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DIFFERENT METHODS FOR DETECTION OF *XANTHOMONAS CAMPESTRIS* PV. *VIGNICOLA* (BURKH.) DYE IN COWPEA (*VIGNA UNGUICULATA* (L.) WALP.) SEEDS AND STUDIES ON HOST RANGE

K. Anitha, Baleshwar Singh, P.C. Agarwal and Ram Nath

National Bureau of Plant Genetic Resources,
Pusa Campus, New Delhi–110 012

Of the four seed health testing techniques viz., seed soaking, seed maceration, blotter test and growing-on test used for the detection of Xanthomonas campestris *pv.* vignicola *(Burkh.) Dye from naturally infected seeds of cowpea, seed soaking test was found to be superior to other methods from quarantine point of view. In the host-range studies,* Glycine max, Pisum sativum *and* Vicia faba *were identified as compatible hosts for the first time and injection-infiltration technique showed superiority over the spray inoculation technique.*

Standardization of techniques for quick detection of seed borne plant pathogens has assumed great relevance in recent years due to increased quantum of exchange of germplasm throughout the world. From quarantine point of view, it is essential to have quick, easy and reliable techniques for detection of seed borne bacteria. Though some work has already been done on the development of different methods for detection of phytopathogenic bacteria from seeds of different crops (Shekhawat and Chakravarti, 1979; Schaad, 1982), limited information is available on these aspects for *Xanthomonas campestris* pv. *vignicola*, causal organism, of bacterial blight of cowpea (Gitaitis and Nilakhe, 1982; Soni and Thind, 1987). Incidence of cowpea blight has been reported to be as high as 62 per cent from an initial inoculum level of 1 per cent infected seed (Shekhawat and Patel, 1977; Therefore, keeping in view the economic and quarantine importance of *X. campestris* pv. *vignicola*, investigations on techniques for detection of this pathogen in cowpea seeds and host range studies were undertaken.

MATERIALS AND METHODS

Mature pods from bacterial blight infected cowpea (EC-244289, imported from Philippines) plants were collected from NBPGR Research Farm, Issapur, Delhi in November, 1990. Seeds collected from pods showing bacterial ooze at peduncle under microscope were considered as infected seeds and were used for these studies. Majority of them were found to be discoloured, deformed and shrivelled. Seeds showing varied symptoms were mixed before use in isolation studies on nutrient agar (NA) medium. Seeds from pods of healthy plants not showing bacterial ooze were used as control. Before use, all the seeds were surface sterilized with rectified spirit for 2 minutes followed by washing in sterile water. Each treatment was replicated five times.

Four methods were used for the detection of *X. campestris* pv. *vignicola*. (i) *Seed soaking*: Ten seeds were soaked in 15 ml sterile water for 3 hr at room temperature (25±1°C). A loopful of the suspension was then streaked on NA plates and incubated at 27±1°C for 6 days, (ii) *Seed maceration*: Ten seeds were soaked in water columns for 3 hr at room temperature and then crushed with pestle and mortar. The paste was transferred to 5 ml sterile water column, stirred well on shaker and allowed to settle down. The supernatant was serially diluted upto 10^{-3} dilutions. One loopful of the suspension from the last dilution was streaked on NA plates and incubated at 27±1°C for 6 days, (iii) *Blotter test:* Ten infected seeds were plated on 4 layers of moist blotters in each plastic petriplate and were incubated at 22±1°C for 8 days. Seedlings showing any symptom were re-incubated for 3 more days for further development of the symptoms. Seeds collected from healthy plants were used as control. (iv) *Growing on test*: Five infected seeds were sown in 6" plastic pots in the green house. The plants were regularly irrigated as well as sprayed with water to provide conducive conditions for disease development.

Similar experiment was conducted with seeds artificially inoculated by *X. campestris* pv. *vignicola* which were produced by soaking healthy seeds in 24 hr old concentrated bacterial suspension for 2 hr followed by drying at room temperature for 1 hr. Similarly, unsoaked seeds from healthy plants as well as seeds soaked in sterile water were used as control.

Hypersentitive Reaction (HR) : The purified, single colony bacterial cultures, isolated in the above mentioned experiments,

were inoculated on fully expanded leaves of tobacco (*Nicotiana tabacum* L.) by injection-infiltration technique (Klement *et al.*, 1964).

Host range : Studies on host range were also conducted with 8 legume crops : *Cyamopsis tetragonoloba* L., *Glycine max* (L) Merr., *Lablab purpureaus* (L) Sweet; *Phaseolus vulgaris* L; *Pisum sativum* L; *Vicia faba* L; *Vigna mungo* (L.) Hepper and *V. radiata* (L.) R. Wilczek using *V. unguiculata* (L.) Walpers as check crop. Five seeds of each of these crops were sown in 15cm plastic pot. Leaves and stem of the healthy seedlings at 3-4 leaf stage were inoculated with 24 hr old bacterial suspension by spraying as well as injection infiltration techniques. Plants treated with sterile water served as control.

RESULTS AND DISCUSSION

Two types of bacterial colonies appeared in seed soaking and maceration tests. One was well-isolated, circular, shiny, mucoid and yellow pigmented which developed on NA after 72 hr while the other one was white type, rough, irregular and developed after 24 hr of streaking. The number of yellow colonies was comparatively higher than white type in seed soaking whereas it was reverse in seed maceration technique.

In blotter test, out of 50 seedlings, only 16 per cent showed minute, water soaked spots on cotyledons and primary leaves on the 7th day which later increased in size and infected area turning yellow. On isolation, similar yellow bacterial colonies, as mentioned earlier, were obtained. No symptoms were observed in control seedlings.

In growing-on test, different types of symptoms were observed in 24 per cent seedlings at varying growth stages on different parts. The symptoms were first recorded as minute, round to irregular, whitish to yellowish green, water soaked spots on leaf margins and lamina after 12-15 days of sowing (first trifoliate leaf stage). On veins, symptoms appeared as whitish water soaked streaks which later increased in size. On stem, similar type streaks appeared (3-4 trifoliate leaf stage) which later extended upwards and downward, turned yellowish and caused stem splitting, drying and ultimately resulted in death of the seedlings (6-7 trifoliate leaf stage). In seedlings raised from artificially inoculated seeds, out of 20 seedlings, only 12 (60%) seedlings showed disease symptoms. On isolation, similar yellow pigmented bacterial colonies were obtained from all types of symptoms. No symptoms were observed on control plants.

All bacterial isolates were purified and single colony cultures were maintained on Yeast Glucose Chalk Agar (YGCA) slants as well as in sterile water column top layered with liquid paraffin (Durgapal, 1980). The single colony isolates were used in the present studies. All isolates were found to be gram-negative and rod shaped.

The yellow isolates showed typical Hypersensitive Reaction (HR) on tobacco leaves whereas white one did not show any reaction.

Koch's postulates were proved for all the yellow isolates indicating their pathogenicity on cowpea plants. Thus the association of *X. campestris* pv. *vignicola* with cowpea seed and the seed borne nature as well as seed transmission of *X. campestris* pv. *vignicola* were confirmed. Thus white isolates did not show any reaction.

The seed soaking technique appears to be superior to all other methods for quick detection of *X. campestris* pv. *vignicola* in cowpea seeds from quarantine point of view. It is a simple method and does not require any specific chemical/medium or instrument, etc. Gitaitis and Nilakhe (1982) also reported the detection of *X. campestris* pv. *vignicola* from cowpea seeds by seed soaking in water but vacuum infiltration and centrifugation were also involved in their technique. This method has been found suitable for the detection of *Pseudomonas syringae* pv. *phaseolcola* from bean seeds (Trigalet and Bidaud, 1978).

Blotter and growing-on tests have been reported for detection of *X. campestris* pv. *vesicatoria* and *X. campestris* pv. *vignicola* from chilli and cowpea seeds, respectively. These techniques require suitable environmental conditions for disease development (Shekhawat and Chakravarti, 1979; Soni and Thind, 1987) and non-availability of suitable incubation conditions may also lead to failure in detection of latent bacterial infection particularly in shrivelled and non- viable seeds.

In host range studies, *Glycine max, Pisum sativum* and *Vicia faba* were recorded to be the new compatible hosts while *C. tetragonoloba, L. purpureus, P. vulgaris, V. mungo* and *V. radiata* were found to be non-compatible. Jindal and Patel (1980) have also reported *L. purpureus, P. vulgaris, V. mungo, V. radiata* and *C. tetragonoloba* as non-compatible hosts for *X. campestris* pv. *vignicola*

and our findings support their observations. However, in respect to *P. vulgaris* and *G. max*, our findings are at variance with the earlier reports. *P. vulgaris* has been reported to be a compatible and common host for this pathogen by Rangaswamy and Gowda (1963), Sabet (1959), Bhatt and Patel (1954), while Jindal and Patel (1980) have recorded *G. max* as non-compatible host. This divergent finding may probably be due to the existence of strain specificity/differential behaviour of the isolates of this pathogen.

The influence of method of inoculation on symptom development was clearly evident in the present study. On *Pisum sativum*, spray inoculation induced inward shrivelling of leaves, yellow discolouration followed by tip drying while no symptoms were observed in *Glycine max*. However, symptoms developed by injection infiltration inoculation in all the three new compatible hosts were different from its natural host (cowpea). On *Pisum sativum*, yellowish, water soaked spots appeared extending upward and downward at inoculated portion of the stem and resulted in withering and wilting of the plants. In *Vicia faba*, dark brown to blackish, water soaked lesions developed around the inoculated portion of leaf and stem, which later extended upward and downward resulting in the death of the plant. In *G. max*, water soaking appeared at inoculated portion on leaf, which later increased in size and turned yellow. No symptoms were developed on control plants. Koch's postulates were proved on cowpea plants with isolates from the three crops. Sabet (1959) also reported the development of different symptoms than normal ones on *Dolichos lablab* and *P. vulgaris* inoculated with *X. campestris* pv. *vignicola*.

CONCLUSIONS

Seed soaking method was proved to be the best method for detection of *X. campestris* pv. *vignicola* from cowpea seed from quarantine point of view. Injection infiltration technique showed superiority over spray inoculation technique for symptom expression. *Glycine max, Pisum sativum* and *Vicia faba* were found to be compatible for *X. campestris* pv. *vignicola* for the first time.

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