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Repository of *Allium* Genetic Resources at ICAR-NBPGR: Prospects and Challenges for Collection and Conservation

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Allium genetic resources repository at the Indian National Genebank (INGB) located at ICAR-National Bureau of Plant Genetic Resources (NBPGR), in India is one of its unique kind which holds germplasm of exotic and indigenous taxa. Under national programme on plant genetic resources, efforts have been made to enrich the repository of *Allium* genetic resources with three components *viz.* seed genebank, *in vitro* and cryogenebank, and field genebank. In this paper the details on the collecting and conservation efforts of *Allium* species are presented, with action points highlighted for the future conservation and crop improvement programmes in the context of the Indian gene centre.

Introduction

Genus *Allium* L. (family Amaryllidaceae) is widely distributed over the warm-temperate and temperate zones of the northern hemisphere with main centre of evolution concentrated along the Irano-Turanian biogeographical region. The new global methods using cryopreservation mainly of vegetatively propagated germplasm of *Allium* have made genebanking effective and cheaper besides enhancing germplasm health, continuing characterization and evaluation to maximize the utilization held in various genebanks.

Global germplasm holdings of *Allium* maintained at main world centers are represented by country-wise large germplasm holdings (Table 1). The intensified efforts for exploration, collection and conservation of *Allium* have resulted in material conserved in various types of repositories. This paper mainly highlights the national collections in *Allium* genetic resources with information on germplasm conserved in repository by the ICAR-National Bureau of Plant Genetic Resources (NBPGR) in the Indian perspective and national efforts on collection and prioritization for future programmes. The gaps in collection and conservation are reflected with reference to future plan of action in the country.

Why Genetic Resource Programme on *Allium*?

There are many gaps in knowledge on potential of *Allium* genetic resources from the Indian region. Utilization of an accession of wild species *A. roylei* Stearn of Indian origin as a potential donor of genes for resistance to powdery mildew and leaf blight to cultivated onion *Allium cepa* L. (de Vries *et al*., 1992), has opened the avenues for search of newer taxa/germplasm (Beetika and Gohil, 2009). Germplasm of cultivated and wild taxa of *Allium* was introduced in late 1980s and was established in ICAR-NBPGR regional station (RS), Bhowali, Uttarakhand. *Allium* genetic resources conservation programme was given high priority under the national programme since 1986 under the leadership of Late Dr KS Negi. Further, intensified efforts towards collection, and conservation of germplasm of cultivated and wild taxa of *Allium* led to establishment of field genebank (FGB) with holding of over 30 species of native and exotic taxa at RS Bhowali (Pandey *et al*., 2022). During planning of National Programmes (NEP 2020-25) the gap areas were included for collection of germplasm.

For the Indian region, the Himalaya hold two distinct centres of diversity for the genus *Allium* – the western Himalaya and the eastern Himalaya, in contiguity with south China and Pakistan. There is drastic loss in habitat due to anthropogenic activities, climate change and species composition in past decades affecting specific niche/habitat and loss of population due to harvest of wild *Allium* for use. This has resulted in rare, endangered and threatened (RET) status of many *Allium* taxa and thus necessitated gathering of native diversity and crop genepools (Sharma *et al.,* 2020). The Indian gene centre constitutes over 4.5-5 per cent diversity globally represented by 35-40 species of cultivated and wild taxa from temperate to alpine regions of the Indian Himalaya and tropical areas that are broadly distributed

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in different agro-ecological regions of India. The Indian *Allium* includes over 10 subgenera, 22 sections and 35-40 taxa distributed in different eco-geographical areas of the temperate and alpine regions of Himalaya sharing many taxa of Chinese origin (Astley *et al*., 1982; Rabinowitch and Brewster, 2018; Pandey *et al*., 2017, 2022).

From Field to Repository

As evidenced through global germplasm holdings of *Allium* species at main centers of the world, the cultivated *Allium* species are well collected, while the wild taxa are meagerly or under-represented in global collections and species distribution areas are not adequately covered. Global germplasm collections of *Allium* of cultivated and wild taxa (over 300 species) have been built-up at IPK, Gatersleben, Germany (Keller and Kik, 2018).

In Indian genebanks, the germplasm of cultivated and wild *Allium* species including exotic germplasm is conserved as *ex situ* collection in seed genebank, *in vitro*/ cryo-repository and in field genebank facilities. Germplasm has been assembled from high hills of Uttarakhand, Himachal Pradesh, Jammu and Kashmir (J&K) of the Western Himalayas and the high altitude regions of north-eastern region (Table 2).

Germplasm Collection and Gap Analysis

Gap analysis of the existing holdings in the repository was based on their performance of the material and its conservation status (common, rare/endangered/ threatened) and other aspects was taken into consideration

Table 1. Global germplasm holdings of *Allium* spp. at main centers*

Country	Institute/Center	Accessions
***Allium cepa*, Total = 4,161**		
Bulgaria	Institute for Plant Genetic Resources (IPGR), Sadovo	398
Poland	Plant Genetic Resources Laboratory of Research Institute of Vegetable Crops, Skierniewice	144
Germany	Genebank, Institute for Plant Genetic and Crop Plant Research (IPK), Gatersleben	348
Hungary	Ministry of Agriculture and Rural Development (MARD), Budapest	305
India	ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi	1,606
Taiwan	Asian Vegetable Research and Development Centre (AVRDC), Tainan	586
USA	United State Department of Agriculture and Development (USDA) Germplasm Resource Network	774
***A. sativum*, Total = 3,705**		
Bulgaria	IPGR, Sadovo	220
China	Vegetable Crop Gene Resources and Germplasm Enhancement (VCGRGE), Ministry of Agriculture, Beijing	417
Germany	IPK, Gatersleben	485
Hungary	MARD, Budapest	51
India	ICAR-NBPGR, New Delhi	913
Czech Republic	Crop Research Institute, Prague	157
South Korea	National Agrobiodiversity Center (NAAS), RDA, Suwon	1,158
USA	USDA Germplasm Resource Network	304
***A. fistulosum*, Total = 68**		
Germany	IPK, Gatersleben	18
Hungary	MARD, Budapest	24
India	ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India	26
***A. ampeloprasum* Total = 111**		
Germany	IPK, Gatersleben	91
India	ICAR-NBPGR, New Delhi	9
Netherlands	Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Department of Vegetable and Fruit Crops, Wageningen	11
***A. proliferum* Total = 154**		
Germany	IPK, Gatersleben	154
***A. chinense* Total = 23**		
India	ICAR-NBPGR, New Delhi	23
***A. tuberosum* Total = 216**		
India	ICAR-NBPGR, New Delhi	29
China	VCGRGE, Ministry of Agriculture, Beijing	174
USA	USDA Germplasm Resource Network	13

*updated from individual websites (December 2021)

Table 2. Status of *Allium* species indigenous collections from different states (1976-2022) #

S.No	Cultivated species	Accessions#	Wild species	Accessions#	Threatened status
1.	*A. cepa* L. var. *cepa* L.	2,847	*A. carolinianum* DC.	25	
2.	*A. sativum* L.	1,913	*A. auriculatum* Kunth	12	Endangered
3.	*A. cepa* var. *aggregatum* G.Don.	85	*A. griffithianum* Boiss.	11	
4.	*A. fistulosum* L.	17	*A. wallichii* Kunth	7	
5.	*A. tuberosum* Rottler ex Spreng.*	13	*A. consanguineum* Kunth	6	
6.	*A. chinense* G.Don	11	*A. przewalskianum* Regel	5	
7.	*A. ampeloprasum* L.	11	*A. humile* Kunth	5	
8.	*A.* x *proliferum* (Moench) Schrad. ex Willd.	10	*A. stracheyi* Baker	3	Vulnerable
9.	*A. hookeri* Thwaites	10	*A. semenovii* Regel	2	
10.	*A. cepa* var. *viviparum* (Metz.) Alef.	4	*A. fasciculatum* Rendle	1	
11.	*A. porrum* L.	3	*A. roylei* Stearn	1	Endangered
12.			*A. prattii* C.H.Wright	1	Rare
13.			*A. victorialis* L.	1	
	Total	4,924		80	

Semwal *et al.* (2021)

to identify under-represented/least represented taxa/ areas of diversity in the national holdings. *Allium* genetic resources in India with a total of 5,004 diverse accessions of cultivated and wild species of *Allium* were collected through explorations undertaken during 1976-2022, which includes mainly common onion and garlic among the cultivated types. Seeds of wild *Allium* species are preferably conserved in the seed genebank, while vegetatively propagated species are conserved in *in vitro*/ cryogenebank repository at New Delhi and field genebank (FGB) at ICAR-NBPGR, Regional Station, Bhowali, Uttarakhand including eight species of rare, endangered and threatened status (Table 3).

Germplasm Conservation

Ex situ conservation strategies for *Allium* germplasm is determined by reproductive biology of the species, mode of multiplication, threat due to different causal factors and status of occurrence (common or less commonly reported, endemic, rare/ vulnerable, etc.) (Chandel and Pandey 1992). *Ex situ* conservation repository at the ICAR-NBPGR has the following components: a) seed genebank, b) *In vitro* genebank and Cryo-genebank and c) Field genebank (Table 3). Flow of material from collection to conservation and use is depicted in flow diagramme (Fig. 1). Seeds of *Allium* species are orthodox and short lived, and have been conserved in seed genebank at ICAR-NBPGR. Due to short longevity of *Allium* seeds, adequate conservation protocols for seed viability have been developed at the ICAR-NBPGR.

For live collections indigenous and exotic wild species are maintained, characterized and evaluated at field genebank (FGB) at Bhowali, Uttarakhand. *In vitro*/cryo genebank was established under a project entitled 'National Facility for Plant Tissue Culture Repository' (NFPTCR) (currently Tissue Culture and Cryopreservation Unit, TCCU) jointly by Indian Council of Agricultural Research (ICAR) and Department of Biotechnology (DBT), India. This facilty conserves *Allium* under a unique and multi-crop repository.

Meager conservation of wild germplasm in genebanks may be attributed to various reasons like niche-specificity, lack of conservation protocols for *ex situ*, rapid loss of seed viability, poor performance/ non-suitability to climatic conditions of FGB. While collecting, asynchronous maturity and seed shattering pose great problems to gather material in sufficient quantity of desired for conservation.

In vitro conservation of *Allium* species is attained under normal growth conditions or subjected to growth-limiting conditions for short- to medium-term conservation in the *In vitro* active genebank (IVAG) and under suspended growth for long-term conservation using cryopreservation techniques in the *In vitro* base genebank (IVBG) (Pandey *et al*., 2018). For short-to medium-term conservation (IVAG), different slow growth *in vitro* conservation strategies developed/refined at TCCU for *Allium* spp. use a single or combination of strategies. Whereas long-term conservation (IVAG and Cryogenebank) using *in vitro* explants (shoot tips) was laid emphasis to cryopreserve all the germplasm conserved *in vitro* in the IVAG. Different cryopreservation techniques – vitrification,

droplet-vitrification, encapsulation-dehydration and encapsulation-vitrification are used. Shoot tip cryopreservation using droplet-vitrification is found to be superior, with a varied degrees of post-thaw regrowth (20-60%) among the different species. So far, 159 accessions belonging to 10 different species of *Allium* are conserved in the form of *in vitro* cryopreserved shoot tips in the IVBG (-196^{o}C, LN in liquid form). Genetic stability has been assessed in cryopreserved germplasm of *Allium* using morphological, cytological and molecular markers, and no significant variation observed in the mother plants and cryopreserved plants. In addition, in order to facilitate pre-breeding in *Alliums* crop improvement and as natural propagules of genetic information, pollen have also been conserved in cryogenebank; pollen cryopreservation protocol was standardized in *A. chinense* and *A. tuberosum.*

Field Genebank at RS Bhowali

The "*Allium* Field Genebank" at ICAR-NBPGR RS Bhowali, Uttarakhand, has germplasm of indigenous as well as exotic species, and was set up by Late Dr KS Negi in 1987. It holds several germplasm collected from difficult areas of Uttarakhand (Niti valley, Milam glacier, and the Valley of Flowers in Western Himalayas), J&K, Himachal Pradesh, Sikkim and Nagaland in the north-eastern region (Negi, 2006). A total of over 150 accessions of *Allium* species are maintained in FGB at RS Bhowali from 1986-2022 and supplied for research to various indentors in the country. Among the major collections *A. tuberosum*, *A. sativum*, *A. negianum*, *A. chinense*, *A. hookerii*, *A. przewalskianum*, *A. schoenoprasum* and *A. ampeloprasum* collected from western and North-eastern Himalayan regions of India are maintained including a few accessions of critically endangered species (*A. stracheyi*). Some of the exotic taxa conserved include *A. tuberosum, A. fistulosum, A. sativum, A. altaicum, A. ledebouranum, A. lineare, A. oreoprasum, A. schaninii, A. pskemense, A. albidum, A. fistulosum, A. ampeloprasum, A. obliquum, A. ramosum, A. schoenoprasum* and *A. angulosum* from Australia, USSR and Switzerland.

Table 3. *Ex situ* conservation of cultivated and wild *Allium* species at ICAR-NBPGR

	Ex situ conservation method				
	Seed genebank	Cryogenebank	*In vitro* genebank	Field genebank	Total
Cultivated *Allium* Species					
A. cepa	991	9		606	1,606
A. sativum		134		779	913
A. fistulosum	16	3	1	6	26
A. chinense		11	9	3	23
A. tuberosum		7	4	18	29
A. hookeri		2	2	12	16
A. ampeloprasum		2		7	9
A. cepa var. *aggregatum*		1		3	4
A. fasciculatum			1	14	15
Sub-total	1,007	169	17	1,448	2,641
Wild *Allium* species					
A. przewalskianum#	1			19	20
A. griffithianum	2	1		8	11
A. carolinianum	3			5	8
A. stracheyi#	2			5	7
A. wallichii#		1		6	7
A. auriculatum	2	1		3	6
A. roylei#	1	1		1	3
A. humile	1			1	2
A. fasciculatum				1	1
A. prattii				1	1
A. victorialis	1				1
Sub-total	13	4		50	67
Total	1,020	173	17	1,498	2,708

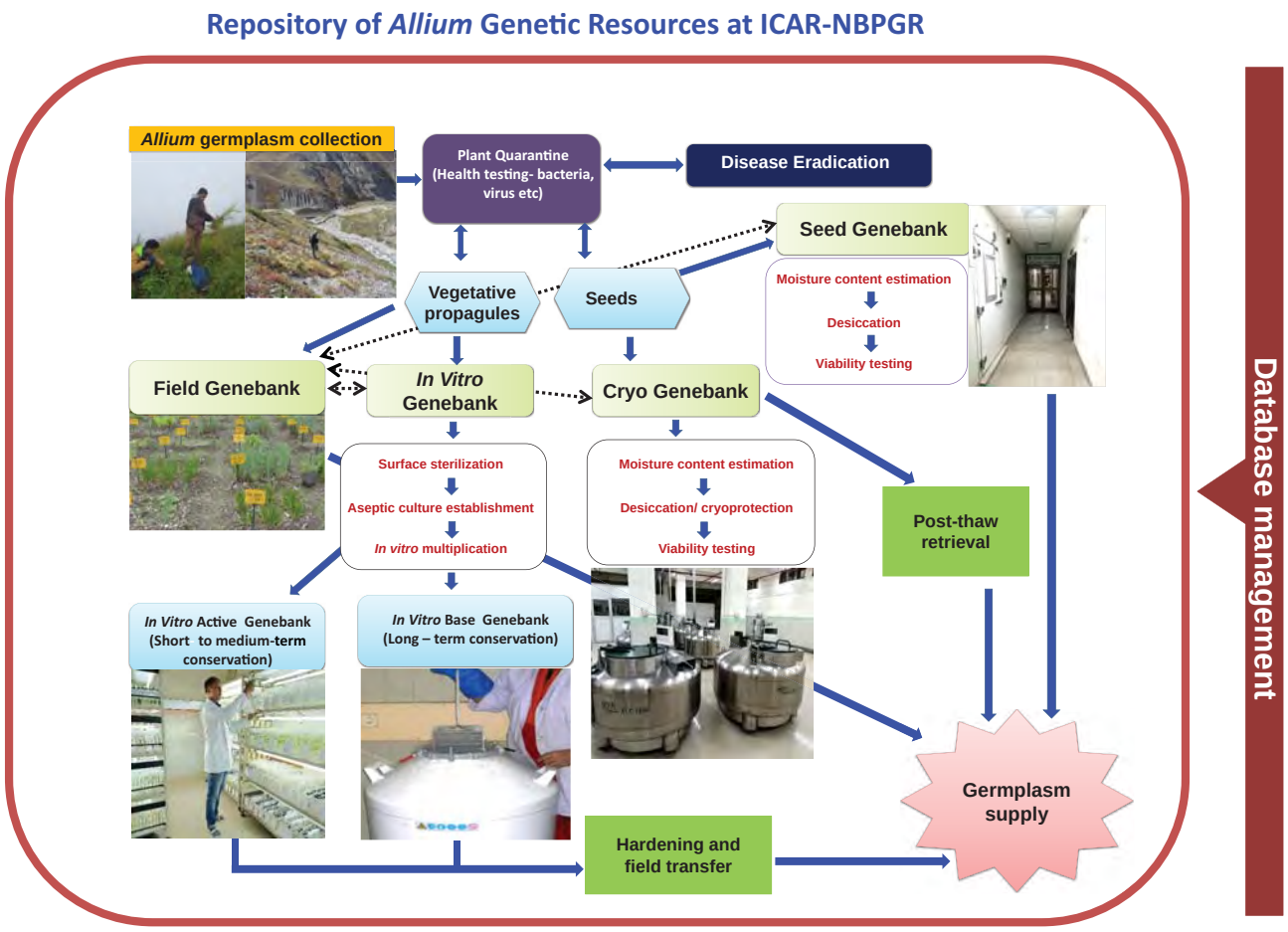
Source: Semwal *et al.* (2021)

Challenges and Action Points

- Difficult access to habitats and wild and threatened species.
- Asynchronized seed maturity/seed shattering of seeds
- Collecting desirable number of seeds/propagules for conservation
- On-spot identification of taxon
- Standardization of cryopreservation protocol
- Limitation in sufficient germplasm for *in vitro* study and lack of manpower/tools for frequent subculturing during *in vitro* conservation
- Establishment problems at FGB and loss of material due to infection/contamination during *in vitro* conservation
- Evaluation and identification/characterization – differential response to agro-climatic conditions leading to no/poor flowering; seed production
- Sexual sterility and poor post-harvest storage response of some species leading to loss of viability during post-harvest process
- Danger of genetic erosion due extensive harvest of the bulbs from wild population.
- Systematics study on identification of genetic resources taxa from subgenus *Cepa* in India
- Identification of *in situ* on-farm sites for conservation

Future Prospective and Action Points Identified

- Prioritization of sites/ areas and underexplored regions for wild *Allium* species
- Focus on wild and hitherto uncollected species diversity including *A. roylei*, *A. farctum*, *A. rhabdotum* which are known for cold hardiness traits and biotic resistance
- Strengthening of repositories: introduction of primary genepool of onion from native areas



- Basic studies on reproductive biology of rare/ endangered taxa- *A. stracheyi, A. wallichii, A. auriculatum, A. humile* and *A. roylei.*
- Protocol establishment: *in situ* conservation, multiplication, evaluation of germplasm; cryo/*in vitro* conservation study
- Identification of sites: niche specific gemplasm (high altitude germplasm that does not establish at lower altitude/ flower/ seed).

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