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Betaine status in wheat in relation to nitrogen stress and to transient salinity stress

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Summary Glycine betaine is readily accumulated in wheat (*Triticum aestivum* cv. Inia) shoots during periods of salinity stress. The ability of the plant to utilize betaine as a source of nitrogen remains unresolved. We, therefore, conducted solution culture experiments in a greenhouse to test the hypothesis that betaine is degraded in wheat shoots under conditions of severe nitrogen deficiency. Betaine concentrations increased in continuously salt stressed plants for only 17 days after salinity was imposed. After this period, concentrations (dry weight basis) decreased steadily until plants died 32 days later. Decreases in betaine concentration were also observed in treatments where salinity stress was removed. The rate of decrease in concentration was greatest in the N-free treatments. These decreases in betaine concentration were the result of dilution by plant growth. Betaine contents (μmol shoot^{-1}) remained unchanged after removal of substrate nitrate. Therefore our results support the hypothesis that betaine is a stable end product of metabolism.

Introduction

The quarternary ammonium compound, glycinebetaine, is actively accumulated by both halophytic and glycophytic species of the Gramineae during periods of environmental stress[11]. There is evidence that, once formed, betaine constitutes an inert end-product that is not further metabolized by the plant[4].

Ladyman *et al.*[9] monitored betaine in barley shoots for 12 days during two cycles of transient drought stress and observed a substantial decrease in betaine concentration in the rewatered plants. Since there was no reduction in total betaine content (μmol shoot^{-1}) throughout the drought-rewatering episodes, these investigators concluded that net degradation of betaine did not occur. The slight decrease in betaine concentration in the leaves of *Spartina alternifolia* following the relief of salt stress was attributed to dilution by plant growth[5]. In contrast, barley shoots not only resumed vigorous growth after the withdrawal of either salinity stress (NaCl) or simulated water deficit (polyethylene glycol) but also continued to synthesize and accumulate betaine at a rate such that the concentration remained

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constant[1]. Hanson and Nelsen[8] found betaine contents of barley leaves (μmol leaf^{-1}) decreased 75% after removal of water stress. The experiment, however, was not designed to differentiate between actual betaine degradation and betaine translocation from the leaf blade. In these investigations, betaine was monitored for relatively short post-stress periods (4–6 days). Furthermore, studies have not included experiments to test the possibility of betaine degradation under severe nitrogen stress.

The objectives of our study were: 1) to evaluate the effects of transient salinity stress on the growth, yield, betaine concentration and total betaine content in Inia wheat and, 2) to determine if, after removal of the stress, betaine can then be degraded by wheat grown in a nitrogen deficient environment. To confirm net degradation of betaine, total content in the shoot must significantly decrease after removal of substrate NO_3^-.

Materials and methods

Plant culture

Wheat seeds (*Triticum aestivum* cv. Inia) were germinated on paper saturated with 0.5 m*M* $CaSO_4$. Two-day-old seedlings were placed on cheesecloth supported between two plastic grids with 1.7 cm^2 openings. The seedlings, separated by the grid partitions, were covered with moist vermiculite. The grid assemblies were transferred to the greenhouse and supported over plastic pots containing 28-liters of aerated nutrient solution. The composition of the nutrient solution was 2.5 m*M* $Ca(NO_3)_2$, 3 m*M* KNO_3, 1.5 m*M* $MgSO_4$, 0.17 m*M* KH_2PO_4, 50 μ*M* Fe (as sodium ferric diethylenetriamine pentaacetate), 23 μ*M* H_3BO_3, 5 μ*M* $MnSO_4$, 0.4 μ*M* $ZnSO_4$, 0.2 μ*M* $CuSO_4$, and 0.1 μ*M* H_2MoO_4. The pH of the solution was maintained between 5.5 and 6.5 with H_2SO_4 and KOH. Six days after germination, the plants were thinned to about 100 seedlings per pot. At this time, solutions were salinated with NaCl:$CaCl_2$ (2:1 on a molar basis) at a rate of 19 mmol l^{-1} day^{-1}, which was calculated to reduce the osmotic potential (OP) of the nutrient solution 0.1 MPa day^{-1}. All solutions were changed weekly.

Experimental design

Experiment I. The first experiment was conducted during March and April 1982. Three treatments were divided among ten pots; two unsalinated controls, four were continuously stressed at – 0.8 MPa, and four were released from – 0.8 MPa stress. Salinity stress was relieved 14 days after salination began at a rate of 0.2 MPa day^{-1} until these cultures consisted solely of the nutrient solution. Shoots were harvested 7, 10, and 14 days after salination was initiated. Harvesting was continued weekly for an additional six weeks. Number of plants harvested was reduced from 10–20 to 5–10 per pot for the last five sample periods.

Experiment II. The second experiment was conducted during March and April 1983. Five treatments were replicated three times. In addition to the three treatments described in experiment I, two additional treatments consisting of control plants and salt stressed plants that were transferred to a N-free medium on the day salinity stress relief began were studied. The culture solution was similar in composition to that described above, but an equivalent amount of sulfate was added to replace the nitrate. The time schedule of the experimental events (salination, stress relief, *etc.*) was as outlined for experiment I, however, plants were salinated to – 0.6 MPa.

Table 1. F-values from two-way analyses of variance of heads/plant and dry weights of both heads and shoots

Treatments compared	Heads/plant	Head dry wt	Shoot dry wt
C and SR	8.49*	4.51*	3.24
S and SR-N	–	–	7.70**
C-N and SR-N	–	–	38.77***

*, **, *** Denote statistical significance at the 5, 1, and 0.5% confidence level, respectively.
– Statistics not conducted.

Hereinafter, treatments will be referred to as: 1) controls (C), 2) controls less nitrogen (C-N), 3) stress release (SR), 4) stress release less nitrogen (SR-N), and 5) continuous stress (S).

Betaine and nitrogen determination

Harvested shoots were weighed and oven-dried at 75–80°C. Samples were ground in a blender and stored in glass vials. Betaine was determined from aqueous extracts of the dry-ground plant material by the modified periodide assay[7]. Total nitrogen was determined by the standard micro-Kjeldahl procedure.

Results and discussion

In both experimental years, the growth of Inia wheat was severely limited by continuous salt stress and the weakened plants died prematurely. When the osmotic potential of the root media was — 0.8 MPa, the plants survived for 35 days; at — 0.6 MPa, for 49 days. At the final harvest of both experiments, the dry weight of the stressed shoots was 14% of the unsalinized control shoots. Since, in general, plant response was similar for both years, only the results of plant growth from experiment II will be presented in detail.

During the early period of growth, Inia wheat was subjected to moderately severe salt stress for two weeks. The stress was then relieved gradually over a three-day period to minimize osmotic shock. The plants recovered without injury except for moderate tip burn on the lower leaves. Vigorous shoot growth ensued, and the growth curve paralleled that of the unstressed control plants (Figure 1). However, shoot dry weights of the two treatments were not significantly different (Table 1). In a study of various salinity regimes on the growth of bean plants, Meiri and Poljakoff-Mayber[10] observed a large increase in leaf area that occurred within 1–2 days following desalination. In wheat, the growth response to stress relief was less abrupt. The relative difference in growth rates of wheat between the control and the SR plants decreased following the removal of stress until day 30; growth rates were equal thereafter. Thus accelerated growth followed the relief of salinity stress. Shoot growth was noticeably reduced after

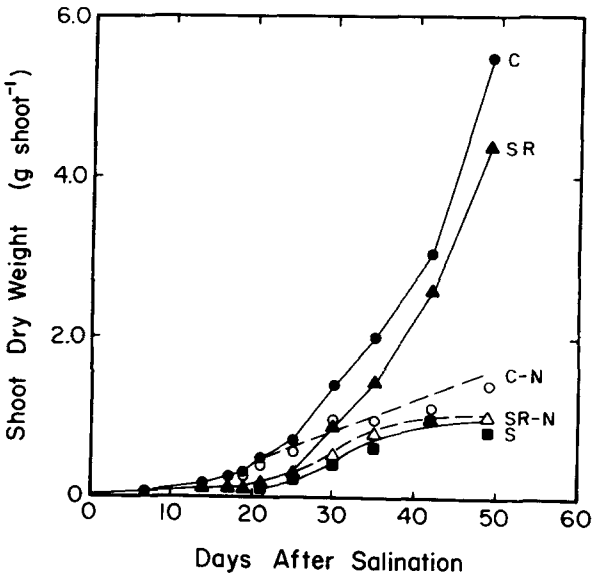

Fig. 1. Effect of salinity and nitrogen stress on dry matter production in Inia wheat shoots. C, control; C-N control less nitrogen; SR, stress release; SR-N, stress release less nitrogen; S, continuous stress.

removal of substrate NO_3 while the growth rates, in general, remained unchanged. Average growth rates for C-N and SR-N treated plants after removal of substrate N were 0.036 and 0.030 g dry wt plant^{-1} day^{-1}, respectively.

Visual observations made three weeks after relief of stress revealed that leaves of SR plants were darker green, wider, and somewhat less erect than those of control plants. In both experiments, the short period of stress also stimulated reproductive growth with respect to the time of head development as well as to the number of heads produced. The enhancement of reproductive growth by salinity has been observed in field studies with safflower[6] as well as wheat[3]. The dry weight of the immature heads from the SR plants was significantly greater ($\alpha < 0.05$) than from the control plants (Tables 1 and 2). The larger weight values were due to the greater number of heads as the absolute weight of each head was equivalent for both treatments. This effect was observed in both experiments.

On the 7th day following salination, betaine concentrations in the stressed shoots were about three-fold higher than in the unstressed control shoots. In the absence of salinity, betaine concentrations were between 20–30 μmol g^{-1} dry wt during the first three weeks. The betaine concentrations in shoots grown in complete nutrient

Table 2. Effect of salt stress release on head development of Inia wheat

Days after initiation of salination	Heads plant^{-1}				Head dry wt (g plant^{-1})			
	42	49	56	63	42	49	56	63
Experiment I								
Control	1.3	1.6	2.0		0.26	0.51	0.9	
Stress release (– 0.8 MPa)	1.7	1.9	2.9		0.41	0.76	1.4	
Experiment II								
Control	0.3	1.5	3.1	4.6	0.06	0.49	1.1	2.9
Stress release (– 0.6 MPa)	0.9	3.0	4.5	5.7	0.24	0.80	1.7	3.5

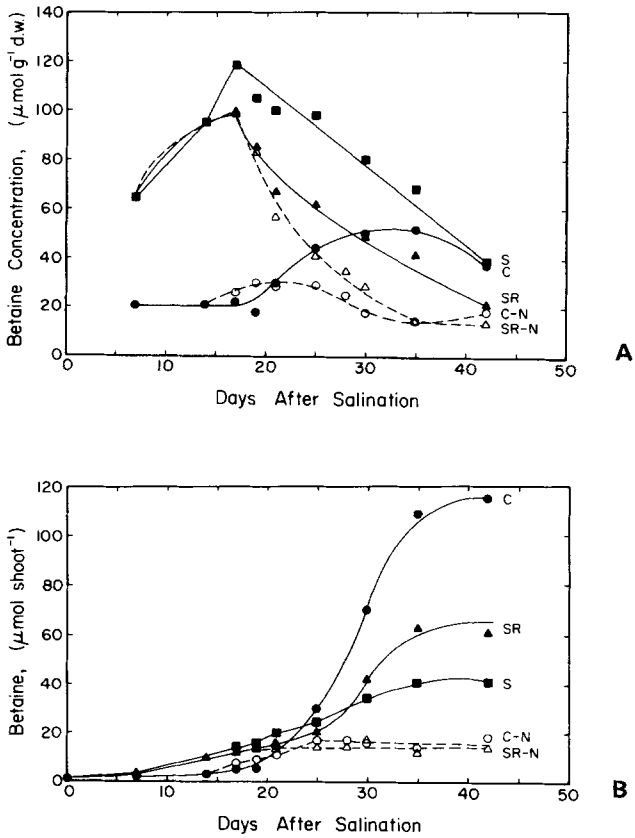

Fig. 2. Time course study of betaine accumulation in Inia wheat shoots. C, control; C-N, control less nitrogen; SR, stress release; SR-N, stress release less nitrogen; S, continuous stress.
A. Betaine concentration, μmol g^{-1} dry weight.
B. Betaine content, μmol shoot^{-1}.

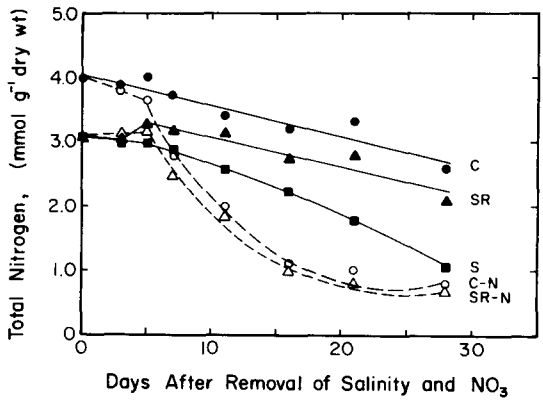

Fig. 3. Time course study of the effect of salinity and nitrogen stress on the total nitrogen content in Inia wheat shoots. C, control; C-N, control less nitrogen; SR, stress release; SR-N, stress release less nitrogen; S, continuous stress.

solution (C) then gradually increased, while the concentration decreased in those shoots grown in the N-free cultures (C-N).

Betaine in the shoot tissue of continuously stressed plants (S) reached the highest concentration (118 μmol g^{-1} dry wt) by the 17th day following salination (Figure 2a). At this time, stress was completely relieved and the betaine in the shoots subjected to both SR and SR-N treatments peaked at 100 μmol g^{-1} dry wt.

After day 17, concentrations in the continuously stressed shoots decreased at a rate of about 3 μmol g^{-1} day^{-1}. Relief of stress caused an even greater reduction in betaine concentration, and this effect was further enhanced by an inadequate supply of nitrogen in the root media, *i.e.*, on the SR-N treatment. This decrease in betaine concentration was probably the result of dilution by plant growth. The unsalinated control shoots of Inia wheat also exhibited elevated betaine concentrations. This increase occurred approximately four-weeks after germination and well in advance of head appearance. Enhanced betaine accumulation has been associated with aging in barley leaves particularly during the earing stage[2].

Total N in wheat shoots significantly decreased ($\alpha < 0.005$) with imposed stress (both salinity and withdrawal of substrate NO_3) as well as with time under treatment (Fig. 3). The effect of salinity on suppressing total N in Mexican dwarf wheat has been observed earlier[12]. Before salinity stress was relieved and NO_3 removed from the media, salinity-treated shoots contained 78% of the total N of the non-salinated controls. As expected, total N concentration was lowest in plants on

-N cultures; differences between C-N and SR-N were small, yet statistically significant ($a < 0.005$).

The betaine content (μmol shoot^{-1}) of the C-N and SR-N treated plants did not decrease significantly after nitrate was removed from the substrate (Fig. 2b). Linear regression correlation coefficients (r) for both treatments were positive (0.309 and 0.059, respectively) from days 25 to 42. Apparently, wheat is incapable of utilizing betaine as a source of available N even under conditions of severe N-stress. Furthermore, our results indicate that betaine represents only a small fraction of the total nitrogen present in Inia wheat shoots. Thus betaine degradation would make an insignificant contribution to the nitrogen status of the plants. These results, therefore, support but do not confirm the hypothesis of Bowman and Rohringer[4], that betaine is an inert end-product of metabolism.

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References

1 Ahmad N and Wyn Jones R G 1979 Glycinebetaine, proline and inorganic ion levels in barley seedlings following transient stress. Plant Sci. Letters 15, 231–237.
2 Ahmad N and Wyn Jones RG 1982 Tissue distribution, proline and inorganic ions in barley at different times during the plant growth cycle. J. Plant Nutrition 5, 195–205.
3 Ayers A D, Brown J W and Wadleigh C H 1952 Salt tolerance of barley and wheat in soil plots receiving several salinization regimes. Agron. J. 44, 307–310.
4 Bowman M S and Rohringer R 1970 Formate metabolism and betaine formation in healthy and rust-affected wheat. Can. J. Bot. 48, 803–811.
5 Cavalieri A J 1983 Proline and glycine-betaine accumulation by *Spartina alterniflora* Loisel. in response to NaCl and nitrogen in a controlled environment. Oecologia (Berlin) 57, 20–24.
6 Francois L E 1964 Salt tolerance of safflower. Agron. J. 56, 38–40.
7 Grieve C M and Grattan S R 1983 Rapid assay for determination of water soluble quarternary ammonium compounds. Plant and Soil 70, 303–307.
8 Hanson A D and Nelson C E 1978 Betaine accumulation and [^{14}C] formate metabolism in water-stressed barley leaves. Plant Physiol. 62, 305–312.
9 Ladyman J A R, Hitz W D and Hanson A D 1980 Translocation and metabolism of glycine betaine by barley plants in relation to water stress. Planta Berlin 150, 191–196.
10 Meiri A and Poljakoff-Mayber A 1970 Effect of various salinity regimes on growth, leaf expansion and transpiration rate in bean plants. Soil Sci. 109, 26–34.
11 Storey R, Ahmad N and Wyn Jones R G 1977 Taxonomic and ecological aspects of the distribution of glycinebetaine and related compounds in plants. Oecologia (Berlin) 27, 319–332.
12 Torres B C and Bingham F T 1973 Salt tolerance of Mexican wheat. I. Effect of NO_3 and NaCl on mineral nutrition, growth, and grain production of four wheats. Soil Sci. Soc. Am. Proc. 37, 711–715.