Dear Author

Here are the proofs of your article.

- You can submit your corrections **online,** via **e-mail** or by **fax**.
- For **online** submission please insert your corrections in the online correction form. Always indicate the line number to which the correction refers.
- You can also insert your corrections in the proof PDF and **email** the annotated PDF.
- For **fax** submission, please ensure that your corrections are clearly legible. Use a fine black pen and write the correction in the margin, not too close to the edge of the page.
- Remember to note the **journal title**, **article number**, and **your name** when sending your response via e-mail or fax.
- **Check** the metadata sheet to make sure that the header information, especially author names and the corresponding affiliations are correctly shown.
- **Check** the questions that may have arisen during copy editing and insert your answers/corrections.
- **Check** that the text is complete and that all figures, tables and their legends are included. Also check the accuracy of special characters, equations, and electronic supplementary material if applicable. If necessary refer to the *Edited manuscript*.
- The publication of inaccurate data such as dosages and units can have serious consequences. Please take particular care that all such details are correct.
- Please **do not** make changes that involve only matters of style. We have generally introduced forms that follow the journal's style.
- Substantial changes in content, e.g., new results, corrected values, title and authorship are not allowed without the approval of the responsible editor. In such a case, please contact the Editorial Office and return his/her consent together with the proof.
- If we do not receive your corrections **within 48 hours**, we will send you a reminder.
- Your article will be published **Online First** approximately one week after receipt of your corrected proofs. This is the **official first publication** citable with the DOI. **Further changes are, therefore, not possible.**
- The **printed version** will follow in a forthcoming issue.

Please note

After online publication, subscribers (personal/institutional) to this journal will have access to the complete article via the DOI using the URL:

`http://dx.doi.org/10.1007/s13353-015-0297-2`

If you would like to know when your article has been published online, take advantage of our free alert service. For registration and further information, go to: http://www.link.springer.com.

Due to the electronic nature of the procedure, the manuscript and the original figures will only be returned to you on special request. When you return your corrections, please inform us, if you would like to have these documents returned.

Metadata of the article that will be visualized in OnlineFirst

Please note: Images will appear in color online but will be printed in black and white.

1	Article Title	**Comparison of redox and gene expression changes during vegetative/generative transition in the crowns and leaves of chromosome 5A substitution lines of wheat under low-temperature condition**	
2	Article Sub- Title		
3	Article Copyright - Year	**Institute of Plant Genetics, Polish Academy of Sciences, Poznan 2015 (This will be the copyright line in the final PDF)**	
4	Journal Name	Journal of Applied Genetics	
5	Corresponding Author	Family Name	**Kocsy**
6		Particle	
7		Given Name	**Gábor**
8		Suffix	
9		Organization	Hungarian Academy of Sciences
10		Division	Agricultural Institute, Centre for Agricultural Research
11		Address	Martonvásár 2462, Hungary
12		Organization	University of Pannonia
13		Division	Doctoral School of Molecular and Nanotechnologies, Research Institute of Chemical and Process Engineering, Faculty of Information Technology
14		Address	Veszprém 8200, Hungary
15		e-mail	kocsy.gabor@agrar.mta.hu
16	Author	Family Name	**Boldizsár**
17		Particle	
18		Given Name	**Ákos**
19		Suffix	
20		Organization	Hungarian Academy of Sciences
21		Division	Agricultural Institute, Centre for Agricultural Research
22		Address	Martonvásár 2462, Hungary
23		e-mail	
24	Author	Family Name	**Carrera**

25		Particle	
26		Given Name	**Dániel Á.**
27		Suffix	
28		Organization	Hungarian Academy of Sciences
29		Division	Agricultural Institute, Centre for Agricultural Research
30		Address	Martonvásár 2462, Hungary
31		e-mail	
32		Family Name	**Gulyás**
33		Particle	
34		Given Name	**Zsolt**
35		Suffix	
36		Organization	Hungarian Academy of Sciences
37		Division	Agricultural Institute, Centre for Agricultural Research
38	Author	Address	Martonvásár 2462, Hungary
39		Organization	University of Pannonia
40		Division	Doctoral School of Molecular and Nanotechnologies, Research Institute of Chemical and Process Engineering, Faculty of Information Technology
41		Address	Veszprém 8200, Hungary
42		e-mail	
43		Family Name	**Vashegyi**
44		Particle	
45		Given Name	**Ildikó**
46		Suffix	
47	Author	Organization	Hungarian Academy of Sciences
48		Division	Agricultural Institute, Centre for Agricultural Research
49		Address	Martonvásár 2462, Hungary
50		e-mail	
51		Family Name	**Novák**
52		Particle	
53		Given Name	**Aliz**
54	Author	Suffix	
55		Organization	Hungarian Academy of Sciences
56		Division	Agricultural Institute, Centre for Agricultural Research

57		Address	Martonvásár 2462, Hungary
58		Organization	University of Pannonia
59		Division	Doctoral School of Molecular and Nanotechnologies, Research Institute of Chemical and Process Engineering, Faculty of Information Technology
60		Address	Veszprém 8200, Hungary
61		e-mail	
62	Author	Family Name	**Kalapos**
63		Particle	
64		Given Name	**Balázs**
65		Suffix	
66		Organization	Hungarian Academy of Sciences
67		Division	Agricultural Institute, Centre for Agricultural Research
68		Address	Martonvásár 2462, Hungary
69		Organization	University of Pannonia
70		Division	Doctoral School of Molecular and Nanotechnologies, Research Institute of Chemical and Process Engineering, Faculty of Information Technology
71		Address	Veszprém 8200, Hungary
72		e-mail	
73	Author	Family Name	**Pál**
74		Particle	
75		Given Name	**Magda**
76		Suffix	
77		Organization	Hungarian Academy of Sciences
78		Division	Agricultural Institute, Centre for Agricultural Research
79		Address	Martonvásár 2462, Hungary
80		e-mail	
81	Author	Family Name	**Galiba**
82		Particle	
83		Given Name	**Gábor**
84		Suffix	
85		Organization	Hungarian Academy of Sciences
86		Division	Agricultural Institute, Centre for Agricultural Research
87		Address	Martonvásár 2462, Hungary

88		Organization	University of Pannonia
89		Division	Doctoral School of Animal and Agricultural Environmental Sciences, Department of Meteorology and Water Management Georgikon Faculty
90		Address	Keszthely 8360, Hungary
91		e-mail	

92		Received	9 April 2015
93	Schedule	Revised	28 May 2015
94		Accepted	4 June 2015

95	Abstract	The aim of our experiments was to investigate the effect of chromosome 5A on the thiol-dependent redox environment and on the transcription of cold- and vernalization-related genes during the transition in crowns and leaves of wheat. Chinese Spring, a moderately freezing-tolerant variety, and its more and less tolerant substitution lines — [CS(Ch5A)] and [CS(Tsp5A)], respectively — with different combinations of vernalization alleles were compared. At low temperature, the amount of cystine and glutathione disulphide and the related redox potentials increased in the crowns but not in the leaves. In the crowns of the substitution lines, the concentration and redox state of thiols were different only at the vegetative and double ridge (start of the generative transition) stages. The expression of the vernalization-related *VRN1* gene increased significantly during the transition both in the crowns and leaves. The transcription of the freezing tolerance-related *CBF14*, *COR14b* and *COR39* genes markedly increased in both organs after 2 weeks at 4 °C when the seedlings were still in the vegetative stage. This increment was greater in CS(Ch5A) than in CS(Tsp5A). The Ch5A chromosome in CS genetic background enhanced the expression of *CBF* regulon even in the generative phase in crown that is the key organ for overwintering and freezing tolerance. At certain developmental stages, both the thiol and the transcript levels differed significantly in the two substitution lines.

96	Keywords separated by ' - '	Chromosome 5A - Freezing tolerance - Glutathione - Redox control - *Triticum aestivum* - Vernalization
97	Foot note information	Communicated by: Andrzej Górny The online version of this article (doi:10.1007/s13353-015-0297-2) contains supplementary material, which is available to authorized users.

Electronic supplementary material

ESM 1
(PDF 163 kb)

ESM 2
(PDF 121 kb)

J Appl Genetics
DOI 10.1007/s13353-015-0297-2

PLANT GENETICS • ORIGINAL PAPER

Comparison of redox and gene expression changes during vegetative/generative transition in the crowns and leaves of chromosome 5A substitution lines of wheat under low-temperature condition

Ákos Boldizsár[1] · Dániel Á. Carrera[1] · Zsolt Gulyás[1,2] · Ildikó Vashegyi[1] · Aliz Novák[1,2] · Balázs Kalapos[1,2] · Magda Pál[1] · Gábor Galiba[1,3] · Gábor Kocsy[1,2]

Received: 9 April 2015 / Revised: 28 May 2015 / Accepted: 4 June 2015

Abstract The aim of our experiments was to investigate the effect of chromosome 5A on the thiol-dependent redox environment and on the transcription of cold- and vernalization-related genes during the transition in crowns and leaves of wheat. Chinese Spring, a moderately freezing-tolerant variety, and its more and less tolerant substitution lines — [CS(Ch5A)] and [CS(Tsp5A)], respectively — with different combinations of vernalization alleles were compared. At low temperature, the amount of cystine and glutathione disulphide and the related redox potentials increased in the crowns but not in the leaves. In the crowns of the substitution lines, the concentration and redox state of thiols were different only at the vegetative and double ridge (start of the generative transition) stages. The expression of the vernalization-related *VRN1* gene increased significantly during the transition both in the crowns and leaves. The transcription of the freezing tolerance-related *CBF14*, *COR14b* and *COR39* genes markedly increased in both organs after 2 weeks at 4 °C when the seedlings were still in the vegetative stage. This increment was greater in CS(Ch5A) than in CS(Tsp5A). The Ch5A chromosome in CS genetic background enhanced the expression of *CBF* regulon even in the generative phase in crown that is the key organ for overwintering and freezing tolerance. At certain developmental stages, both the thiol and the transcript levels differed significantly in the two substitution lines.

Keywords Chromosome 5A · Freezing tolerance · Glutathione · Redox control · *Triticum aestivum* · Vernalization

Communicated by: Andrzej Górny

Electronic supplementary material The online version of this article (doi:10.1007/s13353-015-0297-2) contains supplementary material, which is available to authorized users.

✉ Gábor Kocsy
kocsy.gabor@agrar.mta.hu

[1] Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár 2462, Hungary

[2] Doctoral School of Molecular and Nanotechnologies, Research Institute of Chemical and Process Engineering, Faculty of Information Technology, University of Pannonia, Veszprém 8200, Hungary

[3] Doctoral School of Animal and Agricultural Environmental Sciences, Department of Meteorology and Water Management Georgikon Faculty, University of Pannonia, Keszthely 8360, Hungary

Introduction

Flower primordia are very sensitive to low temperature therefore the appropriate timing of the vegetative/generative transition is very important to avoid injuries of this organ and subsequent yield loss in winter cereals. From this aspect chromosome 5A of wheat plays a special role since several genes affecting the formation of flower primordia and freezing tolerance can be found on this chromosome, as shown by their mapping (Galiba et al. 1995). Among others, vernalization (*VRN*) genes controlling the vegetative/generative transition in the shoot apex, and C-repeat binding transcription factors/dehydration-responsive element binding factors (CBF/DREB1) affecting freezing tolerance were localized on this chromosome (Galiba et al. 2009).

Control of vernalization by the VRN genes

The reduction in temperature during autumn is necessary for vernalization and activates the *VRN* genes, which control the initial development of flower primordia in winter cereals (Distelfeld et al. 2009). In contrast, spring cereals flower without any cold treatment. Three genes play a crucial role in the vernalization process of wheat, *VRN1*, *VRN2* and *VRN3* and the different timing of the vegetative to generative transition derives from differences in their allelic combinations in the various genotypes. In earlier studies, more models were born to explain the connections between these genes. In the first model (described by Shimada et al. 2009) the *VRN1* is an inducer of *VRN3* which inhibits the expression of *VRN2*. In this model, *VRN2* represses *VRN1*. In the second model (exhibited by Distelfeld et al. 2009) we can find a reverse connection between *VRN2* and *VRN3*, so *VRN1* repress *VRN2* that repress *VRN3* and *VRN3* is the inducer of *VRN1*. A good comparison about these models is available in the paper of Distelfeld and Dubcovsky 2010. According to the second model *VRN1*, which is a MADS-box transcription factor, induces flowering by inhibiting *ZCCT1* and *ZCCT2* (Zinc-finger/CONSTANS, CONSTANS-LIKE, TOC1 domain) transcription factors, which are repressors of flowering (Distelfeld et al. 2009). These genes are localized at the *VRN2* locus (Yan et al. 2003; Chen and Dubcovsky 2012). *VRN3* is involved in the induction of flowering and shares significant sequence homology with the *Arabidopsis* FLOWERING LOCUS T (FT), which is a long-distance flowering signal (Yan et al. 2006). The *VRN* genes interact with each other and also affect freezing tolerance (Galiba et al. 2009; Distelfeld et al. 2009). The *VRN1* and *VRN2* loci have been mapped to chromosome group 5 and the *VRN3* locus to chromosome group 7 (Sutka et al. 1999; Yan et al. 2006).

Effect of the combination of VRN1 alleles on vernalization in the examined genotypes

Chinese Spring (CS) is a hexaploid spring wheat genotype. It contains homologous alleles of *VRN1* genes, namely *vrn-A1*, *vrn-B1* and *Vrn-D1* (Supplementary table S1). The only dominant vernalization-insensitive allele is localized on the D genome, but it is sufficient for the evolution of the spring habit (Pugsley 1971). Cheyenne (Ch) carries only the recessive *VRN1* alleles *vrn-A1*, *vrn-B1* and *vrn-D1*. Sears and his colleagues developed a series of nullisomic lines from CS (Sears et al. 1953) and this genetic material was a good commodity for creating single chromosome substitution lines. The first examination of these lines was carried out by Law and Pugsley (Law 1966, 1967; Pugsley 1971; Law et al. 1976), and these researchers demonstrated that if the substituted chromosome 5A was derived from the *T. spelta* genotype, it caused earlier ear-emergence and higher freezing sensitivity than those of CS. This phenomenon caused by the new dominant *VRN1* vernalization-insensitive allele (*Vrn-A1*) in this genotype originated from the donor *T. spelta*. In the case of the CS(Ch5A) substitution line the foreign chromosome originated from the Ch genotype, and on this chromosome there is a recessive vernalization-sensitive allele (*vrn-A1*) which differs from the vernalization-sensitive alleles of CS (Eagles et al. 2011), and this difference can explain the later vegetative/generative transition of CS(Ch5A).

Major cold-responsive genes and their control by VRN genes

The decrease in temperature during autumn is important not only for vernalization but also for cold acclimation, which is necessary for the attainment of the genetically determined freezing tolerance in winter cereals (Sandve et al. 2011). Although low temperature is not necessary to induce the flowering in spring wheat, cold can affect this process in these genotypes as shown among others by its effect on final leaf number (Fowler et al. 1996). Cold affects the expression of both freezing tolerance- and vernalization-related genes. Among the freezing tolerance related genes the cold-inducible CBF transcription factors are well characterized both in *Arabidopsis* and in cereals (Nakashima et al. 2009). Eleven *CBF* genes are localized in the *Fr-2* locus of chromosome 5A in wheat, and *CBF14* has a great influence on freezing tolerance both in wheat and barley (Vágújfalvi et al. 2005; Soltész et al. 2013; Dhillon and Stockinger 2013). The CBF proteins regulate low temperature-dependent changes in the transcript levels of their target genes through binding to the C-repeat elements in their promoter sequence. The group of the CBF-regulated genes form the so-called CBF-regulon. The CBFs may integrate different signals deriving from chloroplast redox state, phytochromes and membrane viscosity and may affect cold acclimation through removal of growth-active gibberellins and control of target genes (Kurepin et al. 2013). Among the genes in CBF-regulon the *cold-regulated 14b* (*COR14b*) gene is well characterized, and its transcript level is different in freezing-tolerant and sensitive wheat and barley genotypes at low temperature (Vágújfalvi et al. 2000; Rapacz et al. 2008). An indirect protective role of COR14b protein through modification of thylakoid membrane was proposed in barley (Rapacz et al. 2008). Similar to *COR14b*, the expression of *COR39* gene is also greatly induced by cold and the encoded protein contains a lysine-rich sequence which facilitates the interaction with other proteins and consequently protects them from low temperature damage (Guo et al. 1992). The expression of cold-regulated

genes is controlled not only by CBFs but also by VRN transcription factors. In mutant plants with high *VRN1* expression, the transcription of *COR* genes was inhibited and freezing tolerance was lower, which indicates the coordinated control of vernalization and freezing tolerance (Galiba et al. 2009; Trevaskis 2010). Due to this coordination the expression of the genes related to freezing tolerance is higher in vegetative state and the transcription of genes related to the flower initiation is greater just before and during the generative transition which is indicated by the appearance of the double ridges. After this transition, during the development of flower primordia a dramatic decrease in freezing tolerance occurs.

Effect of redox changes on the vegetative/generative transition

Like many other different developmental processes, the vegetative to generative transition is also under redox control in plants (Bartoli et al. 2013; Kocsy et al. 2013). The components of the ascorbate-glutathione (ASA-GSH) cycle in interactions with other redox systems and plant hormones may have an important role in the regulation of this process (Kocsy et al. 2013). Thus, in ASA-deficient *Arabidopsis* mutants an alteration in flowering time occurred (Dowdle et al. 2007). Overexpression of γ-glutamlycysteine synthetase, the first enzyme of glutathione synthesis mimicked the effect of low temperature treatment in *Arabidopsis*, since in both cases earlier flowering and increased glutathione disulphide (GSSG) levels were observed (Hatano-Iwasaki and Ogawa 2012). The effect of higher GSSG level on flowering time may be mediated by the oxidative stress2 transcription factor (Blanvillain et al. 2011). The effect of chromosome 5A on the redox state of glutathione and ascorbate was shown during a 3-week hardening period in wheat (Kocsy et al. 2000; Soltész et al. 2011).

Research hypothesis and aims of the experiments

During the vegetative/generative transition the thiol-dependent redox environment and the expression of cold-responsive and vernalization-related genes will be changed according to our hypothesis based on previous results describing the effect of various reductants and oxidants on these parameters (Gulyás et al. 2014). The aim of our experiments was to investigate the effect of chromosome 5A on the thiol-dependent redox environment and on the expression of cold- and vernalization-related genes during the vegetative/generative transition phase in crowns and leaves of wheat plants at low temperature. The moderately freezing-tolerant variety Chinese Spring (CS) and its more [CS(Cheyenne 5A)] and less tolerant [CS(*Triticum spelta* 5A)] substitution lines with different combination of vernalization alleles were compared.

Materials and methods

Plant material and treatments

The plant material consisted of the moderately freezing-sensitive spring wheat (*Triticum aestivum ssp. aestivum*) variety Chinese Spring [CS], and CS chromosome 5A substitution lines where the 5A chromosome originated either from a freezing-sensitive, spring habit spelt wheat (*T. ae. ssp. spelta*) accession [CS(Tsp5A)] or from the freezing-tolerant winter wheat (*T. ae. ssp. aestivum*) variety Cheyenne [CS(Ch5A)]. This experimental system is described in the Supplementary Table S1 and is appropriate to prove whether the various 5A chromosomes in the CS genetic background differently affect the studied parameters during the vegetative/generative transition. After germination in Petri dishes between wet filter papers (1 d 25 °C, 3 d 5 °C, 2 d 25 °C), seedlings were grown with a photoperiod of 16 h, at 260 μmol m^{-2} s^{-1}, 20/17 °C and 70/75 % RH in a growth chamber (Conviron PGV-15; Controlled Env., Ltd., Winnipeg, Canada). The long day growth conditions were chosen in order to eliminate the interactions between different flowering induction pathways. Seedlings were raised in a 2:1:1 (v/v/v) mixture of garden soil, humus and sand in wooden boxes (150 plants in a box). Dimensions of the soil blocks in the boxes were 26*38*10 cm. (length*width*depth), the distance between the plants was 2.5 cm. After 3 weeks the temperature was set immediately to continuous 4 °C (day/night) and the other environmental parameters remained unchanged. Crown and leaf (the second youngest leaves were collected) samples for thiol measurements and gene expression studies were taken before the cold treatment; after 2 weeks at 4 °C when the seedlings were still in the vegetative developmental stage; during the vegetative/generative transition (double ridge stage) and after the appearance of the spikelet primordia (generative phase). Each sampling was started after 6 hours illumination and lasted for 60-90 min. The experiments were repeated three times. In each experiment three samples consisting of a mixture of the crowns and leaves from nine plants were analysed.

Morphology of shoot apices

The developmental stage of the shoot apices, isolated from the crowns of the seedlings, was determined under a Zeiss Stemi

2000-C stereomicroscope (Carl Zeiss Mikroskopie, Jena, Germany) according to the scale of Gardner (Gardner et al. 1985) (Fig. 1). Three developmental stages were distinguished, namely: vegetative (VP, single ridge structure, Gardner's stages 0-1), double ridge (DR, vegetative/generative transition, Gardner's stage 3) and generative phases (GP, initiation of spike primordia, Gardner's stages 4-5).

Determination of thiols

The qualitative and quantitative determination of thiols and the recovery experiments were performed by reverse-phase HPLC (Waters, Milford, MA, USA) connected to a fluorescence detector (W474 scanning fluorescence detector, Waters) as earlier described (Kranner and Grill 1996; Kocsy et al. 2000). The half-cell reduction potential of the thiol/thiol disulphide redox couples was calculated according to Schafer and Buettner 2001.

$$E_{\frac{reduced}{oxidized}}[mV] = E^0 - \frac{RT}{nF}\ln\left(\frac{reduced\ [mmol]^2}{oxidized\ [mmol]}\right)$$
$$= E^0 - \frac{8,314\left[\frac{C\ V}{mol\ K}\right]*298,15\ [K]}{2*96485,34\ [C\ mol]}\ln\left(\frac{reduced\ [mmol]^2}{oxidized\ [mmol]}\right)$$
$$= E^0 - 29,58\ [mV]\log_{10}\left(\frac{reduced\ [mmol]^2}{oxidized\ [mmol]}\right)$$

In this equation, the E^0 is different for the individual thiols. $E^0_{GSH/GSSG}$=-240 mV, $E^0_{Cys/CySS}$=-226 mV and $E^0_{hmGSH/hmGSSG}$=-240 mV (Birtić et al. 2011). The pH was assumed to be 7.0.

Gene expression studies

Total RNA was isolated using the Direct-zol™ RNA Miniprep Kit (Zymo Research) as described by the manufacturer. Reverse transcription was carried out with M-MLV reverse transcriptase and Oligo(dT)$_{18}$ primer (Thermo Scientific) using the method of the supplier. Special indicator genes of the vegetative/generative transition (VRN and HSP genes) and cold acclimation (CBF and COR genes) were selected for gene expression analysis based on previous studies (Koning et al. 1992; Sangster and Queitsch 2005; Galiba et al. 2009; Kocsy et al. 2010). The transcript levels were determined with real-time RT-PCR using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad). The primer sequences were taken from the literature (Guo et al. 1992; Paolacci et al. 2009; Dhillon et al. 2010) or they were designed in our laboratory (Supplementary Table S2). The efficiency values were between 95 and 100 % in the case of all primers so relative transcript levels were calculated with the ΔCt method, using the housekeeping gene similar to phosphoglucanate dehydrogenase protein (unigene identifier: Ta30797) for normalization (Paolacci et al. 2009).

We used the same formula as Chen et al. 2014:

$$= \frac{\left(2^{\left(\overline{C_T HKG} - C_T GOI_1\right)}\right) + \left(2^{\left(\overline{C_T HKG} - C_T GOI_2\right)}\right) + \left(2^{\left(\overline{C_T HKG} - C_T GOI_3\right)}\right)}{3}$$

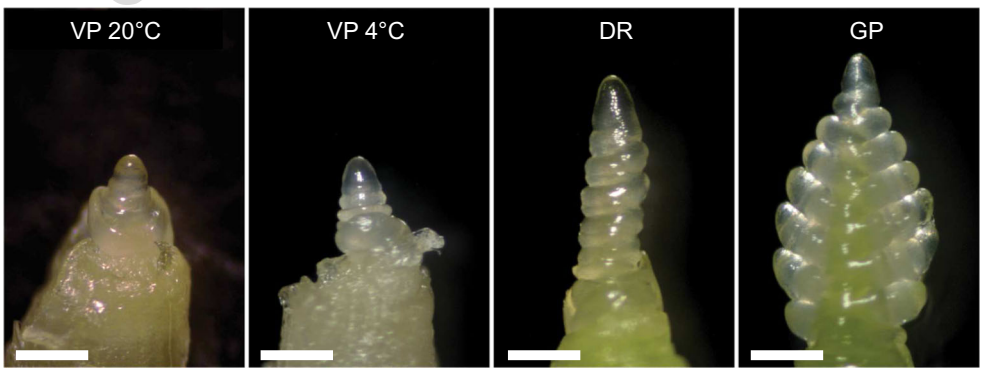

Fig. 1 Morphology of the shoot apices during vegetative/generative transition. The following developmental stages are shown: vegetative phase before the start of cold treatment (growth at 20/17 °C) — VP 20 °C, vegetative phase at 4 °C — VP 4 °C, double ridge stage — DR and generative phase — GP. The apices of Chinese Spring are shown only, since their morphology was similar in the case of the other two genotypes at the individual developmental stages. The three developmental stages on Gardner's scale are the following: VP=Gardner's stages 0-1, DR=Gardner's stage 3 and GP=Gardner's stages 4-5. The white bars indicate 100 μm

where GOI_1, GOI_2, GOI_3 mean the first, second and third technical replicates of the examined sample, thus we could calculate mean and SD from the replicates.

Statistical analysis

For statistical analysis, one-way ANOVA with LSD or Tukey B post hoc test or Mann-Whitney non-parametric test was used by SPSS 16.0. Normality was tested by the Kolmogorov-Smirnov probe and the homogeneity of the variances was tested by Levene's test.

Results

Timing of the vegetative/generative transition

The developmental stage of the plants was checked every week in order to determine the timing of the vegetative/generative transition. The single ridge structures indicated that each genotype was still in the vegetative phase when the cold temperature was applied. Later on, the generative transition was shown by the double ridge formation and plants with spike primordia were already in the generative phase (Fig. 1). Chromosome 5A had a significant effect on the vegetative/generative transition, and the generative phase was observed in CS(Tsp5A) 4 weeks earlier than in CS(Ch5A). The vegetative/generative transition stage occurred 1 week earlier in CS compared to CS(Ch5A), and these two genotypes reached the generative phase after a similar time interval.

Changes in the amount and redox state of thiols during the vegetative/generative transition

In the crowns the amount of cysteine (Cys) and cystine (CySS) and the reduction potential of the Cys/CySS couple ($E_{Cys/CySS}$) were affected by both the temperature and the vegetative/generative transition, since these parameters changed when the seedlings were cultivated for 2 weeks at 4 °C but were still in the vegetative stage and also during the appearance of double ridge on the shoot apices (Fig. 2a, b). In contrast to the increase in the crowns, there was no or small change in CySS levels and $E_{Cys/CySS}$ values in leaves except for CS(Tsp5A) in double ridge stage (Fig. 2c, d). The effect of chromosome 5A on the amount and redox state of cysteine could be observed in the double ridge stage at 4 °C except for the $E_{Cys/CySS}$ value in the leaves.

In the crowns, hydroxymethylglutathione disulphide (hmGSSG) content decreased and hydroxymethylglutathione (hmGSH) content increased during the vegetative/generative transition (Fig. 3a, b). The redox potential of the hmGSH/hmGSSG couple ($E_{hmGSH/hmGSSG}$) showed the same changes as hmGSSG: it was reduced under vegetative/generative transition. In the leaves, hmGSSG concentration exhibited only a decrease until the double ridge stage, the amount of hmGSH decreased through the whole experiment, and there were no or only smaller changes in the $E_{hmGSH/hmGSSG}$ values (Fig. 3c, d). A significant difference in hmGSSG content among the chromosome 5A substitution lines was detected in the vegetative stage at 4 °C and in the double ridge stage in the crowns.

A decrease in the redox potential of the GSH/GSSG couple ($E_{GSH/GSSG}$) and in the GSH content in the vegetative stage at 4 °C was observed in the crowns of the two substitution lines (Fig. 4a and b). The increase in GSSG after transfer to 4 °C was permanent. However, the $E_{GSH/GSSG}$ value did not change or exhibited only small changes, and GSH concentration increased in the leaves (Fig. 4c and d). The effect of chromosome 5A on the amount and redox state of glutathione could be shown in the double ridge stage at 4 °C in the crowns of the two substitution lines.

Expression of the genes related to vegetative/generative transition

The expression of *VRN1* exhibited a large change during the vegetative/generative transition in both organs except for the crowns of CS(Tsp5A) (Fig. 5a and b). Its transcript level was higher before the cold treatment in the crowns of CS(Tsp5A) as compared with the other two genotypes. There was a large difference in the *VRN1* transcript level between CS(Tsp5A) and CS(Ch5A) during double ridge formation.

The expression of *ZCCT2* present in the *Vrn2* locus could not be detected in the present experiment since our plants were 3-week old by the first sampling. The transcription of this gene could be shown only in 1-2 weeks old seedlings (Gulyás et al. 2014).

In the crowns the level of *VRN3* transcripts decreased at 4 °C in CS(Tsp5A), whereas it increased at the double ridge stage in CS, and did not change in CS(Ch5A) (Fig. 5c). In the leaves *VRN3* expression increased at the double ridge stage both in CS and CS(Ch5A), and it was high from the beginning of the experiment in CS(Tsp5A) (Fig. 5d).

The relative expression of the *HSP70* gene in the crowns after the start of cold treatment decreased in CS and CS(Ch5A) genotypes, whereas no changes were observed in CS(Tsp5A). Interestingly, *HSP70* expression increased in the leaves in CS and CS(Tsp5A) genotypes when the cold treatment started. The most intensive change was observed in CS(Tsp5A). In the other developmental stages the expression level returned to baseline levels (Fig. 5e, f).

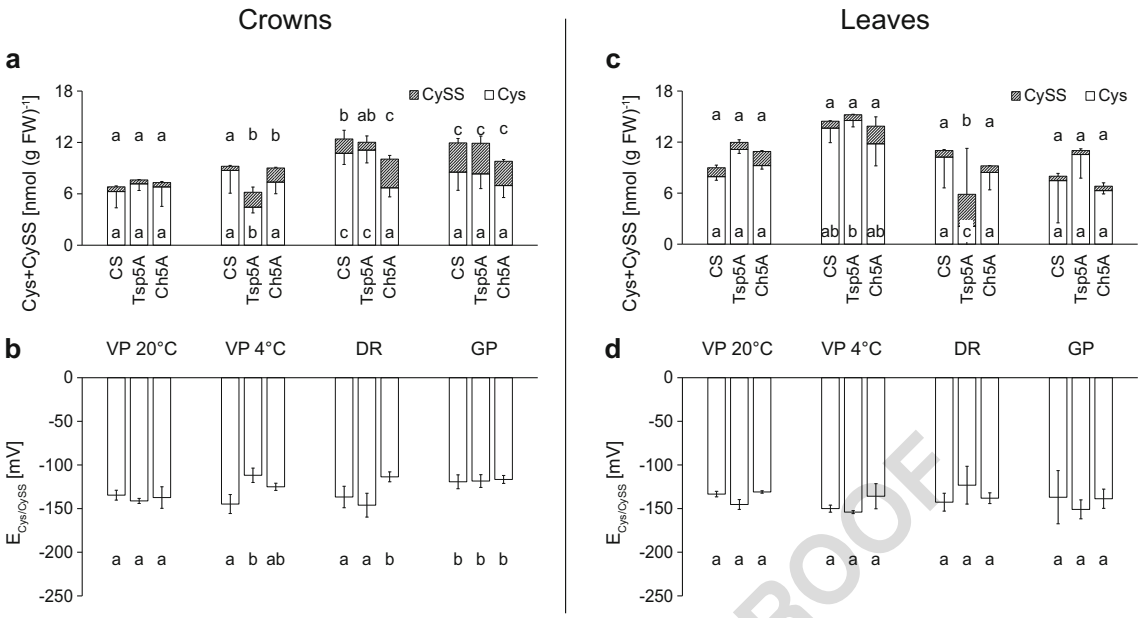

Fig. 2 Concentration and reduction potential of cysteine during vegetative/generative transition. (**a**): Concentration of cysteine (Cys) and cystine (CySS) in crowns; (**b**): Reduction potential of the Cys/CySS couple in crowns; (**c**): Concentration of Cys and CySS in leaves; (**d**): Reduction potential of the Cys/CySS couple in leaves. The developmental stage of the plants is described in the legend of Fig. 1. The values indicated by different letters are significantly different at $p<0.05$ level from those detected at the vegetative stage at 20/17 °C and from each other at a certain developmental stage

The expression of *HSP80* markedly decreased at the beginning of the cold treatment in CS, whereas there were no or only small changes in the crowns of the other two genotypes (Fig. 5g). In the leaves the transcription of *HSP80* only increased in the double ridge stage in CS(Tsp5A) and it was much greater than in the other two genotypes (Fig. 5h).

Transcription of the genes affecting freezing tolerance

The transcription of the *CBF14* gene was strongly induced by cold in all genotypes in both organs, but decreased to the original levels at the double ridge stage (Fig. 6a, b). A significant difference in the *CBF14* expression between the two

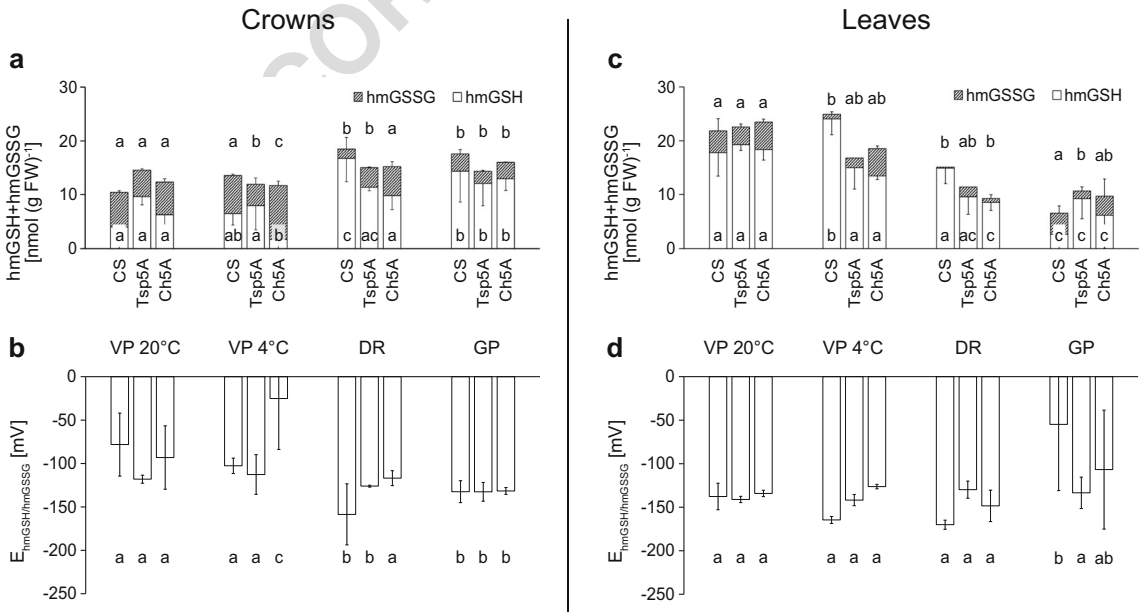

Fig. 3 Concentration and reduction potential of hydroxymethylglutathione during vegetative/generative transition. (**a**): Concentration of hydroxymethylglutathione (hmGSH) and hydroxymethylglutathione disulphide (hmGSSG) in crowns; (**b**): Reduction potential of the hmGSH/hmGSSG couple in crowns; (c): Concentration of hmGSH and hmGSSG in leaves; (**d**): Reduction potential of the hmGSH/hmGSSG couple in leaves. The developmental stage of the plants is described in the legends of Fig. 1. The values indicated by different letters are significantly different at $p<0.05$ level from those detected at the vegetative stage at 20/17 °C and from each other at a certain developmental stage

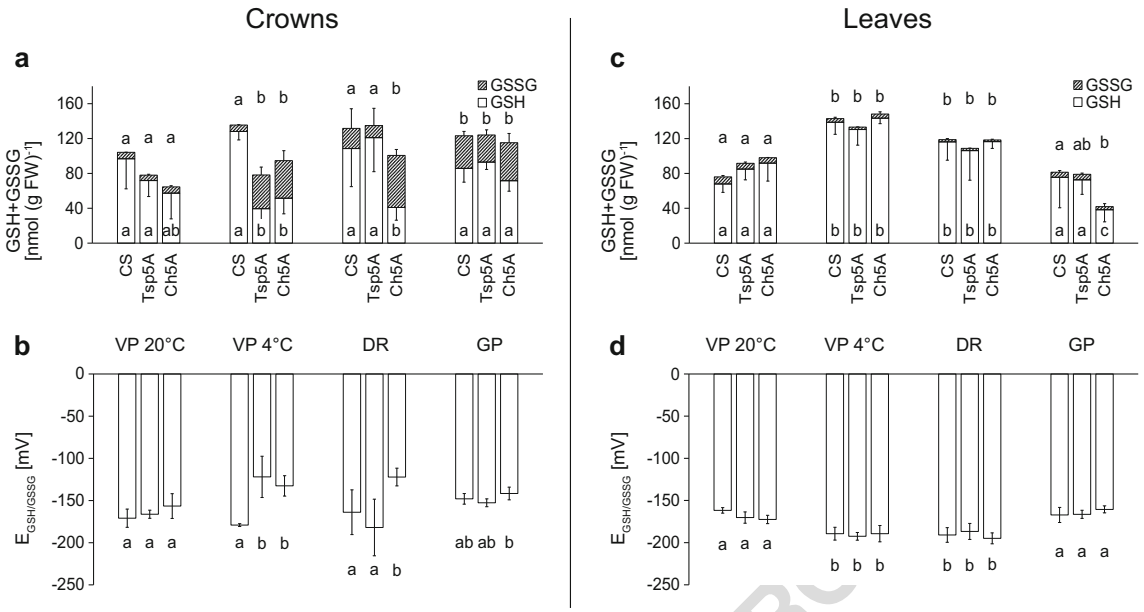

Fig. 4 Concentration and reduction potential of glutathione during vegetative/generative transition. (**a**): Concentration of glutathione (GSH) and glutathione disulphide (GSSG) in crowns; (**b**): Reduction potential of the GSH/GSSG couple in crowns; (**c**): Concentration of GSH and GSSG in leaves; (**d**): Reduction potential of the GSH/GSSG couple in leaves. The developmental stage of the plants is described in the legends of Fig. 1. The values indicated by different letters are significantly different at $p<0.05$ level from those detected at the vegetative stage at 20/17 °C and from each other at a certain developmental stage

substitution lines was observed in the vegetative phase at 4 °C in both organs. More interestingly the relative expression of *CBF14* remained considerable higher in the crown of CS(Ch5A) line even in the generative phase than either in the recipient CS or in the CS(Tsp5A) line. Otherwise the expression of *CBF14* became negligible after the double ridge stage in the leaf samples in all genotypes.

In the case of the *COR14b* transcript level a very strong cold induction was observed in the leaves in each genotype (Fig. 6d). The changes in the expression of this gene show that the cold treatment was effective. In the crowns, similar to the behaviour of *CBF14*, the cold treatment increased the transcript level of *COR14b* significantly only in the most freezing tolerant CS(Ch5A), both in the vegetative and the generative development stages (Fig. 6c).

COR39 expression showed a similar tendency as *CBF14* in both organs, but it also remained higher than the baseline values during the vegetative/generative transition in the leaves (Fig. 6e, f). The effect of chromosome 5A could be seen in the vegetative stage at 4 °C in both organs of the substitution lines.

Discussion

Chromosome 5A affects the timing of the vegetative/generative transition

The observed differences in the timing of vegetative/generative transition in the three genotypes with different 5A chromosomes can be explained by the presence of various *VRN1* alleles encoding an inhibitor of the flowering repressor *ZCCT2*. The *VRN1* allele of CS on chromosome 5A and 5B is vernalization-sensitive (winter growth habit) and that one on 5D is vernalization-insensitive (spring growth habit) and is present in all three genotypes (Whitechurch and Snape 2003; Tóth et al. 2003). Although *vrn-A1* alleles are vernalization-sensitive both in CS and Ch, they differ in one SNP: CS carries the 'Jagger' allele, and Cheyenne carries the 'Wichita' allele (Eagles et al. 2011). This allelic difference explains our observation that the seedlings of the CS(Ch5A) substitution line reached the generative phase one week later than the seedlings of the recipient CS genotype. The *VRN1* allele on chromosome 5A of Tsp is in turn vernalization-insensitive (*Vrn-A1*). This allelic difference can be the main reason for the 4 weeks earlier vegetative/generative transition of CS(Tsp5A) compared to CS and CS(Ch5A).

To exclude the confounding effect of other main regulator genes like *Ppd* (Photoperiod) genes and *phytochrome C* which affect flowering time, long day growth condition (16 h illumination) was applied throughout the experiment (Chen et al. 2014). In fact, the main *Ppd* allele (*Ppd-D1*) locates on 2D chromosome so in this respect there are no allelic differences among the recipient CS and the two 5A substitution lines. However, the *VRN2* locus (containing *ZCCT1* and *ZCCT2* genes) locates on the 5A chromosome so the substitution lines contain different alleles in this locus. The *VRN2* gene is long day dependent, SD can down-regulate the expression of this gene which can cause acceleration of flowering and

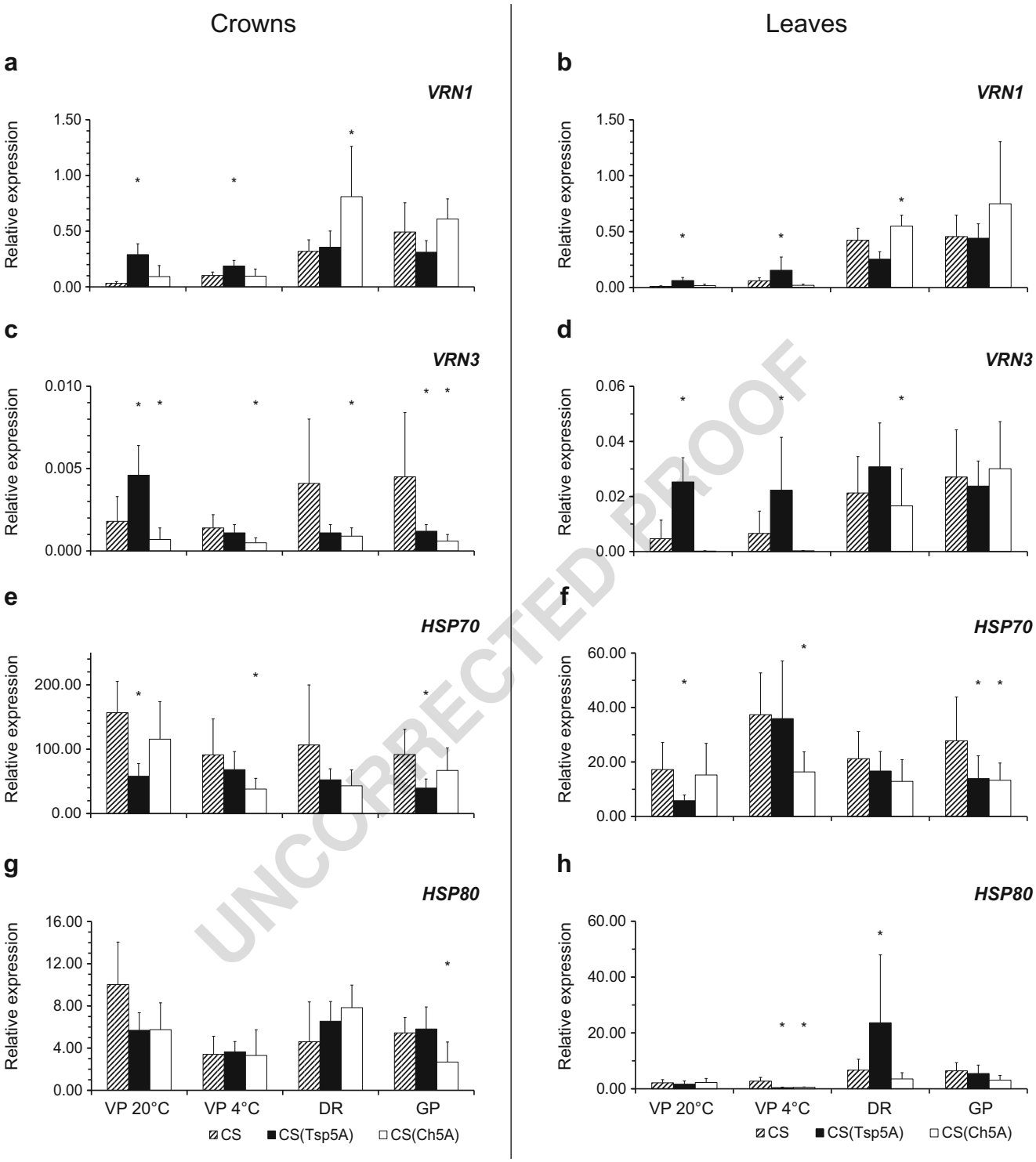

Fig. 5 Relative expression of the genes related to vegetative/generative transition during this process. (**a**) and (**b**): Relative expression of the *VERNALIZATION1* (*VRN1*) gene in the crowns and leaves; (**c**) and (**d**): Relative expression of the *VERNALIZATION3* (*VRN3*) gene in the crowns and leaves; (**e**) and (**f**): Relative expression of the *HEAT SHOCK PROTEIN70* (*HSP70*) gene in the crowns and leaves, (**g**) and (**h**): Relative expression of the *HEAT SHOCK PROTEIN80* (*HSP80*) gene in the crowns and leaves. The values indicated by asterisks are significantly different from the value detected in CS at $p<0.05$ level at a certain developmental stage

elimination of the vernalization requirement (Dubcovsky et al. 2006). So, by applying long day conditions we could avoid this disturbing effect. Similar to our experimental system Laudencia-Chingcuanco et al. (2011) also studied the genome-wide gene expression during the vegetative/generative transition under LD conditions in wheat. The large

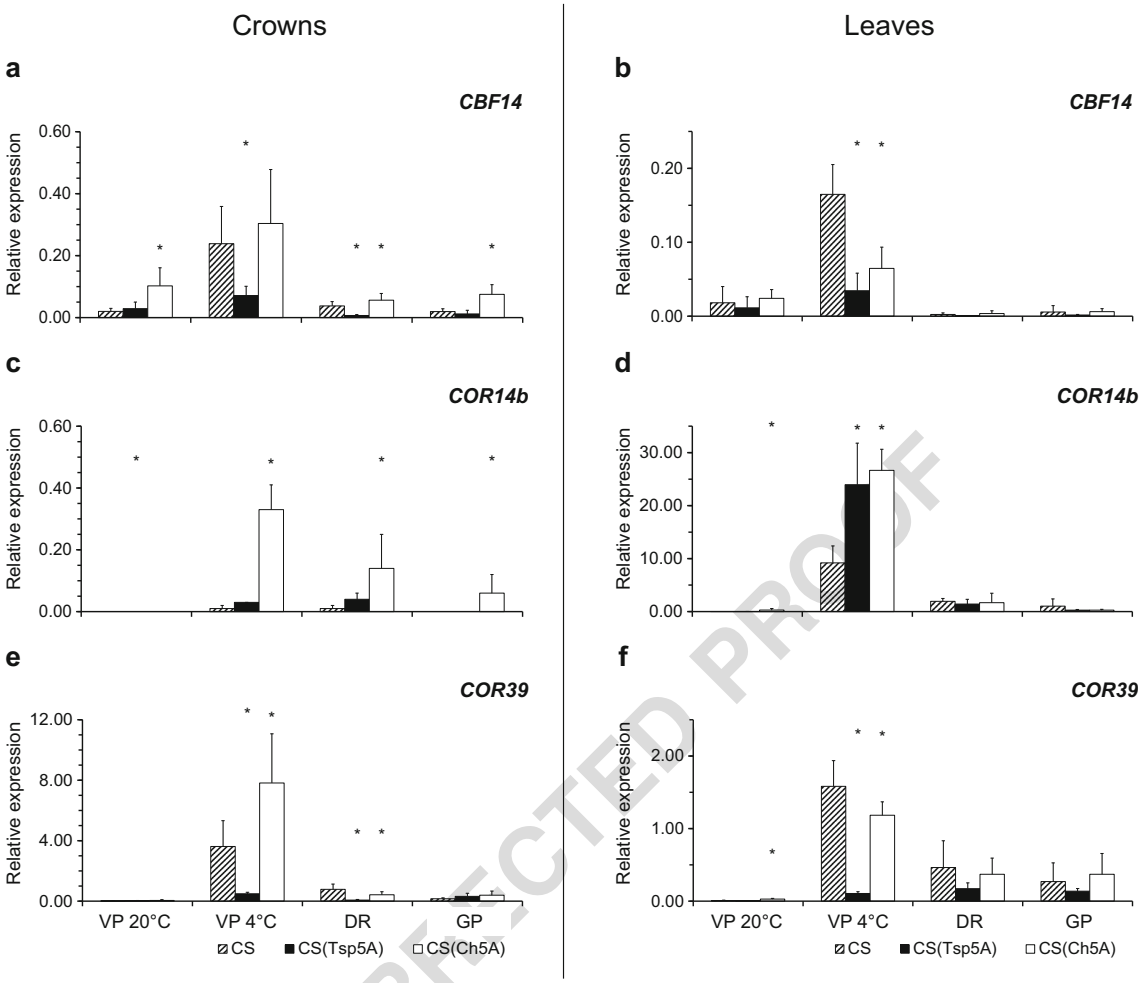

Fig. 6 Relative expression of the genes related to cold acclimation during vegetative/generative transition. (**a**) and (**b**): Relative expression of the *C-REPEAT BINDING FACTOR14* (*CBF14*) gene in the crowns and leaves, (**c**) and (**d**): Relative expression of the *COLD-REGULATED14b* (*COR14b*) gene in the crowns and leaves, (**e**) and (**f**): Relative expression of *COLD-REGULATED39* (*COR39*) gene in the crowns and leaves. The values indicated by asterisks are significantly different from the value detected in CS at $p<0.05$ level at a certain developmental stage

difference in the timing and speed of vegetative/generative transition was also shown in an earlier study using the same genetic system (Soltész et al. 2011).

Redox changes and their suspected control by chromosome 5A during the vegetative/generative transition

The redox changes at low temperature may derive from the altered capacity to use reductants from photosynthesis in various metabolic pathways (Hüner et al. 2012) and can be monitored by the determination of redox state of non-protein thiols. Comparison of Cys, CySS, GSH and GSSG concentrations and their redox potentials in the crowns revealed similar differences between CS, CS(Ch5A) and CS(Tsp5A) (Figs. 2 and 4). The similarities in the alterations of Cys and GSH contents may derive from the regulation of Cys synthesis by GSH (Kocsy et al. 2001). Cultivation at low temperature, which was used for the induction of the vegetative/generative transition in the winter genotypes, resulted in a large increase of CySS and GSSG contents in the crowns but not in the leaves. This is not surprising, as the more oxidizing environment in the crowns could be important for both cold acclimation and vegetative/generative transition (Soltész et al. 2011; Hatano-Iwasaki and Ogawa 2012). The changes in hmGSH and hmGSSG differed from those in the two other thiols, which may indicate their special role in the regulation of stress tolerance and development.

In a previous study the effect of chromosome 5A on thiol levels was observed during a 3-week cold acclimation period (Soltész et al. 2011). Similar to this, in the present study its developmental stage-dependent influence on thiol levels was also shown. During the vegetative/generative transition there was a large difference in the amount and redox state of cysteine and glutathione between the two chromosome 5A substitution lines, which indicates that redox changes mediate the effect of this chromosome on the transition. The importance of the observed differences in this process is also confirmed by

their disappearance during the formation of the spikelet primordia. Chromosome 5A may affect the redox state of the GSH/GSSG couple due to its effect on a glutathione transferase shown by the comparison of the transcriptome profile of CS and the two substitution lines (Kocsy et al. 2010). The chromosome 5A-dependent differences in glutathione metabolism influence the cellular redox environment and the activity of redox-responsive proteins regulating the vegetative/generative transition. Among these proteins, the redox sensitivity of ZCCT2 was shown in our recent experiments (Gulyás et al. 2014).

Expression changes of the genes related to vernalization and their control by chromosome 5A during the vegetative/generative transition

Comparison of the transcriptome profile of chromosome 5A substitution lines indicated that this chromosome affected the expression of many genes, among others the transcription of those which control development (Kocsy et al. 2010). Although the importance of *VRN* genes in the control of flowering was evident from previous studies (for review see Galiba et al. 2009), the developmental stage- and chromosome 5A-dependent differences in their expression have not been shown yet. This is the first study where, using chromosome 5A substitution lines with various *VRN1* alleles, different changes in *VRN1* expression were observed during the vegetative/generative transition. The cold-induced changes in *VRN1* gene expression were much larger in the crowns of CS and CS(Ch5A), where the vegetative/generative transition took place 5 weeks later than in CS(Tsp5A). In CS(Tsp5A), which has two vernalization-insensitive *VRN* alleles (on chromosomes 5A and 5D), *VRN1* transcript levels were high even in the vegetative stage, ensuring the earlier generative transition (Fig. 5). Besides the *VRN* genes, the involvement of *HSP80* in vegetative/generative transition was also shown by the marked increase in its expression in leaves of CS(Tsp5A), a change that was also affected by chromosome 5A. This observation is in accordance with previous experiments in which the preferential expression of *HSP80* was observed in shoot of tomato (Koning et al. 1992). In addition, the control of development by HSPs was also shown in *Arabidopsis* mutants (Sangster and Queitsch 2005). In our experiment the *HSP70* gene was found to be highly cold-inducible, except in the case of the CS(Ch5A) genotype. From the comparison of the two substitution lines it turned out that HSP70 expression increased more intensively in CS(Tsp5A) than in CS(Ch5A) indicating also the regulatory role of chromosome 5A.

Interestingly, the alterations in *VRN1* transcript levels were much more expressed in the leaves as compared to the crowns where the vegetative/generative transition of shoot apices takes place. In addition, in the case of *VRN3* a large difference in transcript levels between the vegetative and generative phase was only observed in the leaves of CS and CS(Ch5A). Similarly, Winfield et al. (2009) also observed a decrease in *ZCCT2* expression only in the leaves of wheat. These findings can be well explained by a recent model proposed by Chen and Dubcovsky (2012). According to this model the regulatory interactions between the VRN proteins take place in the leaves, and VRN3 is transferred from the leaves through the phloem to the shoot apices, where VRN3 induces the vegetative/generative transition.

A relationship between the changes in the amount and redox state of thiols and expression of vernalization-related genes during the generative transition was expected in the present study which was previously observed after pharmacological modification of the redox environment in wheat (Gulyás et al. 2014). However, a parallel change in the gene expression and thiol disulphide levels was only observed in the crowns but not in the leaves. Thus, a general conclusion about the redox control of the vernalization-related genes during the vegetative/generative transition of wheat cannot be drawn.

Transcription of the genes related to freezing tolerance and their regulation by chromosome 5A during the vegetative/generative transition

The difference in the expression of the vernalization-related genes between crowns and leaves during the vegetative/generative transition was observed earlier by the comparison of three other wheat genotypes (Winfield et al. 2009). Such difference was shown not only for these genes but also for those which are involved in cold acclimation and in the control of freezing tolerance in the present experiments. Thus, the expression of *COR14b* and *COR39* gene differed between the crowns and leaves, which can be explained by their specific roles in the individual organs (Fig. 6). Extensive induction of the *CBF14*, *COR14b* and *COR39* genes could only be observed both in crowns and leaves when the seedlings were in the vegetative phase, and their expression decreased to low

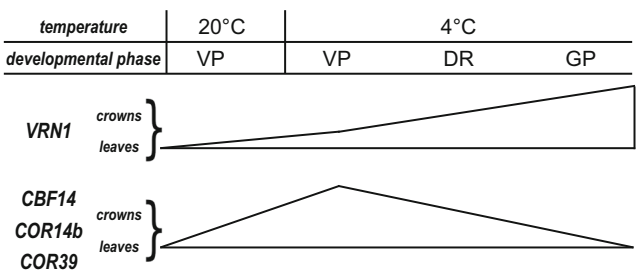

Fig. 7 General trends of changes in the expression of vernalization- and cold responsive genes at low temperature during the vegetative/generative transition. The developmental stage of the plants is described in the legend of Fig. 1

levels during the vegetative/generative transition, when the transcript level of gene controlling flowering (*VRN1*) exhibited large increase (Fig. 7). These results indicate the coordinated control of the cold response and flowering-related genes. The coordination of vernalization and cold acclimation was also studied using *Triticum monococcum* mutants deficient in *VRN1*, but it was not connected to the individual developmental stages (Dhillon et al. 2010). The present study is the first where coordinated expression of the genes controlling these processes in the different developmental stages of the shoot apex is demonstrated.

The above mentioned increased transcript levels of the *CBF14* and *COR39* genes in both organs (leaves and crowns) were observed at the beginning of cold treatment in CS and CS(Ch5A). In these lines the vegetative/generative transition took place 5 weeks later as compared to CS(Tsp5A). The two former genotypes have two vernalization-sensitive *VRN* alleles, whereas the latter has only one, which may contribute to the observed differences in *CBF14* and *COR39* expression due to the coordination of cold acclimation and vernalization. It is worth emphasizing that CS and CS(Ch5A) are more freezing-tolerant than CS(Tsp5A). Identically, marked induction of *COR14b* (Fig. 6) was observed during cold treatment, when apices were still in vegetative phase (at VP 4 °C) in each genotype, but only in leaves. In crowns, which contain the shoot apex, the cold treatment significantly increased the transcript level of *COR14b* only in the most freezing-tolerant CS(Ch5A) line in the vegetative and double ridge stages. It is worth mentioning that the *COR14b* transcript level was still elevated in crown tissues of CS(Ch5A) when the plants had reached the generative phase (Fig. 6c). The same phenomenon was also observed for the *CBF14* transcript levels. Namely, the chromosome 5A of Tsp decreased while the chromosome 5A of Cheyenne increased the expression of *CBF14* in CS genetic background even in the generative phase (Fig. 6). The extended transcription of *CBF14* and *COR14b* (members of CBF-regulon) genes in the CS(Ch5A) line was characteristic only in the crowns but not in the leaves (Fig. 6b and d). Survival of wheat plants after frost damage depends on the intactness of crown tissues (Hoffman et al. 2010). Most likely this is advantageous to the CS(Ch5A) line, and this phenomenon at least partly explains its superior freezing hardiness.

Similar to the vernalization-related genes, the transient changes in the expression of the cold acclimation-related genes in crowns and leaves exhibited no relationship with the continuous cold-induced increase in the thiol disulphide contents observed only in the crowns. Consequently, the hypothesized redox control of these genes was not confirmed in the present experiments.

Conclusions

It can be assumed that chromosome 5A affects both the redox state of thiols and the expression of studied genes in wheat in certain stages of the vegetative/generative transition under low temperature condition. The observed redox and gene expression changes are not associated with each other during the vegetative/generative transition of wheat in the present experiments. If the freezing tolerance-related difference in the accumulation of *COR14b* transcripts can be confirmed in a large set of genotypes it might be a good selection marker for breeding purposes.

Acknowledgments This work was supported by the European Union [FP7-KBBE-2011-5, 289842 – ADAPTAWHEAT], by the Hungarian Research Technology and Innovation Fund [EU BONUS 12-1-2012-0024 and TéT_12_CN-1-2012-0002] and by the Hungarian Scientific Research Fund [OTKA K83642, CNK80781]. Ildikó Vashegyi is Bolyai Fellow of the Hungarian Academy of Sciences.

The authors wish to thank A. Horváth and M. Fehér for their help in plant cultivation and treatment and Gabriella Szalai for help in the HPLC measurement of thiols. Thanks are due to R. Boussicut, F. Taulemesse and V. Allard (INRA, UMR 1095 GDEC, France) for providing the VRN1 and VRN3 primer sequences.

References

Bartoli CG, Casalongué CA, Simontacchi M et al (2013) Interactions between hormone and redox signalling pathways in the control of growth and cross tolerance to stress. Environ Exp Bot 94:73–88. doi:10.1016/j.envexpbot.2012.05.003

Birtić S, Colville L, Pritchard HW et al (2011) Mathematically combined half-cell reduction potentials of low-molecular-weight thiols as markers of seed ageing. Free Radic Res 45:1093–1102. doi:10.3109/10715762.2011.595409

Blanvillain R, Wei S, Wei P et al (2011) Stress tolerance to stress escape in plants: role of the OXS2 zinc-finger transcription factor family. EMBO J 30:3812–3822. doi:10.1038/emboj.2011.270

Chen A, Dubcovsky J (2012) Wheat TILLING mutants show that the vernalization gene *VRN1* down-regulates the flowering repressor *VRN2* in leaves but is not essential for flowering. PLoS Genet 8: e1003134. doi:10.1371/journal.pgen.1003134

Chen A, Li C, Hu W et al (2014) PHYTOCHROME C plays a major role in the acceleration of wheat flowering under long-day photoperiod. Proc Natl Acad Sci U S A 111:10037–10044. doi:10.1073/pnas.1409795111

Dhillon T, Stockinger EJ (2013) *Cbf14* copy number variation in the A, B, and D genomes of diploid and polyploid wheat. Theor Appl Genet 126:2777–2789. doi:10.1007/s00122-013-2171-0

Dhillon T, Pearce SP, Stockinger EJ et al (2010) Regulation of freezing tolerance and flowering in temperate cereals: the VRN-1 connection. Plant Physiol 153:1846–1858. doi:10.1104/pp. 110.159079

Distelfeld A, Dubcovsky J (2010) Characterization of the *maintained vegetative phase* deletions from diploid wheat and their effect on *VRN2* and *FT* transcript levels. Mol Genet Genomics 283:223–232. doi:10.1007/s00438-009-0510-2

Distelfeld A, Li C, Dubcovsky J (2009) Regulation of flowering in temperate cereals. Curr Opin Plant Biol 12:178–184. doi:10.1016/j.pbi.2008.12.010

Dowdle J, Ishikawa T, Gatzek S et al (2007) Two genes in *Arabidopsis thaliana* encoding GDP-L-galactose phosphorylase are required for ascorbate biosynthesis and seedling viability. Plant J 52:673–689. doi:10.1111/j.1365-313X.2007.03266.x

Dubcovsky J, Loukoianov A, Fu D et al (2006) Effect of photoperiod on the regulation of wheat vernalization genes VRN1 and VRN2. Plant Mol Biol 60:469–480. doi:10.1007/s11103-005-4814-2

Eagles HA, Cane K, Trevaskis B (2011) Veery wheats carry an allele of *Vrn-A1* that has implications for freezing tolerance in winter wheats. Plant Breed 130:413–418. doi:10.1111/j.1439-0523.2011.01856.x

Fowler DB, Limin AE, Wang S-Y, Ward RW (1996) Relationship between low-temperature tolerance and vernalization response in wheat and rye. Can J Plant Sci 76:37–42. doi:10.4141/cjps96-007

Galiba G, Quarrie SA, Sutka J et al (1995) RFLP mapping of the vernalization (*Vrn1*) and frost resistance (*Fr1*) genes on chromosome 5A of wheat. Theor Appl Genet 90:1174–1179. doi:10.1007/BF00222940

Galiba G, Vágújfalvi A, Li C et al (2009) Regulatory genes involved in the determination of frost tolerance in temperate cereals. Plant Sci 176:12–19. doi:10.1016/j.plantsci.2008.09.016

Gardner JS, Hess WM, Trione EJ (1985) Development of the Young Wheat spike: A sem study of Chinese Spring Wheat. Am J Bot 72:548–559

Gulyás Z, Boldizsár A, Novák A et al (2014) Central role of the flowering repressor *ZCCT2* in the redox control of freezing tolerance and the initial development of flower primordia in wheat. BMC Plant Biol 14:91. doi:10.1186/1471-2229-14-91

Guo W, Ward RW, Thomashow MF (1992) Characterization of a cold-regulated wheat gene related to *Arabidopsis cor47*. Plant Physiol 100:915–922. doi:10.1104/pp. 100.2.915

Hatano-Iwasaki A, Ogawa K (2012) Overexpression of *GSH1* gene mimics transcriptional response to low temperature during seed vernalization treatment of *Arabidopsis*. Plant Cell Physiol 53:1195–1203. doi:10.1093/pcp/pcs075

Hoffman L, DaCosta M, Ebdon JS, Watkins E (2010) Physiological changes during cold acclimation of perennial ryegrass accessions differing in freeze tolerance. Crop Sci 50:1037. doi:10.2135/cropsci2009.06.0293

Hüner NPA, Bode R, Dahal K et al (2012) Chloroplast redox imbalance governs phenotypic plasticity: the "grand design of photosynthesis" revisited. Front Plant Sci 3:255. doi:10.3389/fpls.2012.00255

Kocsy G, Szalai G, Vágújfalvi A et al (2000) Genetic study of glutathione accumulation during cold hardening in wheat. Planta 210:295–301. doi:10.1007/PL00008137

Kocsy G, von Ballmoos P, Ruegsegger A et al (2001) Increasing the glutathione content in a chilling-sensitive maize genotype using safeners increased protection against chilling-induced injury. Plant Physiol 127:1147–1156. doi:10.1104/pp. 010107

Kocsy G, Athmer B, Perovic D et al (2010) Regulation of gene expression by chromosome 5A during cold hardening in wheat. Mol Genet Genomics 283:351–363. doi:10.1007/s00438-010-0520-0

Kocsy G, Tari I, Vanková R et al (2013) Redox control of plant growth and development. Plant Sci 211:77–91. doi:10.1016/j.plantsci.2013.07.004

Koning AJ, Rose R, Comai L (1992) Developmental expression of tomato heat-shock cognate protein 80. Plant Physiol 100:801–811. doi:10.1104/pp. 100.2.801

Kranner I, Grill D (1996) Determination of glutathione and glutathione disulphide in lichens: a comparison of frequently used methods. Phytochem Anal 7:24–28. doi:10.1002/(SICI)1099-1565(199601)7:1<24::AID-PCA277>3.0.CO;2-2

Kurepin LV, Dahal KP, Savitch LV et al (2013) Role of CBFs as integrators of chloroplast redox, phytochrome and plant hormone signaling during cold acclimation. Int J Mol Sci 14:12729–12763. doi:10.3390/ijms140612729

Laudencia-Chingcuanco D, Ganeshan S, You F et al (2011) Genome-wide gene expression analysis supports a developmental model of low temperature tolerance gene regulation in wheat (*Triticum aestivum L.*). BMC Genomics 12:299. doi:10.1186/1471-2164-12-299

Law CN (1966) The location of genetic factors affecting a quantitative character in wheat. Genetics 53:487–498

Law CN (1967) The location of genetic factors controlling a number of quantitative characters in wheat. Genetics 56:445–461

Law CN, Worland AJ, Giorgi B (1976) The genetic control of ear-emergence time by chromosomes 5A and 5D of wheat. Heredity (Edinb) 36:49–58. doi:10.1038/hdy.1976.5

Nakashima K, Ito Y, Yamaguchi-Shinozaki K (2009) Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. Plant Physiol 149:88–95. doi:10.1104/pp. 108.129791

Paolacci AR, Tanzarella OA, Porceddu E, Ciaffi M (2009) Identification and validation of reference genes for quantitative RT-PCR normalization in wheat. BMC Mol Biol 10:11. doi:10.1186/1471-2199-10-11

Pugsley A (1971) A genetic analysis of the spring-winter habit of growth in wheat. Aust J Agric Res 22:21–31. doi:10.1071/AR9710021

Rapacz M, Wolanin B, Hura K, Tyrka M (2008) The effects of cold acclimation on photosynthetic apparatus and the expression of *COR14b* in four genotypes of barley (*Hordeum vulgare*) contrasting in their tolerance to freezing and high-light treatment in cold conditions. Ann Bot 101:689–699. doi:10.1093/aob/mcn008

Sandve SR, Kosmala A, Rudi H et al (2011) Molecular mechanisms underlying frost tolerance in perennial grasses adapted to cold climates. Plant Sci 180:69–77. doi:10.1016/j.plantsci.2010.07.011

Sangster TA, Queitsch C (2005) The HSP90 chaperone complex, an emerging force in plant development and phenotypic plasticity. Curr Opin Plant Biol 8:86–92. doi:10.1016/j.pbi.2004.11.012

Schafer FQ, Buettner GR (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 30:1191–1212. doi:10.1016/S0891-5849(01)00480-4

Sears AER, The S, Naturalist A, Aug NJ (1953) Nullisomic Analysis in Common Wheat. Am Nat 87:245–252

Shimada S, Ogawa T, Kitagawa S et al (2009) A genetic network of flowering-time genes in wheat leaves, in which an APETALA1/FRUITFULL-like gene, VRN1, is upstream of FLOWERING LOCUS T. Plant J 58:668–681. doi:10.1111/j.1365-313X.2009.03806.x

Soltész A, Tímár I, Vashegyi I et al (2011) Redox changes during cold acclimation affect freezing tolerance but not the vegetative/reproductive transition of the shoot apex in wheat. Plant Biol 13:757–766. doi:10.1111/j.1438-8677.2010.00429.x

Soltész A, Smedley M, Vashegyi I et al (2013) Transgenic barley lines prove the involvement of *TaCBF14* and *TaCBF15* in the cold acclimation process and in frost tolerance. J Exp Bot 64:1849–1862. doi:10.1093/jxb/ert050

Sutka J, Galiba G, Vagujfalvi A et al (1999) Physical mapping of the *Vrn-A1* and *Fr1* genes on chromosome 5A of wheat using deletion lines. Theor Appl Genet 99:199–202. doi:10.1007/s001220051225

Tóth B, Galiba G, Fehér E et al (2003) Mapping genes affecting flowering time and frost resistance on chromosome 5B of wheat. Theor Appl Genet 107:509–514. doi:10.1007/s00122-003-1275-3

Trevaskis B (2010) The central role of the *VERNALIZATION1* gene in the vernalization response of cereals. Funct Plant Biol 37:479–487

Vágújfalvi A, Galiba G, Dubcovsky J, Cattivelli L (2000) Two loci on wheat chromosome 5A regulate the differential cold-dependent expression of the *cor14b* gene in frost-tolerant and frost-sensitive genotypes. Mol Gen Genet 263:194–200. doi:10.1007/s004380051160

Vágújfalvi A, Aprile A, Miller A et al (2005) The expression of several *Cbf* genes at the *Fr-A2* locus is linked to frost

resistance in wheat. Mol Genet Genomics 274:506–514. doi: 10.1007/s00438-005-0047-y

Whitechurch EM, Snape JW (2003) Developmental responses to vernalization in wheat deletion lines for chromosomes 5A and 5D. Plant Breed 122:35–39. doi:10.1046/j.1439-0523.2003.00749.x

Winfield MO, Lu C, Wilson ID et al (2009) Cold- and light-induced changes in the transcriptome of wheat leading to phase transition from vegetative to reproductive growth. BMC Plant Biol 9:55. doi: 10.1186/1471-2229-9-55

Yan L, Loukoianov A, Tranquilli G et al (2003) Positional cloning of the wheat vernalization gene VRN1. Proc Natl Acad Sci U S A 100: 6263–6268. doi:10.1073/pnas.0937399100

Yan L, Fu D, Li C et al (2006) The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. Proc Natl Acad Sci U S A 103:19581–19586. doi:10.1073/pnas.0607142103

AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES.

Q1. Please check corresponding affiliation if correct and appropriate.
Q2. Please check if the affiliations are presented correctly.
Q3. Please check responsible editor if captured name is correct and appropriate.
Q4. Please check if ',' use is correct in numbers used in equations. (298,15 K or 289.15 K) Please note use of '.' in English.