INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
12–448/2013/15–3–493–498
http://www.fspublishers.org

Full Length Article

Effect of Salicylic Acid on Acid Phosphatase Activity during Seed Development and Germination in Pea (*Pisum sativum*)

Ghulam Murtaza[1*] and Rehana Asghar[2]
[1]*Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad 13100, Pakistan*
[2]*Department of Biotechnology, Mirpur University of Science and Technology, Mirpur, Azad Jammu and Kashmir, Pakistan*
*For correspondence: gmurtazaq@hotmail.com

Abstract

In order to assess the effect of salicylic acid (SA) on activities of acid phosphatase (APase, EC 3.1.3.2) in four pea (*Pisum sativum* L.) varieties viz. Meteor, Climax, Green feast and Rondo during seed development and germination the field experiment were conducted using split-split plot design. The seeds were soaked in 0, 0.1 and 0.01 m*M* SA aqueous solutions for six hours. The plants were sprayed at phenological growth stage BBCH 60 with three SA concentrations mentioned previously in the early morning when the plants had their 3rd leaf completely expanded. The highest APase activity was recorded for the variety Greenfeast and for pea plants treated with SA concentration 0.1 m*M*, while the pea plants treated twice i.e., seed priming plus foliar spray revealed maximum APase activity. During seed germination of pea the maximum APase activity was observed for the variety Green feast, SA 0.1 m*M* treated seeds revealed maximum APase activity, while the seeds raised from the pea plants whose seeds were primed plus foliar sprayed exhibited maximum APase activity. © 2013 Friends Science Publishers

Keywords: Salicylic acid; Pea; *Pisum sativum*; Acid phosphatase; BBCH; Seed development

Introduction

One of the most important mineral nutrients for plant growth is phosphorous. The hydrolysis of phosphate esters is a critical process in the energy metabolism and metabolic regulation of plant cells. The ortho phosphate anion (Pi) not only plays a vital functional role in energy transfer and metabolic regulation, but is also an important structural constituent of many biomolecules. Consequently, Pi assimilation, metabolism and storage are of critical importance to plant growth and development. Efficient acquisition and utilization of phosphorous requires phosphatases, which function to hydrolyze Pi from orthophosphate monoesters (Vincent *et al*., 1992). Acid phosphatases are usually glycoproteins, which occur in a wide variety of species and tissues and feature considerable diversity with regard to their physical properties. However, they all appear to be important in the production, transport and recycling of Pi (Duff *et al*., 1994). APases appear to be ubiquitous since have been found in seed development, dormant seeds (Chung and Polya, 1992) and germinating seeds (Nishimura and Beevers, 1978). APases are involved in: providing P during seed germination from stored phytate (Brinch-Pedersen *et al*., 2002); and internal remobilization of P (Duff *et al*., 1991; Baldwin *et al*., 2001). Seed germination is regarded as the best developmental phase which results in a significant induction of APases. Although non-germinated seeds contain a lesser amount of constitutive APase activity, which increases up to 30-fold associated with a decrease in seed organic phosphate reserves usually accompanies germination (Gibson and Ullah, 1988; Biswas and Cundiff, 1991). Other developmental processes that cause APase induction include flowering and senescence (De Leo and Sacher, 1970; Lal and Jaiswal, 1988) and fruit ripening (Kanellis *et al*., 1989).

Salicylic acid (SA) or ortho-hydroxybenzoic acid belongs to a diverse group of plant phenolics and is widely distributed in monocots and dicot plants. SA could be actively transported, metabolized or conjugated and it could also translocate rapidly from the point of initial application to different plant tissues (Popova *et al*., 1997). SA has been reported to have many functions in plants, like it increases stomatal closure in *Vicia faba* and *Commeliana communis* (Nugroho *et al*., 2001). The inflorescence of thermogenic plant and infected plant with necrotizing pathogens were found to have highest levels of SA. It stimulated flowering in *Impatiens, Lemna,* and *Arabidopss thaliana* inhibited the biosynthesis of ethylene, root uptake (Raskin *et al*., 1990; Raskin, 1992; Martínez *et al*., 2004). Exogenous application of SA also partially reverses the inhibitory effect of oxidative (0.5 m*M* paraquat) and heat stress (50°C for 3 h) on seed germination (Alonso-Ramırez *et al*., 2009). SA plays a crucial role in the regulation of physiological and biochemical processes during the entire lifespan of the plant (Vicente and Plasencia, 2011). According to Van de Rhee *et al*. (1990) SA induces peroxidases, superoxide dismutase

To cite this paper: Murtaza, G. and R. Asghar, 2013. Effect of salicylic acid on acid phosphatase activity during seed development and germination in pea (*Pisum sativum*). *Int. J. Agric. Biol*., 15: 493–498

and glycine rich wall protein, all of which are commonly induced by pathogen and thought to function in defense. The SA treatment of wheat plants increased the rate of cell division within the apical meristem of seedling roots causing an increase in plant growth and elevated wheat productivity (Shakirova *et al*., 2003). The aim of the present study was to investigate the effect of SA on the activities of APase during seed development and germination in pea.

Materials and Methods

The field experiments were conducted during the season 2003-2004 and 2004-2005 at Vegetable Seed Production Farm, Chattar Klas and Vegetable Research Station, Jalalabad, District Muzaffarabad, Pakistan. Four pea (*Pisum sativum* L.) varieties i.e., Climax, Green feast, Meteor and Rondo were planted in split-split plot fashion following randomized complete block design (RCBD) with three replicates. The main plots were assigned to pea cultivars, with aqueous SA concentrations, 0, 0.1 and 0.01 m*M* (Murtaza *et al.*, 2010) as subplots and modes of application of SA (seed priming (SD); seed priming plus foliar spray (SPFS) and foliar spray (FS) only) as sub-sub-plots. For seed priming, the seeds were soaked in the SA concentrations for six hours (Benavides-Mendoza *et al*., 2002). The plants were sprayed at phenological stage BBCH 60 (first flower open sporadically within the population) with SA concentrations in the early morning when the plants had their 3rd leaf completely expanded (Gutierrez-Coronado *et al*., 1998).

Samples were collected at three different phenological growth stages i.e., BBCH zero (germination), BBCH seven (fruit development) and BBCH eight (ripening of fruit and seed), respectively (Weber and Bleiholder, 1990; Feller *et al*., 1995). For BBCH 7 and 8, the pods were collected at BBCH 73 (30% of pods reached average maximum length), BBCH 77 (70% of pods reached average maximum length), BBCH 83 (30% of pods ripe, dry and hard) and BBCH 88 (80% of pods ripe, dry and hard). The seeds obtained from the crop were used for germination experiment. The seeds were surface sterilized (70% ethanol for 1 minute and 5 percent sodium hypochlorite for 5 min) and thoroughly rinsed in distilled water. The seeds were sown in plastic trays filled with the same soil that was used for cropping. For phenological growth stage zero, the samples were collected at BBCH 01 (beginning of seed imbibition), at BBCH 03 (seed imbibition complete) and at BBCH 05 (radicle emergence from the seed). All samples were stored at -50°C in a deep freezer till further use.

APase was assayed according to Asghar and DeMason (1992). The seeds were ground in 30 m*M* Tris-Mes buffer (pH 5.0) and extracted for 2 h at 4°C. The slurry was centrifuged at 10,000 g for twenty minutes and the supernatant was used for enzyme assay. APase activity was measured using *p*-nitrophenyl phosphate as the substrate. 0.1 mL of extract was incubated for 15 min with 0.9 mL of 3 m*M* substrate dissolved in 30 m*M* Tris-Mes buffer at pH 5.0. The reaction was stopped by adding 3 ml sodium carbonate (2%). The *p*-nitrophenol released was measured spectrophotometrically at 410 nm. A blank was prepared by adding the enzyme extract after sodium carbonate. Protein content was determined according to Bradford (1976) with BSA as a standard. The data were subjected to analysis of variance using the MSTAT-C program and Duncan's Multiple Range Test was applied to differentiate the means (Steel *et al*., 1997).

Results and Discussion

At all four phonological growth stages a statistically significant difference ($p < 0.001$) was found among varieties in terms of APase activity during years 2003-04 and 2004-05 (Table 1 and Fig. 1). All the varieties revealed highest activity of APase at BBCH 73 in during both growing seasons, whereas, the lowest APase activity was recorded for all varieties at BBCH 88. The variety Greenfeast exhibited the highest APase activity while the Rondo revealed lowest APase activity in both seasons.

The APase activity was influenced by SA concentrations and was significant ($p < 0.001$) at all the four phenological stages during both seasons (Table 1 and Fig. 2). The maximum APase activity was recorded for the plants treated with 0.1 m*M* SA at all the four phonological stages. The interaction between SA and varieties was found significant during the year 2003-2004 at BBCH 77 and during year 2004-2005 at BBCH 73 stages.

During the both years the modes of application of SA were significantly different regarding the APase activities at all the four phenological stages (Table 1 and Fig. 3). The highest APase activity was recorded for the plants whose seeds were primed plus foliar application applied followed by the plants treated with foliar application and the least APase activity for the plants whose seeds were primed. The interaction between varieties and modes of application; SA and modes of application; SA, varieties and modes of application was found nonsignificant in terms of APase activity.

The effect of SA on APase activities were observed at phenological stages BBCH 01, BBCH 03 and BBCH 05 in 2003-2004 and 2004-2005. A statistical significant difference was recorded among four pea varieties ($p>0.001$) in both years (Table 2 and Fig. 4). The Maximum APase activity was observed for the variety Green feast at all three phenological growth stages during both growing seasons.

The effect of SA concentrations was significant ($p>0.001$) in terms of APase activity at all three phenological stages in both seasons (Table 2 and Fig. 5). The highest APase activity was recorded for the plants treated with SA concentration 0.01 m*M*. The interaction between varieties and SA concentrations was found non-significant.

Table 1: Mean squares from the analyses of variance and coefficient of variation (C.V.) of activities of APase (µmol *p*-nitrophenol released min^{-1} mg^{-1} protein) in pea (*Pisum sativum* L.) varieties treated with SA by different modes of application at phenological growth stage BBCH 73 (30% of pods have reached average maximum length), BBCH 77 (70% of pods have reached average maximum length), BBCH 83 (30% of pods ripe, dry and hard) and BBCH 88 (80% of pods ripe, dry and hard) during year 2003-2004 and 2004-2005

Source of variation	df	BBCH 73		BBCH 77		BBCH 83		BBCH 88	
		2003-2004	2004-2005	2003-2004	2004-2005	2003-2004	2004-2005	2003-2004	2004-2005
Replications	2	0.16	1.24	2.45	0.79	3.37	0.41	0.13	1.95
Varieties	3	68.57***	103.29***	118.51***	132.67***	41.05***	72.46***	57.82***	29.53***
Error a	6	1.61	2.58	4.04	1.79	0.90	0.35	1.57	0.86
Salicylic acid	2	45.49***	64.34***	34.13***	21.20***	26.23***	14.89***	21.91***	15.41***
V x S	6	1.96	11.15**	13.24**	2.24	2.18	0.91	1.06	0.82
Error b	16	1.22	2.45	2.17	1.87	1.63	1.28	0.66	1.19
Mode	2	20.95***	109.99***	98.75***	57.87***	80.08***	28.90***	33.04***	21.38***
V x M	6	1.09	0.82	0.27	0.13	0.87*	0.15	0.38	0.08
S x M	4	0.55	2.87*	0.34	0.39	0.55	0.22	0.45	0.17
V x S x M	12	1.35	0.48	0.34	0.19	0.46	0.25	0.11	0.22
Error c	48	1.38	0.80	0.66	0.40	0.34	0.47	0.29	0.23
C.V.		11.40	9.04	8.55	8.51	9.41	8.38	11.05	8.86

Table 2: Mean squares from the analyses of variance and coefficient of variation (C.V.) of the activities of APase in pea (*Pisum sativum* L.) varieties treated with SA concentrations by different modes of application during year 2003-2004 and 2004-2005 at phenological growth stage BBCH 01 (beginning of seed imbibition), BBCH 03 (seed imbibition complete) and BBCH 05 (radicle emerged from seed)

Source of variation	df	BBCH 01		BBCH 03		BBCH 05	
		2003-2004	2004-2005	2003-2004	2004-2005	2003-2004	2004-2005
Replications	2	4.35	6.44	0.85	0.31	11.27*	1.06
Varieties	3	159.13***	179.77***	340.25***	162.37***	147.54***	230.58***
Error a	6	2.82	6.58	4.25	4.78	1.19	4.18
Salicylic acid	2	38.12***	87.61***	125.56***	119.46***	62.13***	129.25***
V x S	6	2.37	5.19	5.40	15.33	3.0	5.73
Error b	16	1.85	2.86	4.68	5.55	1.33	4.81
Mode	2	66.81***	277.53***	257.52***	361.31***	392.79***	262.63***
V x M	6	0.16	0.14	2.87**	0.17	1.90*	3.14**
S x M	4	0.18	0.06	1.35	0.57	20.97	1.21
V x S x M	12	0.22	0.24	0.48	0.55	3.96	0.58
Error c	48	0.40	0.43	40.53	0.64	0.66	0.89
C.V.		5.55	4.84	6.46	4.38	7.02	7.19

*, **, *** significant at P= 0.05, P= 0.01 and P= 0.001, respectively

There was a significant difference between modes of application of SA in terms of APase activity ($p > 0.001$) at all three phenological stages during year 2003-2004 and 2004-2005 (Table 2 and Fig. 6). The maximum APase activity was recorded for the plants whose seeds were treated plus leaf sprayed followed by that of the foliar sprayed. A non-significant interaction between SA and modes of application; between varieties, SA and modes of application was recorded during both years.

Phosphorus is one of the most important mineral nutrient for plant growth but least available. The efficient acquisition and utilization of phosphorus requires a ubiquitous class of enzymes known as phosphatases, which function to hydrolyse Pi from orthophosphate monoesters. Acid phosphatases are enzymes highly expressed in plants and especially in plant tissues such as roots, bulbs, seeds, tubers, coleoptiles and leaves (Yan *et al*., 2001; Tejera Garcia *et al*., 2004). Different developmental processes that cause APase induction include flowering and senescence (De Leo and Sacher, 1970; Lal and Jaiswal, 1988), fruit ripening (Kanellis *et al*., 1989) and seed germination (Gibson and Ullah, 1988). The present study revealed that the specific activity of APase was increased in pea by SA at all four stages of fruit development studied. Pea varieties showed inconsistent results in terms of APase activities during fruit development. At phenological stage BBCH 77 the specific activity was higher than that of phenological stage BBCH 73, while there was a linear decrease in the APase activity at phenological stages BBCH 83 and BBCH 88. Moreover the APase activity was higher in SA concentration 0.1 m*M* and in the STFS plants as compared with ST and FS plants. The highest activity of APase at phenological stage BBCH 73 can be correlated with very high rate of mobilization of phosphorous as needed for plant growth as a structural component of biomolecules. As previously described, the fruit ripening may be one of the cause resulting an increase in the activities of APX during BBCH 77 growth stage. At this stage the pods have attained the maximum length and there is a need of increase in the size of the seed resulting high metabolic rate. This high metabolic rate during the seed development may result in an increase of APase activity.

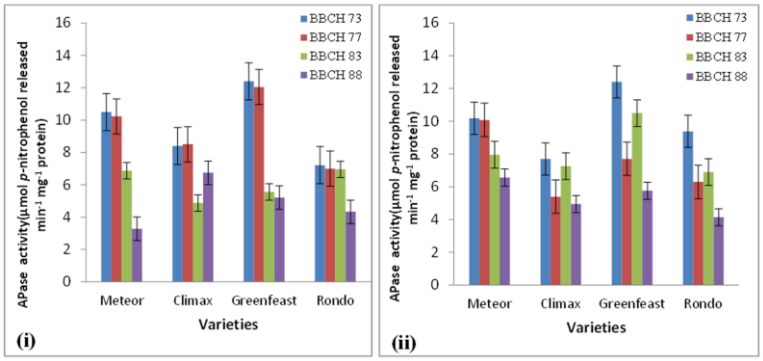

Fig. 1: Effect of SA on the activity of APase in pea varieties at four phenological stages in 2003-2004 (i) and 2004-2005 (ii). The means of three replicates are shown and standard errors are indicated

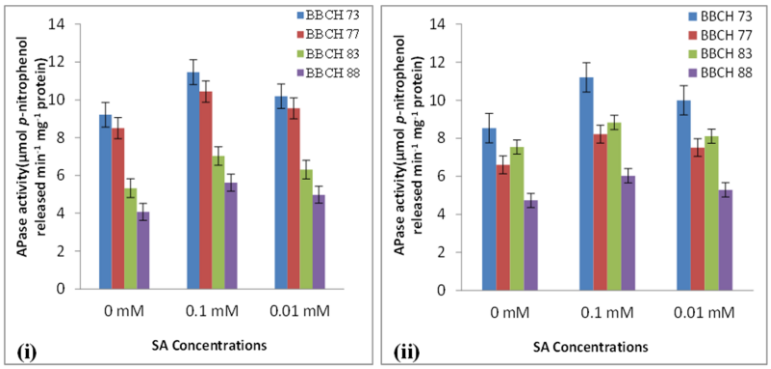

Fig. 2: Effect of SA concentrations on the activity of APase in pea at four phenological stages in 2003-2004 (i) and 2004-2005 (ii). The means of three replicates are shown and standard errors are indicated

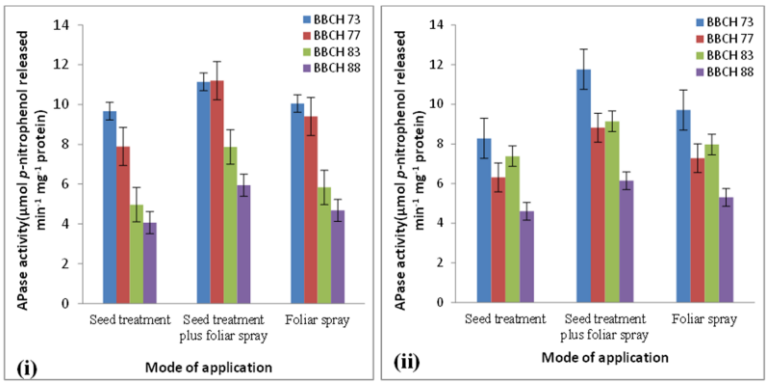

Fig. 3: Effect of modes of application of SA on the activity of APase in pea at four phenological stages in 2003-2004 (i) and 2004-2005 (ii). The means of three replicates are shown and standard errors are indicated

Seed germination is one of the best characterized developmental phase to study the pattern of specific activities of APase. Although ungerminated seeds contain a small amount of constitutive APase activity, a large increase in APase activity (up to 30-fold) concomitant with a decrease in seed organic phosphate reserves usually accompanies germination (Gibson and Ullah, 1988; Biswas and Cundiff, 1991). It has long been recognized that APase activity in plants typically increases when plant becomes phosphorus (Pi) deficient. The increase in APase activity correlates with a low level of Pi in numerous species and plant parts (Chen *et al*., 1990). Salt, water and osmotic stress have also been reported to increase APase activity (Szabo-Nagy *et al*., 1992; Kaur *et al*., 2012). These researches have demonstrated that the induction of APase under osmotic and salt stresses was not accompanied by a decrease in Pi level. Methyl jasmonate markedly induced APase activity in rice leaves and this induction was not caused by a decrease in Pi

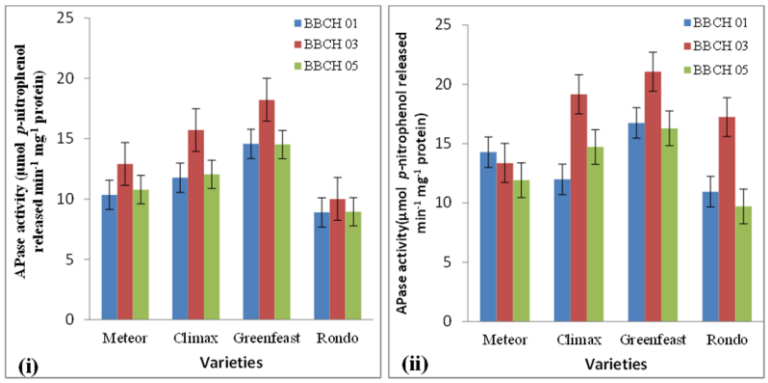

Fig. 4: Changes in activities of APase in the seeds raised from SA treated pea varieties in 2003-2004 (i) and 2004-2005 (ii) at three phenological stages. The means of three replicates are shown and standard errors are indicated

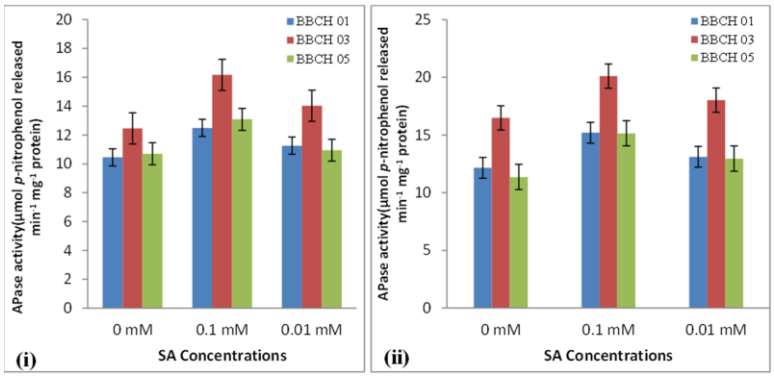

Fig. 5: Effect of different SA concentrations on the activity of APase at three phenological stages in 2003-2004 (i) and 2004-2005 (ii). The means of three replicates are shown and standard errors are indicated

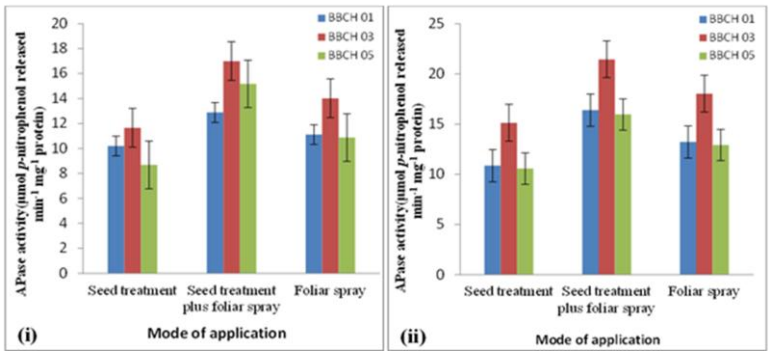

Fig. 6: Effect of different modes of applications of SA on the activity of APase in pea during 2003-2004 (i) and 2004-2005 (ii). The means of three replicates are shown and standard errors are indicated

level (Yeh *et al*., 1995). In the present study the specific activities of APase were lowest at BBCH 01 phenological stage then there was an increase in APase activity at phenological stage BBCH 03 and finally a decline towards the phenological stage BBCH 05 in all the four varieties during seed germination. A statistically significant increase in acid phosphatase activity with SA treatment is consistent with earlier studies (Beβer *et al*., 2000; Law, 2011). The seed raised from SA concentration 0.1 m*M* treated exhibited highest activity of APase, while seeds raised from STFS had maximum value of APase activity. The low activities of APase at phenological stage BBCH 01 may be due to comparatively inactive or very low activity stage where seed is just gaining water for physiological activities. However the decrease in APase at phenological stage BBCH 05 can be correlated to a decrease in seed's organic

phosphate reserve. The results of the present study are in agreement with earlier studies (Shih and Kao, 1998; Beβer *et al*., 2000; Law, 2011).

References

Alonso-Ramırez, A., D. Rodrıguez, D. Reyes, J.A. Jimenez, G. Nicolas, M. Lopez-Climent, A. Gomez-Cadenas and C. Nicolas, 2009. Evidence for a role of gibberellins in salicylic acid-modulated early plant responses to abiotic stress in Arabidopsis seeds. *Plant Physiol*., 150: 1335–1344

Asghar, R. and D.A. DeMason, 1992. Differential activities of acid phosphatase from adaxial and abaxial regions of *Lupinus luteus* (Fabaceae) cotyledons. *Amer. J. Bot*., 79: 1134–1144

Baldwin, J.C., S.K. Athikkattuvalasu and K.G. Raghothama. 2001. *LEPS2*, a phosphorus starvation-induced novel acid phosphatase from tomato. *Plant Physiol*., 125: 728–737

Beβer, K., B. Jarosch, G. Langen and K. Kogel, 2000. Expression analysis of genes induced in barley after chemical activation reveals distinct disease resistance pathways. *Mol. Plant Pathol*., 1: 277–286

Benavides-Mendoza, A., H. Ramirez-Rodriguez, V. Robledo-Torres, J. Hernandez-Davila, J.G. Ramirez-Mezquitic, E. Bacopulos-Tellez, A. Sandoval-Rangel and M.A. Bustamante-Garcia, 2002. Seed treatment with salicylates modifies stomatal distribution, stomatal density and the tolerance to cold stress in pepper seedlings. *Proceedings of the 16th International Pepper Conference*, p: 2. (Tampico, Tamaulipas, Mexico, November 10th-12th, 2002)

Biswas, T.K. and C. Cundiff, 1991. Multiple forms of acid phosphatase in germinating seeds of *Vigna sinensis*. *Photochemistry*, 30: 2119–2125

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgramquantities of proteins utilizing the principle of protein dye-binding. *Anal. Biochem.*, 72: 248–254

Brinch-Pedersen, H., L.D. Sorenson and P.B. Holm, 2002. Engineering crop plants: getting a handle on phosphate. *Trends Plant Sci*., 7: 118–125

Chen, C.T., I.T. Chou and C.H. Kao, 1990. Senescence of rice leaves XX. Changes of protein secretion during senescence. *Plant Sci*., 66: 29–34

Chung, R.P.T. and G.M. Polya, 1992. Copurification and characterization of poppy seed phosphatase and phosphoprotein phosphatase activities. *Plant Sci*., 84: 153–I62

De Leo, P. and J.A. Sacher, 1970. Control of ribnuclease and acid phosphatase by auxin and abscisic acid during senescence of *Rhoeo* leaf sections. *Plant Physiol*., 46: 806–811

Duff, S.M.G., G. Sarath and W.C. Plaxton, 1994. The role of acid phosphatases in plant phosphorus metabolism. *Physiol. Plant*., 90: 791–800

Duff, S.M., W.C. Plaxton and D.D. Lefebvre, 1991. Phosphate-starvation response in plant cells: de novo synthesis and degradation of acid phosphatases. *Proc. Natl. Acad. Sci. USA*, 88: 9538–9542

Feller, C., H. Bleiholder, L. Buhr, H. Hack, M. Hes, R. Klose, U. Meier, R. Stauss, T. Van Den Boom and E. Weber, 1995. Phänologische Entwicklungsstadien von Gemüsepflanzen: II. *Fruchtgemüse und Hülsenfrüchte. Nachrichtenbl. Deut. Pflanzenschutzd*, 47: 217–232

Gibson, D.M. and A.H. Ullah, 1988. Purification and characterization of phytase from cotyledons of germinating soybean seeds. *Arch. Biochem. Biophys*., 260: 503–513

Gutierrez-Coronado, M.A., C. Trejo-Lopez and A. Larque-Saavedra, 1998. Effects of salicylic acid on the growth of roots and shoots in soybean. *Plant Physiol. Biochem.*, 36: 563–565

Kanellis, A.K., T. Solomos and A.K. Mattoo, 1989. Changes in sugars, enzymic activities and acid phosphatase isoenzyme profiles of bananas ripened in air or stored in 2.5% O2 with and without ethylene. *Plant Physiol.*, 90: 251–258

Kaur, S., M. Arora, A.K. Gupta and N. Kaur, 2012. Exploration of biochemical and molecular diversity in chickpea seeds to categorize cold stress-tolerant and susceptible genotypes. *Acta Physiol. Plant*, 34: 569–580

Lal, M. and V.S. Jaiswal, 1988. Modification of flower sex and acid phosphatase activity by phthalimides in female plants of *Morgus nigra* L. *Plant Growth Regul*., 7: 29–37

Law, T.F.B., 2011. *Exploring the Disease Resistance Response (Chitinase, Lysozyme, Acid Phosphatase and Total Phenolic Content) of Kowhai (Sophora microphylla x S. chathamica) to Hormones (Salicylic Acid and Ethylene)*, p: 101. Doctoral dissertation, Auckland University of Technology, New Zealand

Martínez, C., E. Pons, G. Prats and J. León, 2004. Salicylic acid regulates flowering time and links defence responses and reproductive development. *Plant J*., 37: 209–217

Murtaza, G., R. Asghar and S.A. Majid, 2010. Changes in specific activity of ascorbate peroxidase during seed development of pea (*Pisum sativum* L.) treated with salicylic acid. *Afr. J. Biotechnol*., 9: 5333–5337

Popova, L., T. Pancheva and A. Uzunova, 1997. Salicylic acid: properties, biosynthesis and physiological role. *Bulg. J. Plant Physiol*., 23: 85–93

Nishimura, M. and H. Beevers, 1978. Hydrolases in vacuoles from castor bean endosperm. *Plant Physiol*., 62: 44–48

Nugroho, L.H., M.C. Verberne and R. Verpoorte, 2001. Salicylic acid produced by isochorismate synthase and isochorismate pyruvate lyase in various parts of constitutive salicylic acid producing tobacco plants. *Plant Sci.*, 161: 911–915

Raskin, I., 1992. Salicylate, a new plant hormone. *Plant Physiol*., 99: 799–804

Raskin, I., H. Skubatz, W. Tang and B.J.D. Meeuse, 1990. Salicylic acid levels in thermogenic and non-thermogenic plants. *Ann. Bot.*, 66: 376–378

Shakirova, F.M., A.R. Sakhabutdinova, M.V. Bezrukova, R.A. Fatkhutdinova and D.R. Fatkhutdinova, 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci*., 164: 317–322

Shih, C.Y. and C.H. Kao, 1998. Induction of acid phosphatase in detached rice leaves under stress conditions. *Bot. Bull. Acad. Sin*., 39: 29–32

Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1997. *Principles and Procedures of Statistics*, 3rd edition. McGraw Hill, New York, USA

Szabo-Nagy, A., G. Galiba and E. Erdei, 1992. Induction of soluble phosphatases under ionic and non-ionic osmotic stress in wheat. *J. Plant Physiol*., 140: 629–633

Tejera Garcia, N.A., M. Olivera, C. Iribarne and C. Lluch, 2004. Partial purification and characterization of a non-specific acid phosphatase in leaves and root nodules of *Phaseolus vulgaris. Plant Physiol. Biochem.*, 42: 585–591

Yan, X., H. Liao, M.C. Trull, S.E. Beebe and J.P. Lynch, 2001. Induction of a major leaf acid phosphatase does not confer adaptation to low phosphorus availability in common bean. *Plant Physiol.*, 125: 1901–1911

Yeh, C.C., H.S. Tsay, J.H. Yeh, F.Y. Tsai, C.Y. Shih and C.H. Kao, 1995. A comparative study of the effects of methyl jasmonate and abscisic acid on some rice physiological process. *J. Plant Growth Regul.*, 14: 23–28

Van de Rhee, M.D., J.A.L. Van Kan, M.T. Gonzales-Jean and J.F. Bol, 1990. Analysis of regulatory elements involved in the induction of two tobacco genes by salicylate treatment and virus infection. *Plant Cell*, 2: 357–366

Vicente, M.R. and J. Plasencia, 2011. Salicylic acid beyond defence: its role in plant growth and development. *J. Exp. Bot.*, 62: 3321–3338

Vincent, J.B., M.W. Crowder and B.A. Averill, 1992. Hydrolysis of phosphate monoesters: a biological problem with multiple chemical solutions. *Trends Biochem. Sci*., 17: 105–110

Weber, E. and H. Bleiholder, 1990. Erläuterungen zu den BBCH-Dezimal-Codes für die Entwicklungsstadien von Mais, Raps, Faba-Bohne, Sonnenblume und Erbse-mit Abbildungen. *Gesunde Pflanzen*, 42: 308–321

(Received 18 July 2012; Accepted 05 November 2012)