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PLANT GROWTH, METABOLISM AND ADAPTATION IN RELATION TO STRESS CONDITIONS. VII. RESPIRATION, NITROGEN AND PROLINE CONTENTS IN FRENCH BEAN AND MAIZE PLANTS AS INFLUENCED BY SALINITY

By

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ABSTRACT

Carbon dioxide production by seedlings, whole plants and leaves of french bean and of maize showed progressively greater increases with increasing concentrations of Na_2SO_4 added to the basal medium. Also, in response to salinization, marked changes in the total amount and in the relative composition of the nitrogen pool were observed. Thus, whereas proline as well as amino- and total soluble -N contents increased, contents of protein- and total -N decreased; the magnitude of these changes appeared to be a function of the concentration of Na_2SO_4 used. Irregular changes were observed for ammonia- and nitrate-N.

INTRODUCTION

Salt uptake is sometimes, but not always, accompanied by an increased respiration rate, generally referred to as salt respiration (Campbell *et al.*, 1976). In *Xanthium, Phaseolus* and *Atriplex,* respiration increased at low levels of salinity and Schwarz and Gale (1981) interpreted this to be an adaptive response. The decrease in respiration at higher levels of salinity is probably an indication of the commencement of salt damage as shown in *Xanthium* and in *Phaseolus,* whereas in the halophytic *Atriplex* there was no apparent salt damage even when respiration was at its highest level (Schwarz and Gale, 1981).

Besides affecting respiration, salts profoundly alter metabolic activities. Thus the consequent onset of water deficit alters the overall nitrogen budget of crops through effects on both N_2 transport and N_2 assimilation, NO_3^- delivery *via* the xylem to the shoot is depressed and NO_3^- reduction in leaves declines (Shaner and Boyer, 1976; Younis *et al.*, 1987a). In plants under various experimental conditions, free proline as well as aspartic acid, methionine, valine and arginine have been found to accumulate in response to water stress (Tall *et al.*, 1979; El-Shahaby *et al.*, 1987).

The objective of this study was to investigate the possible effects of salinity on respiration rate, nitrogen and proline contents of two plants (*Phaseolus* and *Zea*) of commercial value in Egypt.

MATERIALS AND METHODS

Homogeneous seeds of french bean (*Phaseolus vulgaris* var. Contender) and maize (*Zea mays* var. Giza 2) were used.

The procedures for sterilizing seeds, germination and culturing of seedlings as well as the experimental set-up were described by Hasaneen *et al.* (1989).

Technique used for measuring CO_2 output

The continuous air current method was adopted, in which a moisture saturated stream of CO_2-free air was passed over the experimental material and the resulting respiratory CO_2 was absorbed in a solution of NaOH. This was then quantitatively converted to $BaCO_3$ by the addition of excess $BaCl_2$ and the unreacted NaOH was titrated against HCl. From this, CO_2 produced over a set period could be estimated (Younis *et al.*, 1987b).

Determination of nitrogen and proline

Nitrogenous constituents were extracted by the method of Yemm and Willis (1956). Total-N was determined in the dry powdered tissues by the conventional micro-Kjeldahl method. Aliquots of the extracts were used for estimation of ammonia-N by the method of Delory (1949) using Nessler's reagent. Nitrate-N was estimated by phenol-disulphonic acid method as described by Snell and Snell (1949). Amino-N was measured by the method of Muting and Kaiser (1963). Total soluble-N in the extracts was measured as for total-N. Subtracting the total soluble-N from total-N gave the value for protein-N. The method used for estimation of proline was that of Bates *et al.* (1973).

Each experiment was repeated twice in duplicate, so that the mean obtained was for four replicates with ± standard error where appropriate.

EXPERIMENTAL RESULTS

Changes in respiration

In relation to CO_2 production by control samples, progressively greater increases in CO_2 output throughout 48 hours, were obtained in treated french bean and maize seedlings, whole plants and leaves with an increase in concentration of Na_2SO_4 added to the Hoagland solution (Fig. 1a-f).

Changes in nitrogen contents

a- In seedlings (Table 1a and b)

Nitrate-N contents of the variously salinized french bean seedlings showed markedly higher amounts than those of controls whereas in maize seedlings, salinity induced variable progressive decreases. Ammonia-N contents of the variously salinized french bean seedlings were variable and slightly reduced below those of controls but in maize variable changes were apparent. In the variously salinized bean and maize seedlings, amino- and total soluble-N contents showed progressively greater increases above control values with increase in concentration of Na_2SO_4. Protein- and total-N contents of both seedlings showed progressively greater decreases with an increase of salinity in the Hoagland medium.

b- In whole plants (Table 2a and b)

In contrast to marked progressive decreases in nitrate-, protein- and total-N contents, those contents of ammonia-, amino- and total soluble-N showed progressively greater increases above Hoagland control levels with an increase in concentration of Na_2SO_4 added to the basal Hoagland nutrient media for both bean and maize plants.

c- In leaves (Table 3a and b)

Salinization of bean plants with Na_2SO_4 induced high accumulation of nitrate-N in the leaves; the higher the concentration of Na_2SO_4 used, the lower was the magnitude of accumulation. On the other hand, treatment of maize plants with increasing Na_2SO_4 concentrations induced progressively greater decreases in nitrate-N content in leaves below the control level. Again with an increase in concentration of Na_2SO_4, progressively greater increases of ammonia-, amino- and total soluble-N as well as progressively greater decreases in protein- and total-N contents were obtained in both plant leaves compared to control levels.

Changes in proline content (Table 4a and b)

The proline content of both french bean and maize seedlings, whole plants and leaves showed progressively greater accumulation, above the Hoagland control levels, with increasing concentration of Na_2SO_4 added to the Hoagland culture media.

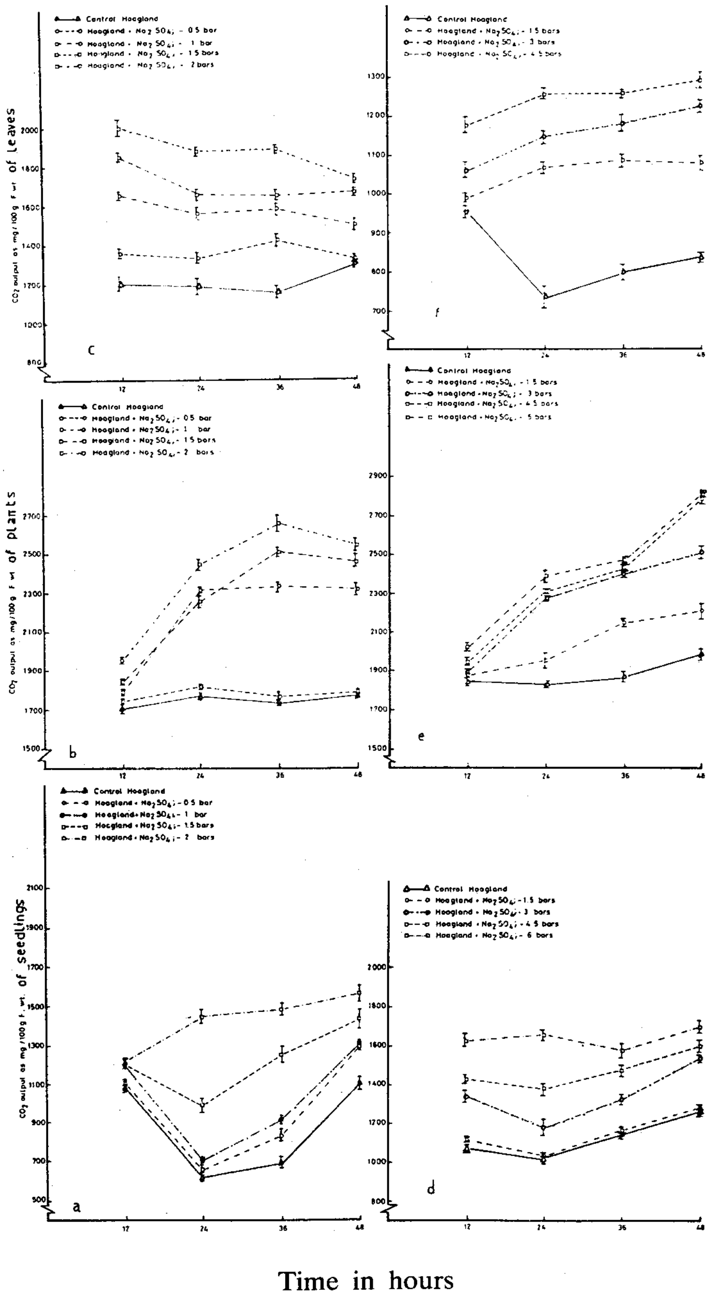

Fig. 1: Effect of increasing Na_2SO_4 concentration on CO_2 production by seedlings, whole plants and leaves of *Phaseolus vulgaris* (a,b and c) and *Zea mays* (d,e and f). Each value is the mean of 4 samples.

Table 1

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

Culture medium	Nitrate-N	Ammonia-N	Amino-N	Total soluble-N	Protien-N	Total-N
a- *Phaseolus vulgaris*						
Control Hoagland	12.0 ±1.5	19.0 ±2.4	2.3 ±0.1	151.8 ±6.8	1188.9 ±58.8	1340.8 ±17.6
Hoagland + Na_2SO_4; -0.5 bar	16.4 ±2.0	17.0 ±2.0	4.3 ±0.1	156.4 ±6.6	1024.4 ±35.3	1180.8 ±47.1
Hoagland + Na_2SO_4; -1 bar	16.5 ±1.9	17.9 ±1.3	4.4 ±0.2	163.4 ±7.2	991.2 ±29.4	1154.6 ±31.8
Hoagland + Na_2SO_4; -1.5 bars	16.7 ±1.6	17.6 ±1.5	4.7 ±0.3	172.2 ±7.1	949.2 ±32.9	1121.4 ±12.3
Hoagland + Na_2SO_4; -2 bars	17.2 ±2.0	16.5 ±1.3	4.8 ±0.3	177.5 ±8.5	864.1 ±37.6	1041.6 ±24.1
b- *Zea mays*						
Control Hoagland	16.1 ±0.7	36.3 ±1.8	4.8 ±0.2	141.8 ±6.5	1135.1 ±20.6	1276.9 ±44.7
Hoagland + Na_2SO_4; -1.5 bars	13.3 ±0.2	32.1 ±1.2	6.3 ±0.2	146.7 ±5.3	990.1 ±38.2	1136.8 ±21.2
Hoagland + Na_2SO_4; -3 bars	13.0 ±0.6	32.9 ±1.2	6.8 ±0.2	184.8 ±2.3	837.1 ±21.8	1021.9 ±12.9
Hoagland + Na_2SO_4; -4.5 bars	12.8 ±0.6	36.1 ±0.6	7.8 ±0.3	199.0 ±6.5	809.8 ±5.8	1008.8 ±5.2
Hoagland + Na_2SO_4; -6 bars	12.5 ±0.3	42.7 ±1.2	10.2 ±0.6	217.1 ±8.0	755.3 ±32.5	972.4 ±42.5

DISCUSSION

Changes in respiration

In relation to CO_2 production by french bean and maize seedlings, whole plants and leaves grown on Hoagland media, CO_2 production by the variously salinized samples, in general, showed progressively greater increases with an increase in concentration of Na_2SO_4 added to Hoagland culture media. In agreement with the present results, the rates of respiration of many plant tissues were stimulated by

Table 2

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

Culture medium	Nitrate-N	Ammonia-N	Amino-N	Total soluble -N	Protein-N	Total-N
a- *Phaseolus vulgaris*						
Control Hoagland	53.8 ±2.2	13.9 ±0.5	3.6 ±0.1	230.9 ±8.8	1709.1 ±59.9	1940.0 ±52.9
Hoagland + Na_2SO_4; -0.5 bar	42.9 ±1.7	13.9 ±0.6	6.2 ±0.1	263.8 ±8.1	1416.6 ±9.8	1680.4 ±50.2
Hoagland + Na_2SO_4; -1 bar	41.9 ±1.1	14.3 ±0.2	6.8*	273.8 ±10.5	1346.7 ±27.5	1620.6 ±12.1
Hoagland + Na_2SO_4; -1.5 bars	37.3 ±1.4	16.0 ±0.6	7.5 ±0.3	293.1 ±7.1	1288.5 ±63.8	1581.6 ±65.3
Hoagland + Na_2SO_4; -2 bars	34.9 ±1.1	20.9 ±1.2	7.8*	326.2 ±15.4	1115.2 ±9.0	1441.4 ±24.4
b- *Zea mays*						
Control Hoagland	51.6 ±0.9	18.5 ±0.9	12.8 ±0.5	249.6 ±5.6	1831.2 ±18.3	2080.8 ±47.1
Hoagland + Na_2SO_4; -1.5 bars	45.5 ±1.8	21.1 ±0.6	14.8 ±0.5	290.2 ±10.1	1450.6 ±41.5	1740.8 ±64.81
Hoagland + Na_2SO_4; -3 bars	37.4 ±0.8	25.7 ±1.2	20.9 ±1.1	320.0 ±11.8	1360.8 ±35.3	1680.8 ±2.9
Hoagland + Na_2SO_4; -4.5 bars	30.0 ±1.2	39.2 ±0.7	22.3 ±0.8	367.0 ±7.1	1273.4 ±43.2	1640.4 ±41.4
Hoagland + Na_2SO_4; -6 bars	28.4 ±0.6	45.6 ±2.3	24.0 ±1.2	389.4 ±17.3	891.3 ±30.2	1280.8 ±47.5

* SE values less than 0.1.

various salinity treatments and the highest rates of respiration were recorded at the highest levels of salt concentration (Nieman, 1962; Divate and Pandy, 1981; Younis *et al.*, 1987b). The present increase in respiration appears to have taken place at the expense of oxidation of metabolites, and the decline in dry matter reported by Hasaneen *et al.* (1989) well support this conclusion.

The stimulatory effects of single salt solutions, particularly the more concentrated ones, on respiration may be due to increased permeability of the cells. Another tentative explanation is that respiration is controlled by the level of phosphate acceptors. Thus ion uptake involves the expenditure of phosphate bond energy and

Table 3

Analysis of nitrogen content in *Phaseolus vulgaris* and *Zea mays* leaves collected from plants treated with increasing Na_2SO_4 concentration. The values listed are given as mg N/100 g F.Wt. Each value is the mean of 4 samples ± standard error.

Culture medium	Nitrate-N	Ammonia-N	Amino-N	Total soluble-N	Protein-N	Total-N
a- *Phaseolus vulgaris*						
Control Hoagland	25.0	14.7	2.6	79.9	873.8	953.7
	±0.6	±0.4	±0.5	±2.3	±35.3	±29.4
Hoagland + Na_2SO_4;	50.2	15.7	3.6	92.7	787.9	880.6
-0.5 bar	±1.2	±0.4	±0.1	±1.6	±41.2	±5.9
Hoagland + Na_2SO_4;	48.6	16.2	3.9*	95.0	645.0	740.0
-1 bar	±1.8	±0.6		±2.9	±26.5	±11.8
Hoagland + Na_2SO_4;	45.3	17.8	4.7	99.0	521.8	620.8
-1.5 bars	±1.4	±0.4	±0.1	±1.8	±12.8	±12.2
Hoagland + Na_2SO_4;	39.8	20.6	6.2	120.2	460.1	580.4
-2 bars	±1.0	±1.0	±0.1	±4.3	±14.8	±17.9
b- *Zea mays*						
Control Hoagland	50.8	14.8	8.2	123.7	668.5	792.2
	±1.7	±0.5	±0.1	±5.3	±17.9	±24.8
Hoagland + Na_2SO_4;	26.0	30.8	8.6	149.0	575.6	724.6
-1.5 bars	±0.6	±1.7	±0.4	±5.3	±23.6	±14.5
Hoagland + Na_2SO_4;	20.3	31.4	14.8	159.0	484.7	643.8
-3 bars	±0.8	±0.8	±0.6	±6.5	±14.2	±25.3
Hoagland + Na_2SO_4;	14.9	34.4	15.6	209.2	364.6	573.8
-4.5 bars	±0.8	±1.8	±0.6	±7.8	±8.1	±19.9

* SE values less than 0.1

hence the breakdown of ATP; the concentration of phosphate acceptors might then rise, and respiration increase (Younis *et al.*, 1970; Lawlor and Fock, 1977). In the present investigation, the increase in respiration due to Na_2SO_4 treatment could be explained on the basis of increased permeability of the cells by the added salt solution which results in a stimulatory effect on respiration. When the loss of permeability is great respiration is depressed by the loss of essential intermediates. However, the involvement of a role of increased phosphate acceptors cannot be ruled out and this must await further investigation.

Table 4

Analysis of proline content in *Phaseolus vulgaris* and *Zea mays* seedlings, whole plants and leaves treated with increasing Na_2SO_4 concentration. The values listed are given as mg/100 g F.Wt. Each value is the mean of 4 samples ± standard error.

Culture medium	Seedlings	Whole plants	Leaves
a-*Phaseolus vulgaris*			
Control Hoagland	12.9 ±0.2	33.0 ±1.0	18.1 ±0.7
Hoagland + Na_2SO_4;-0.5bar	18.7 ±0.7	38.8 ±1.0	20.2 ±0.1
Hoagland + Na_2SO_4; 1 bar	20.4 ±0.8	41.3 ±0.9	24.0 ±0.6
Hoagland + Na_2SO_4;-1.5 bars	25.9 ±0.8	44.2 ±1.4	29.6 ±0.9
Hoagland + Na_2SO_4;-2 bars	30.5 ±1.0	46.0 ±2.1	32.3 ±1.1
b- *Zea mays*			
Control Hoagland	10.8 ±0.4	20.4 ±0.9	10.5 ±0.3
Hoagland + Na_2SO_4;-1.5 bars	12.3 ±0.1	24.2 ±1.0	12.4 ±0.2
Hoagland + Na_2SO_4;-4.5 bars	14.4 ±0.5	27.6 ±1.1	13.5 ±0.1
Hoagland + Na_2SO_4;-4.5 bars	18.6 ±0.8	32.0 ±1.2	14.4 ±0.6
Hoagland + Na_2SO_4;-6 bars	19.7 ±0.5	45.3 ±2.0	— —

Changes in nitrogen content

The eventual decline in the total-N content of both french bean and maize seedlings, whole plants and leaves is mainly consistent with a net loss of dry weight (Hasaneen *et al.*, 1989) due to excessive oxidation of substrates during respiration of the tissues. This observation is clearly supported by Imamul Huq and Larher (1983) and Younis *et al.* (1987a) who found that salinity of various plant tissues led to greater reduction in total-N content below that of untreated plants.

Protein of the variously salinized french bean and maize seedlings, whole plants and leaves showed a decrease by increasing salinity concentration in the media. This, in addition to the observed increase in total soluble-N, is mainly due to more active proteolysis which showed increasing responsiveness to salinity concentration. In support of this conclusion, Kemble and Macpherson(1954) stated that proteolysis and interruption of protein synthesis are generally found to be the results of water stress.

Amino-N of the variously salinized french bean and maize plants showed variable marked accumulations. This, in addition to other variable changes in ammonia- and nitrate-N in both french bean and maize plant tissues, due to salinity influences, may be due to alterations in the intermediary metabolism of nitrogen as reported by Imamul Huq and Larher (1983) and Younis *et al.* (1987a). This most probably may have resulted from an imbalance in inorganic ion status ultimately causing malfuntioning of the enzymes involved. Thus Stewart and Larher (1980) showed that nitrates, amides, free amino acids, proline and amines are among some of the inorganic and organic solutes that show changes in their accumulation under conditions of stress.

Changes in proline content

The present changes in the accumulation of proline content appears to coincide with the decrease in water content of the plant tissues due to salinization with Na_2SO_4. In this connection, Buhl and Stewart (1983) and El-Shahaby *et al.* (1987) reported that free proline often accumulates considerably in several species of seedlings, whole plants or leaves exposed to water stress or to salinity.

A number of roles has been suggested for the proline accumulation during water stress, e.g. removal of ammonia by proline formation (Henckel, 1964), provision of both carbon and nitrogen for post-stress metabolism (Thompson *et al.*, 1966) or improved water relations (Savistakaya, 1967). However, the physiological significance of proline accumulation in plant tissues subjected to water stress or salinization, is still unclear and may be different in different species. In both *Phaseolus* and *Zea* tissues, the increase in free proline due to salinization occurred concurrently with a decrease in protein and polysaccharides (unpublished work). Thus we may suggest that free proline acts as a storage compound for both carbon and nitrogen. In support of this, Barnett and Naylor (1966) have proposed that accumulation of proline is a metabolic adaptation which confers survival value, perhaps acting as a reserve of nitrogen accessible for use upon stress relief. However, the possibility that proline may act as a solute for intracellular osmotic adjustment cannot be ruled out.

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نمو وأيض وملاءمة النباتات لظروف الإجهاد المختلفة
٧ ـ أثر الملوحة على تنفس نباتات الفاصوليا والذرة ومحتـــواها من النيتروجـــين والبـــرولين

محمـــود البـــاز يـــونس - محمـــد نجيب عبد الغني حسانين
و حبيبـــه محمـــد السحـــت

في هذا البحث ، وجد أن إضافة ملح كبريتات الصوديوم إلى الأوساط الغذائية لنباتي الفاصوليا والذرة ، أدت إلى زيادات واضحة في تنفس البادرات والنباتات الكاملة وكذلك الأوراق ، وكانت هذه الزيادات أكثر وضوحاً بزيادة تركيز الملح في الأوساط الغذائية . ولقد أمكن تفسير الزيادة في التنفس إستناداً إلى عمليات الفسفرة داخل الخلايا نتيجة لزيادة إمتصاص أيونات الصوديوم وتراكمها .

ولقد أوضحت نتائج التجارب أن هناك تغيرات ملحوظة في المحتوى الكلي والجزئي للمواد النيتروجينية ، فعلى حين تزايدت كميات البرولين والأحماض الأمينية والنيتروجين الكلي الذائب ، لوحظ تناقصاً في المحتوى البروتيني والمحتوى الكلي للنيتروجين . ولقد لوحظ أن قيمة هذه التغيرات قد اعتمدت على تركيز كبريتات الصوديوم وعلى النبات المستخدم .