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# EFFECT OF TEMPERATURE ON ACCUMULATION OF ABSCISIC ACID AND INDOLE-3-ACETIC ACID IN *TRITICUM AESTIVUM* L. SEEDLINGS

***Kosakivska I. V., L. V. Voytenko, R. V. Likhnyovskiy, A. Y. Ustinova***

*M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine, 2 Tereschenkivska str., 01601, Kyiv-1, Ukraine*

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**Summary:** A high performance liquid chromatography method was used to perform qualitative and quantitative analyses of abscisic acid (ABA) and indole-3-acetec acid (IAA) in leaves and roots of winter wheat under temperature stress. The study aimed to determine the effect of short-term heat and cold stresses on accumulation and distribution of these phytohormones in 7-day-old and 14-day-old seedlings of the heat resistant cultivar Yatran 60. The results indicated that roots accumulated free ABA after short-term cold stress (2 h, +2$^{o}$C) at the early stages of growth (7 days), while at a later stage (14 days), this occurred after short-term heat stress (2 h, +40$^{o}$C). Changes in the amount of free ABA could be a result of stress-induced release from the conjugated form and transition of free ABA from leaves to roots after cold stress and from roots to leaves after heat stress. After heat stress the leaves of 7-day-old seedlings accumulated the conjugated form of IAA, whereas at a later stage – the free active form of the phytohormone. The accumulation pattern of the IAA conjugated form after temperature stresses suggested that reactions of conjugation were involved in maintaining auxin homeostasis in the leaves of 7-day-old seedlings after heat stress and in the roots of 14-day-old seedlings after cold stress. The specific dynamics of changes in ABA and IAA free and conjugated forms at the alarm phase of the reaction indicated that these phytohormones were involved in responses to temperature stress. Both heat and cold stresses induced specific changes in the accumulation of the free and conjugated forms of ABA and IAA, which depended on organs, seedling age and type of stress and correlated with the heat resistance of cultivar Yatran 60.

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**Keywords:** *Triticum aestivum* L.; abscisic acid; indole-3-acetic acid; temperature stress.

**Abbreviations:** ABA – abscisic acid; IAA – indole-3-acetic acid.

*Corresponding author: irynakosakivska@gmail.com

## INTRODUCTION

Phytohormones play a key role in the regulation of growth, development and resistance of plants. Modern molecular physiology emphasizes that the decisive factors in determining the nature of their actions, are the ratio between the individual classes of plant hormones, their concentration and localization in tissues and organs of plants (Davies, 2004; Blum et al., 2012). ABA is one of the major plant hormones involved in plant adaptation (Wilkinson and Davies, 2002). In plant tissues it is present in free and conjugated forms (Zheng-Yi et al., 2014). In response to stress the amount of free (active) ABA increases advantageously thanks to hydrolysis of the conjugated form (Hansen and Dorffling, 1999). The increased level of ABA in tissues triggers the process of stomata closing, which is followed by a decrease in transpiration and an increase in seedling water content (Veselov et al., 2007). ABA activates COR-genes (cold regulated), which include the RAB (ABA-responsive) and DHN (dehydrins), and the LEA (late embryogenesis abundant) family genes products of their activity which are directly involved in the formation of plant resistance to low temperatures (Gusta et al., 2005). IAA is a natural auxin, the major function of which is the regulation of growth processes. In a free form IAA directly affects the mitotic cycle and has a positive effect on the biosynthetic processes (Del Pozo et al., 2005). In a bound state IAA loses its activity. The conjugated form of IAA could be a depot of the phytohormone, and may also be used as a transport form (Bejguz and Piotrowska, 2009).

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

## MATERIALS AND METHODS

*Triticum aestivum* L. cv. Yatran 60 belongs to the short stem, middle-intensive cultivars. It is resistant to lodging and is characterized by high heat and drought resistance. The sterilized calibrated seeds were placed in Petri dishes on moist filter paper and left there for one day at a temperature of +24°C, illuminance 2500 lux, and 16/8 h (day/night) photoperiod. In the absence of fungi infection after 24 h seedlings were transplanted into pots on a mineral substrate, temperature and light conditions were not changed. Every day 100 ml of distilled water were added to the mineral substrate. 7-day-old and 14-day-old seedlings were subjected to short-term (within 2 h) heat (+40°C) and cold (+2°C) temperature stresses. Extraction and determination of free and conjugated forms of ABA and IAA were performed according to the method of Musatenko et al. (2003). Plant material (15 g) was fixed in liquid nitrogen and homogenized in 80% ethanol at 4°C. The homogenate was filtered, evaporated until water residue, frozen, thawed and centrifuged for 20 min at 10000 x *g*. IAA and ABA were extracted from the aliquots of the supernatant with diethyl ether at pH 2.5. To determine conjugated IAA and ABA, after ether extraction the aqueous phase was hydrolyzed with 1N NaOH in 30% ethanol. Hormones were purified by repeated extractions in acid and alkali and by TLC on Silufol UV-254 plates (Kavalier, Czech Republic) in the mixture of chloroform:ethyl acetate:acetic acid (70:30:5). 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 parameters 4.6 x 150 mm and size of particles 5 µm. IAA and ABA were determined at 280 and 254 nm, respectively. Elution of hormones was performed at a rate of 0.5 ml/min in the solvent system methanol:water:acetic acid (59:40:1) in online regime. We used un-labeled IAA and (±) cis-, trans-ABA (Sigma, USA) as internal standards and the standard addition method of quantification. Calculations were made 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

After short-term cold stress the amount of free ABA in roots of 7-day-old winter wheat seedlings increased twice, probably thanks to hydrolysis of the conjugated forms, whose content decreased (Fig. 1). Similar results were obtained after salt stress in roots of bean seedlings (Shevyakova et al., 2013). However, after short-term heat stress the level of free ABA decreased in roots and increased in leaves. At the same time the content of the conjugated forms of ABA in the roots and leaves became lower (Fig. 1). Thus, short-term cold stress caused mobilization of free (active) ABA in roots, whereas heat stress - in leaves of 7-day-

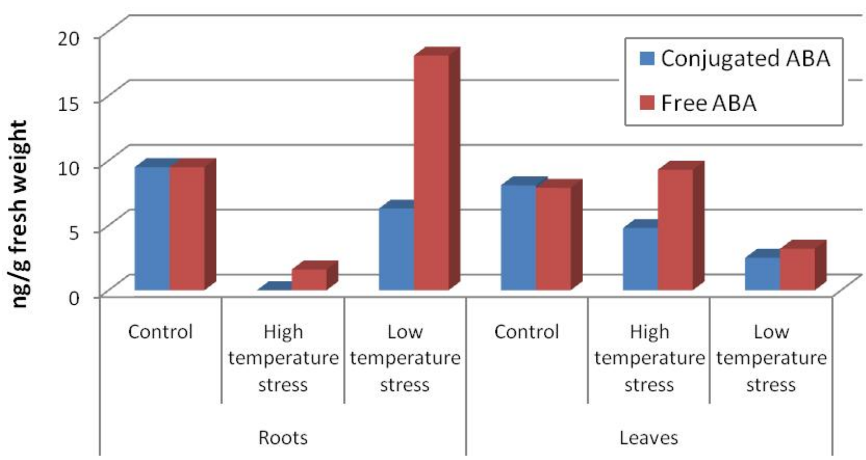


**Figure 1.** ABA content in roots and leaves of 7-day-old winter wheat seedlings subjected to low and high temperature stress.

old winter wheat seedlings. The changes in the amount of free ABA could be a result of stress-induced release from the conjugated form and transit of free ABA from the leaves to roots after cold stress and from the roots to leaves after heat stress.

We observed redistribution of free ABA content from roots to leaves in 14-day-old seedlings under control conditions. The endogenous ABA pool in the roots and leaves of 14-day-old seedlings decreased, being more pronounced in the roots (Fig. 2). Similar results showing that the amount of ABA decreased more actively in wheat roots were obtained by Egorshina et al. (2012). After heat stress the level of free ABA in the roots of 14-day-old seedlings increased fifteen-fold, whereas in the leaves it decreased four times (Fig. 2). The low temperature stress had no effect on the amount of free ABA in roots, while in leaves almost a three-fold decrease was observed. As compared to the control, the level of conjugated ABA in roots and leaves significantly increased after both cold and heat stress (Fig. 2).

It has been shown 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). Also, another study showed that endogenous ABA controlled gene expression of dehydrins during hypothermia of 4-day-old wheat seedlings (Shakirova et al., 2009). The response to cold stress of two wheat cultivars differing in cold tolerance was characterized by a rapid elevation of ABA content and an increased dehydrin synthesis (Kosová et al., 2012). Our study showed that the changes found in free ABA level in roots of 7-day-old seedlings after short-term cold stress and in roots of 14-day-old seedlings after short-term heat stress may

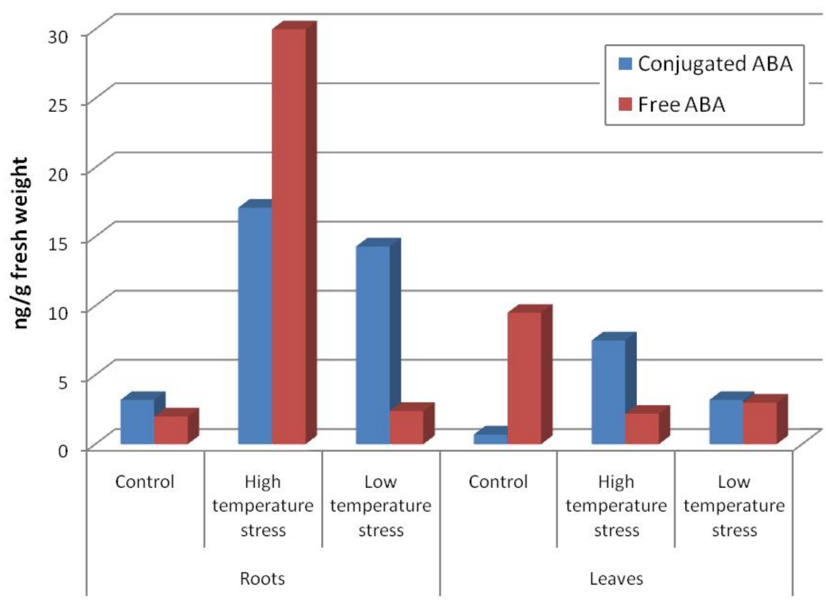


**Figure 2.** ABA content in roots and leaves of 14-day-old winter wheat seedlings subjected to low and high temperature stress.

indicate activation of defense reactions, aiming in particular at the synthesis of stress proteins and thus, maintaining of homeostasis.

It is known that closing of stomata - one of the fastest reactions to abiotic stresses, is directly related with ABA, which is involved in interorganic signal transduction (Agarwal and Jha, 2010). The detected in our study increase of free ABA in leaves of 7-day-old wheat seedlings after heat stress indicates that the activation of protective processes, probably aiming at closing of stomata, occurs. Thus, the obtained data revealed specific features in free and conjugated ABA accumulation in roots and leaves of 7-day-old and 14-day-old seedlings of winter wheat. Our results indicated that roots of the heat resistant cultivar Yatran 60 accumulated free ABA after short-term cold stress at the early stages of growth (7 days), while at a later stage (14 days), this occurred after short-term heat stress.

IAA responses are regulated at 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 desirable in a cell based on cell type, developmental stage, and environmental conditions (Korasick et al., 2013). Our results showed that in leaves of 7-day-old wheat seedlings under control conditions mostly the free form of IAA was present, whereas the conjugated form was found in small quantities. During seedling growth the pool of endogenous IAA in leaves increased due to the accumulation of the conjugated forms. After heat stress the conjugated form dominated in the leaves of 7-day-old seedlings, while in 14-day-old seedlings the free form was predominant (Fig. 3, 4). After cold stress the leaves of 7-day-old seedlings accumulated a great amount

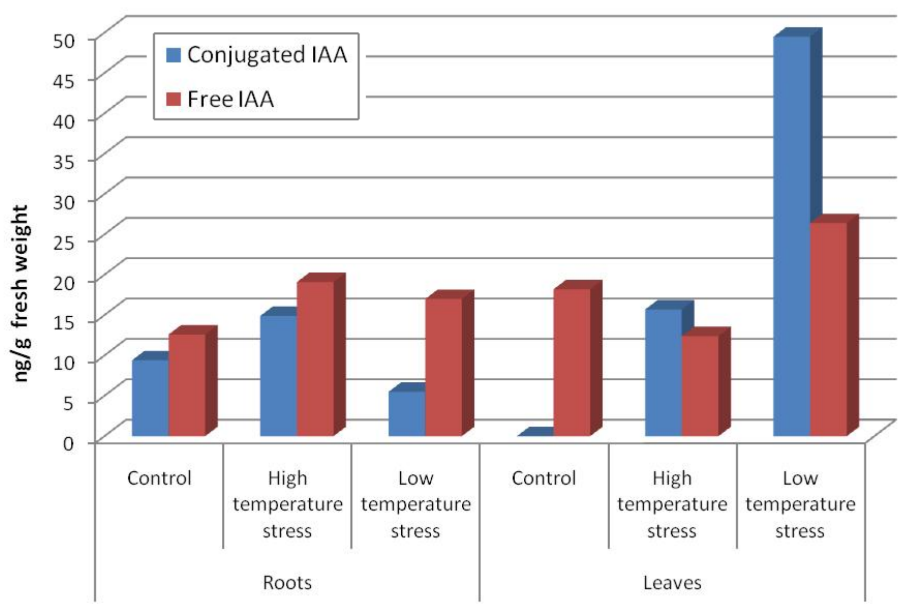


**Figure 3.** IAA content in roots and leaves of 7-day-old winter wheat seedlings subjected to low and high temperature stress.

of the conjugated form of IAA, while the level of free IAA increased 1.4-fold (Fig. 3). An increased level of conjugated IAA after salt stress has been shown by other authors in leaves of *Mesembryanthenum crystallinum* L. (Vedenicheva et al., 2010). In 14-day-old seedlings the pool of endogenous IAA increased as compared with the control, however it was smaller than that observed after heat stress (Fig. 4). Consequently, the changes in the accumulation of IAA in the leaves of 7-day-old seedlings after cold stress showed the same tendencies as during heat stress, but they were much more pronounced.

Our results showed that that during seedling growth the accumulation of the free form of IAA moved from the roots into the leaves (Fig. 3, 4). After heat stress the endogenous pool of IAA in roots increased significantly, especially in 14-day-old seedlings at the expense of the conjugated form (Fig. 4). Accumulation of free IAA was also observed (more than twice). It is known that the accumulation of IAA has a positive effect on the formation of roots in wheat seedlings (Vysotskaya et al., 2007). Taking this into account, it can be assumed that the accumulation of the active form of the phytohormone after heat stress in the roots of winter wheat seedlings initiated their growth, which resulted in the availability of water and nutrients and indirectly increased the resistance to hyperthermia. In the roots of 7-day-old seedlings after short-term cold stress predominance of the free form of IAA was observed. The level of IAA in the roots of 14-day-old seedlings decreased two times, mainly at the expense of the conjugated form. In general, hypothermia had a negative impact on the accumulation of IAA in the roots of 14-day-old winter wheat seedlings (Fig. 4). Thus, our research indicated that IAA was involved in the response to temperature stresses. After cold stress accumulation of IAA, mainly its conjugated form, moved to the

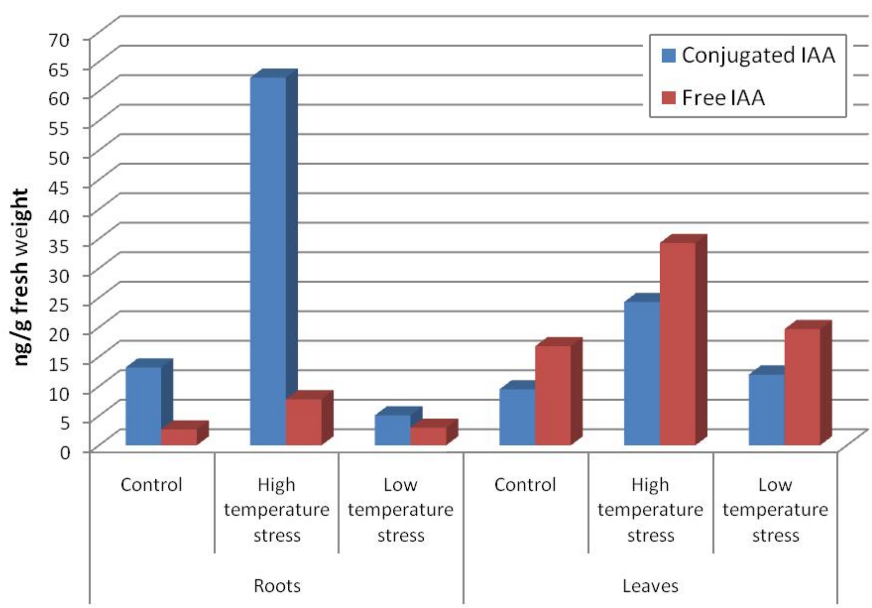


**Figure 4.** IAA content in roots and leaves of 14-day-old winter wheat seedlings subjected to low and high temperature stress.

leaves, and this process was more active at the early stages of development. In contrast, heat stress caused a displacement of IAA accumulation into the roots of the seedlings.

## CONCLUSION

In the present study, short-term temperature stress induced specific changes in the accumulation of free and conjugated forms of ABA and IAA in the heat-resistant winter wheat cultivar Yatran 60, which depended on organs, age of seedlings, and type of stress. The roots of 7-day-old seedlings actively accumulated free ABA and IAA after short-term cold stress, while 14-day-old ones accumulated free ABA and conjugated IAA after short-term heat stress. Accumulation of free IAA in the leaves of 14-day-old seedlings after heat stress correlated with heat resistance of cultivar Yatran 60 and its ability to grow rapidly in conditions of hyperthermia. The accumulation pattern of the IAA conjugated form after temperature stresses indicated that reactions of conjugation were involved in maintaining auxin homeostasis in the leaves of 7-day-old seedlings after heat stress and in the roots of 14-day-old seedlings after cold stress. The specific dynamics of changes in ABA and IAA free and conjugated forms at the alarm phase suggested that these phytohormones actively participated in the defense reactions at the early stages of winter wheat development.

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