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RESEARCH ARTICLE

Allium cepa L. and its Related Taxa in India: Identification, Eco-geographical and Genetic Resources Study

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Allium cepa L. (common onion) and its related taxa were studied to delineate the taxonomic identity of taxa belonging to subgenus *Cepa* in India for distinguishing characters. Study was primarily based on morphological observations recorded during field collection, experimental and herbarium work. Evidences based on findings from molecular and scanning electron microscopy study of seeds of selected taxa in subg. *Cepa* compared with *Allium roylei* and *A. stracheyi* of subg. *Polyprason* supported justification for reconsideration of systematic position of *A. roylei* under subg. *Cepa*. A simple key was prepared to facilitate identification of taxa in subg. *Cepa.*

Key Words: Molecular study, Seed testa, Subgenus *Cepa,* Taxonomic key

Introduction

Genus *Allium* L. with over 800 taxa is distributed mainly in seasonally dry regions of the northern hemisphere with only one species, *Allium dregeanum* Kunth. occurring in South Africa (Friesen *et al*., 2006; Neshati and Fritsch, 2009). The centre of origin of onions especially *Allium cepa* L. (common onion) is debatable due to its non-existence as a wild species (Hanelt, 1990). The common onion belonging to the subg. *Cepa,* is a bulbous plant utilized worldwide primarily for the edible bulbs, with leaves and flowers being used as vegetable, spice and for flavouring. Due to its antioxidant, antimicrobial and anti-diabetic properties, it finds place in pharmacy and food industry. Among all the cultivated taxa, besides some locally important species, *A. cepa* L. represents wide variation in characters of bulb, leaf, scape, and inflorescence/flower (Pandey *et al.*, 2005a, b).

Genus *Allium* has been classified into subgenera and sections based on morphological characteristics such as shape of the rhizome/bulb; shape and size of perianth, filament, pistil, capsule, seed; anatomy of root, leaf, scape, ovary; and chromosome number (Hanelt *et al*., 1992; Friesen *et al*., 2006; Neshati and Fritsch, 2009; Nguyen *et al*., 2008; Choi and Oh, 2011). Based on morphology of rhizome/bulb characters and molecular study *Allium* has been grouped phylogenetically into three main evolutionary lines (Friesen *et al*., 2006; Li *et al*., 2010). Out of the three evolutionary lines, taxa in the subg. *Cepa* belong to the third evolutionary line which is the most advanced line.

Globally the subg. *Cepa* is one amongst the largest cultivated group and has over 30 taxa including many cultivated species distributed among five sections (Friesen *et al*., 2006; Choi and Cota-Sánchez, 2010). The nearest wild relative in the subg. *Cepa* sect. *Cepa* is *A. vavilovii* (Gurushidze *et al*., 2007), which occurs in the Koppet Dag mountains of Turkmenistan. The primary centre of domestication of onion is probably located in the mountainous regions of Turkmenistan and North Iran of South-West Asia due to presence of high concentration of different ecotype variability (Vavilov and Burkinich, 1929). The regions of great diversity in the near east Asiatic and the Mediterranean regions are considered as secondary centres (Fritsch and Friesen, 2002).

The Indian gene centre is rich in diversity of cultivated and wild *Allium* species represented by 35-40 taxa distributed in different eco-geographical regions (Pandey *et al.,* 2008; Pandey *et al.,* 2017). In the Indian context there have been revisionary works on the genus *Allium* besides a few studies on identification, diversity distribution and eco-geography in general and subg. *Cepa* in particular. Based on bulb and leaf characters at sectional level 27 taxa/species of the Indian region have

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been grouped into three sections namely *Schoenoprasum*, *Rhizirhideum* and *Molium* (Baker, 1892). Revisionary works on the Indian *Allium* (Dasgupta, 2006) along with field and herbarium studies (Gohil, 1992; Pandey and Pandey, 2005; Pandey *et al*., 2017) have thrown light on different aspects of the genus. An updated infrageneric classification for genus *Allium* in India has been reported earlier based on distributional data, morphological and herbarium studies grouping the recognized species into ten subgenera and 22 sections (Pandey *et al*., 2017). In the Indian Himalayan region, subg. *Cepa* is represented by four sections, namely sect. *Cepa*, sect. *Annuloprason*, sect. *Seculiferum* and sect. *Schoenoprasum* which occur wild (Pandey *et al*., 2008; 2017).

There are many gaps in knowledge on occurrence of rarer and endemic taxa from the Indian region mainly due to inaccessibility to areas of distribution. Lack of live material available for experimental study and heavy dependence on data from regional floras have led to many identity related issues. Inaccurate identification and limited collection of live material of the closely related species have resulted in inadequate utilisation of the genetic resources for crop improvement. Meagre data on significant characters of bulb or rhizome, leaf anatomy, micro-morphology of ovary and seed and biogeography have resulted in exclusion of Indian *Allium* from the global phylogenetic works.

Hence the present study was attempted with the aim to: 1) understand systematics in subg. *Cepa* in the Indian context through field, morphological, seed micro-morphological studies of selected taxa; 2) reconsider placement of *A. roylei* (subg. *Polyprason*) in subg. *Cepa* based on molecular and Scanning Electron Microscopy (SEM) study; and 3) develop a simple field key to facilitate identification of taxa.

Materials and Methods

Taxa Studied

Taxonomic investigation on the genus *Allium* was undertaken during 2013-2019. Taxa in the subg. *Cepa* of genus *Allium* in the Indian gene centre were studied based on basic field work, and morphological data gathered through experimental and herbarium study. Field studies were undertaken in parts of north eastern hill (mainly Nagaland, Manipur and Arunachal Pradesh) region, Andhra Pradesh, Tamil Nadu and Uttarakhand during germplasm collections. For herbarium studies the authors visited different national herbaria in India at Botanical Survey of India (codes-CAL, BSD), DD, KASH and LWG) and consulted ~200 specimens. Besides, data from online herbaria of Paris (P), Kew (K), Edinburgh (E), Beijing (PE), London (BM, LINN) and Leiden (L) (including type specimens) were used with label information on herbarium specimens which were further validated through global databases and eco-geographical distribution records. Under-represented wild taxa viz., *A. rhabdotum*, *A. farctum*, *A. fedtschenkoanum* var. *fedtschenkoanum*, *A. atrosanguineum* var. *fedtschenkoanum* and *A. semenovii* were studied only through herbarium specimens in Indian and global herbaria (Table 1).

Experimental studies were carried out at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi and field gene bank at Regional Station, Bhowali, Uttarakhand. Selected taxa with confusing identities were subjected to grow-out tests. Cultivated taxa including *A. cepa* var. *cepa*, and the less-common types as *A. cepa* var. *aggregatum* and var. *proliferum* were studied through live samples in the form of bulbs (over 60 samples) collected across entire distribution range as well as plants raised in experimental fields at ICAR-NBPGR, New Delhi and RS Srinagar, J&K.

DNA Sequence Analysis

To support the morphological findings and relatedness among the selected taxa, data were drawn for molecular study. DNA was isolated using the modified cTAB method from young leaves of *A. cepa* (14 accessions), *A. chinensis* (3 accessions), *A. fistulosum* (5 accessions) and one accession each of *A. farctum*, *A. schoenoprasum*, *A. altaicum* and *A. roylei*, amplified at the ITS locus, sequenced and analyzed using MEGA7 (Kumar *et al.,* 2016). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 26 nucleotide sequences. Codon positions containing gaps and missing data were eliminated. There were a total of 596 positions in the final dataset.

Micro-morphological study

Micro-morphological study was undertaken with freshly collected samples as well as herbarium specimens using wet technique. Data were recorded for characters of bulb membrane, anatomy of leaf and scape (cross

Table 1. Diversity distribution and study on the subg. *Cepa* in the Indian region

S. No.	Section (botanical name)	Distribution/habitat	F	H	E	O
			Study source			
1.	**Sect. *Cepa*** (TS - 7: C-05; W-02)					
	Allium cepa var. *cepa*	Cultivated; tropical- subtropical and temperate	√	√	√	√
	Allium cepa var. *aggregatum*		√	√	√	√
	Allium cepa var. *proliferum*	Cultivated (mainly temperate)	√	√	√	√
	Allium fistulosum	Cultivated (mainly temperate)	√	√	√	√
	Allium x cornutum	Cultivated	–	√	–	√
	Allium farctum	Wild; crevices of limestone rocks/ boulders; Western Himalaya (Jammu and Kashmir, Kinnaur district Himachal Pradesh; 2000-3000m)	–	√	–	√
	Allium rhabdotum	Wild; running water, in gravel along the stream edges, open grassy hill side (Bhutan)	–	√	–	√
2.	**Sect. *Sacculiferum*** (TS-1; C-01; W-0)					
	Allium chinense	Cultivated; subtropics-sub-temperate (Khasi hills in north-eastern hills; 1200-1830m)	√	√	√	√
3.	**Sect. *Annuloprason*** (TS-3; C-0; W-3)					
	Allium atrosanguineum var. *atrosanguineum*	Wild; swamp alpine pasture slopes, common on rocky areas (Kashmir, Western Himalaya; 3600-4200 m)	–	√	–	√
	Allium atrosanguineum var. *fedtschenkoanum*	Wild; western Himalaya (Kashmir); frequent on slopes, rocky gravelly soil meadows, high mountain bogs, stream sides (2400-3500m)	–	√	–	√
	Allium seminovii	Wild; northern Himalaya in the forest margin, damp slopes, meadows (above 3000m)	–	√	–	√
4.	**Sect. *Schoenoprasum*** (TS-1; C-0; W-1)					
	Allium schoenoprasum	Wild; meadows, valleys, damp slopes, along streams western Himalaya in Kashmir-Kumaon (2000-3000m)	√	√	√	√

F: field study; H: herbarium study; E: experimental study; O: other sources; TS: total species no.; C-cultivated, W-wild

sections) and flower (stigma and anther characters) using dissection microscope (model Nikon SMZ1500) and hand lens (magnification 10x). Seed morphological characterization of samples of six taxa represented from subg. *Cepa* and subg. *Polyprason* maintained in the National Herbarium of Cultivated Plants (NHCP), ICAR-NBPGR, New Delhi were studied through simple microscopic study at ICAR-NBPGR for seed shape and gross seed size and scanning electron microscopy (SEM) at the Scanning Electron Microscopy Unit in Indian Agricultural Research Institute, New Delhi. For recording data on seed morphological characters the seeds were cleaned and viewed under the 10x and 100x magnifications.

For the SEM study, selected seed samples were washed for 1-2 minutes with absolute alcohol to remove debris. They were further subjected to ultrasonic cleaning by repeated changes in absolute alcohol and then mounted over clean stubs using double sided adhesive tapes. The clean dry seeds were coated with gold film in JEOL JFC 1100E ion-sputtering device. The coated seeds were viewed under the scanning electron microscope (operated at an accelerated voltage of 15 kV) and photographed with JOEL ISM-5500LV.

Result and Discussion

Field, Experimental and Herbarium Study

Characters of bulb shape, colour of bulb coat and other additional data were noted using freshly collected bulbs from live plants for micro-morphological examination of plant parts. The bulb coat in *A. cepa* var. *cepa*, *A. cepa* var. *aggregatum*, *A. chinense*, *A. fistulosum* and *A. schoenoprasum* showed clear distinction in the texture (membranous) in fresh as well as dried samples. The colour of perianth, membrane characters and whether or not leaves channelled were not retained in the herbarium specimens. Loss of these characters in herbarium specimens was also noted by Pandey *et al.* (2019). However, characters of bulb shape and size, bulb if borne singly or in cluster, bulb tunic if membranous, papery/coriaceous; scape length with respect to leaf; arrangement of flower in inflorescence, compactness, inflorescence shape and size; size of anther with respect to perianth if exerted/inserted, and tepal shape were clearly evident in herbarium specimens.

Accessions of *Allium cepa* var. *aggregatum* G. Don collected from Tamil Nadu, Andhra Pradesh, Karnataka and Odisha and Uttar Dinajpur, West Bengal were highly variable in bulb size, bulblets numbers (2-9) and coat colour ranging from red to reddish brown and copper. Cultivars from the north-eastern region were distinct from the south Indian types. White bulb coat in *A. cepa* var. *aggregatum* (aggregate onion) was one of the rarer traits collected in germplasm from Andhra Pradesh. An interesting collection of aggregate onion with elongated bulb (copper coat), locally called 'doona' was collected from four locations in district Pithoragarh and Chamoli (Uttarakhand), and two locations in the north-eastern hill region (Nagaland and Manipur). Grow-out test, herbarium study and validation through micro-morphological study identified the 'doona' as *A. cepa* var. *aggregatum*. Identity confusion with the true shallot *A. ascalonicum* L. was however resolved on the basis of character of leaf and flowers which were very different and distinct. Taxon *A. cepa* var. *aggregatum* group was determined as a variety of the common onion. Bulbs of multiplier onion, *A.* x *proliferum* [*A. cepa* var. *proliferum* (Moench) Regel] collected as a backyard cultigen from Srinagar, Jammu and Kashmir produced many aerial bulbils (HS14255, BLM) under Delhi conditions.

Data from herbarium specimens included both cultivated taxa *viz*., *A. cepa* var. *cepa* L., *A. cepa* var. *aggregatum* G. Don, *A. cepa* var. *proliferum* (Moench) Regel, *A. fistulosum* L., and *A. chinense* G. Don) and wild taxa *viz*., *A. schoenoprasum, A. seminovii*, *A. atrosanguineum* var. *atrosanguineum*, and *A. atrosanguineum* var. *fedschenkoanum* (Table 1). Some of the under-represented wild taxa *A. rhabdotum* Stearn and *A. farctum* Wendelbo were studied through e-images in Indian and global herbaria (Table 1).

Eco-geographical Distribution Allium subg. Cepa (Mill.) Radić.

Eco-geographical data on different species have been found as a useful tool in pin-pointing areas for germplasm collection and conservation (Guarino *et al.*, 2005). In *Allium,* eco-geographic study on diversity distribution, habitat features and source for study material in subg. *Cepa* are given in Table 1 and 2.

Sect. Cepa (Mill.) Prokh.

Sect. *Cepa* is characterized by cylindrical-globose bulb(s) and/or axillary daughter bulbs of large size, condensed rhizome that is reduced to a disc in onion and shallot, and one to several leaf bases covered by a dry thin outer coriaceous coat/tunic and membranous inner one, cylindrical, fistulose opposite leaves formed by leaf sheath, pseudo-stem surrounding the fistulose scape, ovaries with nectariferous pores and two ovules per locule. The sect. *Cepa* is morphologically closer to sect. *Schoenoprasum* Dumort. and through this it is linked to the other species of the subgn. *Rhizirideum* (*A. prezwal*) (Friesen *et al*., 2006). Sect. *Cepa* has two sub-sections: a) *Cepa* alliance: *A. cepa*, *A. vavilovii*; b) *Altaicum* alliance: *A. altaicum*, *A. fistulosum*.

Cultivar *Allium cepa* var. *aggregatum* G. Don also known as ever-ready onion, small onion, multiplier onion, nesting onion, potato onion, shallot and Egyptian ground onion, is globally *cultivated* as a minor bulb crop (Saraswathi *et al*., 2017). It is a crop of the tropical and subtropical regions with tolerance to hot and humid tropical climates with better tolerance to pests and diseases and a longer storage life than the common onion. It differs from *A. cepa* in its perennial habit, in having smaller individual bulbs than regular onions, and with smaller flowers in a loosely arranged inflorescence, and with thin tender leaves often bent and concave on the inner side (Fritsch and Friesen, 2002). The underground bulbs are oblong, semi-cylindrical and clustered, and fertile seeds are produced. It is broadly grown and consumed in the Southern states of India and is famous for its

highly pungent bulbs which are used in preparation of 'Sambar' (pulse curry with vegetables) an important dish in South India. True shallots have a mellow flavour and poor storage, while ever-ready onion has a sharper flavour and better storage.

Allium fistulosum L. commonly known as Welsh onion, bunching onion, long green onion, Japanese bunching onion, and spring onion probably originated in north-western China and is known only in cultivation *(*Rabinowitch and Brewster, 2018)). Its cultivation dates back to at least 200 BC in China where presently it is the most important *Allium* species fulfilling the culinary role of both the common onion and leek in Europe; and now second in importance to the common onion in Japan. The crop is grown throughout the world, but the main area of cultivation remains eastern Asia from Siberia to Indonesia; elsewhere only a home garden crop. The plants usually grow up to more than 50cm tall, as annuals or biennials, with ovoid-oblong bulbs, lateral bulbs few to absent, tunic white-pale reddish brown, leaves thick glaucous with tubular sheath, blade cylindrical, hollow and acute apex, inflorescence a spherical umbel on a long, erect, terete, hollow scape, the umbel composed either of flowers or of bulbils only. In India, *A. fistulosum* is grown to a limited extent in the hilly areas of Himalaya and the Nilgiris. It is a winter hardy early flowering variable species resistant to pests and diseases (Chuda and Adamus, 2009; Pradheep *et al*., 2014). Its closeness with the common onion has been established cytologically (Kohli *et al*., 1984; Kim *et al*., 2003; Nomura *et al*., 1994). It is also reportedly closer to *A. roylei* (Fritsch and Friesen, 2002).

Allium x *proliferum* (Moench) Schrad [syn. *A. cepa* var. *viviparum* (Metzg.) Alef.; *A. cepa* var. *proliferum*] is commonly known as top onion, tree onion, bunching onion or Egyptian onion. It is a spontaneous cultivated hybrid between *A. cepa* (common onion) and *A. fistulosum* (Japanese bunching onion) (Sharma *et al*., 1996). In Kashmir it is the traditionally used onion known by the common name 'Pran' which is a high priced commodity used for culinary seasoning and in preparation of Kashmiri cuisine (Mir *et al.*, 2013). Morphologically the species has a plant height up to 120 cm, with gigantic plant parts, rapid vegetative growth, poorly developed bulb (like parental species *A. fistulosum*), inflorescences possessing sterile flowers with bulbils that often sprout within the umbel (usually 4-8 bulbils per inflorescence), and no seeds. The plant reproduces exclusively through the underground bulbs or aerial bulbils borne on the inflorescence. However, the species resembles *A. cepa* (in leaf characters-inner side flattened), and in the inflorescence having a long spathe. Pathak and Gowda (1993) probably referred to *Allium* x *proliferum* as *A.* x *cornutum*.

Allium x cornutum Clementi ex Vis. is a triploid onion ($2n = 3x = 24$) and a cultigen of Yugoslavian origin. It has characteristically clustered and ovoid-elongated bulbs. It is a minor garden crop in south-eastern Asia, Europe and Canada (Fritsch and Friesen, 2002). The species is sterile and reproduces vegetatively. The taxon was first named *A. cepa* var. *viviparum* (Langer and Koul, 1983; Puizina and Papeš, 1997), but since the name had been already used for viviparous onion it was named as *A. cornutum* (Friesen and Klaas, 1998). Further due to its hybrid origin, its name was changed to *A.* x *cornutum*. However, a study undertaken by Fredotović *et al.* (2014) suggested the grouping of this taxon with the Indian clone 'Pran' (*Allium* x *proliferum*).

The term 'Shallot' is often also used for diploid and triploid viviparous onions namely *A.* x *proliferum* (Moench.) Schrad and *A.* x *cornutum* Clementi ex Vis. (Puizina and Papeš, 1997). The local name shallot has been used for different taxa/species in different regions; the true shallot, being *Allium cepa* (the *aggregatum* group), *Allium fistulosum*, etc.

Fredotović *et al.* (2014) have suggested *A. cepa* L. and *A. roylei* Stearn as two putative parents whereas the identity of the third parental species has remained hitherto unknown. Based on phylogenetic analyses of the internal transcribed spacers ITS1-5.8S-ITS2 of 35S rDNA and the non-transcribed spacer (NTS) region of 5S rDNA of *A*. x *cornutum* and its relatives of the sect. *Cepa* and the wild Asian species *Allium pskemense* B. Fedtsh., *A. pskemense* is therefore inferred to be the third, so far unknown, putative parental species of triploid onion *Allium* x *cornutum*. GISH (genomic *in situ* hybridisation) using DNA of the three putative parental diploids corroborated the results of the phylogenetic study (Fredotović *et al.*, 2014). This taxon has been reported in the adjoining parts of India, in Pakistan and Bhutan (Pradheep *et al*., 2014). In present study, *A.* x *cornutum* was studied from e-herbaria.

Among the wild relatives of onion belonging to sect. *Cepa*, occurring in India, *Allium rhabdotum* Stearn and *A. farctum* Wendelbo are reported from

Fig. 1a. Diversity in *Allium* subg. *Cepa*: *A. cepa* (a-b); *A. cepa* var. *aggregatum* (c-d); *A. proliferum* (e); *A. fitulosum* (f)

Fig. 1b. Diversity in *Allium* subg. *Cepa*: *A. altaicum* (a-b); *A. chinense* (c); *A. schoenoprasum* (d-e); *A. roylei* (f)

the Himalayan region. *A. rhabdotum,* a tropical Asian species, is probably a tertiary relative of common onion (Grierson and Long, 1984; Fritsch and Friesen, 2002; Singh *et al.*, 2013). It is distributed in the Indian subcontinent mainly in Bhutan, in open grassy hills and along streams at altitudes between 3600-3900m (Singh *et al.*, 2013). Morphologically it is closer to *A. fistulosum* and to the *A. altaicum* alliance which occur in the Siberia-Mongolia region. *Allium farctum* was first described from Kalpa valley in Himachal Pradesh, India (type-K.P.Janardanan 47673-BSD). It is reported in the mountains of western Himalaya, West Pakistan and East Afghanistan along steep rock crevices (2000-3000m). It is morphologically allied to *A. galanthum* Karelin & Kirilov in having cylindrical bulbs with a slightly inflated base (Fritsch and Friesen, 2002) but seed coat morphology is closer to the *Oschaninii* alliance which is the secondary genepool of onion (Hanelt, 1990). In the present study distribution of *A. rhabdotum* and *A. farctum* is reported only from herbarium-based work and literature (Stearn, 1960). However, occurrence of *A. rhabdotum* in J&K, India is doubtful (Pradheep *et al*., 2014).

Sect. Seculiferum P.P. Gritz.

Allium chinense G. Don commonly known as Chinese onion, Chinese scallion, glittering chive, Japanese scallion, and Oriental onion is a native to China and Korea. It is a commonly cultivated species in many countries including India in the north-eastern hill region. Floristic records have mentioned its distribution in Meghalaya (1220-1830m), Uttarakhand; and eastwards to Bangladesh, China and Japan (Dasgupta, 2006). In tropical and subtropical areas of China it is recorded

as a cultivated vegetable (http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200027461). *A. chinense* is the only cultivated species among subg. *Cepa* with hollow leaf and solid scape. This species is morphologically and cytologically closer to the common onion; and crossable with *A. tuberosum* with great difficulty (Nomura and Koichi, 1996). During the field study in Nagaland one of the authors recorded two morphotypes of *A. chinense* identified by local names as 'bado-lasun' and 'nano-lasun' having variation in bulb size i.e. bigger bulbs with wider triquetrous channelled (fistulose) leaves and narrower bulb with thin triquetrous leaves respectively. Occurrence of *A. chinense* in wild in the area was not recorded during field study.

Sect. Schoenoprasum Dumort.

Allium schoenoprastum L. has wide ecological and morphological adaptability across different geographical regions of the world (Friesen and Blattner, 2000). The species occurs in the meadows, valleys, alpine slopes and along the streams (2000-3000 m) in the western Himalaya in states of Uttarakhand, Himachal Pradesh, Jammu and Kashmir (Drass), India and extending to south-west Asia, North- and East-Europe and north-America. Observations recorded on several vegetative and reproductive characteristics indicated its distant relation to other taxa of the subg. *Cepa* (Table 1). The most unique characters included, bulb located on condensed oblique rhizome, thin terete leaves, shape of the inflorescence and perianth oblong-lanceolate and anthers length half the perianth. It has been reported to be cytologically allied to *A. fistulosum* but difficult to cross with it (Umehara *et al.*, 2006).

Sect. Annuloprason T.V. Egorova

Taxa of this section are distributed in the meadows, stream banks, moist places and high mountain bogs (2400-5000m) in the Himalayan ranges extending to Afghanistan, China, Kazakhstan, Kyrgyzstan, Pakistan, Tajikistan and Uzbekistan. There are three distinct infraspecific categories, two subspecies *viz.*, *A. atrosanguineum* subsp. *atrosanguineum* Schrenk and *A. atrosanguineum* subsp. *fedtschenkoanum* (Regel) G. Zhu & Turland occur in India and differ in fistulose leaves and perianth colour (Table 2); the third taxon, *A. atrosanguineum* var. *tibeticum* (Regel) G. Zhu & Turland is endemic to China (Zhu and Turland, 2000).

Allium seminovii Regel is an uncommon species distributed in parts of western Himalaya in Himachal Pradesh, Jammu and Kashmir, extending to middle Asia and China. *A. seminovii* has long leaves, a stout, falcate tapering scape bearing sub-globose tapering inflorescence, a lanceolate-ovate and yellow perianth that turns red-purple to red at maturity. The outer perianth lobes are longer than inner ones; are ovate-lanceolate with irregularly denticulate margin, and acuminate apex that make it distinct from *A. atrosanguineum*. *A. atrosanguineum* has characteristic leaves that are equal in size to scape, and globose inflorescence, oblong-ovate, dark purple-red dotted perianth that turns yellow at maturity, and two persistently partite spathe drooping downwards (even visible in herbarium specimens).

Despite the two taxa sharing many characters, in the authors opinion, *A. seminovii* is entirely distinct from *A. atrosanguineum* as was revealed from study of e-images of herbarium specimens located in herbaria (K, P, E) and study of Indian material housed in BSI (specimens from Kashmir-Drass and Tibet areas have distinct vertical fibres and typical perianth representing true *A. seminovii*). However, infra-specific classification within taxon *Allium atrosanguineum* is justified on the basis of characters mentioned above. Therefore, in the present study the three taxa namely *A. atrosanguineum* var. *atrosanguineum*, *A. atrosanguineum* var. *fedtschenkoanum* and *A. seminovii* are being treated separately. Justification for merging *A. atrosanguineum* and *A. seminovii* as suggested by Dasgupta (2006) only on the basis of herbarium study is not agreeable by the authors (Table 2). Contiguous population of these three taxa in the entire eco-geographic range along with support from study of live material may throw more light to support these observations.

Other Taxa With Affinity to Sect. Cepa

Evidence from data on morphology, anatomy, distributional ecology, developmental biology, molecular study, serology and isozyme study have revealed a new taxonomic categorization of the genus *Allium* (Hanelt *et al.*, 1992). Many of the groupings in recent classification have supported relationships among infrageneric groups (subgenera and sections) on the basis of chloroplast and nuclear (ITS) genomic data (Friesen *et al*., 2006). An extensive study on macro- and micro-morphological characters of reproductive parts, anatomy and cytology in combination with molecular data in *Allium* species has helped in understanding character development and geographical distribution (Neshati and Fritsch, 2009; Choi and Cota-Sánchez 2010; Choi *et al*., 2012).

Allium roylei Stearn a member of subg. *Polyprason* Radic' occurs on slopes of Garhwal Himalaya, Mussorie, Himachal Pradesh, Jammu and Kashmir in Gilgit, Ladakh and extends to West-Asia (Afghanistan). Characteristically it has an ovoid bulb not seated on a rootstock (like *A. shoenoprasum*), reddish-brown, scarious outer tunic, leaves which are shorter than scape, filiform, linear, grooved and non-fistular, fistular scape, hemispherical inflorescence, campanulate pale-red flower, and filament longer than perianth. It has been used as a source of powdery mildew resistance (de Vries, 1992). Reports on crossability with *Cepa* subgn. have demanded further investigation to clearly pinpoint stronger phylogenetic linkages (McCollum, 1982). In current classification it has been included as member of sect. *Oreiprason* F.Herm. (with related taxa namely *A. stracheyi* and *A. consanguineum*).

Data from morphological, cytological and molecular studies have shown that *A. cepa* is linked to *A. roylei* through *A. schoenoprasum* especially for characters of protoplast DNA (Li *et al.*, 2010). Evidences from chromosomal and molecular characters of *A. roylei* favour its placement in sect. *Cepa,* but morphological characters of inflorescence, flower parts, and seed testa support exclusion from the sect. *Cepa* (Fritsch and Friesen, 2002).

In the present study, *A. cepa, A. chinense*, *A. fistulosum* and one accession each of *A. farctum*, *A. schenoprasum*, *A. altaicum* and *A. roylei*, were selected to infer phylogenetic relationships using the NJ method (Fig. 2). Sequence analyses of the ITS region attempted on members of the subg. *Cepa* and *A. roylei* indicated that *A. cepa* var. *cepa* was closer to *A. fistulosum* followed by *A. roylei*, and *A. farctum*. *A. schoenoprasum* and *A. chinense* were the most distant taxa used in the study (Fig. 2). These observations were broadly supported by the morphological findings (Table 2) and the seed SEM study. Gurushidze *et al*. (2007) have used molecular tools to infer the phylogenetic relationships of wild and cultivated species of *Allium* sect. *Cepa*.

Seed Micromorphology in Subg. Cepa

In the genus *Allium*, seed morphology of many has served as a useful tool in systematics and taxonomy (Fritsch *et al*., 2006; Neshati and Fritsch, 2009; Celep *et al*., 2012). Study of seed coat patterns provide additional support in identification and delimitation of taxa (Friesen *et al*., 2006; Neshati and Fritsch, 2009; Celep *et al*., 2012). For the taxa of Indian region, there are meager data on

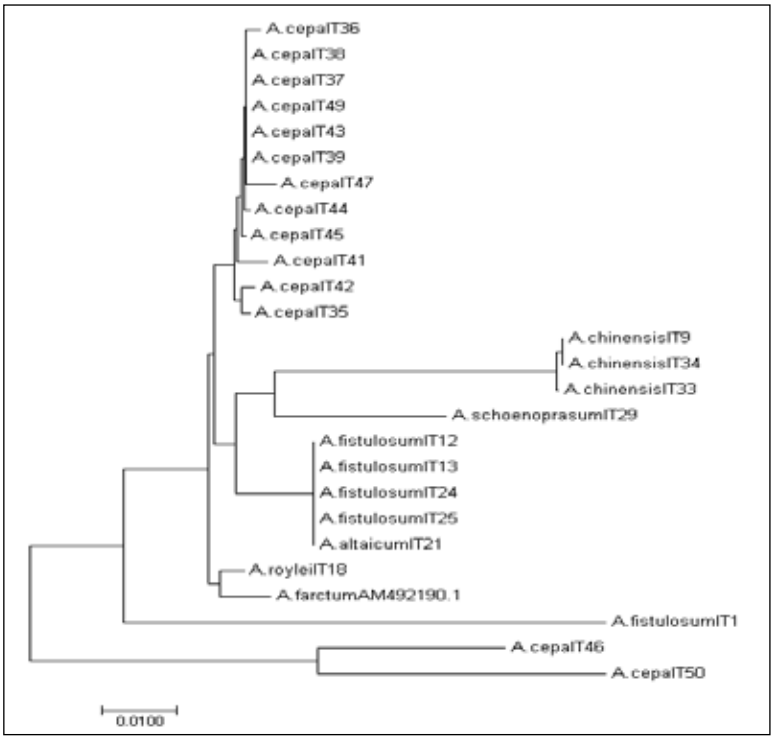

Fig. 2. Relationships amongst the members of the subg. *Cepa* and *A. roylei* (subg. *Polyprason*) based on sequence analyses of the ITS region, inferred using NJ method (numbers in the tree show the distance between different sequences)

Table 2. Qualitative characters used in taxonomy of *Allium* Subgen. *cepa* in India

Character	*A. cepa var. cepa*	*A. cepa var. aggregatum*	*A. cepa* var. *proliferum*	*A. cornutum*	*A. fistulosum*	*A. rhabdotum*	*A. farctum*	*A. chinense*	*A. seminovii*	*A. atrosanguineum* var. *atrosanguineum*	*A.atrosanguineum* var. *fedtschenkoanum*	*A shoenoprasum*
Rhizome	Condensed (discoid)	Condensed (discoid)	Condensed (discoid)	Condensed (discoid)	Condensed (discoid)	Condensed (-)	Condensed (-)	Condensed (-)	Condensed (-)	Condensed (-)	Condensed (-)	Condensed (oblique) bulb not seated on a rootstock
Bulb												
Shape	Solitary	Clustered	Clustred	Solitory-clustred	Solitary/ clustered	Solitary	Solitary	Clustered	Solitary/ Clustred	Solitary/ clustred	Solitary/ clustred	Clustered
Tunic texture	Applanate-globose-cylindric	Ovoid-cylindric-ovoid, semi-cylindric	Elongated to poorly developed, ovoid-cylindric-ovoid	Ovate- pear shaped	Cylindric-ovoid, less developed	Cylindric	Ovoid-oblong	Narrowly ovoid	Cylindric	Cylindric	Cylindric	Ovoid-cylindric
											Coriaceous	Membranous
Colour	Membranous	Membranous	Membranous	Membranous	Membranous	Membranous	Membranous	Membranous	Coriaceous (leathery)	Coriaceous	Blackish	Dark brown
	Purple-red, brown-yellow-pale-white	Purple-red, brown-yellow-pale-white (rare)	Purple-red, brown	Purple-brown	White-pale brown-red	Reddish brown	Reddish brown	White (~tinged purple-pink)	Blackish-brown, striate	Brownish-black		
Leaf blade												
Apex	Acuminate	Acuminate Terete	Acuminate Terete	Acuminate	Acute	Acute/ac	Acute	Acute	Acute	Acute	Acute	Acuminate
Cross section	Terete (flat above) (semi-circular in section)	(round)	(flat above)	Terete (circular)	Terete (perfect circular)	Terete/ filiform	Terete	3-5 angled (triquetrous)	Terete	Terete	Terete	Terete (grooved above)
Pith in cs	Fistulose Half length of scape	Fistulose	Fistulose	Fistulose	Fistulose	Fistulose	Fistulose	Fistulose	Fistulose	Fistulose	Fistulose	Fistulose narrow
Leaf vs scape length	Shorter than scape	Full length of scape	Shoter than scape	Shorter than scape	Equal to scape fistular	Half (shorter than scape)	Half (shorter than scape)	Subequal to scape	Longer than scape	Nearly equal or slightly shorter than scape	Much shorter than scape	Shorter than scape
Scape												
Shape in CS	Terete	Terete	Terete,	Terete	Terete	Terete,	Terete	Terete	Slender (stout)	Terete	Terete,	Terete (thin)
Pith in CS	Fistulose at the basal half	Fistulose (all through)	Fistulose at basal half	Fistulose	Fistulose all length	Fistulose (?)	Non-fistulose	Non-fistulose(filled or solid at basal half of tip)	tapering (my observ from type spec.)	Fistulose	Fistulose	Fistulose
	Hollow-solid above		Hollow-solid above	Flatened at bottom					–			

Character	*A. cepa var. cepa*	*A. cepa var. aggregatum*	*A. cepa* var. *proliferum*	*A. cornutum*	*A. fistulosum*	*A. rhabdotum*	*A. farctum*	*A. chinense*	*A. seminovii*	*A. atrosanguineum* var. *atrosanguineum*	*A.atrosanguineum* var. *fedtschenkoanum*	*A shoenoprasum*
Flower opening	Stellate	Stellate	–					Drooping / laxy		Closed campanulate		
Umbel Shape	Globose Condensed, tough	Globose Loose	Hemispherical,	Globose-semiglobose	Hemi-spherical	Globose (condensed)	Subglobose (condensed)	Subglobose; laxy	Subglobose (tapering) (condensed)	Globose, (condensed)	Globose, (condensed)	Subglobose; (condensed)
Bulbils/ bulblets presence	No Bulblets present	No bulbils	Aerial bulbils present	Bulbils present	Not seen? Bulbils present	-	-	-	-	-	-	bulbils absent two
Spathe valve (no.)	One (splits into 2-4 parts)	One (splits into 2-4 parts)	One (very long; trifid on maturing)	one	Two (1-2 partite)	one	two	two	two (drooping down)	two (soft, not prominent)	two (soft, not prominent)	
Flower/ Perianth Shape	Stelate Oblong-ovate	Stelate oblong-ovate	Campanulate, green stripe oblong-ovate	Stelate Green stripe Obovate	Campanulate, Ovate, acuminate tip (reflexed)	Obovate, round tip	Elliptic-obtuse	Elliptic-suborbicular	Lanceolate-ovate-lanceolate	Oblong-ovate-Blunt tip	Oblanceolate, pointed tip	Oblong -lanceolate
Colour	White	White Prominent' green	-	White	Yellowish-white-(inner longer than outer)	white	White	Pale- purple (inner longer than outer) Light purple	Yellow (turn red-purple red) (inner shorter than outer)	Dark purple- red (dotted, yellow at maturity) Not prominent	Pale yellow	Pale red-reddish pink-deep lilac
Mid vein	Prominent' green		-	Prominent, pale red	Midline faded pale green, Not prominent	very prominent	Not prominent		prominent		Not prominent	Prominent, dark pink
Filament Length wrt. perianth	Equal-slightly longer than to perianth	Equal-slightly longer than to perianth -	Stamen shorter than pistil	Stamen shorter than pistil	Longer than pistil	Exerted	Equal	Much exerted	Shorter	Shorter	Shorter	Shorter than perianth
Tooth present/ absent	Basal end no tooth but bulged prominently		-	-	No tooth	-	present	present	present	present	present	absent
Ovary Shape	Subglobose Pistil lower than stamen	Subglobose Pistil lower than stamen		Oblong	Obovoid		Obovoid, deeply trilobed (4 lobed CAL)	Obovoid-globose with	Subglobose	Style short, obovoid	Globose-oblong Very short	Ellipsoid-subglobose style non-exerted
Style	Slightly exerted	Slightly exerted	Not exerted	Not exerted	Very much exerted	included	included	exerted	included	included		
Nectaries	Pore to form closed cavity				Inconspicuous (open cavity)			concave (hoodlike projection)	concave (hoodlike projection)	-	-	concave (hoodlike projection

"-" : not observed or available in any source:

seed micro-morphology and therefore identifying seeds is very difficult.

Seed Morphological Characterization: Seeds of subg. *Cepa* (var. *cepa*, *aggregatum*, *schoenoprasum*, *fistulosum*) and subg. *Polyprason* (*A. roylei* and *stracheyi*) were distinct for shape of the seed, surface colour and testa sculpture among the section (Fig. 3a, b). The seeds of all species were black with slight variation of dark brown; seed surface varied from dull black to shiny black. The shape was generally ovate. *A. fistulosum* had the smallest and *A. cepa,* the largest seeds.

Seed testa colour ranged from glossy black in *Allium cepa* and *A. roylei* to darkest brown in *A.cepa* var. *aggregatum* and *A. fistulosum* and dull black in *A. schoenoprasum.* The seed shape varied from spherical-crescent, beaked on one side in *A. cepa;* ovalish-round, with shallow beak dull warty texture of seed coat in *A. cepa* var. *aggregatum*; irregularly angled shrunken seed coat with prominent vertical lines in *A. schoenoprasum*; lunar shaped with prominent beak, irregularly angled, beaked in *A. roylei*; widely ovate, sharply beaked, shiny smooth, warty texture seed coat in *A. fistulosum*; and s*eed size* 3-3.3×2.0-2.4 in *A. cepa* and *A.cepa* var. *aggregatum*; 1-3.1 × 0.8-2.2 in *A. fistulosum*; 1-3.3 ×1.2.1 in *A. schoenoprasum* and l 3.3-3.30×1.24-2.4 in *A. roylei.*

Seeds of subg. *Cepa* showed rather simple seed coat cells, flat, granulose (sometimes granula coalescing into small flat verrucae), periclinal walls concave in the center, and straight to curved anticlinal walls covered by a depressed, coarsely striated intercellular region. The seed coat pattern was more variable across the subgenus and different regions on seed coat testa (cells narrow and elongated in depressed region). The predominant shape of the testa cells was irregularly angled tetra- to hexagonal cells with shallow depressed, ± straight. The seed testa cells of subg. *Cepa* sect. *Cepa* (*A. fistulosum*) with densely granulose periclinal walls with no verrucae. These features are typical of testa cells in sect. *Cepa* (Bednorz *et al*., 2011). Our observations were supported by the findings of Bednorz *et al.* (2011).

SEM study: Scanning electron microscopy (SEM) study of seeds in six *Allium* taxa (one from *Polyprason*, five from subg. *Cepa* suggested a close systematic significance. SEM study of seed testa of *A. roylei* with subg. *Cepa* relatives supported justification for its placement with

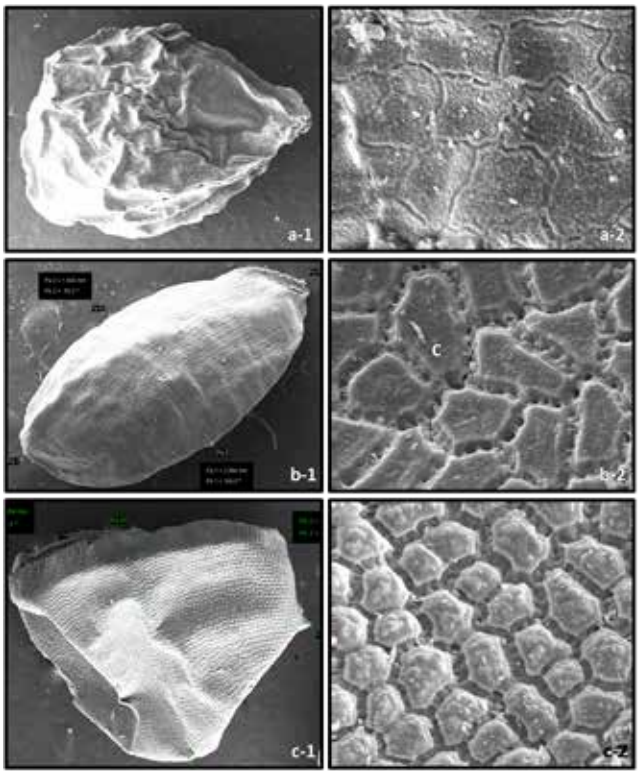

Fig. 3a. SEM scans of *Allium cepa* var. *aggregatum* (a-1,2); *A. schoenoprasum* (b-1, 2); *A. cepa* var. *cepa* (c-1, 2)

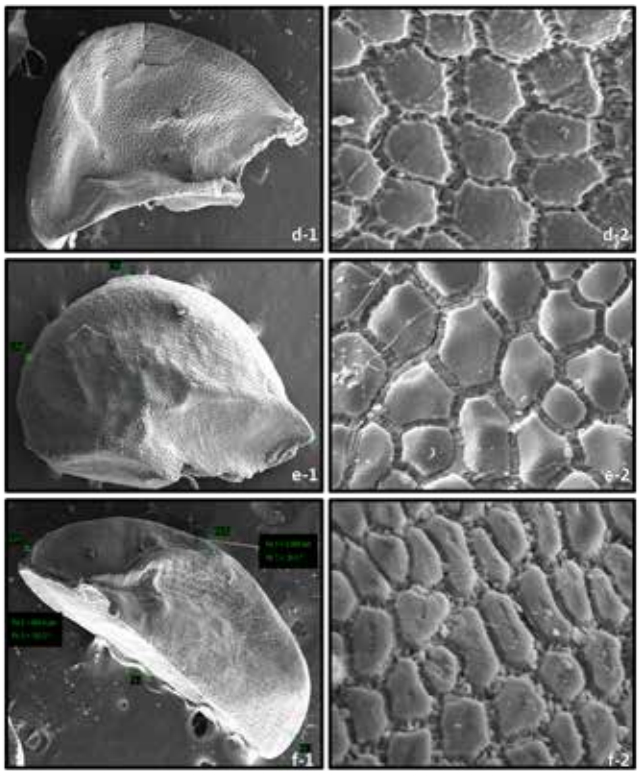

Fig. 3b. SEM scans of *Allium roylei* (d-1,2); *A. fistulosum* (e-1,2); *A. stracheyi* (f-1,2)

subg. *Cepa*. The same was also advocated by Fritsch and Friesen (2002). Seed coat patterns appear to mark different evolutionary levels inside of many taxonomic groups and variation of the testa characters is sufficient to distinguish taxa at sectional level. In present study seed testa ornamentation of selected taxa belonging to subg. *Cepa* and subg. *Polyprason* was used to throw some light on systematic reconsideration of *A. roylei*.

Taxonomic Key to Subg. Cepa

During study data were recorded on shape of rhizome and bulb, tunic texture, structure of leaf and scape, leaf vs scape length, leaf in cross section, shape and size of floral parts to diagnostic tools for grouping at infra-subgenus level. Taxonomic key for identification was prepared based on observations recorded for qualitative characters. Details are provided in Table 3 as follows:

Key to subg. *Cepa*

1. Bulb outer tunic membranous, filament length equal or longer than perianth
   - **2. Leaf fistulose (in cross section), bulb tunic variable**
     - 3. Perianth white, subequal
       - 4. Bulbs clustered or solitary, taxa cultivated
         - 5. Bulb cylindric
           - 6. No aerial bulbils, leaf tip acute, leaves equal to scape, perianth segments with fades pale green mid-vein

             —— ***A. fistulosum***
           - 6. Aerial bulbils present, leaf tip acuminate, leaves shoter than scape, perianth segments with pale green mid-vein

             —— ***A. cepa* var. *proliferum***
         - 5. Bulb ovoid-pear shaped or elliptical
           - 7. Umbel compact flowers
             - 8. Bulb ovate-pear shaped, spathe one valved

               —— ***A x cornutum***
             - 8. Bulb applanate-globose, perianth segments with green mid-vein

               —— ***A. cepa* var. *cepa***
           - 7. Umbel loosely arranged flowers

             —— ***A. cepa* var. *aggregatum***
       - 4. Bulb solitary, taxa occur wild
         - 9. Bulb cylindric, spathe valve one, perianth obovate, round tip

           —— ***A. rhabdotum***
         - 9. Bulb ovoid-oblong, spathe valve two, perianth elliptic-obtuse

           —— ***A. farctum***
     - 3. Perianth purple-red to pale red, inner longer —— ***A. schoenoprasum***
   - **2. Leaf three angled, triquetrous, bulb tunic white or lilac, many in cluster**

     —— ***A. chinense***
1. Bulb outer tunic coriaceous, filament length shorter than perianth
   - 10. Leaf longer than scape, tapering scape,/stout, umbel subglobose (tapering to tip), perianth lanceolate-ovate

     —— ***A. semenovii***
   - 10. Leaf nearly equal to or shorter than scape, scape terete, umbel globose (round tip), perianth oblong-ovate
     - 11. perianth pale yellow, not dotted at maturity, apex pointed/ attenuate

       —— ***A. atrosanguineum* var. *fedtschenkoanum***
     - 11. perianth dark puple-red, dotted yellow at maturity, perianth apex subacute, never attenuate, tip blunt

       —— ***A. atrosanguineum* var. *atrosanguineum***

Conclusions

Study on identification, eco-geographical and genetic resources of *Allium cepa* L. (common onion) and its related taxa in subg. *Cepa* in India has helped in elucidating distinguishing taxonomic traits to facilitate identification of the subg. *Cepa* in the Indian context. Besides macro-morphological data, study on micro-morphology of the floral parts, molecular findings and the SEM of seed has supported placement of *A. roylei* (subg. *Polyprason*) with the subg. *Cepa*. An identification key has been prepared based on most prominent features observed in the field.

Availability of live material particularly of rare and endemic taxa is a bottleneck in studies on taxa belonging to *Allium* in India. Identification of landraces of *A. cepa* var. *aggregatum* (aggregate onion) known by variable common names needs to be resolved for taxonomic confusions. Systamatics on morphotypes especially from southern part of the country and north-eastern and western hills need attention.

The following thrust areas are identified:

- Collection of wild and hitherto uncollected species diversity including *A. roylei, A. farctum, A. rhabdotum* which are known for cold hardiness traits, biotic resistance
- Morpho-molecular study on taxa from subg. *Polyprason* group with subg. *Cepa* to clarify taxonomic placement
- Crop diversification of less-known cultivars *viz.*, *A. chinense* in western Himalaya; introduction of *A. cornutum* from native areas

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