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## IMPACT OF CHILLING ON GROWTH AND HORMONAL HOMEOSTASIS OF *TRITICUM AESTIVUM* AND *TRITICUM SPELTA* DURING INITIAL STAGE OF VEGETATION AND AFTER RECOVERY

### SUMMARY

The effect of short-term chilling (+4°C, 2 h) on hormonal homeostasis and growth parameters was investigated in 14-day-old stressed and 21-day-old recovered plants of *Triticum aestivum* cv 'Podolyanka' and *T. spelta* cv. 'Frankenkorn'. Short-term chilling didn't impact the linear indicators of wheat and spelt shoots and roots but stimulated shoot and root fresh and dry weight growth, while reducing root fresh weight. Spelt plants exhibited better post-stress recovery. Chilling led to a 396% increase in ABA content in wheat and a 74% increase in spelt. Stressed and recovered plants exceeded control plants in ABA content. Wheat exhibited three times higher constitutional SA content than spelt, while spelt exceeded wheat in gibberellins (GA<sub>3</sub> and GA<sub>4</sub>) content. After chilling, SA content increased by 17.3% (wheat) and 18.7% (spelt), while gibberellins decreased by 32.4% (wheat) and 24.4% (spelt). Wheat dominated spelt in constitutional IAA content. Following chilling, IAA content decreased by 62% (wheat) and 72.2% (spelt). Our findings revealed both common characteristics and organ- and species-specific traits in phytohormones accumulation and balance in wheat and spelt plants during the rapid adaptation to chilling and subsequent recovery period. This will contribute to the understanding of how these related wheat species respond to chilling in the early stages of growth. Analyzing differences in constitutional and stress-induced endogenous phytohormone balances may be useful for developing screening methods for resilient genotypes and environmentally friendly technology of stress resistance induction.

**Key words:** *Triticum aestivum*, *Triticum spelta*, phytohormones, chilling, growth, recovery

**Abbreviations:** ABA, Abscisic acid; IAA, indole-3-acetic acid; GA<sub>3</sub> and GA<sub>4</sub>, gibberellins; ROS, reactive oxygen species; SA, salicylic acid

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## INTRODUCTION

Abiotic stresses exert adverse effects on the growth and productivity of cereal crops and account for approximately 50% of the crop losses, with around 7% attributed to low temperature (Kajlaa *et al.*, 2015). Chilling adversely impacts both vegetative and reproductive growth in wheat, causing delayed seed germination and partial plant death due to embryo development disturbances. In seedlings, reduced water and nutrients uptake rates result in cell dehydration, disrupted nutritional processes, and crop losses ranging from 10 to 30% (Ji *et al.*, 2017; Wu *et al.*, 2023). Under chilling, alterations occur in photosynthesis, respiration, and substance transport in wheat plants (Hassan *et al.*, 2021; Yadav, 2010). Chilling triggers the generation of reactive oxygen species (ROS) (Foyer *et al.*, 2002) and lipid peroxidation (Thakur *et al.*, 2010). Within the initial hours of exposure to chilling, a reorganization of the photosynthetic and energy apparatus takes place (Babenko *et al.*, 2019).

Phytohormones play a pivotal role in acquiring cold resistance, serving as components of intricate signaling cascades that regulate growth, initiate morphological and molecular adaptive changes (Eremina *et al.*, 2016; Santner *et al.*, 2009; Tian *et al.*, 2022). The isoprenoid phytohormone abscisic acid (ABA) orchestrates numerous physiological, biochemical, and molecular processes, governing plant growth and development under both normal and stressful conditions (Kishor *et al.*, 2022). Plant tissues experience rapid ABA content elevation under diverse abiotic factors. The hormone interacts with receptors, instigating signaling cascades that trigger plant adaptive response (Rehman *et al.*, 2021; Sah *et al.*, 2016. Mehrotra *et al.*, 2014). ABA's participation in environmental signal integration, in conjunction with other phytohormones, has been established (Parwez *et al.*, 2022). Salicylic acid (SA), a signaling compound governing plant growth and development, plays a pivotal role in fostering plant tolerance to abiotic stresses (Arif *et al.*, 2020; Hu *et al.*, 2022). Fluctuations in endogenous SA dynamics and distribution, along with exogenous application results, indicate the hormone's involvement in mitigating low-temperature effects *via* ABA-dependent or independent pathways, Ca<sup>2+</sup> signaling pathways, mitogen-activated protein kinase pathways, and reactive oxygen and nitrogen species pathways. Activation of these pathways prompts antioxidant production, osmolyte accumulation, cold-responsive protein synthesis (such as LEA and dehydrins), and adjustments in hormonal balance (Miura, Tada, 2014; Saleem *et al.*, 2021). Gibberellins (GAs), a class of hormones crucial in regulating growth and development processes under shifting external conditions, also play a vital role in abiotic stress response formation. Cold, salt and osmotic stress decreased GA biosynthesis and signaling, which leads to growth inhibition, fostering resistance, while enhanced biosynthesis of GA contributes to resistance to shading and submergence (Colebrook *et al.*, 2014). While the role of auxins, particularly indole-3-acetic acid (IAA), in regulating plant growth and development under optimal conditions is extensively studied, their involvement in acquired resistance to abiotic stresses remains less explored (Rahman, 2013).

The homeostasis of endogenous auxins under stressors is determined by changes in hormone transport flow direction and intensity along with metabolic conversions (Korver et al., 2018). Hormonal changes accompanying the response of *Triticum aestivum* plants to cold stress (+4°C) during the “alarm” phase involve increased ABA levels and decreased bioactive auxin and gibberellin contents (Kosova et al., 2012). Additionally, endogenous ABA and H<sub>2</sub>O<sub>2</sub> form a positive feedback loop to mediate SA-induced freezing tolerance in wheat (Wang et al., 2018). Previously, we analyzed the influence of short-term heat stress (+40°C, 2 h) and moderate soil drought (4 days without watering) on growth and endogenous hormonal homeostasis in 14- and 18-day-old plants of winter wheat cv. ‘Podolyanka’ and spelt wheat cv. ‘Frankenkorn’, both during and after a 21-day recovery (Kosakivska et al., 2022; 2023). It is worth noting that majority of scientists now support the idea that whole-grain hexaploid common wheat, *Triticum aestivum* L., is derived from hulled spelt wheat, *Triticum spelta* L. (Faris J. 2014; Luo et al., 2007). This study aims to explore the impact of short-term chilling on growth and hormonal homeostasis of these related wheat species, identifying common and specific features in their response during the initial stages of growth. In our study, we proceeded from the hypothesis that the impact of chilling on wheat and spelt during early growth stages induces specific alterations in the accumulation and distribution of four phytohormone classes. These changes activate stress-protective systems, shaping an adaptive strategy.

## MATERIALS AND METHODS

**Plant material** Fourteen- and 21-day-old plants of winter wheat (*T. aestivum* L.) cv. ‘Podolyanka’ and spelt (*T. spelta* L.) cv. ‘Frankenkorn’ were investigated. These plants were cultivated in laboratory conditions during 2021-2022 years at the M. G. Kholodny Institute of Botany of the NAS of Ukraine (Kyiv). The ‘Podolyanka’ wheat variety is known for its winter and drought resistance, high yield, and adaptability. The ‘Frankenkorn’ spelt variety exhibits frost resistance and ecological flexibility. Wheat and spelt seeds were sterilized with an 80% ethanol for 5 min, rinsed with distilled water, soaked in water in cuvettes for 3 hours, and then germinated at a temperature of +24°C for 21 hours. Sprouted seeds were then planted in 2-liter containers filled with calcined river sand. Plants were grown at a temperature of +20°C, light intensity of 190  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , 16/8 h (day/night) photoperiod, and relative air humidity of 65±5%. Substrate humidity was maintained at 60% of full moisture content, and daily watering was carried out using Knop's solution at a rate of 50 ml per container.

**Chilling stress induction and sampling** Fourteen-day-old plants in the 2-3 leaf stage were divided into two groups. One group was subjected to a temperature of +4°C and light intensity of 190  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for 2 hours (LT-plants), while the other group served as a control (C-plants), continuing growth under the initial experimental conditions. For recovery, plants were grown under controlled conditions until the 21st day, reaching the 3-4 leaf stage. Shoots and roots of 14-

and 21-day-old LT- and C-plants were examined. Morphometric parameters of both species were promptly measured following a short-term chilling.

**Extraction and analysis of phytohormones** Samples of shoots and roots (1,5 g) were frozen and ground in liquid nitrogen using 10 ml of extraction solution – methanol, distilled water, and formic acid in a ratio of 15:4:1. The homogenate was incubated at +4°C for 24 hours in the dark. The extracts 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 minutes and centrifuged again. Internal chemical standards were added to the combined supernatants for identification and calculation of losses (1.6 ng of each substance per microliter). 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 SPE cartridges: C18 Sep-Pak Plus, Waters and Oasis MCX, 6 cc/150 mg, Waters. Elution of IAA, ABA, GA<sub>3</sub>, GA<sub>4</sub> and SA was performed with 100% methanol. This obtained fraction was 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 before analysis.

The aliquots were analyzed through high-performance liquid chromatography using an Agilent 1200 LC/MS series instrument (USA), equipped with a diode-array detector G1315B and single quadrupole mass-detector G6120A. 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). Column ZORBAX Eclipse Plus C18 SS 3.0×150 mm with a sorbent particle size of 3.5 µm was used for the analysis of SA. Agilent OpenLAB CDS ChemStation Edition chromatograph software (rev. C.01.09) was employed for quantitation of the analytes. The content of analytes in the samples was monitored using a mass-detector in the combined mode (electrospray and chemical ionization at atmospheric pressure) with ionization of molecules of analytes in negative polarity during analysis. Detailed chromatographic conditions are described in Kosakivska *et al.*, 2020.

**Statistical analysis** The experiments were conducted with three biological and three analytical replicates. The results were statistically processed using Statistix v. 10.0. Univariate analysis of variance was applied, and differences between mean values were assessed using the ANOVA criterion. Significance was considered at  $P \leq 0.05$ . Our comparative analysis involved stressed 14-day-old plants versus their respective controls, as well as 21-day-old recovered plants versus 21-day-old control plants. Additionally, during result discussion, comparisons were made between 14-day-old stressed plants with 21-day-old recovered plants.

## RESULTS

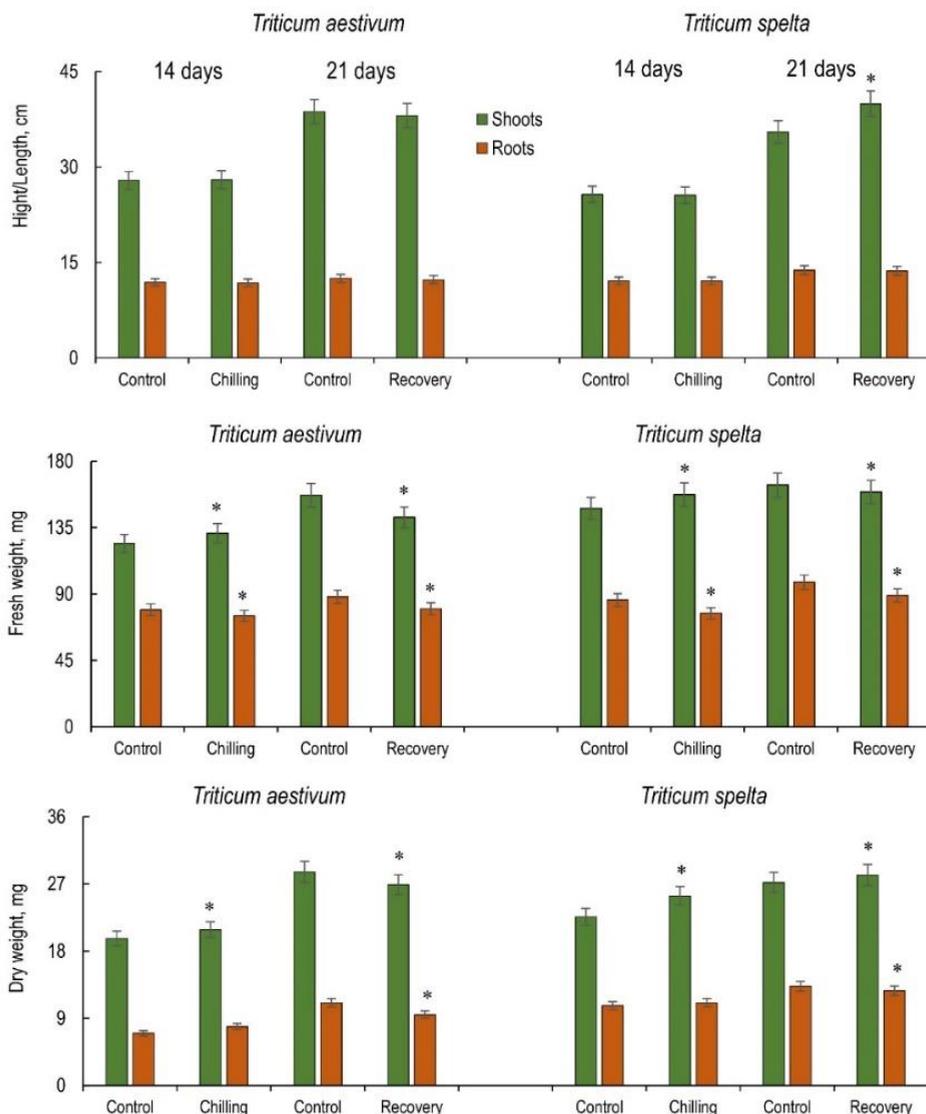
**Growth parameters of wheat and spelt plants after chilling** The short-term exposure to low positive temperature had minimal impact on the linear

parameters of shoots and roots in 14-day-old 'Podolyanka' winter wheat and 'Frankenkorn' spelt wheat plants. Conversely, shoot fresh weight (FW) and dry weight (DW) of stressed wheat plants increased by 5.6% and 6.1%, respectively, while root FW decreased by 5.1% and DW increased by 12.9%. During the recovery phase, the linear parameters of both shoots and roots for 21-day-old stressed wheat plants returned to levels close to unstressed control plants. However, shoot and root FW were 9.6% and 9.3% lower, and DW was reduced by 5.9% and 14.4%, respectively, compared to non-stressed 21-day-old plants (Fig. 1). In stressed 'Frankenkorn' spelt plants, shoot FW and DW experienced increases of 6.1% and 12.4%, respectively, while root FW decreased by 10.5%, and DW has not changed. In the case of 21-day-old spelt wheat plants after recovery, shoot height saw a 12.4% increase the FW and DW of shoots decreased by 3% and 3.6%, respectively, (within the margin of error). Meanwhile, the FW of the roots decreased by 9.2%, and the DW decreased by 4.5%, also within the margin of error (Fig. 1).

Overall, both species displayed considerable resistance to chilling, with spelt plants exhibiting a better recovery.

**ABA accumulation and distribution after chilling** The distribution of endogenous ABA between shoots and roots of 14-day-old winter wheat 'Podolyanka' and spelt wheat 'Frankenkorn' C- plants exhibited similarities. The hormone was predominant in the shoots of both species. By day 21, ABA levels in the shoots of wheat and spelt C- plants increased by 1.6 and 1.3 times, respectively, while changes in hormone content in roots remained within the range of statistical error (Fig. 2). ABA continued to dominate in the shoots of all tested samples of 'Podolyanka' wheat and 'Frankenkorn' spelt. Under the influence of chilling, ABA levels in wheat and spelt LT- plants shoots increased by 4.3 and 1.9 times, respectively. In the roots of wheat, hormone content increased by 5.9 times, while in spelt, it rose by 1.4 times. After the recovery period, ABA levels in both species decreased, yet hormone content in the shoots and roots of wheat remained 1.7 and 3.8 times higher than that of 21-day-old C-plants. In spelt ABA content approached control values (Fig. 2).

The overall ABA content of spelt C- plants was higher than that of wheat C-plants, and this predominance persisted in stressed and recovering wheat plants. The total ABA content in 14-day-old stressed wheat plants saw a 396% increase, totaling  $234.2 \pm 11.7 \text{ ng g}^{-1} \text{ FW}$ . By day 21 of recovery, hormone levels decreased by 35% compared to 14-day-old LT- plants, measuring  $151.9 \pm 7.6 \text{ ng g}^{-1} \text{ FW}$ ; yet, it remained 130% higher than levels observed in 21-day-old C- plants. Following exposure to chilling, the total ABA content in 14-day-old spelt plants rose by 74.1% to  $101.7 \pm 5.1 \text{ ng g}^{-1} \text{ FW}$ . The ABA level in 21-day-old recovered plants reached  $77.9 \pm 3.9 \text{ ng g}^{-1} \text{ FW}$ , which was 23.4% lower than levels found in 14-day-old LT- plants, but 8.2% higher than those observed in 21-day-old control non-stressed plants.

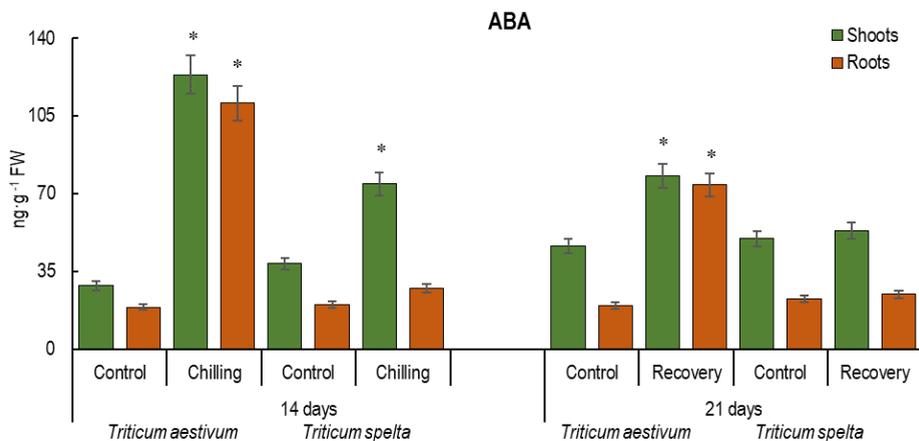


Note \* – significant at  $P \leq 0.05$  compared to control at these stages of vegetation; data are the mean  $\pm$  SE,  $n = 40$

Fig. 1. Effect of chilling ( $+4^{\circ}\text{C}$ , 2 h) on growth parameters of 14-day-old *Triticum aestivum* L. cv ‘Podolyanka’ and *Triticum spelta* L. cv. ‘Frankenkorn’, and on 21-day-old plants after recovery.

**SA accumulation and distribution after chilling** The endogenous SA content in the shoots and roots of 14-day-old winter wheat C- plants exceeded that in spelt C- plants organs by 2.3 and 2.7 times, respectively. The pattern of hormone accumulation in the studied cereal organs on the 21st day of growth exhibited distinct differences. A 1.2-fold increase in SA content was observed in the shoots

of wheat plants, whereas a 1.3-fold decrease hormone concentration was noted in the shoots of spelt plants and in the roots of both species (Fig. 3).



\* – significant difference at  $P \leq 0.05$  vs. control; data are the mean  $\pm$  SE,  $n=9$

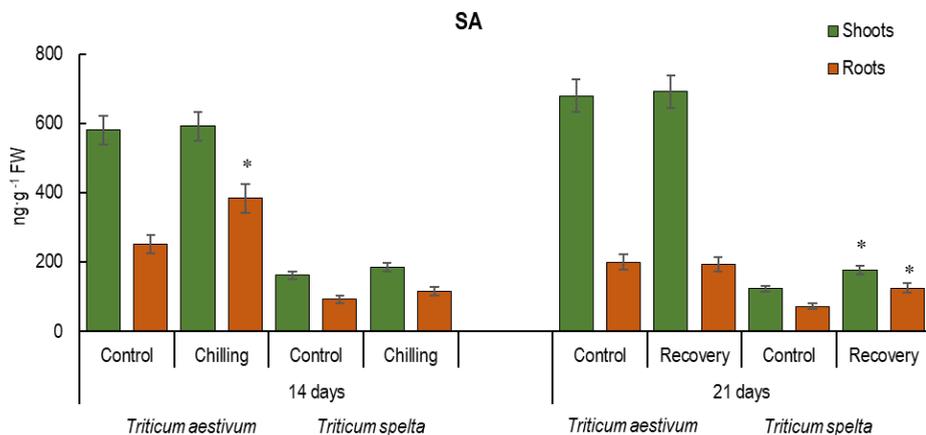
Fig. 2. Accumulation and distribution of endogenous abscisic acid in *Triticum aestivum* L. cv. ‘Podolyanka’ and *Triticum spelta* L. cv. ‘Frankenkorn’ plants under chilling ( $+4^{\circ}\text{C}$ , 2 h) and during the recovery period ( $\text{ng g}^{-1}$  FW)

Under the influence of chilling, the SA content in the roots of winter wheat and spelt wheat LT- plants increased by 1.5 and 1.3 times, respectively, while the changes in the shoots remained within the range of statistical error. Following recovery, in 21-day-old plants, SA levels rose in wheat shoots to  $691.0 \pm 34.6 \text{ ng g}^{-1}$  FW and in spelt roots to  $124.0 \pm 6.2 \text{ ng g}^{-1}$  FW, marking increases of 16.8% and 6.7%, respectively, compared to control unstressed plants. Conversely, in the roots of wheat and shoots of spelt, SA content decreased by 2 and 1.1 times, respectively, during the recovery period. In wheat roots, SA content stayed within the control range at  $192.6 \pm 9.6 \text{ ng g}^{-1}$  FW, whereas in spelt shoots it was 1.4 times higher than that of non-stressed plants, measuring  $175.1 \pm 8.8 \text{ ng g}^{-1}$  FW (Fig. 3).

In essence, the SA content in the organs of winter wheat significantly exceeded that of spelt wheat. The preferred site of hormone accumulation was observed in the shoots of both species. The total SA content in 14-day-old ‘Podolyanka’ wheat plants increased by 17.3% under the influence of chilling, reaching  $975.0 \pm 48.8 \text{ ng g}^{-1}$  FW. After recovery, the hormone concentration decreased by 9.4% to  $883.6 \pm 44.1 \text{ ng g}^{-1}$  FW, well within the parameters of control unstressed 21-day-old plants. For 14-day-old ‘Frankenkorn’ spelt plants, exposure to cold stress resulted in an 18.7% elevation in total SA content, registering at  $300.9 \pm 15.2 \text{ ng g}^{-1}$  FW. While no further changes in hormone levels were observed after recovery, this indicator remained 53.9% higher than that of 21-day-old control unstressed plants.

**GAs accumulation and distribution after chilling** The total content of  $\text{GA}_3 + \text{GA}_4$  in 14-day-old ‘Frankenkorn’ spelt C- plants exceeded that in

‘Podolyanka’ wheat. By the 21st day of growth, the content of GA in the shoots of wheat C- plants remained virtually unchanged at  $32.0 \pm 1.6 \text{ ng g}^{-1} \text{ FW}$ , while in the roots it decreased by 1.2 times, amounting to  $50.8 \pm 2.5 \text{ ng g}^{-1} \text{ FW}$ . In spelt shoots and roots on the 21st day, the accumulation of GA<sub>4</sub> was observed, with its levels being 1.7 and 3.2 times lower than those of GA<sub>3</sub>. Conversely, GA<sub>4</sub> was found only in trace amounts in wheat (Fig. 4).



\* – significant difference at  $P \leq 0.05$  vs. control; data are the mean  $\pm$  SE,  $n=9$

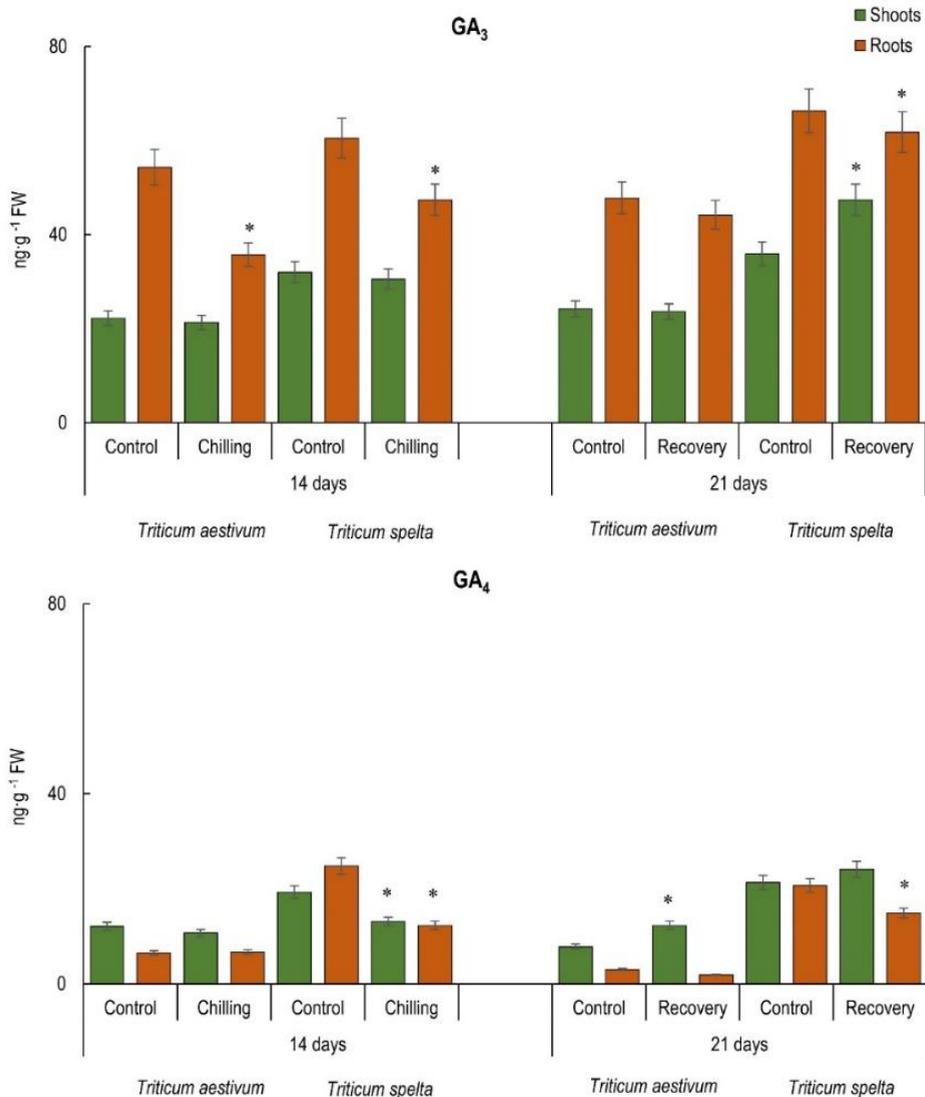
Fig. 3. Accumulation and distribution of endogenous salicylic acid in *Triticum aestivum* L. cv. ‘Podolyanka’ and *Triticum spelta* L. cv. ‘Frankenkorn’ plants under chilling ( $+4^\circ\text{C}$ , 2 h) and during recovery period ( $\text{ng g}^{-1} \text{ FW}$ )

Following exposure to cold stress, the total gibberellin content in 14-day-old ‘Podolyanka’ wheat plants decreased by 32.4%, reaching  $64.4 \pm 3.2 \text{ ng g}^{-1} \text{ FW}$ . Upon recovery by the 21st day, the gibberellins concentration increased by 27.2%, amounting  $81.9 \pm 4.1 \text{ ng g}^{-1} \text{ FW}$ , nearly approaching the values of control unstressed plants. In case of ‘Frankenkorn’ spelt, chilling led to a 24.4% decrease in total GA content, measuring  $103.3 \pm 5.2 \text{ ng g}^{-1} \text{ FW}$ . In recovered plants, hormone levels rose by 43.5%, reaching  $148.2 \pm 7.4 \text{ ng g}^{-1} \text{ FW}$ , a 2.8% increase compared to the 21-day control unstressed plants. GA<sub>4</sub> was primarily accumulated in the shoots of both species, and GA<sub>3</sub> was predominantly stored in the roots (Fig. 4).

In general, GA<sub>3</sub> remained dominant across all experimental samples, with gibberellin accumulation favoring the roots. Under the effects of stress and during the recovery period, more pronounced changes were observed in the accumulation of GA<sub>3</sub> in wheat plants, whereas active fluctuations in GA<sub>3</sub> and GA<sub>4</sub> levels were observed in spelt plants.

**IAA accumulation and distribution after chilling** The content of endogenous IAA in both shoots and roots of 14-day-old ‘Podolyanka’ winter wheat C- plants exceeded that in ‘Frankenkorn’ spelt. Notably, the hormone dominated in wheat roots and spelt shoots. In particular, the IAA level in wheat roots was 3.3 times higher than that in spelt roots, whereas the IAA content in spelt shoots was

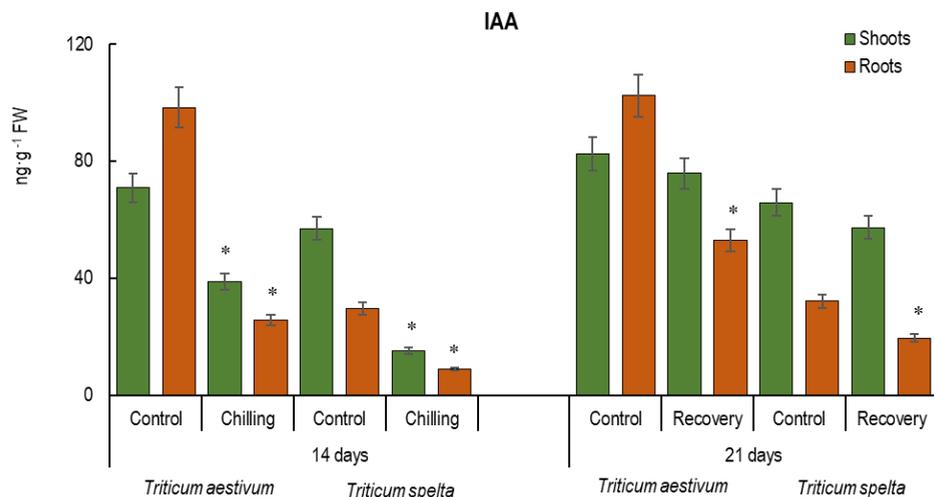
1.2 times lower compared to wheat. By the 21st day of growth, the hormone content exhibited an increase, maintaining a similar quantitative distribution between the shoots and roots as in the 14-day-old wheat and spelt C- plants (Fig. 5).



\* – significant difference at  $P \leq 0.05$  vs. control; data are the mean  $\pm$  SE,  $n=9$

Fig. 4. Accumulation and distribution of endogenous gibberellins GA<sub>3</sub> and GA<sub>4</sub> in *Triticum aestivum* L. cv. 'Podolyanka' and *Triticum spelta* L. cv. 'Frankenkorn' plants under chilling (+4°C, 2 h) and during recovery period (ng g<sup>-1</sup> FW)

Upon short-term exposure to chilling, the IAA level decreased by 1.8 and 3.8 times in the shoots and roots of wheat, respectively, and by 1.9 and 3.3 times in spelt. On the 21st day of recovery, IAA levels increased by 3.8 and 2.2 times in spelt shoots and roots, respectively, and by 2.0 and 2.1 times in wheat. During the recovery period, the endogenous IAA content in the shoots of both wheat and spelt was approached control values, while in the roots it remained 1.9 and 1.6 times lower (Fig. 5).



\* – significant difference at  $P \leq 0.05$  vs. control; data are the mean  $\pm$  SE,  $n=9$

Fig. 5. Accumulation and distribution of endogenous indole-3-acetic acid in *Triticum aestivum* L. cv. ‘Podolyanka’ and *Triticum spelta* L. cv. ‘Frankenkorn’ plants under chilling ( $+4^{\circ}\text{C}$ , 2 h) and during recovery period ( $\text{ng g}^{-1}$  FW)

The total IAA content in 14-day-old ‘Podolyanka’ wheat plants experienced a 62% decrease after cold stress, amounting  $64.4 \pm 3.2 \text{ ng g}^{-1}$  FW. By the 21st day recovery, the IAA levels increased by 99.7%, and reaching  $128.6 \pm 6.4 \text{ ng g}^{-1}$  FW, which was 30.4% lower, then in control non-stressed plants. Similarly, in 14-day-old spelt plants cold stress resulted in a 72.2% reduction in the total IAA content, measuring  $24.0 \pm 1.2 \text{ ng g}^{-1}$  FW. Recovery brought a remarkable 220% increase in hormone content, totaling  $76.8 \pm 3.8 \text{ ng g}^{-1}$  FW, albeit remaining 26.6% less than the IAA content in 21-day-old control unstressed plants. Throughout all experimental variations, ‘Podolyanka’ wheat plants consistently demonstrated higher IAA content.

## DISCUSSION

The combination of winter warming and late spring frosts significantly diminishes the growth and survival of wheat seedlings, causing inhibition of photosynthetic activity, reduced ear count, and grain loss. Early spring frost exposure, however, fosters better survival rates, elevated photosynthetic activity, and increased resistance to subsequent frosts, thus limiting yield loss (Li *et al.*,

2014). The transition from grain-reserve nutrition to external nutrient assimilation through the root system marks a crucial juncture in wheat ontogenesis, particularly evident at the three-leaf stage. One detrimental outcome of global climate change is the rising temperature during of autumn, often accompanied by brief nocturnal frosts. This phenomenon negatively impacts agricultural crops growth and productivity, resulting in significant yield losses. Low-temperature stress induces plant damage, evident at biochemical and ultrastructural levels (Babenko et al., 2019; Ritonga, Chen, 2020). Winter cereals exhibit high cold tolerance and typically necessitate prolonged vernalization to for spring reproduction (Deng et al., 2015). However, sudden chilling during early seedling development poses significant risk, leading to membranes integrity loss, ion leakage, photosynthesis and respiration inhibition, reduced enzymatic activity and carbohydrate metabolism, impaired water and nutrients absorption, assimilate transport disruption, and oxidative stress (Hassan et al., 2021). While the optimum temperature range for growth falls between 16-22° C, germination and tillering can occur at temperature below 5° C (White et al., 1990). Nevertheless, even short-term exposure to low temperatures in unhardened seedlings can be deleterious, resulting in death or hindering subsequent plant development (Kolupaev et al., 2019). The previous work on *Triticum aestivum* L. cv. Yatran 60, a short-stemmed, moderately intensive cultivar resistant to lodging, heat and drought, analyzed the effect of short-term chilling on endogenous phytohormone content and distribution. Results indicated active accumulation of free ABA and IAA in the roots of 7-day-old seedlings, following short-term chilling (+2°C, 2 h) (Kosakivska et al., 2014). Similarly, the impact of short-term chilling (+2°C, 2 h) on hormonal homeostasis was investigated in plants with different ecological strategies, such as *Festuca pratensis*, *Rumex patientia* × *R. tianshanicus*, *Brassica campestris*, and *Amaranthus caudatus* (Kosakivska et al., 2012; 2013). Subsequent studies confirm that two-hour chilling period serves as a stressor for plants. Upon short-term exposure to chilling (+4°C), linear parameters of the shoots and roots in 14-day-old winter wheat 'Podolyanka' and spelt wheat 'Frankenkorn' plants remained unchanged, whereas biomass was affected. Spelt plants exhibited more pronounced changes, with root DW decreasing under chilling and subsequently increasing after recovery on the 21st day. Conversely, 21-day-old wheat plants experienced significant decreases in FW and DW, implying an extended impact of chilling on growth parameters (Fig. 1). A similar reduction in root length, biomass, branching, and surface area was reported in various crop plants by Hussain et al. (2018). Maize plants displayed slower growth and alterations in primary root morphology under low temperature stress (Hussain et al., 2020), with diminished root biomass accumulation (Frey et al., 2020), and reduced branching angles between primary and lateral roots (Nagel et al., 2009). Cold-sensitive rice genotypes exhibited lower DW, shorter and finer root hairs, negatively impacting root area (Rativa et al., 2020). Wu et al. (2023) demonstrated that cold priming (10°C/6°C, 1 day) enhanced cold resistance of wheat seedlings, with the most robust resistant

phenotype observed nine days after priming. Stress memory persisted for 6-12 days in 3-4 leaves following recovery, gradually waning over time.

Changes in growth dynamics paralleled alterations in the balance of endogenous phytohormones. Extensive physiological and genetic studies have illuminated the diverse roles of different hormone classes, revealing intricate signaling pathway crosstalk and interaction (Vanstraelen, Benková, 2012). Among these hormones, abscisic acid serves as principal regulator of plant resistance to abiotic stresses, orchestrating an array on responses related to adaptation and adjustment. (Sreenivasulu *et al.*, 2012). A pivotal role for ABA in inducing cold tolerance has emerged. Guo *et al.* (2023) highlighted that barley plants exhibited disrupted chloroplast ultrastructure, altered starch and sucrose metabolism, reduced antioxidant enzymes activity, and changes in the hormonal regulatory network upon ABA deficiency during early stages (3-leaf phase) exposure to low temperature (0°C, 24 hours). In cold-resistant barley varieties, exposure of chilling (+5°C, one day) significantly increased endogenous ABA content (Ahres *et al.*, 2022).

In response to chilling, the endogenous ABA content exhibited a significant increase in 14-day-old wheat plants, whereas the hormone accumulation in spelt plants was comparatively pronounced. ABA dominated in the shoots of both studied species (Fig. 1). The intensive accumulation of endogenous ABA in the shoots of winter and spring wheat under the influence of low (+4°C) temperature was previously reported by Kosova *et al.* (2012). This study highlighted that the response of winter wheat was more rapid and prominent than that of spring wheat. Alongside the rise in ABA levels, the content of the protective protein dehydrin WCS120 also increased. However, with prolonged exposure to stress (3-7 days), the ABA concentration diminished.

By the 21st day after recovery, the hormone content in both shoots and roots of both wheat and spelt had decreased. Yet, it surpassed the levels observed in control plants, implying a lasting impact of cold priming on ABA accumulation (Fig. 2). Throughout the experimental observations, the endogenous ABA content was consistently twice as high in wheat plants compared to spelt plants, irrespective of the control or experimental conditions. This trend remained consistent even though both genotypes exhibit winter hardiness and frost resistance.

As a crucial stress-related signaling compound, salicylic acid exerts direct or indirect influence on diverse plant growth and development processes. SA plays an important role in the induction of cold tolerance in wheat by regulating ROS formation of (Wang *et al.*, 2018). In ABA-deficient mutant barley, chilling resulted in reduced SA content, albeit still higher than the wild type (*cv Steptoe*) (Guo *et al.*, 2023). The endogenous SA content in winter wheat leaves increased significantly after extended cold exposure (+4°C, 3 days) (Kosova *et al.*, 2012). However, in seedlings of both tolerant and sensitive barley varieties (*Hordeum vulgare* L), the endogenous SA content decreased following prolonged cold stress (+5/7°C, 3 days) (Mutlu *et al.*, 2015). Exogenous SA application was found to alleviate the

adverse effects of chilling (4°C) by triggering the antioxidant system and elevating proline levels in winter wheat plants (Ignatenko et al., 2019). In 14-day-old wheat and spelt plants, the endogenous SA content exhibited an increase approximately 17-19% in response to short-term cold stress. Notably, constitutive and stress-induced SA levels in wheat were consistently higher compared to spelt. The most prominent shifts due to chilling were observed in the roots of the plants (Fig. 3). During the recovery period after cold stress, the SA content in 21-day-old wheat plants returned to levels comparable to control non-stressed plants. Conversely, no notable changes in hormone content were observed in spelt, however, this indicator significantly surpassed that of the control non-stressed plants (Fig. 3).

Gibberellins play a pivotal role in orchestrating plant responses to signals from other phytohormones activated by abiotic stressors (Achard et al., 2006). Notably, the expression of genes encoding enzymes catalyzing main stages of GAs synthesis, such as soluble GA 20-oxidases, typically hinges on environmental cues. Consequently, the endogenous GA content becomes exquisitely responsive to alteration in the external milieu (Colebrook et al., 2014). Our study discerned that chilling engendered a reduction endogenous GA<sub>3</sub> and GA<sub>4</sub> levels in 14-day-old plants of both studied species, with more pronounced changes emerging in the roots. Remarkably, spelt plants exhibited elevated levels of GA<sub>3</sub> and GA<sub>4</sub> comparison to wheat plants. During the recovery period on the 21st day, the hormone content displayed a more distinct elevation in spelt plants, eventually reaching levels akin to unstressed control plants. GA<sub>3</sub> remained dominant across all experimental variations (Fig. 4). Kosova et al. (2012) unveiled that low temperature caused a decline in biologically active GAs in the shoots and roots of winter and spring wheat, with a concomitant rise in inactive hydroxylated forms. Cold stress also triggered a reduction in endogenous GA<sub>4</sub> and GA<sub>7</sub> levels in rice anthers, while their precursor GA<sub>12</sub> remained unaffected (Sakata et al., 2014). When exposed to chilling, wheat leaf growth was impeded, concurrently prompting the accumulation of active GAs, which in turn stimulated cell elongation. This circumstance corresponded to heightened sensitivity thresholds to GA action (Tonkinson *et al.*, 1997). In the quest to bolster stress resilience in agricultural crops, regulation of endogenous GA levels and the employment of synthesis inhibitors stand as pivotal strategies.

Short-term cold stress induced a decrease in IAA content of 14-day-old wheat and spelt plants. The decrease in IAA level was more pronounced in wheat roots (by 73.9%), while in spelt, the converse, was observed in shoots (by 73.5%). The hormone's level in wheat organs was almost threefold higher than that in spelt (Fig. 5). A parallel pattern of changes was noted in leaves and roots of both spring and winter wheat (*Triticum monococcum*) during cold stress (Vanková et al., 2014). A similar decrement in auxin content in the leaves of winter and spring wheat upon initial exposure to chilling (+4°C) was reported by Kosová et al. (2012). In barley mutants deficient in ABA, IAA content increased under chilling, whereas in the wild type (cv Steptoe), it underwent a notable decrease (Guo et al., 2023). After recovery, endogenous IAA accumulated in the shoots and roots of 21-

day-old across both species under study, with spelt plants revealing more pronounced variations (Fig. 5). During the adaptation phase (21 days) of single-grain wheat Vanková *et al.* (2014), documented the accrual of endogenous auxins alongside an elevation in phenolic compounds, integral for stabilizing hormone levels. Garbero *et al.* (2012) demonstrated that short-term cold stress (5°C) led to growth retardation and reduced IAA content in the sensitive *Digitaria eriantha* variety during the recovery period, whereas the resistant variety exhibited heightened hormone levels.

### CONCLUSION

Our research has contributed to comprehending the hormonal systems response in winter wheat and spelt wheat during the initial stage of vegetative growth under chilling stress. These species were chosen due to their genetic relationship, with spelt considered a possible predecessor of wheat. The selected genotypes did not significantly differ in cold resistance. Short-term chilling didn't impact the linear indicators of wheat and spelt shoots and roots but stimulated shoot and root FW and DW growth, while reducing root FW. Spelt plants exhibited better post-stress recovery. Differences manifested in constitutional hormones content and stress-induced changes. Spelt wheat demonstrated higher constitutional gibberellin content, while winter wheat exhibited elevated SA and IAA content. In terms of stress-induced changes, wheat showed more pronounced alterations in ABA and IAA content. Our study revealed that following recovery, the hormonal balance in shoots and roots of stressed plants differed from that in control non-stressed plants, suggesting long-term effects of chilling.

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