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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. prezwalskianum*, *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. prezwalskianum* 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 synonimity 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.	*A. semonovii* 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, Zanskar (N.W. Himalayas)	8-14
2.	*A. schoenoprasum* L.	Bulbs clustered, narrow; scales membranous, head subglobose densifid, flower head companulate, pink or pale purple flower	Western Himalayas; Kashmir to Kumaon hills	8-11
3.	*A. atrosanguineum* Schrenk.	Bulbs narrow, coats membraneous fibrous, leaves fistular, sheaths very long, spathes persistent, head companulate, dark red flowers	Kashmir to Gilgit, Turkestan	11-12
4.	*A. fedschenkoanum* Regel	Leaves 1-2 much shorter than stout scape, head globose, densifid, companulate, 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.	*A. rubellum* 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.	*A. lilacinum* Royle	Bulbs ovoid, coats scarions, red brown, leaves 2-3 scape 8-10 m., fistular head hemispheric, campanulate small pale red flowers	Western Himalayas Garhwal westwards	6-7
7.	*A. carolianianum* D.C. syn. (*A. blandum* 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 Tibbet, Kumaon, to west Nepal. Girthi valley Gori valley, Malari, Milan glacier	13-17
8.	*A. stracheyi* 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, Brahmmathya, Harshil, Gangi, Gangotri, Garba yang Badrinath/ Kedarnath	9-12
9.	*A. consanguineum*, 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.	*A. platyspathum* Schrenk.	Bulb solitary or clustered on a short perpendicular root stock, scales hayline, leaves flat, sheaths hypogeous	Western Tibet	
11.	*A. thomsoni* 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.	*A. bakeri* Regel	Bulbs clustered, 2.5 cm. long, avoid, oblong scales, white membraneous, leaves 2-4, head-laxfid flower campanulate- red-purple	Khasia hills, China and Japan	4-5.5
13.	*A. wallichii* 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.	*A. hookeri* Thuaites	Bulb hardly any, base of stem clothed with long, narrow membraneous sheathes, leaves 12-19, head 2.5-3.0 cm. in diam., Spathe with a long tail, leaves basal, linear, membraneous,		

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.	*A. sikkimense* 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.	*A. prezwalskianum* Regel (*A. jacquemontii* 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, Zanskar (N.W. Himalayas)	16.5
17.	*A. auriculatum* 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.	*A. victorialis* 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.	*A. schrenki* 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.	*A. odorum* L.	Bulb 2.5-10 cm., clustered on an oblique jointed root stock subcylindric or conic, scales finely reti- culate, 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.	*A. tuberosum* 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.	(*A. humile* Kunth.) syn. *A. govanianum* Wall.	Bulbs and foliage as in *A. odorum*; it differes 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.	*A. creoprasum* 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.	*A. clarkei* 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.	*A. atropurpureum* Waldst.	Bulb subglobosely 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.	*A. loratum* Baker	Bulb small, ovoid, outer scales membraneous, 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.	*A. macranthum* 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. shoenoprassum* 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. wallichi* 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. creoprasum* 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 (*Colletrotrichum* 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, B_5 and LS. Based on this analysis, they suggested the modification in the B_5 basal medium as described below (Gamborg *et al.*, 1968). $CaCl_2H_2O$ (150), KNO_3 (2530), NH_4NO_3 (320.16), $NH_4H_2PO_4$ (230.06), $(NH_4)_2$ SO_4 (134), $MgSO_4.7H_2O$ (247), $MNSO_4.4H_2O$ (132), $ZnSO_4.7H_2O$ (2.0), $CuSO_4.5H_2O$ (0.039), KI (0.75), $CoCl_2.6H_2O$ (0.025), H_3BO_3 (3.0), $NaMO_4.2H_2O$ (0.25), $NaH_2PO_4,2H_2O$ (172), $FeSO_4.7H_2O$ (27.85), Na_2EDTA (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-napthyl 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-napthalene 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 ieaves 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) B_5 basal medium supplemented with 0.5 mgl^{-1} isopentenyladenine (2-ip) and 0.1 mgl^{-1} napthaleneacetic acid (NAA). Subsequently, 8 fold increase in shoot number occurred every 6 weeks. Shoots were readily rooted in B_5 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 meritstem 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 B_5 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-Iglesian (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 mgl^{-1} napthalene 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 -napthalene 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 p-chlorophenoxy acetic acid (P-CPA), 2 μm 2, 4-dichloro phenoxy acetic acid (2, 4-D) and 0.5μm 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 eleminated 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 graminaceons 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* x *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 B_5 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, week 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* x *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 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. carolianinum* 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; Khetrapal *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 B_5 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 Lloyed 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 osmaticum (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±2°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°C± 2°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 B_5 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*, differntiation 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 cernum* (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 fragements, 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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