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PLANT GROWTH, METABOLISM AND ADAPTATION IN RELATION TO STRESS CONDITIONS
XII. CARBOHYDRATE AND ACID ACCUMULATION IN *PHASEOLUS VULGARIS* AND *ZEA MAYS* STRESSED WITH SODIUM SULPHATE

By

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ABSTRACT

Phaseolus vulgaris and *Zea mays* seedlings and plants were cultured on quarter-strength Hoagland nutrient medium with or without increasing concentrations of Na_2SO_4. Sodium sulphate increased the levels of reducing sugars whereas sucrose and polysaccharides decreased in the treated seedlings, whole plants and leaves. ∝-ketoglularic and certain carboxylic acids of the Krebs cycle increased in all seedlings and plants in response to the various salt concentrations used, with the exception of puruvate, ∝-ketoglutarate and succinate which decreased in the salinized *Phaseolus vulgaris* seedlings. The present results with Na_2SO_4 are discussed in relation to the effect of salinity in other plant species and appear to be broadly in agreement with those other studies.

INTRODUCTION

In a previous investigation (Hasaneen *et al.*, 1989), Na_2SO_4 was found to induce varied reductions in water content and dry matter accumulation in whole plants and leaves of both *Phaseolus vulgaris* and *Zea mays* seedlings. Those decreases in growth were associated with the acculmulation of Na^+ and Mg^{2+}, which occurred concurrently with a reduction in K^+ and Ca^{2+} contents. Further studies (Younis *et al.*, 1989) have shown progressively greater prodcution of CO_2 by the same tissues of french bean and maize with increasing concentrations of Na_2SO_4. Moreover, marked changes in the total amounts and in the relative composition of the nitrogen pool were observed. The results obtained showed that *Phaseolus vulgaris* is highly sensitive to saliniy compared to *Zea mays* plants; the latter tolerate levels of up to -6 bars compared to -2 bars in the former.

Changes in carbohydrates and acids after salinity treatments have been observed by several workers (Hanson and Hitz, 1982; Handa *et al.*, 1983; Imamul Huq and Larher, 1983) and sometimes these changes show striking similarities to the growth changes (Pheloung and Barlow, 1981; Lambers *et al.*, 1981; Steingrover, 1983, El-Shahaby *et al.*, 1990; Abo-Hamed *et al.*, 1990).

The main purpose of the present study was to investigate further the effects of increasing Na_2SO_4 salinity with respect to carbohydrates and certain acids of the Krebs cycle in the leaves, seedlings and whole plants of *Phaseolus vulgaris* and *Zea mays*.

MATERIALS AND METHODS

The experiments were run with seeds of *Phaseolus vulgaris* (var. Contender) and *Zea mays* (var. Giza 2) of approximately the same size. Pure strains of seeds were kindly supplied by the Agricltural Research Center, Ministry of Agriculture, Giza.

The procedures for sterilizing seeds, germination and culture conditions as well as the experimental set-up were essentially those described by Hasaneen *et al.* (1989).Seedlings (3 days old), whole plants (11 days old) and leaves (19 days old) were sampled after transplantation into culture media for *Phaseolus vulgaris* and after 3, 9 and 15 days respectively for *Zea mays*.

Analytical methods

Estimation of carbohydrates. Soluble sugars were extracted from dried ground materials with 80% (V/V) ethanol. The direct reducing value which was considerd to be equivalent to reducing sugars was determined following the procedure of Nelson (see Bell, 1955). The total reducing value was estimated by determining the optical density at 700 nm after hydrolysis by an adequate amount of invertase. The difference between the total and the direct reducing values is taken as the sucrose content. Polysaccharides were determined in the dry residue left after thorough alcohol extraction of soluble sugars (Younis *et al.*, 1969).

Determination of acids. The method of Freidman and Haugen (1943) was used for the estimation of keto acids. The methods used for the estimation of oxalic and citric acids were essentially those of Snell and Snell (1949), and succinic acid that of Babin (1953) and recoveries of acids added to the extracts were over 95%.

RESULTS

Changes in carbohydrate content

In seedlings, whole plants and leaves of both french bean and maize, reducing sugars showed progressively greater increase with an increase in the concentration of Na_2SO_4 (Tables 1,2 and 3). Unlike reducing sugars, the sucrose and polysaccharide contents were found to decrease in the diffferent tissues of both plants and at different stages of plant development compared to controls. However, the magnitude of decrease of polysaccharides with an increase in concentration of Na_2SO_4 was much more pronounced than with sucrose. As polysaccharides made up approximately 80% of total carbohydrates in controls, it was not suprising to note that changes in polysaccharides closely followed those of total carbohydrates in the tissues and plants under study (see tables 1,2,3).

Table 1

Content of carbohydrates in *Phaseolus vulgaris* and *Zea mays* seedlings treated with increasing Na_2SO_4 concentrations. The values listed are given as mg glucose equivalent/100 g F. Wt. Each value is the mean of 4 samples ± standard error.

Culture medium	Reducing sugars	Sucrose	Polysaccharides	Total carbohydrates
		a. *Phaseolus vulgaris*		
Control (Hoagland)	216.0 ± 9.4	160.0 ± 5.8	1439.4 ± 35.3	1815.4 ± 58.8
$+Na_2SO_4$; -0.5 bar	252.3 ± 7.0	156.3 ± 3.5	499.8 ± 18.2	908.4 ± 4.7
$+Na_2SO_4$; -1 bar	260.9 ± 1.7	150.0 ± 2.9	523.2 ± 13.5	934.1 ± 20.0
$+Na_2SO_4$; -1.5 bars	275.2 ± 5.8	148.7 ± 5.1	552.9 ± 30.6	976.8 ± 41.1
$+Na_2SO_4$; -2 bars	313.9 ± 7.6	140.3 ± 1.7	752.9 ± 29.6	1207.1 ± 33.5
		b. *Zea mays*		
Control (Hoagland)	111.2 ± 3.7	240.8 ± 7.7	1020.0 ± 11.7	1372.0 ± 58.8
$+Na_2SO_4$; -1.5 bars	149.7 ± 5.2	220.6 ± 1.7	970.1 ± 41.2	1340.4 ± 23.7
$+Na_2SO_4$; -3 bars	166.8 ± 3.9	210.4 ± 0.5	890.2 ± 23.5	1267.4 ± 39.6
$+Na_2SO_4$; -4.5 bars	186.5 ± 0.5	198.9 ± 5.2	860.3 ± 35.2	1245.7 ± 26.4
$+Na_2SO_4$; -6 bars	210.3 ± 6.0	180.2 ± 0.7	790.4 ± 8.8	1180.9 ± 17.6

Changes in certain acids of the Krebs cycle

a. In seedlings. In saline treated french bean seedlings, pyruvic acid was found not to change (-0.5 bars) or to decrease **significantly** (with -1, -1.5 and -2 bars) compared to controls (Table 4a). With maize seedlings, Na_2SO_4 of between -3 and -6 bars induced an increase in pyruvic acid content above

Table 2

Content of carbohydrates in *Phaseolus vulgaris* and *Zea mays* plants treated with increasing Na_2SO_4 concentrations. The values listed are given as mg glucose equivalent/100 g F. Wt. Each value is the mean of 4 samples ± standard error.

Culture medium	Reducing sugars	Sucrose	Polysaccharides	Total carbohydrates
		a. *Phaseolus vulgaris*		
Control (Hoagland)	300.3 ± 10.1	225.1 ± 9.4	1702.9 ± 60.5	2228.3 ± 75.4
+Na_2SO_4; -0.5 bar	320.0 ± 11.7	220.3 ± 6.6	1104.0 ± 29.4	1644.3 ± 26.0
+Na_2SO_4; -1 bar	332.0 ± 7.0	216.6 ± 9.7	1000.2 ± 15.9	1548.8 ± 40.4
+Na_2SO_4; -1.5 bars	340.5 ± 9.1	200.2 ± 2.4	990.7 ± 35.7	1531.4 ± 41.9
+Na_2SO_4; -2 bars	390.6 ± 11.1	175.6 ± 3.2	870.6 ± 19.1	1436.8 ± 19.9
		b. *Zea mays*		
Control (Hoagland)	220.3 ± 7.2	340.3 ± 7.0	1990.4 ± 49.1	2551.0 ± 88.2
+Na_2SO_4; -1.5 bars	250.6 ± 6.2	320.2 ± 8.2	1614.3 ± 8.2	2185.1 ± 58.8
+Na_2SO_4; -3 bars	260.7 ± 8.0	300.0 ± 5.2	1230.2 ± 17.8	1790.9 ± 53.5
+Na_2SO_4; -4.5 bars	286.3 ± 9.5	290.0 ± 3.5	990.6 ± 23.9	1566.9 ± 38.8
+Na_2SO_4; -6 bars	340.4 ± 11.9	275.0 ± 6.4	880.4 ± 26.7	1495.8 ± 32.1

that of controls; at -1.5 bars there was no significant difference (Table 4b). On the other hand, α-ketoglutarate appeared, in general, to show varied increases with the various concentrations of Na_2SO_4 used when compared to controls in both plant varieties.

Table 3

Content of carbohydrates in *Phaseolus vulgaris* and *Zea mays* leaves treated with increasing Na_2SO_4 concentrations. The values listed are given as mg glucose equivalent/100 g F. Wt. Each value is the mean of 4 samples ± standard error.

Culture medium	Reducing sugars	Sucrose	Polysaccharides	Total carbohydrates
		a. *Phaseolus vulgaris*		
Control (Hoagland)	180.0 ± 5.2	170.0 ± 5.8	590.2 ± 17.7	940.2 ± 23.6
+Na_2SO_4; -0.5 bar	192.6 ± 7.4	168.4 ± 10.8	570.7 ± 10.9	931.7 ± 18.0
+Na_2SO_4; -1 bar	206.4 ± 3.7	165.7 ± 3.3	540.3 ± 20.7	912.4 ± 30.8
+Na_2SO_4; -1.5 bars	220.4 ± 9.6	160.2 ± 5.4	465.6 ± 15.0	846.2 ± 27.1
+Na_2SO_4; -2 bars	250.3 ± 7.8	130.6 ± 5.0	400.4 ± 4.3	781.3 ± 18.4
		b. *Zea mays**		
Control (Hoagland)	110.3 ± 4.2	100.5 ± 3.2	990.5 ± 20.9	1201.3 ± 59.6
+Na_2SO_4; -1.5 bars	120.4 ± 3.7	95.3 ± 2.5	880.6 ± 31.2	1096.3 ± 56.5
+Na_2SO_4; -3 bars	136.6 ± 3.8	90.6 ± 2.7	660.6 ± 18.8	887.8 ± 25.3
+Na_2SO_4; -4.5 bars	146.4 ± 4.9	80.7 ± 2.1	456.6 ± 15.3	683.7 ± 19.8

* Data for -6 bars saline-treated plants are not available.

Table 4

Content of acids in *Phaseolus vulgaris* and *Zea mays* seedlings treated with increasing Na_2SO_4 concentrations. The values listed are given as mg acid / 100 g F. Wt. Each value is the mean of 4 samples ± standard error.

Culture medium	Pyruvic	α-Ketoglutaric	Citric	Succinic	Oxalic
		a. *Phaseolus vulgaris*			
Control (Hoagland)	17.7 ± 0.3	15.6 ± 0.7	101.5 ± 0.8	208.9 ± 5.2	10.8 ± 0.4
+Na_2SO_4; -0.5 bar	17.2 ± 0.1	19.9 ± 0.7	185.3 ± 6.0	152.1 ± 4.1	13.6 ± 0.5
+Na_2SO_4; -1 bar	15.3 ± 0.2	14.4 ± 0.2	188.7 ± 5.1	106.9 ± 4.0	14.4 ± 0.2
+Na_2SO_4; -1.5 bars	15.4 ± 0.2	16.7 ± 0.4	163.0 ± 1.8	133.8 ± 4.5	15.2 ± 0.5
+Na_2SO_4; -2 bars	16.6 ± 0.3	17.0 ± 0.6	123.3 ± 3.1	185.8 ± 6.7	18.0 ± 0.5
		b. *Zea mays*			
Control (Hoagland)	66.0 ± 3.5	58.4 ± 1.7	198.0 ± 2.9	201.3 ± 0.7	97.2 ± 4.1
+Na_2SO_4; -1.5 bars	66.3 ± 0.7	58.9 ± 2.3	225.2 ± 5.9	220.7 ± 5.2	102.0 ± 1.1
+Na_2SO_4; -3 bars	72.4 ± 1.4	63.2 ± 1.8	242.1 ± 1.2	234.6 ± 8.2	137.2 ± 4.2
+Na_2SO_4; -4.5 bars	89.7 ± 3.5	79.4 ± 2.0	273.2 ± 1.8	246.1 ± 2.3	146.4 ± 3.7
+Na_2SO_4; -6 bars	82.4 ± 1.1	72.1 ± 1.2	292.0 ± 4.2	320.2 ± 1.2	148.8 ± 4.7

In french bean seedlings, marked accumulation of citric acid was observed especially at the lower concentrations of Na_2SO_4. Succinic acid showed varying decreases in response to the various Na_2SO_4 treatments in the french bean seedlings whereas oxalic acid contents showed progressively greater increases with an increase in concentration of the salt used.

With an increase in concentration of Na_2SO_4, progressively greater increases in citric, succinic and oxalic acid contents were observed in maize seedlings.

Table 5

Content of acids in *Phaseolus vulgaris* and *Zea mays* plants treated with increasing Na_2SO_4 concentrations. The values listed are given as mg acid / 100 g F. Wt. Each value is the mean of 4 samples ± standard error.

Culture medium	Pyruvic	α-ketoglutaric	Citric	Succinic	Oxalic
	a. *Phaseolus vulgaris*				
Control (Hoagland)	24.7 ± 0.4	31.3 ± 0.8	206.1 ± 3.6	254.1 ± 8.2	14.0 ± 0.2
+Na_2SO_4; -0.5 bar	24.7 ± 0.8	34.8 ± 1.7	220.5 ± 6.2	254.0 ± 10.2	15.6 ± 0.7
+Na_2SO_4; -1 bar	25.8 ± 0.4	38.1 ± 1.2	235.0 ± 8.8	260.2 ± 5.4	16.4 ± 0.2
+Na_2SO_4; -1.5 bars	32.3 ± 1.3	31.6 ± 1.5	242.0 ± 7.1	276.2 ± 3.6	18.8 ± 0.5
+Na_2SO_4; -2 bars	37.2 ± 0.7	30.8 ± 1.6	265.8 ± 3.4	289.1 ± 6.9	21,2 ± 0.7
	b. *Zea mays*				
Control (Hoagland)	42.6 ± 1.5	40.0 ± 1.2	392.7 ± 13.3	599.6 ± 17.4	67.6 ± 2.5
+Na_2SO_4; -1.5 bars	42.8 ± 1.6	40.0 ± 1.7	460.6 ± 13.2	680.6 ± 11.5	75.6 ± 2.9
+Na_2SO_4; -3 bars	47.2 ± 1.8	45.0 ± 1.8	487.8 ± 8.1	692.4 ± 14.0	91.2 ± 2.4
+Na_2SO_4; -4.5 bars	54.3 ± 2.5	53.1 ± 1.8	498.5 ± 16.7	702.2 ± 18.9	102.4 ± 3.7
+Na_2SO_4; -6 bars	67.1 ± 2.4	58.9 ± 2.3	520.5 ± 12.0	722.3 ± 13.1	147.2 ± 4.2

b. In whole plants (Table 5a and b). In both french bean and maize plants, progressively greater increases in pyruvate contents were obtained with an increase in concentration of Na_2SO_4 compared to controls, except at the lowest concentrations which had no significant effect.

In french bean plants, α-ketoglutarate did not change with -1.5 and -2 bars Na_2SO_4 or increased with -0.5 and -1 bars Na_2SO_4 compared to conrols. In maize plants, the lowest concentration of Na_2SO_4 did not alter the content of α-ketoglutarate whereas the higher concentrations induced progressively greater increases. Progressively greater increases in citric, succinic and oxalic acid were obtained in both plants with an increase in concentration of Na_2SO_4.

c. In leaves (Table 6a and b). With an increase in concentration of Na_2SO_4, progressively greater accumulations of pyruvate, α-ketoglutarate, citrate and succinate were observed, in general, in both leaves. Oxalate, followed the pattern of the other acids in maize leaves, but in french bean leaves it was found either to increase (with -0.5 and -1 bar) or to decrease (with -1.5 and -2 bars).

Table 6

Content of acids in *Phaseolus vulgaris* and *Zea mays* leaves treated with increasing Na_2SO_4 concentrations. The values listed are given as mg acid / 100 g F. Wt. Each value is the mean of 4 samples ± standard error.

Culture medium	Pyruvic	α-ketoglutaric	Citric	Succinic	Oxalic
		a. *Phaseolus vulgaris*			
Control (Hoagland)	14.7 ± 0.7	16.3 ± 0.7	86.2 ± 3.6	150.8 ± 5.7	36.0 ± 1.1
+Na_2SO_4; -0.5 bar	12.8 ± 0.6	14.3 ± 0.2	69.1 ± 2.9	147.6 ± 4.4	39.6 ± 0.9
+Na_2SO_4; -1 bar	18.2 ± 0.7	18.5 ± 0.3	86.1 ± 3.6	160.9 ± 6.4	40.9 ± 1.7
+Na_2SO_4; -1.5 bars	20.0 ± 0.5	20.0 ± 0.0	117.1 ± 4.7	165.3 ± 4.8	34.2 ± 1.8
+Na_2SO_4; -2 bars	28.8 ± 1.1	30.1 ± 0.6	186.6 ± 5.0	175.2 ± 8.9	30.4 ± 1.4
		b. *Zea mays**			
Control (Hoagland)	37.6 ± 1.4	35.2 ± 1.3	200.3 ± 6.0	400.2 ± 12.3	40.0 ± 1.3
+Na_2SO_4; -1.5 bars	40.7 ± 1.6	37.8 ± 1.7	230.6 ± 8.2	490.5 ± 11.4	83.6 ± 2.1
+Na_2SO_4; -3 bars	53.2 ± 1.9	45.6 ± 2.1	270.8 ± 8.1	520.3 ± 11.9	148.4 ± 4.9
+Na_2SO_4; -4.5 bars	54.6 ± 2.6	46.2 ± 1.9	292.8 ± 7.5	552.3 ± 13.1	285.2 ± 8.3

* Data for -6 bars saline-treated plants are not available.

DISCUSSION

The analyses reported in the present investigation are indicative of the extent to which carbohydrates and acid contents were differently affeted by the different salinity levels.

In response to salinity with Na_2SO_4, reducing sugars increased whereas sucrose, polysaccharides and consequently total carbohydrates decreased in the different plant tissues and at the different stages of plant development. The higher the salinity level used, the greater the response. These changes may be attributed to an increase in the turnover of hydrolytic enzymes, but may also result from less utilization of reducing sugars under these conditions of salinity.

Of interest in this connection, Handa *et al.* (1983), using cultured tomato cells adapted to water stress, found that the concentration of reducing sugars in the cells increased with the degree of adaptation, reaching as high as 600 mM in the cells, however, the cells were also found to accumulate some sucrose but the levels of sucrose were 3 to 8 - fold lower than those of reducing sugars. The sucrose concentration increased in moderately adapted cells but did not increase with further adaptation to water stress. Our results are in agreement with these observations, in that the low tolerant *Phaseolus vulgaris* plants were found to accumulate much less reducing sugars than the highly tolerant plants of *Zea mays.*

The observed losses in the various carbohydrate fractions as well as total carbohydrates in seedlings, whole plants and leaves of both genera coincides with the observed decrease in dry matter (Hasaneen *et al.*, 1989) and are far in excess of the concomitant increases in reducing sugars. Thus the losses in carbohydrates of seedlings, whole plants and leaves in various salinized culture media could be primarily due to respiration. This is indeed consistent with the reported increase in the respiration rate of the same tissues (Younis *et al.*, 1989). Thus Na_2SO_4 levels seem to have favoured the utilization of sucrose and starch and the sharp increased rate of CO_2 production seems to be at the expense of losses in carbohydrates. However, conversion of carbohydrates to amino acids is of importance in this connection and cannot be ruled out as supported by the increased levels of organic and amino acids, in particular of proline (Younis *et al.*, 1989).

In both *Phaseolus vulgaris* and *Zea mays* seedlings, whole plants and leaves, α-keto as well as organic acids were, in general, variably increased in response to the various salinity treatments except for pyruvate, α-ketoglutarate and succinate which decreased, in general, in the salinized seedlings of *Phaseolus* plants. The observed increases in the acid components could be explained as follows: acids, in particular keto acids, play a protective role in saline habitats in addition to metabolic functions, they may bind excessive ions absorbed by plants and maintain the electrical neutrality of the cells and finally neutralizing basic compounds (Strogonov, 1970). In support of this explanation, several investigators have shown that in different plant tissues the main carboxylate anions concerned in charge balance during excess cation uptake are malate (Hiatt and Hendricks, 1967; Triplett *et al.*, 1980) and oxalate (Osmond, 1967).

In salinized *Phaseolus* seedlings, the observed decreases in pyruvate, α-ketoglutarate and succinate can be correlated with increased amino acid synthesis (in particular proline) *via* the amination and transamination systems. Our results in this respect support those of Rao and Rao (1979) who found that leaves of salinized groundnut plants showed a sharp decrease in the amounts of α-ketoglutarate, pyruvate and glyoxylate and concomitant increase in glutamic acid, alanine and proline.

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١٢ ـ تراكم المواد الكربوايدراتية والحمضية في الفاصوليا والذرة المعاملة بكبرتيات الصوديوم

محمد نجيب عبد الغني حسنين و محمود الباز يونس
و
حبيبة محمد السحت

تم إنماء بادرات ونباتات الفاصوليا والذرة على محلول هوجلاند المغذى (ربع التركيز الكامل) إما منفرداً أو مضافاً إليه تركيزات متدرجة من ملح كبريتات الصوديوم .
ولقد أدت هذه المعاملات إلى زيادات طردية في السكرات المختزلة بينما لوحظ نقص في السكروز وعديدات التسكر في البادرات والنباتات الكاملة والأوراق .
كذلك أدت معاملة بادرات ونباتات الفاصوليا والذرة بالتركيزات المختلفة من الملح إلى زيادات في حمض الفاكيتوجلوتاريك وكذلك بعض الاحماض الكربوكسيلية لدورة كربس ماعدا حمض البيروفيك وحمض الفاكيتوجلوتاريك وحمض السكسنيك والتي نقصت في بادرات الفاصوليا تحت تأثير التركيزات المختلفة من الملح .
ولقد نوقشت هذه النتائج في ضوء نتائج الآخرين ، وفي ضوء ما هو معروف حالياً عن ميكانيكية التوازن الأزموزي في الخلايا النباتية .