Short Communication

NJ cluster analysis of the *SnRK2*, *PYR/PYL/RCAR*, and *ABF* genes in Tibetan hulless barley

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Genet. Mol. Res. 15 (4): gmr15048840
Received May 31, 2016
Accepted July 21, 2016
Published November 3, 2016
DOI http://dx.doi.org/10.4238/gmr15048840

ABSTRACT. The abscisic acid (ABA) signaling pathway is known as one of the most important signaling pathways in plants and is mediated by multiple regulators. The genes *SnRK2*, *PYR/PYL/RCAR*, and *ABF* are relevant to both ABA-dependent and -independent signaling pathways. To elucidate the profile of these genes from Tibetan hulless barley (*Hordeum vulgare* L. var. *nudum* Hook. f.), we collected available sequences from RNA-Seq data, together with NCBI data from five other model plant species (*Arabidopsis thaliana*, *Brachypodium distachyon*, *Oryza sativa*, *Populus trichocarpa*, and *Sorghum bicolor*). Gene trees of *SnRK2*, *PYR/PYL/RCAR*, and *ABF* were constructed using a neighbor joining (NJ) method. For all genes, we identified a dominant group in which all six species were represented. Three, four, and five groups were found in the NJ trees of *SnRK2*, *PYR/PYL/RCAR*, and *ABF*,

respectively. For each gene, Tibetan hulless barley was divided into three groups. Our analyses indicated that Tibetan hulless barley was associated with *B. distachyon*. The NJ cluster analysis also suggested that Tibetan hulless barley was affiliated with *S. bicolor* (*SnRK2*), *A. thaliana* (*PYR/PYL/RCAR*), and *O. sativa* (*ABF*). These results illustrate a diverse expression of genes *SnRK2*, *PYR/PYL/RCAR*, and *ABF*, and suggest a relationship among the six species studied. Collectively, our characterization of the three components of the ABA signaling pathway may contribute to improve stress tolerance in Tibetan hulless barley.

Key words: *Hordeum vulgare*; Drought-responsive genes; Neighbor-joining tree

INTRODUCTION

The abscisic acid (ABA) signaling pathway is known as one of the most important signaling pathway in plants. Numerous functional aspects of the ABA signaling pathway are available (de Zelicourt et al., 2016). The pathway includes the ABA-bound pyrabactin resistance/regulatory component of ABA receptor (*PYR/RCAR*) proteins (Fan et al., 2016) and SNF1-related kinases *SnRK2* (Tajdel et al., 2016), *ABF* (Yoshida et al., 2015), etc. The transcription factors *SnRK2*, *PYR/PYL/RCAR*, and others that are located in the cytoplasm lead the stomatal response, whereas transcription factors *SnRK2*, *PYR/PYL/RCAR*, *ABF*, and others, which are located in the nucleus, mediate the drought response (Ng et al., 2014). The following gene families have been identified and characterized in *Oryza* (*SnRK2* family) (Saha et al., 2014), *Arabidopsis* (*PYR/RCAR* family) (Merilo et al., 2013), and *Populus* (*ABF* family) (Ji et al., 2013). Georg-Kraemer et al. (2011) analyzed differential gene expression patterns in *Hordeum euclaston*. Zhou et al. (2015) isolated a novel *EsMcsu1* gene encoding a molybdenum cofactor from *Eutrema salsugineum*, which promotes the ABA biosynthesis and increases drought resistance in alfalfa. However, there is still little information about the *SnRK2*, *PYR/PYL/RCAR*, and *ABF* gene families in Tibetan hulless barley (*Hordeum vulgare* L. var. *nudum* Hook. f.).

Among the important grain crops, barley is reasonably tolerant to abiotic stresses and is abundant in many habitats including roadsides and orchards (Zohary and Hopf, 2000). Tibet is one of the domestication centers of cultivated barley (Dai et al., 2012). The Tibetan hulless barley is adapted to the highly stressful conditions of the Tibetan Plateau (Zeng et al., 2015). Yuan et al. (2015) suggested that an up-regulation of the *HbSINA4* gene was induced by drought stress in the Tibetan hulless barley cultivar, Himalaya 10. The ABA signal transduction pathway was found to be significantly different during the drought response process of Himalaya 10 (Zeng et al., 2016). In the present study, we phylogenetically analyzed and compared three drought-resistance related genes *SnRK2*, *PYR/PYL/RCAR*, and *ABF* from Tibetan hulless barley and another five model plant species. These three genes are welled described in other species such as *Arabidopsis thaliana*, *Brachypodium distachyon*, *Oryza sativa*, *Populus trichocarpa*, and *Sorghum bicolor*. There may be a similar biological function of these genes between the affinity species. The objective was to elucidate the profile of these three drought-resistant related genes from Tibetan hulless barley.

MATERIAL AND METHODS

The Tibetan hulless barley cultivar, Himalaya 10, has developed a strong tolerance and adaptation to water deficit. RNA-Seq of Himalaya 10 under different relative soil moisture content levels has been carried out previously (Zeng et al., 2016). Based on results of the ABA signaling pathway relevant to drought stress from the RNA-Seq data, we selected gene sequences of *SnRK2*, *PYR/PYL/RCAR*, and *ABF* from the RNA-Seq data. Following an extensive literature search using search engines (PubMed, Scopus, Google, etc.), we also collected other available sequences from five additional species: *A. thaliana*, *B. distachyon*, *O. sativa*, *P. trichocarpa*, and *S. bicolor* from NCBI (Table 1). The *SnRK2*, *PYR/PYL/RCAR*, and *ABF* sequences were aligned and clustered in MEGA 6.06 (Tamura et al., 2013). Phylogenies of *SnRK2*, *PYR/PYL/RCAR*, and *ABF* were constructed using the neighbor joining (NJ) method. The branch support was assessed using the bootstrap re-sampling method with 1000 bootstrap replicates.

Table 1. Sequence numbers of three genes from six species (*Arabidopsis thaliana*, *Brachypodium distachyon*, *Hordeum vulgare*, *Oryza sativa*, *Populus trichocarpa*, and *Sorghum bicolor*).

Species	Abbreviation	*SnRK2*	*PYR/PYL/RCAR*	*ABF*
A. thaliana	AT	10	14	14
B. distachyon	Bradi	9	16	10
H. vulgare L. var. *nudum*	HVU	14	19	14
O. sativa	Os	11	15	13
P. trichocarpa	POPTR	15	19	16
S. bicolor	Sb	11	18	9
Total number	-	70	101	76

RESULTS

The NJ tree of *SnRK2* of *A. thaliana*, *B. distachyon*, *H. vulgare* L. var. *nudum*, *O. sativa*, *P. trichocarpa*, and *S. bicolor* (Figure 1) showed the formation of three distinct groups. Groups I and III covered approximately the same number of *SnRK2* genes (20 and 19, respectively), whereas group II comprised approximately half (32) the total number of analyzed *SnRK2* sequences. Group I contained two *SnRK2* of *A. thaliana*, six *SnRK2* of *P. trichocarpa*, and three *SnRK2* of each *B. distachyon*, *H. vulgare* L. var. *nudum*, *O. sativa*, and *S. bicolor*. Group II included four *SnRK2* of *B. distachyon*, six *SnRK2* of *P. trichocarpa*, seven *SnRK2* of *H. vulgare* L. var. *nudum*, and five *SnRK2* each of *A. thaliana*, *O. sativa*, and *S. bicolor*. Group III consisted of four *SnRK2* of *H. vulgare* L. var. *nudum*, and three *SnRK2* each of *P. trichocarpa*, *S. bicolor*, *B. distachyon*, *A. thaliana*, and *O. sativa*. Thus, all six species, including the 14 *H. vulgare* L. var. *nudum SnRK2*, were distributed among all three groups in the *SnRK2* phylogeny. Eleven *SnRK2* of *H. vulgare* L. var. *nudum* appeared to exhibit a close relationship to *B. distachyon*, whereas the other three *SnRK2* showed affinities to *S. bicolor*.

The NJ cluster analysis of the *PYR/PYL/RCAR* genes revealed a different pattern (Figure 2), in which the 101 *PYR/PYL/RCAR* sequences of *A. thaliana*, *B. distachyon*, *H. vulgare* L. var. *nudum*, *O. sativa*, *P. trichocarpa*, and *S. bicolor* were assigned to four groups. One group contained over half of the *PYR/PYL/RCAR* genes represented by all analyzed species. The second largest group (group III) contained about one quarter of the *PYR/PYL/RCAR* sequences, whereas about one-tenth of the sequences were found in each of groups II and IV (10 and 7, respectively).

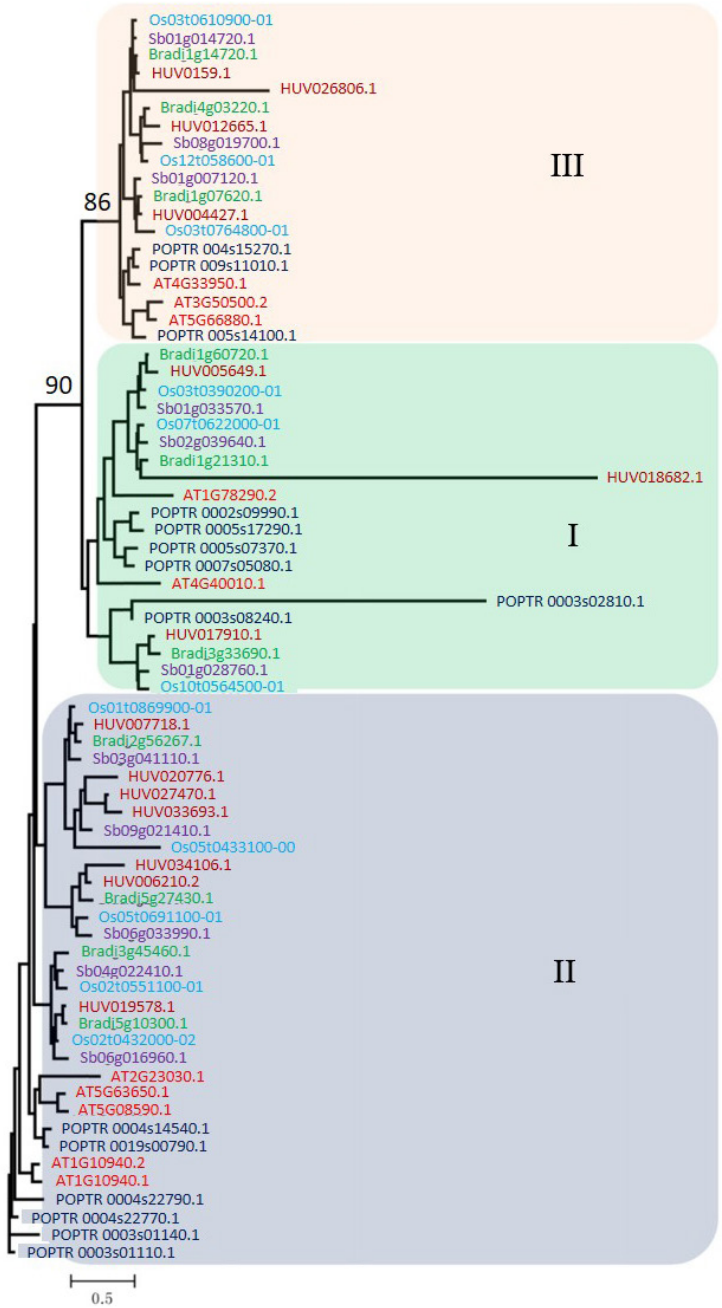

Figure 1. NJ tree of *SnRK2* of *Arabidopsis thaliana*, *Brachypodium distachyon*, *Hordeum vulgare* L. var. *nudum*, *Oryza sativa*, *Populus trichocarpa*, and *Sorghum bicolor*. The colored boxes indicate the three identified *SnRK2* family groups. The scale bar 0.5 indicates substitutions per site in SnRK2. Abbreviations in the sequences are listed here: AT = *A. thaliana*, Bradi = *B. distachyon*, HVU = *H. vulgare* L. var. *nudum*, Os = *O. sativa*, POPTR = *P. trichocarpa*, Sb = *S. bicolor*.

Group I included 6 *PYR/PYL/RCAR* of *H. vulgare* L. var. *nudum*, 8 *PYR/PYL/RCAR* of *O. sativa*, 9 *PYR/PYL/RCAR* of *A. thaliana*, 10 *PYR/PYL/RCAR* of *B. distachyon*, 11 *PYR/PYL/RCAR* of *S. bicolor*, and 14 *PYR/PYL/RCAR* of *P. trichocarpa*. Group II included 2 *PYR/PYL/RCAR* each of *A. thaliana*, *P. trichocarpa*, *B. distachyon*, *O. sativa*, and *S. bicolor*. Group III contained 2 *PYR/PYL/RCAR* of *A. thaliana*, 3 *PYR/PYL/RCAR* of *P. trichocarpa*, 4 *PYR/PYL/RCAR* of *B. distachyon*, 7 *PYR/PYL/RCAR* of *H. vulgare* L. var. *nudum*, and 5 *PYR/PYL/RCAR* each of *O. sativa*, and *S. bicolor*. Group IV consisted of 1 *PYR/PYL/RCAR* of *A. thaliana* and 6 *PYR/PYL/RCAR* of *H. vulgare* L. var. *nudum*. The 19 *PYR/PYL/RCAR* of *H. vulgare* L. var. *nudum* were almost equally distributed among groups I, III, and IV and exhibited a close relationship to *B. distachyon* (group I) and *A. thaliana* (group IV).

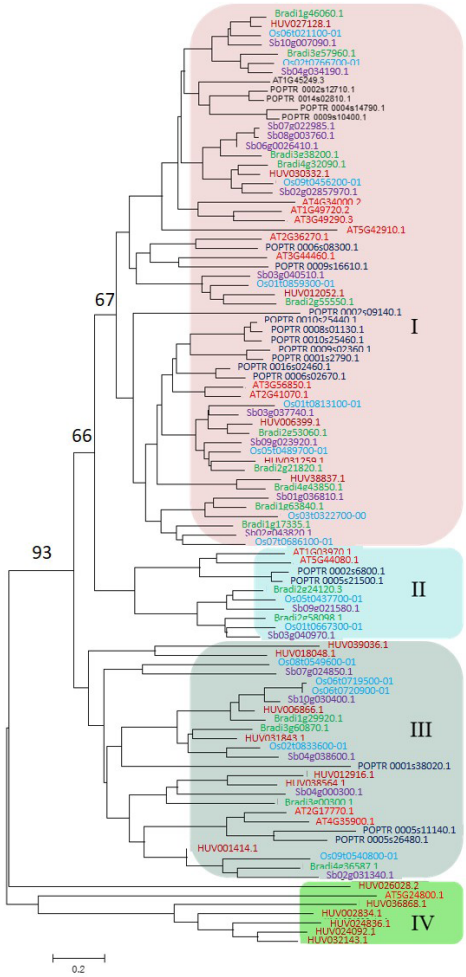

Figure 2. NJ tree of *PYR/PYL/RCAR* family of *Arabidopsis thaliana*, *Brachypodium distachyon*, *Hordeum vulgare* L. var. *nudum*, *Oryza sativa*, *Populus trichocarpa*, and *Sorghum bicolor*. The colored boxes indicate four groups of *PYR/PYL/RCAR*. The scale bar 0.2 indicates substitutions per site in PYR/PYL. Abbreviations in the sequences are listed here: AT = *A. thaliana*, Bradi = *B. distachyon*, HVU = *H. vulgare* L. var. *nudum*, Os = *O. sativa*, POPTR = *P. trichocarpa*, Sb = *S. bicolor*.

Yet another pattern was found in the NJ tree of the *ABF* gene, in which the sequences were distributed into five groups (Figure 3). Group I contained the vast majority (86%) of the *ABF* genes, whereas group II contained a single *P. trichocarpa* gene. Group I included 8 *ABF* of *S. bicolor*, 9 *ABF* of *B. distachyon*, 10 *ABF* of *H. vulgare* L. var. *nudum*, 11 *ABF* of *O. sativa*, and 14 *ABF* each of *P. trichocarpa* and *S. bicolor*. Group III and IV both contained 4 *ABF*; group III included 1 *ABF* each of *B. distachyon*, *H. vulgare* L. var. *nudum*, *O. sativa*, and *S. bicolor*, whereas group IV contained 3 *ABF* of *H. vulgare* L. var. *nudum* and one *ABF* of *O. sativa*. Finally, group V included two *ABF* of *P. trichocarpa*. Similar to that found for *SnRK2* and *PYR/PYL/RCAR*, the 14 *ABF* of *H. vulgare* L. var. *nudum* were also assigned to three groups. *ABF* of *H. vulgare* L. var. *nudum* showed a close relationship to *B. distachyon* (group I) and *O. sativa* (groups III and IV).

Figure 3. NJ tree of *ABF* family of *Arabidopsis thaliana*, *Brachypodium distachyon, Hordeum vulgare* L. var. *nudum*, *Oryza sativa*, *Populus trichocarpa*, and *Sorghum bicolor*. The colored boxes indicate the five *ABF* groups. The scale bar 2 indicates substitutions per site in ABF. Abbreviations in the sequences are listed here: AT = *A. thaliana*, Bradi = *B. distachyon*, HVU = *H. vulgare* L. var. *nudum*, Os = *O. sativa*, POPTR = *P. trichocarpa*, Sb = *S. bicolor*.

DISCUSSION

Based on the importance of the ABA pathway and the available sequences of five other model plant species in NCBI, the gene trees of *SnRK2*, *PYR/PYL/RCAR*, and *ABF* were constructed using an NJ method. For each gene, we identified one dominant group in which all six species were represented. *SnRK2*, *PYR/PYL/RCAR*, and *ABF* from Tibetan hulless barley were scattered into three groups, which is similar to the results found in previous reports. For instance, a phylogenetic analysis of the *ABF* family members found that different plants were distributed in three main groups (Ji et al., 2013). A similar pattern was also observed in a phylogenetic analysis of *SnRK2* from selected higher plants, in which all *SnRK2* genes could be divided into three distinct groups (Wang et al., 2015). Likewise, according to a cluster analysis, *PYR/PYL/RCAR* genes from *Arabidopsis* were divided into three groups when GS was set as 0.32 (Hauser et al., 2011).

Our NJ analysis of *SnRK2* indicated that *H. vulgare* L. var. *nudum* clustered with *B. distachyon* and *S. bicolor*, whereas the phylogenetic tree of *PYR/PYL/RCAR* suggested that there was a close relationship among *H. vulgare* L. var. *nudum*, *B. distachyon*, and *A. thaliana*. Based on the phylogenetic analysis of *ABF*, *H. vulgare* L. var. *nudum* showed a relationship with *B. distachyon* and *O. sativa*. Thus, based on the three gene families, *H. vulgare* L. var. *nudum* is related to *B. distachyon*. Little information about these three genes in Tibetan hulless barley is known prior to the present study. However, an NJ analysis of WRKY domains from *O. sativa*, *A. thaliana*, and *B. distachyon* suggested that the BdWRKY domains were evolutionarily more closely related to the *O. sativa* WRKY domains than those of *A. thaliana* (Wen et al., 2014). Furthermore, a PP2C gene phylogenetic analysis between *B. distachyon* and other plants indicated that the PP2C group in *B. distachyon* was consistent with the PP2C groups found in *Arabidopsis* and *O. sativa* (Cao et al., 2016). A phylogeny of the AP2/EREBP genes suggested that RAV genes in *O. sativa*, *A. thaliana*, and *P. trichocarpa* may share a common ancestor prior to the separation of *Brachypodium* from the other plants (Chen et al., 2016). Together, these results indicate a relationship among *B. distachyon*, *O. sativa*, *A. thaliana*, and *P. trichocarpa*.

The fully sequenced and thoroughly studied *B. distachyon* genome with its relatively small size makes it feasible for matching (International *Brachypodium* Initiative, 2010). Similar to Tibetan hulless barley, *S. bicolor* and *O. sativa* are also monocotyledonous plants. By contrast, *A. thaliana* is a dicotyledon; however, it has a relatively small genome. It was the first plant to have its genome sequenced and the molecular biology underlying many traits are well understood (Genome Assembly, 2016). At least in part, these reasons could explain why Tibetan hulless barley is found to be closely associated with *B. distachyon*, *A. thaliana*, *S. bicolor*, and *O. sativa*.

We phylogenetically analyzed the three genes *SnRK2*, *PYR/PYL/RCAR*, and *ABF*. These three drought-resistance related genes in Tibetan hulless barley were available in the literature of Zeng et al. (2016). Interestingly, we observed that *SnRK2*, *PYR/PYL/RCAR*, and *ABF* of Tibetan hulless barley were all associated with the corresponding genes in *B. distachyon* under certain experimental conditions. It is possible that there is a similarity between the ABA signaling pathway of Tibetan hulless barley and that of *B. distachyon*, because such a mechanism could play a key role in arid environments. Characterization of the three components of the ABA signaling pathway may be used to improve drought tolerance in Tibetan hulless barley.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the following funding sources: the National Program on Key Basic Research Project (#2012CB723006), the National Science and Technology Support Program (#2012BAD03B01), and the Tibet Autonomous Region Financial Special Fund (#2014CZZX001).

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