

**EFFECT OF CONCENTRATION AND APPLICATION METHOD OF GA₃, SALICYLIC
AND ASCORBIC ACIDS ON SEED YIELD OF BROAD BEAN (*Vicia faba* L.)
UNDER SALINE SOIL**

Ali H. Jasim, Naeem S. Matar

Faculty of Agriculture, Babylon University, Iraq

Abstract

An experiment was conducted at Al-Kefel, Babylon, to study the effect of seven acid treatments (GA₃, 50 and 100 mg/l ; Salicylic, 0.5 and 1 mM ; Ascorbic, 50 and 100 mg/l, in addition to the control treatment with water) and its interactions with three application methods (seed priming, foliar, priming+ foliar) on yield parameters of broad bean plants under saline soil (7.5 dsm⁻¹) in 2011-2012 growth season. Randomized complete block design with three replications was used. The results showed as follow: Treatment with GA₃ (100 mg/l) or salicylic acid (1 mM) alleviated salt damage and caused a significant increase in pod numbers/pant (10.6 and 9.6), green pod weight (20.8 and 20.2 gm), weight of 100 seeds (128.2 and 132.2 gm), green pods yield (12.22 and 11.9 ton/hectare) and dry seed yield (3.44 and 3.27 ton/hectare) as compared to control.

Key world: broad bean, saline soil, GA₃, salicylic acid, ascorbic acid, foliar application

INTRODUCTION

Broad bean (*Vicia faba* L.) is an important legume crops grown for its wet and dry seed. And it is cultivated in crop rotations to improve soil properties (Jassim, 2007). Farmers accept planted broad bean because its low cost cultivation and ease of transfer and circulation.

Salinity is major harmful environmental factor that poses a major threat to crop productivity mainly in arid and semiarid region of the world (Munns, 2005). Effects of salinity are more obvious in arid and semiarid regions where limited rainfall, high evaporate transpiration, and high temperature associated with poor water and soil management practices are the major contributing factors (Azevedo Neto et al., 2006). Plant productivity is severely threatened by salinity. About 800 million hectares of land throughout the world are salt-affected (Munns, 2005). Salinity is due to high amount of soluble salts in soils or waters. Elevated levels of soluble salts in the soils as well as water result in restricted growth and yield production in arid and semi-arid regions (Flowers et al., 2010). It has been well practiced that when electrical conductivity (E.C.) exceeds greater than 4 ds.m⁻¹, a sharp decrease in the crop yield has been observed, but the level of reduction depends upon the tolerance potential of crop plants (Sairam et al., 2002). The threshold of broad bean is 1.6 ds.m⁻¹ and the decrease in yield is 9.6% in each increasing 1 ds.m⁻¹ (Ayers and Eberhard, 1960). Photosynthesis, decreases due to salt-induced destruction of photosynthesizing tissues, reduced water potential and high accumulation of Na⁺ and Cl⁻ (Ashraf et al., 2008). The adverse effects of salt stress on photosynthesis as well as plant growth are correlated with oxidative stress induced by generation of excessive amounts of ROS and imbalance in regulation of antioxidative defensive mechanism (Noreen et al., 2010).

GAs are phytohormones known to induce various physiological responses in plants and alter plant metabolism under stress. GA₃ increase photosynthetic rate in leaves of bean (Khan, 2002). GA₃ is important in influencing the photosynthetic activity and consequently the source-sink relations. The

increase in glucose content by GA₃ application shows the efficiency of GA₃ in increasing photosynthesis (Brian et al., 2006).

Salicylic acid (SA) is an endogenous growth regulator, which participates in the regulation of physiological processes in plants. SA mediates the oxidative burst that leads to cell death in the hypersensitive response and acts as a signal for the development of systemic acquired resistance (Shirasu et al., 1997). SA had a major role in modulating the plant response to several abiotic stresses (Senaratna et al., 2000). SA has been reported to ameliorate the adverse effects of salinity by improving the functioning of the photosynthetic pigments and antioxidative enzymes (Idrees et al., 2010; 2011). SA is also considered to be a signaling molecule that plays a key role in growth, development, and defense responses in plants under stress conditions (Cameron, 2000).

Ascorbic acid (AsA) is one of the most important antioxidants abundantly occurring in plants (Smirnoff, 2000). High endogenous AsA in plants is necessary to counteract oxidative stress in addition to regulating other processes of plant metabolism. Endogenous AsA can be increased by exogenous application of AsA through the rooting medium (Chen and Gallie, 2004), as a foliar spray or as seed priming.

Studies showed that application methods affecting the efficiency of growth regulators. Unamba and others (2009) added GA₃ on okra by priming or spraying at low-lying (0-30 mg/l), and reported that both methods were effective, but spraying was the most efficient way than priming in its effect. Akbari and others (2008) added GA₃ by priming , spraying or priming+ spraying at saline stress, they found that priming+ spray was the best in mitigate salt damage and gave the best results.

Nevertheless, there have been no quantitative investigations of the effects of an additional supply of GA₃, salicylic acid and ascorbic acid on broad bean. The objectives of this study were to determine how GA₃, salicylic acid and ascorbic acid affect yield of broad bean under salt stress.

MATERIALS AND METHODS

Experiment was conducted in a private farm in the Al-Kefel area, Babylon governorate/Iraq during 2011-2012 growth season to study the effect of seven treatments: GA₃ at 50 and 100 mg/l, Salicylic Acid (SA) at 50 and 100 mM, Ascorbic Acid (AsA) at 50 and 100 mg/l in addition to water treatment (as control), and its interactions with three application methods: priming, spraying, priming+ spraying, on yield of broad bean plants under salt stress.

Soil was chosen at E.C. 7.5 dsm⁻¹. Complete Randomized Block Design with three replications was used. The seeds were priming for 24 hours according to treatments and seeded in 14/10/2011 in rows 75 cm width , the distance between hills along the row was 20 cm. Plot area was 6.75 m² (3 rows with 4 meters long). Crop and soil service were conducted as recommended in the cultivation of broad bean. Foliar application was carried out twice , the first was done after one month from emergence and the second after one month later the first spray. Average of pods number/plant, seeds number per pods, green pod yield(t/ha) at seed filling stage (from one row of each experimental unit) , 100 dry seeds weight and dry seed yield (t/ha) from the other two rows of each experimental unit , were determined. Statistical analyses were performed with SPSS packet software and the values of least significant differences (LSD_{0.05}) were calculated to compare the means of different treatments.

RESULTD AND DISCUSSION

Table (1) shows that there is significant superiority for GA₃ 100 mg/l compared to control. It was attributed to the increase in vegetative growth (Ali and Matar, 2013_{a,b}). Salinity causes premature

senescence which reduce the amount of assimilate that the plant can produce and a reduction in the assimilate transported to the growing tissue may further limit growth (Munns, 2002) which produce least pod number and weight. Addition of GA₃ will increase the pod number and this was confirmed with (Shah, 2007) in his study on mustard plant when sprayed with 10⁻⁵ mole of GA₃ caused a significant increase in dry weight, leaf area, chlorophyll content and the rate of photosynthesis which led to an increase in pod number. (Alonso-Ramirez et al., 2009) reported that GA₃ by repressing DELLA proteins and changing the SA/JA balance are able to control plant responses to stress. SA had no significant superiority to the rest of the transactions (except the GA₃ 100 mg/l) and this attributed to minimize the falling and abortion flowers and keep flowers from drought and encourage pollination flowers internally before blooming, (Amanullah et al, 2010). And also to its role in the inhibition of ethylene, ABA and enzyme pectin methyl esterase which lead to encourage pod falling (Gupta, 2011). The harmful effects of salinity will alleviate by SA treatment from the possibility of renew sink and improve the rate of accumulation of dry mass in the development of developing productive organs (Davies, 1995). SA contributes in increasing the efficiency and the use of water in addition to protecting pigments and photosynthesis device (Hassanein, 2009). This results agreed with the finding of (Azooz, 2009) on faba bean. The table showed that application methods and the interaction between treatments had no significant effect on plant pod numbers.

Table (1): Effect of GA₃, SA and AsA, and its interactions with application methods on pod numbers in plant

B A	water	GA ₃ 50mg/l	GA ₃ 100mg/l	SA 0.5mM	SA 1mM	AsA 100mg/l	AsA 50mg/l	Mean A
soaking	9.3	10.0	10.0	9.3	10.7	9.3	9.7	9.8
spraying	9.0	9.7	10.7	9.0	11.0	10.0	10.0	9.9
Soaking+ spraying	10.0	10.7	11.0	10.3	9.7	9.0	9.7	10.5
Mean B	9.4	10.1	10.6	9.6	10.4	9.4	9.8	
LSD _{0.05}	LSD A= n.s LSD B=1.179 LSD AB= n.s							

Table (2) Showed that GA₃ 100mg/l and SA 1mM treatments were superior and significantly increased average weight of green pod compared to control and the percentage of increases were (8.12 to 2.16%), respectively. It has been proposed that under salt stress conditions the thickness of the assimilate conducting pathway is reduced (Aldequy and Ibrahim, 2001), and leaves start behaving as sinks rather than sources (Arbona et al., 2005). This causes inhibition of assimilate movement towards the developing reproductive organs, which might be the reason for the observed decrease in pod number, seeds per pod, 1000-seed weight and seed yield per plant. On the other hand, these adverse effects of high salinity were alleviated by the hormone treatment, primarily by rejuvenation of the sink potential and enhancement of the duration or rate of dry mass accumulation in developing reproductive organs (Davies, 1995). GA₃ is known to have a secondary enhancement effect on protein content through the intensification of nitrate reductase activity (Shah, 2004). Stimulation of the

enzyme protein synthesis by GA₃ stimulates the overall protein synthesis (Premabatidevi, 1998). It indicate that the ability of GA₃ to ameliorate all adverse effects of salt stress and rescue the pod weight productivity. SA treatment increased the weight of green pod and it was due to increased in chlorophyll content, modify the content of nutrients elements and reduce the toxic ions content in plant leaves (Ali and Matar, 2013_{a,b}). SA role in increasing the proportion of cytokines and increase the proportion of cell divisions and the number of cells (Sanna et al., 2001). These results are consistent with (Kumar et al., 2000) on soybean and (Zaghlool, 2002) on mung bean. On the other hand AsA had no significant effect, but tended to increase average pod weight. The table showed that method of applications and the interaction had no significant effect on the weight of green pod.

Table (2): Effect of GA₃, SA and AsA, and its interactions with application methods on green pod weight (gm)

B A	water	GA ₃ 50mg/l	GA ₃ 100mg/l	SA 0.5mM	SA 1mM	AsA 100mg/l	AsA 50mg/l	Mean A
soaking	18.0	18.1	20.7	18.17	19.8	17.6	19.2	18.8
spraying	17.7	17.9	20.4	17.9	20.3	17.8	18.6	18.7
Soaking+ spraying	18.1	19.8	21.3	19.9	20.5	18.8	18.7	19.6
Mean B	17.9	18.6	20.8	18.7	20.2	18.1	18.8	
LSD _{0.05}	LSD A=n.s			LSD B= 1.38		LSD AB= n.s.		

Table (3) shows that GA₃ at 50 and 100 mg/l and SA at 0.5 and 1 mM were superior significantly on increasing weight of 100 seeds compared to control. The increases by GA₃ due to its ability to ameliorate all adverse effects of salt stress. Or as a result that GA₃ stimulate to build nucleic acids DNA and RNA, thereby increasing protein building and biological processes within the plant (Al-Asadi, 2006). Under stress conditions beards, the leaves begin to behave like a sink rather than asource. And that these reasons reduce the representation movement towards developing and producing organs(Arbona et al., 2005), that are a major cause of the decline is noticeable in green pod weight. This results was agreed with (Sarkar et al., 2002) who found that 100 seed weight increase in soybean plants with adding GA₃ 100 mg/l. On the other hand, SA led to alleviate salinity damage through the ability to link with amino acids and its role in increasing the production of nucleic acids RNA, DNA in addition. AsA had no significant effect , but tended to mitigation of salt stress damage. The table showed that methods of application and the interaction had no significant effect.

Table(4) showed that GA₃ 100 mg/l and SA 1mM caused a significant increase in green pods yield compared to control, with an increase of 19.6 and 16.5% respectively. The increase due to help the plant to ameliorate the slat stress conditions by improving vegetative growth, pigments content, modify the content of nutrients elements and reduce leaves content of toxic ions and reduce oxidative damage (Jasim and Matar, 2013_{a,b}). it caused increases in photosynthetic potential of plant resulting in more photosynthate production which in turn enhances the sink strength(Iqbal et al., 2011). On the other hand SA alleviate the harmful effect of salt stress (El-Khallal et al., 2009). This results agreed

with (Turkyilmaz et al., 2005) on bean and (Jeyakumar et al., 2008) on mung bean. AsA had no significant effect. AsA had no significant effect. The table showed that application methods and the interaction had no significant effect on green pod yield.

Table (3): Effect of GA₃, SA and AsA, and its interactions with application methods on 100 seeds weight (gm)

B A	water	GA ₃ 50mg/l	GA ₃ 100mg/l	SA 0.5mM	SA 1mM	AsA 100mg/l	AsA 50mg/l	Mean A
priming	119.9	124.8	129.6	126.5	132.1	124.0	124.9	126.0
spraying	122.3	127.7	130.0	127.1	131.6	121.8	124.1	126.4
priming+ spraying	120.0	132.1	129.3	131.4	133.1	125.6	122.1	127.6
Mean B	120.7	128.2	129.6	128.3	132.2	123.8	123.7	
LSD _{0.05}	LSD A= n.s LSD B= 7.17 LSD AB= n.s							

Table (4) : Effect of GA₃, SA and AsA, and its interactions with application methods on green pod yield (ton/ha)

B A	water	GA ₃ 50mg/l	GA ₃ 100mg/l	SA 0.5mM	SA 1mM	AsA 100mg/l	AsA 50mg/l	Mean A
Soaking	10.072	10.901	12.404	10.133	11.831	9.798	11.103	10.984
Spraying	9.701	10.373	11.971	9.973	12.034	10.601	11.132	10.826
Soaking+ spraying	10.803	12.030	12.304	11.630	11.832	10.074	10.733	11.344
Mean B	10.202	11.101	12.223	10.582	11.902	10.161	10.992	
LSD _{0.05}	LSD A= n.s. LSD B= 1.133 LSD AB= n.s.							

Table(5) showed that GA₃, SA at all concentrations caused a significant increase in dry seed yield compared to control. GA₃ 100 mg/l was superior significantly compared to all treatment (except SA 1mM). This increase was due to increases in chlorophyll and vegetative growth, and reduce leaf content of the toxic ions and increasing leaf content of potassium and calcium (Jasim and Matar, 2013_{a,b}). The photoassimilates produced under salt stress are used to support crucial processes such as growth and osmotic adjustment. In general, salinity causes a reduction in sink enzyme activities, leading to an increase in sucrose in source leaves, with a decrease in photosynthesis rate by feedback inhibition (Poljakoff-Mayber and Lerner, 1994). Application of GA is found to induce extracellular

invertase, which acts on sucrose to form hexose. The hexose formed is easily transported to the sink, thereby increasing sink strength. The induction of extracellular invertase by both abiotic and biotic stress stimuli supports the suggestion that extracellular invertase is not only a key modulator of assimilate partitioning, but is also an important component of various stress responses. Similarly, GA increases photosynthetic rate under stress and thus enhances the source potential (Iqbal et al., 2011). These results were agreed with (EL-Shraiy and Hegazi, 2009) on peas and (Gherroucha et al., 2011) on wheat. AsA had no significant effect, but it tend to increase. The table showed that methods of application and the interaction had no significant effect.

Table (5): Effect of GA₃, SA and AsA, and its interactions with application methods on dry seed yield (ton/ha)

B A	water	GA ₃ 50mg/l	GA ₃ 100mg/l	SA 0.5mM	SA 1mM	AsA 50mg/l	AsA 100mg/l	Mean A
Soaking	2.714	2.932	3.352	2.924	3.421	2.897	2.976	3.031
Spraying	2.573	2.808	3.481	2.804	3.210	2.873	2.797	2.935
Soaking+ spraying	2.554	3.314	3.514	3.273	3.199	2.889	2.996	3.106
Mean of B	2.614	3.018	3.449	3.000	3.273	2.886	2.926	
LSD0.05	LSD A= n.s. LSD B= 0.539 LSD AB = 0.313							

CONCLUSION

We concluded that treatment with 100 mg GA₃/l and 1MM SA led to mitigate the damage of salt stress on bean yield, while ASA had no significant effect, but tend to minimize the damage of salt stress. The application of methods and interactions between treatments had no significant effect. We recommend conducting subsequent studies inflict by using higher concentrations and other crops.

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