Salicylic Acid Ameliorates Germination, Seedling Growth, Phytohormone and Enzymes Activity in Bean (*Phaseolus vulgaris* L.) under Cold Stress

Gharib F. A. [1] and Hegazi A. Z. [2]

[1] Botany department, Faculty of science, Helwan University, Egypt.
[2]Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.
*amalhegazi2000@yahoo.com; fgharib_8@yahoo.co.uk

Abstract: An experiment was carried out under laboratory condition to tackle low temperature stress by using salicylic acid (SA). Seeds of six common bean varieties (Polista, Nebraska, Goro, Helda, Duel and Giza 6) were soaked in water or 10^{-4} M aerated solution of salicylic acid (SA) for 6 h. Treated and untreated seeds were germinated at 25°C (optimal temperature) and at 15°C (suboptimal temperature or chilling stress) under dark controlled conditions for 9 and 30 days , respectively. Germination and seedling growth of the six tested varieties were significantly hindered under low temperature. Seed treatments with SA significantly improved germination percentage, germination rate and seedling criteria, compared with control seeds under optimal and low temperature stress conditions. The content of Indolacetic acid (IAA), Gibberellic acid (GA_3) and Abscisic acid (ABA) increased in the different varieties under study, in response to seed soaking in 10^{-4}M SA at 15°C. GA_3/ABA ratio showed maximum increase in Duel and Helda, while the lowest ratio was observed in Giza 6 and Nebraska seedlings. At the same low temperature, catalase activity was decreased, whereas that of polyphenol oxidase increased on using 10^{-4}M SA. Peroxidase isozymes indicated five to three isozymes in seedlings of the six bean varieties. Salicylic acid treatment resulted in detecting (in Duel) and disappearance (in Nebraska) of peroxidase isozymes at Rf: 0.37, which might be responsible for tolerance and sensitivity mechanism, respectively. The present results indicated that, salicylic acid stimulated various growth aspects of bean seedlings perhaps through interference with the enzymatic activities responsible for biosynthesis and/or catabolism of growth promoting and inhibiting substances. Thus, it might be concluded that, SA could eliminate the adverse effects of cold stress in common bean. [Journal of American Science 2010;6(10):675-683]. (ISSN: 1545-1003).

Key words: Common bean, germination, seed soaking, seedling growth, salicylic acid, cold stress, phytohormones, enzymes activity.

1. Introduction:

Common bean (*Phaseolus vulgaris* L.) is grown over a wide range of environments, including sites with low or high soil temperatures at sowing time. Bean plant is sensitive to chilling soil temperatures often encountered during early sowing. Early sown seeds that are subjected to chilling temperatures were smaller, suffered reductions in the rate of emergence and maximal emergence than late sowing (Rodiño *et al*., 2007).

Salicylic acid (SA) is a phenolic compound naturally occuring in plants in very low amounts. Phenolics participate in some way on auxin metabolism by regulating IAA degradation or by controlling the formation of IAA conjugates (El-Mergawi *et al*., 2007). The sustained level of salicylic acid may be a prerequisite for the synthesis of auxin and/or cytokinin (Metwally *et al*., 2003). SA is a common plant-produced signal molecule responsible for inducing resistance to a number of biotic and abiotic stresses (Karlidag *et al*., 2009). SA is involved in establishing the local and systemic disease resistance response of plants after pathogen attack (Kachroo *et al*., 2005).

The role of SA at a certain level with moderate and severe abiotic stress may be different and can be attributed to redox regulations in plant cells (Shu and Hui, 2008) and protection of the cell structure under cold-stress (Zhang *et al*., 2007). SA plays important roles in response to external stimulation and activating defense system in plants. Activation of phospholipase D is an early response to low temperature, involved in the accumulation of free SA and the development of thermotolerance induced by low temperature acclimation in grape berries (Bao *et al*., 2009). Pretreatment with 20 µ g/ml SA significantly improved germination potential and growth criteria of maize seedlings at both 25 and 15 °C (Bedi and Dhingra, 2008). Pepper seed treatment with 10^{-4} M SA and sulfosalicylic acid induced a better growth recovery manifested as tall seedlings and high plant fresh and dry weights in the seedlings

subjected to low temperature (Benavides *et al*., 2002). Application of 3- 5 mM SA increased growth criteria in faba bean but reduced growth in maize plants (El-Mergawi *et al*., 2007). Moreovere, SA could alleviate the injury caused by low temperature in maize (Farooq *et al*., 2009), strawberry (Karlidag *et al*., 2009) and cucumber through the alteration of antioxidant-enzyme activities (Cao *et al*., 2009; Tao *et al*., 2010). Activity of one peroxidase isoform in leaves of red oak seedlings was enhanced modestly by treatment with salicylate (Steven and Jack, 2004). Certain enzymes were activated by SA treatment, while others, like catalase, were inhibited. Catalase seems to be a key enzyme in salicylic acid-induced stress tolerance, since it was shown to bind SA *in vitro* (Chen *et al*., 1993) and was inhibited by SA in several plant species (Conrath *et al*., 1995).

The present study intended to characterize variability for low temperature (15ºC) tolerance in six different common bean varieties (Polista, Nebraska, Goro, Helda, Duel and Giza 6) presoaked in water or 10^{-4}M salicylic acid. Endogenous phytohormones as well as activities of certain related enzymes and peroxidase isozymes were determined in the seedlings (30- day- old) grown at suboptimal temperature.

2. Materials and Methods

Time course experiment

Seeds of six bean (*Phaseolus vulgaris* L.) varieties (Polista, Nebraska, Goro, Helda, Duel and Giza 6) were provided by the Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. The seed indexes, i.e. weight of 100 seeds of the used varieties are shown in the following table:

Varieties	Polista	Nebraska	Goro	Helda	Dule	Giza 6
Wt. of 100 seeds (g)	20.02	54.17	23.92	43.92	15.89	45.20

The seeds were surface sterilized and then washed thoroughly with bidistilled water. Afterwards, seeds of each variety were equally divided into two groups. The first group was soaked for 6 h in SA solution (10^{-4}M). The second group was soaked for 6 h in water to serve as control. The two groups were allowed to germinate in rolled paper towels in controlled cabinet (at Vegetable Crop Seed Production and Technology Department). Fifty seeds replicated four times from each lot and each treatment was planted in the trays according to ISTA rules (2009). The germination percentage (%) and germination rate (days to complete germination) were calculated. At least 10 randomly choice seedlings (9- and 30- day-old grown at 25 or 15 °C, respectively) were taken from each treatment for measurements of different growth criteria (seedling length (cm), seedling root length (cm), fresh and dry weights (g) per seedling). According to ISTA rules, seedling measurements can be taken after 9 days at optimal temperature (25°C), but at suboptimal condition (15°C) it takes 30 days for seedling to reach almost the same criteria.

The experiment for growth criteria was carried out in a split plot design with four replicates. The main plots represented the two treatments (soaking seeds for 6 h in either water or SA). The sub-plots represented the six bean varieties.

The collected samples from each group were either directly kept in a deepfreeze for enzyme extraction or kept in distilled cold ethanol in the refrigerator, ready for hormone extraction and estimation.

Endogenous phytohormones

Seedlings of the six bean varieties presoaked for 6 h in 10^{-4}M salicylic acids or water (control) and grown at 15°C were used. The method of extraction was essentially similar to that adopted by Shindy and Smith (1975)**.** The frozen samples were ground in cold 80% methanol, followed by triple extraction with fresh methanol for 2 hours at 0°C. To estimate the amounts of acidic hormones, the plant hormone fractions and standards were methylated according to Vogel (1975)**,** ready for gas chromatography (GC) analysis. The retention time (RT) and the area of peaks of authentic samples were used for the identification and characterization of peaks of samples under investigation.

Enzyme activities

Catalase and polyphenol oxidase were assayed in seedlings of the six bean varieties presoaked for 6 h in water (control) or 10^{-4}M SA and grown at 15°C, following the method of Kar and Mishra (1976) with some modification as described by Iturbe-Ormaetxe *et al*., (1998). Polyphenol oxidase activity was expressed as the change in optical density g^{-1} fresh weigh $hour^{-1}$ at 430 nm. Catalase activity was expressed as µM H_2O_2 destroyed g^{-1} fresh weight $hour^{-1}$.

Peroxidase isozymes

Extraction, preparation and detection

Peroxidase isozyme patterns were detected in seedlings of the six bean varieties presoaked for 6 h in water (control) or 10^{-4}M SA and grown at 15°C using polyacrylamide gel electrophoresis. Crude extracts were prepared by macerating 300 mg of each sample in 50 µl of the extraction buffer [0.1M Tris- HCl buffer at pH 7.5, containing 0.01 M EDTA, 0.01 M potassium chloride, 0.01M magnesium chloride hexahydrate, 0.1M dithioteritol (DTT), 4% polyvinyl pyrrolidone (PVP) and 10% sucrose] (Gottlieb, 1981). The macerates were placed in a refrigerator for 2 hours, then centrifuged at 14.000 rpm for 20 minutes

at 40ºC. The residue was discarded and the supernatant was used for isozymes study. The separating gel of 10% acrylamide was prepared following the method of Laemmli (1970). Aliquots of 10 µl of the extract were electrophorized in 10% slab polyacrylamide gel at constant voltage of 150 V and 15 mA /gel at low temperature for approximately 2 hours using Hoefer Vertical slab gel unit, Model SE-400. Following the removal of the gel from electrophoretic assembly, it was stained for peroxidase enzyme according to Soltis *et al*. (1983). The gel was incubated in 100 ml staining solution of 0.05 M acetate buffer, pH 5.0, containing 65 mg benzidine dissolved in 1 ml ethanol. Two ml of 0.1 M $CaCl_2$ were added as co-enzyme. Finally, two ml H_2O_2 were added as the substrate in refrigerator until dark brown bands appeared. The stained gels were washed with distilled water and fixed in 50% glycerol, then photographed and diagrammed.

Statistic analysis

The data were tabulated and statistically analyzed, using the analyses of variance method and the treatment means were compared using the Duncan Multiple Range Test (Duncan, 1965).

3. Results and Discussion:

Germination potential and growth parameters

Germination criteria for the six bean varieties under investigation at optimal temperature (25°C) are shown in Table (1). The results demonstrated that there was no significant difference between the main treatments (i.e. soaking in water and SA). But, for seedling fresh and dry weights, soaking in SA caused a significant increase. Nebraska and Helda varieties (as big seeds) recorded higher values in most studied growth criteria. Interaction between treatments and varieties demonstrated that variety Nebraska also showed the highest values, while variety Polista (as a small seed) recorded the lowest values. On the other hand, it is evident from the data presented in Table (2) that SA significantly increased the germination percentage and all the studied growth traits of the six investigated bean varieties grown at suboptimal temperature (15°C). Duel variety, recorded the highest germination percentage and lowest germination rate (i.e. it took lowest number of days for complete germination). In the other growth traits, Nebraska recorded the highest significant values. Interaction between treatments and varieties indicated that salicylic acid could overcome cold stress in bean (Table 2). Soaking in 10^{-4}M SA clearly enhanced germination percentage and subsequent seedling criteria, compared with corresponding control. In this connection, pretreatment of maize seeds with SA significantly improved the percent of germination, emergence and mean days to emergence, primary root and shoot lengths at both 25 and 15 °C. the results obtained herein agreed to a wide extent with those of other authors. Thus, mean days to germination decreased while seedling vigor index and dry weight increased with SA at 15 °C (Farooq *et al*., 2008 and Bedi and Dhingra, 2008). Priming pepper seeds in the presence of 0.1 mM Acetyl SA improved germination performance and resulted in the highest seedling shoot fresh and dry weights at 15 °C (Korkmaz, 2005). Moreover, soaking rice seeds in 5.0 m mol/ liter SA promoted the length of roots and buds and biological yield at low temperature (Ling *et al*., 2001).

Changes in Endogenous phytohormones

It is apparent from the data recorded in Table (3) that the contents of indolacetic acid (IAA), gibberellic acid (GA_3) and abscisic acid (ABA) showed marked increases in the six bean varieties under investigation, in response to presoaking seeds in 10^{-4}M SA, as compared with corresponding controls at 15ºC. The highest concentration of IAA was recorded in Duel and Helda, while the lowest concentration was shown in Giza 6 and Nebraska seedlings, compared to corresponding control. In this connection, spraying faba bean plants with SA at 4 mM tended to cause increases in free IAA (113%), decreases in ester IAA (44%) of control and interfered with IAA-conjugation (El-Mergawi *et al*., 2007).

Cold induced the transcription of a range of genes (Nguyen *et al*., 2009). Gene expression plays multiple roles in abiotic and biotic resistance pathways, as well as in plant growth. The expression of *ZF1* and *GmERF3* showed no obvious changes under cold stress, while was increased as a result of treatments with ABA, SA, GA_3, IAA and H_2O_2 (Chen *et al*., 2009; Yun *et al*., 2010). Furthermore, the expression of *PgSAM* (S-adenosyl-L-methionine synthetase (SAMS) gene) and a *NAC* gene *CarNAC1* (for *Cicer arietinum* L. *NAC* gene 1) was strongly induced by cold, SA, IAA, GA_3, which might help to protect the plants against various abiotic stress responses (Pulla *et al*., 2009; Hui *et al*., 2010). Table (3) shows that, on using 10^{-4}M salicylic acids, both IAA and ABA were generally increased. A more or less similar trend, but to a higher extent was also observed with GA_3. On the bases of these results, it could be concluded that the steep increase in GA_3/ABA ratio seemed to correlate with the pattern obtained with germination and subsequent seedling growth. This ratio showed maximum increase in varieties Duel and Helda, and lowest ratio in Nebraska and Giza 6 seedlings, respectively, using 10^{-4}M salicylic acids compared with corresponding controls.

Table (1): Changes in germination potentials and growth criteria of seedlings (9- day- old) of the six bean varieties as affected by presoaking for 6 h in water (control) or 10^{-4} M salicylic acid (SA). The rate of germination represents time (days) required for maximum germination at 25 ºC. Each value represents the mean of 4 replicates (each of 50 seeds) for germination potential or 10 replicates for growth criteria.

Var.	Germination %			Germination Rate			Seedling Length			Seedling Root Length			Seedling F. wt.			Seedling D. wt.		
	H_2O	SA	mean	H_2O	SA	mean	H_2O	SA	mean	H_2O	SA	mean	H_2O	SA	mean	H_2O	SA	mean
Polista	97.0 ab	95.5 ab	96.25 A	2.083 ab	2.029 c	2.056AB	26.25 g	27.23 fg	26.74 D	11.38 e	12.49 de	11.93 D	1.385 f	1.617 de	1.501 C	0.076 l	0.100 j	0.088F
Nebraska	92.0 ab	96.0 ab	94.00 A	2.123 a	2.061 bc	2.092 A	35.28 ab	36.84 a	36.06 A	16.63 a	17.02 a	16.83 A	2.980 b	3.475 a	3.228 A	0.358 b	0.416 a	0.387A
Goro	93.0 ab	98.0 ab	95.50 A	2.082 ab	2.040 bc	2.062 AB	28.53 ef	29.21 de	28.87 C	12.31 de	13.49 bcd	12.90CD	1.449 ef	1.658 d	1.553 C	0.095 k	0.119 h	0.107 E
Helda	97.0 ab	98.0 ab	97.50 A	2.062 bc	2.020 c	2.041 B	32.81 c	34.03 bc	33.42 B	14.65 bc	14.69 b	14.67 B	2.745 c	3.387 a	3.066 A	0.269 d	0.332 c	0.301 B
Dule	87.0 b	99.0 a	93.00 A	2.052 bc	2.021 c	2.036 B	31.00 d	32.75 c	31.88 B	13.44 cd	14.40 bc	13.92 BC	1.585de	1.689 d	1.637 C	0.112 i	0.123 g	0.117 D
Giza 6	92.0 ab	95.0 ab	93.50 A	2.070 bc	2.053 bc	2.062 AB	29.88 de	30.55 d	30.21 C	12.84 d	14.23 bc	13.53 BC	2.597 c	2.937 b	2.767 B	0.253 f	0.259 e	0.256 C
Mean	93.0 A	96.92 A		2.079 A	2.037 A		30.62 A	31.77 A		13.54 A	14.39 A		2.123 B	2.461 A		0.194 B	0.225 A	

Values within the same column followed by the same letters are not significantly different, using Duncan's Multiple Range Test at 5% level.

Table (2): Changes in germination potentials and growth criteria of seedlings (30- day- old) of the six bean varieties as affected by presoaking for 6 h in water (control) or 10^{-4} M salicylic acid (SA). The rate of germination represents time (days) required for maximum germination at 15 ºC. Each value represents the mean of 4 replicates (each of 50 seeds) for germination potential or 10 replicates for growth criteria.

Var.	Germination %			Germination Rate			Seedling Length (g)			Seedling root Length (g)			Seedling F. W. (g)			Seedling D. W. (g)		
	H_2O	SA	mean	H_2O	SA	mean	H_2O	SA	mean	H_2O	SA	mean	H_2O	SA	mean	H_2O	SA	mean
Polista	75. 0 d	96.0 a	85.50B	7.06 d	5.17 g	6.12 D	16.55 h	20.03 f	18.29 E	9.69 g	11.01 f	10.35 E	1.16 f	1.23 f	1.19 D	0.0998 k	0.111 h	0.106 F
Nebraska	62. 0 ef	88.0 b	75.00C	9.77 a	6.58 e	8.18 A	24.25 c	31.75 a	28.00 A	14.27 b	18.16 a	16.22 A	2.32 c	3.79 a	3.05 A	0.322 b	0.356 a	0.339 A
Goro	66.0 e	96.0 a	81.00B	8.35 c	5.31 g	6.83 C	17.98 g	21.20 e	19.59 D	10.59 f	11.75 e	11.17 D	1.17 f	1.41 e	1.29 D	0.102 j	0.117 g	0.109 E
Helda	84.0 bc	99.0 a	91.50A	6.96 d	5.14 g	6.05 D	20.70 ef	26.79 b	23.74 B	12.50cd	14.05 b	13.27 B	2.26 c	2.81 b	2.54 B	0.249 e	0.296 c	0.273 B
Dule	89.0 b	99.0 a	94.00A	6.17 f	5.02 g	5.59 E	19.79 f	23.21 d	21.50 C	11.95de	12.96 c	12.46 C	1.26 f	1.55 d	1.41 C	0.104 i	0.120 f	0.112 D
Giza 6	58.25 f	82.0 c	70.13C	9.07 b	6.26 f	7.67 B	18.23 g	22.33 d	20.28 D	10.65 f	12.31cde	11.48 D	2.26 c	2.77 b	2.52 B	0.248 e	0.251 d	0.249 C
Mean	72.38 B	93.33A		7.89 A	5.58 B		19.58 B	24.22 A		11.61 B	13.38 A		1.74 B	2.26 A		0.188 B	0.209 A	

Values within the same column followed by the same letters are not significantly different, using Duncan's Multiple Range Test at 5% level

In this connection, the GA_3/ABA ratio might represent the primary hormonal signal. This assumption was based on the postulation that seed germination is regulated by a balance between the relative amounts of endogenous GA and ABA in the seeds and sensitivities of their tissues to these hormones. Bewley (1997) suggested that in tomato seeds, GAs (and/ or cytokinins) move as a chemical signal from the embryonic axis via the cotyledons to the endosperm where they induce *de novo* synthesis of hydrolytic enzymes for germination, whereby this process is repressed by ABA. Thus, the obtained changes in IAA, GA_3 and ABA due to 10^{-4}M salicylic application as a monophenol might be involved in gene expression regulating the signaling activities and/ or levels of growth regulating substances through direct impact on the activities of oxido-reductive enzymes related to the hormonal metabolism. In this connection, Bialczyk *et al*. (1998) have drawn a positive correlation between kinetin level and accumulation of polyphenols at the expense of monophenols in *Rubus* protoplast and leaf blades of tomato.

Table (3): Changes in the contents of endogenous hormone concentrations (µg/1 g fresh wt. equivalents) in seedlings (30- day- old) of the six bean varieties as affected by presoaking seeds for 6 h in water (control) or 10^{-4}M salicylic acid (SA) at 15 ºC.

	Concentrations (µg/1 g fresh wt.)							
	IAA		GA_3		ABA		GA3/ABA	
Solution / Varieties	H_2O	SA	H_2O	SA	H_2O	SA	H_2O	SA
Polista	82.94	88.72	115.89	826.26	24.24	86.43	4.78	9.56
Nebraska	38.43	41.11	176.53	1742.83	70.75	355.74	2.50	4.90
Goro	52.80	56.48	154.11	1006.09	25.78	100.77	5.98	9.98
Helda	86.08	92.08	191.57	1154.24	30.78	77.80	6.23	14.84
Duel	171.14	183.06	81.22	500.19	12.28	20.17	6.62	24.80
Giza 6	34.84	37.27	162.57	1134.14	44.27	206.14	3.67	5.50

Changes in Enzyme activities

The data presented in Fig. (1) indicate a general increase in polyphenol oxidase activity in the six bean varieties under investigation grown at 15 ºC, in response to presoaking seeds in 10^{-4}M SA, as compared with corresponding controls; a more or less opposite trend to that obtained with catalase activity. CATs and other scavenger enzymes such as peroxidase (POD) and superoxide dismutase (SOD) may be coordinately regulated during development, but differentially expressed in response to different stresses for controlling reactive oxygen species homeostasis (Yan *et al*., 2008). The maximum activity of polyphenol oxidase was obtained in cases of Duel and Goro seedlings, whereas the minimum activity was recorded in Nebraska and Giza 6 seedlings, respectively. Similarly, SA improved cold resistance to watermelon and lupine by increasing the activities of POD and SOD. The activities of antioxidative enzymes were more significantly increased in the cold tolerant watermelon germplasm than that of the chilling sensitive germplasm (El-Bahy 2002 and Hua *et al*., 2008). On the other hand, the activities of catalase, were several fold lower, in response to presoaking bean seeds in 10^{-4} M SA, as compared with corresponding controls and this might increase their chilling tolerance. Similarly, SA decreased catalase (CAT) and peroxidase activities and induced chilling tolerance in maize (Horváth *et al*., 2002; Ping and Rui 2007 and Bedi and Dhingra, 2008), winter wheat plants (Tasgn *et al*., 2006) and increased disease defense (Scandalios, 1993 and Conrath *et al*., 1995). On the contrary, SA pretreatment reduced H_2O_2 overproduction, increased catalase activities and participated in enhancing chilling tolerance of banana plants (Zhang *et al*., 2003); maize seedlings (Kun *et al*., 2005) and Manila grass (Wang *et al*., 2009). SA increased CAT and SOD activities and induced heat tolerance in Kentucky bluegrass due to AOS scavenging (He *et al*., 2005). During late embryogenesis in maize, total CAT activity in scutella increased dramatically with SA treatment, which was contributed to the accumulation of CAT2 transcripts (Guan and Scandalios, 1995).

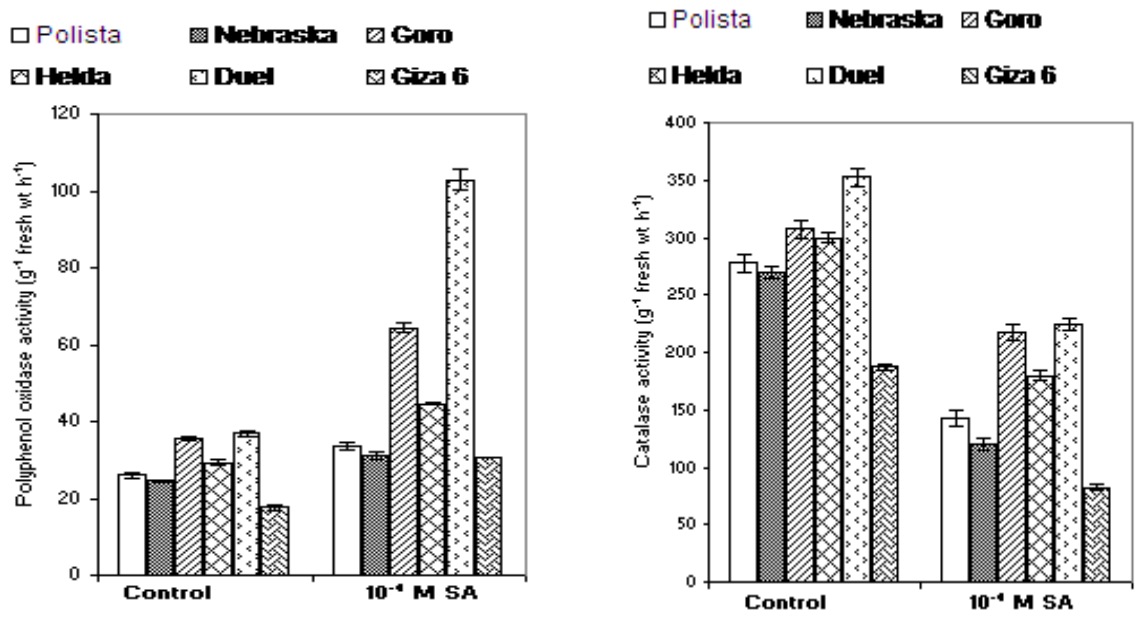

Figure (1): Changes in some enzymatic activities of seedlings (30- day- old) of the six bean varieties as affected by presoaking seeds for 6 h in water (control) or 10^{-4}M salicylic acid (SA) at 15 ºC. The values listed are expressed as changes in enzymatic activity. (g^{-1} fresh weight $hour^{-1}$). Vertical bars represent ± standard deviations.

Peroxidase isozymes

Electrophoretic study of peroxidase isozymes of six bean varieties (Polista, Nebraska, Goro , Helda, Duel and Giza 6) indicated differences in isozyme patterns and intensity by soaking seeds in 10^{-4} M SA, as compared with corresponding control seedlings at 15 ºC. Data in Table (4) and Figure (2) show that five different peroxidase isozymes were detected in the control and salicylic acid treatments in (Polista, Goro and Giza 6 varieties) at Rf: 0.09, 0.24, 0.37, 0.49 and 0.67 with general increase in intensity of peroxidase isozymes at Rf: 0.37 as a result of SA treatment. In this connection, the activity of one peroxidase isoform in leaves of *Quercus rubra* L. seedlings was enhanced due to treatment with salicylate (Steven and Jack, 2004). In contrast, salicylic acid treatment resulted in the disappearance of peroxidase isozymes at Rf: 0.37 in Nebraska (the most sensitive variety) which might be responsible for sensitivity mechanism. In Helda variety, four peroxidase isozymes were detected in the control and salicylic acid treatments at Rfs: 0.09, 0.37, 0.49 and 0.67. Generally, band intensity at Rf: 0.37 recorded the highest concentration being 61.74, 54.88% for the control and SA treated seedlings, respectively. On the other hand, Duel variety showed three isozymes at Rf: 0.09, 0.24 and 0.49. The first and second ones positioned at Rfs: 0.09, 0.24 were found in low concentration in SA seedlings (6.91, 44.46%), respectively compared with (12.31, 69.51%) for their controls. The third isozyme positioned at Rf: 0.49 was present at a higher level (24.20%) in salicylic acid treatment compared with (18.18%) for the control. Moreover, salicylic acid treatments resulted in detecting a new isozyme (Rf: 0.37) with a concentration of 24.43% in Duel variety which might be responsible of tolerance mechanism at low temperature. Similarly, acibenzolar-S-methyl treatment increased peroxidase specific activity and produced two unique isoforms (Bargabus *et al*., 2002). In maize there was, among the peroxidase isoenzymes, a band which could be seen only in SA-treated plants (Janda, *et al*., 1999). Also, dipping tomato roots in solutions of 1.0 µM salicylic hydrazide induced an acidic isozyme of peroxidase (Miyazawa *et al*., 1998).

Table (4): Electrophoretic patterns of peroxidase isozymes (Band intensity %) of seedlings (30-day-old) of the six bean varieties as affected by presoaking seeds for 6 h in water (control) or 10^{-4} M salicylic acid (SA) at 15 ºC.

Variety	Polista		Nebraska		Goro		Helda		Duel		Giza 6	
Soaking solution	H_2O	SA	H_2O	SA	H_2O	SA	H_2O	SA	H_2O	SA	H_2O	SA
Rf												
0.09	12.16	7.59	9.23	8.61	8.53	5.36	6.82	8.98	12.31	6.91	7.76	11.06
0.24	23.03	26.92	43.66	60.44	41.63	38.34	---	---	69.51	44.46	44.72	41.34
0.37	25.16	29.40	10.06	---	21.11	25.01	61.74	54.88	---	24.43	21.68	23.28
0.49	18.08	17.54	18.63	15.48	22.12	22.51	9.04	11.81	18.18	24.20	15.44	15.28
0.67	21.57	18.54	18.41	15.47	6.61	8.79	22.40	24.33	---	---	10.39	9.03
No. of bands	5	5	5	4	5	5	4	4	3	4	5	5

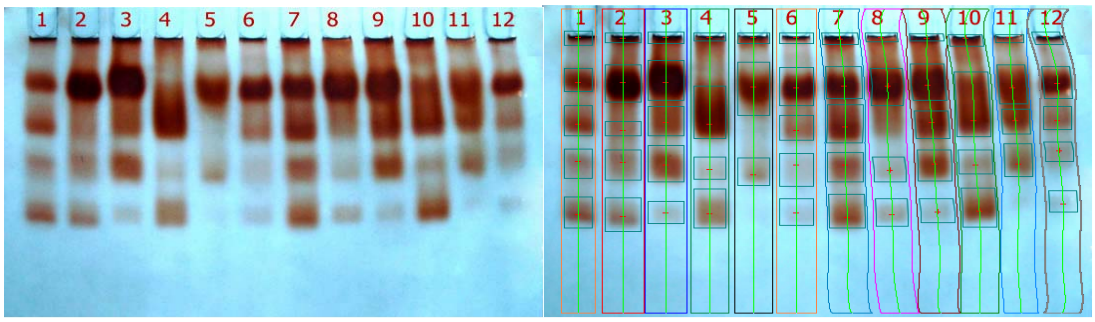

Figure (2): Zymogram analysis of peroxidase isozymes (band intensity %) of seedlings (30- day- old) of six bean varieties [Polista lanes (1, 7); Nebraska lanes (2, 8); Goro lanes (3, 9); Helda lanes (4, 10); Duel lanes (5, 11) and Giza 6 lanes (6, 12)] as affected by presoaking seeds for 6 h in water lanes (1 - 6) or 10^{-4}M salicylic acid (SA) lanes (7- 12) at 15 ºC.

4. Conclusion

Salicylic acid (SA) significantly improved the germination performance of bean at both 25 °C (optimal temperature) and at 15 °C (suboptimal temperature or chilling stress). Presoaking bean seeds in 10^{-4} M SA could be used to eliminate the adverse effects of cold stress and enhance common bean germination at low temperature. Duel was the most promising variety that could germinate at highest rate under cool temperatures (15 °C) while, Nebraska and Giza 6 were the most sensitive varieties.

Corresponding author
Gharib F. A., Hegazi A. Z.
Botany department, Faculty of science, Helwan University, Egypt.
Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.
*amalhegazi2000@yahoo.com;
fgharib_8@yahoo.co.uk

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