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LOCATION OF SEED-BORNE INOCULUM OF *MACROPHOMINA PHASEOLINA* AND ITS TRANSMISSION IN SEEDLINGS OF CUCUMBER

NASREEN SULTANA, TALHA AZEEM AND A. GHAFFAR

Crop Diseases Research Institute, PARC, Karachi University Campus, Karachi-75270, Department of Agribusiness, University of Karachi, Karachi-75270, Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Abstract

Using component plating technique *Macrophomina phaseolina* was isolated from testa, tegmen, cotyledons and embryo of cucumber seeds. The fungus was transmitted to seedling and caused pre- and post-emergence infection which was significantly high under water stress conditions.

Introduction

The seed borne fungi are known to be located in different components of seeds. *Macrophomina phaseolina* have been recovered from testa, tegmen and embryo of sponge gourd seed (Shakir *et al.*, 1995). *M. phaseolina* has been reported from seeds of cucumber, bottle gourd, bitter gourd, pumpkin, Indian gourd, red gourd and sponge gourd (Manthachitra, 1971; Maholay, 1988, 1989; Mathur, 1990; Shakir & Mirza, 1992; Shakir *et al.*, 1995). The fungus has been detected in association with seed coat and cotyledons of melon and was found to penetrate the fruit *via* the peduncle to infect the seed (Reuveni *et al.*, 1983). Recovery from seed coat, tegmen and embryo of sponge gourd has also been reported (Shakir *et al.*, 1995). In germination trials of bottle gourd, *M. phaseolina* has been found to be most devastating fungus causing 53% seed infection (Shakir & Mirza, 1992). The present study describes the location of *Macrophomina phaseolina* in cucumber seeds and its effect on the germination and development of disease.

Materials and Methods

Three samples of cucumber collected from farmer's fields and market's of different places of Pakistan which showed significantly high incidence of *Macrophomina phaseolina* during routine seed health testing techniques, were used to detect the location of fungi by using component plating technique. Seeds of each sample were washed individually with distilled water in a test tube and then soaked for 2 h in distilled water. Soaked seeds were dissected aseptically with a sterilized blade, using a needle and forceps to separate testa tegmen, cotyledons and embryo. Each component was surface disinfected with 2% Na $(OCl)_2$ for 2 minutes and was placed on blotters in Petri dishes (Du-Hyunglee *et al.*, 1984).

For transmission studies, 10 seeds were placed on 3-layered well soaked blotter paper in 9 cm Petri dishes. After six days the lids were removed and dishes put in polyethylene bags for 12-15days. Single seed was placed in test tube of 200 x 20 mm containing 15 ml of 1% water agar at an angle of 60°. The cotton plug was removed as the seedling reached the top. A set of 10 Petri dishes and 100 test tubes was incubated at 24°C under 12 h of alternating cycles of ADL and darkness (Khare *et al.*, 1977). In another experiment, ten seeds were sown in earthen pots containing 3kg of autoclaved soil. Two sets of 10 pots each were used, one set was subjected for water stress and another set was watered regularly.

Result and Discussion

Location of *Macrophomina phaseolina* in cucumber seeds: By component plating of 3 seeds of cucumber, *Macrophomina phaseolina* were recovered from each component of the seed (Table 1) Recovery of *M. phaseolina* was significantly increased in testa (Av. 14.17 ± 12.25) and tegmen (Av. 14.5 ± 11.12) whereas its recovery from cotyledons (Av. 3.0 ± 2.45) and embryo was significantly low (Av.1.0 ± 1.0). The results are in conformity with those of Shakir *et al.,* (1995) who also isolated M. *phaseolina* from testa, tegmen and embryo of sponge gourd seeds. Bhutta *et al.*, (1996) and Dawar (1994) found equal infection level of *M. phaseolina* in the pericarp, endosperm and embryo of sunflower seeds whereas in the present study infection level of *M. phaseolina* decreased with the depth and was very low in cotyledons and embryo.

Seed transmission of *Macrophomina phaseolina i*n cucumber: For transmission studies of *M. phaseolina* in cucumber, the seed samples having > 78% infection was used. The effect of seed infection of *M. phaseolina* on germination and emergence was significantly high on agar slant (Av. 4.2 ± 0.6) and sand medium under water stress conditions (Av. 3.4 ± 0.51). Seeds, which failed to germinate, were found to be covered with sclerotia of *M. phaseolina*. Partially emerged seedlings were found rotted and blackened. Seedling mortality was significantly high in soil medium under water stress condition (Av. 13.2 ± 1.39) than in blotter (Av. 0.96 ± 2.16), agar slant (Av. 0.86 ± 1.9) and sand medium with regular watering (Av. 1.2 ± 2.07) (Table 2). Symptoms in seedling were wilting, blackening of hypocotyls and radical axis and brownish discolouration in root. Seed transmission of *M. phaseolina* in melon was reported earlier (Reuveni *et al*., 1983).

Seed transmissions of pathogen *M. phaseolina* in cucumber was experimentally demonstrated by seedling symptoms test *In vitro* where the pathogen moved from infected seeds to hypocotyls and young seedlings. Further development in seedlings and plant *In vivo* was confirmed by positive serial isolations made up from their roots, hypocotyls and leaves of infected plants. There was a direct correlation between seed infection and loss in germination. Pre-emergence mortality was correlated with internal infection of seeds particularly tegmen and cotyledons infection. The pathogens either extraembryonal or embryonal were able to cause seed rot, seedling mortality and finally death of seedlings. Dhingra & Sinclair (1975) reported that *M. phaseolina* carried on the seed coat either did not show seed germination or produced seedlings that may die soon after emergence due to post-emergence damping off. Reuveni *et al.,* (1983) detected *M. phaseolina* in seed coat and cotyledons of melon and found the fungus to penetrate the fruit *via* peduncle to infect the seed. *M. phaseolina* has been reported to be transmitted from seed to seedling of sunflower (Dawar, 1996; Bhutta *et al.,* 1996) and soybean (Anwar *et al.,* 1995). Seed to seedling transmission of *M. phaseolina* in cucumber in the present study was significantly high in pots subjected to water stress conditions.

Table 1. Recovery of *Macrophomina phaseolina* from seeds of cucumber.

Parts of seed	Mean, SX ±	St. dev
Testa	14.17 ± 12.25	30.01
Tegmen	14.5 ± 11.12	27.25
Cotyledon	3.0 ± 2.45	6.00
Embryo	1.0 ± 1.0	2.45

Table 2. Incidence of *Macrophomina phaseolina* in cucumber grown from highly infected seeds (78%) sown directly on blotter, agar slant and sterilized soil.

Methods	Pre-emergence infection				Post-emergence infection			
	Mean	Std. error	Std. dev	Variance	Mean	Std. error	Std. dev	Variance
Petri plates with moist blotter enclosed in polythene bags.	2.4	0.509	1.14	1.300	5.8	0.969	2.167	4.700
Test tube with agar slant.	4.2	0.663	1.48	2.200	3.2	0.860	19.23	3.700
Pot (Autoclaved soil)								
a) Regularly watered	2.0	0.632	1.41	2.000	5.6	1.20	2.701	7.300
b) Water stress	3.4	0.509	1.14	2.200	13.2	1.30	3.111	9.700
LSD (0.05)	Petri plates = 0.04, Test tube = 0.017, Pot (Regularly watered)= 0.109, Pot (Water stress) = 0.109				Petri plates = 0.12, Test tube = 0.15, Pot (Regularly watered) = 0.15, Pot (Water stress) = -1			

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