ISSN: 2319-6505

Available Online at http://journalijcar.org

International Journal of Current Advanced Research
Vol 5, Issue 10, pp 1320-1322, October 2016

RESEARCH ARTICLE

International Journal of Current Advanced Research

ISSN: 2319 - 6475

ESTIMATION OF CHANGES IN ENZYMATIC ACTIVITY DUE TO INFECTION OF SEED-BORNE BACTERIAL PATHOGENS ASSOCIATED WITH PEA (PISUM SATIVUM L.) SEEDS

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A R T I C L E I N F O

Article History:

Received 18th July, 2016
Received in revised form 2nd August, 2016
Accepted 18th September, 2016
Published online 28th October, 2016

Key words:

Pea seeds, Peroxidase, Polyphenoloxidase, Cellulase, *Pseudomonas syringae* pv. *pisi*, *Xanthomonas campestris* pv. *pisi*.

A B S T R A C T

The objective of this study was to determine the differences in expression of peroxidase, polyphenol oxidase and cellulase activities against seed-borne bacterial blight and leaf spot diseases caused by *Pseudomonas syringae* pv. *pisi* and *Xanthomonas pisi* respectively. The seed samples of pea carrying higher incidence of the respective pathogen as well as healthy seed samples were used for enzymatic activity analysis. In the present study a significant higher enzymatic activity of peroxidase, polyphenol oxidase and cellulase were found in infected seeds as compared to healthy seeds due to infection of bacterial pathogens.

INTRODUCTION

Pea (*Pisum sativum* L.) is an important pulse crop in global agriculture, providing biologically fixed nitrogen, breaking cereal disease cycle and contributing to locally grown food and feed (Martin-Sanz *et al*. 2011, Kotlarz *et al*. 2011 and Kocchar, 2009). Pea seeds are of great nutritional value due to high quality starch, protein and energy content. They have significant amounts of vitamins and minerals and are characterized by a relative high antioxidant activity (Han and Baik, 2008). One of the major constraints in increasing the productivity of pea is seed-borne pathogens. These seed-borne pathogens not only reduce the seed quality and yield but also spread the disease from one season to another. In the present study, two seed-borne bacterial pathogens namely *Pseudomonas syringae* pv. *pisi* and *Xanthomonas pisi* were isolated from infected pea seeds. Plant pathogens produce a large number of pectolytic and cellulolytic enzymes that degrade components of plant cell are the main virulence factor (Sutic and Sinclair, 2000). The interaction between the pathogen and host plant induces some changes in cell metabolism, primarily in the enzyme activities, including peroxidase and polyphenol oxidase (Salari *et al.* 2012 and Ngadze *et al.* 2012). Keeping in the view of above facts, the present study was undertaken to study the effect of seed-borne bacterial pathogens on the enzymatic activity (peroxidase, polyphenol oxidase and cellulase) of pea seeds.

MATERIALS AND METHODS

The seed samples of pea naturally infected with *Pseudomonas syringae* pv. *pisi* (acc. nos. Ps-2529, Ps-2592, Ps-3509 carrying 89.5%, 91.34% and 90% incidence respectively) and *Xanthomonas campestris* pv. *pisi* (acc. nos. Xp-2528, Xp-2542, Xp-2578 carrying 85%, 89.5% and 85% incidence respectively) were selected and used to find out the enzymatic activity in healthy and naturally infected seeds. Colorimetric methods (Malik and Singh, 1980) were used to study the changes in activity of peroxidase, polyphenol oxidase and cellulase.

Peroxidase (PEO)

Three hundred mg incubated seeds (for 7 days using SBM) of pea were ground in 3 ml of 0.1 m solution of phosphate buffer (pH 6.5). The homogenate was centrifuged at 10,000 rpm for 15 min at 4ºC and the supernatant was used for enzyme assay (Malik and Singh, 1980 and Mahatama *et al*. 2008). The reaction mixture in a cuvette contained 3.5 ml of 0.1 M phosphate buffer (pH 6.5). The reaction was initiated with the addition of 0.2 ml of enzyme and 0.1 ml of O-dianisidine solution (1 mg/ml in methanol). Temperature of the mixture was brought to 28-30ºC in a constant water bath and the cuvette was placed in to spectrophotometer which was set at 430 nm 0.2 ml hydrogen peroxide (0.2M) was added to the reaction mixture. Simultaneously a stopwatch was started and the initial absorbance (A_o) was recorded and then after interval of 30s, subsequent absorbance values were recorded upto 3 min. The graph was drawn for different increasing absorbance values against time and the enzyme activity was expressed in terms of increase of absorbance change in OD per unit per mg seed weight.

Polyphenol oxidase (PPO)

Three hundred mg of incubated (for 7 days using SBM) seeds were ground in 3 ml of 0.1 M sodium phosphate buffer, pH

6.0. The homogenate was centrifuged at 10,000 rpm for 15 min at 4ºC and the supernatant was used for enzyme assay (Malik and Singh, 1980, Mahatama *et al*. 2008). The reaction mixture contained 3 ml of catechol (0.01 M catechol in 0.1 M phosphate buffer, pH 6.0). The reaction was initiated with the addition of 0.1 ml of enzyme extract. The change in the colour due to the oxidized catechol was read at 495 nm for 5 min at an interval of every 30s. The enzymatic activity was expressed in terms of increase of absorbance per unit time per mg seed weight.

Cellulase

Three hundred mg of incubated (for 7 days using SBM) seeds were ground in 3 ml of 0.1 acetate buffer, pH 5.4. The homogenate was centrifuged at 10,000 rpm for 15 min at 4ºC and the supernatant was used for enzyme assay (Malik and Singh, 1980 and Mahatama *et al*. 2008). The reaction mixture contained 0.5 ml of 0.1 M acetate buffer (pH 5.4) and 1 ml of enzyme extract. The mixture was equilibrated at 30ºC. 1.5 ml of carboxymethyl cellulose was added and the solution was incubated for 2h at 30ºC. 3 ml of dinitrosalicylic acid was added to the tube and boiled for 3h. The mixture was cooled down and the absorbance was recorded at 560 nm standard curve was prepared by heating known amount of reducing sugar released by cellulose activity and enzymatic activity was expressed in terms of m moles glucose equivalent released in one h.

oxidase activity was recorded in naturally infected seed samples of pea. Polyphenol oxidase enzyme catalyzes the oxygen dependent oxidation of phenols into quinines are ubiquitous among angiosperms (Rai *et al*. 2011). The mean enzyme activity of polyphenol oxidase in the healthy seed samples was 0.244 Δ O.D./min/mg. A significant (P≥0.01) higher enzyme activity was found in both samples infected with *P. syringae* pv. *pisi* (0.980 Δ O.D./min/mg) and *X. campestris* pv. *pisi* (0.953 Δ O.D./min/mg) as compared to healthy samples (Table 1). Delannoy *et al*. (2003) also found significant increased activity of peroxidase enzyme was found after interaction of cotton and bacterial blight pathogen *X. campestris* pv. *malvacearum*.

Cellulase and hemicellulase have been found responsible for hydrolytic degradation of cellulose and hemicelluloses (Heitefuss and Williams, 1976). Cellulase, endoglucanase and protease enzymes have also been reported to be produced by pathovars of *Xanthomonas* namely *X. campestris* pv. *campestris* and *X. oryzae* pv. *oryzae* (Champoiseau *et al*. 2006). In this study, the average activity of cellulase in the healthy seed samples was 0.221 m mole glucose released/h. The enzyme activity was significantly (P≥0.01) higher in both seed samples infected with *P. syringae* pv. *pisi* (0.508 mM glucose released/h) and *X. campestris* pv. *pisi* (0.516 mM glucose released/h) as compared to the healthy samples (Table 1).

Table 1 Estimation of changes in enzymatic activity in seeds infected with pathogen

S. No.	Test seed sample (Acc. nos.)	Peroxidase Δ OD/min/mg seeds	Polyphenol oxidase Δ OD/min/mg seeds	Cellulase mMole glucose released/h
	Check (Healthy samples)			
1.	Acc. No. Pa-2510	0.349	0.227	0.207
	Acc. No. Pa-2522	0.453	0.219	0.245
	Acc. No. Pa-2564	0.371	0.288	0.213
	Mean	0.391	0.244	0.221
	Pseudomonas syringae pv. *pisi*			
2.	Acc. No. Ps-2529	1.159	0.906	0.441
	Acc. No. Ps-2556	1.215	1.108	0.561
	Acc. No. Ps-3509	1.210	0.927	0.523
	Mean	1.194	0.980	0.508
	Deviation (2-1)	+0.803**	+0.736**	+0.287**
	Xanthomonas campestris pv. *pisi*			
3.	Acc. No. Xp-2528	1.189	0.957	0.449
	Acc. No. Xp-2542	1.105	0.989	0.553
	Acc. No. Xp-2578	1.421	0.914	0.546
	Mean	1.283	0.953	0.516
	Deviation (3-1)	+0.892**	+0.799**	+0.295**
	S.Em.	0.61	0.041	0.015

RESULTS AND DISCUSSION

The interaction between the pathogen and host plant induces some changes in cell metabolism, primarily in the enzyme activities, including peroxidase and polyphenol oxidase (Salari *et al*. 2012 and Ngadze *et al*. 2012). Increase in oxygen species such as hydrogen peroxides, superoxide anions and hydroperoxyl radical has been demonstrated to occur in the early resistance response to plant pathogen attack (Do *et al*. 2003). In the present investigation, the mean enzyme activity of peroxidase in healthy seed samples was 0.391 Δ O.D./min/mg. In case of seed samples infected with *P. syringae* pv. *pisi* the peroxidase activity was found 1.194 Δ O.D./min/mg and 1.283 Δ O.D./min/mg in infected seed samples with *X. campestris* pv. *pisi*. The enzyme activity of infected samples was found significantly (P≥0.01) higher than healthy or uninfected samples (Table 1). A higher polyphenol

CONCLUSION

The present investigation showed that the natural infection of seed-borne bacterial pathogens on pea seeds is associated with a significant increase in the enzymatic activity of peroxidase, polyphenol oxidase and cellulase.

References

1. Champoiseau, P., Daugrois, J. H., Pieretti, I., Cociancich, S., Royer, M. and P. Rott (2006). High variation in pathogenicity of genetically closely related strains of *Xanthomonas albilineans*, the sugarcane leaf scald pathogen, in Guadeloupe. Phytopathol. 96(10), pp. 1081-1091.
2. Delannoy, E., Jalloul, A., Assigbetse, K., Marmey, P., Geiger, J. P., Lherminier, J., Daniel, J. F., Martinez, C. and M. Nicole (2003). Activity of class III peroxidases

in the defense of cotton to bacterial blight. Mol. Pl. Microbe Interac. 16(11), pp. 1030-1038.

3. Do, H. M., Hong, J. K., Jung, H. W., Kim, S. H., Ham, J. H. and B. K. Hwang (2003). Expression of peroxidase-like genes, H_2O_2 production, and peroxidase activity during the hypersensitive response to *Xanthomonas campestris* pv. *vesicatoria* in *Capsicum annuum*. Mol. Pl. Microbe Interac.16 (3),pp. 196-205.
4. Han, H. and B. K. Baik (2008). Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and soybeans (*Glycine max*) and their quantitative changes during processing. *Int. J. of Food Sciences and Tech.* 43,pp. 1971-1978.
5. Heitefuss, R. and H. P. Williams (1976). Physiological Plant Pathology. Springer-Verlag, Berlin. Pp. 891.
6. Kochhar, S. L. (2009). Economic Botany in the Tropics. Macmillan India Ltd., Daryaganj, New Delhi. Pp. 658.
7. Kotlarz, A., Sujak, A., Strobel, W. and W. Grzesiak (2011). Chemical composition and nutritive value of protein of the pea seeds-Effect of harvesting year and variety. Veg. Crops Res. Bulletin. 75,pp. 57-69.
8. Malik, C. P. and M. B. Singh (1980). Plant Enzymology and Histo-Enzymology: A text manual. Kalyani Publications, New Delhi/Ludhiana. pp. 434.
9. Martin-Sanz, A., Palomo J. L., Vega M. P. D. L., 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.* 129,pp. 57-69.
10. Ngadze, E., Icishahayo, D., Coutinho, T. A. and J. E. Van der Waals (2012). Role of polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, chlorogenic acid, and total soluble phenols in resistance of potatoes to soft rot. Plant Dis. 96,pp. 186-192.
11. Rai, G. K., Kumar, R., Singh, J., Rai, P. K. and S. K. Rai (2011). Peroxidase, polyphenol oxidase activity, protein profile and phenolic content in tomato cultivars tolerant and susceptible to *Fusarium oxysporum* f. sp. *lycopersici.* Pak. J. Bot. 43(6),pp. 2987-2990.
12. Salari, M., Panjekeh, N., Nasirpoor, Z. and J. Abkhoo (2012). Changes in total phenol, total protein and peroxidase activities in melon (*Cucumis melo* L.) cultivars inoculated with *Rhizoctonia solani*. *African J. Microbiol.* 6(37), pp. 6629-6634.
13. Sutic, D. D. and J. B. Sinclair (2000). Anatomy and physiology of Diseased Plants. CRC Press, Boston. pp. 232.

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