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COLLECTION OF GERMPLASM FROM WESTERN HIMALAYAS

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A germplasm collection mission to the western Himalayas was undertaken jointly by the International Crops Research Institute for the Semi-Arid Tropics(ICRISAT), Patancheru and the National Bureau of Plant Genetic Resources(NBPGR), New Delhi to collect mainly wild Pennisetum, sorghum, finger millet, pulses including pigeonpea and other crops of local importance during October 1991. The hill slopes showed preponderance of primitive agriculture, followed with traditional varieties. Wild Pennisetum were found growing at 500-3100 m. Two identical species of Pennisetum viz., P. orientale L. C. Rich and P. flaccidum Griseb and some more (yet to be identified) were collected. Sorghum with long drooping panicle branches (broom corn), pigeonpea, mostly early-maturing types and finger millet were also collected. In all, a total of 133 samples consisting of Pennisetum(33), sorghum(11), pigeonpea(7), minor millet(30), amaranths(9), cowpea(5), blackgram(8), sesame(6), paddy(4), maize(4) and 16 of other crops were sampled. Good sources for cold tolerance and early maturity are expected to be available in the collected germplasm.

The objectives of the germplasm collection mission from western Himalayas were to collect mainly wild *Pennisetum*, sorghum, minor millets, legumes, amaranths and other locally available crops from their natural habitat and farmers fields; to gather information on special agricultural practices followed in this area; to assess damage by diseases and pests and other constraints for food production; and to record/study the traditional food processing methods. Initial

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planning was done at the NBPGR, New Delhi. Further, the precise areas for collection were identified with the help of Forest Research Institute(FRI), Dehra Dun. Herbarium specimens maintained at FRI were particularly useful in locating the areas of distribution of *Pennisetum*. The collection mission was laid jointly by one scientist each from ICRISAT, Patancheru and NBPGR, New Delhi.

Collection area

The area covered was situated between 29°–31° N latitudes and 79°–81° E longitudes in Uttar Pradesh, India. The altitude ranged from 500 m at Dehra Dun to 3100 m at Mana near Badrinath in Chamoli district. Soil were mostly undeveloped, mixed with sandy to clay loams. However, in the valleys where farming was practiced on terraces, the soil profiles were developed and were fertile.

Agricultural practices

Terrace cultivation on slopes was a common practice. In the valleys, a variety of crops including grain amaranth, finger millet, and various pulses like greengram, blackgram and cowpea were found growing. Pearl millet was cultivated mainly for fodder in the plains. The millet plants were very tall with very thick stems and medium sized heads. Sorghum was also grown under high population density mainly for fodder. On the slopes, tall growing, late maturing sorghums were observed which produced long, loose, drooping panicle branches. A mixture of different crops like finger millet, blackgram, amaranth were also observed which showed a wide maturity range. Finger millet was found grown mixed with upland paddy around Dehra Dun. In all these areas, fields were prepared by ploughing with a wooden plough after the first monsoon rains. Sowing was done by broadcasting or line sowing.

Sampling methods and collection strategy

The area covered and route followed were decided considering the distribution and maturity of the crops and also the distribution of related wild species. Herbarium specimens at the Forest Research Institute, Dehra Dun were examined to identify the areas of distribution for wild species. The areas covered during the present exploration and the route followed is shown in Fig.1. Course grid sampling was followed with an emphasis on collecting random samples. A farmer's field was considered as a unit for sampling and random plants were collected as populations. While collecting samples, information on traditional farming practices and other relevant passport informations were also gathered.

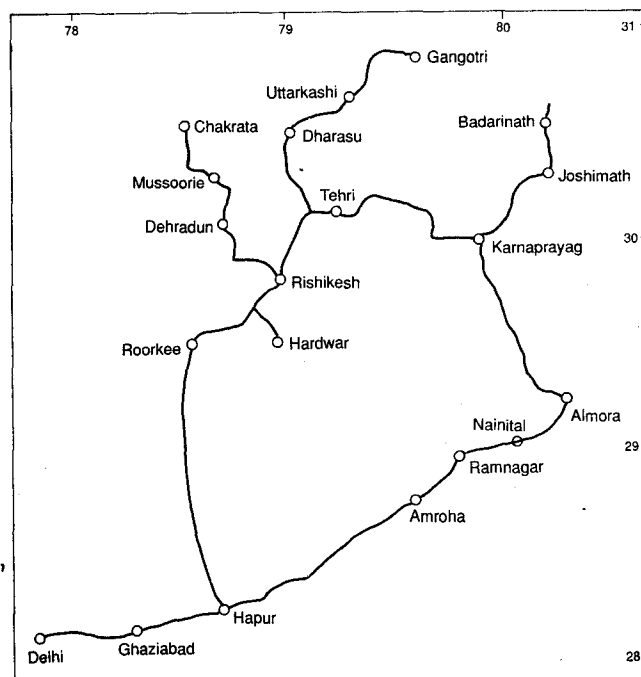


Fig. 1. : Route followed and areas explored for germplasm collected in the western Himalaya.

DIVERSITY IN GERMPLASM COLLECTED

One hundred thirty three samples were collected in pearl millet(2), wild *Pennisetum* spp.(31), sorghum(11), pigeonpea(7), minor millets(30), amaranthus(9), cowpea(5), blackgram(8), sesame(6), paddy(4), maize(4), besides 16 samples of miscellaneous crops. The extent of variability observed and collected in different crops is as under :

Sorghum : Broom corn sorghum for grain, brooms and for forages was commonly grown mixed with finger millet or amaranths. Its panicles were large and loose with drooping branches. Another form with well shaped compact, oval panicles and chalky white grain was also grown for grain.

Pearl millet : It was relatively less important crop of the area, grown mainly in the plains for fodder. Plants were seen over 4 m tall and produced medium size grain. Stems were thick, nodes many with 3-5 basal tillers. Pearl millet was not grown at high elevations.

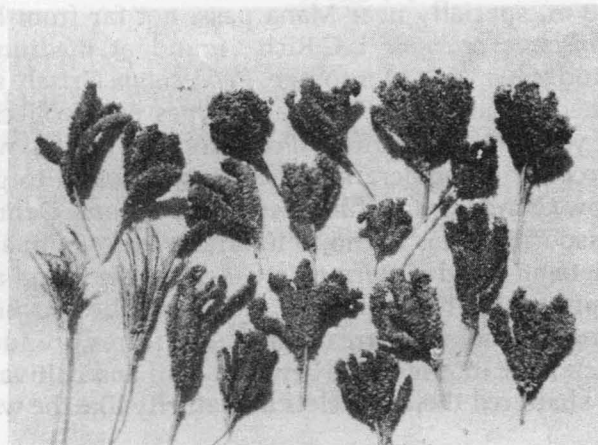
Pigeonpea : It was grown in valleys at the margins of sugarcane fields. Most varieties were relatively early-maturing, grown upto 2 m tall and produced several branches. Considerable variation was observed in number of branches, pods per plant and seeds per pod. Variation was also observed in seed size, colour and shape. Good sources for early maturity, profuse podding and small seed size were observed in this area.

Finger millet : It was extensively grown on terraces, mixed with a variety of legumes like blackgram, greengram or cowpea. It was grown mixed even with rice around Dehradun. Considerable variation was observed in plant height, size and shape of the head and number of fingers(Fig.2). Fingers per head varied from 3-11, and fingers were top curved, incurved, open or compact. Intermediate weedy forms were found in several cultivated fields as a result of introgression between wild and cultivated types. Good sources for large compact heads, white grain and cold tolerance were observed.

Grain amaranths : At higher altitudes amaranths were extensively grown for grain (Fig. 3). They grew over 2 m tall with several branches, and very large inflorescence of different colour (green, red, and a number of intermediate forms). The grain is used as food, tender leaves as vegetables and the stalk for forage. It was usually mixed with legumes.

Legumes : Blackgram(*Vigna mungo* L.) and greengram(*Vigna radiatus* L.) are commonly grown mixed with a variety of crops. Blackgram was twining in habit and produced hairy pods with mottled seeds. Greengram produced clusters of long pods with shining green seeds. In Cowpea(*Vigna unguiculata* (L.) Walp.) long creeping types with profuse branching were commonly grown. Fresh pods were used as vegetable and mature seeds as pulse. It was also an important fodder crop. It was grown mixed with sorghum, amaranths or with other legumes.

Wild relatives : *Sorghum halepense* was found growing almost throughout the area surveyed. However, intermediate shatter canes were not observed. At least four distinct species of *Pennisetum* were collected from the Kumaon and Garhwal hills. Wild *Pennisetum*(Fig.4) were found on bunds and on wastelands upto an altitude of



2



3



4

Fig. 2. : Variability in finger millet germplasm.

Fig. 3. : Variability in grain amaranths.

Fig. 4. : Collection of wild *Pennisetum* at high altitude.

3000 m; specially near Mana pass, not far from Badrinath. *Pennisetum orientale* L.C.Rich., found at medium to high altitudes, was used for forage. *P. flaccidum* Griseb. was found at medium altitudes. Two more types were collected which are yet to be identified. *Pennisetum* collected were good source for cold tolerance. *Eleusine* was found to grow wild at low to medium altitudes, specially around Dehradun and Mussoorie. It was found to hybridize with cultivated finger millet and weedy intermediate forms were found at varying proportions. This clearly indicates the occurrence of introgression in this area. The weedy forms produced heads which were intermediate between wild and cultivated forms and shattered their spikelets at maturity like the wild forms.

GENETIC EROSION

Farmers cultivated only traditional varieties of all crops except for rice, for which improved varieties are used. All the sorghum, pigeonpea and finger millet fields were sown with primitive types. Hence, as such, there appeared to be no threat of replacement of traditional types with improved varieties. However, the wild relatives were threatened because of over grazing by sheeps, goats and cattle.

UTILITY OF THE GERMPLASM

The collected germplasm in general and the wild *Pennisetums*, in particular, should be good sources for cold tolerance as the material was collected at higher altitudes. The pigeonpea material was a good source for early maturity, small grain size and good grain quality; and the finger millet for large finger size and white grain colour.

ACKNOWLEDGMENTS

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DISTRIBUTION, DIVERSITY, USES AND *IN VITRO* CONSERVATION OF CULTIVATED AND WILD *ALLIUMS* — A BRIEF REVIEW

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The genus Allium (Family Alliaceae) comprising both cultivated and wild species is of considerable importance as vegetable, spice or condiments and for medicinal or aromatic value. It comprises some 700 known species of herbaceous nature distributed throughout the world. Indian Gene Centre is considered to be fairly rich in distribution and occurrence of some 30 cultivated and wild species; among which about 4-5 species are cultivated as major crop or as minor kitchen garden plant. The brief review deals with the distribution, diversity and occurrence, particularly of wild Allium species, alongwith their distinguishing taxonomical attributes and potential economic uses. The use of in vitro technology in the micropropagation for rapid clonal propagation, induction of organogenesis and somatic embryogenesis, protoplast culture, and embryo rescue etc. has been reviewed with specific mention of status of in vitro research at NPTCR.

The genus *Allium* has been considered under the family Liliaceae for a very long time and later treated by some Botanists in the family Amaryllidaceae. More recently it has been placed into a separate family Alliaceae (Purseglove, 1972). Interestingly, *Allium* constitutes a very complex genus accounting for well over 700 known species of herbaceous nature. According to Buijsen (1990) genus *Allium* could comprise about 1000 wild species distributed in the world.

Indian gene centre is fairly rich in the distribution and occurrence of some 30 cultivated and wild species. Out of these

only 4-5 species are under cultivation; the major acreage being under onion, garlic and leek. Remaining species occur as wild populations in the natural habitats of Himalayan ecosystem. Biodiversity conservation requires the integration of *in situ* and *ex situ* conservation approaches in a dynamic and flexible manner. This brief review deals with distribution and occurrence, diversity in Indian gene centre and taxonomical classification of the related species followed by detailed accounts on *in vitro* micropropagation and conservation aspects.

DISTRIBUTION AND OCCURRENCE

The distribution of *Allium* species extends westwards to Afghanistan and Turkestan and eastwards to the north eastern region of India and China. The primary distribution of majority of the wild species can be located from Afghanistan eastward to Ladakh (J & K); Lahaul & Spiti, Kinnaur Himalayas (H.P.); and Uttarakhand region (U.P.); parts of Nepal, Sikkim and Bhutan. According to the recent surveys (Buijsen, 1990), all taxa of the genus *Allium* cultivated in south-east Asia are introduced either from China, Central Asia or Europe. Besides, wild species are generally restricted to the northern hemisphere and totally absent in south-east Asia and other tropical areas. The species cultivated in south east Asia which have their origin and secondary centre of diversity in the Sino-Japanese centre may include *A. fistulosum*, *A. chinense* (*A. bakeri*), *A. hookeri* and *A. tuberosum*. The above species are also found in Indian gene centre, some of them still in the wild state. According to Novak *et al.* (1986), many aspects of onion and its allies have been discussed by Jones and Mann (1963). *Allium* is supposed to possess over 600 species in the temperate zone. The domesticated *Allium* species come from the near east, central or eastern Asia and they all have basic chromosome number $x = 8$. The common onion (*A. cepa* L.) and garlic (*A. sativum*) are diploids with $2n = 16$ and are known only in cultivated form. Garden leek (*A. porrum*) is a cultivated form of *A. ampeloprasum* with the tetraploid chromosome number $4x=32$. Onion and leek are propagated by seed, while garlic is an obligate apomictic plant species propagated by planting cloves. The variant *A. cepa* var. *aggregatum* group commonly referred as 'Shallot' is believed to be of north African origin and that of east mediterranean region (Zeven and de wet, 1982) from where it has migrated into all direction through land and sea routes. Among cultivated *Alliums*, only two prominent species, *A. schoenoprasum* and *A. ampeloprasum* have been

introduced by Europeans in South and South-east Asia during the past colonial history of about 400 years.

DIVERSITY IN THE INDIAN GENE CENTRE

The genus *Allium* is very widely distributed in the temperate and alpine regions of Himalayas (Kachroo, 1977). About 30 species have been reported to occur in Indian gene centre (Santapau & Henry, 1973; Babu, 1977). Arora and Nayar (1984) mentioned only three species, *A. rubellum*, *A. tuberosum* and *A. schoenoprasum*, Negi *et al.* (1991) and Negi and Pant (1992) reported that 15 species occur in the temperate and alpine zones of northern Himalaya. Majority of the wild species except *A. rubellum* are considered to be new records. They are also enumerated as little known edible plants of India (Negi, 1988; Negi & Gaur, 1989). Cultivated species grown extensively in the tribal backyards comprise *A. ampeloprasum* L. (syn. *A. prasum* L.) locally known as Leek, Hasgandh or Sidhum, *A. ascalonicum* L. (Shallot), *A. cepa* L. (onion), *A. sativum* L. (garlic) and *A. fistulosum*. Among wild species, mention can be made of *A. semonovii*, *A. stracheyi*, *A. rubellum*, *A. przewalskianum*, *A. carolinianum*, *A. consanguineum*, *A. wallichii*, *A. humile* and *A. victorialis*. Among several valuable reports published on *Alliums*, included work of Hooker (1894), Duthie (1906), Collet (1917), Stewert (1916-17), Jones and Mann (1963) Santapau and Henry (1973), Rau (1975), Mani (1978), Gaur and Senwal (1983), Hazra (1983), Kunkel, (1984), Polunin and Stainton (1984), Rawat *et al* (1985), Uniyal (1989), Negi and Gaur (1991) besides the classical Regel's "*Alliorune monographia*", of Baker (1874).

Recent surveys and plant explorations have confirmed the occurrence of several wild *Allium* species in the northern and north western Himalayan region. The danger of genetic vulnerability, erosion and even extinction have also been sounded. Negi and Pant (1992) reported the occurrence of 10 lesser known wild species of *Allium* from the mountainous regions of northern hills, India and discussed information on their habitat, uses, etc. The prominent species included *A. carolinianum* D.C. (syn. *A. blandum* Wall.), *A. chinense* G. Don (syn. *A. bakeri* Regel), *A. splendens* Willd; *A. exsertum* Baker), *A. consanguineum* Kunth., *A. humile* Kunth. (syn. *A. govanianum* Wall. ex Baker), *A. przewalskianum* Regel, *A. rubellum* M. Bieb., *A. semonovii* Regel, *A. stracheyi* Baker, *A. victorialis* L., and *A. wallichii* Kunth. Baker (1874) suggested seven groups of this

founded upon characters furnished by the bulbs, filaments, spathes and direction of perianth-segments in the expanded flower. Hooker (1894) described the most widely cultivated *Alliums* species in the Indian sub-continent based upon previous works which included *A. ascalonicum* L., *A. ampeloprasum* L., *A. cepa* L. and *A. sativum*. The remaining species occurring wild, as the component of natural ecosystem, were classified, primarily based on the leaves or floral morphology under different sections and groups, although the problems of taxonomic classification, correct nomenclature and synonymity continued to be vague, complex and unresolved (Stearn, 1946). For easy reference, the brief description of 27 species, included in the above treatment, is reviewed in table 1.

Table 1. List of *Allium* species occurring wild in the Indian gene centre

S. No.	Taxa	Salient characteristics	Distribution and Occurrence	Altitudinal Range (000')
1.	<i>A. semonovii</i> Regel	Bulbs tufted, cylindrical, scales membranous, leaves 2-3, stout, head subglobose, densifid, campanulate, pale yellow flowers	Western Himalayas; Kashmir to Garhwal, Singjari (C. Himal.) Dras, Gilgit, Nubra, Satrundi-Chamba, Zaskar (N.W. Himalayas)	8-14
2.	<i>A. schoenoprasum</i> L.	Bulbs clustered, narrow; scales membranous, head subglobose densifid, flower head campanulate, pink or pale purple flower	Western Himalayas; Kashmir to Kumaon hills	8-11
3.	<i>A. atrosanguineum</i> Schrenk.	Bulbs narrow, coats membranous fibrous, leaves fistular, sheaths very long, spathes persistent, head campanulate, dark red flowers	Kashmir to Gilgit, Turkestan	11-12
4.	<i>A. fedschenkoanum</i> Regel	Leaves 1-2 much shorter than stout scape, head globose, densifid, campanulate, pale yellow flowers. Bulbs not formed	Western Himalayas, Kashmir, Turkestan	12

Continued

Table 1 continued

S. No.	Taxa	Salient characteristics	Distribution and Occurrence	Altitudinal Range (000')
5.	<i>A. rubellum</i> M. Bieb.	Bulbs small, ovoid-oblong, sheaths elongate, outer coats striate, inner membranous, leaves 4-6, longer than the scapes. Sheaths elongate, head 1 inch. diam, very small, campanulate rosy flowers	Western Himalayas, Kashmir to Kumaon, westwards to the Ural and Caucasus, Siberia, Frequent in temperate and alpine zones; Budhi, Garbyang Harshil, Mussorrie etc.	1.5-8
6.	<i>A. lilacinum</i> Royle	Bulbs ovoid, coats scarious, red brown, leaves 2-3 scape 8-10 m., fistular head hemispheric, campanulate small pale red flowers	Western Himalayas Garhwal westwards	6-7
7.	<i>A. carolitanianum</i> D.C. syn. (<i>A. blandum</i> Wall.)	Tall, stout, leaves flat broadly linear, head globose very densefid, campanulate pale pink flowers, bulb large, oblong, scales coriaceous entire, spathe ovate	Western Himalayas; Western Tibet, Kumaon, to west Nepal. Girthi valley Gori valley, Malari, Milan glacier	13-17
8.	<i>A. stracheyi</i> Baker	Bulbs small, clustered, narrowly avoid, outer scales fibrous; leaves 3-4, tips rounded, head 2.5 cm., spathes small deltoid head globose or hemispheric dense, campanulate rosy or pale yellow flowers	Western Himalayas; from Kashmir to Kumaon frequent in sub alpine and alpine zones on flat rocks along river banks, Brahmamthya, Harshil, Gangi, Gangotri, Garba yang Badrinath/ Kedarnath	9-12
9.	<i>A. consanguineum</i> , Kunth.	Leaves slender narrowly linear, obtuse flat keeled head hemispheric, Inflorescence Campanulate, Golden yellow flowers	Western Himalayas, Kashmir, Zozila pass, common in sub alpine and alpine zones on rocky substances	8-10

Continued

Table 1 continued

S. No.	Taxa	Salient characteristics	Distribution and Occurrence	Altitudinal Range (000')
10.	<i>A. platyspathum</i> Schrenk.	Bulb solitary or clustered on a short perpendicular root stock, scales hayline, leaves flat, sheaths hypogeous	Western Tibet	
11.	<i>A. thomsoni</i> Baker	Bulbs tufted, narrowly ovoid, outer scales hard, chestnut brown, entire; leaves 4-5, head 2.5-3 cm. dia., spathes short deltoid, leaves rather stout, linear fleshy, head globose, campanulate flowers	Kashmir Himalayas	12
12.	<i>A. bakeri</i> Regel	Bulbs clustered, 2.5 cm. long, avoid, oblong scales, white membranous, leaves 2-4, head-laxfid flower campanulate- red-purple	Khasia hills, China and Japan	4-5.5
13.	<i>A. wallichii</i> Kunth.	Bulbs hardly developed, clustered base of stem thickened, clothed with membranous entire and torn sheaths leaves basal 2-3 ft, long-linear, head lax, 5-7 cm. long, capsule turbinate	Temperate Himalayas, from Kumaon to Sikkim, Gilgit, Common in exposed slopes in rocky temperate region, forest clearing temperate and alpine zones, China peak, Dharahi, Jamnotri, Kodiyabayar Mussorrie, Nanda Devi Sanctuary, Pustara, Realm valley (Central Himalayas), Chor, Gilgit, N. west hills	8-13
14.	<i>A. hookeri</i> Thuaitea	Bulb hardly any, base of stem clothed with long, narrow membranous sheaths, leaves 12-19, head 2.5-3.0 cm. in diam., Spathe with a long tail, leaves basal, linear, membranous,		

Continued

Table 1 continued

S. No.	Taxa	Salient characteristics	Distribution and Occurrence	Altitudinal Range (000')
		head globose laxly many fid, flowers white, capsule abcordate.	Khasia hills at Kala pana, Sri Lanka	5
15.	<i>A. sikkimense</i> Baker	Bulbs tufted, slender cylindric, outer scales of long parallel fibres. Leaves 2-3, leaves basal narrowly linear, head dense fid, pedicel unequal longer or shorter flowers cam- panulate, lilac purple	Sikkim Himalayas (inner ranges)	11-14
16.	<i>A. przewalskianum</i> Regel (<i>A. jacquemontii</i> Regel)	Bulbs tufted, cylindric elongate fibrous coats very finely reticulate, Leaves 3-6, basal filiform, subterate shorter head globose or sub globose pedicels shorter or longer than the lilac Campanulate flowers.	Western Tibet and adjoining region, Dobata, Guge Rakastal (C. Himalayas) Keylong, Lahaul, Khardongla, Zaskar (N.W. Himalayas)	16.5
17.	<i>A. auriculatum</i> Kunth.	Bulbs elongate, narrow, seated on an oblique root stock, scales brown, reticulate, leaves 6-9 head 1.5-2.0 cm., spathes 2-3 short, flower campanulate purplish.	Western Himalayas; Kumaon Hills	
18.	<i>A. victoralis</i> L.	Bulbs 5-7.5 cm. long, clustered on an oblique root stock, sub conic or cylindric, outer scales fibrous reticulate, stem leafy; leaves petialed elliptic to oblong lanceolate obtuse or acute shorter, head dropping in bud then erect laxfid, flowers greenish white or yellowish.	Temperate Himalayas; Kashmir eastwards to Sikkim, N. Asia to Japan, Sella village, chakrata (Central Himalayas). Frequent in under growth of temperate region, forest Binsar, Darma valley, Gangotri, Gori/Nilam valley, Nag tibba	7-13

Continued

Table 1 continued

S. No.	Taxa	Salient characteristics	Distribution and Occurrence	Altitudinal Range (000')
19.	<i>A. schrenki</i> Regel	Bulbs sub cylindrical, inserted on a stout cylindric rootstock outer scales densely fibrous, reticulate and brown; leaves 3-4 linear, flat shorter than the terete striate scape; head globose purplish campanulate perianth	Himalayan mountains, extends to Siberia	10-15
20.	<i>A. odorum</i> L.	Bulb 2.5-10 cm., clustered on an oblique jointed root stock subcylindric or conic, scales finely reticulate, white brown or blackish leaves-many, 6-14, very narrowly linear flatish keeled shorter. Head many or few-fid, 2.5-4 cm. in dia. spathes short.	Western Nepal and adjoining Himalayas, Western Tibet, N. Asia, Japan	10-14
21.	<i>A. tuberosum</i> Roxb.	Bulbs elongate, cylindric, with white flesh root-fibres, scales grey fibrous; leaves 6-12 basal erect narrow linear flat tall compressed. Sometimes concave and twisted; head lax-fid, pedicles much longer than the small white or pink stellate flowers, hemispheric spathes 1-2 small, ovary globosely ovoid deeply 3 lobed, capsule obcordate.	Western Himalayas, Rampur hills, Khasi hills, China, Japan and Thailand	5-6
22.	(<i>A. humile</i> Kunth.) syn. <i>A. govaniianum</i> Wall.	Bulbs and foliage as in <i>A. odorum</i> ; it differs with it due to acutely angled scape, in the larger flowers with narrower sepals leaves many basal sub distichous linear flat obtuse head many fid, flowers white stellate	Temperate Himalayas from Kumaon westwards, Bhyunder valley, Khatling glacier, Nanda Devi Sanctuary, Central and North west Himalayas	8-12

Continued

Table 1 continued

S. No.	Taxa	Salient characteristics	Distribution and Occurrence	Altitudinal Range (000')
23.	<i>A. creoprasum</i> Schrenk.	Bulbs tufted, cylindric or elongate conic, outer scales rigid appressed fibres strongly coarsely reticulated; leaves 8-12 often minutely serrulate, leaves basal, narrowly linear, flatish shorter than the striate scape, head few or many fid pedicels longer than the rosy campanulate flowers, ovary globose, trigonous	Western Tibet, Soongaria, Eastern Turkestan	10-17
24.	<i>A. clarkei</i> Hook.	Bulb small, ovoid 2.5 cm., outer scales closely and finely reticulated pale, leaves 4-8, obtuse flat leaves very many sub basal erect very narrowly linear or filiform shorter than the slender scape head laxfid, pedicels much longer than the stellate white flowers	Kashmir; Skardo	7-11
25.	<i>A. atropurpureum</i> Waldst.	Bulb subglobose ovoid, scales entire, leaves 2-4 basal narrowly linear-oblong or lanceolate shorter than the tall erect, terale fistular scape head large very many and dense-fid. Peridicle much longer than the linear oblong or lanceolate ovary subglobose or depressed	Western Himalayas; from Kashmir, Kistwar, Turkestan and Siberia	8-10

Continued

Table 1 continued

S. No.	Taxa	Salient characteristics	Distribution and Occurrence	Altitudinal Range (000')
26.	<i>A. loratum</i> Baker	Bulb small, ovoid, outer scales membranous, grey, leaves 2-5 narrowed from above the base head 30-50 fid, Spathes 2, head many dense-fid, navicular acute, head globose, with much shorter pedicels and paler flowers. Leaves 3-5, linear lanceolate, flat flaccid ciliolate pedicel short but longer than the campanulate white perianth, ovary globosely triquetrous	Western Himalayas, Kishtwar, Banihal and Tibet	10-14
27.	<i>A. macranthum</i> Baker.	Bulb narrow, coats membranous, leaves 6-9 scapes many, grooved and ribbed robust, leaves many linear, gradually acuminate keeled, head laxfid pedicels much longer than the large campanulate dark purple flowers, ovary deeply 3-lobed	Sikkim Himalayas, (inner ranges)	12-13

(i) Information based (J.D. Hooker, *Alliums*)(ii) On the *Alliums* of India, China and Japan, J.G. Baker (original article)

TAXONOMICAL CLASSIFICATION

Sect. I. Schoenoprasum (Leaves fistular); *A. semonovii* Regel, *A. shoenoprasum* L., *A. strosanguineum* Schrenk, *A. fedschenkcanum* Regel, *A. rubellum* M. Bieb., and *A. lilacianum* Royle.

Sect. II(a) Rhizibidium (Bulb solitary or clustered upon an erect or creeping root stock (Leaves flat); (i) Scales of bulb membranous, not of reticulated fibres; stamens longer than the perianth); *A. blandum* Wall., *A. stracheyi* Baker, *A. consanguineum* Kunth., *A. platyspathum* Schrenk, *A. thomsoni* Baker and *A. bakeri* Regel

Sect. II Rhizibidium (ii) (Stamens equalling or shorter than the perianth); *Sub Section A. wallichii* Kunth, *A. hookeri* Thwaites and *A. sikkimense* Baker *Sub-Section B* (Outer scales of the bulb of reticulated fibres; stamens longer than the perianth); *A. jacquemontii* Regel, *A. auriculatum* Kunth., *A. victoriales* L. and *A. schrunki* Regel. Stamens shorter than the perianth; *A. odorum* L; *A. tuberosum* Roxb; *A. govanianum* Wall., *A. creoprasmum* and *A. clarkei* Hook.

Sect III. Molium (Bulbs not seated on a root stock; leaves flat or keeled. Spathes shorter than the head); *A. stropurpureum* Waldst., *A. loratum* Baker and *A. macranthum*.

POTENTIAL ECONOMIC USES

Allium species have played a very valuable role in the history of mankind providing food, spices, condiments and medicines. Potential uses of some of the *Allium* species have become apparent and significant in the recent years. Among all the *Allium* species, garlic has been traditionally used during the last 4000 years for a variety of purposes and is perhaps the best documented medicinal plant. (Granwald *et al.*, 1992).

In an ethnobotanical study of the Kumaon region of India Shah and Joshi (1970) highlighted that dried leaves of Jumbo (*Allium stracheyi*) are primarily used for flavouring curries and vegetable dishes. Negi and Gaur (1991) further enlisted the occurrence of a few wild species in the Garhwal and Kumaon region of Himalaya confirming the abundance of these wild species. According to them, *A. humile* Kunth. and *A. rubellum* M. Bieb., *A. wallichii* Kunth. and *A. carolianeum* D.Don were locally very important being fragrant and possessing aromatic foliage. Almost every part of the plant is eaten by local inhabitants. Their leaves, and tuberous/fibrous root provide energy and food rich in carbohydrates, vitamins and minerals. Similarly, dried bulb scales of *A. wallichii* are also used for pectoral complaints.

USE OF *IN VITRO* TECHNOLOGY :

Micropropagation of *Allium* species

In *Allium* species, the cell, tissue and organ culture are used both for rapid clonal propagation and for production of pathogen-free materials. The new genetic variation could perhaps be regenerated employing techniques of somaclonal variation as well as the somatic cell hybridization and protoplast fusion integrating

new genes and nuclear contents from diverse sources. The development of new cultivars, hybrids, in all cultivated *Alliums*, is of considerable importance. The techniques of modern plant biotechnology i.e. genetic engineering and plant cell culture may play a key role in the development of new cultivars (Novak *et al.*, 1986).

Onion (*Allium cepa* L.)

Breeding of onion cultivars has been limited due to hybrid sterility and restricted genetic recombinations while attempting interspecific hybridization. Although, development of onion cultivars resistant to fungal, bacterial and viral diseases has been the major objective in the breeding programme so far. Yet onion Smut (*Urocystis* spp.), blight (*Alternaria* spp.), downy mildew (*Peronospora* spp.), pink rot (*Pyrenochaeta* spp.), white rot (*Sclerotium* spp.), basal rot (*Fusarium* spp.), neck rot (*Botrytis* spp.) and smudge (*Colletotrichum* spp) are the prevalent fungal diseases which may cause considerable losses during cultivation and storage.

Novak *et al.* (1986) reviewed the biotechnological work on onion, garlic and leek (*Allium* species). The rapid clonal propagation of onion (*Allium cepa* L.) was reported through tissue culture by Kunimitsu *et al.* (1979) and the application of tissue culture technology to the problem of the multiplication of male sterile plants in common onion was investigated. Explants, each containing two inner scale bases, 2-3 mm. wide and 1.0-1.5 mm long, attached to the disc tissue, 1 mm. thick were found to be the best for the production of adventitious shoots. About 25 pieces of such explants were obtained from one stock bulb, because the bulb has 4 to 6 tillers per shoots which can be further divided into 4-6 sectors according to size.

The explants were grown on Murashige and Skoog (1962) medium with various levels of Kinetin and NAA under a 12 hr. light regime at 20°C. Adventitious shoot formation was strongly dependent on the presence of Kinetin, while NAA only had a modifying effect on the Kinetin influence. Proliferation of adventitious shoots was at its maximum in the medium containing 12 mg/l Kinetin and 0.5 mg/l NAA. It was observed that explants excised from stock bulbs in a post dormant stage were more active in the formation of adventitious shoots than the explant excised in a dormant stage, and produced about 10 shoots per explant in 30 days of incubation. The microscopic study of *in vitro* produced

plants revealed that adventitious shoots were induced directly without intervening callus from the disc tissue adjacent to the scale bases. The divided sections of cultured tissues with one or two adventitious shoots were transferred to Murashige and Skoog (1962) medium containing 0.01 and 0.1 mg/l NAA without Kinetin and kept under a 12 hr light regime at 20°C for 20 days. In the above study (Kunimitsu, 1979), most satisfactory plantlets enduring open culture were produced in the absence of NAA.

Novak *et al.*'s (1986) work on *Allium* is very significant, as they analysed and compared nutrient composition of the different standard medias MS, B₅ and LS. Based on this analysis, they suggested the modification in the B₅ basal medium as described below (Gamborg *et al.*, 1968). CaCl₂H₂O (150), KNO₃ (2530), NH₄NO₃ (320.16), NH₄H₂PO₄ (230.06), (NH₄)₂SO₄ (134), MgSO₄·7H₂O (247), MNSO₄·4H₂O (132), ZnSO₄·7H₂O (2.0), CuSO₄·5H₂O (0.039), KI (0.75), CoCl₂·6H₂O (0.025), H₃BO₃ (3.0), NaMoO₄·2H₂O (0.25), NaH₂PO₄·2H₂O (172), FeSO₄·7H₂O (27.85), Na₂EDTA (37,25), nicotinic acid (1.0), Thiamine HCl (10), pyridoxine HCl (1.0), meso-inositol (100), sucrose (30 g), agar (8 g), pH 5.5. Using the above described medium, authors reported a forty-fold increase in tissue fresh weight.

Shoots and scale-base explants dissected from bulbs of the onion (*Allium cepa*) proliferated numerous shoots on nutrient medium containing 1-4 mg/l 6-benzylaminopurine and 0.12-0.5 mg/l 1-naphthyl acetic acid (Hussey, 1978). Most shoots arose adventitiously from scale explants or leaf bases, but some precocious axillary branching also occurred. *In vitro* shoots eventually formed bulbils which proliferated further axillary and adventitious shoots when trimmed and split in half before sub-culturing to fresh medium. Root formation was also not entirely suppressed by cytokinin and plantlets could be transplanted successfully to compost. The *in vitro* technology in *Allium* spp. can be of special significance, as successful maintenance and multiplication of male-sterile lines for use in hybrid seed production, would also be greatly facilitated by an *in vitro* propagation system. Tissue culture of *Allium* was earlier confined mainly with callus formation for use in breeding and biochemical studies. Bulbing in *Allium cepa* is photoperiodically induced by exposure to long days. 16 h days would have promoted early bulbil formation (Heath and HoldsWorth, 1943). Under short days, *in vitro* bulbil formation would presumably be delayed with possibility of faster shoot proliferation.

Hussey and Falavigna (1980) investigated the origin and production of *in vitro* adventitious shoots in the onion. Adventitious shoots were induced firstly on two scales cut from small bulbs and subsequently on split *in vitro* shoots used as secondary explants, on media containing 6-benzylaminopurine with or without 1-naphthalene acetic acid. More shoots were induced in 16h than in 8h days, but day length had negligible effect on the growth of *in vitro* shoots, dormancy being delayed slightly in short days.

In the *in vitro* produced plants, one tetraploid shoot occurred out of 226 scored for ploidy level; and no polyploids were induced by colchicine treatment of split shoot explants. In above investigations, histological observations revealed that adventitious shoots consistently initiated from atleast two tissue layers, the epidermis and hypodermis, on the abaxial surface of leaves and scales close to the basal plate where the frequency of endopolyploid cells was lowest. In this context, anatomy of the garlic bulbs and factors affecting bulb development were also studied (Mann, 1952). Multicellular origin of the shoots ensures the genetic stability essential for multiplication and storage of elite material.

Meristem culture technique has been useful in *Allium* species for the elimination of viruses. The shoot apex is left within and meristem is located at the bottom of the youngest tubular leaf. A part of the broad stem (generally termed as stem, basal plate, basal disc etc. by different workers) is removed with at least one leaf primordium. According to Novak *et al.* (1986) meristem culture is based on the culturing of meristematic dome and adjacent more or less differentiated tissues (leaf primordia). This technique has been effectively employed for the virus eradication and/or micropropagation of garlic, onion and Welsh onion plants. The smallest isolated piece of garlic meristem used as explant has been 0.1-0.15 mm in length. However, in another report, Bhojwani (1980) used primary explants of 5-8 mm in size, while in onion 10-15 mm long main shoot was used by Hussey (1978). For the purpose of virus eradication, it is beneficial to isolate smaller primary explants size. The viruses and virus diseases of *Allium* species were discussed earlier by Bos (1982).

Garlic (*Allium Sativum* L.)

In vitro micropropagation of garlic (*Allium sativum*) were accomplished through shoot proliferation (Bhojwani, 1980). Shoot buds usually 5-8 mm long, excised from dormant cloves, proliferated both axillary and adventitious shoots on Gamborg's

(1968) B₅ basal medium supplemented with 0.5 mg l⁻¹ isopentenyladenine (2-ip) and 0.1 mg l⁻¹ naphthaleneacetic acid (NAA). Subsequently, 8 fold increase in shoot number occurred every 6 weeks. Shoots were readily rooted in B₅ medium (Gamborg *et al.*, 1968) with addition of +0.01 mg l⁻¹ 2-ip + 0.2 mg l⁻¹ NAA and transferred to pots where about 70 per cent of the formed shoots established as plants. The plants raised by this shoot proliferation method retained the diploid condition of the parents. Subsequently, Bhojwani *et al.* (1982/83) reported production of virus free plants from 6 New Zealand selection of garlic (*Allium sativum* L.) after culturing 0.4-0.9 mm long shoot tips and multiplied using micropropagation technique. Bulbs formed in the first season out of sterile culture were comparable in size and weight to grade-I bulbs raised conventionally by sowing large cloves.

Garlic cultivars being cultivated in U.K. were reported to be infected with two or more viruses. ISEM detection tests indicated (Walkey *et al.*, 1987) that onion yellow dwarf (OYDV), leek yellow stripe and shallot latent viruses (SLV) infect some varieties and accessions in various combinations with other unidentified poty-or carla viruses. They produced virus-free plants of eight garlic cultivars and that of shallot through meristem culture technique. Percentage of virus free garlic plants regenerated was further increased from 25-50 to 85 per cent when infected parent plants were subjected to thermotherapy (38°C) prior to tissue culture. Infact, when cultivars were given high temperature treatment of seven days before the meristem tips were excised for culture, the percentage of virus free plants produced increased from 25 to 85. Preconditioning the parent plant at 30°C before raising the treatment temperature to 36°C and 38°C was an essential requirement of the thermotherapy treatment. In above studies, shallots were more sensitive than garlic to high temperature treatments. Also meristem tips of garlic grew better on a medium containing Gamborg's B₅ mineral salts (Gamborg *et al.*, 1968) than on Murashige and Skoog's (1962) medium. Meristem culture technique coupled with thermotherapy can overcome the problem of small size of meristem to some extent. Ayuso and Pena-Iglesias (1981) reported production of 85 per cent virus free garlic plants using explants of 1-2 mm.

Leek (*A. ampeloprasum* (syn. *A. porrum*))

In order to achieve production of *in vitro* raised shoots of leek, the procedure and protocol was investigated by Dunstan and Short (1977 a, b; 1978 a, b). Large number of shoots were obtained from

the excised basal regions of leek plants cultured on BDS medium. Shoots could be induced optionally upon media within the range of 6.0-8.0 mg l⁻¹ 6 (3 methyl-2 buten-1 yl amino) — purine (2-ip) and 1.0-2.0 mg l⁻¹ naphthalene acetic acid (NAA). Sub culture of induced shoots onto fresh media, from the above hormone ranges, resulted in further multiplication of the callus like region around the bases of induced shoots and adjacent tissue could also result in shoot production. Internally developing shoot primordia occurred in close proximity in meristematic regions; whereas superficially developing primordia did not show distinct affinities, although there was evidence of localized pockets of meristematic cells.

In vitro regeneration system for *Allium* has evidently shown several potential applications. The rapid shoot multiplication potential of the tissue culture technology is considered to be useful to produce clonal materials for genetic and breeding purposes (Hussey, 1978; Hussey and Falavigna, 1980). It is evident from the recent researches that ability to regenerate plants from long term callus cultures permits cellular approaches to *Allium* breeding and genetic studies. Phillips and Hubstenberger (1987) suggested that recovery of useful somaclonal variation, or the traits selected at the cell level, can be of immense importance as somaclonal variation occurring in long term callus or cell cultures may facilitate introgressive breeding. The procedures for micropropagation and plant regeneration from callus were developed by Phillips and Hubstenberger (1987) for *Allium fistulosum* L., *A. altaicum* Pall., *A. galanthum* Kar. & Kir, *A. roylei* Stearn and selected progeny of interspecific crosses of *A. cepa* x *A. fistulosum*, *A. cepa* x *A. galanthum* and *A. cepa* x *A. oschaninii* O. Fedtsch. Each genotype exhibited shoot multiplication in micropropagation systems, and most regenerated plants form callus. The results demonstrated the general applicability of the pictoram based (4 amino 3,5,6, trichloro - 2 pyridine carboxylic acid) tissue culture model for the genus *Allium*. The effects of Pictoram and other auxins in onion was earlier reported by Phillips and Luteyn (1983). Neoformation of bulbils in *Allium porrum* L. cultured *in vitro* was reported by Debergh and Metsenaere (1976). The authors described the procedure developed by them to obtain bulbils on stem explants of leek. The possibilities of root and shoot differentiation were also indicated by inducing callus on the original explant and in this case the auxin was not very critical. NAA (α -naphthalene acetic acid), IAA (indole-3 acetic acid) and IBA (Indole Butyric Acid) were

effective at a concentration higher than 5 mg/l. After a variable period of dark incubation, it was sufficient to transplant the callus on a medium without hormones to obtain shoots and/or roots.

Organogenesis and induction of somatic embryogenesis in *Allium* species

Organogenesis and embryogenesis in callus cultures of garlic was investigated by Mostafa and EL-Nil (1977). Callus cultures were initiated from stem tips, bulb leaf discs and stem segments of garlic by above workers. On AZ medium supplemented with 10 µM p-chlorophenoxy acetic acid (P-CPA), 2 µM 2, 4-dichloro phenoxy acetic acid (2, 4-D) and 0.5 µM kinetin. The callus cultures survived sub culturing into AZ medium containing 1.0 kinetin and 10 µM-3 indole acetic acid (IAA). Organogenesis occurred as shoot formation on modified AZ medium containing 18 µM ammonium nitrogen and 40 µM nitrate nitrogen supplemented with 10 µM kinetin and 10 µM IAA. *In vitro* organogenesis was induced in callus cells cultured on modified AZ medium supplemented with 20 µM kinetin and 10 µM IAA. Filamentous virus particles were eliminated in differentiated shoots or embryoids.

A wide variety of monocot species including cereals, wheat, rice, maize, barley, grasses and other monocots such as sugarcane, banana, lily, garlic and onion have been reported to be amenable to somatic embryogenesis. (Vasil 1985, 1988; Novak *et al.*, 1986; Ahloowalia, 1990). In such investigations, the embryogenic callus which gave rise to somatic embryos is usually compact and nodular and is very much distinguishable. MS medium has been used successfully to induce embryogenic callus culture from a large number of graminaceous species, using MS medium induction of embryogenic callus culture from three *Allium* species employing zygotic embryos as the ex-plant (Valk *et al.*, 1992). The plant regeneration ability of zygotic embryo-derived callus cultures was studied for 12 varieties/accessions of *Allium cepa*, two *A. fistulosum* cultivars and one *A. fistulosum* × *A. cepa* interspecific hybrid and two *A. porrum* varieties. The MS medium was supplemented with 2, 4-dichlorophenoxy acetic acid. The above authors reported that embryogenic calluses of all the above dealt *Allium* species were similar in appearance and plants could be regenerated with high frequency following the somatic embryogenesis pathways, using kinetin supplemented MS medium. Addition of abscissic acid to the regeneration medium stimulated the formation of both somatic

embryos and shoots for a number of varieties. Significant differences occurred between different genotypes/cultivars with regard to shoot regeneration from callus cultures.

Somatic embryogenesis and plant regeneration from the callus cultures of non bulbing onions (*Allium fistulosum* L. 'Japanese Bunching' and *A. fistulosum* x *A. cepa* 'Beltsville Bunching' onion) has been successfully achieved. The seeds were germinated on a modified B₅ medium containing 4.5 M Z, 4-D and 4.6 M kinetin within 2 weeks after seed germination, prolific callus grew from radicles. Callus was embryogenic and formed shoots when transferred to a similar medium containing 7.0 M kinetin (Shahin and Kaneko, 1986). The rooting was successfully achieved by transferring cultures to an IBA fortified medium. Several thousand plants were grown to maturity and selfed seeds obtained from field grown plants were used for field evaluation and testing.

Protoplast culture

Attempts to isolate protoplast in onion (*A. cepa*) were reported about a decade back (Bracha and Sher, 1981) and in garlic (Opatrmy and Havranck, 1977). Novak *et al.* (1986) reported isolation of protoplast from garlic leaves in a mixture of enzymes containing onozuka R-10 (2%), macerozyme (1%) and driselase (1%). According to them, protoplast isolated from the young etiolated leaves formed clumps and did not divide. The presence of two to five nuclei under microscopic examination indicated about the possible fusion during isolation and protoplast obtained from green leaves survived without formation of any clumps. Besides, above *in vitro* techniques, organ fragment culture was initiated in onion using isolated flower heads.

Other *in vitro* Culture Technologies

Successful breeding of varieties through wide hybridization poses formidable challenges. Barriers to crossability among incompatible crosses are expressed at several stages during the complex process of fertilization and embryogenesis. Both pre and/post fertilization barriers act at various stages and result in premature abortion of embryo. Depending upon the nature of crossability barriers different *in vitro* techniques have been utilized to overcome these problems. Wide hybridization is often successful in yielding zygotes of young embryos, but these stop growth and abort before reaching maturity.

Embryo culture technique has proved very valuable in the interspecific hybridization programme, where infertility in F_1 hybrids due to inviability, weak seedling vigour etc. are associated. The isolation of immature fertilized ovule or embryo can help in the establishment of precious and difficult hybrids. The abortion of embryo is caused by genetic defects in the hybrid cells and/or absence or poor development of endosperm which provides nourishment to the growing embryo. Excision of embryo before abortion and culturing in appropriate medium has proved successful in yielding hybrids among a number of species of economic importance. The embryos are excised from ovules before the onset of abortion. The ovules are surface sterilized and the embryos are carefully dissected out and freed of the surrounding tissue before transferring to culture medium under aseptic conditions. Sometimes, embryo may need transfer from one medium to another. Embryo culture in *Allium* spp. has been applied to study physiology of seed germination and to make an assessment of optimal conditions for the growth of isolated embryos (Rijven, 1956; Guha and Johri, 1966). Alternation in the geotropic response of *Allium* spp. seedlings by use of growth regulator was also reported (Guha *et al.*, 1966). Besides, it was also used for wide hybridization to obtain reciprocal interspecific hybrids of *Allium cepa* $\times$ *A. fistulosum* (Dolezel *et al.*, 1980, 1982). According to the authors the number of plants produced through *in vitro* culture was significantly higher as compared to the traditional method. Haploid embryogeny and plant regeneration in unpollinated ovary culture was also reported in *Allium tuberosum* (Tian and Yang, 1989).

STATUS OF *IN VITRO* RESEARCH AT NPTCR/NBPGR

Extensive exploration and germplasm collection programme undertaken by the National Bureau of Plant Genetic Resources resulted in building up of sizeable germplasm collections in onion, garlic and other cultivated and wild *Allium* species. The Regional Station at Bhowali has contributed significantly in the collection of *Allium* species (Negi *et al.*, 1991, Negi and Pant, 1992). Rich germplasm collections were built up at NBPGR from diverse indigenous and exotic sources. Unfortunately, the field maintenance is beset with the problem of garlic mosaic virus (GMV) hitherto not known to be present in India which poses serious constraints.

Among the cultivated *Allium* species, onion (*A. cepa*) is propagated through seeds, although seeds are known to possess

short viability and their germination potential declines rapidly. These are orthodox seeds which can be desiccated to the lower moisture content (5%), thus enabling their conservation at low temperature (-20°C) in genebank. Among other wild species, *Allium tuberosum*, *A. porrum* (*A. ampeloprasum*) and *A. carolinianum* can also be suitably conserved in genebank without much deterioration. Cryopreservation could provide another alternative for long term storage of seeds at the ultra low temperature of liquid nitrogen (-196°C) or using CO_2 refrigeration.

For vegetatively propagated plant species or those which are devoid of seed producing mechanism, tissue culture (*in vitro*) techniques have been integrated in national programme whereby both micropropagation and *in vitro* conservation could be accomplished.

The *in vitro* conservation appears to be feasible, durable, and perhaps economical in the conservation of biological diversity in clonally propagated agri-horticultural crop plants including cultivated *Allium* spp. and their wild relatives.

Germplasm collections of garlic (*Allium sativum* L.) and other *Allium* species were screened for virus infection. (Kumar *et al.*, 1990; Khetrpal *et al.*, 1991). Mosaic type of symptoms were observed in *A. sativum*, *A. ampeloprasum* and *A. ascalonicum*. Leaves and bulbs of garlic produced by micropropagation were found to be infected with garlic mosaic virus (GMV) as determined by DAS-ELISA technique. However, *A. tuberosum* neither showed any virus disease symptoms nor reacted with GMV antiserum in DAS-ELISA. Efforts were initiated to eliminate the viruses from infected germplasm by meristem tip culture. The occurrence of this specific virus (Kumar *et al.*, 1990) brought to the fore an importance of evolving alternate strategies for the maintenance of valuable germplasm resources. This necessitated the urgency and need that germplasm be subjected to rigorous screening for freedom of virus enabling the salvation (cleaning) through tissue culture technique. Micropropagation employing tissue culture technique was initiated on bulbous crops. Among the prominent crops, garlic (*Allium sativum*) was accorded high priority due to the serious problem created by garlic mosaic virus (GMV). *In vitro* technology has now been accorded high priority as a sound strategy to achieve rapid clonal propagation as well as to eliminate virus from garlic germplasm using meristem culture technique.

In case of garlic the dormant shoot buds were cultured on B₅ basal medium as suggested by Gamborg (1968) using shoot bud half as an explant. These subsequently formed single shoot. On media supplemented with hormones (2-ip and NAA), multiple shoots could be formed, both axillary and adventitiously on cultured explants. The number of proliferating shoots ranged from 2 to 10. Rooting occurred on the same medium. Shoots upon transfer to fresh medium either remained single or formed multiple shoots in about two to four weeks. However, upon prolonged culture under 16h photoperiod, *in vitro* bulblets formation also occurred in most of the garlic varieties/accessions. The number of bulblets formed *in vitro* varied from 2-10 and on transfer to the soil under field conditions, plantlets established very well. Currently *in vitro* culture work has been extended to *A. ascalonicum*, *A. tuberosum*, *A. bakeri*, *A. rubellum*, *A. stratechei*, *A. consanguineum*, *A. prezwalskianum*, and *A. carolinianum*.

Among the cultivated species micropropagation protocols reported earlier have been used; in some cases they have been modified and refined as per the need. These species include garlic (*A. sativum*), shallot (*A. ascalonicum*) and Chinese chive (*A. ampeloprasum*). Among the wild *Allium* plant genetic resources in which successful micropropagation has been achieved after development of suitable protocols include : *A. tuberosum* (Pandey *et al.*, 1992), *A. bakeri*, *A. rubellum*, *A. carolinianum*, *A. prezwalskianum*, *A. stratechei*, *A. consanguineum* etc. The *in vitro* production of bulblets could be successfully used for the establishment of plantlets in the field. Healthy bulblets were also produced from field established plants of *A. sativum*, *A. ampeloprasum*. Investigations are further continued on other species and work is at different stages of development.

In vitro Conservation

Recent investigations have clearly shown the feasibility on *in vitro* conservation of diverse plant species (Withers, 1980; Henshaw, 1984; IBPGR, 1985; Ford Lloyd and Jackson, 1986). Different plant parts appear to be amenable to preservation such as shoot tips, meristem, axillary buds, nodal segments, callus, cell suspension cultures, embryos and embryonic axes. Both short and medium term conservation can be achieved employing the techniques of slow growth (through minimal media), through reduction of nutrients (carbon source or the macro/micro nutrients), use of

chemical retardants or the osmoticum (sorbitol/mannitol). The latter has proved to be immensely beneficial particularly the inclusion of mannitol. The conservation of *in vitro* cultures could perhaps be accomplished by combining above protocols with appropriate low temperature regime which is quite variable with different tropical/subtropical and temperate species. Long term storage is accomplished employing preservation in liquid nitrogen (-196°C). Investigations carried out so far clearly suggested a possibility for the conservation of diverse kind of material including *in vitro* culture systems.

Experimental data accumulated so far on *in vitro* conservation of several *Allium* species have demonstrated the distinct feasibility of medium to long term conservation of *Allium* species. Studies carried out at the National Plant Tissue Culture Repository showed that *in vitro* conservation under normal standard culture conditions ($+25\pm 2^{\circ}\text{C}$, 16 h/8 h light dark period) allows survival of cultures upto 8-12 months provided cotton plugs are replaced by polypropylene caps (Balachandran *et al.*, 1990; Sharma *et al.*, 1991; Sharma and Chandel 1992a, b; Pandey *et al.*, 1992), while normal sub culture cycle is about 8-12 weeks. Low temperature of incubation (10°C and 4°C) of shoot culture of garlic (*A. sativum* L.) further enhanced their shelf life upto 14 to 18 months, respectively. The survival percentage varied from 40-70 which is further investigated to upscale it to reasonably high level. Modification of normal nutrient medium by inclusion of high concentration of sugar (4 - 10%) and agar (1.6%) has also proved beneficial in prolonging the subculture duration in our investigations. Cultures after prolonged storage (16 months) could be transferred to field and plantlets exhibited normal growth and morphology.

Effect of reduced temperature upon estimated survival of garlic shoot tip culture after 4, 10 months and 16 months were investigated (El-Gizavy and Ford Lloyed, 1987). In their experiment, shoot tip culture survival was considerably low under controlled temperature $+25^{\circ}\text{C}\pm 2^{\circ}\text{C}$. They reported increase in the rate of survival with decreasing temperature so that after 4 months, 90-100 per cent survival occurred at 4°C and after 16 months storage, only the cultures maintained at 10°C at slightly higher sucrose concentration showed survival. The *in vitro* cultures of different cultivars survived between a range of 40-80 per cent depending on the variety.

In our investigations, garlic shoot tip culture could be preserved for about 4 months without subculture avoiding all deleterious effects. Storage of cultures at low temperatures both 4°C and 10°C enhanced the survival percentage in our experiments also similar to that reported earlier by El-Gizavy and Ford Lloyed, (1987).

In vitro propagation of semi wild *Allium tuberosum* (the Chinese chive) which occurs wild in the north western Himalayas was achieved through shoot proliferation. (Pandey *et al.*, 1992). Halved shoot bases of *Allium tuberosum* proliferated both axillary and adventitious shoots on B₅ medium (Gamborg *et al.*, 1968) supplemented with either 6-benzylaminopurine (0.5 mg/l) or 1-naphthalene acetic acid (0.1 mg/l) and 2-isopentenyladenine (0.5 mg/l). *In vitro* shoots proliferated further upon subculture to fresh medium and rooted spontaneously. Plantlets were transplanted successfully to soil and they retained the diploid condition of the parents. For propagation of a desirable strain, the methods of axillary and adventitious shoot bud proliferation appears to be more appropriate as shown by Pandey *et al.* (1992). It is because they generally offer higher rates of production of phenotypically uniform clones compared to plants regenerated from callus. The limited information on tissue culture of *A. tuberosum* included regeneration of haploid plants from unpollinated ovaries (Hui-quiao and Hong-Yuan, 1989) and the occurrence of apomictic plants from unpollinated ovule cultures (Kojima and Karraguchi, 1989). The regeneration of plantlets from callus was also earlier reported (Zee *et al.*, 1977). In *A. tuberosum*, differentiation of only shoots from callus cultures was observed which was accompanied by karyological instability in callus cells (Roy, 1980). The *in vitro* conservation studies on *A. tuberosum* were carried out recently following the successful micropropagation protocols reported earlier (Pandey *et al.*, 1992). The shoot cultures were successfully maintained for nine months simply by replacing cotton plugs with polypropylene caps that fitted well with Borosil glass tubes and maintained at normal culture conditions (25±2°C). The death of *in vitro* cultures with cotton plugs was attributed mainly due to nutrient depletion and desiccation. Similar results were obtained earlier in ginger and turmeric (Balachandran *et al.*, 1990) and in *Coleus forskohlii* (Sharma *et al.*, 1991). The enhancement of shelf life was further accomplished by reduction in temperature regime, thus *A. tuberosum* cultures could be maintained upto 18 month without any intervening subculture in between.

Chromosomal instability inducing either polyploidy or aneuploidy has been found to occur in the callus derived cultures of *A. cepa* (Yamane, 1975; Sekerka, 1977 a, b and Roy, 1980); *A. cepa* var. *proliferum* (Nandi *et al.*, 1977) and *A. tuberosum* (Roy, 1980). The nature and behaviour of B chromosome in *Allium stracheyii* was shown by Sharma and Aiyanger (1961) and Sen (1974). In our studies, in order to assess the genetic stability of *in vitro* regenerated plants of *A. tuberosum* Rottl. ex Spreng. the assessment of genetic variants (somaclonal variation) was carried out first on field grown material after plants were regenerated and transferred in the field. Subsequently to monitor any changes in the ploidy level or to detect any induced chromosomal abnormalities cytological approach was adopted. Karyological studies of the regenerants indicated that majority (90%) of the cells had shown normal complements.

Meiotic analysis of regenerated plants revealed that by and large they did not differ from the control plants and cytological parameters such as associations, chiasma frequency, terminalization coefficient and pollen stainability showed no significant change (Rao *et al.*, 1992). Interestingly, quadrivalent range and frequency showed a considerable reduction in regenerated plants. C-banding patterns in chromosomes of *Lilium* (Liliaceae) has been also applied for such studies (Synth *et al.*, 1989). Nucleolar activity of B chromosome in *Allium cernuum* (Alliaceae) was demonstrated in a critical study (Bernd Friebe, 1989). It is true that changes in chromosome number are not the only criteria to assess the rate of cytogenetic instability of the *in vitro* produced cultures. The occurrence of chromosome fragments, deletion, ring chromosomes etc. are considered some of the typical features of a long term garlic callus culture (Novak *et al.*, 1986). Accordingly, callus lines with the highest frequency of polyploid mitosis exhibited the maximum rate of chromosomal aberrations and mitotic irregularities (Novak, 1981) comprising disturbances of the mitotic spindle, multipolar mitosis binucleate cells and occurrence of micronuclei. Some alterations in the chromosome length and centromere position in the karyotype of diploid callus cells of *A. cepa* were reported by Sekerka (1977a, b). Similarly, regeneration of cytochimerical plants of *A. sativum* callus culture is probably conditioned by multicellular origin of regenerating shoot meristems. According to Novak *et al.* (1986) the manifestation of somaclonal variation occurred in the long-term garlic callus culture derived plants in the characters such

as plant height, leaf number, leaf position, bulb weight and shape, leaf number inside the bulb and bulb scale colour. In all instances, aerial bulbils, typical for the cultivar used, were formed in the inflorescence. Thus somaclonal variations generated plants and their offspring could perhaps be of considerable importance for the improvement of *Allium* species.

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DISTRIBUTION, DOMESTICATION AND DIVERSITY OF *CUCUMIS* SPECIES COMPLEX IN INDIAN SUB-CONTINENT

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*The sub-continent of India is considered to be the place of origin for cucumber (*Cucumis sativus* L) and a centre of diversity for melon (*C. melo* L). Centres of origin and diversity have long been regarded as valuable sources of both biotic and abiotic plant stresses. A large number of variants are cultivated traditionally in different parts of India, which may have evolved through natural and human selection aided by repeated introgression from wild forms. An account of distribution, of *Cucumis* species complex including wild relatives and their domestication and diversity mainly in Indian sub-continent has been reviewed. Crop variability, genetic erosion status and priorities for *Cucumis* species collection in India and elsewhere are also discussed.*

Indian sub-continent is considered to be the centre of origin for cucumber (*Cucumis sativus* L) and a centre of diversity for melon (*C. melo* L) (Zeven and de Wet, 1982). Diversity is reported to occur in several *Cucumis* species viz., *C. hardwickii* Royle, *C. hystrix* Chakra., *C. setosus* Cogn., *C. trigonus* Roxb. (syn. *C. callosus* Rottl. Cogn.) and *C. prophetarum* L including cultivated cucumber and melon in this region (Arora and Nayar, 1984). The use of wild and cultivated cucurbits in India dates back to pre-historic times. Melon seeds were prominent among the stored materials excavated from the archaeological sites at Harappa and other locations (2000-1700 BC). A strikingly large number of variants evolved gradually through introgression and selection from wild forms, many of which are still cultivated in different parts of India. These landraces contain valuable genes for adaptability to diverse agro-ecological zones, resistance to diseases and pests as well as stress

environments including drought on one side and govern other desirable traits like flavour, taste and flesh characteristics on the other. This paper reviews distribution of cultivated *Cucumis* species (*Cucumis sativus* L and *C. melo* L) and their wild relatives, their domestication and diversity mainly in the Indian sub-continent. Based on distribution of genetic variability, existing collections and genetic erosion status, the priorities for *Cucumis* species collection in India and elsewhere are also discussed.

DISTRIBUTION AND DOMESTICATION

The genus *Cucumis* comprises about 30 different species which are distributed over two geographically distinct areas (Esquinas-Alcazar and Gulick, 1983). South-east of Himalaya is an important region for Asiatic group, of which cucumber (*Cucumis sativus*) is a member. The African group is spread over large parts of Africa and the Middle East to central Asia extending to Pakistan and southern Arabia. Most species with basic number $X=12$ are found in this region with a few tetraploids and hexaploids. Host-parasite and phytogeographic studies led Leppik (1966) to propose North-east Africa, Arabia and the eastern Mediterranean areas as the primary gene centre and south Africa as secondary gene centre of the genus *Cucumis*. The cultivated species include *C. sativus* L. *C. melo* L and *C. anguria* L. Among the wild species, 17 have been identified from southern Africa (Meeuse, 1962) and 13 from tropical eastern Africa (Jeffrey, 1967). Of these, *C. prophetarum* migrated to India, whereas *C. hardwickii* Royle is indigenous to India. For *C. melo*, India, Turkey and Afghanistan have been recorded as secondary gene centres (Whitakar and Bemis, 1976). Cucumber (*Cucumis sativus*) apparently was domesticated in India (de Candolle, 1886). It shares the chromosome number of $2n=14$ with *C. hardwickii*, found wild in the foothills of Himalayas. The free hybridisation with cultivated *sativus*, with no reduction of fertility in F_2 generation suggested that *C. hardwickii* is likely progenitor of cultivated cucumber. However, the absence of semi-wild or wild forms of cucumber unlike other species, like *C. melo* (Seshadri, 1986) does not provide an evidencing support to the hypothesis which has extensive distribution in Africa. At one point $X=7$ was considered primitive in *Cucumis* and was thought to have given rise to $X=12$ through fragmentation of chromosomes (Whitakar, 1933; Ayyangar, 1967 and Bhaduri and Bose, 1947). However, this hypothesis could not be confirmed cytologically and $X=12$ is now regarded as primitive (Yadava *et al.*, 1944). The base number $X=7$ probably evolved from $X=12$ (Singh and Roy, 1974). *Cucumis* taxa with $X=12$ have greater phenotypic variability and

geographic distribution, and despite the higher chromosome number, they have almost the same half chiasma per chromosome value as taxa with $X=7$ (Yadava *et al.*, 1984). These factors suggest that both group of species have a common chromosome complement. The evolution of species with $X=7$ may have been favoured to restrict recombinations by reducing the chromosome number but retaining the same chiasma frequency. This could be a factor contributing to their comparatively restricted phenotypic variability and distribution.

The species of African group of *Cucumis* are mostly diploid with basic chromosome number $X=12$. The Asiatic group is also diploid, but with a chromosome number of $X=7$. The fragmentation theory of Bhaduri and Bose (1947) suggests that *C. melo* with chromosome number 24 was derived from *C. sativus* or a closely related species. Trivedi and Roy (1970) proposed another hypothesis of fusion or translocation of the chromosomes for the origin of *C. sativus* from *C. melo*. Ramachandran and Seshadri (1986) are of the view that the fragmentation theory and their own hypothesis of reduction by haploidy and fragmentation are hardly tenable for establishing a relationship between cucumber and muskmelon.

Interspecific hybridisation provided useful information on species relationships. Deakin *et al.* (1971) presented the first comprehensive data on cross-compatibility relationships of *Cucumis* species and on the pollen fertility of F_1 hybrids. As a result, *Cucumis* species was placed into four groups : (1) the Anguria group includes all the spiny fruited interfertile annual, perennial, and advanced polyploid species; (2) the Melo group, comprising the wild, non-spiny species and the cultivated *C. melo*; (3) Metuliferus group, represented by *C. metuliferus*, having distinct morphology and being cross-incompatible with other *Cucumis* species; and (4) Sativus group, containing the cultivated *C. sativus* and the wild *C. hardwickii* with $2n=14$. Jeffrey (1980) classified the entire genus *Cucumis* into two subgenera :

- (i) Sub-genus *Cucumis* ($X=7$), there are 3-4 Chinese-Himalayan species including *C. sativus*, *C. callosus*, *C. hystrix* Chakra. and *C. bisexualis*.
- (ii) Sub-genus *Melo* ($X=12$), 30 species mostly tropical and south African
 - (a) *anguria* group-dioecious, monoecious or andromonoecious; perennials with yellowish-brown striped fruits, closely related and partially fertile inter-specific

hybrids; *C. anguria*, *C. dispacus*, *C. prophetarum*, *C. myriocarpus* and *C. aculeatus*.

- (b) *melo* group — monoecious, andromonoecious; perennials or annuals with smooth fruits; *C. melo* L, *C. sagittatus* Peyr., *C. dinteri* and *C. humifructus* Stent.
- (c) *metuliferus* group — monoecious annuals with red spiny fruits; *C. metuliferus* Naud.
- (d) *hirsutus* group — dioecious, perennials with smooth orange fruits, *C. hirsutus* Sond.

In India, according to Chakravarty (1982), only 6 species occur. Some are cultivated. Five wild species of *Cucumis* included *C. agrestis*, *C. hystrix*, *C. setosus*, *C. prophetarum* and *C. hardwickii*. *C. callosus* occurs mainly in Punjab, Himachal Pradesh, Rajasthan, Uttar Pradesh, Bihar, Orissa, Tamil Nadu and Karnataka. Wild related species of *C. sativus* are *C. hardwickii* and *C. trigonus*. *C. hardwickii* — a small fruited bitter cucumber with sparse and stiff spines has been found wild in the foothills of north-west Himalayas and southern hills; *C. setosus* is endemic to Maharashtra state; *C. prophetarum* to Sirohi (Abu) of Rajasthan, Gujarat, Maharashtra, Tamil Nadu and Karnataka and *C. hystrix* to Meghalaya, Assam and Mizoram. *C. sativus* L. is the only cultivated species indigenous to India.

DIVERSITY OF CULTIVATED SPECIES AND THEIR WILD RELATIVES

(a) Cucumber (*Cucumis sativus* L)

Cucumber is one of the oldest cultivated vegetable crops. It has been known in history for over 5000 years and is considered to be native to India. It originated in India in the north-western foothills along Nepal Himalayas. Later, it was introduced into Europe, Near East, China and elsewhere, being the secondary gene centre (Zeven and de Wet, 1982). The Near Eastern centre is known for xerophytic types. It carries sources of resistance to powdery mildew, *Sphaerotheca fuliginea* from China and Japan. Cucumber is the only cultivated species in the genus with chromosome number $2n=14$, while other species have $2n=24$ and are mostly African. Cucumber has two forms — creeping one, cultivated in the fields during hot season while climbing forms cultivated during rainy season. Morphologically they differ in fruit size.

Genetic variation present in the cultivated varieties of *C. sativus* does not seem to be sufficient to solve the specific problems connected with the improvement of this plant species. For the increase of genetic variability concerning quantitative inherited characters, and especially as sources of resistance, collection of material from natural habitats of *C. sativus* is essential. *C. sativus* var. *hardwickii* which crosses readily with cucumber since it lacks character for fruit inhibition (Horst and Lower, 1978).

Cucumber is grown throughout India, but maximum genetic diversity occurs in north India with respect to bearing habit, maturity, yield, shape, size, skin colour, skin ornamentation, spines and vine habit etc. Cultivars differ in adaptability/suitability to different seasonal/climatic conditions viz., (a) hot weather type, creeping small fruits, dark-green/sometimes rusty-brown, ovoid with thin and smooth rind, called *Gherkins* grown at the beginning of the spring season under irrigation, less popular but generally preferred for pickling; (b) rainy season crop, early cluster small fruits with scattered black spines, medium green fruits with black spines, long fruits with spines, netted types with cracking skin on ripening. Large fruits with thick rind, suited to wide variety of soils in hills or plains, more popular, used for salads or for cooking in curries, are also available. In north-eastern and western Himalayas, long fruited genotypes occur. Folk selection pressure has resulted in the development of (i) very long fruited types particularly in western India including foothills of Himalayas and (ii) medium and small fruited types in gangetic plains.

In cucumber, two varieties are reported. *C. sativus* var. *sativus* has 3-5 lobed leaves, ovary usually 3-placentiferous with fruits oblong, obscurely trigonus or cylindric. This variety is cultivated all over India and in all tropical and sub-tropical regions. Another variety, *C. sativus* var. *sikkimensis* Hook. f. has 7-9 leaves, ovary often 5-placentiferous, fruits ovoid-oblong, adapted both to temperate and humid climates. *C. hardwickii* is a wild type related to *C. sativus*, adaptable to cold climate and confined to western Himalayas. It has 5-angular ovate leaves, glabrous/tuberculate fruits much smaller than cucumber. The fruits have sparse and stiff spines.

In India, immature and mature fruits are eaten raw as slicing cucumber in salad. It is rarely used as vegetable. Strikingly large number of variants are still cultivated traditionally in different parts of India and these have evolved gradually through human selection aided by repeated introgressions from wild forms. It is estimated that about 95-100 distinct landraces are available in different agro-ecological/eco-geographical zones of India. Locally

adapted landraces included **Sawanariya** (from Sikar, Laxmangarh Shekhawati areas and Chomun in Jaipur); **Balamkakdi** (from Tonk, Kir-ka-kheda, Pindwara and Abu Road); **Balamkhira** (from Nalchha in Dhar district, Malwa region, Madhya Pradesh) and **Blus** (from Bhikangaon/Pati areas in west Nimar region, M.P.) (Fig 1) and many others. The landrace variability in Rajasthan is attuned to dry conditions and adapted under ago-ecological niches with acute water stresses. Amazing diversity in fruits has been sampled. Germplasm collections varied in plant habit (bushy to prostrate), in sex type (monoecious generally to gynomonoecious/ andromonoecious), fruit shape (elliptical, elongated, oblong, globular, stem-end tapered, blossom-end tapered), fruit skin colour (yellowish-white, light-green, medium-green, dark-green, yellow, with uneven colour to dark-green colour at stem-end portion and light-green with whitish splashes on other parts of the fruit), fruit skin mottling, colour of stripes (absent, white, green and yellow), in fruit skin glossiness (dull, glossy), fruit length (15-46.2 cm), fruit width (7.2-30 cm), fruit weight (110-4900g), in stem-end/ blossom-end fruit shape (depressed, flattened, rounded and pointed), flesh texture (smooth, grainy, soft), flesh colour (white, greenish white, green) and in seed coat colour (white, tan, yellow) (Umesh Chandra and Koppar, 1992).

In Madhya Pradesh, variability occurred in cucumber distinctly in two types. One type with fruit length 30-36 cm with diameter of 7-15 cm. The fruits on maturity had smooth, greenish skin with mostly both end of the fruit equally broad. In other type, the fruit length varied from 15-36 cm with range of diameter 15-22 cm, skin was rough with rusty brown colour. Uttar Pradesh collections varied in fruit shape, fruit ribs (generally absent to occasionally superficial), fruit skin colour and flesh texture (Fig 2).

Some of the newly bred cucumber cultivars included Japanese Long Green, Poinsette, Solan Green, Kalianpur Green, Straight Eight, Poona Kheera, Kheera Prome, Sheetal, Balamkhira and F₁ hybrid Pusa Sanyog (CSC, 1985).

(b) Melons (*Cucumis melo* complex)

India is considered as one of the regions of diversity for melon (*C. melo* L.) which is not to be confused with watermelon (*Citrullus lanatus*). *C. melo* (muskmelon) is originated in Africa. The primary centre of diversity in melon is south-west and central Asia, mainly Turkey, Syria, Iran, Afghanistan, north and central India, and Transcaucasia, Turkmenistan, Tadikistan and Uzbekistan. Secondary centre of diversity are China, Korea, Portugal and Spain (Esquinas – Alcazar and Gulick, 1983). Chinese and Japanese melons have small fruits and an unpleasant strong taste.

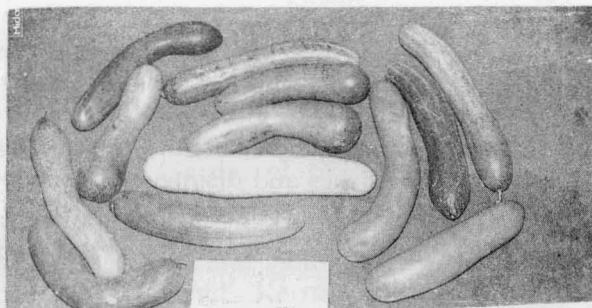


Fig. 1. : 'Balam Kakdi' landrace from Abu Road, Rajasthan

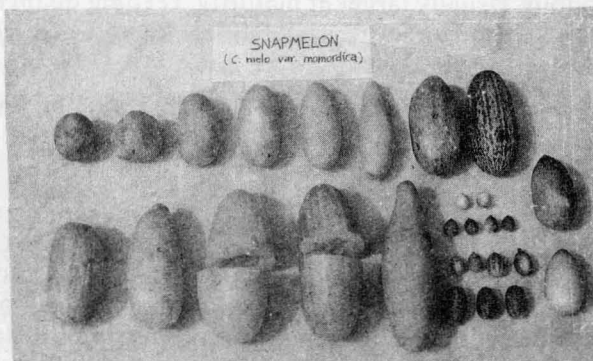


Fig. 2: Snapmelon — variability in shape and size of fruits

Munger and Robinson (1991) suggested 7 horticulturally important melon groups :

1. *C. melo agrestis* (Kachri) : Wild types with slender vines and small, inedible fruits. Probably synonym of *C. melo callosus* (*C. callosus*) and *C. melo trigonus* (*C. trigonus*).
2. *C. melo cantalupensis* Naud. (muskmelon or cantaloupe) : Medium sized fruits with netted, warty, or scaly surface, flesh usually orange but sometimes green, aromatic or musky flavour. Fruits dehiscent at maturity. Usually andromonoecious.
3. *C. melo flexuosus* Naud. (*C. melo* var. *utilissimus* in Indian literature) : snakemelon, snake cucumber, Tar-Kakri; fruit

long and slender, used when immature as an alternative to cucumber, monoecious.

4. *C. melo momordica* (*C. melo* var. *momordica*); **phut** or snapmelon, grown in India and other Asian countries and distinct from any other group. Flesh is white or pale orange, low in sugar and mealy. The smooth surface of the fruit cracks as maturity approaches and disintegrates; monoecious.
5. *C. melo conomon* Mak. (pickling melon or sweet melon): small fruits with smooth skin, white flesh and early maturity, andromonoecious.
6. *C. melo inodorus* Naud. (winter melon): smooth or wrinkled surface with flesh usually white or green and lacking musky odour. Usually larger at maturity, keeping quality longer than muskmelon, and indehiscent at maturity. Mostly andromonoecious.
7. *C. melo dudaim* Naud. (mango melon, pomegranate melon): Fruits small globular, smooth, mottled but not netted, flesh with acid flavour. pickling type.

Out of 7 popular melons, *C. melo agrestis* (syn. *C. melo callosus* or *C. callosus*), *C. melo cantalupensis* (muskmelon); *C. melo* var. *utilissimus* (*C. melo flexuosus*), snakemelon, snake cucumber, **Tar Kakri**; *C. melo* var. *momordica* (**phut** or snapmelon); are available in Indian sub-continent. Snapmelon flourishes well in local regions of Jodhpur, Barmer, Bikaner and adjoining areas (Bhandari, 1967). It is an important cucurbit and is used especially by poor communities in famine. Monoecious types are common in snapmelon. This area, the 'Goldmines' of snapmelon has tremendous diversity in this crop. The germplasm varied in fruit shape, fruit ribs (generally absent but occasionally superficial to deep ridging), fruit skin colour, texture (smooth, grainy, with spines, with stripe, streaked), blossom scar (generally obscure but occasionally conspicuous), fruit length, (2.5-34.0 cm), fruit width (2.5-13.0 cm), fruit weight, (6.8- 149.2g) and flesh colour. **Kachri** melons (*C. melo agrestis*) have more or less uniform diversity in Rajasthan, Madhya Pradesh, Uttar Pradesh and southern states. **Tar Kakri** or snake cucumber (*C. melo* var. *utilissimus*) is grown throughout India and shows a wide variation. Muskmelon occurs more in *tarai* region and western Uttar Pradesh, Chhotanagpur area, West Bengal, Assam, Karnataka and coastal Andhra Pradesh, and possesses interesting variation. In **Kachri**, several fruits of large size are observed in Madhya Pradesh which may be due to introgression of semi-wild *C. melo agrestis* with cultivated melons.

Further studies are needed to establish such facts (Umesh Chandra and Koppar, 1992).

Some of the newly bred varieties included in muskmelon — 'Arka Jeet', an improved variety from local 'Bati' of Lucknow, 'Arka Rajhans' — from Karnataka, Sel. 445 from Rajasthan, 'Allahabad Khajira', 'Lucknow Safeda' from U.P., 'Durgapura Madhu' from Rajasthan, 'Kutana' and 'Punjab Sunheri' from Punjab, 'Bathese' from Andhra Pradesh, 'Sarda' of M.P., 'Gujarat Muskmelon 1', 'Gujarat Muskmelon 2', 'Hara Madhu', 'Pusa Madhuras', 'Sharbat-e-Anar'; 'Hingan', 'Jalbudata', are other promising varieties. 'Pusa Sharbati' and 'Punjab Hybrid' are crossbred varieties. 'Arka Sheetal' and 'Karnal selection' are snakemelon varieties. (Thomas *et al.*, 1983).

(c) Other *Cucumis* species

In *Cucumis*, for *C. hardwickii* and *C. trigonus*, much variability occurs in the Himalaya. While *C. setosus* is restricted to eastern plains, *C. hystrix* extends its range from eastern plains to north eastern hills in Assam, Tura range in Meghalaya and Mishmi hills. Localized variability at species level occurs in western ghats for *C. hystrix* and *C. setosus* in comparison to drier northern plains, where only *C. prophetarum* occurs (Arora and Nayar, 1984; Table 1). Apart from these, *C. aculeatus* Cogn. (Ethiopia), *C. anguria* (Ethiopia and S. Africa), *C. dispacus* Spach. (an ornamental from Africa), *C. ficifolius* A. Rich. (Ethiopia), *C. frageri* Naud. (wild in Nigeria), *C. metuliferus* Naud. cultivated as ornamental and as fruit in Africa and *C. zeyheri* Sond. (from S. Africa), are species of African region. Recently four new species from Somalia viz., *C. jefferyanus*, *C. pubituberculatus*, *C. baladensis* and *C. hastatus* have been added to the genus (Thulin, 1991).

CUCUMIS SPECIES AND DISEASE RESISTANCE

In cucumber, there is lack of resistance to some important pathogens and insects/pests such as cucumber green mottle virus, nematodes etc. *C. hardwickii* possesses resistance to green mottle virus, downy and powdery mildews. *C. hystrix* Chakra. carries resistance to rootknot nematode, powdery mildews and tolerance to green mottle mosaic virus. High level resistance to rootknot nematode occurs in *C. metuliferus*. *C. sativus* var. *sativus* landraces collected from Rajasthan possess resistance to powdery mildews (*Sphaerotheca fuligena*) and tolerance to green mottle mosaic virus (Table 1).

Table 1 : Indian *Cucumis* species, their distribution and useful characteristics

Crops species	Distribution	Useful characteristics
<i>Cucumis sativus</i> var. <i>sativus</i>	Gujarat, Rajasthan, Uttar Pradesh, Cachar (Assam) and Khasi Hills (Meghalaya)	Resistance to powdery mildew (<i>Sphaerotheca fuliginea</i>), tolerance to green mottle mosaic virus
<i>C. sativus</i> var. <i>hardwickii</i>	Foothills of Himalayas, DehraDun and Mussoorie (UP), Himachal Pradesh, Jammu & Kashmir	Resistance to cucumber green mottle mosaic virus, downy (<i>Pseudoperonospora cubensis</i>) and powdery mildews
<i>C. sativus</i> var. <i>sikkimensis</i>	Arunachal Pradesh, Sikkim, Nagaland and Manipur	Adaptable to cold climates; resistance to Anthracnose (<i>Colletotrichum orbiculare</i>)
<i>C. hystrix</i> Chakra.	Plains of eastern India, north-eastern hills and Assam valley western ghats, eastern India Upper gangetic plains, West Bengal and peninsular region	Resistance to root-knot nematode, powdery mildews and tolerance to green mottle mosaic virus
<i>C. setosus</i> Cogn.	Eastern India, upper gangetic plains, West Bengal	Resistance to anthracnose, adapted to high rainfall areas
<i>C. profetorum</i>	Largely distributed in the dry north-western plains, Rajasthan (Sirohi district) and adjoining belt	Resistance to root-knot nematode, drought resistance
<i>C. callosus</i> (syn. <i>C. trigonus</i>)	Widely distributed in eastern and western ghats north-eastern region, extending to Himalayas	Drought resistance
<i>C. melo</i> var. <i>momordica</i>	Rajasthan, Uttar Pradesh, Madhya Pradesh, Khasi Hills (Meghalaya), West Bengal	Drought resistance; resistance to downy mildew.
<i>C. melo</i> var. <i>agrestis</i> (Kachri)	Rajasthan, Madhya Pradesh, Uttar Pradesh and Southern India	Drought resistance

PRIORITIES FOR GERMPLASM COLLECTION

Based on distribution of genetic variability, existing collections and genetic erosion status (Table 2), first priorities included southwestern Asia, from Turkey to Afghanistan (*C. melo*); India, sub-Himalayan tract to north-eastern hills, western ghats, eastern peninsular tract and Indo-gangetic plains (*Cucumis* species wild and cultivated); and Myanmar and southern China (*C. melo*). Second priorities included India, South-east Asia, Southern China (*C. melo* and wild *C. sativus*), tropical Africa, especially Sudan (*C. melo*), tropical and southern Africa (*C. anguria*) and Iberian peninsula (*C. melo*).

Table 2 : Crop variability and genetic erosion status in Indian region

Crop	Cultivated/ wild	General variability status	Genetic erosion status
<i>Cucumis sativus</i> (Cucumber)	Cultivated	Very high	High
<i>C. setosus</i>	Wild	Low	High
<i>C. profetarium</i>	Wild	Low	High
<i>C. hardwickii</i>	Wild	High	High
<i>C. melo</i>	Cultivated	Very high	Medium
<i>C. melo</i> var. <i>momordica</i> (Snampmelon, phut)	Semi-wild/ cultivated	Very high	Low
<i>C. melo</i> var. <i>utilissimus</i> (Snakemelon, Tar-kakri)	Cultivated	Medium	Medium
<i>C. melo</i> var. <i>agrestis</i> (Kachri)	Semi-wild	High	Low

Melons are insect – pollinated; much genetic diversity may, therefore, exist within a population. This diversity may not be obvious in small population; plant collectors should, therefore, sample enough within population diversity by collecting seeds from larger plant populations in the field.

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SELECTION OF DIVERSE PARENTS USING MULTIVARIATE ANALYSIS IN TOMATO (*LYCOPERSICON ESCULENTUM* Mill.)

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*Evaluation of genetic diversity within germplasm collection is important to plant breeders who desire source of genes for particular traits. The twenty five genotypes of tomato (*Lycopersicon esculentum* Mill.) collected from various countries were evaluated for quantitative characteristics in an attempt to differentiate among the lines. The genotypes could be grouped into seven clusters of phenotypic diversity. There was no correspondence between the geographic collection sites of genotypes and their inclusion in particular cluster. Analysis was useful for identification of potentially different sources of desired traits. It was noticed that the entries of the cluster III and VII showed the maximum distance and the crosses between genotypes in these clusters will produce heterotic hybrids. The use of these genotypes in future breeding programme should greatly help to cross the yield plateau in this crop.*

Genetic diversity among parental lines is an important factor for obtaining heterotic hybrids (Moll *et al.*, 1962). Statistical techniques such as D² analysis which quantify the differences among the quantitative traits is an efficient method to evaluate genetic diversity. It is generally agreed that genetically diverse parents will show maximum heterosis and offer the maximum chances of isolating transgressive segregants. Therefore, the present

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study was carried out using D^2 analysis to classify twenty five lines into groups on the basis of quantitative characters.

MATERIALS AND METHODS

The experimental material comprised of twenty five exotic lines of tomato from Australia, Canada, Czechoslovakia, Denmark, England, France, Iraq, Japan, USA, Russia and Yugoslavia were grown at Defence Agricultural Research Laboratory, Hawalbagh during 1990, in randomized block design with three replications. The plots were three meters long and one meter wide, each of them comprising two rows spaced at 50 cm. Plant density was six plants per linear meter. Days to 50 per cent flowering and yield were recorded on plot basis. Five competitive plants were selected from each genotype in each replication to record the observations on plant height, number of clusters per plant, number of fruits per cluster and number of fruits per plant. Mahalanobis (1936) D^2 analysis was used for assessing the genetic divergence between the populations. The genotypes were grouped into a number of clusters by Tocher's method (Rao, 1952).

RESULTS AND DISCUSSION

The twenty five genotypes were highly significant for the characters studied. The coefficient of variation was the maximum for number of fruits per plant followed by yield per plant (Table 1). The coefficient of variation at environmental level also showed

Table 1 : Means and coefficient of variation for different characters

Characters	Days to 50% flowering	Plant height (cm)	No. of clusters per plant	No. of fruits per cluster	No. of fruits per plant	Yield Q/ha
General Mean	55.92 ± 0.74	51.27 ± 1.34	16.40 ± 2.70	3.21 ± 0.42	23.20 ± 4.88	117.80 ± 15.40
C.V. (%)	2.31	4.55	8.52	2.79	12.74	12.47
C.D. 5%	2.12	3.83	7.67	1.20	13.89	43.98
C.D. 1%	2.83	5.12	10.24	1.60	18.53	58.68

similar pattern but magnitude of the values were less than corresponding genotypic values. The genotypes were grouped into seven clusters (Table 2). Cluster I contained the maximum number of genotypes (8). These genotypes were of early to medium flowering duration. Cluster II comprised five genotypes of similar duration of flowering to that of Cluster I but comparatively higher number of fruits and yield per plant. Four genotypes of cluster III were dwarf in comparison to genotypes in other clusters but having the highest number of fruits per plant thereby producing the highest yield too. Cluster IV and V each contained three genotypes of medium duration and plant height. Cluster VI and VII comprised of single genotype by and large of late duration, tall type with large number of fruits per cluster.

Table 2 : Grouping of 25 entries of tomato in different clusters

Cluster	No. of entries	Accessions (with source country)
I	8	Red star UFN (Canada), Fire ball (Canada) Heinz (Canada), Mountain Pride VF (Canada) EC 10303 (Japan), EC 6199 (USA), New York Speical (USA), EC 13553 (USA)
II	5	EC 8590 (England), EC 9689 (France), EC 8973 (England), Star Shot (Canada), EC 7787 (Yugoslavia)
III	4	EC 16787 (Australia), EC 16788 (Australia) Cold Set (Canada), EC 7261 (Iraq).
IV	3	Pilgrim VF (Canada), Lemon boy (Canada), EC 8821 (Denmark)
V	3	EC 10302 (Japan), EC 10310 (Japan), EC 14167 (Russia).
VI	1	Olympic Pink (Canada)
VII	1	EC 11847 (Czekoslovakia)

The genetic divergence of the genotypes is in no way influenced by geographic distribution. In this experiment, there are various examples of genotypes having same origin but falling in different groups. Similarly the genotypes namely 'Red Star VFN' from Canada, 'EC 10303' from Japan, 'New York Special' from USA

having different origin fell in the same group. These results are in agreement with the findings of Balasch *et al.* (1984).

The intra-cluster distance varied from 0 to 4.83, while inter-cluster distance ranged from 6.18 to 25.86 (Table 3). Intra-cluster distances were smaller than the inter-cluster distance which revealed that the genotypes within cluster had greater similarity. High inter-cluster distance between III and VII and I and VII indicated greater divergence between the genotypes belonging to these clusters. Character means (Table 4) showed appreciable variation among the seven clusters grouped according to D² analysis. The differences among cluster means though observed for all characters but were more pronounced for plant height, number of clusters per plant, number of fruits per plant and yield per plant. The cluster III showed the highest yield and number of fruits per plant. The genotypes belonging to cluster VII were late in flowering but had highest number of clusters per plant. Twenty five genotypes included in this experiment exhibited wide genetic variation for all six characters studied especially for number of fruits per plant confirming earlier report by Singh *et al.* (1978). The genotypes in the present experiment were not well adapted to the environment in which the study was conducted.

Table 3 : Inter and intra-cluster value in tomato accessions

Clusters	Accessions						
	I	II	III	IV	V	VI	VII
I	2.30	6.83	7.71	6.18	8.04	10.30	18.05
II		3.52	8.58	10.13	6.49	6.80	16.71
III			3.03	7.86	9.48	13.95	22.21
IV				4.29	7.98	14.79	18.36
V					4.83	15.99	20.92
VI						0	25.86
VII							0

Table 4 : Cluster means for different clusters

Characters	I	II	III	IV	V	VI	VII
Days to 50% flowering	51.62	54.74	54.33	55.44	48.78	60.33	60.00
Plant height (cm)	55.03	65.99	37.73	38.03	52.47	70.97	73.47
No. of cluster per plant	14.76	16.67	16.33	17.67	19.00	12.00	25.33
No. of fruits per cluster	2.67	3.20	3.42	3.64	3.45	3.67	4.33
No. of fruits per plant	18.91	23.27	25.08	23.89	24.34	10.67	23.33
Yield (Q/ha)	93.24	138.42	182.52	78.60	134.33	123.33	84.33

The utility of classifying germplasm for selection of diverse parents for hybridization has long been appreciated (Bhatt, 1970, Arunachalam, 1981). It was noticed that the lines of the cluster III and VII ($D = 19.18$) and I and VII ($D = 15.75$) showed the maximum distance. Obviously, the crosses between genotypes in these clusters might be useful for fixing transgressive segregants. However, the selection of more than one genotypes from cluster III may pose some other problems. In that context, apart from genetic distance, the performance of genotypes for yield and yield contributing characters should be given due consideration. It was found expedient to select parental entries with considerable genetic distance between them especially for the characters, viz., number of fruits per plant for heterosis breeding. In this study, genotypes EC 16787, 16788, Cold Set and EC 11847 appeared to be desirable for further improvement programme. Inclusion of these parents in crossing programme is expected to prove more rewarding.

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STUDIES ON BIOSYNTHESIS OF QUALITY PARAMETERS IN PEARL MILLET

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Studies have revealed significant variation in genotypes with respect to biosynthesis of various quality parameters. Soluble sugars in general decreased while starch, soluble protein and lipid content increased with the advancement of seed development. Different genotypes had different rates of accumulation of quality constituents. A stage, 21 days after anthesis, has been identified as the critical stage, when biosynthesis of all the quality parameters is at peak for exploiting and manipulating the variability for developing good quality genotypes.

Pearl millet is considered to be physiologically highly efficient plant type as it transfers almost all the nutrients from plant tissue to the grain. It has been indicated that there is wide range of variation in this regard. (Gupta *et al.*, 1980). This variation was quantitative and seemed to be controlled by additive gene effects. The present investigations were undertaken with a view to have better understanding with regard to variation in the biosynthesis of various quality parameters viz., soluble sugar, starch, soluble proteins, and total lipids contents in the developing grains of pearl millet. In addition to this, an attempt has also been made to study the rate of synthesis of fatty acids during the development of pearl millet grain.

MATERIALS AND METHODS

Samples of developing pearl millet grains were collected at 6, 14, 21, 28 days after flowering and at grain maturity from twelve inbred genetic stocks of pearl millet. The samples were analysed

for soluble sugars, starch, soluble protein and total lipids content. The soluble sugars and starch content were estimated by the method of Yemm and Willis (1954), soluble protein by the method of Lowry *et al.*, (1951) whereas total lipid content was estimated by cold extraction method (Folch *et al.*, 1957). In addition to the above constituents, rate of fatty acid synthesis was also studied by incorporating 1-C^{14} sodium acetate into total lipids as described earlier (Stumpf, 1977).

RESULTS AND DISCUSSION

The studies have revealed that soluble sugars decreased while starch, soluble proteins and lipid content increased during seed development in all the genotypes, however, the rate of fatty acid synthesis was more in the earlier stages as compared to the later stages (Fig. 1).

The analysis of variance for different biochemical constituents at different stages of development revealed highly significant genotypic differences for all the traits at all the stages except for starch content at 28 days after anthesis. The estimates of genotypic and phenotypic coefficients of variation and heritability revealed less influence of environment during grain development. Significance of variances due to genotype $\times$ stage interaction suggested differential rate of utilization of soluble sugars and accumulation of soluble protein.

The mean values for different biochemical constituents at different stages of development are presented in Table 1. The highest amount of soluble sugars at first stage was observed in L 10 (19.3%) followed by A1 (18.9%) and L24 (18.6%). At maturity, maximum amount of soluble sugars was found in L 20 (8.6%). Starch content at first stage was maximum in L5 (58.6%) closely followed by L6 (58.1%). At maturity, it was maximum in L6 (61.1%) followed by L24 (60.7%). For these two parameters i.e., soluble sugars and starch the genotypes showed greater variability at early stages of development as compared to later stages of development. Singh and Julliano (1977) in rice and Yazdi-Samadi *et al.* (1977) and Adams *et al.* (1980) in soybean have also reported that changes in the soluble sugar content closely follow the changes in the rate of starch accumulation during seed development. With the increase in starch content, there is decrease in the soluble sugars content.

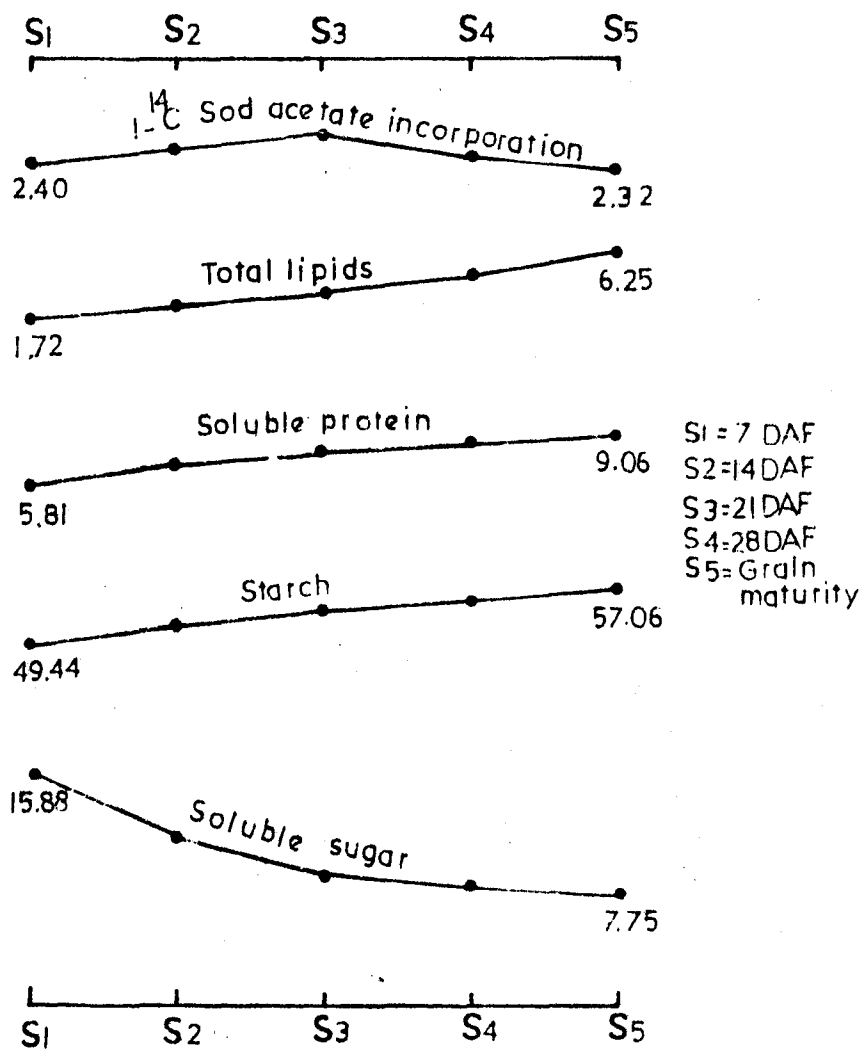


Fig. 1. Trend in biosynthesis of various biochemical constituents in pearl millet grain

Table 1 : Mean values for soluble sugars content during grain development in pearl millet

Genotypes stages	Soluble sugars (%)					Starch (%)					Soluble protein (%)					Total lipids (%)					¹⁴ C sodium acetate incor- poration (mol/g fresh wt)				
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₁	S ₂	S ₃	S ₄	S ₅	S ₁	S ₂	S ₃	S ₄	S ₅	S ₁	S ₂	S ₃	S ₄	S ₅	S ₁	S ₂	S ₃	S ₄	S ₅
L 5	12.3	9.3	8.3	8.0	7.0	56.8	58.2	59.4	59.6	59.6	6.5	7.1	8.7	9.9	9.9	1.8	2.1	4.3	4.6	5.5	2.2	3.0	3.6	2.9	1.8
L 6	14.9	9.1	8.9	7.9	7.8	56.1	58.4	59.0	60.6	61.1	6.5	7.1	7.3	8.1	8.2	1.8	2.6	3.7	5.2	6.2	3.0	3.5	4.5	3.4	2.8
L 7	17.4	10.1	8.9	8.3	8.3	44.6	51.4	52.6	52.9	53.9	6.5	6.9	7.1	8.5	8.5	1.9	2.9	3.7	4.5	6.5	2.8	3.7	4.7	2.4	1.3
L 10	19.3	14.3	10.3	8.5	7.9	51.1	52.0	53.2	53.4	53.8	6.6	6.9	7.7	7.9	8.3	1.9	2.5	3.3	4.5	6.9	1.8	2.7	4.9	2.7	2.4
L 11	16.7	12.3	8.9	8.3	8.1	51.0	52.4	53.6	55.1	57.8	5.9	6.2	6.7	8.0	8.9	1.8	2.5	3.7	4.3	5.5	2.5	3.9	4.6	3.4	2.8
L 19	15.9	10.4	10.1	8.8	7.4	54.0	55.0	56.5	57.7	57.9	5.7	7.0	7.1	7.5	10.4	1.8	2.8	4.1	4.2	6.2	1.3	2.3	2.6	1.7	1.5
L 20	13.4	11.8	10.8	9.9	8.6	50.7	51.3	56.6	57.4	57.9	5.0	5.5	5.7	6.3	7.4	1.9	2.0	3.6	4.8	5.9	3.6	5.9	7.7	6.8	4.9
L 24	18.6	12.1	9.8	8.8	8.3	50.9	52.3	56.4	56.4	60.7	5.2	6.7	6.7	6.9	9.0	1.3	2.7	2.4	4.5	6.3	2.9	4.8	5.0	2.5	2.0
L 46	12.4	11.4	9.2	8.8	8.3	49.3	52.1	57.0	57.1	55.6	5.5	8.9	9.1	10.1	11.7	1.5	2.5	3.1	4.0	6.0	2.8	3.7	6.0	3.7	2.8
A 1	18.9	13.8	11.6	8.9	8.3	41.1	46.4	49.3	52.6	56.6	5.1	5.7	7.1	7.5	7.9	1.8	2.2	3.4	4.4	6.4	1.9	3.2	3.6	2.5	1.9
A 6	15.6	9.3	6.5	6.4	6.4	40.4	46.4	49.3	51.1	53.2	5.3	8.2	8.3	9.3	9.8	1.3	2.2	3.4	4.2	7.3	2.1	3.8	4.8	2.8	2.0
A 7	13.6	11.0	9.4	8.6	6.6	47.5	49.4	51.7	55.0	56.8	5.7	7.1	7.4	8.3	8.7	1.8	2.4	3.3	3.7	6.3	1.9	2.5	3.1	1.9	1.6
Mean	15.8	11.2	9.4	8.4	7.8	49.4	52.1	54.6	55.7	57.1	5.8	6.9	7.4	8.2	9.1	1.7	2.5	3.5	4.4	6.3	2.4	3.6	4.6	3.1	2.3
S.E.	0.7	0.5	0.4	0.2	0.2	1.5	1.1	1.0	0.8	0.8	0.2	0.3	0.3	0.3	0.3	0.06	0.08	0.14	0.11	0.15	0.19	0.27	0.39	0.38	0.28
Range	12.4	9.1	6.5	6.4	6.4	40.4	46.4	49.3	51.1	53.2	5.0	5.5	5.7	6.3	7.4	1.3	2.0	2.4	3.7	5.5	1.3	2.3	2.6	1.7	1.3
to	to	to	to	to	to	to	to	to	to	to	to	to	to	to	to	to	to	to	to	to	to	to	to	to	to
19.3	14.3	11.6	9.9	8.3	8.3	56.8	58.4	59.4	60.6	61.1	6.6	8.5	9.1	10.1	11.7	1.9	2.9	4.3	5.2	7.3	3.6	5.9	7.7	6.8	4.9

The soluble protein content was maximum in L10 (6.6%) followed by L5, L6 and L7 (6.5%) each at the first stage of seed development. However, at the grain maturity, maximum protein content was observed in L 46 (11.7%). This genotype had low protein content at the first stage but because of the faster rate of protein accumulation, this genotype could accumulate more protein compared to other genotypes. Similar findings have been reported earlier in pearl millet grain (Gupta *et al.*, 1981).

Earlier studies have revealed that pearl millet grain contains 5 to 8 per cent lipids. In the present study, attempt has been made to study the biosynthesis of these lipids. It is observed that at earlier stages of grain development, the lipid accumulation is less while at later stages, it is more. Such trend has also been reported in oil seeds grown under different conditions (Bhatia and Sukhija, 1971; Gurr *et al.*, 1972; Norton and Harris, 1975).

Studies regarding rate of fatty acid syntheses revealed that in all the lines, the acetate incorporation into total lipids was considerably low at the first stage of grain development (i.e. 7 DAF). However, it increased rapidly and reached a maximum level by the time of third stage (i.e. 21 DAF). Thereafter rate of incorporation gradually decreased, as the seed advanced towards maturity.

The low initial uptake of acetate could be attributed partly to the seed being engaged in the biogenesis of various sub cellular organelles and partly to the non-availability of adequate precursors like ATP, NADPH and glycerol which are needed for the synthesis of lipids (Gurr *et al.*, 1972, 1974). Norton and Harris (1995) have also reported low lipid content and high content of soluble sugars and starch in the initial stages of seed development. It can be said that though the amount of soluble sugar was high at the initial stages yet their catabolism to yield ATP, NADPH, glycerol and acetate was probably low. In addition, the lower activity of acetyl Co A-carboxylase, which catalyses the first committed step in long chain fatty acid biosynthesis (Bhatia *et al.*, 1978), could also attribute to lower lipid synthesis at the initial stages.

The maximum 1-¹⁴C-sodium acetate incorporation into total lipids at 21 days after anthesis, a stage where the grains display maximum oil filling, has indicated that the tissue was geared up for the optimal synthesis of fatty acids during the brief developmental span. It is, therefore, suggested that the stage of 21

days after flowering may be considered as the critical stage for exploiting and manipulating variability for developing genotypes with better quality.

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**DIFFERENT METHODS FOR DETECTION OF
XANTHOMONAS CAMPESTRIS PV. VIGNICOLA
(BURKH.) DYE IN COWPEA (VIGNA UNGUICULATA
(L.) WALP.) SEEDS AND STUDIES ON HOST RANGE**

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Of the four seed health testing techniques viz., seed soaking, seed maceration, blotter test and growing-on test used for the detection of Xanthomonas campestris pv. vignicola (Burkh.) Dye from naturally infected seeds of cowpea, seed soaking test was found to be superior to other methods from quarantine point of view. In the host-range studies, Glycine max, Pisum sativum and Vicia faba were identified as compatible hosts for the first time and injection-infiltration technique showed superiority over the spray inoculation technique.

Standardization of techniques for quick detection of seed borne plant pathogens has assumed great relevance in recent years due to increased quantum of exchange of germplasm throughout the world. From quarantine point of view, it is essential to have quick, easy and reliable techniques for detection of seed borne bacteria. Though some work has already been done on the development of different methods for detection of phytopathogenic bacteria from seeds of different crops (Shekhawat and Chakravarti, 1979; Schaad, 1982), limited information is available on these aspects for *Xanthomonas campestris* pv. *vignicola*, causal organism, of bacterial blight of cowpea (Gitaitis and Nilakhe, 1982; Soni and Thind, 1987). Incidence of cowpea blight has been reported to be as high as 62 per cent from an initial inoculum level of 1 per cent infected seed (Shekhawat and Patel, 1977; Therefore, keeping in view the economic and quarantine importance of *X. campestris* pv. *vignicola*, investigations on techniques for detection of this pathogen in cowpea seeds and host range studies were undertaken.

MATERIALS AND METHODS

Mature pods from bacterial blight infected cowpea (EC-244289, imported from Philippines) plants were collected from NBPGR Research Farm, Issapur, Delhi in November, 1990. Seeds collected from pods showing bacterial ooze at peduncle under microscope were considered as infected seeds and were used for these studies. Majority of them were found to be discoloured, deformed and shrivelled. Seeds showing varied symptoms were mixed before use in isolation studies on nutrient agar (NA) medium. Seeds from pods of healthy plants not showing bacterial ooze were used as control. Before use, all the seeds were surface sterilized with rectified spirit for 2 minutes followed by washing in sterile water. Each treatment was replicated five times.

Four methods were used for the detection of *X. campestris* pv. *vignicola*. (i) *Seed soaking*: Ten seeds were soaked in 15 ml sterile water for 3 hr at room temperature ($25\pm 1^{\circ}\text{C}$). A loopful of the suspension was then streaked on NA plates and incubated at $27\pm 1^{\circ}\text{C}$ for 6 days, (ii) *Seed maceration*: Ten seeds were soaked in water columns for 3 hr at room temperature and then crushed with pestle and mortar. The paste was transferred to 5 ml sterile water column, stirred well on shaker and allowed to settle down. The supernatant was serially diluted upto 10^{-3} dilutions. One loopful of the suspension from the last dilution was streaked on NA plates and incubated at $27\pm 1^{\circ}\text{C}$ for 6 days, (iii) *Blotter test*: Ten infected seeds were plated on 4 layers of moist blotters in each plastic petriplate and were incubated at $22\pm 1^{\circ}\text{C}$ for 8 days. Seedlings showing any symptom were re-incubated for 3 more days for further development of the symptoms. Seeds collected from healthy plants were used as control. (iv) *Growing on test*: Five infected seeds were sown in 6" plastic pots in the green house. The plants were regularly irrigated as well as sprayed with water to provide conducive conditions for disease development.

Similar experiment was conducted with seeds artificially inoculated by *X. campestris* pv. *vignicola* which were produced by soaking healthy seeds in 24 hr old concentrated bacterial suspension for 2 hr followed by drying at room temperature for 1 hr. Similarly, unsoaked seeds from healthy plants as well as seeds soaked in sterile water were used as control.

Hypersensitive Reaction (HR) : The purified, single colony bacterial cultures, isolated in the above mentioned experiments,

were inoculated on fully expanded leaves of tobacco (*Nicotiana tabacum* L.) by injection-infiltration technique (Klement *et al.*, 1964).

Host range : Studies on host range were also conducted with 8 legume crops : *Cyamopsis tetragonoloba* L., *Glycine max* (L) Merr., *Lablab purpureus* (L) Sweet; *Phaseolus vulgaris* L; *Pisum sativum* L; *Vicia faba* L; *Vigna mungo* (L.) Hepper and *V. radiata* (L.) R. Wilczek using *V. unguiculata* (L.) Walpers as check crop. Five seeds of each of these crops were sown in 15cm plastic pot. Leaves and stem of the healthy seedlings at 3-4 leaf stage were inoculated with 24 hr old bacterial suspension by spraying as well as injection infiltration techniques. Plants treated with sterile water served as control.

RESULTS AND DISCUSSION

Two types of bacterial colonies appeared in seed soaking and maceration tests. One was well-isolated, circular, shiny, mucoid and yellow pigmented which developed on NA after 72 hr while the other one was white type, rough, irregular and developed after 24 hr of streaking. The number of yellow colonies was comparatively higher than white type in seed soaking whereas it was reverse in seed maceration technique.

In blotter test, out of 50 seedlings, only 16 per cent showed minute, water soaked spots on cotyledons and primary leaves on the 7th day which later increased in size and infected area turning yellow. On isolation, similar yellow bacterial colonies, as mentioned earlier, were obtained. No symptoms were observed in control seedlings.

In growing-on test, different types of symptoms were observed in 24 per cent seedlings at varying growth stages on different parts. The symptoms were first recorded as minute, round to irregular, whitish to yellowish green, water soaked spots on leaf margins and lamina after 12-15 days of sowing (first trifoliate leaf stage). On veins, symptoms appeared as whitish water soaked streaks which later increased in size. On stem, similar type streaks appeared (3-4 trifoliate leaf stage) which later extended upwards and downward, turned yellowish and caused stem splitting, drying and ultimately resulted in death of the seedlings (6-7 trifoliate leaf stage). In seedlings raised from artificially inoculated seeds, out of 20 seedlings, only 12 (60%) seedlings showed disease symptoms. On isolation, similar yellow pigmented bacterial colonies were obtained from all types of symptoms. No symptoms were observed on control plants.

All bacterial isolates were purified and single colony cultures were maintained on Yeast Glucose Chalk Agar (YGCA) slants as well as in sterile water column top layered with liquid paraffin (Durgapal, 1980). The single colony isolates were used in the present studies. All isolates were found to be gram-negative and rod shaped.

The yellow isolates showed typical Hypersensitive Reaction (HR) on tobacco leaves whereas white one did not show any reaction.

Koch's postulates were proved for all the yellow isolates indicating their pathogenicity on cowpea plants. Thus the association of *X. campestris* pv. *vignicola* with cowpea seed and the seed borne nature as well as seed transmission of *X. campestris* pv. *vignicola* were confirmed. Thus white isolates did not show any reaction.

The seed soaking technique appears to be superior to all other methods for quick detection of *X. campestris* pv. *vignicola* in cowpea seeds from quarantine point of view. It is a simple method and does not require any specific chemical/medium or instrument, etc. Gitaitis and Nilakhe (1982) also reported the detection of *X. campestris* pv. *vignicola* from cowpea seeds by seed soaking in water but vacuum infiltration and centrifugation were also involved in their technique. This method has been found suitable for the detection of *Pseudomonas syringae* pv. *phaseolcola* from bean seeds (Trigalet and Bidaud, 1978).

Blotter and growing-on tests have been reported for detection of *X. campestris* pv. *vesicatoria* and *X. campestris* pv. *vignicola* from chilli and cowpea seeds, respectively. These techniques require suitable environmental conditions for disease development (Shekhawat and Chakravarti, 1979; Soni and Thind, 1987) and non-availability of suitable incubation conditions may also lead to failure in detection of latent bacterial infection particularly in shrivelled and non-viable seeds.

In host range studies, *Glycine max*, *Pisum sativum* and *Vicia faba* were recorded to be the new compatible hosts while *C. tetragonoloba*, *L. purpureus*, *P. vulgaris*, *V. mungo* and *V. radiata* were found to be non-compatible. Jindal and Patel (1980) have also reported *L. purpureus*, *P. vulgaris*, *V. mungo*, *V. radiata* and *C. tetragonoloba* as non-compatible hosts for *X. campestris* pv. *vignicola*.

and our findings support their observations. However, in respect to *P. vulgaris* and *G. max*, our findings are at variance with the earlier reports. *P. vulgaris* has been reported to be a compatible and common host for this pathogen by Rangaswamy and Gowda (1963), Sabet (1959), Bhatt and Patel (1954), while Jindal and Patel (1980) have recorded *G. max* as non-compatible host. This divergent finding may probably be due to the existence of strain specificity/differential behaviour of the isolates of this pathogen.

The influence of method of inoculation on symptom development was clearly evident in the present study. On *Pisum sativum*, spray inoculation induced inward shrivelling of leaves, yellow discolouration followed by tip drying while no symptoms were observed in *Glycine max*. However, symptoms developed by injection infiltration inoculation in all the three new compatible hosts were different from its natural host (cowpea). On *Pisum sativum*, yellowish, water soaked spots appeared extending upward and downward at inoculated portion of the stem and resulted in withering and wilting of the plants. In *Vicia faba*, dark brown to blackish, water soaked lesions developed around the inoculated portion of leaf and stem, which later extended upward and downward resulting in the death of the plant. In *G. max*, water soaking appeared at inoculated portion on leaf, which later increased in size and turned yellow. No symptoms were developed on control plants. Koch's postulates were proved on cowpea plants with isolates from the three crops. Sabet (1959) also reported the development of different symptoms than normal ones on *Dolichos lablab* and *P. vulgaris* inoculated with *X. campestris* pv. *vignicola*.

CONCLUSIONS

Seed soaking method was proved to be the best method for detection of *X. campestris* pv. *vignicola* from cowpea seed from quarantine point of view. Injection infiltration technique showed superiority over spray inoculation technique for symptom expression. *Glycine max*, *Pisum sativum* and *Vicia faba* were found to be compatible for *X. campestris* pv. *vignicola* for the first time.

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STRATEGY FOR THE PRODUCTION OF VIRUS-FREE PLANT GERMPLASM UNDER *IN-VITRO* CONSERVATION AND EXCHANGE — A BRIEF REVIEW

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With the increase of national and international exchange of plant germplasm, the potential risk involved in distribution of plant pathogens, especially plant viruses in previously unaffected areas has increased. Realising the need for developing an efficient system for maintaining and exchanging virus-free germplasm, work has been initiated at National Plant Tissue Culture Repository of NBPGR. The strategy for production of virus-free germplasm is discussed in this brief review.

Conservation and exchange of the plant genetic resources is of considerable importance for sustained crop improvement activities. However, the germplasm infected by any pathogen impedes the progress, eventually resulting in waste of time, resources and even the valuable germplasm. Further, exchange of infected germplasm may lead to the introduction of hazardous pathogens or races into new areas (Lange, 1985). Therefore, it is very essential that adequate measures be taken for control of the pathogens associated with germplasm particularly with the accessions maintained in *in-vitro* repositories, gene banks and plant introduction stations.

National Plant Tissue Culture Repository (NPTCR), NBPGR, New Delhi, since its inception in 1986, has continued to conserve the germplasm diversity in several economically important vegetatively propagated plant species employing *in-vitro*

techniques. For facilitating its future utilization in breeding programmes and national and international exchange, elimination of virus and virus-like agents assumes special significance. Considering the importance of the diseases caused by these pathogens in the crops under *in-vitro* conservation programme currently being studied at NPTCR and in view of the necessary quarantine guidelines for exchange of germplasm, a programme on production of virus-free germplasm for conservation and exchange was initiated at NPTCR in 1990. Information on virus and virus-like diseases associated with the crops under the programme and procedure for the production of virus-free germplasm is highlighted in this presentation.

Virus diseases and their prevention

Pathogens such as fungi, bacteria, nematodes, mycoplasma like organisms, viruses, viroids etc. are the common causal agents of plant diseases. The level of success in controlling diseases depends entirely upon the types of etiological agents involved in evoking the disease and the exact mechanism by which the pathogens invade the host system. Effective chemotherapeutical control measures are available for the diseases caused by fungi, bacteria and nematodes, whereas attempts to control virus diseases in plants by using antiviral compounds have met with only limited success, because of close association of virus multiplication and host metabolism (Karthi, 1986; Long and Cassells, 1986). The only way of virus disease control is, therefore, prevention of infection.

Among all the preventive measures, removal or avoidance of source of infection is recognised as the most effective method of control. Usually the infected propagules serve as initial source of virus inoculum. Therefore, the virus diseases can be effectively controlled; if the plants are raised from the virus free planting materials. In crops, where the plants are propagated through seed, virus-free plants may be generated by using the seed harvested from the healthy plants of the population (Lange, 1985). But in the case of vegetatively propagated crops, systemically infected plants carry the viruses from one generation to another and usually remain infected in their entire life span. Thus, elimination of inoculum from such plants is the only way of virus disease control in vegetatively propagated plants (Henshaw and O'Hara, 1984).

Over the last two decades, *in-vitro* culture of plant tissue, especially meristem-tip culture or a combination of thermotherapy and meristem-tip culture has established its credibility as an efficient method for elimination of viruses from the infected plants and the method has been successfully used in more than 35 plant species (Quak, 1977; Bhojwani and Razdan, 1983). This technique is now employed as a routine procedure in most of the genetic resources centres engaged in the conservation and distribution of virus-free germplasm, particularly for clonally propagated materials (Kartha, 1986; Love *et al.*, 1987; IBPGR, 1988).

Virus and virus-like diseases associated with plant species handled at NPTCR

A number of germplasm lines belonging to tuberous crops — *Ipomoea batatas* (sweet potato), *Dioscorea* species (yams); bulbous crops — *Allium sativum* (garlic) and related wild and semi wild *Allium* species; fruit crops — *Citrus* spp., *Musa* spp. (banana); spice crops — *Zingiber officinale* (ginger), *Curcuma* spp. (turmeric), *Piper* spp.; medicinal and aromatic plants and endangered plant species — *Coleus forskholii* (Coleus), *Rauwolfia serpentina* (sarpagandha), *Sassurea lappa* (kuth), *Tylophora indica* (antamul) are being conserved in *in-vitro* form at NPTCR.

A survey of literature revealed the association of several important virus and virus-like diseases with some of the plant species under *in-vitro* conservation (Table 1). For example, *Citrus* spp. are attacked by more than 25 viruses (Garnsey, 1988). In banana, bunchy top virus disease is threatening the production in one quarter of the world's banana growing areas (Dale, 1987). Sweet potato is attacked by several viruses, out of which, sweet potato feathery mottle virus, is prevalent wherever the crop is grown (Moyer and Salazar, 1989). This virus has also been found occurring in Indian germplasm (Kumar *et al.*, 1990). Garlic mosaic virus disease is considered to be important in garlic in Taiwan, Japan, Phillippines, Germany, France (Chen and Ko, 1979; Ahmed and Benigno, 1985; Lee *et al.*, 1979; Graichen *et al.*, 1985; Messiaen *et al.*, 1981). This diestase is quite prevalent in India and has received considerable attention in recent years (Ahlawat, 1974; Ahlawat and Chakraborty, 1990; Kumar *et al.*, 1990; Khetarpal *et al.*, 1991).

Table 1 : Important virus and virus-like diseases associated with the plant species handled at NPTCR *In-vitro* conservation and exchange

Plant species	Disease	Reference
<i>Allium sativum</i>	* Garlic mosaic	Ahlawat, 1974; Chen and Ko, 1979; Kumar <i>et al.</i> , 1990.
	Garlic latent	Graichen and Leistner, 1987.
	Garlic yellow (chlorotic) streak	Xie <i>et al.</i> , 1981; Mohamed and Young, 1981.
	Onion yellow dwarf	Chen and Ko, 1979; Graichen and Leistner, 1987.
<i>Citrus</i> spp.	* Tristeza	Bridges, 1966; Nariani and Raychaudhuri, 1968.
	* Exocortis (viroid)	Nariani <i>et al.</i> , 1968 Duran-Vila <i>et al.</i> , 1986.
	* Greening (Procaryote)	Raychaudhuri, 1981; Garnier and Bove, 1983.
	Xyloporosis	Childs <i>et al.</i> , 1955.
	* Infectious variegation	Singh and Chakraborty, 1975; Catara, 1984.
	Stubborn (Spiroplasma)	Fawcett, 1946; Rahimian, 1983.
	* Citrus mosaic	Murty and Reddy, 1975; Usugi <i>et al.</i> , 1986.
<i>Ipomoea batatas</i>	** Sweet potato feathery mottle	Moyer and Kennedy, 1978; Kumar <i>et al.</i> , 1990.
	Sweet potato latent	Green <i>et al.</i> , 1988.
	Sweet potato mild mottle	Hollings <i>et al.</i> , 1976.
	Sweet potato vein mosaic	Nome, 1973.
	Sweet potato yellow dwarf	Chung <i>et al.</i> , 1986.
	Sweet potato caulimo-like	Atkey and Brunt, 1987
<i>Musa</i> spp.	* Banana bunchy top	Vakili, 1969; Summanwar and Marathe, 1982; Dale, 1987; Wu and Su, 1990.
	* Cucumber mosaic or Infectious chlorosis	Magee, 1940; Patel and Mali, 1986; Shong <i>et al.</i> , 1990.
	Banana streak	Lockart, 1986.
	Abaca mosaic	Celino, 1956.
<i>Tylophora indica</i>	** Cucumber mosaic	Kumar <i>et al.</i> , 1991.

* Reported from India

** Identified in India for the first time from NPTCR laboratory.

Investigations undertaken at NPTCR during the period 1990-92 revealed the presence of sweet potato feathery mottle virus, garlic mosaic virus and cucumber mosaic virus diseases in *Ipomoea batatas*, *Allium sativum* and *Tylophosra indica* germplasm collections, respectively (Kumar *et al.*, 1990, 1991a & b). Besides the above mentioned diseases, banana bunchy top and sweet potato mild mottle diseases are suspected to occur in germplasm collections based on the symptoms observed. Above all, the presence of some other diseases in latent form in different germplasm collections can not be ruled out. This necessitates critical screening of germplasm for the freedom from viruses and salvaging of the infected material by *in-vitro* techniques.

Procedure for the production of virus-free Germplasm

The production of virus-free germplasm includes mainly three aspects i.e. routine virus indexing, selection of virus-free mother plants and elimination of viruses from the infected mother plants by meristem-tip culture combined with various treatments (Fig. 1). Routine virus indexing plays a key role in the production of virus-free plants, as only after testing the plants for specific viruses, they could be identified as virus-free or infected. Often it becomes necessary to repeat the process to ensure virus-free status of the mother plants selected from the field and in meristem-tip culture raised plants.

In order to develop routine virus indexing techniques, first of all the viruses are detected, identified and purified, and then the detection techniques are standardized. In certain cases where the virus detection kits are readily available, they may be procured and used in routine virus indexing.

The following detection techniques are generally applied in routine virus indexing :

- i) Infectivity assays — These methods are based on the transmission of viruses by artificial means to the susceptible indicator or test plants and visual examination for the symptoms produced on them after a certain incubation period. Most frequently employed tests are mechanical sap inoculation and graft transmission (Noordam, 1973).
- ii) Serological tests — The basic principle involved in these tests is detection of viruses in the infected plants on the basis of visible or measurable reaction between virus (antigen) and its homologous antiserum (poly or monoclonal antibodies). In

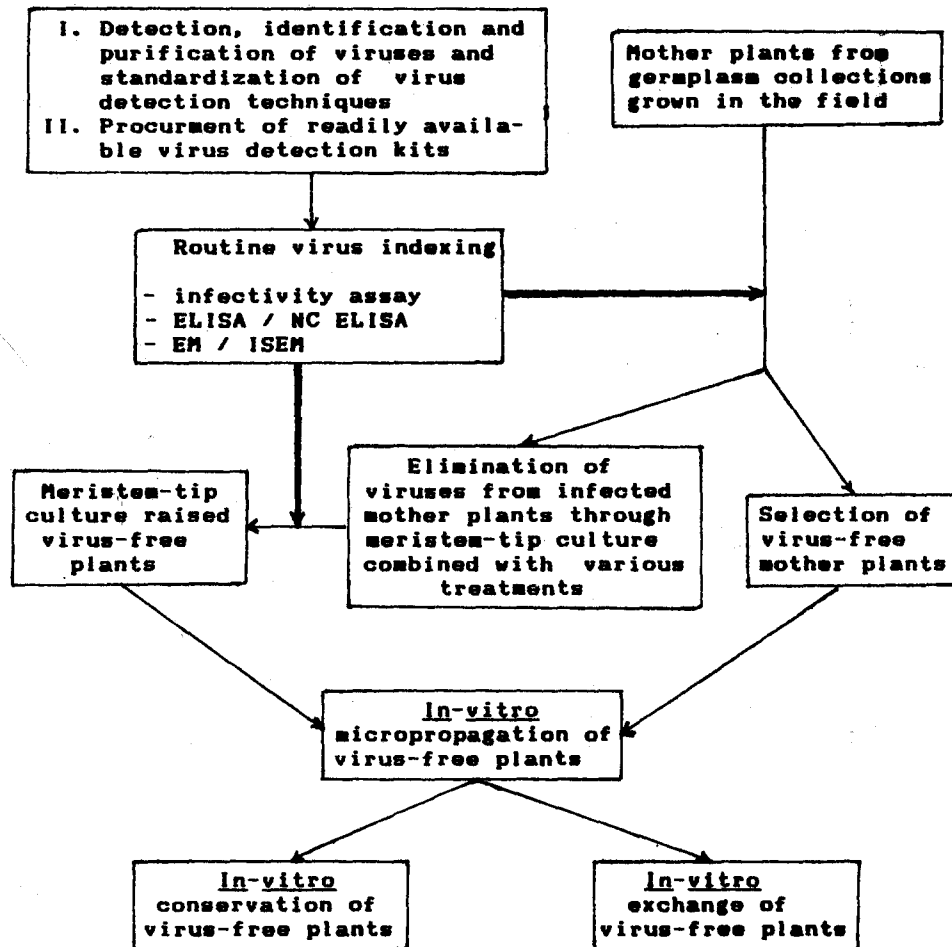


Fig. 1. : Diagrammatic scheme for the production of virus-free germplasm under *in-vitro* conservation and exchange.

recent years, the serological detection methods, such as enzyme linked immunosorbent assay (ELISA) (Clark *et al.*, 1988) and dot-immunobinding assay (DIBA) or nitrocellulose ELISA (NC ELISA) (Powell, 1987), which are sensitive and specific, are commonly used in routine virus indexing.

- iii) Electron microscopy (EM) — Using this technique viruses can be detected in the tissues of infected materials directly by visualizing the virus particles. (Hitchborn and Hills, 1965; Horne, 1967). EM coupled with serology, known as immunosorbent electron microscopy (ISEM), is employed for distinguishing serologically unrelated virus particles in mixed infections (Milne and Lesemann, 1984).

Apart from the above mentioned techniques, several other molecular and biochemical techniques such as ELISA with monoclonal antibodies to epitopes that are highly conserved between viruses of a given taxon (Carter and ter Meulen, 1984; Halk and De Boer, 1985); isolation and analysis of dsRNA by gel electrophoresis (Kurppa and Martin, 1986); nucleic acid hybridization with cRNA or cDNA probes (Garger and Turpen, 1988; Palukaitis, 1988), and polymerase chain reaction (PCR) (Langeveld *et al.*, 1991; Lopez-Moya *et al.*, 1992) have been developed in recent years and they are all potentially very useful for routine virus indexing.

It is a well known fact that in nature, some genotypes exhibit resistance to viruses and sometimes within the population a part of the stock may remain virus-free. In such cases the plants grown in the field are tested several times for viruses and subsequently virus-free mother plants are selected for *in-vitro* micropropagation. Where the entire population of the clone is infected, the virus-free mother plants are obtained by eliminating the viruses through *in-vitro* culture techniques.

Several *in-vitro* culture methods such as meristem-tip, callus, nucellar, floral, ovule and protoplast culture can be used for obtaining virus-free plants with varying degree of success (Nadgauda and Mascarenhas, 1985; Kartha, 1986). Among all these techniques meristem-tip culture technique combined with thermotherapy has been found to be the most efficient technique for the production of virus-free plants, as the regenerated plants usually retain their genetic characteristics due to the more uniformly diploid nature of the cells (Kartha, 1986). Since, the main aim is to conserve/exchange the germplasm without disturbing its genetic

stability, only this technique is generally used to eliminate the viruses.

It is important to recognise that even after following all precautions to excise small meristem tips and subjecting them to various treatments favouring virus eradication, only a small proportion of the cultures may yield virus-free plant. Therefore, each meristem-tip culture plant has to be tested for specific viruses before using it as a virus-free mother plant (Bhojwani and Razdan, 1983; Kartha, 1986). Virus-free mother plants are then micropropagated in *in-vitro* and the healthy stocks are transferred to the medium and long-term *in-vitro* conservation. Such *in-vitro* cultures are used in national and international exchange of germplasm.

CONCLUSION

Several important virus diseases often occur in the germplasm of vegetatively propagated crop plants under field gene banks. The detection of a few hitherto unreported viruses in germplasm collections at NPTCR clearly indicates the contamination of existing collections with the viruses. Based on symptoms observed, there is a possibility of occurrence of some more viruses and their related strains in the germplasm collections. Absence of the disease symptoms does not always ensure that plants are free from viruses, as they may be present in latent form. Screening of germplasm collections against the viruses by using various sensitive biochemical and molecular techniques such as ELISA, ISEM, isolation of dsRNA, cDNA, PCR, thus become absolutely essential to ensure the virus-free status of germplasm.

In recent years *in-vitro* culture systems have assumed greater importance in the global scheme for germplasm conservation and exchange, as they have many advantages including elimination of pests and pathogens from the germplasm collections. However, *in-vitro* culture alone cannot guarantee a pathogen free, especially virus-free status of plants (Henshaw and O'Hara, 1984; IBPGR, 1988). Production of virus-free germplasm, therefore, requires a special procedure, where meristem-tip culture and virus indexing techniques are combined. The scheme is depicted by a flow diagram (Fig. 1). The approach dealt with above would eventually help to reduce the risk of viral contamination in the clonally propagated germplasm diversity being conserved and exchanged.

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EVALUATION AND UTILIZATION OF AROMATIC RICE (*Oryza sativa* L.) GERMPLASM FOR AGRONOMIC TRAITS

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The development of semi-dwarf varieties of rice capable of responding to higher doses of fertilizers and irrigation in the sixties, made the vertical choice with respect to consumer quality grades abruptly narrow, thereby shifting the emphasis in food production from quality to quantity. As a result the traditional low yielding but high quality rices started vanishing from the scene. However, the strong liking of the Indian consumers for superfine scented rices together with their increasing demand for export purposes kept the interest in basmati rices alive. Further, lately there has been considerable emphasis on quality improvement of the high yielding rice varieties. The present studies were carried out to assess the genetic potential of some of the exotic and aromatic rices for possible utilization as donors of agronomic traits.

The experimental materials comprised 120 genotypes of aromatic rice collected from diverse geographical sources (Bangladesh-2, China-5, India-3, Indonesia-4, Iran-2, IRRI-14, Myanmar-2, Nepal-1, Pakistan-46, Philippines-8, Thailand-1 and Vietnam-2). Planting was done in a randomized complete block design in two replications during kharif, 1990 at the Crop Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar under irrigated conditions in two row plots of 3 meters length. Row to row distance was kept at 25 cms, while plants in a row were spaced 15 cm apart. One month old seedlings were transplanted in well puddled field @ one seedling/hill. The crop was grown under good management conditions and all the recommended package of practices for basmati group of varieties were followed. Data were recorded on days to 50% flowering, plant height (cm), number of effective tillers/plant, panicle length (cm),

number of grains/panicle, spikelet fertility (%), panicle exertion (scored on 1-9 scale), 1000 grain weight (g) and grain yield/plant (g). The standard statistical methods were used to estimate various parameters.

Analysis of variance of means for different characters showed highly significant differences among genotypes. The range of treatment means presented in Table 1 along with the general mean for various characters showed a wide range of variability for days to 50% flowering. The germplasm included a mutant line of Basmati 370 which flowered in 92 days and some photosensitive land races took as long as 157 days to flower. The plant height ranged from 74.4 cm to 176.0 cm and most of the lines were tall as evidenced by the general population mean of 132.7 cm. The dwarf cultures were either very late in flowering (Kalimunchh mutant) or showed very poor panicle exertion (Balugyun and Macunting) and suffered in grain yield. Dwarf and early cultures possessing basmati grain characteristics did not exist in the germplasm.

The number of effective tillers per plant also showed a wide range of variation in the germplasm screened. However, majority of the cultures screened were low tillering type yet a few moderately high tillering types were available e.g. Basmati 376, (17.0), Mikhudeb (15.0), Sitasail (13.0), Basmati Surkh 81-15 (13.0), Sipala (13.0) which could be exploited in breeding programme aimed at increasing tiller number.

The spikelet fertility in the germplasm ranged from 18.3% to 97.0% with a general mean of 81.0%, while the number of filled grains/panicle ranged from 20.0 to 265.0 (Badshahbhog) with a general mean of 105.2. Delayed flowering exposed the crop to lower atmospheric temperatures and was mainly responsible for reduced fertility of the spikelets. Two lines, namely Khao Dawk Mali and Hawm Mali which showed very poor spikelet fertility (18.3% and 20.3%, respectively) took more than 150 days to flower. Decrease in fertility percentage with increasing length of cooling treatment has been reported by Satake and Koike (1983). However, Badshahbhog was an exception. It flowered in 150 days and possessed maximum number of filled grains (265 grains/panicle) with 76.9% spikelet fertility.

Panicle length in the germplasm ranged from 20.6 cm (Kalimunchh mutant) to 39.4 cm (Basmati 410). There were many genotypes in the germplasm which possessed significantly longer panicles than Basmati 370 and T3 (Table 1). Only a few tall genotypes like Latai, Ramjiwain, Kalh Joha and UPRH 22 had short panicles. In general, the short panicles also showed poor panicle

Table 1 : Range, mean and promising cultures for different agronomic traits in the aromatic rice germplasm.

Sl.No.	Character	Mean	Range	Promising cultures
1.	Days to 50% flowering	114.9	92.0-157.5	Basmati 208 (95.0), Basmati 334 (95.0), Ayepyauung (95.0), Dwarf Basmati (92.0), Mussairene (95.0)
2.	Plant height (cm)	132.7	74.4-176.1	Guinata (94.0), Kinandang Pula (97.0), Kalimunch Mutant (80.7), Kunsan-Woo-Shan-Gon (96.5), Balugyun (81.2), Macunting (74.4), Dwarf Basmati (86.0)
3.	Number of tillers/plant	8.3	3.2-17.5	Basmati 370 (11.0), Sitasail (13.0), Basmati 208 (11.0), Basmati 372(12.0), Basmati 376(17.0), Basmati Surkh 81.15(13.0), Mikhudeb (15.0), Ayepyauung (13.0), Sipala (13.0), New Sabarmati (13.0), Kasturi (11.0), Dwarf Basmati (12.0)
4.	Panicle length (cm)	30.1		Basmati 122(37.2), Basmati 372(36.2), Basmati 410(39.4), Basmati 611(36.1), Pageleoh (37.2), N 12 (37.2), Type 3 (37.2)
5.	Number of filled grains/panicle	105.2	20.0-265.0	Kinandang Pula (185), Basmati 123 (181), Basmati 376(188), Lakshmivilas(176), Badshahbhog (265)
6.	Spikelet fertility (%)	81.0	18.3-97.0	Kinandang Pula (95.5), Dinorado (95.4), 6 Palawa Yi-Lu-Hsiang (95.5)
7.	Panicle exertion score (1-9 scale)	3.7	1.0-8.0	Basmati 370(1), Type 3(1), Sitasail (1), Basmati 123(1), Basmati 138 1), Basmati 372 (1), Basmati 376(1), Basmati 613(1)
8.	1000 grain weight (g)	19.8	8.3-31.6	Kinandang Patong (31.6), Milfor 6-2 (31.6), Tarome (26.5), Cempocelak (26.8), New Sabarmati (26.1), Palwan (28.3), Ku 76-1 (30.1)
9.	Grain yield/plant (g)	16.8	2.7-59.9	Bindli (30.6), Sitasail (30.6), Basmati 123 (39.5), Basmati 376 (59.9), Yi-Lu-Hsiang(35.5), New Sabarmati (39.5)

exsertion. However, some genotypes like Basmati 136, Basmati 6113, Basmati 436 and Basmati 6129 showed poor exsertion inspite of long panicles. Delayed flowering has been reported to be one of the reasons for poor panicle exsertion (Gupta, 1988).

The magnitude of variability was very high in case of grain yield/plant as indicated by the range (2.7-59.9 g). In the germplasm screened, the highest yielding genotype Basmati 376 produced as many as 17.5 productive tillers/plant (population mean 8.3) and longer (35.6 cm) panicles though the 1000 grain weight was at par with Basmati 370. Its spikelet fertility (84.1%) was also better than the spikelet fertility of Basmati 370 (73.5%). The next high yielding genotype Basmati 123 possessed moderate number of tillers/plant (10.5) but had very high (93.6%) spikelet fertility in addition to longer panicles (35.0 cm). Thousand grain weight of this variety was also higher than that of Basmati 370. The importance of the above yield components for increased production has been highlighted by Suyambulingam and Jebarani (1979). However, the main defect of these genotypes was their tallness which made them susceptible to lodging.

The 1000 grain weight ranged from 8.3 g (Rajbhog) to 31.6 g (Kinandang Patong) with a general mean of 19.8 g. Kasturi (21.1 g) and Ratna (20.3 g) were at par in 1000 grain weight but superior to Basmati 370 (18.1 g).

The present results pinpoint definite genotypes for use as donors in various breeding programmes aimed at incorporating single or multiple traits but these do not separate out the genotype x environmental interaction. Therefore, further evaluation of the germplasm at increased number of locations and over different years will be helpful in selecting stable genotypes.

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**EFFECT OF ELECTROMAGNETICALLY PULSED
NUTRIENT MEDIUM ON GERMINATION AND
TUBE GROWTH OF THE *IMPATIENS*
BALSAMINA L. POLLEN***

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The effect of magnetic flux on the plants have been investigated by Pitman (1963), Pitman and Ormrod (1970). Alexander and Ganeshan (1990) and Alexander and Rajasekharan (1990), using the male gametophyte (pollen) of papaya, obtained increased germination and tube length over control. The authors, during their investigations on bio-electromagnetism, found that pulsed nutrient medium promotes germination and tube length in unpulsed balsam (*Impatiens balsamina* L.) pollen during *in vitro* growth and the finding is reported here.

The source of electromagnetic field was double Helmholtz coil (radius 15 cm; 200 turns of 21 gauge enameled copper wire in each coil). The power supply consisted of a 2A step down transformer (0,3,6,12,15,18,21,24V) and a 5A bridge rectifier. The DC supply was regulated at 1.5A (20 ± 1 Oersted) with a 2A DC meter. The circuit was completed in a clockwise direction. The coil was positioned and aligned along the Earth's magnetic north. The platform of the coil was aluminium ($24 \times 14 \times 0.1$ cm). The centre of the coil was marked on the Aluminium plate to place the medium (place of uniform force).

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The medium consisted of 5 per cent sucrose (Merck analytical grade) supplemented with 100 ppm (0.8306×10^{-3} MgSO₄); 30 ppm (0.2967×10^{-3} N) KNO₃ and 30 ppm (1.4556×10^{-3} N) boric acid. Three ml of the medium in a 5 ml glass vial each was placed on the platform of Helmholtz coils for treatment, and as control. Balsam pollen collected on a sunny day (R. H. 80 ± 2 per cent) at 10.30 A.M. was used as experimental material. Aliquots (0.1 ml) of control, and treatment medium (zero hr) were drawn to prepare hanging drop mounts according to standard procedures (Ganeshan, 1985), before the power supply was switched on. The power supply was switched on immediately after zero hr., and aliquots were drawn at intervals of 5, 10, 15, 30, 60 and 90 minutes from the control and treated medium to prepare hanging drop mounts. The control and treated mounts were incubated for 20 minutes $25 \pm 1^\circ$ C; R.H. 100 per cent and stained with Alexander's stain (Alexander, 1980). There were three replicates for each of the time intervals and controls. The observations on pollen germination were recorded from 10 randomly selected microscopic fields following the procedure reported by Ganeshan (1985). The tube length was measured from 10 randomly selected germinated pollen grains. The data were analysed using analysis of variance and treatments were compared using L S D ($p = 0.05$).

The results obtained with respect to per cent germination and tube length (in μ) of the control and treated medium are given in Table 1. All treatments other than pulsing the medium for 15 minutes (ignoring the Earth's magnetic field) were statistically non-significant. However, treatment of the medium for 15 minutes (20×1 Oersted) produced 23 per cent ($p = 0.05$) higher germination over that of control and 178.7 per cent ($p = 0.1$) increase in tube length over the control. Photomicrographs of control and pollen germinated in the medium exposed to 20 ± 1 Oersted for 15 minutes in hanging drop mounts are given in Fig. 1 and 2. These data showed that exposing the nutrient medium (containing macro and micro elements) for 15 minutes to a magnetic flux (20 ± 1 Oersted) significantly promoted germination and tube growth of balsam pollen.

Table 1 : Germination percentage and tube length of Balsam (*Impatiens balsamina* L.) pollen *in vitro* in the control and treated nutrient media.

(Values in parenthesis show percent change over control)

Treatment of nutrient medium (in minutes)*	Germination (per cent)		Tube length (in μ)	
0	49.53 ab	—	113.62 a	—
5	53.92 bc	(8.7)	100.33 a	(-11.7)
10	50.67 ab	(2.3)	85.67 a	(-0.6)
15	61.11 d	(23.4)	316.67 b	(+178.7)
30	56.54 bcd	(14.2)	96.00 a	(-15.5)
60	51.82 bcd	(4.6)	87.67 a	(-22.3)
90	41.17 a	(-16.9)	82.00 a	(-27.8)

The treatment preceded by the same alphabet are not significantly different.

* At 20 minutes exposure significant decrease in germination and tube length were observed from those of 15 minutes exposure, hence deviated from regulated time interval of 5 minutes to 30,60 and 90 minutes exposures to assess the spectrum of action of prolonged exposures of pulsing.

Seed pulsed in static magnetic field produced increased germination and yield (Pitman, 1963; Pitman and Ormrod, 1970). Permanent magnets and electromagnets produce static magnetic field. Alexander and Rajasekharan (1990) observed many windows which promote germination and tube length of papaya pollen over control. Out of these windows only one is the 'Response Window' which gave maximum germination and tube length. The biological systems were energised in the magnetic field by earlier investigators. However in this study the pollen nutrient medium was pulsed. The energisation showed many windows which

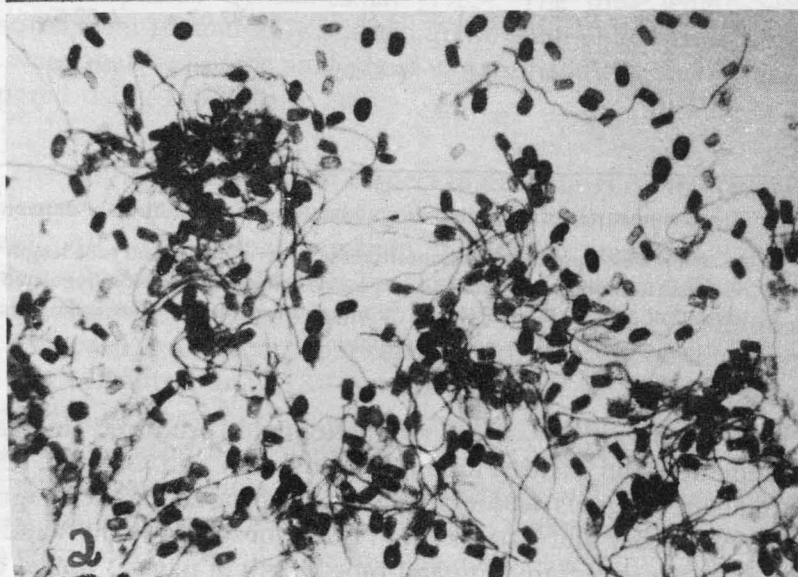


Fig. 1. Photomicrograph showing germination and tube length of balsam (*Impatiens balsamina* L.) pollen cultured in control nutrient medium.

Fig. 2. Photomicrograph showing germination and tube length of balsam (*Impatiens balsamina* L.) pollen cultured in nutrient medium pulsed for 15 minutes.

boosted the germination of balsam pollen. Out of these windows pulsing for 15 minutes produced maximum germination (23.38 per cent increase over control). In the case of tube length different pattern has been observed. Exposure of nutrient medium to 15 minutes produced 178.7 per cent increase of tube length over control. However, exposure to all other time intervals produced lower values than control. This indicate that in the time interval taken there is only one response window for tube length.

The response in the increase in germination and tube length by energising nutrient medium in static magnetic field is paramagnetic. All paramagnetic materials are expected to behave alike under all strength of magnetic field. The explanation for this observed unique phenomenon with no parallel in literature led investigations in magnetobiology using NMR/ESR studies of the nutrient medium before and after exposure in the magnetic field. Hence the present study be treated as a preliminary report.

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**REACTION OF INTERSPECIFIC HYBRIDS OF
SUGARCANE TO SHOOT BORER, *CHILO*
INFUSCATELLUS SNELLEN INCIDENCE
UNDER SALINE CONDITIONS**

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Early shoot borer, *Chilo infuscatellus* Snellen (Crambidea : Lepidoptera) is one of the major pests in all the sugarcane growing areas of sub-tropical India. The pest is more predominant during pre-monsoon period. The problem of soil and irrigation water salinity is on the increase throughout the world. Observations on early shoot borer incidence were recorded in interspecific hybrids (ISH) which were being evaluated for their relative performance under saline water irrigation conditions.

Twenty ISH-clones of sugarcane (involving *Saccharum officinarum*, *S. robustum*, *S. barberi* and *S. spontaneum*) along with three standard checks were planted on 23.03.91. at the Regional Centre Of Sugarcane Breeding Institute, Karnal. Irrigation was done with tubewell water alone (E.C. 0.4 dS/m) or 120 meq. salts mixture added per litre of it. The chloride type salts mixture had Na: Ca: Mg as 6:2:2 and Cl:SO₄ as 8:2 on meq. basis. E.Ce. of the soil with saline water irrigation was 12 ± 0.5 dS/m. Split plot design was used taking saline environment in the main plot and hybrids in the sub-plot. Each clone was replicated thrice with a plot and hybrids in the sub-plot. The plot size was 10.2 m² each. Shoot borer incidence was recorded after 90 days of planting. Analysis was done using sariied 't' test, taking percent incidence as unit and Keeping the replications intact for 69 pairs of observations. Split plot analysis was done to study the interaction amongst various factors (Table 1).

Table 1 Percent incidence of shoot borer in different inter-specific hybrid (ISH) sugarcane clones under saline and non-saline conditions.

ISH-clone number	Percentage	Per cent Incidence		Mean	Percent increase
		Saline	Non-saline		
130	H × S	36.8	35.9	36.3	2.5
132	H × S	73.3	36.3	54.8	101.9
134	H × S	73.7	48.6	61.1	51.6
136	H × S	44.6	42.0	43.3	6.2
146	H × S	42.6	33.8	38.2	26.0
147	H × S	66.1	41.5	53.8	59.2
150	H × S	47.8	36.9	42.3	29.5
152	H × B	44.5	22.2	33.3	100.0
153	H × B	53.5	50.1	51.8	6.7
156	H × B	62.7	41.1	51.9	52.5
157	H × B	68.5	54.7	61.6	25.2
164	H × B	54.9	38.6	46.7	42.2
168	H × B	35.5	31.5	33.5	42.7
173	H × B	72.7	52.7	62.7	37.9
175	H × B	67.2	38.5	52.8	74.5
103	H × O	72.0	27.2	49.6	164.7
107	H × O	56.7	38.3	47.5	48.0
120	H × O	61.0	26.7	43.8	128.4
23	H × (O × R	52.7	36.3	44.5	45.1
38	H × (O × R	49.2	42.6	45.9	15.4
Standard checks					
Co-1148		62.9	42.3	52.6	48.7
Co-7717		63.3	46.4	54.8	36.4
Co-J-64		57.2	55.3	56.2	3.4
Mean		57.3	39.9		
L.S.D. at 5%					
	Between saline and non-saline			= 10.59	
	Between varieties			= 18.67	
	Between (one) hybrid in saline and non-saline			= 26.40	
	Between hybrids in saline or non-saline			= 27.33	

* Percent increase in incidence under saline over non-saline control
H-Hybrid; S-spontaneum; B-barberi; O- officinarum and R-robustum.

The incidence of shoot borer was significantly higher ($t=5.86^{**}$) under saline than non-saline conditions as revealed from Paired 't' test. Reduced shoot growth under saline condition may be presumed as one of the factors contributing towards more shoot borer attack. A perusal of the Table 1 shows the following effects of environments and clones.

Main effects : The mean shoot borer incidence was significantly higher under saline conditions (57.3%) compared to non-saline conditions (39.9%). ISH-clones differed amongst themselves in shoot borer incidence. Highest mean incidence of 62.7% was recorded in ISH-173 which was significantly higher than those of the clones ISH-120, 136, 150, 146, 136, 168 and 152; the last two recorded the lowest incidence of 33.5% and 33.3% respectively. Commercial cultivars did not differ significantly amongst themselves.

Effect of clones : In a given environment, hybrids differed significantly from one another in shoot borer incidence. Under saline environment, per cent incidence ranged from a minimum of 35.5% in ISH-168 to a maximum of 73.7% in ISH-134. Clones ISH-130, 136, 146, 152 and 168 recorded incidence lower than 45% while ISH-103, 132, 134 and 173 recorded incidence higher than 72%; the former group differed significantly from the latter.

Under nonsaline conditions, per cent incidence was a highest of 55.3% in the standard Co J-64 while ISH-103, 120 and 152 recorded incidence lower than 27%, the difference being statistically significant (Table 1).

The present studies using ISH-clones thus confirm our earlier work with 40 promising genotypes of sugarcane that early shoot borer attack is generally more under saline than nonsaline conditions (Sardana and Kumar, 1992). ISH-130, 146, 152 and 168 clones had an average incidence of lesser than 40% compared to a maximum of 63% in ISH-173. Further, ISH-130 and 168 had similar incidence under saline and nonsaline conditions. These preliminary observations suggest that these two clones may be used in breeding programmes for resistance to shoot borer.

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FLORAL POLYMORPHISM IN *VALERIANA* *JATAMANSI* JONES

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Valeriana belonging to family *Valerianaceae*, is represented by 210 species where most of them are temperate to alpine ornaments. Species of this genus are characterised by sexual polymorphism i.e. male, female and hermaphrodite flowers occurring on separate plants. This character makes it a cross-pollinated crop with the result that a lot of variability is encountered in its populations.

V. jatamansi Jones (syn. *V. wallichii* DC), commonly known as Mushkbala or Tagar has considerable medicinal properties. Its root is used as incense and in perfumes. Medicinally the root is also used as stimulant, carminative, anti-spasmodic and useful in hysteria, epilepsy, cholera, shell shock and neurosis. Roots also contain monoterpenic derivative known as valepotriates used as tranquillizers and sedatives (Chopra *et al.*, 1956; Anon, 1986). This species is widespread in sub-temperate and temperate regions of Himachal Pradesh. A review of literature reveals that the plants of *V. jatamansi* are dioecious with male and female flowers occurring on separate plants (Collett, 1921, Blatter, 1928-29, Lawrence, 1960).

However, on the basis of extensive surveys in Himachal Pradesh it has been observed that although sexual polymorphism is encountered in with plants but in nature mostly two types of plants viz. hermaphrodite and female flowers are found. No male plant could be observed. The hermaphrodite flowers are larger with well developed male and female parts as compared to female flowers which are characterised by well developed pistil only apart from rudimentary anthers. This phenomenon, known as 'gynodioecism' has been earlier reported from any species of

Valeriana but the exact name of the species has not been listed (Weberling 1989). The purpose of this communication is to put on record the phenomenon of gynodioecism in *Valeriana jatamansi* Jones.

Apart from the earlier mentioned two types, few plants bearing both female and hermaphrodite flowers on the same plant (composed in the same inflorescence) were also observed (gynomonoecism). So, there are three types of plants in *V. jatamansi* i.e. : (i) Female only, (ii) hermaphrodite, and (iii) female and hermaphrodite both on the same plant. Hence in *V. jatamansi*, both the phenomenon of gynodioecism and gynomonoecism are encountered although the frequency of gynomonoecism is very low. Since *V. jatamansi*'s roots are important medicinally it would be interesting to compare the root chemistry of all the three types of plants. If any noticeable differences are observed, then the flower composition will have a maker value. Efforts in this direction are continuing in this department.

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SCREENING OF CLUSTERBEAN (*CYAMOPSIS TETRAGONOLOBA* (L.) (TAUB) GERMPLASM FOR TRANSPIRATIONAL COOLING

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Clusterbean, popularly known as *guar* (*Cyamopsis tetragonoloba* (L.) Taub) is one of the major rainfed *kharif* crops of the arid and semi-arid plains of North-West India. Its production is largely dependent on the rainfall, which is not only scanty but very erratic in this region during the crop season. This is the major challenging problem in increasing the production of this important industrial crop. To overcome this problem, some ways and means have to be developed by which uncertainty or reduction in productivity can be minimized during the drought years.

Breeding for drought tolerance is a tedious process. Only a few germplasm lines can be agro-physiologically evaluated for drought tolerance at a time due to lack of simple and quick screening techniques (Parsons, 1979; Jhonson, 1980; Sagar and Kapoor, 1986). Little is known about the genetics and stability of grain yield and other quantitative characters under water stress in guar. Until the information on nature and genetics of drought characterisation in gaur are known more precisely, plant improvement programmes would have to rely on screening techniques that are based on plant responses to drought stress (Fisher and Maurer, 1978; Stafford, 1983; Stafford and Mc Michal, 1991). Transpirational cooling (ambient air temperatures minus canopy temperature) has been reported to be a useful indirect indicator of plant water stress and has been indirect indicator of plant water stress and has been found to be highly correlated with seed yield in chickpea and mustard (Singh *et al.* 1990). By rapid measurement of air and canopy temperature by infrared thermometry, it is possible to screen large number of crop cultivars. Lines thus selected can be subjected to detailed agro-physiological evaluation for drought tolerance.

Therefore, during *kharif*, 1990, mid-day (11.00-13.00h.) canopy and air temperatures were recorded by infrared thermometer, at pod initiation stage on two hundred twenty eight seed types and one

hundred twenty vegetable types of clusterbean at NBPGR Research Farm, Issapur (New Delhi), with a view to have an idea of transpirational cooling in this crop. This, in turn, could serve as a basis for field testing of selected germplasm lines for drought tolerance. The mean soil (5cm below surface) and air temperatures at the time of recording observations were 34.2°C and 40.6°C respectively.

The plant canopy of seed types of guar was found to be cooler than the ambient air in the range of 0.5°C (CH-99-1) to 8.3°C (DCB-190), while two guar genotypes (DCB-190 and CB-200-2) exhibited more than 8°C transpirational cooling during mid-day, ten others viz, CH-200-1, CH-214-2, CHA 23-1, CHA 83-4, CHA 97-1, DCB-223, DCB-258, DCB-116, DCB-148 and DCB-90 maintained their canopies cooler than the ambient air in between 7°C and 8°C.

The vegetable types of clusterbean showed transpirational cooling in the range of 0.2°C (IC-41156) to 7.2°C (IC-113880). Canopy temperatures of four guar genotypes viz; IC-40620, IC-40633, CP-167 and CP-277, were observed to be less than the prevailing air temperature by 6°C to 7°C.

Thus, out of the 228 seed types and 120 vegetable types of clusterbean screened for Transpirational cooling, twelve seed types and five vegetable type exhibited relatively higher amount of transpirational cooling and merit detailed evaluation under drought conditions.

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COLLECTING AROIDS DIVERSITY IN NORTH EASTERN INDIA

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The north eastern region of India lies between 21° 57' and 29° 28' N 89° 40' and 97° 25' E and includes the states of Assam, Meghalaya, Arunachal Pradesh, Nagaland, Manipur, Mizoram and Tripura. The aroids belonging to *Colocasia*, *Xanthosoma*, *Amorphophallus* and *Alocasia* genera are mostly used as staple or subsistence food. The corms and cormels are rich in starch and used as vegetables. The leaves and petioles of a *Colocasia* and *Xanthosoma* are cooked and eaten as vegetables. Different aroids have medicinal and ornamental importance also. Ample scope exists for exploring the potentialities of these crops. An assembly of diverse genetic stocks and understanding the nature and magnitude of variability among these genetic stocks would help in identifying the desirable cultivars for commercial cultivation. An attempt has been made to describe species distribution, their collection, maintenance and morphological diversity observed in aroids grown under shifting cultivation [Jhuming by the tribals of north east region of India (Table 1 and 2)]. General of aroids under cultivation belong to *Colocasia* spp. (Taro), *Xanthosoma* spp. (Tannia or Co-or Yam), *Alocasia* spp. (Giant Taro) and *Amorphophallus* spp. (Elephant foot Yam); occupy fourth rank after rice, maize and millets in the diet of tribal people. Medicinal uses and improvement techniques have also been discussed.

DIVERSITY COLLECTED

TARO (*Colocasia esculenta* (L.) Schoot.)

Four species namely *esculenta*, *affinis*, *fallax* and *manii* are grown in NE states of India. *Colocasia esculenta* is extensively grown

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for its edible tubers. The other species of this genus normally grow in wild habitat and are rich in oxalic acid contents. *Colocasia esculenta* is highly polymorphic in vegetative and tuber characters and different strains of this are found in the north east and known by various local names like Bish Katchu, Man Katchu, Dudh Katchu etc. The 216 accessions of taro cultivars that have been collected from various parts of NEH region (Hore and Sharma, 1986-92) show variability in their tuber shape (Cylindrical, oblong, round and tubular), petiole colour (Green, black, violet, greenish black), colour of leaf margin (green, black and brown) and petiole attachment.

Table 1 : Distribution of aroids in north-eastern region of India (After Hooker, 1894, except *Xanthosoma* spp.)

Species	Distribution
<i>Colocasia esculenta</i> (L.) Schott.	In all NE states
<i>Colocasia affinis</i> Schott.	Meghalaya and Assam
<i>Colocasia fallax</i> Schott.	Meghalaya
<i>Colocasia mannii</i> Hook. f.	Assam
<i>Amorphophallus campanulatus</i> (Roxb.) Blume ex Decne	In all NE States
<i>Amorphophallus bulbifera</i> Blume	Meghalaya
<i>Xanthosoma sagittifolium</i> (L.) Schott.	In all NE states
<i>Xanthosoma violaceum</i> Schott.	In all NE states
<i>Alocasia cucullata</i> (Lour.) Schott.	Meghalaya (Khasi Hills)
<i>Alocasia fornicata</i> Schott.	Assam
<i>Alocasia navicularis</i> Koch. & Bouche	Meghalaya (Khasi Hills)
<i>Alocasia fallax</i> Schott.	Meghalaya (Khasi Hills)
<i>Alocasia macrorrhiza</i> (L.) Schott.	In all NE states
<i>Alocasia indica</i> (Roxb.) Schott.	NE states

Table 2 : Germplasm collection of Aroids from the region and their maintenance at NBPGR, Shillong

Species	Assam	Meghalaya	Manipur	Nagaland	Mizoram	Arunachal Pradesh	Tripura	Total
<i>Colocasia esculenta</i> (L.) Schott.	19	48	01	22	10	15	29	144
<i>C. affinis</i> Schott.	12	04	-	10	05	04	02	37
<i>C. fallax</i> Schott.	02	13	-	04	02	01	01	23
<i>C. mannii</i> Hook. f.	04	05	-	-	-	01	02	12
<i>Amorphophallus campanulatus</i> (Roxb.) Bl. ex Decne	-	02	-	-	-	-	-	02
<i>Xanthosoma sagittifolium</i> (L.) Schott.	02	04	-	-	-	-	04	10
<i>X. violaceum</i> Schott.	-	01	-	-	-	-	01	02
<i>A. macrorrhiza</i> (L.) Schott.	01	02	-	-	-	-	-	03
TOTAL	40	79	01	36	17	21	39	233

In NEH region, this species is generally cultivated in *Jhum* as mixed crop. Monoculture of the species is scarcely seen. Rhizomatous tuberous type are much preferred in hills while in Assam plains corm types are more prevalent. In some of the remote hilly places of this region, the taro is consumed as staple food in scarcity days, when cereals are not available. The raw and boiled stems are much relished by the domesticated swings. In Mizoram, the stems are dried and baked and used further in vegetable preparation. Processing and production of silage from Taros is unknown to the local people.

TANNIA OR NEW CO-COYAM (*XANTHOSOMA* SPP.)

Two species occur in this region viz. *Xanthosoma sagittifolium* and *X. violaceum*. The commonly available *X. sagittifolium* existing in this region has different forms. Specific distinctions of Tannia (*Xanthosoma*) can be determined by its flesh colour. In Assam and Tripura, they are called as "Dudh/Dahl Katchu" due to the presence of white latex in the stem. In Meghalaya, it grows in kitchen garden sporadically. Large scale cultivation of the species in this region was hardly seen. The species prefers moist, shady places for its proper growth. Shillong regional station is having 12 collections of Tannia. This species is much relished by the local people. In Assam and Tripura region it is much preferred to *Colocasia*. The harvested stems 4-5 in number, tied together in a bundle are sold in the market.

GIANT TARO (*ALOCASIA* SPP.)

Six species are known to occur in north-east India. Among them *Alocasia macrorrhiza* (Man Katchu), *A. Indica* and *A. Cucullata* are collected for their edible stem and root stalks. The species are grown in shady wasteland area or near the small streams. The growth of the plant is vigorous during the rainy season. The station has collected 3 samples from Assam and Meghalaya. The presence of needle like crystals of calcium oxalate in the tissues of *Alocasia* spp. make the taste acrid. Hence the local people boil the same in water for prolonged period, drain out water and then prepare the vegetable. Since the corm is large, cylindrical (sometimes more than 12 kg. or more in wt.) it is made into pieces and sold in the local market. The larger leaves are sometimes used as umbrella during the rainy season.

ELEPHANT FOOT YAM (*AMORPHOPHALLUS CAMPANULATUS* (ROXB.) BL. EX DECNE

To species are commonly distributed in NE Region. They are *Amorphophallus campanulatus* and *A. bulbifera*. *A. campanulatus* is a herbaceous plant bears herispherical corm and a large solitary mottled leaf on a long petiole. This species produced five to ten corms from each corm at the end of growing season. The corms of wild plants are irritating due to the presence of raphides (Calcium oxalate). In cultivated forms, raphides are less. The corms are used for edible purpose after thorough washing and prolonged cooking.

USES

Colocasia, *Xanthosoma* and *Amorphophallus* are cultivated more extensively. *Alocasia* spp. are grown on a limited scale by certain tribes in north eastern region. In order to depend less on rice and maize the aroids act as staple food. *Amorphophallus* corms are usually eaten as vegetables either after boiling or baking. In *Alocasia*, the stem and tuber portions are peeled off and eaten as vegetable after thorough cooking. These crops have high medicinal value. The acrid juice of *Alocasia macrorrhiza* Schott. and *Amorphophallus peltatus* Hook were used as contact poison (Gimlette, 1929), boiled petiole exudate used as mild laxative (Watt, 1889) and also as stimulant on the skin for removing fever blotches (Burkill, 1935). Petioles of *Colocasia esculenta* have been used for curing wounds and snake bites. *Xanthosoma* spp. decoctions have been used for reducing the body temperature (Burkill and Haniff 1930). The burned petiole in poultice form had been used as a cure for sprained muscle (Maxwell, 1960).

FUTURE RESEARCH FOR IMPROVEMENT

Tuber crops mostly constitute a group of highly cross pollinated and vegetatively propagated plants. Aroids in general, possess very little genetic variability due to the continuous vegetative methods of propagation and lack of sexual reproduction. This makes the crop improvement difficult. Most of the cultivated varieties are non flowering or shy flowering besides possessing high amount of sterility. Extreme protogyny in relation to opening of spathe and failure of spathe to separate at the neck region have been identified as the causes preventing natural seed set in aroids. By hand pollination seed set could be achieved in some cultivars of *Amorphophallus* and *Colocasia* that flowered under natural

conditions. The production of true seeds and seedlings in this way can help in producing high degree of genetic variability. Gibberellic acid treatment could induce flowering in non flowering types in *Colocasia* (Unnikrishnan and Sreekumari, 1984). They also reported that with 0.2 percent calchicine treatment of *Amorohophallus* for 16 hours a tetraploid plant was obtained which showed vigorous growth and broader leaves. Using the techniques of selection, hybridization, polyploidy and irradiation, early maturing high yielding genotypes with desirables plants and tuber characters (good cooking qualities, acrid free taste, blight resistant) suitable for water logged areas can be produced.

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INTERCEPTION OF KARNAL BUNT IN WHEAT FROM RUSSIA

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Exchange of seed materials of various crops at international level has become greatly helpful in increasing the crop yield. Seeds are also the most important source of survival and long distance dissemination of plant pathogens and therefore, there is always a risk of introducing exotic pests and pathogens alongwith such imports into areas where they were not known to occur earlier. Karnal bunt or partial bunt or Indian bunt of wheat caused by *Neovossia indica* (Mitra, 1931) Mundkur, was first reported in India from Karnal (Mitra, 1931). At present it has been occurring in many states of India. In recent years, its occurrence has been recorded in Mexico, Afghanistan, Pakistan, Iraq, and Nepal (Duran, 1972; Locke and Watson, 1955; CMI, 1974; Singh *et al.*, 1989). It was intercepted for the first time in India during 1970 in wheat samples received from Mexico and in later years in seed lots imported from Lebanon, Syria, Turkey and Sweden (Lambat *et al.*, 1983).

During quarantine processing of four samples of spring wheat received from Krasnodar (Russia), a single seed in a sample of cv. Budinir showed the presence of sorus along the furrow. The sorus showed black powdery mass of spores covered by a thin membrane. The teliospores were dark brown in colour, spherical to oval, with reticulations on the episporangia which appears as curved spines and measured 22.0-25.0 μm in diameter. The teliospores had truncate projections surrounded by a delicate membranous sheath. Based on morphological characteristics and the symptoms in seed, the pathogen was identified as *Neovossia indica* (Mitra, 1931) Mundkur. So far as the published records are concerned, Karnal bunt disease does not appear to have been reported from Russia. This interception constitutes the first record of its occurrence in that country. This interception also indicates that the disease has a wider

geographical distribution than was assumed in the past. *Neovossia indica* has been listed as a high risk pathogen (A-1) for European and Mediterranean region and for USA (EPPO, 1979).

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SECOND ANNOUNCEMENT

INDIAN SOCIETY OF PLANT GENETIC RESOURCES ISPGR DIALOGUE ON PLANT GENETIC RESOURCES : DEVELOPING NATIONAL POLICY

New Delhi : December 1-2, 1993

India holds a prominent position among the world's well documented centres of origin and regions of crop plants. It has also developed a strong system for collection, exchange, evaluation and conservation (*in situ*, *ex situ*, *in vitro* and field genebanks) of its plant wealth. There is, however, an urgent need to evolve a national policy on Plant Genetic Resources (PGR) since there are no clear guidelines and binding regulations that should comprehensively direct all the ongoing activities of different central and state government departments, research institutions, private sector agencies and non-governmental organisations.

With the advent of Breeders' Rights, Farmers' Rights and also Intellectual Property Rights (IPR), unrestricted availability of plant genetic resources for research use across the nations is becoming increasingly concerned. Recent advances in biotechnology, permitting the use of new patent tools of molecular biology and genetic engineering, have added another dimension to this debate since candidate genes for genetic manipulation are to be found in germplasm collections of cultivated plants and their wild relatives that are still evolving under natural selection pressures.

1992 Convention on Biological diversity (CBD) has recognised sovereign rights of nations over their biological resources and in determining access to them by linking their transfer to transfer of technology and sharing of benefits. The FAO Commission of PGR is now engaged in harmonising the International Undertaking on PGR with provisions of the CBD. With the lead initiative taken by IBPGR, the International Agricultural Research Centres under CGIAR have offered to place their global germplasm collections under auspices of the FAO. GATT negotiations based on Draft Final Act also appear to be moving towards conclusion. It is high time that the National Policy on PGR is drafted and discussed with a view to evolving a consensus.

Responding to these developments, the Indian Society of Plant Genetic Resources (ISPGR) is organising a **Dialogue on Developing National Policy on Plant Genetic Resources**. A 2-day programme is proposed to be held at the IARI Auditorium, New Delhi on

December 1-2, 1993 comprising four technical sessions and a well planned poster session. Each session will have 8 panelists including lead experts drawn from India and abroad (FAO, IBPGR and ICRISAT) who will make brief presentations of 10 minutes each and initiate discussions. The Poster Session will facilitate display of information and viewpoints relevant to plant genetic resources activities and theme of the Dialogue.

Registration :

The Indian Society of Plant Genetic Resources cordially invites you for participation enclosed registration form may be filled in and sent alongwith the registration fee of Rs. 250/- for members and Rs. 350/- for others by 26th November 1993, through Demand Draft drawn in favour of ISPGR payable at New Delhi to Dr. B.P. Singh, General Secretary, ISPGR, Germplasm Exchange Division, NBPGR, Pusa Campus, New Delhi-110 012.

Weather :

Delhi has cold weather in December, minimum temperature falling to around 5°C. Winter clothing is advised.

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