INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
12–626/2013/15–4–787–790
http://www.fspublishers.org

Full Length Article

Molecular Characterization of Pea for Resistance to *Pseudomonas syringae* pv *pisi*

Sheikh Muhammad Iqbal[1]*, Arshad Javaid[2], Ahmad Bakhsh[1] and Shahid Riaz Malik[1]
[1]*National Agricultural Research Centre, Islamabad, Pakistan*
[2]*Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan*
*For correspondence: iqbalsh65@hotmail.com

Abstract

One hundred and ten pea (*Pisum sativum* L.) germplasm lines were screened under artificial disease conditions for their resistance against *Pseudomonas syringe* pv *pisi*, the cause of bacterial blight disease. Different strains corresponding to the two races of *P. syringae* pv *pisi* (race 2 and 3) were used for inoculation. The race 2 showed more virulence behaviour than race 3 on pea. Thirty pea lines (27.3%) were found resistant to race 2, while 49 lines (44.5%) to that of race 3. Twenty pea lines were selected among the genetic stock for Simple Sequences Repeat (SSR) analysis. Based on SSR analysis, two major groups A and B were identified. In the group A, there were four genotypes viz. ATC909, ATC613, ATC912 and ATC1641, whereas all other genotypes were placed in group B. The resistant genotypes reported in the present study are suggested for used in breeding program to develop disease resistant pea cultivars.

Keywords: Bacterial blight; Pea; SSR markers; Germplasm

Introduction

Pea is one of the main sources of vegetable protein (21–32%) in various parts of the world. It is used as a green vegetable in Asian countries and as dry seed in Australia, Europe, America and in Mediterranean regions (Nasir *et al.*, 2006). *Pseudomonas syringe* pv *pisi* is reported to cause bacterial blight of pea over a wider range of environmental conditions (Martín-Sanz *et al.*, 2011). *Pseudomonoas syringae* pv *pisi* is primarily a seed-borne pathogen, but infected pea trash can be an important source of inoculum (Hollaway *et al.*, 2007). All above ground plant parts are attacked by the pathogen throughout the growing season. The affected stipules show fan-shaped, water-soaked lesions, which turn brown and papery (Benlioglu *et al.*, 2010; Richardson and Hollaway, 2011). The disease causes widespread damage to pea crop in all the continents (Hollaway *et al.*, 2007). On the basis of the differential response of eight pea genotypes, seven pathogenic races of *P. syringe* pv *pisi* have been identified (Bevan *et al.*, 1995). The frequency of each race varies between regions. However, race 2 and 6 predominate worldwide (Hollaway *et al.*, 2007). However, Race 3 predominates in Australia because of widespread cultivation of pea cultivars susceptible to Race 3, but resistant to Race 2 (Hollaway *et al.*, 2007). Recently, race 8 of this pathogen has also been suggested by Martín-Sanz *et al.* (2011).

Bacterial blight of pea can be controlled effectively by using resistant host varieties (Martín-Sanz *et al.*, 2011). The identification of markers associated with different disease resistance genes has been achieved by the use of bulked segregant analysis (BSA) in combination with molecular markers in various crop plants. There are molecular markers available for marker-assisted selection (MAS) for disease resistance. Some of these markers including SCAR (sequence characterized amplified region) are specific and widely used for MAS. Therefore, molecular markers linked to resistant genes must be developed for enhancing breeding pace for developing disease resistant cultivars in limited time. The objective of present investigation was to develop molecular markers that could be used for pea breeding program to develop bacterial blight resistance in pea.

Materials and Methods

Screening of Pea Germplasm against Bacterial Blight

One hundred and ten germplasm accessions of *Pisum sativum* were obtained from South Australian Research and Development Institute, University of the Adelaide, Australia and screened against bacterial blight under artificial disease conditions. The experimental material was planted in composted soil in seed trays and kept at 20°C/17±2°C day/night with temperature for two days with supplementary lighting to give a 12-h day length. Different types of strains corresponding to the two races (race 2 and 3) of *Pseudomonas syringae* pv *pisi* were used for inoculation (Taylor *et al.*, 1989; Bevan *et al.*, 1995). For inoculum

To cite this paper: Iqbal, S.M., A. Javaid, A. Bakhsh and S.R. Malik, 2013. Molecular characterization of pea for resistance to *Pseudomonas syringae* pv *pisi*. *Int. J. Agric. Biol*., 15: 787–790

production, the isolates were subcultured onto the medium.

Pea seedlings were inoculated after two weeks of sowing (Malik *et al*., 1987). The isolates were scraped from the surface of plate with the help of a sterile pin and pierced into the main stem at its junction with the stipules at the youngest node for stem inoculation. Further the young leaves were wounded with the same entomological pins used for stem inoculation and sprayed with a bacterial suspension (ca 10^9 cfu mL^{-1}) prepared in sterile tap water. Manoxol was added as wetting agent and the concentration was maintained at 0.025% in the bacterial suspension, which was sprayed thoroughly to wet the leaf surfaces. Inoculated plants were kept in a mist chamber for 48 h after inoculation and then transferred to the glasshouse. Stem and leaf responses of pea to bacterial inoculation were recorded after one week of inoculation. The disease responses were recorded in three categories, (1) a typical susceptible response (S) showed an area of water soaking spreading from the site of inoculation, (2) a typical resistant response (R) resulted in necrosis localised at the point of inoculation, and (3) Incomplete expression with a combination of resistant and susceptible symptoms (MS), localised necrosis surrounded by a limited water-soaked area.

Microsatellite Polymorphism

The DNA was isolated from young seedling grown under a glasshouse conditions using the methodology explained by Doyle and Doyle (1990). Among twenty genotypes used for the present study, each of five was either resistant or susceptible to race 2 and race 3.

The sequences of pea genome were obtained from Genbank/EMBL database. Computer software FINDPATTERNS, developed by the GCG Wisconsin Package (Genetics computer Group, 575 Science Drive, Madison, WI53711, USA) was used to search for all possible dinucleotide and trinucleotide SSR motifs. On the bases of various complementary and permuted sequences for a given sequence of the simple, ten trinucleotide four-repeat motifs and four dinucleotide five-repeat motifs were explored. Among these, ten sequences having up and down stream information of the SSR were chosen for designing flanking primers. Software AMPLIFY (Bill Engels, USA) was used to check the primers and the primers were got synthesized by OLIGO Express (Burstin *et al.*, 2001).

Each PCR reaction contained 0.2 µm of each primer, 20 ng of template DNA, 1.5 mM $MgCL_2$, 0.2 mM dNTP, 1 U Taq polymerase in a total volume of 5 µL, and covered by paraffin oil. PCR was accomplished in a PTC-100 thermocycler with 35 cycles of 30 sec denaturation at 94°C, 30 sec annealing and 30 sec elongation at 72°C and 5 min elongation step at 72°C. The PCR products were electrophoresed for 120 min at 1500 V on 5.7% acrylamide/0.3% bis-acrylamide denaturing gels, and were silver stained using 1 g L^{-1} $AgNO_3$. The bands were scored visually and genetic distances among the 20 genotypes were estimated by the multilocus Rogers' distance (Rogers, 1972) for the various polymorphic markers, except PsBlox13.1 due to the account for the distance computation as already there is a marker (Burstin *et al.*, 2001).

Results and Discussion

Data regarding the screening of 110 pea lines against bacterial blight under artificial disease conditions in greenhouse is presented in Table 1. The race 2 was more virulent than race 3. Thirty lines (37.3%) were resistant and 31 (28.2%) were susceptible to race 2, while 49 (44.5%) showed intermediate response. In case of race 3, sixteen lines (14.5%) were resistant and 64 (58.2%) were susceptible, while 30 lines (27.3%) showed intermediate response. Elvira-Recuenco and Taylor (2001) reported varying reactions by different races of bacteria on Spanish pea. Twenty pea cultivars were selected to screen against the race-2 and race-3 of *P. syringe* pv *pisi* under the artificial disease conditions for precise investigation of SSR markers for further use in MAS. The results regarding screening against both the races of bacterial blight are presented in Table 2. Initially four primers (PEA0M14A,

Table 1: Disease reaction of pea germplasm lines to bacterial blight under artificial disease conditions

Disease reaction	Race-2		Race-3	
	No. of lines	Percentage of total	No. of lines	Percentage of total
Resistant (R)	30	27.3	16	14.5
Moderately resistant (MR)	49	44.5	30	27.3
Susceptible (S)	31	28.2	64	58.2

Table 2: Pea cultivars randomly selected for SSR analysis (showing resistant and susceptible to race 2 and race 3 of bacterial blight)

Cultivar	Disease Reaction	
	Race-2	Race-3
ATC 758	R	-
Alma	R	-
ATC 1644	R	-
ATC 1641	R	-
ATC 812	R	-
ATC 754	S	-
ATC 756	S	-
ATC 966	S	-
ATC 967	S	-
ATC 975	S	-
ATC 1744	-	R
ATC 775	-	R
ATC 954	-	R
ATC 742	-	R
ATC 762/P1	-	R
ATC 613	-	S
ATC 909	-	S
ATC 912	-	S
ATC 525	-	S
ATC 1191	-	S

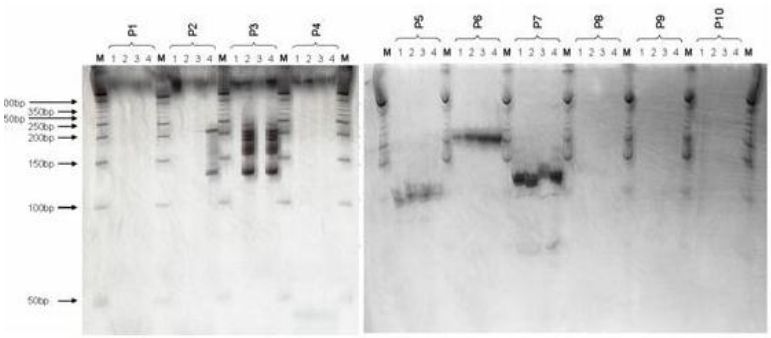

Fig. 1: SSR maker indicating polymorphism for pea genotypes

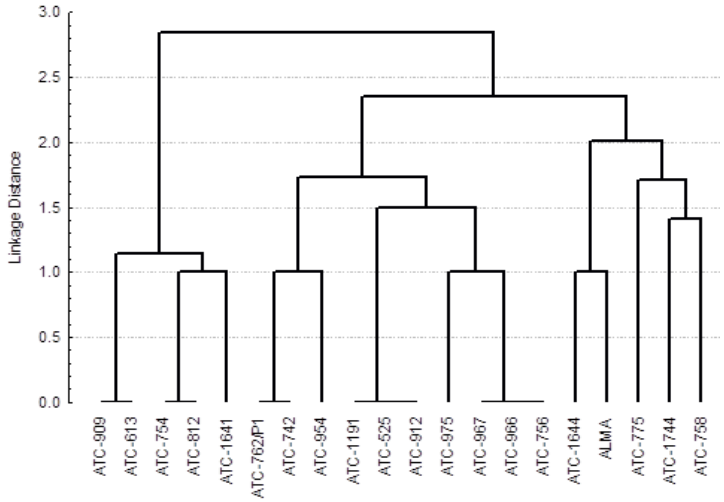

Fig. 2: Dandrogram of 20 pea genotypes are showing phylogenetic relationship

Table 3: Genetic distances among 20 pea genotypes based on SSR markers

Variable	Alma	ATC-1644	ATC-1641	ATC-812	ATC-574	ATC-756	ATC-966	ATC-967	ATC-775	ATC-1744	ATC-976	ATC-954	ATC-742	ATC-762P1	ATC-613	ATC-909	ATC-912	ATC-525	ATC-1191
ATC758	1.41	2.0	3.16	3.32	2.65	2.65	2.65	2.65	2.45	1.41	2.0	2.83	2.65	2.65	3.61	3.46	2.65	2.65	2.65
Alma		1.41	2.83	3.00	2.24	2.24	2.24	2.24	2.00	2.00	2.45	2.45	2.24	2.24	3.32	3.16	2.24	2.24	2.24
ATC1644			2.45	2.65	1.73	1.73	1.73	1.73	2.00	2.45	2.00	2.00	1.73	1.73	3.0	2.83	1.73	1.73	1.73
ATC1641				1.00	2.65	2.65	2.65	2.65	2.83	3.46	3.16	3.16	3.00	3.00	1.73	1.41	2.65	2.65	2.65
ATC812					2.45	2.45	2.45	2.45	2.65	3.61	3.32	3.00	2.83	2.83	1.41	1.00	2.45	2.45	2.45
ATC754						0.00	0.00	0.00	1.00	2.65	2.24	1.73	1.41	1.41	2.45	2.65	1.41	1.41	1.41
ATC756							0.00	0.00	1.00	2.65	2.24	1.73	1.41	1.41	2.45	2.65	1.41	1.41	1.41
ATC966								0.00	1.00	2.65	2.24	1.73	1.41	1.41	2.45	2.65	1.41	1.41	1.41
ATC967									1.00	2.65	2.24	1.73	1.41	1.41	2.45	2.65	1.41	1.41	1.73
ATC775										2.45	2.45	2.00	1.73	1.73	2.65	2.83	1.73	3.00	3.00
ATC1744											1.41	2.45	2.24	2.24	3.61	3.74	3.00	2.65	2.65
ATC976												2.00	1.73	1.73	3.32	3.46	2.65	1.73	1.73
ATC754													1.00	1.00	2.65	2.83	1.73	2.00	2.00
ATC742														0.00	2.83	3.00	2.00	2.00	2.00
ATC762P2															2.83	3.00	2.00	2.45	2.45
ATC613																1.00	2.45	2.24	2.24
ATC909																	2.24	0.00	0.00
ATC912																		0.00	0.00
ATC525																			0.00

PEAATPSYND, PSRBCS3C and PSARGDECA) were tested for amplification and setting up the protocol. As the results were not very much clear thus ten more markers (PEA0M14A, PEAATPSYND, PSRBCS3C, PSARGDECA, PSGDPP, PEAPHTAP, PSBT2AGEN, PSY17134, PSZINCFIN and PSAJ3318) were investigated

on four cultivars (ATC 758, ATC 967, ATC 1744 and ATC 909). The SSR reaction of these cultivars is depicted in Fig. 1.

Based on polymorphic data of SSR markers, two major groups were observed at linkage distance of 2.5 comprising of four genotypes (ATC909, ATC613, ATC912 and ATC1641) in the group A and all others in the group B (Fig. 2). These groups were further categorized in to six clusters. The group A consisted of one cluster, whereas group B comprised of five clusters at 50% linkage distance. The cluster II consisted three genotypes (ATC762P1, ATC742, ATC754), whereas cluster III consisted of eight genotypes, viz., ATC1191, ATC525, ATC912, ATC975, ATC967, ATC966, ATC756 and ATC754. The cluster IV consisted of two genotypes (ATC976 and ATC1744), cluster V consisted of one genotype (ATC1745) and two genotypes were grouped in cluster VI. It was observed that nine genotypes were grouped at maximum similarity, whereas the highest genetic distances were observed for the genotypes ATC912 vs ATC1744, ATC758 vs ATC613 and ATC976 vs ATC613 (Table 3). It is yet to be confirmed that the information on SSR markers are attributed toward race specificity or simple diversity among two races. Although the present information does not reveal clear understanding of bacterial blight races to be employed for MAS, however these results could be a bench mark for further investigations related to MAS or these could be used for fingerprinting of pea cultivars (Bevan et al., 1995; Burstin *et al.*, 2001). In order to apply molecular techniques in breeding program of any crop, particularly for the development of resistant cultivar, one needs to know the level of polymorphism as the first step. The resistant genotypes reported in the present study can be used in disease resistant cultivar development program.

References

Benlioglu, K., U. Ozyilmaz and D. Ertan, 2010. First report of bacterial blight caused by *Pseudomonas syringae* pv. *pisi* on pea in Turkey. *Plant Dis.*, 94: 232

Bevan, J.R., J.D. Taylor, I.R. Crute, J.P. Hunter and A. Vivan, 1995. Genetics of specific resistance in pea (*Pisum sativum*) cultivars to seven races of *Pseudomonas syringae* pv *pisi*. *Plant Pathol.*, 44: 98–108

Burstin, J., G. Deniot, J. Potier, C. Weinachter, G. Aubert and A. Barranger, 2001. Microsetillite polymorphisim in *Pisum sativum. Plant Breed.*, 120: 311–317

Doyle, J.J. and J.L. Doyle, 1990. Isolation of plant DNA from freshtissues. *Focus*, 12: 13–15

Elvira-Recuenco, M. and J.G. Taylor, 2001. Resistance to bacterial blight (*Pseudomonas syringae* pv. *pisi*) in Spanish pea *(Pisum sativum)* landraces, *Euphytica*, 118: 305–311

Hollaway, G.J., T.W. Bretag and T.V. Price, 2007. The epidemiology and management of bacterial blight (*Pseudomonas syringae* pv. *pisi*) of field pea (*Pisum sativum*) in Australia: a review. *Aust. J. Agric. Res.*, 58: 1086–1099

Malik, A.N., A. Vivian and J.D. Taylor, 1987. Isolation and partial characterisation of three classes of mutant in *Pseudomonas syringae* pv *pisi* with altered behaviour towards their host *Pisum sativum. J. Gen. Microbiol.*, 133: 2393–2399

Martín-Sanz, A., J.L Palomo, de la M.P. Vega and C. Caminero, 2011. Identification of pathovars and races of *Pseudomonas syringae*, the main causal agent of bacterial disease in pea in North-Central Spain, and the search for disease resistance. *Eur. J. Plant Pathol.*, 29: 57–69

Nasir, M., A. Ghafoor, M.R. Khan and A.S. Qureshi, 2006. Screening of *Pisum sativum* (L.) germplasm against *Erysiphe pisi* Syd. *Acta Biol. Cracoviensia Series Bot.*, 48: 33–37

Richardson, H.J. and G.J. Hollaway, 2011**.** Bacterial blight caused by *Pseudomonas syringae* pv. *syringae* shown to be an important disease of field pea in south eastern Australia. *Aust. Plant Pathol.*, 40: 260–268

Rogers, J.S., 1972. *IV. Measures of Genetic Similarity and Genetic Distance*, Vol. VII, pp: 145–153. In: Studies in genetics University of Texas Publication, Austin

Taylor, J.D., J.R. Beven, I.R. Crute and S.L. Reader, 1989. Genetic relationship between races of *Pseudomonas syringae* pv *pisi* and cultivars of *Pisum sativum*. *Plant Pathol.*, 38: 364–375

(Received 07 August 2012; Accepted 10 January 2013)