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EFFECTS OF *ALTERNARIA HELIANTHI* TOXIN ON SUNFLOWER POLLEN

R.L. Ravikumar

Sorghum Scheme, University of Agricultural Sciences, Dharwad - 580 005, India

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SUMMARY

In the present study an attempt has been made to study the effect of *Alternaria helianthi* toxin on pollen germination and reaction of pollen of different genotypes to the toxin. The addition of toxin to pollen germination media led to a reduction in pollen germination. However, the inhibition of pollen germination was not uniform in all genotypes. The genotypes which are moderately tolerant to the disease at sporophytic level also produced pollen that germinated better in the presence of toxin than did pollen from highly susceptible genotypes suggesting the sporothytic gametophytic association. A strategy to utilise pollen assay to differentiate genotypes in resistant/susceptible and pollen selection in developing disease resistant populations in sunflower is discussed.

Key words: *Alternaria helianthi*, pollen, germination, phytotoxin, susceptible, tolerance.

INTRODUCTION

Alternaria leaf blight, caused by *Alternaria helianthi* (Honsf.) Tubaki and Nishihara, is potentially a destructive disease in India and elsewhere (Zimmer and Hoes, 1987; Hiremath *et al.*, 1990). Although genetic resistance to Alternaria leaf blight would be the most economic means of reducing yield loss, the information available so far indicate that high level of resistance to this disease is not available in cultivated and related taxa (Ravikumar *et al.*, 1995).

Many investigations have shown that the genes imparting resistance to many biotic and abiotic stresses such as herbicides, salinity, low temperature, phytotoxin (Ottaviano and Sarigorla, 1993; Sarigorla *et al.*, 1994; Frova *et al.*, 1995) etc., are expressed at pollen level. The possibility of using large populations of haploid genome makes pollen selection a potentially efficient strategy to enrich the frequencies of desirable genes (Ottaviano and Sarigorla, 1993). Exposure of pollen to stress during its formation, germination, tube growth and/or fertilization may lead to selection of tolerant pollen resulting in the accumulation of resistant genes in the progeny. The development of a reliable technology capable of identifyng differences

in pollen graines is a prerequisite for application of gametophytic selection in plant breeding. In the present study, an attempt has been made to study the effect of *Alternaria helianthi* toxins on sunflower pollen germination and reaction of pollen of different genotypes to the toxin. Further, we discuss the possibilities of using pollen reaction in differentiating resistant and susceptible genotype and pollen selection in developing Alternaria - resistant populations of sunflower.

MATERIAL AND METHODS

Pollen germination: Sunflower pollen can be successfully grown in a liquid medium containing sucrose (150 g/l), boric acid (200 mg/l), potassium nitrate (200 mg/l), magnessium sulphate (200 mg/l), calcium nitrate (200 mg/l) and PEG 6000 (223.6 g/l) along with the extract of one stigma (Keshavamurthy *et al.*, 1994) in cavity slides.

Plant material: Four genotypes including one highly susceptible (L 101) and three moderately tolerant (Acc. No. 180, 873 and 1229) genotypes were selected for this study (Ravikumar *et al.*, 1995). Forty plants of each genotype were grown in the field during winter 1995 with uniform spacing (60 x 30 cm). At flowering, pollen grains of sunflower genotypes were brushed onto petri plates soon after dehiscence (800 - 0900 hrs) and incubated in humid chamber (70% RH) for 20 minutes. Such pollen grains were used for germination study.

Toxin culture filtrate: The pathogen *Alternaria helianthi* isolated from diseased plants in the field was used in this study. The organism was maintained on potato dextrose agar (PDA) at room temperature. Twenty-day old culture was used to inoculate potato dextrose broth (PDB). Individual disks (1 cm dia) of sporulating cultures were placed in each 250 ml conical flask containing 75 ml of PDB. Control flasks were not inoculated. The flasks were incubated at room temperature for one month. The cultured and control flasks were harvested by filtering through cheese cloth and Whatman no 1 filter paper. The dry weight of the fungus remaining on the cheese cloth was recorded. The presence of toxin in the filtrate was confirmed by inoculating the sunflower leaves with the filtrate by pin prick.

Experiment 1: The pollen growth media with fungal extract (PGM + FE) were prepared by dissolving the components of pollen growth media (Keshavamurthy *et al.*, 1994) in the fungal culture filtrate instead of water. Similarly, control pollen growth medium was prepared in the control culture filtrate. The fungal filtrate used in this study had 0.248 g (dry weight) of fungus in 75 ml. The pollen grains of different genotypes were placed in cavity slides containing 100 ml of pollen growth medium with stigma extract. The cavity slides were kept in a humid chamber (70-80% RH) for 30 minutes. Four cavities for each treatment/genotype were used and about 400 pollen grains were scored for germination. The percent germination (evident as buds) and percent pollen grains with tubes (where the pollen grains had tubes with length at least equal to its diameter) were assessed.

Experiment 2: The fungal filtrates (dry weight of the fungus 0.261 g) were freeze dried overnight. The residue was extracted in methanol and redried by rotary evaporation at 36°C and redissolved in 6 ml of water. Such toxin stock solutions were stored at 4°C in dark. A series of PGM containing 0.02, 0.04, 0.06, 0.10, 0.15 and 0.2 ml of toxin stock solution/ml were prepared. The control was withouth toxin stock solution. The genotype Acc. No. 873 was chosen for this study. The pollen germination and pollen grains with tubes were recorded as in Experiment 1.

RESULTS AND DISCUSSION

Pollen germination of the four genotypes ranged from 68.80% in L 101 to 95.56% in Acc. No. 180 while pollen grains with tubes ranged from 57.58% in L 101 to 92.26% in 180 in the absence of culture filtrate (Table 1.) Pollen tube growth was also prominent (Figure 1) in the controls. The addition of culture filtrate led to a reduction in pollen germination and tube growth. However, the inhibition of pollen germination and tube growth was not uniform in all the genotypes. The highest inhibition of pollen germination (60.44%) and tube growth (72.42%) was observed in the highly susceptible genotype L 101 while it was only 44.20 and 42.16%, respectively, in the tolerant genotype Acc. No. 1229. The genotypes which are moderately tolerant to Alternaria leaf blight at sporophytic also produced pollen that germinated better in the presence of toxin than did pollen from the highly susceptible genotype. Apparently pollen from susceptible plants was more sensitive to toxins than pollen from tolerant plants suggesting the sporophytic level gametophytic association. The differential sensitivity of pollen grains to Alternaria toxin may be due to the expression of resistant genes in the male gametophyte. Effects of pathotoxin on pollen were also described in many other pathogen plant systems (Hodgkin and Mac Donald, 1986; Bino *et al.*, 1988). These results demonstrate that mechanisms involved in disease resistance are active in both vegetative and generative tissues of several plant species.

Table 1: Germination and pollen tube growth of different genotypes in control PGM and pathogen culture extract pollen germination media (PGM+FE)

	Control PGM		PGM + FE		Inhibition (%)	
Genotype	Pollen germination (%)	Pollen with tubes (%)	Pollen germination (%)	Pollen with tubes (%)	Pollen germination (%)	Pollen with tubes (%)
Acc. No. 180	95.56	92.60	41.54 (43.40)	35.54 (38.22)	55.6	61.78
Acc. No. 873	75.34	66.04	35.00 (46.46)	27.33 (42.10)	53.54	57.90
Acc. No. 1229	81.15	71.20	45.75 (55.80)	41.18 (57.84)	44.20	44.16
Acc. No. L101	68.80	57.58	23.09 (33.56)	16.80 (27.58)	60.44	72.42

Values in parentheses are percentage pollen germination and tube growth with respect to their control

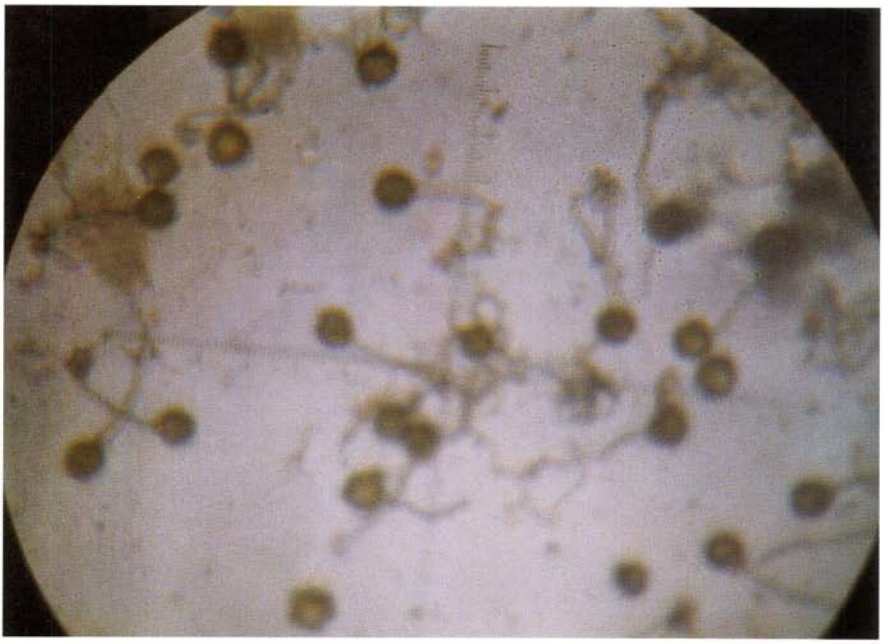

Figure 1. Pollen germination and tube growth in control

Figure 2. Inhibition of pollen germination by pathotoxin

The germination and tube growth of pollen of the genotype Acc. No. 873 was tested over a range of toxin concentrations. The percent germination and tube growth were found to be sensitive to increasing toxin concentration (Table 2). The percentage germination and tube growth decreased with increasing toxin concentracion and at high toxin levels the pollen germination was drastically reduced (Figure 2). The effective toxin level for 50% pollen inhibition and tube growth for this genotype under study was found to be around 0.1 ml. The determination of effective dose for 50% inhibition for different genotypes and their association with sporophytic reaction, if established, then pollen assay can be used an easy and effective tool to differentiate plants into resistant/susceptible. Further, the gametophytic sporophytic relationship gives an opportunity to select pollen for resistance and tolerance to phytotoxins which can be exploited in developing resistant populations. The potential of this selection method, however, depends on the refinement of methodology such as selection conditions, methods for separation and concentration of selected pollen and techniques for insuring fertilization with selected pollen. Currently, efforts are being made to establish the relationship between sporophyte and gemetophytic reaction involving several genotypes.

Table 2: The effect of varying concentrations of toxin on pollen germination and tube growth in Acc. No. 873

Treatment (ml tss/ml PGB)	Pollen germination (%)	Pollen with tubes (%)
Control	78.02 (100.00)	74.02 (100.00)
0.02 ml/ml	76.06 (97.49)	74.84 (100.00)
0.04 ml/ml	68.46 (87.74)	66.84 (90.29)
0.06 ml/ml	62.58 (80.21)	58.39 (78.88)
0.1 ml/ml	46.38 (59.44)	43.97 (59.40)
0.15 ml/ml	28.45 (37.47)	24.23 (32.73)
0.2 ml/ml	9.69 (12.41)	6.17 (8.33)

Values in parentheses are percentage pollen germination and tube growth with respect to their control
tss = toxin stock solution, PGM = pollen growth medium

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REFERENCES

Bino, R.J., Franken, J., Witsenboer, H.M.A., Hille, J. and Doons, J.J.M., 1988. Effects of *Alternaria alternata* f. sp. *lycopersici* toxins of pollen. Theor. Appl. Genet., 76: 204-208.

Frova, C.P., Portaluppi, M. Villa and Sarigorla, M., 1995. Sporophytic and gametophytic components of thermotolerance affected by pollen selection. J. of Heredity, 86(1): 50-54.

Hiremath, P.C., Kulkarni, M.S. and Lokesh, M.S., 1990. An epiphytotic Alternaria blight of sunflower in Karnataka. Karnataka J. Agric. Sci., 3(3&4): 277-278.

Hodgkin, T. and Mac Donald, M.V., 1986. The effect of a phytotoxin from *Alternaria brassicicola* on Brassica pollen. New Phytol, 104: 631-636.

Keshavamurthy, M.N., Nanja Reddy, Y.A., Virupakshappa, K. and Umashanker, R., 1994. Development of suitable germination medium for trinucleate pollen grains: An illustration with sunflower. J. Oilseeds Res., 11(2): 304-307.

Ottaviano, E. and Sarigorla, M., 1993. Gametophytic and sporophytic selection. In : Plant Breeding : Principles and Prospects (Ed). Hoyward, H.D., N.D. Bosemark and I. Ramagosa, Chapman Hall, London, 333-352.

Ravikumar, R.L., Doddamani, I.K. and Kulkarni, M.S., 1995. Reaction of selected germplasm lines and *Helianthus tuberosus* derived introductions to *Alternaria helianthi*. Helia, 18(23): 67-72.

Sarigorla, M., Ferrario, S., Frascoroli, E., Frova, C., Landi, P. and Villa, M., 1994. Sporophytic response to pollen selection for alchlor tolerance in maize. Theor. Appl. Genet., 88(6/7): 812-817.

Zimmer, D.E. and Hoes, J.A., 1978. Diseases. In: Sunflower Science and Technology, ASA, Monograph (Ed) American Society of Agronomy, Madison, WI. 505 pp.

EFECTOS DE LA TOXINA DE *Alternaria helianthi* SOBRE EL POLEN DE GIRASOL

RESUMEN

En el presente estudio se ha hecho un intento de estudiar el efecto de la toxina de *Alternaria helianthi* sobre la germinación y reacción del polen de diferentes genotipos a la toxina. La adición de la toxina a medio de germinación del polen condujo a una reducción de la germinación es éste. Los genotipos que fueron moderadamente tolerantes a la enfermedad a nivel esporofítico también produjeron polen que germinó mejor en presencia de la toxina que el polen de los genotipos altamente susceptibles sugiriendo una asociación del esporofítico y gametofito. Se discute una estrategia para utilizar la prueba del polen para diferenciar genotipos en resistentes y susceptibles y resistentes y la selección de polen en el desarrollo de poblaciones resistentes en girasol.

EFFETS DE LA TOXINE *D'Alternaria helianthi* SUR LE POLLEN DE TOURNESOL

RÉSUMÉ

Dans cette étude un essai a été réalisé pour étudier l'effet de la toxine d'*Alternaria helianthi* sur la germination du pollen et la réaction du pollen de différents génotypes à la toxine. L'apport de toxine au milieu de germination du pollen conduit à une réduction de la germination du pollen. Cependant l'inhibition de la germination n'est pas uniforme pour tous les génotypes. Les génotypes dont le sporophyte est modérément tolérant à la maladie, sont aussi ceux qui produisent du pollen qui germe mieux en présence de toxine, par opposition au pollen de génotypes très sensibles, suggérant une association de type sporophytique gametophytique. Une stratégie est discutée pour l'utilisation d'un test pollinique dans la différenciation des génotypes résistants on sensibles et la sélection du pollen pour la création de populations résistantes à la maladie.