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Comparison of salt-induced osmotic adjustment and trigonelline accumulation in two soybean cultivars

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Abstract

21-d-old seedlings of the soybean (*Glycine max*) cvs. Essex and Forrest were treated with NaCl in a step-wise manner over 9 d (3 d 30 mM, 3 d 70 mM, and 3 d 100 mM) and maintained under 100 mM NaCl for an additional 14 d. During salt treatment, osmotic potential decreased more in cv. Forrest relative to cv. Essex. In non-stressed leaf tissue, cv. Forrest contained more trigonelline (TRG) relative to cv. Essex. During salt treatment, TRG amounts increased in cv. Forrest but were unchanged in cv. Essex. Both cvs. osmotically adjusted in response to salt stress; the maximal osmotic adjustment was 0.80 and 0.18 MPa in cv. Forrest and cv. Essex, respectively.

Additional key words: *Fabaceae*, *Glycine max*.

Introduction

Living cells, in order to maintain turgor, must be able to develop an osmotic potential at least equivalent to the osmotic potential exerted by the extracellular solution. In response to declining leaf water potential (ψ_w), many plant species experience a similar decline in osmotic potential (ψ_π) (Morgan 1984). To maintain osmotic balance under osmotic-stress, the majority of organisms rely upon low molecular mass organic solutes that are "compatible" with normal metabolism (Brown and Simpson 1972). A small number of molecules, in both prokaryotic and eukaryotic cells, function as organic compatible solutes and include sugars, amino acids, and

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Abbreviations: OA - osmotic adjustment; QAC's - quaternary ammonia compounds; RWC - relative water content; TRG- trigonelline; ψ_π - osmotic potential.

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methylamines such as trigonelline (for reviews see Crowe *et al.* 1992, Csonka 1989, Rhodes and Hanson 1993).

Trigonelline (TRG), nicotinic acid betaine, has been shown to stabilize enzyme activity *in vitro* (Shomer-Ilan *et al.* 1991) and is postulated to function as a compatible solute in response to salinity- and water deficit-stress (Shomer-Ilan *et al.* 1991, Naidu *et al.* 1992). TRG is present in the seeds (Tramontano *et al.* 1986) and leaves (Tramontano and Jouve 1997) of *Glycine max*. We are interested in the biochemical responses of *G. max* to environmental stresses, particularly the accumulation of low molecular mass compatible solutes, with the long-term goal of genetically mapping the genes involved in trigonelline biosynthesis. The present experiments were undertaken to determine the variation of salt-induced TRG accumulation and osmotic adjustment between the contrasting soybean cultivars Essex and Forrest.

Materials and methods

Plants: The soybean [*Glycine max* (L.) Merr.] cultivars Essex (Smith and Camper 1973) and Forrest (Hartwig and Epps 1973) were obtained from Dr. D.A. Lightfoot (Southern Illinois University, Carbondale, IL, USA). Soybean seedlings were grown under controlled conditions (14-h photoperiod, day/night temperature 29/23 °C, irradiance of 200 μmol m^{-2} s^{-1} from a mixed light source), watered daily and fertilized once per week with a modified Hoagland's solution (Wood *et al.* 1996). 21-d-old seedlings were treated with NaCl in a step-wise manner over 9 d in order to avoid salt-shock (3 d 30 mM, 3 d 70 mM, and 3 d 100 mM) and maintained under 100 mM NaCl for an additional 14 d. The V1 leaf (*i.e.* the first leaf beneath the first unfolded trifoliate leaf) was harvested on day 9, 16, and 23, and analyzed for TRG as described below.

Determination of water relations: Relative water content (RWC) was measured by placing leaf tissue into tared flasks containing distilled water and calculated according to the formula

$$\text{RWC} = [(\text{fresh mass - dry mass})/(\text{water saturated mass - dry mass})] \times 100.$$

A second leaf was sealed in a polyethylene freezer bag, frozen in liquid nitrogen and stored at -20 °C. Leaf tissue was thawed, centrifuged at 1 200 *g* for 25 min at 4 °C to extract cell sap. Osmotic potential (ψ_π) of the collected cell sap was measured using a *Wescor Model 5520* vapor pressure osmometer (*Wescor*, Logan, USA). Osmotic potential was corrected, according to Tyree (1976), for the dilution of symplastic sap by apoplastic water that occurs when expressed sap is collected from frozen and thawed tissue. Osmotic potential at maximum pressure potential ($\psi_{\pi 100}$) was calculated according to the formula

$$\psi_{\pi 100} = \psi_\pi (\text{RWC} - \text{AWC})/(1.0 - \text{AWC})$$

where AWC was 11 %. Osmotic adjustment was calculated as the difference between $\psi_{\pi100}$ values estimated for salt-stressed and non-stressed soybean leaves.

Extraction, isolation and analysis of trigonelline: Leaf tissue (0.1 - 0.5 g) was extracted in methanol at 4 °C in the dark, and phase separated by the addition of distilled water and chloroform (5:3:2, respectively v/v/v). The aqueous phase was removed, air-dried and resuspended in distilled water. TRG was purified by *Dowex-1-OH*$^-$ and *Dowex-50-H*$^+$ ion exchange chromatography. Reduced sugars and quaternary ammonia compounds (QACs) were eluted from *Dowex-1-OH*$^-$ with distilled water while amino acids were eluted by 2.5 M HCl. QACs (including TRG) and amino acids were eluted from a *Dowex-50-H*$^+$ column with 6 M NH_4OH. The purified fractions were air-dried and resuspended in 1 cm^3 of H_2O (for details see Wood *et al.* 1996). TRG was measured spectrophotometrically at 264 nm (UV-VIS spectrophotometer *Lambda 12*, *Perkin-Elmer*, Norwalk, USA) as described by Yuyama and Suzuki (1985) and quantified using authentic trigonelline standards (*Sigma*, St. Louis, USA). The identity of TRG was confirmed by plasma desorption mass spectrometry using a *BIOION 20R Plasma Desorption Mass Spectrometer* (*BIOION KB*, Uppsala, Sweden) as described Bonham *et al.* (1995).

Results and discussion

Water status of soybean seedlings in response to salinity-stress: Cultivars Essex and Forrest contrast for yield (Njiti *et al.* 1997), resistance to disease (Chang *et al.* 1997), manganese (Kilo and Lightfoot 1996) and aluminum toxicity (Miur and Sabbe 1993), and drought-tolerance (Miur and Sabbe 1993). Prior to salt treatment, 21-d-old seedlings were essentially indistinguishable based upon plant height (*ca.* 45 cm) and number of expanded leaves (11) (data not shown). RWC was measured on fully expanded leaves (Fig. 1*A*). Non-stressed plants of both cultivars maintained a RWC > 95 % throughout the experiment. RWC of salt-stressed plants was indistinguishable from non-stressed plants during the first 9 d. Over the next 14 d, RWC declined linearly for both cultivars and cv. Forrest maintained higher RWC relative to cv. Essex on both day 15 and day 23.

Trigonelline accumulation and osmotic adjustment: Prior to the initiation of stress, $\psi_{\pi100}$ was identical in both cultivars (Fig. 1*B*). During NaCl treatment, $\psi_{\pi100}$ decreased on both cvs. indicating an active accumulation of solute; cv. Forrest developed a lower ψ_π relative to cv. Essex. Shamad and Shadad (1997) have demonstrated that cv. Forrest can tolerate NaCl stress up to an ψ_π of -1.5 MPa. Under those conditions, the decrease in ψ_π was attributed primarily to the accumulation of soluble proteins, proline and calcium. The contribution of TRG, and other QACs, to osmotic adjustment (OA) in *G. max* was not determined in that study.

To more closely study the individual solutes contributing to OA, we analyzed soybean seedlings for the presence of QACs. PD-MS analysis demonstrated the only QAC prevalent in soybean is TRG (data not shown). In non-stressed leaf tissue, cv. Forrest contained more TRG relative to cv. Essex (Fig. 1*C*). During salt stress, TRG amounts were essentially unchanged in cv. Essex. In cv. Forrest, TRG amounts increased linearly to 500 μg g^{-1}(d.m.) (day 16) and then declined to amount indistinguishable from cv. Essex. The QACs glycine betaine and nicotinic acid betaine (TRG) are derived from choline and nicotinic acid, respectively (Rhodes and Hanson 1993). The biosynthesis of these compatible solutes is intimately linked to primary metabolism and TRG is considered a storage form of nicotinic acid. Unlike glycine betaine which is not appreciably catabolized in higher plants (Ladyman *et al.* 1980). TRG can be catabolized by demethylation to nicotinic acid. Under prolonged stress (16 - 23 d) in cv. Forrest, TRG content declined indicating an increased demand for nicotinic acid.

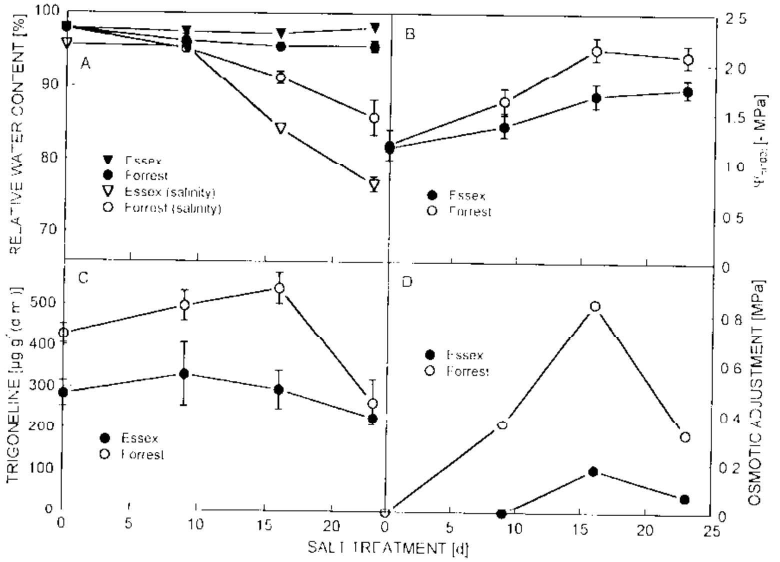

Fig. 1. Comparison of relative water content (RWC) (*A*), osmotic potential at maximum pressure potential ($\psi_{\pi 100}$) (*B*), trigonelline (TRG) accumulation (*C*), and osmotic adjustment (OA) (*D*) within 21-d-old soybean seedlings of cvs. Forrest (*open circles*) and Essex (*closed circles*) during salinity treatment. In A data of controls are also presented (*triangles*). Each data point represents the mean measurement on 3 individual plants and the *error bars* indicate SE.

The maximal OA was 0.80 MPa in cv. Forrest (16 d) and 0.18 MPa in cv. Essex (16 d) (Fig. 1*D*). In both cvs., OA declined during prolonged salt stress (16 - 23 d) and ψ_π remained constant (Fig. 1*B*) as RWC declined (Fig. 1*A*). Cells no longer actively accumulated additional solutes and OA decreased as RWC declined, withdrawing more water from the cell. TRG content increased in cv. Forrest (Fig. 1*C*) and accounted for 0.25 % of the maximal measured OA (16 d) (data not shown). TRG content did not increase in cv. Essex (Fig. 1*C*) but accounted for 5.1 %

of the maximal OA (day 16) (data not shown). The maximal OA of cv. Forrest is 4-fold greater than cv. Essex. TRG contributes to OA in soybean under salt stress. However, the ability of cv. Forrest to develop greater OA relative to cv. Essex is not due to an increased accumulation of TRG. The modest contribution of TRG to OA in salt-stressed soybeans suggests that its role as a compatible solute is limited unless TRG is sequestered within organelles or specific tissues. Targeted accumulation of compatible solutes may represent an important protective mechanism that allows sensitive cellular components to survive stress (Rhodes and Hanson 1993). An alternative hypothesis for the role of TRG in plant stress responses has been suggested by Berglund (1994). The biosynthesis of TRG, by the methylation of nicotinic acid, depletes the S-adenosyl-methionine pool and may serve as a hypomethylating reaction, thereby limiting oxidative stress-induced DNA methylation.

The physiological importance of OA as an adaptive trait to drought or salinity is still an open question (Hare *et al.* 1998). We are interested in evaluating the role of TRG in soybean yield stability under drought- and salt-stress by evaluating quantitative trait loci (QTLs). An cv. Essex × cv. Forrest F_5 derived population of 100 soybean recombinant inbred lines has been developed (Chang *et al.* 1997) that is ideal for identifying genes underlying the biochemical basis of yield, and yield under stress. The variation in TRG amount and OA in the parental lines of this population (*i.e.* cv. Forrest and cv. Essex), presented in this report, will allow the genetic mapping of loci associated with TRG accumulation. When compared with molecular map data, we will be able to identify loci conditioning high yields under stress and evaluate the role of TRG accumulation (and OA) in the phenotype.

References

Berglund, T.: Nicotinamide, a missing link in the early stress response in eukaryotic cells: a hypothesis with special reference to oxidative stress in plants. - FEBS Lett. **351**: 145-149, 1994.

Bonham, C.C., Wood, K.V., Yang, W.-J., Nadolska-Orczyk, A., Samaras, Y., Gage, D.A., Poupart, J., Burnet, M., Hanson, A.D., Rhodes, D.: Identification of quaternary ammonium and tertiary sulfonium compounds by plasma desorption mass spectrometry. - J. Mass Spectroscopy **30**: 1187-1194, 1995.

Brown, A.D., Simpson, J.R.: Water relations of sugar-tolerant yeasts: The role of intracellular polyols. - J. gen. Microbiol. **72**: 589-591, 1972.

Chang, S.J.C., Doubler, T., Kilo, V., Suttner, R., Klein, J., Schmidt, M.E., Gibson, P.T., Lightfoot, D.A.: Association of loci underlying field resistance to soybean sudden death syndrome (SDS) and cyst nematode (SCN) race 3. - Crop Sci. **37**: 965-971, 1997.

Crowe, J.H., Hoekstra, F.A., Crowe, L.M.: Anhydrobiosis. - Annu. Rev. Plant Physiol. **54**: 579-599, 1992.

Csonka, L.N.: Physiological and genetic responses of bacteria to osmotic stress. - Microbiol. Rev. **53**: 121-147, 1989.

El-Samad, H.M.A., Shadad, M.A.K.: Salt tolerance of soybean cultivars. - Biol. Plant. **39**: 263-269, 1997.

Hare, P.D., Cress, W.A., Van Staden, J.: Dissecting the roles of osmolytes during stress. - Plant Cell Environ. **21**: 535-553, 1998.

Hartwig, E.E., Epps, J.M.: Registration of Forrest soybeans. - Crop Sci. **13**: 287, 1973.

Kilo, V., Lightfoot, D.A.: Loci underlying resistance to manganese toxicity mapped in soybean recombinant inbred lines. - Soybean Genet. Newslett. **23**: 155-157, 1996.

Ladyman, A.R., Hitz, W.D., Hanson, A.D.: Translocation and metabolism of glycine betaine by barley plants in relation to water stress. - Planta **150**: 191-196, 1980.

Miur, J.H., Sabbe, W.E., Soybean nutrition on acid soils. - Agron. Abstr. **85**: 280, 1993.

Morgan, J.M.: Osmoregulation and water stress in higher plants. - Annu. Rev. Plant Physiol. **35**: 299-319, 1984.

Naidu, B.P., Paleg, L.G., Jones, G.P.: Nitrogenous compatible solutes in drought-stressed *Medicago* spp. - Phytochemistry **31**: 1195-1197, 1992.

Njiti, V., Schmidt, C., Schmidt, M., Lightfoot, D.A.: Mapping loci underlying yield in Illinois. - Soybean Genet. Newslett. **24**: 136-138, 1997.

Rhodes, D., Hanson, A.D.: Quaternary ammonium and tertiary sulfonium compounds in higher plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **44**: 357-384, 1993.

Shomer-Ilan, A., Jones, G.P., Paleg, L.G.: *In vitro* thermal and salt stability of pyruvate kinase are increased by proline analogues and trigonelline. - Aust. J. Plant Physiol. **18**: 279-286, 1991.

Smith, T.J., Camper, G.M.: Registration of Essex soybeans. - Crop Sci. **13**: 495, 1973.

Tramontano, W.A., McGinley, P.A., Ciancaglini, E.F., Evans L.: A survey of trigonelline concentrations in dry seeds of the *Dicotyledoneae*. - Environ. exp. Bot. **26**: 197-205, 1986.

Tramontano, W.A., Jouve, D.: Trigonelline accumulation in salt-stressed legumes and the role of other osmoregulators as cell cycle control agents. - Phytochemistry **44**: 1037-1040, 1997.

Tyree, M.T.: Negative turgor pressure in plant cells: fact or fallacy. - Can. J. Bot. **54**: 2738-2746, 1976.

Wood, A.J., Saneoka, H., Joly, R.J., Rhodes, D., Goldsbrough, P.B.: Betaine aldehyde dehydrogenase in *Sorghum bicolor*: Molecular cloning and expression of two related genes. - Plant Physiol. **110**: 1301-1308, 1996.

Yuyama, S., Suzuki, T.: Isolation and identification of N1-methylnicotinic acid (trigonelline) from rat urine. - J. Nutr. Sci. Vitamol. **31**: 157-167, 1985.