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RESEARCH ARTICLE

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In silico characterization of boron transporter (BOR1) protein sequences in *Poaceae* species

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ABSTRACT

Boron (B) is essential for the plant growth and development, and its primary function is connected with formation of the cell wall. Moreover, boron toxicity is a shared problem in semiarid and arid regions. In this study, boron transporter protein (BOR1) sequences from some *Poaceae* species (*Hordeum vulgare* subsp. *vulgare*, *Zea mays*, *Brachypodium distachyon*, *Oryza sativa* subsp. *japonica*, *Oryza sativa* subsp. *indica*, *Sorghum bicolor*, *Triticum aestivum*) were evaluated by bioinformatics tools. Physicochemical analyses revealed that most of BOR1 proteins were basic character and had generally aliphatic amino acids. Analysis of the domains showed that transmembrane domains were identified constantly and three motifs were detected with 50 amino acids length. Also, the motif SPNPW EPGSYDHWTVAKDMFNVPPAYIFGAFIPATMVAGLYYFDHSVASQ was found most frequently with 25 repeats. The phylogenetic tree showed divergence into two main clusters. *B. distachyon* species were clustered separately. Finally, this study contributes to the new BOR1 protein characterization in grasses and create scientific base for *in silico* analysis in future.

Key words: BOR1, Boron, *in silico* analysis, *Poaceae*, protein.

Introduction

Boron (B) is crucial for plants and its accessibility in soil and irrigation water affects agricultural yields and crop quality (Tanaka & Fujiwara, 2008). Boron deficiency has effects on many processes in vascular plants such as root elongation, carbohydrate metabolism, pollen germination, nitrogen fixation, and nucleic acid synthesis in plants (Blevins & Lukaszewski, 1998). Besides, boron toxicity is dangerous problem that can restrict plant growth arid and semi-arid soils in the world (Nable et al., 1997). Boron plays important role in occurring cell wall structure and up to 90% of the cellular boron has been used in the cell wall (Loomis & Durst, 1992). Also, boron has functions at the membrane level (Shelp et al., 1995), the cells need boron to maintain membrane integrity (Cakmak et al., 2005), and some enzymes require boron compounds in plants for catalytic process (Power & Woods, 1997).

In last decade, a few B-uptake/transport related genes have been found in various plant organisms. *Arabidopsis thaliana* BOR1 was the first gene related to B-transport, which decreases boric acid in the cell for xylem loading and it is crucial for preventing B-deficiency in shoots (Takano et al., 2002). Six BOR1-like genes were identified in *Arabidopsis* and some plant species have similar BOR1 genes. *Oryza sativa* BOR1-like gene (OsBOR1) is similar to AtBOR1 with its three paralogs (OsBOR2, OsBOR3 and OsBOR4) (Nakagawa et al., 2007). In Algerian barley landrace Sahara 3771 (Sahara), Bot1 gene was identified as AtBOR1 ortholog for the boron-toxicity tolerance (Sutton et al., 2007). NIP5-1 belongs to the major intrinsic protein family that regulates B-uptake for plant development under B-limitation (Takano et al., 2006). Also, NIP6-1 is a similar gene to NIP5-1 that promotes boric acid transportation via the membrane (Tanaka et al., 2008).

Today, the most efficient method to analyze the gene function is to investigate sequence similarity of the protein or DNA. Particularly, homolog protein sequences indicate enormous amount of evolutionary information. The aim of this study was to investigate the protein sequences of boron transporter protein (BOR1) from different *Poaceae* plants by using bioinformatics tools. Results support opportunities to understand the characteristics and phylogenetic relationship of boron transporter proteins in major cereal species.

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Materials and Methods

Sequence data

AtBOR1 (NP_850469) protein sequence has been published and this protein sequence was obtained from NCBI (http://www.ncbi.nlm.nih.gov) and used as a reference. We performed database searches against protein sequences (Blastp) and searching parameters as follows: E-value $\leq e^{-10}$ and other parameters were defaulted. All the sequences of boron transporter protein were downloaded in FASTA format from NCBI. A total of 20 protein sequences were used from different *Poaceae* species for *in silico* analysis.

The sequences were aligned using the ClustalW program (http://www2.ebi.ac.uk/clustalw; Larkin et al., 2007). Phylogenetic analysis was performed by using MEGA 5.1 software (Tamura et al., 2011) by bootstrap analyses with 1000 replications (Felsenstein, 1985). Bootstrapping is a generally used method to obtain reliable trees by resampling the dataset many times (Soltis & Soltis, 2003). The evolutionary history was evaluated using the Neighbor-Joining (NJ) method and distance matrix data as input that determines the distance between each pair of taxa (Saitou & Nei, 1987). Physicochemical analyses (molecular weight, pI and amino acid composition) were done by ProtParam, which is an online tool available on ExPASy proteomics server and it permits the calculation of various physical and chemical parameters (Gasteiger et al., 2005). Analysis of domain and conserved protein motifs was conducted using MEME (http://meme.sdsc.edu/meme/meme.html), which can be used discovering and comparing motifs related DNA or protein sequences (Bailey et al., 2009). The analyses of protein domain were characterized using SMART (Simple Modular Architecture Research Tool; http://smart.embl-heidelberg.de/) which allows the identification and annotation of genetically mobile domains and the analysis of domain architectures (Letunic et al., 2012). The subcellular distribution and N-glycosylation sites of the BOR1 proteins were predicted by using WoLF PSORT (http://wolfpsort.org/) (Horton et al., 2007) and the NetNglyc 1.0 (http://www.cbs.dtu.dk/services/NetNGlyc/) servers, respectively.

Results and Discussion

In present study, we performed *in silico* analysis of boron transporter protein sequences from some *Poaceae* species by bioinformatics applications that one reference sequence (*A. thaliana* as outgroup species) and twenty *Poaceae* species sequences were used (Table 1 and Figure 1). Physicochemical analysis revealed that the most abundant amino acid residues were Ala, Leu, Ile, Ser, and Val. Especially, aliphatic amino acids (alanine, leucine, isoleucine, valine, etc.) were identified most frequently in the protein sequences. An average length of protein sequence and molecular weight of BOR1 protein sequence were found 679 amino acids and 75982.152 Da, respectively. Isoelectric point (pI) refers net charge of proteins. Computed pI value lower than 7 (pI<7) indicates that proteins were acidic or higher than 7 (pI>7) indicates that proteins were with basic character.

Table 1. *List of boron transporter protein sequences in some Poaceae species.*

Species	**NCBI accession number**
Arabidopsis thaliana	NP_850469
Hordeum vulgare subsp. *vulgare*	BAJ97664, BAK06588, ABS83564, ABS83562, BAJ87955
Zea mays	NP_001151747, NP_001147798, NP_001167745
Brachypodium distachyon	XP_003579069, XP_003569518, XP_003568821
Oryza sativa subsp. *japonica*	NP_001067049, AAQ02664, ABG22050, ABD78951, ABD78950
Oryza sativa subsp. *indica*	EEC69514, EEC78613
Sorghum bicolor	XP_002455089
Triticum aestivum	ABX26206

The computed pI value of NP_850469, NP_001067049, AAQ02664, NP_001151747, NP_001167745, EEC78613, BAJ97664, BAK06588, ABG22050, EEC69514, ABD78951, XP_003579069 (pI>7) were considered as basic, while NP_001147798, XP_003569518, XP_002455089, ABD78950, ABX26206, ABS83564, ABS83562, XP_003568821, and BAJ87955 (pI<7) were considered as acidic. The most acidic and basic protein sequences were ABX26206 (*Triticum aestivum*, pI: 5.87) and EEC69514 (*Oryza sativa* subsp. *indica*, pI: 9.14), respectively (Table 2).

A total of three different conserved motifs were detected by using MEME software and the repeat numbers were ranging from 21 to 25 with width and best possible match amino acid sequences are shown in Table 3 and Figure 2.

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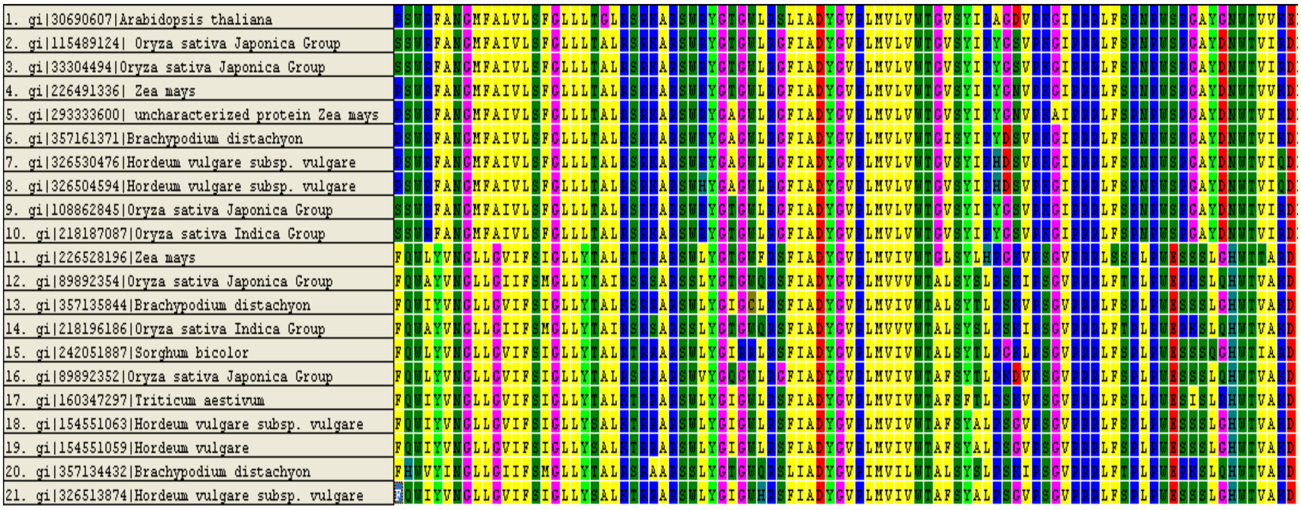

Figure 1. *Multiple alignment of the amino acid sequences of boron transporter protein 98-198.*

Sequence of particular residue types was known as a pattern, motif, signature or fingerprint thought to important conserved regions that infer protein structure and sequence evolution history (Sahay & Shakya, 2010). Our findings were consistent with this hypothesis and three conserved regions were found in protein sequences with 50 amino acids length. Therefore, these conserved regions may prove BOR1 protein stability in plants. Based on combine block diagram of BOR1 proteins, all protein sequences had motif I, motif II, and motif III, respectively. It can be suggested that these motifs were related to transport activity and demonstrating stability of the proteins.

Protein domains are part of protein sequences and many proteins have one or more domains, which performed different biochemical functions (Zhang et al., 2012). Domain analysis of BOR1 protein sequences revealed the presence of transmembrane domain consistently (Table 4). Transmembrane domains play important role on some biological processes such as anion exchange, ion transport etc. BOR1 gene indicates significant homology to animal bicarbonate (HCO^{-3}) transporters (Takano et al., 2002). Thus, domain analysis of BOR1 protein sequences supports this homology. Also, predicted subcellular localizations revealed that all BOR1 proteins were located as integral membrane proteins and some members were located in cytoplasma, nucleus, vacuole, and endoplasmic reticulum, respectively. Hence, these proteins could provide transport functions. Predicted N-glycosylation sites were detected in all BOR1 protein sequences of grasses and the number of N-glycosylation sites ranged from 1 to 3 (Table 4). Except from three sequences, the others contain two or more glycosylation sites. 279 NWTV, 391 NASL, and 143 NASN were found with highest numbers, whereas *Zea mays* (NP_001147798), *Brachypodium distachyon* (XP_003569518), and *Sorghum bicolor* (XP_002455089) had one site with 143 ASN. Glycosylation is an important post-translational modification, and could affect protein folding, localization and trafficking, as well as protein solubility. The N-X-S/T consensus sequence was accepted as a general recognition element (Schwarz & Aebi, 2011). These glycosylation sites may cause to new catalytic functions in BOR1 proteins for regulating transport activities in grasses.

To understand the evolutionary relationships of plant boron transporter proteins from *Poaceae* family, a phylogenetic analysis was performed by using MEGA 5.1. (Figure 3). The evolutionary history was evaluated based on the Neighbor-Joining method by bootstrap technique. The phylogenetic tree separated into two divergent groups with 100% value and reference AtBOR1 protein sequence (as outgroup species) was located in main subgroup II. *Zea mays* and *Sorghum bicolor* were clustered together with the highest bootstrap value 100% and this result is in agreement with taxonomic data that *Z. mays* and *S. bicolor* belong to same subfamily *Panicoideae*. Also, this result might explain that these plants have similar evolutionary dynamics. Except boron transporter 4-like protein *B. distachyon* (XP_003569518); *B. distachyon*, *H. vulgare* and *T. aestivum* protein sequences were clustered together in main subgroup I and *B. distachyon* and *H. vulgare* were clustered together in main subgroup II.

Table 2. *Parameters computed using Expasy's ProtParam tool in BOR1 proteins including sequence length, molecular weight (MW), and pI values.*

Organism and protein name	**Accession Number**	**Sequence lenght**	**MW**	**pI**
[*Oryza sativa* Japonica Group]	NP_001067049	711	79335.8	9.07
Boron transporter [*Oryza sativa* Japonica Group]	AAQ02664	711	79335.8	9.07
[*Zea mays*]	NP_001151747	709	78968.	9.13
Uncharacterized protein LOC100381433 [*Zea mays*]	NP_001167745	702	78327.7	9.00
PREDICTED: probable boron transporter 2-like [*Brachypodium distachyon*]	XP_003579069	712	79252.7	8.88
Predicted protein [*Hordeum vulgare* subsp. *vulgare*]	BAJ97664	712	79267.6	8.72
Predicted protein [*Hordeum vulgare* subsp. *vulgare*]	BAK06588	712	79218.5	8.60
Boron transporter 1, putative, expressed [*Oryza sativa* Japonica Group]	ABG22050	637	71538.1	8.73
Hypothetical protein OsI_38753 [*Oryza sativa* Indica Group]	EEC69514	588	65787.6	9.14
Boron transporter-like protein 2 [*Zea mays*]	NP_001147798	664	74278.4	6.17
Boron transporter [*Oryza sativa* Japonica Group]	ABD78951	677	76296.0	8.63
PREDICTED: boron transporter 4-like [*Brachypodium distachyon*]	XP_003569518	666	74438.8	6.75
Hypothetical protein OsI_18656 [*Oryza sativa* Indica Group]	EEC78613	673	75847.4	8.04
Hypothetical protein SORBIDRAFT_03g004180 [*Sorghum bicolor*]	XP_002455089	670	74924.6	6.42
Boron transporter [*Oryza sativa* Japonica Group]	ABD78950	672	75582.9	6.24
Boron transporter 2 [*Triticum aestivum*]	ABX26206	666	74429.5	5.87
Boron transporter [*Hordeum vulgare* subsp. *vulgare*]	ABS83564	666	74460.8	6.48
Boron transporter [*Hordeum vulgare*]	ABS83562	666	74544.9	6.37
PREDICTED: boron transporter 4-like [*Brachypodium distachyon*]	XP_003568821	678	76620.1	6.82
Predicted protein [*Hordeum vulgare subsp. vulgare*]	BAJ87955	666	74564.9	6.40

This finding supports taxonomic data that *Brachypodium*, *Triticum* and *Hordeum* belong to the same subfamily *Pooideae*. *Oryza* species generally were grouped together both in main subgroup I and subgroup II. *Oryza* clades can be explained that rice BOR1 genes diverged recently from monocots during evolutionary history and protein sequences of rice may prove this genetic background. Orthologs are genes in different organisms and orthologous proteins have similar regulation, and usually the same specificity in close organisms (Makarova et al., 1999). Phylogenetic analysis revealed that protein sequences of close plant species clustered together and it was thought that ortholog protein sequences of different close species may cause observed phylogenetic relationships (Figure 3).

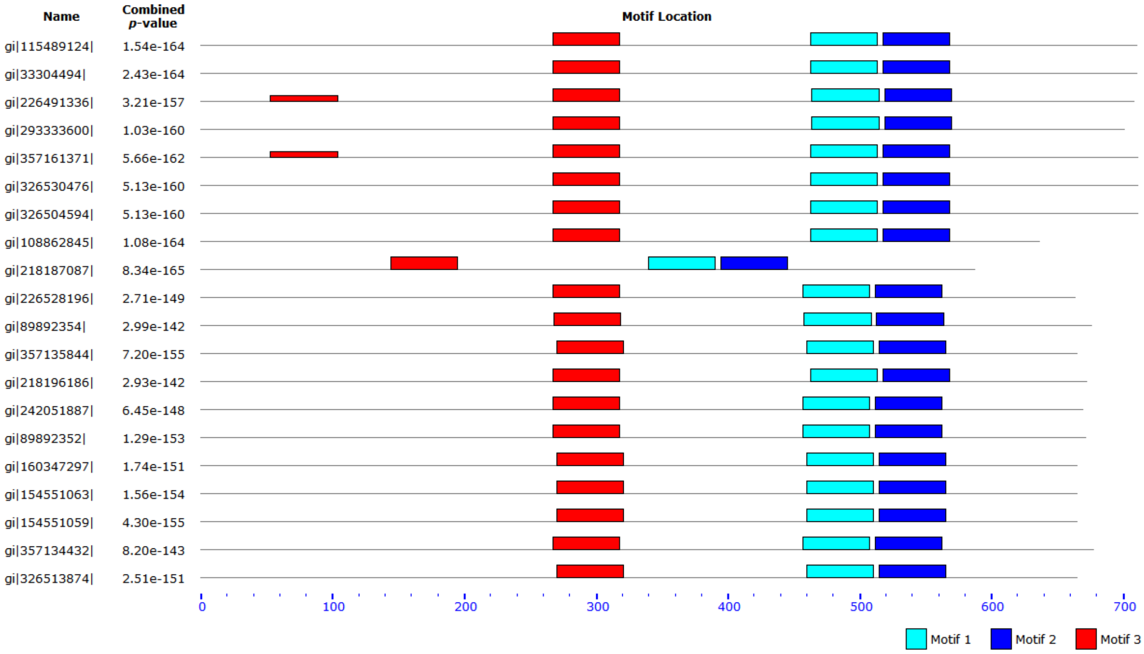

Figure 2. *Combined block diagrams of the conserved protein motifs in Poaceae BOR1 proteins.*

Table 3. *Different motifs commonly observed in boron transporter protein sequences with best possible match amino acid sequences.*

Motif number	Width Sequence	Protein sequences	Repeat numbers
1	50	EQRLSNLLQSTMVGGCVGAMPVIKKIPTSVLWGYFAYMAIDSLPGNQFWE	23
2	50	FTAPSRRYKVLEEYHTTFVETVPFKTIAMFTIFQTVYLLICFGITWIPIA	21
3	50	SPNPWEPGSYDHWTVAKDMFNVPPAYIFGAFIPATMVAGLYYFDHSVASQ	25

Sequence divergence infers evolutionary history of genes and its product proteins that occurs by point mutations and small deletions and insertions (Krylov et al., 2003). According to sequence similarity matrix analysis (data not shown), BOR1 protein sequences of *B. distachyon* (3 sequences), Z. *mays* (3 sequences), *H. vulgare* (5 sequences), *O. sativa* subsp. *japonica* (5 sequences), and *O. sativa* subsp. *indica* (2 sequences) showed sequence similarity ranging from 49.4% to 68.1%, from 51.5% to 95%, from 49.2% to 99.7%, from 51.4% to 99.8%, and 39.3%, respectively. *A. thaliana* sequence was the most similar to *O. sativa* subsp. *japonica* (76.9%, NP_001067049), *T. aestivum* sequence was the most similar to *H. vulgare* (90%, ABS83562), and *S. bicolor* sequence was the most similar to *Z. mays* (91.7%, NP_001147798). Interestingly, the lowest sequence similarity was found in *O. sativa* subsp. *indica* sequences (39.3%) while the highest sequence similarity was found in *O. sativa* subsp. *japonica* (99.8%). Protein families were grouped as ancient proteins, middle-age proteins, and modern proteins. Ancient proteins were used as metabolism enzymes mostly (Doolittle et al., 1986.) BOR1 is probably a member of a family of highly conserved membrane proteins in plants (Takano et al., 2002). Also, BOR1 proteins were detected both in prokaryote and eukaryote organisms, thus BOR1 proteins may be ancient proteins in plants.

Table 4. *Predicted domain, subcellular localization, and N- glycosylation sites of boron transporter protein sequences.*

Protein name and organism	**Accession number**	**Predicted domain**	**Predicted subcellular localization**	**Predicted N-glycosylation sites**
[*Oryza sativa* Japonica Group]	NP_001067049	Transmembrane	Integral membrane protein	279 NWTV, 391 NASL
Boron transporter [*Oryza sativa* Japonica Group]	AAQ02664	Transmembrane	Integral membrane protein, endoplasmic reticulum	279 NWTV, 391 NASL
[*Zea mays*]	NP_001151747	Transmembrane	Integral membrane protein, vacuole, endoplasmic reticulum	279 NWTV, 391 NASL
Uncharacterized protein LOC100381433 [*Zea mays*]	NP_001167745	Transmembrane	Integral membrane protein, endoplasmic reticulum	279 NWTV, 391 NASL
PREDICTED: probable boron transporter 2-like [*Brachypodium distachyon*]	XP_003579069	Transmembrane	Integral membrane protein, cytoplasm	279 NWTV, 391 NASL
Predicted protein [*Hordeum vulgare* subsp. *vulgare*]	BAJ97664	Transmembrane	Integral membrane protein, cytoplasm	279 NWTV, 391 NASL
Predicted protein [*Hordeum vulgare* subsp. *vulgare*]	BAK06588	Transmembrane	Integral membrane protein, endoplasmic reticulum	279 NWTV, 391 NASL
Boron transporter 1, putative, expressed [*Oryza sativa* Japonica Group]	ABG22050	Transmembrane	Integral membrane protein, vacuole, endoplasmic reticulum	279 NWTV, 391 NASL, 634 NSTC
Hypothetical protein OsI_38753 [*Oryza sativa* Indica Group]	EEC69514	Transmembrane	Integral membrane protein, vacuole, endoplasmic reticulum	156 NWTV, 268 NASL
Boron transporter-like protein 2 [*Zea mays*]	NP_001147798	Transmembrane	Integral membrane protein, cytoplasm, vacuole	143 NASN
Boron transporter [*Oryza sativa* Japonica Group]	ABD78951	Transmembrane	Integral membrane protein, cytoplasm	61 NDTD, 329 NPSA, 392 NASS
PREDICTED: boron transporter 4-like [*Brachypodium distachyon*]	XP_003569518	Transmembrane	Integral membrane protein, cytoplasm	143 NASN
Hypothetical protein OsI_18656 [*Oryza sativa* Indica Group]	EEC78613	Transmembrane	Integral membrane protein, vacuole	60 NDTD, 328 NPSA, 397 NASS
Hypothetical protein SORBIDRAFT_03g004180 [*Sorghum bicolor*]	XP_002455089	Transmembrane	Integral membrane protein, endoplasmic reticulum	143 NASN
Boron transporter [*Oryza sativa* Japonica Group]	ABD78950	Transmembrane	Integral membrane protein, cytoplasm, vacuole	143 NASN, 668 NLSE
Boron transporter 2 [Triticum aestivum]	ABX26206	Transmembrane	Integral membrane protein, nucleus, cytoplasm	60 NETD, 143 NASN
Boron transporter [*Hordeum vulgare* subsp. *vulgare*]	ABS83564	Transmembrane	Integral membrane protein, cytoplasm, nucleus	60 NETN, 143 NASN
Boron transporter [*Hordeum vulgare*]	ABS83562	Transmembrane	Integral membrane protein, cytoplasm	60 NETN, 143 NASN
PREDICTED: boron transporter 4-like [*Brachypodium distachyon*]	XP_003568821	Transmembrane	Integral membrane protein, nucleus, cytoplasm	328 NPSA, 393 NCSE, 615 NESI
Predicted protein [*Hordeum vulgare* subsp. *vulgare*]	BAJ87955	Transmembrane	Integral membrane protein, nucleus, cytoplasm	60 NETN, 143 NASN

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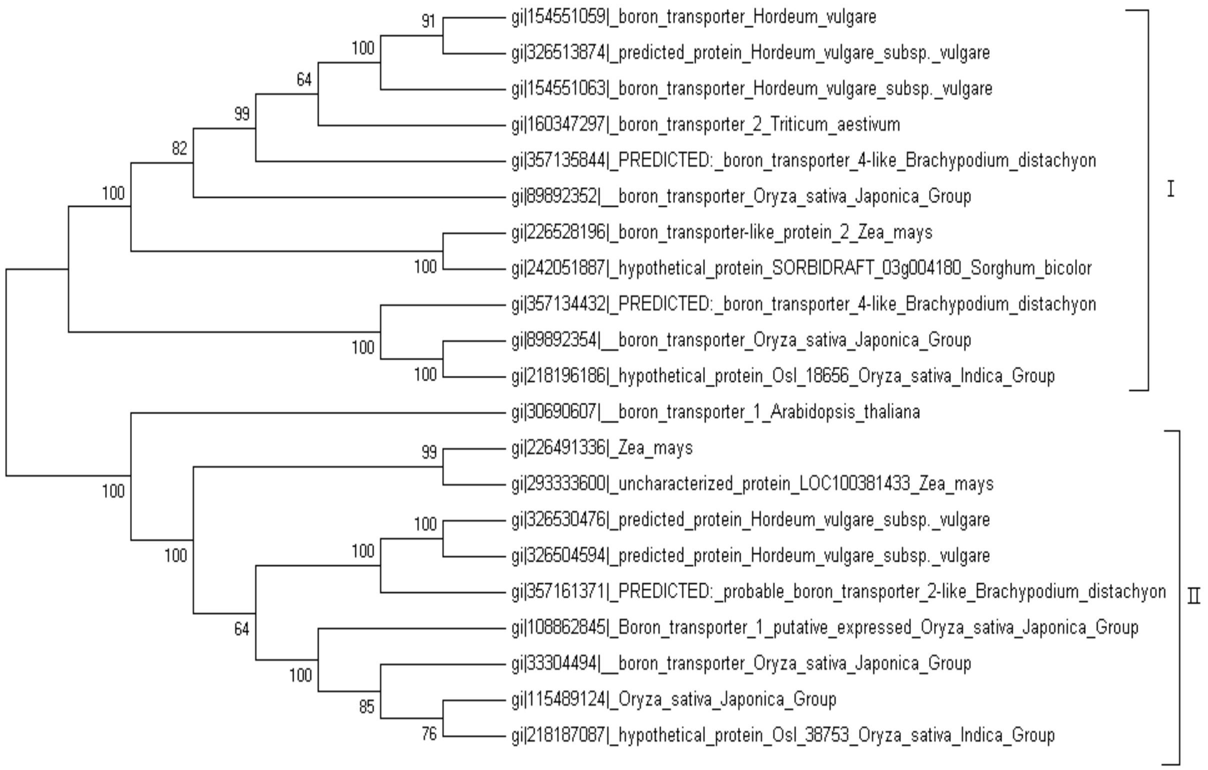

Figure 3. *Phylogenetic consensus tree of boron transporter protein sequences from different Poaceae species based on Neighbor-Joining method.*

As a result, we have carried out the comprehensive sequence analysis of BOR1 protein sequences by bioinformatics tools and this study results may help characterization of new plants BOR1 proteins in grasses.

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