

## PREBREEDING AND ALTERING THE GENETIC ARCHITECTURE OF INDIAN SUNFLOWERS USING WILD SUNFLOWERS

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### Abstract

The narrow genetic base of the Indian sunflower cultivars demands concerted efforts to develop newer germplasm by way of incorporation of additional genetic variability in terms of resistance to biotic and abiotic stresses, oil quality and sources of cytoplasm from wild sunflowers. Attempts were made to improve Indian sunflower cultivars through introgression of desirable traits from wild sunflowers. Leaf spot incited by *Alternaria helianthi* is a major disease in India and nine perennial species of all three ploidy groups were found to be tolerant to the pathogen in an in vitro assay. The genes from the primary gene pool could easily be transferred through conventional breeding while the introgression of traits of interest from the species of the secondary gene pool required several manipulations using recent techniques since many of the interspecific hybrids were sterile due to ploidy differences, genomic incompatibility, cytoplasmic imbalances or other factors causing sterility. Some 350 promising prebreeding germplasms with extensive variability for both qualitative and quantitative traits were developed using annual diploid species. These stabilized recombinant interspecific inbred lines (RIILs) also served as a reliable source of resistance to other biotic stresses, like rust and downy mildew. Cytological analysis of pollen mother cells and molecular characterization using sunflower specific simple sequence repeat markers (SSRs) confirmed the presence of the wild genome in the RIILs. The breeding value of the interspecific derivatives is discussed.

### Introduction

The sunflower crop was introduced to India in the early 1970s. The crop by virtue of its wide adaptability has established itself as a major oilseed crop in the southern part of the country and lately has made a dent in nontraditional areas of Northern India in the spring season. Due to its short growing season, it is cultivated for 2-3 growing seasons per year under assured irrigation and is identified as an ideal crop in various intercropping systems and cropping sequences. However, sunflower production in India is severely constrained by the heavy yield losses due to diseases like *Alternaria* leaf spot, downy mildew, rust and sunflower necrosis disease. Further, susceptibility of this crop to water stress in rainfed cultivation results in low yields. Hence, recent years have witnessed a decline in the acreage under the crop mostly in the traditional sunflower growing regions like Karnataka, Maharashtra and Andhra Pradesh states.

Under these circumstances there is a need to broaden the genetic base of Indian sunflower to circumvent the constraints in sunflower production in India. Among the various approaches

to manage the biotic stresses, host plant resistance is the most reliable and economical to the end users. With its large potential for export as confectionery/non-oilseed, there is a need to develop genotypes with specific quality characteristics. Plant breeding efforts to develop varieties/hybrids with the desired economic characteristics are limited by the narrow genetic base of the cultivated sunflower. Cultivation is dominated by hybrids that are based on the *H. petiolaris* (PET1) cytoplasm and varieties that are either the earlier introductions or selections from the same lineage. Hence, concerted efforts are required to incorporate additional genetic variability from reliable sources by integrating modern biotechnological tools and conventional breeding methods. Wild sunflowers are rich sources of genetic variability in terms of resistance to biotic and abiotic factors, and oil quality, and continue to serve as sources of cytoplasm and restorers (Thompson et al., 1981; Seiler, 1992). The present investigation aims to introgress desirable traits from wild sunflowers into cultivated sunflower and to develop prebreeding germplasm.

### Materials and Methods

Thirty-six *Helianthus* species were collected from Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia and USDA-ARS, USA and assembled in the in vitro and in vivo *Helianthus* garden. The collection includes species from three ploidy groups (diploids, tetraploids and hexaploids) and two habit groups (annuals and perennials). The desirable traits available in this species collection for exploitation in the genetic improvement of cultivated sunflower for Indian conditions are listed in Table 1. Flowering in perennial species was very late after a long period of vegetative phase. As these species were propagated either through micropropagation or transplanting suckers/runners obtained from already established plants from the *Helianthus* garden, sowing of the sunflower cultivars for use as parent in the interspecific hybridization was adjusted accordingly to synchronize the flowering to facilitate effective crossing. Crossing was attempted in both directions by hand emasculation and pollination when the wild species were used as the female parent, while the cultivars were treated with GA at the star bud stage and used as the female parent. The number of florets pollinated was limited by the fewer number of flower heads produced by each species. Seed set was observed in several cross combinations while a few others showed degeneration of pollinated flowers. Interspecific hybrids were successfully produced between cultivated and wild diploid annual sunflowers, viz., *H. annuus* L.(wild), *H. argophyllus* T. and G., *H. praecox* Engelm. and A. Gray, *H. petiolaris* Nutt., *H. debilis* Nutt., *H. niveus* (Benth.) Brandege and *H. neglectus* Heiser using conventional hybridization. Trispecific hybrids were produced with the objective of converging desirable genes simultaneously from two diploid wild species into cultivated sunflower. The crosses include (*H. praecox* x *H. petiolaris*) x sunflower cultivars; (*H. argophyllus* x *H. annuus* [wild]) x sunflower cultivars and (*H. argophyllus* x *H. petiolaris*) x sunflower cultivars. To eliminate the undesirable wild characters from the progenies, limited numbers of backcrosses were made with the cultivar as the recurrent pollen parent and were advanced to further generations. Stable variants with desirable traits were selected in advanced generations and evaluated. Pollen mother cells were studied at various stages of meiosis using 1% acetocarmine for chromosome association and behaviour. Molecular diversity of the RIILs was assessed using 100 sunflower specific simple sequence repeats according to the procedure of Yu et al., 2002.

Table 1. *Helianthus* species available at DOR for improvement of Indian cultivated sunflower.

Sl. No	Trait of interest	Source of wild sunflower(s)
1.	Drought	<i>H. argophyllus</i> , <i>H. deserticola</i>
2.	Salinity	<i>H. paradoxus</i> , <i>H. debilis</i> , <i>H. annuus</i> (wild)
3.	Rust	<i>H. praecox</i> ssp. <i>praecox</i> , <i>H. annuus</i> (wild), <i>H. praecox</i> ssp. <i>runyonii</i> , <i>H. praecox</i> ssp. <i>hirtus</i> , <i>H. argophyllus</i> , <i>H. petiolaris</i> ssp. <i>fallax</i> , <i>H. petiolaris</i> ssp. <i>petiolaris</i> , <i>H. atrorubens</i> , <i>H. angustifolius</i> , <i>H. hirsutus</i>
4.	<i>Alternaria helianthi</i>	<i>H. maximiliani</i> , <i>H. occidentalis</i> ssp. <i>plantagineus</i> , <i>H. mollis</i> , <i>H. divaricatus</i> , <i>H. simulans</i> , <i>H. decapetalus</i> , <i>H. pauciflorus</i> , <i>H. resinosus</i> , <i>H. tuberosus</i>
5.	Downy mildew	<i>H. praecox</i> ssp. <i>praecox</i> , <i>H. annuus</i> (wild), <i>H. praecox</i> ssp. <i>runyonii</i> , <i>H. praecox</i> ssp. <i>hirtus</i> , <i>H. argophyllus</i> , <i>H. maximiliani</i> , <i>H. occidentalis</i> ssp. <i>plantagineus</i> , <i>H. giganteus</i> , <i>H. nuttallii</i> ssp. <i>rydbergii</i> , <i>H. mollis</i> , <i>H. grosseserratus</i> , <i>H. decapetalus</i> , <i>H. hirsutus</i> , <i>H. pauciflorus</i> , <i>H. rigidus</i> , <i>H. tuberosus</i>
6.	Novel variants	<i>H. divaricatus</i> *, <i>H. occidentalis</i> , <i>H. atrorubens</i>
7.	Cytoplasm	<i>H. argophyllus</i> , <i>H. petiolaris</i>
8.	Oil quality High linoleic acid  High oleic acid  Reduced saturated fatty acids (stearic & palmitic)	<i>H. simulans</i> , <i>H. laevigatus</i> , <i>H. strumosus</i> , <i>H. debilis</i> ssp. <i>tardifolius</i>  <i>H. praecox</i> ssp. <i>runyonii</i> , <i>H. atrorubens</i> , <i>H. hirsutus</i> , <i>H. argophyllus</i> , <i>H. annuus</i> (wild), <i>H. resinosus</i> , <i>H. debilis</i> ssp. <i>cucumerifolius</i>  <i>H. pauciflorus</i> , <i>H. giganteus</i> , <i>H. annuus</i> (wild)
9.	Oil content	<i>H. anomalus</i> , <i>H. petiolaris</i> , <i>H. deserticola</i> , <i>H. niveus</i> ssp. <i>canescens</i>
10.	Seed protein	<i>H. paradoxus</i> , <i>H. debilis</i> ssp. <i>debilis</i> , <i>H. maximiliani</i> , <i>H. laevigatus</i> , <i>H. atrorubens</i> , <i>H. giganteus</i>

\* Novel genetic variants have been developed.

## Results and Discussion

**Evaluation of Prebreeding Lines.** Some 350 promising single plant selections from five cross combinations involving *H. argophyllus*, *H. petiolaris*, wild *H. annuus*, *H. debilis* and a trispecific cross involving *H. argophyllus*, *H. annuus* (wild) and cultivated sunflower were evaluated for various growth and yield characters. Most of the lines exhibited high uniformity and were stable in character expression. The prebred lines expressed wide variability for various qualitative traits like stem thickness, leaf angle and arrangement, leaf colour, shape, texture, hairiness, number of leaves, petiole and stem pigmentation, disc colour, stigma colour and ray floret morphology. Significant variation was recorded for growth and yield characters (Table 2). Among the prebred materials, 15 lines recorded seed yield > 50g/plant and most of them were derived from the cross involving *H. petiolaris* with sunflower and the trispecific cross. The lines with earliness, short plant type, and resistance to various diseases, including the new viral disease have been included in the population improvement and inbred

development programmes. A new source of CMS with *H. argophyllus* cytoplasm having stable maintainers and complement fertility restoration system has been identified and characterized. The new genetic variability created through interspecific hybridization will not only be utilized directly as a cultivar but plants will also serve as donors in the national sunflower improvement programme. The prebreeding lines are being utilized by various researchers for the identification of maintainer and/or restorers for different sterile cytoplasm.

Table 2. Evaluation of prebreeding sunflower lines.

Cross combination*	No of Lines	LAI	SPAD (Chlo. content)	Pl. height	Days flow.	Head dia.	100-seed weight	Yield/pl(g)
PS lines	20	2.1-4.2 (3.37)	30.4-40.1 (34.2)	88-154.2 (117.9)	55-73 (61.6)	8.8-21.0 (14.1)	3.1-6.9 (4.75)	6.5-80.2 (26.5)
ARG-SF	79	2.0-6.6 (3.71)	25.1-38.9 (32.4)	53.6-150.2 (111.7)	53-74 (62.6)	8.2-22.0 (13.2)	2.2-7.4 (4.55)	0.8-43.7 (18.6)
PET-SF	50	2.1-5.8 (3.77)	28.4-41.5 (34.1)	54.6-181.4 (125.8)	52-74 (61.3)	7.6-19.0 (13.9)	2.7-7.1 (4.7)	1.4-77.3 (27.8)
ANN-SF	22	1.9-4.9 (3.4)	28.7-38.1 (33.1)	56.5-148.4 (106.5)	52-74 (64.2)	9.0-16.0 (12.3)	2.4-7.1 (4.03)	2.1-51.7 (17.2)
ARG-ANN-SF	81	1.5-5.0 (3.33)	24.4-39.5 (32.4)	44.8-225.2 (104.2)	52-74 (61.6)	8.2-22.7 (13.9)	2.3-7.1 (4.6)	1.8-73.2 (20.11)
SF-DEB	28	1.6-3.9 (2.87)	26.8-40.3 (34.4)	95-146.6 (113.2)	55-68 (60.6)	9.6-16.2 (13.4)	2.5-7.0 (4.7)	9.9-42.2 (23.2)
Overall mean		3.48	33.1	112.2	61.9	13.5	4.57	21.2

No of lines evaluated = 350

\* PS – physiologically superior plant types; SF – cultivated sunflower; ARG – *H. argophyllus*; PET – *H. petiolaris*; ANN – *H. annuus* wild; DEB – *H. debilis*

Figures in parentheses represent mean values

**Cytological and Molecular Characterization.** The prebreeding sunflower lines were highly fertile (> 98%) and the somatic chromosome number was 34. Five stable inbreds were selected in each of the five interspecific cross combinations, viz., ARG-SF, PET-SF, ANN-SF, ARG-ANN-SF and SF-DEB, and the meiotic chromosome associations at diakinesis were studied in 100 PMCs for each inbred line. While cultivated sunflower revealed the formation of 17 regular bivalents, the prebred lines regardless of the cross combination showed higher (trivalents, quadrivalents) and lower (univalents) configurations. Trivalents (1-2) were observed only in the lines derived from the PET-SF combination. The number of quadrivalents varied from 1 to 3 with a mean association of 0.25 to 1.0. The number of univalents was 2 to 6 with a mean association of 0.10 to 1.35. Though the stabilized prebred sunflower lines showed morphological similarities with the cultivated sunflower, the presence of distinct chromosome associations in spite of backcrosses and sib mating clearly indicate the presence of the wild genome in these RIILs, and hence, serve as an useful stock for gene mapping studies. Forty prebreeding lines were selected based on genetic diversity and were screened along with two check cultivars for molecular diversity using sunflower specific SSRs. Variation in allelic lengths was detected with a maximum of up to 8 alleles per locus. Maximum molecular diversity was detected in the trispecific cross involving *H. argophyllus*, *H. annuus* (wild) and cultivated sunflower.

**Rust Resistance.** Rust incited by *Puccinia helianthi* Schw. is a problem during spring in the southern parts of India. Wild sunflowers, particularly the diploids, are reported as potential sources of rust resistance. Hence, under severe natural epiphytotic conditions, twenty-two wild *Helianthus* species, their interspecific hybrids and 380 recombinant inbred lines derived from interspecific crosses between cultivated sunflower and four diploid annuals (*H. petiolaris*, *H. argophyllus*, wild *H. annuus*, and *H. debilis*) and three diploid perennials (*H. occidentalis* Riddell, *H. maximiliani* Schrader, and *H. divaricatus* L.) along with the cultivars were studied for their reaction to rust. The disease incidence ranged from 0 to 100 % in the recombinant inbred lines (RIILs) while none of the wild species or their F1 hybrids revealed susceptibility to rust. In the cultivar germplasm the disease incidence was 100% but the disease severity was low and varied between 3.1-9.4. The disease severity in the RIILs varied between 0 and 40. The mean disease severity was low in lines derived from diploid annuals as compared to diploid perennials. One line (PS 1089) derived from *H. argophyllus* x cultivar and two lines (PS 2011, PS 2032) derived from *H. petiolaris* x cultivar crosses were found to be immune. The study indicates the possibility for exploitation of the prebred sunflower lines derived from diploid annuals for resistance to rust but detailed investigations are needed to determine the nature of resistance and the number of genes involved in the resistance reaction.

**Alternaria Resistance.** Leaf spot caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara is economically important in India and other tropical countries. The disease is reported to reduce seed and oil yields by 27 to 80% and 17 to 33% respectively, besides leading to germination losses varying from 23 to 32%. Wild sunflowers were screened for their resistance to *A. helianthi*. Reliable sources of resistance were found in 9 perennial species of all three ploidy groups (Sujatha et al., 1997). Diploid and hexaploid species were utilized in the programme on breeding for *Alternaria* resistance. Of the five diploid perennials showing resistance to *A. helianthi*, viz., *H. mollis* L, *H. occidentalis*, *H. maximiliani*, *H. divaricatus* and *H. simulans* E. E. Wats., hybrids were obtained when cultivated sunflower was crossed with these species, except for *H. mollis*. The interspecific hybrid involving *H. divaricatus* and the cultivated sunflower was highly fertile. It set seeds by self and open pollinations. The meiosis in the interspecific hybrids was normal with the formation of 17 II at metaphase I followed by equal anaphase separation. Similar results were also obtained in the interspecific hybrid combinations involving *H. occidentalis* and *H. maximiliani* with cultivated sunflower. Unlike other interspecific hybrids dominated by the wild parent's traits, these hybrids showed many of the traits of the cultivar parent used. This has led to doubts of the materials being hybrids. Interestingly, the putative hybrid plants have shown distinct reactions to *Alternaria* with each plantlet varying from the other in the degree of infection by the disease. Hence, isozyme analysis and RAPDs using Operon primers have been attempted to confirm the hybridity. Since the above three interspecific hybrid combinations were highly pollen fertile and showed distinct reactions against *Alternaria*, individual F1 plants were selfed and the F2 progenies were studied. Based on the reaction to leaf spot and desirable plant characters, several single plants were selected and raised as F3 families. The evaluations of these progenies led to the identification of several promising lines which are being maintained through intermating in order to avoid loss of desirable genes introgressed from the wild species. Interestingly, a few derivatives from the *H. divaricatus* cross had shown a few plants with unique characteristics, viz., white pollen and double head (Y type branching). These plants were selfed, sib mated and cross pollinated with other normal types to evaluate

the genetics of these traits and also to stabilize these traits in these lines. Further evaluation of these progenies and other prebred lines with much promise for agronomic performance is in progress. Interspecific hybrids involving *H. simulans* were produced for the first time (Prabakaran and Sujatha, 2004). They showed a highly resistant reaction to *A. helianthi*, but were sterile. These hybrids were multiplied on a large scale through tissue culture and the hybrid sterility barrier was circumvented by resorting to in vitro and in vivo chromosome doubling for development of amphidiploids.

Interspecific hybridization involving the hexaploid species, viz., *H. resinosus* Small and *H. tuberosus* L. showing high levels of resistance has been undertaken. As backcrossing of these hybrids resulted in triploid derivatives that were sterile, anther culture of the F1 hybrids involving hexaploid species was undertaken and several plantlets were developed from both the cross combinations. Further investigations in terms of characterization and evaluation are in progress.

Considerable progress has been made to widen the genetic base of Indian cultivated sunflower using wild sunflowers. The future focus is on improving cultivar tolerance to drought and salinity and resistance to *Alternaria* leaf spot. Simultaneously, programmes are being undertaken for development of appropriate mapping populations for desirable agronomic attributes.

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