Journal of Applied Sciences Research, 9(8): 4889-4895, 2013
ISSN 1819-544X
This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLES

Responses of Photosynthetic Pigments and Amino Acids Content of Moringa Plants to Salicylic Acid and Salinity

[1]Hussen, M.M., [2]Lobna, S. Taha, [2]Rawia, A. Eid and [2]Soad, M.M. Ibrahiem

[1]Water Relations and Irrigation Dept., National Research Center, Cairo, Egypt.
[2]Ornamental and woody trees Dept., National Research Center, Cairo, Egypt.

ABSTRACT

Responses of photosynthetic pigments and amino acids content of moringa plants to salicylic acid and salinity were studied in a pot experiment conducted in the greenhouse of the National Research Center, Dokki, Cairo, Egypt during the summer season of 2011. Salicylic acid was sprayed in the ratio of 100 and 200 ppm more than distilled water as a control and plants irrigated by 2500 , 5000 ppm diluted sea water and control plants irrigated by fresh water. The concentration of Chl.a increased parallel to the increase in salt concentration in water of irrigation, while, carotenoids concentrations gave its higher values with 2500 ppm salt concentration treatment and decreased with the higher salt concentration but still more than the control. Meanwhile, both salt concentrations induced approximately the same effect on Chl.b concentration. Methionine and tyrosine concentration decreased as the concentration of salts increased in irrigation solution. The reverse was true approximately for lucine, glutamic acid, arginine and lysine concentrations. Concerning the concentration of glycine, valine and phenylalanine concentrations, it could be noted that the concentration of 2500 ppm decreased its concentrations and tended to increased with the highest salt solution used. Histidine and isolycine concentrations increased only by 5000 ppm salts in water of irrigation but the aspartic acid concentration did not showed any clear response to the different diluted seawater. A positive relationship was observed between the concentration of aspartic acid, thrionine, serine, glutamic acid, valine, isoleucine, lucine, phenylalanine and histidine and the concentration of salicylic acid exogenous application. However, arginine, glycine and methionine decreased with the moderate concentration of salts but tended increased with the high salts in the diluted seawater treatment.

Key words: Moringa(*Moringa oleifara L*am) –Salinity –Salicylic acid -Growth-Chlorophyll-Carotenoids-Amino acids.

Introduction

Moringa (*Moringa oleifara* L.) is mirace tree having tremendous uses like medicinal, water purification, alley cropping, biopesticides, biogas, vegetable and biofuel (Ashfoq, *et al*., 2012 and Nouman, *et al.,* 2012). Anwar, *et al* (2007) and Siddhurasu and Becker (2003) mentioned that moringa is an important food commodity as a plant parts such as leaves, flowers, fruits and immature pods can be used as a highly nutritive vegetable. In addition, moringa is believed to have multiple medicinal uses (Morimitsu, 2000).

Salinization of agricultural lands is occurring throughout the world, but especially in regions where irrigation water has a high salt concentration and water evaporates rapidly from the soil surface. Salts becomes progressively concentrated in the root zone because the plant roots absorb water but very little salt (Kozlowski, 1997). Salinity affected badly growth of moringa as reported by Nouman, *et al* (2012).

Salicylic acid (SA), is one of the phenolic compounds produced in the plant. It has shown many important functions in the plant and can change physiological behavior of plant (Khandaker, *et al.,* 2011). Salicylic acid has a direct physiological effect through the alteration of antioxidant enzyme activities, induces flowering, increase flower life, retards senescence and increases cell metabolic rate. The sustained level of salicylic acid may be a prerequisite for the synthesis of auxin and/or cytokinin (Metwally, *et al*. 2003).

Salicylic acid is a naturally plant hormone, occurring an important signal molecule known to have diverse effects on biotic and abiotic stresses tolerance in plants (Khan, *et al*., 2010). Several researchers used salicylic acid to alleviate salt stress in plants among of them Shakirova, et al (2003); Hussein, *et al*., (2007); Salihi, *et al* (2011) and Hussein, *et al* (2012).

Amino acids are not only building blocks of proteins but also participate in many metabolic networks that control growth and adaptation to the environment. In young plants, amino acid biosynthesis is regulated by a compound metabolic network that links nitrogen assimilation with carbon metabolism. This network is strongly

Corresponding Author: Hussen, M.M., Ornamental and woody trees Dept., National Research Center, Cairo, Egypt.
E-mail: lobnasalah82@yahoo.com

regulated by the metabolism of four central amino acids, namely glutamine, glutamate, aspartate, and asparagine (Gln, Glu, Asp, and Asn), which are then converted into all other amino acids by various biochemical processes. Amino acids also serve as major transport molecules of nitrogen between source and sink tissues, including transport of nitrogen from vegetative to reproductive tissues. Amino acid metabolism is subject to a concerted regulation by physiological, developmental, and hormonal signals. This regulation also appears to be different between source and sink tissues (Galili, *et al* 2008). Photosynthesis is one of the major metabolic process in the plant and pigments from the important conistituents of this process. Salinity adversely affected these processes. Therefore, this work designed to investigate the effect of salicylic acid on photosynthetic pigments and amino acids and to alleviate salt tolerant in moringa plants.

Materials and Methods

A pot experiment was conducted in the greenhouse of the National Research Center to evaluate the effect of salt stress and /or salicylic acid on photosynthetic pigments and amino acids content of moringa plants. The treatments were as follows:

Salinity: Irrigation by diluted sea water: tap water, 285 ppm, 2500 and 5000 ppm . The analysis of seawater used in irrigation was illustrated in Table (1).

Antioxidants: Salicylic acid in the rate of 100 and 200 ppm and control plants were sprayed with the same quantity of distilled water.

The experiment included 9 treatments, 3 salinity treatments in combination with 3 salicylic acid treatments in factorial experiment. The statistical design was split plot in 8 replicates.

Moringa seeds (*Morirga oleifera* L.) were sown in April,10, 2011 and earthenware filled with clay loam soil mixed with beet moth (1:1) and every pot received 1.80 g of ammonium sulphate, 1.5 g calcium super phosphate and 0.5 g Potassium sulfate after 21 days from sawing seedlings were transolocated to the pots (50 cm) width) contained 8 Kg soil . The physical and chemical properties of used soil were illustrated in Table (2). Plants thinned twice after one and two weeks after transplanting and left one plant/pot in 8 replicates for treatment. Plants irrigated with saline water and control with fresh water was started at 45 days from sowing. Salicylic acid was sprayed at 45 and 6o days from sawing.

Table 1: Analysis of seawater used in irrigation.

Source	pH	EC dSm^{-1}	Soluble cations (mM)				Soluble anions(mM)			
			Na^+	K^+	Mg^{++}	Ca^{++}	CO^{--}_3	HCO^-_3	Cl-	$SO^{=}_4$
Seawater	7.94	50	475.000	9.700	56.000	10.000	2,500	2.300	536.000	28.000

Total soluble salts = 32.000 mg/L

Table 2: Some physical and chemical properties of studied soil A and B

A . Soil mechanical analysis

Sand		Silt	Clay	Soil
Course >200 µ %	Fine 200-20µ %	20-2 µ %	< 2 µ %	Texture
9.70	16.75	35.22	38.33	Clay loam

B. Soil chemical analysis

pH 1:2.5	EC dSm^{-1} 1:5	$CaCO_3$ %	CEC C mole Kg^{-1}	OM %	Soluble cations and Anions meq/100 g soil							
					Na^+	K^+	Ca^{2+}	Mg^{2+}	CO^{-3}	HCO^{-3}	Cl^{-1}	SO^{-2}
7.50	1.45	2.65	30.56	1.35	1.95	0.36	2.68	1.57	0.0	0.97	1.8	3.79

Available macro-nutrients %			Available micro-nutrients ppm			
N	P	K	Zn	Fe	Mn	Cu
0.49	0.27	0.93	3.5	5.4	7.8	4.2

At 90 day from sowing, 3 plants from every treatment were picked and cleaned, dried in electric oven at 70 ^oC, and ground in stainless steel mill.

Samples of green leaves were collected from every treatment for photosynthetic pigments and determination using the method described by Saric *et al.* (1967).

Amino acid were determined using amino acid analyzer according to Baily (1967). Dry and defatted samples containing 25 mg protein where hydrolysed with 6 N HCl at 105 ^{o} for 24 hr in a sealed tube. After cooling and filtering , the residue left after filtration was washed with distilled water and the combined filtrates were completed to 25 ml in a volumetric flask . A portion of the filtrate (5 ml) was evaporated to dryness at room temperature in a desiccators under vacuum . The residue was dissolved in 5 ml buffer (0.2 N sodium citrate , pH 2.20) and the solution was filtered through 0.22 µm membrane. Twenty microliters of the final filtrate were injected in the instrument capsule for quantitative determination of the amino acids(Spachman, *et al*. ,1958). The cationic exchange resins UL-tropac sodium and special programmed buffer system were used

(citrate buffers 0.2 N with three different pH , at 3.2, 4.25 and 6.45 at a rate of 35 ml/hour). The effluent was met by a stream of ninhydrin reagent at a rate of 25 ml/hour. The quantitative estimation of the amino acids depends on the colorimetric determination of the blue colour (Muhler, 1964). Data collected were subjected to the proper statistical analysis described by Snedecor and Cochran (1980).

Results and Discussion

Photosynthetic pigments:

Effect of salinity:

The concentration of Chl.a increased parallel to the increase in salt concentration in water of irrigation, while, carotenoids concentrations gave its higher values with 2500 ppm salt concentration treatment and decreased with the higher salt concentration but still more than the control. Meanwhile, both salt concentrations induced approximately the same effect on Chl.b concentration. Elanz, *et al* (2011) subjected sunflower plants to different salinity levels (0 - 200 mM NaCl) and found that chlorophyll content was decreased. Saker and Arafa (2009) noticed that the high salinity level 14.6 dS/m decreased photosynthetic pigments of canola plants. Also, Nouman, *et al* (2012) emphasized that moringa plants survived up to 8 dS/m with a slight reduction in its chlorophyll a content. B-carotene and chlorophyll b were increased significantly up to 12 dS/m. Farahbakhsh and Saiid (2011) reported that Chlorophyll content were adversely affected with increasing salt concentration and about 20.42 % reduction appeared at 12dS/m salinity. Such a result was obtained by Misra, *et al* (1997) who reported 71% reduction in chlorophyll of phaseolus seedlings exposed to NaCl. They concluded that salinity treatment is known to be effect photosynthetic pigments due to the reduction of their synthesis or the induction of degradation during salt stress.

Table 3: Effect of salicylic acid and irrigation by diluted seawater on photosynthetic pigments of moringa plants (mg/ 100 g f.w)

Treatments	Chl.a	Chl.b	Carotenoids	Chl.a+Chl.b	Chl.a:Chl.b	Chl.a+Chl. b:carotenoids
Control	157.9	48.2	165.7	206.1	3.28	1.24
SA1	321.2	117.1	279.3	438.3	2.74	1.57
SA2	304.4	100.6	239.1	405.0	3.03	1.69
Sa1	177.0	75.7	202.4	252.7	2.34	1.25
Sa2	208.4	78.4	191.5	286.8	2.66	1.50
Sa1+SA1	188.3	64.3	121.3	252.6	2.93	2.08
Sa1+SA2	189.8	70.2	176.3	260.0	2.70	1.48
Sa2+SA1	138.4	62.5	126.4	200.9	2.21	1.59
Sa2+SA2	217.7	95.4	228.5	313.1	2.28	1.37
L.S.D at 5%	19.80	12.78	15.28	21.06	…….	…….

Sa1= Irrigation by 2500 ppm diluted seawater
Sa2=Irrigation by 5000 ppm diluted seawater
SA1=Spraying by 100 ppm salicylic acid
SA2= Spraying by 200 ppm salicylic acid
L.S.D. =Least significant difference

Effect of salicylic acid:

Chlorophyll a and chlorophyll b concentrations were significantly increased with application of salicylic acid via leaves compared to the control treatment which sprayed with distilled water. Total chlorophyll as well as total carotenoids showed the same response. Moreover, the increment caused by spraying salicylic acid in the concentration of 100 ppm exceeded those obtained from use of 200 ppm.

Noreen, *et al* (2009) found that application of SA improved chl.a and chl.b pigments. They stated that photosynthetic pigments such as chl.a and chl.b chief compounds of photosynthesis, during photosynthesis mechanism and hence growth induce of biomass production or yield. A possible conclusion of photosynthetic efficiency with chl.a and chl b. has been observed. They added that a strong negative correlation between leaf Na+ and each leaf chlorophyll. Purcarea and Borbely (2011) noticed that chl.a and chl.b increased non-significantly with the low SA level but significantly with the high level of this bio-regulator. Zheo, *et al* (1995) and Sinha, *et al* (1993) confirmed these results. Faridaddin, *et al* (2003) reported that *Brassica juncea* plant sprayed with SA showed 20 percent chlorophyll highest than those sprayed with water only. By contrast no change in chlorophyll content was observed in corn and soybean plants exogenously sprayed with acetyl salicylic acid (Khan, *et al* 2003). SA activated the synthesis and the rate of de-epoxidation but decreased the level of chlorophyll pigments in both wheat and mung bean plants. Also, the rates of chl.a and chl.b. were increased in wheat plants (Purcarea and Borbely, 2011). SA also increased chl.a and carotenoids contents in

maize plants. (Sinha, *et al* 1993). Enhancing effect of SA on photosynthetic capacity can be attributed to its stimulatory effects on Rubisco activity or pigments contents (Purcarea and Borbely, 2011).

Effect of interaction:

The interactive effect of salicylic acid spraying and salt stress on the concentration of chlorophyll and carotenoids were reported in Table (3). The highest concentration of these pigments were obtained by application of 100 ppm salicylic acid and irrigated by fresh water but the lowest were showed in plants sprayed by distilled water and irrigated regularly with fresh water. Faridaddin, *et al* (2003) concluded that the reduction in chlorophyll contents may occur due to acceleration in chlorophyll degradation or reduce in chlorophyll synthesis. However, it has been reported that in presition of salt stress causes deterioration in the structure of chloroplast e.g., thylakid membranes and plastid (Mittler, 2002). The treatment with 0.1 mM SA solution increased chlorophyll and carotenoides in unstressed plants than that stressed ones (Purcarea and Borbely, 2011).

Amino acids concentration:

Effect of salinity:

Data presented in Table (4) indicated that methionine and tyrosine concentration decreased as the concentration of salts increased in irrigation solution. The reverse was true approximately for lucine, glutamic acid, arginine and lysine concentrations. Concerning the concentration of glycine, valine and methionine concentrations, it could be noted that the concentration of 2500 salts decreased its concentrations and tended to increased with the highest salt solution used. Histidine and isoleucine concentrations increased only by 5000 ppm salts in water of irrigation but the aspartic acid concentration did not showed any clear response to the different diluted seawater.

Data in Table (4) cleared that the essential amino acid decreased slightly with the first salt level and increased with the highest level of salts in diluted seawater used in irrigation. Meanwhile, the nonessential amino acid as well as total amino acids was pronouncedly decreased with the highest salt treatment.

Cusido, *et al* (1987) showed that deficit of K induced by salinity increased the levels of free amino acids, especially of aspartic acid, glutamic acid and proline. On the other hand, the levels of nicotine in leaves of treated plants were lower than controls. In contrast, treatment of the plants with 100 m*M* NaCl induced in general an increase of nicotine in the roots. These results indicate that there was an unclear effect of salinity either on synthesis or on translocation of nicotine from roots to leaves. Effect of salinity on soluble protein, free amino acids and nicotine contents in *Nicotiana rustica* L. was studied by Hussein, *et al* (2005) and demonstrated that phenylalanine, serine, alanine and glutamic acids increased in grains of plants subjected to 200 ppm and tended to decrease with the highest salt level. Hussein, *et al* (2007) revealed that all amino acids concentration lowered by salinity except proline and glycine in maize plants. Zushi and Matsuzoe (2006) found that glutamate content on a fresh weight basis was unaffected by water stress, but increased 2-fold under salinity stress. This increase may contribute to an improvement in fruit taste. Under water stress on a dry weight basis, proline and γ-aminobutyrate were increased, sparagines, glutamine and arginine were decreased, and other amino acids were unaffected. In contrast, under salinity stress, all amino acids were increased. We conclude that a net accumulation of fruit acids occurred in tomato fruit under salinity stress, but not under water stress. Furthermore, the accumulation of proline and γ-aminobutyrate was a common response in tomato fruit under water and salinity stresses. Therefore, these two amino acids may help to defend against water and salinity stresses in tomato fruit. Monique, *et al* (2013) noticed that significant increases in the free amino proline and glutamate and significant decreases in sparagines, glutamic acids, jmine and gamma-amino-butyric-acid were found with increasing salinity. Abd El-Qodos (2009) found that the lowest levels of salinity increased significantly total free amino acids content as compared with control. Increasing salinity to 8000 ppm reduced total free amino acids. Furthermore, Sarwat and El-Shrief (2007) indicated that amino acids appeared to be decreased with salinity depending on tolerance concerned amino acids response in barley.

Effect of salicylic acid:

Data illustrated in Table (4) pointed out that a positive relationship between the concentration of aspartic acid, thrionine, serine, glutamic acid, valine, isoleucine, lucine, phenylalanine and histidine and the concentration of salicylic acid exogenous application. However, arginine, glycine and methionine decreased

with the moderate concentration of salts but tended to increase with the high salts in the diluted seawater treatment. Data in the same table indicated that essential amino acids increased as the concentration of SA increased. The nonessential amino acid and total amino acids showed approximately the same trend. Hussein, *et al* (2007) emphasized that SA application in the rate of 200 ppm increased the concentration of all amino acids except methionine in maize plants.

Effect of interaction:

Data in Table (4) showed that the total amino acids increased with the application of salicylic acid under both salinity treatment or in the control. The increases in AA caused by application of SA under irrigation with 2500 ppm salt solution exceeded those show with this spraying treatment irrigated with diluted seawater contains 5000 ppm salt. The essential, nonessential and total amino acids higher values were shown when plants sprayed by 200 ppm either plants irrigated by water contains 2500 or 5000 ppm salts. The highest values of these parameters were shown with the application of 200 ppm SA and irrigation by 2500 ppm salts in diluted seawater treatment.

Hussein, *et al* (2007) pointed out that under 4000 ppm salinity, SA spraying improved the concentration of arginine, glutamic, lysine and serine in maize plants. Purcarea and Borbely (2011) reported that in the case of determination of amino acids, salt stress reduced significantly the content in amino acids. Treatment with SA determined an enhancement in free amino acids values in comparison with salt stressed plants. The highest value of enhancement was registered in root of salt stressed plantlets treated with 0.1 mM SA solution. In cases of proline, under stress, with and without SA treatments , the proline content increased non-significantly but in case of increase of SA treated seedlings leaves the increase of proline content was higher than untreated seedlings leaves (Purcarea and Borbely, 2011).

Table 4: Effect of salicylic acid and salt stress on amino acid concentrations of moringa plants (g/100g protein).

Treatments / *Amino acid*	Control	SA1	*SA2*	Sa1	Sa2	Sa1+SA1	Sa1+SA2	Sa2+SA1	Sa2+SA2
Aspartic	0.97	1.57	2.26	0.98	1.82	1.82	2.50	0.97	1.68
Thrionine	0.17	0.50	1.31	0.26	0.95	0.98	1.03	0.59	0.78
Serine	0.14	0.72	1.34	0.32	0.96	0.88	1.20	0.71	0.99
Glutamic	0.75	1.77	3.50	1.06	2.53	1.62	3.02	1.54	2.70
Glycine	0.55	0.41	0.77	0.36	0.63	0.22	0.65	0.26	0.52
Alanine	0.70	1.49	2.23	0.89	1.57	1.77	2.52	0.86	1.65
Valine	0.62	1.23	1.38	0.34	1.09	0.93	1.81	0.45	1.05
Methionine	0.75	0.27	0.83	0.26	0.25	0.14	0.34	0.01	0.22
Isoleucine	0.30	0.53	0.83	0.28	0.82	0.23	0.72	0.25	0.73
Lucine	0.88	2.09	2.84	0.93	2.21	0.93	2.47	0.81	2.15
Tyrosine	1.62	1.32	1.68	1.18	1.39	0.88	1.48	0.65	1.12
phenylealanine	1.06	1.82	2.03	0.84	1.79	1.06	2.11	0.73	1.52
Histidine	103	1.39	1.51	1.04	1.52	0.77	1.36	0.23	0.70
Lysine	0.38	1.50	1.32	0.65	1.53	0.75	1.94	0.65	0.94
NH4	4.92	1.75	1.25	1.88	2.03	1.39	1.97	1.58	1.75
Arginine	0.48	1.20	1.20	0.74	2.46	0.83	2.64	0.76	1.52
Essential AA	5.19	11.33	12.05	4.60	13.09	4.99	11.78	3.99	8.09
Nonessential AA	5.21	8.48	12.98	5.53	10.46	7.82	14.01	5.75	10.18
Total AA	10.40	19.81	25.03	10.13	23.55	12.81	25.79	9.74	18.27

Sa1= Irrigation by 2500 ppm diluted seawater
Sa2=Irrigation by 5000 ppm diluted seawater
SA1=Spraying by 100 ppm salicylic acid
SA2= Spraying by 200 ppm salicylic acid
L.S.D. =Least significant difference

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