

Amelioration of Salinity-Induced Metabolic Changes in Soybean by Weed Exudates

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ABSTRACT

Root exudates of three weed plants, jungle rice, cocklebur and purslane, were used as foliar spray into NaCl-stressed soybean seedlings to test their possible ameliorative effects on NaCl-induced injury. Exudates of jungle rice roots exhibited highest level of kinetin and gibberellic acid and lowest concentration of indole acetic acid as well as abscisic acid. Cocklebur root exudates contained highest level of IAA or ABA while purslane one showed lowest amount of GA₃ or Kinetin. NaCl imposition decreased contents of protein, oil, K, Mg, P, and NO₃, whereas increased contents of free proline, alkaloids, Na and Cl. The activity of nitrate reductase was reduced while that of protease was stimulated. Translocation rate of carbon assimilates was also inhibited by salt exposure. The highest contents of protein, oil, nitrate reductase activity, translocation efficiency (¹⁴CO₂-assimilates), NO₃, P, K, and Mg ions and the lowest contents of free proline, total alkaloids, protease activity, Na, and Cl ions in salinized soybean were found as a result of application of root exudates of jungle rice weed plant. This may be due to its highest inclusion of growth regulators that mitigate NaCl deleterious effects. Other possible mechanisms that explain alleviation effects of weed root exudates against NaCl stress are discussed.

Key Words: Root exudates; Kinetin; ¹⁴CO₂; Gibberellic acid; Salinity

INTRODUCTION

Soybean (*Glycine max* L. Merr.) is one of great important leguminous plant. It is also considered as a good source of vegetable protein and oil since it has the highest level of protein in comparison with the other leguminous plants. In Egypt, it is not feasible to expand the area for such valuable crop due to high competition since cotton, rice and maize. Therefore, it is necessary to investigate certain abiotic factors that may limit the soybean yield, for example, drought and salinity. Eisa and Hanna (1997) and Frederick *et al.* (2001) showed that drought stress reduced growth pattern in soybean. Marques (1982) reported that soybean plants are most susceptible to drought stress during the reproductive period particularly pod formation and filling.

NaCl stress negatively influence the protein content and proline oxidase activity while increased protease capacity (Muthukumarasamy *et al.*, 2000). Saker and EL-Ashal (1995) found that salinity stress increased the content of alkaloids. NaCl treatment increased Na⁺ and Cl⁻ level whereas decreased K⁺ and Mg²⁺ level (AL-Wakeel *et al.*, 1995) in fenugreek plants. The present work was undertaken to investigate whether the injurious effects induced by salinity could be mitigated using exudates of certain weed roots as natural tools.

MATERIALS AND METHODS

Plant material. Uniform seeds of soybean (*Glycine max* L.

Merr.) cv. Giza 35 were obtained from the Crop Institute, Agricultural Research Center, Giza, Egypt.

The seeds were surface sterilized using 1.0 g L⁻¹ HgCl₂ for 15 minute and rinsed thoroughly with distilled water. The seeds were then germinated in Petri dishes in the dark at 24°C. The pregerminated seeds were transferred into 25 cm diameter black polyethylene pots containing sand culture. The water holding capacity was 12-15% and the pH of the soil extract was about 7.0. Five seedlings were planted in each pot. The pots were irrigated with 1/4-strength Hoagland's nutrient solution (Hoagland & Arnon, 1950). A 250 mL of the nutrient solution were supplied to each pot twice daily. After 35 days of seedling emergence salt stress treatments were imposed. The seedlings were treated with full Hoagland's solution containing NaCl levels of 1.2, 5.7, 10.3 and 19.4 dS m⁻¹ which are equivalent to 0.0, 50, 100 and 200 mM, respectively. The plants were subjected to these salt levels for 90 d. The pots were rinsed with water once a week to avoid salt accumulation.

Collecting the root exudates. Sixty uniform plants of the three weeds; jungle rice (*Echinochloa colonum* L.), cocklebur (*Xanthium* sp.) and purslane (*Portulaca oleraceae* L.) were gently pulled with their intact root system in the vegetative stage from the soil of field practices in the Horticultural Research Center, Giza. The plants of each weed were washed carefully with distilled water several times. The weeds were kept inside clean flasks full of 1 L distilled water for 3 days at 22-23°C and light intensity of 1500 Lux. The resulting solution in each flask was filtered and evaporated to 200 mL under vacuum at 25°C. Then, the

insoluble materials were removed by centrifugation at 2000 rpm for 30 min according to the methods of El-Habasha and Behairy (1977). The exudates of the three weeds were collected and divided into two groups. The first (20 mL) was used for determination of growth regulators using HPLC following the procedure of Shindy and Orrin (1975). The second (180 mL) was added to Tween 20 to fix solutions to the plant leaf surface. The solutions were sprayed once into the leaves in the morning at the flowering stage after 45 days using a manual pump. All determinations were carried out at the harvest stage (90-day-old). All treatments were replicated 3 times.

The translocation efficiency ($^{14}\text{CO}_2$ -assimilates). It was assayed according to (Moussa, 2001). A pot from each treatment was placed under Bell-jars, which were used as a photosynthetic chamber. Radioactive $^{14}\text{CO}_2$ was generated inside the chamber by a reaction between 10% HCl and 50 $\mu\text{C NaH}^{14}\text{CO}_3 + 100 \text{ mg Na}_2\text{CO}_3$ as carrier. Then the illumination was afforded by tungsten lamp. After 30 min., which was considered as zero time, the plants were removed from the chamber and left for 2 and 4 h in the normal air to assimilate CO_2 . After the assigned period had elapsed the leaves were quickly detached from the stem, weighed and frozen for 5 min. to stop the biochemical reactions. Then the leaves were subjected to extractions by 80% hot ethanol. The ethanolic extracts were taken for ^{14}C assay in soluble compounds using Bray Cocktail by means of Liquid Scintillation Counter (LSC2-Scaler Ratemeter SR7, Nuclear Enterprises).

Protein, proline, oil and alkaloids contents. Protein content was quantified according to (Bradford, 1976). Following the method of A.O.A.C. (1975) percentage of seed crude oil level was estimated. Extraction and determination of alkaloids were carried out following the method of Koul *et al.* (1982). The procedure of Bates *et al.* (1973) was applied to estimate free proline concentration.

Nitrate reductase and protease activity. Nitrate reductase activity was assayed according to the method of Ferrario *et al.* (1998). Protease enzyme activity was measured as described by Gallop *et al.* (1957).

Elemental analysis. According to the procedure of Cataldo *et al.* (1975), the content of NO_3 was calculated. Chloride concentration was determined as described by Lutts *et al.* (1996). The method of Prokopy (1995) was applied for phosphorus estimation. Potassium, sodium and magnesium levels were determined using an AAS-1-N Atomic Absorption Spectrophotometer (Zeiss, Jena, Germany).

RESULTS AND DISCUSSION

Root exudates of the three examined weeds showed various levels of growth regulating substances (Fig. 1). In this respect, roots of jungle rice exuded the highest amount of cytokinins (kin), and gibberellic acid (GA_3) and low levels of auxins (IAA) and abscisic acid (ABA), while exudates of cocklebur roots had the highest levels of auxins

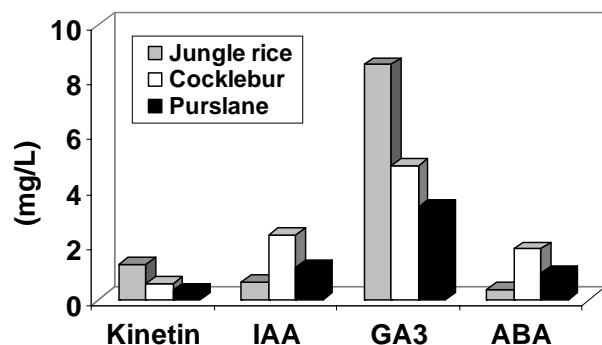
(IAA), ABA and moderate level of cytokinins (kin). Purslane roots exuded the lowest amount of GA_3 and kin and moderate levels of IAA or ABA. These results are consistent with those reported by Arshad and Frankenberger (1991), Behairy *et al.* (1998) and Hamed (2000).

Protein and oil content. Soybean plants exposed to 100 and 200 mM NaCl showed significant decrease in their protein content by 20.3% and 41.7% below those of control samples (Table I). Muthukumarasamy *et al.* (2000) reported remarkable decrease in the protein content of salt stressed radish plants. The interactive effect of NaCl (100 & 200 mM) and foliar spray of extracts of (a) jungle rice, (b) cocklebur and (c) purslane into soybean plants, induced marked increases in protein accumulation by: 14.9 and 21.1% for (a), 10.6 and 8.98% for (b) and 2.6 and 14.45% for (c), respectively, as compared with samples received only salt treatments (Table I). These findings were in agreement with those indicated by Datta *et al.* (1997) who reported that damage to the apparatus of protein synthesis by salinity was prevented by kinetin, which led to better plant growth. Stefanov *et al.* (1998) also indicated that application of GA_3 and phenylurea increased protein and total nitrogen contents of maize seedlings. Soybean plants treated with 100 or 200 mM NaCl exhibited significant decreases in their oil contents by 45.5 or 81.3%, respectively, when compared with that of untreated samples (Table I). This inhibition in the oil content is similar to that reported by Nouredin *et al.* (2002) who found a reduction in the oil yield of soybean plants under salt stress. Foliar spraying of weed extracts: jungle rice (a), cocklebur (b) and purslane (c) in presence of NaCl 100 and 200 mM substantially increased the oil content by 60.2 and 156.8%

Table I. Effect of foliar applications of weed extracts on alkaloids (mg g⁻¹DW), free proline ($\mu\text{mol g}^{-1}\text{FW}$), protein and oil contents of stressed soybean plant after 90 days

Weed extract	NaCl (mM)	Free proline	Total alkaloids	Seed % Protein	Oil
0.0	0.0	198.5	18.5	43.9	23.5
	50	230.1	23.4	40.2	19.1
	100	258.4**	30.9**	35.0**	12.8**
	200	295.6**	41.6**	25.6**	4.4**
Jungle rice	0.0	186.8	17.1	51.6	30.1
	50	205.4	16.8	47.4	27.4
	100	235.6**	24.6**	40.2**	20.5**
	200	267.5**	31.5**	31.0**	11.3**
Cocklebur	0.0	190.1	17.5	48.8	31.0
	50	210.3	17.0	47.5	21.4
	100	227.4**	21.4**	38.7*	14.5 ^{NS}
	200	270.2**	25.8**	27.9*	7.0*
Purslane	0.0	180.6	17.7	45.0	27.8
	50	221.4	14.8	42.1	22.6
	100	239.2**	10.5**	35.9 ^{NS}	16.4*
	200	261.3**	11.3**	29.3*	8.6**

*Significant; **Highly significant; NS: Non significant. Each value is the mean of three replicates

Fig. 1. The content of growth regulator in the three weed extracts

for (a) 13.3 and 59.1% for (b) and 28.13 and 95.45% for (c), respectively, above those of salinized plants (Table I). The increase in oil content may be due to a reduction in lipase activity induced by a hormone presents in the extracts (Sodek & Wright, 1983).

Proline and alkaloid content. Stress imposed by 100 and 200 mM NaCl increased the free proline content of soybean plants by 30.18 and 48.92%, respectively, in comparison with control samples (Table I). The increase in proline content could be attributed to a decrease in proline oxidase activity in saline condition as indicated by Muthukumarasamy *et al.* (2000) and Rajesh *et al.* (1999). Proline concentration markedly decreased following treatment soybean seedlings with 100 and 200 mM NaCl and extracts of (a) jungle rice (b) cocklebur and (c) purslane by 8.8 and 9.51% for (a), 11.99 and 8.59% for (b) and 7.4

Table II. Effect of foliar applications of weed extracts on enzyme activities of stressed soybean plant after 90 days

Weed extract	NaCl (mM)	Nitrate reductase (U g ⁻¹ FW)	Protease (U g ⁻¹ FW)
0.0	0.0	170	248
	50	156	269
	100	128	307
	200	101	341
Jungle rice	0.0	178	245
	50	170	230
	100	158	261
	200	132	215
Cocklebur	0.0	181	243
	50	172	239
	100	135	258
	200	116	219
Purslane	0.0	168	246
	50	159	221
	100	140	243
	200	108	226
LSD	1%	11.6	13.5
	5%	7.1	8.4

Table III. Effect of foliar applications of weed extracts on ion contents (mg g⁻¹DW) of stressed soybean plant after 90 days

Weed extract	NaCl (mM)	Na	Cl	NO ₃	P	K	Mg
0.0	0.0	53.5	61.4	105.6	63.2	96.8	27.4
	50	63.9	78.9	90.2	50.1	80.2	20.3
	100	79.4	92.4	77.5	43.0	65.4	13.2
	200	96.8	123.6	51.4	30.2	41.6	5.0
Jungle rice	0.0	50.0	62.4	123.5	78.9	118.6	40.2
	50	51.6	63.6	115.1	70.1	105.1	31.5
	100	60.2	50.2	98.3	62.5	90.2	25.3
	200	43.8	38.1	72.4	46.4	81.1	15.7
Cocklebur	0.0	52.1	58.9	125.6	83.4	123.6	38.5
	50	49.2	60.4	110.3	74.6	101.2	32.7
	100	47.8	51.7	102.1	61.8	97.4	21.8
	200	46.3	41.2	70.1	49.2	75.3	13.2
Purslane	0.0	51.4	61.0	119.4	75.1	120.0	35.0
	50	50.0	65.1	106.5	68.3	90.3	25.8
	100	58.2	57.3	95.1	57.1	78.6	17.4
	200	47.9	39.2	67.4	40.2	67.1	10.1
LSD	1%	3.8	2.9	8.1	4.5	10.5	5.8
	5%	2.4	1.7	5.0	2.7	6.7	3.7

Each value is the mean of three replicates

and 11.6% for (c) as compared with plants received salt alone (Table I). This decrease in proline could be accounted for decreasing ABA level in salinized plants, which sprayed with growth regulators as reported by Hathout (1996). ABA has been shown to increase proline content in salt stressed plants (Younis *et al.*, 1994). NaCl stress caused significant increases in the alkaloid content of soybean plants when compared with control samples (Table I). Spraying the salinized soybean plants with the tested weed extracts significantly decreased their alkaloid contents compared with only NaCl-treated plants (Table I). Salinized *Hyoscyamus* plants exhibited high level of alkaloid fraction, which may be induced by K deficiency and proline accumulation in saline environment Saker and El-Ashal (1995).

Nitrate reductase and protease activity. NaCl (100 & 200 mM) treated-soybean samples exhibited significant reduction in the activity of nitrate reductase (NR) enzyme by 24.71 and 40.59% and increase in protease capacity by 23.79 and 37.5% in comparison with control plants (Table II). Abd El-Baki *et al.* (2000) reported that application of salinity decreased NR activity in maize. Application of weed extracts to soybean plants increased their NR activity relative to salinized controls (Table II), which could be explained by effect of kinetin and GA₃ of weed extracts. Kinetin and GA₃ have been reported to increase NR activity in maize leaves (Sinha *et al.*, 1994). Taiz and Zeiger (1998) indicated that cytokinin regulates NR by controlling transcription of NR gene. Consistent with our results is that NaCl increased protease activity in radish plants (Muthukumarasamy *et al.*, 2000) and in *Ceriops roxburghiana* plants (Rajesh *et al.*, 1999). Protease activity decreased in presence of NaCl and foliar spray of soybean

Table IV. Effect of foliar application of weed extracts on translocation rate (TR) of stressed soybean plant after 90 days

Weed extracts	NaCl (mM)	Zero time (30 min)	Translocation rate (2 h)		Translocation rate (4 h)	
		Specific activity (*dpm mg ⁻¹ FW)	Specific activity (dpm mg ⁻¹ FW)	TR (%)	Specific activity (dpm mg ⁻¹ FW)	TR (%)
0.0	0.0	53887	38799	28	24249	55
	50	50146	37108	26	25073	50
	100	45602	35569	22	25993	43
	200	38114	32396	15	25917	32
Jungle Rice	0.0	55813	35162	37	16743	70
	50	54066	35142	35	20004	63
	100	51841	35770	31	23328	55
	200	45422	34066	25	25436	44
Cocklebur	0.0	52988	34442	35	15366	71
	50	48342	32872	32	19336	60
	100	42825	30834	28	20556	52
	200	33614	25882	23	20168	40
Purslane	0.0	54128	35724	34	17320	68
	50	52617	35253	33	20521	61
	100	48101	35594	26	25493	47
	200	40398	30702	24	25855	36

FW = fresh weight; *Disintegration per minute

plants with weed extracts (Table II). Growth regulators have been reported to reduce protease activity in cotton and wheat (Renu & Goswami, 1995; Hegazi *et al.*, 1995).

Elemental analysis. Salinity treatment (100 and 200 mM NaCl) significantly declined the content of NO₃, P, K and Mg of soybean plants while increased the content of Na and Cl as compared with control plants (Table III). Other reports indicate that salinity stress increased Na and Cl whereas decreased K, Mg and P in different plant species (Al-Wakeel *et al.*, 1995; Gadallah, 1996; Briendra & Bijandra, 1996). The contents of NO₃, P, K and Mg of soybean plants treated with 100 and 200 mM NaCl and sprayed with weed extracts increased the values of these ions than those of salinized plants, meanwhile lower contents of Na and Cl as compared with those receive NaCl alone (Table III).

Salinity adversely affected the NO₃ content in soybean plants concomitantly with reduction in NR activity. These results could be interpreted on the basis that decreasing NO₃ content inhibits the biosynthesis of NR enzyme. Moreover, it has been demonstrated that nitrate reductase is substrate inducible enzyme and thus its level should be an index of reduced nitrogen available for protein synthesis in plants (Heldt, 1997). These effects of weed extracts through their inclusion of growth regulators are consistent with the data of Niharika *et al.* (1998), Irving *et al.* (1992), Hegazy and Abdel Wahab (1966) who indicated an increase in K, P as a result of kinetin and GA₃ treatment. Excess Na might cause problems with membranes, enzyme inhibition, or general metabolic dysfunction (Hopkins, 1995). Photosynthesis, on the other hand, is inhibited when high concentrations of Na and/or Cl accumulate in chloroplasts (Taiz & Zeiger, 1998).

Exogenous application of GA increased the growth and yield of salt-stressed plants. GA reduced the net accumulation of Na and Cl and maintained a high level of K in salinized (Prakash & Prathapasanen, 1990). The previous data are agreement with our results presented in Table III.

Salt stressed soybean plant showed inhibitory influence on the translocation rate of labeled soluble compounds by 26, 22 and 15% after two hours and by 50, 43 and 32% after four hours in response to 50, 100 and 200 mM NaCl, respectively as compared with control plants (Table IV). Application of weed extract: (a) jungle rice, (b) Cocklebur and (c) purslane to NaCl (50, 100 and 200 mM) treated soybean plants, considerably increased their translocation rate by 35, 31 and 25% for (a), 32, 28 and 23% for (b) and 33, 26 and 24% for (c), after two hours, respectively, as compared with salinized plants (Table IV). Also, that application of weed extract: (a) jungle rice, (b) Cocklebur and (c) purslane and NaCl (50, 100 and 200 mM) to soybean plants, considerably increased their translocation rate by 63, 55 and 44% for (a), 60, 52 and 40% for (b) and 61, 47 and 36% for (c), after four hours, respectively, relative to salinized plants. It seems that weed extracts application might exert some sort of initiating steps to favour the translocation presumably through increasing the contents of either K or P (Mengel & Viro, 1974; Yamada *et al.*, 1997).

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