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Effect of drought and waterlogging on saccharides and amino acids content in potato tubers

*Matyáš Orsák[1], Zora Kotíková[1], František Hnilička[2], Jaromír Lachman[1]**

[1]*Department of Chemistry, Faculty of Agrobiology, Food and Natural Resources, University of Life Sciences Prague, Prague, Czech Republic*

[2]*Department of Botany and Plant Physiology, Faculty of Agrobiology, Food and Natural Resources, University of Life Sciences Prague, Prague, Czech Republic*

**Corresponding author: lachman@af.czu.cz*

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Abstract: The study was focused on the effect of drought and waterlogging stresses in two-year pot experiments in the peat substrate on the content of glucose, fructose and sucrose and free amino acids in potato tubers of four cultivars (yellow-fleshed Laura, Marabel, Milva and blue-fleshed Valfi) after 71 days of exposure to stresses conditions (BBCH 909). Drought and waterlogging increased levels of fructose, glucose, and sucrose in three potato cultivars except for cv. Laura. Drought stress increased L-proline (+248.4%), L-hydroxyproline (+135.3%), L-arginine (+29.97%), L-glutamic acid (+29.09%) and L-leucine (+22.58%) contents in all analysed cultivars. Moreover, the high effect of drought stress on an increase of L-phenylalanine, L-histidine, L-threonine, and total free amino acids content of the cvs. Laura, Valfi and Marabel has been observed. A comparison of the effects of drought and waterlogging stresses on the content of total amino acids showed an increase under drought and a decrease under waterlogging conditions. On average, of all cultivars, waterlogging stress caused an increase of L-tyrosine content, whereas drought stress decrease. In addition, drought stress caused a significant increase of L-proline in all cultivars while waterlogging its decrease. Obtained results confirmed different responses of susceptible or resistant cultivars to abiotic stresses.

Keywords: *Solanum tuberosum* L.; long-term abiotic stresses; soluble sugars; protein building units

Potatoes (*Solanum tuberosum* L.) are the fourth most important food crop in the world after rice, wheat and maize (Ezekiel et al. 2013). They are promising plants in developing countries, which produce more than half of the total world potato production (Monneveux et al. 2013). Potatoes are an excellent source of saccharides, proteins, and other health benefit phytonutrients, therefore are highly desirable in the human diet.

Under sufficient humidity conditions, potato yields more food per unit of water than other major crops. Potato for every m^3 of water applied to the crop produces 5 600 kcal of dietary energy, compared to 3 860 in maize, 2 300 in wheat and 2 000 in rice (Monneveux et al. 2013). However, current climate changes, with more fluctuations in temperature and precipitation levels, significantly affect the yield and quality of potato tubers.

Drought stress is one of the major yields and quality limiting abiotic stress worldwide. Potato is sensitive to drought due to its shallow root system. When drought occurs in spring and early summer, the quality and quantity of the tubers might be considerably reduced (Bündig et al. 2017).

Water stress influences potato growth and production by reducing the amount of productive foliage, by decreasing the rate of photosynthesis per unit of leaf area, and by shortening the vegetative growth period with respect to potato under well-watered conditions. Potato yield under water stress depends on the time, duration, and severity of the stress, as well as on genotype (Hirut et al. 2017). Because a selection for water stress-tolerant genotypes in the field is time-consuming, cost-intensive and difficult to reproduce, it was proposed that screening

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genotypes for their response to stress conditions *in vitro* might be an alternative (Bündig et al. 2017).

The need for more tolerant potato cultivars that can withstand fluctuation in water availability and deliver good quality tubers resulted in the present study, which focused on: (*i*) change of the content of soluble saccharides; (*ii*) change of amino acid content; (*iii*) change of L-proline content (special amino acid involved in abiotic stresses) under long-term drought stress (DS) and waterlogging stress (WLS).

MATERIAL AND METHODS

Plant material and potato growing in the greenhouse two-year 2017 and 2018 experiment. Four cultivars of potatoes are characterised in Table 1 (they have approximately the same growth period – medium early). Potato plants were grown in partially controlled temperature and humidity conditions of the greenhouse of the Department of Botany and Plant Physiology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague. Potato plants were grown in 5 L pots in the substrate Hawita baltisches Trysubstrat (dispensing 45025, pH_{CaCl_2} 5.8, white peat 70%, black peat 30%, structure fine, HAWITA Gruppe GmbH, Vechta, Germany). 2 g of NPK 8-24-24 fertiliser were added to the pot before starting the experiment. In each pot, one plant of potato was grown. The experiment was performed in four replicates for each year (a total of eight replicates; in both years, the same experimental conditions were ensured). The pots were placed in the greenhouse according to the Latin square method. Plants were grown under the natural light mode, where the length of the day was 13 h and dark 11 h. The temperature mode was set at 22 °C by day and 17 °C by night at 70% air relative humidity. The experimental scheme included three variants of the experiment; the first variant consisted of irrigated control, the second variant represented the water deficit, and the third variant the permanent waterlogging. The control plants were irrigated with distilled water at regular three-day intervals where the amount of water was 450 mL per pot. In the second variant, the water deficit was induced by the method of gradual natural drying out of the substrate. In the third variant, the plants and the substrate were kept in anoxia by placing the pots in a bath with water. Plant stress in the second and third variant of the experiment was induced in the development phase BBCH 109. Plants of all experimental variants were twice fertilised with 3% NPK 8-24-24 solution in 450 mL water for watering in the developmental stages 406 and 703 BBCH. In the nutrient solution, 2.40 g of N, 3.14 g of P and 5.98 g of K in pure nutrients were added. Tuber sampling was carried out in the developmental step according to BBCH 909 (harvested product, 71 days of stress).

Preparation of sample for the determination of saccharides. About 2.5 g of a lyophilised potato sample (Lyovac GT2, Steris, Inc., Hurth, Germany) was weighed into a 100 mL Erlenmeyer flask. Further, 50 mL of 80% ethanol was added, and the flask was sealed with parafilm. The mixture was shaken for 30 min on a shaker (150 rpm, GFL 3006, Burgwedel, Germany). Subsequently, filtration followed, and then 25 mL of the filtrate was evaporated at 40 °C for 20 min under vacuum (Rotavapor R-200; Büchi Labortechnik, AG, Flawil, Switzerland). The resulting residue was dissolved in 5 mL of deionised water, followed by microfiltration through a nylon microfilter (0.45 μm) and transfer of the sample to the vial. Finally, 1 drop of isopropanol was added as a preservative, and the sample was subjected to

Table 1. Characteristics of analysed potato cultivars (Orsák et al. 2020)

Cultivar	Origin of tubers/year	Maturity	Skin color	Flesh color	Shape of tubers	Origin	Resistance to biotic stress
Laura	Austria 1976	medium-early	red	dark yellow	oval	Saskia × MPI 49 540	medium-high
Marabel	Germany 1993	medium-early/ early	yellow	yellow	oval	Nena × MA 75 304 2	high
Milva	Germany 2002	medium-early/ early	yellow	yellow	oval to tear drop	Nena × Dunja	medium-high
Valfi	Czech Republic 2005	medium-early/ medium-late	purple-lilac	dark blue to black	round to oval	clone selection from British Columbia Blue heritage cultivar	medium less

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HPLC-RI analysis. All samples were analysed in three replicates.

Chromatographic analysis of saccharides by HPLC-RI. For the determination of saccharides in potatoes, Waters 2695 High-Pressure Liquid Chromatograph (Sciencix, Burnsville, USA) with Refractometric Detector 2414 (Sciencix, Burnsville, USA) was used. Isocratic elution mode was used with the mobile phase consisting of 75% acetonitrile and 25% deionised water; the mobile phase flow rate was 1.25 mL/min, the chromatographic column temperature 35 °C and the detector temperature 40 °C. The separation was carried out on a Luna® 5 µm NH_2 100 Å column (250 × 4.6 mm, Phenomenex Inc., Lane Cove, Australia), and the injected volume was 10 µL at 12.5 min time analysis. Quantification of D-glucose, D-fructose and sucrose was performed by external calibration (five-point calibration, concentration range 2–20 µg/mL).

Preparation of sample for the determination of amino acids. 0.5 g of lyophilised potato tubers was weighed into a 15 mL falcon tube, and then 5 mL of extraction mixture methanol/water (1:1) was added. The mixture was shaken for 30 min on a shaker. Subsequently, the sample was centrifuged (5810R, Eppendorf, Ltd., Hamburg, Germany) at 8 228 rcf for 5 min. The supernatant was diluted 100 times with water. The sample thus prepared was filtered through a nylon microfilter (0.45 µm) and analysed on LC-MS/MS.

Chromatographic analysis of amino acids by LC-MS/MS. A liquid chromatography (UltiMate 3000 RS, Thermo Fisher Scientific, Waltham, USA) coupled with a mass detector (A hybrid triple quadrupole with linear ion trap, 3200 QTRAP AB Sciex, Inc., Framingham, MA, USA) was used to analyse the amino acids. The separation of individual amino acids under RP-HPLC conditions was achieved by ion-pair chromatography. Heptafluorobutyric acid (HFBA) was added to the mobile phase as an ion-pairing agent.

Chromatography was provided on the analytical column: ZORBAX SB-C18, 3.0 × 150 mm, 5 µm (Agilent Inc., Santa Clara, USA). Chromatography conditions were as follows: column temperature 25 °C, autosampler temperature 10 °C, mobile phase A 20 mmol HFBA in water, B 20 mmol HFBA in methanol (gradient elution), 0–0.5 min 20% B isocratic elution, 0.5–9 min 60% B linear grade elution, 9–10 min 60% B isocratic elution, 10–11 min 20% B linear gradient elution, 11–15 min 20% B isocratic elution, analysis time 15 min, flow rate 0.3 mL/min, spray volume 1 µL.

Quantification of all protein-forming amino acids (including Pro and Hyp) was performed by external calibration.

Dry matter determination. The batches containing potato quarters were freeze-dried for 100 h to reach constant weight. The batches were weighed before and after the process, and the dry matter was calculated as a loss of water.

Statistical analysis. The data were processed by Chromeleon (Thermo Fisher Scientific, Inc., Waltham, USA), Analyst 1.4 (AB Sciex, Canada) and Excel (Microsoft, Redmond, USA). Statistical evaluation was performed using the Statistica software (ver. 12; StatSoft, Inc., Tulsa, USA). The influence of cultivar, drought/waterlogging stress and their interaction on the saccharides and amino acids was evaluated by factorial ANOVA ($P \leq 0.05$). Tukey's Post Hoc *HSD* (honestly significant difference) test was used for detailed statistical evaluation.

RESULTS AND DISCUSSION

Saccharide profile and quantity in potato cultivars were affected by DS and WLS. DS increased the content of fructose, glucose, and sucrose in all potato cultivars. Similarly, WLS caused an increase of all analysed saccharides, except for the cv. Laura, where a decrease of glucose was observed. DS showed a higher impact on the increase of saccharide concentration with the exceptions of cv. Laura for sucrose and cv. Valfi for glucose and sucrose, where the impact of DS on their concentration was lesser by comparison with the effect of WLS. The effect of DS and WLS on the content of saccharides in potato tubers of cvs. Laura, Milva, Marabel, and Valfi are given in Figure 1. On average, of all cultivars, the highest increase under DS was observed for fructose content (by +261.1%), followed by glucose (by +121.7%) and sucrose (by +111.2%). Under WLS, the increases were lower, for sucrose by +98.21%, fructose by +93.68% and glucose by +57.83% on average of all examined cultivars.

Drought is one of the most important factors that inhibit photosynthesis significantly. Glucose induces stomatal closure and enhances plants' adaptability (Sami et al. 2016). Different strategies to avoid or tolerate stress effects were observed in alfalfa (Maghsoodi et al. 2017) and wheat (Song et al. 2017). However, in the present study, generally, fructose, glucose and sucrose increased to varying degrees related to cultivars. Sugar accumulation reduces the rate of photosynthesis, main-

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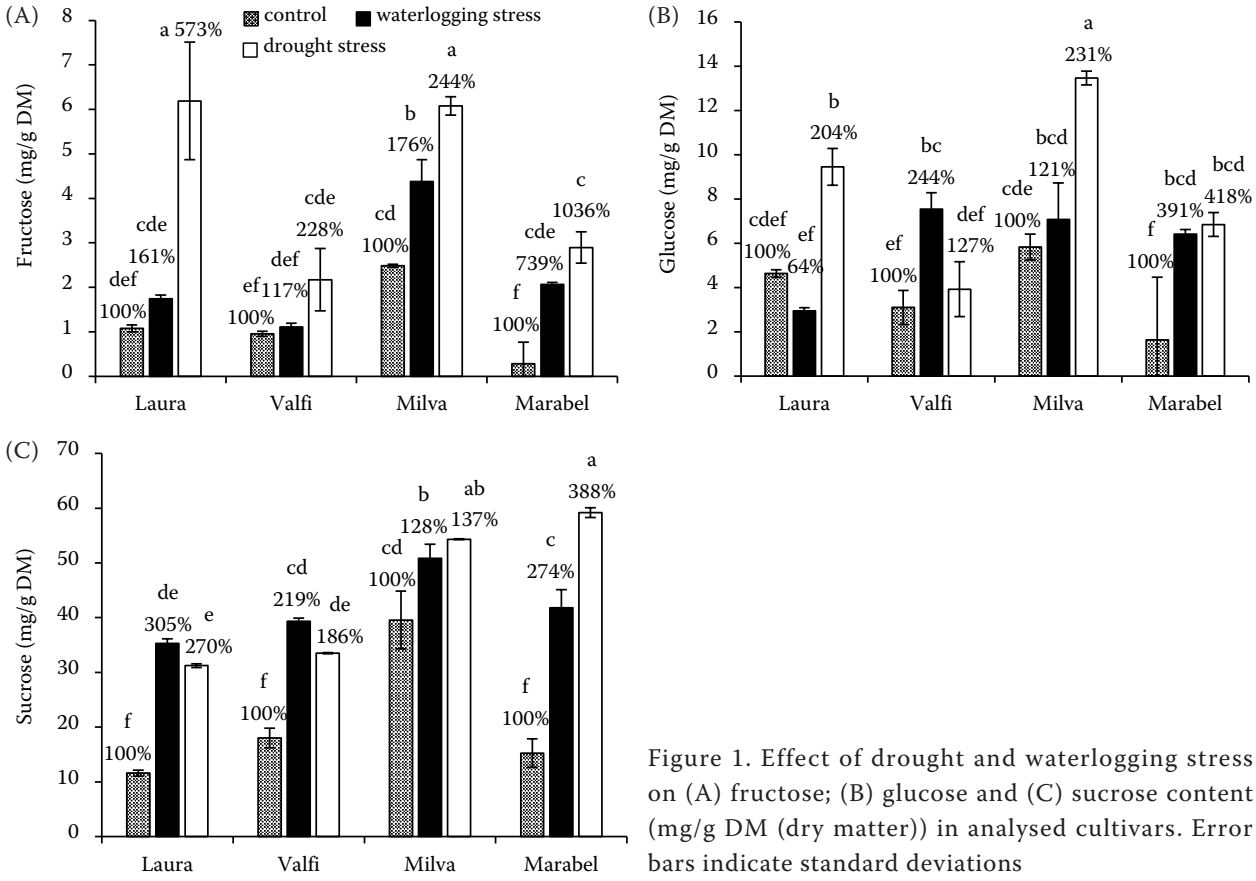

Figure 1. Effect of drought and waterlogging stress on (A) fructose; (B) glucose and (C) sucrose content (mg/g DM (dry matter)) in analysed cultivars. Error bars indicate standard deviations

tain the turgidity of leaves, and prevents dehydration of membrane proteins. Hence, the accumulation of soluble sugars like glucose, fructose and sucrose leads to activation of stress genes resulted in maintaining the integrity of membranes, and proteins, biosynthesis of osmolytes, maintaining of osmotic and ionic homeostasis, regulation of cell division and growth inhibition and in the final step to abiotic stress tolerance (Sami et al. 2016). The major role of these sugars during stress is membrane protection and scavenging of free radicals (Krasenky and Jonak 2012).

Glucose is the primary energy provider for the most important sugar-signaling molecules. Therefore, monitoring intracellular glucose contents is an essential step for glucose response in abiotic stresses. Under DS, glucose content increased, only for cv. Valfi, the increase was statistically insignificant. In addition, in rice plants, the abiotic elicitors like osmotic stress, salinity and extreme temperature induced the intracellular glucose increases (Zhu et al. 2017). Sucrose, glucose, and fructose are recognised as the main sensing molecules and elicit sugar sources in sink organs.

Sucrose is the main form to assimilate transport, and its concentrations increased under DS in our experiments in potatoes. This agrees within kale study, where in leaves sucrose concentrations increased with DS (Pathirana et al. 2017). Similarly, as in our study, DS increased total soluble sugars, sucrose, fructose, and glucose in star fruit (*Averrhoa carambola* L.) (Wu et al. 2017).

The increase or decrease of fructose, glucose and sucrose is affected by the severity of DS and WLS as well as by potato genotypes. In the present study, the conditions of used stress were characterised by high intensity of severity according to average relative water content in leaves RWC 73.23% for DS and 87.10% for WLS. In both milder or severer conditions of two years, Wegener et al. (2017) observed an increase of sucrose and a decrease of glucose and fructose in three potato genotypes.

Effect of DS and WLS on amino acids (AAs) of potato cultivars. DS increased on average contents of Arg (+29.97%), L-proline (Pro) (+248.4%), L-hydroxyproline (Hyp) (+135.3%), L-glutamic acid (Glu) (+29.09%) and L-leucine (Leu) (+22.58%) in all

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analysed cultivars. High effect of DS on the increase of L-phenylalanine (Phe) (+45.66%), L-histidine (His) (+32.52%), L-threonine (Thr) (+36.15%) and total amino acids contents (+17.76%) in cvs. Laura, Valfi and Marabel were also determined. DS increased levels of 15 amino acids (and total amino acid content) in cvs. Laura and Marabel, 14 amino acids in cv. Valfi and 9 in cv. Milva. In contrast, a decrease was recorded in all cultivars for L-tyrosine (Tyr) under DS, and likewise, L-aspartic acid (Asp), L-tryptophane (Trp), L-methionine (Met), and L-glutamic acid (Glu) decreased in the Laura and Milva cultivars (Table 2).

Table 2. Effect of drought (DS) and waterlogging stress (WLS) on the levels of amino acids in potato cultivars (mg/g dry matter)

Amino acid	cv. Laura			cv. Valfi			cv. Milva			cv. Marabel		
	control	WLS	DS	control	WLS	DS	control	WLS	DS	control	WLS	DS
His	0.75 ± 0.03^{g}	0.88 ± 0.02^{de}	0.96 ± 0.03^{d}	1.08 ± 0.05^{b}	1.19 ± 0.03^{b}	1.42 ± 0.02^{a}	0.85 ± 0.02^{ef}	0.79 ± 0.02^{gef}	0.78 ± 0.03^{fg}	0.84 ± 0.03^{ef}	0.42 ± 0.02^{h}	1.51 ± 0.07^{a}
Phe	0.74 ± 0.01^{e}	0.82 ± 0.02^{cd}	0.88 ± 0.05^{c}	0.59 ± 0.00^{h}	0.97 ± 0.03^{b}	0.72 ± 0.01^{ef}	0.73 ± 0.02^{e}	0.78 ± 0.01^{de}	0.66 ± 0.02^{fg}	0.61 ± 0.01^{gh}	0.40 ± 0.01^{i}	1.63 ± 0.04^{a}
Arg	2.86 ± 0.03^{f}	4.73 ± 0.18^{d}	3.37 ± 0.07^{e}	6.40 ± 0.22^{b}	6.10 ± 0.08^{b}	7.83 ± 0.16^{a}	2.18 ± 0.02^{g}	2.64 ± 0.01^{f}	2.20 ± 0.01^{g}	2.83 ± 0.08^{f}	1.48 ± 0.02^{h}	5.14 ± 0.18^{c}
Tyr	1.29 ± 0.03^{b}	1.84 ± 0.05^{a}	1.00 ± 0.05^{c}	0.98 ± 0.02^{c}	1.38 ± 0.03^{b}	0.92 ± 0.05^{c}	0.68 ± 0.01^{d}	0.57 ± 0.00^{e}	0.31 ± 0.02^{g}	0.60 ± 10.0^{de}	0.49 ± 0.01^{f}	0.50 ± 0.03^{f}
Ala	0.63 ± 0.03^{h}	0.82 ± 0.03^{def}	0.92 ± 0.04^{bc}	0.88 ± 0.04^{cd}	0.86 ± 0.01^{ef}	0.77 ± 0.01^{ef}	0.65 ± 0.02^{gh}	0.62 ± 0.03^{h}	0.74 ± 0.05^{fg}	1.37 ± 0.05^{a}	0.40 ± 0.02^{i}	0.99 ± 0.01^{b}
Ser	0.77 ± 0.01^{b}	0.61 ± 0.02^{cd}	0.95 ± 0.06^{a}	0.58 ± 0.03^{de}	0.58 ± 0.01^{de}	0.56 ± 0.02^{def}	0.50 ± 0.02^{f}	0.58 ± 0.01^{de}	0.51 ± 0.03^{ef}	0.67 ± 0.01^{c}	0.32 ± 0.04^{g}	0.76 ± 0.01^{b}
Pro	0.21 ± 0.01^{f}	0.11 ± 0.01^{f}	2.75 ± 0.02^{b}	0.77 ± 0.01^{e}	0.14 ± 0.01^{f}	1.62 ± 0.05^{d}	0.13 ± 0.01^{f}	0.10 ± 0.00^{f}	2.42 ± 0.05^{c}	2.62 ± 0.07^{bc}	0.07 ± 0.00^{f}	6.16 ± 0.26^{a}
Val	2.14 ± 0.04^{de}	2.01 ± 0.07^{ef}	3.09 ± 0.08^{a}	2.88 ± 0.09^{bc}	2.93 ± 0.12^{bc}	2.78 ± 0.15^{c}	1.65 ± 0.05^{g}	1.25 ± 0.06^{h}	1.84 ± 0.05^{fg}	2.37 ± 0.08^{d}	1.30 ± 0.03^{h}	3.19 ± 0.09^{a}
Thr	0.62 ± 0.02^{cdef}	0.62 ± 0.04^{cde}	0.71 ± 0.04^{bc}	0.46 ± 0.06^{g}	0.74 ± 0.02^{g}	0.56 ± 0.01^{defg}	0.63 ± 0.03^{cd}	0.56 ± 0.02^{defg}	0.52 ± 0.02^{efg}	0.51 ± 0.01^{fg}	0.31 ± 0.02^{h}	1.23 ± 0.08^{a}
Leu	0.49 ± 0.01^{d}	0.51 ± 0.01^{d}	0.61 ± 0.00^{b}	0.56 ± 0.02^{c}	0.69 ± 0.01^{a}	0.62 ± 0.02^{b}	0.23 ± 0.01^{g}	0.30 ± 0.00^{f}	0.31 ± 0.02^{f}	0.30 ± 0.00^{f}	0.14 ± 0.01^{h}	0.41 ± 0.02^{e}
Asp	1.12 ± 0.07^{c}	0.53 ± 0.05^{i}	0.89 ± 0.01^{def}	1.36 ± 0.07^{b}	1.01 ± 0.05^{cd}	1.52 ± 0.10^{a}	0.90 ± 0.01^{de}	0.75 ± 0.04^{fg}	0.81 ± 0.02^{efg}	0.56 ± 0.02^{hi}	0.49 ± 0.02^{i}	0.69 ± 0.00^{gh}
Lys	0.72 ± 0.01^{gh}	0.76 ± 0.03^{g}	0.79 ± 0.03^{fg}	1.60 ± 0.07^{b}	1.87 ± 0.04^{a}	1.46 ± 0.02^{c}	1.17 ± 0.06^{d}	0.72 ± 0.02^{gh}	0.63 ± 0.02^{h}	1.00 ± 0.03^{ef}	0.69 ± 0.03^{h}	0.87 ± 0.02^{g}
Gly	0.00 ± 0.00^{bcd}	0.00 ± 0.00^{bcd}	0.00 ± 0.00^{bcd}	0.00 ± 0.00^{bc}	0.01 ± 0.00^{a}	0.00 ± 0.00^{bc}	0.00 ± 0.00^{bc}	0.00 ± 0.00^{d}	0.00 ± 0.00^{bcd}	0.01 ± 0.00^{ab}	0.00 ± 0.00^{de}	0.00 ± 0.00^{bcd}
Glu	1.10 ± 0.02^{g}	1.57 ± 0.04^{def}	1.73 ± 0.09^{d}	2.29 ± 0.02^{bc}	2.21 ± 0.07^{bc}	2.37 ± 0.09^{b}	1.67 ± 0.02^{de}	2.18 ± 0.08^{c}	1.75 ± 0.02^{d}	1.54 ± 0.06^{ef}	1.43 ± 0.03^{f}	2.66 ± 0.11^{a}
Hyp	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}	0.00 ± 0.00	0.02 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}	0.00 ± 0.00	0.02 ± 0.00^{a}
Ile	1.00 ± 0.01^{d}	0.96 ± 0.02^{d}	1.21 ± 0.02^{b}	1.24 ± 0.06^{b}	1.54 ± 0.02^{a}	1.14 ± 0.02^{c}	0.60 ± 0.01^{f}	0.51 ± 0.01^{g}	0.69 ± 0.02^{e}	0.94 ± 0.01^{d}	0.52 ± 0.01^{g}	1.00 ± 0.02^{d}
Trp	0.26 ± 0.00^{d}	0.41 ± 0.01^{b}	0.20 ± 0.01^{ef}	0.35 ± 0.01^{c}	0.54 ± 0.03^{a}	0.36 ± 0.00^{c}	0.23 ± 0.01^{d}	0.20 ± 0.00^{ef}	0.09 ± 0.01^{g}	0.18 ± 0.01^{f}	0.38 ± 0.01^{bc}	0.17 ± 0.00^{f}
Met	0.89 ± 0.03^{c}	0.88 ± 0.03^{c}	0.82 ± 0.02^{cd}	0.79 ± 0.03^{de}	1.24 ± 0.03^{a}	1.09 ± 0.01^{b}	0.73 ± 0.03^{e}	0.71 ± 0.03^{e}	0.54 ± 0.04^{f}	0.75 ± 0.03^{de}	0.50 ± 0.01^{f}	0.75 ± 0.03^{de}
Asn	20.64 ± 0.49^{e}	23.56 ± 1.26^{cd}	22.67 ± 1.09^{de}	26.68 ± 0.29^{b}	23.59 ± 0.75^{cd}	31.84 ± 1.39^{a}	23.85 ± 0.33^{cd}	21.95 ± 0.74^{de}	22.18 ± 0.47^{de}	25.41 ± 0.80^{bc}	9.68 ± 0.48^{f}	31.53 ± 0.20^{a}
Gln	3.69 ± 0.25^{gh}	4.81 ± 0.29^{ef}	3.31 ± 0.23^{h}	8.18 ± 0.35^{c}	11.14 ± 0.07^{a}	10.72 ± 0.39^{ab}	7.92 ± 0.10^{c}	6.44 ± 0.23^{d}	5.40 ± 0.26^{e}	6.67 ± 0.22^{d}	4.33 ± 0.20^{fg}	10.11 ± 0.51^{b}

Different letters in the lines mean significant differences among individual amino acid contents at each cultivar and stress (DS and WLS) at $P \leq 0.05$

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Comparison of the effects of DS and WLS on the content of total free AAs content (control 48.18 mg/g DM) showed an increase under DS (to 56.73 mg/g DM) and a decrease under WLS (to 42.55 mg/g DM).

Hydroxy amino acid Tyr decreased in all cultivars under DS (on average by 23.11%), but WLS caused an increase in cvs. Laura and Valfi while a decrease in cvs. Milva and Marabel have been observed (Table 2). Responses to the stresses pointed to the same genetic ancestor of Marabel and Milva cultivars (Nena cultivar). On average of all cultivars, Tyr content as compared with control (0.889 mg/g DM), under WLS increased (to 1.069 mg/g DM) and conversely under DS decreased (to 0.683 µg/g DM).

From the literature is known that DS increases total amino acid content (Hildebrandt et al. 2015). However, only a few papers have examined the factors affecting the amino acid contents in potato. To date, no paper has looked at the complex amino acid profile in this crop after DS and WLS. Amino acids have various prominent functions in plants. They are used in the process of protein biosynthesis and represent building blocks for signaling processes, and, in addition, are involved in plant stress response (Hildebrandt et al. 2015).

Under different abiotic stresses, like salt stress, proteolysis increases and consequently, the concentrations of amino acids and their metabolites are increased. Total free amino acids (AAs) under our DS increased (Figure 2A). This agrees with the results of some studies of other authors (Muttucumaru et al. 2015, Wegener et al. 2017). The results have shown that total amounts of free total AAs significantly increased due to DS, where it was reported by Wegener et al. (2017) in the average range by 26.6% and 16.9%. Free AAs and their derivatives serve as osmolytes, and therefore they can help maintain cell volume and stabilise proteins and other molecules, to adapt the cells to DS. In our case, a significant increase of total AAs was found for cvs. Laura, Valfi and Marabel, but a significant decrease has been observed for cv. Milva (Figure 2A). It is possible that some AAs are partially incorporated into enzymes or proteins needed for adaptive responses and subsequently reduces. DS induces changes in free AAs, which are dependent on the stress intensity and severity (Wegener et al. 2017).

Special proteogenic amino acid Pro (it is an osmolyte, antioxidant, chaperone and stabiliser of membranes and enzymes) and its derivative L-hydroxyproline (Hyp) in the present experiments showed significant changes in all cultivars under stress conditions (Figure 2B). DS caused a significant increase of Pro content in all cultivars (on average by 248.4%), while WLS caused its decrease (by 88.67%). Such increase under DS was also reported in several previous studies (Muttucumaru et al. 2015, Wegener et al. 2017). It can be supposed that extreme Pro increase went on the cost of the other AAs depending on the stress intensity. Likewise, Hyp under DS increased by 139.3%, but under WLS, its values were not detected. One of the adaptive metabolic responses to drought or salt stresses is the accumulation of Pro (Bandurska et al. 2017). Proline acts as an osmolyte and a chemical chaperone and therefore is accumulated by plants under various stress conditions (Szabados and Savouré 2009). Higher Pro accumulation in drought-susceptible than drought-resistant potato genotypes has been found (Schafleitner et al. 2007). However, our results showed that Pro and Hyp concentrations increased in tubers of all examined cultivars under DS. Thus, we can confirm that the accumulation of Pro is one of the striking metabolic responses of potato plants to DS likewise as was also reported in other plants (Per et al. 2017). In a link between Pro accumulation and ABA may also be involved sugars; sucrose has been found to show an inhibitory effect on ABA-induced Pro accumulation. In Pro accumulation may also be involved other phytohormones such as bioactive cytokinins, gibberellins, ethylene, salicylic acid, or nitric oxide (Iqbal et al. 2014).

L-Glutamine (Gln) in the present study decreased in cvs. Laura and Milva (draught sensitive) while increased in cvs. Valfi and Marabel (drought tolerant) (Figure 2E). Such a trend could be related to the decrease of enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT) under DS (Xia et al. 2020). L-Asparagine (Asn) increased in cvs. Laura, Valfi and Marabel and decreased in cv. Milva (Figure 2D). An increase in glasshouse experiment was reported in both drought-tolerant and sensitive cultivars, where an important factor was a range of stresses with extreme severity (Muttucumaru et al. 2015, Wegener et al. 2017).

Hydrophobic amino acid Leu increased under DS in all cultivars (Figure 2C) likewise as L-valine (Val) and isoleucine (Ile) with the except for cv. Valfi, where an insignificant decrease has been found. Such increase was assigned by Muttucumaru et al. (2015) to drought-tolerant cultivars. Sulphur amino acid L-methionine (Met) increased in cv. Valfi, cv. Marabel was under DS comparable with control

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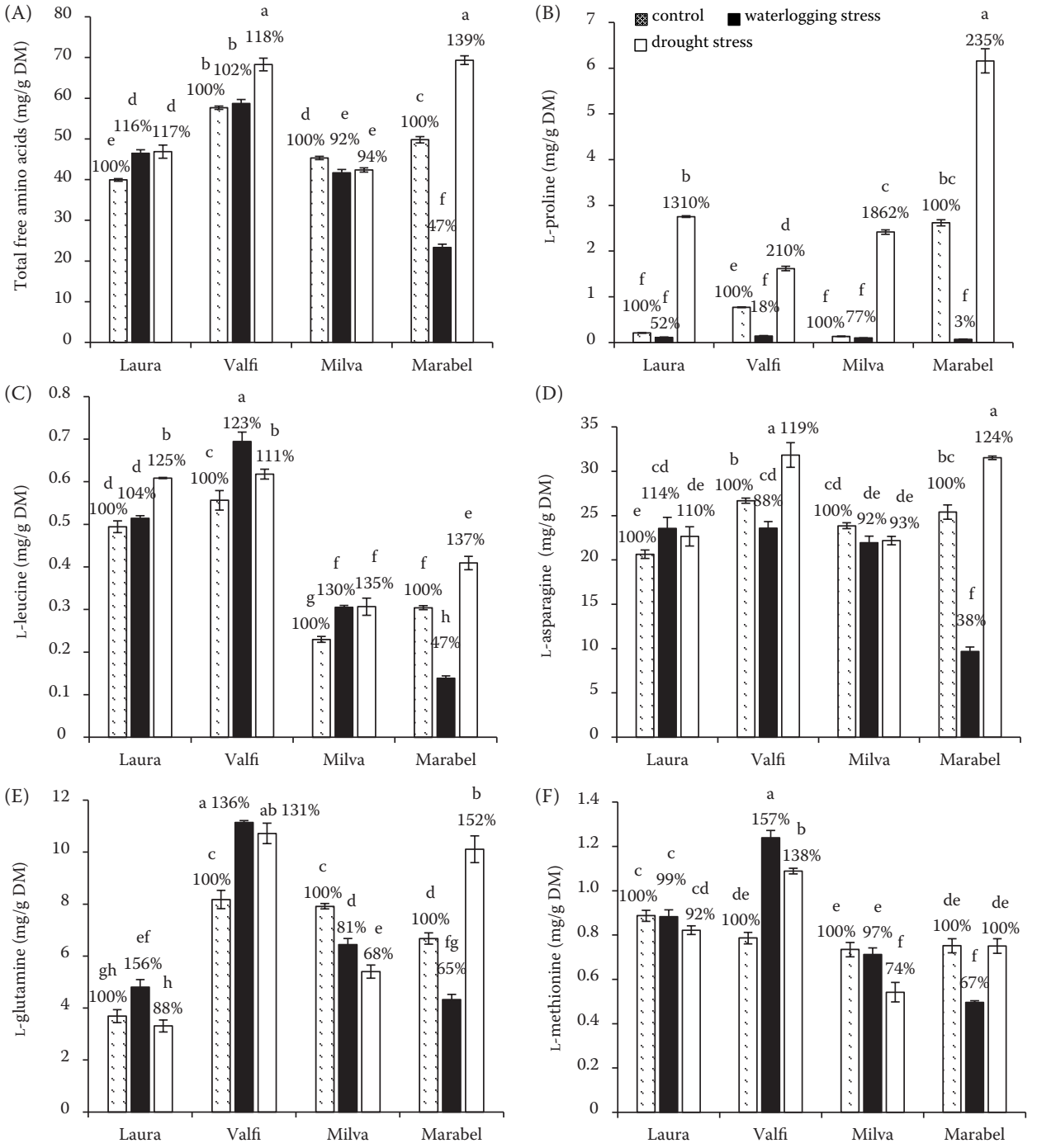

Figure 2. Effect of drought and waterlogging stress on (A) total free amino acids; (B) L-proline; (C) L-leucine; (D) L-asparagine; (E) L-glutamine, and (F) L-methionine (mg/g dry matter (DM)) in analysed cultivars. Error bars indicate standard deviations

and decreased in cvs. Laura and Milva (Figure 2F). Another sulphur amino acid, L-cysteine (Cys), was not in free form in analysed cultivars detected.

A study of the metabolic response of *Arabidopsis* to abiotic stresses revealed that Lys and Thr are induced under different stress situations (Obata and Fernie 2012). Moreover, branched-chain amino acids are also induced during various stresses (Joshi et al. 2010). This was confirmed in our case for Leu. DS also increased substantially levels of Arg, Pro, Hyp, Glu, Phe, His, Thr and total amino acids content in most cultivars except for cv. Milva.

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The present study shows that the greatest number of amino acids, which concentration increased (including Pro and Hyp), comes from α-glutarate family. Yoon et al. (2017) reported gamma-aminobutyric acid (GABA) and glutamate contents in greenhouse-grown spinach exposed to cold stress. Plants also accumulate high levels of GABA in response to different environmental stresses like salt stress (Häusler et al. 2014), or GABA, Pro and Met under enhanced Zn concentration (Pavlíková et al. 2014). Special roles in protective mechanism against DS have Pro and Hyp that amounts under stress significantly increased.

DS resulted in a decrease of Tyr in all examined cultivars. This can be due to a significant increase of tyrosine hydroxylase and L-DOPA decarboxylase activities, which was reported in potato leaves cv. Desirée under DS by Świędrych et al. (2004). Increased activity of these enzymes leads to hydroxylation of Tyr to L-dihydroxy phenylalanine (L-DOPA) and subsequently its decarboxylation to catecholamine biosynthesis. Also, a moderate increase Tyr decarboxylase levels producing L-tyramine were observed in *Arabidopsis* seedlings under drought stress, and this points to a vital function of Tyr decarboxylase in the integration of a multitude of environmental stresses (Lehmann and Pollmann 2009). Different responses of cultivars to WLS (Milva and Marabel decreased Tyr, while Laura and Valfi increased Tyr) could depend on different enzyme activities in the shikimate and aromatic acid biosynthesis pathways based on genotype.

WLS decreased levels of most amino acids in cvs. Marabel (except for L-tryptophan Trp) and Milva (except for Arg, Glu, Leu, Phe and Ser). Contrariwise, His, Phe, Tyr, Val, Thr, Leu, Asp, Lys, L-glycine (Gly), Ile, Trp, Met and Gln increased in cv. Valfi and likewise His, Phe, Arg, L-alanine (Ala), Ser, Pro, Val, Thr, Leu, Lys, Gly, Glu, Hyp, Ile, and Asn in cv. Laura. Differences in responses point to the strong role of cultivar that was also confirmed with the change of total AAs content – it increased in cvs. Laura and Valfi, while *vice versa* in cvs. Marabel and Milva decreased.

In summary, this study aimed at a better understanding of drought and waterlogging tolerance of potato cultivars in response of saccharides and amino acids to these abiotic stresses. We characterised the effects of DS and WLS on contents of the individual (fructose, glucose and sucrose) and total saccharides and free total and individual AAs in tubers of four potato cultivars (Laura, Marabel, Milva, Valfi). DS and WLS increased the content of fructose, glucose, and sucrose in three potato cultivars except for cv. Laura. On average, of all cultivars, the highest increase was caused under DS in fructose content, followed by sucrose and glucose. In WLS, such increases were lower.

DS increased Pro, Hyp, Arg, Glu and Leu levels in all analysed cultivars. High effect of DS on Phe, His, Thr and total free AAs content increase in the cvs. Laura, Valfi and Marabel has been observed. Comparison of the effects of DS and WLS on the content of total AAs showed an increase under DS and contrariwise a decrease under WLS. Pro and Hyp showed a significant increase under DS and a decrease in WLS in all cultivars. Likewise, DS decreased Tyr content in all cultivars, but WLS only in the Milva and Marabel cultivars. Results showed the high impact of potato genotypes and cultivars on different responses against stresses related to their sensitivity or tolerance.

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