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Full Length Article

Role of Jasmonic Acid and Gamma Radiation in Alleviating Salt Stress in Moringa

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Abstract

The present investigation studied the effect of two doses of γ-rays (20, 40 Gy) or foliar spraying of jasmonic acid 0.2 and 0.3 µM on moringa plants under salt stress condition (4000 mg/L NaCl). Foliar spray by jasmonic acid especially (0.3 µM) gave the highest peroxidase and polyphenol oxidase enzymes activity under normal or stress condition. Gamma rays and jasmonic acid treatments caused changes in activities, the band concentration of peroxidase and polyphenol oxidase isozymes and increased amino acid contents. Under salt stress condition, Cl, Fe, K and Na percentage increased significantly and of Ca, Mg and S decreased. The greatest level of K were also observed in samples treated by γ- rays or sprayed by JA under normal or salt stress condition. Gamma irradiation with dose 20 Gy and spraying with jasmonic acid (0.3 µM) increased moringa resistance to salt stress during growth under 4000 mg/L level of salt stress.

Keywords: Salinity; Gamma radiation; Moringa; Jasmonic acid; Amino acid; Minerals

Introduction

High salinity is one of abiotic stresses that represent a major reduction in crop productivity. Salinity changes physiological and cellular process of plant, which negatively affects its growth and development (Munns, 2011). In addition to osmotic, ionic imbalance and toxicity salt stress also induces oxidative stress in plants (Rout and Shaw, 2001), which in turn, initiates antioxidant system of the plants to cope up with oxidative damage caused by the stress.

Recent reports have underlined the capability of γ-rays as devices for seed priming a procedure utilized as a part of seed industry to upgrade plant resistance to biotic/abiotic stress. A synergistic treatment by γ--rays and NaCl enhanced oxidative stress and activate antioxidant mechanisms, which reinforce cell components, subsequently being perfect with plant survival (Macovei *et al*., 2014).

Gamma rays have been demonstrated as practical and viable when contrasted with other ionizing radiations on account of its simple accessibility and the force of entrance. Gamma irradiation especially low doses accelerates cell division, growth, stress tolerance and yield production (Chakravarty and Sen, 2001; El-Beltagi *et al.,* 2011). Another approach to relieve adverse effects of salt stress has been the use of methyl jasmonate (Me JA). Yoon *et al.* (2010) reported that Me JA significantly relieved the adverse effects of NaCl stress. Jasmonates are produced from oxidation of linolinic acid by the action of lipoxygenase (Vick and Zimmerman, 1984) and one of the most important plant growth regulators, which occur in plants and affect morphology, physiology and biochemistry of plants, especially under salt stress (Yoon *et al.,* 2010). Moreover, it increases plant resistance to environmental stress such as heavy metals and elemental toxicity, drought, low temperature and salt stress (Anjum *et al.*, 2011; Enteshari and Jafari, 2013). Jasmonic acid (JA) is derived from the release of fatty acid of cell membrane by the action of lipase in response to stress (Anderson, 1989; Farmer and Ryan, 1992).

Moringa (*Moringa oleifera*) is from foothills of the Himalayas in northwestern India and used in multiple purpose (Olson, 2010) and the most widely cultivated species of Moringaceae family due to its easy propagation, fast growth and its numerous economic uses (Fahey, 2005). In addition, moringa have multiple medicinal uses, highly nutritious leaves, highly contents of vitamin A, vitamin C and natural antioxidants (Anwar and Bhanger, 2007). Also, their seeds are a good resource of high quality oil used in cooking, cosmetics and lubrication (Lala and Tsaknis, 2003). Alatar (2011) added that it can be grown well in salt affected soils and considered NaCl resistant at germination and emergence stage (Elhag and Abdalla, 2012). Despite its inherent salt tolerance, inducing further resistance would make moringa even more useful for planting in high-salinity environments. This work used γ- rays and Jasmonic acid to evaluate moringa performance after 50 days from germination under salt stress condition.

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Materials and Methods

Seeds of moringa were obtained from the Agriculture Research Center, Ministry of Agriculture, Giza, Egypt.

Radiation Treatments

Dry seeds irradiation was performed at national center for research and technology (NCRRT). Cairo, Egypt. By using (Co^{60}) as source of γ-rays at dose levels 20 and 40 Gy with dose rate of 7.5 rad/sec.

Experimental Design

Treated and control seeds were sown in plastic bags (12*20 cm) filled by equal ratio of sand and compost 3 kg per each. Equal amount of water was added to each bag every 5 days to keep the soil at field capacity and considered control group. Second group was irrigated by NaCl solution with concentration 4000 mg/L, After 10 days from germination and considered saline group. The experiment was carried out in a wire house at NCRRT, Cairo, Egypt and stated in complete randomized design with ten replications at season 2014. Data was recorded after 50 days from germination.

Jasmonate Application

After ten days from germination a part of untreated control plants sprayed by two levels of Jasmonic Acid (JA). Jasmonate sprays typically contain small amounts of solvents (ethanol) to dissolve it. The typical concentration of JA sprays was 0.2 and 0.3 µM, repeated every 10 days.

Determination of Peroxidase Activity

Fresh and young leaf samples were homogenized in distilled water (50 mg/ mL). The homogenates were centrifuged at 8000 rpm for 15 min at 5ºC in a cold centrifuge. The supernatants were kept in deep freezer till use. Peroxidase activity was measured according to Vetter *et al.* (1958). In 200 µL of sample, the following reagent (1 mL of 1% o-phenylenediamine in 95% ethyl alcohol, fresh every four hours) plus 1 mL of 0.3% hydrogen peroxide was added. After 5 min of the reaction allowed to react, the time stopped by adding 2 mL of saturated sodium bisulfite. The blank of each sample was done by adding the dye, followed by sodium sulphite and then hydrogen peroxide. The enzyme activity stopped by the sulfite when hydrogen peroxide is added. The enzyme activity was expressed as the change in absorbance at 430 nm (ΔOD_{430})/minute/g fresh weight.

Determination of Polyphenol Oxidase Activity

Polyphenol oxidase activity was measured according to modification of Ishaaya (1971). For it, 0.5 mL phosphate buffer (0.1 M, PH7) was added to 200 µL enzyme solution and 200 µL catechol solution (2%). The substance and other reagent were separately incubated at the optimum temperature of the reaction (25ºC) before the initiation of reaction. Enzyme reaction was determined by adding catechol solution, then after 1 min, the optical density was determined. Zero adjustment was against sample black. The polyphenol oxidase activity was determined as ΔO. D. Unit x at an absorbance of 405 nm.

Native-Polyacrylamide Gel Electrophoresis (Native–PAGE)

This was performed according to Stegemannet *et al.* (1985). Fresh leaves samples (0.5 g) were homogenated in 1 mL of 10% glycerol. Then a mixture at 10000 rpm for 5 minutes was centrifuged. 40 µL extract of each sample was mixed with 20 µL sucrose and 10 µL bromophenol blue, then 50 µL from each mixture was applied to each well. The run was performed at 150 volt until the bromophenol blue dye has reached the separating gel and then the voltage was increased to 200 volt. After electrophoresis, the gels were stained according to their enzyme systems with the appropriate substrate and chemical solution then incubated at room temperature in dark for complete staining. Incubation was done for 1 to 2 h.

Amino Acid Composition

Weight of 50 mg of powdered leaves in tube then 5 mL of 6 N HCl was added. The tubes were closed and kept in oven at 110ºC for 24 h for complete digestion (AOAC, 1990). The samples were evaporated and dissolved in sodium citrate then filtered and analyzed (Baxter, 1996). The system used was the High Performance Amino Acid Analyzer, Biochroma 20.

Mineral

Minerals were measured on Energy Dipersive X-Ray Model (Oxford) attached to a scanning electron microscope (JEOL-JSM 5400). In this analysis, the characteristic X-ray radiation emitted from each element when the specimen is bombarded with high energic electrons-is utilized to determine the kind of the elements that exists in the specimen surface and their percentage. The elements estimated were calcium (Ca), chloride (Cl), iron (Fe), potassium (K), magnesium (Mg), sodium (Na) and sulphur (S).

Statistical Analysis

The means of three replicate of data were analyzed using the least significance difference test at 0.05 (Steel *et al.*, 1997).

Results

Peroxidase and Polyphenoloxidase Activities

The activity of peroxidase enzyme decreased significantly with 40 Gy dose in both normal and saline conditions but

increased with 20 Gy. Foliar spraying with JA especially (0.3 µM) gave the highest activity of peroxidase enzyme under normal or stress condition. The other concentration (0.2 µM) increased activity of peroxidase enzyme significantly under saline condition compared with control. Plants irradiated with 20 Gy under salt stress condition showed a significant increase in peroxidase activity compared to unstressed plants subjected to 20 Gy irradiations. However, irradiation with 40 Gy showed no significant difference in the enzyme activity between normal or stress condition.

Maximum polyphenol oxidase activity was recorded in plants under normal or stressed conditions, treated with 0.3 µM JA than 0.2 µM JA treatment (Fig. 1). Gamma irradiation at level of 20 Gy significant increased polyphenol oxidase activity under saline irrigation compared with the corresponding control although no effect was observed when a higher radiation dose used.

Electrophoretic Pattern of Peroxidase and Polyphenol Oxidase Isozymes

Peroxidase isozymes: Electrophoretic pattern of peroxidase showed eight bands (Fig. 2). It was noticed that most of separated bands from samples grown under normal conditions were faint (9 bands) or very faint (4 bands) but, in saline irrigation plants, 13 band very dark and 9 bands were dark in particular, bands 3 and 6 were very dark for all treatments under high salinity conditions. This result suggests that high salinity condition increased concentration of peroxidase in moringa plants. Several bands (either new or with increased intensities; A, C, E, F, G and H) were visible in samples from plants grown under saline condition compared with normal control.

Bands A, E, G and H were only clearly visible in different treatments grown under saline condition and can be considered as positive markers for salinity. In contrast, band D can be considered a negative marker for salinity as it was present in control samples subjected to non -saline but was absent in samples subjected to saline irrigation.

Polyphenol oxidase isozyme: A comparison of polyphenol oxidase band intensities in moringa plants grown under water or saline solution (4000 mg/L) (Fig. 3) showed presence of up to nine bands. One faint band (E) and one very faint band (I) was visible in plants treated with saline solution. Although weak, it can be considered positive marker for salinity as it appeared in most of the different treatments.

Amino Acids Contents

Total amino acid content was higher for control plants grown under saline conditions compared with water (Table 1). Levels of proline and methionine were notable higher for control plants grown under saline conditions compared with plant watered normally (Table 1).

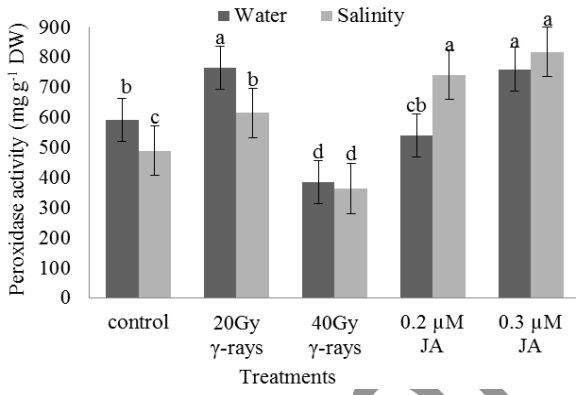

Fig. 1: peroxidase activity in moringa plants as affected by γ-rays and sprayed by Jasmonic acid under normal or saline irrigation**.** Data were statistically analyzed using the least significance difference. Each value is the mean of three replicates. Different letters indicate significant variation

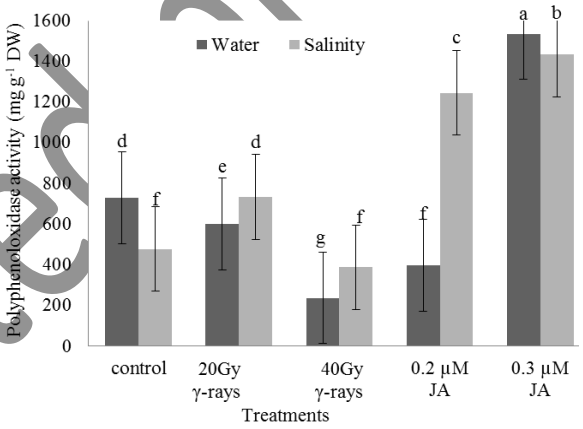

Fig. 2: Polyphenoloxidase activity in moringa plants as affected by γ-rays and sprayed by Jasmonic acid under normal or saline irrigation. Data were statistically analyzed using the least significance difference. Each value is the mean of three replicates. Different letters indicate significant variation

Total amino acids contents of plants were higher following gamma radiation of seeds under both normal and salinity condition when compared with the non-irradiated control.

Mineral Composition

Mineral investigated in moringa were shown in Table 2. Chlorine, ferrous, potassium and sodium percentage increased significantly under salt stress while levels of calcium, magnesium and sulfur decreased. The highest levels of potassium were observed in samples treated by γ-rays or sprayed by JA, under normal or salt stress condition.

Levels of calcium, chlorine, ferrous, magnesium, and sodium in samples watered normally or with saline solution and sprayed by 0.2 or 0.3 µM JA mostly decreased as compared with the corresponding control.

Table 1: Amino acids concentration (mg g^{-1} DW) of moringa plants grown from seeds treated with different doses of γ-rays or plants sprayed with JA under normal or saline irrigation

Irrigation	Water					Salinity (4000 mg/L)				
Treatment		Dose/Gy			JA/ µM		Dose/Gy			JA/µM
Amino acid	control	20	40	0.2	0.3	control	20	40	0.2	0.3
Aspartic acid	14.7	12.6	16.8	16.8	16.8	14.7	14.7	16.8	14.7	16.8
Threonine	14.7	12.6	16.8	14.7	14.7	14.7	12.6	12.6	12.6	16.8
Serine	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6	10.5	15.5
Glutamic acid	12.1	12.6	16.8	16.8	16.9	14.7	14.7	14.7	12.6	12.6
Proline	1.1	2.1	2.2	1.1	1.5	4.2	1.1	4.0	2.0	4.8
Glycine	10.5	10.5	12.6	10.5	10.5	10.5	10.5	10.5	8.4	8.4
Alanine	8.4	10.5	10.5	8.4	8.8	8.4	8.4	10.5	8.4	4.4
Cystine	2.1	2.1	2.1	2.1	2.4	4.2	2.1	4.2	2.1	2.1
Valine	10.5	12.6	14.7	10.5	10.5	10.5	10.5	12.6	10.5	10.5
Methionine	2.4	4.2	4.2	2.0	2.0	8.4	2.1	2.1	2.0	2.0
Isoleucine	10.5	10.5	12.6	10.5	10.5	12.6	8.4	10.5	8.4	8.4
Leucine	51.3	56.7	60.9	50.4	52.5	58.8	48.3	56.7	54.1	50.5
Tryosine	8.4	12.6	16.8	8.4	8.0	10.5	10.5	10.5	10.5	10.5
Phenylalanine	12.6	16.8	18.9	12.6	12.0	14.7	12.6	14.7	12.6	12.6
Histidine	10.5	12.6	14.7	14.7	14.7	10.5	10.5	12.6	10.5	10.5
Lysine	14.7	16.9	23.1	18.9	17.8	14.7	16.8	21	16.8	16.8
Arginine	14.7	14.7	23.1	27.3	22.5	14.7	14.7	16.8	18.9	18.9
Total	201.2	235.2	279.4	238.3	234.7	239.4	211.1	243.4	215.6	222.1

Table 2: Mineral percentage of moringa plants grown from seeds treated with different doses of γ-rays or plants sprayed by Jasmonic acid under normal or saline irrigation

Irrigation	Treatments	Ca	Cl	Fe	K	Mg	Na	S
Water	control	51.06^{A}	11.99^{G}	6.64^{A}	11.33^{I}	6.47^{C}	5.32^{E}	7.19^{D}
	20Gy	21.73^{C}	6.05^{I}	0.86^{B}	42.68^{E}	7.64^{A}	5.57^{D}	15.48^{A}
	40Gy	13.86^{F}	13.90^{F}	0.70^{CBD}	51.47^{C}	6.91^{B}	7.42^{B}	5.74^{E}
	0.2µM JA	11.34^{G}	6.84^{H}	0.63^{CD}	62.04^{B}	3.89^{F}	4.40^{HG}	10.86^{B}
	0.3µM JA	11.50^{G}	6.91^{H}	0.71^{CBD}	62.50^{A}	3.90^{F}	4.51^{G}	10.00^{C}
	control	32.00^{B}	23.6^{D}	6.60^{A}	21.20^{H}	5.64^{D}	6.10^{C}	5.20^{FE}
Salinity	20Gy	14.00^{F}	31.32^{A}	0.73^{CBD}	34.14^{G}	5.99^{D}	11.31^{A}	2.51^{G}
4000 mg/L	40Gy	20.59^{D}	22.67^{E}	0.80^{CB}	40.28^{F}	$5,73^{D}$	4.31^{H}	5.62^{E}
	0.2µM JA	15.26^{E}	25.17^{C}	0.59^{D}	43.63^{D}	5.20^{E}	4.95^{F}	5.21^{FE}
	0.3µM JA	14.70^{E}	26.00^{B}	0.55^{D}	43.73^{D}	5.13^{E}	4.91^{F}	4.89^{F}

Data were statistically analyzed using the least significance difference. Each value is the mean of three replicates. Different letters indicate significant variation

Discussion

This work studied the effect of γ- rays and Jasmonic acid on moringa plant performance under salt stress condition. Foliar spray by Jasmonic acid especially (0.3 µM) gave the highest peroxidase and polyphenol oxidase enzymes activity under normal or stress condition. Gamma rays and Jasmonic acid treatments caused changes in activities, the band concentration of peroxidase and polyphenol oxidase isozymes and increase amino acid contents. Under salt stress condition, Cl, Fe, K and Na percentage increased significantly, and of Ca, Mg and S decreased. The greatest level of K observed in samples treated by γ- rays or sprayed by JA under normal or salt stress condition. Plant hormones play important roles in regulation of developmental processes and signaling networks in plants under abiotic stress (Khan *et al.,* 2013). One of known classical plant hormones is Jasmonic acid (JA), have been recently integrated and shown as potential implement in enhancing tolerance of plants to abiotic stress (Nazar *et al.,* 2011). JA is biochemically activated by abiotic stresses and capacity as vital signaling molecule responsible for resistance reactions in plants (Khan *et al.,* 2012).

Reactive oxygen species (ROS) are toxic molecules, which considered as signaling molecule to control gene expression of antioxidant defense system (Neill *et al.,* 2002; Vranova *et al.,* 2002). To balance the danger of reactive oxygen species, plants improved the activity of antioxidant enzymes especially superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and peroxidase (pod), which in turn increased stress tolerance (Jiang and Zhang, 2002). In accordance with our results Abou-Zeid and Abdel-Latif (2011) observed that antioxidant enzymes activities significantly increased in wheat leaves with increasing gamma rays (1-5 k Gy).

Polyphenol oxidase (PPO) activity is inducible by volatile or liquid jasmonates in many plants (Doan *et al.,* 2004). Plants elicit a molecular response to prevent oxidative damage due to ROS production and adjust to the

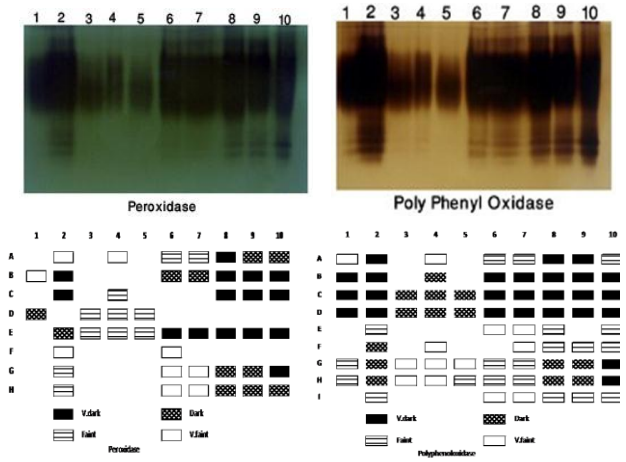

Fig. 3: Peroxidase and polyphenol oxidase isozymes of moringa plants treated by gamma rays or sprayed with Jasmonic acid under normal (1, 2, 3, 4, 5) or saline (6, 7, 8, 9, 10) irrigation
1: Control; 2: 0.2 µM JA; 3: 0.3 µM JA; 4: 20 Gy; 5: 40 Gy;
6: Control salinity; 7: 0.2 µM JA salinity; 8: 0.3 µM JA salinity;
9: 20 Gy salinity; 10: 40 Gy salinity

oxidative stress (Baxter *et al.*, 2007; Yaycili and Alikamanoğlu, 2012; Demirkaya, 2014). They have built up a multifaceted antioxidant defense with enzymatic molecules, including catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and peroxidase (POD). All these molecules act as scavenging ROS molecules formed during oxidative damage (Erdal and Çakirlar, 2014; Petrić *et al.*, 2014). The results obtained by Kawasaki *et al.* (2001) suggested that APX is a chief enzyme involved in H_2O_2 scavenging to overcome oxidative stress under salt stress.

Peroxidase and polyphenol oxidase PPO were induced in tomato plants 48 h after treatment with 0.5 – 10 mM JA (Thaler, 2002), or 72 h after spraying plants with 7.5 mMor 10 mM MeJA (Boughton *et al.*, 2005).

Tariq *et al.* (2011) reported that MeJA treatment enhanced the activities of catalase, peroxidase and superoxide dismutase enzymes. By improving the ROS scavenging mechanism, methyl jasmonate increases the antioxidant activity to remove the toxic effect of free radicals increasing plant resistance.

Amino acid levels increased in different plant under salt stress condition (Muthukumarasamy *et al.*, 2000; Wang and Nii, 2000). Hamideldin and Hussein (2009) demonstrated that gamma radiation raised the total amino acid contents in normal and salt treated plant. Hussein *et al.* (2012) observed that amino acid contents markedly increased with 20, 40 and 80 Gy as compared with control plants during two season of experiment. In addition, irradiated seed with gamma rays and then sown at different salinity levels increased total amino acid. Abdelgawad *et al.* (2014) showed that MeJA increased free amino acid content of maize plant compared to the corresponding untreated plant.

Salt stress induced harmful effect on moringa plant performance induced nutrient disorders during first stage of growth. Treated plants with salt caused nutrient imbalance and deficiencies. This may be due to excessive accumulation of Na^+ and reduce uptake of other mineral such as K^+, Ca^{2+} and Mn^{2+} (Karimi *et al.*, 2005). Hussein and Abou-Baker (2014) revealed that mineral contents (N, P, K, Na and Ca) of moringa plant decreased with increasing salinity level with significant difference in N and without significant difference in other nutrients. However, the relation between salinity and minerals nutrition of crops are very complex. Numerous studies were conducted and reported the disorder in nutrients as a result of salt stress (Hasanuzzaman *et al.*, 2013). On the other hand, Carter *et al.* (2005) found that plant accumulates Cl more than Na at lower salinity concentration where at high concentration plant accumulate high amount of both elements.

Gamma irradiation increased Na, K and Ca percentage in damsisa plant comparing to control under different salinity conditions. High salinity concentration reduced Mg percentage in irradiated plants (Hussein *et al.*, 2012).

Sheteawi (2001) confirmed that treatment of soybean by Jasmonic acid increase N, P and K level under irrigated with 50 mM NaCl salt stress.

Conclusion

Gamma irradiation with dose 20 Gy and spraying with jasmonic acid (0.3 µM) increased moringa resistance to salt stress during growth under 4000 mg/L level of salt stress.

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