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Mycorrhizal association in mungbean (*Vigna radiata*) and its impact on disease resistance

U. K. DUTTA AND P. M. BHATTACHARYA
Department of Plant Pathology,Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal, 736 165.

Among the pulse crops mungbean [*Vigna radiata* (L.) Wilczek], commonly known as green gram, has been cultivated in India since time immemorial in almost all the states and occupies about 3.08 mha with an annual production of 1.31 mt and productivity of 4.25 q/ha. In India this crop is grown all around the year in three crop seasons ; Pre-kharif, Kharif and Post-kharif and is known for high rhizosphere microbial activity. A set of 27 mungbean germplasms were screened for their mycorrhizal association under Terai agro-climatic region of West Bengal and an attempt was taken to relate their ability to form mycorrhiza with biochemical parameters and disease incidence. The genotypes were divided into four different mycorrhizal categories depending on their per cent root length infection. Phenolic concentration was found lower in plants with high mycorrhizal association, whereas peroxidase activity and protein level were found to be higher in the same plants at an early stage. Per cent disease index (PDI) for Cercospora-leaf spot was correlated with different parameters and it was found that degree of mycorrhizal association was inversely related with PDI in all the resistance categories of the genotypes studied. Phenolics in plants with high mycorrhizal association imparting high level of resistance was found lower than that in plants with lower mycorrhizal association having moderate resistance indicating a different mechanism of resistance in mycorrhizal plants. However, protein concentration increased with mycorrhizal infection and was found to be higher in plants with lower PDI.

Key words : Mungbean, mycorrhizal, biochemical changes, Cercospora-leaf spot

INTRODUCTION

Interactions of mycorrhizas and the mycorrhizal fungi with other rhizosphere microorganisms, particularly the root pathogenic microorganisms have received considerable attention. The mycorrhizal fungi as members of the rhizosphere can compete with the other members of the population to have a unique niche and sometimes, can dominate over others (Harley and Smith, 1983). The dominance is expressed as suppression of diseases caused by root pathogens, often interpreted as resistance or tolerance of mycorrhizal plants against root diseases. Induction of defensive reactions in roots by hyphae during the formation of normal mycorrhizal associations has been reported for both VAM and ECM fungi (Albrecht *et al.,* 1994; Lambis and Mehdy, 1995; Vierheiling *et al.,* 2000). VAM fungi elicit phytoalexins, but this does not constitute a full defence response (Koide and Schreiner, 1992). Partial defence induction may result because mycorrhizal fungi cannot fully evade plant defence, or because initial stages of colonization are not fully balanced associations. The induction of defence reaction would occur at some cost to the plant, but may increase its resistance to subsequent pathogenic invasion (Brundett, 2004).

Thus an attempt has been made to study the mycorrhizal relation of green gram (*Vigna radiata*) under the Terai ecological condition with objective to screen mungbean germplasms in relation to Cercospora leaf spot disease and relate it with its efficiency to form mycorrhiza.

MATERIALS AND METHODS

The field experiments were conducted at Pundibari Research Farm and lab experiments were done in Research laboratory, Dept. of Plant Pathology, Uttar

Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar.

Peroxidase activity was estimated by using pyrogallol as substrate to the enzyme with the help of spectrophotometric methods. The total phenol content was estimated using Folin - Ciocalteau Reagent by following method of Malick and Singh (1980). The OD phenol was estimated by the method of Mahadevan and Sridhar (1982). Protein was estimated following Lowry's method (1951) using Bovine Serum Albumin (BSA) as standard.

The root samples preserved in FAA for at least seven days were stained. Alkaline hydrolysis of root samples with 10% potassium hydorxides was done at 121°C in an autoclave for 1-3 minutes depending upon root thickness. Clearing of root cortex and subsequent stain penetration were better by this method. Alkaline hydrogen peroxide was used for 5-10 minutes to bleach coloured root pieces. The roots were then washed with several changes of water and then treated with 1 (N) hydrochloric acid for 10 minutes and ultimately stained with 0.1% Trypan blue in lactophenol. Stained root segments of 1 cm length kept in de-staining solution for about 5 minutes and mounted in lactophenol were observed under Kombistereo Binocular Microscope (Leica, MZ3) for root infection enumeration. Per cent root length infection was determined by estimating length of roots showing vesicles and/or arbuscules by microscopic measurement with ocular micrometer. For each sample, 5x10, 1 cm root pieces were examined.

RESULTS AND DISCUSSION

Out of 16 genotypes studied for mycorrhizal association, disease severity was recorded with particular reference to Cercospora-leaf spot, result of which is presented in Table 1. Disease intensity varied from 6.5-50.4% showing a wide variation in resistance reaction within the germplasms tested. On the basis of disease reaction in terms of per cent disease index (PDI) the genotypes were grouped into 4 categories namely resistant (<20% PDI), moderately resistant (20-30% PDI) moderately susceptible (30-40% PDI) and susceptible (>40% PDI). The average of PDI in each category was correlated with mycorrhizal status, phenol and OD phenol and protein concentration. The results from study are presented in Table 1 and Figs. 1-4.

Disease severity in relation to mycorrhizal association

Results are presented in Table 1, Fig. 1 and Fig. 2. Results showed a trend of decline in mycorrhizal in-

Table 1 : Mycorrhizal infection and biochemical parameter in relation to increasing disease intensity (PDI)

Genotype	Per cent disease Index(%)	Mycorrhizal Infection(%)	Phenol Concentration (mg/g)	OD-Phenol Concentration (mg/g)	Peroxidase Activity (change/min/g)	Protein Concentration (mg/g)
SLM-70	6.54	39.78	21.85	5.42	0.6	37.4
A-12-4(3)	9.88	41.93	21.52	7.19	1.7	44.08
A-12-4/1	9.88	33.83	35.18	12.64	1.2	52.98
A-300	12	29.93	10.14	4.97	0.7	39.63
Pusa-93-33-2	19.94	62.44	15.34	5.71	2	50.75
Mean	11.65	41.58	20.81	7.19	1.24	44.97
A-224	21.94	30.04	24.45	8.52	0.6	30.72
HUM-6	22.94	23.02	23.15	9.11	1	44.08
Pdm-84-143	25.08	16.1	27.06	14.57	1.3	39.62
Pusa-95-31	25.81	43.99	22.18	11.47	2.4	41.85
A-267	27.72	28.57	20.23	9.4	1.2	26.23
SML-264	28.18	33.4	18.6	8.22	2.8	32.95
Mean	25.95	29.02	27.13	12.26	1.86	35.91
SML-190	34.04	18.86	26.73	11.47	0.7	37.4
NBM-100	35.84	22.47	15.99	6.89	1.6	38.75
Mean	34.94	20.67	21.36	9.18	1.15	38.07
A-33(3)	45.38	19.51	13.72	5.12	1.4	46.31
Mld-95-21(3)	48.84	22.08	19.58	9.7	1.1	24.05
NBM-36	50.39	19.61	20.23	10.73	1.5	37.4
Mean	48.20	20.40	17.84	8.52	1.33	35.92

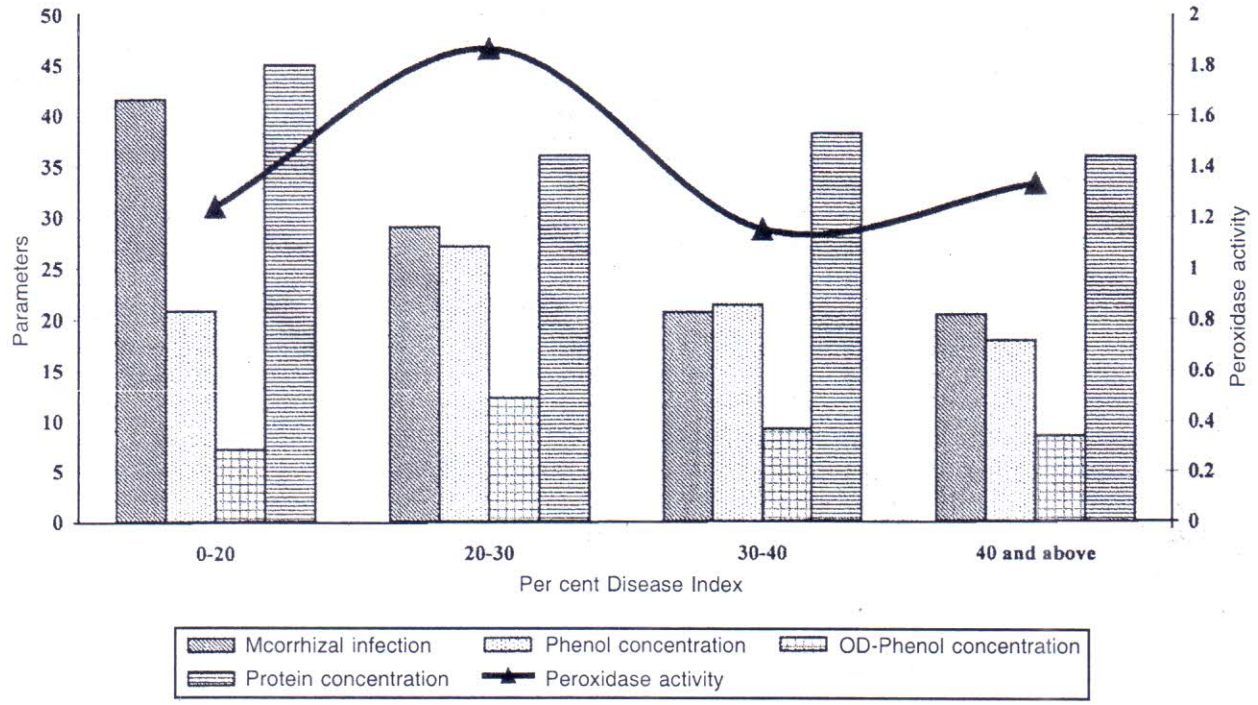

Fig. 1 : Mycorrhizal infection and biochemical parameters in relation to increasing disease intensity (PDI).

fection with increasing disease severity Simple correlation presented in Fig. 1 revealed the degree of mycorrhizal association was negatively correlated with disease severity with high correlation co-efficient (0.87). The result indicated that mycorrhizal associations had an inverse relation with disease which might be due to induction of partial resistance on infection by myco-symbiont. The partial resistance in compatible host-symbiont combination might be due to biochemical changes as elevated lytic or oxidative enzymes and phytoalexin as discussed by Koide and Schreiner, (1992) and Albrecht *et al.*, (1994).

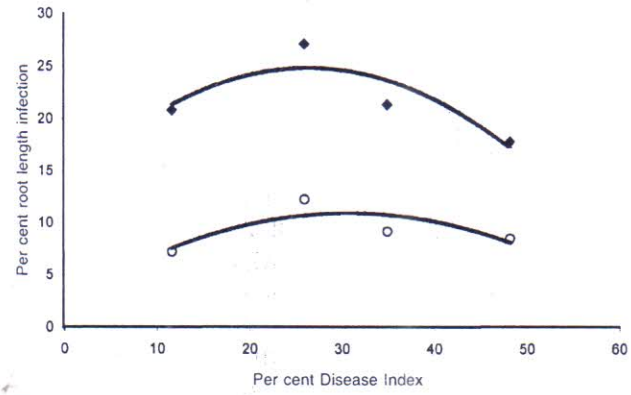

Fig. 3 : Relation of phenolics concentration to increasing disease intensity.

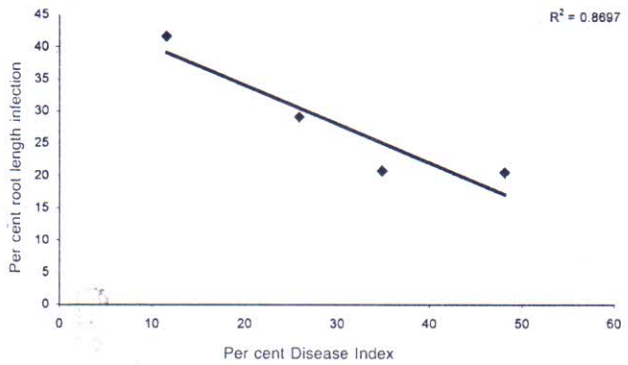

Fig. 2 : Relation of mycorrhizal infection to increasing disease intensity.

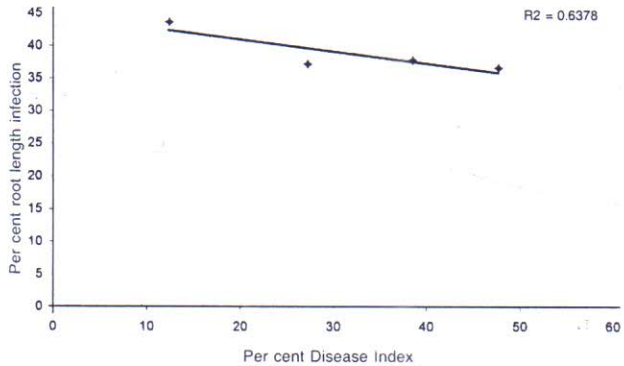

Fig. 4 : Relation of protein concentration to increasing disease intensity.

Phenolics as basis for disease resistance

Phenolics, namely total phenol or OD phenol concentration was estimated for all the 16 genotypes through out the growth period and it was found that phenolics reached its highest concentration at 45-50 DAS (Table 1 and Fig. 3). Phenol concentration was highly variable with genotypes. Phenol concentration was found higher in resistant genotypes and lower in susceptible ones (Sindhan *et al.,* 1999) with exception of the genotypes with lowest PDI and highest mycorrhizal association indicating release of biotic stress on mycorrhization. Phenolic compounds remain in the plant cells as phytoanticipins or may be synthesized after infection. In case of release of biotic stress by any other mechanism may lead to down regulation of phenolic metabolism as observed in plants with high mycorrhizal infection.

Protein concentration in relation to disease severity

Soluble protein concentration was estimated at 40 DAS for all the genotypes under study and the result is presented in Table 1 and Fig.4. Results indicated that protein concentration was highest in resistant genotypes and lowest in susceptible genotypes. The data were put to simple correlation analysis and the result indicated a negative correlation with disease severity with high correlation coefficient (0.64) following the trend of mycorrhizal association. It was also found that there is a positive correlation of soluble protein with mycorrhizal association. The resistance might have resulted from enhance protein accumulation especially stress related ones, due to high mycorrhizal infection in plant at an early stage of growth indicating unbalanced state of symbiosis (Brundett 2004).

Peroxidase activity in relation to disease severity

Peroxidase activity was estimated by spectrophotometric method and expressed as change in absorbance per unit time per unit fresh weight. The results presented in Table 1 and Fig. 1 indicated a similar trend with that of phenolics. The data revealed that higher mycorrhizal infectivity down regulated the stress metabolism, however, provided defense by some other mechanism, and that might have its base in excess protein accumulated or induced other than peroxidase. Here, chitinase or cell wall bound peroxidase induction due to mycorrhizal (Albrecht, 1994) interaction might play a key role in plant defense, which requires further investigation.

Levels of mtcorrhizal association in different mungbean genotype were highly variable and degree of infection may play a role in partial defense against disease. This induction of defense reaction may be due to protein accumulation on mycorrhization other than peroxidase. Thus, mycorrhizal association in crop plants in general and legumes in particular may be useful by virtue of its capacity to induce partial defense beside improving plant health by enhancing nutrient uptake especially, phosphorus.

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