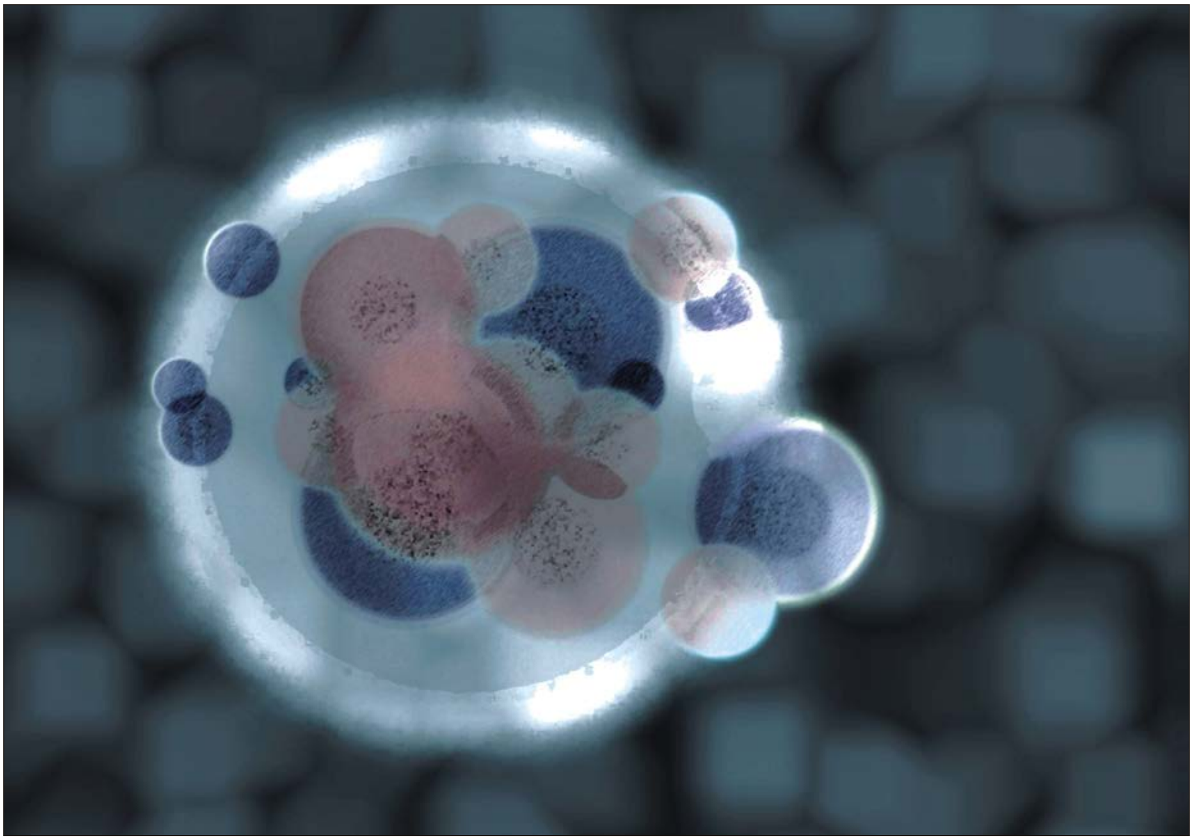

Journal of Biological Sciences

ISSN 1727-3048

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Journal of Biological Sciences 8 (1): 171-175, 2008
ISSN 1727-3048

Response of Salt Stressed *Ricinus communis* L. To Exogenous Application of Glycerol and/or Aspartic Acid

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Abstract: Changes caused by salinity and glycerol or aspartic acid on growth, oil contents, endogenous glycerol and recinine alkaloids of *Ricinus communis* plant have been studied. Growth, oil contents and recinine alkaloids in roots of *Ricinus communis* plant were significantly lowered with the salinization effect using NaCl. On other hand, oil contents and recinine alkaloids in shoots were increased. However, endogenous glycerol in shoots and roots increased with increasing salinization level. Growth, oil contents, recinine alkaloids and endogenous glycerol in the different organs of plant originating from seeds soaked in glycerol or aspartic acid were generally increased over the control. However, recinine alkaloids in roots decreased. Soaking seeds in glycerol or aspartic acid counteracted the adverse effect of salinity on growth and secondary products of *Ricinus communis* L. Plant.

Key words: Aspartic acid, castor, glycerol, NaCl, ricinine, salinity, alkaloids, *Ricinus communis*

INTRODUCTION

Ricinus communis L. (Euphorbiaceae) commonly called as castor bean grows wild in Egypt and other parts of the world. Castor bean is cultivated for the production of castor oil which is used mainly for technical purposes and also in medicine as a purgative. The leaves have been used for treatment of rheumatic pains and as antibacterial and anti-inflammatory (Luseba *et al*., 2007; de la Paz *et al*., 2006). The leaf, root and seed oil of this plant have been used for laxative and the treatment of inflammation and liver disorders (Ilavarasan *et al*., 2005; Capasso *et al*., 1994; Visen *et al*., 1992). The natural products obtained from this species include, oils, alkaloids and various phenolics (Luseba *et al*., 2007).

The major alkaloid in castor seeds is recinine, a pyridine-based alkaloid which is biosynthesized from glycerol or aspartic acid as precursors that are also involved in pyridine nucleotides cycle (Noctor *et al*., 2006). The production of recinine, like other pyridine-based alkaloids, is enhanced by stress (Noctor *et al*., 2006). They are involved in many other defence and signaling reactions, for example, production of nitric oxide and metabolism of reactive lipid derivatives (Crawford and Guo, 2005; Mano *et al*., 2005). Salinity, however, is an important stress encountered by plants including *R. communis*. The effects of salinity on the oil, glycerol and ricinine contents of castor bean were studies by Ali (2002).

Exogenous application of nicotinic acid, an intermediate in pyridine nucleotide synthesis, was successful in ameliorating the adverse effect of NaCl on growth of *R. communis* (Ali, 2002). Labelling studies have shown that nicotinate, nicotinamide, quinolinate and NAD are all effective sources of the pyridine ring for ricinine synthesis (Noctor *et al*., 2006).

No work could be obtained on the effect of exogenously applied glycerol or aspartic acid on growth and secondary products of *R. communis* under saline conditions. So, the present work was undertaken to study the effect of glycerol or aspartic acid on growth, recinine alkaloids, oil and endogenous glycerol in *R. communis* under the influence of NaCl.

MATERIALS AND METHODS

Seeds of *R. communis* were obtained from the National Center for Agricultural Research, Giza, Egypt and transferred to the Botany Department Research Lab during 2006. Seeds were soaked for 48 h in different concentrations of glycerol (10, 25 and 50 mM), aspartic acid (5, 10 and 15 mM), mixture of glycerol and aspartic acid (10+5, 25+10 and 50+15 mM), which were chosen based on preliminary studies. Seeds of control plants were soaked in distilled water. Thereafter, the seeds were grown in quartz sand culture, one plant per pot, in a greenhouse and watered daily with 100 mL of Hoagland's nutrient solution (Hewitt, 1966) with 0, 50 and 100 mM

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NaCl. Five replicates were used for each set of experiments. Plants were grown for 6 weeks and harvested, dried at 60°C to constant mass and then weighted.

Determination of oil content: Oil was extracted from shoots and roots with chloroform:methanol (2:1) by volume and total oil estimated colormeterically by the methods of Marsh and Weinstein (1966).

Determination of alkaloid: Ricinine alkaloid was extracted according to Lee and Waller (1972) by homogenization of 5 g tissue with 200 mL chloroform. The residue was further extracted with 75% methanol until the residue was free of soluble pigments. The chloroform and methanol-water extracts were combined and concentrated under reduced pressure to about 25 mL, cooled and the aqueous solution decanted. The residue was rinsed twice with water. The aqueous fraction was evaporated to dryness. The residue was then extracted with hot methanol. The collected methanol extract was concentration and used for calorimetrically estimation of the alkaloids using calibration curve, contents of alkaloid calculated as mg ricinine/g dry mass (DM).

Determination of glycerol: Endogenous glycerol was measured according to Youmis *et al*. (1987). Extraction of glycerol was carried out by boiling a known weight of sample in water, macerated in a mortar and then centrifuged. The supernatant was collected in a fresh tube, made up to volume and used for the estimation of glycerol. To 10 mL of extract, 5 mL of 7.5% potassium dichromate and 30 mL sulphuric acid (50% v/v) were added. The contents were vortexed, boiled for 15 min and then left to cool at room temperature. The volume was made up to 50 mL and intensity of the colour developed was measured at 6000 nm.

All the present experiments were repeated five times and data were analyzed by applying the least means significant difference (LSD) at 5 and 1% levels of probability.

RESULTS

Growth as in Table 1 reveal that fresh-dry mass (DM) of *R. communis* plants originating from seeds soaked in glycerol, aspartic acid, mixture of glycerol and aspartic acid were significantly raised with increasing the concentration of any of these compounds. On the other hand, plants originating from seeds soaked only in distilled water and irrigated with saline solutions (50 and 100 mM NaCl) the fresh-dry mass (DM) per plant were lowered than of non salinized plants. Seeds soaked in various levels of glycerol or aspartic acid and irrigated with saline solution progressively alleviated the inhibitory effect of salinization on fresh-dry mass (DM). The

Table 1: Effect of glycerol, aspartic acid or mixture of both on fresh and dry mass (DM) (g plant^{-1}) *R. communis* L. at various NaCl concentrations

Treatments		Fresh mass		Dry mass	
NaCl (mM)	Glycerol (mM)	g plant^{-1}	Control (%)	g plant^{-1}	Control (%)
0	0	6.59	100.00	1.32	100.00
	10	7.18**	109.00	1.50*	113.60
	25	9.30**	141.10	1.82**	137.90
	50	10.22**	155.10	2-17.00**	164.40
50	0	5.64**	85.56	1.24	93.94
	10	5.82**	88.32	1.34	101.50
	25	8.99**	136.40	1.78*	134.90
	50	6.43	97.57	1.35	102.30
100	0	3.83	58.12	0.78**	59.09
	10	4.81	72.99	0.98**	74.24
	25	6.84	103.80	1.46	110.60
	50	6.08	92.26	1.37	103.80
LSD at 5%		0.49		0.16	
LSD at 1%		0.74		0.93	
Treatments		Fresh mass		Dry mass	
NaCl (mM)	Aspartic acid (mM)	g plant^{-1}	Control (%)	g plant^{-1}	Control (%)
0	0	6.59	100.00	1.32	100.00
	5	10.27**	155.80	1.94**	147.00
	10	9.73**	147.70	1.88**	142.40
	15	9.03**	137.00	1.79**	135.60
50	0	5.64**	85.58	1.24	93.94
	5	8.16**	123.80	1.72**	130.30
	10	8.37**	127.00	1.75**	132.60
	15	8.37**	127.00	1.63**	123.50
100	0	3.83	58.12	0.78**	59.09
	5	4.64**	70.41	1.28	96.97
	10	7.64**	115.90	1.71**	129.60
	15	4.61**	69.95	1.01**	76.52
LSD at 5%		0.61		0.15	
LSD at 1%		0.93		0.22	
Treatments		Fresh mass		Dry mass	
NaCl (mM)	Glycerol+ Aspartic acid (mM)	g plant^{-1}	Control (%)	g plant^{-1}	Control (%)
0	0	6.59	100.00	1.32	100.00
	10+5	11.50**	174.50	2.36**	178.79
	25+10	10.55**	160.10	2.14**	162.12
	50+15	9.83**	149.20	1.91**	144.70
50	0	5.64*	85.58	1.24	93.94
	10+5	7.62*	115.60	1.59*	120.45
	25+10	9.01**	136.70	1.88**	142.42
	50+15	7.64*	115.90	1.76**	133.33
100	0	3.83**	58.12	0.78**	59.09
	10+5	5.15**	78.15	1.63*	123.48
	25+10	7.97**	120.90	1.21	91.67
	50+15	6.08	92.26	1.21	91.67
LSD at 5%		0.86		0.22	
LSD at 1%		1.30		0.33	

*: Significant differences as compared with the control, **: Highly significant differences as compared with the control, DM = Dry Mass

Table 2: Effect of glycerol on oil, ricinine alkaloids and endogenous glycerol of *R. communis* at various NaCl concentrations

Treatment		Shoots						Roots					
		Oil		Ricinine		Glycerol		Oil		Ricinine		Glycerol	
NaCl (mM)	Glycerol (mM)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)
0	0	54.64	100.00	0.16	100.00	2.50	100.00	37.59	100.00	2.95	100.00	8.00	100.00
	10	84.02**	153.77	0.17	106.25	5.36*	214.41	39.66	105.51	3.44**	116.61	17.79**	222.38
	25	86.70**	158.67	0.17	106.25	6.23**	249.20	70.16**	186.65	2.61**	88.47	22.23**	277.88
	50	89.85**	164.44	0.17	106.25	9.56**	382.40	63.77**	169.65	2.24**	75.93	22.23**	277.88
50	0	87.72**	160.54	0.14	87.50	10.67**	426.80	41.62*	110.72	2.04**	69.15	20.46**	255.75
	10	86.17**	157.70	0.12	75.00	9.34**	373.60	38.40	102.15	2.16**	73.22	19.12**	239.00
	25	90.88**	166.33	0.12	75.00	9.34**	373.60	33.67*	89.57	1.41**	47.80	14.23**	177.88
	50	96.76**	177.09	0.11*	68.75	7.56**	302.40	29.40**	78.21	1.27**	43.05	13.01**	162.63
100	0	55.40	101.39	0.14	87.50	8.00**	320.00	29.30**	77.95	0.46**	15.59	11.56**	144.50
	10	100.87**	104.61	0.08**	50.00	9.34**	373.60	30.91**	82.23	1.28**	43.39	12.45**	155.63
	25	73.67**	134.83	0.07**	43.75	9.34**	373.60	30.17**	80.26	0.77**	26.10	12.45**	155.63
	50	72.45**	132.60	0.06**	37.50	11.56**	462.40	26.67**	70.95	0.63**	21.36	13.78**	172.25
LSD at 5%		5.15		0.05		2.21		2.94		0.10		10.47	
LSD at 1%		7.89		0.07		3.34		4.45		0.15		2.23	

*: Significant differences as compared with the control, **: Highly significant differences as compared with the control, DM = Dry Mass

Table 3: Effect of aspartic acid on oil, ricin alkaloids and endogenous glycerol of *R. communis* at various NaCl concentrations

Treatment		Shoots						Roots					
		Oil		Ricinine		Glycerol		Oil		Ricinine		Glycerol	
NaCl (mM)	Aspartic acid (mM)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)
0	0	54.64	100.00	0.16	100.00	2.50	100.0	37.59	100.00	2.95	100.00	8.00	100.00
	5	88.76**	162.41	0.22**	137.50	6.23*	249.2	46.61**	123.00	2.23*	75.59	16.45**	205.63
	10	86.28**	157.91	0.19*	118.75	9.43**	373.6	66.92**	178.03	1.80	61.02	17.34**	216.75
	15	110.92**	203.00	0.27**	168.75	9.43**	373.6	49.18**	130.82	0.31**	10.51	14.23**	177.88
50	0	87.72**	160.54	0.14	87.50	1067.00**	426.8	41.62**	110.72	2.05**	69.02	20.46**	255.75
	5	75.11**	137.46	0.19**	118.75	13.11**	524.4	39.66**	105.51	1.13**	44.41	14.67**	183.38
	10	82.36**	150.73	0.14	87.50	16.01**	640.4	44.45**	108.25	0.97**	32.88	16.45**	205.63
	15	87.66**	160.43	0.07**	43.75	17.78**	711.2	41.37**	110.06	0.94**	31.86	18.67**	233.38
100	0	55.40	101.39	0.14	87.50	8.00**	320.0	29.30**	77.95	0.16**	5.42	11.56**	144.50
	5	53.60	98.10	0.15	93.75	8.00**	320.0	22.08**	58.74	0.24**	8.14	19.56**	244.50
	10	58.53	107.12	0.09**	56.25	18.23**	729.2	21.75**	57.86	0.60**	20.34	18.67**	233.38
	15	61.29**	112.17	0.03**	18.75	12.48**	499.2	91.03**	50.63	0.24**	8.14	19.56**	244.50
LSD at 5%		4.17		0.03		3.68		3.43		0.53		3.19	
LSD at 1%		6.31		0.04		5.57		5.19		0.78		4.82	

*: Significant differences as compared with the control, **: Highly significant differences as compared with the control, DM = Dry Mass

Table 4: Effect of glycerol + aspartic acid on oil, ricin alkaloids and endogenous glycerol of *R. communis* at various NaCl concentrations

Treatment		Shoots						Roots					
		Oil		Ricinine		Glycerol		Oil		Ricinine		Glycerol	
NaCl (mM)	Glycerol+ Aspartic acid (mM)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)	DM ($mg\ g^{-1}$)	Control (%)
0	0	54.64	100.00	0.16	100.00	2.50	100.0	37.59	100.00	2.95	100.00	8.00	100.00
	10+5	114.50**	209.55	0.18	112.50	5.78*	231.2	77.46**	206.07	1.87**	63.39	18.24**	228.00
	25+10	126.11**	230.80	0.17	106.25	4.89	195.6	71.04**	188.99	0.53**	17.97	27.57**	344.63
	50+15	113.26**	207.28	0.15	93.75	4.46	178.4	67.92**	180.69	0.40**	13.56	30.24**	378.00
50	0	87.72**	160.54	0.14	87.50	10.67**	426.8	41.62*	110.72	2.04**	69.15	20.46**	255.75
	10+5	139.13**	254.63	0.13	81.25	11.57**	462.8	40.04	106.52	1.87**	63.39	16.45**	205.75
	25+10	93.56**	171.23	0.12*	75.00	6.67**	266.8	32.90	87.52	1.93**	65.42	24.90**	311.25
	50+25	87.33**	159.83	0.11*	68.75	6.67**	266.8	33.71	89.68	2.13**	72.20	26.64**	333.00
100	0	55.40	101.39	0.14	87.50	8.00**	320.0	29.30**	77.95	0.16**	5.42	11.56	144.50
	10+5	65.46*	119.80	0.13	81.25	10.67**	426.8	20.13**	53.55	0.40**	13.56	15.80**	197.50
	25+10	68.50**	125.37	0.13	81.25	10.67**	426.8	16.96**	45.12	0.85**	28.81	22.23**	277.88
	50+15	73.86**	135.18	0.10	62.50	7.68**	307.2	15.80**	42.03	1.43**	48.47	23.03**	287.87
LSD at 5%		7.6		0.04		3.13		4.41		0.55		4.53	
LSD at 1%		11.5		0.06		5.01		6.66		0.83		5.47	

*: Significant differences as compared with the control, **: Highly significant differences as compared with the control, DM = Dry Mass

maximum increase in fresh-dry mass (DM) was observed when plants treated with a mixture of glycerol and aspartic acid.

The results revealed that glycerol, aspartic acid, or mixture applications leads to significant increase in oil contents, ricinine alkaloids and endogenous glycerol in the different organs of *R. communis* (Table 2-4). On the other side, the ricinine alkaloids in roots decreased. In plants originating from seeds soaked in distilled water and irrigated with saline solutions (50 and 100 mM NaCl) the oil contents and endogenous glycerol in shoots were significantly raised in comparison to those of control plant, but the ricinine alkaloids remained more or less unchanged. In case of roots the oil contents and ricinine alkaloids significantly decreased with increasing salinity accompanied by increase in endogenous glycerol content. The oil contents in shoots of plants originating from seeds soaked in various levels of glycerol, aspartic acid or mixture and then treated with salinity was raised significantly when the corresponding plants treated with either NaCl or non-salinized plant. However, the oil content and ricinine alkaloids in roots were significantly lowered when compared with those of plant treated with either NaCl or control plant. Endogenous glycerol contents in shoots and roots were significantly raised when compared with those of NaCl treated or nonsalinized plant.

DISCUSSION

Precursors involved in pyridine nucleotide cycle compounds (glycerol, aspartic acid or mixture of glycerol+aspartic acid) applied at different levels significantly elevated the fresh-dry mass (DM) per plant. These are in agreement with those, obtained by Garcia-Jiménez *et al.* (1998) and Marian *et al.* (2000). On the other hand, in plants originating from seeds soaked only in distilled water and irrigated with saline solutions (50 and 100 mM NaCl) the fresh-dry mass (DM) per plant was significantly lowered compared to that of nonsalinized plants. This reduction in fresh-dry mass (DM) in *R. communis* is agreement with the results obtained by other authors (Schurr *et al.*, 2000; Ali, 2000, 2002).

The soaking of seeds in various levels of glycerol, aspartic acid or mixture and irrigated with saline solution progressively alleviated the inhibitory effect of salinization on fresh-dry mass (DM) of *R. communis*. The results also indicated that glycerol, aspartic aid, mixture of glycerol and aspartic acid was capable of inducing a significantly increase in oil content, ricinine alkaloids and endogenous glycerol in shoots and roots in *R. communis*. However, in roots ricinine alkaloids decreased. The metabolic relationships of pyridine nucleotide cycling have been examined and found to be implicated in the biosynthesis of ricinine alkaloids by *R. communis* L. (Noctor *et al.*, 2006). Pyridine nucleotides are key players in signaling through Reactive Oxygen Species (ROS) since they are crucial in the regulation of both ROS-producing and ROS-consuming systems in plants (Mittler *et al.*, 2004; Foyer and Noctor, 2005). Since ROS is considered a symptom of salinity stress, resynthesis of consumed pyridine nucleotide maintain nucleotide pools (Noctor *et al.*, 2006).

In plants originating from seeds soaked in distilled water and irrigated with saline solution, the oil and endogenous glycerol in shoots were significantly raised in comparison to these of the control plants. However, the ricinine alkaloids decreased with increasing salinity. Such a promotion in oil and endogenous glycerol content of various salinized plants was also recorded by some other authors (Ali, 2002). In case of roots the oil content and ricinine alkaloids significantly lowered with increasing salinity accompanied by increase in endogenous glycerol content. It appears that NaCl in root accelerated the breakdown of oils to fatty acids and glycerol which plays an important role in osmotic adjustment (Marian *et al.*, 2000).

Seeds soaked in various levels of glycerol, aspartic acid or mixture and treated with salinity exerted a significant increase in the oil content of shoots when compared with those of the corresponding plants treated with either NaCl or non salinized plant. However, the contents of oil and ricinine alkaloids in roots decreased significantly. The present experiments suggest that there may be at least one mechanism by which NaCl inhibits growth of *R. communis*, probably through reducing one of more of the endogenous levels of glycerol or aspartic acid which in turn adversely affect various cellular processes. It has been hypothesized that if an increase in one or more of endogenous glycerol or aspartic acid, through soaking seeds may play specific protective role in plants adapted to extreme environment.

Finally, the adequate supply of glycerol or aspartic acid is essential for normal plant growth and development. This may explain why exogenous application of such compounds help *R. communis* overcomes the effect of salinity stress on growth. It can be concluded that soaking seeds of *R. communis* in glycerol or aspartic acid could be considered of great importance for plant growth, oil and ricinine alkaloids.

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