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DIFFERENT PATHOGENICITY ASSAYS TO EVALUATE VIRULENCE OF *PSEUDOMONAS SYRINGAE* PV.*PISI* ON COMMERCIAL PEA (*PISUM SATIVUM* L.) VARIETIES

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ABSTRACT

Pea (*Pisumsativum* L) is a major legume grown in Pakistan which used both as fresh vegetable and canned food. However, pea production in Pakistan is under stress due to various abiotic and biotic factors. Among biotic, the most destructive is bacterial blight caused by *Pseudomonassyringae* pv *pisi*(*Ppi*). Therefore, in present study different pathogenicity assays were performed to observe the innate immunity present in different commercial pea cultivars against *Ppi* isolated from pea seeds. The results of pathogenicity assays showed that leaf detach method was best method for symptom development as it took 2 days in comparison to foliar injection and soil drenching methods where incubation period prolonged for 6-8 days respectively. Pea varieties viz. DMR-4, DMR-7 Green forest, Rondo and Dasan showed susceptibility against *Ppi* isolates in all three methods of inoculations. Commercially growing pea germplasm is not showing resistance against *Ppi* which considered a looming threat to pea productions all over the world. Therefore, an extensive screening of commercial pea germplasm against local bacterial isolates needed to be performed to avoid future crop failure.

Keywords: Pea, *Pisumsativum* L, *Pseudomonas syringae*pv*pisi*, Bacterial blight

INTRODUCTION

Pea (*Pisumsativum* L) is a major crop of temperate region but due to high demand both as fresh and canned food it is grown in different agro-ecological zones of the world. However, pea cultivation all over the world is under stress due to various abiotic and biotic stresses. Among biotic stresses, bacterial blight caused by *Pseudomonas syringae*pv. *pisi(Ppi)* is major constraint to pea production (Hollaway*et al*., 2007; Martín-Sanz*et al*., 2011; Rihardson and Hollaway, 2011) all over the world. Characteristic symptoms of pea blight are brown spots on infected leaves which later coalesced and resulted in a complete death of plant (Hollaway*et al*., 2007). Though, pathogen is majorly seed borne but can also survive in soil and in plant debris as well. Management of infected plant is an uphill task, however, disease free seed, screening and subsequent cultivation of resistant varieties seemed most practical options to curb inoculum build up in field. In Pakistan, *Ppi* was reported both from seeds and pea fields but disease is not regularly monitored (Akhtar and Aslam, 1985; Ali *et al*., 2015). However, exotic pea lines were screened against exotic strains of *Ppi* by using stem inoculation or wounding pea leaves which were later sprayed with *Ppi* inoculum (*Iqbal et al*., 2013). Screening of local commercial pea varieties under favourable conditions against local isolates is helpful to assess varietal field fitness against prevailing bacterial pathogens in field. Different types of pathogenicity assays used for artificial inoculation of bacterial pathogens depending upon the host and microbe involved. The most common methods used for bacterial inoculations include pressure infiltration, foliar spray,leaf detach method,soil drenching method (Schaad*et al*., 2013).

Therefore, aim of present study was to evaluate the innate immunity of different commercial pea varieties against *Ppi* by using different methods of inoculation such as leaf detach, pressure infiltration in stem and soil drenching method. The results of research will not only be helpful in evaluating most resistant variety but also provide the information about the best method for inoculation for varietal screening.

MATERIAL AND METHODS

Bacteria

For pathogenicity assay, bacterial culture *Ppi* which was previously isolated and

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characterized from pea seeds (*Pisumsativum* L) in Department of Plant Pathology, PMAS-Arid Agriculture was used. Before inoculation bacterial culture was streaked on KB medium and incubated at 25 ^{0}C for 24 hours. Individual bacterial colonies were removed and bacterial suspension was adjusted 10^8cfu ml^{-1} prior to inoculations.

Plant Material

Different commercial Pea varieties i.e. Rondo, DMR-7, DMR-4, Dasan and Green Forest were grown in pots containing sterilized potting mixture. Soil was sterilized with 37% formaline with 1:9 ratios (1 part formaline and 9 parts soil). Treated soil was covered with a polythene sheet and kept in sun light for 3-4 days after which sheet was removed and soil was kept open for 5 days to release fumes. Twenty-one days old seedlings were used for performing different pathogenicity assays.

Pathogenicity Assays

i. Leaf detach method:

In leaf detach method, fully expanded leaves of peas were washed and sterilized with 1% chlorox and dipped in sterilized distilled water to remove bleach effect. Later on, leaves were placed in Petri plates containing double filter paper to retain the moisture level. Inoculum was applied on the leaves with the help of 1cc syringe needle. Control leaves were inoculated with sterilized water only. The leaves were incubated at 28 ˚C under high humid conditions and were observed for appearance of symptoms (Winstead and Kelman, 1952).

ii. Stem Inoculation method

In case of stem inoculation a drop of inoculum was placed at the junction of a leaf petiole and the stem with the help of 1cc syringe needle. The entry of inoculum into plant was facilitated by minor pricking into the stem with common pin and plants were closely monitored for disease symptom development (Winstead and Kelman, 1952).

iii. Soil Drenching Method

Pea plants were not watered for a day before inoculation to reduce moisture contents in rhizosphere. Roots of pea seedlings were slightly injured by inserting a scalpel in the pots in order to facilitate bacterial entry into plants through wounds. A bacterial suspension (10ml) was poured into soil of each pot. The ratio between bacterial suspension and potting mixture was almost about 1:10 (v/v) respectively and inoculations were performed in the afternoon. Plants were regularly watered after inoculation and were kept at temperature range from 28 ˚Cto 30 ˚Cat 90% relative humidity (Winstead and Kelman, 1952).

Disease Measurement

In all methods of inoculation disease severity was recorded based on slight modification of Winsted and Kelment's scores (Winsted &Kelman, 1952). For soil drenching and stem inoculation methods they were

Disease ratting scale	Soil drenching/Stem inoculation	Leaf detach method
0	No symptom	No symptom
1	Partial necrosis	Partial yellowing
2	Localized necrosis	Complete chlorosis
3	Plant collapsed and dead	Leaflet collapsed and leaves withered.

RESULTS AND DISCUSSION

Pathogenicity Assay

Pea varieties i.e. Rondo, DMR-7, DMR-4, Dasan and Green Forest were screened out by three different methods viz. leaf detached method, stem inoculation method and soil drenching method. Although all methods differed significantly at 0.05 level of significance for the appearance of symptoms (Figure 1, 2, 3) but leaf detach method (Figure. 1) was most significant in development of disease symptom in which symptoms were evident after 2 days of inoculation while in case of stem inoculation and soil drenching method symptoms appeared after 6 and 8 days of post inoculation respectively (Figure. 2 and 3). Leaf detach method also used by previous workers for evaluation of pathogenicity of phytopathogenic bacteria on many crops (Randawa and Civerolo 1985: Yessad*et al*., 1992).There are certain advantages of using detach leaf assay over other methods to assess virulence of bacterial pathogen as it is very convenient and second it could be performed at any time of year provided disease free parent

plants are available. Furthermore, resident saprophytic microflora could be avoided by dipping leaves in 1% chlorox solution. Results showed that DMR-4 was the most susceptible variety. It is pertinent to mention that no pea variety showed complete resistance once pea plants were challenged with bacterial pathogens (Figure 4).

Similar results were reported when exotic Australian pea germplasm was tested against different races of *Ppi* under favourable conditions (Iqbal *et al*., 2013). The pea germplasm showed susceptibility against race 2 and 3 out of seven races of *Ppi*. It is plausible to mention that *Ppi* can be differentiated from other pathovars on the basis of symptom development. Still bacterial blight caused by *Ppi* sometimes confused with identical or similar symptoms caused by *Pseudomonas syrinage* pv. *syringae* and *Pseudomonas viridiflava* (Taylor and Dye, 1972) on pea plantation. These two pathovars cause disease under more restricted environmental conditions and are sometimes associated with frost damage. It is, therefore, essential to confirm the presence of *P. s*.pv. *pisi* by isolation and identification (Taylor and Dye, 1972). The situation is alarming as currently we have no clue about which race of bacterial blight pathogen is prevailing in the pea seeds and fields in Pakistan as race 6 devoid of any avirulence genes (Hollaway*et al*., 2007). In addition, available commercial pea germplasm in country seemed susceptible against bacterial pathogen prevailing in pea seeds which could pose serious threat to pea production.Therefore, a comprehensive screening of pea varieties is needed.

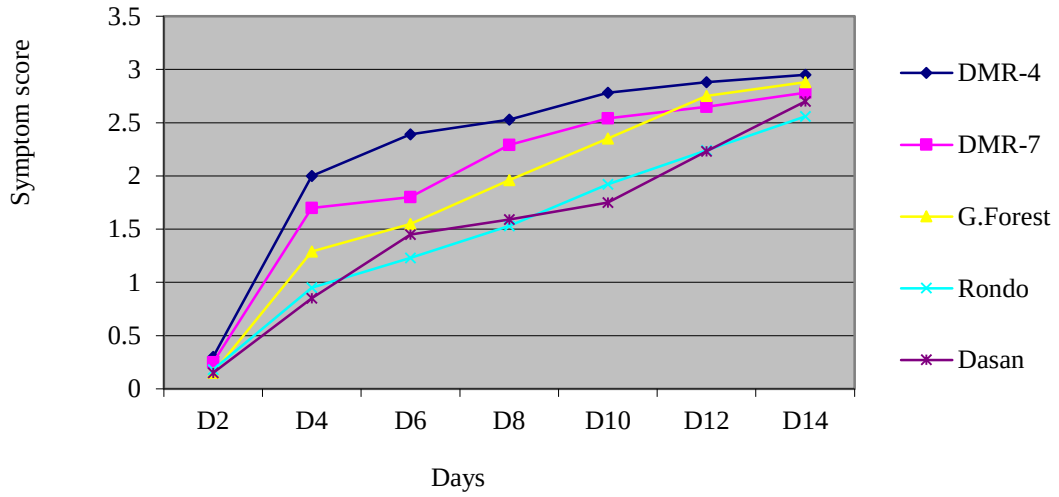

Figure 1. Bacterial blight progress in pea varieties after inoculation into detach leaves

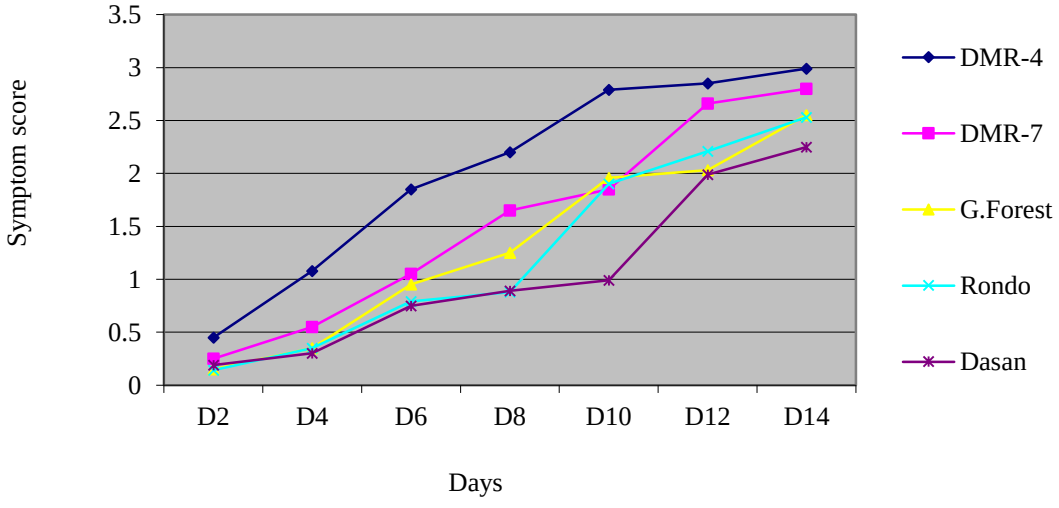

Figure 2. Bacterial blight progress in pea varieties after stem inoculation

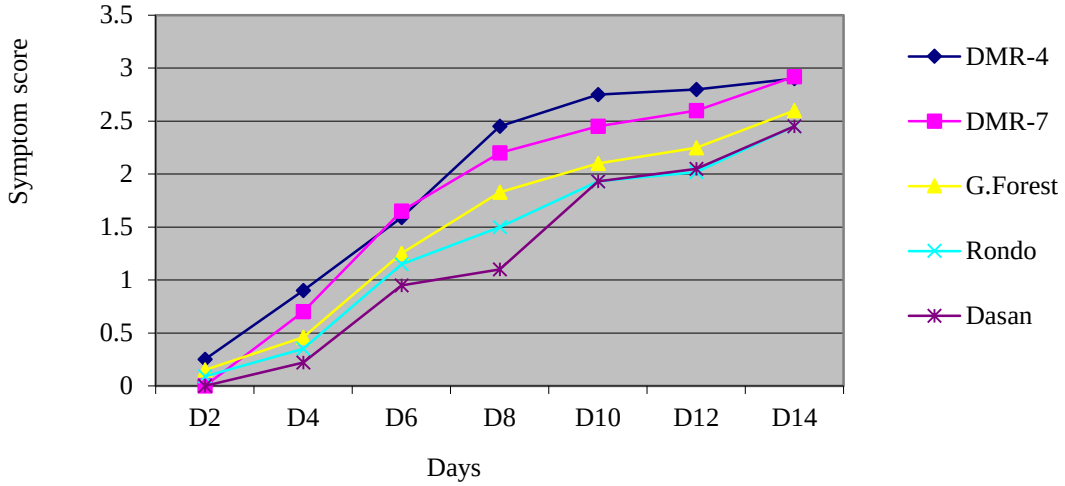

Figure 3. Bacterial blight progress in pea varieties in soildrenching

Figure 4. Pea plant showing blight symptoms after challenged by *Pseudomonas syringae*pv*pisi*

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