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Expression of *IRT1* gene in barley seedlings under zinc deficiency at optimal and low temperatures

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Expression of *IRT1* gene in barley seedlings under zinc deficiency at optimal and low temperatures

Abstract: The deficiency or excess of zinc (Zn) cause negative effect on plant metabolism and development. Therefore, plants have established a tightly controlled system, including protein transporters to balance the uptake and utilization of metal ions. In this study, the relative expression of *HvIRT1* gene, encoding the transmembrane protein IRT1 was analyzed in shoots and roots of barley (*Hordeum vulgare* 'Nur') under zinc deficiency at optimal (22 °C) or low (4 °C) temperatures. The Zn deficiency (0 μmol) caused an increase in *HvIRT1* gene expression under both optimal temperature condition and cold. Although, the difference in mRNA content of *HvIRT1* gene in roots of barley under optimal and low temperature was not observe. However, the *HvIRT1* expression in leaves was higher at optimal temperature compare with cold condition. Moreover, long-term (7 days) of low temperature influence along with zinc deficiency leads to a significant decrease in the amount of *HvIRT1*transcripts in leaves, that corresponds to a decrease of photosynthesis rate and biomass accumulation. Overall, these findings suggest that *HvIRT1* gene play an important role in plant's response to zinc deficiency under optimal temperatures condition as well as at cold.

Key words: *IRT1*; *Hordeum vulgare*; zinc deficiency; low temperatures

Izražanje *IRT1* gena v sejankah ječmena ob pomanjkanju cinka pri optimalnih in nizkih temperaturah

Izvleček: Pomanjkanje ali prebitek cinka (Zn) povzročata negativne učinke na presnovo in razvoj rasttlin. Zaradi tega so rastline razvile dobro nadzorovan sistem, vključno s proteinskimi transporterji za uravnavanje privzema in porabe kovinskih ionov. V raziskavi je bilo analizirano izražanje *HvIRT1* gena, ki kodira transmembranski protein *IRT1* v poganjkih in koreninah ječmena (*Hordeum vulgare* 'Nur') ob pomankanju zinka pri optimalni (22 °C) in nizki (4 °C) temperaturi. Pomanjkanje cinka (0 μmol) je povzročilo povečano izražanje *HvIRT1* gena pri optimalni kot pri nizki temperaturi. Razlika v vsebnosti mRNK *HvIRT1* gena v koreninah ječmena v optimalnih razmerah in pri nizki temperaturi ni bila opažena, a kljub temu je bilo izražanje gena *HvIRT1* v listih večje pri optimalni temperaturi v primerjavi s hladnimi rastnimi razmerami. Daljša izpostavitev (7 dni) nizki temperature je ob pomanjkanju cinka povzročila značilno zmanšanje transkriptov *HvIRT1* v listih, kar ustreza upadu fotosinteze in akumulacije biomase. Ta odkritja nakazujejo, da igra *HvIRT1* gen pomembno vlogo pri odzivu rastlin na pomanjkanje cinka tako v optimalnih razmerah kot pri nizkih temperaturah.

Ključne besede: *IRT1*; *Hordeum vulgare*; pomanjkanje cinka; nizke temperature

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1 INTRODUCTION

Zn deficiency has been recognized as an important factor affecting crop production. Cell transmembrane proteins from ZIP family (*zinc-iron-regulated transporter*) play an important role in providing plants of the necessary amount of zinc (Pedas et al., 2008; Lee and An, 2009; Yamunarani et al., 2013). The IRT1 (*iron-regulated transporter1*) proteins, belonging to the ZIP family, were firstly discovered in cereals. ZIP proteins are able to transport various divalent cations, such as Fe^{2+}, Zn^{2+}, Cu^{2+}, $Mn^{2+}$from the rhizosphere through the plasma membrane into the cytoplasm of root cells, as well as from xylem vessels in leaf mesophyll cells (Palmer and Guerinot, 2009). It has been reported that zinc deficiency leads to increase in the activity of IRT1 protein and *IRT1* gene expression in parallel with high accumulation of Zn in roots and shoots of rice, maize and *Arabidopsis* (Ishimaru, 2006; Pedas et al., 2008; Yamunarani et al., 2013; Kabir et al., 2017 etc.). Therefore, IRT1 protein play essential role in Zn uptake, translocation and storage of Zn in plant cells especially under Zn deficiency. Although, most of the evidence from these studies was performed on plants under optimal temperature conditions, however, in nature plants are often exposed to low temperatures during the growing season, that caused in decrease in supply of nutrients to root cells, that result in their deficiency in plants (Hacisalihoglu et al., 2001). Perhaps this effect may be associated with a decrease in the activity of transport proteins (Guerinot, 2000; Hacisalihoglu et al., 2001). Despite this data, the effect of cold on *IRT1* gene expression and IRT1 protein activity is still unclear. Some reports demonstrated that the IRT1 protein activity regulated at the both translation and transcription level (Shin et al., 2013; Brumbarova et al., 2015). According these findings, we studied the expression of the *IRT1* gene in the roots and leaves of barley under zinc deficiency at optimal and low temperatures.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL AND GROWTH CONDITIONS

Seeds of barley (*Hordeum vulgare* 'Nur')were purchased from the Tula Research Institute of Agriculture, Tula, Russia. Seedlings were cultivated in a growth chamber with 14 h photoperiod, a photo-synthetic photon flux density of 180 μmol m^{-2} s^{-1}, a temperature of 22 °C and a relative humidity of 60 - 70 % on Hoagland-Arnon nutrient solution (pH 6.2 to 6.4) with optimal (variant Zn 2 μmol + 22 °C) zinc content or its deficiency (variant Zn 0 μmol + 22 °C). Seven-day-old seedlings (initial level) were separated. One part of the plants of both variants was exposed to low temperature (4 ° C) (variants Zn 2 μmol + 4 °C and Zn 0 μmol + 4 °C), and the other part was left under the optimal temperature during 7 days. All parameters were measured at day 0 (initial level) and 1, 3, 7 days after treatments.

2.2 BIOMASS AND NET PHOTOSYNTHETIC RATE DETERMINATION

For biomass determination plants were collected, their shoots and roots separated and dried in an oven at 85 °C for 24 h. The net photosynthetic rate (P_N) was measured during a day using portable photosynthesis system HCM-1000 (Walz, Effeltrich, Germany).

2.3 GENE EXPRESSION

The expression pattern of *HvIRT1* gene in leaves and roots was monitored by real-time PCR. Frozen roots and leaf tissues were homogenized with liquid nitrogen. Total RNA was extracted using a TRizol reagent (*Evrogen*, Moscow, Russia) as instructed by them anufacturer. The total RNA was treated with RNase free DNase (*Syntol*, Moscow, Russia) to remove genomic DNA. The purity of RNA samples and their concentrations were determined spectrophotometrically (*SmartSpecPlus*, *Bio-Rad*, Hercules, USA): samples withA260/A280 ratios within 1.8 - 2.0 were used for further analysis. The total RNA (1 μg) was reverse-transcribed using a *MMLV* RT kit (*Evrogen*) following the supplier's recommendations. Real-time quantitative PCR was performed using the *iCycler iQ* detection system (*Bio-Rad*). Analyzes were performed using a *SYBR Green* PCR kit (*Evrogen*). The PCR conditions consisted of denaturation at 95 °C for 5 min followed by 45 cycles of denaturation at 95 °C for 15 s, annealing at 56 °C for 30s, and extension at 72 °C for 45 s. A dissociation curve was generated at the end of each PCR cycle to verify that a single product was amplified using *iCycler iQ*. To minimize sample variations, mRNA expression of a target gene was normalized relative to the expression of a housekeeping gene *actin*. The mRNA content of target gene (*HvIRT1)* were quantified in comparison to the *actin* by the ΔΔCt method (Livak and Schmittgen, 2001). Primers were designed (using the *Primer Design* program): *HvActin* (U21907) ATGTTTTTTTCCAGACG (direct) and ATCCAAGCCAACCCAAGT (reverse), *HvIRT1 (*EU54802) GTGCTTCCACCAGATGTTTGAG (direct) andGGATGCCGACGACGATGA (reverse).

2.4 STATISTICAL ANALYSIS

All data are presented as means ± standard errors (SEs) from at least three independent replicates. Significant differences between variants and relative to the initial level were calculated by two-way analysis of variance (ANOVA) using Microsoft Excel 2010. Student's *t*-test was applied to compare statistical significance at level of $p < 0.05$.

3 RESULTS AND DISCUSSION

Table 1 shows the effect of Zn deficiency under optimal and low temperatures on plant dry mass (DM) accumulation and net photosynthetic rate (P_N). Under optimal temperature conditions Zn deficiency did not significant affect the DM and P_N parameters compare with plants grown with optimal Zn concentration. However, after 7 days Zn deficiency (Zn 0 µmol + 22 °C) caused slightly reduction root DM compare with variant Zn 2 µmol + 22 °C.

Despite the Zn concentration the low temperature leads to reduce DM accumulation and P_N parameter. Although, after 7 days Zn deficiency in combination with low temperature (Zn 0 µmol + 4 °C) leads to significant decrease in root DM and photosynthesis activity compare with variant Zn 2 µmol + 4 °C.

Under optimal growth conditions (variant Zn 2 µmol + 22 °C) the transcript level of *HvIRT1* gene gradually increase in roots and leaves of barley during 7 days (Fig.).While, at the initial level, the *HvIRT1* gene expression was 3-fold higher in roots seedlings grown with Zn deficiency (Zn 0 µmol + 22 °C). Further *HvIRT1* gene mRNA content in variant Zn 0 µmol + 22°C slightly increased in compare with variant Zn 2 µmol+ 22 °C. At the initial level there was no significant difference in mRNA content of *HvIRT1* gene in leaves between Zn 2 µmol + 22 °C and variant Zn 0 µmol + 22 °C variants (Fig.). However, leaves of barley exposed to Zn deficiency (Zn 0 µmol + 22 °C) showed higher the *HvIRT1*mRNA content within 1 day and slightly decrease on seventh day in compare with Zn 2 µmol + 22 °C variant.

Low temperature caused an increase in *HvIRT1* gene expression in roots along with time of exposure in both variants (Zn 2 µmol + 4 °C and Zn 0 µmol + 4 °C) (Fig.). However, on 7th day of experiment the amount of *HvIRT1* gene transcripts in roots of barley variant Zn 0 µmol + 4 °C were greater than in variant Zn 2 µmol + 4 °C. Low temperature resulted in *HvIRT1* gene transcript accumulation in leaves of barley variant Zn 2 µmol + 4 °C. After 1 day of cold impact the *HvIRT1* gene expression was 4-fold higher in variant Zn 2 µmol + 4 °C compare with initial level and 10-fold higher on seven day of experiment. In variant Zn 0 µmol + 4 °C the *HvIRT1* gene mRNA content increased after 3 days of exposure to low temperature and significant decreased on seven day of experiment.

In general, our results demonstrated a high tolerance of barley 'Nur' to Zn deficiency. It was shown that under conditions of zinc deficiency, the accumulation of biomass and photosynthetic activity remained stable until the end of the experiment. Similar data were described previously (Hajiboland and Beiramzadeh. 2008; Kabir et al., 2017). The capability of plants to grow under Zn deficiency mostly depend on metal transporters activity, including protein IRT1 (Suzuki et al., 2012; Yamunarani et al., 2013; Kabir et al., 2017). Zn deficiency under optimal temperature leads to increase in *HvIRT1* transcript amount in roots and leaves of barley that resulted in activation of transport metal ions in cells and kept growth and photosynthetic activity. This also support the fact that after 7 days the gene expression of *HvIRT1* decreased along with photosynthetic activity. The negative effect of Zn deficiency on photosynthesis process was described previously in *Oryza sativa* L. (Hajiboland and Beiramzadeh, 2008), *Zea mays* L. (Liu et al., 2016), *Sorgum bicolor* (L.) Moench (Li et al., 2013), however the transport protein activity was not studied.

There are fragmentary data about influence of low temperature on metal transporters activity. The negative effect of 0°C temperature on gene expression, encoding protein transporters ZIP1 and ZIP3 was described previously (Grotz et al., 1998). We have also shown the increase in *HvIRT1* gene expression in barley under chilling (Kaznina et al., 2019). It is supposed that due to negative influence of chilling on nutrient transport into cells the increase in *HvIRT1* gene expression in roots that we reported can be a result of requirement of mesophyll cells of leaves in nutrients that are necessary for photosynthesis process. There are no data about activity of transporter proteins under chilling and Zn deficiency condition. Thus considering the results described above for barley under low temperature and Zn deficiency during 7 days in leaves the *HvIRT1* mRNA content significantly decreased. It seems to be a result of decrease in requirement in nutrients caused by slowdown of photosynthetic activity and growth of seedlings under stress conditions. Additionally, it can be a result of interruption in signal transduction from leaves to roots that was demonstrated previously in plants under chilling and optimal nutrient level (Giehl et al., 2009; Romera et al., 2011).

Table 1: The effect of zinc deficiency on the root and shoot dry biomass and photosynthesis rate of barley plants 'Nur' at optimum (22 °C) and low (4 °C) temperatures

	Time, days			
Variant	0 (initial point)	1	3	7
		Dry root biomass, mg		
Zn 2+22°C	5.51 ± 0.55 aA	6.72 ± 0.48 aA	7.29 ± 0.42 bA	8.21 ± 0.60 bA
Zn 0 +22°C	5.10 ± 0.45 aA	6.51 ± 0.41 aA	6.92 ± 0.38 bA	7.96 ± 0.46 bA
Zn 2+4°C	5.51 ± 0.55 aA	6.20 ± 0.47 aA	6.26 ± 0.48 aB	7.36 ± 0.54 bAB
Zn 0+4°C	5.10 ± 0.45 aA	6.13 ± 0.29 aA	6.23 ± 0.35 aB	6.36 ± 0.46 aB
		Dry shoot biomass, mg		
Zn 2+ 22°C	20.13 ± 1.88 aA	20.89 ± 1.21 aA	27.21 ± 1.48 bA	31.32 ± 2.41 bA
Zn 0 +22°C	18.99 ± 1.89 aA	19.51 ± 1.09 aA	24.06 ± 1.62 bA	32.13 ± 1.32 bA
Zn2+4°C	20.13 ± 1.88 aA	20.00 ± 1.51 aA	20.87 ± 1.50 aB	24.77 ± 1.39 bB
Zn 0 +4°C	18.99 ± 1.89 aA	19.63 ± 1.16 aB	18.42 ± 0.95 aC	19.28 ± 1.21 aC
		Net photosynthetic rate, μmol CO_2 $m^2 \cdot s^{-1}$		
Zn 2+ 22°C	6.82 ± 0.16 aA	7.77 ± 0.17 bA	6.54 ± 0.50 aA	6.53 ± 0.10 aA
Zn 0 +22°C	6.95 ± 0.14 aA	7.69 ± 0.14 bA	6.46 ± 0.49 aA	6.27 ± 0.12 aA
Zn2+4°C	6.82 ± 0.16 aA	4.65 ± 0.20 bB	4.86 ± 0.12 bB	4.16 ± 0.09 cB
Zn 0 +4°C	6.95 ± 0.14 aA	4.83 ± 0.12 bB	4.35 ± 0.15 bB	3.82 ± 0.17 cC

Different lowercase letters indicate significant differences in columns (between variants), uppercase letters - in rows (relative to the initial level) ($p < 0.05$).Values perform mean ± SE (n = 10)

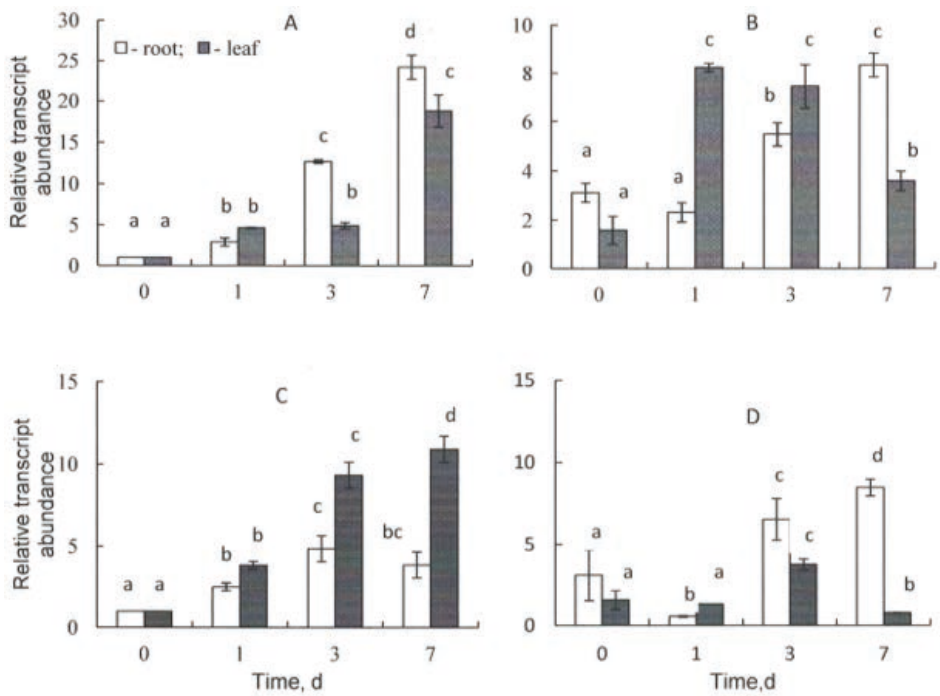

Figure 1: The effect of zinc optimum (a, c) and zinc deficiency (b, d) on *HvIRT1* gene transcription in the roots and leaves of barley plants 'Nur' at 22 °C (a, b) and 4 °C (c, d). Different lowercase letters indicate significant differences relative to the initial level ($p < 0.05$)

4 CONCLUSIONS

According to our results it was shown that Zn deficiency caused in increase in *HvIRT1* gene expression in leaves of barley not only under optimal temperature as was shown in another reports but also under cold. For the first time we demonstrated that long-term exposure (7 days) to low temperature leads to significant decrease in *HvIRT1* mRNA amount in leaves in parallel to slow down in photosynthetic activity and growth. Taken together, the results presented here illustrate the participation of the *HvIRT1* gene in adaptation to Zn deficiency under optimal and low temperatures conditions.

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