

Influence of adding ascorbic acid and yeast on growth and yield and Rhizobium of snap bean (*Phaseolus vulgaris* L.) under irrigation with saline water

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Abstract: A field experiment was carried out in Horticulture Department / Collage of Agriculture/University of Baghdad to study influence of adding ascorbic acid(asa) and bread yeast extract in snap bean cv.primel under irrigation with saline water using sodium chloride salt (NaCl) during spring season of 2016 .A factorial experiment using Randomized Complete Block Design(RCBD) with three replications were conducted . The first factor includes three treatments of salinity which were tap water (S0), 4ds.m⁻¹(S1) and 8ds.m⁻¹ (S2) . The second factor includes three treatments which were control treatment without any adding (C),ascorbic acid 0.3g.l⁻¹(A) and yeast extract 12g.l⁻¹(Y). Results showed significant and gradually decreases in all studied traits of vegetative growth , yield , leaves content of prolien and rhizobia viability by increasing salinity level. The superiority of yeast extract (Y) adding was observed in root nodules/plant ,dry weight/plant, pods number/plant, pod weight, pods yield/plant,prolien content and rhizobia viability while highest value observed in both of plant height and leaf area due to ascorbic acid .The correlations among all the studied traits were significant and positive except in prolien content with other traits were negative and significant .

Keywords: Bean production, Rhizobium, Salinity stress, Yeast.

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I. Introduction

The bean (*Phaseolus vulgaris* L.) is one of the important vegetables crops grown in Iraq that occupies a great importance in local consumption and it is an important source of dietary protein in many developing countries but is considered as sensitive species to salinity compared to other legumes [7] . Salinity constitutes a major obstacle in the production and plant growth especially in the regions where the water for irrigation is loaded with salt and it induces the reduction of growth and yield of sensitive varieties [30]. The adverse effects of high concentration of salts for plants are due to the osmotic relation of water and to specific ionic effects (e.g., Na⁺ and Cl⁻) on the protoplasm combined with oxidative damage in tissues [41] . The bean can resist electrical conductivity in irrigation water (EC) up to 0.7 dSm⁻¹ without affecting yield and quality while at an EC of 1 and 2.4 dSm⁻¹ a yield loss of 10 and 50 percent would be expected, respectively [23] . Salinity also affects the absorption of water and nutrients and the physical and chemical properties of soil and lead to a decrease the efficiency of photosynthesis . [18,26,27] . The relationship between root nodulation bacteria and legume host is complicated and usually determined by several factors and its ability to symbiosis with rhizosphere organisms was affected by salinity. The use of soil and irrigation water with a high content of soluble salts is a major limiting factor for crop productivity in the semi-arid areas of the world. While important physiological insights about the mechanisms of salt tolerance in plants have been gained, the transfer of such knowledge into crop improvement has been limited. The identification and exploitation of soil symbiotic microorganisms promotes a beneficial physiological effects include improved nutrient and water uptake, growth promotion, and alteration of plant hormonal status and metabolism and there was an abundant literature on how symbiotic nitrogen-fixing bacteria affect legume responses to salt stress [6,11,12,13,24,34]. The diversity of microbial properties capable of promoting plant growth makes it difficult to be sure about the importance of particular mechanisms within specific plant-microbe interactions in saline environments. Nevertheless, the range of organisms existing in the rhizosphere of halophytic plants [28] may provide a valuable resource for this alternative way of improving crop tolerance to salinity. Samra et al [33] and Yadegari and Rahmani [38] referred to that the presence of *Rizobia* bacteria effectively and the emergence of symbiotic relationship may increase plants resistance to the stress conditions, and with expansion of the geographical area of saline soil it was necessary to add chemical fertilizers containing nitrogen to increase the probability Resistance of the plant, but the negative effects of chemical fertilizers led to the search for other methods with positive impact on the soil microorganisms. The studies have indicated that bread yeast fungi resistant the high levels of temperature and salinity [37]. Abou- El-Yazied and Mady [3] referred to some important chemical characters of yeast extract

analysis (Table 1.) .Ascorbic acid (AsA) is an important antioxidant in plants which accumulates in plants as an adaptive mechanism to environmental stress such as salinity, AsA regulates stress response as a result of a complex sequence of biochemical reactions such as activation or suppression of key enzymatic reactions, induction of stress responsive proteins synthesis, and the production of various chemical defense compounds and it had a protective role in plant cells from the adverse effects of salt stress[5,19,39]. So research for improvement the tolerance of plants and its symbiotic relationship to high levels of salinity becomes an imperative for agricultural production. This study is to investigate the role of adding Ascorbic acid (AsA) and yeast extract on improvement the growth, yield and Rhizobium bacteria activity of snap bean under irrigation with different levels of saline water.

II. Materials And Methods

The factorial experiment was conducted at the fields of department of horticulture and landscape gardening, collage of Agriculture, university of Baghdad during the spring season of 2016 using snap bean cv. Primel to study the influence of adding ascorbic acid 0.3 g.l⁻¹ (A) and yeast extract 12 g. l⁻¹ (Y) after emergence of plants every two weeks and with four times, in addition of control treatment without any adding (C) under three different levels of irrigation with saline water using sodium chloride salt (NaCl). Salinity treatments were tapwater (S0), 4 dS.m⁻¹ (S1) and 8 dS.m⁻¹ (S2). Plastic pots of 30cm diameter and 25 cm depth were used each one provided with outlet in the bottom and filled with 14 kg of sterilized soil and peatmoss with ratio 1:1. The pots were arranged in factorial experiment in a randomized complete blocks design (3×3) with three replicates. Each replicate contained 36 pots. Ten seeds were sown in each pot on 1 March the plants were then thinned to four healthy plant for each pot. The pots were washed every 20 days and non-saline and salinity treatments were reapplied in order to prevent further increase in electrical conductivity (EC) due to adding of saline water. Each pot was fertilized with ammonium sulfate (20.6% N) at a rate of 2.8 g/pot, calcium superphosphate (15.5% P₂O₅) at a rate of 2.8 g/pot, and potassium sulfate (48% K₂O) at a rate of 1.4 g/pot. These fertilizers were applied at two equal doses; the first was added after 3 weeks and the second after 5 weeks from sowing. After 52 days from sowing, three samples from each treatment were taken for measuring vegetative growth parameters plant height, nodules number/plant, leaves area, dry weight/plant. At a maturity period all plants from each pot were harvested to determine pods yield, pods number/ plant, pod weight. To determine free proline level content in leaf tissue 0.5 g of leaf samples from each group were homogenized in 3% (w/v) sulphosalicylic acid and then filtered through a filter paper [Bates et al]. After addition of acid ninhydrin and glacial acetic acid, the mixture was heated at 100 °C for an hour in water bath., Reaction was then stopped by ice bath. The mixture was extracted with toluene and the absorbance of fraction with toluene aspired from liquid phase was read at 520 nm. Proline concentration was determined using calibration curve and expressed as μmol proline g⁻¹ FW according to the method described by Bates et al. [9]. Viable count data of *Rhizobium* from Effective root nodules of three plants from each treatment were carried out by using viable count method according to Beck et al [10]. Data were analyzed based on experimental design model. Means comparison was performed based on least significant difference (LSD) test (P≤0.05). All statistical analyses were performed using GenStat (V.12) software.

Table 1. Chemical analysis of bread yeast extract.

Amino acid%		Vitamins (mg/100 g DW)		Minerals	
Alanine	1.69	Vit.B1	23.33	Nitrogen %	6.88
Arginine	1.49	Vit.B2	21.04	Phosphorus%	0.66
Aspartic acid	2.32	Vit.B6	20.67	Potassium%	0.95
Cystine	0.63	Vit.B12	19.17	Magnesium%	0.19
Glutamic acid	3.76	Thiamin	23.21	Calcium%	0.17
Glycine	1.45	Riboflavin	27.29	Sulfur%	0.48
Histidine	0.71	Inositol	20.43	Iron(ppm)	107
Isoleucine	0.85	Biotin	20.04	Zinc(ppm)	77
Leucine	1.91	Nicotinic acid	73.92	Copper(ppm)	5
Lysine	1.13	Panthenic acid	38.43	Others	
Phenyl alanine	1.18	P amino benzoic acid	29.49	Manganese(ppm)	43
Proline	1.29	Folic acid	26.22	Crude Protein%	2.2
Serine	1.98	Pyridoxine	22.09	Crude Fat%	33.21
Threonine	1.54			Carbohydrates%	7.2
Tryptophan	0.25			Crude Fiber%	3.8
Tyrosine	0.99				
Valine	1.4				
Methionine	0.4				

III. Results And Discussion

3.1.1. Effect of salinity on growth and yield parameters

From Table 2. we can find that there was significant and gradually decreases in plant height, root noules /plant, leaf area/plant, dry weight/plant, pods number /plant, pod weight /plant and pods yield/plant, by increasing salinity level .Similar growth reduction due to salinities were also reported by Lovelli et al. [22] and Krouma et al[20] on bean. Previous studies reported that depressing effect of saline water on plant growth may be attributed to the effect of increasing soil soluble salt content and raising the osmotic pressure of the soil solution and as a result, less water flows from the soil into the plant [21,40], consequently less water is available for normal growth and development. Moreover the inhibited effects of saline water may be due to its effects on cell division and cell elongation . The salinity stress effected a reduced in vegetative growth therefore the availability of photosynthesis decreased during the reproductive phase which lead to decreasing pods number and yield at harvest period. These results are in agreement with [2,35] .

3.1.2 Effect of salinity on proline and Rhizobium viable count

Data in Table 2. also indicated that salinity induced a significant increase in proline accumulation in leaves of snap bean cv.primel .The proline may act not only as an osmolyte, but it may also help the cells to overcome oxidative stress in salt stressed plants and it can protects the cell by balancing the osmotic potential of cytosol with that of vacuole and external environment [15,31]. The data of Table 2. also refered to significant and gradually decreases in Rhizobium viable count in Effective root nodules of plants that most microorganism in Iraqi soils suffer and affected by salinity, that results were agreement with [1,2] and similar results were founded by Delgado et al. [14] which mentioned that salinity was also responsible for a decrease in cytosolic protein of nodules, specifically nodule leghemoglobin ; this effect was more pronounced in pea and bean nodules than in soybean and faba-bean nodules as well as Salinity may inhibited O₂ uptake from nodules bacteroids of each legume and Under severe stress the reduction in nodule leghemoglobin may be involved in salt-induced inhibition of viability in Rhizobium.

Table 2. Effect of salinity on growth, yield, proline and Rhizobium viable count .

Salinity Levels ds.m ⁻¹	Plant Hight cm	Nodules Numbere /plant	Leaves Area cm ²	Dry Weight g	Pods Number/plant	Pod Weight g	Pods Yield/plant g	Prolien μmol .g ⁻¹ FW	Rhizobium in 1ml x10 ⁶
S0	51.9	7.79	928	42.97	17.89	3.422	61.62	0.7267	3.017
S1	39.54	4.98	904.8	35.8	10.33	3.144	33.03	2.2556	1.3341
S2	35.62	4.18	892.9	32.81	8.68	2.944	25.72	4.8956	0.8338
LSD 5%	2.755	0.93	29.16	3.759	1.184	0.2107	6.586	0.10826	0.01968

3.2.1.Effect of ameliorative substances on growth and yield parameters

Data in Table 3. show that adding ascorbic acid and yeast extract compared with control were significantly improved plant growth and yield parameters plant height, root noules /plant, leaf area/plant, dry weight/plant, pods number /plant, pod weight /plant and pods yield/plant. The positive effect of yeast extract was observed and it was the highest that may be due to the ability of yeast extract by using bread baker yeast (*Saccharomyces cereviciae*) to produce indol acetic acid (IAA) and gibberellic acid (GA3) in addition to cytokinin [29,36] and those products lead to use yeast extract in improving growth and productivity in some vegetable crops under conditions of a biotic stress [16]. Also table 2. referred to the positive role of ascorbic acid in the increasing of leaf area and that due to stimulate growth rate and cell division and/or cell enlargement and this, in turn, improves plant growth. These results agreement with [19].

3.2.2. Effect of ameliorative substances on proline and Rhizobium viable count

Table 3. referred to significant effects of adding both of yeast extract and ascorbic acid on leaves content of prolien and rhizobia of effective root nodules in plants. The highest values of prolien content and rhizobium were in yeast extract treatment that due to yeast content of prolien and that may lead to further increase in plant leaves., Previous studies mentioned that root colonization by symbiotic microorganisms which promotes by using of yeast can induce a major changes in the relative abundance of the major groups of organic solutes such as modifying the composition of carbohydrates and inducing accumulation of specific osmolytes such as proline thus facilitating osmotic adjustment [32]. These results are agreement with [4,17].

Table 3. Effect of applied ameliorative substances on growth, yield, proline and Rhizobium viable count .

ameliorative substances	Plant Height cm	Nodules Number /plant	Leaves Area cm ²	Dry Weight/plant g	Pods Number/plant	Pods Weight g	Pods Yield/plant g	Prolien $\mu\text{mol} \cdot \text{g}^{-1}$ FW	Rhizobium in 1ml x10 ⁶
C	39.14	4.33	875.6	35.06	9.78	2.956	29.51	2.77	1.137
A	44.87	5.64	928.1	37.86	13.34	3.267	44.9	2.01	1.813
Y	43.06	6.97	921.9	38.67	13.78	3.289	45.97	3.09	2.233
LSD 5%	2.025	0.546	18.97	1.433	1.175	0.276	3.041	0.0449	0.0197

3.3.1. Effect of the interaction on growth an yield parameters

The results in Table 4. showed that interaction between the two studied factors had significant effect on plant growth and yield parameters .Using ascorbic acid and yeast treatment improved parameters of growth and yield in the salt stressed plants. Promotive effects of AsA and yeast indicated that could play a role in alleviating the adverse effect of salin water on metabolic activities relevant to growth and consequently plant yield. These results are agreement with [17] on common plants.

3.3.2. Effect of the interaction on proline and Rhizobium viable count

Data in Table 4. indicate that interaction between salinity and a meliorative substances induced a significant increase in proline content in leaves and Rhizobium bacteria from effective root nodules .The superior value of Rhizobium viable count and prolien content was in yeast treatment this result refers to that using soil microorganisms especially rhizosphere bacteria and mycorrhizal fungi which interact with plants by alleviating stress opens new alternative strategy against salinity. A similar results were agreement [6,11,13] .

Table 4. Effect of salinity and amelioratives interactions on growth , yield , proline and Rhizobium viable count

Salinity and Substances interactions	Plant Height cm	Nodules Number plant	Leaf area cm ²	Dry Weight/plant g	Pods Number/plant	Pods Weight g	Pods Yield plant g	Prolien $\mu\text{mol} \cdot \text{g}^{-1}$ FW	Rhizobium in 1ml x10 ⁹
S0C	46.00	6.30	899.60	39.50	14.67	3.167	46.47	0.810	2.210
S0A	52.93	7.43	958.30	43.07	18.67	3.667	68.20	0.730	3.040
S0Y	56.77	9.63	926.00	46.33	20.33	3.433	70.20	0.640	3.801
S1C	36.43	3.77	869.20	34.00	8.33	2.967	24.70	2.833	0.802
S1A	42.53	5.23	915.70	37.07	11.33	3.067	35.73	1.800	1.400
S1Y	39.67	5.93	929.30	36.33	11.20	3.400	38.67	2.133	1.800
S2C	35.00	2.93	858.00	31.67	6.33	2.733	17.37	4.670	0.400
S2A	39.13	4.27	910.30	33.43	10.03	3.067	30.77	3.500	1.001
S2Y	32.73	5.33	910.40	33.33	9.67	3.033	29.03	6.516	1.1002
LSD 5%	3.51	1.056	34.76	3.782	1.853	0.4142	6.887	0.1105	0.03102

3.4. Correlation coefficients among the studied traits

The data of Correlations in Table 5. indicated to that all correlations among the traits were positive and significant except correlations of prolien content with other traits which were negative and significant. The highest positive correlation was observed between pods number per plant and pods yield per plant reached (0.981). These result are agreement with [17,25] .

Table 5. Correlation coefficients among the studied traits

Traits	Plant Height cm	Nodules /plant	Leaf area cm ²	Dry Weight g	Pods number /plant	Pod weight /plant g	Pods yield /plant g	Prolien $\mu\text{m} \cdot \text{g}^{-1}$ FW	Rhizobium in 1ml x10 ⁶
Plant height cm	1								
Nodules /plant	.827**	1							
Leaf area cm ²	.550**	.575**	1						
Dry weight g	.921**	.858**	.555**	1					
Pods number/plant	.914**	.898**	.601**	.911**	1				
Pod weight /plant g	.615**	.672**	.610**	.655**	.675**	1			
Pods yield /plant g	.924**	.909**	.642**	.914**	.981**	.767**	1		
Prolien $\mu\text{m} \cdot \text{g}^{-1}$ FW	-.829.**	-.616.**	-.418.*	-.757.**	-.736.**	-.550.**	-.739.**	1	
Rhizobium in 1ml x10 ⁶	.929**	.938**	.637**	.929**	.969**	.703**	.971**	-.744.**	1

** , * : significant at p<0.01 and p<0.05, respectively

IV. Conclusion

The present study reported a positive effects of using bread yeast (*Saccharomyces cerevisiae*) extract in snap bean plants cv. Primel under irrigation with different levels of saline water and the important role of plant alleviation and tolerance to salinity in high level due to yeast adding and its contents of proteins, vitamins and its ability to produce hormones such as (Cytokinins, IAA, GA3) which significantly effect on both plants and symbiotic microorganism. Ascorbic acid was known as antioxidant and it simulates growth of plants and promotes the tolerance of a biotic stress such as salinity and its effect.

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