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Title

PEP-carboxylase activity supports organic acid metabolism of maize (*Zea mays*) under salt stress

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Introduction

Plant's reaction to salt stress is characterized by two phases (Munns 1993). The first phase is determined by osmotic stress whereas sodium toxicity leads to decreased growth rates in the second phase. Studies with newly developed resistant maize genotypes (Schubert and Zörb 2005) documented a better plant growth due to maintenance of cell wall acidification and thus cell extension growth (Pitann et al. 2009). Kumar (unpublished results) found an increase in PEP-carboxylase activity in young shoots of resistant maize hybrids but not in salt-sensitive species. PEP carboxylase (EC 4.1.1.31), which catalyzes the irreversible carboxylation of phosphoenolpyruvate, plays an essential role in C₄ carbon assimilation (Chollet et al. 1996). Furthermore, Davies (1973) assigned a central role to PEP-carboxylase in the plant pH-stat system. This study investigates the reason for enhanced PEP-carboxylase activity in maize in regard to salt stress during the first phase. To identify effects on C₄ carbon metabolism sucrose concentrations in maize were analyzed under two different light intensities and compared with results for wheat as a representative of C₃ plants. Effects on organic acid metabolism and pH regulation were evaluated by analyzing alkalinity and malate concentration.

Material and methods

Wheat (*Triticum aestivum* L. cv. Thasos) and maize SR 03 hybrid (*Zea mays*, Schubert and Zörb 2005) were grown in two climate chambers at 26°C for 16 h and 18°C for 8 h. Four replicates of each genotype were grown in nutrient solution under control conditions (1 mM NaCl) and saline conditions (100 mM NaCl) in parallel under 330 Wm⁻² and 200 Wm⁻² light intensity. On 13th day after germination, NaCl treatment was started with 25 mM NaCl and was raised in 25 mM NaCl increments every day till 100 mM NaCl concentration was reached on day 16. The plants were harvested on 20th day. The shoot fresh weight was determined immediately after the harvest. PEP-carboxylase activity was analyzed using an enzymatic method (Bender 1984). For sugar concentrations an enzymatic test combination from Biopharm was used. Alkalinity as a parameter of organic anions was analyzed with the method of Jungk (1968). The malate test-kit (Biopharm) was used for the quantification of malate concentrations.

Statistical analyses

Means and standard errors (SE) were calculated from four replicates. Significant differences between treatments were calculated using the t-test.

Results and discussion

Shoot fresh weight

Both the wheat genotype and the maize genotype showed a significant and comparable reduction in shoot fresh weight under saline conditions. For wheat a reduction by 30% was detected, whereas maize maintained a shoot fresh weight of 60% under salinity. Similar results were obtained for low-light conditions (data not shown). Since there were no significant differences in shoot weight reduction between wheat and maize it can be concluded that in the first phase of salt stress both genotypes exhibit salt resistance at a similar level. Fresh weight reductions under salinity suggest osmotic stress, which characterizes the first phase of salt stress (Munns 1993). Growth reduction can be ascribed to a reduced cell extension growth due to a lower apoplastic acidification caused by osmotic stress (Van Volkenburgh and Boyer 1985, Zörb et al. 2005, Pitann et al. 2009).

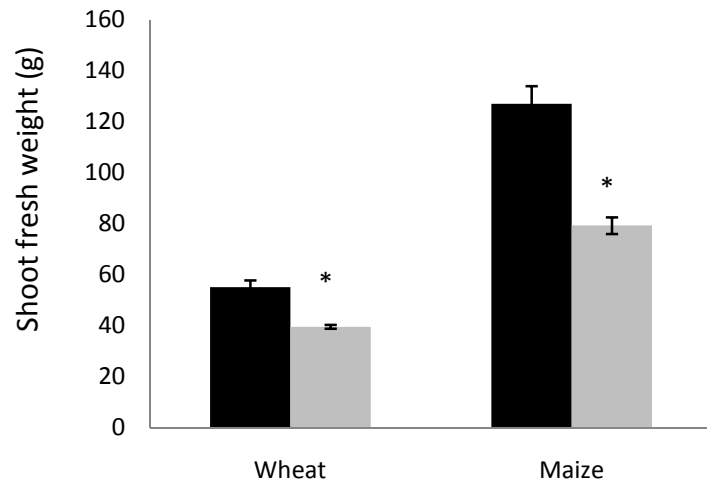


Fig. 1 Effect of salt stress on shoot fresh weights of wheat and maize under normal light conditions (330 Wm^{-2}). The values are means \pm SE, significant differences between treatments at $*P = 5\%$; black columns: control, gray columns: 100 mM NaCl treatment.

PEP-carboxylase activity

Under saline conditions Kumar (unpublished results) showed an increase in PEP-carboxylase activity in two different maize genotypes in the young shoots, whereas activity in old shoot parts and roots was identical for control and salt-stress conditions. For this reason, in this experiment, PEP-carboxylase activity and the following parameters are only shown for young shoots. Under control as well as under saline conditions the wheat genotype revealed no variation in PEP-carboxylase activity while the maize genotype showed a significant increase under salinity (Fig. 2). In the normal light treatment PEP-carboxylase activity in salt-treated maize plants reached about 160% relative to the control plants. Under low light condition similar results were obtained. Maize plants, which had grown under 200 Wm^{-2} showed a PEP-carboxylase activity nearly 140% of the control. An increase in enzyme activity only in maize led to the deduction that PEP-carboxylase activity may improve sugar and/or organic acid synthesis under saline conditions in maize but not in wheat.

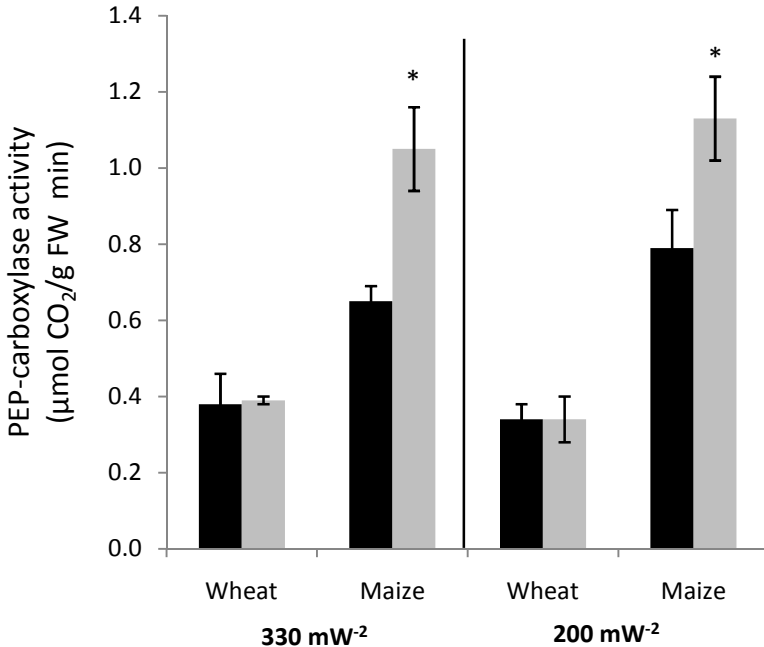


Fig. 2 Effect of salt stress on PEP-carboxylase activity of wheat and maize under normal (330 Wm⁻²) and low light conditions (200 Wm⁻²). The values are means ± SE, significant differences between treatments at *P = 5%; black columns: control, gray columns: 100 mM NaCl treatment.

Sugar concentrations

In the young shoot, both species showed a significant increase in sucrose concentration under saline conditions. As sucrose concentration in wheat rose up to 225% maize showed an increase up to 185% under salinity. Increased sugar concentration in salt-treated plants can be explained in terms of accumulation effects caused by water stress and reduced fresh weight production. This assumption agrees with results of Scherer et al. (1982) who found an increase of sugar concentrations in aerial parts of wheat plants under potassium deficiency. Schubert et al. (1995) found that water stress caused decreased dry weight production and increased sugar concentrations in shoots of alfalfa. Similarly, drought stress effected the accumulation of solutes in young leaves of grain legumes (Amede et al. 2003). It may be argued that increased PEP-carboxylase activity in maize under salt stress has a function in the C₄ metabolism to increase sugar concentration. However, wheat accumulated more sucrose and the effect was independent of light intensity although PEP-carboxylase was not increased (Fig.3). It can thus be concluded that light intensity of 200 Wm⁻² did not limit photosynthesis neither in wheat nor in maize.

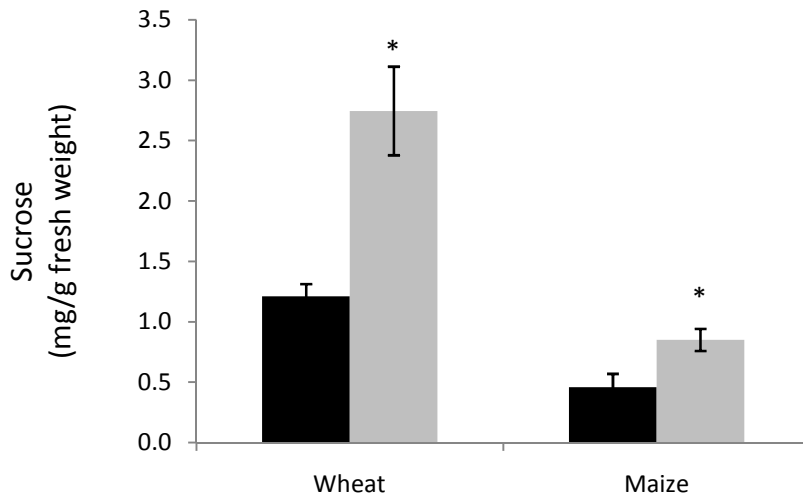


Fig. 3 Effect of salt stress on sucrose concentrations in wheat and maize under normal light conditions (330 Wm^{-2}). The values are means \pm SE, significant differences between treatments at * $P = 5\%$; black columns: control, gray columns: 100 mM NaCl treatment.

Alkalinity

In the young shoot, wheat exhibited a significant reduction in alkalinity under saline conditions (Fig. 4). Under normal light conditions a reduction of 80% was detected. In contrast, in comparison with control plants the 100 mM NaCl-treated maize plants showed no significant differences in alkalinity neither under normal light nor under low-light condition. Alkalinity is a parameter for the concentration of organic anions in plant tissue (Jungk 1968). As maize showed an increase in PEP-carboxylase activity under saline conditions and contrary to wheat exhibited no decrease in alkalinity it can be supposed that organic acid concentration is supported by enhanced enzyme activity in maize. Davies (1973) suggested PEP carboxylase as an essential part in the biochemical pH-stat system. Upon cytosolic pH increase, PEP is carboxylated to oxaloacetate, which is directly reduced to malate by malate dehydrogenase. Malate is therefore an important intermediate metabolite in the biochemical pH-stat system.

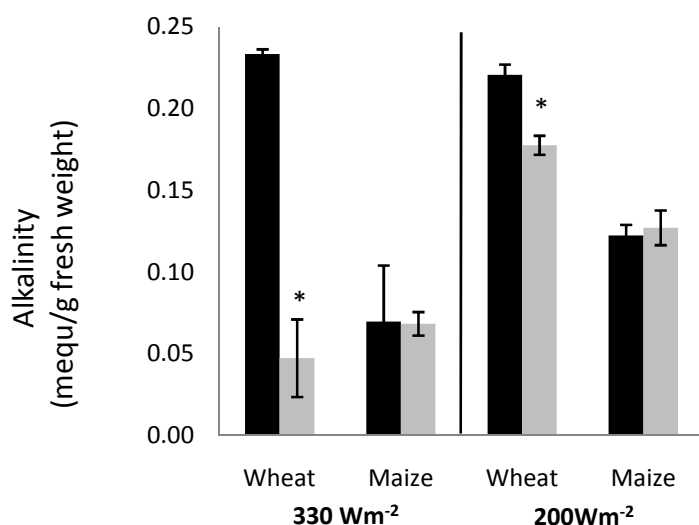


Fig. 4 Effect of salt stress on alkalinity in wheat and maize under normal (330 Wm⁻²) and low light conditions (200 Wm⁻²). The values are means \pm SE, significant differences between treatments at *P = 5%; black columns: control, gray columns: 100 mM NaCl treatment.

Malate

Malate showed a significant decrease in the young shoots of wheat under normal and low light conditions analogously to the alkalinity results. Compared to the control, malate concentration was reduced by about 55%. In maize, malate concentration was maintained both under low and high light intensity (Fig. 5).

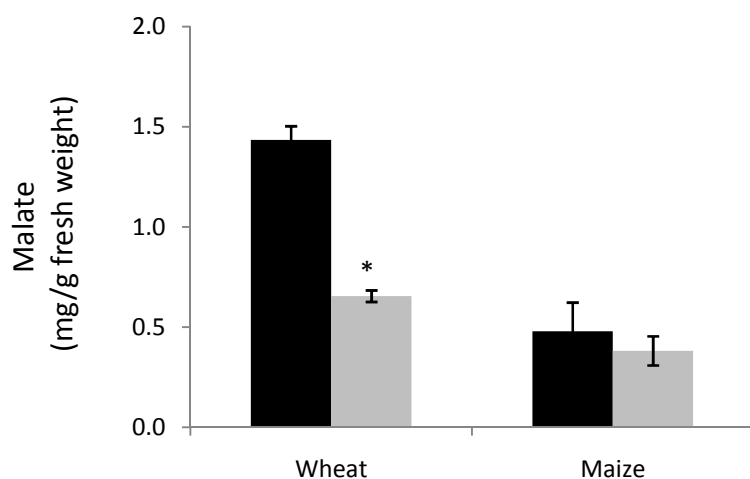


Fig. 5 Effect of salt stress on malate concentration in wheat and maize under normal (330 Wm⁻²) and low light conditions (200 Wm⁻²). The values are means \pm SE, significant differences between treatments at *P = 5%; black columns: control, gray columns: 100 mM NaCl treatment.

Conclusion

PEP carboxylase may have a function for the salt resistance of maize during the first phase of salt stress. The results indicate that it is not the C₄ pathway that requires higher PEP-carboxylase activity during salt stress but organic acid synthesis. The reason for organic anion requirement under salt stress remains obscure. Besides cytosolic pH regulation organic anions may serve as counter ions to prevent toxic chloride accumulation.

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