PERGAMON

Phytochemistry 52 (1999) 1235–1238

PHYTOCHEMISTRY

# Trigonelline concentrations in salt stressed leaves of cultivated *Glycine max*

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Received 12 August 1998; received in revised form 4 May 1999

**Abstract**

Concentration of trigonelline (nicotinic acid betaine) and relative water content were determined in nonstressed and salt stressed leaves of 30-day old seedlings of *Glycine max* among 17 cultivars representing eight maturity groups. Soybean seedlings were treated with NaCl in a step wise manner over 9 days (3 days 30 mM, 3 days 70 mM, and 3 days 100 mM). Trigonelline concentrations ranged from 63.8 to 162.4 μg g $DW^{-1}$ in nonstressed leaf tissue and from 75.4 to 218.7 μg g $DW^{-1}$ in salt stressed leaf tissue. During salt treatment, trigonelline concentrations increased in 10 cultivars, declined in five cultivars and remained unchanged in two cultivars. Trigonelline concentration was not significantly correlated with maturity group. 

*Keywords: Glycine max*; Fabaceae; Salt stress; Soybean; Trigonelline

## 1. Introduction

The ability to modify essential metabolic processes is key to adapting to adverse environmental conditions (Yancey, 1982). The active accumulation of solutes compatible with cellular metabolism is thought to play a central role in osmotic adjustment (Yancey, 1994). Trigonelline (nicotinic acid betaine), is the *N*-methyl conjugate of nicotinic acid and is synthesized by *S*-adenosyl-L-methinoine:nicotinic acid-*N*-methyltransferase (EC 2.1.1.7) (NNMT) (Upmeier, Gross, Koster & Barz, 1988). NNMT is a soluble enzyme that catalyzes the transfer of the methyl group from *S*-adenosylmethionine to nicotinic acid. Trigonelline has been shown to stabilize enzyme activity in vitro (Shomer-Ilan, Jones & Paleg, 1991), to act as a cell cycle regulator (Evans & Tramontano, 1984; Tramontano & Jouve, 1997) and is postulated to function as a compatible solute in response to salinity and water deficit stress (Shomer-Ilan et al., 1991; Naidu, Paleg & Jones, 1992). Trigonelline is present in the seeds, leaves, roots, stems and pods of *Glycine max* (Evans & Tramontano, 1984; Naidu et al., 1992; Tramontano, McGinley, Ciancaglini & Evans, 1986) and trigonelline concentrations were measured in various tissues of the soybean cultivar Amsoy 71 during seedling ontogeny (Shomer-Ilan et al., 1991). Trigonelline is known to accumulate in leaves of salt stressed *G. max* (Tramontano & Jouve, 1997). However, members of the Fabiflorae (including *G. max*, *Vicia faba*, *Phaseolus vulgaris* and *Pisum sativum*) have comparatively high trigonelline concentrations in both dry seeds (Evans & Tramontano, 1984; Tramontano et al., 1986) and nonstressed leaves (Shomer-Ilan et al., 1991; Evans & Tramontano, 1984).

The ability of *G. max*, as a species, to accumulate trigonelline has been clearly established (Evans & Tramontano, 1984; Tramontano & Jouve, 1997; Tramontano et al., 1986); however, the variation amongst cultivars within the species has not been addressed. *G. max* is photoperiod sensitive and varieties are placed into 13 maturity groups (i.e. 000 to *X*) based

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0031-9422/99/$ - see front matter 
PII: S0031-9422(99)00410-0

Table 1
Leaf RWC[a] and trigonelline concentration in 30-day old seedlings of 17 cultivars of *Glycine max* after nine days of salt stress (values are mean ± sd of three replicates)

| Cultivar | MG[b] | Relative water content (%) | | | | | Trigonelline concentration (μg gDW$^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control | | Treatment | | Ratio | Control | | Treatment | | Ratio |
| | | Mean | sd[c] | Mean | sd | | Mean | sd | Mean | sd | |
| Manitoba Brown | 00 | 90 | 7.4 | 89 | 9.5 | 0.99 | 137.3 | 5.1 | 218.7[d] | 75.5 | 1.59 |
| Mandarin | I | 91 | 9.1 | 89 | 7.1 | 0.98 | 95.1 | 18.4 | 138.1[d] | 31.1 | 1.45 |
| Richland | II | 96 | 0.7 | 89[d] | 4.1 | 0.93 | 86.8 | 15.0 | 97.9 | 4.8 | 1.13 |
| Dunfield | III | 98 | 2.1 | 92 | 3.3 | 0.94 | 87.4 | 23.0 | 75.4 | 11.6 | 0.86 |
| Illini | III | 92 | 8.7 | 90 | 7.3 | 0.98 | 92.4 | 4.2 | 149.2[d] | 41.4 | 1.61 |
| Lincoln | III | 96 | 5.2 | 98 | 4.1 | 1.02 | 162.4 | 9.0 | 182.3 | 23.7 | 1.12 |
| A.K. (FC30761) | IV | 96 | 1.5 | 89[d] | 4.6 | 0.93 | 100.0 | 9.0 | 87.7 | 17.4 | 0.88 |
| Patoka | IV | 94 | 7.8 | 91 | 5.7 | 0.97 | 112.1 | 7.4 | 101.7 | 17.5 | 0.91 |
| Union | IV | 92 | 6.7 | 89 | 10.2 | 0.97 | 93.2 | 24.7 | 147.8[d] | 55.6 | 1.59 |
| Essex | V | 93 | 4.0 | 97 | 0.5 | 1.04 | 106.0 | 14.4 | 123.1[d] | 5.8 | 1.16 |
| Forrest | V | 90 | 9.3 | 95 | 3.5 | 1.06 | 97.6 | 11.7 | 154.0[d] | 56.1 | 1.58 |
| Arksoy | VI | 92 | 4.1 | 91 | 0.5 | 0.99 | 131.3 | 28.8 | 131.2 | 24.8 | 1.00 |
| Haberlandt | VI | 92 | 6.0 | 91 | 6.3 | 0.99 | 114.3 | 33.5 | 83.7 | 10.4 | 0.73 |
| Ogden | VI | 98 | 5.1 | 89 | 9.8 | 0.91 | 140.2 | 29.3 | 136.4 | 29.5 | 0.97 |
| CNS | VII | 91 | 5.4 | 99[d] | 0.1 | 1.09 | 153.2 | 62.5 | 171.8 | 64.4 | 1.12 |
| Roanoke | VII | 95 | 3.4 | 92 | 3.0 | 0.97 | 93.3 | 13.6 | 93.4 | 2.9 | 1.00 |
| Tokyo | VII | 96 | 1.5 | 94 | 4.6 | 0.98 | 63.8 | 21.9 | 136.7[d] | 44.9 | 2.14 |
| Mean | | 94 | 2.6 | 92 | 3.5 | – | 109.8 | 26.5 | 131.1 | 38.6 | – |
| $LSD_{0.05}$ | | 6 | | 7 | | – | 50.1 | | 72.2 | | – |

[a] Relative water content = [(fresh weight − dry weight)/(turgid weight − dry weight)] × 100.
[b] MG: maturity group.
[c] sd: standard deviation.
[d] Means are significant at 0.05 level of probability as compared between control and treatment within each cultivar.

upon the length of darkness required to trigger the transition from vegetative to reproductive growth (Palmer, Hymowitz & Nelson, 1996). The present experiments were undertaken to determine the variation of trigonelline accumulation within cultivated *G. max* across eight maturity groups. We are interested in the biochemical responses of *G. max* seedlings to environmental stresses, particularly the accumulation of low molecular weight compatible solutes, with the long term goal of genetically mapping the genes involved in trigonelline biosynthesis. This survey will help define future physiological studies and, more importantly, allow the construction of recombinant inbred populations segregating for trigonelline accumulation.

## 2. Results and discussion

The trigonelline concentration in leaves of nonstressed and salt stressed seedlings of 17 soybean cultivars was determined at 30 days as described (see Section 3). A variety of soybean tissues are capable of accumulating trigonelline; however, Evans & Tramontano (1984) have demonstrated that trigonelline is detectable only in leaves of seedlings and preflowering plants (i.e. 20- to 50-days old). Prior to salt treatment (see Section 3), 21-day old seedlings of the cultivars were essentially indistinguishable based upon plant height (ca. 45 cm) and number of expanded leaves (11) (data not shown). The leaf relative water content (RWC) of nonstressed plants varied between 90 and 98% (Table 1). In response to a 9-day salt treatment, RWC of salt stressed plants varied between 89% and 99% and was indistinguishable from nonstressed plants.

All of the cultivars tested, which represented a broad spectrum of the genetic diversity within *G. max*, contained measurable amounts of trigonelline in 30-day old seedlings. PD MS analysis demonstrated that the only quaternary compound(s) prevalent in soybean were trigonelline ($m/z$: 138 $[M + H]^+$) and its adduct ion ($m/z$: 160 $[M + Na]^+$) (data not shown). In nonstressed leaves, mean trigonelline concentrations ranged from 63.8 to 162.4 μg g DW$^{-1}$, a 2.5-fold difference (Table 1). The mean value of the nonstressed concentrations was $109.8 \pm 26.5$ μg g DW$^{-1}$. In salt stressed leaves, mean trigonelline concentrations ranged from 75.4 to 218.7 μg g DW$^{-1}$, a 2.9-fold differ-

ence (Table 1). The mean value of the salt stressed trigonelline concentrations was $131.2 \pm 38.6$ μg g $DW^{-1}$. In salt stressed leaves, 11 cultivars with trigonelline concentrations $< 138.1$ μg g $DW^{-1}$ were significantly lower than Manitoba Brown (218.7 μg g $DW^{-1}$) ($P < 0.05$). During salt treatment, trigonelline concentrations increased significantly ($P < 0.05$) in seven cultivars (Manitoba Brown, Mandarin, Illini, Union, Essex, Forrest and Tokyo) (Table 1). Cultivars within a maturity group did not respond similarly (viz. trigonelline accumulation) to salt treatment. For example, in maturity group IV trigonelline concentrations increased in one cultivar (Union) and remained unchanged in two (A.K. and Patoka). Similarly, in maturity group VII, trigonelline concentrations increased in one cultivar (Tokyo) and remained unchanged in two (CNS and Roanoke). Trigonelline concentration in both nonstressed and salt stressed leaves was not significantly correlated with maturity group (data not shown) which suggests trigonelline biosynthesis and accumulation is independent of photoperiod sensitive development and maturation.

The 17 cultivars exhibited continuous variation for trigonelline concentration that suggests that the character, i.e. trigonelline biosynthesis and accumulation, is a polygenic trait. Based upon this survey, the capacity to synthesize trigonelline is uniformly distributed in cultivated soybean. In contrast to other compatible solutes such as glycine betaine (Rhodes & Hanson, 1993), trigonelline is present in relatively high concentrations within nonstressed plants (Shomer-Ilan et al., 1991; Evans & Tramontano, 1984). Trigonelline is postulated to function as a compatible solute in response to salinity stress and may contribute to NaCl-induced osmotic adjustment, particularly if trigonelline 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 & Hanson, 1993). Alternatively, since the biosynthesis of trigonelline depletes the *S*-adenosyl-methionine pool it may serve as a hypomethylating reaction, thereby limiting oxidative stress-induced DNA methylation (Berglund, 1994). The physiological importance of osmotic adjustment as an adaptive trait to drought or salinity is still an open question (Hare, Cress & van Staden, 1998) and we are interested in evaluating the role of trigonelline in soybean yield stability under drought and salt stress by evaluating quantitative trait loci. The potential enhancement of soybean stress tolerance by altering trigonelline accumulation, either by traditional plant breeding or biotechnology, does not require the introduction of a de novo biosynthetic pathway into agronomically important soybean cultivars.

## 3. Experimental

### 3.1. Plant material and NaCl treatment

All *G. max* cultivars were obtained from USDA Soybean Germplasm Collection (Urbana, IL) except Essex and Forrest. Seeds were surface sterilized and three seeds were germinated in a single pot with fertilizer premixed soil ($N : P_2O_5 : K_2O = 0.07 : 0.01 : 0.03\%$). Seedlings were watered daily and grown under controlled conditions (14 h photoperiod, 29°C day, 23°C night, 200 μmol $m^{-2}$ $s^{-1}$ from a mixed light source). Twenty one-day old seedlings were treated with NaCl in a stepwise manner over nine days in order to avoid salt shock (3 days 30 mM, 3 days 70 mM, and 3 days 100 mM). RWC was measured by placing 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, Saneoka, Joly, Rhodes & Goldsbrough, 1996).

### 3.2. Extraction, isolation and analysis of trigonelline

Leaves of nonstressed and salt stressed seedlings were harvested 30 days after planting. 0.1–0.5 g of leaf tissue was extracted in MeOH at 4°C in the dark and quaternary ammonia compounds were isolated as described in Ref. (Wood et al., 1996). Trigonelline was purified by Dowex-1-$OH^-$ and Dowex-50-$H^+$ ion exchange chromatography. The purified fractions were air dried and resuspended in 1 ml of purified $H_2O$. Trigonelline was measured spectrophotometrically (UV–Vis spectrum ($H_2O$) nm: $\lambda_{max}$ ($\varepsilon$) 264 (2700)), essentially as described (Yuyama & Suzuki, 1985) and quantified using authentic trigonelline standards (Sigma, St. Louis, MO, USA). The identity of trigonelline, $m/z$: 138 $[M + H]^+$, 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).

### 3.3. Data analysis

Data were analyzed for mean separations using Fisher's least significant difference (LSD). Pearson correlation coefficients were calculated to determine relationships among characters, using PROC CORR procedure in SAS (SAS Institute, Cary, NC).

## Acknowledgements

The authors would like to thank Dr. David Rhodes (Purdue University, West Lafayette, IN, USA) for assisting with the PD MS analysis. This work was sup-

ported in part by grants to AJW from the Illinois Soybean Program Operating Board and the Office of Research and Development Administration, Southern Illinois University.

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