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ORIGINAL PAPER

Changes in hormonal status of winter wheat (*Triticum aestivum* L.) and spelt wheat (*Triticum spelta* L.) after heat stress and in recovery period

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Abstract
The dynamics and distribution of endogenous cytokinins (CKs), gibberellic (GA_3) and salicylic (SA) acids in wheat (*Triticum aestivum* L., 'Podolyanka') and spelt wheat (*Triticum spelta* L., 'Frankenkorn') plants was analyzed using HPLC–MS. Fourteen-day-old plants that had been exposed to short-term heat stress (+40 °C, 2 h) and 21-day-old plants after recovery were studied. Heat stress induced rapid changes, both specific and nonspecific, in hormone levels and distribution. The level of GA_3 decreased in the shoots and roots of both winter and spelt wheat. A reduction in SA content was observed in wheat, while an increase was observed in spelt. The pool of CKs significantly increased in wheat, while in spelt—it decreased more than twofold. After recovery, an increase in GA_3 content occurred in both species, but not to the levels measured in control plants. More active accumulation of GA_3 was observed in the roots. The content of SA in the shoots of wheat continued to decrease, while in the roots it increased. In spelt, hormone concentration decreased, but it remained higher than in 21-day-old control plants. In shoots of both plants the pool of CKs decreased, while in wheat roots it did not change, and in spelt roots it decreased. The total CKs content in stressed wheat plants was twice as high as in spelt. In general, we established significant hormonal fluctuations, which indicate a direct involvement of endogenous cytokinins, gibberellic and salicylic acids in wheat and spelt response to heat stress. Screening of stress-resistant genotypes of cereals may benefit from quantitation of CKs, GA_3, and SA.

Keywords *Triticum aestivum* L. · *Triticum spelta* L. · Gibberellic and salicylic acids · Cytokinins · Heat stress

Abbreviations

GA_3	Gibberellic acid
SA	Salicylic acid
CKs	Cytokinins
iP	Isopentenyladenine
iPa	Isopentenyladenosine
t-Z	*trans*-Zeatin
t-ZR	*trans*-Zeatin riboside
t-ZOG	*trans*-Zeatin-*O*-glucoside
HPLC–MS	High-performance liquid chromatography-mass spectrometry
ROS	Reactive oxygen species
FW	Fresh weight
DW	Dry weight

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Introduction

More than 40% of worldwide areas where wheat is grown are exposed to high temperature stresses and an increase by 1 °C from the average of 23 °C reduces yield by approximately 10% (Narayanan 2018). Heat stress causes negative changes in the water regime (Hasanuzzaman et al. 2013), inhibits photosynthetic activity (Ashraf and Harris 2013) and metabolic processes (Farooq et al. 2011), induces the accumulation of reactive oxygen species (ROS) (Wang et al. 2011), and changes in hormonal status (Abhinandan et al. 2018). The initial stages of wheat ontogenesis are especially sensitive to high temperatures (Abid et al. 2018).

Phytohormones are signaling biomolecules with different chemical structures and physicochemical properties, which act in nanomolar concentrations to regulate physiological and metabolic processes in plants. More that 130 forms of gibberellins exist, but physiological activity is characteristic only of certain gibberellic acids (GAs) (GA_1, GA_3, GA_4, GA_5, GA_6 and GA_7), while others constitute

their precursors and inactive forms (Sponsel and Hedden 2010). The main functions of GAs are the regulation of seed germination, coordination of cell division and elongation, sex determination, pollen and flower development, flowering induction, seed and fruit formation (Gantait et al. 2015). Following a reduction in the endogenous GAs content, plant growth is stunted, whereas enhanced hormone biosynthesis prevents stress damage (Colebrook et al. 2014). Cytokinins (CKs)—another important component of the phytohormone complex—are present as free bases: isopentenyladenine (iP), dihydrozeatin, *cis*-zeatin, and *trans*-zeatin (*t*-Z) and their conjugates (ribosides and nucleotides). *t*-Z and its derivatives are the most active dominant forms (Kieber and Schaller 2018). CKs are involved in regulation of cell division, formation of meristems, photosynthesis, aging, absorption of macro- and microelements and responses to the negative effects of the environment (Ha et al. 2012; Veselov et al. 2017; Cortleven et al. 2019). In the early phases of heat stress, CKs stimulate the opening of the stomata and transpiration and activate the antioxidant system (Prerostova et al. 2020). Salicylic acid (SA) is a phenolic compound involved in the regulation of such important physiological processes as photosynthesis, respiration, transpiration and thermogenesis (Janda and Ruelland 2015), and enhances plant resistance to a wide range of abiotic and biotic stressors (Kang et al. 2014; Kumar 2014; Jayakannan et al. 2015). SA induces stress resistance by promoting the accumulation of osmolites and antioxidant defense, as well as through cross-talk with other hormones (Khan et al. 2015; Janda 2019).

The winter wheat *Triticum aestivum* L., which is a main grain crop in Ukraine, is cultivated on almost 240 million hectares. An important element of its cultivation technology, which affects productivity, involves the use of high-yielding genotypes tolerant to biotic and abiotic stressors (Morgun et al. 2016). Spelt wheat (*Triticum spelta* L.) is a husked-wheat species, is considered a wild precursor of winter wheat, has the same genomic composition, is easily crossed and used as a donor of valuable agricultural traits (Babenko et al. 2018). High nutritional qualities and adaptability to organic farming make spelt an attractive option in many European countries (Lacko-Bartošová et al. 2010; Escarnot et al. 2012).

Here, we quantify and compare the dynamics and distribution of endogenous gibberellic acid GA_3, CKs and SA in shoots and roots of wheat and spelt plants and to identify specific and nonspecific traits of phytohormonal status associated with response to heat stress and recovery. The study of adaptation mechanisms provides useful information for the selection of stress-resistant varieties of cereals in light of expected future climate change.

Materials and methods

Plant material

Experiments were performed at the M. G. Kholodny Institute of Botany of the NAS of Ukraine in 2018–2019. Plants of wheat (*T. aestivum* L., cultivar 'Podolyanka') and spelt wheat (*T. spelta* L., cultivar 'Frankenkorn') were studied. Spelt seeds were obtained from the collection of the National Centre for Plant Genetic Resources of Ukraine in Kharkiv, and wheat seeds—from the collection of the Institute of Plant Physiology and Genetics of the NAS of Ukraine (Kyiv). The seeds were calibrated, sterilized in 80% ethanol, washed with distilled water, and placed for 3 h in a cuvette with water. Then, the seeds were planted in 2-L vessels. River sand sterilized by calcination was used as the substrate. Plants were grown under controlled conditions at a temperature of + 20/17 °C (day/night), light intensity 190 μmol·m^{-2}·s^{-1}, photoperiod 16/8 h (day/night), relative air humidity—70 ± 5%, substrate humidity of 60% from full moisture content. The plants were watered with 50 ml of Knop solution per vessel daily.

Abiotic stress treatments and sample collection

To simulate temperature stress, 14-day-old plants were placed in a thermostat at a temperature of + 40 °C for 2 h, under the illumination of 190 μmol·m^{-2}·s^{-1}. For recovery, the plants were grown to 21-day-old in standard conditions (see above). Shoots and roots of 14- and 21-day-old plants were collected for further study.

Extraction of GA_3, SA, and CKs

Samples of shoots and roots (2 g) were frozen and ground in liquid nitrogen using 10 ml of extraction solution—methanol, distilled water, and formic acid (15:4:1 ratio). The homogenate was incubated at + 4 °C for 24 h in the dark. Extracts containing plant hormones were obtained by 30 min centrifugation at 15,000 RPM and + 4 °C and separation of the supernatant. The precipitate was resuspended in 5 ml of extraction solution. The suspension was incubated for 30 min and centrifuged again. The combined supernatants were evaporated to an aqueous residue under reduced pressure in a vacuum evaporator at + 40 °C. Further purification was performed on two solid-phase SPE cartridges: C18 Sep-Pak Plus, Waters and Oasis MCX, 6 cc/150 mg, Waters (Kosakivska et al. 2020). The C18 Sep-Pak Plus cartridge was used to remove lipophilic substances, proteins and pigments. Sorption and separation of phytohormones of different classes were performed on the Oasis MCX cartridge.

Elution of GA_3 and SA was performed with 100% methanol (first fraction). The second fraction with cytokinins was obtained using an alkaline solution of methanol (60 ml of methanol, 2.5 ml of 25% ammonia and deionized water to 100 ml). The obtained fractions were evaporated to dryness in concentrator flasks using a vacuum rotary evaporator at a temperature not exceeding + 40 °C. Each dry residue was dissolved to 200 µl with 45% methanol prior to analysis.

Quantification of GA_3, SA, and CKs

Analytical determination of phytohormones was performed using high-performance liquid chromatography on Agilent 1200 LC/MS series instrument (USA) with diode-array detector G1315B and single quadrupole mass detector Agilent G6120A. Phytohormone content was analyzed and calculated using Agilent OpenLAB CDS ChemStation Edition chromatograph software (rev. C.01.09). Chromatographic separation was carried out using an Agilent ZORBAX Eclipse Plus C18 column 4.6 × 250 mm with a lipophilic-modified sorbent, particle size 5 µm (reverse phase chromatography).

To determine the content of SA, 10 µl aliquots of the first fraction was separated using a solvent system (acetonitrile, deionized water, acetic acid in a volume ratio of 45:54.9:0.1) and SA was detected at an analytical wavelength of 302 nm. The flow rate of the mobile phase was 0.8 ml/min. After separating an aliquot of 20 µl of the first fraction with a solvent system (acetonitrile, deionized water, acetic acid—30:69.9:0.1), GA_3 was quantitatively detected by the mass spectrometer signal. The flow rate of the mobile phase during the GA_3 detection was 0.5 ml/min.

The 20 µl aliquots of the fraction with cytokinins were separated using a system of solvents (methanol, deionized water, acetic acid), the detection was performed at a wavelength of 269 nm. A step gradient system was used to elute the cytokinins, namely: 0 min: CH_3OH/0.5% solution of CH_3COOH in deionized water (37/63)—25 min: CH_3OH/0.5% solution of CH_3COOH (70/30)—35 min: CH_3OH/0.5% CH_3COOH solution (100/0) at a constant flow rate of 0.5 ml per minute. The duration of the column equilibration after analysis (post-run) was 15 min.

Unlabeled GA_3, SA, *trans*-zeatin-*O*-glucoside (*t*-ZOG), (*t*-Z), *trans*-zeatinribozid (*t*-ZR), iP, and isopentenyladenosine (iPa) manufactured by Sigma-Aldrich (USA) were used as chemical standards for the creation of calibration tables in the chromatographic methods of the instrument software. The content of analytes in the samples was monitored using a mass spectrometer in the combined mode (electrospray and chemical ionization at atmospheric pressure) with ionization of molecules of analytes in negative polarity and positive—for cytokinins during analysis. For quantitation of GA_3, the molecular weight of which is 346, we used the signal of the mass detector in SIM (setting 50% of the scan time for single ion monitoring of the 345 m/z value $[346\text{-H}]^-$).

Statistical analyses

All measurements were performed with three biological and three analytical replicates. Phytohormonal content was analyzed and calculated using Agilent OpenLAB CDS ChemStation Edition (rev. C.01.09), a program for controlling the HPLC/MS (high-performance liquid chromatography–mass spectrometry) instrument and processing the data). Statistical analysis was carried out in *Statistica*, version 6.0 (StatSoft Inc.). One-way analysis of variance (ANOVA) was conducted, with tests at $P \leq 0.05$ considered statistically significant (Van Emden 2008).

Results

Morphometric parameters of *T. aestivum* and *T. spelta* after short-term hyperthermia and in recovery period

A short-term high temperature stress caused minor changes in shoot height and primary root length of wheat and spelt plants. Some changes were observed in the fresh (FW) and dry weight (DW) of organs. FW and DW of wheat shoots did not change appreciably. On the other hand, in roots an increase in FW was accompanied by a decrease in DW, as a result of the elevated hydration of root cells and the intensification of water transport to shoots. In spelt wheat, FW of shoots and roots declined, while DW of roots increased. The negative effects of stress manifested on the 21st day. Neither species was able to completely recover its morphometric parameters. The height of the shoots remained less than in control. The growth of spelt roots was slower than control. A decrease in DW and FW of stressed plants organs was observed (Table 1).

Thus, the negative effects of short-term hyperthermia were manifested as a decline in morphometric parameters in wheat and spelt plants on the 21st day after recovery. Wheat plants were more resistant.

Dynamics and distribution of endogenous gibberellic acid in *T. aestivum* and *T. spelta* after short-term hyperthermia and in recovery period

We observed that GA_3 concentration in the roots of fourteen and twenty one-day-old control plants of wheat exceeded the corresponding indicators in shoots by 2.7- and 2.3-fold, respectively. Temperature stress caused a decrease in GA_3 levels. Roots were more sensitive to high temperature:

Table 1 Effect of short-term high temperature stress (+40 °C, 2 h) on shoot and root morphometric parameters of 14-day-old *Triticum aestivum* L. 'Podolyanka' and *Triticum spelta* L. 'Frankenkorn' plants and on 21-day-old plants after recovery

Option experiment	Shoot height (cm)	Fresh/dry shoot weight (mg)	Root length (cm)	Fresh/dry root weight (mg)
Triticum aestivum cv. Podolyanka (seed germination 98%)				
Control, 14th day	29.6 ± 1.2	165.2 ± 4.6[a] 20.8 ± 0.6[b]	7.8 ± 0.4	47.7 ± 2.6[a] 8.2 ± 0.9[b]
Heat stress, 14th day	30.8 ± 1.5	167.8 ± 8.4[a] 23.0 ± 0.7[b]	7.9 ± 0.4	63.6 ± 3.2[a*] 9.2 ± 0.7[b]
Control, 21st day	44.0 ± 2.2	323.5 ± 16.2[a] 55.8 ± 0.8[b]	16.6 ± 0.8	98.7 ± 4.9[a] 14.7 ± 0.7[b]
Recovery, 21st day	42.7 ± 2.1	312.2 ± 15.6[a] 50.1 ± 0.7[b]	14.9 ± 0.7	81.5 ± 4.1[a] 11.4 ± 0.7[b]
Triticum spelta cv. Frankenkorn (seed germination 92%)				
Control, 14th day	29.1 ± 1.6	192.0 ± 9.6[a] 26.6 ± 0.7[b]	12.3 ± 0.6	112.6 ± 5.6[a] 24.5 ± 1.1[b]
Heat stress, 14th day	28.9 ± 1.4	171.5 ± 8.6[a*] 23.3 ± 0.7[b]	12.1 ± 0.6	106.5 ± 5.3[a] 25.2 ± 1.2[b]
Control, 21st day	44.0 ± 2.2	231.7 ± 11.6[a] 43.4 ± 0.6[b]	15.4 ± 0.8	146.4 ± 7.3[a] 25.3 ± 0.8[b]
Recovery, 21st day	42.8 ± 2.1	229.0 ± 11.5[a] 42.0 ± 0.6[b]	12.5 ± 0.6	128.1 ± 6.4[a*] 24.4 ± 0.b[2]

[a]Average fresh weight

[b]Average dry weight of plant organ. $P < 0.05$

$^{*}P < 0.05$; $^{**}P < 0.01$;$^{***}P < 0.001$ compared to control at this stages of vegetation

the amount of GA_3 decreased by 37.3%, while in shoots it decreased by 23.2%. On the 21st day after recovery, an increase in hormone content was seen, but the recorded values were below control. GA_3 accumulation after recovery was more intensive in wheat roots (Fig. 1).

At the same time, endogenous GA_3 dominated in shoots of 14-day-old control spelt plants and in roots of 21-day-old plants. During growth, the content of endogenous GA_3 in shoots and roots of control 21-day-old spelt plants decreased 3.7- and 1.6-fold, respectively. In shoots and roots of post-stress spelt plants the level of endogenous GA_3 decreased by 17% and 48%, respectively. In the roots of 21-day recovery plants, GA_3 content increased in relation to post-stress plants 17% and exceeded the control 21-day-old plants by 27%. In shoots of 21-day-old post-stress plants, the GA_3 content was 20.7% higher than in

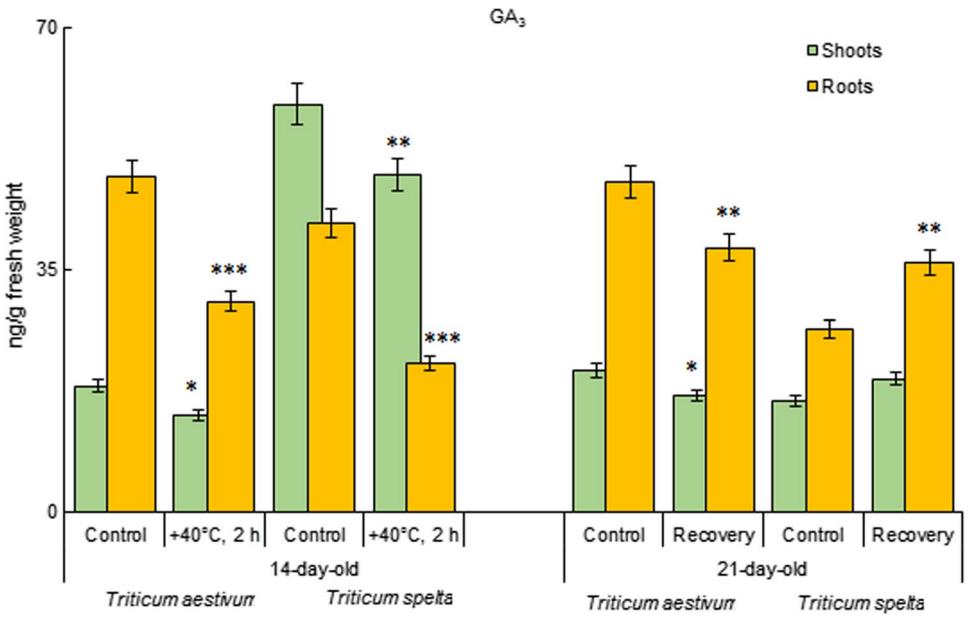

Fig. 1 Dynamics and distribution of endogenous gibberellic acid in the organs of 14-day-old plants of *Triticum aestivum* L. cv. Podolyanka and *Triticum spelta* L. cv. Frankenkorn after short-term hyperthermia (+40 °C, 2 h), and on the 21st day after recovery (ng/g FW); differences between the mean values were evaluated using Bonferroni-corrected ANOVA, considered to be significant at $P < 0.05$;$^{*}P < 0.05$; $^{**}P < 0.01$;$^{***}P < 0.001$ compared to control at this stages of vegetation

control, but 2.5-fold lower than in 14-day-old post-stress plants (Fig. 1).

Thus, endogenous GA_3 dominated in the roots of control and post-stress wheat plants, while in spelt the site of hormone accumulation was in the shoots. Heat stress inhibited the accumulation of hormones. After recovery, GA_3 accumulated in the roots of both species.

Dynamics and distribution of endogenous salicylic acid in *T. aestivum* and *T. spelta* after short-term hyperthermia and in recovery period

The ratio of SA content between shoots and roots of 14- and 21-day-old wheat plants was 3:1 and 6:1, and spelt 2.5:1 and 1.9:1, respectively. On the 21st day, the amount of hormone in the wheat shoots increased by 24.1% and in the roots—decreased by 35%. Maximum accumulation of SA was recorded in the roots of 14-day-old and shoots of 21-day-old wheat plants. During growth, the amount of SA in spelt plants decreased. On the 21st day, its level decreased almost fourfold in shoots and threefold in roots.

After heat stress, in shoots and roots of 14-day-old wheat plants SA content decreased by 30.0% and 46.7%, respectively. During the recovery period on the 21st day, the level of SA in shoots continued to decrease, while in roots increased 2.1-fold compared to control and 2.6-fold compared to post-stress plants (Fig. 2).

Dynamics and distribution of SA in post-stress spelt plants differed from those in wheat. In shoots, the SA content increased by 16%, and in roots—by 13%. On the 21st day after recovery, the amount of hormone decreased, but was higher than in 21-day control plants (Fig. 2).

Therefore, the accumulation of endogenous SA in control wheat plants occurred much more intensely than in spelt. In both species, SA dominated in shoots. Heat stress inhibited the accumulation of the hormone in wheat. After recovery, the site of SA accumulation was in the shoots of both species.

Dynamics and distribution of endogenous cytokinins in *T. aestivum* and *T. spelta* after short-term hyperthermia and after recovery

Among the studied forms of CKs in wheat we identified *t*-Z, *t*-ZOG and iP. *t*-ZR and iPa were present in trace amounts. After heat stress the content of *t*-Z increased 1.3-fold, *t*-ZOG—4.7-fold and iP—2.4-fold in wheat roots, whereas in shoots the content of *t*-Z decreased 1.4-fold, and iP and *t*-ZOG rose 2.3-fold and 2.6-fold, respectively. Total CKs content in shoots after heat stress rose 1.7-fold, and in roots—2.5-fold. On the 21st day after recovery in shoots of wheat, the pool of endogenous cytokinins decreased 1.4-fold, while in roots it did not change. The content of iP in shoots declined almost 13-fold, while, conversely, in roots, it increased 3.9-fold. In roots *t*-Z content rose 1.7-fold (Table 2).

We found five forms of CKs in spelt. After heat stress in the roots, the content of *t*-Z and iP increased 2.3-and twofold, and the content of *t*-ZOG significantly (7.4-fold) decreased. A decrease in the content of *t*-Z (1.3-fold), *t*-ZR (2.2-fold), iPa (up to trace amounts) and *t*-ZOG (2.8-fold) was detected in shoots. The total content of CKs in shoots after heat stress decreased 2.4-fold, and in roots 2.2-fold. On the 21st day after recovery, the pool of endogenous cytokinins decreased 1.5-fold in shoots, and 1.8-fold—in

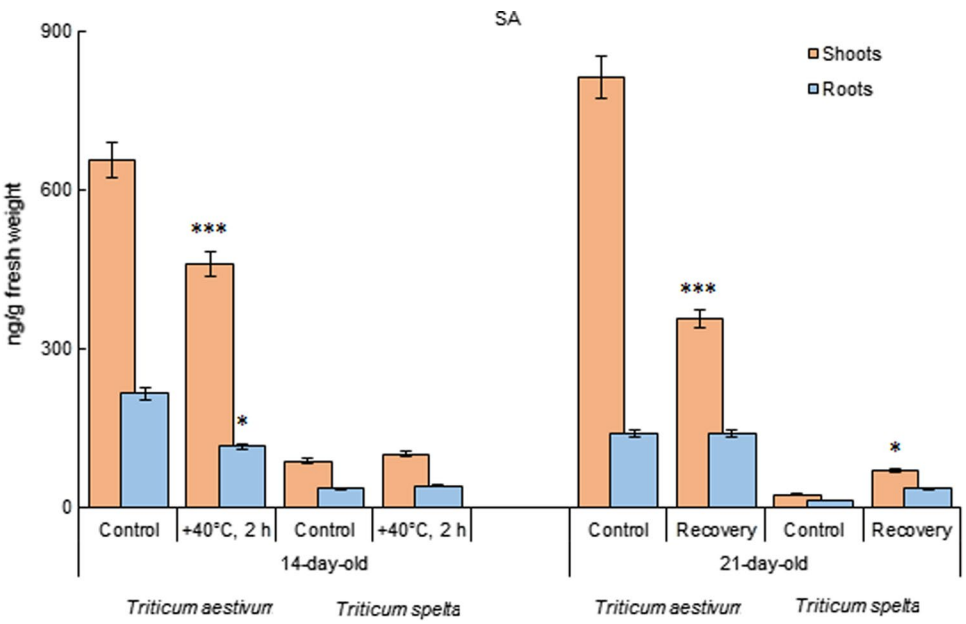

Fig. 2 Dynamics and distribution of endogenous salicylic acid in the organs of 14-day-old plants of *Triticum aestivum* L. cv. Podolyanka and *Triticum spelta* L. cv. Frankenkorn after short-term hyperthermia (+40 °C, 2 h) and on the 21st day after recovery (ng/g FW); differences between the mean values were calculated using Bonferroni-corrected ANOVA, considered to be significant at $P<0.05$;*$P<0.05$; **$P<0.01$;***$P<0.001$ compared to control at this stages of vegetation

Table 2 Effect of short-term high temperature stress (+40 °C, 2 h) on cytokinins content in shoots and roots of 14-day-old *Triticum aestivum* L. 'Podolyanka' plants and on 21-day-old plants after recovery, ng/g FW

Sample	*t*-Z	ZR	iPa	iP	ZOG	∑
14-day-old plants						
Shoot control	151.1 ± 7.6	Traces	Traces	267.6 ± 13.4	27.7 ± 1.4	446.4
Shoot stress	104.3 ± 5.2**	Traces	Traces	604.9 ± 30.2***	71.4 ± 3.6**	780.6
Root control	115.4 ± 5.8	Traces	Traces	13.1 ± 6.6	Traces	128.5
Root stress	157.6 ± 7.9**	Traces	Traces	62.4 ± 3.1***	96.7 ± 4.8***	316.7
21-day-old plants						
Shoot control	210.7 ± 10.5	Traces	Traces	283.2 ± 14.2	291.2 ± 14.6	785.1
Shoot stress	189.2 ± 9.5**	Traces	Traces	21.9 ± 1.1***	330.9 ± 16.5***	542.1
Root control	74.0 ± 3.7	Traces	Traces	4.7 ± 0.2	283.2 ± 14.2	361.9
Root stress	43.4 ± 2.2***	Traces	Traces	18.2 ± 0.9**	302.8 ± 15.1*	364.4

t-Z—*trans*-zeatin, ZR—zeatin riboside; iPa—isopentenyladenosine, iP—isopentenyladenine, ZOG—zeatin-*O*-glucoside; traces—less than 0.5 ng/g FW. $P < 0.05$;*$P < 0.05$; **$P < 0.01$;***$P < 0.001$ compared to control at this stages of vegetation

roots. In shoots, the levels of *t*-Z (2.3-fold), ZR (twofold) and ZOG (1.6-fold) also decreased, while the amount of iPa (2.3-fold) and iP (1.2-fold) increased. In roots, the content of all CK forms declined significantly: *t*-Z and ZR 4.4-fold, iPa 13-fold, iP to trace amounts, while the content of *t*-ZOG almost did not change (Table 3).

Discussion

Since the beginning of the century the ambient temperature has been steadily rising and is expected to rise further. At higher temperatures, the duration of all stages of ontogenesis, photosynthetic activity, stability of cell membranes, relative water content and leaf area index, total biomass and wheat yield decrease (Narayanan 2018). One of the critical periods of wheat ontogenesis is the three-leaf stage, when a transition from nutrition drawn from grain reserves to the absorption of nutrients from the outside through the root system occurs. High-yielding modern wheat genotypes, local species and wild precursors are characterized by specific temperature optima and some anatomical-morphological and biochemical differences. As compared to wild species, modern wheat varieties have higher stability of the photosynthetic apparatus, and wider ranges of optimal temperatures, which contribute to increased productivity throughout the growing season (Brestic et al. 2018). Genes involved in the regulation of phytohormone biosynthesis under stress conditions affect the ontogenesis and resistance of agricultural crops. In particular, changes in the metabolism and signaling of gibberellins and brassinosteroids determine stress tolerance, while yields are regulated mainly by cytokinins (Nadolska-Orczyk et al. 2017). The expression of genes encoding enzymes involved in GAs synthesis is regulated by external signals. Under the influence of negative factors, GA synthesis is regulated by *GA2ox* genes, which encode GA2-inactivating enzymes, as well as the *DELLA RGL3* gene, which encodes a growth suppressor (Colebrook et al. 2014; Minguet et al. 2014). We have found that in the initial stages of ontogenesis, the nature of the accumulation and distribution of gibberellic acid in the organs of wheat and spelt follow specific patterns. The content of GA_3 in spelt

Table 3 Cytokinins content in *Triticum spelta* L. cv. Frankenkorn after short-term hyperthermia (+40 °C, 2 h) and on the 21st day after recovery, ng/g FW

Sample	*t*-Z	ZR	iPa	iP	ZOG	∑
14-day-old plant						
Shoot control	25.2 ± 1.3	537.7 ± 26.9	6.4 ± 0.3	Traces	341.7 ± 17.1	911.0
Shoot stress	19.6 ± 1.0*	238.2 ± 11.9**	Traces	Traces	119.1 ± 10.0***	376.9**
Root control	22.3 ± 1.1	Traces	Traces	89.7 ± 4.5	588.1 ± 29.4	700.1
Root stress	51.3 ± 2.6**	Traces	Traces	185.0 ± 9.3***	79.5 ± 4.0***	315.9***
21-day-old plant						
Shoot control	13.0 ± 0.7	231.6 ± 11.6	22.7 ± 1.1	15.9 ± 0.8	52.8 ± 2.6	336.0
Shoot stress	5.6 ± 0.3*	113.9 ± 5.7*	52.9 ± 2.6**	19.1 ± 1.0	32.2 ± 1.6*	223.7*
Root control	14.5 ± 0.7	210.0 ± 10.5	73.8 ± 3.7	66.5 ± 3.3	116.0 ± 5.8	480.8
Root stress	3.3 ± 0.2***	144.7 ± 7.2*	5.7 ± 0.3***	0.8 ± 0.04***	108.4 ± 5.4	262.9**

t-Z—*trans*-zeatin, ZR—zeatin riboside; iPa—isopentenyladenosine, iP—isopentenyladenine, ZOG—zeatin-*O*-glucoside; traces—less than 0.5 ng/g FW. $P < 0.05$;*$P < 0.05$; **$P < 0.01$;***$P < 0.001$ compared to control at this stages of vegetation

shoots was more than threefold higher than its content in wheat shoots. On the other hand, the amount of GA_3 in roots of both species was approximately the same, with a slight predominance in wheat. The response of wheat and spelt to hyperthermia was not species-specific. In 14-day post-stress wheat and spelt plants, GA_3 accumulation was inhibited. However, the preservation of the distribution of the hormone between the organs turned out to be specific. In wheat, GA_3 dominated in roots, and in spelt—in shoots. After short-term hyperthermia, the biomass of wheat roots increased by 33%, while the biomass of spelt roots decreased by 5% (Table 1). The architecture of the root system is the main interface between the plant and abiotic factors, as it identifies and responds to negative influences, and helps overcome them. Length and density of the main and lateral roots play a crucial role in tolerance acquiring. Thus, an increase in density and diameter of the main root and development of lateral roots improved access to moisture at greater soil depths, increased hydration, photosynthetic activity and stomatal conductance, growth and drought resistance of rice and corn plants (Lynch et al. 2014; Zhan et al. 2015). Gibberellins have a positive effect on root growth. A recent study showed that HDT1/2 (histone deacetylases) mediates the early transition from root tip cell division to their elongation by inhibition the transcription of the *GA2ox2* gene, a gibberellin inactivator (Li et al. 2017). On the 21st day after recovery, the amount of GA_3 in wheat did not reach control levels, while in spelt it exceeded control levels. GA_3 was predominant in the roots of both species. On the 21st day, the weight of the roots of post-stressed wheat plants was 17%, and spelt—12% less than that of control plants (Table 1).

Heat stress in general had a negative effect on the accumulation of GA_3 in the organs of 14-day-old plants, but a sufficiently high level of the hormone in wheat roots induced an increase in their biomass and an acquisition of tolerance. In spelt roots, active accumulation of GA_3 and growth of biomass wad observed after recovery on the 21st day, indicating a successful completion of post-stress adaptation.

The mechanism of stress tolerance under the action of SA is quite complex and not clearly understood. It includes the production of osmolites, induction of antioxidant activity and interaction with other hormones (Khan et al. 2015). We found that in the initial stages of ontogenesis after the transition from heterotrophic to autotrophic nutrition, SA accumulated in shoots of 14-day-old plants. The content of SA in wheat shoots was threefold higher than in roots. A similar distribution was observed in spelt, where the level of the hormone in shoots was 2.5-fold higher than in roots. The amount of SA in wheat shoots was 7.6-fold higher than in spelt, similar results were obtained for roots where hormone content was 6.3-fold higher. In 14-day-old post-stress wheat plants, the amount of SA decreased significantly, while in spelt organs, on the contrary, it increased slightly. On the 21st day after recovery, the SA content in spelt shoots and roots was threefold higher than in control, while in wheat it decreased in shoots 2.2-fold and doubled in roots. An accumulation of endogenous SA was observed in response to water stress in soybean plants (Hamayun et al. 2010), in rice plants under salt stress (Sawada et al. 2006), and in wheat under heavy metal (zinc) pollution (Kosakivska et al. 2019). It should be noted that SA content in plants under abiotic stresses, as a rule, grows more slowly than does the generation of ROS (reactive oxygen species). Because of this, SA is considered to be a signaling molecule involved in the perception, amplification and transduction of primary ROS signals (Larkindale and Huang 2004).

Our study has shown that during the formation of wheat and spelt reaction to short-term heat stress, a complex modifications of the cytokinins hormones occur, the nature of which depends on the species and organ of the plant. Indeed, after heat stress, the pool of CKs in wheat shoots and roots increased significantly. In roots, *t*-Z, *t*-ZOG and iP accumulated, while in shoots—*t*-Z content decreased, and iP and *t*-ZOG content increased. On the other hand, the total content of CKs in spelt shoots and roots more than halved. The content of *t*-Z and iP in spelt roots rose and the level of t-ZOG significantly declined. In shoots, a decrease in the content of *t*-Z, *t*-ZR, iPa and *t*-ZOG was recorded.

It should be noted that spelt plants significantly surpassed wheat plants by the total quantitative content of cytokinins in the control conditions. At the same time, the pool of cytokinins in wheat shoots was higher than in spelt in post-stress plants. On the 21st day after recovery in shoots of wheat and spelt, the pool of endogenous cytokinins decreased, while in wheat roots it did not change, and in spelt roots it decreased. The total CKs content in stressed wheat plants was twice as high as in spelt. Earlier we showed that after short-term heat stress in the roots of 14-day-old wheat plants of frost-resistant Volodarka variety, the CKs pool increased due to a significant accumulation of endogenous *c*-Z and iPa. Conversely, in the shoots, the general level of CKs was halved (Kosakivska et al. 2016).

The effect of high temperature on the balance of cytokinins has been studied mainly in the reproductive period of cereal development (Cheikh and Jones 1994; Wang et al. 2020). In rice inflorescences and roots under the action of high temperature the content of active cytokinins forms decreased (Wu et al. 2017). Under short-term heat stress, changes in the content of certain forms of cytokinins have been recorded (Farkhutdinov et al. 1997; Todorova et al. 2005). At a temperature of + 40 °C for the first 30 min, the level of active forms of cytokinins increased in the leaves and decreased in the roots, and after 2 h of stress reduction of cytokinins content was detected both in the leaves and in the roots of arabidopsis plants (Dobrá et al. 2015). Zeatin and zeatinribozid play a key role in the regulation of

growth processes of higher plants, while in shoots they act as positive growth regulators, and in roots—as growth suppressors. Therefore, it is plausible that the changes in the dynamics and distribution of cytokinins after short-term hyperthermia revealed in this work, are aimed at reducing the growth activity of wheat and spelt. The simultaneous surge in the accumulation of iP and *t*-ZOG in wheat is due to continued cytokinins biosynthesis (as its primary products), and a shift in the synthesis of the hormone under the action of stress on conjugation to *O*-glucoside. At the same time, in spelt plants, accumulation of iP was recorded in shoots, while the content of *t*-ZOG decreased significantly in both shoots and in roots.

Plants integrate ecological and endogenous signals through a complex network of phytohormonal interactions, forming reaction and regulating growth and development. The involvement of cytokinins, gibberellins and SA in plant responses to stresses is broadly appreciated, and evident from the results of exogenous applications of hormones. Thus, priming with solutions of gibberellic and salicylic acids induced the germination of rye grains Secale montanum, increased the germination index and coefficient of germination rate in drought conditions. After treatment with phytohormones, the content of antioxidant enzymes catalase and ascorbate peroxidase increased (Ansari et al. 2013). Treatment with a SA solution contributed to the formation of stress resistance of wheat to hyperthermia (Asif et al. 2019), maintained photosynthetic activity, reducing oxidative damage to photosynthetic membranes due to the production of antioxidants glutathione and ascorbate (Chen et al. 2016), induced proline accumulation, which promoted the normalization of hydration (Khan et al. 2013). A positive effect of cytokinin treatment was revealed for wheat (Gupta et al. 2003) and barley (Hosseini et al. 2008). The mechanism of this action is related to the ability of these hormones not only to stimulate cell division, but also to increase the attracting ability of seeds and prolong the period of active photosynthesis (Hönig et al. 2018).

Our study showed that heat stress induced rapid changes, both specific and nonspecific, in the hormonal balance in 14-day-old wheat and spelt plants, the nature of which depended on the species and organ of plant. Our data also revealed a prolonged effect of heat stress on phytohormones accumulation and distribution in the organs of 21-day-old wheat and spelt post-stress plants. It is worth noting that in control conditions the content of endogenous SA was significantly higher in wheat, while the content CKs was higher in spelt. These fluctuations indicate a direct involvement of endogenous cytokinins, gibberellic and salicylic acids in the formation of a response to heat stress, and may be useful in screening of stress-resistant genotypes of wheat and spelt taking into account future extreme climatic changes.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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