POLLEN SELECTION AND PROGENY EVALUATION FOR *ALTERNARIA* LEAF BLIGHT RESISTANCE IN SUNFLOWER (*HELIANTHUS ANNUUS* L)

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SUMMARY

High level of resistance to leaf blight caused by *Alternaria helianthi*, the important disease of sunflower is not reported either in the cultivated or related taxa. Therefore, recurrent selection to improve resistance has been proposed, which is cumbersome and time consuming. Several researchers have examined the possibility of increasing the efficiency of plant breeding for biotic and abiotic stress through selection of microgametophyte. In sunflower, there was correspondence between the reaction of the pollen and sporophyte to selective agent and a procedure that ensures pollen on the stigma has been reported. Selection at the pollen stage could increase the performance of the resultant progeny. In this context, the present study was conducted for application of selection pressure against *Alternaria* leaf blight on pollen grains to achieve preferential transmission of selected trait from gametophytic generation to next generation. Four heterozygous genotypes showing different levels of susceptibility to leaf blight were chosen as male parents and two genotypes CMS 234A and L101 as female. Toxin stress on the female style tissue was imposed uniform application of the culture filtrate on the stigma and style one hour before pollination. Distilled water applied styles and stigma served as control. Following this the female heads were pollinated with pollen grains of the selected parents producing two sets of 7 hybrids- one set with selected pollen grains while the other with non selected pollen grains. These hybrids were evaluated for their reaction to *Alternaria* leaf blight in two replications in field under natural epiphytotic conditions. The susceptibility of each genotype was assessed on ten randomly selected plants per replication at 2 stages after flowering. The progenies derived from pollen selection showed better tolerance to the disease compared to the progeny obtained through non-selected pollen. The selection of resistant pollen on the stigmatic surface resulted in the corresponding increase in the progeny resistance. However, the improvement through pollen selection was not very high, as the parental lines used are not highly resistant to this disease. Hence, it is required to practice more number of repeated cycles of selection to achieve useful level of resistance in case sunflower.

INTRODUCTION

Alternaria leaf and stem blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara is the most devastating disease causing significant yield losses in all the sunflower growing countries (Hiremath *et al*., 1990; Kochman and Kong, 1992). Inbuilt resistance would be the most economic means of reducing yield losses and studies so far indicate that a useful level of resistance is not available either in the cultivated or related taxa (Morris et.al.,1983; Kochman and Kong, 1992; Ravikumar *et al*., 1995). Hence, the available moderate level of resistance has to be improved for the success of development of resistant varieties/hybrids. Several researchers have shown the male gametophytic selection as an alternate efficient technique for the improvement of traits such as biotic and abiotic stress tolerance in several plant species (Hormaza and Herrero, 1992; Jourden *et al*., 1996). In sunflower, correspondence has been reported between pollen germination on the pathogen-stressed stigma and the sporophytic reaction of the pollen parent to *Alternaria* leaf blight (Ravikumar and Chikkodi, 1998). It is presumed that only those pollen grains having resistant alleles would overcome the toxin effect and germinate on the stressed stigma leading to selective fertilisation by resistant pollen grains achieving preferential transmission of a selected trait from the gametophytic generation to the next generation.

MATERIAL AND METHODS:

<u>*Selection of parents*</u>: Five genotypes including one susceptible (L 101), three moderately tolerant (Acc. Nos. 180, 873 and 1229) and one cytoplasmic male sterile (CMS 234A) genotypes were selected for this study. Of these, the moderately tolerant origins with some heterozygosity were used as male parents and CMS 234A and L 101 were used as female parents. Before crossing, L 101 was made male sterile, using a growth regulator (Seetharam and Kusuma kumari, 1976).

<u>*Toxin culture filtrate*</u>: One disc of sporulating pathogen, *Alternaria helianthi* grown on Potato Dextrose Agar was placed in each of 250 ml flask containing 75 ml of Potato Dextrose Broth. Control flasks were not inoculated. After incubating for one month at room temperature the flasks were harvested by filtering through cheesecloth followed by Whatman NO. 1 filter paper. The culture filtrate inhibited *in vitro* pollen germination.

<u>*Pollen selection and progeny evaluation*</u>: CMS 234A and L 101 were used as female and the three moderately resistant genotypes were used as male parents. The pathogen culture filtrate was applied uniformly on the stigma and style one hour before pollination, which formed the stressed stigma leading to pollen selection. The heads treated with control flask extracts formed the control, i.e. no pollen selection. The pollen grains collected from each of the male parent were dusted abundantly on the treated heads which were covered with muslin cloth. The control and stress induced pollinations were continued for 6-8 days until the flowers in the heads were pollinated. The seeds were harvested separately and two sets of seven hybrids - one from pollen selection and the other from no pollen selection were obtained.

The two sets of seven hybrids (Table 3) were grown in the field following a factorial randomised block design with two replications, during rainy season, which is ideal for the

development of epiphytotics. The susceptibility of each genotype was assessed on ten randomly selected plants per replication at two stages viz., at the beginning of flowering (stage I) and at physiological maturity (stage II). The observations on susceptibility were recorded on a 0-9 scale following Mayee and Datar (1986).

Data on seven crosses with two main treatments were analysed using a factorial randomised block design with two factors- pollen selection / no pollen selection and cross.

RESULTS AND DISCUSSION:

The analysis of variance and comparison of mean squares indicate that pollen selection treatment overall had a significant effect on the progeny disease reaction (table 1). The progenies derived through pollen selection treatment had a lower mean susceptibility values (3.46 and 6.28 respectively) compared to progeny obtained through no pollen selection (4.58 and 7.84 respectively) at stage I and II of disease scoring (table 2). The progeny obtained through pollen selection showed significant improvement for resistance indicating that the selection for resistant pollen on the stigmatic surface leads to corresponding increase in the progeny disease resistance. Numerous studies in different plants (Hormaza and Herrero, 1992; Jourden *et al*., 1996) have also shown a response to pollen or male gametophytic selection and skewing of the progeny towards desirable trait for several biotic and abiotic stresses.

Table-1: Mean squares from analysis of variance of the pollen selection treatment.

Source of variation	d.f.	Stage 1	Stage 2
Pollen selection treatment	1	8.86**	17.02**
Hybrids	6	2.88**	3.61**
Hybrids x pollen treatment	6	0.15	0.97

* Significant at 5% level.

Table-2: Effect of pollen selection on mean progeny disease score.

Pollen selection treatment	Disease score	
	Stage-1	Stage-2
Selected	3.46	6.28
Non-selected	4.58	7.84

The improvement made through a single cycle of pollen selection did not vary from cross to cross (Table-3). However, the gain achieved through one cycle of pollen selection was not very high. This could be due to non-availability of high level of resistance in the parental lines and polygenic inheritance of resistance to leaf blight in sunflower (Ravikumar *et al.* 1995). In polygenically controlled characters the interaction effects many not be same in both gametophytic and sporophytic generations leading to non-correspondence of selection from pollen to progeny. However, pollen selection could allow the choice of rare favourable

allelic combination, which would be difficult to detect on the sporophyte. The results indicated that pollen selection was effective in improving the tolerance of the progeny. It is required to practice more number of repeated cyclic selections including male parents producing pollen segregating for resistance to achieve high level of resistance. Attempts are being made to combine pollen and plant selections alternatively to achieve high level of resistance.

Table-3: Effect of pollen selection on progeny disease score of different crosses

Crosses	Stage-1		Stage-II	
	Selected pollen	Non-selected	Selected pollen	Non selected
234A x 873	2.59	3.78	5.94	6.80
234A x L 101	3.05	4.05	6.54	7.40
234A x 1229	3.33	4.60	4.76	7.84
L101 x 873	3.68	4.06	6.65	7.89
L101 x L101	5.20	6.45	9.00	9.00
L101 x 1229	3.20	48.8	5.60	7.80
L101 x 180	3.15	4.27	6.49	8.14

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