Short Communication

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

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

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 sequences 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).

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

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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* 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* var. *nudum*, *O. sativa*, and *S. bicolor*, whereas group IV contained 3 ABF of *H. vulgare* 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* var. *nudum* were also assigned to three groups. ABF of *H. vulgare* 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* 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* 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 \textit{SnRK2}, \textit{PYR/PYL/RCAR}, and \textit{ABF} were constructed using an NJ method. For each gene, we identified one dominant group in which all six species were represented. \textit{SnRK2}, \textit{PYR/PYL/RCAR}, and \textit{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 \textit{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 \textit{SnRK2} from selected higher plants, in which all \textit{SnRK2} genes could be divided into three distinct groups (Wang et al., 2015). Likewise, according to a cluster analysis, \textit{PYR/PYL/RCAR} genes from \textit{Arabidopsis} were divided into three groups when GS was set as 0.32 (Hauser et al., 2011).

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

The fully sequenced and thoroughly studied \textit{B. distachyon} genome with its relatively small size makes it feasible for matching (International Brachypodium Initiative, 2010). Similar to Tibetan hulless barley, \textit{S. bicolor} and \textit{O. sativa} are also monocotyledonous plants. By contrast, \textit{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 \textit{B. distachyon}, \textit{A. thaliana}, \textit{S. bicolor}, and \textit{O. sativa}.

We phylogenetically analyzed the three genes \textit{SnRK2}, \textit{PYR/PYL/RCAR}, and \textit{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 \textit{SnRK2}, \textit{PYR/PYL/RCAR}, and \textit{ABF} of Tibetan hulless barley were all associated with the corresponding genes in \textit{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 \textit{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.

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