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Identification of soybean strains resistant to *Xanthomonas campestris* pv. *glycines*

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Summary

Soybean germplasm was screened for resistance to bacterial pustule disease. The etiological agent, *Xanthomonas campestris* pv. *glycines,* was isolated from the leaves of field grown soybean in Maharashtra, India. The screening of soybean stocks was carried out by excised leaf inoculation method. A differential susceptibility to the pathogen was observed in the tested stocks. Two stocks P-4-2 and P-169-3 were found to be completely resistant to the pathogen and displayed an incompatible reaction. Four cultivars, EC-34160, Bragg, Kalitur and PK-472 displayed moderate resistance and the remaining stocks were susceptible to the attack of the pathogen. The stocks P-4-2 and P-169-3 remained resistant even to a high concentration of 10^9 colony forming units (cfu)/ml of the pathogen.

Introduction

Soybean (*Glycine max* L. Merr) cultivation in India is rapidly increasing as an oil seed crop to overcome the shortage of vegetable oils. Meal after oil extraction has equally important use for export and domestic food industry. The crop is now grown over a sizable area in the states of Madhya Pradesh and Maharashtra. There are two major bacterial diseases prevalent in soybean viz. bacterial blight of soybean caused by *Pseudomonas syringae* pv. *glycinea* and the bacterial pustule disease caused by *Xanthomonas campestris* pv. *glycines* (Xcg). The latter causes premature defoliation and consequent reduction in seed weight. About 40% reduction in crop yield has been reported due to this disease (Parthuangwong & Amnuaykit, 1987). *Pseudomonas*-soybean interactions show gene-for-gene complementarity, and its genetics is being vigorously pursued (Keen, 1990; Keen et al., 1991). On the other hand so far *Xanthomonas*-soybean interactions have been poorly investigated as very little information is available in literature. Considerable damage to soybean crop by the bacterial pustule disease has been reported and recently soybean germplasm has been tested for resistance under field conditions (Verma, 1990). The development of multiple disease resistance in crop plants has become a major priority, since this is a cost effective way to assist resource-poor farmers (Nene, 1988). The assessment of available genetic resources is a normal pre-requisite for programs aimed at improving disease resistance. There are no reports of either isolation and characterization of Xcg from India, or a systematic screening of available soybean strains for resistance under laboratory condi-

tions. Recently, we have isolated and characterized *Xanthomonas campestris* pv. *glycines* strains from Maharashtra. In this paper we describe screening and identification of soybean stocks showing resistance to this pathogen.

Materials and methods

Organism and culture conditions. Xanthomonas campestris pv. *glycines* AM2 was isolated from the infected field material collected from the Regional Research Centre, Amravati, Maharashtra. The details concerning isolation and characterization of this pathogen are being published elsewhere. The culture was propagated on a starch agar medium containing per litre potato starch 10.0 g; $K_2HPO_4 \cdot 3H_2O$, 3.0 g; KH_2PO_4, 1.5 g; $(NH_4)_2SO_4$, 2.0 g; L-methionine, 0.5 g; Nicotinic acid 0.25 g; L-glutamic acid, 0.25 g; Agar 20.0 g; pH 6.8–7.0, the plates or slants were incubated at ambient temperature (25 ± 3° C) in an airconditioned laboratory for three days. For propagation in liquid culture either starch medium without agar or LB medium was used.

Plant inoculations. For inoculation of plants, an overnight culture was centrifuged and pellet resuspended in sterile water. When required the suspension was serialy diluted in sterile water and the total viable count as the number of colony forming units (cfu/ml) was determined by plating on starch agar. The plants were grown in pots under ambient conditions (25 ± 3° C), 70% RH) and white light (1000 lux/m^2, 12 h/d). Leaves of 10 day old plants were infiltrated with the bacterial suspension using a tubulin syringe without needle.

Excised leaf inoculation. Twenty soybean cultivars were screened for resistance against the most virulent isolate Xcg AM2 using excised leaves for assay. The cultivars/stocks included Acme, Bragg, CO-1, EC-34160, EC-39025, Flambeau, Kalitur, MACS-13, Merit, Monetta, P-4-2, P-169-3, Panjab-1, PK-472, TAS-38, TAS-42, TAS-60, TAS-90, TAS-91, and UVM-21. The non-host plants included were *Phaseolus vulgaris* HUR-15, *Vigna radiata* TAP-7 and *Vigna mungo* TAU-1. For the excised leaf assay the second or third leaf from the top of field grown plants were excised and used for the screening as described by Reddy et al. (1987). The leaves were then inoculated in triplicate with the pathogen using a tubulin syringe without needle or were painted with a mixture of bacterial suspension mixed with celite. The leaves were incubated under controlled conditions (21 ± 1° C, 70% RH, 4100 lux/m^2, 12 h/d) in a plant growth chamber. After incubating for 7 days, the degree of susceptibility was assessed by visually observing the intensity and area of chlorotic lesions on the three leaves produced by the pathogen and assigning arbitrary units (+). The cultivars showing resistance were selected for further screening in potted plants.

Results

Figure 1 shows the leaves of field grown soybean cv. Monetta infected with the bacterial pustule disease. A number of putative *Xanthomonas* strains were isolated from this material and screened for virulence on the susceptible cultivar Monetta. The isolate *Xanthomonas campestris* pv. *glycines* AM2 was found to display the highest virulence. Distinct chlorotic lesions developed within 48 h of inoculation with this strain. The soybean stocks tested in this study displayed differential reaction to this pathogen. On the basis of screening the soybean germplasm could be divided into three classes as shown in Table 1. Interestingly, two genetic stocks P-4-2 and P-169-3 showed no symptoms of disease upon inoculation of this organism. Among other cultivars the degree of susceptibility to this pathogen varied. As shown in Fig. 2 lesions were more pronounced in cultivar Monetta than in PK-472 indicating that the latter cultivar was less susceptible to the attack of this pathogen. Like PK-472, Bragg, Kalitur and EC-34160 were also found to display lower susceptibility to this pathogen. As shown in Fig. 3 the severity of lesions increased with the increase in incubation period from 7–14 days. Infection was equally severe when the pathogen mixed with celite was painted onto the leaves. Heat (100° C/5 min) and gamma radiation (20 kGy, 0.5 kGy/min, Gamma chamber, AECL, Canada

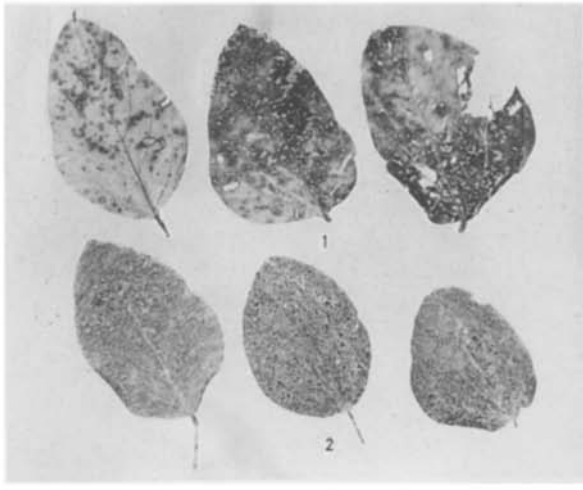

Fig. 1. Leaves of cultivar Monetta from the field showing damage due to bacterial pustule disease.

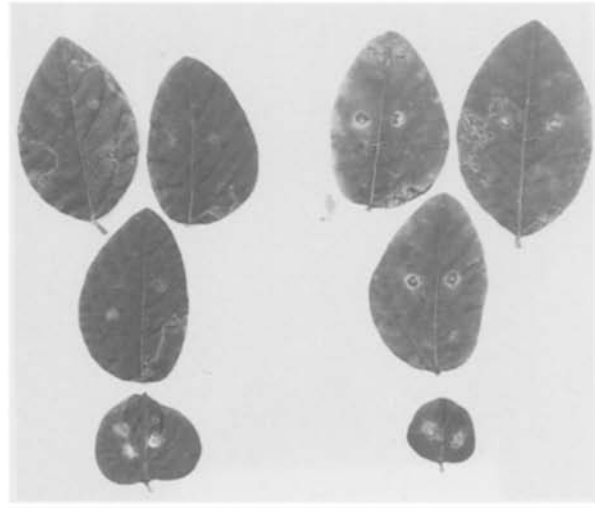

Fig. 2. Excised leaf inoculation assay, PK-472 (left), Monetta (right); Top three leaves from field grown soybean, bottom leaves from potted plants. Note the lesions are more pronounced in Monetta compared to PK-472. Inoculum, 10^5 cfu/ml.

Ltd.) killed organism failed to form any lesions as shown in the figure. Figure 4 shows the degree of chlorotic lesions on six stocks of soybean and a non-host plant, *Phaseolus vulgaris* HUR-15, with different concentrations (10^5–10^9 cfu/ml) of the organism in inoculum. Susceptible cultivars Merit and Monetta showed prominent lesions at the lowest concentration of the inoculum, whereas in moderately resistant cultivars lesions could only be seen when higher concentration of inoculum were used. As shown in Table 2, cultivars Bragg, PK-472 and EC-34160 either required higher inoculum or longer incubation for the display of lesions. The two genetic stocks P-4-2 and P-169-3 did not show characteristic chlorotic lesions even when inoculated with a high cell concentration (10^9 cfu/ml) of the isolate Xcg AM2 and therefore displayed complete immunity toward the pathogen. A weak confluent hypersensitive reaction was also noticed in these cultivars.

Discussion

The extensive screening of the available soybean germplasm showed that all except two cultivars were susceptible to this pathogen. The degree of sensitivity to this pathogen varied in susceptible cultivars. This could probably be due to different levels of horizontal resistance in these stocks. The two stocks which displayed complete immunity against this organism, namely P-4-2, and P-169-3, showed no symptoms of disease even after challanging with a high concentration of the pathogen. These two cultivars also displayed a light brown

Table 1. Soybean germplasm screened for bacterial pustule disease

Reaction	Cultivar/Stock
Resistant (Immune reaction)	P-4-2, P-169-3
Moderately resistant	Bragg, EC-34160, Kalitur, PK-472
Susceptible	Acme, CO-1, EC-30295, Flambeau, Harosoy, MACS-13, Merit, Punjab-1, TAS-38, TAS-42, TAS-60, TAS-90, TAS-91, UVM-21

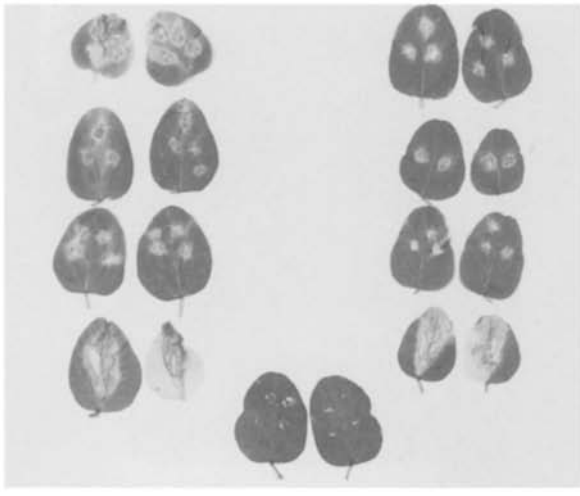

Fig. 3. Effect of incubation on lesion development in PK-472 (right) and Monetta (left), from top, row 1,2 & 3, 14, 10 and 7-day old lesions respectively. Row 4, infection by painting of pathogen-celite mixture. Bottom row, heat and radiation killed cells.

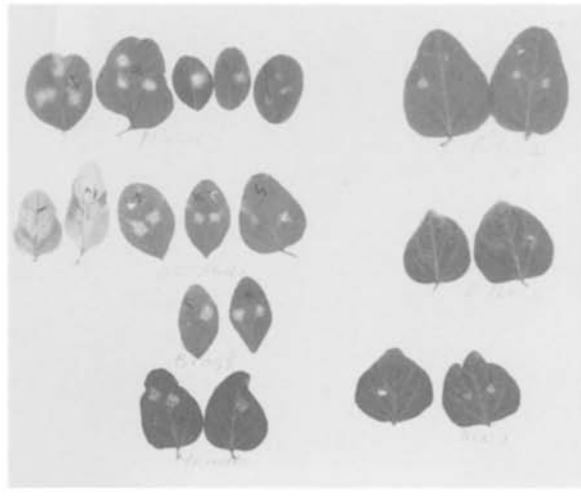

Fig. 4. Effect of inoculum on lesion development in cultivars; left top & 2nd row, Merit and EC-34160, inoculum, 10^9, 10^8, 10^7, 10^6, & 10^5 cfu/ml from left to right. Left 3rd and 4th row, Bragg with 10^7 & 10^6 cfu/ml and Monetta with 10^6 & 10^5 cfu/ml, respectively. On the right hand side top, P-4-2; middle, P-169-3; and bottom, non-host *Phaseolus vulgaris* displaying complete immunity to the pathogen.

confluent hypersensitive response typical of *avrBs2-BS2* interaction at the site of infection (Kearny & Staskawicz, 1990). Xcg is reported to possess DNA homologous to avrBs2 but does not elicit HR on pepper cultivar ECW20R homozygous for *Bs2* resistance gene (Kearny & Staskavicz, 1990). This raises hopes of possible interplay of gene-for-gene system in this interaction much on the lines of other well recognized gene-for-gene interactions (Keen, 1990). High field resistance to this pathogen has recently been reported in cultivars

Table 2. Relative resistance of soybean cultivars/stocks to Xcg isolate AM2

Cultivar/stock	Inoculum concentration (cfu)ml)		
	10^7	10^6	10^5
Monetta/Merit	++(2)	++(3)	+(5)
Bragg/EC-34160/PK-472	++(5)	+(5)	+(5)
P-4-2/P-169-3	HR	HR	HR

+, Arbitrary unit of virulence (chlorotic lesions); Figures in parentheses represent days after infiltration of leaves; Lines P-4-2 and P-169-3 were also inoculated with higher concentrations (10^8 and 10^9 cfu/ml) of the pathogen and were found to be completely resistant displaying a weak confluent HR; cfu = colony forming unit.

Bragg and PK-472 by Verma (1990). However, these cultivars were found to be only moderately resistant to this isolate in the present studies. Resistance to bacterial pustule disease was first recognised in the soybean cultivar CNS by Hartwig & Lehman (1951) and has since been incorporated into many commercial cultivars today. The resistance was found to be recessive and attributed to a major gene pair which has been designated *rxp*. Under field conditions this resistance is expressed as near immunity with occasional small chlorotic spots (Hartwig & Lehman, 1951). However, substantial pathogen growth was observed on artificially inoculated plants of resistant cultivars and they became severly diseased (Chamberlain, 1962; Fett, 1984). Groth & Braun (1986) reported that Xcg grew equally well in the leaves of resistant and susceptible cultivars, although a six fold inoculum was needed to initiate infection in resistant plants. It is significant to note in this context that the stocks P-4-2 and P-169-3 gave an immune reaction even to a 10^4 fold increase in inoculum. These results indicate that gene(s) providing this resistance may be different from the known *rxp* gene.

Further it would be interesting to know if the Xcg

AM2 interaction with the cultivars P-4-2 and P-169-3 involves formation of phytoalexins as a confluent HR is evident. It has earlier been shown that soybean phytoalexins were not responsible for resistance of cultivar Clark 63 containing *rxp* gene, to a virulent strain of Xcg (Fett, 1984).

Investigations are in progress to study the nature of resistance in stocks P-4-2 and P-169-3 by genetic analysis and will be reported later.

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