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Sunflower genetic resources management at DOR

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The article discusses about the strength of genetic resources available at DOR, Hyderabad, evaluation of 350 sunflower accessions carried out during 2013; screening of the high oleic acid accessions; augmentation of germplasm and wild species including their derivatives introduced during last five years; status of germplasm lines supplied during the last five years and future line of action for sunflower genetic resource management in India. The PGR include primitive forms of cultivated plant species and landraces, modern cultivars, obsolete cultivars, breeding lines and genetic stocks, weedy types and related wild species of crop plant (IPGRI 1993). The *Helianthus* L. germplasm collection in the National Gene

Bank is held at the Directorate of Oilseeds Research, Rajendranagar, Hyderabad and maintains 2322 accessions. The sunflower collection is composed of accessions of the domesticated species *Helianthus annuus* and its wild relative taxa along with associated passport and other information. Sunflower germplasm is augmented, regenerated, characterized and distributed to conduct a basic and applied research in country through AICRP on sunflower in India. In this paper we provide a summary of germplasm availability and other activities carried out in the National Gene Bank at DOR.

New germplasm is obtained by augmentation mostly from European countries. After augmentation of the germplasm accessions the procedure of conservation and maintenance is described. The evaluation of 350 sunflower accessions was carried out during *kharif*2013 in augmented block design along with three checks DRSF 108;DRSF 113 and Morden at Narkhoda farm. For the evaluation of high oleic acid accessions nine genotypes with low and high oleic acid content were used to constitute the panel (KBSH-44, DRSH-1, CMS 17 A, 6D-1, TSG-12, TSG-17, TSG-38, UK-90, DOP-80). Genomic DNA was isolated from 10 mg leaf tissue by CTAB method (Doyle and Doyle, 1987). For the screening of promising accessions for high oleic acid content the reported microsatellite marker already by Nagarathna *et al.* (2011) and the procedure to amplify the high oleic allele is followed.

Present status of sunflower genetic resources in National Gene Bank at DOR

The availability of genetic resources in the National Gene Bank is summarized in table 1. The wild species maintained at DOR along with the number of accessions is given in table 2.

Table 1. Availability of genetic resources in national gene bank at DOR

Germplasm Accessions	Number
GMU	1200
Exotic collections (EC)	350
DRSI (Inbreds)	69
DRSF (Populations)	5
PS (Prebred sunflower)	154
CMS (A and B)	25
Restorers	72
Back crosses (CMS conversion)	15
Gene pool (GP) for high oil, yield and autogamy	390
Wild species	42 (503 accessions)
Total	2322

Table 2. *Helianthus* species established at DOR, Hyderabad

Species	Habit	Ploidy	No of accessions	Disease resistance trait
<i>H. annuus</i> (wild)	Annual	Diploid	45	Rust and downy mildew
<i>H. praecox</i>	“	“	11	Rust and downy mildew
<i>H. praecox</i> ssp. <i>praecox</i>	“	“	1	Rust and downy mildew
<i>H. praecox</i> ssp. <i>runyonii</i>	“	“	25	Rust and downy mildew
<i>H. praecox</i> ssp. <i>hirtus</i>	“	“	1	Rust, downy mildew and brown stem canker
<i>H. argophyllus</i>	“	“	22	Rust, downy mildew, brown stem canker, <i>Orobanche cumana</i>
<i>H. neglectus</i>	“	“	14	-
<i>H. niveus</i> ssp. <i>canescens</i>	“	“	18	-
<i>H. petiolaris</i>	“	“	88	Rust and brown stem canker
<i>H. petiolaris</i> ssp. <i>fallax</i>	“	“	1	Rust and brown stem canker
<i>H. petiolaris</i> ssp. <i>petiolaris</i>	“	“	1	Rust
<i>H. debilis</i> ssp. <i>cucumerifolius</i>	“	“	18	-
<i>H. debilis</i> ssp. <i>vestitus</i>	“	“	1	Ornamental
<i>H. debilis</i> ssp. <i>silvestris</i>	“	“	15	Ornamental
<i>H. debilis</i> ssp. <i>tardifolius</i>	“	“	1	Brown canker

<i>H. maximiliani</i>	Perennial	“	31	<i>A. helianthi</i> , downy mildew, brown stem canker, <i>Sclerotinia sclerotiorum</i>
<i>H. occidentalis</i> ssp. <i>plantagineus</i>	“	“	3	<i>A. helianthi</i> , downy mildew
<i>H. atrorubens</i>	“	“	4	Rust
<i>H. paradoxus</i>	“	“	12	
<i>H. angustifolius</i>	“	“	1	Rust
<i>H. cusikii</i>	“	“	1	-
<i>H. giganteus</i>	“	“	33	Downy mildew, brown stem canker, <i>Sclerotinia sclerotiorum</i> , <i>Verticillium dahliae</i>
<i>H. salicifolius</i>	“	“	4	Downy mildew, brown stem canker, <i>S.</i>
<i>sclerotiorum</i>				
<i>H. microcephalus</i>	“	“	8	Downy mildew, powdery mildew
<i>H. pumilis</i>	“	“	1	Downy mildew
<i>H. glaucophyllus</i>	“	“	1	Downy mildew and powdery mildew
<i>H. nuttallii</i> spp. <i>rydbergii</i>	“	“	57	Downy mildew
<i>H. mollis</i>	“	“	11	<i>A. helianthi</i> , downy mildew, brown stem canker, <i>Sclerotinia sclerotiorum</i>
<i>H. divaricatus</i>	“	“	20	<i>A. helianthi</i> , brown stem canker
<i>H. grosseserratus</i>	“	“	18	Downy mildew
<i>H. simulans</i>	“	“	3	<i>A. helianthi</i> , , brown stem canker
<i>H. decapetalus</i>	“	Tetraploid	17	<i>A. helianthi</i> , downy mildew, brown stem canker, powdery mildew
<i>H. hirsutus</i>	“	“	11	Tolerant to <i>Alternaria</i> , resistant to downy mildew and rust
<i>H. pauciflorus</i>	“	“	18	<i>A. helianthi</i> , downy mildew
<i>H. laevigatus</i>	“	“	2	Downy mildew, <i>Phoma macdonaldi</i>
<i>H. smithii</i>	“	“	1	Downy mildew, powdery mildew, stem canker
<i>H. ciliaris</i>	“	“	1	Downy mildew and <i>Phoma macdonaldi</i>
<i>H. resinosus</i>	“	Hexaploid	8	<i>A. helianthi</i> , powdery mildew and brown stem canker
<i>H. strumosus</i>	“	“	13	<i>Phoma macdonaldi</i>
<i>H. rigidus</i>	“	“	9	Downy mildew
<i>H. tuberosus</i>	“	“	18	<i>A. helianthi</i> , downy mildew, brown stem canker, <i>Sclerotinia sclerotiorum</i>
<i>H. eggerti</i>	“	“	1	Downy mildew
<i>H. californicus</i>	“	“	1	Prohibited species in India

Conservation of the genetic resources

Immediately after receiving accessions, sowing of the germplasm was taken up at Narkhoda farm either in single row or in two rows of 4 m length. Each individual head was sib mated regularly after the onset of flowering. The mature head will be harvested separately and two sets will be made. One set of germplasm accessions is deposited and conserved for a period of 2-3 years under the short term cold storage facility of DOR (Temp. 8° C; RH-15 %) to retain the true to type of characters of the accession. Another set is maintained at Germplasm Management Unit (GMU). Similar type of conservation procedure for ICRISAT mandate crops was earlier reported by Upadhyaya *et al.* (2008).

Maintenance and regeneration of accessions

The regeneration of cultivated *H. annuus* accessions is done at Narkhoda farm of DOR, by direct seeding into rows. Sunflower accessions are hand pollinated after bagging the main capitulum (selfing) in order to maintain the genetic purity and to avoid cross pollination with honey bees or wind. Bulk pollen is collected from the each head/plant in the petriplate and subsequently dusted with brush to each head of the same accession (sib mating). After sib mating of every accession, hands along with brush are cleaned with surgical sprit. Harvesting of the individual head is carried out at proper maturity stage.

Augmentation of new germplasm collection

The new germplasm accessions added in National Gene bank during the last five years received from USDA, USA and IFVCNS, Serbia are listed in table 3. During this period, germplasm activities were intensified with particular focus on procurement and identification of trait specific germplasm. Accordingly, wild *Helianthus* species were obtained for exploitation in breeding programme aimed at resistance to powdery mildew. Cultivar germplasm (120 accessions) were imported from USDA, USA. Similar type of augmentation procedure for ICRISAT mandate crops was earlier reported by Upadhyaya *et al.* (2008).

Table 3 New collections added in gene bank within last five year

Material Received	No. of Accessions	Source	Remark
Downy mildew differentials (prevalent in India)	14	USDA, Northa Dakota	Multiplied and gave one set to Latur
Germplasm	58	USDA,ARS Ames	High test weight, high oleic acid, early maturity and Dwarf stature
Wild species	93	USDA,ARS Ames	—
CMS	6	IFVCNS, Serbia	High seed yield, high oil used in national crossing programme
Germplasm	62	USDA,ARS Ames	High test weight, High Oleic acid, early maturity and dwarf stature
Wild species	33	IFVCNS, Serbia	Annual and perennials

Evaluation of sunflower accessions

For evaluation of 350 sunflower germplasm accessions three Checks DRSF 108, DRSF113 and Morden were used for comparison. Out of these accessions evaluated, seed yield per plant was above 25 g/plant in four accessions and reported (Table 4). Oil content was recorded above 38 per cent in 3 accessions and at par with the check DRSF113 (38.0). Fifteen accessions were identified as early (<75 days maturity) and at par with the short duration check, Morden (75) and can be utilized in the development of short duration hybrids in sunflower. Similar results were reported by Dudhe *et al.* (2011) which confirm this finding. The promising accessions identified for various traits are presented in table 4.

Table 4. Promising germplasm accessions identified for different traits

S. No.	Character	Accessions
1.	50% flowering (Days)	EC-601708-1 (48), EC-601886 (48), EC-601977 (48), EC-601669 (49), EC-601666 (50)
2.	Days to maturity	EC-601886 (72), EC-601985 (73), EC-601619 (74), EC-601631 (74), EC-601610 (75), EC-601610-1 (75), EC-601617 (74), EC-601630 (76), EC-601683 (73), EC-601672 (73), EC-601680 (74), EC-601681 (74), EC-601682 (74), EC-601676-1 (74), EC-601671 (75)
3.	Plant height (cm)	EC-601654 (60.8), EC-601756 (62.3), EC-601727-1 (67.3)
4.	No. of leaves per plant at maturity	EC-601871 (23), EC-601609 (25.4), EC-601677 (25.5)
5.	Head diameter (cm)	EC-601659 (15.8), EC-601615 (16), EC-601770 (17), EC-601677 (17.5)
6.	100-seed weight (g)	EC-601801 (4.2), EC-601809 (4.8), EC-601901 (5.6), EC-601909 (5.8), EC-601926 (6.0)
7.	Seed yield per plant (g)	EC-602083 (20.7), EC-601625 (22.5), EC-602046 (22.6), EC-602015 (23.3), EC-602014 (25.9), EC-602075 (28.5), EC-601861 (29.5), EC-601609 (29.7)
8.	Oil content (%)	EC-601923 (38.2), EC-601938 (38.7), EC-601750 (38.5)

Molecular confirmation of high oleic acid traits

The *fad2* primers were used to detect the high oleic specific amplicon in the high oleic lines. Nine genotypes with low and high oleic acid content were used to constitute the panel (KBSH-44, DRSH-1, CMS 17 A, 6D-1, TSG-12, TSG-17, TSG-38, UK-90, DOP-80). The high oleic specific allele was detected only in TSG-17 and DOP-80 and presented in Figure 1. Similar observation for high oleic acid accessions were reported by Nagarathna *et al.* (2011).

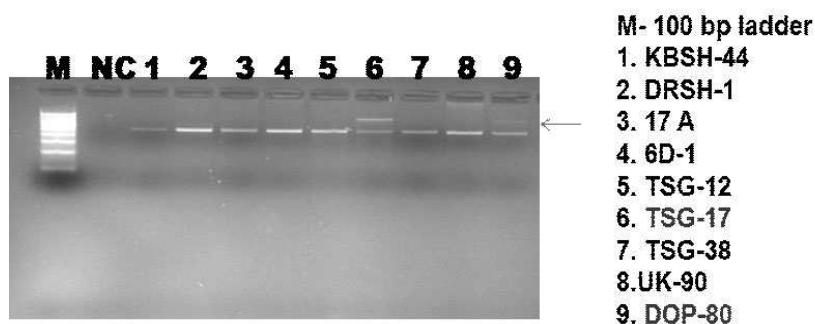


Figure 1: Confirmation of high oleic specific allele in TSG-17 and DOP-80

Organization of Germplasm Field Day

Organization of field day is a regular activity so that the AICRP breeders will get an idea about the germplasm accessions conserved and maintained in the National Gene Bank. In the germplasm day breeders keenly observe the variability among the sunflower accessions and select the promising accessions for future use in breeding programme.

Table 5. Germplasm field day organized at DOR and number of participated scientists and number of accessions supplied from Gene Bank

Host Institution	Year	Number of scientist participated	Number of accessions raised	Characteristics of the material	Number of accessions supplied
DOR,Hyderabad	2009-2010	14	250	High yield, high oil content	400
DOR,Hyderabad	2010-2011	11	300	All Genetic stock, exotic collection	300
DOR,Hyderabad	2011-2012	12	350	Early maturity, PM tolerance, Oleic acid, High autogamy	250
MAU, Latur	2012-2013	15	>2000	NBPGR 1000 exotic collection, DM tolerant/ resistant accessions, Genetic stock, Germplasm available at centre	200
UAS, Bangalore	2013-2014	-	-	-	560

Regular supply of germplasm accessions

Supply of germplasm accessions to the concerned researcher is another regular activity of Gene Bank. Either the AICRP researchers or academicians from the SAU's will send their material requirement to the Project Director, DOR. The requested material will be supplied after signing the MOU between the centre and DOR, Hyderabad. The total number of accessions supplied during the last five years is presented in Table 5.

Progresses towards germplasm documentation and e catalogue preparation

Till date, the data pertaining to 2000 sunflower accessions on 34 DUS descriptor characteristics is documented in SPSS 15.2 version. Also, under the evaluation activity, data on 1750 accessions on eight agronomic characteristics viz., days to 50 per cent flowering, days to maturity, head diameter, plant height, number of leaves per plant, 100-seed weight, seed yield per plant, oil content per cent is documented.

Future line of action for sunflower genetic resource management in India

Some of the future perspectives to enhance the use of genetic resources in sunflower improvement in India are

1. To characterize and catalogue all the available germplasm accessions and elimination of duplicates from the available collection.
2. Identification of trait specific germplasm lines from available collection in National Gene Bank and to incorporate insect pest and disease resistance in high yielding varieties with broad agronomic base.
3. Development of superior gene pools and core collection utilizing exotic variability in sunflower is immediate need.
4. The introgression of desirable traits from wild sunflowers to cultivar base through ploidy manipulations needs special attention for sunflower improvement in India.

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