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Effect of Nicotinic Acid and Nicotinamide Adenine Dinucleotide on Growth and Content of Oil, Glycerol and Ricinine Alkaloids of Salinity stressed *Ricinus communis L.*

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Summary

ALI R. M. 2002. Effect of nicotinic acid and nicotinamide adenine dinucleotide on growth and content of oil, glycerol and ricinine alkaloids of salinity stressed *Ricinus communis L.* – Phyton (Horn, Austria) 42 (2): 269–277. – English with German summary.

The interactive effects of salinity and soaking of seeds of *Ricinus communis L.* in nicotinic acid or NAD on growth and secondary production were studied. Fresh and dry mass, oil, ricinine alkaloids and endogenous glycerol in the different organs of plant originating from seeds soaked in nicotinic acid or NAD were generally higher compared to the control. However, ricinine alkaloids in roots decreased. Salinity provoked a decrease in growth fresh and dry mass, oil contents, ricinine alkaloids in roots and its also dramatically enhanced oil contents and ricinine alkaloids in shoots. However, endogenous glycerol in shoots and root increased with increasing salinization level. Soaking seeds in nicotinic acid or NAD counteracted the adverse effects of salinity on growth and secondary products of *Ricinus communis*.

Zusammenfassung

ALI R. M. 2002. Die Wirkung von Nikotinsäure und Nikotinamid-Adenin-Dinucleotid auf das Wachstum und den Gehalt an Öl, Glycerin und Ricinalkaloide bei salzgestresstem *Ricinus communis L.* – Phyton (Horn, Austria) 42 (2): 269–277. – Englisch mit deutscher Zusammenfassung.

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Die wechselseitigen Auswirkungen von Salinität und dem Anquellen in Nikotinsäure oder NAD auf Wachstum und Sekundärproduktion wurden bei Ricinussamen untersucht. Frisch- und Trockenmasse, Ölgehalt, Ricinalkaloide und Glycerin waren in den Organen von Pflanzen, welche von Samen stammten, die in Nikotinsäure bzw. NAD ausgequollen wurden, allgemein höher als die der Kontrollen. Der Ricingehalt in den Wurzeln sank jedoch ab. Salzeinfluss führte zu einer Abnahme im Wachstum, Frisch- und Trockengewicht, Ölgehalt und Ricinalkaloide der Wurzeln, erhöht jedoch stark den Ölgehalt und Ricinalkaloide im Spross. Im Spross und in den Wurzeln steigt jedoch der Glyceringehalt mit höherer Salinität an. Das Anquellen von Samen in Nikotinsäure oder NAD wirkte den nachteiligen Auswirkungen der Salinität auf Wachstum und Sekundärprodukte von *Ricinus communis* entgegen.

Introduction

Castor bean (*Ricinus communis L.*) is cultivated for its economic and medicinal importance. The natural products obtained from this species include, oils, ricinine alkaloids and various phenolics (RISK 1986). The effects of salinity on the oil and glycerol content of castor bean were studies by AHMED & al. 1979, HEIKAL & al. 1980 and YOUNIS & al. 1987. Glycerol as a compatible solute plays an important role in osmoregulation in algae (HELLEBUST 1976).

The metabolic relationships of nicotinic acid and NAD have been examined previously and these compounds have been implicated in the biosynthesis of ricinine by *Ricinus communis* (WALLER & al. 1966, HILES & BYERRUM 1969).

Furthermore, exogenous application of nicotinic acid was successful in ameliorating the adverse of NaCl on growth of some plants (ZIDAN 1991, SHADDAD & HEIKAL 1982).

No work could be obtained on the effect of exogenously applied nicotinic acid NAD on growth and secondary products of *Ricinus communis* under saline conditions.

Therefore, the present work was undertaken to study the effect of nicotinic acid and NAD, obligatory members of the pyridine nucleotide cycle on growth, ricinine alkaloids, oil and endogenous glycerol in *Ricinus communis* under the influence of NaCl.

Material and Methods

Seeds of *Ricinus communis* were soaked for 48 hours in different concentrations of nicotinic acid (5, 10 and 15 mM) and NAD (0.5, 1.5 and 3 mM), which was 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 100mM 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 colorimetrically by the method of MARSH & WEINSTEIN 1966.

Determination of alkaloid

Ricinine alkaloids were extracted according to LEE & WALLER 1972. Alkaloids were extracted by homogenization of 5 g tissue with 200 ml chloroform. The residue was further extracted with methanol-water (3 /1) by volume 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 taken to dryness under reduced pressure. The residue was then extracted with hot methanol. The collected methanol extract was concentrated and used for colorimetrically estimation of the alkaloids.

Determination of glycerol

Endogenous glycerol was measured by method of MANSOUR 1972 and modified by YOUNIS & al. 1987. Extraction of glycerol was carried out by boiling a known weight of sample in water grinding in a mortar and then centrifuging the mash. The supernatant was filtered, made up to volume and used for the estimation of glycerol. To 10 cm^3 of water extract, in a boiling tube, 5 cm^3 of 7.5 percent potassium dichromate followed by 30 cm^3 sulphuric acid (50% v/v) were added. The contents were mixed, boiled for 15 min and then cooled. The mixture was then made up to 50 cm^3 and intensity of the colour developed was measured at 600 nm.

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

Experimental Results

The data presented in Table (1) reveal that fresh-dry mass of *Ricinus communis* plants originating from seeds soaked in nicotinic acid or NAD were significantly raised with increasing 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 per plant were lower than that of nonsalinized plants. Seeds soaked in various levels of nicotinic acid or NAD and irrigated with saline solution progressively alleviated the inhibitory effect of salinization on fresh-dry mass of *Ricinus communis*.

The results indicated that nicotinic acid or NAD application led to a highly significant increase in oil contents, ricinine alkaloids and endogenous glycerol in the different organs of *Ricinus communis* (Table 2, 3). On the other hand, 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

Table 1.
Effect of NaCl and nicotinic acid or NAD on fresh and dry mass (g/plant) of *Ricinus communis*.

Treatments		Fresh mass		Dry mass		Treatments		Fresh mass		Dry mass	
NaCl (mM)	Nicotinic Acid (mM)	(g/plant)	% control	(g/plant)	% control	NaCl (mM)	NAD (mM)	(g/plant)	%control	(g/plant)	% control
0.0	0.0	6.56	100	1.32	100	0.0	0.0	6.56	100	1.32	100
	5	8.70**	132.6	1.67**	126.5		0.5	6.87	104.7	1.59**	120.5
	10	10.15**	154.7	2.05**	155.3		1.5	7.43**	113.3	1.64**	124.2
	15	8.48**	129.3	1.71**	129.6		3	7.66**	116.8	1.81**	137.1
50	0.0	5.64*	86	1.24	94	50	0.0	5.64**	86	1.24	94
	5	6.41	97.7	1.34	102.5		0.5	7.00*	106.7	1.61**	122
	10	6.84	104.3	1.49	112.9		1.5	7.07*	107.8	1.61**	122
	15	7.21**	109.9	1.64**	124.3		3	7.05*	107.5	1.65**	125
100	0.0	3.83**	58.4	0.78**	59.1	100	0.0	3.83**	58.4	0.78**	59.1
	5	4.09**	62.5	1.03**	78.1		0.5	6.65	101.32	1.53*	116
	10	4.16**	63.4	1.06**	80.3		1.5	6.70	102.1	1.49*	112.9
	15	6.59	100.5	1.41	106.8		3	6.36	97	1.38	104.6
L.S.D.at 5%		0.74		0.25		L.S.D.at5%		0.37		0.17	
L.S.D.at 1%		1.11		0.37		L.S.D.at1%		0.56		0.26	

* Significant differences as compared with the control.
** Highly significant differences as compared with the control.

Table 2.
Effect of NaCl and nicotinic acid on oil,ricinine alkaloids and endogenous glycerol of *Ricinus communis*.

Treatments		Shoots						Roots					
NaCl	Nicotinic Acid	Oil		Ricinine		Glycerol		Oil		Ricinine		Glycerol	
(mM)	(mM)	$mg.g^{-1}$ DM	% Control	$mg.g^{-1}$ DM	% Control	$mg.g^{-1}$ DM	% Control	$mg.g^{-1}$ DM	% Control	$mg.g^{-1}$ DM	% Control	$mg.g^{-1}$ DM	% Control
	0.0	54.64	100	0.16	100	2.50	100	37.95	100	2.95	100	8.00	100
0.0	5	56.24	102.9	0.25**	156.3	7.56**	302.4	87.47**	232.7	2.03**	68.8	18.90**	263.3
	10	57.75**	105.7	0.21	131.3	11.12**	444.8	72.41**	192.6	2.10**	71.2	26.68**	333.5
	15	74.75**	136.8	0.21	50	3.56	142.4	70.63**	187.9	2.00**	67.8	31.57**	394.6
50	0.0	87.72**	160.5	0.08*	31.3	10.67**	426.8	41.62	110.7	2.00**	67.8	20.46**	255.7
	5	98.02**	179.4	0.05**	25	10.88**	435.2	40.43	107.6	1.08**	36.6	18.67**	233.4
	10	121.17**	221.2	0.04**	25	11.56**	462.4	42.73*	113.7	0.83**	28.1	16.01**	200.1
	15	99.78**	182.6	0.04**	37.5	11.11**	444.4	45.22*	120.3	0.69**	23.4	17.68**	221
100	0.0	55.40	101.4	0.06**	56.3	8.00**	320	29.30*	78	0.16**	5.4	11.56*	144.5
	5	56.08	102.6	0.09*	50	9.78**	391.2	28.20**	75	1.05**	35.6	22.23**	277.9
	10	59.92**	109.1	0.08*	31.3	9.78**	391.2	27.85**	74.1	1.95**	66.1	18.67**	233.4
	15	74.52**	136.4	0.05**		8.00**	320	26.55**	70.6	0.81**	27.5	9 **	112.5
L.S.D.at 5%		2.94		0.06		1.23		5.39		0.07		2.45	
L.S.D.at 1%		4.45		0.09		1.86		8.10		0.11		3.71	

* Significant differences as compared with the control.
** Highly significant differences as compared with the control.

Table 3.
Effect of NaCl and NAD on oil ,ricinine alkaloid and endogenous glycerol of *Ricinus communis*.

Treatments		Shoots						Roots					
NaCl (mM)	NAD (mM)	Oil		Ricinine		Glycerol		Oil		Ricinine		Glycerol	
		$mg.g^{-1}$ DM	% Control	$mg.g^{-1}$ DM	% Control	$mg.g^{-1}$ DM	% Control	$mg.g^{-1}$ DM	% Control	$mg.g^{-1}$ DM	% Control	$mg.g^{-1}$ DM	% Control
	0.0	54.64	100	0.16	100	2.50	100	37.95	100	2.95	100	8.00	100
0.0	0.5	94.50**	173	0.18	112.5	10.23**	409.2	63.19**	168.1	1.86**	63.1	21.57**	269.6
	1.5	90.27**	165.2	0.15	93.8	7.56**	302.4	81.60**	217.1	2.41**	81.7	28.57**	357.13
	3	89.64**	164.1	0.18	100	6.67**	266.8	87.66**	227.2	2.33**	79	23.57**	294.6
50	0.0	87.72**	160.5	0.08**	50	10.67**	426.8	41.62	110.7	2.05**	69.5	20.46**	255.8
	0.5	91.91**	168.2	0.12*	75	7.11**	284.4	49.98**	133	1.16**	39.3	21.34**	266.8
	1.5	81.38**	148.9	0.13	81.3	8.00**	320	46.65**	124.1	1.57**	53.2	22.23**	277.9
	3	80.90**	148.1	0.17	106.3	6.67**	266.8	44.05**	117.2	1.40**	47.5	24.23**	302.9
100	0.0	55.40	101.4	0.06**	37.5	8.00**	320	29.30**	78	0.16**	5.4	11.56*	144.5
	0.5	59.82	109.5	0.08**	50	12.00**	480	35.97	95.7	0.27**	9.2	16.89**	211.1
	1.5	65.53**	119.9	0.08**	50	11.11**	444.4	20.13**	53.6	0.39**	13.2	17.78**	222.3
	3	63.30**	115.9	0.05**	31.3	11.11**	444.4	19.32**	51.4	0.43	14.6	17.78**	222.3
L.S.D.at 5%		5.39**		0.04		1.96		4.90		0.10		2.70	
L.S.D.at 1%		8.16		0.05		2.97		7.42		0.14		4.45	

* Significant differences as compared with the control.
** Highly significant differences as compared with the control.

shoots were significantly raised in comparison to those of control plant, but the ricinine alkaloids decreased with increasing salinity. 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 nicotinic acid or NAD and then treated with salinity was raised significantly when compared with the corresponding plants treated with either NaCl or nonsalinized plant. However, the oil content and ricinine alkaloids in roots were significantly lowered when compared with those of plants 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 non-salinized plant.

Discussion

Nicotinic acid or NAD applied at different levels significantly elevated the fresh-dry mass per plant. These findings are in agreement with those obtained by SHADDAD & HEIKAL 1982 and ZIDAN 1991. Also, YAMAMOTO 1963 and HAGEDORN & PHANG 1986 observed that the endogenous level of NADP is a rate-limiting factor and plays a key role in the control of metabolism in plants. 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 per plant was significantly lowered compared to that of nonsalinized plants. This reduction in fresh-dry mass in *Ricinus communis* is in agreement with the results obtained by some other authors (AHMED & al. 1979, HEIKAL & al. 1980, JESCHKE & al. 1997 and SCHURR & al. 2000, ALI 2000).

Soaking of seeds in various levels of nicotinic acid or NAD and irrigated with saline solution progressively alleviated the inhibitory effect of salinization on fresh-dry mass of *Ricinus communis*. ZIDAN 1991 observed that when salt stressed wheat plants were sprayed with nicotinic acid, the growth rate was comparatively raised. Also, SHADDAD & HEIKAL 1982 reported that exogenous application of nicotinic acid was successful in ameliorating the adverse effect of NaCl on growth of pea, sesame and wheat plants. ALI 1994 reported that in *Pisum sativum* plants treated with tryptophane, the endogenous nicotinic acid increased in shoots or roots, which counteracted the adverse effect of NaCl.

The results in this work indicate that nicotinic acid or NAD is capable of inducing a significant increase in oil content, ricinine alkaloids and endogenous glycerol in shoots and roots in *Ricinus communis*. However, in roots ricinine alkaloids decreased. The metabolic relationships of nicotinic acid and NAD have been examined and found to be implicated in the biosynthesis of ricinine alkaloids by *Ricinus communis* L. (WALLER & al. 1966, HILES & BYERRUM 1969). Also, NAD was incorporated into ricinine as results of having been first hydrolyzed to nicotinamide and nicotinic acid, both of which can be incorporated into ricinine alkaloids (HILES & BYERRUM 1969).

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 salinised plants was also recorded by other authors (AHMED & al. 1979, HEIKAL & al. 1980, YOUNIS & al. 1987). 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 (YOUNIS & al. 1987). Glycerol as a compatible solute in *Ricinus communis* plays an important role in osmotic adjustment (BEN-AMOTZ & AVRON 1973, HELLEBUST 1976). Also CUSIDO & al. 1987 mentioned that plants often react to stress by leading intermediary compounds to secondary metabolism. This author reported that treating *Nicotiana restica* L. plants with NaCl decreased the levels of nicotinic acid in leaves and increased these levels in roots.

Seeds soaked in various levels of nicotinic acid or NAD 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 nonsalinized 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 *Ricinus communis*, probably through reducing in nicotinic acid and or NAD, which in turn adversely affects various cellular processes. It has been hypothesized that if an increase in nicotinic acid or NAD through soaking seeds may play a specific protective role in plants adapted to extreme environment (SHADDAD & HEIKAL 1982, ZIDAN 1991, ALI 1994).

Finally an adequate supply of nicotinic acid or NAD is essential for normal plant growth and development. This may explain why exogenous application of such compounds helps the plant *Ricinus communis* overcome the effect of salinity stress on growth. From the above results, soaking seeds of *Ricinus communis* in nicotinic acid or NAD could be considered of great importance for plant growth, oil and ricinine alkaloids.

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