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EFFECTS OF SALICYLIC ACID ON SUCROSE SYNTHASE ACTIVITY DURING SEED DEVELOPMENT AND GERMINATION IN PEA (*PISUM SATIVUM* L.)

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Key words: *Pisum sativum*, Pea, Salicylic acid, Sucrose synthase, Seed development, Germination

Abstract

Effects of 0, 0.1 and 0.01 mM salicylic acid (SA) on activities of sucrose synthase (Sus) in four varieties of pea (*Pisum sativum* L.) *viz.*, Meteor, Climax, Green feast and Rondo during seed development and germination were studied. Sus activity was highest in the variety Meteor and for the varieties treated with SA (0.1 mM), while the varieties treated twice i.e. seed treated plus foliar spray revealed maximum Sus activity. During germination, the maximum Sus activity was observed for the variety Green feast. Seeds treated with SA 0.1 mM revealed maximum Sus activity, while the seeds raised from the varieties and whose seeds were treated plus foliar sprayed exhibited maximum Sus activity.

Introduction

Salicylic acid (SA) is a ubiquitous phenolic compound occurring in plants in very low amounts and has been reported to regulate the physiological processes in plants such as nutrient uptake, stomatal closure, inhibition of ethylene biosynthesis, chlorophyll synthesis, protein synthesis, photosynthesis and transpiration (Khan *et al.* 2003, Raskin 1992, Shakirova *et al.* 2003). The expression of pathogenesis related protein genes has been regulated by SA, suggesting its key role as a signal molecule by providing resistance against pathogen attack (Raskin 1992). The application of salicylic acid revealed an increase in number of pods and yield in mung bean (Sing and Kaur 1980).

The developing seed is a sucrose sink on the intact plant and during germination it becomes a source plus a sink as the cotyledons produce sucrose and the young embryonic plant consumes sucrose. The sucrose synthase (Sus) is a key enzyme of metabolism of carbohydrate in all the plant species. Sus is thought to be responsible for the mobilization of sucrose in sink organs including pea embryos, and to be determinant of sink strength in these organs (Zrenner *et al.* 1995, Edwards and Rees 1986a,b). Sus is implicated in a wide variety of processes, including starch synthesis (Ricard *et al.* 1998) and phloem transport (Geigenberger *et al.* 1993). The activity of Sus in pea testa is proposed to help the interconversion of sucrose and starch and thus buffering supply of carbohydrates to the immature embryo (Rochat and Boutin 1992). The present study was undertaken to examine the effects of SA on the activity of Sus during seed development and germination in pea.

Materials and Methods

The field experiments were carried out during the growing seasons of 2003 and 2005 at Vegetable Seed Production Farm, Chattar Klas, Muzaffarabad, Azad Kashmir, Pakistan. Four varieties of pea (*Pisum sativum* L.) i.e. Meteor, Climax, Green feast and Rondo were obtained

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from Ayub Agricultural Research Institute (AARI), Faisalabad. The crops were planted in split-split plot fashion with three replicates. The main plots were assigned to pea cultivars, with salicylic acid (0, 0.1 and 0.01 mM in water) as subplots and modes of application of salicylic acid (seed treatment, seed treatment plus foliar spray and foliar spray only) as sub-sub-plots. The soil was silt loam with pH 7.2. After thoroughly preparing, the soil was added with N: P: K @ 45: 90: 90 kg/ha as urea, single super phosphate (SSP) and sulphate of potash (SOP). Half of the amount of urea was added at soil preparation and other half at 45 days after germination. The main plot was divided into subplots and sub-subplots. Each sub-subplot measured 3 × 1.8 m^2 (5.4 m^2) with plant to plant distance 15 cm and row to row distance 90 cm (Hussain and Badshah 2002). There were two rows in each sub-subplot and 20 plants in each row. Weeding, hoeing and other agronomic practices were carried out uniformly as and when needed. For germination experiments, the seeds obtained from the crop were sown in plastic trays filled with the same soil that was used for growing the crop in the field. The germination experiments and the enzyme assays and protein estimations were carried out in the Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan.

Salicylic acid treatment and sampling: Salicylic acid (Sigma) (MW 138.1) was dissolved in a few drops of dimethylsulfoxide and added to a ultimate volume one liter of distilled water. The pH was adjusted at 5.5 with KOH (1.0 N). Three concentrations of SA (0, 0.1 and 0.01 mM) were maintained. The seeds were soaked in SA concentrations for six hours. The plants were sprayed at phenological stage after Biologische Bundesanstalt, Bundesortenamt and Chemical Industry, BBCH 60 (first flower open sporadically within the population) with aqueous solutions SA concentrations during early morning when the 3rd leaf of the plants completely expanded. In all cases spraying were carried out using a manual pump (Gutierrez-Coronado *et al.* 1998). Pods were sampled at three different phenological growth stages i.e. BBCH zero (germination), BBCH 7 (fruit development) and BBCH 8 (ripening of fruit and seed), respectively (Feller *et al.* 1995, Weber and Bleiholder 1990). For BBCH 7 and 8, the pods were collected at BBCH 73 (30 per cent of pods reached average maximum length), BBCH 77 (70 per cent of pods reached average maximum length), BBCH 83 (30 per cent of pods were ripe, dry and hard) and BBCH 88 (80 per cent of pods ripe, dry and hard). The seeds obtained from the crop were used for germination experiment. The seeds were surface sterilized (70 per cent ethanol for 1 minute and 5 per cent sodium hypochlorite for 5 minutes) and thoroughly rinsed with distilled water. 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.

Sucrose synthase extraction and assays: Sucrose synthase was extracted using the method of Rochat and Boutin (1992). Sus activity was assayed for sucrose cleavage by monitoring the synthesis of UDP-glucose following the method of Weber *et al*. (1996). Protein content was determined according to Bradford (1976) with BSA as a standard.

Results and Discussion

A statistically significant difference ($p < 0.001$) was found among varieties in terms of Sus activity at all the four phenological stages (Table 1, Fig. 1). Over all, the varieties revealed highest activity of Sus at BBCH 73 in the both seasons, whereas, the lowest Sus activity was recorded for all the varieties at BBCH 88. The highest Sus activity was observed for the variety Meteor and the lowest Sus for the variety Green feast in both the seasons.

The effect of SA concentrations on Sus activity was significant ($p < 0.001$) at all the four phenological stages during both seasons (Table 1, Fig. 2). The maximum Sus activity was

recorded for the plants treated with 0.1 mM SA at all the four stages. The interaction between SA and varieties was non-significant.

The modes of application of SA were significantly different in terms of Sus activities at all the four phenological stages (Table 1, Fig. 3). The maximum Sus activity was recorded for the plants whose seeds were treated plus foliar application applied followed by the plants treated with foliar application and the least Sus activity for the plants whose seeds were treated only. A significant interaction ($p > 0.001$) between varieties and modes of application was recorded at BBCH 73 in both the seasons, while at BBCH 73 and BBCH 77 the interaction was significant in 2004-2005

Table 1. Mean squares from the analyses of variance and coefficient of variation (CV) of activities of Sus (µmol min/mg/protein) in pea varieties treated with salicylic acid by different modes of application at different phenological stages.

Source of variation	Df	Phenological stage							
		BBCH 73		BBCH 77		BBCH 83		BBCH 88	
		2003-'04	2004-'05	2003-'04	2004-'05	2003-'04	2004-'05	2003-'04	2004-'05
Replications	2	1.70	3.46	10.33*	0.95	3.55	5.41	1.24	12.76**
Varieties (V)	3	76.20***	198.59***	69.26***	47.10***	55.44***	100.07***	58.85***	102.42***
Error a	6	2.60	4.13	1.25	1.35	1.92	3.89	2.19	1.14
Salicylic acid (S)	2	60.02***	68.25***	65.99***	52.89***	81.89***	39.81***	39.54***	29.05***
V × S	6	2.83*	4.39	0.99	0.59	2.10	1.77	2.18	3.52
Error b	16	1.01	3.00	1.32	0.35	1.51	1.57	1.27	1.31
Mode (M)	2	179.68***	215.29***	69.86***	90.73***	38.84***	53.75***	38.76***	60.45***
V × M	6	4.12***	6.68***	0.25	0.65*	0.55**	0.15	0.20	0.18
S × M	4	4.52***	6.35**	0.02	0.93*	0.66**	0.56*	0.19	0.53
V × S × M	12	0.79	1.17	0.20	0.19	0.24	0.20	0.10	0.36
Error c	48	0.42	1.42	0.13	0.27	0.13	0.16	0.14	0.19
CV		4.46	7.93	2.52	3.69	3.11	3.45	4.63	4.56

*, **, *** Significant at p = 0.05, 0.01 and 0.001, respectively.

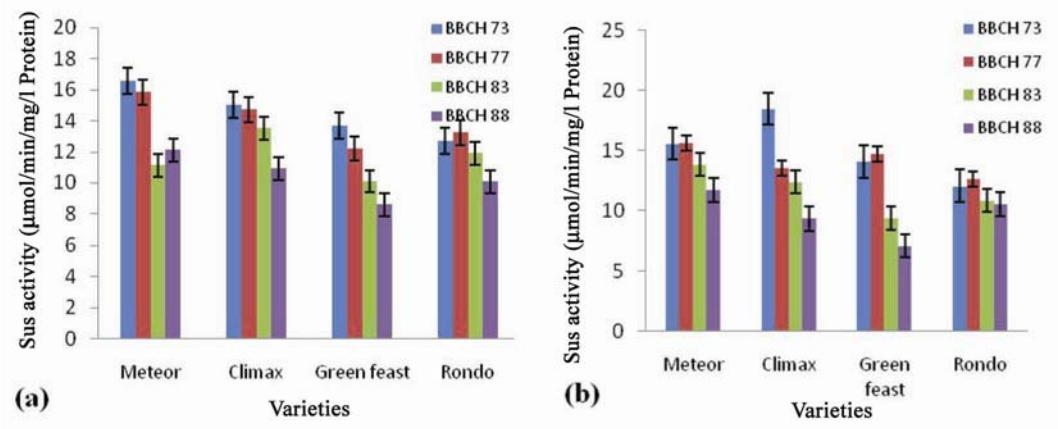

Fig. 1. Effect of SA on the activity of sucrose synthase in pea varieties at four phenological stages in 2003-2004 (a) and 2004-2005 (b). The means of three replicates are shown and standard errors are indicated.

and 2003-2004, respectively in terms of Sus activity. At all the remaining phenological stages, the interaction between varieties and modes of application was nonsignificant. A significant interaction was also recorded between SA concentrations and modes of application at BBCH 73,

BBCH 77 and BBCH 83, while it was found nonsignificant at BBCH 88. The interaction between varieties, SA concentrations and modes of applications were statistically nonsignificant.

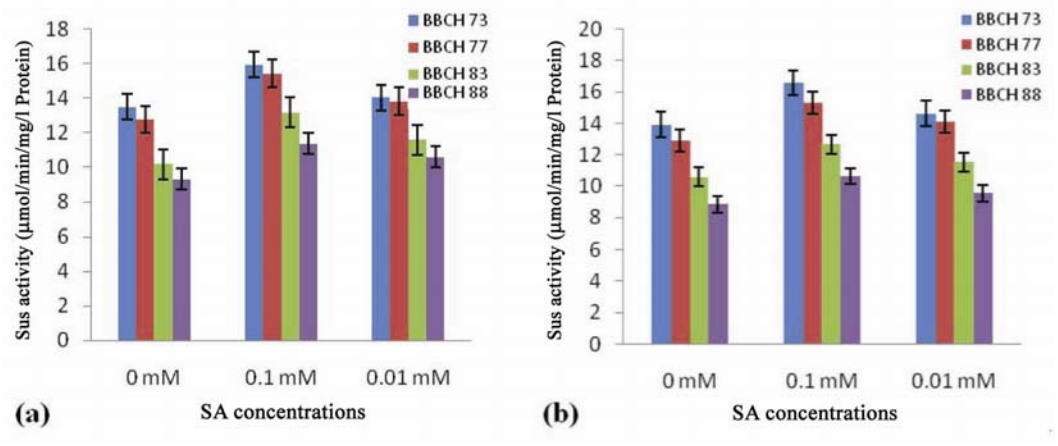

Fig. 2. Effect of salicylic acid (SA) concentrations on the activity of sucrose synthase in pea at four phenological stages in 2003-2004 (a) and 2004-2005 (b). (n = 3).

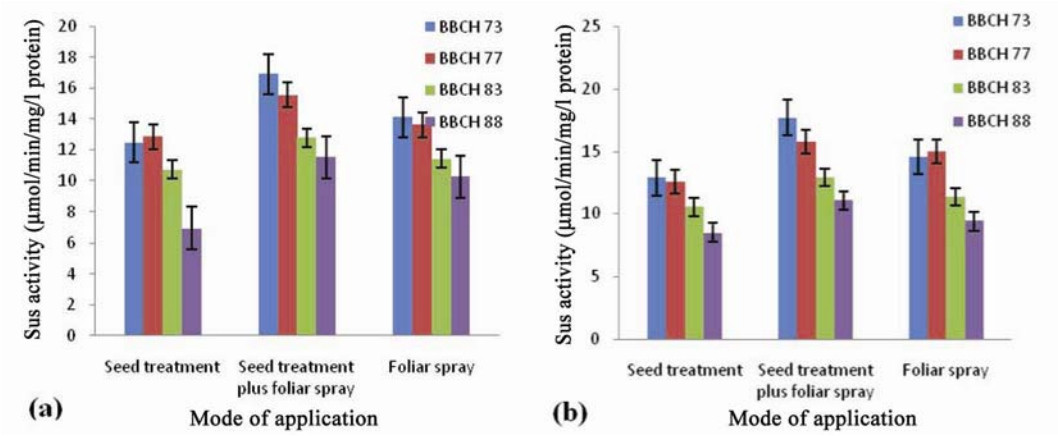

Fig. 3. Effect of modes of application of SA on the activity of sucrose synthase in pea at four phenological stages in 2003-2004 (a) and 2004-2005 (b). (n = 3).

The changes in the activities of Sus induced by SA were observed at phenological stages BBCH 01, BBCH 03 and BBCH 05 in 2003-04 and 2004-05. A significant difference was recorded among the four pea varieties ($p > 0.001$) in both the years (Table 2, Fig. 4). Maximum Sus 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 Sus activity at all the three phenological stages in both the seasons (Table 2, Fig. 5). The maximum Sus activity was recorded for the plants treated with SA concentration 0.01 mM. The interaction between varieties and SA concentrations was found nonsignificant.

Table 2. Mean squares from the analyses of variance and coefficient of variation (CV) of the activities of Sus (µmol min/mg/protein) in pea varieties treated with salicylic acid (SA) concentrations by different modes of application during year 2003-2004 and 2004-2005 at phenological stages.

Source of variation	Df	BBCH 01		BBCH 03		BBCH 05	
		2003-2004	2004-2005	2003-2004	2004-2005	2003-2004	2004-2005
Replication	2	0.12	0.01	0.98	0.28	2.46	3.02
Variety	3	6.47***	7.76***	100.76**	243.37***	277.56**	214.15***
Error a	6	0.22	0.25	4.98	3.00	12.76	6.72
Salicylic acid	2	5.29***	3.37***	45.27***	71.88***	52.27***	261.83***
V × S	6	0.04	0.17	3.17	2.03	11.37	2.91
Error b	1 6	0.12	0.17	1.99	2.03	4.60	2.91
Mode	2	9.60***	12.44***	104.69***	145.54***	252.88***	235.71***
V × M	6	0.12	0.10	0.58	0.21	3.18*	1.11
S × M	4	0.18*	0.44**	0.92	0.35	0.18	0.36
V × S × M	1 2	0.05	0.07	0.63	0.86	1.52	0.54
Error c	4 8	0.07	0.09	0.99	0.44	1.13	0.67
CV		10.67	9.74	14.24	7.16	10.77	7.42

*, **, *** Significant at p = 0.05, 0.01 and 0.001, respectively. BBCH 01 (beginning of seed imbibition), BBCH 03 (seed imbibition complete) and BBCH 05 (radicle emerged from seed).

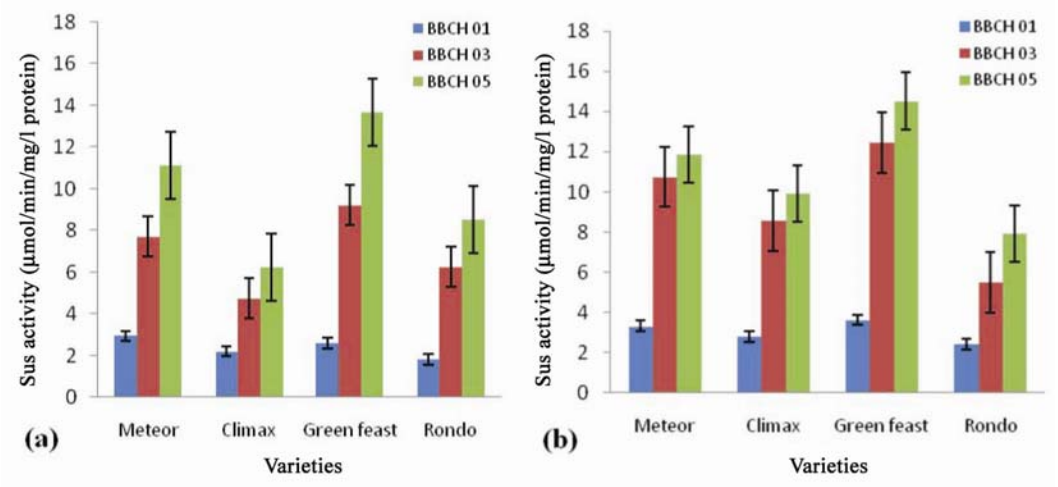

Fig. 4. Changes in activities of sucrose synthase in the seeds raised from SA treated pea varieties in 2003-2004 (a) and 2004-2005 (b) at three phonological stages. (n = 3).

There was a statistical difference between modes of application of SA regarding Sus activity (p > 0.001) at BBCH 01, BBCH 03 and BBCH 05 in 2003-04 and 2004-05 (Table 2, Fig. 6). Highest Sus activity was recorded for the plants whose seeds were treated plus leaf sprayed followed by that of the foliar sprayed. A nonsignificant interaction between varieties and modes of application was recorded. A significant interaction between SA and modes of application was recorded during both growing seasons at BBCH 01, while it was nonsignificant at BBCH 03 and BBCH 05. Interaction between varieties, SA concentrations and modes of application was statistically nonsignificant.

In the present study, the seeds of the variety Meteor showed the highest activity of Sus as compared to all other varieties. Sus activities were higher at the phenological stages BBCH 73 and BBCH 77, while it declined gradually toward the phenological stages BBCH 83 and BBCH 88 (Fig. 1). Furthermore, Sus activities were higher in the plants treated with SA concentration 0.1 mM as compared with 0 mM and with the plants treated with SA concentration 0.01 mM (Fig. 2).

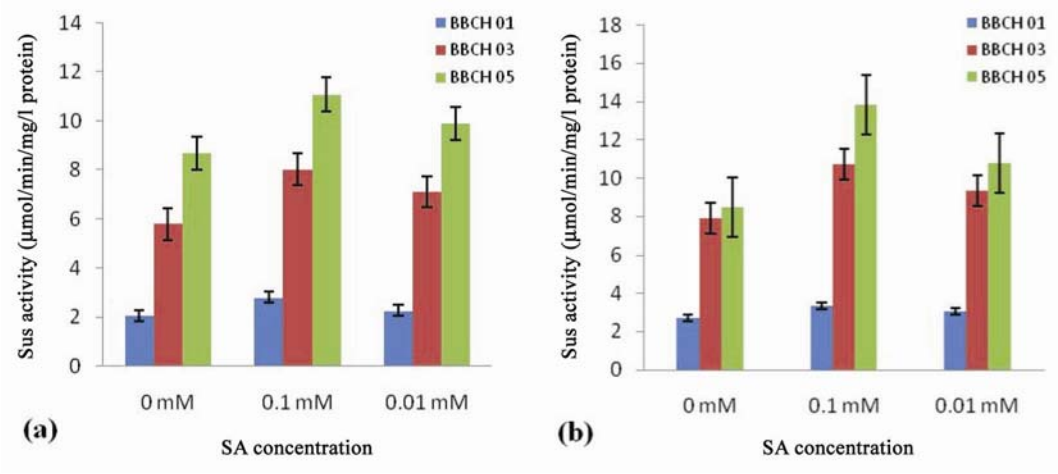

Fig. 5. Effect of different SA concentrations on the activity of sucrose synthase at three phenological stages in 2003-04 (a) and 2004-05 (b). (n = 3).

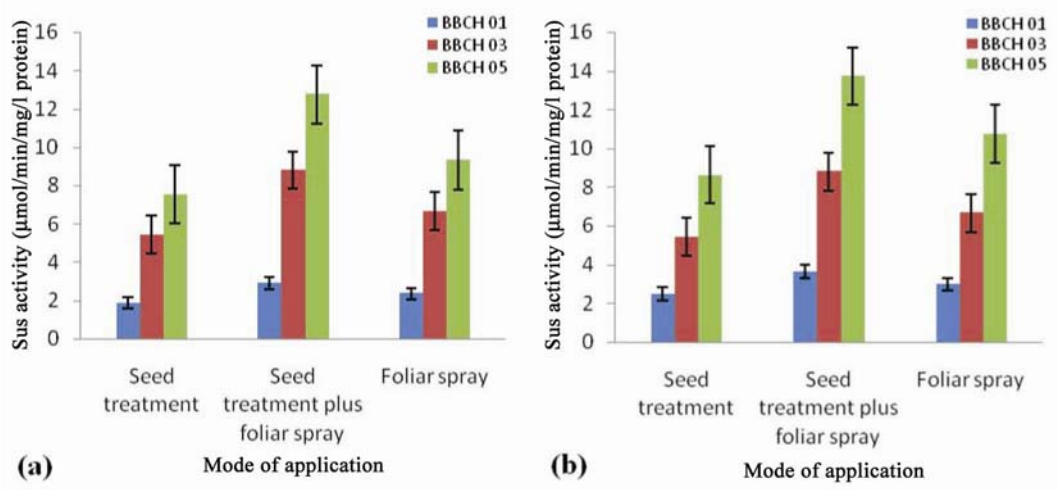

Fig. 6. Effect of different modes of applications of SA on the activity of sucrose synthase in pea during 2003-04 (a) and 2004-05 (b). The means of three replicates are shown and standard errors are indicated.

The higher activities of Sus at phenological stages BBCH 73, BBCH 77 and BBCH 83 indicated that main metabolic activity of pea seed was devoted to the biosynthesis of starch from the phloem derived sucrose resulted in active storage of starch in the cotyledons. The sequentional development of pea seed required that sink size was more or less fixed before storage takes place (Ambrose *et al.* 1987). During the present study the higher rate of Sus clearly reflected the activity

of sink as was suggested by Ho (1988). Comparatively low Sus activity at the phenological stage BBCH 88 can be correlated with the reduction in uploading of phloem assimilates. The activity of Sus is positively correlated with the starch synthesis.

Sucrose is the most important primary nutrient essentially for all the higher plant cells. When sucrose is broken down by sucrose synthase pathway, both hexoses from sucrose can feed into glycolysis. In the present study, there was a gradual increase in Sus activities starting from the phenological stage BBCH 01 to the phenological stage BBCH 05 in all the four varieties (Fig. 4). The maximum Sus activity was observed for the variety Green feast while Rondo exhibited the lowest Sus activity at all the three stages of germination. The increase in Sus activity may be due to increasing demand for cellulose synthesis and preparation for the nodulation at the later stages of seed germination. The activities of Sus were also higher in the seed raised from pea plants treated with SA concentration 0.1 mM and seeds raised from seed treated plus foliar sprayed (STFS) plants. Over all, the Sus activity was induced by SA and this induction was more distinct in the pea plants that received two doses of SA as seed treatment plus foliar spray. The results of the present study are in general agreement with earlier studies (Dejardin *et al.* 1997, Ambrose *et al*. 1988, Ho 1988).

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