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WILD *Helianthus* SPECIES USED FOR BROADENING THE GENETIC BASE OF CULTIVATED SUNFLOWER IN INDIA

Sujatha, M.*

Directorate of Oilseeds Research, Rajendranagar, Hyderabad 500 030, INDIA

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SUMMARY

The present investigation has been undertaken to introgress desirable traits from wild sunflowers to cultivated sunflower. Using conventional methods of crossing, backcrossing and selection, several pre-bred lines with altered plant architecture, high yield and oil content, maturity duration and inbuilt tolerance to major biotic stresses have been developed from crosses involving diploid annuals. These recombinant interspecific inbred lines are being utilized in the national sunflower network program for development of inbred lines and heterosis breeding. The pre-bred lines were characterized through cytological and molecular techniques using sunflower specific SSRs. Diploid perennial and hexaploid species have been utilized in the program aimed at introgression of resistance to *A. helianthi* through various ploidy manipulation procedures. The utility of the prebreeding material in the sunflower network program in India is presented.

Key words: ***Alternaria helianthi, Helianthus* spp, pre-breeding, resistance, sunflower necrosis disease**

INTRODUCTION

Sunflower with an area of 2.1 million hectares and production of 1.25 million tons is one of the most important oilseed crops of India. India's share in total world production of sunflower is about 6%, accounting for 10.0% of world acreage. Sunflower is grown year round as a sole crop and it also fits well as an intercrop with legumes, in double cropping and in three-crop rotations. Despite the premier position the crop holds in the vegetable oil economy of the country, the average productivity level is low (629 kg/ha) as compared with the world's productivity (1240 kg/ha). After the introduction of the crop in India in the early 1970s, a need for hybrids was recognized. Experimental hybrids were developed in 1974-75 using the *cms*

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* Corresponding author, Phone: 91-040-4015345, Fax: 91-040-4017969,
e-mail: mulpurisujata@yahoo.com

and restorer lines introduced from USA. The first hybrid from public sector was released for commercial cultivation in 1980. Since then the hybrid breeding program has been quite successful and 29 productive hybrids were developed by both public (18) and private sectors (11). These hybrids are intended for different agro-production situations, which are occupying 95% of the crop-grown area. Despite these successes with the crop, the major problems threatening sunflower productivity in India are the stagnating and unstable yields and vulnerability to various biotic stresses.

The major diseases attacking sunflower crop in India are *Alternaria* leaf spot (*Alternaria helianthi*), rust (*Puccinia helianthi* Schw.), downy mildew (*Plasmopara halstedii* (Farl.), *Rhizopus* head rot and a sunflower necrosis disease (a disease of recent origin). They are causing very high yield losses (Table 1).

Table 1: Potential and actual losses estimated for the major diseases of sunflower in India

Disease	Potential (%)	Actual (%)
Alternaria blight	90	10-40
Downy mildew	80	2-25
Rust	35	1-10
Soil borne	30	1-10
Seed borne	23	2-7
Sunflower necrosis disease	90	5-70

The crop is affected at all growth stages. Disease incidence is highly unpredictable and it tends to fluctuate from year to year, season to season and location to location, thus necessitating region-specific management strategies. Among the various approaches to manage these diseases, host plant resistance is the most reliable and economical to the end users. Plant breeding efforts to develop varieties/hybrids with inbuilt tolerance to the major diseases are constrained by the narrow genetic base of the cultivated sunflower. Sunflower is an introduced crop and the varieties are selections from these introductions. Hybrids are also developed from inbreds derived from the same narrow gene pool. A gene bank with about 1000 accessions is available in the country but it is characterized by unacceptable levels of intra-accessional heterogeneity in many lines thus restricting the scope for utilization of the material in breeding programs. Most studies on breeding for disease resistance were confined to screening of the available cultivar germplasm against the diseases under natural conditions and hence, these sources could not be converted into usable forms. With the exception of downy mildew disease, the released cultivars rated as tolerant/resistant are based on their field reaction to the pathogen. However, none of these were bred from parents that were classified as genetically resistant to a particular disease.

Wild *Helianthus* species serve as potential sources of novel genetic variability and several desirable characteristics such as resistance to biotic and abiotic stresses, cytoplasmic male sterility, fertility restorer genes and oil quality have been successfully introgressed into cultivated sunflower (Thompson *et al.*, 1981; Seiler, 1992). The growing needs for additional genetic variability to improve the cultivated sunflower makes it necessary to collect, maintain, characterize, evaluate and utilize the wild sunflower germplasm. Concerted efforts are required to incorporate additional genetic variability from reliable sources by integrating modern biotechnological tools and conventional breeding methods. The present study was undertaken to broaden the genetic base of cultivated sunflower for various agronomically desirable traits using diploid annuals and to transfer resistance to *Alternaria helianthi* using perennial species.

MATERIAL AND METHODS

A collection comprising of 36 wild *Helianthus* species obtained from Novi Sad, Yugoslavia, and USDA, USA, was established at DOR, Hyderabad. As most of the wild species are self-sterile and seed set is difficult, a rapid *in vitro* micropropagation protocol has been standardized for all the wild species which are being maintained in an *in vitro* collection (Sujatha and Prabakaran, 1997). Screening against *Alternaria helianthi* was done according to the method of Sujatha *et al.* (1997).

RESULTS AND DISCUSSION

1. Pre-breeding

Interspecific hybrids were successfully produced between cultivated sunflower and other wild diploid annual sunflowers *viz., H. annuus* (wild), *H. argophyllus, H. praecox, H. petiolaris, H. debilis, H. niveus* and *H. neglectus*. The interspecific hybrids were studied for pollen fertility and meiotic chromosome behavior to understand genomic relationships (Table 2). All cultivars were backcrossed and in each generation plants showing reduced height without branching and yellow disc color were selected for further generation advancement. Early lines with short plant type and resistance to various diseases have been included in the population improvement and inbred development programs.

As a prelude to mapping the sunflower population, 40 diverse pre-bred sunflower lines from 5 interspecific cross combinations (*H. argophyllus* × SF, *H. petiolaris* × SF; *H. annuus (wild)* × SF, *H. argophyllus* × *H. annuus (wild)* × SF, SF × *H. debilis)* were selected after subjecting the quantitative data of 250 pre-bred lines to diversity analysis. Molecular characterization using 100 sunflower specific SSRs covering the 17 linkage groups and 20 sequence tagged site (STS) markers was carried out according to the method of Yu *et al.*, 2002). Twenty-eight lines showed polymorphism and the primers revealing polymorphism varied from 1 to 9. Maximum genetic diversity was obtained for the line PS 2048, which was derived from the cross involving *H. petiolaris* and cultivated sunflower while maximum phenotypic diversity was detected in PS 4083 and PS 4093 derived from the tri-specific cross involving *H. argophyllus, H. annuus* (wild) and cultivated sunflower. Maximum numbers of unique alleles were observed in lines derived from the cross involving *H. petiolaris* and cultivated sunflower.

Table 2: Interspecific hybrids between sunflower and annual diploid species

Hybrid combination	Pollen fertility (%)	Traits of interest	Number of pre-bred lines developed
H. debilis × H. annuus	23.1	Oil quality (linoleic and oleic) and seed protein	31
H. annuus × H. debilis	0-2.3		
H. praecox × H. annuus	12.5	Resistance to rust and downy mildew, high oleic acid	--
H. petiolaris × H. annuus	99.0	Source of *cms*, resistance to rust, high linoleic acid	56
H. argophyllus × H. annuus	35.4	Source of *cms*, tolerance to drought, resistance to rust, downy mildew, high oleic acid	80
H. annuus × H. argophyllus	0-18.6		
H. annuus × H. annuus (wild)	98.0	Fertility restoration genes, resistance to rust and downy mildew	25
H. annuus (wild) × H. annuus	97.0		
H. argophyllus × H. annuus (wild)	27.5	Used in trispecific crosses with cultivated sunflower	90
H. argophyllus × H. petiolaris	40.0		
H. praecox × H. petiolaris	37.8		

A total of 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 pre-bred lines expressed wide variability for various qualitative traits like stem thickness, leaf angle and arrangement, leaf color, shape, texture, hairiness, number of leaves, petiole and stem pigmentation, disc color, stigma color and ray floret morphology. Significant variation was recorded for growth and yield characters and promising lines for desirable traits have been identified (Table 3). Among the pre-bred materials, 15 lines recorded seed yield > 50 g/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 programs. The sunflower breeders in the AICRP network are successfully utilizing the pre-bred lines developed in this program. At DOR, the pre-bred sunflower (PS) lines are being used as a source of maintainers and restorers for different cytoplasms and in heterosis breeding program. At TNAU, Coimbatore, standard heterosis of 151.6% was reported; the lines PS 1012, PS 1034, PS 1065 and SANS

03 were identified as good combiners, the line ARSS 01 is in AYT and 46 PS lines are used in the heterosis breeding program. At Latur and Akola, lines with high yield were identified. Thus, introgression can open up a novel and unknown treasure source of gene combinations.

Table 3: Promising material developed from diploid species

Trait	Lines
Rust (immune)	1089, 2011, 2032
Alternaria (tolerance)	2036
Alternaria (susceptible- useful for genetic studies)	2046, 3035
cms	*Arg* cytoplasm
Restorers for *Arg* cyto	P73, 2026, 5017, 5016
Superior inbreds/conversion	48 out of 80 lines
Drought endurance	1066, 2005, 2036, 4091
Phenotypic variants	2046, 4005 (Susc but High yield)
Agronomically superior lines	White pollen, double heads from crosses involving sunflower and *H. divaricatus*

Lines in the 1000 series are derived from crosses between *H. argophyllus* and sunflower, 2000 series from *H. petiolaris* and sunflower, 3000 series from *H. annuus (wild)* and sunflower, 4000 series from trispecific cross involving *H. argophyllus, H. annuus (wild)* and sunflower, 5000 from sunflower and *H. debilis.*

***Cms* from *H. argophyllus*.** Sunflower is one of the few crops in which the phenomenon of heterosis has been widely exploited. More than 95% of the area under sunflower crop in India is covered by commercial hybrids based on the *H. petiolaris* (*Pet 1)* cytoplasm. Attempts were made to identify new sources of *cms* from wild *Helianthus* collection by studying reciprocal cross combinations. Interestingly, such a nuclear-cytoplasmic interaction has been identified in combinations where *H. argophyllus* (designated as *Arg* cytoplasm) was used as ovule parent and several lines with the same cytoplasmic background and different phenotypic expressions have been developed and are presently being tested for fertility restoration and combining ability. The main constraint for exploitation of diverse cytoplasmic male sterile lines is the limited availability of fertility restoring genes in the inbred populations. However, in the case of *Arg* cytoplasm, we have identified 46 restorer lines from various breeding materials. Work on tagging the fertility restorer gene(s) for the *Arg* cytoplasm using molecular markers has been initiated using the method of bulk segregant analysis (BSA). A total of 506 RAPD primers were tested with the DNA isolated from parents and F_2 bulks of fertile and sterile plants of which 10 gave polymorphism and 2 of these were found to co-segregate with the trait of interest. The polymorphism generated with primer OPH11 was validated on all the individual plants and it gave a distinct band of 1375 bp in the fertile plants. Further work on conversion of the marker to SCAR for its subsequent validation on fertility restorers is in progress.

2. Introgression of resistance to *Alternaria helianthi*

Intensive screening of the *Helianthus* species against *A. helianthi* has resulted in the identification of reliable genetic variability for resistance to this pathogen. Nine perennial species *viz., H. maximiliani, H. mollis, H. divaricatus, H. simulans* and *H. occidentalis* (diploids), *H. pauciflorus* and *H. decapetalus* (tetraploids) and *H. resinosus* and *H. tuberosus* (hexaploids) were found to be highly resistant while the annuals were susceptible (Sujatha *et al.,* 1997). Among these, the perennial diploids and hexaploids were chosen for the breeding program aimed at introgression of resistance to *A. helianthi.* This is mainly because the pollen and ovule sterility resulting from abnormalities in meiosis due to dissimilar ploidy status of the parental species could be avoided in case of diploid perennials while the hexaploid species are easily crossable with cultivated sunflower. Before embarking upon a large-scale resistance-breeding program using wild relatives there is a need to discriminate between host and non-host resistance because host plant resistance is genetically controlled.

The biochemical basis of resistance to the leaf spot/blight pathogen *A. helianthi* was compared in six wild *Helianthus* species of three ploidy levels (diploid, tetraploid and hexaploid), possessing different degrees of resistance to leaf spot/blight pathogen, and cultivated sunflower (*H. annuus* cv. CO-4, susceptible check). The comparison involved sugars, phenols and isozymes of peroxidase, polyphenol oxidase and chitinase. The cultural characteristics and histochemical variations following infection with *Alternaria helianthi* were also studied in these six wild *Helianthus* species. Of these, the species, *H. occidentalis* and *H. tuberosus* were found to be highly resistant while *H. hirsutus* was moderately resistant. Media supplemented with leaf extracts of wild species, with the exception of *H. grosseserratus,* supported less growth and sporulation of *A. helianthi* than media supplemented with leaf extract of cultivated sunflower. Reduced infectivity of the pathogen was recorded when grown on leaf extract media of *H. occidentalis, H. hirsutus* and *H. tuberosus*. Furthermore, abnormalities in the shape of the conidia were noticed on media supplemented with leaf extracts of *H. occidentalis* and *H. tuberosus*. Histochemical studies showed restriction of the pathogen to epidermal cells in resistant wild sunflowers as well as higher accumulation of phenols. The resistant species (*H. tuberosus, H. occidentalis*) of wild sunflowers possessed higher levels of constitutive as well as induced total phenols and total sugars as compared with cultivated sunflower and susceptible wild sunflowers. Polyacrylamide gel electrophoretic (PAGE) isozyme analysis of defense-related enzymes showed a positive correlation of resistance with chitinase and polyphenol oxidase and a negative correlation with peroxidase.

Among the five diploid species, interspecific crosses were successful with *H. simulans, H. divaricatus, H. maximiliani* and *H. occidentalis.* The first success at interspecific hybridization between cultivated sunflower (*H. annuus*) and a diploid perennial species, *H. simulans* is reported (Prabakaran and Sujatha, 2004). The

F_1s from both direct and reciprocal crosses exhibited dominance of the wild species phenotype and were pollen sterile. Meiosis was irregular in the F_1 hybrids and both univalents and multivalents were observed. The problem of poor crossability with the diploid perennials was overcome by resorting to repeated forced pollinations and employing the amphidiploidization route circumvented the F1 hybrid sterility in some of the crosses. The amphiploids serve as fertile bridges and facilitate interspecific gene transfer through conventional breeding (Prabakaran and Sujatha, 2004).

Cross-compatibility between sunflower and the two hexaploid species *viz*., *H. resinosus* and *H. tuberosus*, was very high. The interspecific hybrids were tetraploids having three genomes from hexaploid species and one from cultivated sunflower. The pollen fertility of these hybrids was moderately low. Meiotic analysis revealed the formation of quadrivalents, bivalents and univalents. Backcrosses were attempted on the tetraploid F_1s and the BC_1F_1s were triploid and sterile. Fertile diploid progenies were obtained through manipulation of ploidy by following the anther culture technique. Pollen fertility of the anther plantlets varied between 0 and 58.3%. The progenies thus derived from various cross combinations including lines derived from diploid annuals are presented in Table 4.

Table 4: *Alternaria*-tolerant lines from various cross combinations

Helianthus species involved in the cross with cultivated sunflower	Numberof lines tolerant to *A. helianthi*	Incidence of *Alternaria* (%)	Lines susceptible to rust/ SND	Promising lines
H. argophyllus	10	5-20	4 (10-20%)	6
H. petiolaris	5	5-10	4 (3-10%)	1
H. annuus (wild)	4	2-20	3 (2-20%)	1
H. arg × *H. ann (w)* (trispecific)	9	5-20	5 (5-10%)	4
H. debilis	5	5-20	5 (3-20%)	0
H. maximiliani	26	2-20	19	7
H. mollis	2	15	--	2
H. decapetalus	6	--	3	3
H. occidentalis	46	2-20	28	18
H. neglectus	19	0-5	13	6
H. simulans	3	0-3		
H. divaricatus	60	0-20	40	20
H. tuberosus (anther culture plants)	74	0-5	--	74
H. resinosus (anther culture plants)	31	10	--	31

Wild diploid annuals were found to be susceptible to *A. helianthi* under laboratory screening assay using high inoculum load (Sujatha *et al.*, 1997). However, none of the species showed susceptibility to the pathogen in the field even under high epiphytotic conditions. Interestingly, some of the interspecific derivatives derived from diploid annuals showed high field tolerance to *A. helianthi.* Some of the *Alternaria* tolerant lines showed susceptibility to rust and various other stresses and hence, were not included in the breeding program for resistance to *A. helianthi.*

Sunflower necrosis disease. Sunflower necrosis disease (SND) transmitted by thrips and caused by tobacco streak virus was first observed in 1997 near Bangalore (Karnataka) in South India and later it spread at an alarming rate to the neighboring states posing a serious threat to sunflower cultivation. Unlike other diseases where the disease intensity does not have a 1:1 relation to yield loss, the SND incidence can be directly translated to yield loss. The disease occurs at all phenological growth stages of the crop (Figure 1).

Figure 1: Symptoms of Sunflower Necrosis Disease (SND) at various phenological stages

A sap inoculation technique has been optimized for large scale screening of sunflower genotypes against SND. About 500 genotypes comprising of the released cultivars, diverse inbreds, *cms* and restorer lines, germplasm accessions and few derivatives of wild sunflower species were screened against SND and the degree of severity varied among the lines tested. Twenty perennial wild *Helianthus* species were screened against SND and none of the tested species produced the symptoms probably due to the indeterminate and perennating habit of the species studied. Systematic studies have been undertaken for identification of reliable sources of resistance to SND in wild sunflowers. The virus has been characterized and the gene has been cloned but the limitations in plant regeneration and transformation protocols for sunflower need to be circumvented so as to tackle the problem through transgenic approach.

The study clearly demonstrated the potential of wild *Helianthus* species and also the advantages of integrated approaches in terms of conventional breeding methodologies and modern techniques for successful exploitation of the genetic diversity available in the wild species for the genetic improvement of sunflower. The advanced breeding lines possessing the vast genetic variability of the wild genomes have benefited the sunflower research programs under the All India Co-ordinated Program (AICRP) on sunflower. The future objectives are:

- Identification of sources of resistance to sunflower necrosis disease.
- Broadening the genepool for resistance to *Alternaria helianthi* and designing appropriate breeding strategies to transfer resistance to *A. helianthi*, which is a polygenically controlled trait.
- Diversification of *cms* sources and synthesis of diverse and heterotic gene pools.
- Enhancing cultivar tolerance to drought and salinity.

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ESPECIES SILVESTRES DEL GÉNERO *HELIANTHUS* UTILIZADAS PARA LA AMPLIACIÓN DE LA BASE GÉNICA DEL GIRASOL CULTIVADO EN INDIA

RESUMEN

Esta investigación fue emprendida con el fin de realizar el traspaso de las características favorables de las especies silvestres en el girasol cultivado. Utilizando los métodos de cruzamiento estándares, el cruzamiento reversible y la selección, varias líneas de preselección con la arquitectura alterada y período de vegetación de la planta, el alto rendimiento contenido de aceite y la tolerancia hacia los estreses bióticos principales agregada, han sido engendradas por cruzamientos, que incluían las especies diploides anuales. Esas líneas interespecies consanguíneas recombinantes se utilizan en la red nacional para la investigación de girasol para la formación de las líneas consanguíneas y selección heterotrófica. Las líneas de preselección se caracterizan por las técnicas citológicas y moleculares sobre la base de los marcadores específicos SSRs. Las especies diploides y hexaploides perennes son utilizadas en el programa de introducción de la resistencia hacia *A. helianthi* mediante diferentes métodos de manipulación con los niveles de ploidía. En el trabajo se demuestra el grado de utilización del material de preselección en los programas de selección de girasol en India.

ESPÈCES DE TOURNESOL SAUVAGE UTILISÉES DANS LE BUT D'ACCROÎTRE LA BASE GÉNÉTIQUE DU TOURNESOL CULTIVÉ EN INDE.

RÉSUMÉ

Cette recherche a été entreprise dans le but d'intégrer des caractéristiques du tournesol sauvage au tournesol de culture. En utilisant les méthodes courantes de croisement, de rétrocroisement et de sélection, on a développé plusieurs lignées présélectionnées avec architecture végétale et durée de végétation altérées, rendement et contenu d'huile élevés et tolérance au principaux stress biotiques à partir de croisements incluant des espèces diploïdes annuelles. On utilise ces lignées autogames interspécifiques recombinantes dans le programme du tournesol du réseau national pour développer des lignes autogames et pour la sélection hétérosis. Les lignées présélectionnées ont été caractérisées par les techniques cytologiques et moléculaires utilisant des marqueurs spécifiques SSRs. Les vivaces diploïdes et hexaploïdes ont été utilisées dans un programme destiné à l'intégration d'une résistance à l'*A. helianthi* par différents procédés de manipulation au niveau de la ploïdie. L'article présente l'utilité du matériel présélectionné dans le programme de sélection du tournesol en Inde.