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Response of Maize Leaf Sucrose Phosphate Synthase to Salinity

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Abstract: Crops grown in salt affected soils may suffer from drought stress, ion toxicity and mineral deficiency leading to reduced growth and productivity. In this work, the effect of three NaCl levels (30, 60 and 90 mM) on growth, sucrose levels as well as SPS activity of Maize were investigated. Growth of seedlings was sensitive to salinity, as indicated by relative growth rate (RGR) which was inhibited to about 50% in plants subjected to 90 mM NaCl. Salinity at 30,60 and 90 mM NaCl caused a significant increase in sucrose contest throught the experimental period, also sucrose phosphate synthase activity in leaves was increased after salt treatments.The results suggest that the increased carbon remobilization, indicated by increased sucrose levels, is attributed to the enhanced SPS activity when Maize was subjected to salt stress.

Keywords: Maize, leaf sucrose, phosphate synthase and salinity

INTRODUCTION

Salinity is a major problem in today's irrigation agriculture, as millions of tons of salt are annually dumped on to the soil from the irrigation water. For crop plants differences in salt resistance exist not only among different genera and species, but even within a species which may on the whole be considered salt sensitive[4,9]. The reduction in yield of many crops by salinity is well documented[8,5,7]. The growth of plants may be reduced under salt stress because of (a) an osmotic stress due to lowering of the external water potential or (b) effects of specific ions on metabolic processes ranging from the absorption of nutrients to enzyme activation or inhibition. The ability to synthesize sucrose is a widespread, possibly universal, characteristic of higher plants cells. Sucrose is derived from hexose phosphates through the combined activities of UDP-glucose pyrophosphorylase (E.C 2.7.7.9), sucrose phosphate synthase (E.C 2.4.1.14) and sucrose phosphatase (E.C 3.1.3.24). Sucrose-p synthase appears to be a major control point in sucrose formation during photo assimilation of CO_2, it catalysis the reaction: UDP-glucose + fructose 6+ phosphate ® sucrose 6-phosphate + UDP.

This study was designed to examine the role of sucrose in maintaining the cellular osmotic equilibrium of Maize during salt stress. The results strongly support the conclusion that SPS is an important control point in the regulation of sucrose synthesis.

MATERIALS AND METHODS

Plant materials: Maize (*Zea mays* L., CV 875) seeds were germinated in plates covered by two layers of sheath clothes, then planted in 10x10x15-cm plastic pots containing lecaton (f2-4mm) as a soil additive. The pots were placed in a large plastic bowl which holds 20 pots. The irrigation liquid is placed in the bowl to a definite mark and was always completed when necessary. Seedlings were grown in growth chamber with irradiance 60 w/m^2, temperature (27±2°C), RH (60±5%), photoperiod (12-h d/12-h night cycle) and watering with half strength Hoagland nutrient solution; salt treatment started seven days after panting.

Sodium chloride was added in four concentrations of 0, 30, 60 and 90 mM to the nutrient solution. These corresponded to electrical conductivities of 0.01, 2.83, 4.82 and 6.03 dSm^{-1} respectively the control was irrigated with NaCl-free nutrient solution.

Relative Growth Rate: In order to monitor the potential for growth under stress, relative growth rates were determined weekly for the 21 days of salt treatment. Harvests were made at weekly intervals consisting of 5 seedlings per treatment. At harvest, roots were rinsed briefly through several changes of dist. H_2O. The harvested plants were dried at 68°C and dry weights were obtained. The relative growth rate was determined according to the formula of West *et al.,*[12].

$$RGR = \frac{InW_2 - InW_1}{t_2 - t_1}$$

Where W_1 and W_2 are dry weights obtained from the first and second harvests, respectively, and t_2-t_1 is the time interval in days between those harvests.

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The RGR represents that growth obtained relative to the amount of tissue present, expressed as a daily average. This is a reflection of growth potential under the conditions imposed.

Determination of Sucrose Content: This was made by the enzymatic test as described by Bergmeyer and Bernt (1974). The method is used for the determination of glucose, fructose and sucrose.

Enzyme Assay: Fresh leaf material was ground in cold mortar and pstle in an extraction buffer (8 mL buffer/g fresh weight) containing 25 mM Hepes/KOH (pH 7.5), 5 mM $MgSO_4$, 2 mM sodium diethyldithio carbamate, 2 mg/mL PVP (soluble), and 5 mM freshly added b-mercaptoethanol. The extract was filtered through eight layers of cheesecloth and the filtrate was centrifuged at 12.000 rpm for 10 min. A 1-mL aliquot of supernatant was passed through a prespum 5-mL G-50 Sephadex column packed into a 5-mL syringe. This column was centrifuged at 475 g for 30S, and the eluant was used for enzyme assay.

Sucrose–p synthase was assayed by measurement of sucrose produced from fructose 6-p plus UDPG. The reaction mixture contained 250 mL leaf extract and 50 mL 100 mM fructose 6-p, 100 mM UDPG, 40 mM Hepes/KOH (pH 7.5) and 10 mM $MgSO_4$. Mixtures were incubated at 25°C and the reactions were terminated by mixing a 50-mL aliquot of enzyme preparation with 50 mL of 30% KOH. Hexoses were destroyed by placing the tubes in boiling water for 10 min. The tubes were cooled, 1 mL of anthrone reagent (76 mL H_2SO_4, 30 mL H_2O and 150 mg anthrone) was added, the mixture was incubated at 37°C for 20 min, and the A_{620} was measured.

Determination Protein Content: Protein content was determined using coomassive Brilliant Blue G-250 (Bio-rad reagent) as described by Bradford[2].

RESULTS AND DISCUSSIONS

Results:

Maize Sucrose p-synthase: Leaf samples were taken at one week interval to measure SPS activity. The extractable SPS activity of the control plants increased slightly through the experiment period. The activity of SPS in extracts prepared from Maize leaves was essentially constant throughout the day; average enzyme rates during day 7 of treatment were about 7.3 m mol product (mg protein main)$^{-1}$ in control plants and increase slightly (15%) at the end of experiment (Table 1).

Salt treatment resulted in an increase in sucrose p-synthase activity throughout the whole experimental period. The increase in SPS activity in seedlings exposed to 90 mM NaCl for 2 weeks was about 42% of the control plants.

Relative Growth Rate: The calculated PGR values are presented in table 2. In addition, the RGR values obtained for plants under salt stress are expressed as percentage of the controls to reflect the inhibition of growth potential caused by this treatment. The data show that plants grown under high NaCl conc were significantly more suppressed overall in relation to the control especially in the 3rd week.

The change in sucrose content of Maize leaves in response to salinity induced by different NaCl conc. (0, 30, 60, and 90) is shown in Fig (1). The sucrose content increased significantly in response to salinity. At the end of experiment sucrose content in Maize treated with 90 mM NaCl reached nearly 40% of the control plants.

Discussion: Recently, attention has focused on the potential regulatory role of sucrose phosphate synthase. Lines of evidence suggest that this enzyme may contribute to the control of sucrose production in leaves, as there is a close correlation between the rate of sucrose synthesis and the extractable activity of sucrose phosphate synthase[6].

This correlation is observed between species, between genotypes within a species, and in plants in which the rate of sucrose synthesis is experimentally manipulated by varying factors such as photoperiod, temperature, salinity and nutritional status. In this work, an enhanced SPS activity was closely associated with an increase of sucrose accumulation in leaves of Maize subjected to different concentrations of NaCl. The result supports the proposal that SPS is a key enzyme in the synthesis of sucrose, the transported form of carbon assimilates in plants. These results agrees with the findings of Yang *et al.*[13], who concluded that the enhanced SPS activity was closely correlated with the increase of sucrose accumulation in stems of rice.

Recent work by Chen *et al.*[3], showed that, in the presence of NaCl, the production of total carbohydrates decreased whereas cellular reducing sugars, water-soluble sugars and sucrose content and SPS activity increased in *Microcoleus vaginatus* reaching a maximum in the presence of 200 mM/L NaCl. The growth of Maize was impaired by salt stress as indicated by the relative growth rate (RGR). Part of this growth inhibition may have been due to a greater osmotic shock, reduced leaf expansion or other factors. We believe that in this case, salinity affected general metabolism, causing reduces photosynthesis.

Table 1: SPS activity of Maize seedlings subjected to different NaCl concentrations in the nutrient medium. Measurements were taken at 7, 14 and 21 days after imposing stress.

Treatment (salt conc. mM)	Sucrose-p synthase activity (nmol sucrose. Min^{-1}. mg $protein^{-1}$)		
	DAS		
	7	14	21
0	7.3±1.1	8.9±1.2	8.6±1.1
30	9.2±1.3	11.7±1.4	13.0±1.9
60	13.3±1.2	17.9±1.5	20.2±1.8
90	14.6±1.5	20.0±1.8	25.3±2.1

a- DAS days after salt treatment

b- Differences were significant in a one-tailed *t*- test at a = 0.05.

Table 2: Relative growth rate of maize grown in 0, 30, 60, 90 mM NaCl.

DAS	NaCl concentrations (mM)						
	0	30	%	60	%	90	%
7	0.0832	0.071	85	0.066	79	0.065	78
14	0.0982	0.085	87	0.073	74	0.057	58
21	0.108	0.094	87	0.082	76	0.053	49

*Control plants were grown in half strength Hoagland solution.

*DAS: days after salt treatment.

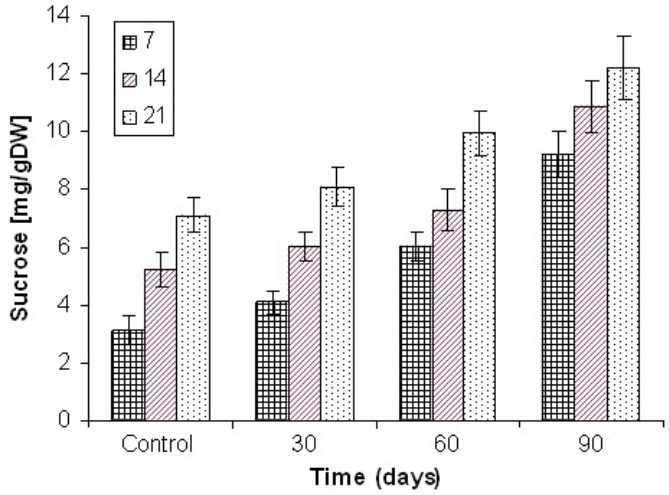

Fig. 1: Sucrose content of 7, 14 and 21 day old Maize seedlings grown under different salinity levels.

Growth of Maize was sensitive to salinity, as indicated by leaf dry weight and RGR. Reduction in dry weight of plant tissues reflects the increased metabolic energy cost and reduced carbon gain, which are associated with salt adaptation. It also reflects salt impact on tissues, reduction in photosynthetic rates per unit of leaf area and attainment of maximum salt concentration tolerated by the leaves[11]. The mechanisms of salt resistance in plants are largely unknown. Our understanding of the regulation of the only established pathway of sucrose production is limited almost exclusively to photosynthetic tissues. In Maize leaves, changes in the levels of metabolites when the rate of sucrose synthesis is varied indicate that this pathway is regulated, at least partly, by SPS. However, the relative contribution of this enzyme to the regulation of sucrose synthesis is still only poorly understood.

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