Curative effect of vitamin C and brassinolide on growth of mungbean (*Vigna radiata* (L.)Wilczek) under salinity stress

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Abstract:

A field experiment was carried out during 2018growing season, to investigate the influence of foliar application of vitamin C at concentration of 0, 60 and 120mg.l^{-1} in addition to brassinolide 0,2 and 4mg.l^{-1} on vegetative growth parameters of mungbean plants subjected to salt stress (NaCl) at four levels (0.9,5.5,10and15dS.m^{-1}). The experiment was conducted in factorial Randomized Completely Block Design (R.C.B.D) with three replicates and means were compared at Least Significant Difference (LSD) at probability of 0.05. Results indicated that salt stress levels from0.9 to15dS.m^{-1} reduced significantly the average of vegetative growth parameters (plant height, leaf area, dry weight and chlorophyll content). Moreover, application of vitamin C and brassinolide increased significantly the average of vegetative growth parameters above, and had reduced the adverse effect of salt stress particularly 120 mg.l^{-1} vitamin C and 4mg.l^{-1} brassinolide.

Key words: mungbean, salt stress, vitamin C, brassinolide

Introduction:

Vigna radiata (L.)Wilczek is a summer crop that returns to the legume family. It also has a short life 75 to 90 day, and needs a small amount of water compared to other crops (**1**). The salt stress is defined as increasing the concentration of soluble salts in the media of plant growth, and effect on the growth of the plant by increasing the osmotic pressure and ionic imbalances and the decrease of water absorption by the plant to show the morphology symptoms of reducing growth indicators: fresh and dry

weights, leaf area and length of roots (**2**). Also, effect on metabolic pathways such as: respiration and photosynthesis with increased reactive oxygen species (ROS) or so-called oxidative stress (**3**).

There was a significant decrease in plant height, dry weight and chlorophyll content of mungbean irrigate with saline concentrations (**4**). Also, significant decrease was found in plant height, dry weight and leaf area of two varieties of mungbean irrigate with saline concentrations (0, 50, and 75) mmol (**5**), while reduction was observed in the indicators of the morphology growth of three cultivar of mungbean grown in the saline media 5dS.m^{-1}(**6**). Similar results showed in other plants such as chickpea (**7**) and pea (**8**). Vitamin C (Vit.C) has an important role in plant growth to maintain an antioxidant system that protects the plant from ROS damage result from various stresses (**9**). It was observed that foliar spraying of mungbean with concentrations of vitamin C (0.5, 1.0 and 2.0)mmol resulted in a significant increase in plant height, dry weight and chlorophyll content (**10**). Also, the concentration 0.4 gm.l^{-1} of vitamin C showed a significant increase in vegetative growth parameters of Faba Bean (**11**). Brassinosteroids (BL) are groups of plant hormones that have an important role in plant growth and metabolism (**12**). It was observed that foliar spraying with a concentration of 10^{-8}M from 24-epibrassinolide significantly increased plant height, dry weight and chlorophyll content of mungbean (**13**). Machado *et al*.(**14**) found that 0.4µ mol of BL caused a significant increase in plant height, dry weight and chlorophyll content of Cowpea. Lalotra *et al*. (**15**) noticed that 0.05mmol of BL gave a significant increase in plant height, leaf area and chlorophyll content in mungbean plants compared with non-treated plants. Thus, this investigation aimed to study the effect of vitamin C in combination with brassinolide at different concentrations on some growth parameters of the local variety of mungbean irrigated with saline water.

Materials and Methods:

The field experiment was conducted at the Biology department, College of Education (Ibn Al-Haithum), University of Baghdad during the growing season 2018, to study the effect of salt stress (NaCl) at four levels (0.9(tap water), 5.5, 10 and 15) dS.m^{-1}, three vitamin C concentrations (0, 60 and 120) mg.l^{-1}, three brassinolide concentrations (0,50 and100) mg.l^{-1} and their interaction on some growth parameters of mungbean crop cv. Local. The soil was brought from the field of department and cleaned by filtering through 2mm sieve and table (1) showed the some physical and chemical characters of soil (**16**).

Table (1): Some physical and chemical characters of the investigated soil.

Soil Particles $g.kg^{-1}$			Texture	O.M $g.Kg^{-1}$	pH	E.C. $ds.m^{-1}$	N $mg.Kg^{-1}$	P ppm	K ppm
Clay	Silt	Sand	Slity loam clay						
481.9	337.6	108.5		0.91	8.10	2.80	20.00	0.81	33.81

The seeds were planted on 1/4/2018 and placed 3-5seeds in one pit and the soil was irrigated with water on 100% of soil viability water by the Haygro-Moisture Meter (**17**). After germination, the seedlings were reduced to one for pit, and become the number of plants in the experimental unit 15 plants. The superphosphate triphosphate fertilizer (P_2O_5% 46) was added at a rate of 75kg.ha^{-1} at one dose before planting. Nitrogen fertilizer (urea) was added at a rate of 40kg.ha^{-1} in twice times, the first added before planting and the second after one month of the first adding (**18**).After 30 days from planting, plants were irrigated with saline water levels mention above and after 40days from planting plants were sprayed with vitamin C and brassinolide concentrations mention above. Spraying processes were carried out during the morning until the solutions were run off all plants by using a manual sprayer. The harvest was taken after 45days from sowing (four plants) to calculate the vegetative growth characteristics: plant height (cm), leaf area (dm^2) (**19**), dry weight (g), chlorophyll concentration ($\mu g.cm^{-2}$). The field experiment was designed according to the Randomized Completely Block Design (R.C.B.D) and means were compared at least significant difference (LSD) at probability of 0.05(**20**).

Results and Discussion:

-Plant height:

The results in table (2) revealed that there was a significant decrease in the average of plant height with increase of salt levels without Vit.C and BL sprayed and the salt level 15dS.m^{-1} gave the lowest value was 35.55 cm compared to control (tap water).The apparent decrease in the height of the mungbean plant by increasing the salinity levels may be due to several reasons, like the competition of sodium and chloride ions with other ions on the absorption sites, and then weak growth and decrease plant height (**21**) or increased photorespiration accompanied by energy consumption and the accumulation of free radicals in the organs (**22**).The accumulation of sodium and chloride ions in the plant affects the enzymatic efficiency and metabolic processes such as photosynthesis, respiration and building of nucleic acids, protein and carbohydrates. Moreover decreased cell division in merstiamtic zones (**23**).

In related to vitamin C sprayed treatments, a significant increase in vitamin C concentrations from (0 to 120) mg.l^{-1} in the average of plant height at rate 11.51%. The increase in plant height may be due to the role of vitamin C in stimulating cell division and increasing the mitotic index (**24**), as well as its role in regulating the process of mitosis (**25**).

Table (2): Effect of NaCl salt, vitamin C (Vit.C) and brassinolide (BL) and their interaction on plant height (cm) of mungbean.

Vit.C (mg.l^{-1})	BL (mg.l^{-1})	NaCl (dS.m^{-1})				Vit.C x BL
		0.9	5.5	10.0	15.0	
0	0	48.03	46.55	37.94	40.57	40.57
	2	49.75	48.38	38.55	42.60	42.60
	4	51.12	48.47	39.74	44.06	44.06
60	0	50.23	47.21	43.96	34.80	44.05
	2	50.82	48.18	48.16	37.31	46.12
	4	53.26	50.76	49.21	39.55	48.20
120	0	52.36	50.43	46.10	34.71	45.90
	2	55.58	53.68	45.93	35.22	47.60
	4	53.14	52.94	49.22	38.07	48.35
NaCl		51.59	49.62	44.31	35.55	Vit.C x BL=0.56
L.S.D 0.05		NaCl=0.37	NaCl xVit.Cx BL=1.13			
NaCl x Vit.C						Vit.C
0		49.63	47.80	38.75	33.44	42.40
60		51.43	48.72	47.11	37.22	46.12
120		53.69	52.35	47.08	36.00	47.28
L.S.D 0.05		NaCl x Vit.C=0.65				Vit.C=0.32
NaCl x BL						BL
0		50.21	48.07	42.67	33.09	43.51
2		52.05	50.08	44.22	35.42	45.44
4		52.51	50.73	46.06	38.17	46.86
L.S.D 0.05		NaCl x BL=0.65				BL=0.32

Furthermore, the effect of spraying brassinolide affected positively on average of plant height and at concentration gave 4mg.l^{-1} highest value of 46.86cm compared to the 0. The increase in plant height may be resulted from the role of brassinolide in stimulating the plant to increase the absorption of nutrients from the soil which in turn increased its permeability across the stem tissues in the shoot (**26**).

The results in Table (2) also showed that all two interactions were significant. The treatment (0.9dS.m^{-1}and120mg.l^{-1}Vit.C) was significantly superior to give height average of plant height 53.69 cm. Moreover the interaction between salt levels and brassinolide concentrations and (0.9dS.m^{-1} and4mg.l^{-1} BL) treatment was significantly superior to other treatments by give average of plant height 52.51cm.

In correlation of the concentration of vitamin C and brassinolide, the results in the table (2) showed significantly increased in average of plant height and the treatment (120mg.l^{-1} Vit.C and 4mg.l^{-1} BL) was given highest 48.35cm.

A significant difference was found at the three interactions among NaCl, vitamin C and brassinolide. Plants irrigated with the tap water and sprayed with 120mg.l^{-1} Vit.C and 2mg.l^{-1} BL was superior to give the highest of the plant height 55.58cm and differed significantly from other plants, while the lowest plant height in plants irrigated with 15ds.m^{-1} and non-sprayed with vitamin and brassinolide hormone gave 29.75cm. This confirms the harmful effect of salt stress on decrease plant height and dwarfism, but plants sprayed with vitamin C (120mg.l^{-1}) and brassinolide hormone (4mg.l^{-1}) with the same level of saline previous (15dS.m^{-1}) gave 39.55cm. The spraying of vitamin C and brassinolide contributed to the harmful effect of salt stress because the increase in the plant height.

- Leaf area:

The results of Table (3) showed that the mean of leaf area decreased significantly when increasing salt levels and the level of 15dS.m^{-1} gave lowest leaf area 33.41dm^2 compared to the saline level 0.9dS.m^{-1} 43.89 dm^2. The reduction in leaf area by increasing sodium chloride levels has been attributed to reduction in mitotic division of merstiamtic cells in the leaf tops, as well as sodium and chlorides ions competition with other ions in absorption site therefore the leaves become wilted and suffering Chlorosis(**21**).

The spraying of vitamin C resulted in an increase in the average of leaf area at the concentrations used. All of them reached a significant level. The spraying treatment exceeded 120 mg.l^{-1} of vitamin C by giving the highest leaf area 41.43dm^2 and at rate of 14.48% compared to control. The increase in leaf area was due to the role of vitamin C in activating cell expansion, accelerating cell division and increasing vegetative growth, including leaf area and number of leaves (**27**).

The results of the same table also showed that spraying of the plants with the concentrations of brassinolide all achieved a significant increase in the average of leaf area and a concentration of 4mg.l^{-1} gave the highest leaf area 41.62dm^2 and at rate of 17.34% compared to control. The leaf area increased with sprayed brassinolide hormone was due to its synergistic role with the action of Indol acetic acid (IAA) in elongation and increased cell division of leaves (**28**).

The results indicated in Table (3) also showed that all two interactions were significant. The treatment (0.9dS.m^{-1} and 120mg.l^{-1} Vit.C) was significantly superior to other treatments by give average of leaf area 47.35dm^2. Moreover the interaction between salt levels and brassinolide concentrations and treatment (0.9dS.m^{-1} and 4mg.l^{-1} BL) was significantly

superior to other treatments by give average of leaf area 45.96dm^2. The results of the two interactions between the concentration of vitamin C and brassinolide showed significantly increased in average of leaf area and the treatment (120mg.l^{-1} Vit.C and 4mg.l^{-1} BL) was given highest 43.69 dm^2.

The results of the triple interaction in the same table showed significant differences between the plants .The plants irrigated with the tap water and sprayed with 120mg.l^{-1} Vit.C and 2mg.l^{-1} BL exceeded the leaf area of the plant by 50.20dm^2 and differed significantly from other plants. While the lowest leaf area in plants irrigated with 15dS.m^{-1} and non-sprayed with vitamin and brassinolide hormone that gave 26.61dm^2. This confirms the harmful effect of salt stress on lack of enlargement of leaves and leaf area reduction, but plants sprayed with vitamin C (120mg.l^{-1}) and brassinolide hormone (4mg.l^{-1}) with the same level of saline previous (15dS.m^{-1}) gave 39.61dm^2. The spraying of vitamin C and brassinolide contributed to the harmful effect of salt stress because the increase in the leaf area.

Table (3): Effect of NaCl salt, vitamin C (Vit.C) and brassinolide (BL) and their interaction on leaf area (dm^2) of mungbean.

Vit.C (mg.l^{-1})	BL (mg.l^{-1})	NaCl(dS.m^{-1}) 0.9	5.5	10.0	15.0	Vit.C x BL
0	0	38.41	35.44	31.78	33.06	33.06
	2	40.57	38.71	35.58	36.65	36.65
	4	43.72	40.06	37.58	38.87	38.87
60	0	40.72	38.74	33.52	28.31	35.32
	2	42.99	40.62	37.47	33.93	38.75
	4	46.50	44.91	42.72	39.61	43.43
120	0	44.19	40.44	36.15	31.33	38.03
	2	50.20	45.65	41.63	37.26	43.69
	4	47.66	44.34	40.52	37.74	42.56
NaCl		43.89	40.99	37.44	33.41	Vit.C x BL=0.27
L.S.D 0.05		NaCl=0.18	NaCl x Vit.C x BL=0.55			
NaCl x Vit.C						Vit.C
0		40.90	38.07	34.98	30.82	36.19
2		43.41	41.42	37.90	33.95	39.17
4		47.35	43.48	39.44	35.45	41.43
L.S.D 0.05		NaCl x Vit.C=0.31				Vit.C=0.16
NaCl x BL						BL
0		41.11	38.21	33.82	28.75	35.47
2		44.59	41.66	38.23	34.31	39.70
4		45.96	43.10	40.27	37.16	41.62
L.S.D 0.05		NaCl x BL=0.31				BL=0.16

Dry weight:

The results of table (4) showed that the average of dry weight decreased gradually and significantly by increasing at sodium chloride in irrigation water compared to the control treatment which irrigated only with tap water. At salt level 15dS.m^{-1} was gave the lowest value 14.85g while the highest 22.37g in control. The decrease in the dry weight of the shoot is due to salt stress in raising the osmotic potential and increasing the toxicity of sodium and chloride ions in plant tissues and nutritive imbalance with reflected in the metabolic processes (**29**).

Davidson and Campbell (**30**) confirmed that dry matter is the net production of photosynthesis and depends on the balance between photosynthesis and respiration, also salt stress increase oxidative stress and increase the production of free radicals (**31**).

Table (4): Effect of NaCl salt, vitamin C (Vit.C) and brassinolide (BL) and their interaction on dry weight (g) of mungbean.

Vit.C (mg.l^{-1})	BL (mg.l^{-1})	NaCl(dS.m^{-1})				Vit.C x BL
		0.9	5.5	10.0	15.0	
0	0	14.91	11.20	10.10	11.17	11.17
	2	16.08	12.54	11.74	12.34	12.34
	4	18.42	14.56	12.32	13.84	13.84
60	0	18.32	16.25	14.04	12.25	15.21
	2	22.73	19.32	17.29	14.80	18.54
	4	25.01	23.00	19.65	15.95	21.15
120	0	27.85	25.94	22.99	19.73	24.13
	2	29.54	28.01	24.83	20.83	25.80
	4	28.48	26.58	25.01	22.50	25.64
NaCl		22.37	19.82	17.55	14.85	Vit.C x BL=0.14
L.S.D 0.05		NaCl=0.10	NaCl x Vit.C x BL=0.29			
NaCl x Vit.C						Vit.C
0		16.47	12.77	11.37	9.19	12.45
2		22.02	19.86	16.99	14.34	18.30
4		28.62	26.85	24.28	21.02	25.19
L.S.D 0.05		NaCl x Vit.C=0.17				Vit.C=0.08
NaCl x BL						BL
0		20.36	17.80	15.71	13.49	16.84
2		22.78	19.96	17.95	14.88	18.89
4		23.97	21.71	18.99	16.18	20.21
L.S.D 0.05		NaCl x BL=0.17				BL=0.08

The spraying of vitamin C resulted in a significant increase in the average of dry weight and 120mg.l^{-1} treatment gave highest mean 25.19g compared to the control.

Vitamin C increases cell division and the efficiency of enzymes, including photosynthetic enzymes (**32**). It improves the efficiency of photosynthesis and increases metabolic material, and acts as co-factor to enzymes in chloroplasts (**33**).

The results in table (4) showed that the dry weight was significantly increased by the use of brassinolide and all concentrations. The $4mg.l^{-1}$ treatment was gave highest average of dry weight 20.21g compared to the control. The spraying of the hormone brassinolide increased the dry weight of the plant by increasing the efficiency of photosynthesis by activating the enzyme RuBisco, which increases the representation of CO_2 in the leaves, which is the basic unit for building carbohydrates (**12**).

All two interactions between salt levels and concentrations of vitamin C, salt levels and concentrations of brassinolide, concentrations of vitamin C and brassinolide hormone were significant, and ($0.9dS.m^{-1}$and $120mg.l^{-1}$Vit.C),($0.9dS.m^{-1}$ and $4mg.l^{-1}$BL)and ($120mg.l^{-1}$Vit.C and $2mg.l^{-1}$ BL) treatments were gave the highest average of dry weight (28.62, 23.97 and 25.80)g respectively.

The triple interaction between the three studied factors had a significant effect in this character and plants irrigated with tap water and sprayed with $120mg.l^{-1}$ of vitamin C and $2mg.l^{-1}$ of brassinolide superior to other plants because that gave heights dry weight 29.54g, but the lowest dry weight in plants irrigated with $15dS.m^{-1}$ and without spraying the vitamin and hormone that gave 8.47g, this also confirms the negative effect of salt stress in decrease dry weight, while the spraying of vitamin C and brassinolide in overcoming the negative effect of stress through the increase in dry weights, but plants sprayed with vitamin C($120mg.l^{-1}$) and brassinolide($4mg.l^{-1}$) with the same level of saline previous($15dS.m^{-1}$) gave 22.50g.

- Chlorophyll content:

The results in table (5) showed significant differences between salt levels in the average chlorophyll content and the level of $15dS.m^{-1}$ gives lowest mean of this character is $36.65\mu g.cm^{-2}$ and at rate25.49% compared to control. This decrease in chlorophyll content may be justified by effect of sodium chloride, in absorption of the elements involved in building the chlorophyll like nitrogen. Also, high salinity causes deformation the membranes of plastids or inhibition enzymatic activity responsible for building pigments or increasing the activity of the enzymes Chlorophylase and Peroxidase responsible for the analysis the pigment and increase free radicals that inhibit the building of proteins in pigments (**34**).

All concentrations of vitamin C (Table 5) affected positively on the average of chlorophyll content compared to control and 120mg.l^{-1} treatment highest value of this pigment was 47.87µg.cm^{-2}. The increases in chlorophyll content has been attributed to several factors, including the contribution of vitamin C to the increase in photosynthesis pigments concentration and reflect the accumulation of dry matter in the plant (**35**), or perhaps to its role in increasing root growth and increasing absorption of elements involved in the build this pigment (**36**).

Furthermore the concentrations of the brassinolide caused a significant increase in chlorophyll content at treatment 4mg.l^{-1} highest value was 44.94µg.cm^{-2}. The increase in chlorophyll content is due to the effect of brassinolide in inhibiting the Chlorophylase enzyme responsible for the analysis of the pigment (**37**) or possibly stimulating the NADH-protochlorophyllid reductase enzyme responsible for build this pigment (**38**).

The interactions between salt levels and concentrations of vitamin C, salt levels and concentrations of brassinolide, concentrations of vitamin C and brassinolide hormone were significant, and (0.9dS.$m^{-1}$and 120mg.L^{-1}Vit.C), (0.9dS.$m^{-1}$and 2mg.l^{-1} BL) and (120mg.l^{-1}Vit.C and 2mg.l^{-1} BL) treatments were gave the highest average of chlorophyll content (53.27,50.71 and 50.51)µg.cm^{-2} respectively.

The triple interaction between the three studied factors had a significant effect in this character and plants irrigated with tap water and sprayed with 120mg.l^{-1} of vitamin C and 2mg.l^{-1} of brassinolide superior to other plants because that gave chlorophyll content 57.01µg.cm^{-2}, but the lowest chlorophyll content in plants irrigated with 15ds.m^{-1} and without spraying the vitamin and hormone that gave 29.77 µg.cm^{-2}, this also confirms the negative effect of salt stress in decrease dry weight, while the spraying of vitamin C and brassinolide in overcoming the negative effect of stress through the increase in chlorophyll content, but plants sprayed with vitamin C (120mg.l^{-1}) and brassinolide(4mg.l^{-1}) with the same level of saline previous (15dS.m^{-1}) gave42.70 µg.cm^{-2} .

Table (4): Effect of NaCl salt, vitamin C (Vit.C) and brassinolide (BL) and their interaction on chlorophyll content (µg.cm^{-2}) of mungbean.

Vit.C (mg.l^{-1})	BL (mg.l^{-1})	NaCl(dS.m^{-1})				Vit.C x BL
		0.9	5.5	10.0	15.0	
0	0	44.23	38.14	35.44	36.90	36.90
	2	46.27	40.26	37.31	39.02	39.02
	4	47.91	42.22	38.62	40.62	40.62
60	0	46.55	43.91	40.73	34.60	41.45
	2	48.87	46.75	43.22	37.34	44.04
	4	49.11	47.58	45.05	41.19	45.73
120	0	50.17	47.46	44.17	38.15	44.99
	2	57.01	55.18	48.25	40.17	50.15
	4	52.63	51.58	46.94	42.70	48.46
NaCl		49.19	45.90	42.19	36.65	Vit.C x BL=0.09
L.S.D 0.05		NaCl=0.06	NaCl x Vit.C x BL=0.18			
NaCl x Vit.C						Vit.C
0		46.14	40.20	37.12	31.91	38.84
2		48.17	46.08	43.00	37.71	43.74
4		53.27	51.41	46.45	40.34	47.87
L.S.D 0.05		NaCl x Vit.C=0.11				Vit.C=0.05
NaCl x BL						BL
0		46.98	43.17	40.11	34.17	41.11
2		50.71	47.39	42.93	36.58	44.40
4		49.88	47.13	43.53	39.20	44.94
L.S.D 0.05		NaCl x BL=0.11				BL=0.05

This study concluded that treatment (120mg.l^{-1} Vit.C and 4mg.l^{-1} BL) has reduced the negative effects of salt stress in mungbean plant.

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التأثير العلاجي لفيتامينC والبراسينولايد على نمو الماش (*Vigna radiata* (L.)Wilczek) تحت الاجهاد الملحي

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الخلاصة :

نفذت تجربة حقلية اثناء موسم النمو2018 بهدف استجابة نبات الماش لتراكيز فيتامينC(0, 60 و120) ملغم.لتر$^{-1}$ والبراسينولايد(0 ,2و4)ملغم.لتر$^{-1}$ تحت تاثير مستويات الاجهاد الملحي (كلوريد الصوديوم)(0.9 , 5.5 ,10 و15)ديسيسمنز.م$^{-1}$ وأثرها في مؤشرات النمو الخضـــري لنبات الماش, وفق تصميم الـــقطاعات المعشاة الكاملة وبثلاث مكررات. حللت البيانات إحصائيا طبقاً للتصميم اعلاه وقورنت متوسطات المعاملات على أساس اختبار أقل فرق معنوي بمستوى احتمال5%. اشارت النتائج ان زيادة مستويات الاجهاد الملحي (0.9 الـــى15) ديسيســـمنز. م$^{-1}$ ادى الى خفض معنوي للمتوسطات مؤشرات النمو(ارتفاع النبات, المســـاحة الورقيـــة, الـــوزن الجاف, محتوى الكلوروفيل), وكان للرش بفيتامينC والبراسينولايد تـــاثيرا ايجابـــا فـــي زيـــادة متوسطات مؤشرات النمو اعلاه وفي ازالة التاثير السلبي للاجهاد الملحي لاسيما فـــي المعاملـــة 120ملغم. لتر$^{-1}$ فيتامينC و 4ملغم.لتر$^{-1}$ البراسينولايد.