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Effect of long-term drought and waterlogging stress on photosynthetic pigments in potato

*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: In potato tubers of four cultivars, the impact of drought and waterlogging stress on total carotenoid content (TCC) and individual carotenoids profile variance and total carotenoids, chlorophylls (*a*, *b*, *a* + *b*) content in leaves during period 71 days of stress in two-year pot greenhouse experiments were investigated. The different response between potato cultivars was observed after exposure to drought stress (an increase of TCC was in Marabel and Laura cultivars) and after waterlogging stress (in Milva and Marabel cultivars). On average, of all cultivars, both drought and waterlogging stresses caused total carotenoid content to increase with a higher impact of drought stress. In potato tubers, eight carotenoids were identified with the most represented violaxanthin, luteoxanthin, antheroxanthin and lutein. Significant differences in individual carotenoid amounts between cultivars have been determined. On average, drought and waterlogging stresses of all cultivars significantly increased contents of violaxanthin, 9'-(*Z)*-neoxanthin and luteoxanthin, while antheraxanthin, lutein and zeaxanthin levels decreased. In leaves, both abiotic stresses decreased chlorophyll contents (*a*, *b*, *a* + *b*) with a very destructive impact of drought stress. While carotenoids accumulated in tubers as a result of stress, the opposite trend was characteristic in leaves, where their content decreased depending on the duration of stress.

Keywords: *Solanum tuberosum* L.; tuberous crop; water deficiency; resistance; absorbance; long-term abiotic stresses

Potato is one of the four major crops in the world. It is planted in 156 countries and regions worldwide. Potato is a typical crop of temperate climate, sensitive to water deficiency. Abiotic stresses, such as drought or waterlogging stress, can reduce photosynthesis, promote stomatal closure, and induce photosynthetic pigments loss. However, up until now, there have been no profound studies on the drought and waterlogging resistance in potatoes. In physiology and biochemistry are involved apart of osmolytes (saccharides, amino acids, and antioxidants) also, and active pigments (chlorophylls and carotenoids) participate in photosynthetic reactions (Li et al. 2017).

Chlorophyll consists of a porphyrin ring with magnesium and the phytol side chain. Phytol is a diterpene with two potential isomeric forms, (2*E*,7*R*,11*R*)-3,7,11,15-tetramethyl-hexadec-2-en-1-ol (*E*-phytol) and (2*Z*,7*R*,11*R*)-3,7,11,15-tetramethyl-hexadec-2-en-1-ol (*Z*-phytol). Chlorophyll *a* and chlorophyll *b* are two types of chlorophyll, to produce energy in photosystem I and II. Chlorophyll *a* (green pigment) and chlorophyll *b* (yellow pigment) differ in structure only at carbon 3; chlorophyll *a* possess at this position bound -CH_3 group while chlorophyll *b* is an aldehyde with -CHO group attached to this position. Chlorophyll *a* is the primary photosynthetic

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pigment absorbing energy at 675 nm in the energy phase. It is the reaction center of the antenna array made up of core proteins that bind chlorophyll with carotenoids. Chlorophyll *b* absorbs energy from wavelengths at 675 nm and is the accessory pigment that collects energy and passes it on to chlorophyll *a* (Roca et al. 2016).

Carotenoids are yellow, orange, and red pigments responsible for the colour of most fruits and vegetables. They are C_{40} isoprenoids with a long-conjugated polyene chain that is responsible for their colour and biological activities. Carotenoids play an important role in plants in both photosynthetic and non-photosynthetic tissues, where they assist in light harvesting and photoprotection or act as signalling molecules, precursors of volatile compounds, colourants, and pollinator attractants (Nisar et al. 2015). Carotenoids are divided into carotenes (hydrocarbons) and xanthophylls (oxygen derivatives of carotenes). Carotenes and xanthophylls are both located in various types of plastids. They accumulate high levels in chloroplasts (photosynthetic tissue) and chromoplasts found in mature fruit, vegetable, and flower tissues. In chloroplasts, carotenoids are associated with proteins in pigment-protein complexes located in thylakoid membranes. In chromoplasts, carotenoids are deposited in multiple lipoprotein structures, which differ among plant species and tissues. Fibrillary and tubular lipoprotein structures contain xanthophylls in the form of fatty acid esters located on the α- or β-ionone ring (Howitt and Pogson 2006).

Keeping in mind the important health benefits of carotenoids and high potato consumption per capita, the objective of this study was: (*i*) to assess the effect of long-term drought (DS) and waterlogging stress (WS) on the content and profile of carotenoids in potato tubers of different cultivars; (*ii*) to evaluate the effect of drought and waterlogging stresses on the pigments – chlorophyll *a*, chlorophyll *b*, total chlorophyll (*a* + *b*), and total carotenoids content in potato leaves; and (*iii*) to compare the impact of stresses on pigment levels.

MATERIAL AND METHODS

Plant material and potato growing in the greenhouse two-year 2017 and 2018 experiments. Four cultivars of potatoes (*Solanum tuberosum* L.) are characterised in Table 1.

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 Baltisches Substrat (HAWITA Gruppe GmbH, Vechta, Germany). Baltisches Trysubstrat (dispensing 45025, pH value 5.8, white peat 70%, black peat 30%, structure fine, the content of salts 1.5 g/L $CaCl_2$, and fertilisers 1.2 kg/m^3). Before starting the experiment, two grams of NPK 8-24-24 fertiliser was added to the pot. In each pot, one plant potato was grown. The experiment was performed in four replicates. The pots were placed in the greenhouse according to the Latin square method.

Plants were grown under the natural light mode, where the day length was 13 h and dark 11 h. The temperature mode was set at 22 ± 2 °C by day and 17 ± 2 °C by night at 70 ± 10% air relative humidity. The experimental scheme included three variants of the experiment; the first variant consisted of

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

Cultivar	Origin of tubers/year	Maturity	Skin colour	Flesh colour	Shape of tubers	Origin	Resistance to biotic stress
Laura	Austria 1976	medium-early	red	dark yellow	oval	Saskia × MPI 49 540 2	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

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. Rehydration was induced in the drought experiment in developmental phases 406–509 BBCH (27–32 days of stress) and 703–805 BBCH (53–60 days of stress). 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 variants of the experiment was induced in the development phase BBCH 109.

Plants of all experimental variants were fertilised twice with 3% NPK solution in 450 mL water per pot for watering in the developmental stages: 406 and 703 BBCH. Tuber sampling was carried out in the developmental step according to BBCH scale 909 (harvested product, 71 days of stress).

Determination of carotenoids in potato tubers. Detailed methodology for sample extraction, chromatographic separation and carotenoid identification is described in Kotíková et al. (2016). Briefly, 2 g of lyophilised sample were extracted twice with 12 mL of ethanol/acetone/hexane mixture (1:1:2, *v/v/v*). 18 mL of the extract was evaporated under a vacuum at 40 °C (Rotavapor R-200, Büchi Labortechnik, AG, Flawil, Switzerland). The dry residue was reconstituted with 2 mL of ethanol/acetone (3:2, *v/v*) solution containing 0.2% BHT, filtered through a syringe filter (PVDF, 0.45 μm) and analysed by HPLC-DAD. The analysis was conducted using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, USA) coupled with a diode array detector. Gradient elution on a YMC Carotenoid C30 column (150 mm × 3.0 mm, particle size = 3 μm, YMC Co., Kyoto, Japan) was used for carotenoid separation. The tertiary mobile phase consisted of methanol (A), water (B) and *tert*-butyl methyl ether (C). Gradient: initial conditions of 90% A, 10% B and 0% C were kept for 1 min, then increased to 90% A, 0% B and 10% C at 6 min, reaching 40% A, 0% B and 60% C at 22 min following column flush and re-equilibration for 11 min. The operating conditions were as follows: flow rate 0.6 mL/min; column temperature 25 °C; autosampler temperature 10 °C; injection volume 10 μL; detection at λ = 445 nm (spectral acquisition 300–700 nm). Peak area and external calibration (0.05–10 μg/mL per analyte) were used for analyte quantitation. All analyses were performed in triplicates, and the results were expressed on a dry weight (DW) basis. The sum of all carotenoid components is called total carotenoid content (TCC).

Determination of chlorophylls *a* and *b*, total chlorophylls (*a* + *b*) and total carotenoids in leaves. Porra et al. (1989) and Lichtenthaler and Bushmann (2001) slightly modified methods were used. Briefly, each leaf was cut with the corkscrew disc of an area of 1 cm^2. The disc was transferred into the tube with 1 mL of N, N'-dimethylformamide, covered with parafilm and black tissue and was agitated overnight on a laboratory shaker Biosan Orbital Shaker 3D (Biosan, LtD., Vráble, Slovak Republic) in a laboratory hood. The next day the extract was poured into spectrophotometric micro-cuvettes of volume 1.5 mL for VIS spectrum (Verkon, Ltd., Prague, Czech Republic), and absorbance was measured on spectrophotometer Evolution 201 UV-Vis (Thermo Fisher Scientific, Ltd., Madison, USA) at wavelengths λ = 480, 646.8, 663.8, and 710 nm, respectively. We set the maximum for TCC determination at λ = 480 compared to 470 nm (Lichtenthaler and Bushmann 2001). Individual carotenoids' absorption maxima differ (violaxanthin 473 nm, neoxanthin 469 nm, lutein 477.5 nm, zeaxanthin 483 nm or β-carotene 482 nm, respectively, Ashenafi et al. 2023). For the calculation of chlorophyll *a* and chlorophyll *b,* and carotenoids, the equations according to Porra et al. (1989) and Liechtenhalter and Buschmann (2001) were used:

(1) Chlorophyll *a*:

$$Ca = 12.0 \times A_{663.8} - 3.11 \times A_{646.8}$$

(2) Chlorophyll *b*:

$$Cb = 20.78 \times A_{646.8} - 4.88 \times A_{663.8}$$

(3) Total chlorophyll (*a* + *b*):

$$C_{tot} = 7.12 \times A_{663.8} - 17.67 \times A_{646.8}$$

(4) Total carotenoids in leaves

$$Car = (1\,000 \times A_{480} - 1.12 \times Ca - 34.07 \times Cb)/245$$

Results were in Figure 2 expressed in nmol/0.5 cm^2 and converted in the text to nmol/cm^2. All manipulations had to be carried out in diffused light, and the samples had to be covered with a black cloth.

Relative water content and relative water saturation deficit. From a leaf blade without a middle rib, fifteen discs were cut out using a 1 cm diameter cork and weighed (fresh matter). Thereafter they were placed in a Petri dish with distilled water. Petri dishes were placed in a room with laboratory temperature and diffuse irradiation environment. Samples were saturated for at least

3 h. Then they were weighed and dried. After drying, the dry matter was weighed. The obtained weight values were set in the following equation:

$$\text{RWSD} = \frac{\text{matter after saturation} - \text{fresh matter}}{\text{mater after saturation} - \text{dry matter}} \text{ x } 100\ [\%]$$

Relative water content (RWC), given in percent, is an indicator of the water balance of a plant that expresses the total amount of water contained by the plant. The amount of water missing from the plant to maximum saturation is given by the relative water saturation deficit (RWSD), given in percent. The gradual natural drying of the substrate-induced drought stress. To monitor the level of stress and subsequent rehydration, the water saturation deficit was monitored (Larcher 2003). The RWC and RWSD values are shown in Table 2.

Statistical analysis. The data were processed by Chromeleon (Thermo Fisher Scientific, Inc., Waltham, USA) and Excel (Microsoft, Redmond, USA). Statistical evaluation was performed using the Statistica software (ver. 12; StatSoft, Inc., Tulsa, USA). Tukey's Post Hoc *HSD* (honestly significant difference) test was used for detailed evaluation.

RESULTS AND DISCUSSION

Carotenoid contents and profiles and their evolution under drought and waterlogging stress in tubers of four potato cultivars. The highest total carotenoid content was found in control tubers of cv. Laura (14.17 μg/g DW), followed by cv. Milva (8.515 μg/g DW), cv. Marabel (7.713 μg/g DW) and cv. Valfi (2.338 μg/g DW), as it is shown in Figure 1A. Tukey's *HSD* test showed that these four potato cultivars differed significantly from each other in their total carotenoid contents at $P \leq 0.05$. Drought stress (after 71 days) caused an increase of TCC in Marabel, Laura and Valfi cultivars in contrast with its decrease in the Milva cultivar. Waterlogging stress increased TCC in cvs. Milva, Marabel and Valfi, but decreased it in cv. Laura. Thus, individual potato cultivars have a different response; the highest-increase in TCC was recorded after exposure to DS in Marabel and Laura cultivars and after WS in cvs. Milva and Marabel.

In control, DW and WS potato tubers, eight carotenoids were identified and quantified: violaxanthin, 9*Z*-neoxanthin, luteoxanthin, antheraxanthin, lutein, zeaxanthin, neoxanthin and β-carotene (Figures 1B–E). Moreover, in DS and WS tubers of cv. Valfi little amounts of α-carotene have been determined (Figure 1E). In terms of representation of individual carotenoids, on average of all cultivars, the most contained was violaxanthin (3.125 μg/g DW), followed by luteoxanthin (2.225 μg/g DW), antheraxanthin (1.025 μg/g DW), lutein (0.968 μg/g DW), 9*Z*-neoxanthin (0.725 μg/g DW), neoxanthin (0.717 μg/g DW), β-carotene (0.207 μg/g DW) and zeaxanthin (0.198 μg/g DW). This tendency was followed especially in Laura and Marabel cultivars, but high violaxanthin and luteoxanthin were also characteristic in the Milva cultivar. Drought and waterlogging

Table 2. Relative water content (RWC) and relative water saturation deficit (RWSD) (%) depending on the variant and time

Day	RWC			RWSD		
	C	DS	WS	C	DS	WS
0	81 ± 3	81 ± 3	81 ± 3	19 ± 3	19 ± 3	19 ± 3
10	80 ± 4	78 ± 3	82 ± 4	20 ± 4	22 ± 3	18 ± 4
17	81 ± 2	77 ± 3	82 ± 3	19 ± 2	23 ± 3	18 ± 3
27	82 ± 3	76 ± 2	80 ± 2	18 ± 3	24 ± 2	20 ± 2
32	82 ± 2	75 ± 4	82 ± 2	18 ± 2	25 ± 4	18 ± 2
39	81 ± 2	75 ± 4	83 ± 3	19 ± 2	25 ± 4	17 ± 3
46	82 ± 3	75 ± 4	81 ± 4	18 ± 3	25 ± 4	19 ± 4
53	81 ± 2	75 ± 4	82 ± 3	19 ± 2	25 ± 4	18 ± 3
60	80 ± 3	74 ± 3	81 ± 2	20 ± 3	26 ± 3	19 ± 2
66	81 ± 2	73 ± 4	81 ± 3	19 ± 2	27 ± 4	19 ± 3
71	81 ± 2	73 ± 4	82 ± 4	19 ± 2	27 ± 4	18 ± 4

C – control; DS – drought stress; WS – waterlogging stress

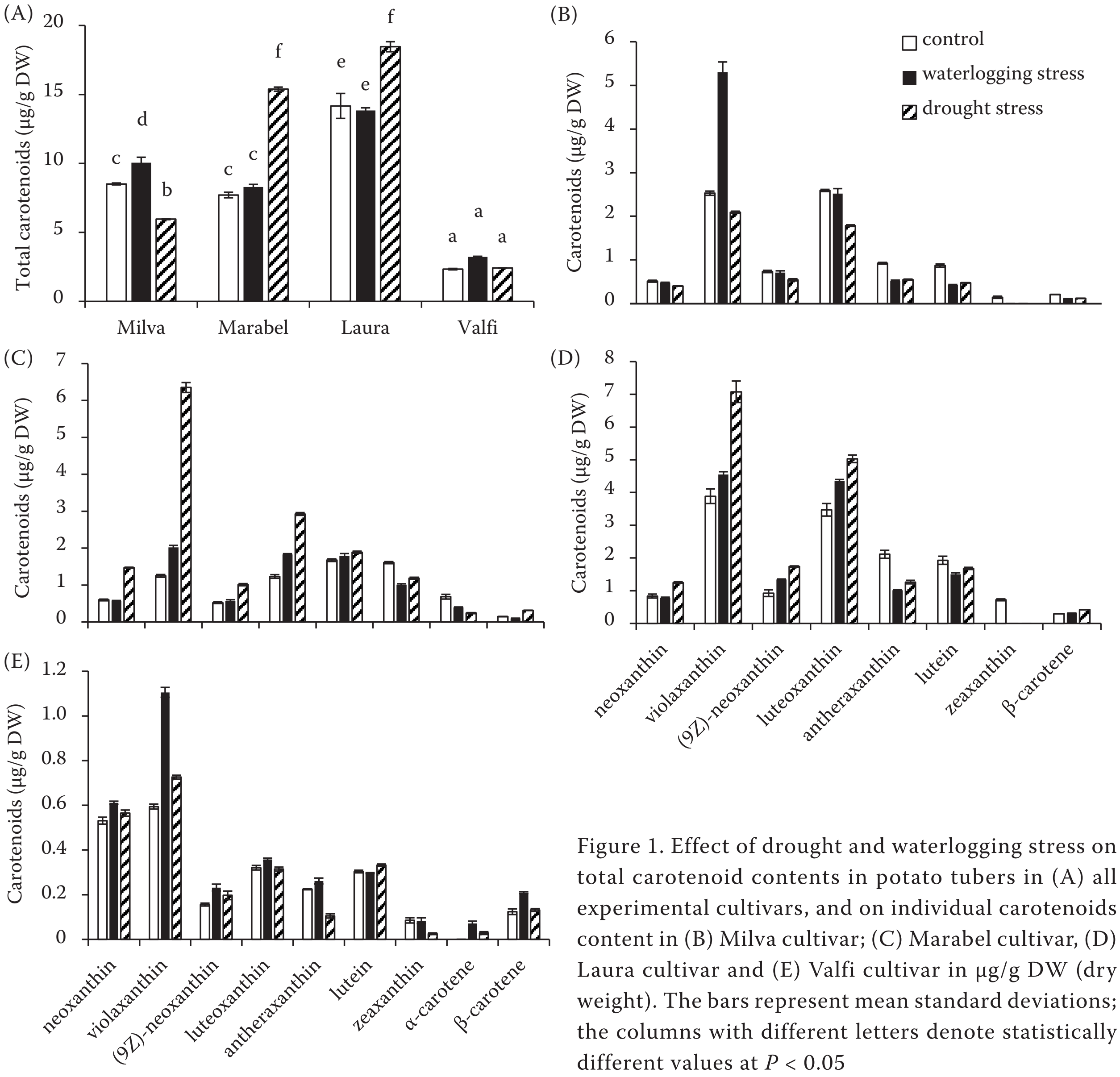

Figure 1. Effect of drought and waterlogging stress on total carotenoid contents in potato tubers in (A) all experimental cultivars, and on individual carotenoids content in (B) Milva cultivar; (C) Marabel cultivar, (D) Laura cultivar and (E) Valfi cultivar in μg/g DW (dry weight). The bars represent mean standard deviations; the columns with different letters denote statistically different values at $P < 0.05$

stresses (on average of all cultivars) increased significantly contents of violaxanthin (from 2.064 μg/g DW in control to 3.246 μg/g DW in WS and 4.062 μg/g DW in DS), 9Z-neoxanthin (from 0.584 μg/g DW in control to 0.714 μg/g DW in WS and 0.873 μg/g DW in DS) and luteoxanthin (from 1.904 μg/g in control to 2.266 μg/g DW in WS and 2.514 μg/g DW in DS). The highest increase of carotenoid content was observed after DS in cvs. Marabel, Valfi, and Laura, with a lower increase after WS in cvs. Valfi, Marabel, and Laura. The exception was the Milva cultivar, where both stresses caused a significant decrease in carotenoids.

Amounts and composition of carotenoids, along with levels of carotenogenic genes, vary in response to abiotic and biotic stresses (Hannoufa and Hossain 2012). Increased TCC was observed in the regulated deficit irrigation with 40% and 50% reduction in the leaf water potential in common and cherry tomato cultivars (Coyago-Cruz et al. 2017), with differences between cultivars. Such differences between tomato cultivars may be caused by SIMX1 encoding a MIXTA like MYB transcription factor that enhanced carotenoid accumulation and drought resistance in tomatoes (Ewas et al. 2016). Thus, the elevation of TCC in individual cultivars might be related to the drought stress resistance of the cultivar. Potato is often considered a drought-sensitive crop. Cultivars with higher carotenoid content and other phytochromes as key regulators in abiotic stress responses may be

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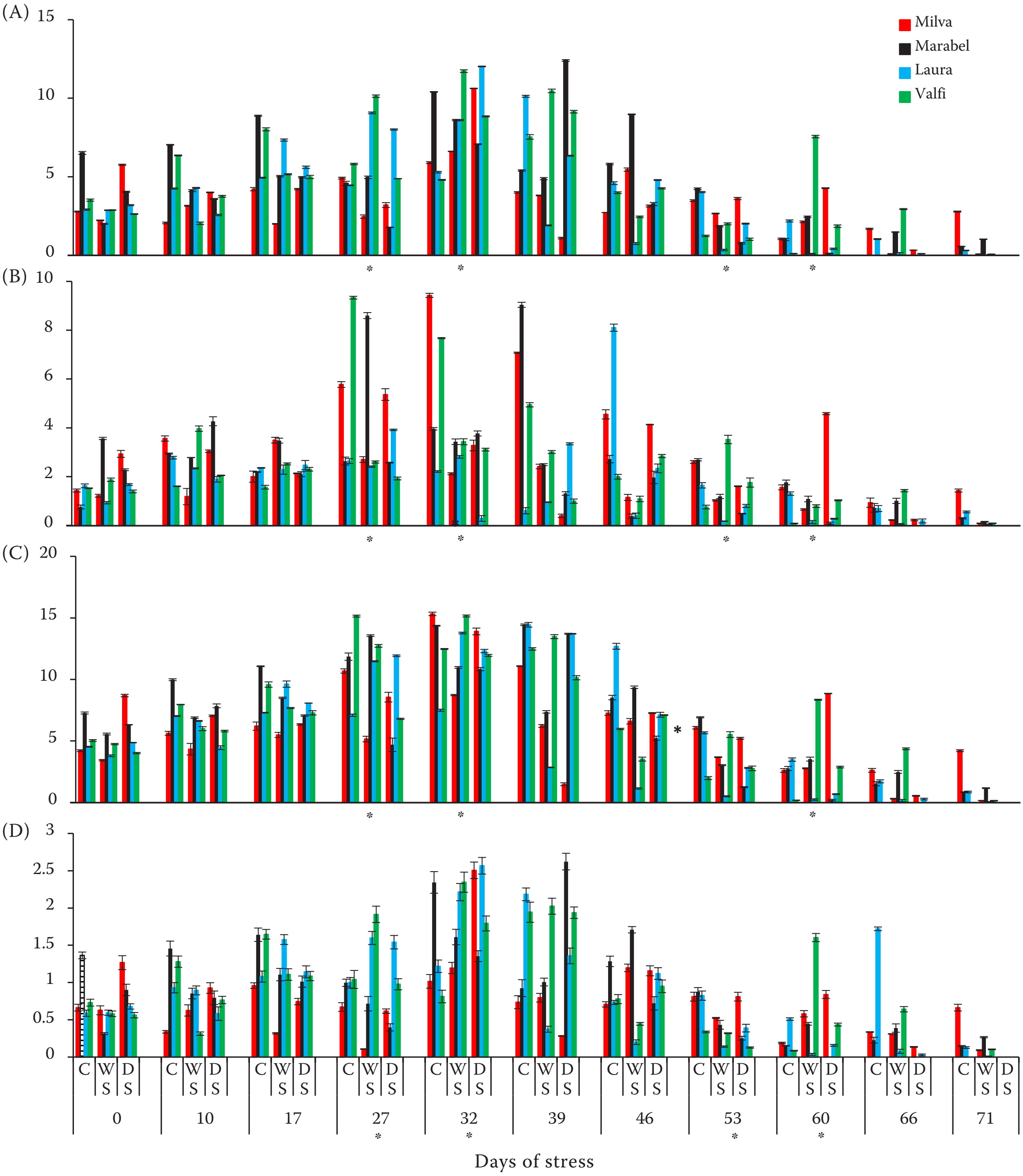

Figure 2. Contents of (A) chlorophyll *a*; (B) chlorophyll *b*; (C) total chlorophyll (*a* + *b*), and (D) total carotenoids in leaves of four analysed potato cultivars under drought and waterlogging stress in 0–71 days in nmol/cm^2. DS – drought stress; WS – waterlogging stress; *under drought stress between 27th–32nd days rehydration (5 days) and between 53rd–60th days rehydration (7 days). The bars represent mean standard deviations

an alternative route toward developing drought-tolerant potatoes (Gavassi et al. 2017).

Chlorophylls *a* and *b* content in potato leaves affected by DS and WS. Phytochromes are the best-characterised and most frequently studied plant photoreceptors, which modulate levels of pigments in leaves. Between them, the most important pigments are chlorophylls and carotenoids (Gavassi et al. 2017).

Drought and waterlogging stresses' effect on potato leaves was studied in a period of 71 days (Figures 2A–C). On average of all examined dates and examined potato cultivars, it was determined that chlorophyll *a* content decreased in comparison with control (8.024 nmol/cm^2) to 6.822 nmol/cm^2 under DS (85.02%) and to 7.274 nmol/cm^2 (90.65%) under WS. Similarly, chlorophyll *b* content decreased on average for all cultivars and dates of stress action in comparison with control (5.936 nmol/cm^2) to 3.642 nmol/cm^2 (61.36%) under DS and to 3.632 nmol/cm^2 (61.19% under WS). A similar situation also applies to total chlorophyll (*a* + *b*) content at the end of the experiment (71 days) in all cultivars. The impact of waterlogging stress was not so severe. The trend of chlorophyll increase has been recorded in the stresses' period 0–39 days, with the maximum at 32–39 days (also in control, the highest in DS for cvs. Marabel and Laura and in WS for cv. Valfi), while in the following period of 41–71 days, a decrease and destruction of chlorophylls have been observed with complete destruction under DS at 71 days.

Potato cultivars differed in the representation of chlorophyll *a* and chlorophyll *b* in control samples, but chlorophyll *a* prevailed over chlorophyll *b* in all cultivars. The highest average ratio was in cv. Laura (3.006), followed by cvs. Marabel (2.393), Valfi (1.99) and Milva (1.191). The average ratio of chlorophyll *a*/*b* (average of all examined cultivars and drought stress terms was higher in drought stress (3.353) and waterlogging stress (2.909) by comparison with control (2.012), indicating that chlorophyll *b* was more decomposed by stress. However, contrary, in peanut leaves by the stress induced by polyethylene glycol, the reduction in chlorophyll *a* level was higher than that of chlorophyll *b* (Meher et al. 2018). Ratio chlorophyll *a*/*b* depends on many factors, such as intensity of the radiation (Beneragama and Goto 2010), high temperature (Shon et al. 2015), nitrogen availability (Maina and Wang 2015) and plant species (Flores-de-Santiago et al. 2016).

Effects of drought stress on chlorophyll *a* fluorescence in two rubber tree clones was recently studied (Falqueto et al. 2017). A decrease of chlorophyll *a* fluorescence was found at critical 38 days of drought stage. A similar effect of drought stress on chlorophyll fluorescence in dry beans (*Phaseolus vulgaris* L.) or *Aloe vera* L. was also confirmed (Mathobo et al. 2017). It is interesting that heat-induced senescence in bentgrass, which could be under certain conditions like DS, reduced chlorophyll content due to accelerated chlorophyll degradation caused by increased gene expression levels of chlorophyllase and higher pheophytinase activity while chlorophyll-synthesising genes and enzymatic activities were not differentially by the stress altered (Jespersen et al. 2016). Reducing chlorophyll *a*, chlorophyll *b* or chlorophyll (*a* + *b*) as a result of drought and water stress, as it is shown in the present study could, might be useful for the prediction of potato yield and susceptibility to the stress of different cultivars (Ramírez et al. 2014). Their findings suggest that chlorophyll content could be associated with an oxidative stress occurrence that ultimately reduces yield during senescence.

Carotenoid levels in potato leaves affected by drought and waterlogging stress. Total carotenoid content decreased during potato leaves' stresses, similar to chlorophylls (Figure 2D). On average of all cultivars and in terms of effects of stresses, the drought stress has been shown to be the most efficient, decreasing total carotenoid content to 1.608 nmol/cm^2 (92.63%) and waterlogging stress to 1.612 nmol/cm^2 (92.86%) when compared with control (1.736 nmol/cm^2). The destruction of carotenoids seems to be lower than that of chlorophylls. However, at the end of the experiment, the situation was similar, showing no carotenoids in all cultivars in 71 days of DS and Marabel and Valfi in 66 days of stress. Likewise, in another study, severe water deficit decreased the leaf contents of total chlorophyll (35%) and carotenoids (29%) in three genotypes of the wild desert potato *Solanum kurtzianum* Bitter & Wittm. – the potato species best adapted to desert and semi-desert conditions (Ibañez et al. 2021). Transgenic sweetpotato (*Ipomoea batatas* L.) plants with higher drought and salt stress contained higher levels of total carotenoid and β-carotene, associated with lower membrane permeability and higher photosynthetic rate. Elevated carotenoid accumulation in transgenic plants mitigated the reduction in leaf photosystem II efficiency and chlorophyll induced by drought stress (Ke et al. 2019). A lycopene cyclase gene *IbLCYB2* increases carotenoid content and abiotic stress tolerance in transgenic sweetpotato (Kang et al. 2018). Higher chlorophyll and carotenoid content in potato-resistant cultivars allowed plants to tolerate water deficit.

Carotenoids and their derivatives participate in signalling plant developmental responses to abiotic stress (Shumskaya and Wurtzel 2013). In contrast, to the increase of total carotenoid contents in carrot roots after drought stress, the impact of stress conditions in leaves led to a decrease of less than

50% of carotenoid contents compared to the control (Perrin et al. 2017). Except for α-carotene, carotenoid contents were correlated with chlorophyll *a*, chlorophyll *b* and total chlorophyll contents (from 0.67 to 0.93). Moreover, chlorophyll contents were also correlated between them (> 0.9). In the present study, correlation coefficients for chlorophyll *a*/chlorophyll *b*, chlorophyll *a*/total chlorophyll, chlorophyll *b*/total chlorophyll, chlorophyll *a*/total carotenoids, chlorophyll *b*/total carotenoids and total chlorophyll/total carotenoids were $R = 0.800$, $R = 0.943$, $R = 0.948$, $R = 0.968$, $R = 0.748$ and $R = 0.898$ ($P \leq 0.05$), respectively. Overexpression of genes in transgenic potatoes associated with tolerance to abiotic stresses was overviewed in several reviews with the aim of biotechnology of enzymes enhancing the carotenoid levels in resistant potato cultivars (Nowicka et al. 2018). Abiotic stresses are also elicitors of the biosynthesis of plant secondary metabolites in plants which acquire plant defence mechanisms and can alter the concentration of bioactive compounds possessing human health benefits such as antioxidative and anti-inflammatory properties (Sharma et al. 2022).

Relative water saturation deficit and relative water content. In potato leaves sampled at BBCH phenological growth phases 105 (5th leaf of main stem unfolded), 406 (60% of total final tuber mass reached) and 805 (berries in the 1st fructification ochre-coloured or brownish) RWSD showed an increase (%) in all analysed varieties, and RWC decreased under DS, while under WS no significant changes were recorded. The increase was positively related to the timing of the stress period, with a higher increase caused by drought stress (Table 2).

In conclusion, the present study gives new insight into different responses of potato cultivars growing under DS and WS conditions. The analysed potato cultivars differed significantly from each other in total carotenoid content in tubers – Laura > Milva > Marabel > Valfi. The different response between potato cultivars has also been observed after exposure to DS (the highest increase was in Marabel and Laura cultivars) and after WS (in Milva and Marabel cultivars). On average, of all cultivars, both DS and WS caused TCC to increase with a higher impact of DS. In potato tubers, eight carotenoids were identified with the most contained violaxanthin, luteoxanthin, antheroxanthin and lutein. Also, significant differences between cultivars have been determined in individual carotenoid levels. Both DS and WS, on average of all cultivars, significantly increased contents of violaxanthin, 9'-(*Z*)-neoxanthin and luteoxanthin. In leaves, long-term abiotic stresses in periods 0–39 days increased chlorophyll *a*, chlorophyll *b*, chlorophyll (*a* + *b*) and TCC levels. In 41–71 days, their contents decreased with their contents a very destructive impact on DS. Thus, while carotenoids accumulated in tubers due to stresses, the opposite trend was characteristic in leaves, where their content decreased depending on the duration of stress. Higher accumulated levels of photosynthetic pigments, chlorophylls and carotenoids contribute to the resistant properties of potato cultivars. In addition, higher levels of carotenoids are one class of secondary metabolites appreciated for their antioxidant and health-beneficial properties.

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