Integration of low-temperature and long-day flowering responses in cereals

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ABSTRACT

Temperate cereals perceive seasonal cues to synchronise flowering with optimal conditions in spring. Two seasonal cues that promote flowering of temperate cereals are prolonged exposure to low temperatures (vernalization) and long days. Vernalization is a pre-requisite for the long-day response, so long-day promotion of flowering occurs after winter as days lengthen in spring. By examining interactions between genes controlling vernalization requirement and daylength responses we have been able to describe how low-temperature and long-day flowering responses are integrated in cereals. The low-temperature response is mediated by *VERNALIZATION1* (*VRN1)*, a *FRUITFULL*-like MADS box gene. *VRN1* is induced by prolonged exposure to low temperatures and acts in the shoot apex to promote inflorescence initiation. *VRN1* also acts in leaves to allow vernalized plants to respond to long days. We will present data to support our model of integration of flowering pathways in cereals and briefly compare the regulatory mechanisms controlling flowering time in cereals with those of Arabidopsis.

INTRODUCTION

Early studies showed that germinating seeds at low temperatures for extended periods can promote flowering of some wheat and barley varieties.[1] Without cold pre-treatment the same varieties grow vegetatively for extended periods and often fail to flower. It was concluded that these cereal varieties have a cold requirement that must be met before flowering can occur.[1]

Subsequently, Purvis[2] showed that cold treatment of germinating rye seeds promotes rapid flowering only when plants are grown in long days after cold treatment. When plants are germinated at low temperatures then grown in short days, the shoot apex develops part way towards flowering but stem elongation does not occur and development of the shoot apex aborts. Purvis concluded that lengthy exposure to low-temperatures during germination exerts an "after effect", which subsequently allows long-days to promote stem elongation and inflorescence development during growth at normal temperatures. Purvis also showed that when plants are germinated at low temperatures then grown in short-days for several weeks they retain the capacity to flower rapidly when shifted to long days, suggesting that cold treatment of seeds is remembered and has a lasting influence on development.[2]

Genes that control flowering time have now been isolated from temperate cereals. These include *VERNALIZATION1 (VRN1)*, *VRN2*, *FT1* (*VRN3*) and *PHOTOPERIOD1* (*PPD1*). *VRN1* encodes a *FRUITFULL*-like MADS box transcription factor, [3,4,5] related to genes that promote inflorescence development in many plant species. *VRN2*[6] and *PPD1*[7] encode proteins similar to those that coordinate circadian rhythm and daylength perception to control activity of the daylength flowering-response pathway. Similarly, *FT1* acts in the daylength flowering response. In Arabidopsis[8] and rice[9] the FT protein is produced in leaves in inductive day-lengths and is then transported to the shoot apex to promote reproductive development. *FT1* is likely to have the same conserved function in temperate cereals.[7]

We have examined how genetic interactions between *VRN1*, *VRN2*, *PPD1* and *FT1* influence vernalization requirement and the daylength flowering-response. This has allowed us to describe the molecular mechanisms that integrate vernalization and daylength flowering responses in cereals[10]. In this paper we re-examine the vernalization studies of Gassner[1] and Purvis[2]. We show that cold treatment induces expression of *HvVRN1* (*Hordeum vulgare VRN1*) in germinating seeds, that expression of *HvVRN1* is subsequently maintained after vernalization, and that this is a prerequisite for the long-day flowering response. The implications of these data for molecular genetic models of flowering time pathways in cereals are discussed.

METHODS AND MATERIALS

Seeds (cv, Sonja, *HvVRN1/HvVRN2/PPD-H1*) were imbibed in foil covered pots and placed at 4 °C for up to 9 weeks. Pots were then moved to glasshouse conditions after different durations of cold treatment and grown in either short days (8 hours light/16 hours dark) or long days (16 hours light/8 hours dark). Apex dissection was performed using binocular dissecting microscopes. RNA extraction and quantitative PCR was performed according to methods which have been described previously.[10]

RESULTS

Germination at low temperatures accelerated development of the shoot apex during subsequent growth at normal temperatures (Figure 1). Plants grown from cold treated seeds also flowered earlier and showed lower final leaf numbers than plants grown from seeds germinated without cold treatment.

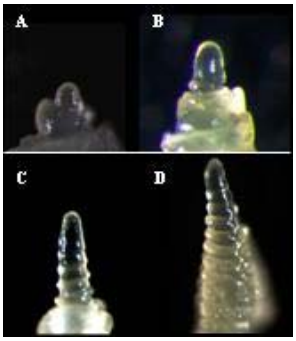

Figure 1. Apex development after different germination and daylength treatments. (A) The shoot apex after 9 weeks cold treatment. (B) The shoot apex at the three leaf stage from a plant grown in long-days without prior cold treatment (C) The shoot apices at the three leaf stage from plants grown in either short (C) or long days (D) from seeds subjected to 9 weeks cold treatment.

Germination at low temperatures promoted apex development to the greatest extent when plants were subsequently grown in long days (Figure 1). In short-days the shoot apex elongated more in plants grown from cold treated seeds than in control plants, and the apex began to produce double ridges by the third leaf stage, but apex development did not progress rapidly beyond this stage and the stem did not elongate. Consequently flowering was delayed. A similar effect was observed when vernalized plants were grown in long days until the double ridge stage then shifted to short days; development of the shoot apex slowed, stem elongation did not occur and flowering was delayed. This shows that long days are required for stem elongation and post-initiation inflorescence development.

Expression of *HvVRN1* was examined during germination in the cold. *HvVRN1* expression increased in seedlings during cold treatment; 4 weeks cold treatment was sufficient to activate expression of *HvVRN1*, but longer cold treatments induced *HvVRN1* expression to higher levels (Figure 2).

Elevated expression of *HvVRN1* was maintained during subsequent development at normal glasshouse temperatures. At the three leaf stage, approximately 2 weeks after the end of cold treatment, *HvVRN1* was expressed at high levels in the shoot apex of vernalized plants, irrespective of daylength (Figure 3a). *HvVRN1* expression also remained high in leaves after germination at low temperatures (Figure 3b).

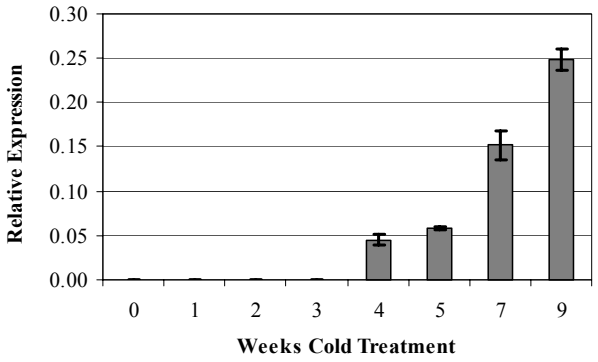

Figure 2. Expression of *HvVRN1* in seedlings germinated at low temperature. *HvVRN1* expression ,relative to *ACTIN*, in seeds during vernalization treatment. Seeds were imbibed (0) then sown in pots at 4^{o}, and harvested for gene expression analysis after different lengths of cold treatment. Error bars show standard error.

Daylength did not influence *HvVRN1* expression in the leaves of plants grown from seeds exposed to cold for 7 weeks during germination, but in plants grown from seeds exposed to cold for 9 weeks during germination expression of *HvVRN1* was higher in the leaves of plants grown in long days (Figure 3B). This suggests that, in addition to the low-temperature response, expression of *HvVRN1* can be further induced in leaves when plants that have been vernalized for long periods are grown in long days.

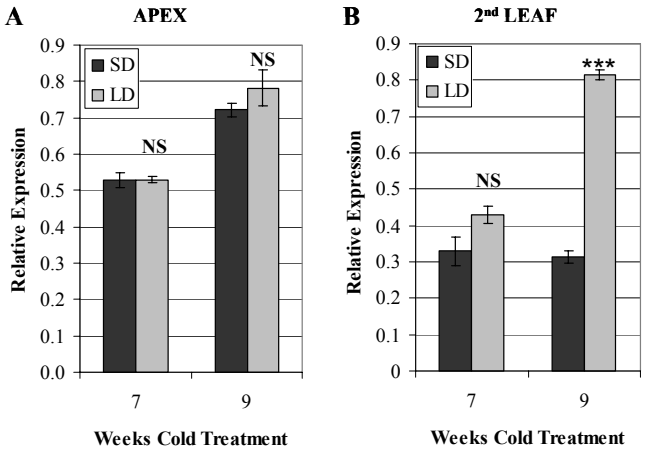

Figure 3. Expression of *HvVRN1* in leaves of vernalized plants. A. Expression of *HvVRN1* relative to *ACTIN*, in the apices of plants harvested at the third leaf stage in either short or long days, after 7 or 9 weeks cold treatment during germination. B. Expression of *HvVRN1* relative to *ACTIN*, in the second leaf of plants harvested at the third leaf stage in either short or long days, after 7 or 9 weeks cold treatment during germination. Error bars show standard error. (Students T-Test: NS non significant difference, ***: $p<0.001$).

DISCUSSION

Exposing germinating barley seeds to lengthy periods of cold exerts an "after effect" which promotes inflorescence initiation when plants are returned to normal growth conditions. The effect of cold is strongest when plants are subsequently grown in long days, but also occurs when plants are grown in short-days. Following germination at low-temperatures, long days also promote stem elongation, inflorescence development and flowering. Thus, vernalization accelerates inflorescence initiation, and allows long days to further accelerate both inflorescence initiation and subsequent stages of inflorescence development. These results are consistent with those of similar experiments using a winter rye[2].

A likely explanation for the after effects of cold is stable activation of *HvVRN1*. When seeds are germinated at low temperatures *HvVRN1* is induced in seedlings and expression levels correlate with the length of cold applied. When plants are shifted to normal growth temperatures *HvVRN1* expression levels remain high in the shoot apex and leaves. We have shown previously that alleles of *HvVRN1* that are expressed at high levels without cold treatment have the same effect on reproductive development as seed vernalization.[10] Thus, the lasting influence of low-temperature treatment on plant development can be explained by stable activation of *HvVRN1* transcription.

Expression of *HvVRN1* probably promotes inflorescence initiation by controlling the identity of cells in the shoot apical meristem. In addition to this role in the shoot apex, *HvVRN1* is likely to act in the leaves to promote long-day induction of *HvFT1*, and thereby promote the long-day flowering response. Hence, *HvFT1* is not expressed in leaves of plants grown from seeds germinated without cold treatment, but is expressed in leaves of plants grown in long days from cold treated seeds where *HvVRN1* is expressed.[10] One possible mechanism by which *HvVRN1* might activate this daylength response is by down-regulating *HvVRN2*, a repressor of *HvFT1*.[10,11]

Long-days caused further up-regulation of *HvVRN1* in leaves of plants that had been vernalized for 9 weeks. This might be mediated by *HvFT1*. In Arabidopsis, *FT* acts through *FLOWERING LOCUS D*, to activate expression of *FRUITFULL* (the Arabidopsis *VRN1* equivalent) in the leaves of long day grown plants.[12] A similar pathway might operate in cereals,[13] but in vernalization responsive barleys this occurs after low-temperature induction of *HvVRN1*. In barley, this response might be limited to leaves as daylength did not influence *HvVRN1* expression in the shoot apex of vernalized plants.

So how do low temperatures activate expression of *HvVRN1*? This is unlikely to involve *HvVRN2* or *FT1*, as neither of these genes is active in seeds germinated in the dark. One possible explanation for cold induction of *HvVRN1* is that histone modifications at the *HvVRN1* locus maintain the gene in a transcriptionally inactive chromatin state, and that these modifications are removed during prolonged cold treatment resulting in a mitotically stable increase in *HvVRN1* activity. Such a mechanism could account for the "after effect" of vernalization observed by Purvis.

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