

## Breeding for resistance to various biotic stresses in sunflower through wide hybridization and biotechnological tools

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- Sunflower production in India is constrained by the susceptibility of the released varieties/hybrids to a wide array of biotic stresses viz., *Alternaria helianthi*, powdery mildew, downy mildew, sunflower necrosis disease besides, the epidemics of *Spodoptera litura* and rust. Continuous cultivation of the crop subjected it to disease pressure with overlapping disease cycles. Host plant resistance is considered as one of the most viable options for enhancing the productivity.
- Sources of resistance were identified through screening of germplasm which included the wild *Helianthus* species, core germplasm (comprising of 112 accessions from different countries), exotic germplasm, interspecific derivatives (derived from crosses of cultivated sunflower with annual and perennial species), etc, through natural and artificial assays. In case of diseases for which genetic resources are inadequate, genetic transformation protocols are developed.
- Reliable sources of resistance to *A. helianthi* were found in perennial species; to *Spodoptera litura* and rust in annual and perennial species and also the interspecific derivatives from crosses involving *H. argophyllus* and *H. petiolaris*; to powdery mildew in diploid perennials and annuals. For sunflower necrosis disease (SND), few wild species with indeterminate growth habit were resistant but transfer to cultivated sunflower with determinate growth habit was unsuccessful. Hence, transgenic approach for introgression of resistance to SND is attempted by deploying the tobacco streak virus coat protein (TSV-CP) gene for which highly efficient and reproducible protocols of tissue culture and transformation were developed by circumventing the problems of low regeneration frequency, genotype dependence and precocious flowering.
- The studies indicate the possibility for widening the genetic base of cultivated sunflower through introgression of desirable genes from wild species and their derivatives and also through biotechnological tools. The identified sources are being used for development of appropriate mapping populations as a prelude for marker assisted breeding programmes. The genetic transformation protocol being optimized could be successfully used for development of sunflower transgenics for several other agronomically desirable traits.

**Key words:** disease resistance, interspecific hybridization, ploidy, transgenics, wild sunflowers,

## INTRODUCTION

Sunflower with an area of 1.0 million hectares and production of 0.65 million tonnes is one of the most important oilseed crops of India. Sunflower is grown round the year as a sole crop and fits well as an intercrop with legumes, in double cropping and in three crop sequences as well. Despite the premier position the crop holds in the vegetable oil economy of the country, the average productivity levels are low (650 kg/ha) as compared to the world's productivity (1327 kg/ha). The major problems confronting sunflower productivity in India are the stagnating and unstable yields and vulnerability to various biotic stresses at all crop growth stages. The major biotic stresses attacking sunflower crop in India are *Alternaria* leaf spot (*Alternaria helianthi*), downy mildew (*Plasmopara halstedii* (Farl.), powdery mildew (*Golovinomyces cichoracaerum*), rust (*Puccinia helianthi* Schw.), sunflower necrosis disease and *Spodoptera litura* causing very high level of yield losses. Disease incidence is highly unpredictable and tend 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. 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 resistant to a particular disease.

Wild *Helianthus* species, interspecific derivatives and exotic germplasm 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 present study reports the attempts made at identification of sources of resistance to various biotic stresses and their introgression into the cultivar background. In diseases like sunflower necrosis disease where reliable sources of resistance are limited, genetic transformation protocols were developed for deployment of the TSV-coat protein gene.

## MATERIAL AND METHODS

A species garden comprising of 43 wild *Helianthus* species obtained from Novisad, Yugoslavia and USDA, USA was established at DOR, Hyderabad, India. As most of the wild species are self-sterile and seed set is difficult, a rapid *in vitro* micropropagation protocol for all the wild species has been standardized and the wild species are being maintained in an *in vitro* species garden. Screening against *Alternaria helianthi* was according to the method of Sujatha et al. (1997). In addition to these, the core set of germplasm (112 accessions) obtained from USDA-ARS, Ames, exotic germplasm (4 accessions) obtained from USDA-ARS, North Dakota, USA and interspecific derivatives developed at the institute (350 lines) and obtained from USDA-ARS, North Dakota (35 accessions) were used in the study. For *A. helianthi*, sunflower necrosis disease, powdery mildew and *S. litura*, preliminary screening was under natural field condition and promising material was subjected to artificial assays. Screening for rust was done under epidemic conditions.

## RESULTS AND DISCUSSION

*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. Interestingly, only the perennial species like *H. maximiliani*, *H. mollis*, *H. divaricatus*, *H. simulans* and *H. occidentalis* (diploids), *H. pauciflorus* and *H. decapetalus* (tetraploids) and *H. resinusus* and *H. tuberosus* (hexaploids) were found to be highly resistant while the annuals were susceptible (Sujatha et al., 1997). Among these the diploid perennials and hexaploids were chosen for the breeding programme aimed at introgression of resistance to *A. helianthi* as 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 cultivated sunflower forms hybrids easily with the hexaploid species.

Before embarking upon a large-scale resistance-breeding programme 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, *A. helianthi* and cultivated sunflower (*H. annuus* cv. CO-4, susceptible check) in terms of sugars, phenols and isozymes of peroxidase, polyphenol oxidase and chitinase. 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 to cultivated sunflower and susceptible wild sunflowers. Polyacrylamide gel electrophoretic (PAGE) isozyme analysis of defence-related enzymes showed a positive correlation of resistance with chitinase and polyphenol oxidase and a negative correlation with peroxidase (Madhavi et al., 2005a).

The cultural characteristics and histochemical variations following infection with *Alternaria helianthi* was studied in six wild *Helianthus* species of three ploidy levels (diploid, tetraploid and hexaploid) possessing different degrees of resistance to the pathogen and cultivated sunflower (*H. annuus* cv. CO-4, susceptible check). Of these, the species, *H. occidentalis* and *H. tuberosus* were found to be highly resistant while *H. hirsutus* was moderately resistant (Madhavi et al., 2005b). 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 supplemented media 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.

Among the five diploid species, interspecific crosses were successful with *H. simulans*, *H. divaricatus*, *H. maximiliani* and *H. occidentalis*. It is worth mentioning that the interspecific hybrid involving *H. simulans* (both directions) and cultivated sunflower is the first report of successful hybridization (Prabakaran and Sujatha, 2004). The F<sub>1</sub>s from both direct and reciprocal directions exhibited dominance of the wild species phenotype and were pollen sterile. Meiosis was irregular in the F<sub>1</sub> hybrids and both univalents and multivalents were observed. Multiplication of the interspecific hybrids was achieved through *in vitro* culture of nodal sections on Murashige & Skoog medium supplemented with 0.5 mg l<sup>-1</sup> benzyladenine. Fertility of the interspecific hybrids was improved by subjecting the *in vitro* proliferating shoots to 0.001% colchicine incorporated in the shoot multiplication medium. The amphiploids serve as fertile bridges and facilitate interspecific gene transfer through conventional breeding. Thus, the problem of poor crossability with the diploid perennials was overcome by resorting to repeated forced pollinations and the F<sub>1</sub> hybrid sterility in some of the crosses was circumvented by employing the amphidiploidization route.

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. Fertile diploid progenies were obtained through manipulation of ploidy by following the anther culture technique (Sujatha and Prabakaran, 2006). The progenies thus derived from various cross combinations are being evaluated for their reaction to *A. helianthi* and for their utility in *Alternaria* breeding programme.

**Rust:** Thirty wild *Helianthus* species, a few interspecific hybrids and 380 recombinant interspecific inbred lines (RIILs) derived from interspecific crosses between cultivated sunflower and four diploid annuals (wild *H. annuus*, *H. argophyllus*, *H. petiolaris* and *H. debilis*) and three diploid perennials (*H. occidentalis*, *H. maximiliani* and *H. divaricatus*) were studied for their reaction to rust caused by *Puccinia helianthi* under severe natural epiphytotic condition (Sujatha et al., 2003). The disease incidence ranged from 0 to 100% in the stable interspecific derivatives while none of the wild sunflowers and their F<sub>1</sub> hybrids revealed susceptibility to rust. The disease severity in the RIILs varied between 0 and 40%. The mean disease severity was low in lines derived from diploid annuals (6.0-10.4%) as compared to those derived from diploid perennials (23.1-31.1%). One line (PS 1089) derived from *H. argophyllus* x cultivated sunflower and two

lines (PS 2011 and PS 2032) derived from *H. petiolaris* x cultivar crosses were found to be immune. Disease severity and disease incidence were correlated with various physiological traits.

**Powdery mildew:** Powdery mildew is world wide in distribution and has become a serious disease in India during the past 2-3 years. During the first year of its appearance, it was observed only in the spring season but now it has assumed serious proportion and is found attacking the crop regardless of the season. Genetic resistance is rather limited in the released cultivars and the parental lines of hybrids. Hence, screening of wild *Helianthus* species, back cross inbred lines, interspecific derivatives, core germplasm, inbred lines and few exotic accessions were screened under field conditions by simulating the conditions followed by rescreening under artificial inoculation conditions. Several methods described for bringing infection were tested of which dusting of spores on to the healthy leaves of plant has proven to be the most effective and convenient method of infection. Based on the differential response of the accessions derived from diverse genetic backgrounds a scale for obtaining reliable estimate of the disease has been devised taking into consideration the infection on lower middle and top leaves from flowering till harvest stages at 10 days interval. The DSI ranged from 15 to 100 and AUDPC ranged from 104 to 648. Sources of resistance were identified in 3 annual wild species (*H. argophyllus*, *H. agrestis*, *H. debilis*), 6 perennials (*H. angustifolius*, *H. atrorubens*, *H. rigidus*, *H. salicifolius* Dietr., *H. pauciflorus* Nutt and *H. resinosus* Small), two interspecific derivatives (ID-16, ID-25), and two exotic lines with multiple resistance (TX16R, EC-53725).

**Sunflower necrosis disease:** Sunflower necrosis disease (SND) transmitted through thrips and caused by tobacco streak virus was first observed during the year 1997 near Bangalore (Karnataka) in South India and later spread at an alarming rate to the neighbouring 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. A sap inoculation technique has been optimized for large scale screening of sunflower genotypes against SND. About 1500 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. Systematic studies have been undertaken for identification of reliable sources of resistance to SND in wild sunflowers. About 80 accessions comprising annual and 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. The virus has been characterized and the gene has been cloned but the limitations in plant regeneration and transformation protocols for sunflower were the major bottlenecks through which transgenics could be developed. Hence, an efficient protocol for shoot regeneration and transformation was developed for development of plants with inbuilt resistance to sunflower bud necrosis through deployment of the coat protein gene of the tobacco streak virus (Figure 1).

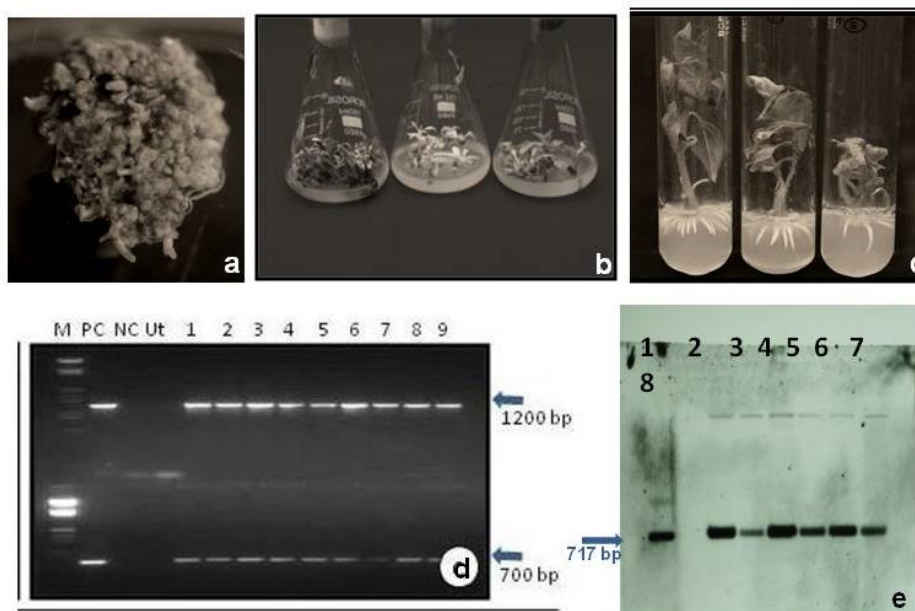


Figure 1: Regeneration and transformation in sunflower. A. Adventitious shoot regeneration from cotyledons derived from mature seeds; b. Selection of transformed shoots on kanamycin, c. Rooting of putative transformants, d. PCR analysis of transformants obtained with pCAMBIA 2301 showing the presence of *UdA* and *npt* genes, e. Southern analysis of putative transformants harbouring the *TSV-CP* gene showing the hybridization signal at 717 bp.

***Spodoptera litura*:** *S. litura* (Fabr.), the tobacco caterpillar, is a major defoliator on sunflower in the tropics. Genetic variability for resistance to *S. litura* is limited in the cultivar germplasm of sunflower. 43 accessions of 17 wild *Helianthus* species of the annual and perennial habit groups were evaluated along with cultivated sunflower under field and no-choice conditions in the laboratory for resistance to this pest (Sujatha and Lakshminarayana, 2007). Under field conditions, *H. occidentalis* and *H. argophyllus* were found to be immune with no leaf damage and few accessions of the species belonging to section Divaricati were found to be resistant. Laboratory bioassays against neonate, two and four-day-old larvae confirmed resistance both in terms of high larval mortality and low larval weight gain in eight species viz., *H. occidentalis*, *H. argophyllus*, *H. tuberosus*, *H. maximiliani*, *H. mollis*, *H. simulans*, *H. divaricatus* and *H. hirsutus*. Intra-accessional variability was observed and accessions of few species showed varied reaction (resistant, partially resistant to susceptible) to the target pest. Field evaluation of 224 backcross derived inbred lines from five cross combinations involving diploid species under high natural pest incidence revealed low damage in plants derived from crosses involving *H. argophyllus* and *H. petiolaris*.

The study clearly demonstrated the potential of wild *Helianthus* species and exotic germplasm 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 and cultivar germplasm for the genetic improvement of sunflower. The future thrust is on Identification of sources of resistance to sunflower necrosis disease; broaden the genepool for resistance to *Alternaria helianthi*, design appropriate breeding strategies to transfer resistance to *A. helianthi*, which is a polygenically controlled trait; deploy marker assisted breeding strategies for transfer of resistance to powdery mildew and *A. helianthi* from the identified sources to the cultivar background.

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