked flowers as informed in Auhar and Dagli villages in Bilaspur district. Roots of three months old seedlings (45-60 cm long) were said to be used for pickle making and sparingly sold in market of Punjab and parts of Kangra district of Himachal Pradesh. For this, after one and a half month in nursery, seedlings were headed back to 30 cm high so as to enable root growth for another one and a half month.

Study on seedling establishment and some considerations on conservation

Though seeds are produced in large quantity, natural regeneration of moringa seems to be poor on natural habitats *i.e.* a very few young plants were located in those areas. Bosch (2004) stated that *M. oleifera* is probably extinct in its natural state, although which is not evident in the present exploration-based study. In the present experiment, the collected germplasm showed about 60-90% germination (data not presented) which is in line with that of cultivated material (Parrotta, 2009). Seedling establishment in pots was about 80-90%, which is quite satisfactory except in KP/PKS/RC/10-13 and 14. These two accessions failed to establish in pots owing to fungal damage in seed coat immediately after germination (radicle emergence) The survived seedlings revealed variable growth rates and some

variation with respect to leaflet size and shape among the accessions studied. It was apparent from the present study that seedlings suffer less mortality under care. Nduwayezu *et al*. (2007) reported that provenances having seeds with big-sized kernel yielded high plant survival and growth rates in *M. oleifera* under Botswana conditions. This statement is corroborated to some extent in this work too but needs more fine-tuning of experimental conditions to have a meaningful conclusion.

Throughout the areas explored, though wild *Moringa* plants were not felt as so important (probably due to the availability of alternatives), villagers never cut the trees. Thus, the probable reasons for poor natural regeneration might be rapid decline in seed viability, poor competition by seedlings for light and soil moisture with associated vegetation particularly grasses, pressure from browsing animals and urbanization. Seeds have no dormancy period and lose viability within three months (Sharma and Raina, 1982; Morton, 1991). In natural conditions, seeds are shed during April-May and monsoon begins during July, i.e. at least after 60 days only conducive situation might occur, by the time, majority of the seeds might lose their viability. Authors also observed that fallen fruits are highly prone to attack by termites.

Conclusion

In view of increasing importance of *M. oleifera* in terms of vegetable and industrial value in Indian and world markets, respectively, this under-utilized species deserves renewed attention for identification of the desirable traits, for which collection of germplasm from its native areas is of utmost importance. This species responds well to vegetative propagation, hence, providing an opportunity for the breeders/germplasm curators to secure the genetic variation created/collected. Studies on factors affecting natural regeneration of this species and seed conservation are required to be investigated.

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Genetic Diversity Analysis of Antirrhinum (*Antirrhinum majus* L.) Inbreds Using RAPD Markers

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Genetic diversity analysis of 15 *Antirrhinum majus* L. inbreds was carried out using RAPD markers to assess genetic diversity present amongst them. A total of 80 DNA amplicons were scored using nine primers. Inbreds shared 63% genetic similarity among themselves. Inbreds were distinguishable from each other based on their banding pattern which is evident by the fact that none of the inbreds shared 100% similarity between each other. UPGMA cluster analysis grouped the inbreds into four clusters at 85% similarity. The co-phenetic correlation coefficient (r) was 0.9322, exhibiting best fit between dendrogram and similarity matrix based on RAPD data. The polymorphism information content (PIC) was in the range of 0.00 to 0.962, while average expected gene diversity ranged from 0.00 to 0.425. Jaccard's pair wise similarity coefficient ranged from 55 to 98.8%.

Key Words: Antirrhinum; Cluster; Genetic diversity; Polymorphism information content; RAPD

Introduction

Snapdragon (*Antirrhinum majus*) native of the Mediterranean region, from Morocco and Portugal north to Southern France and east to Turkey and Syria is amongst most beautiful ornamental flowering plants. The genus *Antirrhinum* ($2n = 16$) has >20 species, is mostly found around the Mediterranean Sea and in North America (Stubbe, 1966). Genetic variability considered to be the basis of plant breeding (Simmond 1983) is the essential pre-requisite for initiation of breeding work as improvement of any crop is possible only when wide genetic variability exists in the population of that species. Molecular marker analysis is a powerful tool for distinguishing one individual from another on the basis of DNA banding pattern using molecular markers and RAPD molecular markers discovered by Welsh and McClelland (1990) and Williams *et al*. (1990) can be used for assessment of genetic variability in populations as it is simple, fast, requires small quantity of template DNA and involves no radioactivity or southern blotting and hybridization for distinguishing individuals. Thus, present investigation was taken up to distinguish *Antirrhinum majus* inbreds from each other and to assess genetic variation among them using RAPD molecular markers.

Materials and Methods

Plant Material

A total of 15 *Antirrhinum majus* L. inbred lines were sampled from Model Floriculture Centre (MFC), GB Pant University of Agriculture and Technology (GBPUAT), Pantnagar, India (Table 1). Twelve inbreds were from flower seed companies located in India at New Delhi, Lucknow, Guwahati and Dimapur in North-East and three inbreds *viz.* SA-1, Sant-11 and Anti-331 were introduced from Thompson and Morgan (Ipswich, United Kingdom). These inbreds were selfed for six generation to maintain their genetic purity.

DNA Isolation

DNA was extracted using the procedure reported by Torres *et al.* (1993) with some modifications. Leaf material (500 mg) was ground to a fine powder in liquid nitrogen, mixed with 8 ml of extraction buffer [CTAB (Cetyl trimethyl Ammonium bromide) (2% w/v), Tris-HCl (100 mmol/l pH 8.0), EDTA (20 mmol/l pH 8.0), NaCl (1.4 mol/l), sodium bisulfite (0.1% w/v) and 2-mercaptoethanol (0.4% v/v)] and incubated at 60°C for 45 min. The samples were mixed with an equal volume of chloroform-iso amyl alcohol (24:1) and centrifuged at 9,000 xg for 5 minutes under

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Table 1. Details of inbreds of *Antirrhinum majus* used for DNA fingerprinting

Sl. No. (Sample code)	Name of Genotype	Source of collection
1.	65 G	New Delhi, India
2.	135 G	Lucknow, Uttar Pradesh, India
3.	109 G	New Delhi, India
4.	98 G	Guwahati, Assam, India
5.	Sant 11	Ipswich, United Kingdom
6.	97 G	Lucknow, Uttar Pradesh, India
7.	128 G	New Delhi, India
8.	92 G	Lucknow, Uttar Pradesh
9.	Anti-331	Ipswich, United Kingdom
10.	328 G	Dimapur, Nagaland, India
11.	113 G	Guwahati, Assam, India
12.	99 G	Lucknow, Uttar Pradesh
13.	SA-1	Ipswich, United Kingdom
14.	90 G	New Delhi, India
15.	61 G	Dimapur, Nagaland, India

refrigeration. The DNA was precipitated by adding one volume of chilled isopropanol for 1 h. The DNA pellet was washed in ethanol (70%). Finally, the DNA pellet was air-dried and suspended in an appropriate volume of 1 X TE buffer and quantified using UV spectrophotometer.

PCR Amplifications and Electrophoresis

PCR amplifications were performed in a volume of 25 µl reaction containing 1µl template DNA (100 ng/µl), dNTPs (2.5 µM each) 2 µl, *Taq* polymerase (3 U/µl) 0.25 µl, Reaction buffer (10 X) 2.5 µl, Primer 1 µl. Amplifications were performed in a PCR thermal cycler (Eppendorf, Hamburg, Germany). The thermal cycles performed were programmed as follows: 3 cycles of 94°C (2 min)/36°C (1 min 30 s)/72°C (2 min) and 41 cycles of 94°C (20 s) /36°C (40 s)/72°C (2 min) and final extension at 72^0C (6 min). RAPD primers were same as those used by Gomez *et al.,* 2002 and were custom synthesized from Ocimum Biosolutions Pvt. Ltd., Hyderabad, India (Table 2). DNA Fragments generated by amplification were separated on 1.5% agarose gel run in 1 X Tris-borate EDTA buffer, stained with ethidium bromide (0.5 µg/ml), and visualized with ultraviolet light and photographs. "Low range DNA ruler" (Bangalore Genei) was included in the gels as a molecular size reference.

Molecular Markers Data Analysis

DNA banding pattern were scored for the presence (1) or absence (0) of bands of various molecular sizes in the form of binary matrix. Data were analyzed to obtain Jaccard's similarity coefficients among the inbreds by using NTSYS-PC (version 2.02e; Exeter Software, Setauket, NY, USA; Rohlf, 1990). Dendrogram was constructed using the Unweighted Pair Grouping Method with Arithmetic Averages (UPGMA) and Sequential Agglomerative Hierarchical and Nested (SAHN) clustering methods. The reliability of the dendrogram was tested by co-phenetic correlation coefficient between similarity and co-phenetic value matrix based on RAPD data using Mxcomp of NTSYS-PC (version 2.02e; Rohlf, 1990).

Average expected Gene Diversity (H_j)

Bands were scored in the gel and converted to numbers. For this, each of the band (in the same row) was scored and transformed to 1, if it was present or to 0, if it was absent. The presence of band corresponds to the dominant/ heterozygous genotype (AA/Aa) and the absence to the recessive genotype (aa) for dominant marker and were scored 1 and 0, respectively. On the basis of this scoring pattern, allelic frequencies were calculated.

Polymorphism Information Content

The genetic diversity of each RAPD locus was estimated using Polymorphism Information Content (PIC) that refers to the value of a marker for detecting polymorphism within a population of inbreds, depending on the number of detectable alleles and the distribution of their frequency (Anderson *et al.,* 1993) as follows

$$PIC = 1 - \sum_{j=1}^{n} P^2_{ij}$$

Where, P_{ij} is the frequency of j^{th} allele in the i^{th} primer and summed up over *n* alleles.

Results

A total of 10 primers were used for genetic diversity analysis of *Antirrhinum majus* inbreds. Out of 10 primers, nine produced reproducible polymorphic bands. Each of the primers varied greatly in their ability to resolve variability among the inbreds. The individual primer produced bands in a range of 2 (Primer- OP-020) to 15 (Primer- UBC-543) bands. A total of 80 DNA amplicons were scored using these nine primers with an average of 8.90 bands per primer (Table 2). The amplified fragments ranged from 100 bp to 3500 bp. The polymorphism percentage was in the range of 0.00% to 100% for these primers (Nadarajan *et al.,* 1999). All primers based on their % polymorphism and unique band amplification were considered as highly informative primers. A moderate

to high proportion of polymorphic amplified products were detected. PIC values ranged from 0.00 for primer 6 to as high as 0.962 for primer 10, with an average of 0.338 for nine primers (Table 2). A gel scoring was done using the 0-1 matrix for band profile of 80 DNA amplicons produced by 9 polymorphic RAPD primers, which clearly distinguished *Antirrhinum majus* inbreds from each other at molecular level. The results clearly showed that 15 *Antirrhinum majus* inbreds were genetically diverse from each other at molecular level in spite of having similarities at morphological level, which is evident by similarity coefficient matrix between inbreds as none of the inbreds shared 100% similarity with each other (Table 3) and by the dendrogram generated using similarity matrix as well (Fig. 2).

Average Expected Gene Diversity

The average expected gene diversity ranged from 0.00 for primer UBC-540 followed by 0.156 for primer OP-020 to a maximum of 0.425 for primer UBC-543 followed by 0.415 for primer UBC-557 with an average of 0.216 for all the random primers among all the inbreds at all the locus studied (Table 2).

Jaccard's Similarity Coefficient

Pair-wise Jaccard's similarity coefficient for the genetic similarities among 15 inbreds is presented in Table 3. The value of the coefficients were estimated on the basis of nine primers which ranged from 55% between inbreds 98G and 328G followed by 56.3% between inbreds 98G and 113G and by 57.5% between inbreds 109G and Anti-331

Table 2. Number of RAPD loci, primer sequence, amplified fragment size, PIC and average expected gene diversity generated by 9 random primers

Primer Code	Primer Name	Nucleotide sequence (5′-3′)	Tm (^{0}C)	Fragment size range	PIC	Average expected gene diversity
109761	UBC-507	AGA CGT ACT C	30.0	250-3250	0.045	0.062
109762	UBC-511	GAA TGG TGA G	30.0	350-3050	0.509	0.425
109763	UBC-514	CGG TTA GAC G	32.0	275-3050	0.102	0.041
109764	UBC-523	ACA GGC AGA C	32.0	300-3050	0.442	0.400
109766	UBC-540	CGG ACC GCG T	34.0	350-1400	0.000	0.000
109767	UBC-557	GTG TAG AGC C	32.0	450-3050	0.589	0.415
109768	UBC-543	CGC TTC CGG T	34.0	150-2750	0.272	0.218
109769	OP-001	GGC ACG TAA G	32.0	500-2500	0.124	0.232
109770	OP-020	ACA CAC GCT G	32.0	1500-2000	0.962	0.156

Table 3. Jaccard's similarity coefficient between fifteen *Antirrhinum majus* inbreds

	65G	135G	109G	98G	Sant11	97G	128G	92G	Anti-331	328	113G	99G	SA-1	90G	61G
65G	1.000-														
135G	0.863	1.000													
109G	0.700	0.788	1.000												
98G	0.675	0.763	0.975	1.000											
Sant11	0.788	0.750	0.688	0.663	1.000										
97G	0.800	0.738	0.650	0.625	0.938	1.000									
128G	0.763	0.700	0.613	0.588	0.925	0.963	1.000								
92G	0.813	0.750	0.638	0.613	0.925	0.988	0.950	1.000							
Anti-331	0.813	0.725	0.613	0.588	0.875	0.888	0.875	0.900	1.000						
328	0.825	0.738	0.575	0.550	0.763	0.775	0.763	0.788	0.888	1.000					
113G	0.863	0.750	0.588	0.563	0.800	0.863	0.850	0.875	0.875	0.913	1.000				
99G	0.875	0.763	0.600	0.575	0.763	0.825	0.813	0.838	0.838	0.900	0.963	1.000			
SA-1	0.863	0.750	0.638	0.613	0.875	0.888	0.850	0.900	0.925	0.838	0.850	0.888	1.000		
90G	0.850	0.738	0.625	0.600	0.888	0.875	0.863	0.888	0.913	0.825	0.838	0.875	0.988	1.000	
61G	0.850	0.763	0.625	0.600	0.863	0.850	0.838	0.863	0.888	0.775	0.813	0.825	0.938	0.950	1.000

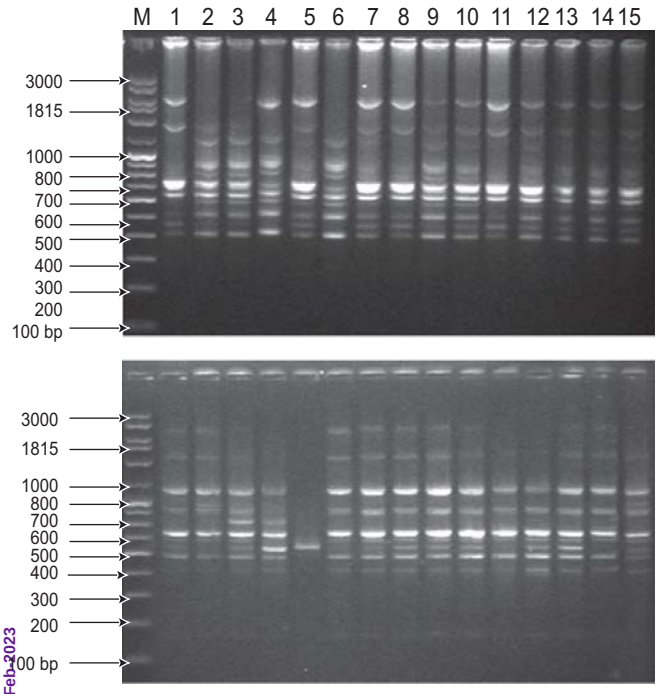

Fig. 1. DNA profiles of 15 *Antirrhinum majus* inbreds obtained with primers (a)UBC-523 and (b)UBC-511. Marker Lane corresponds to low range DNA rular as fragment size marker

to a maximum of 98.8% between inbreds SA-1 and 90G followed by 97.5% between inbreds 109G and 98G and by 96.3% between inbreds 97G and 128G and between 113G and 99G. This maximum similarity between these inbreds indicates the relatedness of these inbreds by ancestry. These similarity coefficients, thus, also indicate that sufficient amount of variability is present between the fifteen inbreds and that the inbreds were different from one another.

Cluster Analysis

The UPGMA cluster analysis method was followed for the construction of the phylogenetic tree (Fig. 2). The result showed that at 0.85 similarity coefficient four major clusters were formed. Out of these clusters, two clusters were having two inbreds. These genotype clusters were Cluster I (inbreds 65G and 135G) and IV (inbreds 109G and 98G) clearly indicating distinctness of these inbreds from the others. Cluster II was having seven inbreds *viz*. inbreds (Sant-11, 97G, 92G, 128G, Anti-331, SA-1, 90G and 61G) while Cluster III was having three inbreds (328G, 113G and 99G). This dendrogram analysis has grouped inbreds into different clusters based on their similarity coefficient and thereby on the basis of genetic variability. Inbreds included in single cluster are genetically closer

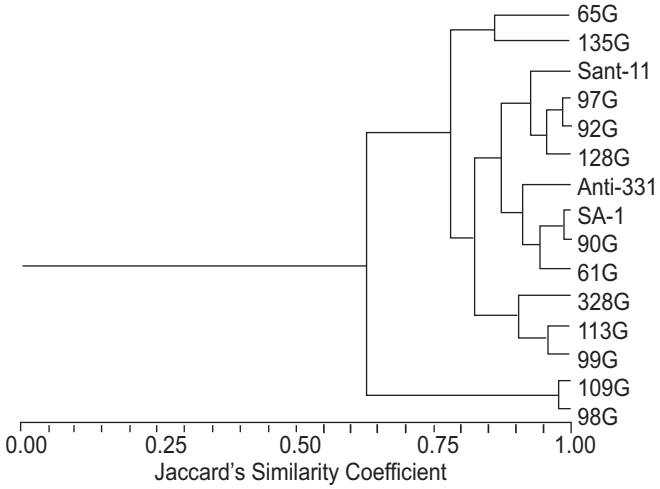

Fig. 2. UPGMA dendrogram of 15 inbreds of *Antirrhinum majus* based on Jaccard's similarity coefficient

than the inbreds of other clusters. The co-phenetic correlation coefficient (r) was 0.9322, exhibiting best fit between similarity and co-phenetic value matrix based on RAPD data.

Discussion

Out of the 10 primers used for diversity analysis of *Antirrhinum majus* inbreds nine produced reproducible amplifications. These primers revealed variability among the *Antirrhinum majus* inbreds at DNA level. The polymorphism percentage exhibited by the RAPD primers was in the range of 0.00% to 100% for these primers (Nadarajan *et al.,* 1999 reported 77.4% polymorphism between seven *Japonica*, two *Indica* and one tropical *Japonica* rice varieties by RAPD markers). All primers based on their % polymorphism and unique band amplification were considered as highly informative primers. A moderate (*A. majus*, 58.04%; this study) to high (*A. microphyllum*, 90%; Torres, 1999) proportion of polymorphic amplified products were detected. As expected, the PIC distributions revealed that, in terms of genetic distance, dominant markers had lower levels of polymorphism as compared to codominant markers, which agrees with the results published by Becker *et al.* (1995); Russell *et al.* (1997) and Pejic *et al.* (1998). The average expected gene diversity ranged from 0.00 to a maximum of 0.425 with an average of 0.216 for all the random primers among all the inbreds at the entire locus studied (Lu *et al.,* 2005).

Pair-wise Jaccard's similarity coefficient for the genetic similarities among 15 inbreds presented in Table 3 ranged from 55% between inbreds 98G and 328G to a maximum of 98.8% between inbreds SA-1 and 90G.

Similar values of similarity coefficients were obtained among rapeseed inbreds (Cartea *et al*., 2005). This maximum similarity between these inbreds indicates the relatedness of these inbreds by ancestry. These similarity coefficients, thus, also indicate that sufficient amount of variability is present between the fifteen inbreds. The genetic relationship among the inbreds using UPGMA cluster analysis based on the RAPD-derived data was studied. Our results indicated that it is easy to distinguish all the snapdragon inbreds from others by using molecular markers. Similar studies were also done by many other workers e.g. in *Antirrhinum* by Torres *et al.* (2003) and in wheat inbreds by Wei *et al.* (2005). The cluster analysis based on Jaccard's similarity coefficient clearly indicated distinctness of inbreds grouped in single cluster from the others. This dendrogram analysis has grouped inbreds into four different clusters based on their similarity coefficient and thereby on the basis of genetic variability (Jimenez *et al.,* 2002). Inbreds included in single cluster are genetically closer than the inbreds of other clusters. The co-phenetic correlation coefficient (r) was 0.9322, exhibiting best fit between similarity and cophenetic value matrix based on RAPD data (Uma *et al.,* 2008).

In a wider context, the speed, efficiency and reliability of the RAPD methodology are important considerations for the development of strategies for effective management of germplasm collection in terms of identification of duplicates, the estimation of diversity, monitoring genetic erosion and enhancing the use of these collections. RAPD markers have been used for the DNA fingerprinting for identification of cultivars and for assessment of genetic relationships among cultivars (Li and Nelson, 2002). The results suggest that RAPD molecular markers can be used efficiently for analyzing genetic diversity and phylogenetic analysis (Ni *et al.,* 2003; Nagaraju *et al.,* 2002).

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On-farm Dynamics of Wheat Genetic Resources in Nainital District of Uttarakhand

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Current status of wheat genetic resources and their management was studied in eight blocks of Nainital district of Uttarakhand. A total of 13 wheat varieties comprising of six traditional types and seven improved high yielding types (HYVs) were found under cultivation. Over four decades, 66.43% of the area of traditional varieties has been replaced by HYVs. Rye (*Secale cereale*) locally known as *Russi gehun* is also under sporadic cultivation. The selection of varieties was varied depending upon the topography, agro-climatic niches and resources of the area. Two traditional varieties *viz*. *Naulia* and *Daulatkhani* were found to have rare distribution. Significant variability for some characteristics between and among the traditional varieties were observed. Assured grain yield, good straw quantity, drought tolerance, grain quality, taste and resistance to birds and animal damage were some of the farmers' criteria for selection of the varieties.

Key Words: Genetic resources, Wheat, Uttarakhand

District Nainital is situated in Central Himalayan region between 80º14' East longitude and 29º5' North latitude. The region is a well known centre of crop diversity (Arora 1990; Khushoo 1992). The total geographical area of district is 3422 km^2. Geographically, it is divided in to two zones viz. hilly and the plains. The conservation of crop diversity is the major concern of today. After green revolution, many high yielding varieties (HYVs) particularly of wheat and rice were introduced in this region for increasing of production. The introduction of high yielding varieties, initially free of cost, has encouraged the farmers to adopt them. Consequently farmers abandoned their old traditional varieties, which were locally adapted and catering to farmers' needs. As a result, many traditional crop varieties were disappeared. A particular worry is about the substitution of diverse set of genetically variable crop landraces by a few genetically uniform modern varieties (Brush 1991; Harlan 1992; National Research Council, 1993). In contrast to modern agriculture, traditional farming system not only serves to preserve the diversity of local varieties, but also the human knowledge and behaviour practices that have shaped this diversity (Bellon, 1996). The present study aimed to understand the on-farm dynamics of traditional and high yielding varieties of wheat (*Triticum aestivum* L.) in district Nainital of Uttarakhand.

Materials and Methods

Data of wheat (*Triticum aestivum* L.) genetic resources under cultivation in district Nainital were collected from primary sources by using a structured questionnaire accompanied with interview schedule at individual farm household level during *rabi* 2008-09. Sample villages were randomly selected from all eight blocks of the district. All the regions representing distinct agro-climatic niches were covered for the study. A total of 32 villages and 109 households were randomly selected as respondent farmers for documentation of wheat varieties under cultivation in their villages. A non participant observation method was also employed while recording the information.

The information on erosion, shift in varietal diversity and changes were obtained by using participatory rural appraisal (PRA) technique. Current status was validated by recording observations in the fields for wheat cultivars diversity. All possible care was taken to determine the consistency in farmers naming and describing wheat cultivars by comparing information from farm households, different social groups etc. Information obtained was authenticated with the help of knowledgeable farmers and agricultural extension and development workers engaged in the region.

Results and Discussion

The present study revealed that six traditional varieties (TVs) are being grown by the local farmers of which

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Munda, Naulia and *Daulatkhani* are the awnless (*Munda*) varieties while *Ryat, Syat* and *Chanosi* are awned (*Jhusia*). High yielding varieties viz. RR 21, S 227, VL 616, VL 738, VL 829, UP 2382, UP 2554 and PBW 343 are also under cultivation. *Chanosi* is dominant in all blocks except in Ramnagar, where none of the TV is under cultivation as land is irrigated and well connected to National highway.

Local farmers informed that before 1970s they used to grow TVs only, none of the HYVs were present in the region. Presently HYVs have occupied a large area (66.43%) of wheat cultivation. It is evident that the erosion of the TVs is a result of state sponsored 'Green Revolution', which pushed the HYVs, chemical fertilizers, pesticides etc. free of cost (Maikhuri *et al.*, 2004). The present study revealed that replacement of TVs in different development blocks varies from 24 to 100%. The least replacement is observed in Betalghat and Kotabagh blocks as these are not well connected with high ways. In Bhimtal, Dhari, Haldwani, Okhalkanda and Ramgarh blocks most of the TVs have been replaced. Haldwani development block is also well connected with road networks. Consequently TVs of crops including wheat have been replaced under the onslaught of developments and mushrooming of construction works. Development block Okhalkanda is backward in agriculture hence government agencies have provided input resources in the form of fertilizer, HYVs etc. to the farmers for agricultural development. As a result 63.74% of the TVs' area have been occupied by HYVs.

Among the TVs, *Naulia* and *Daulatkhani* are the worst affected and almost disappeared from the farmers' fields. Their presence in the study area is mere 2.78% of each. Among TVs *Ryat gehun* occupies the highest area (25.0%) followed by *Munda* (22.23%), *Chanosi, Syat gehun* (19.42% each) and *Russi gehun* (*Secale cereale*) (8.34%). The reduction of area under traditional varieties is also evident in other regions and countries. In terms of genetic diversity, the introduction of HYVs has increased the diversity of wheat genetic resource in the region as most of the high yielding varieties have diverse germplasm from various sources in their pedigree.

Grain yield, straw yield, drought tolerance, grain quality, taste and resistance to birds and animal damage (because of presence of awns) are the major criteria adopted by farmers for selection of wheat varieties and their area share of cultivation. Assured yield is the most important feature of the variety. Awned TVs, *Chanosi, Ryat* and *Syat* are ranked first in view point of assured yield followed by HYVs and awnless TVs. Traditional awnless varieties are found suitable for drought conditions, reported the farmers. A study conducted in district Almora of Uttarakhand by Tiwari and Das (1996) also revealed the same trend. The grain quality and taste of *Chapati* are also very important criteria for adopting a variety in the region. Awnless TVs are good in grain quality and *chapatti* taste particularly *Daulatkhani*, once a very popular cultivar in Kumaon hills as reported by farmers. Similar findings were also reported by Mehta *et al.* (2009).

In Central Himalayas the traditional agro-biodiverstiy is complex with strong linkage between crop plants, animals and forests (Palni *et al.*, 1998) and straw a byproduct of crops is an important source of fodder for the domestic animals. The farmers normally prefer TVs which have good quality straw alongwith grain yield. The study revealed that in spite of possessing all the desirable traits, the awnless TVs have been replaced by the farmers because they were very prone to birds and animal damage. Consequently the overall area share of the awnless TVs has been reduced to the lowest rank (Table 1). Jarvis *et al.* (2000) also reported that for selection of variety, it is important to understand how farmers make use of their crops and agro morphological characteristics in different capacities particularly selecting among the plants in the crop populations to maintain the desirable traits and to increase the prevalence of other valued traits in the population overtime.

Variations in size, shape and colour of grains were observed among and within the local cultivars

Table 1. Replacement of traditional varieties by HYVs in different development blocks of district Nainital

Development block	Area (%) of wheat varieties under cultivation	
	TVs	HYVs
Betalghat	76.00	24.00
Bhimtal	51.20	48.80
Dhari	10.00	90.00
Haldwani	4.00	96.00
Kotabagh	71.00	29.00
Okhalkanda	36.26	63.74
Ramgarh	20.12	79.88
Ramnagar	0.00	100.00
Total	33.57	66.43

Table 2. Grain character of traditional varieties grown in district Nainital

Name of traditional varieties	Grain length (mm)	Grain width (mm)	100-seed weight (gm)	Grain colour	Remarks
Naulia	5.9	3.30	4.02	Amber	Awnless, tall
Ryat gehun	5.81	2.98	2.71	Red	Awned, tall, drought tolerant
Syat gehun	5.98	3.11	3.04	Amber	Two variants: awned and awnleted
Munda	6.22	2.83	3.47	Variants white, amber and red	Awnless, bold grains
Daulatkhani	5.77	3.02	3.67	Amber	Awnless, bold grains
Chanosi	6.25	3.30	4.17	Amber	Awned, plant height medium, bold grain

(Table 2). In *Syat gehun* two variants – amber and reddish grain colour with awned and awnleted spikes were recorded. *Ryat gehun* grains are of medium size and red (light to dark red) in colour. There were two variants in *Chanosi* long and bold with white grain and medium size with amber grain. In *Munda*, three variants – red, amber and white grain colour were recorded. *Naulia* a bold grain wheat, two colour variants amber and white were found under cultivation in some pockets of Bhimtal block. Three variants of grain colour in *Daulatkhani* were collected during the study and collection mission. The intra cultivar diversity observed in on-farm managed populations could be explained by size in the number of landraces cultivated during the last 2-3 decades. In the North-western Himalayas, farmers plant several landraces in the same or in nearby fields (Bisht *et al.*, 2007). Further, at local level there is frequent exchange of landraces among farmers. The seed selection is normally very uncommon and the size of the population is relatively large which avoid substantial loss of intra-varietal diversity from generation to generation through genetic drift. Increase in intra-varietal diversity over continuous cultivation has also been reported in other studies as well (Benzancan *et al.*, 2008; Bary *et al.*, 2008).

The overall diversity of traditional wheat cultivars are of paramount importance to cater the subsistence requirement of the local farmers in rainfed agro-ecosystems of the Himalayas. The traditional wheat cultivars were giving better yields under low input rainfed conditions. The abandoning of TVs and adopting of HYVs without considering any pros and cons in the Himalayan region particularly in rainfed marginal farming systems has negative impact on agricultural production. Before developing of HYVs, it is very essential to study and understand the topography, micro climate, pedology and resource conditions of the target region. The traits of traditional cultivars, for which they are popular among the local farmers must be incorporated in the new improved varieties. It is also important to take care of the traditional cultivars and those should be collected and conserved for the future generations while introducing the improved high yielding varieties in traditional agro-ecosystems, so that the process of sustainable development may also be continued.

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Molecular Diversity Analysis of Traditional Rice Varieties of Kerala Using RAPD Markers

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Thirty traditional landraces collected from nine ecological zones of Kerala were subjected to molecular studies. Twenty oligonucleotide primers used for the study produced 222 amplicons with a polymorphism of 82%. The amplification products derived with random primers had size ranging from 2.0 kb to less than 0.5 kb. Of the twenty primers OPF-04 gave maximum number of polymorphic products and also produced two unique positive products in the accession Cheruvirippu (size between 1.0 kb and 1.5 kb) and in the accession Njavara yellow (size of less than 0.5 kb). This primer has the highest resolving power (24.52) and Effective Multiplex Ratio (15.0) and so is selected as the best primer for distinguishing rice varieties in this study. Clustering based on Jaccard's similarity coefficient revealed the highest similarity between the accessions Chettivirippu and Pokkali (0.825). Both these accessions are suited to the saline sodic soils of Kerala. The Njavara group of accessions (medicinal rice), Njavara yellow and Njavara black, clustered at a similarity value of 0.707. The primer OPB-05 and OPF-01 could distinguish the Njavara accessions from others. OPB-05 produced unique product with a size of less than 1.0 kb and OPF-01 produced product at size of less than 0.5 kb unique for the Njavara accessions. Even though these accessions were from different ecological zones location specific clustering was not observed pointing the uniqueness of the collection at the micro level.

Key Words: Diversity, RAPD, Rice

Introduction

Kerala is considered as one of the centres of diversity of rice and the antiquity of rice cultivation here dates back to 3000 BC (Manilal, 1990). Rice covers a vast array of ecological niches and a vast diversity of germplasm of both cultivated and wild rice exist in Kerala (Kumary and Francies, 2002). Diversity at ecosystem level, inter-specific levels in addition to ethnic diversity is the highest in this stretch of land. These traditional varieties may be containing favourable genes, especially genes for resistance to biotic and abiotic stresses. Conservation and characterization of these varieties is essential for future genetic improvement of rice.

Characterization based only on morphological and physiological parameters with clear cut features of distinctness are not always possible. The morphologies reflect not only the genetic constitution of the individual but also the interaction of the genotype with the environment within which it is expressed (Patterson and Weatherup, 1984). Characterization with molecular markers is more reliable and DNA based molecular markers are in abundance and clearly allow the comparison of genetic material avoiding any environmental influence on gene expression. RAPD (Randomly amplified polymorphic DNA) markers (Williams *et al*., 1990) are PCR based and is widely in use for molecular analysis of plant genome. RAPD analysis is a simple technique with lower cost using a small amount of DNA and has the advantage of hitting the genome randomly.

The present investigation was done to assess the molecular diversity of the thirty genotypes collected from different ecological zones of Kerala using RAPD markers.

Materials and Methods

The experimental material consisted of 30 traditional rice accessions of Kerala chosen from the material collected under NATP project on Sustainable Management of Biodiversity, at KAU (Table 1). The seed material was procured from Rice Research Station, Moncompu, Alappuzha.

Isolation of Genomic DNA

Isolation of genomic DNA was done from all the 30 samples following the procedure of Regowsky *et al.* (1991) with required modifications.

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Table 1. Morphological characters of the selected 30 rice accessions

S. No.	Name	Source	Leaf			Days to heading	Panicle		
			Length (cm)	Width (cm)	Blade colour		Length (cm)	Type	Secondary branching
1	Njavara (yellow)	Allepey	49	0.7	Green	58	18.5	Compact	Light
2	Njavara (black)	Palakkad	61.6	1.0	Pale green	60	26.0	Intermediate	Light
3	Cheeravithu	Palakkad	51.5	1.1	Purple blotch	91	24.5	Intermediate	Light
4	Jeerakasala	Wayanad	35	1.1	Pale green	92	36.5	Compact	Heavy
5	Chempan	Trivandrum	48.5	0.9	Pale green	104	29.5	Intermediate	Light
6	Kannikayama	Kannur	52.5	0.8	Green	96	27.2	Compact	Light
7	Thowan	Kannur	42.2	1.0	Green	102	23.3	Compact	Light
8	Kozhivalan	Kannur	41.7	1.3	Pale green	121	23.4	Compact	Heavy
9	Kururayima	Kannur	64.51	1.2	Green	107	27.2	Compact	Light
10	Karuthacheera	Perumbavur	53.5	0.9	Green	89	26.7	Compact	Light
11	Kalladi Aryan	Kannur	35.5	0.7	Green	101	22.5	Compact	Light
12	Veluthittaryan	Palakkad	59	1.5	Green	126	22.5	Intermediate	Light
13	Athikiramundakan	Allepey	45.5	1.2	Green	116	20.6	Intermediate	Light
14	Anakodan	Palakkad	39.1	1.2	Green	125	22.3	Compact	Light
15	Veluthakattamodan	Kannur	63.7	1.1	Green	90	28.8	Intermediate	Light
16	Kattamodan	Palakkad	79.4	1.1	Pale green	100	27.3	Compact	Light
17	Allikannan	Kannur	40.5	1.0	Green	104	25.2	Compact	Light
18	Parambuvattan	Palakkad	32.5	0.6	Dark green	94	23.3	Compact	Light
19	Vellakkoli	Thrissur	45.2	0.7	Green	89	23.5	Intermediate	Light
20	Vellamundakan	Allepey	35.9	0.8	Dark green	76	17.4	Compact	Light
21	Cheruvirippu	Ernakulam	58.5	1.3	Green	103	26.0	Open	Light
22	Choottupokkali	Ernakulam	65	1.4	Green	104	27.5	Intermediate	Light
23	Chenthadi	Wayanad	31.5	0.6	Green	87	23.1	Intermediate	Light
24	Kazhama	Kannur	54.3	1.1	Pale green	96	29.8	Intermediate	Light
25	Mundon	Malapuram	57.5	0.8	Dark green	88	27.2	Intermediate	Light
26	Chettivirippu	Ernakulam	43	1.0	Dark green	100	26.5	Intermediate	Clustering
27	Pokkali 3	Ernakulam	48	0.9	Dark green	102	23.5	Intermediate	Light
28	Ponkuruka	Ernakulam	49.5	1.2	Dark green	95	25.2	Compact	Light
29	Pandivella	Trivandrum	52	1.1	Green	99	28.0	Compact	Heavy
30	Thavalakannan	Palakkad	43.2	1.1	Green	105	24.0	Compact	Light

Random Amplified Polymorphic DNA (RAPD)

The procedure of Williams *et al*. (1990) was used for the amplification of DNA. The amplification was done using 20 reported arbitrarily designed primers, which included primers from different Operon primer series *viz.* OPA-5, OPB-05, OPB-08, OPB-10, OPC-07, OPC-15, OPD-03, OPD-18, OPF-01, OPF-03, OPF-04, OPF-05, OPF-06, OPF-13, OPG-18, OPG-19, OPH-19, OPK-14, OPK-19 and OPL-17 [Barooah and Sarma (2004); Raghunathachari *et al.* (2000); Saker *et al.* (2005)].

The reaction was carried out in 25 µl reaction mixture containing 20 ng template DNA, 2.5µl of 10x PCR buffer, 2 µl of 2.5 mM $MgCl_2$, 1 µl of 10 mM dNTP mix, 1 unit of Taq DNA polymerase and 1 µl of 10 pmoles primer. Amplification was done in a thermocycler (MJ Research Inc., USA) with PCR cycle as follows:

An initial denaturation at 94ºC for 5 min followed by 40 cycles of denaturation at 94ºC for 1 min, annealing at 36ºC for 1 min and extension at 72˚C for 90 sec. The synthesis step of final cycle was extended further by 7 min. Finally the products of amplification were stored at 4ºC. Amplified products were separated by agarose gel electrophoresis using 1.4% gel as described earlier and photographed using gel documentation system.

Data Analysis

The reproducible bands were scored for their presence (1) or absence (0) for all the genotypes studied. A genetic similarity matrix was constructed using the Jaccard's coefficient method (Jaccard, 1908).

Each RAPD band was treated as an independent character and was scored as present (1) or absent (0). The resulting binary data matrix was used to compute a parwise distance matrix using the DICE coefficient utilizing the software WINBOOT (Yap and Nelson 1996).The distance matrix was subjected to cluster analysis employing UPGMA using SAHN (Sequential Aglomerative Heirarchial and nested cluster) module of the software NTSYS pc (Rohlf 2002).The robustness of the clusters was estimated by performing a bootstrap analysis on 1000 data replicates using WINBOOT (Yap and Nelson 1996). Principal coordinate analysis was conducted using the EIGEN procedure of NTSYS pc in order to cluster the genotypes. The value of each of the 20 primers was assessed using two indices PIC which

Table 1. continued

S. No	Name	Grain							Maturity days
			Lemma and palea		100-grain wt	Seed coat colour	Endosperm		
		Awning	Colour	Pubescence			Type	Scent	
1	Njavara (yellow)	Absent	Brown furrows on straw	Hairs on upper portion	2.01	Brown	Non waxy	Non scented	80
2	Njavara (black)	Absent	Black	Hairs on upper portion	2.18	Brown	Non waxy	Non scented	98
3	Cheeravithu	Absent	Brown furrows on straw	Hairs on upper portion	2.65	Brown	Non waxy	Non scented	109
4	Jeerakasala	Absent	Straw	Hairs on upper portion	2.27	White	Non waxy	Lightly scented	123
5	Chempan	Absent	Straw	Hairs on upper portion	2.52	Brown	Non waxy	Non scented	121
6	Kannikayama	Absent	Brown furrows on straw	Hairs on upper portion	3.5	Brown	Non waxy	Non scented	122
7	Thowan	Absent	Straw	Short hairs	3.33	Red	Non waxy	Non scented	132
8	Kozhivalan	Absent	Straw	Hairs on upper portion	2.36	Brown	Non waxy	Non scented	148
9	Kururayima	Absent	Gold and/or gold furrows on straw background	Short hairs	2.7	Brown	Non waxy	Non scented	133
10	Karuthacheera	Absent	Straw	Short hairs	2.65	Brown	Non waxy	Non scented	109
11	Kalladi Aryan	Absent	Brown furrows on straw	Hairs on upper portion	2.51	Brown	Non waxy	Non scented	109
12	Veluthittaryan	Absent	Gold and/or gold furrows on straw background	Hairs on upper portion	2.86	Red	Non waxy	Non scented	150
13	Athikiramundakan	Absent	Brown	Hairs on lemma keel	2.24	Brown	Non waxy	Non scented	147
14	Anakodan	Absent	Straw	Hairs on lemma keel	2.41	Red	Non waxy	Non scented	147
15	Veluthakattamodan	Long and fully awned	Gold and/or gold furrows on straw background	Hairs on upper portion	3.25	Brown	Non waxy	Non scented	132
16	Kattamodan	Absent	Brown furrows on straw	Short hairs	2.14	Light brown	Non waxy	Non scented	130
17	Allikannan	Absent	Gold and/or gold furrows on straw background	Hairs on upper portion	3.03	White	Non waxy	Non scented	135
18	Parambuvattan	Long and partly awned	Black	Hairs on upper portion	2.84	Light brown	Non waxy	Non scented	120
19	Vellakkoli	Absent	Brown furrows on straw	Short hairs	2.45	Brown	Non waxy	Non scented	130
20	Vellamundakan	Absent	Brown furrows on straw	Hairs on upper portion	2.62	Red	Non waxy	Non scented	117
21	Cheruvirippu	Short and partly awned	Brown furrows on straw	Hairs on upper portion	3.36	Brown	Non waxy	Non scented	135
22	Choottupokkali	Absent	Straw	Hairs on upper portion	2.72	Brown	Non waxy	Non scented	135
23	Chenthadi	Absent	Straw	Hairs on upper portion	3.32	Brown	Non waxy	Non scented	127
24	Kazhama	Absent	Gold and/or gold furrows on straw background	Hairs on upper portion	1.52	Brown	Non waxy	Non scented	135
25	Mundon	Absent	Straw	Hairs on upper portion	2.33	Brown	Non waxy	Non scented	129
26	Chettivirippu	Absent	Straw	Hairs on upper portion	1.8	Red	Non waxy	Non scented	122
27	Pokkali 3	Absent	Straw	Short hairs	2.63	Brown	Non waxy	Non scented	136
28	Ponkuruka	Short and partly awned	Brown	Hairs on upper portion	2.38	Brown	Non waxy	Non scented	121
29	Pandivella	Absent	Gold and/or gold furrows on straw background	Hairs on upper portion	1.91	Red	Non waxy	Non scented	122
30	Thavalakannan	Absent	Gold and/or gold furrows on straw background	Hairs on lemma keel	2.91	Light brown	Non waxy	Non scented	134

* Data obtained from NATP on Sustainable Management of Biodiversity [Principal Investigator, Dr S Leenakumary]

is same as diversity index (DI) and Rp (Prevost and Wilkinson 1999).PIC was estimated as PIC= ∑ (1-pi ^{2})/n where n is the number of band positions analyzed in the set of accessions, pi is the frequency of the ith pattern. The ability of the primers to distinguish between accessions was assessed by calculating their resolving power as, Rp= ∑Ib where Ib is the band informativeness and Ib=1- (2x0.5-pi) where pi is the proportion of individuals containing the band (Prevost and Wilkinson, 1999). Marker Index was calculated as the product of DI and EMR (Effective multiplex ratio). EMR of a primer is defined as the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay (Milbourne *et al*., 1997).

Results and Discussion

The 20 oligonucleotide primers randomly selected produced multiple band profiles with a number of amplified fragments ranging from 2.0 kb to less than 0.5 kb with mean number of 11.10 amplicons per primer. The primer OPF-04 gave the maximum number of amplicons (15) (Fig. 1) while the lowest number (7) was amplified by the primer OPG-18 (Table 2).

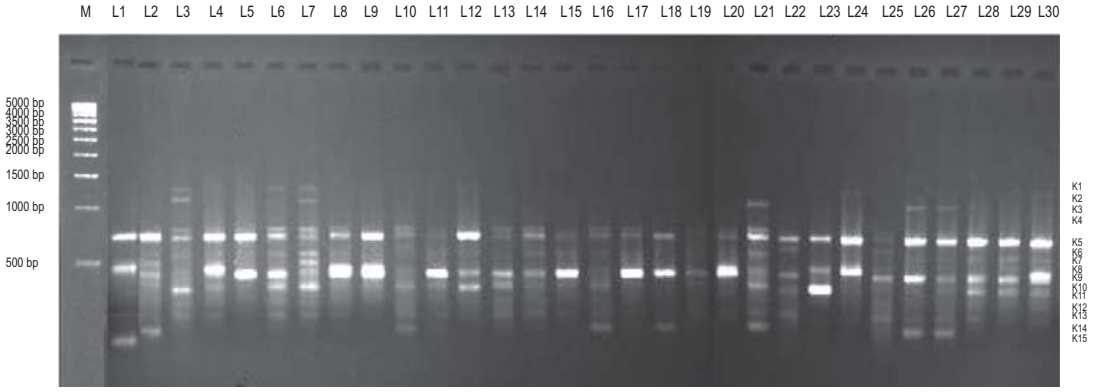

Fig. 1: Amplification profile of the DNA of 30 rice accessions using the primer OPF-04

L1 = Njavara yellow, L2 = Njavara Black, L3 = Cheeravithu, L4 = Jeerakasala, L5 = Chempan, L6 = Kannikayama, L7 = Thowan, L8 = Kozhivalan, L9 = Kururayima, L10 = Karuthacheera, L11 = Kalladi Aryan, L12 = Veluthittaryan, L13 = Athikiramundakan, L14= Anakodan, L15 = Veluthakattamodan, L16 = Kattamodan, L17 = Allikannan, L18 = Parambuvattan, L19 = Vellakkoli, L20 = Vellamundakan, L21= Cheruvirippu, L22 = Choottupokkali, L23 = Chenthadi, L24 = Kazhama, L25 = Mundon, L26 = Chettivirippu, L27 = Pokkali, L28 = Ponkuruka, L29 = Pandivella, L30 = Thavalakannan

Table 2. Base sequence of RAPD primers, number of amplicons and percentage of polymorphism in rice genomic DNA

Primer name	Sequence	Number of amplicons	Number of polymorphic amplicons	Number of monomorphic amplicons	Polymorphism (%)	PIC	Rp	EMR
OPA-05	AGGGGTCTTG	9	6	3	66.66	0.42	7.46	3.96
OPB-05	TGCGCCCTTC	12	10	2	83.33	0.59	15.50	8.30
OPB-O8	GTCCACACGG	9	8	1	88.88	0.52	6.71	7.04
OPB-10	CTGCTGGGAC	11	6	5	54.54	0.29	6.42	3.24
OPC-07	GTCCCGACGA	10	9	1	90.00	0.66	12.48	8.10
OPC-15	GACGGATCAG	12	10	2	83.33	0.52	12.42	8.30
OPD-03	GTCGCCGTCA	8	6	2	75.00	0.39	6.34	4.50
OPD-18	GAGAGCCAAC	11	10	1	90.90	0.57	12.70	9.00
OPF-01	ACGGATCCTG	14	12	2	85.71	0.58	18.52	10.20
OPF-03	CCTGATCACC	9	6	3	66.66	0.37	6.76	3.96
OPF-04	GGTGATCAGG	15	15	0	100.00	0.65	24.52	15.00
OPF-05	CCGAATTCCC	10	9	1	90.00	0.67	13.48	8.10
OPF-06	GGGAATTCGG	14	13	1	92.85	0.65	18.58	11.96
OPF-13	GGCTGCAGAA	10	6	4	60.00	0.32	6.42	3.60
OPG-18	GGCTCATGTG	7	5	2	71.42	0.43	6.14	3.55
OPG-19	GTCAGGGCAA	11	9	2	81.81	0.46	10.32	7.25
OPH-19	CTGACCAGCC	14	13	1	92.85	0.53	14.88	11.96
OPK-14	CCCGCTACAC	12	7	5	58.33	0.41	9.72	4.07
OPK-19	CACAGGCGGA	14	13	1	92.85	0.70	15.02	6.96
OPL-17	AGCCTGAGCC	10	9	1	90.00	0.81	6.90	8.10

The average number of polymorphic bands per primer was found to be 9.10. A total of 222 amplicons were produced which showed a polymorphism of 81.98%. The polymorphism produced by the different primers ranged from 100% to 54.54%. Forty cultivated varieties of rice were evaluated for polymorphism after amplification with thirty six decamer RAPD primers by Ravi *et al.* (2003) and a total of 499 RAPD markers were produced with a polymorphism percentage of ninety. He *et al*. (2004) had reported 69.4% polymorphism while using 12 RAPD primers in assessment of genetic diversity of allelopathic rice germplasm.

Of the 20 primers studied, 13 gave a polymorphism of above 80%. This shows the effectiveness of the selected primers in genetic analysis of the different accessions. Primer OPF-04 gave 100% polymorphism in the studied accessions. Barooah and Sarma (2004) had reported 100% polymorphism for the primer OPK-19. In the present study, this primer produced 92.85% polymorphism. Barooah and Sarma (2004) had reported that the primer OPK-14 gave the least percentage of polymorphism (33.33) in the genetic diversity analysis of traditional Sali rice. In the present study this primer produced 58.33% polymorphism.

The 20 primers used in this study produced a total of 222 amplicons with mean number of 11.10 bands per primer. Raghunathachari *et al*. (2000) reported an average of 11.4 bands per primer in the study of genetic variability in Indian scented rice germplasm using RAPD analysis. Saker *et al.* (2005) had reported on an average 10.90 bands per primer in the study of RAPD analysis in Egyptian rice genotypes.

In this RAPD analysis of rice accessions the amplicons produced had a molecular weight ranging from 2.0 kb to less than 0.5 kb. Raghunathachari *et al*. (2000) had reported amplicons of size ranging from 4.0 kb to 0.5 kb. Wu *et al*. (2002) had reported amplicons of size 2.5 kb to 0.5 kb. Neeraja *et al*. (2002) reported amplicons of size 3.9 kb to 0.25 kb whereas Monna *et al*. (1994) had reported amplicons of size 2.0 kb to less than 0.1 kb.

Nine unique positive amplicons were obtained by the primers OPC-15, OPD-03, OPF-03, OPF-04, OPF-05, OPG-18 and OPK-19 (Table 3). These bands could distinguish Njavara yellow, Njavara black, Cheeravithu, Chempan, Thowan, Cheruvirippu and Allikannan. These markers can be used as fingerprints for these accessions. Primers OPB-05 and OPF-01 produced amplicons for the Njavara group alone at less than 1000 bp. Njavara group includes Njavara yellow and Njavara black, two ecotypes of the medicinal rice Njavara.

Comparative ability of different primers to detect diversity in 30 accessions was depicted from the PIC, Rp and EMR values (Table 2). The mean PIC value for the twenty primers was 0.5 .The primer OPB-10 showed the least PIC value and primer OPF05 showed the highest PIC value (0.673).The mean Rp value for the twenty primers was 11.56 with OPF 04 having the highest (24.52) and OPG 18 (6.14) the lowest. EMR (Effective Multiplex ratio) of the primers ranged from 15 (OPF 04) to 3.55 (3.55) and that value was also highest for OPF 04. Among the 20 primers studied OPF 04 is the best primer for distinguishing the varieties with highest Rp value, high PIC value and highest EMR.

Table 3. Unique positive and negative markers identified in the RAPD analysis of the 30 accessions of rice

Accession	Unique positive markers			Unique negative markers			
	Size of marker (bp)	Primer	Total No. of markers/variety	Size of marker (bp)	Primer	Total No. of markers/ variety	Grand total
Njavara yellow	< 500	OPF-04	1	-	-	-	2
	500 - 1000	OPK-19	1	-	-	-	
Njavara black	< 500	OPD-03	1	-	-	-	1
Cheeravithu	~ 1000	OPF-05	1	-	-	-	1
Chempan	< 1000	OPF-05	1	-	-	-	1
Thowan	500 - 1000	OPG-18	1	-	-	-	1
Kozhivalan	-	-	-	< 500	OPH-19	1	1
Athikiramundakan	-	-	-	~ 500	OPL-17	1	1
Allikannan	~ 500	OPF-03	1	< 1000	OPL-17	1	2
Vellakkoli	-	-	-	< 500	OPG-19	1	2
	-	-	-	< 1000	OPL-17	1	
Cheruvirippu	1000-1500	OPF-04	1	-	-	-	2
	~ 1500	OPC-15	1	-	-	-	
Choottupokkali	-	-	-	~ 1000	OPD-03	1	1
Chenthadi	-	-	-	< 500	OPK-19	1	1

In the similarity indices, worked out based on Jaccard's coefficient analysis, accessions Chettivirippu and Pokkali 3 had the highest similarity index of 0.825. These accessions formed a cluster with maximum similarity in UPGMA clustering (Fig. 2). These two accessions are the landraces of Ernakulam district suited especially for saline sodic soils of Kerala (Pokkali). The least similarity coefficient (0.451) was observed between Njavara yellow and Choottupokkali. Njavara yellow is the medicinal rice and Choottupokkali is an accession suited for saline soil. Njavara yellow and Njavara black, the two ecotypes of the medicinal rice Njavara, had a similarity coefficient of 0.707. The clustering of these two accessions had a high bootstrap value of 85.

Jaccard's similarity coefficient values were used for the nested clustering of the genotypes to develop dendrogram (Fig. 2). At a similarity coefficient of 59% the accessions clustered into two main clusters. The first cluster (I) consisted of three accessions *viz.* Mundon, Choottupokkali and Vellakkoli while all the other twenty seven accessions formed the second cluster (II).

In the dendrogram, Jeerakasala and Chempan formed a cluster with 80% similarity. These two accessions were collected from two ecological zones of Kerala state, Wayanad and Trivandrum. The two accessions Kannikayama and Kozhivalan from Kannur district clustered at 78% similarity and these accessions also had similar morphological characters. Cheruvirippu and Chettivirippu, both from Ernakulam district suited to saline soils also clustered at 78%. Even though Karuthacheera and Kalladi Aryan clustered at 78% these are from two different ecological zones *viz.*

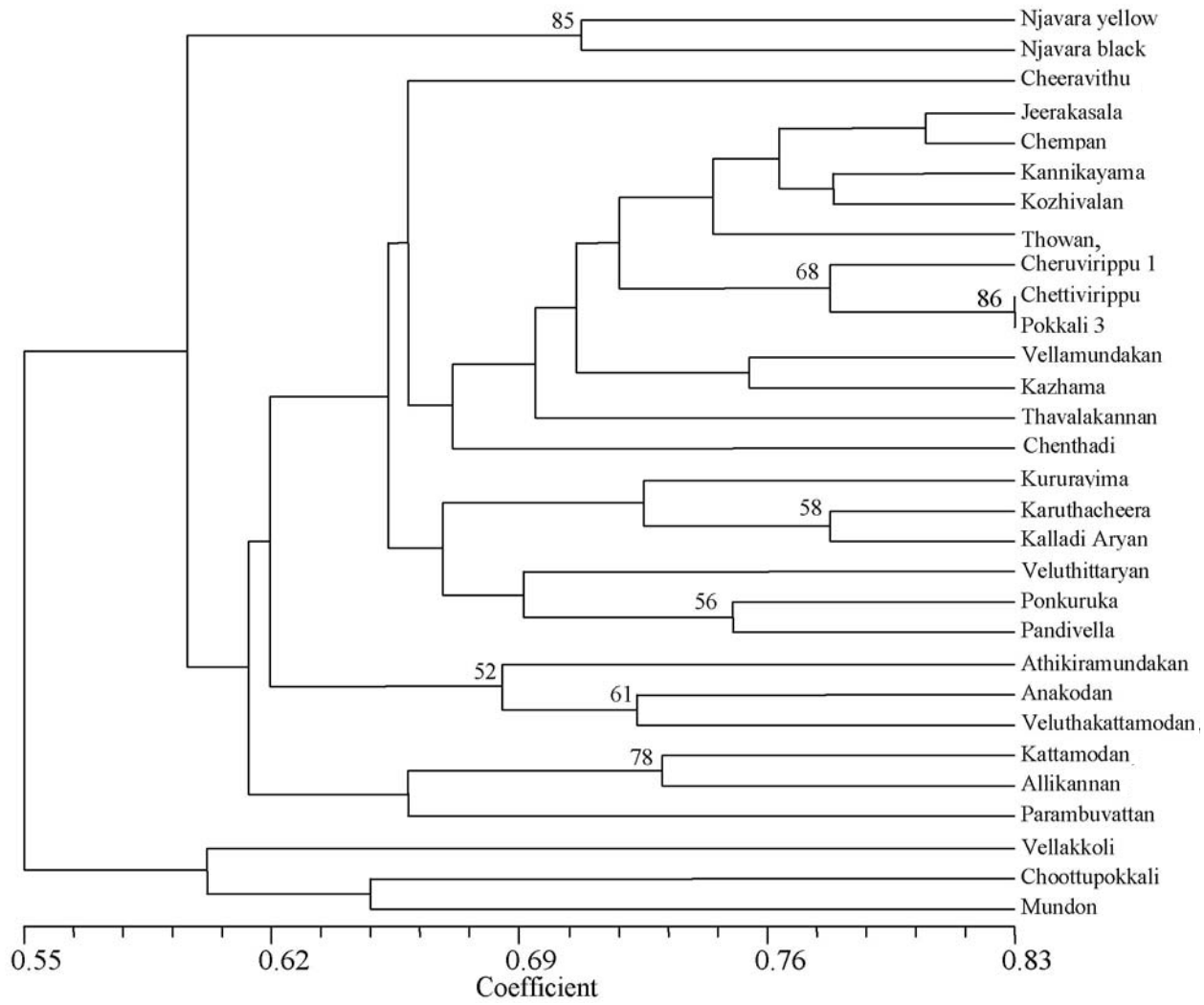

Fig. 2. Dendrogram of rice cultivars based on RAPD markers (Bootstrap values above 50 written at the nodes)

Perumbavur (Central Kerala) and Kannur (North Kerala). Ponkurukka and Pandivella which clustered at 75% are from nearby locations, Ernakulam and Trivandrum. At 73% similarity Kururayima from Kannur district clustered with Karuthacheera from Perumbavur and Kalladi Aryan from Kannur. But the other accession from Kannur district did not show much similarity with these. Accessions Kattamodan and Allikannan, which clustered at 73% with a bootstrap value of 78, were also from two different ecological zones, Palakkad and Kannur. Anakodan and Veluthakattamodan clustered at 72%. These accessions are also from Palakkad and Kannur, respectively. Vellamundakan from Allepey district and Kazhama from Kannur district clustered at 76%. But Athikiramundakan the other accession from Allepey apart from Njavara Yellow, the medicinal rice from Allepey, was distant from Vellamundakan. This shows that the accessions from a single location were distinct from each other and the collections were representative of the ecological zone at microlevel.

The principal co-ordinate analysis gave a better picture of the groupings. In this the medicinal rice accessions njavara yellow and njavara black were distinctly placed (Fig. 3)

Location Specific Similarity

In order to assess the location specific similarity of the accessions the similarity indices of the 30 accessions were grouped. The 30 accessions were collected from nine locations in Kerala State (Fig. 4). Of this Thrissur, Perumbavur and Malappuram had only one accession each. Wayanad and Trivandrum had two accessions each. Similarity indices within each location with more than two accessions were grouped for analysis. The two accessions from Wayanad, Jeerakasala and Chenthadi, showed a similarity of 67.6%. The accession from Trivandrum, Pandivella and Chempan, showed a similarity of 65.2%. Within the accessions from Ernakulam district only Pokkali 3 and Chettivirippu had above 80% similarity. Within the accessions of Kannur district Kannikayama and Thowan, Kannikayama and Kozhivalan, Thowan and Kozhivalan, Thowan and Kazhama, and Kozhivalan and Kazhama had above 70% similarity. Among the accessions within Palakkad and Allepey district, none had similarity above 70%. In this study no marker was obtained for grouping the accessions within the location.

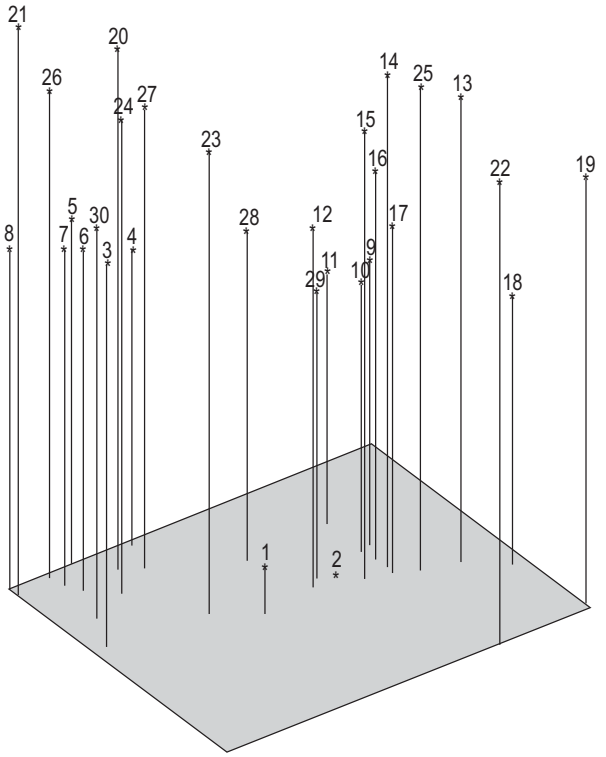

Fig. 3. Ordination 3D Projection of the rice genotype

A1 = Njavara yellow, A2 = Njavara black, A3 = Cheeravithu, A4 = Jeerakasala, A5 = Chempan, A6 = Kannikayama, A7 = Thowan, A8 = Kozhivalan

A9 = Kururayima, A10 = Karuthacheera, A11 = Kalladi Aryan, A12 = Veluthittaryan, A13 = Athikiramundakan, A14 = Anakodan, A15 = Veluthakattamodan,

A16 = Kattamodan, A17 = Allikannan, A18 = Parambuvattan, A19 = Vellakkoli, A20 = Vellamundakan, A21 = Cheruvirippu, A22 = Choottupokkali

A23 = Chenthadi, A24 = Kazhama, A25 = Mundon, A26 = Chettivirippu, A27 = Pokkali 3, A28 = Ponkuruka, A29 = Pandivella, A30 = Thavalakannan

RAPDs for Kernel Colour

Kernel colour or seed coat colour in rice is a complex character and the accessions in this study included 19 accessions with brown seed coat colour, two with white, three with light brown and six with red seed coat colour (Table 1) Vanaja *et al*. (2008) reported that with primer OPK-14 a bright thick amplicon of size 1000 bp was detected in 50% of white kernelled varieties, which were totally absent in red kernelled varieties. In the present study with the primer OPK-14 an amplicon at 1000 bp was detected but the total absence of the amplicon in red kernelled accessions was not seen (Fig. 5). The variation in seed coat colour as red, brown, light brown and white shows

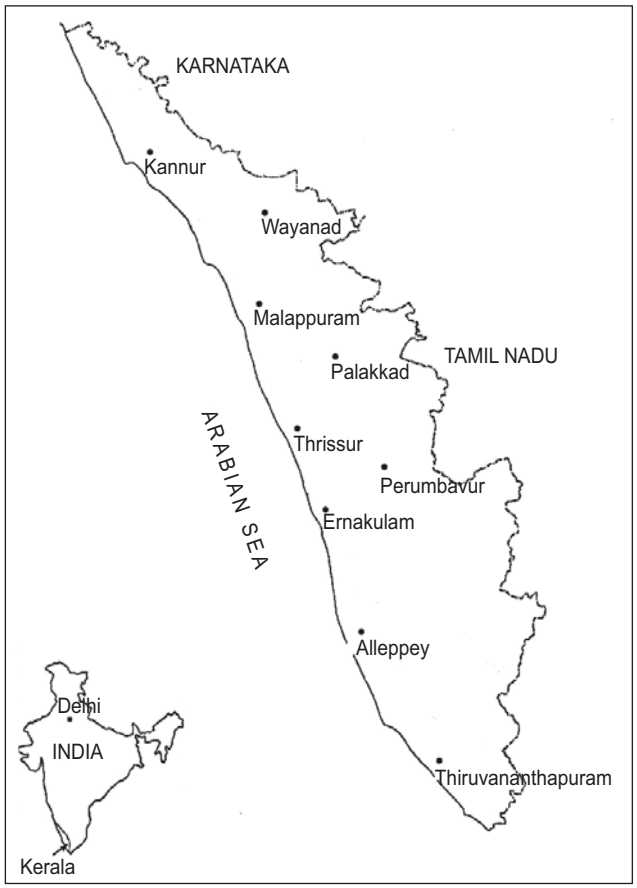

Fig. 4. Locations of the sites of germplasm collections used in Kerala

that this character kernel colour in rice is controlled by more than one gene or alleles so tagging this gene requires more studies by screening large germplasm with robust markers.

A good germplasm collection can offer diverse sources of gene donors in plant breeding programmes and thereby result in higher level of diversity in the cultivated varieties and agricultural systems. The 30 genotypes used in this study were collected from different locations of Kerala under project on "Sustainable Management of Biodiversity". The molecular diversity analysis of these accessions showed that the accessions were representative of the location specific micro environment and the molecular diversity is high enough for use in crop improvement programmes.

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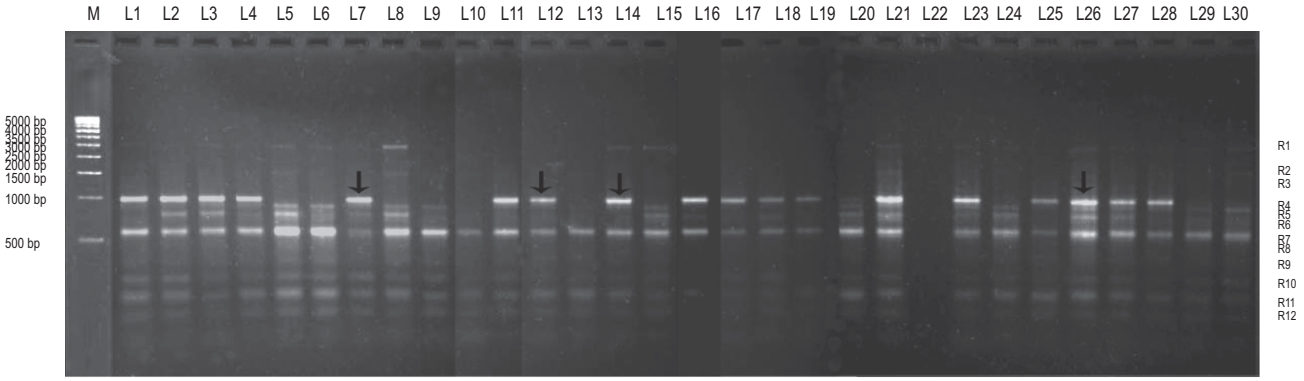

Fig. 5. Amplification profile of the DNA of 30 rice accessions using the primer OPK-14

↓– Red Kernelled accessions with amplicon at 1000bp

L1 = Njavara yellow, L2 = Njavara Black, L3 = Cheeravithu, L4 = Jeerakasala, L5 = Chempan, L6 = Kannikayama, L7 = Thowan, L8 = Kozhivalan, L9 = Kururayima, L10 = Karuthacheera, L11 = Kalladi Aryan, L12 = Veluthittaryan, L13 = Athikiramundakan, L14= Anakodan, L15 = Veluthakattamodan, L16 = Kattamodan, L17 = Allikannan, L18 = Parambuvattan, L19 = Vellakkoli, L20 = Vellamundakan, L21= Cheruvirippu, L22 = Choottupokkali, L23 = Chenthadi, L24 = Kazhama, L25 = Mundon, L26 = Chettivirippu, L27 = Pokkali, L28 = Ponkuruka, L29 = Pandivella, L30 = Thavalakannan

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Pathogenic Fungi and Bacterium Intercepted in Oilseed Crops Introduced During 1999-2008

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A large number of germplasm and trial material of oilseed crops are being introduced each year to develop better crop varieties. There is always an inherent risk of introduction of a new pathogen(s) or race(s) or virulent strain(s) into the country along with such imports. During 1999-2008, a total of 10,450 germplasm samples of different species of oilseed crops viz., brassicas, crambe, linseed, safflower, sesame, soybean and sunflower were imported from several countries. Seed health testing of these samples for quarantine clearance resulted in the detection of pathogenic fungi in 1,300 samples which included – *Alternaria brassicae*, *A. brassicicola*, *Botrytis cinerea*, *Phoma lingam* in *Brassica* spp.; *A. brassicicola* in *Crambe* spp.; *B. cinerea*, *Fusarium solani*, *Puccinia carthami* in *Carthamus* spp.; *Cercospora kikuchii*, *Colletotrichum dematium*, *F. oxysporum*, *F. solani*, *Macrophomina phaseolina*, *Peronospora manshurica* in *Glycine* spp.; *A. helianthi*, *P. helianthi* in *Helianthus* spp.; *A. lini* in *Linum* spp.; *A. sesami*, *C. sesami*, *M. phaseolina*, *Rhizoctonia bataticola* in *Sesamum* spp. and a bacterium *Xanthomonas campestris* pv. *campestris* in *Brassica* spp. Significance of interception of pathogenic fungi and the bacterium is discussed.

Key Words: Germplasm, Interceptions, Oilseed crops, Pathogenic fungi, Pathogenic bacterium, Quarantine, Salvaging

Introduction

India spends enormous foreign exchange on imports of edible oil and during 2007-08 the imports were 6.3 million tones of vegetable oils worth ₹ 25,000 crores. The country has been importing exotic germplasm of oilseed crops to develop high yielding varieties by their utilization under various breeding programmes aimed to increase oil production. Such imports carry risk of inadvertent introduction of exotic pests like pathogenic fungi and bacteria. A large number of economically important pathogenic fungi were intercepted in introduced germplasm at the National Bureau of Plant Genetic Resources, New Delhi, from time to time including *Peronospora manshurica* (Naum.) Syd. Ex Gaum. on soybean which is not yet reported from India; *Puccinia carthami* Corda which are known to possess a number of virulent races; *Botrytis cinerea* Pers.: Fr., *Fusarium solani* (Mart.) Sacc. and *Macrophomina phaseolina* (Tassi) Goid which have a wide host range (Agarwal *et al.*, 2006; Khetarpal *et al*., 2001; Singh *et al*., 2007). In this paper pathogenic fungi and a bacterium intercepted on germplasm of various oilseed crops imported from several countries during 1999-2008 is presented and economic importance of important ones is discussed.

Materials and Methods

During 1999-2008, a total of 10,450 germplasm samples of brassicas, crambe, linseed, safflower, sesame, soybean and sunflower were received from several countries for quarantine processing. All the seeds were first examined visually and then under stereo-binocular microscope for the presence of fungal fructifications, rust pustules and spores adhered on the seed surface, crusts of soybean downy mildew etc., for symptoms such as discolouration/spots. Seed samples of soybean found treated with microbial cultures or suspected to carry traces of crusts of oospores of downy mildew were subjected to washing test (Agarwal *et al.*, 2006a). The unhealthy-looking seeds of all the crops were subjected to blotter test. The seeds were placed on 3 layers of moist blotters in plastic Petri plates (seeds/plate varied from 10 to 25, depending on the size of the seed and the sample) and incubated for 7 days at 20 ± 1^0C under alternating cycles of 12 h light and darkness. Observations for the associated pathogenic fungi and/or bacteria were recorded on the 8th day under stereo-binocular microscope. Slides were also prepared and observed under compound microscope, wherever needed. The fungi intercepted were identified as per the characteristics described in IMI descriptions of fungi and bacteria. For detection of *Xanthomonas*

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campestris pv. *campestris* in brassicas, seedlings showing 'V' shaped lesions in the blotter test were cut with a sharp blade, to see the bacterial ooze from vascular bundles indicated the bacterial association with infected portion (Shekhawat *et al*., 1982).

Infected seed samples were salvaged by hot water treatment, alcohol + sand wash and fungicidal treatment techniques (Agarwal *et al.*, 1990; Dev *et al*., 1999) before their release to the indenters.

Results and Discussion

Eighteen seed-borne fungi and one bacterium of quarantine significance were intercepted during 1999-2008 on introduced germplasm of different crops from several countries (Table 1). During visual and stereoscopic observations *P. manshurica*, the causal agent of downy mildew was intercepted as crust of oospores on soybean (*Glycine max* (L.) Merr.) seeds from Brazil, Japan, Taiwan, Thailand and USA. The interception of this downy mildew is of high quarantine significance to India as it is not yet reported from the country and it had a number of physiological races as well as its oospores could remain viable for 8 years. Crop losses of 10 to 11.8% due to downy mildew have been reported (Agarwal *et al.,* 2006a). *Puccinia carthami* Corda, rust of safflower (*Carthamus tinctorius* L.) was intercepted in *Carthamus* spp. from USA. The teleutospores of *P. carthami* carried on the seed surface were found responsible for the serious epiphytotics of safflower rust reported in USA (CAB International, 2007). Yield losses can approach 100% on susceptible varieties (http://wiki.bugwood.org/HPIPM:Safflower_Rust). *P. helianthi* Schwein., rust of sunflower (*Helianthus annuus* L.) was intercepted in *H. annuus* from Australia, Bulgaria, China, Egypt, France and USA. Yield losses in sunflower as high as 50% due to a reduction in the capitulum and seed size and in oil content have been reported in Canada and Argentina (Siddiqui and Brown, 1977). Among the insect pests and diseases, diseases of sunflower alone cause 10% yield loss in the field itself (http://orissagov.nic.in/e-magazine/Orissareview/2008/feb-march-2008/engpdf/83-84.pdf).

Observations on seeds incubated on moist blotters revealed presence of a large number of economically important fungi. *Alternaria brassicae* (Berk.) Sacc., Alternaria blight, leaf blight fungus of crucifers was intercepted in *Brassica juncea* from Australia, Canada, and Korea; in *B. napus* from Australia; in *B. nigra* from Canada; in *B. oleracea* from the Netherlands and Taiwan; in *B. oleracea* var. *capitata* from the Netherlands; in *B. rapa* from Sweden; in *Brassica* spp. from Canada and in *Crambe abyssinica* var. *galacticia* from UK. In a field experiment conducted in Himachal Pradesh (India), *B. rapa* variety yellow sarson suffered maximum yield loss (27.53%), followed by *B. rapa* variety brown sarson (25.01%) and *B. juncea* (20.28%) due to Alternaria blight (Kumar, 1997). Seed yield losses due to *A. brassicae* may be greater than 50% and even the occurrence of a moderate disease may cause losses of 20% (Szopinska *et al*., 2007). Yield losses ranging from 10-75% have been reported from different parts of India. The losses were higher in yellow sarson (38-45%) followed by brown saron (26%) and mustard (17-18%) (Saharan and Mehta, 2002).

A. brassicicola (Schwein.) Wiltshire, the causal agent of Alternaria blight, black spot of crucifers and brown rot of cabbage was intercepted in a number of brassicas from several countries (Table 1); in *C. abyssinica* from USA and in *C. abyssinica* var. *galacticia* from UK. Reductions in seed yield and oil content of 38 and 14%, respectively, have been reported due to this pathogen on *B. juncea* (CAB International, 2007). It is of worldwide economic importance resulting in yield reductions upto 50% in affected crops (http://www.seedquest.com/News/releases/2007/september/20346.htm).

A. helianthi (Hansf.) Tubaki and Nishihara, causal agent of leaf blight of sunflower was intercepted in sunflower seeds from France and USA. The disease is reported to reduce seed and oil yields by 27 to 80% and 17 to 33%, respectively, leading to germination losses varying from 23 to 32% (Balasubrahmanyam and Kolte, 1980).

A. lini (Dey), the causal agent of leaf and bud blight in linseed, was intercepted in linseed seeds from France and USA. The loss due to the disease is estimated to vary from 28 to 60% in Uttar Pradesh (Singh *et al.,* 2005)

A. raphani (Groves & Skolko) [syn. *A. japonica* (Yoshii)], the Alternaria black spot fungus of crucifers was intercepted in *B. rapa* from Australia. *A. japonica* in association with *A. brassicae* can cause yield losses between 40 and 70% in certain rapeseed cultivars; alone it caused yield losses of 34-42% (CAB International, 2007).

Table 1. Pathogenic fungi and bacterium intercepted in oilseed crops during 1999-2008

Fungi/bacterium	Crop	Source/ country	No. of samples	
			Received	Found infected
Alternaria brassicae	*Brassica juncea*	Australia	263	7
		Canada	488	28
		Korea	146	4
	B. napus	Australia	287	5
	B. oleracea	Netherlands	472	11
		Taiwan	1	1
	B. oleracea var. *capitata*	Netherlands	167	6
	B. rapa	Sweden	14	2
	Crambe abyssinica var. *galacticia*	UK	2	2
A. brassicicola	*Brassica* spp.	Australia	57	11
		Belgium	1	1
		Canada	12	3
		China	4	1
		Germany	35	16
		Hungary	25	1
		Netherlands	179	13
		USA	53	24
	B. juncea	Australia	440	126
		Canada	569	367
		Korea	146	6
	B. napus	Australia	287	12
		Russia	2	1
	B. nigra	Australia	57	4
		Canada	26	1
	B. oleracea	Netherlands	484	77
		Taiwan	2	1
		UK	80	6
	B. oleracea var. *capitata*	Netherlands	167	10
	B. rapa	Australia	4	3
		Sweden	9	1
	C. abyssinica	USA	72	1
	C. abyssinica var. *galacticia*	UK	2	2
A. helianthi	*Helianthus annuus*	France	477	2
		USA	52	3
A. lini	*Linum* spp.	Russia	4	2
A. raphani	*B. rapa*	Australia	4	2
A. sesami	*Sesamum* spp.	Japan	12	1
		Sudan	1	1
		Uganda	4	1
	S. indicum	Brazil	3	2
		USA	329	1
Botrytis cinerea	*B. juncea*	Canada	80	1
		Germany	8	1
	B. oleracea var. *capitata*	Netherlands	167	1
	Carthamus spp.	Germany	9	2
Cercospora kikuchii	*Glycine max*	USA	47	1
C. sesami	*Sesamum* spp.	Uganda	4	1

Contd.

Table 1. Contd.

Fungi/bacterium	Crop	Source/ country	No. of samples	
			Received	Found infected
Colletotrichum dematium	*C. abyssinica* var. *galacticia*	UK	2	2
	G. max	Australia	2	1
		Taiwan	205	5
	H. annuus	USA	12	5
Fusarium oxysporum	*G. max*	Brazil	48	1
F. solani	*B. juncea*	Canada	50	2
	Carthamus spp.	Germany	9	1
	G. max	Taiwan	68	4
Macrophomina phaseolina	*G. max*	Taiwan	205	2
	Sesamum spp.	Sudan	1	1
Peronospora manshurica	*G. max*	Brazil	48	33
		Japan	15	1
		Taiwan	1566	21
		Thailand	9	3
		USA	2245	131
Phoma lingam	*B. juncea*	Australia	170	24
		Canada	468	7
Puccinia carthami	*Carthamus* spp.	USA	275	33
P. helianthi	*H. annuus*	Australia	80	10
		Bulgaria	2	1
		China	6	6
		Egypt	5	5
		France	1166	171
		USA	641	33
Rhizoctonia bataticola	*G. max*	Taiwan	1000	1
	Sesamum spp.	Sudan	1	1
Xanthomonas campestris pv *campestris*	*B. juncea*	Australia	313	29
		Canada	568	30
		Germany	8	7
		USA	4	1
	B. napus	Australia	287	3
		Germany	21	5

A. sesami (Kaw) Mohanty and Behera, the Alternaria blight, leaf blight fungus of sesame was intercepted on *Sesamum indicum* from Brazil and USA; in *Sesamum* spp. from Japan, Sudan and Uganda. The disease has been reported in epiphytotic form in Orissa in 1957 and Maharashtra in 1975 (Deshpande and Shinde, 1976). In Karnataka, the disease caused yield losses ranged from 0.1 to 5.7 g seeds/capsule (Siddaramaiah *et al*., 1981; Agarwal *et al*., 2006b).

Botrytis cinerea Pers. Fr. [=*Botryotinia fuckeliana* (de Bary) Whetzel], Botrytis rot, grey mould-rot fungus was intercepted in *B. juncea* from Canada, Germany; in *B.oleracea* var. *capitata* from the Netherlamds and in *Carthamus* spp. from Germany. It is difficult to assess the damage caused by *B. cinerea*. However, economic losses of >50% may occur in many crops, depending on the prevailing environmental conditions (CAB International, 2007). Grey mould can lead to 40% loss in stored cabbage (http://www.brassicastoday.com/news.aspx?id=3bed9072-5046-46b4-a805-be8081c77189).

Cercospora kikuchii (Matsu & Tomoyasu) Gardner, a purple blotch fungus was intercepted on soybean from USA. Most of the economic losses cased by *C. kikuchii* are due to the purple seed stain phase of the disease. The incidence of purple seed stain can be as high as 50% of a seed lot (Bowers and Russin, 1999).

C. sesami (Zimm.) causes leaf spot in sesame was intercepted in *Sesamum* spp. from Uganda. Singh *et al.* (2005) reported the estimated yield losses about 5 to 20% due to Cercospora leaf spot in sesame.

Colletotrichum dematium (Pers.) Grove, the causal agent of leaf spot, leaf blight, stem spot, dieback and anthracnose was intercepted in *C. abyssinica* var. *galacticia* from UK. The anthracnose disease

causes damage in warm humid areas, resulting in reduced stand, seed quality and loss of yields in soybean ranged from 26% to 100% in certain areas of Brazil and India (Sinclair and Backman, 1989). In field studies, yield loss in soybean estimated due to anthracnose was 17% for Kansas, 23% for cv. Cosoy 79 and 30% for cv. Williams 82 (Khan and Sinclair, 1992).

Fusarium oxysporum Schlecht. ex Fr. causes wilt disease was intercepted in *G. max* from Brazil. Lange *et al.* (2007) while compared the yield of wilt susceptible (cv. Canterra 1604) and resistant (cv. Cougar CL) rapeseed in Canada reported that the susceptible variety was severely affected and yielded 44% of resistant variety. Hanif *et al*. (1999) reported that chickpea wilt caused by *F. oxysporum* f. sp. *ciceris* is the most devastating disease resulting 10-50% crop loss every year in Pakistan.

F. solani (Martius) Sacc. [=*Nectria haematococca* (Wollenw.) Gerlach], the causal agent of tuber rot, sudden death syndrome of soybean, foot rot of peas and beans was intercepted in *B. juncea* from Canada; *Carthamus* spp. from Germany and *G. max* from Taiwan. *F. solani* causes substantial economic losses in all crops world over. Root rot, caused by *F. solani* f. sp. *phaseoli*, considered among the most serious and wide spread soil-borne diseases of bean with yield losses of up to 84% is attributed to this pathogen (Park and Tu, 1994).

Macrophomina phaseolina (Tassi) Goid (Syn.-*Rhizoctonia bataticola* (Taubenh.) E.J. Butler; *Sclerotium bataticola* Taubenh.), the causal agent of charcoal rot, ashy stem blight and root rot in a variety of crops was intercepted in *G. max* from Taiwan and in *Sesamum* spp. from Sudan. Yield losses of 36.8 to 79.2% have been reported in sunflower from Venezuela. Yield losses due to *M. phaseolina* in sesame have been estimated up to 57% (Singh *et al.*, 2005). A decrease of grain yield by 31 to 38% has been reported in sorghum from India due to this fungus (CAB International, 2007).

Phoma lingam (Tode ex Fr) Desm. [=*Leptosphaeria maculans* (Desm.) Ces. & De Not.], the causal agent of black leg in crucifers was intercepted in *B. juncea* from Australia and Canada. The yield losses due to *P. lingam* were usually 0-10% but could often reach 30-50% in individual fields (Zhou *et al*., 1999). The major economic losses caused by Phoma are from the stem canker disease phase. It is these cankers which can give yield loss of up to 50% in extreme cases, and frequently 0.5-1 t/ha, largely as a result of limiting seed fill due to restricted water movement from the roots and weakening the collar, causing premature ripening and also lodging (http://www.bayercropscience.co.uk/docushare/SRC/Proline_OSR_SRC_M15097.pdf).

Xanthomonas campestris pv. *campestris* (Pammel) Dowson, the causal agent of black rot in crucifers, was intercepted in *B. juncea* from Australia, Canada, Germany and USA; *B. napus* from Australia, Germany; *B. oleracea* from the Netherlands, UK; and *B. rapa* from Australia. Black rot caused 23 to 57% losses in susceptible cabbage cultivars in some regions of Russia. It is also reported that the disease appeared annually in Manipur (India) near the end of February and its effects were more severe (up to 50% losses) in susceptible cultivars of cabbage. The pathogen could survive in seeds upto 3 years (CAB International, 2007).

Infected seed samples of brassicas were salvaged through Hot Water Treatment at 50^0C for 20 min.; safflower and sunflower seeds against rust through ethyl alcohol wash for 1 min. and air dried (Agarwal *et al.*, 1990). Rest of the samples of crambe, linseed, safflower, sesame, soybean and sunflower found infected with various pathogens were salvaged by treating the seeds with Thiram + Bavistin (1:1) mixture (Dev *et al.*, 1999).

All the samples of soybean found infected with downy mildew fungus were rejected and incinerated. During examination of plates after incubation, in some cases, more than one pathogen was detected on single seed in the sample and 8.97% samples were found infected with pathogens. Data analysis on Pathogen-wise interception indicated that *A. lini* and *A. raphani* were intercepted in 50% samples followed by *A. brassicicola* (25.35%), *C. sesami* (25%), *P. carthami* (12%) and *P. helianthi* (11.89%). *R. bataticola* caused the least infection (0.19%) (Table 2 and 3).

Country-wise analysis of data on interceptions (Table 2) indicated that samples received from Belgium, Egypt and Sudan were found 100% infected; from Bulgaria and Russia with 50% infection, though the number of samples received from these countries were very less. Brazil showed 36.36% infection while over

Table 2. Country-wise distribution of pathogenic fungi intercepted in oilseed crops introduced during 1999-2008

Source/ ↓ Pathogen →	A.b	A.bc	A.hel	A.lin	A.rap	A.ses	B.c	C.d	C.k	C.s	F.o	F.s	M.p	P.m	P.l	P.c	P.h	R.b	X.c c	Total infected samples (Total samples processed)	Total infected samples (%)
Australia	12 (550)	156 (845)			2 (4)			1 (2)							24 (170)		10 (80)		34 (604)	239 (2255)	10.59
Belgium		1(1)																		1 (1)	100.00
Brazil						2(3)					1(48)			33(48)						36 (99)	36.36
Bulgaria																	1(2)			1 (2)	50.00
Canada	28 (488)	371 (607)					1(80)					2 (50)			7(468)				31 (580)	440 (2273)	19.35
China		1(4)															6(6)			7 (10)	7.00
Egypt																	5(5)			5 (5)	100.00
France			2(277)														171 (1166)			173 (1443)	11.98
Germany		16(35)					3 (17)					1(9)							18 (54)	38 (115)	33.04
Hungary		1(25)																		1 (25)	4.00
Japan						1 (12)								1(15)						2 (27)	7.40
Korea	4(146)	6(146)																		10 (292)	3.42
Netherlands	17 (639)	100 (830)					1(167)												12 (200)	130 (1836)	7.08
Russia		1(2)		2(4)																3 (6)	50.00
Sudan						1 (1)							1(1)					1(1)		3 (3)	100.00
Sweden	2 (14)	1 (9)																		3 (23)	13.04
Taiwan	1 (1)	1 (2)						5 (205)				4 (68)	2 (205)	21 (1566)				1 (1000)		35 (3047)	1.14
Thailand														3(9)						3 (9)	33.33
Uganda						1 (4)				1 (4)										2 (8)	25.00
UK	2 (2)	8(82)						2 (2)											1(80)	13 (166)	7.83
USA		25 (125)	3 (52)			1 (329)		5 (12)	1 (47)					131 (2245)		33 (275)	33 (641)		25(57)	257 (3783)	6.79
Total infected samples/ Total samples processed	66 (1840)	688 (2713)	5 (529)	2 (4)	2 (4)	6 (349)	5 (264)	13 (221)	1 (47)	1 (4)	1 (48)	7 (122)	3 (206)	189 (3883)	31 (638)	33 (275)	226 (1900)	2 (1001)	121 (1575)	1402 (15628)	8.97
Total % infected samples	3.58	25.35	0.94	50.00	50.00	1.71	1.89	5.88	2.12	25.00	2.08	5.73	1.45	4.86	4.85	12.00	11.89	0.19	7.68	8.97	

Figures outside parenthesis are No. of samples found infected and in parenthesis are total number of samples processed

A.b=*Alternaria brassicae*; A.bc= *A. brassicicola*; A.hel= *A. helianthi*; A.lin= *A. lini*; A.rap= *A. raphani*; A.ses= *A. sesami*; B.c= *Botrytis cinerea*; C.d= *Colletotrichum dematium*; C.k= *Cercospora kikuchii*; *C.s= C. sesami*; F.o= *Fusarium oxysporum*; F.s= *Fusarium solani*; M.p= *Macrophomina phaseolina*; P.m= *Peronospora manshurica*; P.l= *Phoma lingam*; P.c= *Puccinia carthami*; P.h= *P. helianthi*; R.b= *Rhizoctonia bataticola*; X.c c= *Xanthomonas campestris* pv. *campestris*

Table 3. Crop-wise distribution of pathogenic fungi intercepted in oil-seed crops introduced during 1999-2008

Crop/ ↓ Pathogen →	A.b	A.bc	A.hel	A.lin	A.rap	A.ses	B.c	C.d	C.k	C.s	F.o	F.s	M.p	P.m	P.l	P.c	P.h	R.b	X c c	Total infected samples (Total samples processed)	Total infected samples (%)
Brasica spp.	64 (1838)	685 (2639)			2(4)		3 (255)					2 (50)			31 (638)				121 (1575)	908 (6999)	12.97
Crambe spp.	2 (2)	3 (74)						2 (2)												7 (78)	8.97
Carthamus spp.							2 (9)					1(9)				33 (275)				36 (293)	12.28
Glycine spp.								6 (207)	1 (47)		1 (48)	4 (68)	2 (205)	189 (3883)				1 (1000)		204 (5458)	3.73
Helianthus spp.			5(529)					5 (12)									226 (1900)			236 (2441)	9.66
Linum spp.				2(4)																2 (4)	50.00
Sesamum spp.						6 (349)				1 (4)			1 (1)					1(1)		9 (355)	2.53
Total infected samples/ Total samples processed	66 (1840)	688 (2713)	5 (529)	2 (4)	2 (4)	6(349)	5 (264)	13 (221)	1 (47)	1 (4)	1 (48)	7 (127)	3 (206)	189 (3883)	31 (638)	33 (275)	226 (1900)	2 (1001)	121 (1575)	1402 (15628)	8.97
Total % infected samples	3.58	25.35	0.94	50.00	50.00	1.71	1.89	5.88	2.12	25.00	2.08	5.73	1.45	4.86	4.85	12.00	11.89	0.19	7.68	8.97	

Figures outside parenthesis are No. of samples found infected and in parenthesis total number of samples processed
A.b=*Alternaria brassicae*; A.bc= *A. brassicicola*; A.hel= *A. helianthi*; A.lin= *A. lini*; A.rap= *A. raphani*; A.ses= *A. sesami*; B.c= *Botrytis cinerea*; C.d= *Colletotrichum dematium*; C.k= *Cercospora kikuchii*; *C.s= C. sesami*; F.o= *Fusarium oxysporum*; F.s= *Fusarium solani*; M.p= *Macrophomina phaseolina*; P.m= *Peronospora manshurica*; P.l= *Phoma lingam*; P.c= *Puccinia carthami*; P.h= *P. helianthi*; R.b= *Rhizoctonia bataticola*; X.c.c= *Xanthomonas campestris* pv. *campestris*

33% infection was detected in samples from Germany and Thailand. Samples from Taiwan showed the least infection *i.e.* 1.14%.

Crop-wise analysis of data (Table 3) indicated that highest infection of 50% samples of *Linum* spp., though the number of samples received was very less. *Brassica* spp. samples showed 12.97% infection followed by *Carthamus* spp. with 12.28%. *Sesamum* spp. samples showed least infection of 2.53% samples.

Interception of a number of pathogenic fungi and a bacterium indicated that international movement of planting material has played an important role in the spread of many seed-borne pathogens *viz*., *X. campestris* pv. *campestris* from Europe to USA in seeds of kale and cauliflower, *X. axonopodis* pv. *phaseoli* from the Netherlands to Canada in bean seeds and *Septoria linicola* from Canada to Australia etc. (Neegaard, 1977). A number of pathogenic fungi including *P. manshurica*, the downy mildew fungus of soybean which is not yet reported from India; *B. cinerea*, *C. dematium*, and *F. solani*, which have a wide host range and *P. carthami* that possesses a number of virulent races and a bacterium, *X. campestris* pv. *campestris* that also possesses a number of virulent races, were intercepted on a large number of crops during 1999-2008 while processing germplasm for quarantine clearance. If such pathogens get introduced and establish in the country, they may cause a great loss to the crop production. A thorough and critical examination of seeds and other planting material has prevented their entry into the country and is being done with utmost care.

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Variation and Association among Fodder Yield and other Traits in Germplasm of Forage Sorghum (*Sorghum bicolor* (L.) Moench)

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An evaluation of 105 forage sorghum germplasm, for days to 50% flowering, plant height, number of leaves/plant, leaf length, leaf breadth, stem girth, leaf stem ratio and green and dry fodder yield/plant in Augmented Block Design during 2008-09 and 2009-10, revealed highly significant difference amongst the accessions. The phenotypic coefficient of variation was higher than the corresponding genotypic coefficients for all the characters, denoting environmental factors influenced their expression. Broad sense heritability was moderate (61.21% for number of leaves/plant) to high (92.49 for stem girth) confirming that genotypic variance has contributed substantially to the total variance. The association and path coefficient analysis revealed that stem girth, number of leaves/plant, plant height, leaf length and leaf breadth were the important characters and may be selected to increase the fodder yield. Based on the results of the means of the two years considering together the various traits, the accession IS-3665 and EJ-16 was found to be superior for days to 50% flowering, RAJ-21 for plant height and leaf characters, IS-2887 and IS-3266 for green fodder yield. Therefore, these accessions may be utilized in further breeding programme for developing superior varieties.

Key Words: Forage Sorghum, Germplasm, Heritability

Introduction

Forage crops are important for the economy of India as these crops provide major nutritional base for the livestock. With the increase in the world human population and economic growth, the demand of animal products such as milk, meat and eggs in the human diet is bound to increase. Improvement in the quality of cattle and buffalo depends upon the adequate supply of required feed and fodders (Patil and Singh, 2006). In northern Gujarat, livestock is an important component of rural and urban economy. However, the low productivity of livestock is a matter of concern, which is primarily due to insufficient fodder and feed resources and non availability of quality fodder. To sustain the wealth and provide nutritional security for animal health the large quantity of quality green fodder is required (Anonymous, 2006). Sorghum (*Sorghum bicolor* (L.) Moench) because of its high tolerance to high temperature and better ability to withstand drought is suited for arid and semi-arid regions of Gujarat and can play a vital role for the uplift of socio-economic status of the farmers. Sorghum is preferred over maize in *kharif* and summer season because of its high tolerance to various stress and its superiority to pearlmillet in having lower oxalate and fiber content (Prakash *et al*., 2010). The production potential of the sorghum varieties is low in Gujarat. The majority of the farmers in this area grow sorghum for fodder purpose and harvest these landraces along with the ear head at the time of maturity to feed the cattle.

Plant genetic resources play an important role in generating new crop varieties with the high yield potential and resistance to biotic and abiotic stresses. It is well established fact that the progress in improvement of a crop depends on the degree of variability in the desired character in the base material *vis-à-vis* germplasm collection. The study of relationships among quantitative traits is important for assessing the feasibility of joint selection of two or more traits and hence for evaluating the effect of selection for secondary traits on genetic gain for the primary trait under consideration. A positive genetic correlation between two desirable traits makes the job of the plant breeder easy for improving both traits simultaneously. The path coefficient analysis allows partitioning of correlation coefficient into direct and indirect contributions (effects) of various traits towards dependent variable and thus, helps in assessing the cause-effect relationship as well as effective selection. In the present study, therefore, variability for fodder yield and related attributes was estimated in a collection of 105 accessions over the two years.

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Materials and Methods

One hundred five forage sorghum genotypes collected from Directorate of Sorghum Research, Hyderabad and local landraces of Gujarat were used for the present study. The trial was grown in Augmented Block Design for two seasons *kharif* 2008 and *kharif* 2009 at Sorghum Research Station, Sardarkrushinagar Dantiwada Agricultural University, Deesa (Gujarat). In both seasons these accessions were divided in to the seven blocks and each block consisted of 15 accessions with three check varieties *viz*., GJ-39, GFS-5 and CSV-21 (F). Deesa is situated at latitude of 24.5º N and longitude 72º E and at an elevation of 136 M msl. The soil of the field was sandy in texture with pH value of 7.5 to 8.00 having good physical and chemical properties (Organic Carbon= 0.23, EC dsm^{-} = 0.232, K_2O= 259.9 kg/ha and P_2O_5= 46.2 kg/ha). The experimental unit was a single-row plot of 6.75 m long, spaced at 0.45 m apart. NPK 120:40:00 fertilizer was applied as half basal dose of nitrogen and full dose of phosphorus at the time of sowing and half nitrogen applied after one month of sowing. Plots were thinned down after two weeks of crop emergence and plant-to-plant distance of 0.10 m was maintained. The experimental years showed different temperature regimes, humidity, rain fall and sunshine hours during the crop durations. The all other recommended agronomical practices were followed to raise a good crop in both the seasons. Data were taken on days to 50% flowering, plant height (cm), number of leaves/plant, leaf length (cm), leaf breadth (cm), stem girth (cm), leaf stem ratio, green fodder yield/plant(g) and dry fodder yield/plant (g). Statistical analysis was done according to the standard statistical procedures (Federer, 1996; Burton, 1952; Johnson *et al*., 1955; Al-Jibouri *et al*., 1958; Dewey and Lu, 1959).

Results and Discussion

In an augmented design, the evaluation of the checks is first done to get an estimate of error, which is used for deriving adjusted values of entries. The data obtained from both seasons was significant for most of the traits indicating the effects of season x genotype interactions. Hence, the ANOVA was done season wise. Evaluation of 105 accessions in both the season showed significant differences for the traits *viz*., days to 50% flowering, plant height, number of leaves/plant, leaf length, stem girth, green fodder yield and dry fodder yield suggesting the estimate of significant variation among the entries. These results support the selection programme for high yielding genotypes. Similar pattern of variability in germplasm evaluation of different sizes have been reported by earlier workers (Alhassan *et al*., 2008; Warked *et al*., 2008; Jain *et al*., 2009).

The genetic constants for the characters revealed that the magnitude of phenotypic coefficient of variation (PCV) was higher than the corresponding genotypic coefficient of variation (GCV) for all the traits denoting environmental factors influencing their expression to some degree or other (Table1). Wide differences between PCV and GCV implied their susceptibility to environmental fluctuation, whereas narrow differences between PCV and GCV suggested their relative resistance to environmental alterations. The estimate of GCV and PCV alone is not much helpful in determining the heritable portion. The amount of advance to be expected from selection can be achieved by estimating heritability along with coefficient of variability. Burton (1952) also suggested that GCV and heritability estimate would give better information about the efficiency of selection. The heritability in broad sense was observed to be high 61.21 to 92.49 for all the traits which had significant differences among the accessions in both the seasons. The high degree of heritability estimates for most of the traits suggested that the characters are under genotypic control. Similar result was also reported by earlier workers (Sharma *et al*., 2006; Jain *et al*., 2009). High heritability coupled with high genetic advance and GCV were noticed for days to 50% flowering, green fodder yield/plant, dry fodder yield/plant and plant height. From the study of heritability and genetic advance it is inferred that simple selection among germplasm accessions can bring about significant improvement in these traits as the heritability and estimated genetic advance were high. The expected genetic advance might have been biased upward as it is based on the estimates of heritability in broad sense, secondly in the augmented design the estimation of mean square due to the error is based on the check variety only, and hence, it might have given the high estimates of genetic variances in it.

The genotypic and phenotypic correlation coefficients worked out among different characters including green and dry fodder yield/plant revealed that in general

Table 1. Range, mean, coefficient of variation, heritability and expected genetic advance for green fodder yield other characters in sorghum

Characters	Years	Genotypic coefficient of variation	Phenotypic coefficient of variation	Heritability (broad sense) (%)	Expected genetic advance % of Mean
Days to 50% flowering	I	13.52	12.36	83.56	17.40
	II	12.51	13.21	80.21	17.40
Plant height	I	29.95	36.90	92.17	67.87
	II	28.21	37.21	91.52	65.54
Number of leaves/plant	I	36.60	29.91	61.79	4.44
	II	37.41	32.01	61.21	4.10
Leaf length	I	13.88	9.00	90.00	0.17
	II	14.21	10.21	91.41	0.31
Leaf breath	I	24.06	21.69	81.24	1.85
	II	24.51	23.21	82.54	2.10
Stem girth	I	49.67	47.77	92.49	2.90
	II	44.21	46.54	91.42	3.21
Leaf: stem ratio	I	43.29	35.99	69.09	0.21
	II	42.10	36.21	66.54	0.32
Green fodder yield/plant	I	50.73	45.87	81.75	109.50
	II	52.10	44.21	80.41	100.2
Dry fodder yield/plant	I	54.89	48.69	78.70	47.90
	II	52.31	41.54	75.21	42.21

Table 2. Phenotypic correlation coefficient on the basis of unadjusted values between different characters in sorghum over two years

Character	Year	Plant height	No. of leaves/ plant	Leaf length	Leaf breadth	Stem girth	Leaf stem ratio	Green fodder yield/plant	Dry fodder yield/ plant
Days to 50 % flowering	I	0.2139	0.2147	0.2132	0.2952	-0.2048	-0.0107	-0.1204	0.0913
	II	0.1594	0.1939	0.1717	0.2381	-0.2135	-0.016	-0.1258	0.0924
Plant height (cm)	I	-	0.4342**	0.2742	0.2162	0.0736	-0.2435	0.3494**	0.1967
	II	-	0.4151**	0.2715	0.089	0.1233	-0.1525	0.425**	0.2385
No. of leaves/plant	I		-	0.4405**	0.3323*	0.0537	-0.2104	0.4138**	0.2046
	II		-	0.3905**	0.3074*	0.0596	-0.1557	0.4382**	0.2107
Leaf length	I			-	0.4866**	0.1077	-0.3277*	0.1819	0.2922*
	II			-	0.4784**	0.144	-0.3065*	0.3222*	0.2827*
Leaf breadth	I				-	-0.2195	-0.3696*	-0.2243	-0.0082
	II				-	-0.2566	-0.317*	-0.3233*	-0.0356
Stem girth	I					-	-0.0042	0.6992**	0.4723**
	II					-	0.005	0.6698**	0.4799**
Leaf stem ratio	I						-	0.0065	-0.1112
	II						-	-0.0029	-0.103
Dry fodder yield/ plant	I							-	0.7982
	II							-	0.8121

* Significant at 5% and ** Significant at 1%

the phenotypic correlation coefficient were similar to genotypic correlation coefficient (Table 2). In some cases the phenotypic correlation was slightly higher then the genotypic correlation coefficients, which may be a result of modifying effect of environments on the association of the characters. The green fodder yield/ plant showed positive and significant correlation with stem girth, number of leaves/plant, plant height and leaf breadth in both the seasons while with the leaf length although a positive association was observed in both

Table 3. Direct (diagonal) and indirect (non diagonal) effects of different characters on green fodder yield in sorghum at genotypic level over two years

Characters		Days to 50% flowering	Plant height	No. of Leaves/ plant	Leaf length	Leaf breadth	Stem girth	Leaf stem ratio	Dry fodder Yield/ plant
Days to 50 % flowering	I	**-0.0871**	-0.0186	-0.0187	-0.0186	-0.0257	0.0178	0.0009	-0.0079
	II	**-0.1176**	-0.0188	-0.0228	-0.0202	-0.028	0.0251	0.0019	-0.0109
Plant height	I	0.0104	**0.2487**	0.0211	0.0133	0.0105	0.0036	-0.0119	0.0096
	II	0.008	**0.2502**	0.0209	0.0136	0.0045	0.0062	-0.0077	0.012
No. of leaves/plant	I	-0.0004	-0.0008	**0.2018**	-0.0008	-0.0006	-0.0001	0.0004	-0.0004
	II	0.0003	0.0007	**0.2016**	0.0006	0.0005	0.0001	-0.0002	0.0003
Leaf length	I	0.0107	0.0138	0.0221	**0.0502**	0.0244	0.0054	-0.0165	0.0147
	II	0.0136	0.0216	0.031	**0.0794**	0.038	0.0114	-0.0243	0.0224
Leaf breadth	I	-0.0384	-0.0282	-0.0433	-0.0634	**-0.1302**	0.0286	0.0481	0.0011
	II	-0.03	-0.0112	-0.0388	-0.0603	**-0.1261**	0.0324	0.04	0.0045
Stem girth	I	-0.0718	0.0258	0.0188	0.0377	-0.0769	**0.3504**	-0.0015	0.1655
	II	-0.0598	0.0345	0.0167	0.0403	-0.0719	**0.2801**	0.0014	0.1344
Leaf stem ratio	I	-0.0006	-0.0136	-0.0118	-0.0183	-0.0207	-0.0002	**0.056**	-0.0062
	II	-0.0009	-0.0082	-0.0083	-0.0164	-0.017	0.0003	**0.0535**	-0.0055
Dry fodder yield/plant	I	0.0568	0.1224	0.1273	0.1817	-0.0051	0.2937	-0.0691	**0.6219**
	II	0.0605	0.1561	0.138	0.1851	-0.0233	0.3142	-0.0675	**0.6548**

Residual effects: 0.449 (I Year), Residual effects: 0.456 (II Year)

the seasons the value was significant only in the II year. Green fodder yield exhibited negative correlation with leaf breadth; however, the value was significant in II year only. It is also noticed that characters *viz*., number of leaves/plant, plant height, leaf length and stem girth exhibited positive associations with fodder yield have also showed positive associations among themselves in both the years. Out of these traits some traits like plant height and stem girth are having the high broad sense heritability and high expected genetic advance should be used in selection programme. Similar views have been reported by earlier workers (Anup *et al*., 2000; Sankarapandian, 2010).

Correlation co-efficient indicates only the general associations between any two traits without tracing any possible causes of such associations. In such situations, the path coefficient analysis at phenotypic level (Table 3) is done to partition the correlation coefficients in to direct and indirect effects. Green fodder yield/plant was taken as dependent variable while computing the path coefficient. The path coefficient analysis based on both the seasons revealed that the characters like stem girth, number of leaves/plant, plant height and leaf length which had positive significant association with green fodder yield also exerted positive and high direct effects on green fodder yield (Table 3). This confirms the role of these traits in determining the green fodder yield and therefore, their values in constructing the selection criterion. Whereas leaf breadth showed positive significant association with green and dry fodder yield in both the seasons. But the direct effect of leaf breadth was negative with fodder yield in both the season, which may be a result of the indirect effect of this trait *via* other traits.

The conclusion that can be reached from the variability, correlations and path coefficient is that number of leaves/plant, leaf length, leaf breadth and stem girth are the most important component traits for green and dry fodder yield/plant. Based on the results of the means of the two seasons some of the accessions identified superior for the different morphological characters were IS-3665 and EJ-16 was for earliness, RAJ-21 for plant height and leaf characters, IS-2887 and IS-3266 for fodder yield (Table 3). Therefore, these accessions could be utilized in further breeding programme for developing superior varieties of fodder sorghum.

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Table 4. Top ranking accessions selected on the basis of average of different characters over two years (2008-09 and 2009-10) in forage sorghum

S.No.	Days to 50 % flowering	Plant height (cm)	Number of leaves/plant	Leaf length (cm)	Leaf breadth (cm)	Stem girth (cm)	Green fodder yield/plant (g)	Dry fodder yield/plant (g)
1	IS-3265 (46.5)	RAJ-21 (285)	E-3 (14.75)	RAJ-21 (75.75)	RAJ-21 (7.75)	IS-3309 (4.9)	IS-2887 (290.05)	IS-3309 (142.6)
2	EJ-16 (49.0)	IS-3316 (250)	RAJ-21 (14.35)	IS-3922 (73.95)	RAJ-30 (7.2)	IS-2887 (4.8)	IS-3266 (288.9)	IS-3266 (113.5)
3	EJ-15 (49.0)	IS-3260 (245)	RAJ-25 (14.2)	E-14 (70.80)	RAJ-7 (6.6)	IS-3260 (4.7)	IS-3922 (278.7)	IS-3922 (105.7)
4	EJ-17 (51.0)	IS-3328 (243)	IS-3338 (14.1)	IS-6065 (72.75)	EP-59 (6.55)	IS-3215 (4.6)	IS-3309 (250.25)	IS-3342 (97.5)
5	IS-3203 (53.5)	IS-3338 (237)	IS-3336 (13.55)	E-13 (72.25)	RAJ-19 (6.55)	IS-3336 (4.45)	IS-3342 (234.6)	IS-3328 (92.9)
6	IS-3267 (54.5)	E-14 (235)	EP-59 (13.55)	RAJ-25 (72.1)	RAJ-20 (6.5)	IS-3328 (4.4)	IS-3328 (218.3)	IS-2887 (88.1)
7	IS-3250 (57.0)	IS-6014 (232)	RAJ-20 (12.95)	RAJ-20 (71.75)	RAJ-15 (6.4)	IS-3321 (4.4)	IS-3284 (202.5)	IS-3321 (83.75)
8	E-12 (58.5)	RAJ-20 (233)	IS-3267 (12.75)	IS-3306 (71.35)	EP-56 (6.35)	IS-3922 (4.35)	IS-3341 (200.25)	IS-3341 (77.5)
9	IS-3223 (59.0)	EJ-42 (223)	EP-139 (12.4)	IS-3371 (70.7)	EP-58 (6.35)	IS-700 (4.30)	IS-3321 (197.25)	IS-698 (71.75)
10	E-3 (59.0)	RAJ-7 (223)	EP-56 (12.35)	EP-56 (70.5)	RAJ-16 (6.15)	IS-3267 (4.30)	IS-3374 (194.75)	IS-3274 (71.75)
11	IS-3266 (59.5)	RAJ-25 (222)	RAJ-30 (12.35)	IS-4015 (69.6)	EP-61 (6.10)	IS-3241 (4.30)	IS-697 (184.5)	IS-3365 (70.5)
12	EJ-18 (61.0)	E-3 (221)	RAJ-15 (12.25)	IS-3342 (69.75)	RAJ-8 (6.15)	IS-4266 (4.0)	IS-703 (180.7)	IS-703 (68.15)
13	E-4 (62.5)	IS-697 (220)	IS-3276 (12.20)	IS-3336 (68.9)	SEVS-5 (5.85)	IS-3267 (4.0)	IS-3274 (179.9)	IS-3284 (65.15)
14	IS-6065 (63.5)	IS-3199 (220)	IS-3279 (12.2)	IS-3222 (68.5)	SEVS-4 5.8)	E-2 (3.95)	IS-3365 (179.0)	IS-3374 (42.4)
15	IS-18676 (63.5)	E-12 (220)	RAJ-8 (12.35)	IS-3301 (68.4)	SEVS-6 (5.65)	IS-606 (3.85)	IS-698 (177.7)	IS-697 (27.8)

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Exploiting Heterosis for Yield and Horticultural Traits in Cucumber (*Cucumis sativus* L.)

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Hybrids in cucumber have several well known advantages over the open-pollinated varieties. To find out more appropriate combinations for developing superior hybrids in cucumber, the crosses among eight parents (including two gynoecious lines) were attempted in half diallel fashion. Twenty-eight F_1s, eight parents and one check were grown in a Randomized Block Design with three replications at two different locations *viz*. Nauni (L1) and Chambaghat (L2). Sufficient genetic diversity among parents and F_1s for all the traits infers the scope of improvement through selection in the parental material. Among the parents, G_2 took minimum value for number of days to first female flower appearance, node number of first female flower and days to marketable maturity. Heterobeltiosis and standard heterosis for earliness were maximum in EC173934 x LC-40 and G_2 x Gyn1, respectively. The cross K-90 x G_2 exhibited maximum heterobeltiosis and standard heterosis for yield and number of fruits.

Key Words: Cucumber, Gynoecious line, Half diallel, Heterobeltiosis, Heterosis breeding

Introduction

Introduction of hybrids and their success in cucumber (*Cucumis sativus* L.), a member of family Cucurbitaceae has made it imperative for the breeders to find out more appropriate combinations to develop superior hybrids as these have several well known advantages over the open-pollinated varieties. Heterosis is of direct relevance for developing hybrids and effective tool in the hands of breeders for effective improvement in yield, earliness and quality. In cucumber too, success of F_1 hybrids has encouraged the breeders to develop hybrids which are early, vigorous, high yielding, tolerant to diseases and insect-pests and more efficient in the use of water and fertilizers. Moreover, use of gynoecious lines as a parent in producing cucumber hybrids ensures high yield level in the resultant hybrids

Currently, very few hybrids have been developed by public sector and the farmers are purchasing hybrid seeds from the private sector companies, who are charging exorbitantly. Stability of genotype is also important for its wider adaptation. To tide over the situation, there is a need to make concentrated efforts to develop F_1 hybrids and making their seed available to the farmers at a reasonable price. So, the present investigations were conducted to exploit hybrid vigour for developing the best suitable combination which can replace the conventional varieties as well as hybrids from private sector and also to make F_1 hybrids seed production cost effective with the use of gynoecious lines.

Materials and Methods

The present investigations were carried out at two locations *viz.* Experimental Farms, Nauni (L1) and Chambaghat (L2) of the Department of Vegetable Science, Dr. YS Parmar University of Horticulture and Forestry, Solan (Himachal Pradesh). Crosses among eight parents were attempted in a half diallel fashion. All the parents except two gynoecious lines were of monoecious type. The materials comprising twenty eight F_1s, eight parents and one check (Pusa Sanyog F_1) were grown in a Randomized Block Design with three replications at both the locations. Spacing was 1.25x 1.00 m. Data were recorded on five randomly selected plants/plot at each location for yield and quantitative

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characters. At each location, data were recorded on 555 plants for days to first female flower appearance, node to first female flower, days to marketable maturity, internodal length (cm), number of fruits/plant, yield/plant (Kg), fruits weight (g), fruit length (cm), fruit width (mm), TSS (^{o} B) and flesh to seed cavity ratio. Mean values were subjected to statistical analysis according to Fisher (1938) for analysis of variance. Heterotic effects of F_1s were worked out over better parents and over check and expressed as per cent increase or decrease over mean values.

Results and Discussion

Analysis of variance (Tables 1 and 2) indicated sufficient genetic diversity among parents and F_1s for all the traits at both the locations which infers scope of improvement through selection in the parental material.

Among the parents, G_2 took minimum value for number of days to first female flower appearance, node number of first female flower and days to marketable maturity followed by Gyn1 while EC173934 as well as LC-40 exhibited maximum values for these traits (Table 3). Very few crosses exhibited heterobeltiosis for days to first female flower appearance, node number of first female flower appeared and days to marketable maturity being maximum in EC173934xLC-40 at both locations (Tables 5 and 6). Heterosis over check variety was maximum in G2xGyn1 followed by K-90xG2 for these traits. Similar results on heterosis were reported by Pyzhenkov and Kosareva (1981) and Musamade and Kale (1986) for days to first female flower appearance; and by El-Shawaf (1980) for node number of first female flower and days to marketable maturity. In accordance with present findings, Doligibh and Sidorova (1983) and Singh *et al.* (1999) observed heterosis for earliness. Genotype or cross combination producing female flower earlier are desirable because ultimately the fruit is to develop from female flower. Similarly, appearance of female flowers on lower nodes is an indicative of earliness. Uniformity in results at different locations gives an indication of the authenticity of heterotic combinations.

G_2 recorded minimum internodal length. Among F_1s, LC-40xGyn1 observed minimum internodal length closely followed by K-90xG_2 at L1 and K-90xG2 closely followed by LC-40xGyn1 at L2. At L1, Heterobeltiosis and standard heterosis were maximum in LC-40xGyn1 while heterosis over better parent was maximum in PoinsettexLC-40 and over check variety in K-90xG2 at L2. Minimum internodal length means more number of nodes/vine for fruit bearing.

Table 1. Analysis of variance for plant traits in cucumber

Source of variation	Df	Character											
		Days to first female flower appearance		Node to first female flower appearance		Days to marketable maturity		Internodal length		No. of fruits/plant		Yield/plant (Kg)	
		L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
Genotype	35	508.72*	396.22*	24.12*	32.36*	531.33*	436.21*	10.95*	8.31*	8.72*	12.35*	0.90*	0.91*
Error	70	1.67	1.29	0.68	0.61	1.69	1.33	0.82	1.07	0.34	0.45	0.004	0.03

* Significant at 5% level of significance
L1-Nauni; L2-Chambaghat

Table 2. Analysis of variance for fruit traits in cucumber

Source of variation	Df	Character									
		Fruit weight (g)		Fruit length (cm)		Fruit width (mm)		TSS (^{o}B)		Flesh to seed cavity ratio	
		L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
Genotype	35	3936.12*	3579.39*	11.62*	14.04*	1.24*	12.40*	0.055*	0.075*	0.004*	0.003*
Error	70	187.07	147.70	0.012	0.27	0.007	0.068	0.004	0.018	0.0001	0.0001

* Significant at 5% level of significance
L1-Nauni; L2-Chambaghat

Table 3. Mean performance of parents and F_1s for plant traits in cucumber

Crosses/ Parents	Days to first female flower appearance		Node no. of first female flower		Days to marketable maturity		Internodal length (cm)		No. of fruits/ plant		Yield/plant (kg)	
	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
K-90	71.33	63.00	9.00	10.00	78.33	70.33	13.33	13.90	7.67	8.33	2.70	3.07
G_2	40.33	35.33	1.00	1.00	46.67	42.67	7.67	9.00	11.67	12.67	2.61	2.83
Poinsette	60.33	53.33	6.00	6.67	68.00	61.33	15.83	15.03	6.34	7.00	1.81	1.96
EC173934	85.33	76.67	11.67	12.67	94.67	85.00	14.10	13.93	7.67	8.33	1.79	1.99
K-75	63.00	56.33	8.33	10.67	72.33	64.33	15.00	15.20	6.68	7.00	2.15	2.70
LC-11	80.33	70.67	8.00	11.33	88.67	79.00	13.00	13.67	5.67	6.33	1.50	1.68
LC-40	85.33	73.33	12.67	13.33	93.67	80.33	12.67	13.70	5.67	6.33	1.77	2.08
Gyn_1	42.00	38.00	1.33	1.33	49.00	45.00	12.43	12.37	10.67	12.33	2.32	2.64
Pusa Sanyog (check)	52.67	45.00	4.33	5.67	60.33	52.33	10.66	11.93	10.61	11.67	2.71	3.03
K-90x G_2	47.00	41.33	3.33	4.33	54.33	48.00	10.66	10.30	13.33	14.67	3.75	4.05
K-90xPoinsette	59.33	54.33	5.67	8.67	67.00	62.00	11.67	13.00	8.33	8.67	2.04	2.29
K-90xEC173934	67.68	60.00	7.33	7.33	74.67	68.00	11.40	12.13	6.32	7.00	2.02	2.18
K-90xK-75	77.66	71.33	9.68	10.67	84.67	79.33	14.70	15.23	7.33	8.33	1.90	2.20
K-90xLC-11	82.33	73.33	7.34	9.33	90.33	81.00	16.00	16.00	6.67	7.67	2.02	2.33
K-90xLC-40	71.33	63.33	7.33	9.00	78.67	71.33	14.60	12.67	6.33	7.33	1.74	2.06
K-90x Gyn_1	51.33	43.67	4.33	4.33	57.32	50.67	12.67	12.00	11.67	12.33	3.20	3.42
G_2 xPoinsette	54.00	51.67	4.33	6.00	63.68	59.00	13.00	12.00	8.67	9.67	2.08	2.38
G_2 x EC173934	66.00	57.33	5.68	7.00	74.00	65.67	12.30	13.13	7.33	7.67	1.70	1.87
G_2x K-75	55.33	46.33	3.67	4.33	63.66	53.67	13.50	11.57	10.33	11.00	2.97	3.08
G_2 x LC-11	57.67	50.67	5.34	6.67	64.65	58.00	11.83	11.47	8.33	8.67	2.63	2.75
G_2 x LC-40	73.67	73.33	6.68	8.67	81.33	70.67	11.13	11.63	6.33	7.67	1.41	1.81
G_2 x Gyn_1	43.34	40.33	2.67	3.00	52.00	47.33	11.83	11.60	10.67	11.33	2.58	2.88
Poinsette x EC173934	73.33	68.33	6.34	7.67	82.00	76.67	11.93	12.33	7.33	8.33	1.81	2.12
Poinsettex K-75	64.33	57.33	6.68	8.00	72.00	65.67	17.27	16.00	7.32	8.33	2.31	2.67
Poinsettex LC-11	65.68	58.00	5.33	9.33	73.34	65.67	16.20	15.90	7.34	8.00	1.48	1.72
Poinsettex LC-40	69.00	63.00	6.67	8.33	76.68	71.00	12.07	11.53	6.66	7.67	1.80	2.06
Poinsettex Gyn_1	68.33	61.67	4.68	6.00	76.67	69.67	14.83	14.07	8.67	9.67	2.62	2.98
EC173934x K-75	74.00	68.00	10.00	13.33	81.00	76.00	15.37	14.27	6.33	7.33	1.51	1.83
EC173934x LC-11	81.00	74.00	6.33	8.33	88.66	82.00	14.37	13.77	6.33	7.00	1.90	2.13
EC173934x LC-40	75.32	66.33	7.00	7.33	84.00	74.33	13.03	14.83	7.34	7.67	1.87	2.00
EC173934x Gyn_1	72.68	61.67	9.68	6.67	80.67	69.67	13.00	14.53	6.66	7.33	1.47	1.68
K-75x LC-11	77.68	70.67	8.33	9.00	85.67	78.67	12.73	13.63	7.33	7.67	1.82	2.03
K-75x LC-40	81.34	72.67	7.33	7.33	90.00	80.33	11.67	12.00	6.33	7.00	1.76	1.97
K-75x Gyn_1	50.00	45.00	4.33	5.00	57.68	52.33	13.76	13.57	10.66	11.33	2.98	3.05
LC-11x LC-40	87.00	80.00	13.33	14.67	95.00	87.67	14.40	13.33	5.33	6.33	1.73	1.94
LC-11x Gyn_1	53.00	48.33	3.68	4.33	60.00	55.67	12.67	11.67	8.67	8.67	2.84	3.13
LC-40x Gyn_1	69.00	59.00	6.33	12.67	75.67	66.67	10.00	10.93	6.67	7.66	1.68	2.02
SE±	**1.06**	**0.93**	**0.68**	**0.64**	**1.06**	**0.94**	**0.74**	**0.85**	**0.47**	**0.55**	**0.05**	**0.14**
$CD_{0.05}$	**2.10**	**1.85**	**1.35**	**1.28**	**2.11**	**1.87**	**1.47**	**1.69**	**0.94**	**1.09**	**0.10**	**0.28**

L1-Nauni; L2-Chambaghat

Table 4. Mean performance of parents and F_1s for fruit traits in cucumber

Crosses/Parents	Fruit weight (g)		Fruit length (cm)		Fruit width (mm)		TSS (°B)		Flesh to seed cavity ratio	
	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
K-90	318.33	316.67	19.73	19.17	6.80	6.97	3.00	3.03	0.21	0.20
G_2	226.67	231.67	12.65	12.27	5.47	5.57	2.90	2.93	0.19	0.20
Poinsette	268.33	261.67	16.47	16.40	4.32	4.40	2.77	2.80	0.25	0.25
EC173934	243. 32	250.00	14.17	14.07	6.31	6.17	3.30	3.47	0.22	0.21
K-75	305.00	296.67	16.43	16.90	6.16	6.10	2.99	3.03	0.21	0.21
LC-11	280.00	283.33	18.30	18.90	6.17	6.06	2.98	3.07	0.24	0.23
LC-40	281.66	290.00	14.63	14.90	6.60	6.43	3.03	3.13	0.20	0.21
Gyn_1	241.67	238.33	20.30	23.30	4.61	4.80	2.88	2.87	0.27	0.27
Pusa Sanyog (check)	268.33	266.67	16.67	16.83	5.27	5.20	3.23	3.33	0.21	0.22
K-90x G_2	251.66	260.00	14.28	14.20	5.53	5.23	2.95	2.93	0.18	0.19
K-90x Poinsette	246.67	256.67	17.62	17.80	5.54	5.40	3.27	3.30	0.18	0.17
K-90x EC173934	323.33	330.00	17.33	17.37	5.60	5.53	2.99	2.97	0.18	0.18
K-90x K-75	265.00	273.33	19.50	19.17	6.75	6.57	3.03	3.10	0.21	0.23
K-90x LC-11	378.32	356.67	18.15	18.43	6.63	6.13	2.94	2.97	0.17	0.18
K-90x LC-40	280.00	285.00	19.27	19.67	5.18	5.63	3.06	3.10	0.21	0.19
K-90x Gyn_1	250.00	253.33	18.25	18.23	5.37	5.50	2.98	2.97	0.20	0.20
G_2 xPoinsette	240.00	223.33	18.63	19.10	4.65	4.63	3.02	3.03	0.18	0.17
G_2 x EC173934	233.33	228.33	17.83	18.80	6.25	6.63	3.05	3.13	0.19	0.18
G_2x K-75	275.00	280.00	18.47	18.80	6.20	6.80	3.15	3.23	0.20	0.18
G_2 x LC-11	301.66	303.33	15.65	15.77	5.35	5.40	3.07	3.10	0.19	0.18
G_2 x LC-40	210.00	230.00	16.82	17.10	5.33	5.80	3.23	3.00	0.21	0.21
G_2 x Gyn_1	213.33	221.67	19.25	19.27	5.90	6.07	3.14	3.17	0.19	0.19
Poinsette x EC173934	263.33	273.33	19.38	17.93	5.47	5.63	3.13	3.27	0.32	0.32
Poinsettex K-75	291.66	303.03	16.65	15.80	5.75	5.50	3.23	3.23	0.17	0.17
Poinsettex LC-11	308.33	306.67	17.77	17.63	4.70	4.90	2.97	3.03	0.18	0.21
Poinsettex LC-40	228.33	230.00	20.10	20.13	5.73	6.27	2.78	2.93	0.25	0.25
Poinsettex Gyn_1	266.67	255.33	17.13	17.07	4.78	4.37	3.13	3.10	0.27	0.25
EC173934x K-75	265.00	271.67	17.40	17.20	5.85	6.03	2.87	2.87	0.21	0.21
EC173934x LC-11	305.00	316.67	18.97	18.90	6.08	6.17	2.98	3.04	0.26	0.26
EC173934x LC-40	248.33	241.67	18.37	18.27	5.28	5.77	3.00	3.00	0.22	0.22
EC173934x Gyn_1	231.66	228.33	18.75	18.60	5.32	5.40	3.12	3.03	0.21	0.22
K-75x LC-11	276.66	270.00	19.38	19.43	6.51	6.67	3.04	2.87	0.17	0.18
K-75x LC-40	271.67	268.33	18.43	18.67	5.25	5.20	3.20	3.30	0.18	0.18
K-75x Gyn_1	276.66	271.67	18.07	18.00	5.13	5.37	2.83	2.78	0.18	0.19
LC-11x LC-40	316.66	320.00	18.47	18.67	5.15	5.03	3.04	3.07	0.20	0.19
LC-11x Gyn_1	311.67	311.67	19.17	19.23	5.16	5.63	3.15	3.13	0.18	0.19
LC-40x Gyn_1	228.33	235.00	17.30	17.13	5.35	5.50	3.28	3.40	0.20	0.19
SE±	**11.17**	**9.92**	**0.09**	**0.18**	**0.07**	**0.21**	**0.05**	**0.11**	**0.009**	**0.008**
$CD_{0.05}$	**22.27**	**19.79**	**0.18**	**0.36**	**0.14**	**0.42**	**0.10**	**0.22**	**0.018**	**0.017**

L1-Nauni; L2-Chambaghat

Table 5. Heterotic response of F_1s for plant traits in cucumber at Nauni (L1)

Crosses	Percentage increase or decrease for											
	Days to first female flower appearance		Node no. of first female flower		Days to marketable maturity		Internodal length (cm)		No. of fruits/ plant		Yield/plant (kg)	
	Over BP	Over Check	Over BP	Over Check	Over BP	Over Check	Over BP	Over Check	Over BP	Over Check	Over BP	Over Check
K-90 x G_2	16.53	-10.76	233.33	-23.08	16.43	-9.94	39.13	0.00	14.40	25.18	38.77	38.25
K-90 x Poinsette	-1.66	12.66	-5.57	30.77	-1.47	11.05	-12.50	9.37	8.70	-21.88	-24.32	-24.60
K-90 x EC173934	-5.14	28.47	-18.52	69.23	-4.68	23.76	-14.50	6.87	-17.39	-40.63	-25.31	-25.58
K-90 x K-75	23.28	47.47	16.00	123.08	17.05	40.33	10.25	37.81	-4.35	-31.25	-29.51	-29.77
K-90 x LC-11	15.42	56.33	-8.33	69.23	15.32	49.72	23.08	50.00	-13.04	-37.50	-25.06	-25.34
K-90 x LC-40	-0.47	34.81	-18.52	69.23	0.43	30.39	15.26	36.87	-17.39	-40.63	-35.56	-35.79
K-90 x Gyn_1	22.22	-2.53	225.00	0.00	17.01	-4.97	1.88	18.75	9.38	9.38	-18.52	18.08
G_2 x Poinsette	33.88	2.53	333.33	0.00	36.43	5.52	69.57	21.87	-25.71	-18.75	-20.54	-23.37
G_2 x EC173934	63.64	25.32	466.67	30.77	58.57	22.65	60.44	15.31	-37.14	-31.25	-34.82	-37.15
G_2 x K-75	37.19	5.06	266.67	-15.39	36.43	5.52	76.09	26.56	-11.43	-3.13	13.52	9.47
G_2 x LC-11	42.98	9.49	433.33	23.08	38.57	7.18	54.35	10.93	-28.57	-21.87	0.51	-3.08
G_2 x LC-40	82.65	39.87	566.67	53.85	74.29	34.81	45.22	4.37	-45.71	-40.63	-46.05	-47.97
G_2 x Gyn_1	7.44	-17.72	166.67	-38.46	11.43	-13.81	54.35	10.93	-8.57	0.00	-1.40	-4.92
Poinsette x EC173934	21.55	39.24	5.56	46.15	20.59	35.91	-15.37	11.87	-4.35	-31.25	0.00	-33.21
Poinsettex K-75	6.63	22.15	11.11	53.85	5.88	19.34	15.11	61.87	10.00	-31.25	7.75	-14.51
Poinsettex LC-11	8.84	24.68	-11.11	23.08	7.84	21.55	24.62	51.87	15.79	-31.25	-18.23	-8.49
Poinsettex LC-40	14.37	31.01	11.11	53.85	12.75	27.07	-4.74	13.12	5.26	-37.50	-0.55	-33.58
Poinsettex Gyn_1	62.70	29.75	250.00	7.69	56.46	27.07	19.30	39.06	-18.75	-18.75	13.09	-3.32
EC173934 x K-75	17.46	40.51	20.00	130.77	11.98	34.25	8.98	44.06	-17.39	-40.63	-29.77	-44.28
EC173934 x LC-11	0.83	53.79	-20.83	46.15	0.00	46.96	10.51	34.68	-17.39	-40.63	6.15	-29.89
EC173934 x LC-40	-11.72	43.04	-40.00	61.54	-10.32	39.23	2.90	22.18	-4.35	-31.25	4.28	-31.12
EC173934 x Gyn_1	73.02	37.98	625.00	123.08	64.63	33.70	4.56	21.87	-37.50	-37.50	-36.69	-45.88
K-75 x LC-11	23.28	47.47	4.17	92.31	18.43	41.99	-2.05	19.37	10.00	-31.25	-15.19	-32.72
K-75 x LC-40	29.10	54.43	-12.00	69.23	24.42	49.17	-7.90	9.37	-5.00	-40.63	-17.98	-34.93
K-75 x Gyn_1	19.05	-5.06	225.00	0.00	17.69	-4.42	10.72	29.06	0.00	0.00	28.49	9.84
LC-11 x LC-40	8.30	65.19	66.67	207.69	7.14	57.46	13.68	35.00	-5.88	-50.00	-2.26	-36.29
LC-11 x Gyn_1	26.19	0.63	175.00	-15.38	22.45	-0.55	1.88	18.75	-18.75	-18.75	22.73	4.92
LC-40 x Gyn_1	64.29	31.01	375.00	46.15	54.42	25.14	-19.57	-6.25	-37.50	-37.50	-27.34	-37.88

Cross combination K-90 x G_2 produced the maximum yield/plant and number of fruits/plant. The same cross recorded maximum heterosis over better parent as well over check variety for yield at both the locations and for number of fruits/plant at L1 only. PoinsettexK-75 recorded highest heterobeltiosis for number of fruits followed by K-90xG_2 while standard heterosis was maximum in K-90xG_2. However, among parents, K-90 and G_2 recorded the highest yield/plant and number of fruits/plant, respectively, at both locations. Similar findings for number of fruits were reported by Om *et al.* (1978), Solanki *et al.* (1982), Lower *et al.* (1982), Musamade and Kale (1986) and Singh *et al.* (1999).

Positive and significant heterosis for yield was reported by many workers (Belik and Koziner 1968; Ghaderi and Lower 1978; Om *et al.* 1978; Solanki *et al.* 1982; Hormuzdi and More, 1989). Bairagi *et al.* (2002) also found heterosis for yield related traits. As evident from the results, heterotic crosses combinations performed stably for yield and contributing traits at both the locations.

Table 6. Heterotic response of F_1s for plant traits in cucumber at Chambaghat (L2)

Crosses	Percentage increase or decrease for											
	Days to first female flower appearance		Node no. of first female flower		Days to marketable maturity		Internodal length (cm)		No. of fruits/ plant		Yield/plant (kg)	
	Over BP	Over Check	Over BP	Over Check	Over BP	Over Check	Over BP	Over Check	Over BP	Over Check	Over BP	Over Check
K-90 x G_2	16.98	-8.15	333.33	-23.53	12.50	-8.28	14.44	-13.69	15.78	25.71	32.07	33.52
K-90 x Poinsette	1.88	20.74	30.00	52.94	1.09	18.47	-6.47	8.94	4.00	-25.71	-25.22	-24.40
K-90 x EC173934	-4.76	33.33	-26.27	29.41	-3.32	29.94	12.71	1.68	-16.00	-40.00	-28.80	-28.02
K-90 x K-75	26.63	58.52	6.67	88.24	23.32	51.59	9.59	27.65	0.00	-28.57	-28.26	-27.47
K-90 x LC-11	16.40	62.96	-6.67	64.71	15.17	54.78	17.07	34.08	-8.00	-34.29	-24.02	-23.19
K-90 x LC-40	0.53	40.74	-10.00	58.82	1.42	36.31	-7.54	6.15	12.00	-37.14	-32.94	-32.20
K-90 x Gyn_1	14.91	-2.96	225.00	-23.53	12.59	-3.19	-2.97	0.56	0.00	5.71	11.41	12.64
G_2 x Poinsette	46.23	14.82	500.00	5.88	38.28	12.74	33.33	0.56	-23.68	-17.14	-15.88	-21.43
G_2 x EC173934	62.26	27.41	600.00	23.53	53.91	25.48	45.93	10.06	-39.47	-34.29	-33.88	-38.24
G_2 x K-75	31.13	2.96	333.33	-23.53	25.75	2.55	28.52	-3.07	-13.16	-5.71	8.82	1.65
G_2 x LC-11	43.40	12.59	566.67	17.65	35.94	10.83	27.41	-3.91	-31.58	-25.71	-2.94	-9.34
G_2 x LC-40	107.55	62.96	766.67	52.94	65.63	35.03	29.26	-2.51	-39.47	-34.29	-36.00	-40.22
G_2 x Gyn_1	14.15	-10.37	200.00	-47.06	10.94	-9.55	28.89	-2.79	-10.53	-2.86	1.77	-4.95
Poinsette x EC173934	28.13	51.85	15.00	35.29	25.00	46.50	-11.48	3.35	0.00	-28.57	6.01	-30.22
Poinsette x K-75	7.50	27.41	20.00	41.18	7.07	25.48	6.43	34.08	19.05	-28.57	-1.24	-12.09
Poinsette x LC-11	8.75	28.89	40.00	64.71	7.07	25.48	16.34	33.24	14.29	-31.43	-12.24	-10.33
Poinsette x LC-40	18.13	40.00	25.00	47.06	15.76	35.67	-15.81	-3.35	9.52	-34.29	-1.28	-32.20
Poinsette x Gyn_1	62.28	37.04	350.00	5.88	54.81	33.12	13.75	17.88	-21.62	-17.14	12.75	-1.87
EC173934 x K-75	20.71	51.11	25.00	135.29	18.14	45.22	2.39	19.55	-12.00	-37.14	-32.10	-39.56
EC173934 x LC-11	4.72	64.44	-26.47	47.06	3.80	56.69	0.73	15.36	-16.00	-40.00	6.85	-29.67
EC173934 x LC-40	-9.55	47.41	-42.00	29.41	-7.47	42.04	8.27	24.30	-8.00	-34.29	-4.00	-34.07
EC173934 x Gyn_1	62.28	37.04	400.00	17.65	54.82	33.12	17.52	21.79	-40.54	-37.14	-36.24	-44.51
K-75 x LC-11	23.44	57.04	-15.63	58.82	22.28	50.32	-0.24	14.25	9.52	-34.29	-24.82	-33.08
K-75 x LC-40	28.99	61.48	-31.25	29.41	24.87	53.50	-12.41	0.56	0.00	-40.00	-27.04	-35.06
K-75 x Gyn_1	18.42	0.00	275.00	-11.77	16.30	0.00	9.70	13.69	-8.11	-2.86	12.96	0.55
LC-11 x LC-40	13.20	77.78	29.41	158.82	10.97	67.52	2.44	11.73	0.00	-45.71	-6.72	-35.93
LC-11 x Gyn_1	27.19	7.41	225.00	-23.53	23.70	6.37	-5.66	-2.24	-29.73	-25.71	18.69	3.30
LC-40 x Gyn_1	55.26	31.11	850.00	123.53	48.15	27.39	-11.59	-8.38	-37.84	-34.29	-23.49	-33.41

K-90 recorded maximum fruit weight among parents while K-90xLC-11 produced significantly higher fruit weight (Tables 7 and 8). The same cross combination recorded maximum heterobeltiosis as well as standard heterosis. Ghaderi and Lower (1978), Solanki *et al.* (1982), Musamade and Kale (1986) and Hormuzdi and More (1989a) also observed heterosis for this trait.

Among the parents, Gyn1 produced significantly longer fruits at both the locations (Table 4). Maximum heterbeltiosis (Tables 7 and 8) was exhibited by G_2xEC 173934 followed by EC 173934xLC-40 (L1) and PoinsettexLC-40 (L2). However, maximum heterosis over check variety by PoinsettexLC-40 followed by K-90xK-75 at L1 and EC 173934xLC-40 followed by K-90 x LC-40 at L2. Maximum fruit width was produced by K-90. Heterobeltiosis was maximum in G_2xGyn1 at L1 and LC-11xLC-40 at L2 while standard heterosis was maximum in K-90xK-75 followed by K-90xLC-11 at L1 and G2xK-75 followed by K-75xLC-11 and K-90xK-75 at L2.

Table 7. Heterotic response of F_1s for fruit traits in cucumber at Nauni (L1)

Crosses	Percentage increase or decrease for									
	Fruit weight (g)		Fruit length (cm)		Fruit width (mm)		TSS (^oB)		Flesh to seed cavity ratio	
	Over BP	Over Check	Over BP	Over Check	Over BP	Over Check	Over BP	Over Check	Over BP	Over Check
K-90x G_2	-20.94	-6.21	-27.62	-14.30	-18.68	4.80	-1.67	-8.87	-10.00	-14.28
K-90x Poinsette	-22.51	-8.08	-10.69	5.74	-18.59	4.93	9.12	1.13	-28.00	-15.63
K-90x EC173934	1.57	20.50	-12.76	4.00	-17.65	6.13	-9.39	-7.53	-15.39	-14.04
K-90x K-75	-16.75	-1.24	-1.18	17.00	-0.69	27.99	1.22	-6.19	-1.59	-3.13
K-90x LC-11	18.75	40.99	-8.02	8.90	-2.45	25.71	-1.78	-8.97	-27.78	-18.75
K-90x LC-40	-12.04	4.35	-2.37	15.60	-23.82	-1.83	1.10	-5.36	3.23	0.00
K-90x Gyn_1	-21.47	-6.83	-21.67	9.50	-21.09	1.71	-0.67	-7.94	-24.39	-3.13
G_2 xPoinsette	-10.56	-10.56	13.16	11.80	-14.93	-11.81	4.02	-6.70	-29.33	-17.19
G_2 x EC173934	-4.11	-1304	25.88	7.00	-0.95	18.45	-7.58	-5.67	-12.31	-10.94
G_2x K-75	-9.84	2.48	12.37	10.80	0.76	17.56	5.35	-2.47	-4.76	-6.25
G_2 x LC-11	7.74	12.42	-14.48	-6.10	-13.30	1.33	2.79	-5.16	-22.22	-12.50
G_2 x LC-40	-25.44	-21.74	14.92	0.90	-19.28	1.01	6.83	0.00	3.33	-3.13
G_2 x Gyn_1	-11.73	-20.50	-17.38	15.50	7.86	1.81	8.28	-2.89	-29.27	-9.38
Poinsette x EC173934	-1.86	-1.86	17.71	16.30	-13.31	3.66	-5.05	-3.09	26.67	48.44
Poinsettex K-75	-4.37	8.69	-4.96	-6.10	-6.61	8.97	7.91	-0.10	-32.00	-20.31
Poinsettex LC-11	10.12	14.91	-2.91	6.60	-23.84	-10.99	-0.56	-8.25	-26.67	-14.06
Poinsettex LC-40	-18.94	-14.91	22.07	20.60	-13.18	8.65	-8.04	-13.92	1.33	18.75
Poinsettex Gyn_1	-0.62	-0.62	-26.47	2.80	3.61	-9.41	8.67	-3.09	-2.44	25.00
EC173934x K-75	-13.12	-1.24	5.88	4.40	-7.24	10.93	-13.03	-11.24	-4.62	-3.13
EC173934x LC-11	8.93	13.67	3.64	13.80	-3.59	15.29	-9.60	-7.73	8.33	21.88
EC173934x LC-40	-11.83	-7.45	25.51	10.20	-20.09	0.00	-9.09	-7.22	0.00	1.56
EC173934x Gyn_1	-4.79	-13.67	-19.53	12.50	-15.74	0.76	-5.56	-3.61	-21.95	0.00
K-75x LC-11	-9.29	-3.11	5.92	16.30	5.57	23.37	1.45	-6.08	-27.78	-18.75
K-75x LC-40	-10.93	1.24	12.17	10.60	-20.44	-0.44	5.73	-1.03	-15.87	-17.19
K-75x Gyn_1	-9.29	3.11	-22.46	8.40	-16.73	-2.84	-5.57	-12.58	-32.93	-14.06
LC-11x LC-40	12.43	18.01	0.91	10.80	-21.96	-2.34	0.55	-5.88	-18.06	-7.81
LC-11x Gyn_1	11.31	16.15	-17.74	15.00	-16.27	-2.14	5.59	-2.58	-32.93	-14.06
LC-40x Gyn_1	-18.94	-14.91	-25.75	3.80	-18.98	1.39	4.48	1.55	-28.05	-7.81

TSS has direct relevance to sweetness in cucumber and was maximum in parent EC 173934 at both locations (Table 4). Heterobeltiosis and standard heterosis were found low at each location, being maximum in K-90 x Poinsette and LC-40xGyn1, respectively (Tables 7 and 8). Flesh to cavity ratio was found maximum in Gyn1 (among parents) and Poinsette x EC 173934 (among cross combination) at each location. Maximum flesh to seed cavity is desired. The same cross combination recorded maximum heterobeltiosis as well as standard heterosis (Tables 5 and 6).

Extent of heterosis over better parents for yield and most of the horticultural characters was medium to high. It may be concluded that for developing high yielding and early maturing varieties, hybridization is the appropriate method. Cross combination K-90xG_2 was found best in terms of yield and number of fruits while G_2xGyn1 and EC 173934xLC-40 were earliest in maturity at both locations.

References

Bairagi SK, DK Singh and HH Ram (2002) Studies on heterosis for yield attributes in cucumber (*Cucumis sativus* L.) *Prog. Hort.* **33**: 178-183.

Table 8. Heterotic response of F_1s for fruit traits in cucumber at Chambaghat (L2)

Crosses	Percentage increase or decrease for									
	Fruit weight (g)		Fruit length (cm)		Fruit width (mm)		TSS (°B)		Flesh to seed cavity ratio	
	Over BP	Over Check	Over BP	Over Check	Over BP	Over Check	Over BP	Over Check	Over BP	Over Check
K-90x G_2	-17.90	-2.50	-25.91	-15.64	-24.88	0.64	-3.29	-12.00	-6.67	-13.05
K-90x Poinsette	-18.95	-3.75	-7.13	5.74	-22.49	3.85	8.79	-1.00	-29.73	-20.00
K-90x EC173934	4.21	23.75	-9.39	3.17	-20.57	6.41	-14.42	-11.00	-14.29	-16.92
K-90x K-75	-13.68	2.50	0.00	13.86	-5.74	26.28	2.19	-7.00	6.25	4.62
K-90x LC-11	12.63	33.75	-3.83	9.51	-11.96	17.95	-3.26	-11.00	-22.86	-16.92
K-90x LC-40	-10.00	6.88	2.61	16.83	-19.14	8.33	-2.13	-8.00	-7.94	-10.77
K-90x Gyn_1	-20.00	-5.00	-27.93	8.32	-21.05	5.76	-2.19	-11.00	-28.05	-9.23
G_2 xPoinsette	-14.65	-16.25	16.46	13.47	-16.77	-10.90	3.41	-9.00	-29.73	-20.00
G_2 x EC173934	-8.67	-14.38	33.65	11.68	2.70	21.80	-9.62	-6.00	-12.70	-15.39
G_2x K-75	-5.62	5.00	11.24	11.68	11.47	30.77	6.59	-3.00	-15.63	-16.92
G_2 x LC-11	7.06	13.75	-16.58	-6.34	10.99	3.85	0.00	-8.00	-21.43	-15.39
G_2 x LC-40	-20.69	-13.75	14.77	1.58	-9.85	11.54	-4.26	-10.00	1.59	-1.54
G_2 x Gyn_1	-6.99	-16.88	-23.85	14.46	8.98	16.67	7.96	-5.00	-31.71	-13.85
Poinsette x EC173934	4.46	2.50	9.35	6.54	-8.65	8.33	-5.77	-2.00	28.38	46.15
Poinsettex K-75	2.25	13.75	-6.51	-6.14	-9.84	5.77	6.59	-3.00	-29.73	-20.00
Poinsettex LC-11	8.24	15.00	-6.70	4.75	-19.23	-5.77	-1.09	-9.00	-13.51	-1.54
Poinsettex LC-40	-20.67	-13.75	22.76	19.60	-2.59	20.51	-6.38	-12.00	2.70	16.92
Poinsettex Gyn_1	-2.42	-4.25	-32.54	1.39	-9.03	-16.03	8.14	-7.00	-8.54	15.39
EC173934x K-75	-8.43	1.88	1.78	2.18	-2.16	16.03	-17.13	-14.00	0.00	-1.54
EC173934x LC-11	11.77	18.75	0.00	12.28	0.00	18.59	-12.50	-9.00	12.86	21.54
EC173934x LC-40	-16.67	-9.38	22.59	8.52	-10.36	10.90	-13.46	-10.00	3.18	0.00
EC173934x Gyn_1	-8.67	-14.48	-26.48	10.50	-12.43	3.85	-12.50	-9.00	-20.73	0.00
K-75x LC-11	-8.99	1.25	2.82	15.45	9.29	28.21	-6.52	-14.00	-21.43	-15.39
K-75x LC-40	-9.55	0.63	10.45	10.89	19.17	0.00	5.32	-1.00	-15.63	-16.92
K-75x Gyn_1	-8.43	1.88	-28.85	6.93	12.02	3.21	-8.24	-16.50	-31.71	-13.85
LC-11x LC-40	10.35	20.00	-0.18	12.08	21.76	-3.21	-2.13	-8.00	-17.14	-10.77
LC-11x Gyn_1	10.00	16.88	-23.98	14.26	-7.14	8.33	2.17	-6.00	-31.71	-13.85
LC-40x Gyn_1	-18.96	-11.88	-32.62	1.78	-14.51	5.77	8.51	2.00	-29.27	-10.77

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SHORT COMMUNICATION

Eliminating Smut (*Moesziomyces penicillariae*) from Pearl Millet Seeds under Transboundary Movement

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Smut of pearl millet caused by *Moesziomyces penicillariae* (Bref.) Venky is a seed-transmitted disease and fungicide seed treatment is not effective in inhibiting viability of smut spores present on seed surface. In the present study sodium hydroxide (NaOH), potassium hydroxide (KOH) and ethanol were tested at three different concentrations (5, 10 and 15%) to prevent the spore ball germination. NaOH and KOH were effective at 10 and 15% concentrations. None of these treatments could completely inhibit germination of spore balls. Spore ball elimination was tried by stirring infected pearl millet seeds for 1, 2 and 3 min with sterilized sand and ethanol. This seed stirring treatment for 2 min. was effective in complete elimination of smut spore balls from pearl millet seed surface. This simple method can be used as a routine treatment in quarantine processing of germplasm and seed certification programmes to prevent spread of pearl millet smut through seed.

Key Words: Ethanol, External contamination, Quarantine, Seed treatment, Spore ball

Smut caused by *Moesziomyces penicillariae* (Bref.) Venky, is a wide spread disease of pearl millet second only to downy mildew causing yield losses up to 10% (Leslie, 2003). Smut is an important disease of pearl millet in northern India particularly in the states of Haryana, Punjab, Gujarat and Rajasthan (Thakur and King, 1988). Bhomik and Sundaram (1971) reported that 50-70% of the crop was infected with smut in some fields with damage up to 100% in individual panicle.

Smut is a floral disease and infection is confined to individual spikelets, often scattered to near base of the earhead. In the field, infected florets are converted into sori, which break at maturity and release brown or black spore balls and healthy seeds get surface-contaminated. These surface-contaminated seeds when used for sowing, the spore balls act as the primary source of inoculum. Fungicides were tried to control pearl millet smut with limited success (Thakur *et al.*, 1992; Yadav and Duhan, 1999). The disease has lot of implications in export of germplasm as countries such as Nigeria and Zimbabwe seek additional declaration that consignment is free from smut. During 2004-2008, a total of 6,253 pearl millet samples have been exported to 31 countries all over the world. Pearl millet smut was also intercepted from the Philippines, Niger, USA and Zimbabwe during import quarantine processing (Chakrabarty *et al.*, 2004). Keeping the importance of the disease in view, the present study was aimed to develop a suitable method for eliminating surface contamination of smut spore balls either by affecting the spore ball germination or by eliminating the spore balls from seed surface so as to facilitate germplasm exchange across the countries.

Infected pearl millet panicles were collected from smut experimental nursery at International Crops Research Institute for the Semi-Arid Tropics, Patancheru. The infected sori were ruptured and spore balls were collected in a glass petri dish under aseptic conditions.

Aqueous solutions of chemicals *viz*., sodium hydroxide, potassium hydroxide and ethanol were prepared at 5, 10 and 15% concentrations to study their effect on spore ball germination. Spore balls and healthy pearl millet seeds were soaked in each concentration of chemical for 2 min. Spore balls were filtered and thoroughly washed in sterile distilled water. These spore balls were suspended in sterile

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distilled water and incubated at 30^0C for 48 h to record the germination. Spore balls soaked in sterile distilled water served as control. Treated pearl millet seeds were sown in sterilized soil to observe effect of these chemicals on seed germination.

Seeds @ 400 for each treatment of healthy pearl millet hybrid, HHVBC Tall-B1 were surface sterilized with 2% Clorax for 2 min. Fifteen smut sori from infected panicles were ruptured on 400 healthy seeds in a Petri plate and mixed thoroughly to get adherence of spore balls on seed surface. Then the seeds in batches were put into a test tube @ 50 per tube along with equal volume of sterilized sand. Five ml of ethanol was added to the test tube and stirred for 1, 2 and 3 min. in a vortex mixture. Seeds were thoroughly washed with sterile distilled water and plated on potato dextrose agar medium amended with carbendazim and thiram mixture (1:1) @ 1g/l and plates were incubated for five days at 30^0C. Observations on colony growth of *M. penicillariae* were recorded using stereo-binocular microscope.

Data presented in Table 1 revealed that seed treatment with NaOH resulted in minimizing spore germination to 11.3, 5.7 and 5.2% at 5, 10 and 15%, respectively, as compared to 15.2% in control. Although KOH at 5% increased spore germination as compared to control, at higher concentrations of 10 and 15%, the spore germination was reduced to 9.7 and 4.4%. Spore germination was more at all concentrations of ethanol (22.5-31.4%). It was observed that pearl millet seed germination was not hampered significantly due to chemical treatment except in 15% NaOH (90%) as compared to control (95%).

In spore elimination study using ethyl alcohol plus sand, no smut spores were found adhering to the seed surface in seed samples stirred for 2 and 3 min., whereas smut colonies appeared on media in seed samples stirred for one min. (10%) and control (90%). The colonies were light yellowish in colour and slimy containing budding sporidia.

Literature review indicated that no fungicide could completely inhibit the viability of smut spores of pearl millet (Yadav and Duhan, 1999). Thiram and captan could reduce spore ball viability from 19.7 to 10.7% and 27.0 to 14.1%, respectively, even after storage for eight months (Yadav and Duhan, 1999). Thakur *et al.* (1992) have reported that carbendazim or vitavax treatment @ 2.5 g/kg of seeds was effective in controlling the inoculum. In this study also complete inhibition of viability of spore balls was not achieved with different chemical treatments. However, no smut spores were found adhering to the seed surface when stirred using ethyl alcohol and sand.

Table 1. Effect of different chemicals on smut spore ball germination

Treatment	Spore ball germination (%)	Seed germination (%)
KOH 5%	17.8	95.0
KOH 10%	9.7	98.3
KOH 15%	4.4	93.3
NaOH 5%	11.3	98.3
NaOH 10%	5.7	95.0
NaOH 15%	5.2	90.0
Ethanol 5%	22.5	98.3
Ethanol 10%	29.7	98.3
Ethanol 15%	31.4	95.0
Control	15.2	95.0
Mean	15.3	95.6
LSD (5 %)	9.13	6.66
F (P)	< 0.001	NS

Thus, seed samples mixed with sand and ethanol and stirred for 2 min. can be recommended for complete elimination of surface contamination of smut spores from pearl millet seed samples. Instead of water, ethanol was used as stirring medium to avert problem of drying of seeds after treatment. Ethanol treatment facilitated quick drying of seed without using seed drier. Similar results were observed by Agarwal *et al.* (1990) for elimination of rust spores from safflower seeds. This simple method can effectively be used especially for germplasm exchange and seed certification programmes.

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SHORT COMMUNICATION

Screening for Resistance against Northern Corn Leaf Blight (*Exserohilum turcicum* (Pass.) K.J. Leonard & Suggs) in Temperate Maize Lines

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Ten early duration maize lines, namely, CM 128, V 327, V 335, V 13, V 128, V 17, V 336, CM 129, V 53 and V 27, were screened against northern corn leaf blight (*Exserohilum turcicum* (Pass.) K.J. Leonard & Suggs) under field as well as under artificial epiphytotic conditions. Five inbreds, *viz*., V 335, V 13, V 336, V 53 and V 27 were identified as resistant while rest five *viz.*, CM 128, V 128, V 327, V 17 and CM 129 were found susceptible. In the pooled disease scores across the three seasons, the highest mean score of 4.0 was recorded for V 128, indicating high level of susceptibility, whereas, lowest mean score of 1.5 recorded for V 335, showed high level of resistance across the locations. The promising inbreds can be utilized as a resistant donor in breeding programme. These inbreds possess resistance to northern corn leaf blight, can be used successfully in developing high yielding early maturing varieties for the hill region.

Key Words: ***Exserohilum turcicum*, Maize, Resistance, Screening, Turcicum leaf blight**

The production of the maize in hilly areas (Kumaoun hills) of India is low as compared to the other area of the nation. The major cause of low yield is Northern corn leaf blight which causes a wide range (28-91%) of yield losses in maize (Sharma and Aujla, 1968; Pant *et al.,* 2000; Singh *et al.,* 2003).

Success of any crop improvement programme depends on the nature and magnitude of genetic variability present in the crop. The effectiveness of selection largely depends on the amount of heritability for traits under study. Therefore, present study was undertaken to reduce the loss due to Turcicum leaf blight (*Helminthosporium turcicum* Pass. or *Exserohilum turcicum* (Pass.) K.J. Leonard & Suggs) through selecting early maturing resistant maize material. An attempt has been made to evaluate the variability in present material of maize.

Turcicum leaf blight is the most common as well as chronic problem of maize in the hills. Passerini, from Italy was first to describe the Turcicum leaf blight in 1876. In India, Butler *et al.* (1920), first observed this disease in maize. It is the most prevalent form in all the major maize growing regions of India during rainy (*kharif*) as well as winter (*rabi*) season (Lal, 1991). The disease can substantially reduce the grain yield of maize over a wide range from 28% to 91% (Ullstrup, 1951; Chenulu and Hora, 1962; Sharma and Aujla, 1968; Pant *et al.,* 2000). Severe disease conditions prevailing at the time of ear formation may cause total grain loss.

This disease has also been observed to be the most important in the hills, mainly in Jammu and Kashmir, Himachal Pradesh, Sikkim, West Bengal, Meghalaya, Tripura, Assam and Uttarakhand. The symptoms first develop on lower leaves and successively progress to upper leaves of the plant under favourable conditions. In severe disease condition, the entire foliage generally gets killed, giving the appearance of frosted leaves. The ears and kernels are seldom infected, but some lesions do develop in the outer husk. The typical symptoms seen on a susceptible host are long elliptical (4 to 20 cm long and 1 to 5 cm wide), grayish-green to brown (tan) in color. Spores are produced in the lesions, which are olive-green to black, and usually produce concentric rings, giving the entire spot a target like appearance. Spores from the primary lesions re-infect the host producing secondary cycle of the disease (Partridge, 1997). The presence and absence of this type of lesions are governed by host-pathogen interaction (Carlos De Leon, 1987).

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Ten maize inbred lines with different genetic background and origin were evaluated against Turcicum leaf blight under artificial epiphytotic conditions during *kharif* 1998 and 1999 at Hawalbagh, Almora and *rabi* 1998-99 at Amberpet, Hyderabad. Heavily infected leaves with Turcicum leaf blight collected during last season and were ground to powder and stored at room temperature. The powdered inoculum was inoculated as per procedure. A pinch of the leaf powder was dropped into the whorl of each plant, commencing from 20 days after planting. The inoculation was preferably done late in the afternoon till the pre-tasseling stage four times at weekly intervals. Spray of spore suspension with spore concentration of $2.5x10^4$/ml was done on plants, commencing from 20 days after planting till pre-tasseling stage at 5-7 days interval. The disease severity was recorded at flowering stage and dry silk stage in terms of intensity following the rating scale of 1.0 to 5.0 with 0.5 intermediate rating (Payak and Sharma, 1982). Intermediate ratings between two numbers (1.25, 1.5, 1.75, 2.25, 2.75, 3.25, 3.5, 3.75, 4.25, 4.5 and 4.75) was also recorded. Row-to-row spacing of 60 cm and plant-to-plant of 25 cm was maintained at both the locations. Fertilizer doses @ of 160 Kg N, 60 Kg P_2O_5 and 40 Kg K_2O were applied to the crop at both the locations.

Mean score for Turcicum leaf blight recorded during *kharif* 1998 and 1999 are presented in Table 1. Mean score of turcicum leaf blight during *kharif* 1998 ranged from 1.8 (V 53) to 4.0 (V 17) while, during *kharif* 1999 it varied from 1.9 (V 13) to 4.5 (V 17). The five inbreds, *viz*., CM 128, V 327, V 128, CM 129 and V 17 exhibited above 3.0 score and were identified as susceptible while rest of five inbreds, V 335, V 13, V 336, V 53 and V 27 with disease of score of less than 2.5, were found to posses resistance. V 17 recorded the maximum disease score of 4.5 during *kharif* 1999, was highly susceptible, whereas the minimum disease score of 1.8 was recorded for V 53 during *kharif* 1998, which indicated high level of resistance for Turcicum leaf blight.

The disease mean scores for Turcicum leaf blight recorded during *rabi* 1998-1999 are presented in Table 2. The highest mean score (3.9) was recorded for V 17, while the lowest (1.5) for V 335. Out of ten inbreds tested during the season, five, *viz.*, V 335, V13, V 336, V 53 and V 27 indicated resistance for Turcicum leaf blight, while remaining five (CM 128, V 327, V 128, V 17 and CM 129) showed susceptibility.

The disease reaction for Turcicum leaf blight was pooled across three seasons in two environments and is presented in the Table 3. The highest mean score

Table 1. Mean disease reaction of maize inbreds Turcicum leaf blight, *kharif* 1998 and 1999 at Hawalbagh, Almora

Inbred lines	1998	1999	Mean	Type of reaction
CM 128	3.5	3.8	3.7	S
V 327	3.8	4.1	3.9	S
V 335	2.0	2.0	2.0	R
V 13	2.3	1.9	2.1	R
V 128	3.8	4.1	3.9	S
V 17	4.0	4.5	4.3	S
V 336	2.0	2.3	2.1	R
CM 129	3.0	3.5	3.3	S
V 53	1.8	2.0	1.9	R
V 27	2.3	2.5	2.4	R

S-susceptible (2.6 to 5.0), R-resistant (1.0 to 2.5)

Table 2. Mean disease reaction of maize inbreds for Turcicum leaf blight, *rabi* 1998-99 at Amberpet, Hyderabad

Inbred lines	1998-99	Type of reaction
CM 128	3.0	S
V 327	3.3	S
V 335	1.5	R
V 13	1.7	R
V 128	4.0	S
V 17	3.9	S
V 336	2.0	R
CM 129	3.5	S
V 53	2.2	R
V 27	2.0	R

S-susceptible (2.6 to 5.0), R-resistant (1.0 to 2.5)

Table 3. Pooled mean disease reaction of maize inbreds Turcicum **leaf blight (Hawalbagh and Hyderabad)**

Inbred lines	1998 *(Kharif)*	1998-99 *(Rabi)*	1999 *(Kharif)*	Pooled	Type of reaction
CM 128	3.5	3.0	3.8	3.4	S
V 327	3.8	3.3	4.1	3.7	S
V 335	2.0	1.5	2.0	1.8	R
V 13	2.3	1.7	2.0	2.0	R
V 128	3.8	4.0	4.1	4.0	S
V 17	4.0	3.9	4.5	4.1	S
V 336	2.0	2.0	2.3	2.1	R
CM 129	3.0	3.5	3.5	3.3	S
V 53	1.8	2.1	2.0	2.0	R
V 27	2.3	2.0	2.5	2.3	R

S-susceptible (2.6 to 5.0), R-resistant (1.0 to 2.5)

of 4.0 was recorded for V 128, indicating highest level of susceptibility and lowest mean score (1.8) was recorded for V 335 indicating highest level of resistance across the environment. The other susceptible inbred lines on the basis of pooled score were, CM 128 (4.0), V 327 (3.7), V 128 (4.0), V 17 (4.1) and CM 129 (3.3), while inbreds having score of 1.8 (V 335), 2.0 (V 13), 2.1 (V 336), 2.0 (V 53) and 2.3 (V 27) were resistant to Turcicum leaf blight.

In a similar study, Singh *et al.* (2003), identified 17, 27, 111 and 10 inbred lines developed at Almora as resistant, moderately resistant, susceptible and highly susceptible to Maydis leaf blight caused by *Bipolaris maydis* (*Cochliobolus heterostrophus*). Their study, which involved about 65 genotypes, also indicated susceptibility of CM 128, V 327, V 128, V 17 and V 27, while V 335, V 13, V 336 and V 53 showed resistance reaction.

Elliott and Jenkins (1946) screened 200 inbred lines, 126 crosses and 184 double crosses against the Turcicum leaf blight and found that NC34 was the most resistant while CI123, K715, KY114, MO21A, T49B, T105, BK115, CI15 and TX116 showed traces of infection and the resistance to Turcicum leaf blight was transmitted to hybrid progeny. In a similar study, Gowda and Kaisar (1990) tested 37 maize lines for resistance to *Exserohilum turcicum*. Of these 10 were resistant and 12 were moderately resistant. Rathee *et al.* (1999) reported eight genotypes having high level of disease resistance.

The inbred lines identified to possess resistance to Turcicum leaf blight in the present study, can be used successfully in developing high yielding early maturing varieties for the hill region having high level of resistance to Turcicum leaf blight.

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SHORT COMMUNICATION

Factor Analysis of Components of Yield and some Growth Parameters in Urdbean (*Vigna mungo* (L.) Hepper)

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Factor analysis was carried out on 64 genotypes of urdbean to assess the extent of variation for different characters. Data collected on seventeen quantitative traits were subjected to Principal Factor analysis. There were only seven factors which accounted for maximum percentage to the total variance. In factor one about 50 percent variation was associated with grain yield, harvest index, pods/plant, pod bearing branches/plant, plant height, leaf area and leaf growth rate. Factor 2 accounted almost 20% variation and traits included were harvest index and net assimilation rate. Factor 3, 4, 5 and 7 contained variation for some physiological traits including few yield component characters.

Key Words: Factor analysis, Growth traits, Multivariate analysis, Urdbean, Yield traits

Urdbean (*Vigna mungo* (L.) Hepper) is an important self-pollinating diploid (2n=2x=22) crop. It forms a significant component of the dietary requirement of people in India. The factor component analysis indicate the extent of genetic diversity of germplasm accessions based on the evaluated characters. Further, univariate statistical analysis are not adequate as they ignore the correlation among the variables and sometimes the conclusion may be misleading, while the multivariate analysis technique taking into account the interdependence and relative importance of various characters involved yield more meaningful genetic information. The aim of multivariate analysis is to reduce the number of variables by employing suitable linear transformation and to choose a few linear combinations in some optimum manner. However, minor genes, whose effects could be identified by plant morphology, have been exploited for the genetic improvement of urdbean, but this has not led to major gains in the yield potential. The analysis of constraints to increasing genetic potential of these crops reveals the existence of wide difference between the actual biological potential and the highest levels achieved through the present available base. However, the selection of diverse parents and desirable traits belonging to distant sources also led to a wide spectrum of gene and gene-combinations for morpho-physiological traits. In determining the potential of genetically different lines and cultivars, breeders have to observe many different characters that influence yield. Accurate evaluation of these characters is made difficult by the genotype-environment interaction. This study was undertaken in order to determine the dependence relationship between yield components and some physiological traits of several genotypes of urdbean using factor analysis.

The genetic materials for the present investigation consist of 64 genotypes (promising accessions and some advance breeding lines) included in the study. These genotypes were evaluated for their performance at the Regional Research Station, Himachal Pradesh Agricultural University, Bajaura (31^0 48' N, $77^0$00 E, 1099 msl). The experiment was conducted in Randomized Block Design with three replications under rainfed conditions. Data were recorded on ten randomly selected plants/replication on X_1= seed yield/plant (g), X_2= harvest index (%), X_3= 100-seed weight (g), X_4= seeds/pod, X_5= pods/plant, X_6= biological yield/plant (g), X_7= plant height (cm), X_8 = pod bearing branches/plant, X_9 = days to 50% flowering, X_{10}= days to maturity, X_{11}= leaf area/plant, X_{12}= crop growth rate (g day-^{1}), X_{13}= leaf growth rate (cm^2 day-^{1}), X_{14}= net assimilation rate (mg dm-^{2}), X_{15}= specific leaf weight (mg day-^{1}), X_{16}= leaf area ratio (dm-^{2} g-^{1}) and X_{17}= leaf weight ratio. Estimates of factor loadings were based on data from all replications for all populations. Factor analysis calculations were performed using SPSS factor analysis programme. Simple correlation coefficients were calculated for 17 characteristics of 64 genotypes. Thus factor analysis is a multivariate analysis method which aims to explain the correlation between a large set of variables in terms of a small number of underlying

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independent factors. It is assumed that each of the variables measured, depends upon the underlying factors but is also subject to random errors. The principal factor analysis method explained by Harman (1976) was followed in the extraction of the factor loadings. The array of communality, the amount of the variance of a variable accounted by the common factors together, was estimated by the highest correlation coefficient in each array as suggested by Seiller and Stafford (1985). The number of factors was estimated using the maximum likelihood method of Rao (1952). The varimax rotation method (an orthogonal rotation) was used in order to make each factor uniquely defined as a distinct cluster of inter correlated variables (Rao, 1952). The factor loadings of rotated matrix, the percentage variability explained by each factor and the communalities for each variable were determined. The results obtained on principal factor matrix of the present investigation have been presented below.

The total variance explained by factors is indicated in Table 1. Only the first seven factors which account for almost 75% of the total variance are important. A principal factor matrix after orthogonal rotation for these seven factors given in Table 2. The values in the table, or loadings, indicate the contribution of each variable to the factors. For the purpose of interpretation only those factor loadings greater than 0.5 were considered important, these values are highlighted in the bold in Table 2. Factor 1, which accounted for about 50% of the variation, was strongly associated with seed yield, harvest index, pods/plant, biological yield/plant, plant height, pod bearing branches/plant, leaf area/plant and leaf growth rate. This factor was regarded as productivity/plant factor, since it included several traits which are component of yield including some desirable growth traits. All majority of variables had positive loadings in factor 1. The sign of the loading indicates the direction of the relationship between the factor and the variable. Factor 2, which account for about 20% of the variation was named a reproductive (fertility) factor since it contained the harvest index, days to flowering and net assimilation rate which are associated with fertility. Again all these variables had positive loadings. The third factor was named as efficiency factor since it consisted leaf area/plant and leaf growth rate. This of course indicates the efficiency of the plant to convert the available resource into economic produce. Factors 3, 4, 5 and 7 also accounts for variation, since it consisted some growth parameters and couple of yield traits. Likewise, factor 6 does not contain any positive loading to be considered in the selection. Seiller and Stafford (1985) indicated that seeds/pod and 100-seed weight regarded as yield/unit area factor in the guar crop. However, in this study 100-seed weight was not included in any of the factors since it had very low loadings (less than 0.50). Seeds/pod were regarded as an economic factor in cluster bean by Rao and Paroda (1982). In this study, however factors grouped with the desirable phenological, yield components and physiological traits (factor 1 and 2). This study indicating that selection of variables in the productivity/plant and reproductive factor could enable breeders to select better genotypes/traits for desired increment in seed yield of urdbean. It is also expected that knowledge of the groups and sub-groups identified may be used to breed new genotypes for specific purposes. Assembling of the accessions provides preliminary answer to the identification of parental lines and specific characters for future urdbean breeding programmes. Similar observations were also reported by Walton (1991), Woldeamlak and Alelign (2007) and Ramesh *et al.* (2011).

Table 1. Total variance explained for each factor based on 17 different characters in 64 urdbean genotypes

Factor	% of variance	Cumulative %
1	21.89	21.89
2	13.50	34.88
3	11.67	46.55
4	8.09	54.65
5	7.10	61.75
6	6.62	68.35
7	5.90	74.88
8	5.21	79.48
9	4.89	84.32
10	4.40	88.70
11	3.56	92.45
12	3.70	95.85
13	1.90	97.76
14	1.47	99.20
15	0.60	99.80
16	0.30	99.92
17	0.23	100.00

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Table 2. Principal factor matrix after varimax rotation for 17 characters of 64 genotypes of urdbean. Number in bold are those with factor loadings greater than 0.50

Variables	Factor						
	1	2	3	4	5	6	7
Seed yield/ plant	**0.868**	-0.231	-0.383	-0.100	0.030	0.132	0.045
Harvest index	**0.599**	**0.747**	0.242	0.122	0.032	0.348	0.263
100- seed weight	0.416	0.164	-0.180	-0.125	-0.342	-0.031	-0.021
Seeds/pod	-0.128	0.263	-0.425	-0.051	**0.547**	0.421	0.193
Pods/ plant	**0.785**	-0.463	-0.090	-0.017	-0.129	-0.035	0.145
Biological yield /plant	**0.732**	0.392	-0.422	-0.098	0.036	-0.087	0.032
Plant height	**0.534**	0.374	-0.036	-0.311	0.054	-0.026	0.208
Pod bearing branches/plant	**0.706**	-0.471	-0.051	-0.333	0.035	-0.012	-0.042
Daya to 50% flowering	0.214	**0.622**	0.122	0.480	0.008	0.244	-0.190
Days to maturity	0.106	0.236	-0.399	0.031	0.067	-0.103	0.329
Leaf area/plant	**0.547**	0.138	**0.746**	0.032	0.287	-0.098	-0.034
Crop growth rate	0.277	-0.102	0.153	0.080	-0.329	-0.076	-0.123
Leaf growth rate	**0.551**	0.160	**0.754**	-0.421	0.259	-0.023	0.024
Net assimilation rate	-0.055	**0.501**	0.233	0.065	-0.296	0.223	**0.611**
Specific leaf weight	-0.121	0.272	0.149	0.080	-0.225	-0.023	0.106
Leaf area ratio	-0.056	-0.106	-0.089	**0.677**	**0.603**	0.252	0.231
Leaf weight ratio	0.116	0.158	-0.061	0.432	-0.031	0.342	-0.025

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SHORT COMMUNICATION

Study on Yield Stability of Wheat Genotypes under Rainfed Environments in Intermediate Zone of J&K

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Sixteen wheat genotypes were evaluated to study the stability of yield and its components by growing them during rabi season for three years from 2006 to 2009 at Regional Agricultural Research Station, Rajouri, J&K. Significant mean square due to genotypes revealed the presence of wide variability among the genotypes for all the traits studied. The component of genotypes x environments interaction was observed significant for all the characters except spike length, effective tillers/plant and biological yield. Based on stability parameters, genotypes UP 2964, HPW 230 and VL 895 exhibited higher mean grain yield and least deviation from zero indicating wide stability for yield. These varieties could be recommended for commercial cultivation for this region.

Key Words: G x E interaction, rainfed, stability, wheat

Wheat production is about 80.6 million tons during 2009-10 in India and counts for approximately 12% of world production. India being the second largest in population is also the second largest in wheat consumption after China, with a huge and growing wheat demand. Depending on the population and income growth, poverty alleviation and the rate of urbanization, a demand-supply gap may open at a rate of about 1 to 2% per year which is equivalent to 0.7 to 1.4 mt of wheat, requiring additional production of such quantity growing over the years. Plant breeders while developing improved cultivars, are often confronted with problem of interpreting genotypes x environment interactions evidenced by differences in relative rankings of crop cultivars when compared over a series of environments. G x E interaction for quantitative traits tends to reduce the usefulness of genotypic means, the correlation between genotype and phenotype and progress from selection. In wheat, most of the present day popular varieties are introduction and they usually show inconsistent yield and lack of stability under fluctuating environments over the years at a given location. Therefore, the present study was undertaken to identify the most stable genotypes of wheat for this rainfed area to increase the wheat production per unit area as well as total production.

The present investigation was carried out at the Regional Agricultural Research Station, (SKUAST-J), Rajouri. The experimental material for the study consisted of 16 diverse genotypes. All the genotypes were evaluated in Randomized Block Design with three replications during *rabi* 2006 to 2009 under rain fed environment. Each plot consisted of six rows of 5 m length, spaced 25 cm apart and maintaining an inter-plant distance of 10 cm within rows. All the recommended agronomic practices were followed to raise the good crop. The crop experienced moisture stress during early development in November- December. However, the crop recovered well following sufficient rains from the month of January onwards. Observations were recorded on ten characters. Observations on plant height, spike length, number of grains per spike and1000-grain weight were recorded by randomly selecting five plants in each plot and in each replication, whereas observations on days to 50% flowering, days to maturity, biological yield, harvest index, effective tillers per plant and grain yield were recorded on plot basis. Stability analysis was done by using Eberhart and Russel model (1966).

The pooled analysis of variance observed in Eberhart and Russel, model is presented in Table 1. The mean squares due to genotypes were highly significant for all the traits indicating wide variability among the genotypes. Similar results were also reported by Kheiralla *et al.,* 1993; Ahmad *et al.*, 2003 and Crossa and Joshi, 1998. Highly significant mean squares due to G x E interaction were observed for all the traits except spike length, effective tillers/plant and biological yield. This suggests that these traits were least influenced by the environments (Mohammad *et al.*, 2009). The liner components of G x E interaction was highly significant for days to 50% flowering, days to maturity, plant height, 1000-grain weight, number of grains/spike, biological yield and harvest index. Similar findings were

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Table 1. Analysis of variance for different characters for stability parameters in wheat

Sources of variation	d.f.	Days to 50 % flowering	Days to maturity	Plant height (cm)	Spike length	Effective tillers/plant	1000-grian weight (g)	No. of seeds/ spike	Biological yield (kg)	Harvest index (%)	Grain yield (kg)
Varieties	15	85.896 **	314.986 **	95.994 **	1.351 **	30.785 **	0.239 **	187.48 **	0.622 **	77.591 **	0.49 **
Environments	2	2735.262 **	2697.563 **	19.852 *	6.103 **	8.086	0.78 **	166.10 **	0.004	1.9 **	0.037
Genotype X Env.	30	19.960 **	73.980 **	8.636 *	0.192	4.172	0.042*	22.53 **	0.008	6.4 **	0.041*
Env.+ (Var. x Env.)	32	189.667 **	237.954 **	9.337 *	0.562 **	4.417	0.057 *	31.50 **	0.007	6.131**	0.04
Environments (Lin.)	1	5470.523 **	5395.127 **	39.704 **	12.207 **	16.172 *	0.556 **	332.20 **	0.007	3.99**	0.074
Var.* Env.(Lin.)	15	35.086 **	131.073 **	13.628 **	0.237	4.935	0.062 *	39.71 **	0.011 *	12.59**	0.012
Pooled Deviation	16	4.532 **	15.831 **	3.416	0.138	3.196	0.021	5.021	0.004	0.206	0.065 **
Pooled Error	90	1.082	1.405	2.023	0.216	1.838	0.052	8.31	0.096	9.197	0.018

*,**Significant at 5% and 1% levels , respectively

reported by Jagadish *et al.* **(**1997). However, pooled deviation (non-linear) responses of cultivar as measured by deviation from liner regression were highly significant for days to 50**%** flowering, days to maturity and grain yield per plot, indicating lack of simple or liner relationship of genotype for different years for these traits.

A genotype is considered to be stable in performance if it has high mean performance (xi) unit regression coefficient (bi=1) and least deviation from regression (s^2di= 0) according to Eberhart and Russel (1966). The estimates of stability parameters *viz.* mean (Xi), liner sensitively coefficient (bi) and non-linear sensibility coefficient (S^2di) are given in Table 2. A linear response i.e. b>i was estimated. However, in certain genotypes the bi values were found negative for some traits. Stability parameters (Table 2) showed that all the genotypes revealed average performance across the environments and nine genotypes showed non-significant mean square deviations (s^2d) from zero and could be considered as the stable genotypes across the different environment for seed yield. Three genotypes (UP 2964, HPW 230 and VL 895) had the high mean values and non significant deviation suggesting good stability of the genotypes. Variety HS 365 has the regression coefficient value near to unity but it has low mean value and significant deviation. Similarly another variety VL 898 has high mean performance but significant deviation hence both the varieties cannot be considered stable. For 1000-grain weight varieties VL 872, HS 240, HPW 255 and VL 900 have high mean values and non-significant deviation indicating good stability. Varieties HPW 249, HPW 230, Sonalika and VL 898 for spike length and varieties VL 900 and UP 2962 for tillers per plant recorded high mean values and non significant deviation from zero indicating good stability. Three varieties VL 870, VL 872 and VL 804 also had high mean values but showed significant deviation for 1000-grain weight, therefore, cannot be considered as stable varieties.

Genotype x environment interaction has important effect on the yield components. Therefore, thorough understanding of the variations among cultivars in their response to environment would further improve the probability of predicting and identifying cultivars with superior yield attributes. Also, suitable selection strategies to accommodate the significant G × E interactions need to be developed.

Wheat is the major crop of Jammu and Kashmir during winter, therefore, high yielding genotypes with stability

Table 2. Estimates of stability parameters of some wheat varieties

Genotypes	Days 50% flowering			Days to maturity			Plant height (cm)			Spike length (cm)			Effective tillers/plant		
	μMean	βi	σ²di	μMean	βi	σ²di	μMean	βi	σ²di	μMean	βi	σ²di	μMean	βi	σ²di
HPW-249	130.8	1.03	-1.04	181.1	0.98	-0.94	98.4	1.79	-1.93	10.2	2.07	-0.16	19.6	-3.85	-0.93
HPW-230	138.3	0.92	13.52**	174.2	1.56	23.37**	100.8	1.82	-1.87	9.6	-0.05	0.08	21.0	1.54	0.26
VL-895	130.2	1.34	0.67	174.7	1.21	6.34	105.7	-1.19	2.64	7.9	1.32	-0.21**	16.6	2.29	-1.46
Sonalika	142.2	0.96	4.03	183.3	0.99	5.58	96.6	3.11	3.13	9.9	0.64	-0.13	17.2	1.16	-1.95
VL-870	144.3	0.71	-0.87	193.4	-0.13	38.91	100.2	1.86	-1.86	9.0	1.01	-0.16	22.6	3.1	8.79**
HPW-267	146.6	0.43	-0.64	205.9	-0.07	11.25*	92.9	-0.3	-0.36	9.6	0.05	-0.11	18.7	1.88	5.32**
VL-898	135.9	1.33	2.61	178.0	0.93	-1.19	98.3	0.62	2.68	9.8	0.94	0.03	15.8	2.11	-2.01
UP-2964	144.2	0.74	-1.16	172.1	1.25	8.33	97.4	-0.06	-0.52	8.3	1.3	-0.21**	20.6	1.89	-2
VL-872	135.2	1.55	-0.73	164.2	1.93	95.41**	84.6	-2.45	2.53	8.7	1.71	-0.12	22.1	1.85	3.93*
HPW-255	137.6	1.15	-0.35	177.3	1.53	20.49**	100.5	-1.54	-0.72	9.1	0.85	0.15	20.1	1.65	-2.01
HS-240	135.2	1.18	-1.09	174.7	1.57	6.79	93.7	-3.25	6.46**	9.4	0.67	-0.20**	16.0	3.4	0.3
VL-738	143.0	1.02	0.53	183.1	0.67	9.58	97.2	2.09	-1.83	9.4	0.6	-0.19*	13.6	-1.32	0.76
VL-804	135.7	1.39	2.05	170.0	1.46	-1.36	99.3	3.74	4.69*	8.6	1.15	0.12	23.4	-3.1	8.79**
HS-365	147.0	0.67	2.74	193.2	-0.07	11.25*	83.7	3.27	-1.66	8.2	1.59	-0.02	17.0	-1.61	3.38*
HS-295	142.0	1.03	24.57**	176.2	1.06	-1.34	98.4	1.29	-0.27	9.4	1.17	-0.08	20.6	2.7	-0.39
VL-900	144.0	0.56	9.19**	180.8	1.14	-1.38	95.3	5.22	12.66**	8.8	0.97	-0.21**	25.4	2.31	-1.79

*5% Significant, **1% Significant

Table 2. Estimates of stability parameters of some wheat varieties

Genotypes	1000-grain weight (g)			No. of grains/spike			Biological yield (kg)			Harvest index (%)			Grain yield/plot		
	μMean	βi	σ²di	μMean	βi	σ²di	μMean	βi	σ²di	μMean	βi	σ²di	μMean	βi	σ²di
HPW-249	45.8	1.19	-0.02	68.3	1.54	-7.99*	5.8	-13.89	-0.07	32.1	8.38	-9.88*	2.246	-0.49	-3.05**
HPW-230	45.4	0.58	0.01	35.5	1.65	-7.22*	6.1	10.67	-0.04	37.8	-2.38	-9.99*	2.418	-0.71	0.14
VL-895	46.1	-0.93	-0.03*	42.5	2.43	-4.55	5.9	0.88	-0.09*	32.0	6.03	-9.91*	2.327	1.69	-0.01
Sonalica	45.2	1.31	0.03*	47.9	2.81	0.4	5.7	3.02	-0.09*	26.2	-1.69	-10**	1.663	4.24	5.38**
VL-870	49.1	0.71	-0.04**	37.9	0.98	-7.99*	4.7	1.26	-0.09*	42.6	-2.64	-10**	2.303	-2.75	0.08
HPW-267	50.0	0.59	-0.05**	57.5	3.18	18.57**	4.6	0.63	-0.08*	35.6	4.63	-9.9*	1.651	0.17	-3.01**
VL-898	50.6	-1.51	-0.03*	41.8	1.52	-2.98	5.5	3.9	-0.06*	39.3	1.23	-9.96*	2.373	0.35	0.03*
UP-2964	50.9	0.26	-0.04**	41.8	-1.66	-6.89*	5.6	-1.13	-0.09*	44.2	2.04	-9.85*	2.476	2.07	-0.02
VL-872	53.6	1.56	-0.04**	47.9	1.2	-6.75*	5.0	0.38	-0.09*	34.0	-5.79	-9.27*	1.504	2.8	0.02
HPW-255	45.5	2.48	-0.01	49.0	1.48	-8.01*	5.7	1.38	-0.05	30.5	-1.98	-9.39*	1.551	1.67	4.82**
HS-240	45.4	0.3	-0.01	44.5	1.31	-5.85*	5.4	-3.52	-0.09*	33.1	-0.99	-9.99**	1.727	0.85	0.05
VL-738	51.7	1.57	-0.05**	48.3	-1.54	3.21	5.1	3.14	-0.09*	29.6	-0.26	-10**	1.482	1.72	-4.02**
VL-804	50.5	0.83	-0.03*	50.9	0.71	-7.84*	5.9	2.39	-0.08*	30.9	20.98	-8.94*	1.851	1.64	0.01
HS-365	52.7	0.26	-0.05**	47.8	-0.39	9.49**	5.1	1.63	-0.09*	28.3	3.81	-10**	1.317	0.97	-0.41*
HS-295	51.7	3.63	-0.05**	41.1	-0.3	-5.45*	5.1	1.63	-0.09*	38.1	-10.17	-9.7*	1.536	2.04	0.06
VL-900	52.5	3.17	-0.04**	51.6	1.07	-8.01**	5.0	3.64	-0.09*	37.2	-5.19	-9.94*	2.194	-0.28	0.03

*5% Significant ,**1% Significant

under fluctuating agro-climatic environments would be a great importance for the farmers. The temperature and moisture availability play a major role in the expression of a genotypes in sloppy and undulating field conditions under hilly region of the state. Further, the genotypes with low water requirements and tolerant to low temperature will also enhance the yield potential of wheat crop. The genotypes identified as stable in the present investigation can be utilised in future crossing programme of wheat breeding for the development of desirable genotypes. These genotypes can also be utilised as donor parents to get desirable transgressive -segregants in later generations. The yield stability character is not a single gene controlled it depends on genotypic expression and various environmental factors, therefore, to utilize the potential of these genotypes in wheat breeding programme a suitable breeding method should be followed.

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SHORT COMMUNICATION

Combining Ability Analysis for Yield and Other Quantitative Traits in Soybean (*Glycine max* L. Merril)

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Genetic analysis was carried out using 5x5 diallel set of soybean crosses along with the parents for estimation of combining ability. The analysis revealed that the parents and crosses differ significantly for general combining ability and specific combining ability effects. The *gca* and *sca* components suggested predominance of additive gene effects for days to 50% flowering, days to maturity and plant height and non-additive gene effects for all the traits. Parents JS 335 and RAUS 5 were the good combiners for grain yield/plant whereas parent Bragg was best combiner for days to 50% flowering and days to maturity. The crosses JS 93-05 x PS 562, RAUS 5 x Bragg and PS 562 x RAUS 5 were found to be the best specific combiners for grain yield/plant while cross PS 562 x RAUS 5 for days to 50% flowering, days to maturity and plant height.

Key Words: Combining ability, Quantitative traits, Soybean, Yield components

Soybean [*Glycine max* (L.) Merril] is world's most important oil seed crop and is recognized as miracle bean due to its high nutritive value and numerous uses. It is rich in oil (18-22 %) and protein (38-42%). In India area under soybean cultivation is about 8.87 mha and production is about 9.46 mt whereas, its contribution to total production of oil seeds and total oil availability from 9 major oilseeds would be about 37% and 25%, respectively (Anonymous, 2008).

The productivity of soybean is very low in India (1.0 ton/ha) as compared to world's average yield of 2.24 tones/ha. So the present strategies and investigations for development of early maturing and high yielding soybean genotypes need further strengthening that requires adequate genetic information about this crop. Genetic information about the combining ability of parents and nature of gene effects helps in proper understanding of inheritance of characters, selection of suitable parents for hybridization and in isolating the promising early generation hybrids for further exploitation in breeding programmes. Such information is quite limited to soybean. In the present investigation efforts have been made to generate information on these aspects for different quantitative characters in soybean to help breeders in designing suitable breeding strategy to enhance the production potential of this crop.

Five genetically diverse genotypes originating from various agro-climatic zones of the country, namely JS 93-05, PS 562, JS 335, RAUS 5 and Bragg were included in investigation. These lines were crossed in diallel fashion in all possible combinations excluding reciprocals. Thus, the experimental material consisted of 15 entries (5 parents and 10 F_1's) and were grown during *kharif* 2007-08 in Randomized Block Design with three replications at Agricultural Research Farm, Andro, under Central Agricultural University, Imphal. The row-to-row and plant-to-plant distance were 45 and 10 cm, respectively. Observations were recorded on five randomly selected plants from each entry in each replication in respect of nine important characters namely days to 50% flowering, days to maturity, branches/plant, plant height (cm), number of pods/ plant, number of nodules/plant, grain yield/plant (g), 100-seed weight (g) and oil content (%). The combining ability analysis was carried out following method 2, model I (Griffing, 1956).

The knowledge on the mechanisms that control the main quantitative traits of agronomic interest of soybean is fundamental for genetic improvement and can be acquired through methodologies of diallel crosses. The values associated to the general combining ability (*gca*) of each parent and to the specific combining ability (*sca*) of each cross are estimated. The significant mean squares due to *gca* and highly significant mean squares due to *sca* for all the traits except number of branches/plant (Table 1)

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suggested that both additive and non-additive gene effects were important for the characters under investigation. Estimates of variances due to *gca* and *sca* suggested preponderance of additive gene effects for days to 50% flowering, days to maturity and plant height whereas non-additive gene effects for all the traits *viz*., days to 50% flowering, days to maturity, plant height (cm), number of pods/plant, number of nodules/plant, grain yield/plant, 100-seed weight and oil content (%).

Estimates of *gca* effects (Table 2) revealed that parent, Bragg was best combiner for days to 50% flowering and days to maturity. For grain yield/plant JS 335 and RAUS 5 exhibited positive and significant *gca* effects whereas, parent PS 562 was good combiner for number of nodules/plant. Significant positive *gca* effect for plant height was expressed by RAUS 5. The genotype JS 93-05 was best general combiner for days to maturity. Agrawal *et al*., (2006) reported the preponderance of non-additive gene effects for these traits in soybean. Sharma *et al.* (1997) also reported the preponderance of non-additive gene effects for days to maturity, plant height while non-additive effect for other traits. Singh *et al.* (1974); Gadag *et al*. (1999) have emphasized the importance of both additive and non-additive type of gene action for days to 50% flowering, days to maturity, plant height, 100-seed weight and grain yield/plant. Dominance of non-additive gene effects were also reported for pods/plant, seeds/plant and grain yield (Tawar *et al.*, 1989; Li *et al*., 1991).

The crosses with high *sca* effects for different characters are presented in Table 3. Crosses JS 93-05 x PS 562, RAUS 5 x Bragg and PS 562 x RAUS 5 were high specific combiners for grain yield/plant. Amongst these, the cross PS 562 x RAUS 5 for days to 50% flowering, days to maturity and plant height had high specific combiner for these characters in addition to its being best combiner for grain yield/plant. The crosses JS 93-05 x Bragg and PS 562 x JS 335 showed maximum *sca* effects for 100-seed weight and best cross combiners for days to 50% flowering and number of pods/plant, respectively. The cross combination PS 562 x RAUS 5 exhibited best combiner for days to 50% flowering, days to maturity and plant height.

In the present study (Table 3), the parents involved in the crosses exhibited as general combiners as high x low or/ low x high and low x low in which the former combinations were more frequent. In crosses involving the parents in the combination of high x low and low x low performance, the genetic interactions might be of

Table 1. Analysis of variance for combining ability for nine traits in 5x5 diallel crosses of soybean

Source of variance	d.f.	Days to 50% flowering	Days to maturity	Number of branches/ plant	Plant height (cm)	No. of pods/ plant	No. of nodules/ plant	Grain yield/ plant	100-seed weight	Oil content (%)
gca	4	8.021 **	20.503 **	0.114	12.584 **	30.052	20.932 *	4.594 *	0.787 *	0.150
sca	10	4.535 **	19.574 **	0.266	14.510 **	41.212 **	85.224 **	4.655 **	1.466 **	1.181 **
Error	28	0.374	0.798	0.131	2.960	13.195	7.472	1.212	0.234	0.223
σ^2g		1.092	2.815	-0.002	1.375	2.408	1.923	0.483	0.079	-0.010
σ^2s		4.161	18.776	0.135	11.550	28.017	77.752	3.443	1.231	0.958
σ^2g / σ^2s		0.263	0.150	-0.018	0.119	0.086	0.025	0.140	0.064	-0.011

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

Table 2. Estimates of gca effects of the parents for different characters in 5x5 diallel cross of soybean

S.No.	Parents	Days to 50% flowering	Days to maturity	Number of branches/ plant	Plant height (cm)	No. of pods/ plant	No. of nodules/ plant	Grain yield/ plant	100-seed weight	Oil content (%)
1.	JS 93-05	-0.495 *	1.467 **	0.171	-0.352	-0.790	1.276	-0.521	0.174	-0.033
2.	PS 562	-0.829 **	-1.343 **	-0.019	-2.162 **	0.686	2.276 *	-0.240	0.327	0.229
3.	JS 335	-0.781 **	-1.057 **	-0.033	0.695	1.352	-1.581	0.841 *	-0.420 *	-0.033
4.	RAUS 5	0.410	-1.295 **	-0.176	1.267 *	1.971	-0.390	0.850 *	0.217	0.01
5.	Bragg	1.695 **	2.229 **	0.057	0.552	-3.219 *	-1.581	-0.930 *	-0.298	-0.176
	S.E. (gi)	0.206	0.301	0.122	0.581	1.227	0.924	0.372	0.163	0.159
	S.E. (gi-gj)	0.326	0.477	0.193	0.909	1.941	1.461	0.588	0.258	0.252

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

Table 3. Estimates of sca effects of the parents for different characters in 5x5 diallel cross of soybean

Crosses	Days to 50% flowering	Days to maturity	Bran ches/ plant	Plant height (cm)	No. of pods/ plant	No. of nodules/ plant	Grain yield/ plant (g)	100-seed weight (g)	Oil content (%)
JS 93-05xPS 562	0.63	0.69	-0.39	-2.39	-12.65 **(LxL)	-1.17	3.99 **(LxL)	-1.78	-1.04
JS 93-05xJS 335	-0.41	-5.58 **(HxL)	0.68 *	0.08	0.68	-4.98	0.02	-0.76	1.55
JS 93-05xRAUS 5	-0.60	-0.01	-0.34	-4.82 **(LxH)	1.06	-4.58	0.45	0.19	0.17
JS 93-05xBragg	1.11 *(LxH)	1.12 *(HxH)	0.09	-1.77	-0.74	-5.65	-0.77	1.21 *(LxL)	0.53
PS 562xJS 335	-0.41	-1.11	0.04	-3.11	4.20 *(LxL)	-0.98 1.01	1.48 *(LxH)	-1.37	
PS 562xRAUS5	3.06 **(LxL)	7.46 **(LxL)	-0.64 *	3.31 *(LxH)	-5.08 **(LxL)	-7.50 **(HxL)	1.87 *(LxH)	-1.89 *(LxL)	-0.58
PS 562x Bragg	-2.55 *(LxH)	-5.39 **(LxH)	0.28	0.69	2.44	14.01 **(HxL)	0.54	-0.41	-0.39
JS 335x RAUS 5	-1.65 *LxL)	-1.82 *(LxL)	0.19	-5.54 **(LxH)	2.92	8.68 **(LxL)	1.25	0.19	-0.82
JS 335x Bragg	-3.27 **(LxH)	-3.34 **(LxH)	0.29	2.50	6.44 **(LxH)	13.54 **(LxL)	1.19	0.23	-0.13
RAUS 5x Bragg	0.87	0.88	-0.55 *	-2.06	-4.84 *	-6.31 *(LxL)	2.64 *(HxL)	-0.26	-0.68
S.E. (Sij)	0.53	0.77	0.31	1.50	3.17	2.38	0.96	0.42	0.41
S.E. (Sij-Sik)	0.80	1.16	0.47	2.25	3.36	3.57	1.44	0.63	0.62

*, ** significant at 5% and 1% level, respectively; L: Low *gca* of parents, H: High *gca* of parents

additive x dominance and dominance x dominance type, respectively.

The category of combination for high x low *gca* effect play an important role in the expression of positive and significant *sca* effects. The cross combinations PS 562 x RAUS 5 and RAUS 5 x Bragg which involved one parent with desirable and significant *gca* effect for grain yield and other with poor or even negative *gca* effects. Such crosses showing high *sca* effects with at least one general combiner could produce desirable transgressive segregants in subsequent generations. Langhan, (1961) also suggested that high x low crosses might produce better transressive segregants in later generations.

In the present study, cross JS 93-05 x PS 562 was found to be in category of low x low *gca* effects. Such cross could be utilized in production of homozygous high yielding lines (Cho and Roy, 2000). It is evident that the crosses exhibited desirable *sca* effects involved parents with good, average or poor *gca* effects, indicating thereby influence of non-additive interactions in these crosses. Further, *gca* and *sca* of the parents and crosses, respectively, for grain yield largely depend on their respective combining abilities for one or more yield components. Parents JS 335, RAUS 5 and Bragg having been isolated as good general combiners for different traits may be suitably used in breeding programme.

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SHORT COMMUNICATION

Allium roylei Stearn – An Uncharted Precious Gene Reservoir of North-West Himalayas

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Allium roylei Stearn belonging to family Alliaceae is a lesser known plant of economic importance. The species is reportedly a repository of genes imparting resistance to various harmful fungal diseases. As per Hooker, the species in India is distributed along the Himalayan, sub-Himalayan ranges and Garhwal Himalayas. In Jammu and Kashmir, except for some plants growing along Gilgit slopes of Ladakh, there were no reports of its occurrence till 2002. Since then, explorations to several regions have unfolded two more populations from the state. The germplasm base of the taxon is likely to widen if extensive explorations are undertaken in Jammu and Kashmir (J&K) and its adjoining regions.

Key Words: *Allium cepa*, *A. roylei*, Downy mildew, Translocation heterozygotes

Allium roylei Stearn belonging to family Alliaceae is an important wild species. Commonly known as *panchali gajna* or *jungli pyaz*, its leaves and inflorescences are used by tribals for seasoning dishes. Bulbs are also consumed for getting relief from headache. In addition bulbs are also given to horses for relief from colic pain (Kohli and Gohil, 2009a). Herbarium specimens of *A. roylei* bearing voucher number 10065 and 10066 were submitted in the botanical herbarium of University of Jammu. It harbours genes imparting resistance against dreadful fungal diseases (downy mildew, leaf blight and anthracnose). Being sexually compatible with *A. cepa*, it has been successfully used as a donor of these genes for improving the common onion (Scholten *et al*., 2007).

In India, the taxon has been reported from the Himalayan and sub–Himalayan ranges; Garwhal westwards between 6000–7000 ft. Surprisingly, the species finds no mention in the floras of Jammu, Srinagar and Udhampur (Sharma and Kachroo, 1981; Dhar and Kachroo, 1983; Swami and Gupta, 1998). It, however, figures in the flora of Ladakh, according to which it is distributed in Gilgit slopes (Murti, 2001). From Jammu province, Sharma and Gohil (2002) reported its occurrence for the first time from Bani (district Kathua) and Mendhar (district Poonch). Since then, some other areas of its distribution have been recorded from this province. A new population was collected from Gourwan (district Reasi) (Kohli, 2007) and another was reported from Kishtwar High Altitude National Park (Kumar and Hamal, 2009). A map depicting areas of collection of various populations of this species is shown in Fig.1. With more explorations, there is every likelihood of the distributional range of this species getting widened.

Perusal of the literature brings forth interesting features associated with this plant species. *A. roylei* is a wild perennial herb (Figs 2a-d). Its preference for culinary purposes by locals, wherever it is found wild, hastens its rate of exploitation and may lead to total extinction in these regions. This exploitation has led to its being assigned the status of a rare species in western Himalaya (Pandey *et al*., 2008). Not withstanding its enormous potential, the species has remained unexplored in North-West Himalayas of which J&K forms a part.

A. roylei, therefore, faces various pressures on account of extrinsic and intrinsic factors. While the former can largely be attributed to the anthropogenic stress, intrinsic causes have been brought to light when three of the four populations reported from Jammu province were subjected to extensive morphological and cytological analyses (Sharma and Gohil, 2003; Kohli, 2007; Kohli and Gohil, 2009b). These studies revealed that the plants of all the three populations are complex translocation heterozygotes (Fig. 2e), unlike the normal diploids reported from Mussoorie. Complex translocations have imposed sexual sterility. As a result of anthropogenic pressure and bottlenecks in the sexual reproduction, the species is likely to become extinct.

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Fig. 1. Map of Jammu province: The letters highlighted and in bold represent the areas from which collections of *A. roylei* were made. Asterisk represents the region from which another population of *A. roylei* was reported but not collected.

Luckily, like most other species of genus *Allium*, *A. roylei* also propagates vegetatively through underground bulbs (Fig. 2b). Vegetative propagation provides an efficient means of survival for all these taxa. A single plant of *A. roylei* produces 4–5 new underground bulbs/bulblets. Under experimental conditions in the Jammu University Botanical Garden, healthy plants were raised separately from each such bulblet. This means that germplasm of *A. roylei* can be increased, maintained and utilized for various purposes.

Considering its importance, incomplete surveys, unknown distributional range and paucity of detailed cytological data, the present communication is an attempt to prompt botanists to undertake extensive explorations of North-West Himalayas and its adjoining areas. These explorations will be of both academic and applied interest. Besides widening the germplasm base of this important gene reservoir, these would help in establishing its actual status *vis-à-vis* conservation, cytological nature and the evolutionary strategies adopted by the plant. Additions to the germplasm base may help isolating collections with other novel genes for plant breeders to exploit.

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Fig. 2. *Allium roylei* a) Plant; b) Bulbs; c) Inflorescence; d) Individual flower; e) A pmc at metaphase-I with 2VIII

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