

# Expression of *cor* genes in *Lolium multiflorum*/*Festuca arundinacea* introgression forms under cold acclimation conditions

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## Introduction

The mechanisms of cold acclimation have not been fully understood in the agronomically important group of forage grasses, including species of *Lolium-Festuca* complex. These species are characterized by complementary agronomic traits. Among them, *L. multiflorum* (Italian ryegrass) possesses a high forage quality and productivity but a low tolerance to environmental stresses, including frost tolerance. *Festuca arundinacea* (tall fescue) is characterized by a higher level of tolerance to a wide range of environmental stresses, including drought and frost (Kosmala et al. 2007, 2012). One of the ways to combine the complementary traits of both species is the introgression, which involves the transfer of the selected genes from one species to another during backcrossings of intergeneric hybrid with one of its parental species. The *L. multiflorum*/*F. arundinacea* introgression forms have been already proved to be good models to recognize some crucial components of molecular mechanisms involved in cold acclimation (CA) of forage grasses (Kosmala et al. 2007; Augustyniak et al. 2018).

## Methods

Two tetraploid introgression forms of *L. multiflorum*/*F. arundinacea*, analyzed earlier and also selected for the research presented here, were the high frost tolerant (HFT) and low frost tolerant (LFT) genotypes. The plant frost tolerance and changes in physiological parameters (electrolyte leakage, chlorophyll fluorescence, and gas exchange) during CA were evaluated according to Larsen (1978) and Augustyniak et al. (2018), respectively. The HFT and LFT introgression forms were used as models to recognize the expression profiles (at transcript level) of the selected *cor* (cold-regulated) genes, *cor14b* and *Wcor80*, under 3 weeks of CA using real time qRT-PCR.

## Electrolyte leakage [EL]

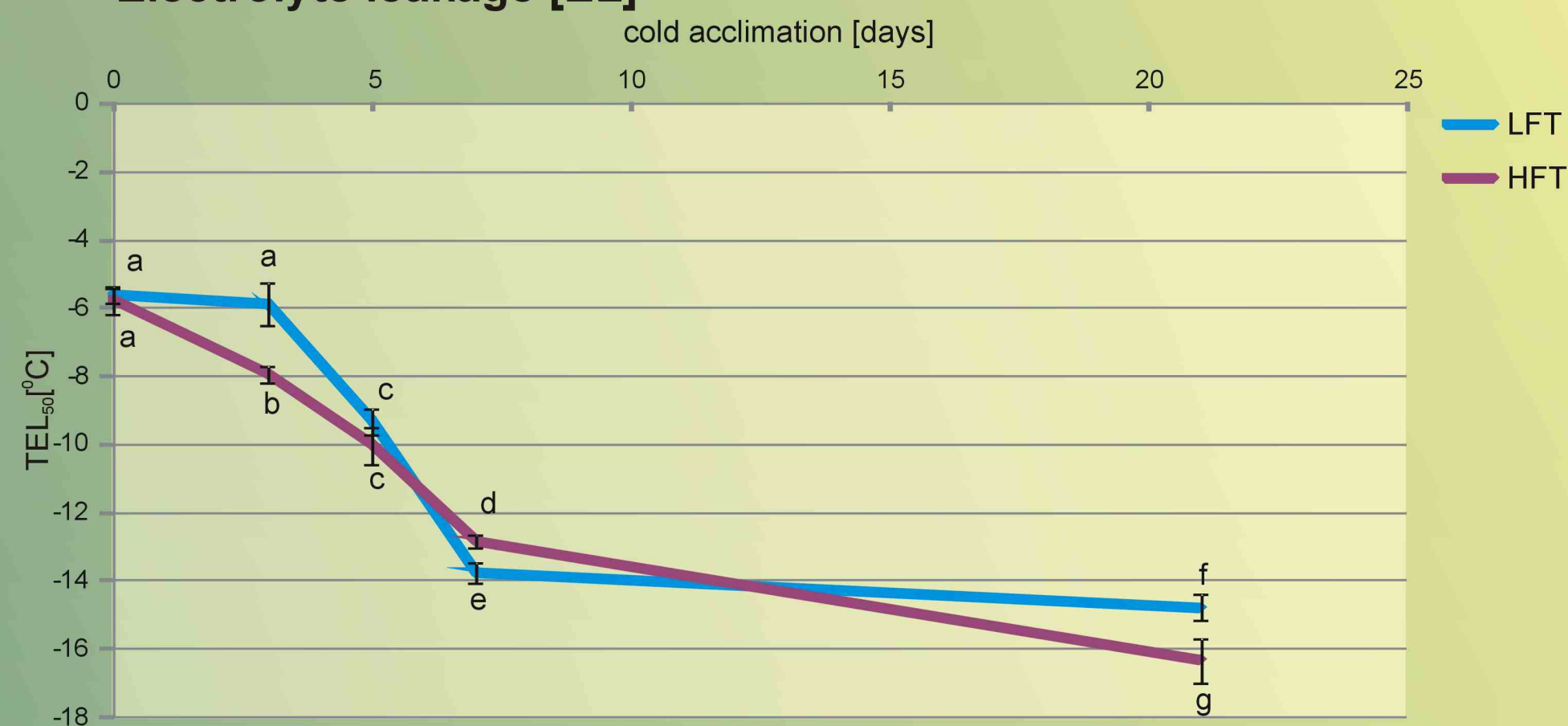


Fig. 1. A relative electrolyte leakage (EL) observed during 21 days of cold acclimation in *L. multiflorum*/*F. arundinacea* introgression forms. Standard errors are indicated. Homogeneity groups are denoted by the same letters (Tukey's test;  $P < 0.05$ ).

## Results

The selected HFT and LFT genotypes differed significantly with respect to their ability to prevent electrolyte leakage (Fig. 1) and other physiological parameters, including chlorophyll fluorescence (Fig. 2), and gas exchange (data not shown), following CA progression (Augustyniak et al. 2018). Cold acclimation process significantly and progressively increased cellular membrane tolerance to freezing in two analyzed forms, however, after three weeks of CA, the HFT introgression form showed a lower level of the electrolyte leakage parameter ( $-16.31^{\circ}\text{C}$ ), compared to the LFT form ( $-14.78^{\circ}\text{C}$ ) (Augustyniak et al. 2018) (Fig. 1). All the analyzed and calculated parameters of chlorophyll fluorescence showed clear differences between both analyzed introgression forms at the majority of the experimental time points, in the control conditions and under CA (Augustyniak et al. 2018) (Fig. 2). The HFT introgression form revealed a significantly higher stomatal conductance, higher transpiration rate and lower water use efficiency under control conditions, compared to the LFT form (Augustyniak et al. 2018) (data not shown). The differences in a transcript abundance of two analyzed here genes between the HFT and LFT genotypes, were clearly observed. The HFT was shown to increase the abundance of *Wcor80* transcript level earlier during cold hardening and the LFT increased it at more advanced time points of CA. Until the 5<sup>th</sup> day of cold treatment the abundance of *Wcor80* transcript was significantly higher in the HFT form, however, its accumulation level dropped on the 7<sup>th</sup> day of CA. The impact of cold hardening was also apparent in the *cor14b* gene expression. The presented results showed a significantly higher accumulation level of *cor14b* transcript in the HFT introgression form, during 3 weeks of low temperature treatment. Although the transcript accumulation level of *cor14b* in the LFT form was significantly lower under acclimation conditions, compared to the HFT form, it also increased slightly during the 1<sup>st</sup> week of stress treatment. However, after 2 weeks of CA its level returned to the control values.

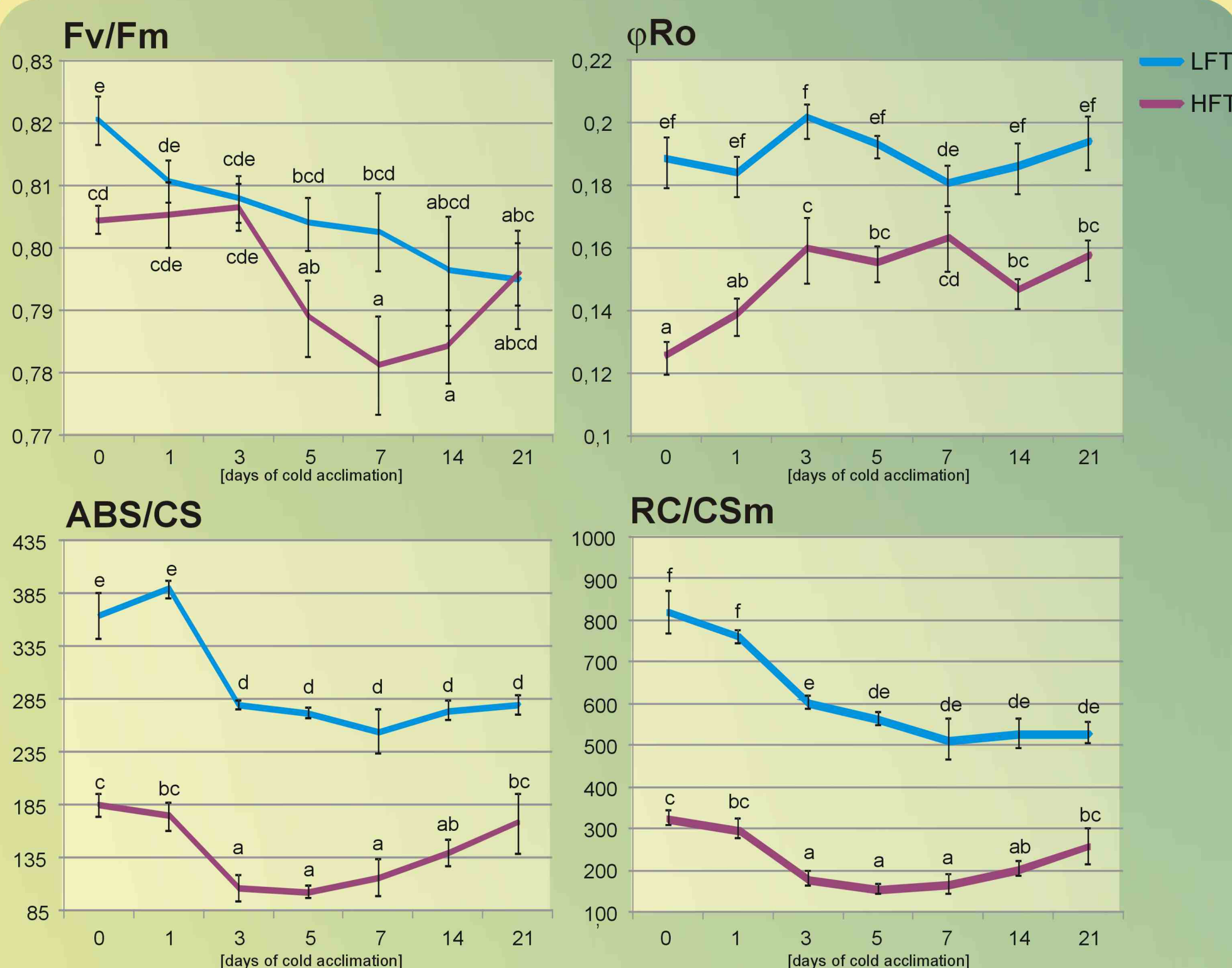


Fig. 2. The selected parameters of chlorophyll fluorescence observed during 21 days of cold acclimation in *L. multiflorum*/*F. arundinacea* introgression forms. Standard errors are indicated. Homogeneity groups are denoted by the same letters (Tukey's test;  $P < 0.05$ ).

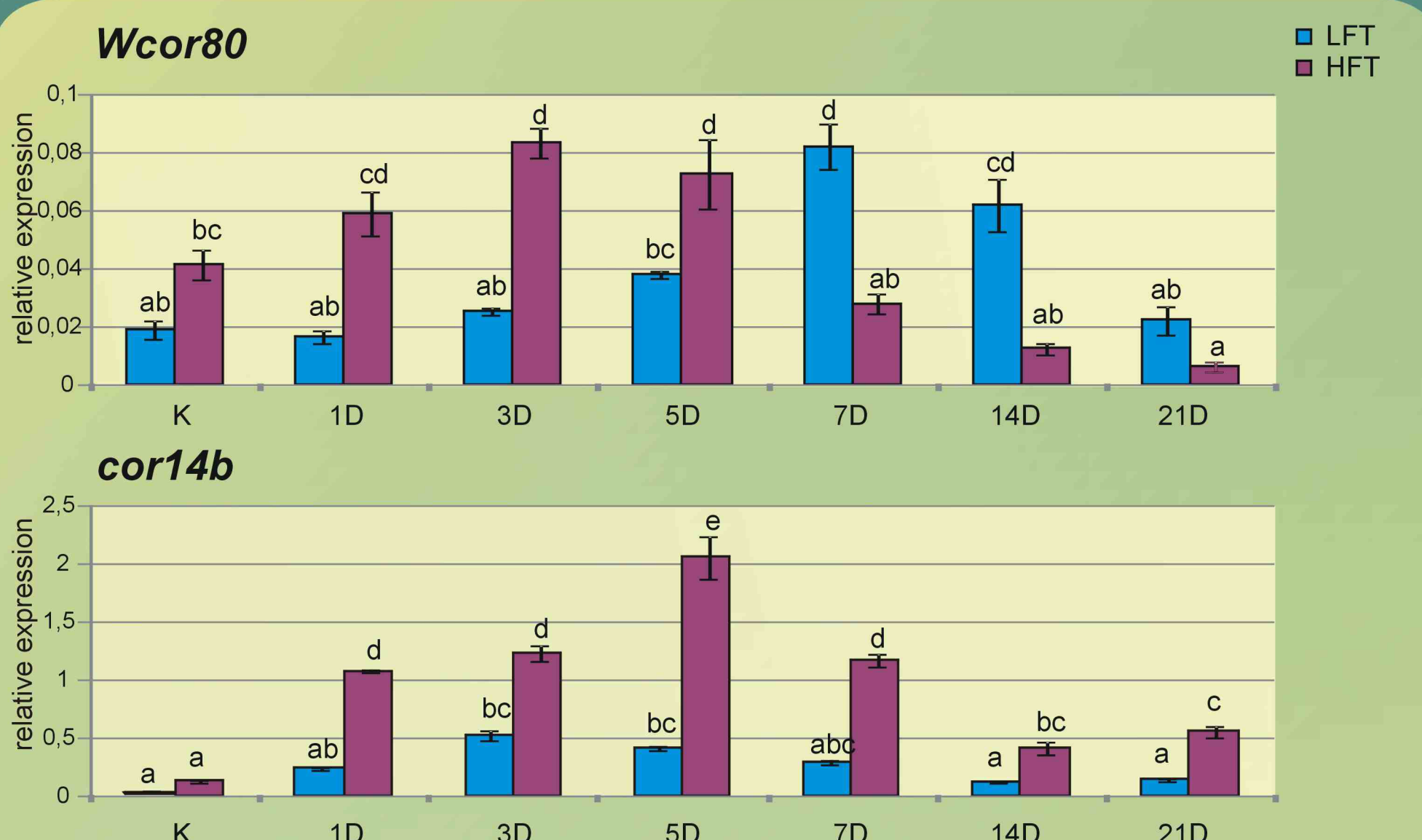


Fig. 3. A relative expression profiles of cold regulated genes *Wcor80* and *cor14b* in *L. multiflorum*/*F. arundinacea* LFT and HFT introgression forms, during 21 days of cold acclimation. The transcript level of actin was used as reference. Error bars represent the standard errors of three biological and two technical replicates. Homogeneity groups are denoted by the same letters (Tukey's test;  $P < 0.05$ ).

## Conclusions

We suggest that the analyzed transcript accumulation profiles could be good molecular markers linked to the potential of frost tolerance in *Lolium-Festuca* forage grasses. The *Wcor80*, from *Triticum aestivum* (wheat) *wcs120* gene family, codes a dehydrin (Sarhan et al. 2008). The *COR14b* protein, from *Hordeum vulgare* (barley), protects thylacoid membranes against photoinhibition and was proved to be an important chloroplast component to develop frost tolerance (Rapacz et al. 2008). The obtained results clearly showed that in fact the expression patterns of both genes were significantly different between the HFT and LFT *L. multiflorum*/*F. arundinacea* genotypes. Moreover, the observed accumulation profiles were strongly associated with the cold acclimation progression. The detail function of the analyzed proteins in the mechanisms of tolerance to low temperature and protection against photoinhibition in forage grasses, has yet to be deeply analyzed.

## References

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