Plant Omics Journal POJ

POJ 15(01):13-16 (2022) ISSN:1836-3644
doi: 10.21475/POJ.15.01.22.p3466

Research Note

Oxophytodienoic acid reductase 1 (*HvOPR1*) is differentially expressed during spike development of barley

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Abstract

Interest is increasing towards revealing genes network underlying cereals developmental stages. Pattern of *HvOPR1* gene expression during spike developmental stages has remained unknown. In this study, the relationship between HvOPR1 and spike development in barley was identified. Transcriptomic levels of *HvOPR1* gene were quantified using qRT-PCR. Expression analysis revealed that *HvOPR1* was differentially regulated during spike development. The lowest expression levels were scored for Milk Development and Dough Development stages. One the other hand, the highest expression of *HvOPR1* was noticed during Heading. Our data showed an evidence of a possible regulatory role of HvOPR1 during booting, heading and pollination stages.

Keywords: Barley, spike development, oxophytodienoic acid reductase, gene expression.
Abbreviations: OPR_oxo-phytodienoic acid reductase; JA_jasmonic acid; qRT-PCR _quantitative real-time PCR.

Introduction

The multigene family of oxo-phytodienoic acid reductases (OPRs) catalyze the reduction of double bonds in α,β-unsaturated aldehydes or ketones. They relate to the octadecanoid pathway, which converts linolenic acid to jasmonic acid (JA). Members of this family can be grouped into two classes according to their substrate specificity; OPRI and OPRII (Schaller et al., 1998). OPRI class members preferentially reduce cis-(-) OPDA over cis-(+) OPDA. While members that catalyze the reduction of cis-(+) OPDA, such as OPR3 in *Arabidopsis thaliana*, are directly related to jasmonic acid biosynthesis and belong to OPRII class (Schaller et al., 1998). Recently, an alternative pathway of JA biosynthesis through a peroxisomal OPR3-independent pathway have been described, where 4,5-didehydro-JA is reduced in the cytosol by Arabidopsis OPR2 (OPRI member) in the *atopr3* mutant plants (Chini et al., 2018).

OPR gene family was investigated in many plants, examples include the six OPR genes that were identified in *Arabidopsis thaliana,* and the three genes in tomato (Strassner et al., 2002; Stintzi and Browse, 2000), 13 OPR genes that were reported in rice genome (Agrawal et al., 2004), six OPR genes were characterized in pea (Matsui et al., 2004), and the 48 OPR genes that were recently identified and characterized in wheat (*Triticum aestivum* L.) (Mou et al., 2019).

OPR gene family has been studied well in terms of their role in plant development and response toward biotic and abiotic stresses (Al-Momany and Abu-Romman, 2016). The physiological role in alleviation of photooxidative stress of OPR family members has been thoroughly investigated in Arabidopsis (Biesgen and Weiler, 1999; Stintzi and Browse, 2000; Chini et al., 2018). Also, in *Astragalus sinicus, AsOPR1* Was found to in the regulation of nodule formation and development, as well as affect endogenous JA metabolism (Wei et al., 2017). In a transcriptome analysis of maize inbred lines, differing in their drought tolerance capability performed recently by Zheng et al. (2020), three members of OPRI subgroup (ZmOPR1, 2 and 3), were upregulated in maize roots when drought-sensitive seedlings where subjected to water deficiency (drought) stress for 24, 48 and 72h. Furthermore,

ZmOPR1 and *ZmOPR2* where upregulated when drought-tolerant seedling where subjected to similar conditions.

Individual OPR family members may have distinct functions and expressions in response to specific environmental cues. In barley, the expression profile of two OPRI genes in suggested a role in plant responses and defense to abiotic stresses (Abu-Romman, 2012; Al-Momany and Abu-Romman, 2014). Expression of HvOPR1 was found to be differentially regulated during leaf development (Abu-Romman, 2012). While both enzymes were up-regulated in response to drought, hydrogen peroxide and wounding were increased in response to jasmonic acid and salicylic acid (Abu-Romman, 2012; Al-Momany and Abu-Romman, 2014). However, when treating seedlings with abscisic acid, *HvOPR1* gene was induced, while *HvOPR2* was not affected (Abu-Romman, 2012; Al-Momany and Abu-Romman, 2014). In the current work, the role of HvOPR1 in spike development will be highlighted.

Results and Discussion

According to Zodaks scale of development (Zadoks et al., 1974), cereals undergo ten stages (stage 0 - stage 9) during their lifespan; those are in respect: Germination, Seedling growth, Tillering, Stem elongation, Booting, Heading (Inflorescence emergence), Pollination (Anthesis), Milk Development, Dough Development and Ripening. Each of the aforementioned stages includes several substages. In this experiment, several substages were selected from each of Booting, Heading (Inflorescence emergence), Pollination (Anthesis), Milk Development and Dough Development. Spikes of barley were sampled in 16 spike developmental substages between boots swollen (GS 45) and kernels at hard dough (GS 87).

Interest is increasing globally towards revealing genes network underlying cereals developmental stages. Recent studies have investigated gene expression in selected developmental stages of spike and inflorescence in barley under normal and stress conditions (Liu et al., 2020; Thabet et al., 2020). However, pattern of *HvOPR1* gene expression during spike developmental stages is still remained unknown. In this study, the relationship between HvOPR1 and spike development in barley was identified. Transcriptomic levels of *HvOPR1* gene were quantified using qRT-PCR and presented as fold difference of *HvOPR1* transcripts normalized to barley *α–Tubulin* reference gene using the $2^{-\Delta\Delta Ct}$ method.

Genetic control of different pre-anthesis (pre-heading) stages in wheat and barley is largely unknown and only few studies covered the genetic control of this phase (Borràs-Gelonch et al., 2010). Here, relative expression levels of *HvOPR1* gene during the Booting stage (appearance of the sheath of the flag leaf) of barley spike development were measured in three substages: boots swollen, sheath opening and awns emergence. *HvOPR1* levels reached 14.5 folds at 'awns emergence' to be the highest among booting stage, while the expression levels were 9.7 and 5.4 folds in 'boots swollen' and 'sheath opening' respectively. These data show a possible regulatory effect of HvOPR1 at boots swollen and awns emergence.

Inflorescence architecture in cereals is highly diverse regarding the arrangement of spikelets and flowers (Wang et al., 2018; Liu et al., 2020). In wheat and barley, the molecular network that regulate inflorescence architecture and thus grain yield is an important research focus. In the current study, *HvOPR1* relative expression was detected during the Heading (Inflorescence emergence) stage, in each of the following substages: 1/4 of head emerged, head half emerged, 3/4 of head emerged and full head emerged. Levels of *HvOPR1* were significantly increased in this stage and score 19.5, 18.7 and 21.2 folds in '1/4 of head emerged', 'head half emerged' and '3/4 of head emerged' respectively, then slightly decreased to 16.5 folds when the plant's heads were fully emerged. In addition, transcript levels of *HvOPR1* gene stabled around 15.7 during Pollination (Anthesis) stage when quantified at the 'half anthesis' and 'anthesis complete' substages. Our data showed an evidence of a possible role of HvOPR1 during Heading and Pollination stages. Nevertheless, the coordination between *HvOPR1* gene and other co-expressed genes during these stages need to be revealed.

Interestingly, *HvOPR1* levels were significantly decreased when Milk Development stage began and continued to decrease with time. In 'water ripe' substage *HvOPR1* levels were 6 folds, then reached 4.6 folds at 'early milk' and 3.5 folds at 'medium milk'. At 'late milk', *HvOPR1* expression scored 2.3 folds. Also, during Dough Development stage, *HvOPR1* expression levels continued to decrease in the same manner. At the 'early dough' and 'soft dough' substages, *HvOPR1* levels were 3.3 and 2.8 folds, respectively, while at the 'hard dough', *HvOPR1* expression levels were the lowest level among the group, and among the entire experiment. These data indicated that the role of HvOPR1 declined in late stages of barley's spike development.

Materials and Methods

Plant materials and treatments

Seeds of barley (*Hordeum vulgare*) cv. 'Rum' were planted under greenhouse conditions as previously described (Abu-Romman et al., 2011). Following the Zadoks decimal growth code (Zadoks et al., 1974), spikes of barley were sampled in 16 spike developmental stages between boots swollen (GS 45) and kernels at hard dough (GS 87). Sample collection were performed according to Abu-Romman and Al-Hadid (2017) and collected spikes were quickly frozen in liquid nitrogen.

RNA extraction and HvOPR1 expression analysis

Approximately 60 mg of spike tissue was used for total RNA extraction using Total RNA Purification Kit (Jena Bioscience, Germany). Total RNA concentration was determined by using a spectrophotometer at 260 nm. Synthesis of cDNA was performed with PrimeScript™ RT Master Mix (TaKaRa, Japan) according to manufacturer's instructions using 2 µg of total RNA.

KAPA SYBR® FAST qPCR Kit (KAPA BIO, USA) was used to quantify the relative *HvOPR1* expression by quantitative real-time PCR (qRT-PCR). Data of qRT-PCR were presented as fold difference of *HvOPR1* transcripts normalized to barley *α–Tubulin* reference gene using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008). Table (1) shows primer sequences used in expression analysis.

Table 1. Primers used for the expression analysis of *HvOPR1*.

Gene	Accession	Sequence (5'–3')	Amplicon size (bp)
HvOPR1	JN797728	F-CATGTCCACCAAACTCAACG	188
		R-AACCCTCTGTGACCACCTTG	
α–Tubulin	U40042	F-AATGCTGTTGGAGGTGGAAC	164
		R-GAGTGGGTGGACAGGACACT	

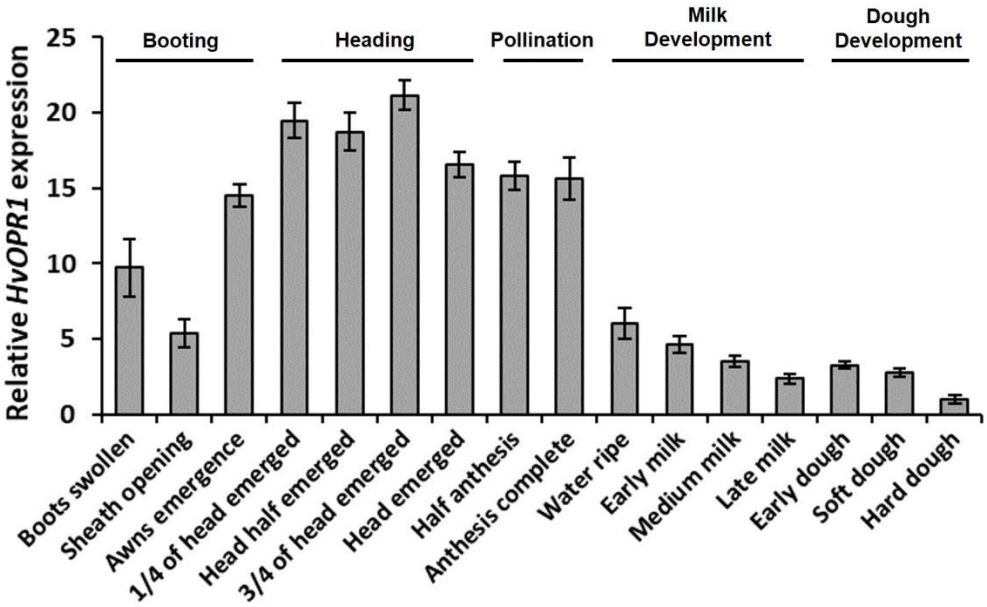

Figure 1. Relative expression levels of *HvOPR1* during spike developmental stages. Relative *HvOPR1* expression was quantified using qRT-PCR. Data of qRT-PCR were presented as fold difference of *HvOPR1* transcripts normalized to barley *α–Tubulin* reference gene using the $2^{-\Delta\Delta Ct}$ method. Each value represents the mean ± standard error of three biological replicates.

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