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ASSOCIATION BETWEEN FIELD AND *In vitro* REACTION TO *Alternaria* LEAF BLIGHT IN SUNFLOWER GENOTYPES

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SUMMARY

Five genotypes showing differential field reactions to *Alternaria* leaf and stem blight were tested for their reaction to pathogen culture filtrate under *in vitro* conditions. The pathogen culture filtrate reduced *in vitro* germination, root and shoot growth and reduction was not uniform in all the genotypes. There was significant negative association between *in vitro* root growth and field disease score of genotypes suggesting the reliability of this trial for *in vitro* evaluation. The germination and shoot length *in vitro* did not show any definite trend to be reliable. Similarly, the detached leaf evaluation under *in vitro* did not show definite trend of association with field disease score. It is suggested to test a large number of genotypes with varying degree of resistance/susceptibility to confirm the results.

Key words: ***Alternaria helianthi*, culture filtrate, resistance**

INTRODUCTION

The occurrence of natural epiphytotics of *Alternaria* leaf and stem blight significantly reduce both seed yield and oil content besides leading to germination losses (Reddy and Gupta, 1977; Hiremath *et al.,* 1990). The control of the disease using fungicides is not practicable under Indian conditions. In such a situation, the development and cultivation of resistant cultivars offers the most economic means of disease management. However, high level of resistance has not been reported in sunflower so far. Further, the disease development is highly dependent on environmental condition and growth stages of the crop causing epidemics during rainy season (Bhaskaran and Kandaswamy 1980; Allen, 1983a; Jeffrey *et al.,* 1984). Hence, it is required to develop a fast and reliable screening technique independent of seasonal conditions. The pathogen *A.helianthi* produces a specific toxic metabolite in

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culture which produces a typical symptom of the disease when inoculated on the leaves (Kumar *et al.,* 1991). It is also reported that the toxin inhibits the seed germination as well as root and shoot growth under *in vitro* conditions (Islam and Marić, 1980). Although studies have been conducted using different techniques (Sujatha *et al.,* 1993; Kumar *et al.,* 1991; Chattopadhyay, 1999), the association between field screening and *in vitro* evaluation has not been clearly established. Keeping this in view, an attempt has been made to study the association between *in vitro* and *in vivo* reaction of selected genotypes of sunflower.

MATERIAL AND METHODS

Sixty-two genotypes derived from different interspecific crosses and populations were tested for their reaction to *Alternaria* leaf spot during *kharif* 1999. Although high level of resistance was not observed among the genotypes, five genotypes showing differential reaction to disease were selected for this study (Table 1).

Table 1: Field reaction of selected genotypes of sunflower to *Alternaria* leaf and stem blight

Genotype	PDI (stage I)	PDI (stage II)
ARANSS-02F	21.83	84.53
ARANSS-03F	27.77	93.07
EC-413060	38.87	61.60
180-34	30.37	70.10
Morden	24.43	71.33

Culturing of the pathogen

The pathogen *Alternaria helianthi* isolated from susceptible plants in the field was used in this study. The organism was grown for 20 days on potato dextrose agar at room temperature and the spores were harvested for detached leaf testing.

For culture twenty-day old culture was used to inoculate flasks containing 75 ml of PDB. The control flasks were not inoculated. The flasks were incubated at room temperature for 1 month. The culture and the control flasks were harvested by filtering through cheese cloth and Whatman N^{o} 1 filter paper. After confirming the presence of toxin in filtrate by inoculating sunflower leaves, the culture filtrate was used for seed germination studies.

Detached leaf testing against *A.helianthi*

Third leaves from the bottom excluding the cotyledons were detached with the petiole from thirty days old seedlings. The leaves with petiole were immersed in a spore suspension (4 × 105 spores/ml) prepared from two-week old cultures and transferred to petri plates containing two layers of moist filter paper with one set of respective controls (treated with distilled water). Disease severity was recorded by visually examining each leaf after three and seven days of inoculation following the

pictorial key of Allen *et al.* (1983b). Each treatment comprised of two leaves in three replicates and the experiment was repeated thrice.

Effect of culture filtrate on seed germination and seedling vigor

One hundred-fifty healthy sunflower seeds of the selected five genotypes were soaked in 50 ml of culture filtrate separately for 24 h. They were then spread on blotting paper moistened with culture filtrate. Equal number of healthy seeds were soaked in non-inoculated potato dextrose extract on 3rd and 7th day after treatment. After seven days, the root and shoot length of the seedlings were recorded. The experiment was repeated three times.

Statistical analysis

The following parameters were derived from *in vitro* studies.

$$\text{Germination (percent control)} = \frac{\text{Percent germination in toxin}}{\text{Percent germination in control}} \times 100$$

Similarly for root length and shoot length, the percent of control was calculated.

The rank root correlation between *in vitro* parameters *viz*., germination (percent control), root length (percent control), shoot length (percent control), disease severity of detached leaf and *in vitro* field susceptibility values were determined using Spearman's rank correlation coefficients (Sigel, 1956).

Table 2: *In vitro* germination, seedling vigor and affected leaf area of different genotypes

Genotype	Germination percent		Seedling vigor				Leaf area affected (*in vitro*)
	7thday		Control		Toxin		
	Control	Toxin	Root (cm)	Shoot (cm)	Root (cm)	Shoot (cm)	7thday
ARANSS-03F	94	86	4.73±2.62	3.12±1.1	1.21±0.91	2.06±1.1	30%
ARANSS-02F	95	88	3.30±0.39	2.66±0.5	2.08±0.75	1.84±0.26	25%
EC-413060	92	90	2.49±0.26	1.87±0.17	1.34±0.16	0.93±0.12	2%
180-34	100	80	5.09±0.69	3.37±0.13	1.81±0.32	1.15±0.18	2%
Morden	98	86	3.23±0.38	1.85±0.14	0.96±0.85	2.68±0.03	30%

Table 3: Correlation between *in vitro* germination, seedling traits and affected leaf area in sunflower

In vitro parameters	Field PDI	
	Stage I	Stage II
Germination (% of control at 7th day)	0.1000	-0.1000
Root length (% of control)	-0.5000*	-0.2000
Shoot length (% of control)	-0.5000*	0.6000*
Infected leaf area	-0.5500*	0.8000*

* Significant at 5%

RESULT AND DISCUSSION

The results indicate that seed germination, root and shoot were highest in control and reduced on application of toxin in all the genotypes indicating the effect of toxin on seed germination and seedling growth (Tables 2 and 3, Figure 1).

Figure 1: Effect of pathogen culture filtrate on seedling growth

Such deleterious effect of pathogen culture filtrate on seed germination and root and shoot length was observed by Islam and Marić (1980). However, the reduction was not uniform in all genotypes. The highest inhibition of germination was found in 180-34 followed by Morden while the least was in EC 413060. The highest inhibition of root growth was recorded in 180-34 and the least in ARANSS-02F (Table 3) indicating varying degree of effect of toxin on different genotypes or the differential tolerance of genotypes at seedling stage to the culture filtrate. Further, the selected genotypes were tested for their reaction to disease *in vitro* using detached leaf technique (Sujatha *et al.,* 1997). The genotypes showed differential reaction at the same spore inoculum level (Table 3). The genotypes EC-413060 and 180-34 showed the lowest level of infection of 2% while, ARANSS-03F and Morden showed the highest infection of 30%. Such differential reaction of genotypes under *in vitro* detached leaf was observed by Sujatha *et al.* (1997).

The correlation analysis indicates that there was significant negative association between PDI (stage I) and *in vitro* root and shoot growth. The genotypes with high disease values at stage I showed increased inhibition of root and shoot length suggesting usefulness of these parameters in assessing the disease at stage I. The negative relationship between *in vitro* infected leaf area and PDI (stage I) indicates the non-reliability of this parameter in assessing the field reaction of the genotype. The association of *in vitro* parameters with PDI (stage II) also did not show definite trend. There was a desirable negative association between seed germination, root length with PDI (stage II) indicating the reliability of this parameter to assess he disease at stage II. Further, the *in vitro* leaf area affected and PDI (stage II) recorded significant positive association suggesting its dependability. Sujatha *et al.* (1997) also observed similar results. However, the shoot length (% of control) showed positive association with PDI (stage II), suggesting its non-conformity with field reac-

tion. The results showed that although the culture filtrate had inhibitory effect on seed germination and seedling traits under *in vitro* conditions, the association between field and *in vitro* reaction did not show a definite trend. It has to be tested over a large number of genotypes showing a wide variation for disease reaction.

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CORRELACIÓN ENTRE LA REACCIÓN DEL CAMPO Y LA REACCIÓN *IN VITRO* EN ALTERNARIOSIS DE LA HOJA EN GENOTIPOS DE GIRASOL

RESUMEN

Cinco genotipos con reacción diferencial a alternariosis de la hoja y del tallo en el campo, se han investigado para determinar su reacción en el filtrado del cultivo del patógeno en las condiciones *in vitro*. El filtrado disminuyó significativamente la plantación *in vitro* y el crecimiento de la raíz y de los brotes, y la disminución no ha sido uniforme en todos los genotipos. Se ha determinado también una significante correlación negativa entre el crecimiento *in vitro* de la raíz y la calificación de la enfermedad en genotipo en el campo, lo que indica la confiabilidad de este ensayo para la evaluación *in vitro*. En la plantación *in vitro* y la longitud de brotes, la correlación no ha sido expresada suficientemente para la evaluación de confiabilidad. Semejante a ello, tampoco en la evaluación *in vitro* de las hojas cogidas no había existido una suficientemente fuerte correlación con la calificación de la enfermedad en el campo. Para la confirmación de estos resultados, se recomienda el *test* de un mayor número de genotipos con diferentes niveles de resistencia.

CORRÉLATION ENTRE LES RÉACTIONS À LA ROUILLE DES FEUILLES DANS LES CHAMPS ET *IN VITRO* DANS LE GÉNOTYPE DU TOURNESOL

RÉSUMÉ

Cinq génotypes à réaction différentielle à la rouille des feuilles et de la tige dans les champs ont été examinés dans le but d'établir leur réaction à un filtrat de culture de pathogènes dans des conditions *in vitro*. *In vitro*, le filtrat a diminué de façon importante la faculté germinative et la croissance de la racine et de la pousse, toutefois, la diminution n'était pas uniforme dans tous les génotypes. Une corrélation négative importante a été établie entre la croissance de la racine *in vitro* et l'évaluation de la maladie dans les génotypes des champs, ce qui montre la sûreté de cette expérience pour l'évaluation *in vitro*. La corrélation pour la faculté germinative et la longueur de la pousse n'était pas suffisamment marquée pour qu'on puisse en évaluer la certitude. De manière semblable, dans l'évaluation *in vitro* des feuilles cueillies il n'y avait pas de corrélation suffisamment grande avec l'évaluation de la maladie dans les champs. On recommande des tests sur un plus grand nombre de génotypes ayant différents niveaux de résistance pour la confirmation de ces résultats.