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Sodium Chloride Effects on the Sugar Metabolism of Several Plants

By

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With 2 tables

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Summary

Two salt tolerant plants, *Atriplex nummularia* L. and *Zygophyllum album* L., and a sensitive one, *Capsicum frutescens* L. were grown in sand culture irrigated with nutrient solutions of different NaCl concentrations. Monthly samples of shoots were dried and the free and bound sugars were estimated. Glucose, fructose, sucrose, and cellobiose were the free sugars commonly present in the three species. In addition, *Z. album* and *C. frutescens* contained free ribose and maltose, while *A. nummularia* contained free raffinose. *A. nummularia* had the least level for each free sugar compared with the other two species. The level of free ribose decreased significantly in *C. frutescens* with 0.1 M NaCl, but that of sucrose showed a significant increase in *Z. album* with 0.15 M NaCl. The bound sugars detected in each species were xylose, arabinose, rhamnose, glucose, and galactose. *Z. album* was characterized by higher xylose levels than the other two species. The level of bound-xylose, arabinose, and glucose increased significantly in *Z. album* with 0.15 M, while that of rhamnose increased with higher significance in *C. frutescens* by 0.1 M NaCl. *A. nummularia* had the least total free and bound sugars which showed significant increase in *Z. album* with 0.15 M NaCl.

Zusammenfassung

Sandkulturen von *Atriplex nummularia* L. und *Zygophyllum album* L. (salztolerant) sowie *Capsicum frutescens* L. (salzempfindlich) wurden mit Nährlösungen verschiedenen NaCl-Gehaltes versorgt und der Gehalt an

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freien und gebundenen Zuckern in monatlichen Abständen bestimmt. Glucose, Fructose, Saccharose und Cellobiose wurden in allen drei Arten frei gefunden; *Z. album* und *C. frutescens* enthielten zusätzlich freie Ribose und Maltose, *A. nummularia* freie Raffinose. Unter Salzeinfluß nahm bei *C. frutescens* der Gehalt an freier Ribose ab (0,1 M NaCl), in *Z. album* stieg der Saccharosegehalt mit 0,15 M NaCl an. An gebundenen Zuckern wurden Xylose, Arabinose, Rhamnose, Glucose und Galactose in allen drei Arten gefunden. 0,10 M NaCl erhöhte den Rhamnosegehalt von *C. frutescens*, in *Z. album* nahmen durch 0,15 M NaCl sowohl die freien wie die gebundenen Zucker zu. Die genannten Unterschiede sind auf dem 95% bzw. 99%-Niveau gesichert, im übrigen traten keine signifikanten Unterschiede auf. Die Ergebnisse werden im Hinblick auf die Salztoleranz und die Synthese von Hemicellulose kurz diskutiert. (Editor)

Introduction

There are important differences among species in the effect of salt stress on sugar metabolism. AKOPIAN (1957), and AZIZBEKOVA and BOBAEVA (1962) reported a decrease in monosaccharide with an increase in disaccharide concentration of leaves by salinity. ULRICH and OHKI (1956) showed that chlorine is necessary for top and root growth of sugar-beets and stimulating sugar formation rather than sugar utilization. TULLIN (1954) indicated that chloride accumulation in sugar beets, due to salinity, induced the formation of galactan, one of the constituents of the cell-wall hemicellulose polysaccharides. Increased synthesis of hemicellulose in the leaves was proposed by ALEKSEEVA (1964) as an adaptive reaction for tolerance to dryness and high temperature, and GAFF and CARR (1961) suggested that the more hydrated and thicker cell-wall the greater would be the buffering effect against dryness.

The aim of the present study is to relate differences in free and bound sugars between *Atriplex nummularia*, *Zygophyllum album*, and *Capsicum frutescens* shoots with tolerance to NaCl stress.

Materials and Methods

Atriplex nummularia L., *Zygophyllum album* L., and *Capsicum frutescens* L. plants, having different degrees of salt tolerance were grown from seeds in sand culture in the glasshouse. *C. frutescens* seeds were obtained from the Seed-Research Specialists Inc. (variety California Wonder), those of *A. nummularia* were collected from their cultivated plants at Ras El-Hikma Experimental Station, on the Western Egyptian Coast, and those of *Z. album* were collected from their naturally growing plants at the coastal salt-marshes of the Western desert. Initially, ten seeds from each species were planted at the same time in each 35 × 25 cm diameter glazed culture-pot containing pure sand of less than one mm diameter, and irrigated with distilled water until emergence of seedlings. Emergence took place on early May, 5—10 days after sowing.

After complete emergence, plants were thinned to uniform seedlings; two for each of *C. frutescens* and *A. nummularia*, and five for *Z. album*, then irrigated with a base nutrient solution for one month, followed by nutrient solutions containing either 0, 0,05, 0,1, or 0,15 M NaCl. The base nutrient solution (Hoagland and Arnon 1950) composed of: 5 × 10^{-4}M KH_2PO_4, 2,5 × 10^{-3} M KNO_3, 2,5 × 10^{-3} M $Ca(NO_3)_2.4H_2O$, 10^{-3} M $MgSO_4.7H_2O$, 2,3 × 10^{-5} M H_3BO_3, 4,5 × 10^{-6} M $MnCl_2.4H_2O$, 3,5 × 10^{-7} M $ZnSO_4.7H_2O$, 1,6 × 10^{-7} M $CuSO_4.5H_2O$, 8 × 10^{-8} M $(NH_4)_6Mo_7O_{24}.4H_2O$, and 8 × 10^{-5} M iron citrate. The pH of the solution was adjusted at 6,5. Cultures were irrigated with enough solution to assure relatively uniform salt distribution, and excess solution was allowed to drain through an outlet opening at the bottom of each pot. For each species, each of the control and the three NaCl levels was represented by 24 pots. The pots were arranged statistically so that each would receive equal conditions for growth. The midday temperature inside the glasshouse was near 45° in summer and 30° C in winter.

One month from the application of saline solutions (July 13), plants of each treatment were removed from two pots, and a pooled sample of shoots with similar age were excised, dried in an aerated-oven at 70°C until constant weight, then ground into a fine powder by a micromill. Sampling was repeated monthly throughout the different growth stages of each variety, which continued for eleven months (July—May) for each of the two perennial species; *A. nummularia* and *Z. album*, and for 7 months (July—Jan.) for *C. frutescens*. A dry sample of about 1 g was transferred to a Soxhlet apparatus for lipid and pigment extraction, by using an equivalent mixture of petroleum ether and diethyl ether for 24 hrs. The remaining residue was dissolved in 50 ml 80% ethanol in a 250 ml flask, fitted with a reflux condenser, and heated on a water-bath for 2 hrs., then filtered. The filtrate containing the free sugars was concentrated to 5 ml for subsequent sugar determination. The remaining residue was then hydrolysed for 4 hrs with 30 ml N HCl in a 250 ml flask, refluxed on a hot plate, then filtered. Excess HCl was removed from the filtrate under vacuum until a residue was left, which was dissolved in 10 ml 80% ethanol for bound-sugar analysis. The alcohol-soluble extracted sugars were separated by the ascending one-dimentional method of paper chromatography using Whatman No. 1 paper. The solvent system used was iso-propanol: n-butanol: water, 7 : 1 : 2 (Smith 1962). The chromatographic separation was carried out in a stainless steel chamber of 12-sheet capacity. For chromatographic separation, three applications were conducted for each sample under the same conditions. The time required to attain complete separation (35 cm beyond the point of application) was about 66 hrs at room temperature (25°C). The sugar spots were made visible by treating the chromatograms with aniline phthalate reagent (Partridge 1949). The chromatograms were then dried at room temperature, then transferred to an oven at 105°C for 5 minutes to make the separated spots visible. Sucrose spots were made visible by heating the chromatograms 10 minutes further at 120°C (Aso *et al.* 1951). The sugars of the unknown mixture were identified by comparison with standards in an artificial mixture composed of sugars of R_g values developed on the same chromatogram. An UV lamp of 240 nm was used to trace the area boundaries of each developed spot. The concentration of each sugar was estimated by following a method devised by

Fisher, Parsons and Morrison (1948), relating the spot area (mean of three) with the logarithm of sugar concentration. For convenience, chromatographic maps of different concentrations were developed for each standard sugar, covering the range 5 to 150 μg.

Statistical methods used (Steel and Torrie 1960) involved comparison of all monthly means with the control to test the effect of NaCl levels on sugar concentration in each species by applying Dunnett's two-sided procedure for 0,05 and 0,01 levels.

Table 1

Effect of NaCl on the free sugar contents of shoots (mg/g dry wt.)

NaCl concentration (M)	Ribose	Glucose	Fructose	Sucrose	Maltose	Cellobiose	Raffinose
			C. frutescens [2]				
0	3.7	19.6	19.8	52.0	3.8	5.2	0
0.05	2.4	13.4	14.7	44.8	7.6	5.4	0
0.10	1.6 [1]	10.8	10.9	48.0	4.1	4.4	0
			A. nummularia				
0	0	1.2	3.0	6.6	0	0.64	1.5
0.05	0	1.5	3.4	5.6	0	0.55	1.9
0.10	0	1.7	2.9	8.1	0	0.50	1.5
0.15	0	2.4	3.8	9.9	0	0.77	1.9
			Z. album				
0	3.6	9.5	24.3	22.2	3.1	2.8	0
0.05	3.6	10.8	28.9	31.8	3.2	2.6	0
0.10	3.4	11.6	26.2	36.3	4.2	2.4	0
0.15	4.1	15.1	33.3	45.0 [1]	3.1	2.7	0

[1]) Significant at 0.05 lèvel with respect to the control (Dunnett's two-sided test).

[2]) *C. frutescens* plants survived for two months only under 0.15 M NaCl.

Results

The species included in the present study were characterized by specific variations in sugar types. The common free sugars present were glucose, fructose, sucrose, and cellobiose (Table 1). In addition, ribose and maltose were present in both *C. frutescens* and *Z. album*, and raffinose in *A. nummularia*. In each species, glucose, fructose, and sucrose were the free sugars found in appreciable amounts compared with other sugars. The concentration of these three sugar were relatively lower in *A. nummularia* than in either *C. frutescens* or *Z. album*. Higher levels of fructose were detected in *Z. album*, lower ones in *A. nummularia*, while intermediate concentrations were present in *C. frutescens*. Sucrose was relatively lower in *A. nummularia* compared to either *C. frutescens* or *Z. album*. Sodium chloride decreased the concentration of free ribose in *C. frutescens*, but induced an increase in free sucrose in *Z. album*. Statistical analyses indicated that the effect of 0.1 M NaCl level was significant on the free ribose concentration in *C. frutescens*, and that of 0.15 M NaCl level only was significant on the free sucrose concentration in *Z. album*. However, none of the other free sugars was affected significantly by NaCl, and also none of the NaCl treatment mean of any free sugar of *A. nummularia* was statistically significant with respect to the control.

The bound sugars resulted from the hydrolysis of the residue left after free sugar extraction were xylose, arabinose, rhamnose, glucose, and galactose (Table 2). *A. nummularia* was characterized by the least levels of bound

Table 2

Effect of NaCl on bound sugars and the total free and bound sugar contents of shoots (mg/g dry wt.).

NaCl concentration (M)	Xylose	Arabinose	Rhamnose	Glucose	Galactose	Total free and bound sugars
			C. frutescens [3]			
0	29.9	9.1	17.1	22.9	14.6	197.7
0.05	33.7	13.0	25.9	19.0	16.4	201.3
0.10	27.8	9.7	42.8 [1]	18.1	15.7	193.9
			A. nummularia			
0	16.8	9.0	11.8	8.0	11.5	70.0
0.05	13.4	7.6	9.4	9.1	11.8	64.3
0.10	14.5	7.7	13.8	8.6	10.3	69.6
0.15	13.4	6.9	10.5	7.3	11.2	68.1
			Z. album			
0	43.2	10.0	15.0	7.0	12.2	152.9
0.05	49.1	11.5	21.3	7.7	12.0	182.5
0.10	57.4	14.7	25.3	7.8	15.3	204.6
0.15	72.1 [2]	16.1 [2]	26.9	10.5 [2]	16.3	245.2 [2]

[1] Significant at 0.01 level with respect to the control.

[2] Significant at 0.05 level with respect to the control (DUNNETT's two-sided test).

[3] *C. frutescens* plants survived for two months only under 0.15 M NaCl.

sugars compared with the other two species. In both *A. nummularia* and *Z. album* xylose was present in relatively higher concentrations with respect to other bound sugars, and its concentration was appreciably higher in *Z. album*. Lower levels of bound glucose were detected in *Z. album* and of bound glucose and arabinose in *A. nummularia*, and of bound arabinose in *C. frutescens*. Sodium chloride increased the concentration of bound rhamnose in *C. frutescens* and of bound xylose, arabinose, and glucose in *Z. album*. Statistical analyses showed that the effect of 0.1 M NaCl level was highly significant on the bound rhamnose concentration in *C. frutescens*, and that of 0.15 M NaCl level only was significant on bound xylose, arabinose, and glucose in *Z. album*. However, none of the other bound sugars was affected significantly by NaCl, and none of the NaCl treatment means of any bound sugar of *A. nummularia* was statistically significant with respect to the control.

A. nummularia showed the least concentration of total free and bound sugars. Sodium chloride increased the concentration of total free and bound sugars in *Z. album*, but was without effect in either *A. nummularia* or *C. frutescens*. Statistical analyses indicated that the effect of 0.15 M NaCl only was significant on the total free and bound sugar concentration in *Z. album*. None of the NaCl treatment means of total free and bound sugar concentration of either *C. frutescens* or *A. nummularia* was statistically significant with respect to the control.

Discussion

Decreased free ribose in the 0.1 M NaCl-affected *C. frutescens* plants must be accounted for greater utilization in nucleotide formation. Atkinson and Polya (1967) detected an activation of soluble ATP-ases from several plants by NaCl, and Morozovskii and Kabanov (1968) found that the basic pathway of ATP formation by oxidative phosphorylation is not inhibited in pea leaves by chloride salinization, and proposed a stimulation of the pentose phosphate pathway. The effect of salinity on respiration was reported by several authors including Laties (1954, 1959), Nieman (1962), and Brouwer (1965). Since the effect of NaCl on respiration was found to be generally greater and occurred at lower levels of salt in the more sensitive species (Nieman 1962), an active mechanism of salt absorption with more demand for nucleotide formation would be mostly prevailing under the effect of NaCl in the least tolerant species *C. frutescens*.

The presence of relatively lower concentrations of free sugars in *A. nummularia*, while higher levels in *Z. album*, suggests the operation of two different osmotic mechanisms for salt tolerance. The latter, which is a succulent plant and contained more water (Ahmed, Higazy, El-Shourbagy, and Hegazy 1970), tended to be more sugar dependent than the former species. In this regard, Rikhter (1927) detected considerable amounts of carbohydrates and comparatively little salts in *Artemisia maritima salina* and *Anabasis salasa*, while the reverse was true in *Salicornia herbacea*, *Suaeda maritima*, and *Halocnemum strobilaceum*. Increased concentration of sucrose in the 0.15 M NaCl-treated *Z. album* plants agreed with work reported by AKOPIAN (1957) who abserved an increase in disaccharides concurrently with a decrease in monosaccharides in the leaves of *Goebelia alopecuroides* tree grown under saline conditions.

Increased synthesis of bound xylose, arabinose, and glucose in the 0.15 M NaCl-treated *Z. album* plants, but of bound rhamnose in the 0.1 M NaCl-affected *C. frutescens* plants could be attributed to increased synthesis of cell-wall hemicelluloses in both species. However, differences between the two must exist with regard to the nature of the building blocks. ALEKSEEVA (1964) proposed that increased synthesis of hemicelluloses in the leaves is an adaptive reaction for tolerating dryness, and GAFF and CARR (1961) suggested that the thicker the cell-wall the greater would be the buffering effect against drying forces.

Increased total free and bound sugars with NaCl in *Z. album* is supported by STROGONOV (1964) and BERNSTEIN and HAYWARD (1958) who reported higher carbohydrate content in plants growing under saline conditions.

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