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SHORT COMMUNICATIONS

Host response of *Vigna radiata* genotypes to powdery mildew infection

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Powdery mildew *(Erysiphe polygoni* DC) is one of the important foliar disease in mungbean *(Vigna radiata* L. Wilczek). Earlier, a resistant source was identified in RUM lines which remains free from any disease symptoms up to 40 days after inoculation (DAI) with TI isolate from Trombay, both under a controlled environment and in the field (21). The genetic studies on inheritance indicated that the powdery mildew resistance is governed by two dominant genes designated as *Pm1* and *Pm2* (22). Comparative studies on compatible and incompatible reactions of powdery mildew disease have been carried out in barley (13) and in peas (20,24). However, this information was not available in mungbean. In the present investigation, developmental stages of the pathogen as well as host cellular responses to pathogen were investigated in *Pm1/Pm2,* and *pm1/pm2* genotypes.

Disease screening

The plants namely RUM-5 and TPM-1 showing differential reactions to the powdery mildew isolate were grown in pots kept in a net house. Fully expanded third trifoliate leaves were excised from 25-day-old plants a little above the pulvinus. They were inoculated by spraying with a spore suspension of the *Erysiphe polygoni,* in water (3.5×10^6 conidia/ml). The leaves were kept in controlled environment chambers maintained at 20±1°C and 4136 Lux/m^2 with fluorescent white lights as described previously (21).

Development stages of the pathogen

Conidial germination was followed using a technique described by Cirulli *et al.* (7). Strips of cellotape were pressed on the leaf surface and peeled off Conidia and germ-tubes were clearly visible on the removed cellotape after staining with a few drops of alcoholic lactophenol cotton blue solution (50 ml lactophenol; 1g cotton blue and 49ml of 95% ethanol). Germination of conidia on the leaf surface was studied by observing 250 conidia in five random samples at 2, 4, 6, 8, 24, 48, 72 and 96 hours after inoculation (HAI). The infected leaves were cut into 3 × 1cm pieces and fixed in 70% ethanol for 24 h. The leaf segments were cleared as described by Shipton and Brown (23). Cleared leaf pieces were stained with alcoholic lactophenol cotton blue solution. Measurements of hyphal lengths were made using an ocular micrometer. The observations were based on the following developmental stages of the pathogen from 2-96 HAI; 1) germination of the conidia 2) formation of a normal germ-tube 3) appressorium formation 4) penetration peg extension 5) haustorium formation 6) growth of hyphae. The observations on browning and collapse of epidermal, and mesophyll cells, autofluoresence and papillae were recorded between 24 and 48 HAI. The infected leaves of RUM-5 (R) and TPM-1 (S) plants as well as uninoculated control leaves collected at 24 and 48 HAI were immediately boiled in alcoholic lactophenol for 5 min and mounted in lactophenol after several rinses with water (19). The observations were made from 250 infection sites in 5 replications. Observations of Autofluorescence, and papillae of epidermal and mesophyll cells and cell wall fluorescence were recorded using a UV fluorescence microscope (Zeiss) with filter combination BP.546; FT.580; LP.590 rd BP.450-490; FT.510; LP.520.

Lignin, Phenolic compounds and Callose

The presence of lignin and phenolic compounds was investigated using the Toluidine blue 0 and Phloroglucinol HCI tests and for callose, the Aniline blue fluorescence tests were made (11,25).

The genotypes showing R and S reactions did not show any differences in percentages of germination of conidia between 2-96 HAI. The extension of penetration pegs, haustorium formation and hyphal growth were not observed in RUM-5. However, in TPM-1 the ex-

tension of penetration pegs in 94%, haustorium formation in 92% and the hyphal growth in 92% of the germinated conidia was observed at 2-96 HAI. In TPM-1 (S) the hyphal growth was 843μ at 48h and no hyphal growth was seen in RUM-5 (Fig. 1). The cellular responses in leaves during the course of primary penetration by penetration pegs in RUM-5(R) and TPM-1 (S) included aggregation of cytoplasm and browning of epidermal cells, followed by their collapse and autofluorescence. Papillae formation was seen in both

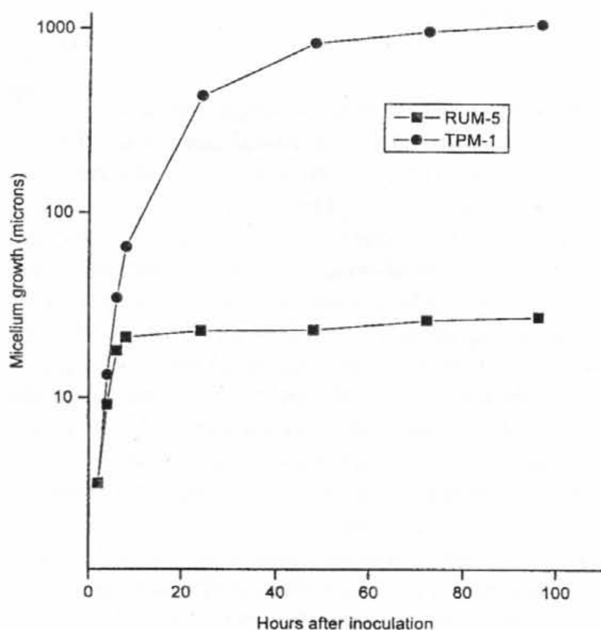

Fig. 1. Mycelium growth on genotypes RUM-5 (RO), TPM-1(S),

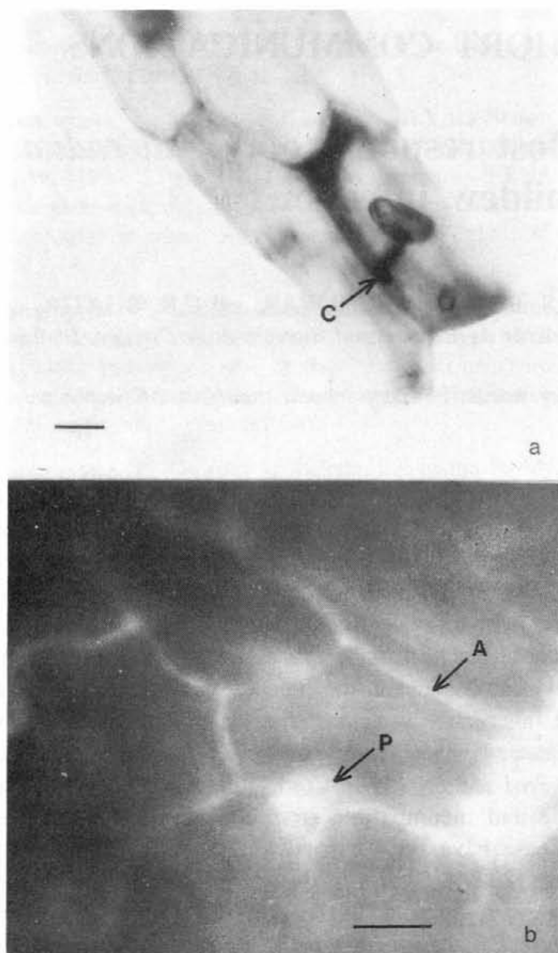

Fig. 2. Response to *Erysiphe polygoni* infection on *Vigna radiata* in (RUM-5 (RO)). a) cytoplasmic aggregation in epidermal cell below a penetration site (C), b) autofluroescence of infected cell walls (A) and in papillae (P). (Scale bar = 10μm).

Table 1. Response of epidermal and mesophyll cells to powdery mildew infection in resistant(RUM-5) and susceptible (TPM-1) genotypes of *Vigna radiata.*

Parameters	Geimotypes			
	RUM-5		TPM-1	
	24HAI	48HAI	24HAI	48HAI
Browning				
Epidennal cells	50.0±0.00	50.0±0.00	50.0±0.00	50.0±0.00
Mesophyll cells	43.9±0.71	48.0±0.71	31.6±1.21	37.0±2.63
Collapse				
Epidennal cells	28.6±1.99	36.0±2.12	1.6±0.51	2.6±0.51
Mesophyll cells	13.6±0.93	17.0±2.17	00.0±0.00	00.0±0.00
Autofluorescence				
Epiderinal cells	48.0±0.71	50.0±0.00	41.6±1.36	46.0±1.38
Mesophyll cells	46.6±1.54	48.6±0.75	32.0±2.59	35.6±1.72
Papillae				
Epidermal cells	46.6±0.93	48.6±0.51	31.6±2.16	36.6±1.57

resistant and susceptible reaction at 24 HAI. At the penetration sites, aggregation of cytoplasm was found in RUM-5 between 8-24 HAI (Fig.2 a). No such changes were observed in TPM-1. In both RUM-5 and TPM-1 browning of epidermal and mesophyll cells was observed at the penetration sites at 48 HAI. However, in RUM-5 the entire cell turned brown in colour and the intensity of colour was much higher when compared to TPM-1. Browning of adjacent epidermal and mesophyll cells was also observed but the number of such cells was 86-96% in RUM-5 and 61-74% in TPM-1 (Table 1). Collapse of the epidermal cells (56-72%) was observed in RUM-5 following penetration peg penetration. Very few epidermal cells had collapsed in TPM-1. Mesophyll cells adjacent to the penetration sites also collapsed in 26-34% of the sites examined in RUM-5. No such collapse of mesophyll cells was observed in TPM-1 at 48 HAI (Table 1). Fluorescence microscopic studies revealed the presence of yellow autofluorescence in the epidermal cell walls of infected RUM-5 (Fig.4b) and TPM-1 . Such fluorescence was not observed in the cell walls in uninoculated RUM-5 and TPM-1 leaf cells. In RUM-5, autofluorescence was seen in through out the collapsed epidermal cell. The autofluorescence is attributed to the production and accumulation of polyphenolic compounds. In TPM-1, although weak autofluorescence was observed in the cell walls, very few epidermal cells had collapsed at 48 HAI (Table 1).

Autofluorescence was present in the papillae of both RUM-5 (Fig. 2b) and TPM-1 cells. However, the autofluorescence was concentrated around penetration sites in RUM-5 and was weak and not concentrated around papilla in TPM-1. In cleared leaf segments after staining with Toluidine blue showed that cell walls and papillae stained blue in RUM-5. No such staining was observed in TPM-1. The positive colour reaction indicated the presence of either lignin or phenolic compounds in RUM-5 cells. The phloroglucinol-HCI test did not show any positive reaction either in RUM-5 or in TPM-1, indicating the absence of lignin in the cells and papillae. The specimens were observed with Aniline blue test for callose induced fluorescence. The fluorescence was mostly confined to the central core of papillae in RUM-5 while it was not in TPM-1. Weak callose fluorescence was observed in the cell walls of both resistant and susceptible genotypes.

Differences were not observed for germination percentages of conidia on the mungbean genotypes showing resistant and susceptible reactions. Similar observations were reported in peas (20,24) and in barley. (15). Powdery mildew resistant reaction mechanism in barley dose not operate at the level of spore germination. Analysis of different growth stages of powdery mildew disease in barley indicated that the formation on of penetration pegs and successful penetration into the host cell was more common in susceptible than in resistant genotypes (13). Differences in mycelial growth of *E. pisi* were observed in resistant and susceptible leaves of peas (24). Significant differences in resistant and susceptible genotypes were also observed in the formation of penetration pegs and mycelial growth in the present studies. In barley, induction of cytoplasmic aggregation in *Egraminis* was reported in the cells of resistant genotypes between 8 and 16 HAI (5,6,14). In our studies cytoplasmic aggregation was observed at 8 HAI in resistant types. However, it was not observed in the susceptible types. Cytoplasmic aggregation is the initial response in resistant genotypes in response to fungal infection. Browning of epidermal and mesophyll cells were observed in resistant and susceptible reactions against powdery mildew in barley (28). In the present studies similar browning in epidermal cells of resistant and susceptible genotypes was seen. The intensity of browning was more in resistant as compared to the susceptible genotypes.

Papillae are wall like deposits formed in the host in response to pathogen penetration pegs which completely encase the haustoria (1,5). The papillae arrest the fungal growth either a physical barrier in barley (8) or act as a chemical barrier in *Phaseolus vulgaris* (12) or by accumulation of toxins in barley (9). It has been observed that the initiation of papilla formation is earlier in resistant than susceptible genotypes (2). In our studies we did not detect any such difference in papilla formation. However, the papillae were effective in arresting the pathogen growth in the resistant (RO) reaction. Phenolic compounds found in papillae have been implicated in disease resistance (16,18). In our studies autofluorescence indicated the presence of phenolic compounds in the host cells of the resistant genotypes. Occurrence of callose in the form of B1-3 glucans has been reported in the papillae (10, 25,26,27). In our studies callose was concentrated in papillae of resistant and scattered in the cells of susceptible reactions. The papillae also contain UV absorbing compounds (3,4,17,19) which gives autofluorescence. In our studies autofluorescence was observed in both resistant and susceptible reaction. The intensity was higher in the collapsed epidermal cells of the resistant genotypes when compared to susceptible types.

In conclusion, the resistant genotype (R) reacts earlier (8-16h) to pathogen invasion than the susceptible genotype. The resistant mechanism is probably due to a combination of i) initiation of early cytoplas-

mic aggregation and papillae formation ii) collapse of epidermal and mesophyll cells and iii) Presence of autofluorescence and of phenolic compounds.

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