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THE RELATIONSHIP BETWEEN GROWTH REGULATORS AND THE OXIDATIVE STRESS INDUCTION IN SOYBEAN NODULES GROWN UNDER SALINE CONDITIONS

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ABSTRACT

Effect of pre-soaking of soybean seeds in GA_3 or IAA treatments on number, dry weight, nitrogen, protein, leghaemoglobin, MDA contents and SOD activity in nodules of soybean *Glycine max* (L.) Merr. cv. Crawford was studied under different salinity levels (control, 6 and 12 dS/m) in a greenhouse experiment.

The studied parameters were severely inhibited due to irrigation with saline water and the impact was more obvious at the vegetative stage. Application of growth regulators, reduced the determined effect of salinity on nodulation, nitrogen, protein and leghaemoglobin contents and decreased the extent of membrane damage which was monitored by measuring the MDA content. On the contrary, the SOD activity was not affected by IAA or GA_3 treatments. The treatment of 1.14 mM IAA was the most effective.

Keywords: soybean, salinity stress, GA_3, IAA, oxidative stress, nodules.

Abbreviations:

Lb = Leghaemoglobin SOD = Super-oxide dismutase AOS = Active oxygen species
MDA = Malondialdehyde TBA = 2-Thiobarbutyric acid ROS = Reactive oxygen species

INTRODUCTION

Salinity in soil or water is of increasing importance to agricultural productivity because it causes a stress condition to plant growth.

One of the biochemical changes possibly occurring when plants are subjected to harmful stress is the production of activated oxygen species (AOS). The chloroplasts and mitochondria of plant cells are important intracellular generators of activated oxygen species. Electrons leaked from electron transport chains can react with O_2 during normal aerobic metabolism to produce activated oxygen species such as superperoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-). These cytotoxic oxygen species are highly reactive and in the absence of any protective mechanism they can seriously disrupt normal metabolism through oxidative damage to lipids, proteins and nucleic acid. (Gossett *et al*., 1994; Smirnoff, 1993; Zhang *et al*., 1995).

Fortunately plants possess endogenous protective mechanism including a number of antioxidant enzymes such as superoxide dismutase, glutathione reductase, ascorbate peroxidase, peroxidase, catalase and carotenoids that protect them against the damaging effects of activated oxygen species by scavenging and removing toxic products (Halliwell and Hutteridge 1989).

Biological nitrogen fixation performance is highly sensitive to salt stress. The delicate balance existing between the host plant and its symbiont get disturbed even under mildly adverse conditions (Singleton *et al*., 1981).

Nitrogen fixing micro-organisms, as well as the whole plants, have developed mechanisms to avoid the potential risk of oxygen derived radicals (Salin, 1991 and Dalton, 1995). Most of the oxygen consumed during respiration of bacteroids and mitochondria is reduced to water, but activated O_2 species (AOS), including the superoxide radical (O^-_2)and hydrogen peroxide (H_2O_2) are always formed as by-products. Also, legume root nodules have a high potential for producing reactive oxygen species because they contain abundant oxygen-labile proteins, leghaemoglobin and Fe which can become available for catalysing free radical formation (De Lorenzo *et al*., 1994; Dalton 1995; Gogorcena *et al*., 1995). In addition, nodules contain small molecules that may act as AOS scavengers (Kumet and Foyer 1994).

Growth regulators, particularly GA_3 and IAA have been considered essential factor for nodule initiation (Boissya and Gogoi, 1987) and for rhizobial penetration (Schmidt *et al.,* 1988).

Little information is available on the role of growth regulators in nodule formation and nitrogen fixation under salinity condition in legumes. Therefore, an attempt has been made to study the role of GA_3 and IAA on nodulation, nitrogen fixing efficiency and the relationship between nitrogen fixation and oxidative stress induction in nodules of salt-treated soybean plant (irrigated with different concentrations of saline solution).

MATERIALS AND METHODS

The present investigation was conducted in pots during the two growing seasons of 1998 and 1999 at the National Research Centre greenhouse. Surface sterilized soybean seeds (Crawford cv) were pre-soaked separately for four hours in distilled water (control) and in various plant growth regulators of different concentrations (GA_3 : 0.29 and 0.58 mM and IAA : 0.57 and 1.14 mM).

Soybean seeds were sown in pots (5 seeds/pot) of 35 cm diameter and 30 cm depth, filled with 15 kg sandy soil of 8.19 % clay, 6.40 % silt, 83.30 % sand and 0.38 % organic matter. For inoculation procedures, 10 ml aliquots of *Bradyrhizobium japonicum* broth culture contained 7.5 x 10^6 viable cell/ml were added directly in the seed bed at sowing time then covered with a thin layer of sandy soil. Three healthy plants were maintained in each pot and after 30 days, salt stress was induced by irrigating treated plants with either 0, 60 or 120 mM NaCl saline solution (corresponding to 0, 6 or 12 dS/m salinity levels). Each treatment was replicated five times and arranged in a complete randomized design.

Sampling was done twice at the vegetative (35 days-old) and flowering (cum-pod initiation stage), for estimating nodulation criteria. Two sets of homogenous plant samples at each stage were collected, the first one for nodules number and its dry weight determination and the second was used for studying the other criteria..

Leghaemoglobin content of nodules was estimated by the method of Hartee (1955) based on conversion of hematin to pyridine hemochromogen.

Total Nitrogen content was determined using modified micro-kjeldahl method as described by Peach and Tracey (1956).

Lipid peroxidation was measured as the amount of MDA determined by TBA reactants as described by Heath and Packer (1968). SOD activity was estimated according to Stewart and Bewley (1980).

Data were statistically analyzed according to Snedecor and Cochran (1980) where treatment means were compared by L.S.D. at 5 % level of probability.

RESULTS AND DISCUSSION

Number and dry weight of nodules

Increasing salinity levels, severely decreased number of nodules/plant and nodules dry weight at vegetative and flowering stages, but the inhibition was more severe at vegetative stage as presented in Tables (1 & 2). In growth regulators free treatments salinity reduced number of nodules / plant at the vegetative stage by 38.5 % and 79.5 % at 6 and 12 dS/m respectively, while the nodule dry weight reduced by 20 % at 6 dS/m and 60 % at 12 dS/m, as compared to the control (irrigated with tap water). The reduction percentages in flowering stage ranged between 20.5 to 48.6%.

In Table (2) salinity was less effective on nodules dry weight. Surivan et al., (1985) stated that nodule inhibition was most severe at vegetative stage, due to the effect of salinity which may induce obstruction in nodule initiation process. Also, Ghandorah and Sliman (1989), found that total number of nodules and total plant dry weight of soybean were decreased by increasing salinity. Delay in nodule initiation and development under salinity may be attributed mainly to harmful effect of salt on rhizobial population, infection process and establishment of infection thread (Kumar and Garg, 1978).

Pre-soaking of soybean seeds in IAA or GA_3 solutions, significantly reduced the impact of salinity hazard on nodulation, which resulted in a remarkable increase in number of nodules and their dry weight. IAA was more effective than GA_3, the most successful results were obtained at 1.14 mM IAA. These results are in agreement with those of Singh and Kumar (1989).

Nitrogen content in root nodules

Increasing salinity level up to 12 dS/m, reduced nitrogen content in plant roots by 58 % as compared to the control (Table 3). The inhibitive effect of salinity on nitrogen fixation in soybean root nodules could be due to the adverse effect of salts on leaf osmotic potential and expansion more than its direct effect on nitrogenase system (Singleton et al., 1981). Also, salinity may reduce the nitrogen fixing efficiency either directly by affecting the microsymbionts and nodules function or indirectly by reducing growth of soybean plants (Singleton et al., 1981).

Pre-Soaking of seeds in IAA or GA_3 enhanced nitrogen content of root nodules by 35 % at high salinity stress using 1.14 mM IAA and by 16%

using 0.58 mM GA_3 at vegetative stage. At flowering stage, nitrogen content in root nodules was reduced by 45 % at 12 dS/m, salinity level, while using IAA increased N_2 content by 22 % and GA_3 by 20 % (Table 3). Thus, growth regulator treatments might help in maintaining the anaerobic conditions in the vicinity of nitrogenase system. Improvements in nodulation and N_2-fixing system resulted in increasing N_2 content in root nodules. (Zaidi and Singh, 1993). Also, Abdel-Aziz *et al*. (1985) found that IAA up to 50 ppm stimulated germination, N-concentration and uptake in soybean seedling grown under saline condition. In general, there are no great differences between the diverse concentrations within the same growth regulator regarding N_2-content of soybean root nodules.

Protein percent in root nodules

Table (4) show that high salinity level (12 dS/m) severely reduced protein content of root nodules where the reduction percent reached 57.97 % in comparison with control. Inclusion of growth regulators (IAA & GA_3) reduced the adverse effect of salinity on protein content of soybean root nodules particularly with IAA. The same trend was obtained by Premabatidevi (1998).

Table (4) : Effect of growth regulators on protein percent of soybean root nodules grown under saline conditions.

Salinity levels dS/m / Growth regulators (mM)	Protein % (Vegetative stage)		
	0	6	12
Control	13.8	10.0	5.8
IAA			
0.57	14.7	12.0	9.9
1.14	14.8	12.1	10.5
GA_3			
0.29	14.1	10.75	7.81
0.58	14.25	10.75	7.87

Leghaemoglobin content in root nodules

Salinity treatment resulted in a 14.2 % and 57.1 % decrease in Lb content of nodules at low (6 dS/m) and high salinity levels (12 dS/m) respectively during the vegetative stage as shown in Fig. 1 (a & b). The decline in Lb content in root nodules during the flowering stage was less than that of vegetative stage. Leghaemoglobin decreased by 7.1 % at low salinity and by 21.4 % at high salinity level. Similar results were obtained by Comba *et al*., (1998), where they found that salt treatment resulted in 50 % decrease in Lb content of soybean root nodules.

On the other hand, little is known about the effect of growth regulators on Lb content in soybean root nodules grown under saline condition. It was clear in Fig. 1 (a&b) that IAA was more effective than GA_3 on Lb degradation as the Lb content at high salinity level was decreased by 21 % in the presence of 1.14 mM IAA and by 28.5 % with 0.58 mM GA_3 during the vegetative stage.

1

2

3

(a)

Fig. (1) : The combined effect of growth regulators and various salinity levels on leghaemoglobin content of soybean root nodules at vegetative (a) and flowering (b) stages.

Lipid peroxidation

With increasing salinity levels, the MDA content was increased, thus indicating an increase in lipid peroxidation as presented in Fig. (2). The extent of membrane damage was monitored by measuring MDA content which increased at low salinity level (6 dS/m) by 12 % and increased by 45 % compared to the control at high salinity level during the vegetative stage.
On the other hand, use of growth regulators slightly decreased the MDA content in soybean nodules at low and high salinity levels by 10 % and 12.5% respectively compared to the above mentioned results.

On the contrary, Comba *et al*. (1998) found that an increment of MDA content was not observed in soybean nodules grown under salinity stress and attributed their unexpected results to a consequence of natural plant ageing.

Fig. (2) : Impact of growth regulators and various salinity on MDA content levels in root nodules of soybean.

Superoxide dismutase (SOD)

Fig. (3) shows that a clear decline in SOD activity in root nodules with increasing the magnitude of salinity stress. The SOD was reduced by 9 % at 6 dS/m and by 18 % at 12 dS/m. Singha and Choudhury (1990) found that salinity decreased the SOD activity in leaves, chloroplasts and mitochondria of pea plants and stated that inhibition of SOD activity under salt stress is a consequence of an altered synthesis and accumulation of less active enzymes in salt treated plants cannot be entirely ruled out.

On the other hand, the growth regulators treatment did not alter any change in SOD activity in root nodules under saline condition. Premabatidevi (1998) found that the catalytic property of the enzyme is unaffected by the growth regulators, but hormones enhance synthesis of enzymes.

Fig. (3) : Effect of growth regulators and different salinity levels on SOD content in root nodules of soybean.

In conclusion, salt stress creates oxidative stress through the formation of reactive oxygen species (ROS), might be in excess than the amount present under normal physiological conditions.

ROS species attack lipids, proteins and nucleic acids causing lipid peroxidation and protein denaturation. To prevent such damage plant cells are equipped with an antioxidant system and protective enzymes. In the present investigation, the (SOD) which act as an superoxide radical scavengers which would remove the superoxide anion generated under salt stress.

On the other hand, application of growth regulators proved to be beneficial by accelerating the nitrate assimilation process and consequently productivity. But, it was not clear in the present investigation that the growth regulators increased protection against oxidative damage which may be due to earlier oxidative damage which might occur in the period over which the symbiosis becomes established as suggested by Comba et al. (1997).

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العلاقة بين منظمات النمو والإجهاد الأكسيدى فى عقد نبات فول الصويا النامي تحت الظروف الملحية

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*** قسم النبات ، ** قسم الميكروبيولوجيا الزراعية ، المركز القومى للبحوث- الدقى – القاهرة**

أجريت تجربة أصص بصوبة المركز القومى للبحوث لدراسة تأثير نقع بذور فول الصويا فى كل من حمض جبريليك (GA_3) وحمض الاندول أسيتيك (IAA) بتركيزات مختلفة (0.29 ، 0.58 ، ممول للأول ، 57 ، 1.14 ممول للثانى) لمدة أربع ساعات على كل من العدد، والوزن الجاف ومحتوى كل من النتروجين والبروتين والليجهيموجلوبين، والمالون الدهيد ونشاط إنزيم السوبر أوكسيد ديسميوتيز فى عقد نبات فول الصويا النامى تحت المستويات المختلفة من الملوحة، حيث كان التوصل الكهربى للمحاليل المستخدمة تعادل صفر ، 6 ، 12 dS/m

وقد أظهرت النتائج المتحصل عليها أن أغلب الصفات قد ثبطت عند التركيز العالى من الملوحة (dS/m12) خاصة أثناء مرحلة النمو الخضرى. كما وجد أن إستخدام منظمات النمو السابق ذكرها قد قلل من تأثير الملوحة الضار على تكوين العقد الجذرية ونموها، كما ساعدت على زيادة محتواها من كل من النيتروجين والبروتين والليجهيموجلوبين.

بالإضافة إلى ذلك فقد ساهمت منظمات النمو فى تقليل الضرر الناشىء على غشاء الخلية والذى يقاس بمحتوى (MDA). وأما نشاط أنزيم السوبر أكسيد ديسميوتيز فلم يتأثر باستخدام منظمات النمو أدى إستخدام تركيز 1.14 ممول من حمض الاندول اسيتيك إلى الحصول على أفضل النتائج.

Table (1) : Effect of growth regulators on number of nodules/plant in soybean grown under various levels of saline solution.

Salinity levels dS/m / Growth regulators (mM)	Vegetative stage			Flowering stage		
	0	**6**	**12**	**0**	**6**	**12**
Control	21.0	12.9	4.3	79.0	62.8	40.6
IAA						
0.57	38.7	22.8	20.0	89.7	79.3	47.6
1.14	43.1	32.7	27.1	102.3	88.6	61.1
L.S.D at 5 % level	S = 2.1	G. = 2.8	S x G = N.S.	S = 2.0	G. = 2.2	S x G = N.S.
GA_3						
0.29	38.2	22.5	14.9	93.0	83.7	53.0
0.58	27.1	28.5	11.9	85.7	78.2	41.0
L.S.D. at 5 % level	S = 2.3	G = 3.1	S x G = N.S	S = 3.4	G. = 3.4	S x G = 4.9

S : Salinity & G : growth regulator & N.S : Not significant

Table (2): Influence of growth regulators and various salinity levels on root nodules dry wt. (g/plant) of soybean

Salinity levels dS/m / Growth regulators (mM)	Vegetative stage			Flowering stage		
	0	**6**	**12**	**0**	**6**	**12**
Control	0.5	0.4	0.2	0.85	0.80	0.4
IAA 0.57	0.50	0.45	0.40	1.2	1.3	0.7
1.14	0.90	0.70	0.60	1.4	1.3	0.5
L.S.D at 5 % level	S = 0.12	G = 0.11	S x G = N.S.	S = 0.11	G = 0.12	S x G = 0.2
GA_3 0.29	0.50	0.45	0.40	1.50	1.10	0.50
0.58	0.30	0.30	0.20	0.75	0.50	0.40
L.S. D. at 5 % level	S = 0.2	G = 0.2	S x G = N.S.	S = 0.9	G = 0.81	S x G = 1.7

S : Salinity & G : growth regulator & N.S : Not significant

Table (3): The combined effect of growth regulators and different salinity levels on N_2 content (mg/g dr.wt) in root nodules of soybean.

Salinity levels dS/m / Growth regulators (mM)	Vegetative stage			Flowering stage		
	0	**6**	**12**	**0**	**6**	**12**
Control	22.1	16.1	9.3	25.8	19.4	14.2
IAA						
0.57	23.5	19.5	15.7	26.7	22.1	18.5
1.14	23.7	19.5	16.9	26.9	22.9	19.6
L.S.D at 5 % level	S = 0.5	G. = 0.55	S x G = 1.1	S = 0.3	G = 0.4	S x G = 0.6
GA_3						
0.29	22.7	17.2	12.5	25.9	21.6	18.1
0.58	22.8	17.2	12.6	26.1	21.7	19.2
L.S.D. at 5 % level	S = 0.46	G = 0.51	S x G = 0.85	S = 0.4	G = 0.4	S x G -= 0.75

S : Salinity & G : growth regulator