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Screening for powdery mildew (*Erysiphae polygoni* DC.) resistance in mungbean (*Vigna radiata* (L.) Wilczek) using excised leaves

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Abstract. A simple, reliable method for assessing powdery mildew reaction on excised mungbean leaves has been developed and used for screening germplasm accessions. Third trifoliate leaves from 21–25 day old field-grown plants were cultured in enamel trays filled with tap water at 21 ± 1°C, 12 h/day photoperiod (4136 lux/m^2 from incandescent and white fluorescent light). After 9–12 days such leaves showed rooting from petiole and substantial root growth followed. Excised leaves could be maintained upto 40 days. Leaves were sprayed with conidial suspension (3·5 × 10^6 conidia/ml) of a local isolate of powdery mildew fungus. In susceptible types, powdery mildew lesions appeared 8–10 days after inoculation, enlarged and coalesced to cover the entire leaf area in 20 days. Fifty five germplasm accessions were tested using this method; all except 7 RUM accessions were susceptible. These RUM accessions showed resistant reaction even after two reinoculations. They were also resistant in the field under natural powdery mildew infection.

Keywords. Mungbean; *Vigna radiata*; powdery mildew; *Erysiphae polygoni*; resistance.

1. Introduction

Powdery mildew (*Erysiphae polygoni* DC.) is a serious foliar disease of mungbean (*Vigna radiata* (L.) Wilczek). Grain yield losses ranging from 20–40% due to this disease have been reported (Soria and Quebral 1973; Legaspi *et al* 1978). The losses are much higher when the pathogen infects the crop before flowering. Though certain cultivars are reported to have moderate field resistance (AVRDC 1978; Catedral and Lantican 1978), to the best of our knowledge, no resistant types have been identified so far. This paper reports (i) a simple, reliable method of screening for powdery mildew reaction using excised leaves under controlled environment and (ii) identification of a highly resistant source from germplasm collection.

2. Materials and methods

The foundation seed of 55 germplasm accessions used in this study were obtained from late Prof. S Ramanujam, Indian Agricultural Research Institute, New Delhi; Dr Satyanarayana Rao, Regional Agriculture Research Station, Lam (AP) and Dr Hyo Guen Park, Asian Vegetable Research and Development Centre, Shanhua, Taiwan. All accessions were grown and maintained in field. *E. polygoni* conidia were collected from infected leaves of the crop grown in field during 1985. The pathogen was initially maintained on mungbean plants grown in pots and later on detached leaves in growth chamber.

2.1 *Excised leaf method*

Mungbean leaves were obtained from 21–25 day old field grown plants. Third trifoliate leaf from the base which was fully expanded at this age was excised a little above the pulvinus. The leaves were washed with tap water and petioles trimmed to maintain a uniform length of about 5 cm. Petioles were inserted through a series of holes in opaque plastic sheet (2 mm thick) and held in position with cotton plugs. Plastic sheets with the inserted leaves were placed over enamel trays (30 × 30 × 5 cm) filled with tap water upto a level so that the lower 2 cm of petiole was always immersed. The leaves remained above the plastic sheet (figure 1). The trays were kept in growth chamber maintained at 21 ± 1°C, and 12 h/day illumination of 4136 lux/m^2. This growth temperature was arrived at after initial testing from 20–28°C. After 24 h, the excised leaves in trays were inoculated by spraying with conidial suspension ($3{\cdot}5 \times 10^6$ conidia/ml) in water containing 0·001% Tween 80. The number of conidia was estimated using hemocytometer. The spray volume was 10 ml/900 cm^2.

2.2 *Screening of germplasm*

Ten leaves of each stock were cultured and inoculated as described above. Disease symptoms were scored at 10 and 20 days after inoculation (DAI). The resistant types were reinoculated 11 and 15 days after first inoculation. Susceptible and resistant stocks were tested in 5 independent replicates.

3. Results

3.1 *Rooting and survival of detached leaves*

Roots developed from the petiole after 9–12 days and substantial root growth followed (figure 3). At 22°C, the uninoculated leaves survived upto 40 days. At temperatures higher than this, senescence was rapid and survival decreased. At 28°C only 10% of the leaves survived.

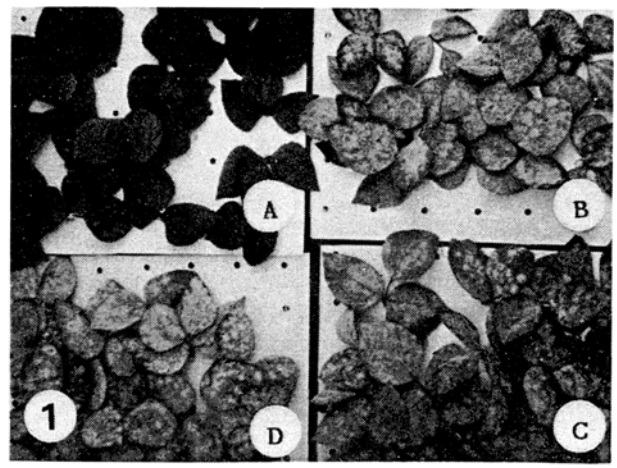
3.2 *Disease development*

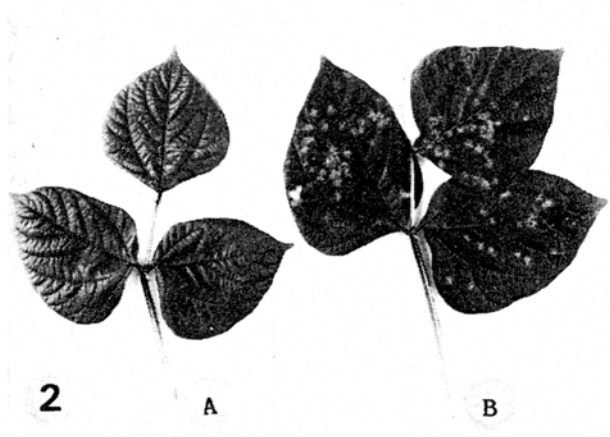
Powdery mildew lesions were observed on the susceptible types between 8–10 DAI (figure 2B). Disease lesions enlarged, coalesced and almost the entire leaf area was covered by the conidia, 20 DAI (figure 3B).

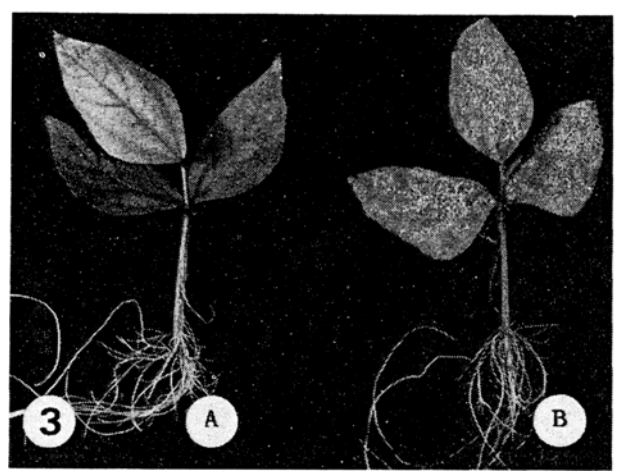
3.3 *Identification of resistant source*

Fiftythree germplasm accessions listed in table 1 were screened for powdery mildew reaction. All accessions except the RUM lines showed susceptible reaction at 10 and 20 DAIs the entire leaf lamina was covered with conidia (figure 3B). The RUM lines (table 1) were highly resistant and inspite of two reinoculations, these accessions did not show any sign of fungal growth (figure 3A).

All the accessions were also tested under natural infection in field during 1986.







Figures 1–3. **1.** Trays with plastic sheets supporting excised mungbean leaves. Powdery mildew resistant (**A**) and susceptible (**B**, **C** and **D**) types. **2.** Resistant (**A**) and susceptible (**B**) leaves 10 days after inoculation. Distinct disease lesions are seen in the susceptible type. **3.** Resistant (**A**) and susceptible (**B**) leaves 20 days after inoculation. Susceptible leaf is fully covered with conidia. This is also to show profuse rooting from basal region of petioles.

Table 1. Mungbean germplasm lines screened for powdery mildew (*E. polygoni*) disease.

Resistant cultures	RUM-1, RUM-5, RUM-7, RUM-11, RUM-20, RUM-22, RUM-33
Susceptible cultures	G-65, G-182, Jawahar-45, K-851, Khandhar-1, M-72, M-872, ML-1, ML-3, ML-4, ML-5, ML-9, ML-12, ML-24, ML-26, ML-33, ML-53, ML-62, ML-65, MM, Moong culture-1, Moong culture-2, PHLV-18, PIMS-1, PIMS-2, PIMS-3, PS-2/11, PS-10, PS-16, Pusa baisakhi, R-192-1, R-288-8, S-8, S-9, SSM-1, T-1, T-21, T-44, TAM-8, TAM-20, TAP-7, Tainan-1, TT-1-E, TT-2-B, TT-8-B, 4 × 9, 5 × 10, 7 × 10.

Except the 7 RUM lines, all the remaining accessions were heavily infected. The RUM lines remained free from powdery mildew symptoms in the field, as in growth chamber testing using detached leaves.

4. Discussion

A simple, reliable method of identifying resistant plants is a pre-requisite for an efficient breeding programme aiming to develop disease resistant cultivars. Excised leaves have been used for disease resistance screening in barley (Edwards 1983), tobacco (Rufty *et al* 1987), peanuts (Foster *et al* 1980) and beans (Tu 1986). The method offers several advantages in genetic and breeding experiments as outlined by Tu (1986). The technique provides a reliable screening method for foliar diseases in a small controlled environment area. Single plants from segregating populations can be screened independently for different diseases or various physiological races of the same pathogen. The method reported here on excised mungbean leaves is useful for screening resistance against powdery mildew.

Rooting from excised leaves has been reported in several leguminous species including mungbean (Lie 1971). Rooting prevents the senescence of excised leaves and permit normal disease development. In previous experiments, plant growth regulators (PGRs) like IAA and IBA were used in the media. PGRs are reported to alter powdery mildew reaction of detached barley leaves (Edwards 1983). In the present experiment, no PGRs were used and profuse rooting of excised leaves was observed in tap water.

No systematic studies have been conducted in mungbean to screen the germplasm for powdery mildew resistance in controlled environment. Moderate field resistance was reported in the cultivars ML-5 and ML-3 (AVRDC 1978). However, these were found to be susceptible in the present experiment. This could be due to the presence of different physiological races of the pathogen. No information on physiological races of *E. polygoni* is available. High degree of resistance observed in RUM-1, RUM-5, RUM-7, RUM-11, RUM-20, RUM-22 and RUM-33 to the Trombay isolate of *E. polygoni* will be useful in further resistance breeding programme. These stocks, however, have several undesirable features like day length sensitivity, black seed coat colour and susceptibility to *Cercospora* leaf spot and yellow mosaic virus. Genetic studies and breeding experiments using RUM lines have been initiated and the results on the inheritance will be reported later.

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