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The *mvp2* mutation affects the generative transition through the modification of transcriptome pattern, salicylic acid and cytokinin metabolism in *Triticum monococcum*

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ABSTRACT

Wild type and *mvp2* (maintained vegetative phase) deletion mutant *T. monococcum* plants incapable of flowering were compared in order to determine the effect of the deleted region of chromosome 5A on transcript profile and hormone metabolism. This region contains the vernalization1 (*VRN1*) gene, a major regulator of the vegetative/generative transition. Transcript profiling in the crowns of *T. monococcum* during the transition and the subsequent formation of flower primordia showed that 306 genes were affected by the mutation, 198 by the developmental phase and 14 by the interaction of these parameters. In addition, 546 genes were affected by two or three factors. The genes controlled by the deleted region encode transcription factors, antioxidants and enzymes of hormone, carbohydrate and amino acid metabolism. The observed changes in the expression of the gene encoding phenylalanine ammonia lyase (*PAL*) might indicate the effect of *mvp2* mutation on the metabolism of salicylic acid, which was corroborated by the differences in 2-hydroxycinnamic acid and cinnamic acid contents in both of the leaves and crowns, and in the concentrations of salicylic acid and benzoic acid in crowns during the vegetative/generative transition. The amount and ratio of active cytokinins and their derivatives (ribosides, glucosides and phosphates) were affected by developmental changes as well as by *mvp2* mutation, too.

1. Introduction

The exact flowering time is of the key importance for perennial grasses since their reproductive organs are highly sensitive to low temperature. Winter wheat necessitates an exposure to low temperature to fulfil its vernalization requirement and ensure its transition from the vegetative to the reproductive phase. Without cold treatment, winter wheat genotypes are incapable of flowering, while spring genotypes do not have such demands. If the transition to the generative phase occurs too early, even a milder frost may result in great yield loss due to the considerable decrease of freezing tolerance.

Flowering time is controlled by three well characterized gene families (Laurie, 1997). Photoperiod response genes (*Ppd*) sense day length and usually long day (LD) conditions induce their expression. The second gene family contains 'earliness *per se*' factors, which take part in the initiation of floral primordia and in the determination of the numbers of vegetative and generative primordia independently of environmental conditions (Worland, 1996). The third family consists of the vernalization genes *VRN1*, *VRN2* and *VRN3*. Their allelic differences and interactions are important in the timing of the vegetative/generative transition (see for review Distelfeld et al., 2009; Galiba et al., 2009). During vernalization at low temperature, *VRN1* is induced, which inhibits the flowering repressor, *VRN2*. In consequence, the inhibition imposed by

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Abbreviations

Genotypes:
Tm wt or wt *Triticum monococcum* wild type
mvp2 *Triticum monococcum* maintained vegetative phase mutant

Salicylic acid metabolism:
CA cinnamate
oHCA 2-hydroxy-cinnamic acid (*ortho*-hydroxy-cinnamic acid)
BA benzoate
SA salicylate

Light conditions:
Ppd photoperiod response gene
SD short day
LD long day

Development:
VP 20 °C vegetative phase at 20 °C, single ridge structure of the apices
VP 4 °C vegetative phase at 4 °C
DR double ridge, this phenophase shows the vegetative/generative transition during the development
SI initiation of spike primordia

Cytokinins:
CK cytokinin
tZ *trans*-zeatin
DHZ dihydrozeatin
iP isopentenyladenine
cZ *cis*-zeatin
tZR *trans*-zeatin riboside
DHZR dihydrozeatin riboside
iPR isopentenyladenosine
cZR cZ riboside
tZR5′MP tZR5′- monophosphate
DHZR5′MP DHZR5′-monophosphate
iPR5′MP iPR5′-monophosphate
cZR5′MP cZR5′-monophosphate
tZ9G tZ-N^9-glucoside
DHZ9G DHZ-N^9-glucoside
iP9G iP-N^9-glucoside
cZ9G cZ-N^9-glucoside
tZOG tZ-O-glucoside
tZROG tZR-O-glucoside
DHZOG DHZ-O-glucoside
DHZROG DHZR-O-glucoside
cZOG cZ-O-glucoside
cZROG cZR-O-glucoside

VRN2 on *VRN3*, an activator of flowering, is terminated, and the vegetative/generative transition occurs. The role of *VRN1* region in the induction of flowering was demonstrated by using maintained vegetative phase (*mvp2*) mutant that never flowers (Shitsukawa et al., 2007b). The effect of *mvp2* mutation on transcriptome was investigated after one-week cold period when the seedlings were still in the double ridge stage (Diallo et al., 2014). Genes related to transcriptional regulation, sugar metabolism, oxidative and biotic stresses were affected by the mutation. However, no transcriptomic data are available during the formation of spikelet primordia. Detailed analysis revealed that besides the *VRN1* gene, the *AGAMOUS-LIKE GENE 1* [*AGLG1;* control of fruit development (Yan et al., 2003)], the *CYSTEINE PROTEINASE (CYS;* degradation of proteins) and *PHYTOCHROME-C (PHY-C)* genes were also deleted in the *mvp2* mutant. The latter gene encodes a photoreceptor that also affects flowering in a light-dependent manner (Distelfeld and Dubcovsky, 2010; Chen et al., 2014).

Besides the above mentioned major regulators of vernalization, the expression of a large gene set changes during the process of low temperature induced flower initiation as shown by transcriptome analysis in wheat (Gulick et al., 2005; Monroy et al., 2007; Winfield et al., 2009; Majláth et al., 2012). Comparison of the transcriptome profile in a spring and winter wheat genotype during cold treatment showed different expression of genes encoding among others protein kinases, putative transcription factors and Ca-binding proteins (Gulick et al., 2005). Winfield et al. (2009) monitored the developmental-phase-dependent gene expression changes and identified several MADS-box genes, which may play an important role in the onset of flowering. The investigation of cold-induced transcript profile changes in chromosome 5A substitution lines ensured the possibility of obtaining more information about the control of flowering since the *VRN1* gene is localised on this chromosome. The alterations of the transcriptome of plants in vegetative stage have been compared in winter and spring line, and expression of the gene encoding *Dem* (deficient embryo and meristem) protein affecting the development of apical meristem was proved to be different (Kocsy et al., 2010).

Plant growth regulators, especially gibberellins (Mutasa-Gottgens and Hedden, 2009), play an important role in the control of flowering. The auxins also regulate flowering through the members of the AINTEGUMENTA-LIKE/PLETHORA transcription factor family (Krizek, 2011). The role of methyl jasmonate in this process was recently shown in *T. monococcum*, where its level was higher, and many jasmonate-responsive genes were affected in non-flowering *mvp2* mutant (Diallo et al., 2014). In addition, treatment with this hormone delayed flowering with simultaneous downregulation of *VRN1* and *VRN3* genes. Moreover, salicylic acid (SA) participates also in the control of flowering time since SA-deficient plants flower later and UV-C stress activates the vegetative/generative transition in *Arabidopsis* through this hormone (Martínez et al., 2004). The involvement of cytokinins as a long-distance signal of the floral transition process has also been recently shown (Bernier, 2013). Transient cytokinin maximum was observed at the onset of vegetative-generative transition both in *Brassica napus* (Tarkowská et al., 2012) and *Triticum monococcum* (Vanková et al., 2014).

Despite the intensive study of the regulation of flowering processes, the transcriptional and hormonal control during the initial development of flower primordia in wheat plant is still poorly understood. In the present experiments the possible involvement of the *mvp2* mutation-dependent changes of transcriptome profile and the SA and cytokinin metabolism in the control of vegetative/generative transition have been studied.

2. Materials and methods

2.1. Plant material and treatments

Triticum monococcum KU 104-1 strain and its *mvp2* mutant were analysed in this study. The *mvp2* mutant was generated by ion beam radiation and has a large deletion that includes *VRN1* (Shitsukawa et al., 2007b) and the other three genes (Distelfeld and Dubcovsky, 2010).

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 (light cycle started at 2:00 and finished at 18:00), at 260 μmol m^{-2} s^{-1}, 20/17 °C and 75/65% RH in a