Effects of Drought Stress and Storage on the Metabolite and Hormone Contents of Potato Tubers Expressing the Yeast *Trehalose-6-phosphate Synthase 1* Gene

Zsófia Juhász[1], Dirk Balmer[2], Anita Sós-Hegedűs[1], Armelle Vallat[3], Brigitte Mauch-Mani[2] & Zsófia Bánfalvi[1]

[1] NARIC Agricultural Biotechnology Institute, Gödöllő, Hungary

[2] Faculty of Sciences, University of Neuchâtel, Neuchâtel, Switzerland

[3] Chemical Analytical Service of the University of Neuchâtel, Neuchâtel, Switzerland

Correspondence: Zsófia Bánfalvi, NARIC Agricultural Biotechnology Institute, P.O. Box 411, Gödöllő, Hungary. Tel: 36-28-526-153. E-mail: banfalvi@abc.hu

Abstract

Comparative studies on the tuber yield and quality of commercial potato cultivars were conducted. White Lady was the wild-type (WT) accession used, and transgenic lines in this background expressing the yeast *trehalose-6-phosphate synthase 1* (*TPS1*) gene were analysed. The plants were grown in a greenhouse under well-watered and drought stress conditions, and the metabolite and hormone contents of freshly harvested and stored tubers were tested. Periodic drought resulted in an average 50% yield loss in WT and a 30% yield loss in *TPS1* plants. However, the average tuber mass remained higher in WT than in *TPS1* plants. Stress elevated the abscisic acid, proline, asparagine, and phenylalanine levels and significantly affected the levels of an additional 12 compounds in tubers. In contrast to abscisic acid, the salicylic acid levels in stressed tubers were reduced. In general, storage and stress had similar effects on the metabolite and hormone concentrations in both WT and *TPS1* tubers. Interestingly, storage increased the mannose, phenylalanine, and abscisic acid concentrations and decreased the salicylic acid concentrations only in the tubers of well-watered plants. *TPS1* tubers had a longer dormancy period than WT tubers and exhibited alterations in the concentrations of 13 metabolites.

Keywords: drought stress, hormones, metabolic profiling, *Solanum tuberosum* L., sprouting, tuber

Abbreviations: ABA: abscisic acid, CK: cytokinin, ET: ethylene, GA: gibberellic acid, GC-MS: gas chromatography-mass spectrometry, JA: jasmonic acid, JA-ILE: jasmonic acid-isoleucine, IAA: indole-3-acetic acid, TPP: trehalose-6-phosphate phosphatase, TPS1: trehalose-6-phosphate synthase 1, SA: salicylic acid, UHPLC-MS/MS: ultra-high-pressure liquid chromatography-tandem mass spectrometry, WT: wild-type

1. Introduction

Drought is by far the most important environmental stress in agriculture, and improving crop yields under drought stress is a major goal of plant breeding. To date, various approaches have been used to produce drought-tolerant plants. One of the approaches is the engineering of transgenic cultivars expressing osmolyte biosynthesis genes (Cattivelli et al., 2008).

Drought-tolerant transgenic rice lines showing tissue-specific or stress-inducible accumulation of the osmolyte trehalose, which has no negative effects, have been isolated previously (Garg et al., 2002). Based on this work, an attempt to improve the drought tolerance of the potato cv. White Lady was made by increasing the level of trehalose via transformation with the yeast *trehalose-6-phosphate synthase 1* (*TPS1*) gene driven by the promoter of the drought-inducible potato gene, *StDS2* (Dóczi et al., 2002). Using a marker-free transformation method, two independent *TPS1* transgenic potato lines, T1 and T2, were isolated. In contrast to the expected drought-induced expression, only very low, constitutive *TPS1* expression was detected in the transgenic lines, likely because of chromosomal position effects. This expression pattern, however, was sufficient to alter the plants' drought responses. Detached leaves of the T1 and T2 plants showed an eight-hour delay in wilting compared to the non-transformed wild-type (WT) control. Potted T1 and T2 plants retained water for six days longer than WT plants and maintained high stomatal conductance as well as a satisfactory rate of net photosynthesis. Under

optimal growth conditions, however, the transgenic plants grew more slowly, had a lower CO_2 fixation rate, and exhibited a 35% reduction in stomatal density (Stiller et al., 2008). To understand the molecular basis of this phenomenon, the transcriptomes and metabolites of WT and *TPS1* transgenic plants were compared using microarray analysis and gas chromatography-mass spectrometry (GC-MS). In total, 99 genes were differentially expressed in the mature source leaves of *TPS1* transgenic plants compared with WT. Although the trehalose concentration did not change, the malate, inositol, and maltose levels were higher in the *TPS1* leaves than in the WT leaves (Kondrák et al., 2011). Transcriptomic and metabolic profiling also revealed that the levels of several mRNAs and metabolites were altered in the leaves of drought-stressed plants. In total, 379 genes of known function showed at least a 2-fold expression change between genotypes, between stress conditions, or both. The majority of the genes with altered expression were implicated in photosynthesis and carbohydrate metabolism and were downregulated in both WT and *TPS1* plants upon drought stress. The fructose, galactose, and glucose contents were increased and decreased in drought-stressed WT and *TPS1* leaves, respectively, while the amounts of proline, inositol, and raffinose were highly increased in both WT and *TPS1* leaves under drought conditions (Kondrák et al., 2012).

Leaf alterations can influence tuber development and quality. For example, the ectopic expression of the *Arabidopsis* phytochrome B gene, which encodes a photoreceptor in leaves, can increase tuber yield, and this effect is largely due to increased leaf stomatal conductance (Thiele et al., 1999). In contrast, drought stress induces stomatal closure, which limits photosynthesis and leads to reduced assimilate production and canopy growth, which in turn results in lower tuber yield and quality (Gregory & Simmonds, 1992).

After they are produced, tubers undergo a period of dormancy, and the length of this dormancy period is under genetic and environmental control. Temperature, water availability, and photoperiods during growth and storage are important environmental factors that regulate sprouting behaviour. Although dormancy is defined as the absence of visible growth, dormant meristems are metabolically active, and several transcripts and proteins unique to either dormant or growing meristems have been identified (Bachem et al., 2000). Dormancy is also under hormonal regulation. Gibberellins (GAs) and cytokinins (CKs) are generally considered growth promoters, whereas abscisic acid (ABA) and ethylene (ET) are believed to inhibit sprout growth. Recently, it was shown that the reactivation of meristem activity and sprout growth requires both CK and GA and that free indole-3-acetic acid (IAA) might shorten dormancy by stimulating bud growth (S. Sonnewald & U. Sonnewald, 2013). Tuber cells must be metabolically competent before plant hormones can exert their growth control. This was demonstrated by expressing the *Escherichia coli OtsB* gene, which encodes trehalose-6-phosphate phosphatase (TPP), in tubers, which was found to promote tuber sprouting (Debast et al., 2011). In contrast, the expression of an *E. coli* trehalose-6-phosphate synthase (*OtsA*) in potatoes resulted in a considerable delay in tuber sprouting (Lytovchenko et al., 2005; Debast et al., 2011).

Based on these findings, we investigated the metabolite and hormone compositions of tubers of potato plants expressing the yeast *TPS1* gene. To examine environmental effects, the plants were grown under well-watered and drought stress conditions, and tubers were analysed shortly after harvest and after three months of storage at room temperature.

2. Materials and Methods

2.1 Plant Materials and Growth Conditions

The potato (*Solanum tuberosum* L.) cv. White Lady and the *TPS1*-expressing transgenic lines, T1 and T2 (Stiller et al., 2008), were vegetatively propagated from cuttings on RM medium (MS medium without vitamins) (Murashige & Skoog, 1962) containing 2% (w/v) sucrose at 24 °C with a photoperiod of 16 h of light (170 µmol m^{-2} s^{-1}) and 8 h of darkness.

Six-week-old plants obtained by tissue culture were transferred into pots containing sterile A260 soil (Stender AG, Schermbeck, Germany; 1,000 g/pot) and grown under greenhouse conditions at 18-28 °C. The soil water content was determined gravimetrically (g of water per g of soil) at a depth of 5-7 cm. Optimal growth conditions were provided by regular watering (70-80% soil humidity). Pesticides and fungicides were applied when needed. Four weeks after planting, the plants were divided into two groups. Seven plants per line were continuously irrigated to maintain 70% soil moisture content, and eight plants per line were exposed to drought stress by withholding irrigation for 7-10 days while the water content of the soil decreased to 30%. Seven dry cycles were created within a growing season. Between the dry cycles, the plants were irrigated with an optimal amount of water for one week. The tubers were harvested at full maturity four months after planting. The entire experiment was repeated twice.

2.2 Non-Targeted Analysis of Polar Metabolites

The peeled tubers were chopped in an electric blender and ground into a fine powder in liquid nitrogen. Metabolite extraction was performed according to a previously published method (Schauer et al., 2005) by mixing 125 mg of tuber powder with 700 µl of methanol; 60 µl of the internal standard (0.2 mg ml^{-1} ribitol in water) was subsequently added. The mixture was extracted by shaken at 1,000 rpm for 15 min at 70 °C, and mixed vigorously with 1 volume of water. To separate the polar and non-polar metabolites, 375 µl of chloroform was added, and the mixtures were vortexed. After centrifugation at 13,000 rpm for 15 min, 150 µl from the upper methanol/water phase was removed and reduced to dryness in a vacuum. For methoxyamination, 40 µl of methoxyamine hydrochloride (MEOX) dissolved in 20 mg ml^{-1} pyridine was added to the dried extract and agitated for 90 min at 37 °C. N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was used for derivatisation (60 µl, 30 min, 37 °C). The samples were analysed in the splitless mode in a quadrupole-type GC-MS system (Finnigan Trace/DSQ, Thermo Electron Corp., Austin, TX, USA) equipped with a 30 m capillary column (Rxi-5 ms, 0.25 mm ID, 0.25 µm df, Restek, Bellefonte, PA, USA). Sample aliquots (1 µl) were injected with a split ratio of 30 ml min^{-1} using the hot-needle technique. The injection temperature was 230 °C, and the temperature of the interface and the ion source was set to 250 °C. The carrier gas was helium, and a constant flow rate of 1 ml min^{-1} was used. The temperature program included 12.5 min of isothermal heating at 90 °C for 2 min, followed by a 5 °C min^{-1} oven temperature ramp to 330 °C. The system was temperature equilibrated for 2 min at 90 °C prior to the injection of the next sample. The detection was performed in the total ion chromatogram (TIC) positive mode. The identification and quantification of the 33 compounds listed in Table 1 were achieved using the NIST 11 mass spectral database. In addition, the sugars and amino acids presented in Figure 2 were identified based on comparisons of the retention times and mass spectra with those of standards analysed under identical conditions.

2.3 Extraction and Assays of Trehalose-6-phosphate (T6P) and Trehalose by GC-MS

T6P was extracted from tuber tissues using the method described by Lunn et al. (2006) with modifications. Aliquots (250 mg fresh weight) of the frozen tuber powder were transferred to pre-cooled 2-ml Eppendorf tubes, vortexed with 500 µl of ice-cold chloroform/methanol (3:7, v/v), and incubated at -20 °C for 2 h with occasional mixing. Water-soluble components, including T6P, were extracted from the chloroform phase by adding 400 µl of water. After centrifugation at 13,000 rpm for 4 min at 4 °C, the aqueous-methanol phase was transferred to a 5-cm Petri dish and maintained at 4 °C. The extraction was repeated with 400 µl of cold water, and the second extract was combined with the first extract. The methanol was evaporated in a laminar flow cabinet, and the aqueous solution was supplemented with water to 1 ml. High molecular mass components were removed from the samples by applying an Amicon Ultra-4 centrifugal filter device (Millipore) and centrifuging at 4,000 g for 20 min at 4 °C. An aliquot (100 µl) of the extract was dried with 20 µl of ribitol (20 mg ml^{-1}). Methoxyamination, derivatisation, and GC-MS analysis were carried out as described above for non-targeted metabolite analysis except that the detection was performed in the selected ion monitoring (SIM) positive mode. The selected ions had m/z values of 271, 315, and 387. The T6P concentrations of the tubers were calculated using a standard (Sigma Cat. No. T4272).

Detection of trehalose was attempted in the tubers following the methods of Kim et al. (2012). The parameters of the GC-MS analysis were the same as those described above, and a standard (Sigma Cat. No. T5251) was used to identify the trehalose in the total ion chromatogram.

2.4 Plant Hormone Quantification

Hormones were quantified simultaneously in single samples using an optimised UHPLC-MS/MS analysis method as previously described (Glauser et al., 2014). Briefly, hormones were isolated from tissue samples (100 mg fresh weight) using a 99.5:0.5 (v/v) ethanol acetate:formic acid extraction solvent. During the extraction, an internal standard solution with isotopically labelled hormones was added to the samples. The hormones were quantified based on a calibration equation obtained by linear regression from a calibration curve for each analyte. The peak areas of the hormones measured in the samples were normalised to the internal standard before applying the calibration equation.

2.5 RNA Isolation and Analysis

Total RNA was extracted from leaves and tubers according to the method of Stiekema et al. (1988) and quantified using a NanoDrop spectrophotometer. DNaseI-treated total RNA (2 µg) was reverse-transcribed with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). The obtained cDNAs were diluted 10-fold, and the RT-PCR assays were performed with *TPS1*-specific primers (5'-ATTCTGGATCGTTCA-3' and 5'-GATGAAATCGCAGACTTACA-3') and 18S rRNA primers (5'-GGG CAT TCG TAT TTC ATA GTC AGA G-3' and 5'-CGG TTC TTG ATT AAT GAA AAC ATC CT-3').

2.6 Statistical Analysis

Student's *t* test was used to determine significant differences in the relative tuber number, yield, and T6P level at $P < 0.05$ and $P < 0.01$.

Using the SPSS software package (SPSS Inc., an IBM Company), one-way MANOVA was applied to determine differences ($P < 0.05$) between the metabolite compositions of independent groups (i.e., tubers of well-watered plants vs. stressed plants, fresh tubers vs. stored tubers, and WT tubers vs. *TPS1* transgenic tubers).

3. Results

3.1 Effects of Drought Stress on Yield and Tuber Dormancy

WT control plants and the *TPS1* transgenic lines T1 and T2 were grown under well-watered and periodic drought conditions in two independent experiments, as described in the Materials and Methods section. Under well-watered conditions, the number of tubers per plant and the total tuber biomass were reduced in both *TPS1* lines compared to the WT controls (Figure 1A & 1B). The average tuber number per plant was similar in the two experiments (3.9 and 4.0 tubers per well-watered WT plant), although the average yield was quite different between the two experiments (11.8 g and 17.6 g of tubers per well-watered WT plant). However, both parameters were always lower in the *TPS1* transgenic lines than in WT. Under well-watered conditions, the average reductions in yield were 60 and 50% for the T1 and T2 plants, respectively, compared to WT. Periodic drought caused an increase in the number but a reduction in the size of the WT tubers and, in general, resulted in a 50% yield loss. Drought had a milder effect on the *TPS1* transgenic plants and tubers; the increase in the number of tubers was less pronounced than the increase in WT, and the reduction in yield generally did not exceed 20-40% (Figure 1A & 1B).
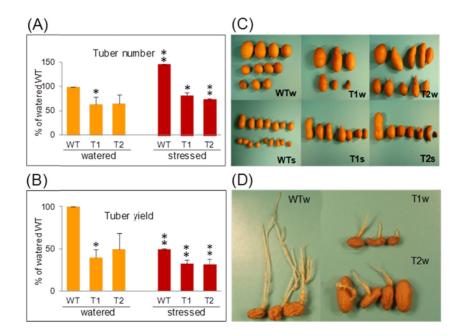

Figure 1. Tuber numbers and tuber yields of wild-type (WT) and *TPS1* (T1 and T2) plants under well-watered (w) and drought-stress (s) conditions. (A) Relative tuber number per plant. (B) Relative tuber yield per plant. (C) Tubers at 12 weeks after harvest stored at room temperature in darkness. (D) Tubers at 6 months after harvest stored at room temperature in darkness. Bars and error bars represent the mean ± SD from two independent experiments. In each experiment, the tubers were harvested from seven well-watered and eight stressed plants per line. The average numbers of tubers per well-watered WT plant in the two independent experiments were 3.9 and 4.0, while the tuber yields were 11.8 g and 17.6 g. These values are considered 100% for comparisons with the other samples in the same experiments. *$P < 0.05$, **$P < 0.01$ (Student's *t* test)

The T1 and T2 tubers were longitudinal in shape, and the WT tubers were oval (Figure 1C). The tubers were stored at room temperature (20-22 °C) in the dark and were assessed visually on a weekly basis for sprouting. WT tubers started sprouting in a highly synchronised manner 12 weeks after harvest. In contrast, sprouting in the T1 and T2

tubers was significantly delayed compared with sprouting in the WT. By week 12, only a few tubers had small sprouts, and full sprouting was achieved only at approximately 18 weeks after harvest. Sprout development was slow in the T1 and T2 plants compared to WT. After six months of storage, the sprouts on the T1 and T2 tubers were 1-5 cm in length, while those on the WT tubers reached 15-20 cm (Figure 1D).

The sprouting behaviour of well-watered plants did not differ substantially from that of drought-stressed plants in our experiments.

3.2 Metabolite Content of Tubers

The metabolite content of tubers was analysed by GC-MS. A total of 33 compounds, primarily including amino acids, organic acids, sugars, and sugar alcohols, were identified (Table 1). Significant changes induced by stress, storage, the combination of stress and storage, or the expression of *TPS1* were detected for 28 metabolites (Figure 2 & Supplementary Figure 1). The largest changes were detected in the concentrations of fructose, glucose, galactose, mannose, proline, asparagine, and phenylalanine (Figure 2). The tuber content of sucrose, which is the most abundant sugar in tubers, is also shown in Figure 2.
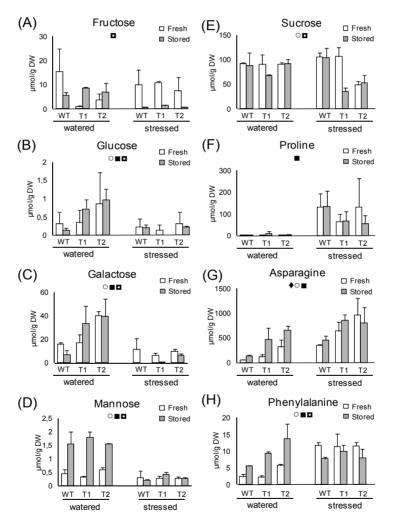

Figure 2. Metabolites detected by GC-MS. The bars and error bars represent the mean ± SD of two groups of tubers derived from two independent experiments. In each experiment, the tubers were harvested from seven well-watered and eight stressed plants per line. Freshly harvested tubers and tubers stored for 12 weeks at room temperature in darkness were analysed. Tubers larger than 2 cm in diameter were selected, peeled, and grouped. The number of tubers per group was 6-13 and the tubers weighed 20-180 g. Significant differences (one-way MANOVA, $P < 0.05$) between the tubers of well-watered and drought-stressed plants are indicated by ■, those between fresh and stored tubers are indicated by ○, and those between WT and *TPS1* tubers are indicated by ♦. Interactions between stress and storage are indicated by ◘. WT, wild-type; T1 and T2, *TPS1* lines; DW, dry weight

Table 1. Compounds detected in tubers by non-targeted GC-MS analysis

Compound class	Metabolites
Amino acid	ß-alanine, asparagine, aspartic acid, γ-aminobutyric acid (GABA), glutamic acid, glutamine, glycine, isoleucine, phenylalanine, proline, serine, threonine, tryptophan, 5-oxoproline
Sugar	fructose, galactose, glucose, maltose, mannose, raffinose, sucrose
Organic acid	*cis*-aconitic acid, isocitric acid, fumaric acid, galactaric acid, glyceric acid
Sugar alcohol	galactinol, inositol, mannitol, sorbitol
Fatty acid	palmitic acid, stearic acid
Other	glucose-6-phosphate

Storage led to changes in the levels of 17 compounds (Supplementary Table 1), including the sugars glucose, galactose, mannose, and sucrose (Figure 2B-2E) and the amino acids asparagine and phenylalanine (Figures 2G & 2H). Stress affected the amounts of 14 compounds (Supplementary Table 1). The highest increases induced by drought were in the concentrations of proline, asparagine, and phenylalanine (Figure 2F-2H). The *TPS1* transgene significantly influenced the amounts of 13 metabolites: asparagine (Figure 2G), ß-alanine, aspartic acid, GABA, galactaric acid, glutamine, glycine, isocitric acid, mannitol, 5-oxoproline, serine, stearic acid, and threonine (Supplementary Table 1 & Supplementary Figure 1). Similar to what was observed for asparagine (Figure 2G), higher concentrations of 11 metabolites were detected in transgenic tubers compared to WT tubers. In general, the metabolite contents of *TPS1* transgenic tubers and WT tubers were similarly affected by storage.

3.3 Hormone Content of Tubers

The same set of tubers that were tested to determine their metabolite contents were also tested to determine their hormone contents using ultra-high-pressure liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Our study focused on ABA, SA, and JA because of their roles in the mediation of stress responses (Acharya & Assmann, 2009). IAA was also measured. As expected, the ABA concentration in tubers harvested from stressed plants was higher than that of tubers harvested from well-watered plants. Unexpectedly however, although the concentration of ABA increased upon storage in the tubers of well-watered plants, it remained at the same level in tubers of stressed plants (Figure 3A). The changes in SA showed an opposite trend. SA was present in higher amounts in the tubers of well-watered plants than in the tubers of stressed plants, and it decreased in the tubers of well-watered plants only upon storage (Figure 3B). JA and its active form JA-ILE were present in very low amounts in freshly harvested tubers, and their concentrations decreased over time (Figures 3C & 3D). The IAA concentration was higher in tubers of *TPS1* transgenic plants than in WT plants (Figure 3E).

3.4 TPS1 Gene Expression and T6P Content in Tubers

In the *TPS1* transgenic lines T1 and T2, the transgene is expressed under the control of the promoter region of the *S. tuberosum DS2* gene, which was shown to be activated by drought in an ABA-independent manner in leaves (Dóczi et al., 2002). Nevertheless, it was found that in the transgenic lines, *TPS1* was expressed at a very low level in leaves, even under optimal growth conditions, and it was induced only moderately by drought (Stiller et al., 2008). *TPS1* expression in tubers was not tested in previous experiments. Figure 4A shows that *TPS1* was expressed in freshly harvested tubers of well-watered plants at a low level and increased slightly under drought stress. Figure 4A also shows that the amount of *TPS1* mRNA decreased upon storage.

To assess whether transgene expression affects the T6P content in tubers, the T6P level was measured in samples from transgenic tubers and WT control tubers. As shown in Figure 4B, a significant difference (*$P < 0.01$) was detected in only one transgenic sample compared with the freshly harvested, well-watered WT control. Nevertheless, the T6P level in the transgenic line was lower than that in WT; thus, it may reflect the heterogeneity of the tubers rather than the effect of *TPS1* expression.

Plant cells contain a wide array of non-specific phosphatases; thus, T6P may be converted to trehalose without the simultaneous stimulation of TPP activity (Elbein et al., 2003). To test this hypothesis, we attempted to measure the trehalose concentrations of tubers by GC-MS. However, the tuber trehalose content was below the detection level (< 0.05 µmol g^{-1} DW).
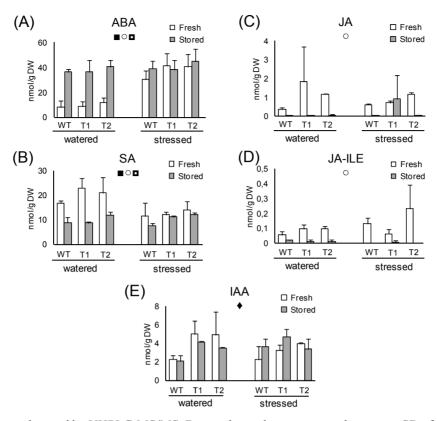
Figure 3. Hormones detected by UHPLC-MS/MS. Bars and error bars represent the mean ± SD of the same groups of tubers described in the legend of Figure 2. Significant differences (one-way MANOVA, $P < 0.05$; Supplementary Table 2) between the tubers of well-watered and drought-stressed plants are indicated by ■, and those between fresh and stored tubers are indicated by ○. Interactions between stress and storage are indicated by ▫. WT, wild-type; T1 and T2, *TPS1* lines; DW, dry weight

Figure 4. *TPS1* expression and T6P levels in tubers. (A) RT-PCR analysis of *TPS1* transgene expression in tubers of well-watered and drought-stressed plants. RT-PCR analysis of 18S RNA was used as a control. (B) Relative T6P levels of the tubers. RNA and T6P were isolated from the same groups of tubers described in the legend of Figure 2. The average T6P concentrations in freshly harvested tubers of well-watered WT plants in the two independent experiments were 255 and 123 nmol g^{-1} DW. These values are considered 100% for comparisons with the other samples in the same experiments. The error bars denote the SD; *$P < 0.01$ (Student's *t* test); WT, wild-type; T1 and T2, *TPS1* lines; DW, dry weight

4. Discussion

4.1 Effect of Stress on Tuber Yield and Metabolite Composition

In this study, the tuber yield and quality of the commercial cv. White Lady and *TPS1* transgenic lines in this background, T1 and T2, were compared. Drought stress reduces photosynthesis, plant biomass, and tuber yield almost proportionally to the rate of water consumption (Monneveux et al., 2013). In our study, periodic drought resulted in an average yield loss of 50% in WT and 20-40% in *TPS1* transgenic plants. Nonetheless, the average tuber mass of WT plants remained higher than that of the T1 and T2 plants. This result might be due to the lower CO_2 fixation rate and lower fresh mass of the *TPS1* transgenic plants compared to WT (Stiller et al., 2008).

Periodic drought reduced the size but increased the number of tubers, especially for WT plants. The large number of small tubers indicates that tuber formation was not inhibited. This phenotype is similar to that of sugar-storing starch-deficient mutants, which are unable to convert sucrose to starch in the tuber and have low sink strength (Müller-Röber et al., 1992). However, we did not observe a significant increase in sucrose concentration (Figure 2E), suggesting that factors other than sugar-storing ability also influence sink strength.

Drought stress increased the proline content of tubers from a barely detectable level to 130 μmol g^{-1} DW (Figure 2F). An increase in proline content triggered by stress in the leaves of various plant species is a well-documented response (Obata & Fernie, 2012), and proline accumulation was found to be induced by high salinity, drought, and selenium in tubers (Teixeira & Pereira, 2007; Maggio et al., 2008; Jezek et al., 2011). Glutamine synthetase plays a key role in nitrogen metabolism and has been implicated in the regulation of proline levels in plants (Brugiere et al., 1999). This enzyme is activated in growing tubers in response to drought (Teixeira & Pereira, 2007). In a field experiment, glutamine and glutamate showed the greatest changes in response to various treatments (Maggio et al., 2008). We also observed 2-fold increases in glutamine and glutamate in stressed tubers (Supplementary Figure 1). Asparagine accumulation appears to be another general response of tubers to water limitation because it was detected in the cvs. Agria and Merit (Maggio et al., 2008) as well as in cv. White Lady (Figure 2G). Phenylalanine showed an approximate 5-fold increase in freshly harvested tubers of stressed plants compared to well-watered plants (Figure 2H) that can be transformed into lignins, phenolic acids, flavonoids, stilbenes, and lignans (Boudet, 2007).

The amino acids proline, asparagine, and phenylalanine, which showed the greatest increases during drought stress, are synthesised via various pathways. These pathways, however, are interconnected via carbon metabolism (Figure 5). The *Arabidopsis ASN1* gene encodes asparagine synthase, which synthesises asparagine and glutamate from aspartate and glutamine, respectively, and is one of the genes that is most affected by sugar. Proline metabolism is similarly regulated through the transcriptional regulation of the *P5CS* gene, which encodes Δ1-pyrroline-5-carboxylate synthetase. In *Arabidopsis*, both genes are regulated by the transcription factor bZIP11, which is repressed by sucrose. Interestingly, it was also shown that increased bZIP11 expression leads to increased phenylalanine levels (Hanson et al., 2008). Although the regulatory circuits are not well known in potatoes, it appears likely that glutamine is mobilised to tubers, where it is converted into asparagine by the asparagine synthase StAst1 (Chawla et al., 2012). Interestingly however, we could not detect any change in the sucrose level upon drought stress in either leaves (Kondrák et al., 2012) or tubers of WT plants (Figure 2E). Thus, we concluded that the activity of the transcription factor(s) controlling proline, asparagine, and phenylalanine synthesis in potato may not depend on sucrose.

In addition to proline, raffinose levels were greatly increased (11-fold) upon drought stress in leaves (Kondrák et al., 2012) but not in tubers of the same plants (Supplementary Figure 1), indicating that source and sink organs respond to the same stress through mechanisms that are at least partially different.

Drought stress increased the ABA concentration while decreasing the SA concentration of tubers (Figure 3A & 3B). Thus, the condition-specific positive/negative interaction between ABA and SA that exists in other plant organs (Acharya & Assmann, 2009) may also exist in tubers.
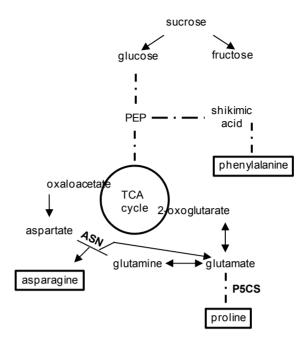
Figure 5. Metabolic pathways leading to the biosynthesis of asparagine, proline, and phenylalanine. Dashed lines symbolise multistep reactions, and solid lines symbolise one-step reactions. ASN, asparagine synthase; P5CS, Δ1-pyrroline-5-carboxylate synthetase

4.2 Effect of Storage on the Metabolite and Hormone Compositions of Tubers

The length of the dormancy period depends on both the genetic background and the environmental conditions during tuber development and storage. GAs and CKs are generally considered to be growth promoters, whereas ABA and ET are believed to inhibit sprout growth (S. Sonnewald & U. Sonnewald, 2013). Although the level of GAs in tuber tissues is below the detection limit (Lytovchenko et al., 2005; Morris et al., 2006), we attempted to estimate the level of CKs in WT and *TPS1* transgenic tubers using a bioassay based on chlorophyll formation in cucumber cotyledons (Fletcher & McCullagh, 1971). Unfortunately, the level of CKs was under the detection level of 0.1 μg ml^{-1} 6-benzylaminopurine (data not shown). In contrast, ABA was present at detectable levels in all tuber samples. Surprisingly however, we observed a 4-fold increase in the ABA concentration of stored tubers harvested from well-watered plants (Figure 3A). Previously, a continuous decline in the ABA content of tubers of various potato varieties was detected during storage. However, this decline did not correlate with the sprouting behaviour of the tubers (Suttle, 1995; Biemelt et al., 2000). Thus, our finding may reflect a cultivar-specific difference or indicate a specific period, namely the early phase of sprout development, when the ABA level may be increased due to tuber water loss.

Similar to stress, storage increased the tuber asparagine concentration (Figure 2G). A massive increase in the asparagine level was previously noted in the Pentland Dell and Bintje varieties when they were stored for a long period of time (Brierley et al., 1997; De Wilde et al., 2005). The asparagine content of Bintje reached 17 mg g^{-1} DW (De Wilde et al., 2005), which is comparable to the 101 μmol g^{-1} DW (Figure 2G) (equivalent to 13.2 mg g^{-1} DW) observed in stored tubers of well-watered White Lady plants.

One of the most interesting findings of this study was that the levels of two metabolites, mannose and phenylalanine, and two hormones, ABA and SA, were different in tubers of stressed and non-stressed plants upon storage. While the concentrations of mannose, phenylalanine, and ABA in tubers of well-watered plants increased upon storage, they remained the same or even decreased in the stored tubers of stressed plants (Figures 2D & 2H; Figure 3A). However, while the SA concentration in tubers of well-watered plants decreased upon storage, it did not change significantly in the stored tubers of stressed plants (Figure 3B). The physiological importance of these differences remains obscure.

4.3 Effect of TPS1 Expression on the Dormancy and Metabolite Composition of Tubers

The expression of yeast *TPS1* resulted in a longer dormancy period and altered the concentrations of 13 metabolites compared to their levels in WT tubers. However, the general changes in metabolite concentrations in

stored WT and *TPS1* tubers were very similar and depended mainly on the environmental conditions in which the plants were grown rather than on the genotype of the plants. This result indicates that the differences detected between the freshly harvested and stored tubers are more likely to reflect the tuber aging process rather than the developmental stage of sprouting.

The expression of the *E. coli* TPS-encoding gene *OtsA* driven by the strong tuber-specific patatin promoter *B33* significantly delayed tuber sprouting compared with WT. The delay in the sprouting of the *B33-OtsA* lines correlated with the T6P content of the tubers, which reached 46 nmol g^{-1} fresh weight (FW) in the transgenic line with the highest level of O*tsA* expression. Thus, it was concluded that the T6P levels either directly or indirectly affect tuber dormancy (Debast et al., 2011). In the T1 and T2 transgenic lines, the yeast *TPS1* gene was expressed from the drought-inducible *StDS2* promoter. However, due to "leaky" transcriptional regulation, the *TPS1* gene was also expressed in the tubers of well-watered plants (Figure 4A). Although this expression did not result in a significant increase in the T6P level, which was between 123 and 255 nmol g^{-1} DW (approximately 20-50 nmol g^{-1} FW) in WT tubers (Figure 4B), the dormancy period of the transgenic tubers was prolonged. Thus, although our result supports the previous finding that TPS has a role in the maintenance of dormancy, it indicates that T6P is unlikely to have an effect on the duration of dormancy.

5. Conclusion

Expression of yeast *TPS1*, even at a low level, has a pleiotropic effect on potato plants at phenotypic, transcriptional, and metabolic levels in both leaves and tubers. Weak expression of *TPS1* does not increase the concentration of T6P or trehalose in either leaves or tubers. Thus, it is not the amounts of these metabolites per se that are responsible for the pleiotropic effects, including delayed leaf wilting and prolonged dormancy of tubers compared to WT. Previously, it has been shown that cauliflower TPS and *Arabidopsis* TPS5, TPS6, and TPS7 can bind to 14-3-3 proteins if the Ser[22] and Thr[49] residues are phosphorylated (Moorhead et al., 1999; Harthill et al., 2006). Because the TPS1 protein of yeast is 40% identical to TPS5 and TPS1 contains Ser and Thr residues in the same locations as the Ser and Thr residues in *Arabidopsis* TPS isoforms, we postulate that phosphorylation of yeast TPS1 and its interaction with 14-3-3 proteins may also occur in potato. Binding of yeast TPS1 to 14-3-3 proteins can influence the activities of housekeeping proteins such as nitrate reductase and may lead to an imbalance in ion homeostasis and hormone signalling, processes in which 14-3-3 proteins have well-understood functions (Oecking & Jaspert, 2009). Taken together, these processes may result in the alterations that were detected in *TPS1* transgenic potatoes.
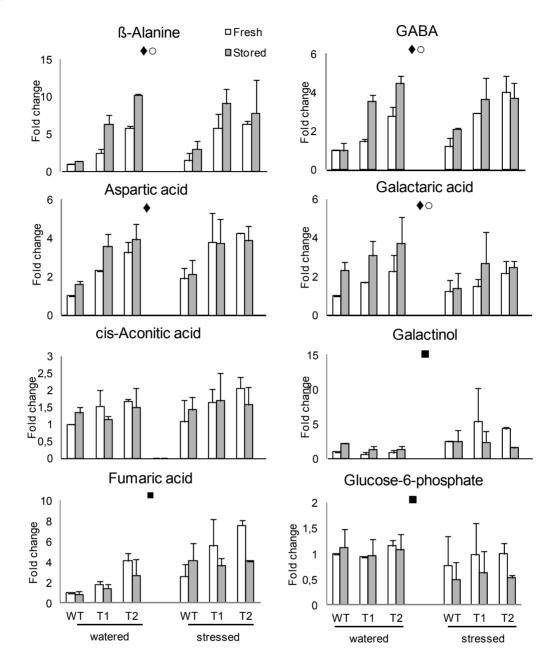
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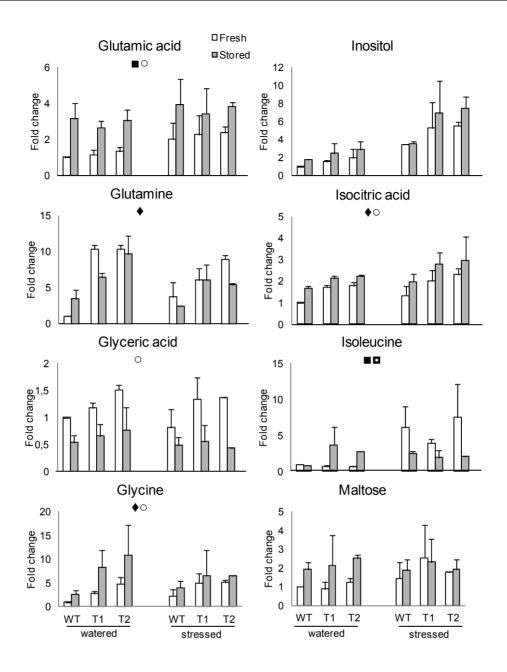
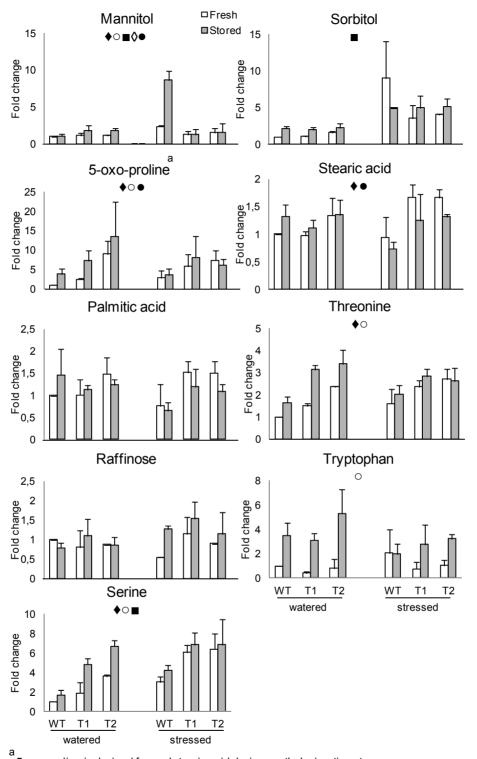responsiveness to hormones during sprouting. *Plant Physiology, 156*, 1754-1771. http://dx.doi.10.1104/pp.111.179903

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Appendixs

a 5-oxoproline is derived from glutamic acid during methyloximation step.

Supplementary Figure 1. Metabolites detected by GC-MS. The bars and error bars represent the mean ± SD of the same groups of tubers described in the legend of Figure 2. Significant differences (one way MANOVA, $P <$ 0.05) between tubers of well-watered and drought-stressed plants are indicated by ■, those between fresh and stored tubers are indicated by ○, and those between WT and *TPS1* tubers are indicated by ♦. Interactions between stress and storage ◘, genotype and storage ◊, and genotype and stress are indicated by ●. WT, wild-type; T1, T2, *TPS1* lines; DW, dry weight

Table S1. Significant differnces (P<0.05) are highlighted by colours

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Genotype									
	Fructose (217, 307)	1.899E14	2	9.494E13	1.780	.210	.229	3.559	.300
	Mannose	3.128E12	2	1.564E12	.146	.866	.024	.291	.068
	Galactose	3.728E15	2	1.864E15	.072	.931	.012	.144	.059
	Glucose	1.319E15	2	6.594E14	.833	.458	.122	1.666	.160
	Sorbitol	3.592E14	2	1.796E14	2.696	.108	.310	5.391	.433
	Inositol	4.072E17	2	2.036E17	1.975	.181	.248	3.949	.329
	Sucrose	4.043E17	2	2.021E17	.588	.571	.089	1.176	.126
	Maltose	1.441E12	2	7.207E11	.596	.567	.090	1.191	.127
	L-Isoleucine (158)	2.269E13	2	1.134E13	.334	.723	.053	.667	.092
	L-Proline (142)	3.161E16	2	1.581E16	1.109	.362	.156	2.218	.200
	Glycine (174)	1.284E14	2	6.422E13	17.724	.000	.747	35.448	.998
	Glyceric acid (189,292)	3.048E10	2	1.524E10	7.022	.010	.539	14.044	.846
	Fumaric acid (245)	2.426E12	2	1.213E12	5.807	.017	.492	11.614	.769
	L-Serine (204, 218)	3.428E14	2	1.714E14	21.727	.000	.784	43.453	1.000
	L-Threonine (218, 291)	4.016E12	2	2.008E12	15.182	.001	.717	30.364	.994
	beta-Alanine (248)	1.007E13	2	5.036E12	24.873	.000	.806	49.747	1.000
	Aspartic acid (232)	4.871E15	2	2.436E15	27.981	.000	.823	55.961	1.000
	5-oxo-Proline	3.096E15	2	1.548E15	48.531	.000	.890	97.062	1.000
	GABA (304,174)	9.287E16	2	4.643E16	38.114	.000	.864	76.228	1.000
	Glutamic acid (246)	6.948E15	2	3.474E15	1.046	.381	.148	2.092	.191
	L-Phenylalanine (218,192)	7.370E14	2	3.685E14	5.333	.022	.471	10.667	.731
	L-Asparagine (188, 231)	4.343E17	2	2.172E17	20.388	.000	.773	40.776	.999
	cis-Aconitic acid (229, 375)	1.622E12	2	8.112E11	3.100	.082	.341	6.200	.488
	Glutamine (156)	2.765E16	2	1.383E16	38.095	.000	.864	76.190	1.000
	Isocitric acid (273)	1.948E18	2	9.740E17	8.206	.006	.578	16.413	.899
	D-Mannitol (319)	1.818E15	2	9.088E14	19.752	.000	.767	39.505	.999
	Palmitic acid (313, 314)	8.188E12	2	4.094E12	3.458	.065	.366	6.915	.534
	Galactaric acid (333)	7.076E12	2	3.538E12	9.934	.003	.623	19.869	.947

L-Tryptophan 202	2.012E13	2	1.006E13	2.691	.108	.310	5.382	.432
Stearic acid (341,117)	1.931E13	2	9.657E12	10.810	.002	.643	21.619	.962
Glucose-6-phosphate (387)	2.356E12	2	1.178E12	.536	.599	.082	1.072	.119
Raffinose (437)	1.625E12	2	8.125E11	1.062	.376	.150	2.124	.193
Galactinol (204)	2.935E12	2	1.468E12	.038	.963	.006	.077	.055
Storage								
Fructose (217, 307)	2.499E14	1	2.499E14	4.686	.051	.281	4.686	.512
Mannose	1.070E15	1	1.070E15	99.664	.000	.893	99.664	1.000
Galactose	4.192E17	1	4.192E17	16.159	.002	.574	16.159	.957
Glucose	2.842E16	1	2.842E16	35.911	.000	.750	35.911	1.000
Sorbitol	2.524E13	1	2.524E13	.379	.550	.031	.379	.088
Inositol	5.245E16	1	5.245E16	.509	.489	.041	.509	.101
Sucrose	4.355E18	1	4.355E18	12.672	.004	.514	12.672	.904
Maltose	6.187E12	1	6.187E12	5.113	.043	.299	5.113	.547
L-Isoleucine (158)	6.623E13	1	6.623E13	1.948	.188	.140	1.948	.251
L-Proline (142)	1.116E14	1	1.116E14	.008	.931	.001	.008	.051
Glycine (174)	7.217E13	1	7.217E13	19.918	.001	.624	19.918	.983
Glyceric acid (189, 292)	1.798E11	1	1.798E11	82.841	.000	.873	82.841	1.000
Fumaric acid (245)	4.960E11	1	4.960E11	2.374	.149	.165	2.374	.295
L-Serine (204, 218)	9.901E13	1	9.901E13	12.552	.004	.511	12.552	.901
L-Threonine (218, 291)	1.769E12	1	1.769E12	13.377	.003	.527	13.377	.918
beta-alanine (248)	2.624E12	1	2.624E12	12.962	.004	.519	12.962	.910
Aspartic acid (232)	2.057E14	1	2.057E14	2.363	.150	.165	2.363	.293
5-oxo-Proline	5.432E14	1	5.432E14	17.032	.001	.587	17.032	.966
GABA (304,174)	1.710E16	1	1.710E16	14.035	.003	.539	14.035	.930
Glutamic acid (246)	2.345E17	1	2.345E17	70.608	.000	.855	70.608	1.000
L-Phenylalanine (218,192)	1.129E15	1	1.129E15	16.342	.002	.577	16.342	.959
L-Asparagine (188, 231)	1.860E17	1	1.860E17	17.463	.001	.593	17.463	.969
cis-Aconitic acid (229, 375)	3.238E10	1	3.238E10	.124	.731	.010	.124	.062
Glutamine 156	1.528E15	1	1.528E15	4.211	.063	.260	4.211	.471
Isocitric acid (273)	1.370E18	1	1.370E18	11.543	.005	.490	11.543	.876
D-Mannitol (319)	1.009E15	1	1.009E15	21.925	.001	.646	21.925	.990

Palmitic acid (313, 314)	8.157E11	1	8.157E11	.689	.423	.054	.689	.119
Galactaric acid (333)	7.096E12	1	7.096E12	19.925	.001	.624	19.925	.983
L-Tryptophan (202)	2.155E14	1	2.155E14	57.620	.000	.828	57.620	1.000
Stearic acid (341, 117)	1.272E12	1	1.272E12	1.424	.256	.106	1.424	.196
Glucose-6-phosphate (387)	7.229E12	1	7.229E12	3.289	.095	.215	3.289	.386
Raffinose (437)	2.081E12	1	2.081E12	2.720	.125	.185	2.720	.330
Galactinol (204)	5.553E13	1	5.553E13	1.449	.252	.108	1.449	.198
Stress								
Fructose (217, 307)	6.184E13	1	6.184E13	1.159	.303	.088	1.159	.168
Mannose	1.280E15	1	1.280E15	119.272	.000	.909	119.272	1.000
Galactose	8.712E17	1	8.712E17	33.586	.000	.737	33.586	1.000
Glucose	3.410E16	1	3.410E16	43.090	.000	.782	43.090	1.000
Sorbitol	3.412E15	1	3.412E15	51.203	.000	.810	51.203	1.000
Inositol	5.159E17	1	5.159E17	5.003	.045	.294	5.003	.538
Sucrose	5.422E17	1	5.422E17	1.578	.233	.116	1.578	.212
Maltose	1.684E12	1	1.684E12	1.392	.261	.104	1.392	.192
L-Isoleucine (158)	3.789E14	1	3.789E14	11.143	.006	.481	11.143	.865
L-Proline (142)	3.821E17	1	3.821E17	26.807	.000	.691	26.807	.997
Glycine (174)	2.376E11	1	2.376E11	.066	.802	.005	.066	.056
Glyceric acid (189, 292)	5.527E9	1	5.527E9	2.547	.136	.175	2.547	.312
Fumaric acid (245)	3.929E12	1	3.929E12	18.807	.001	.610	18.807	.978
L-Serine (204, 218)	2.178E14	1	2.178E14	27.615	.000	.697	27.615	.998
L-Threonine (218, 291)	1.282E11	1	1.282E11	.970	.344	.075	.970	.148
beta-alanine (248)	4.504E11	1	4.504E11	2.225	.162	.156	2.225	.279
Aspartic acid (232)	6.049E14	1	6.049E14	6.950	.022	.367	6.950	.678
5-oxo-Proline (156)	3.740E13	1	3.740E13	1.173	.300	.089	1.173	.170
GABA (304,174)	7.345E15	1	7.345E15	6.029	.030	.334	6.029	.617
Glutamic acid (246)	6.610E16	1	6.610E16	19.904	.001	.624	19.904	.983
L-Phenylalanine (218,192)	2.133E15	1	2.133E15	30.875	.000	.720	30.875	.999
L-Asparagine (188, 231)	6.058E17	1	6.058E17	56.871	.000	.826	56.871	1.000

	cis-Aconitic acid (229, 375)	3.009E11	1	3.009E11	1.150	.305	.087	1.150	.167
	Glutamine (156)	2.416E15	1	2.416E15	6.656	.024	.357	6.656	.659
	Isocitric acid (273)	8.620E17	1	8.620E17	7.263	.019	.377	7.263	.697
	D-Mannitol (319)	1.285E15	1	1.285E15	27.924	.000	.699	27.924	.998
	Palmitic acid (313, 314)	6.170E11	1	6.170E11	.521	.484	.042	.521	.102
	Galactaric acid (333)	1.739E12	1	1.739E12	4.882	.047	.289	4.882	.529
	L-Tryptophan (202)	7.088E12	1	7.088E12	1.895	.194	.136	1.895	.245
	Stearic acid (341,117)	9.369E11	1	9.369E11	1.049	.326	.080	1.049	.157
	Glucose-6-phosphate (387)	2.544E13	1	2.544E13	11.573	.005	.491	11.573	.877
	Raffinose (437)	1.314E12	1	1.314E12	1.718	.214	.125	1.718	.227
	Galactinol (204)	5.568E14	1	5.568E14	14.528	.002	.548	14.528	.937
Genotype * Storage	Fructose (217, 307)	3.106E14	2	1.553E14	2.911	.093	.327	5.822	.462
	Mannose	3.659E13	2	1.829E13	1.704	.223	.221	3.408	.289
	Galactose	6.927E16	2	3.463E16	1.335	.300	.182	2.670	.234
	Glucose	4.665E15	2	2.333E15	2.948	.091	.329	5.896	.467
	Sorbitol	2.950E14	2	1.475E14	2.214	.152	.270	4.427	.364
	Inositol	2.003E17	2	1.002E17	.971	.406	.139	1.943	.180
	Sucrose	1.051E18	2	5.253E17	1.529	.256	.203	3.057	.263
	Maltose	2.221E11	2	1.110E11	.092	.913	.015	.184	.061
	L-Isoleucine (158)	7.783E13	2	3.892E13	1.145	.351	.160	2.289	.206
	L-Proline (142)	1.359E16	2	6.797E15	.477	.632	.074	.954	.111
	Glycine (174)	6.983E12	2	3.492E12	.964	.409	.138	1.927	.179
	Glyceric acid (189, 292)	1.570E10	2	7.851E9	3.618	.059	.376	7.236	.554
	Fumaric acid (245)	1.257E12	2	6.286E11	3.009	.087	.334	6.018	.476
	L-Serine (204, 218)	6.904E12	2	3.452E12	.438	.655	.068	.875	.105
	L-Threonine (218, 291)	2.527E11	2	1.264E11	.955	.412	.137	1.911	.178
	beta-Alanine (248)	5.280E11	2	2.640E11	1.304	.307	.179	2.608	.229
	Aspartic acid (232)	3.678E13	2	1.839E13	.211	.813	.034	.422	.076
	5-oxo-Proline (156)	8.627E13	2	4.314E13	1.353	.295	.184	2.705	.236
	GABA (304,174)	3.876E15	2	1.938E15	1.591	.244	.210	3.181	.272

Glutamic acid (246)	6.407E15	2	3.203E15	.965	.409	.139	1.929	.179
L-Phenylalanine (218,192)	3.201E14	2	1.601E14	2.316	.141	.279	4.633	.379
L-Asparagine (188, 231)	2.833E16	2	1.416E16	1.330	.301	.181	2.660	.233
cis-Aconitic acid (229, 375)	7.924E11	2	3.962E11	1.514	.259	.201	3.028	.260
Glutamine (156)	1.839E15	2	9.193E14	2.533	.121	.297	5.066	.410
Isocitric acid (273)	1.072E16	2	5.362E15	.045	.956	.007	.090	.055
D-Mannitol 319	1.079E15	2	5.395E14	11.727	.002	.662	23.454	.974
Palmitic acid (313, 314)	3.431E12	2	1.715E12	1.449	.273	.194	2.897	.251
Galactaric acid (333)	3.436E11	2	1.718E11	.482	.629	.074	.965	.111
L-Tryptophan (202)	3.087E13	2	1.543E13	4.127	.043	.408	8.254	.613
Stearic acid (341,117)	1.768E12	2	8.839E11	.989	.400	.142	1.979	.183
Glucose-6-phosphate (387)	1.653E12	2	8.266E11	.376	.694	.059	.752	.097
Raffinose (437)	2.208E11	2	1.104E11	.144	.867	.023	.289	.067
Galactinol (204)	8.743E13	2	4.371E13	1.141	.352	.160	2.281	.205
Genotype * Stress								
Fructose (217, 307)	1.393E14	2	6.965E13	1.306	.307	.179	2.611	.229
Mannose	8.052E12	2	4.026E12	.375	.695	.059	.750	.097
Galactose	3.162E16	2	1.581E16	.610	.560	.092	1.219	.129
Glucose	2.607E15	2	1.303E15	1.647	.233	.215	3.294	.280
Sorbitol	3.716E14	2	1.858E14	2.788	.101	.317	5.577	.446
Inositol	3.197E17	2	1.598E17	1.550	.252	.205	3.100	.266
Sucrose	5.927E15	2	2.964E15	.009	.991	.001	.017	.051
Maltose	2.233E12	2	1.117E12	.923	.424	.133	1.846	.173
L-Isoleucine (158)	9.373E13	2	4.687E13	1.378	.289	.187	2.757	.240
L-Proline (142)	4.012E16	2	2.006E16	1.407	.282	.190	2.815	.245
Glycine (174)	9.490E12	2	4.745E12	1.310	.306	.179	2.619	.230
Glyceric acid (189, 292)	4.341E9	2	2.171E9	1.000	.396	.143	2.000	.184
Fumaric acid (245)	7.130E9	2	3.565E9	.017	.983	.003	.034	.052
L-Serine (204, 218)	1.836E13	2	9.178E12	1.163	.345	.162	2.327	.208
L-Threonine (218, 291)	2.500E11	2	1.250E11	.945	.416	.136	1.890	.176
beta-Alanine (248)	9.419E11	2	4.709E11	2.326	.140	.279	4.652	.380

Aspartic acid (232)	6.142E12	2	3.071E12	.035	.965	.006	.071	.054
5-oxo-Proline (156)	6.518E14	2	3.259E14	10.219	.003	.630	20.438	.952
GABA (304,174)	1.443E15	2	7.217E14	.592	.568	.090	1.185	.126
Glutamic acid (246)	4.444E14	2	2.222E14	.067	.936	.011	.134	.058
L-Phenylalanine (218,192)	8.796E14	2	4.398E14	6.365	.013	.515	12.731	.807
L-Asparagine (188, 231)	1.594E16	2	7.970E15	.748	.494	.111	1.497	.148
cis-Aconitic acid (229, 375)	1.004E11	2	5.019E10	.192	.828	.031	.384	.073
Glutamine (156)	2.767E15	2	1.384E15	3.812	.052	.389	7.624	.577
Isocitric acid (273)	6.559E16	2	3.280E16	.276	.763	.044	.553	.084
D-Mannitol (319)	2.757E15	2	1.379E15	29.962	.000	.833	59.924	1.000
Palmitic acid (313, 314)	9.451E12	2	4.725E12	3.991	.047	.399	7.982	.598
Galactaric acid (333)	1.127E11	2	5.635E10	.158	.855	.026	.316	.069
Malonic acid 245	1.968E13	2	9.841E12	.736	.499	.109	1.473	.147
L-Tryptophan 202	4.361E12	2	2.180E12	.583	.573	.089	1.166	.125
Stearic acid (341, 117)	1.488E13	2	7.438E12	8.325	.005	.581	16.650	.903
Glucose-6-phosphate 387	2.791E12	2	1.395E12	.635	.547	.096	1.270	.132
Raffinose 437	9.483E11	2	4.742E11	.620	.554	.094	1.240	.130
Galactinol 204	9.494E13	2	4.747E13	1.239	.324	.171	2.477	.219
Storage * Stress								
Fructose (217, 307)	5.863E14	1	5.863E14	10.991	.006	.478	10.991	.860
Mannose	8.788E14	1	8.788E14	81.855	.000	.872	81.855	1.000
Galactose	6.528E17	1	6.528E17	25.167	.000	.677	25.167	.996
Glucose	2.615E16	1	2.615E16	33.043	.000	.734	33.043	.999
Sorbitol	8.509E13	1	8.509E13	1.277	.281	.096	1.277	.180
Inositol	1.022E17	1	1.022E17	.991	.339	.076	.991	.151
Sucrose	8.924E18	1	8.924E18	25.968	.000	.684	25.968	.996
Maltose	3.628E12	1	3.628E12	2.998	.109	.200	2.998	.357
L-Isoleucine (158)	4.710E14	1	4.710E14	13.854	.003	.536	13.854	.927
L-Proline (142)	1.719E15	1	1.719E15	.121	.734	.010	.121	.062
Glycine (174)	1.428E13	1	1.428E13	3.940	.070	.247	3.940	.447
Glyceric acid (189, 292)	7.893E8	1	7.893E8	.364	.558	.029	.364	.086
Fumaric acid (245)	9.397E9	1	9.397E9	.045	.836	.004	.045	.054

L-Serine (204, 218)	2.198E13	1	2.198E13	2.787	.121	.188	2.787	.336
L-Threonine (218, 291)	6.183E11	1	6.183E11	4.675	.052	.280	4.675	.511
beta-Alanine (248)	4.666E10	1	4.666E10	.230	.640	.019	.230	.073
Aspartic acid (232)	2.556E14	1	2.556E14	2.936	.112	.197	2.936	.351
5-oxo-Proline (156)	2.859E14	1	2.859E14	8.963	.011	.428	8.963	.785
GABA (304,174)	3.722E15	1	3.722E15	3.055	.106	.203	3.055	.363
Glutamic acid 246	1.186E15	1	1.186E15	.357	.561	.029	.357	.085
L-Phenylalanine (218,192)	2.639E15	1	2.639E15	38.198	.000	.761	38.198	1.000
L-Asparagine (188, 231)	1.470E16	1	1.470E16	1.380	.263	.103	1.380	.191
cis-Aconitic acid (229, 375)	2.219E10	1	2.219E10	.085	.776	.007	.085	.058
Glutamine (156)	1.091E14	1	1.091E14	.301	.594	.024	.301	.080
Isocitric acid (273)	3.715E16	1	3.715E16	.313	.586	.025	.313	.081
D-Mannitol (319)	4.370E14	1	4.370E14	9.499	.010	.442	9.499	.807
Palmitic acid (313, 314)	3.620E12	1	3.620E12	3.057	.106	.203	3.057	.363
Galactaric acid (333)	1.374E12	1	1.374E12	3.857	.073	.243	3.857	.439
L-Tryptophan (202)	3.381E13	1	3.381E13	9.041	.011	.430	9.041	.788
Stearic acid (341,117)	9.122E12	1	9.122E12	10.211	.008	.460	10.211	.834
Glucose-6-phosphate (387)	5.440E12	1	5.440E12	2.475	.142	.171	2.475	.305
Raffinose (437)	1.752E12	1	1.752E12	2.290	.156	.160	2.290	.286
Galactinol (204)	3.235E14	1	3.235E14	8.440	.013	.413	8.440	.760
Genotype * Storage* Stress Fructose (217, 307)	2.307E14	2	1.154E14	2.163	.158	.265	4.326	.357
Mannose	1.642E13	2	8.212E12	.765	.487	.113	1.530	.151
Galactose	1.701E16	2	8.506E15	.328	.727	.052	.656	.091
Glucose	3.727E14	2	1.864E14	.235	.794	.038	.471	.079
Sorbitol	4.110E14	2	2.055E14	3.085	.083	.340	6.169	.486
Inositol	2.091E17	2	1.045E17	1.014	.392	.145	2.028	.186
Sucrose	3.293E16	2	1.646E16	.048	.953	.008	.096	.056
Maltose	4.586E11	2	2.293E11	.189	.830	.031	.379	.073
L-Isoleucine (158)	4.431E13	2	2.215E13	.652	.539	.098	1.303	.135
L-Proline (142)	1.251E16	2	6.254E15	.439	.655	.068	.877	.105
Glycine (174)	1.166E13	2	5.828E12	1.608	.241	.211	3.217	.274

Glyceric acid (189, 292)	3.535E9	2	1.767E9	.814	.466	.120	1.629	.158
Fumaric acid (245)	4.609E11	2	2.305E11	1.103	.363	.155	2.206	.199
L-Serine (204, 218)	2.060E13	2	1.030E13	1.306	.307	.179	2.612	.229
L-Threonine (218, 291)	1.709E11	2	8.543E10	.646	.541	.097	1.292	.134
beta-Alanine (248)	2.379E11	2	1.190E11	.588	.571	.089	1.175	.126
Aspartic acid (232)	4.206E13	2	2.103E13	.242	.789	.039	.483	.080
5-oxo-Proline (156)	4.363E13	2	2.181E13	.684	.523	.102	1.368	.139
GABA (304,174)	8.965E15	2	4.483E15	3.680	.057	.380	7.359	.561
Glutamic acid (246)	2.047E14	2	1.024E14	.031	.970	.005	.062	.054
L-Phenylalanine (218,192)	1.225E14	2	6.126E13	.887	.437	.129	1.773	.168
L-Asparagine (188, 231)	3.210E16	2	1.605E16	1.507	.261	.201	3.013	.259
cis-Aconitic acid (229, 375)	1.399E11	2	6.994E10	.267	.770	.043	.535	.083
Glutamine (156)	2.676E15	2	1.338E15	3.687	.056	.381	7.374	.562
Isocitric acid (273)	2.174E16	2	1.087E16	.092	.913	.015	.183	.061
D-Mannitol (319)	1.608E15	2	8.039E14	17.473	.000	.744	34.946	.998
Palmitic acid (313, 314)	3.140E11	2	1.570E11	.133	.877	.022	.265	.066
Galactaric acid (333)	3.418E11	2	1.709E11	.480	.630	.074	.960	.111
L-Tryptophan (202)	5.857E12	2	2.928E12	.783	.479	.115	1.566	.153
Stearic acid (341,117)	2.456E11	2	1.228E11	.137	.873	.022	.275	.067
Glucose-6-phosphate (387)	9.204E9	2	4.602E9	.002	.998	.000	.004	.050
Raffinose (437)	1.087E12	2	5.433E11	.710	.511	.106	1.420	.143
Galactinol (204)	3.187E13	2	1.593E13	.416	.669	.065	.832	.102

Table S2. Significant differnces (P<0.05) are highlighted by colours

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	SA	2997.663[a]	11	272.515	3.907	.014	.782	42.979	.906
	IAA	407.497[c]	11	37.045	1.694	.189	.608	18.638	.509
	ABA	220760.507[d]	11	20069.137	12.614	.000	.920	138.759	1.000
	JA	165.977[e]	11	15.089	1.483	.254	.576	16.309	.448
	JA-ILE	6.059[f]	11	.551	3.276	.026	.750	36.035	.839
Intercept	SA	45948.117	1	45948.117	658.775	.000	.982	658.775	1.000
	IAA	5716.681	1	5716.681	261.468	.000	.956	261.468	1.000
	ABA	1066412.740	1	1066412.740	670.292	.000	.982	670.292	1.000
	JA	195.684	1	195.684	19.228	.001	.616	19.228	.980
	JA-ILE	4.808	1	4.808	28.591	.000	.704	28.591	.998
Genotype	SA	472.494	2	236.247	3.387	.068	.361	6.774	.525
	IAA	200.193	2	100.096	4.578	.033	.433	9.156	.661
	ABA	4600.755	2	2300.377	1.446	.274	.194	2.892	.250
	JA	35.494	2	17.747	1.744	.216	.225	3.488	.295
	JA-ILE	.424	2	.212	1.261	.318	.174	2.521	.223
Stress	SA	349.549	1	349.549	5.012	.045	.295	5.012	.539
	IAA	3.015	1	3.015	.138	.717	.011	.138	.064
	ABA	69711.671	1	69711.671	43.817	.000	.785	43.817	1.000
	JA	.872	1	.872	.086	.775	.007	.086	.058
	JA-ILE	.275	1	.275	1.636	.225	.120	1.636	.218
Storage	SA	1001.729	1	1001.729	14.362	.003	.545	14.362	.935
	IAA	35.316	1	35.316	1.615	.228	.119	1.615	.216
	ABA	108934.442	1	108934.442	68.471	.000	.851	68.471	1.000
	JA	71.775	1	71.775	7.053	.021	.370	7.053	.684
	JA-ILE	3.390	1	3.390	20.162	.001	.627	20.162	.984
Genotype * Stress	SA	20.883	2	10.441	.150	.863	.024	.299	.068
	IAA	34.035	2	17.018	.778	.481	.115	1.557	.152
	ABA	2332.282	2	1166.141	.733	.501	.109	1.466	.146
	JA	.187	2	.094	.009	.991	.002	.018	.051
	JA-ILE	.355	2	.178	1.056	.378	.150	2.112	.193
Genotype * Storage	SA	79.406	2	39.703	.569	.581	.087	1.138	.123
	IAA	57.848	2	28.924	1.323	.303	.181	2.646	.232
	ABA	3452.445	2	1726.222	1.085	.369	.153	2.170	.197

	JA	10.447	2	5.224	.513	.611	.079	1.027	.116
	JA-ILE	.562	2	.281	1.671	.229	.218	3.342	.284
Stress * Storage	SA	806.966	1	806.966	11.570	.005	.491	11.570	.877
	IAA	72.296	1	72.296	3.307	.094	.216	3.307	.387
	ABA	30576.610	1	30576.610	19.219	.001	.616	19.219	.980
	JA	9.547	1	9.547	.938	.352	.073	.938	.145
	JA-ILE	.562	1	.562	3.342	.092	.218	3.342	.391
Genotype * Stress * Storage	SA	266.636	2	133.318	1.911	.190	.242	3.823	.320
	IAA	4.793	2	2.397	.110	.897	.018	.219	.063
	ABA	1152.304	2	576.152	.362	.704	.057	.724	.095
	JA	37.655	2	18.828	1.850	.199	.236	3.700	.310
	JA-ILE	.491	2	.245	1.460	.271	.196	2.919	.252

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