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Effect of Seed Priming with Salicylic Acid on Seed Germination and Seedling Growth of Broad bean (*Vicia faba* L).

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Seed priming with growth regulators, especially salicylic acid, is a promising alternative seed primer because it is an important inducer of resistance against diseases and pests, as well as acting significantly on quality and seed yield. The exogenous salicylic acid SA applied at 0.0, 0.5, 1.0, and 3.0mM SA concentrations to faba bean seeds showed significant increase in seed germination and seedling growth except at high concentrations. Seed germination decreased by increasing of SA up to 3.0mM. As, SA at high concentrations 1.0 and 3.0 mM had inhibitory effects on germination as well as it reduced seedling growth ,where the lengths of plumule and root (radical) were reduced compared to control .Moreover ,these highest concentrations of SA shown negative result for fresh root weight. **.**Meanwhile, SA at low concentrations had no significant effect at ($P>0.05$) on all tested growth parameters. It can be noted seed priming of faba bean with low concentration of SA will speed up the germination time and enhance the establishment of seedlings as well as powerful tool in enhancing the growth and productivity of such crop especially in areas with saline irrigation water resources. Moreover; primed seeds will be less susceptible to soil-borne pathogens.

Key words*:* salicylic acid, germination, seed vigor, growth regulator**.**

Introduction

Faba bean (*Vicia faba* L.) is beneficial for human nutrition perspectives because they are good source of energy, protein and dietary fiber Ma *et al.,* (2005). Seed vigour is a very good indicator of the potential field performance followed by the field planting value. Growth regulators play an important role in increasing germination rate of seeds, employed for rooting, vegetative propagation and overall yield of several plants as well as they mostly used for improving seed germination and the productivity of a large number of agricultural crops Jamieson *et al.,* (2002).

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Salicylic acid SA is a natural compound that plays a central role in certain physiological processes and defense responses in plants Shi and Zhu, (2008). It can affect seed germination, cell growth, stomatal opening, expression of genes associated with senescence, and fruit production Klessig *et al*., (2009). Salicylic acid plays an important role for increasing resistance to environmental stress Raskin, (1992). It has been reported that SA increases salinity tolerance Jamshidi Jam *et al*., (2012) and resistance to water deficit in seedlings Bezaukova *et al*. (2001).The use of these regulators increases plant tolerances to abiotic and biotic stresses.

Many researchers have evaluated the processes involved in seed germination, and how they are affected by plant hormones in a range of plant families Hermann *et al*., (2007). In drought condition, using some plant growth regulators such as salicylic acid and cycocel might be an effective strategy to prevent destructive effect of drought and provide plant compatibility. These regulators are known as a strategy to prevent the harmful effect of environmental stresses. These stresses include: heat, cold, heavy metals and drought. Salicylic acid is part of a signaling pathway that is induced by a number of biotic and abiotic stresses. A lot of data exist on the protective effect of SA against ultraviolet light Yalpani *et al*., (1994), salinity Shakirova *et al*., (2003), drought Singh and Usha, (2003), heavy metal toxicity Metawally at al., (2003) and mediating plant defense against pathogens Raskin (1992).

Materials and Methods

The experiments were carried out under laboratory conditions at Department of Biology, Faculty of Science, Yanbu Branch, Girls Sections, Taibah University, Kingdom of Saudi Arabia during February and March 2016.

Source of Seed Samples and Chemical Substance (SA):

Seeds of Faba bean (*V. faba* L.) were purchased from a local supermarket in Yanbu, Kingdom of Saudi Arabia. Broad bean seeds were first surface sterilized with 0.2% mercuric acid for 10 min and followed by washed repeatedly with sterile water to remove all traces of $HgCl_2$. Salicylic acid :(Sigma, Aldrich) is obtained from Laboratory of the Department of Chemistry, Faculty of Science, Yanbu Branch, Girls Sections, Taibah University, Kingdom of Saudi Arabia .

Germination Bioassay:

The seeds of faba bean_were soaked in distilled water to test their viability, and then the precipitated seeds were air dried at room temperature.

Healthy seeds of the recipient species were disinfected with 0.2% mercuric acid for 10 min and followed by washed repeatedly with sterile water to remove all traces of $HgCl_2$.

Different concentrations of exogenously applied Salicylic acid SA i.e., 0.5 mM , 1.0 mM and 3.0 mM) are used as a substrate medium for the germination experiment. Fifteen healthy seeds (in three replicates) for the recipient species during the experimental period were allowed to germinate in the different concentrations of SA under normal laboratory conditions with day temperature ranging from 20-22^{o}C and night temperature from 14-16^{o}C. Sterilized petri-dishes (150×20 mm diameter) were used for germination test. Fifteen seeds of nearly equal size were placed in each Petri dishes double laid with Whatman No1 filter paper. Fifteen to twenty ml of each level of the donor treatment solutions were added to each of the petri-dishes respectively for different concentration. Distilled water was used as a control.

Petri dishes were incubated in a lit room at an average temperature of about 20-22^{o}C for 9 days. The experiment was replicated 3 times. Germination inhibition or stimulation percentage was calculated for each concentration treatment according to the general equation. Germination percentage was determined according to recommended methods by (ISTA, 1985). Number of germinated seeds was counted starting from the 1st day after germination when roots appeared till the 9thday, which is the last day. Emergence of radical is considered as an indicator of germination. *Germination Percentage was calculated as follows* GP _ Number of germinated seeds / Number of total seeds x 100

Seed Viability and Seedling Vigour index

Seed germination tests were conducted for each solution of Salicylic acid SA as maintained above as follows: Standard, Root length, plumule length and Vigour index determined following method of Abdul Baki and Anderson (1973). Seedling vigour was estimated by measurement of the length of germinated roots and shoots daily from the 3rd day after planting till the 9th day, then adding the recorded measurement taken during the experimental time and multiplying with germination percentage. Seedling vigour index was determined as the product of the percentage germination and that of seedling length. *Seedling vigor index (SVI) was calculated by the following formula: SVI _ Seedling length cm x Germination percentage/100 (Abdul-Baki and Anderson, 1973)*

Determination of Fresh matter

Remove seedlings from Perti-dishes and wash off any filter paper remains. Blot plants gently with soft paper towel to remove any free surface moisture. Weigh immediately (plants have a high composition of water, so waiting to weigh them may lead to some drying and therefore produce inaccurate data).

Statistical analysis

Data concerning the effect of different concentrations of salicylic acid on faba bean_ seed germination and seedling growth was subjected to standard analysis of variance (ANOVA) using SPSS V.16 and with the least significant difference was used to compare means of traits ($p < 0.05$).

Results

Seed Viability and Vigor Index Test:

Different concentrations of Salicylic acid SA were applied as seed primer of faba bean. Results in Table 1 showed that the Effect of the four SA concentrations on vigour index of faba bean at the end of priming period (9 days). The results indicate that al tested concentrations of SA (0.5 mM , 1.0 mM and 3.0 mM) affected vigour index of faba bean. 0.5mM and 1.0 mM of SA the cause highest records of vigour index of faba bean .

Meanwhile, high concentration of SA (3.0 mM) cause a significance decrease in vigor index when compared with control treatments. As concentration of SA increased the vigour index significantly decreased. Maximum vigour index recorded in 0.5mM SA compared to control sets (2277 ±4.198); whereas the maximum inhibitory effect of the donor treatment (SA) for seed vigor index was recorded in 3.0 mM SA (309 ± 59.91).

Table 1: Effect of Different Concentrations of Salicylic acid SA on the Seed Vigor Index (SVI) of Germinated faba bean_Seeds.

Salicylic acid SA Concentration	**Seed Vigor Index /Days**				
	1st day	3rd day	5th day	7th day	9th day
Control	0	86 a	280 a	839.2 b	1520 c
0.5 mM	0	108 b	533 b	119.5 a	2277 b
1.0 mM	0	63.84 a	240 a	596.5 b	708.3 a
3.0 mM	0	40 a	114.4 a	253.1 a	309.7 a

Figures with the same letter are not significantly different (p=0.05)

Seed germination:

Effect of different concentrations of SA on germination and inhibition percentage (GP or IP) of faba bean_was represented in (Table 2). The results indicate that maximum percentage of germination was reported in the concentration 0.5mM of SA ,which was (93.3±16.13145%) compared to that of all the concentrations and control treatments which was reported (80 ±14.661%).

Table 2: Effect of Different Concentrations of Salicylic acid SA on the Percentage of Germination (GP) of faba bean Seeds.

Salicylic acid SA Concentration	**Germination Percentage (GP) /Days**				
	1st day	3rd day	5th day	7th day	9th day
Control	0	20 a	40 b	66.6 c	80 c
0.5 mM	0	33.3 b	53.3 b	73.3 c	93.3 c
1.0 mM	0	26.6 b	40 b	46.6 b	46.6 b
3.0 mM	0	20 a	26.6 a	33.3 a	33.3 a

Figures with the same letter are not significantly different ($p=0.05$)

Whereas; the minimum percentage of germination (Maximum inhibition) of seed germination was found on higher concentration of SA showing only 33.3±6.17451% germination.

Root Length (RL):

Compared to the control, a gradual decrease in radicle (RL) lengths of faba bean seedling roots was noticed along the gradual SA concentrations (Table 3). Obviously, all concentration levels had significantly decrease of faba bean seedling roots RL The maximum root length recorded for 0.5mM SA was (13.1 ± 2.35 cm) compared to control which (10 ± 1.757cm) and it was followed by 1mM SA as (7.2 ± 1.54cm) respectively while a minimum root length was found at highest SA concentration 3mM (4.8 ±. 1.81cm). As the concentration of SA decreased, there was an increment in root length.

Table 3: Effect of Different Concentrations of Salicylic acid SA on Root Lengths (RL) (cm) of faba bean Seedlings.

Salicylic acid SA Concentration	Root Length (cm) /Days				
	1st day	3rd day	5th day	7th day	9th day
Control	0	1.8 b	3.0 a	6.0 b	10.0 b
0.5 mM	0	2.0 b	6.0 b	8.0 b	13.1 b
1.0 mM	0	1.4 a	4 .0a	7.5bc	7.2 a
3.0 mM	0	1.2 a	3 a	4.3 a	4.8 a

Figures with the same letter are not significantly different (p=0.05)

Plumle Length (PL):

Results in (Table 4) showed that primed faba bean seeds with SA increased the plumule length of faba bean seeds compared with high concentrations and control treatments control. Whereas, the minimum plumule length was found at highest concentration (3.0 mMSA) which was (4.5± 0.238cm) while the maximum was given by 0.5 mM SA (11.3± 2.13cm). As the concentration of SA decreased, there was an increment in plumule length.

Table 4: Effect of Different Concentrations of Salicylic acid SA on Plumle Lengths (PL) (cm) of faba bean Seedlings.

Salicylic acid SA Concentration	Plumle Length (cm) /Days				
	1st day	3rd day	5th day	7th day	9th day
Control	0	2.5 c	4.0 c	6.6 b	9.0 c
0.5mM	0	1.2 a	4.0 c	8.3 c	11.3 c
1.0 mM	0	1.0 a	2.0 b	5.3 b	8.0 b
3.0 mM	0	0.8 a	1.3 a	3.3 a	4.5 a

Figures with the same letter are not significantly different (p=0.05)

Root Fresh Weight (gm):

Seedling fresh weight of broad bean are showed in (Table5). Data reveals that; the inhibitory effect was concentration dependent, as a gradual inhibition in root fresh weight obtained as concentration increased . Results of root fresh weight reveals that priming *faba bean* seeds with SA decreased the root weight as compared to control, Whereas; at the end of priming period (9 days) the highest root weight (11.3± 2.133gm) was recorded by 0.5mM SA in comparison with control priming (9± 1.567mg) and it was followed by 1mM SA, and 3mM SA (8±1.482 gm, 4.5±. 0.768gm) respectively. On the other hand, minimum root fresh weight was given by 3mM SA (4.5±. 0.768gm).

Table 5: Effect of Different Concentrations of Salicylic acid SA on Root Fresh Weight (gm) of faba bean Seedlings.

Salicylic acid SA Concentration	**Root Fresh Weight (gm) /Days**				
	1st day	3rd day	5th day	7th day	9th day
Control	0	2.5 c	4.0 b	6.6 c	9.0 c
0.5 mM	0	1.2 a	4.0 b	8.3 d	11.3 d
1.0 mM	0	1.0 a	2.0 a	5.3 b	8.0 b
3.0 mM	0	0.8 a	1.3 a	3.3 a	4.5a

Figures with the same letter are not significantly different (p=0.05)

In conclusion, According to the results of this study and the ANOVA analysis it could be noted that, no statistically significant between 0.5 and 1.0 mM of SA concentrations at the 9th day after sowing. Based on the results, it can be concluded that low concentrations of SA have more positive effects on faba bean germination than medium and high concentrations. This means that seed priming of faba bean with this low concentration of SA will speed up the germination time and enhance the establishment of seedlings.

Discussion

The results of this study clearly show that, the effect of salicylic acid on the seed germination and seedling growth of faba bean was stated to be variable, promoting some parameters and inhibiting others depending on its concentration. As, Maximum vigour index recorded in 0.5mM SA compared to control. Whereas the maximum inhibitory effect of (SA) for seed vigor index was recorded in 3.0 mM . In our treatments with increased SA concentration the percentage of germination and seedling growth parameters in the different treatments during 9 days of faba bean seeds were decreased. On the contrary, SA enhanced the emergence in lower concentrations; that appeared harmless for both seed germination and establishment of the seedling.

The promotive effect of salicylic acid could be attributed to its bioregulator effects on physiological and biochemical processes in plants such as:- Promoting effect of SA is due to increased level of Cell division, cell differentiation and cell elongation within the apical meristem of seedling root, and increment in biosynthesis of organic foods which caused an increase in seedling growth (Abd El-Wahed *et al*., 2006; El-Khallal *et al.,* 2009, and Delavari *et al.,* 2010). Ion uptake, transport and the availability and movement of nutrients could result in stimulating different nutrients in the leaves (Raskin, 1992).Habibi and Abdoli (2013) reported that low concentrations of SA increased the germination percentage, but this increase was not significant compared to the control treatment. Similar, Farahbakhsh (2012) reported that the concentration of 0.25 and 0.5 mM of salicylic acid on measured traits was more effective compared with the other levels. There are conflicting reports about the effects of salicylic acid on seed germination. Sharafizad *et al*. (2013) reported that after the control treatment (priming with distilled water), the highest percentage of germination was for low concentrations of Salicylic acid (0.7 mM) and germination percentage decreased with increasing concentrations of SA. These in agreement with our results. SA induced several effects depending on the concentration applied and quite high doses were required to observe symptoms; in all cases, the first effect observed was a delay in seed germination (Metraux *et al*., 1990). Above a concentration of 0.5mM, the seed germination process was dramatically impeded.

Our results also indicate that, the minimum percentage of germination (Maximum inhibition) of seed germination was found on higher concentration of Saying this respect ,Several workers reported that stimulating effects of SA on germination are concentration dependent (Rajjou *et al*., 2006 and Singh *et al*., 2010). SA significantly stimulated the activities of enzymes involved in germination such as transkelolase, enolase, malate dehydrogenase, phosphoglycerate kinase, glyceraldehyde 3-phosphate, dehydrogenase, fructose 1,6-diphosphatase, and pyruvate decarboxylase. In addition; seeds germinated in SA as substrate media showed abundant levels of isocitrate lyase and malate synthase (key enzymes of glyoxylate cycle) (Eastmond and Graham, 2001 and Rajjou *et al*., 2006). (Rajjou *et al*., 2006) hypothesized that detoxification mechanism in germinating seeds counteract by exogenous SA treatments

SA significantly increases plant growth either in stress or without stress conditions. This effect probably related to SA inhibition of Cl- and Na+ absorption and its help for Mg, Fe, Mn, N and Cu absorption or results from its effect on lipid peroxidation and membrane permeability (Gunes *et al.*, 2007).Lower SA could promote sink/source regulation, enzymatic activities, protein synthesis and photosynthetic activity as well as increase the antioxidant capacity of plants (Raskin, 1992; Blokhina *et al.*, 2003 and El-Tayeb, 2005).It is possible that SA stimulates the seed germination via bio-synthesis of gibberellic acid and acts as thermogene inducers Shah, (2003). Eastmond and Graham, (2001 reported that isocitrate lyase and malate synthase were found to be more abundant in seeds germinated in the presence of SA than in its absence .Isocitrate lyase and malate synthase are two key enzymes of the glyoxylate cycle that play a crucial role in the synthesis of carbohydrates from storage lipids during seed germination and seedling establishment. An elicitation of these metabolic pathways by SA would favor the transition from a metabolically quiescent state to a metabolically active state during germination, thereby accounting for the observed improvement in seed vigor in the presence of this molecule.SA caused marked increments in IAA, GA3, zeatin and zeatin riboside, in the meantime decrease in ABA content comparing with untreated controls (Shehata *et al.,* (2001) and Zaghlool, (2002). On the other hand, the decrease in ABA content attributed to the shift of the common precursor isopentenyl pyrophosphate to biosynthesis of cytokinins and/or gibberellins instead of ABA (Hopkins and Huner, 2004).Findings of Eisavand *et al.* (2011) showed an increase in respiration activities, ATP production, induced RNA activity and protein synthesis in primed seeds, as well as enhanced radicle length and weight of primed sunflower seed.

Results in this study are in agreement with several reports which related to applications of SA at higher levels could reduce emergence index. Similar results have been reported for maize embryo germination, for which high doses of SA, in the range of 3 to 5 mM, completely inhibited germination (Guan *et al.*, 2007). Above a concentration of 0.5 mM, this compound entailed a strong retardation of growth and plants appeared bleached, most presumably because at these high concentrations SA induced an oxidative stress (Rao *et al*., 1997). Improvement in germination characteristics of primed seeds could be results of increasing the antioxidant profile of treated seeds (Ansari and Sharif-Zadeh, 2012).Increasing SA concentration caused many changes in physiological pathways during seed germination and seedling growth. From these changes:-When exogenous SA is more than 1 mm, plants usually trend to oxidative burst and cell death. On the basis of evidence that high levels of SA can bind and inhibit H_2O_2 removing enzymes, ascorbate peroxidase (APX) and catalase (CAT) (Durner and Klessig, 1995). Findings of Nun *et al.* (2003) showed that SA can inhibit the activity of catalase. Reduced catalase activity leads to increased hydrogen peroxide (H_2O_2) product which stops germination processes.At the higher levels of SA; sucrose which come from scutellum independtaly to seedlings during germination is inhibited. As a result ketoaldehydes, H_2O_2 and various free radicals are generated and inhibit seedling growth (Russell *et al*, 2002).Enhance ABA synthesis, which can stop the seed germination (Wu *et al.*, 1998; Ahmad *et al.* 2012).In addition, NO (Nitric Oxides) can chemically react with superoxide, generating the cytotoxic compounds peroxynitrite and hydroxyl radical, therefore inducing oxidative burst and cell death (Van Camp and Van Montagu, 1998).SA is an effective inhibitor of ethylene biosynthesis and does it via effect on ACC-synthethas enzyme (Raskin *et al.*, 1992). Results represented in this research; the decrease in germination percentage may be result of ethylene biosynthesis inhibition. Mazaherie Tirani and Manouchehri (2005) reported that high concentrations of SA reduced Brassica napus seed germination compared to hydro-priming which is in *accordance with the findings of the present study*. Whereas, When using 0.5 mM salicylic acid, Szepesi *et al.* (2005) found increased percentage of germination in tomato seeds. Data agree with those found by Maia *et al.* (2000), who have found in soybean that salicylic acid used alone has increased the lengths of shoots and roots and fresh weight at concentrations of 50 and 100 mg.kg-1.Reduction in physiological parameters previously reported in our study may be a result of higher dosages of SA used where toxic effects are more pronounced than beneficial effects of SA and this in agreement with (Barkosky and Einhellig 1993).

Conclusion and Recommendation

From this study we can be concluded that priming of faba bean with low concentrations of SA (0.5 mM) is a simple, cheap and unsophisticated tool that has a practical importance and could be recommended to farmers to achieve higher germination and uniform emergence under field conditions to accelerate potential crop yield in near future. In future, the exogenous application of this phytohormone might act as powerful tool in enhancing the growth and productivity of more biomass and photosynthetic capacity, especially in areas with saline irrigation water resources at planting stage. Moreover; the planted seeds will be less susceptible to soil-borne pests and diseases, and this has positive consequences on management of this crop at planting and germination stage.

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