

ORIGINAL ARTICLE

## Effect of Temperature on *Triticum aestivum* L. Seedlings Growth and Phytohormone Balance

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The study aimed to determine the effect of short-term heat and cold stresses on growth, accumulation and distribution of abscisic acid (ABA) and indole-3-acetic acid (IAA) in 7 and 14-day-old *Triticum aestivum* L. seedlings of the frost resistant cultivar Volodarka. A high performance liquid chromatography method has been used to provide a qualitative and quantitative analysis of hormones in leaves and roots. Biometric analysis showed that at the early stage of vegetation (7 days) seedlings were more resistant to temperature stresses, especially to cold, than at the later ones (14 and 21 days), that correlates with the frost resistance of cultivar. The results indicated that at the early stages of growth, after a short-term cold stress (2 h., +2<sup>0</sup>C), the amount of free ABA substantially increased in roots, while at the later ones, it occurred after a short-term heat stress (2 h., +40<sup>0</sup>C) in leaves. After a heat stress the leaves of 7-day-old seedlings accumulated a conjugated form of IAA. Heat stress caused the accumulation of free IAA in roots of 14-day-old seedlings. Cross stress (cold+heat) caused some increase in the pool of endogenous ABA both in roots and leaves while the amount of free IAA increased only in leaves. Changes in the accumulation of the free and conjugated forms of ABA and IAA depended both on the organs and the age of seedlings, and the type of stress and correlated with frost resistance of the cultivar Volodarka.

*Key words:* abscisic acid, indole-3-acetic acid, temperature stress, *Triticum aestivum* L.

Phytohormones play a key role in the regulation of growth, development and resistance of plants. Abscisic acid is one of the major plant hormones involved in plant adaptation (Wilkinson, Davies, 2002). In plant tissues it is present in free and conjugated forms (Zheng-Yi *et al.*, 2014). In higher plants the ABA active cis-isomer is dominant while the ABA trans-isomer presents an inactive form of the hormone (Piotrowska, Bajguz, 2011). It was revealed that under drought conditions the accumulation of active ABA provoked stomata closure with a subsequent decrease in transpiration, preservation of the water status that reduces a drought action (Nejad, Meeteren, 2007). ABA is involved in the formation of responses as a trigger which activated signal cascades (Osakabe *et al.*, 2014). Changes in endogenous ABA concentrations are a signal for gene expression and biosynthesis of proteins sensitive to a cold stress (Beck *et al.*, 2007; Shinozaki, Yamaguchi-Shinozaki, 2007; Shakirova *et al.*, 2009). ABA activates COR-genes and genes of the LEA family, whose products are directly involved in the formation of plant resistance to low temperature (Gusta *et al.*, 2005).

Indole-3-acetic acid – a natural auxin, the major function of which is the regulation of growth processes. In a free form IAA directly affects the mitotic cycle, produces a positive effect on biosynthetic processes (Del Pozo *et al.*, 2005). In a bound state IAA loses its activity. A conjugated form of IAA could be a depot of phytohormone, and may also be used as a transport form (Veselov *et al.*, 2007;

Bejguz, Piotrowska, 2009). Our previous studies demonstrated a considerable increase of the amount of conjugated IAA in leaves of heat resistant winter wheat cultivar Yatran 60 after a cold stress (Kosakivska *et al.*, 2014).

The aim of our study was to investigate the influence of temperature stresses on the growth process, accumulation and distribution of free and conjugated forms of ABA and IAA between the roots and leaves of frost resistant 7 and 14-day-old winter wheat seedlings to elucidate the role of these hormones in the regulation of plant resistance. We supposed that the specific changes in content of free and conjugated forms of ABA and IAA in leaves and roots, which took place after temperature stresses, correlated with frost resistance and could be used as biomarkers in screening and selection of new cultivars.

## MATERIALS AND METHODS

*Triticum aestivum* L. cv. Volodarka belongs to the short stem, high-intensive cultivars. It is resistant to lodging, characterized by frost and drought resistance, has a high level of ecological plasticity. This is a new selection product obtained using chromosome engineering techniques. Its genome includes rye translocations. Crop capacity of this cultivar reaches 100 centner per hectare (genetic potential is up to 115 – 124 centner per hectare) under optimal climatic conditions and intensive technologies of cultivation (Morgun *et al.*, 2008).

The sterilized calibrated seeds were placed in Petri dishes on moist filter paper (20 seeds per one

dish) and left there at temperature +24°C, illuminance 2500 lux, photoperiod of 16: 8. Distilled water was added into the dishes daily. 7-day-old and 14-day-old seedlings were subjected to short-term (within 2 hours) heat (+40°C) and cold (+2°C) temperature stresses. To produce a cross stress, the 14-day-old seedlings, which had been subjected to a short-term heat stress at the 7-day-old stage, were exposed to a short-term cold stress and vice versa. To characterize seedlings biometrically, the length and weight of their leaves and roots were measured on 7, 14 and 21 days in controls and after temperature stresses. Extraction of hormones and determination of free and conjugated forms of ABA and IAA were performed according to the method (Kosakivska et al., 2014). Qualitative and quantitative analyses of ABA and IAA were performed using high performance liquid chromatography (HPLC) on a liquid chromatograph Agilent 1200 LC system with diode array detector G 1315 B (USA), column Eclipse XDB-C 18, with the parameters 4.6 x 150 mm, size of particles - 5 microns. IAA and ABA were determined at 280 and 254 nm, respectively. Elution of hormones was performed at a rate 0,5 ml/min in the solvent system methanol: water: acetic acid (59: 40: 1) in online mode. We used unlabeled IAA and ( $\pm$ ) cis-, trans-ABA (Sigma, USA) and the standard addition method of quantification. Chromatograms were calculated using the software Chem Station (version 3.1 V.) in offline mode. Experiments were carried out in three biological and five analytical replicates. Digital materials were processed statistically using the programs MS Excel 2002 and Origin 6.0. Significant differences were

assessed by Student's criterion, using a 5% level of significance ( $P \leq 0,05$ ).

## RESULTS AND DISCUSSION

It was shown that after short-term temperature stresses applied to 7-day-old seedlings the growth of 14-th and 21-day-old seedlings was inhibited. Response to the heat stress was more evident. Nevertheless, on the 21-st day after a cold stress seedling leaves length was practically similar to that of control (Fig. 1). When 14-day-old seedlings were exposed to temperature stresses we also observed inhibition on the 21-st day after the stresses, however, no substantial differences between responses to cold and heat stresses were revealed. Cross stresses were followed by some decrease in seedling leaves length, although responses to both stresses were practically the same. In general, based on the biometric studies results it may be concluded that the acclimatization of seedlings exposed to temperature stresses at the age of 7 days was more successful than under stresses at 14-day age and under cross stresses. At the early vegetation stages the Volodarka cultivar seedlings were more resistant to temperature stresses, especially to cold, than at the later ones and that correlates with the cultivar frost resistance.

We have shown that after a short-term cold stress the amount of free ABA increased in roots of 7-day-old seedlings, probably partly thanks to hydrolysis of a conjugated form, the content of which decreased. Totally, the endogenous ABA pool in roots increased six times. However, after a short-term heat stress the pool of endogenous ABA in roots decreased, while in leaves it increased, but was lower than after a cold

stress. (Fig. 2). Similar results were obtained after a salt stress in roots of bean seedlings (Shevyakova *et al.*, 2013). At the same time the content of the conjugated form of ABA in the roots and leaves after a heat stress was revealed to reduce, whereas in leaves the amount of conjugated ABA following a cold stress sharply enhanced (Fig. 2). Thus, the short-term cold stress caused the mobilization of free (active) ABA both in roots and leaves of 7-day-old winter wheat seedlings, but in roots the process of accumulation was more active. Changes in the amount of free ABA could be a result of stress-induced release from a conjugated form and transit of free ABA from the leaves to roots after a cold stress. Other authors showed that conjugated form of ABA is splitted in the leaf apoplast that involves production of a free form transferred to the mesophyll (Osakabe *et al.*, 2014).

Like in our previous study using seedlings of the heat resistant winter wheat cultivar Yatran 60 (Kosakivska *et al.*, 2014), we observed the redistribution of the free ABA accumulation from roots to leaves in 14-day-old seedlings under control conditions. Pool of endogenous ABA in roots and leaves of 14-day-old seedlings decreased. It was more pronounced in roots (Fig. 2). Similar results, which showed that decrease in the amount of ABA occurred more actively in the wheat roots, were obtained by (Egorshina *et al.*, 2012). The level of free ABA in roots of 14-day-old seedlings was shown to decrease one and half time after a heat stress, whereas in leaves it increased two times. The low temperature vice versa had no effect on the amount of free ABA in leaves, while in roots there was observed

some increase. As compared to the control after a cold stress the level of conjugated ABA in roots increased, and in leaves it decreased after both stresses. Cross stress (cold+heat) caused some increase in the pool of endogenous ABA both in seedling leaves and roots. With an opposite stress combination (heat+cold) there occurred a decrease in the pool of endogenous ABA, however, roots displayed dominance of a free form of the phytohormone while in leaves there dominated a conjugated one (Fig. 2).

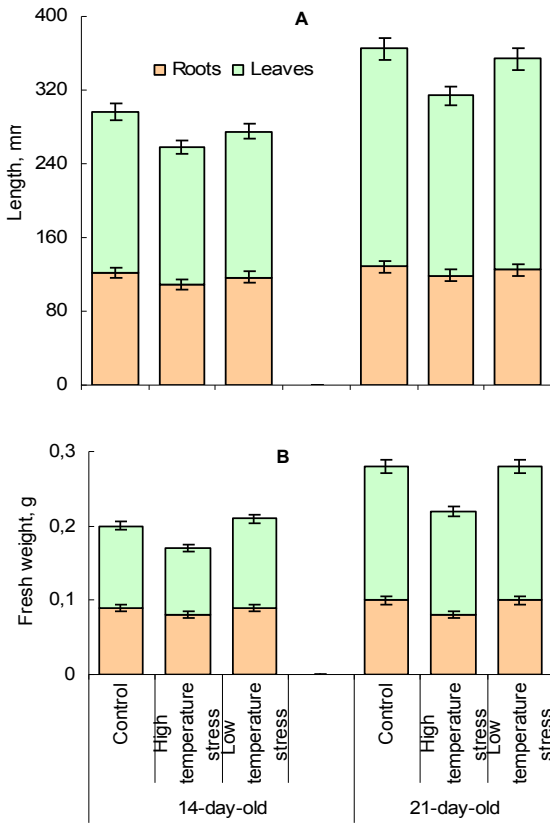
The other authors' reports showed that cold resistance of wheat seedlings at the early stages of the hardening is associated with gene expression of stress proteins and transcription factor which occurs with the participation of ABA (Talanova *et al.*, 2011). In addition, study of 4-day-old wheat seedlings indicated that endogenous ABA controlled gene expression of dehydrins during hypothermia (Shakirova *et al.*, 2009). The cold stress response of two wheat cultivars, differing in cold tolerance, is characterized by a rapid elevation of ABA content and an increase of dehydrin synthesis (Kosová *et al.*, 2012). Changes found in the free ABA level in roots of 7-day-old seedlings after a short-term cold stress and in leaves of 14-day-old seedlings after a short-term heat stress may indicate that defense reactions, aimed in particular at the synthesis of stress proteins and the maintaining of homeostasis, are activated. As is known, closing of stomata - one of the fastest reactions to abiotic stresses, is directly related with ABA, which is involved in interorganic signal transduction (Agarwal, Jha, 2010). Our results

indicated that roots of the frost resistant cultivar Volodarka accumulated a great amount of free ABA after a short-term cold stress at the early stages of growth (7 days). An active accumulation of free ABA in roots under low temperature impact enables winter wheat seedlings to adapt to cold conditions after germination and perhaps this is one of the factors that provide frost resistance of Volodarka cultivar.

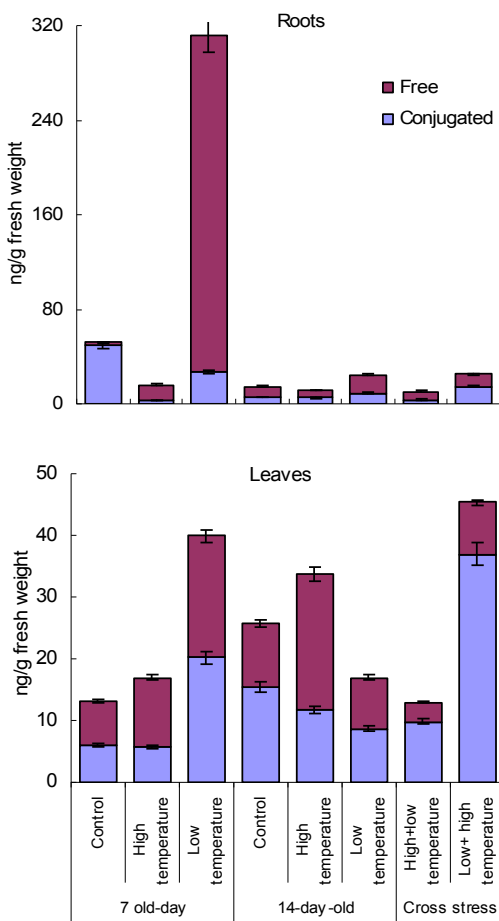
IAA responses are regulated at the three interdependent levels: homeostasis, directional transport, and signaling. The general term homeostasis includes biosynthesis, oxidative degradation, and IAA modifications. Combined with transport, these factors maintain the precise levels of IAA required for a cell depending on the cell type, developmental stage, and environmental conditions (Korasick *et al.*, 2013). We have shown that in roots of 7-day-old seedlings under control conditions there was present mostly free form of IAA, whereas in leaves the amounts of free and conjugated forms of the hormone were practically the same. During the growth of seedlings the pool of endogenous IAA in roots increased due to the accumulation of the conjugated forms. After a heat stress in leaves of 7-day-old seedlings the conjugated form dominated, while in the 14-day-old ones a high temperature led to a sharp increase in the amount of free IAA. After a cold stress leaves and roots of 14-day-old seedlings displayed an increase in the level of free IAA (Fig.3) that correlates with the biometric study data demonstrating resistance of growth processes to low temperature effects. Cross stress (cold+heat) caused

an increase in the endogenous IAA pool in seedling leaves. With an opposite cross stress (heat+cold) the endogenous IAA pool reduced and there appeared a conjugated form of hormone (Fig.3). After a heat stress the IAA accumulation, namely a conjugated form, was transferred to the leaves of 7-day-old seedlings. A heat stress led to the transfer of the IAA accumulation, inversely, to roots of 14-day-old seedlings. The pattern of IAA conjugated form accumulation after temperature stresses suggested that to maintain the auxin homeostasis under conditions of hypothermia, leaves of 7-day-old seedlings of the Volodarka winter wheat cultivar activated conjugation reaction. IAA conjugation is a widely used mechanism of regulating free IAA levels in plants. IAA conjugates can function as a form of free IAA storage. Generally, IAA conjugates are biologically active in an indirect manner since free IAA is released from IAA conjugates by hydrolysis (Bajguz, Piotrowska 2009; Ludwig-Muller, 2011).

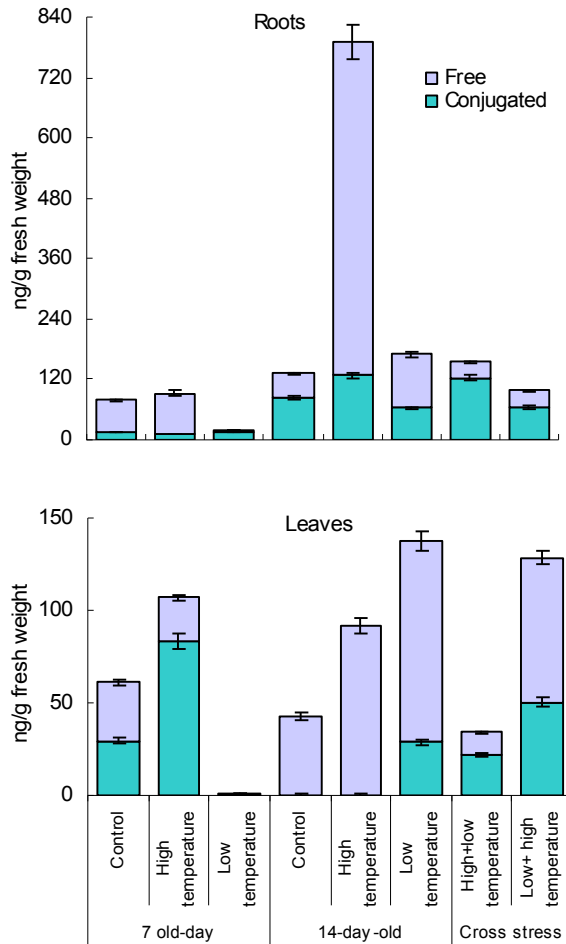
Changes in the phytohormone balance in 7-day-old seedling roots after a cold stress displayed in a sharp increase of free ABA amount against the decrease of free IAA, whereas in seedling leaves a heat stress caused an increase in conjugated IAA against the decrease of endogenous ABA that occurred mostly at the expense of conjugated form of hormone. At the later growth (14-day-old) stages after a heat stress substantial changes in the phytohormone balance were observed in roots where an increase of free IAA amount occurred.



**Figure 1.** Biometric characteristic of 14 and 21-day-old seedlings of *Triticum aestivum* L. cv. Vololodrka subjected at 7-day-old stage to high (+40°C, 2h) and low (+4°C, 2h) temperature stress (a - length, mm, b - fresh weight, g )



**Figure 2.** ABA content in roots and leaves of 7 and 14-day-old seedlings of *Triticum aestivum* L. cv. Volodarka subjected to low and high temperature stress



**Figure 3.** IAA content in roots and leaves of 7 and 14-day-old seedlings of *Triticum aestivum* L. cv. Volodarka subjected to low and high temperature stress

## CONCLUSION

Thus, short-term temperature stresses induced specific changes in the accumulation of the free and conjugated forms of ABA in the Volodarka frost-resistant winter wheat cultivar, which depended both on the organs and the age of seedlings, and the type of stress. Roots of 7-day-old seedlings actively accumulated free ABA after a short-term cold stress. Free ABA accumulation in roots after low temperature effects at the early development stages enables winter wheat seedlings to adapt to cold conditions following germination and this is one of the factors that provide frost resistance of the Volodarka cultivar. After a heat stress 14-day-old seedlings accumulated substantial

amounts of free IAA in roots that caused activation of growth processes. A cross stress (cold+heat) led to an increase of the endogenous ABA pool both in seedling roots and leaves while the amount of free IAA increased only in leaves.

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