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Screening of Sunflower Genotypes for Resistance Against *Alternaria* Blight

[1]C.V.C.M. Reddy, [2]A.V.V. Reddy, [1]B. Sinha and [2]M. Shanta Lakshmi
[1]Department of Genetics and Plant Breeding, Institute of Agricultural Sciences,
Banaras Hindu University, Varanasi 221005, India
[2]Division of Genetics and Plant Breeding, Directorate of Oilseeds Research, Hyderabad, India

Abstract: In this study, genotypes were screened on field and lab environments to evaluate genotypes for resistance to *A. helianthi*. Disease severity was recorded by visually examining each leaf after seven days after inoculation as percentage of leaf area occupied by the sporulating fungus using the pictorial key in each replication and the average computed. The disease intensity for hybrids ranged from 3.73 to 52.33%. RHA 587 and ARG × RHA 587 were found to be resistant to alternaria blight both under field and lab conditions and therefore have the potential to reduce yield losses because of this disease in the field.

Key words: Sunflower, resistance, leaf spot, *Alternaria*

INTRODUCTION

Sunflower is infected by a large number of disease causing pathogens resulting in severe economic losses in yields. Occurrence of wide spread diseases was identified as one of the major constraint for low productivity of sunflower. Alternaria leaf blight caused by *Alternaria helianthi* (Hansf) Tubaki and Nishihara which induce pre and post emergence mortalities and seed lining blight/rots in the field (Shobharani and Ravikumar, 2003). By and large the incidence and intensity of the diseases are influenced by season, varieties/hybrids grown, stage of the crop, region and climatic conditions etc. Although the exact estimate of yield losses due to these diseases of sunflower is not known, the potential and actual losses estimated for the major diseases revealed that in case of alternaria blight it was 90% (Agrawat *et al*., 1979; Balasubramanyam and Kolte, 1980). So the present investigation helps to identify resistant genotypes and improve disease resistant cultivars.

MATERIALS AND METHODS

Field screening: During field screening programme, the two CMS lines (CMS PET-1, Helianthus petialaris, CMS ARG Helianthus argophyllus) and twenty eight testers (Table 1) along with susceptible check L-101 were screened for their reaction against alternaria blight under natural conditions in field during *Kharif* season in 2004.

The intensity of disease in the field was estimated from five randomly selected plants in each genotype which were tagged with labels, at the flowering stage of the crop. On an average 60 leaves were selected at random from the selected plants and disease severity was recorded by visually examining each leaf for leaf area damaged using pictorial key of Allen *et al.* (1983) in each relationship and the average was computed. The scale adopted to indicate the degrees of resistance in sunflower genotypes is furnished below (Table 3).

Lab screening: During evaluation of F_1s (Table 2) along with parents (Table 1 and 2), detached leaf technique (Prasad *et al*., 1996; Sujatha *et al*., 1997) was employed to identify resistance against alternaria blight under laboratory conditions in *rabi* season in 2004. These lines were artificially inoculated with pathogen isolated from leaf blight affected plants and disease severity was recorded. Leaves of forty hybrids and their 14 parental lines (respective B lines of four CMS lines and ten testers) grown in DOR Farm, Hyderabad during *rabi* season in 2004 were detached with petiole from third node of newly emerged branch at similar physiological maturity.

Table 1: Testers used in the investigation of sunflower genotypes against *Alternaria* blight

Testers		
RHA 271	RHA587	SF 211
RHA 273	RHA859	SF 216
RHA274	RHA6D-1	BLC P 6
RHA 297	HAM161	PARRUN1329
RHA298	HAM174	RES 834-1
RHA 341	HAM175	RCR 8297
RHA344	HAM180	R83R6
RHA345	SF 206	NDLR 1
RHA346	SF 207	
RHA356R	SF 208	

Corresponding Author: C.V.C.M. Reddy, Research Scholar, Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005, India

Table 2: Investigated crosses of sunflower genotypes against *Alternaria* blight

Crosses			
PET-1 × RHA 271	PET-1 × HAM 174	ARG × RHA 271	ARG × HAM 174
PET-1 × RHA 273	PET-1 × HAM 175	ARG × RHA 273	ARG × HAM 175
PET-1 × RHA274	PET-1 × HAM 180	ARG × RHA274	ARG × HAM 180
PET-1 × RHA 297	PET-1 × SF 206	ARG × RHA 297	ARG × SF 206
PET-1 × RHA 298	PET-1 × SF 207	ARG × RHA 298	ARG × SF 207
PET-1 × RHA 341	PET-1 × SF 208	ARG × RHA 341	ARG × SF 208
PET-1 × RHA 344	PET-1 × SF 211	ARG × RHA 344	ARG × SF 211
PET-1 × RHA 345	PET-1 × SF 216	ARG × RHA 345	ARG × SF 216
PET-1 × RHA 346	PET-1 × BLC P 6	ARG × RHA 346	ARG × BLC P 6
PET-1 × RHA 356R	PET-1 × PARRUN 1329	ARG × RHA 356R	ARG × PARRUN 1329
PET-1 × RHA 587	PET-1 × RES 834-1	ARG × RHA 587	ARG × RES 834-1
PET-1× RHA 859	PET-1 × RCR 8297	ARG × RHA 859	ARG × RCR 8297
PET-1 × RHA 6D-1	PET-1 × R 83 R6	ARG × RHA 6D-1	ARG × R 83 R6
PET-1 × HAM 161	PET-1 × NDLR 1	ARG × HAM 161	ARG × NDLR 1

Table 3: Scale adopted to indicate degree of resistance against *Alternaria* blight

S.No.	Disease severity (%)	Category
1	0	Immune (I)
2	<1	Highly resistant (HR)
3	1-5	Resistant (R)
4	5-25	Moderately resistant (MR)
5	25-50	Susceptible (S)
6	>50	Highly susceptible (HS)

Detached leaves were immersed in spore suspension of 10^3 conidia mL^{-1} of *Alternaria helianthi* prepared from 15 day old culture grown on SLEM. The inoculated leaves were transferred to petriplates (15 cm diameter) containing two layers of filter paper with one set of respective controls. For each genotype three replications were maintained. The petriplates containing inoculated leaf sample were incubated at 27±1 °C in culture room for a week. Disease severity was recorded described above.

The general laboratory techniques followed for the present study was described by Aneja (1993) and Dhingra and Sinclair (1993) for sterilization, isolation and preparation of media and maintenance of fungal cultures.

Preparation of culture media (SLEM): Sunflower Leaf Extract Medium (SLEM) was prepared by using the leaves of sunflower cv. CO-4 were obtained from Research Farm, DOR, Hyderabad. The leaves of fifty six F_1s developed during *kharif*, 2004 along with parents were used for screening against *Alternaria* blight during *rabi*, 2004. Chopped leaf bits (200 g) of sunflower cv. CO-4 were boiled in 500 mL of water for about 10-15 min. In another 500 mL of water, 20 g of agar was melted. Both these were filtered through the double-layered muslin cloth in to a third container and 20 g sucrose was added to the filtrate. The final volume of medium was made upto one litre by adding water and the medium was autoclaved.

Isolation of the pathogen: The pathogen *Alternaria helianthi* was isolated from infected sunflower leaves collected from fields of the DOR, Hyderabad. The leaves were washed thoroughly with sterilized distilled water and small pieces of (3 mm^2) diseased leaf spots adjoined with some healthy tissue were cut with the help of a scalpel and surface sterilized by immersing them in 0.1% mercuric chloride solution for 30 sec in order to eliminate surface contaminants. The leaf bits were washed thoroughly in sterilized distilled water and excess moisture was removed by blotting using sterilized blotting paper. These leaf bits were transferred aseptically on to sterilized petriplates containing culture medium PDA (Potato 200 g, Dextrose 20 g, agar 20 g and distilled water 1 L.) and incubated at 27±1 °C. The mycelia growth emerging from diseased leaf bits was transferred directly into fresh PDA plates. The culture was purified by single spore isolation technique. The pure culture was then transferred into culture medium (SLEM) with the help of sterilized needle.

Identification of pathogen: The fungus associated with blight disease of sunflower was identified as *Alternaria helianthi* based on the descriptions as given by Ellis (1976).

Pathogenicity tests: Pathogenicity tests were carried out using alternaria blight susceptible sunflower cv. CO-4. The seeds of the cultivar were sown in 18 cm diameter earthen pots containing a mixture of soil and farm yard manure in 4:1 ratio and maintained on glasshouse bench. Three plants were raised in each pot. The inoculum (conidial suspension) of *A. helianthi* was prepared by gentle scraping of the 15 day old culture grown on SLEM in petriplates with the help of a camel hair brush by adding 5 mL of sterile distilled water to each petriplate. The spore suspension was filtered through three layers of muslin cloth and Tween 20 (polyoxyethylene sorbiton monoleate) was added at the rate of 0.1% to the suspension to enable uniform spread of inoculum on the sprayed leaves. The resultant conidial suspension was

diluted to get a spore load of 1×10^3 conidia mL^{-1} by using haemocytometer. The conidial suspension was sprayed with a hand atomizer over the detached leaves and inoculated for symptom production.

RESULTS

Field screening: Thirty genotypes were screened for their reaction against alternaria blight along with susceptible check L-101 under field conditions.

All the thirty genotypes showed significant differences for disease severity (%) and it ranged from 3.33 to 67.00%. Based on disease severity (%), all the parental lines were grouped into three categories i.e., resistant (1-5%), moderately resistant (5-25%) and susceptible (>50%) are presented in Table 4. Of the thirty genotypes, one inbred RHA 587 (3.33%) showed resistance, four genotypes RHA 273 (23.67%), RHA 274 (22.67%), SF 207 (20.67%) and RCR 8297 (23.67%) showed moderate resistance and the remaining genotypes PET-1 (33.33%), ARG (28.33%), RHA 271 (33.67%), RHA 297 (34.00%), RHA 298 (36.33%), RHA 341 (34.67%), RHA 344 (29.67%), RHA 345 (36.67%), RHA 346 (32.67%), RHA 356R (56.00%), RHA 859 (28.33%), RHA 6D-1 (36.00%), HAM 161 (38.67%), HAM 174 (41.33%), HAM 175 (43.33%), HAM 180 (35.00%), SF 206 (41.33%), SF 208 (29.00%), SF 211 (47.33%), SF 216 (27.67%), BLC P 6 (45.00%), PARRUN 1329 (67.00%), RES 834-1 (35.67%), R83 R 6 (38.33%), NDLR 1 (28.33%) showed susceptible reaction to *Alternaria* blight when compared with susceptible check L-101.

Identification of the pathogen: Isolation of the pathogen *Alternaria helianthi* (Hansf.) Tubaki and Nishihara was made from the infected leaves of sunflower cv. Co-4 showing typical leaf spot/blight symptoms on leaf extract medium of cultivated sunflower cv. CO-4 (SLEM) and purified by single spore method and maintained on Sunflower Leaf Extract Medium.

Growth of the fungus was observed after two days of plating, when incubated at 27±1°C on sunflower leaf extract medium. The rate of growth of the culture was very slow (growth rate in mm) on the medium. Maximum colony growth was obtained 21 days after plating. The culture was greyish white with brown pigmentation. Sporulation was observed at the center of the colony after 21 days of incubation.

Microscopic examination of the fungal culture revealed branched, septate, smooth and olivaceous brown coloured mycelium. Conidiophores were cylindrical, either solitary or in fascicles, pale grey to brown in colour, strait or curved, simple or branched, separate, upto 120 μm long and 8-11 μm broad. Conidia were solitary, straight or slightly curved, cylindrical long, ellipsoid, rounded at ends, sub hyaline to golden brown, smooth, with 2-12 transverse and occasionally one or more longitudinal septa, often constricted at the septa, 45-145 μm long, 10-30 μm thick in the broadest part. On the basis of cultural and morphological characteristics, the fungus associated with spot/blight disease in sunflower was identified as *A. helianthi* (Hansf.) Tubaki and Nishihara and the characteristic features tallied with CMI description of *A. helianthi* (Ellis, 1976).

Pathogenicity tests: Pathogenicity was proved by spray inoculation with conidial suspension (1×10^3 conidia mL^{-1}) of the pathogen on susceptible sunflower cv. CO-4. The initial symptoms were observed 48 h after inoculation on leaves in the form of oval to circular spots surrounded by yellowish halo measuring 1-2 mm in diameter. These spots gradually increased in size, which coalesced forming bigger spots of about 3-5 mm in diameter with angular margins. The pathogen could be reisolated from the infected leaves and the fungus obtained resembled the original isolate.

Screening of parents and F_1s for resistance against alternaria blight *in vitro*: Fifty six F_1s along with parents (respective B lines of two CMS lines and twenty eight testers) were screened under laboratory conditions using detached leaf technique to search for resistant genotypes against alternaria blight. All the eighty six genotypes showed significant differences for disease severity (%) and it ranged from 3.73% (ARG × RHA 587) to 52.33% (PET-1 × RHA 345). These genotypes grouped into four categories i.e., resistant (1-5%), moderately resistant (5-25%), susceptible (25-50%) and highly susceptible (50-65%) and presented in Table 4. Among the eighty six genotypes screened, two genotypes (RHA 587 and ARG × RHA 587) exhibited resistant, while only eight genotypes, of which five are parental testers, RHA 273 (21.00%), HA 274 (22.00%), SF 207 (19.00%), SF 216 (23.67%), RCR 8297 (18.00%) and three hybrids ARG × HAM 161 (24.67%), ARG×RCR 8297 (20.33%) and ARG × NDLR (21.67%) were moderately resistant, while seventy three genotypes were susceptible, among them twenty three parental lines showing susceptibility. PARRUN 1329 (52.67%), PET-1× RHA 345 (52.33%) and PET-1× RHA 356 R (50.67%) were highly susceptible. None of the genotypes neither parents nor hybrids exhibited immune or highly resistant reaction to the disease.

Table 4: Grouping of 86 genotypes based on disease severity (%) against *Alternaria* blight in sunflower

Category	Genotypes
Resistant (1-5%)	RHA 587, ARG × RHA 587
Moderate resistant (5-25%)	RHA 273, RHA 274, SF 207, SF 216, RCR 8297, ARG × HAM 161, ARG × RCR 8297, ARGx NDLR 1
Susceptible (25-50%)	PET-1, ARG, RHA 271, RHA 297, RHA 298, RHA 341, RHA 344, RHA 345, RHA 346, RHA 859, RHA 6D-1, HAM 161, HAM 174, HAM 175, HAM 180, SF 206, SF 208, SF 211, BLC P6, RES 834-1, R 83 R6, NDLR 1, PET-1 × RHA 271, PET-1 × RHA 273, PET-1 × RHA 274,PET-1 × RHA 297, PET-1 × RHA 298, PET-1 × RHA 341, PET-1 × RHA 344, PET-1 × RHA 346, PET-1 × RHA 587, PET-1 × RHA 859, PET-1 × RHA 6D-1, PET-1 × HAM 161, PET-1 × HAM 174, PET-1 × HAM 175, PET-1 × HAM 180, PET-1 × SF 206, PET-1 × SF 207, PET-1 × SF 208, PET-1 × SF 211, PET-1 × SF 216, PET-1 × BLC P6, PET-1 × PARRUN 1329, PET-1 × RES 834-1, PET-1 × RCR 8297, PET-1 × NDLR 1, ARG × RHA 271, ARG × RHA 273, ARG × RHA 274, ARG × RHA 297, ARG × RHA 298, ARG × RHA 341, ARG × RHA 344, ARG × RHA 345, ARG × RHA 346, ARG × RHA 356R, ARG × RHA 859, ARG × RHA 6D-1, ARG × HAM 174, ARG × HAM 175, ARG × HAM 180, ARG × SF 206, ARG × SF 207, ARG × SF 208, RG × SF 211,ARG × SF 216, ARG × BLC P6, ARG × PARRUN 1329, ARG × RES 834-1, ARG × R 83 R6
Highly susceptible (>50%)	PARRUN 1329, PET-1 × RHA 345, PET-1 × RHA 356R,

DISCUSSION

Among the thirty genotypes tested under field conditions, one showed resistance and four showed moderate resistance and the rest of the twenty five were found to be susceptible. Under laboratory conditions, one of the parental lines and one hybrid was resistant, while five parental lines and three hybrids recorded moderate resistant reaction. Selected lines showed varied level of resistance and none exhibited either immune or highly resistant reaction to the disease. Only two parental genotypes and one hybrid were highly susceptible at field level (Table 4). RHA 587 and ARG × RHA 587 found to be resistant and can be used in back cross breeding for transferring resistant gene to other cultivars. The results are in accordance with Morris *et al.* (1983) Valazhahan *et al.* (1991, 1994) Mirza and Hoes (1996), Chattopadhyay (1999), Amares and Nargund (2000), Ahmed *et al.* (2001) and Shobharani and Ravikumar (2002, 2003). The differences in disease reaction of genotypes under field and laboratory conditions were due to environmental conditions and availability of inoculum load.

This shows that genes for resistance to alternaria blight are dispersed differently in genotypes and in hybrid combinations, the favorable alleles get accumulated to give resistant disease reaction. Resistance to alternaria blight was polygenically controlled and dominance phenomenon was expressed in hybrids. The present findings are in agreement with the earlier investigations of Nagaraju *et al.* (1992), Leite *et al.* (1999) and Ahmed *et al.* (2001) who suggested that hybrids performed better in response to their resistance to leaf spot and such resistance was derived either from the CMS or restorer lines used in their production. Our present study concludes that resistance to alternaria blight was controlled by polygenes and showed dominance effect, it can be exploited in hybrid seed production in sunflower for disease resistance.

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