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Trigonelline concentration in field-grown soybean in response to irrigation

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Abstract

Trigonelline (TRG) is a conjugate of nicotinic acid, and is postulated to function as a compatible solute in response to salinity- and water deficit-stresses. TRG concentrations and several agronomic characteristics were measured under irrigated field and non-irrigated field conditions within 18 soybean (*Glycine max*) genotypes using leaves taken from different growth stages (vegetative, flowering and pod development). Under irrigation, relative water content (RWC) ranged from 90.0 to 99.6 %. Under non-irrigation, RWC ranged from 86.3 to 97.5 %. TRG concentration ranged from 364 to 555 μg g^{-1}(d.m.) under irrigation, and from 404 to 570 μg g^{-1}(d.m.) under non-irrigation. TRG concentrations increased in the majority of genotypes (15 of 18) under non-irrigation even though RWC did not significantly differ in many genotypes between treatments. TRG decreased as plants progressed to pod development and seed filling. Mean seed yield under non-irrigated conditions declined 55 % relative to the irrigated controls. TRG concentrations among all genotypes were significantly correlated with seed yield.

Additional key words: betaine, compatible solute, *Glycine max*, water deficit-stress.

Introduction

Trigonelline (TRG), nicotinic acid betaine, is the N-methyl conjugate of nicotinic acid and is synthesized by S-adenosyl-L-methionine nicotinic acid-N-methyltransferase (EC 2.1.1.7) (see Wood *et al.* 2000 for review). Many plant species accumulate organic compatible solutes, such as proline, sugar alcohols (polyols) and several different betaines, including TRG, when subjected to drought or salinity (Smirnoff and Cumbes 1989, Rhodes and Hanson 1994, Nuccio *et al.* 1999). These solutes, such as proline (Yoshiba *et al.* 1997) and glycine betaine (Sakamoto and Murata 2000), are postulated to protect plant growth and development.

The ability to synthesize TRG is widely distributed in cultivated *Glycine max* (Cho *et al.* 1999), and intracellular accumulation of TRG has been studied in various tissues of *G. max* by Evans and Tramontano (1984), Tramontano and Jouve (1997), and Cho *et al.* (1999). TRG is highly concentrated in leaves (Evans and Tramontano 1981, Evans and Tramontano 1984, Shomer-Ilan *et al.* 1991) as well as dry seeds (Tramontano *et al.* 1986) of *G. max*, *Vicia faba*, *Phaseolus vulgaris* and *Pisum sativum*. In response to germination, TRG is released from the seeds of seven *Medicago* species and is more abundant than other betaines such as stachydrine (Phillips *et al.* 1995). NaCl-stress has been shown to increase the TRG concentration in leaves of *G. max* (Tramontano and Jouve 1997, Cho *et al.* 1999) and *Medicago sativa* (Tramontano and Jouve 1997).

Crop improvement for drought- and salt-tolerance is a critical issue for the future of agriculture in the semi-arid and arid regions. 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 TRG biosynthesis (Cho *et al.* 1999, Wood *et al.* 2000, Pfeiffer *et al.* 2001,

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Abbreviations: RWC - relative water content; TRG - trigonelline.

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Cho *et al.* 2001). We have characterized alterations in TRG concentration at different soybean growth stages under consecutive irrigated and non-irrigated field conditions in order to examine the relationship between TRG concentration and agronomically important plant characteristics such as seed yield, and to evaluate the potential role of TRG in stress adaptation.

Materials and methods

Plants and field experiment: Six soybean (*Glycine max* L.) cultivars (Essex, Forrest, Hutcheson, Spry, Stressland and Young), two PIs (PI 416937 and PI 471938) and 10 recombinant inbred lines from the cross of Essex by Forrest ($F_{5:13}$; Njiti *et al.* 1998) (Table 1) were planted on July 13, 1999 in a randomized complete block design with three replicates at two environments. One test was performed under weekly based well-irrigated field conditions during the growing season from early vegetative (V3) to seed filling stages (RS) at the Southern Illinois University Agronomy farm (Carbondale, IL). Irrigation was started on July 30, 1999 two weeks before a first sampling for trigonelline concentrations. The other test was conducted under non-irrigated field conditions at the same location. Volumetric water contents of soils in irrigated and non-irrigated fields were measured weekly during vegetative and reproductive growth stages using time domain reflectometry (*Trase Systems Corp.*, Santa Barbara, USA) with a 30 cm long buriable waveguide designed for physical contact with the soil being measured (Stan *et al.* 1999). Two-row plots were used, with rows planted 0.75 m apart and 3.0 m long. Plots were planted with approximately 17 seeds m^{-1}. Data were recorded for seed yield, seed mass and other traits at approximately the same time in both environments.

RWC was measured by placing a fully expanded leaf tissue into tared flasks containing distilled water and calculated according to the formula RWC = (fresh mass - dry mass)/(water saturated mass - dry mass) × 100 (Wood *et al.* 1996).

Extraction, isolation and analysis of trigonelline: For determining TRG concentration leaf samples were taken at early vegetative (V4), flowering (R1) and full pod development (R4) stages from each plot in two environments. Approximately 0.5 g of fresh leaf tissue was extracted in methanol at 4 °C in the dark and quaternary ammonia compounds (QACs) were isolated as described by Cho *et al.* (1999). TRG was purified by *Dowex-1-OH⁻* and *Dowex-50-H⁺* ion exchange chromatography. Reduced sugar and quaternary ammonia compounds were eluted from *Dowex-1-OH⁻* with deionized water and amino acids eluted by 2.5 M HCl. The purified fractions were air-dried and resuspended in 1 cm^3 of H_2O. TRG was measured spectrophotometrically at 264 nm (UV-VIS spectrophotometer *Lambda 12*, *Perkin-Elmer*, Norwalk, USA), essentially as described by Yuyama and Suzuki (1985) and quantified using TRG standards (*Sigma*, St. Louis, MO, USA).

Data analysis: Statistical analysis was done using the *General Linear Models* (GLM) procedure (SAS Institute 1992). A separate analysis was done for each test, followed by a combined analysis. Data were analyzed for mean separations using Fisher's least significant difference (LSD) at the 0.05 level of probability. Pearson correlation coefficients were calculated to determine relationships among characters, using *PROC CORR* procedure.

Results

Relative water content under different field environments: Soil moisture contents ranged from 21 to 32 % during the growth stages V4, R1 and R4 under irrigation, while under non-irrigation they ranged from 9 to 28 % (Fig. 1). Soil moisture contents decreased sharply after August 9, 1999 under non-irrigated conditions and were clearly different between two fields. Significant differences in RWC between treatments (*i.e.* drought and irrigation) were found in early vegetative stage of two genotypes, Stressland and 80 EXF23. At the R1 stage in four genotypes (Stressland, EXF23, EXF76, EXF80) RWC ranged from 97.3 to 99.6 % and were significantly higher under irrigation relative to the lowest RWC under non-irrigation. Prior to the seed filling stage, none of genotypes (with the exception of EXF89) were significantly different for RWC compared to those at different stages (Table 1). RWC values at individual growth stages were significantly different in some genotypes (Table 1). Young had the lowest mean RWC across three growth stages among all 18 genotypes under both treatments (Table 1).

Trigonelline concentrations during development and in response to drought: At the V4 stage, TRG concentrations among genotypes ranged from 499 to 850 μg g^{-1}(d.m.) under irrigation and from 505 to

848 µg g^{-1}(d.m.) under non-irrigation (Fig. 2). TRG concentrations were significantly different for four genotypes (Forrest, Stressland, PI 416937 and EXF76) under non-irrigation as compared with irrigation. At the R1 stage (Fig. 2), TRG concentrations among the 18 genotypes ranged from 304 to 568 µg g^{-1}(d.m.) under irrigation, and from 320 to 605 µg g^{-1}(d.m.) under non-irrigation, demonstrating a general increase in TRG concentrations in response to drought. TRG concentrations significantly increased in four genotypes (Forrest, EXF7, EXF23, EXF44) under non-irrigation with Forrest having the highest concentration. At the pod development stage, TRG concentrations among the 18 genotypes ranged from 203 to 479 µg g^{-1}(d.m.) under irrigation, and from 240 to 480 µg g^{-1}(d.m.) under non-irrigation.

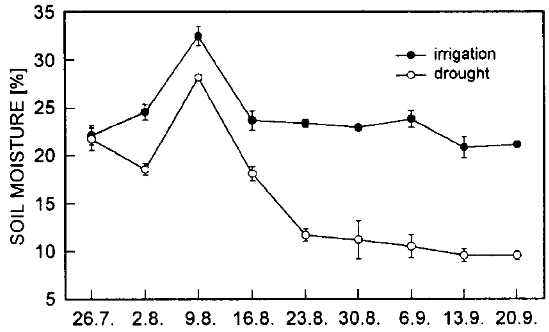

Fig. 1. Soil moisture content in irrigated and non-irrigated field conditions in southern Illinois during late-planted soybean growth.

Seed yield, seed mass and maturity: Mean maturity day across all genotypes was 118 d (range from 109 to 124 d) under irrigation, and 117 d (range from 108 to 121 d) under non-irrigation (Table 2). Three genotypes (Essex, Young and EXF89) were significantly different for maturity day when compared between the two environments. Among the 18 genotypes, seed mass significantly differed in only 3 of 18 genotypes. However, mean seed yields under non-irrigated field conditions declined 55 % relative to irrigated field conditions, and some genotypes decreased more than 130 %. In 14 of 18 genotypes, seed yields significantly decreased (Table 2). TRG concentration was correlated with seed yield in both irrigated and non-irrigated plants ($r = -0.23$; $P = 0.01$).

Table 1. Relative water content (RWC) [%] at early vegetative (V4) and reproductive (R1, R4) growth stages of soybean plants grown under irrigated and non-irrigated field conditions. Means ± SE, $n = 3$, * - significant at $P = 0.05$ within each soybean genotype under irrigation and non-irrigation.

Genotype	Irrigation V4	R1	R4	Non-irrigation V4	R1	R4
Essex (E)	96.7 ± 3.2	95.6 ± 3.8	96.5 ± 3.1	95.3 ± 1.0	97.1 ± 0.5	94.8 ± 2.7
Forrest (F)	95.6 ± 3.2	97.0 ± 2.1	95.8 ± 2.5	94.9 ± 0.7	96.6 ± 0.1	96.8 ± 0.5
Hutcheson	92.3 ± 1.6	94.1 ± 1.3	94.5 ± 3.5	96.1 ± 0.6	89.2 ± 4.4	94.1 ± 3.1
PI416937	96.3 ± 3.6	90.0 ± 1.5	95.6 ± 2.1	95.9 ± 0.5	89.6 ± 2.4	88.3 ± 4.2
PI 471938	93.6 ± 3.1	92.5 ± 3.7	92.6 ± 4.1	93.1 ± 2.8	94.4 ± 1.5	92.5 ± 3.9
Spry	94.8 ± 3.0	92.6 ± 4.8	92.5 ± 1.2	88.3 ± 3.5	95.5 ± 0.2*	89.3 ± 1.1
Stressland	97.6 ± 2.2*	97.3 ± 2.4*	95.3 ± 1.6	89.4 ± 2.8	95.1 ± 1.6	95.0 ± 2.0
Young	93.2 ± 1.0	88.3 ± 1.7	91.9 ± 0.6	89.0 ± 2.7	89.1 ± 0.3	92.1 ± 3.0
EXF7	95.6 ± 2.6	95.0 ± 3.7	95.2 ± 3.4	95.0 ± 1.6	92.3 ± 3.4	94.9 ± 2.9
EXF18	92.7 ± 1.4	93.5 ± 5.5	95.1 ± 4.8	93.2 ± 4.8	89.6 ± 0.2	90.0 ± 4.0
EXF23	99.5 ± 0.1*	98.7 ± 1.2*	96.4 ± 1.4	96.6 ± 0.7	90.6 ± 3.5	93.1 ± 3.0
EXF44	95.9 ± 4.0	93.9 ± 5.4	92.2 ± 4.9	92.8 ± 5.1	93.8 ± 3.9	94.5 ± 2.9
EXF59	95.3 ± 3.4	95.3 ± 2.5	98.1 ± 1.2	89.9 ± 2.5	92.7 ± 2.8	93.4 ± 4.1
EXF67	91.0 ± 4.5	92.6 ± 4.1	91.7 ± 1.6	88.1 ± 3.1	89.6 ± 4.1	96.9 ± 0.1
EXF76	96.3 ± 1.7	99.6 ± 0.1*	92.0 ± 1.4	95.4 ± 2.6	93.8 ± 1.6	96.7 ± 0.6
EXF80	93.7 ± 4.2	97.3 ± 1.9*	95.6 ± 3.8	88.4 ± 4.3	94.0 ± 0.6	86.6 ± 4.0
EXF85	95.2 ± 4.7	95.4 ± 3.7	97.9 ± 2.0	93.0 ± 2.6	89.4 ± 1.1	94.6 ± 2.8
EXF89	93.9 ± 3.3	95.0 ± 3.2	97.4 ± 1.5*	94.5 ± 3.4	94.2 ± 1.9	86.3 ± 4.5

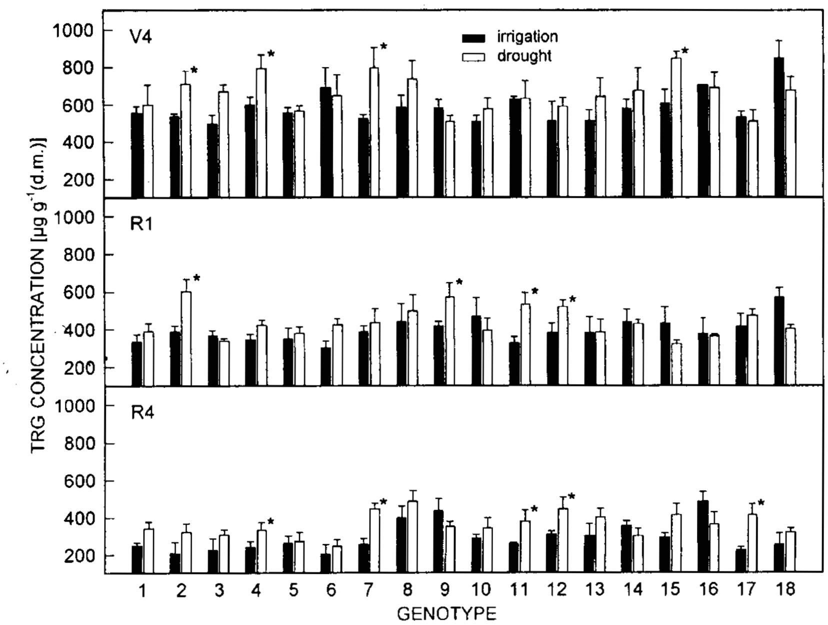

Fig. 2. Trigonelline (TRG) concentration in the combined and individual growth stages among 18 soybean genotypes in two environments. TRG concentration at the V4 stage, Rl stage, and R4 stage. * - significant differences at $P = 0.05$ as compared with the other environment. 1, Essex; 2, Forrest; 3, Hutcheson; 4, PI416937; 5, PI471938; 6, Spry; 7, Stressland; 8, Young; 9, EXF7; 10, EXF18; 11, EXF23; 12, EXF44; 13, EXF59; 14, 260 EXF67; 15, EXF76; 16, EXF80; 17, EXF85; 18, EXF89.

Table 2. Growth and seed characteristics in various soybean genotypes under irrigated and non-irrigated field conditions. Means ± SE, $n = 3$, * - significant at $P = 0.05$ within each soybean genotype under irrigation and non-irrigation.

Genotype	Maturity [d] irrigation	non-irrigation	Seed mass [g seed^{-1}] irrigation	non-irrigation	Seed yield [kg ha^{-1}] irrigation	non-irrigation
Essex (E)	123 ± 0.1*	120 ± 0.9	0.10 ± 0.003*	9.6 ± 0.7	4137 ± 416*	1720 ± 155
Forrest (F)	121 ± 0.9	119 ± 0.6	0.09 ± 0.004	9.3 ± 0.7	4096 ± 692*	2888 ± 624
Hutcheson	120 ± 1.3	121 ± 0.3	0.10 ± 0.003*	11.4 ± 0.3	3256 ± 948	2765 ± 603
PI416937	117 ± 2.1	116 ± 0.3	0.13 ± 0.008*	11.5 ± 0.1	2519 ± 724*	1167 ± 94
PI 471938	120 ± 2.1	120 ± 0.3	0.10 ± 0.009	10.2 ± 0.6	3533 ± 522*	1597 ± 395
Spry	111 ± 0.9	111 ± 1.2	0.12 ± 0.009	12.2 ± 0.4	4116 ± 216*	2949 ± 177
Stressland	109 ± 1.5	108 ± 0.6	0.12 ± 0.002	11.9 ± 0.2	3973 ± 74*	2294 ± 468
Young	124 ± 1.1*	121 ± 0.9	0.09 ± 0.008	8.3 ± 0.5	2150 ± 676*	819 ± 160
EXF7	121 ± 1.2	118 ± 1.2	0.10 ± 0.006	9.6 ± 0.5	5079 ± 753*	2314 ± 348
EXF18	116 ± 2.7	116 ± 0.9	0.11 ± 0.001	11.0 ± 0.2	4854 ± 403*	3338 ± 396
EXF23	112 ± 2.3	112 ± 1.2	0.10 ± 0.001	10.0 ± 0.2	5529 ± 492*	3461 ± 411
EXF44	121 ± 0.1	120 ± 0.6	0.10 ± 0.002	10.0 ± 0.1	5140 ± 357*	2683 ± 195
EXF59	117 ± 0.3	116 ± 1.3	0.10 ± 0.003	10.2 ± 0.5	5837 ± 530*	3645 ± 598
EXF67	121 ± 0.6	120 ± 0.9	0.08 ± 0.001	8.4 ± 0.1	3338 ± 550*	2621 ± 54
EXF76	119 ± 0.7	119 ± 0.6	0.10 ± 0.002	9.5 ± 0.4	3830 ± 143*	3195 ± 35
EXF80	118 ± 0.3	118 ± 1.2	0.09 ± 0.009	9.2 ± 0.2	2949 ± 908	2969 ± 74
EXF85	122 ± 1.2	119 ± 0.7	0.10 ± 0.001	9.8 ± 0.2	3440 ± 488	2888 ± 216
EXF89	120 ± 0.3*	118 ± 0.3	0.10 ± 0.006	9.6 ± 0.4	2765 ± 763	2150 ± 606

Discussion

We examined a collection of soybean cultivars, PI lines and recombinant inbred lines for developmental and drought-stress inducible alterations in TRG concentration under field conditions. Previous work has established the presence of TGR in leaves and salt-induced accumulation of TRG in soybeans grown under controlled conditions (Tramontano and Jouve 1997, Cho *et al.* 1999, Wood 1999). The ability to accumulate TRG is widely distributed in cultivated soybean (Cho *et al.* 1999). Under non-irrigation TRG concentrations increased in the majority of genotypes (15 of 18) even though RWC did not significantly differ in many genotypes between environments (see Table 1). Soil moisture content was a critical factor because non-irrigated plants maintained RWC but decreased yields of more than 35 %.

Three putative physiological roles for the *in vivo* function of TRG have been proposed: *1*) TRG as an osmoprotectant, *2*) TRG as a regulator of the cell cycle, and *3*) TRG as a storage form of nicotinic acid (Evans and Tramontano 1981, Wood 1999, Wood *et al.* 2000, Cho *et al.* 2001). The observed increase in TRG concentration is not in response to a decline in RWC and is therefore, by definition, not participant in osmotic adjustment (*i.e.* active accumulation of solutes in response to a decline in leaf water potential). The ability of TRG to specifically affect a cellular process (*i.e.* promote cell cycle arrest in G2) has been clearly demonstrated (Evans and Tramontano 1984, Evans *et al.* 1984, Lynn *et al.* 1982, Mazzuca *et al.* 2000, Tramontano *et al.* 1982, 1985). During development in all soybean genotypes analyzed TRG content declined, indicating an increased demand for nicotinic acid in primary metabolism, most likely as precursor for NADH, NADPH, and/or nucleotides. TRG translocation has been described in leguminous planu (Evans and Tramontano 1984, Evans *et al.* 1984). TRG stored in mature seeds is translocated to the root meristem of developing seedlings, and TRG that has accumulated in leaves during development is translocated to the developing pod and eventually to seeds during maturation. The *Arabidopsis thaliana AtProT2*, which encodes a proline transporter identified by functional complementation in yeast, has been shown to also efficiently transport TRG (Breitkreuz *et al.* 1999) and may mediate the export/import of TRG across the plasma membrane and play a key role in TRG translocation.

Leaf TRG concentrations among all genotypes were significantly, and negatively, correlated with seed yield and seed mass. Our results suggest TRG is acting as an inhibitor of cell division and/or storage form of nicotinic acid, rather than as a compatible solute. Detailed biochemical analysis may yield greater insight to the regulatory pathways common to both phenotypes. Metabolic engineering of the biosynthetic pathway to alter TRG accumulation (Nuccio *et al.* 2000, Cho *et al.* 2001) will allow the direct assessment of the ability of reduced TRG concentrations to ameliorate the deleterious effects of drought-stress upon plant productivity, and evaluate TRG biosynthesis as an adaptive response to enhancing vegetative drought-stress tolerance.

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