

Bioinformatics Prediction and Comparison of *Artocarpus heterophyllus* BRI1 Family Members

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Molecular Plant Breeding, 2020, Vol.11, No.16 doi: [10.5376/mpb.2020.11.0016](https://doi.org/10.5376/mpb.2020.11.0016)

Received: 14 Aug., 2020

Accepted: 18 Aug., 2020

Published: 04 Sep., 2020

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Preferred citation for this article:

Yang X.R., Yu X.D., Wu F.H., Cai Z.P., Luo J.J., and Cao P.N., 2020, Bioinformatics prediction and comparison of *Artocarpus heterophyllus* BRI1 family members, Molecular Plant Breeding, 11(16):1-11 (doi: [10.5376/mpb.2020.11.0016](https://doi.org/10.5376/mpb.2020.11.0016))

Abstract Full-length trantogenic sequencing (PRJNA579273) of jackfruit seedling stems and leaves was performed. On this basis, biological information analysis was performed on Jackfruit (*Artocarpus heterophyllus*) BR INSENSITIVE 1 (BRI1) family members *AhBRI1*, *AhBRL1* and *AhBRL2* to translate genes into proteins. Using online analysis tools, jackfruit, Arabidopsis (*Arabidopsis thaliana*), Sichuan mulberry (*Morus notabilis*) and Poplar (*Populus trichocarpa*) were used to analyze and obtain different physicochemical properties data and the secondary and tertiary structure of the protein. The bioinformatics prediction and comparison between the BRI1 protein families of different species were obtained. The results showed that the amino acid residues were 1 195, 1 170 and 985, and the theoretical isoelectric points were 6.32, 5.77 and 7.08. The protein was weakly acidic, hydrophilic and stable. There are secretory pathway signal peptides or chloroplast transport peptides with transmembrane structure. The main components of the secondary structure are irregular coil and -helix, and the tertiary structure is spiral tubular structure. This study provides a basis for further functional studies on members of the BRI1 protein family of jackfruit.

Keywords *Artocarpus heterophyllus*; BRI1; Protein family

BRI1 belongs to leucine-rich repeats receptor-like kinase (LRR-RLK), and is the receptor of the plant hormone brassinosteroids (BRs). Phytohormone brassinosterol is a kind of multi-hydroxyl sterol derivative, which is widely distributed in plants. BRs can promote cell elongation and division, regulate plant development, and enhance plant resistance to adversity (Yang et al., 2011).

As a BRs receptor, BRI1 is a leucine-rich repetitive receptor kinase, consisting of extracellular ligand-binding domain, transmembrane domain and intracellular kinase domain (Oh et al., 2011). The AtBRI1 extracellular domain consists of 25 leucine-rich repeats (LRRs), with 24 amino acids per unit length. Between the 21st and 22nd LRR, there are 70 amino acids which do not meet the same sequence and are named 70-amino acid Island (Li and Chory, 1997). The island of 70 amino acids can be folded back inside the superhelix to form a hydrophobic surface capsule for binding to BRs (Hothorn et al., 2011; She et al., 2011). The intracellular kinase domains of BRI1 were mainly divided into 12 conserved domains (Li and Chory, 1997). When BRs is deficient, BRI1 kinase inhibitor 1 (BKII) inhibits kinase domain activity by binding to BRI1. When BRI1 receives the BRs signal, BKII is dissociated from BRI1 (Wang and Chory, 2006; Tang et al., 2008). BRI1 cascades phosphorylation-mediated signals through binding and identification of BRs and recruitment of BRI1 associated receptor-like kinase 1 (BAK1). After a series of protein interactions, bri1-EMS Suppressor 1 (BES1) and BRassinin-suppressor 1 (BZR1), which are non-phosphorylated, enter the nucleus and regulate the expression of a series of related genes (Chao Sun & Jia Li, 2017; She et al., 2011; Li and Chory, 1997). In addition, the cognate

protein Brassinosteroid Insensitive 1 Like 1 (BRL1) and Brassinosteroid Insensitive 1 Like 3 (BRL3) of BRI1 also participate in the signal transduction function of BR (Cano et al., 2004; Zhou et al., 2004).

Artocarpus heterophyllus is one of the common woody plants in tropical areas and has high economic and scientific value. Jackfruit is an evergreen tree of the *Artocarpus* family Moraceae. Its pulp is rich in sugars, proteins and other nutrients needed by the human body. It has medicinal effects such as lowering blood sugar, anti-oxidation and accelerating wound healing, and is of high economic and scientific value (Zhang Tao and Poon Yonggui, 2013, *Guangdong Agricultural Science*, 40(4): 94-96; Zhang Jindong, 2019).

According to statistics, the BRI1 homologous gene data of 88 plants have been included in NCBI database, among which 54 are herbaceous and 34 are woody, indicating that there are relatively few woody plants. In the previous study, our research group obtained full-length transcriptomic data of the stems and leaves of three generations of jackfruit seedlings (Pan Min et al., 2020, Private Communication), based on which we analyzed the BRI1 protein family and provided guidance for the functional research of the BRI1 protein family of jackfruit.

1 Results and Analysis

1.1 Homology analysis of BRI1 protein in jackfruit

In order to explore the interspecies homology of the BRI1 family members of jackfruit, Isoform 507 (*AhBRI1*), Isoform 1684 (*AhBRL1*) and Isoform 1269 (*AhBRL2*) with the highest homology were selected from the full-length transcriptome data of stem and leaf of jackfruit (PRJNA579273), and their amino acid sequences were predicted for comparison. Comparing the 3 proteins of jackfruit with members of the BRI1 protein family of the model plant, the evolutionary tree is made up of 3 branches, with *AhBRI1*, *AhBRL1* and *AhBRL2* in each branch (Figure 1).

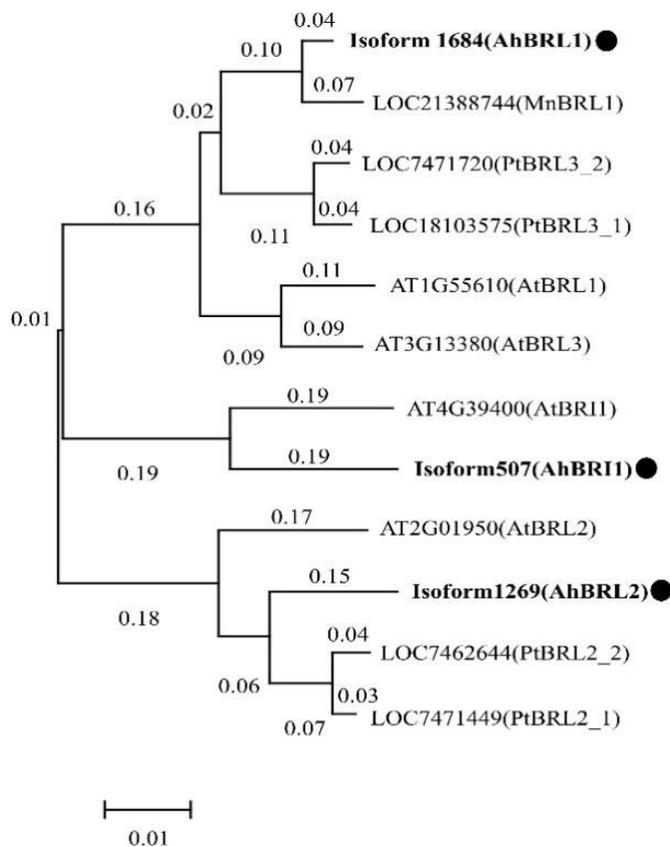


Figure 1 Member of the BRI1 protein family sequence NJ tree

Homology comparison of BRI1 protein sequences of jackfruit, *Arabidopsis thaliana*, Sichuan mulberry and Chinese hair fruit tree showed that AhBRI1 was most similar to AtBRI1 (69%), AhBRL1 and MnBRL1 had the highest similarity (90%), and AhBRL2 had higher homology with PtBRL2_1 and PtBRL2_2 (78% and 77%) (Table 1).

Table 1 Comparison of BRI1 protein homology between *A. heterophyllum* and three other plants

Specise	Protein name	Molecular weight (kD)	Homology (%)			LOC number	Chromosome
			AhBRI1	AhBRL1	AhBRL2		
<i>A. heterophyllum</i>	AhBRI1	130.624	100	49	46	Isoform_507	--
	AhBRL1	126.829	50	100	47	Isoform_1 684	--
	AhBRL2	107.665	48	47	100	Isoform_1 269	--
<i>A. thaliana</i>	AtBRI1	130.543	69	49	44	AT4G39 400	C4
	AtBRL1	127.424	50	67	47	AT1G55 610	C1
	AtBRL2	118.314	45	48	55	AT2G01 950	C2
	AtBRL3	126.661	51	67	47	AT3G13 380	C3
<i>M. notabilis</i>	MnBRL1	130.677	48	90	46	LOC21 388 744	--
<i>P. trichocarpa</i>	(BRI1-like_1)						
	PtBRL2_1	123.520	47	49	78	LOC7 471 449	C8
	(BRI1-like_2)						
	PtBRL2_2	124.019	46	53	77	LOC7 462 644	C10
	(BRI1-like_2)						
<i>P. trichocarpa</i>	PtBRL3_1	133.201	50	74	45	LOC18 103 575	C11
	(BRI1-like_3)						
	PtBRL3_2	133.089	50	75	45	LOC7 471 720	C1
	(BRI1-like_3)						

Note: '--' Indicates that it is currently unknown

1.2 Analysis of protein components and physicochemical properties

Physicochemical parameters of BRI1 protein in jackfruit, *Arabidopsis thaliana*, Sichuan mulberry and Poplar were compared using online analysis tool "ProtParam", and the results showed that the physicochemical properties of BRI1 protein in all plants were similar (Table 2). The theoretical isoelectric points were distributed between 5.77 and 7.08 and were stable proteins. The total mean hydrophobicity was negative, indicating that the protein had certain hydrophilicity. Acidic amino acids were more than basic amino acids (8.25%~10.12% acidic amino acids, 7.52%~9.36% basic amino acids), indicating that BRI1 protein was weakly acidic. Leu in protein contains the main amino acids (leucine) content were the highest (13.4% ~ 15.9%), Ser (Ser) times (10.5% ~ 12.9%), Gly (glycine) 3 (7.5% ~ 9.2%), the Asn (asparagine) 4 (6.3% ~ 7.8%); Different from the proportion of main amino acids in other proteins, the Asn content in AhBRL2 (7.9%) was higher than that in Gly (7.6%). In AtBRL2, Gly content was the third (7.5%), Ile (isoleucine) was the fourth (7.2%). The difference may be due to differences in species.

"ProtScale" was used to analyze the hydrophilicity and hydrophobicity of the BRI1 protein family members of jackfruit. The results showed that AhBRI1 had the highest score of 3.278 at the 13th place and the lowest score of 3.656 at the 822nd place (Figure 2A). AhBRL1 had the highest score of 3.633 at 797 and the lowest score of 3.400 at 817 (Figure 2B). AhBRL2 had the highest score of 3.244 at 711 and the lowest score of -2.944 at 931 (Figure 2C). The prediction results of the three proteins all showed that the hydrophilic region was larger than the hydrophobic region, indicating that the BRI1 family protein of jackfruit was hydrophilic protein. Similar results were obtained by predicting the hydrophilicity/hydrophobicity of other BRI1 protein family members.

Table 2 Physical and chemical properties of members of the BRI1 protein family

Protein name	Number of amino acids (aa)	Isoelectric point	Major amino acids (%)	Acidic amino acids (%)	Basic amino acids (%)	Aliphatic index	Grand average of hydropathicity	Instability index
AhBRI1	1 195	6.32	Leu 14.4, Ser 12.6, Gly 7.8, Asn 7.3	9.46	8.70	96.04	-0.031	34.51 stable
AhBRL1	1 170	5.77	Leu 15.6, Ser 12.7, Gly 8.4, Asn 7.8	9.32	7.86	98.85	-0.033	38.33 stable
AhBRL2	985	7.08	Leu 15.9, Ser 11.4, Asn 7.9, Gly 7.6	8.93	8.93	103.77	-0.050	33.44 stable
AtBRI1	1 196	5.99	Leu 14.1, Ser 11.5, Gly 8.5, Asn 6.8	10.12	8.95	92.78	-0.086	34.64 stable
AtBRL1	1 166	6.97	Leu 13.4, Ser 10.5, Gly 9.2, Asn 7.0	9.09	9.01	94.79	-0.056	31.27 stable
AtBRL2	1143	6.56	Leu 13.8, Ser 11.3, Gly 7.9, Ile 7.0	9.71	9.36	97.50	-0.125	38.39 stable
AtBRL3	1 164	6.30	Leu 14.8, Ser 11.6, Gly 9.0, Asn 6.5	9.54	8.94	96.71	-0.062	31.07 stable
MnBRL1	1 205	6.14	Leu 15.0, Ser 12.9, Gly 8.3, Asn 7.5	8.96	8.13	97.27	-0.014	38.94 stable
PtBRL2_1	1 134	5.83	Leu 15.3, Ser 11.2, Gly 7.8, Asn 6.8	9.79	8.47	101.29	-0.043	30.92 stable
PtBRL2_2	1 135	5.94	Leu 15.4, Ser 10.7, Gly 7.7, Asn 6.3	9.78	8.63	101.98	-0.015	30.57 stable
PtBRL3_1	1 224	6.37	Leu 15.2, Ser 12.2, Gly 8.4, Asn 6.8	8.58	8.01	95.74	-0.077	39.31 stable
PtBRL3_2	1 224	6.22	Leu 15.6, Ser 12.1, Gly 8.6, Asn 7.4	8.25	7.52	97.96	-0.038	37.57 stable

1.3 Peptide prediction and analysis

Leaderpeptide is a recognition protein used to guide newly synthesized peptide chains into different organelles (Dong Jiao et al., 2010). The "TargetP 1.1 Server" was used to predict the sequence of all BRI1 family members, and a Specificity of 0.95 was selected. The results were as follows: in the 3 proteins of jackfruit, the prediction reliability of AhBRI1 guiding peptide was level 1, and there was a guiding peptide cleavage site at the 21st position of the protein sequence, the signal peptide region was the residues of the first 21 amino acids, and the mature protein started from the 22nd site (Table 3). The possible presence of secretory pathway signal peptides was analyzed. AhBRL2's guide peptide prediction reliability is level 3, which may have signal peptide, and it is predicted that there is a guide peptide cleavage site at the 28th position of the protein sequence, the signal peptide region is between 1-28 amino acid residues, and the initiation site of the mature protein is the 29th site. However, the prediction score of AhBRL1 secretory pathway signal peptide was low, so the guide peptide of AhBRL1 might be a chloroplast transport peptide different from that of AhBRI1 and AhBRL2. MnBRL1, PtBRL2_1, PtBRL2_2 and 3 Arabidopsis thaliana proteins AtBRI1, AtBRL1 and AtBRL3 have high prediction reliability, and all have cleavage sites, indicating that they are likely to have secretory pathway signal peptides. However, AtBRL2, PtBRL3_1 and PtBRL3_2 have low reliability and have not found their leading peptide splitting sites, so it is impossible to determine whether they have leading peptides.

Signal peptide is a polypeptide chain located at the N end of a peptide chain. It is a type of peptide. The main function is to guide the newly synthesized peptide chain into the endoplasmic reticulum (Wei et al., 2006). In order to verify the accuracy of the above prediction results for the guiding peptide of the BRI1 protein family members of jackfruit, the tool "Signal P 4.1 Server" was used for online prediction. According to the predicted

results, ACCORDING to y-Score and S-Score, AhBRL1 is consistent with the above predicted results -- there is no signal peptide (Figure 3B). The Cleavage site of AhBRI1 was Cleavage site between pp.22 and 23. The Cleavage site of mature protein was Cleavage site between pp.22 and 23. The Cleavage site of mature protein was Cleavage site between pp.22 and 23. The cleavage site of AhBRL2 is between the 28th and 29th sites, the mature protein is activated from the 29th site, and the signal peptide region is composed of 1-28 amino acid residues (Figure 3C). It can be seen that although there are differences between the predicted results of "TargetP 1.1 Server" website, they are generally similar. In conclusion, jackfruit 2 proteins AhBRI1 and AhBRL2 direct newly synthesized peptide chains to the endoplasmic reticulum, while AhBRL1 may direct new peptide chains into

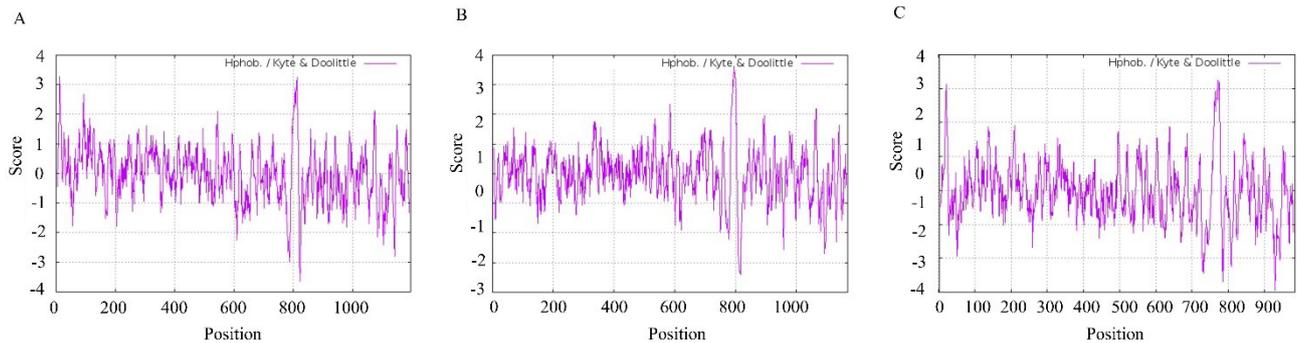


Figure 2 Hydrophilic/hydrophobic prediction of amino acid sequences of members of the BRI1 protein family of jackfruit
 Note: A: AhBRI1; B: AhBRL1; C: AhBRL2

Table 3 Peptide prediction of members of the BRI1 protein family

Name	Chloroplast signal peptide	Mitochondrion signal peptide	Signal peptide	Other	Loction	RC	TPlen
AhBRI1	0.009	0.025	0.961	0.049	S	1	21
AhBRL1	0.961	0.024	0.004	0.078	C	1	41
AhBRL2	0.001	0.153	0.751	0.256	S	3	28
AtBRI1	0.005	0.097	0.960	0.009	S	1	31
AtBRL1	0.006	0.042	0.950	0.034	S	1	21
AtBRL2	0.213	0.596	0.003	0.042	*	4	-
AtBRL3	0.007	0.152	0.887	0.017	S	2	23
MnBRL1	0.001	0.020	0.845	0.031	S	1	27
PtBRL2_1	0.013	0.027	0.948	0.031	S	1	30
PtBRL2_2	0.013	0.034	0.974	0.024	S	1	26
PtBRL3_1	0.158	0.684	0.010	0.010	*	3	-
PtBRL3_2	0.056	0.740	0.025	0.012	*	2	-

Note: "*" Indicates that it is currently unknown; '-' indicates that no peptide cleavage site has been found; 'S' Indicates the secreting pathway signal peptide; 'C' Indicates chloroplast transit peptide; 'Reliability' value is smaller, the more reliable

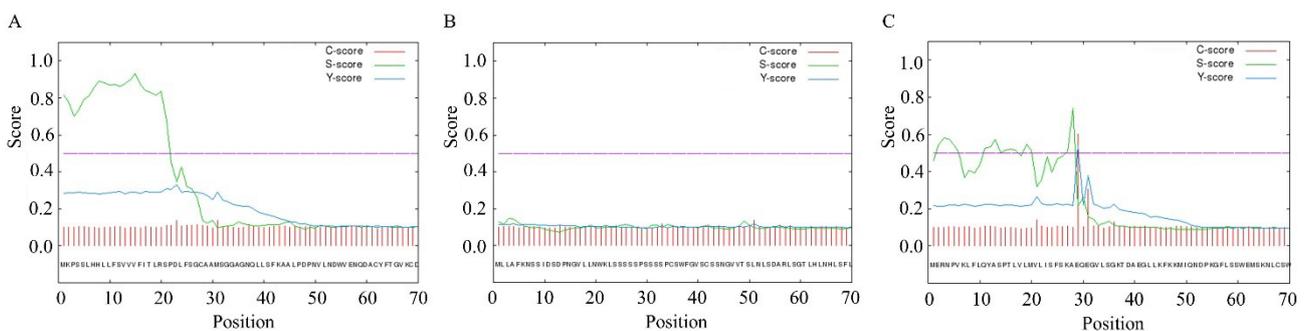


Figure 3 Prediction of signal peptides in members of jackfruit BRI1 protein family
 Note: A: AhBRI1; B: AhBRL1; C: AhBRL2

1.4 LRR prediction and analysis

Li and Chory (1997) divided the AtBRI1 extracellular domain into 25 LRR and an island structure consisting of 70 amino acid residues. By comparing 3 BRI1 family members of jackfruit with AtBRI1 (Figure 4), the amino acid islands in the 3 proteins are all composed of 68 amino acids. In AhBRI1, AhBRL1 and AhBRL2, amino acids at the 655 and 687 amino acids were missing. AhBRL2 is missing the 656 amino acid and the 638 amino acid. Compared with AtBRI1, the missing fragments were mainly distributed between LRR1 and LRR7, among which AhBRL2 was the most missing fragment and concentrated distributed between LRR1 and LRR5. In addition, the remaining domains have a high degree of consistency.

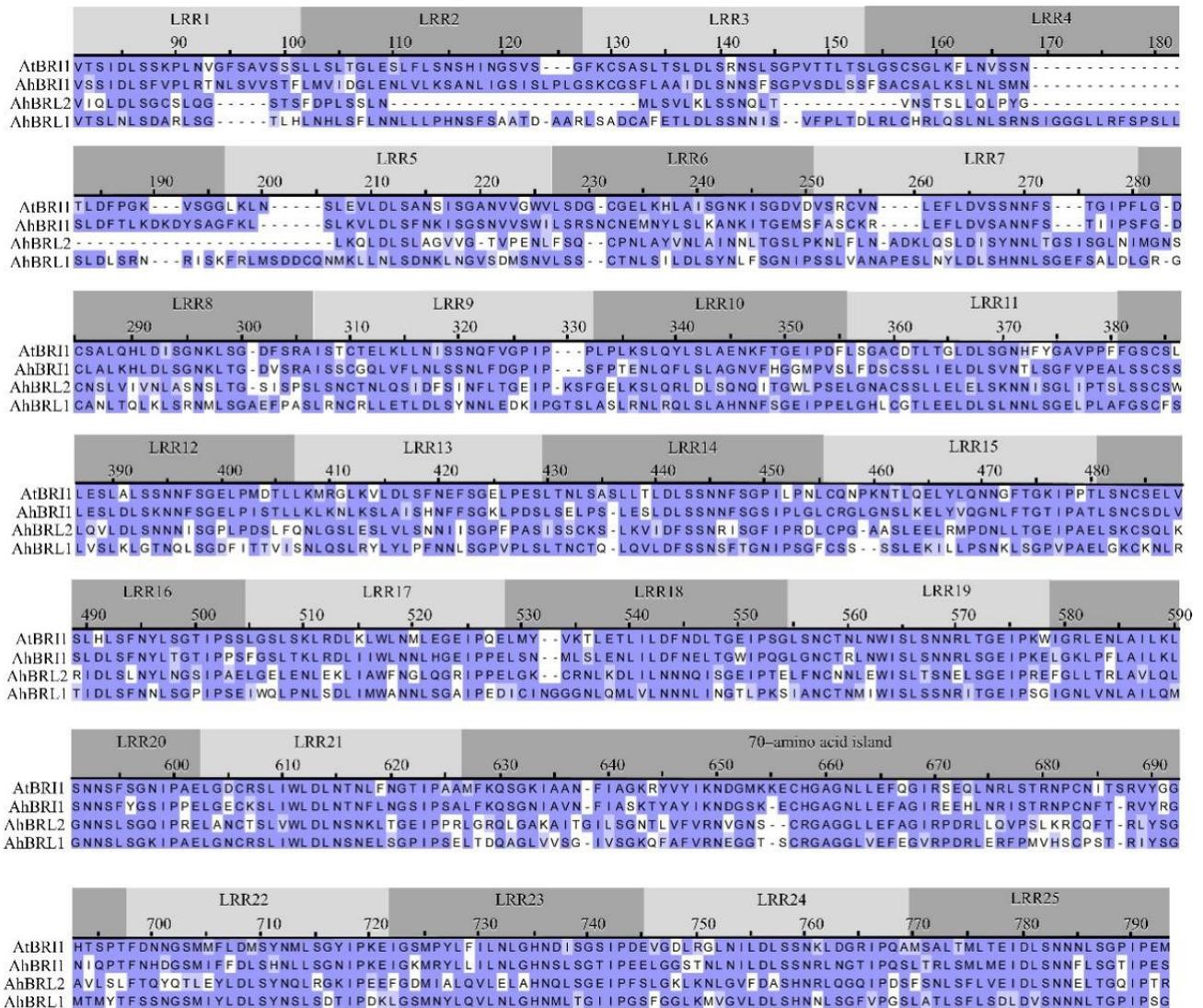


Figure 4 Multiple sequence alignment results of Lrr domain of 4 protein sequences

1.5 Prediction and analysis of transmembrane domain

"TMHMM Server V. 2.0" was used to analyze the transmembrane structure of BRI1 protein family of jackfruit online. The results showed that the predicted location of the AhBRI1 transmembrane region was located at the amino acid residues at positions 795 ~ 817 (Figure 5A). The AhBRL1 transmembrane region was located at amino acid residues in positions 784-806 (Figure 5B). The predicted location of AhBRL2 transmembrane helical region is located at the amino acid residue at positions 755-777 (Figure 5C). Therefore, all 3 jackfruit proteins were transmembrane proteins.

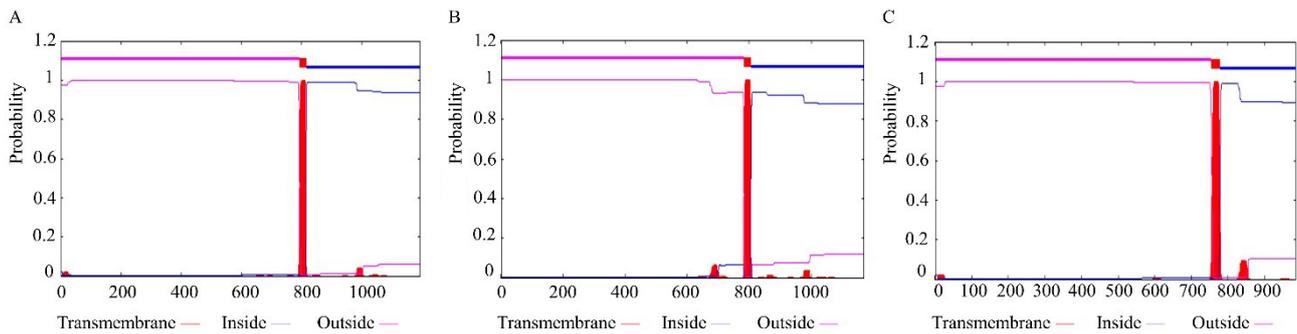


Figure 5 Transmembrane structure prediction of members of the jackfruit BRI1 protein family
 Note: A: AhBRI1; B: AhBRL1; C: AhBRL2

1.6 Kinase domain prediction and analysis

The kinase domain of LRR-RLKS can transmit signals to downstream factors through substrate phosphorylation (Tang et al., 2008). In the study of Li and Chory (1997), by comparing AtBRI1 with CLV1, ERECTA, TMK1, RLK5 and Xa21 (Hanks and Quinn, 1991), it was found that conserved amino acid residues existed in eukaryotic protein kinases, and the regions where these conserved amino acid residues clustered were divided into 12 sub-domains. Compare 3 jackfruit proteins with AtBRI1 (Figure 6). The sequence was divided into 12 parts and the conserved amino acid residues were circled. The 15 invariant amino acids in all protein kinases were represented by *, and the amino acid residues circled by the dashed line indicated that the conserved amino acid of AtBRI1 was mutated here. For example, the 985 bit was mutated from Leu to Ile. The mutation of Tyr (tyrosine) to Phe (phenylalanine) at position 1,053. The mutation of position 1077 from Asp (aspartic acid) to Asn; The 1214 mutation from Leu to Phe. Moreover, compared with the kinase domain, AhBRL1 was short of the 1178th bit, the 1030th to 1032th bit (Gly, Asn and Gly), the 1151st bit (Asp) and the 1168th bit (Ser). Lack of structure in AhBRL2 domain VII~XI, more than 1 031 (Glu) and 1 032 (Arg arginine) amino acids.

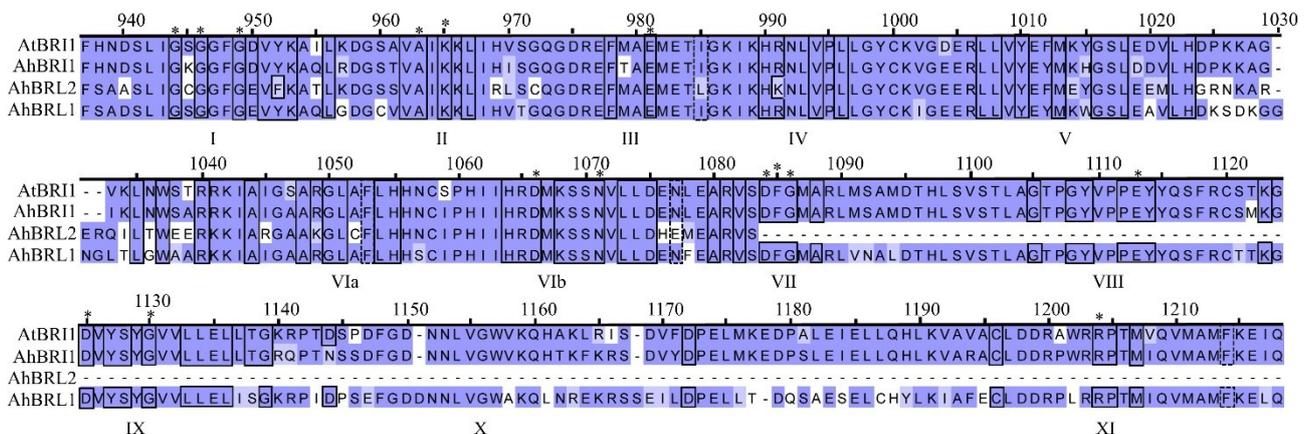


Figure 6 Multiple sequence alignment results of 4 protein sequence kinase domains
 Note: □ refers to conservative amino acids; '*' refers to 15 invariant amino acids in all protein kinases; In the dotted box is the amino acid AtBRI1 which is different from other comparison sequence conserved regions in the reference

1.7 Prediction of secondary structure of protein

The secondary structure of a protein is a regular repeated conformation, and the hydrogen bond between the carbonyl and amide groups on the skeleton is the main maintenance force (Shen Chen et al., 2014). The secondary structure of RII protein family of jackfruit was analyzed by SOPMA (Table 4). The results showed that irregular coil and -helix were the main secondary structural elements (irregular coil accounted for 48.20%~49.57%, α -helix for 33.76%~35.53%), followed by extension chain and -folding (extension chain accounted for 13.50%~13.81%, β -folding accounted for 2.44%~3.35%). Analysis of the remaining protein family members revealed similar results.

1.8 Prediction of tertiary structure of protein

The conservative domain mapping tool in Swiss-Model was used to MODEL and analyze the three-level structure (Figure 7). In the figure, the third-order structure of jackfruit BRI1 is a curved spiral tubular structure. The tubular end is in a right-handed spiral shape relative to the starting position around the central axis. It conforms to the typical characteristics of BRI1 (Liu Mingyue et al., 2018).

Table 4 Secondary structure prediction of members of the BRI1 protein family

Name	Alpha helix		Beta turn		Extended strand		Random coil	
	Quantity	Proportion(%)	Quantity	Proportion(%)	Quantity	Proportion(%)	Quantity	Proportion(%)
AhBRI1	414	34.64	40	3.35	165	13.81	576	48.20
AhBRL1	395	33.76	37	3.16	158	13.50	580	49.57
AhBRL2	350	35.53	24	2.44	134	13.60	477	48.43
AtBRI1	452	37.79	34	2.84	146	12.21	564	47.16
AtBRL1	423	36.28	23	1.97	141	12.09	579	49.66
AtBRL2	444	38.85	32	2.80	137	11.99	530	46.37
AtBRL3	408	35.05	32	2.75	162	13.92	562	48.28
MnBRL1	437	36.27	36	2.99	154	12.78	578	47.97
PtBRL2_1	434	38.27	33	2.91	128	11.29	539	47.53
PtBRL2_2	392	34.54	40	3.52	146	12.86	557	49.07
PtBRL3_1	421	34.40	30	2.45	169	13.81	604	49.35
PtBRL3_2	391	31.94	31	2.53	175	14.30	627	51.23

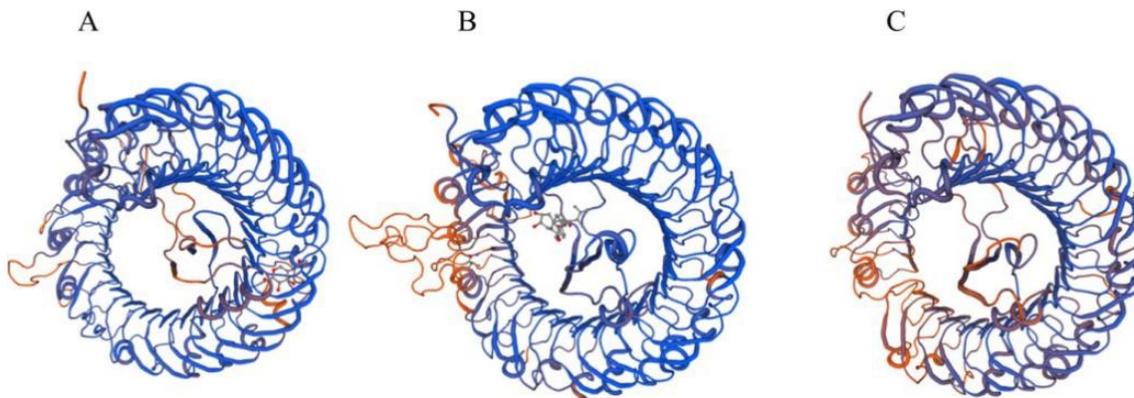


Figure 7 Tertiary structural modeling of members of the BRI1 protein family

Note: A: AhBRI1; B: AhBRL1; C: AhBRL2

2 Discussion

In this study, the AhBRI1 gene of jackfruit was extracted, encoding 1195 amino acids, which is different from the BRI1 gene of *Eucommia Ulmoides* (encoding 1203 amino acids) (Liu et al., 2018), which is caused by different species. In this study, biological software was used to predict the physicochemical properties and structure of BRI1 protein family members of jackfruit. The physicochemical properties of the protein sequence showed that the protein was acidic and stable. The results showed that AhBRI1 and AhBRL2 may have secretory pathway signaling peptides, while AhBRL1, unlike these two proteins, has chloroplast transport peptides and may have specific functions. The prediction of signal peptides also confirmed that AhBRI1 and AhBRL2 had signal peptides, and AhBRL1 could not detect the characteristics of relevant signal peptides. The hydrophilic/hydrophobic prediction of the protein family members showed that the hydrophilic region was larger than the hydrophobic region, so it was speculated that the three proteins were all hydrophilic proteins. The transmembrane domains of 3 jackfruit proteins were predicted, which were consistent with the characteristics of transmembrane proteins. The prediction of three-stage structure modeling showed that the BRI1 protein family of jackfruit conforms to the

general BRI1 protein characteristics. Compared with the analysis results of Liu Mingyue et al. (2018), the similarity is higher.

BRI1 has multiple roles, which can participate in the immune regulation of biological stress and influence the development of pollen and anthers at different developmental stages (Wang et al., 2019, *Anhui Agricultural Sciences*, 47(4): 26-29, 33; Lei and Xu, 2019). When comparing the BRI1 protein family members of jackfruit and AtBRI1, the four proteins were found to be partially different in the 70-amino acid island region. When comparing the AhBRL2 and AtBRI1 kinase domains, it was found that AhBRL2 was missing longer fragments, which may affect its specific functions. There is also evidence that the amino acid island structure sequence of *Osmunda japonica* BRL2 protein is different from that of other BRI1 protein families in *Arabidopsis thaliana*, which may be the result of BR receptor evolution (Fan et al., 2015), so the difference of AhBRL2 island region may also be caused by evolution.

In this study, the physicochemical properties and structure of the BRI1 protein family members of jackfruit were predicted and analyzed, and it was found that there were differences among the three BRI1 proteins. For example, the guiding peptide types of AhBRL1 were different from AhBRI1 and AhBRL2. Compared with AhBRI1 and AhBRL1, AhBRL2 lacks longer kinase region fragments. These will provide guidance for further studies on the function of members of the BRI1 protein family of jackfruit.

3 Materials and Methods

3.1 Material

The full-length transcripts of stem and leaf of three generations of Jackfruit (*Artocarpus heterophyllus*) used in this study were obtained from the results completed by our research group (SRA database, PRJNA579273) (Pan Min et al., 2020, Private Communication).

3.2 Homology analysis of multiple species

TAIR from *Arabidopsis thaliana* genome database (<https://www.arabidopsis.org/>) was extracted by *Arabidopsis* AtBRI1, AtBRL1, AtBRL2 and AtBRL3 protein sequence and nucleotide sequence. Search from the NCBI web site (<https://www.ncbi.nlm.nih.gov/pubmed/>), *Sichuan comospore* Yang MnBRL1, PtBRL2_1, PtBRL2_2, PtBRL3_1 and PtBRL3_2 protein sequence and nucleotide sequence. With the nucleotide sequences of each species as reference, bioEdit software was used to carry out local BLAST, and the imported data was the full-length transcriptome of stem and leaf of Jackfruit. The result of the analysis in each group before selecting 10 high homologous genes, remove the repeated ID, extracted from jackfruit stem leaf total length of the transcriptome data retrieved from gene nucleotide sequence, using NCBI online tools (<https://www.ncbi.nlm.nih.gov/orffinder/>) to predict protein amino acid sequence. Using mega_x_10.0.5 software, the BRI1 protein family members of *Arabidopsis*, jackfruit, *Sichuan mulberry* and *Populus hairy* were compared and the evolutionary tree was constructed. Using NCBI BLAST function (<https://blast.ncbi.nlm.nih.gov/BlastAlign.cgi>) to compare protein homology; BRI1, BRL1 and BRL2 of jackfruit were analyzed by reference to the classification of *Arabidopsis thaliana* BRI1-LRR and kinase domain (Li and Chory, 1997). Jalview software was used to optimize the alignment, deepen the matching amino acids, and align the amino acids with "-".

3.3 Analysis of physical and chemical properties of proteins

Using NCBI BLAST function (<https://blast.ncbi.nlm.nih.gov/BlastAlign.cgi>) to compare protein homology; Protein peptide prediction using guide website (<http://www.cbs.dtu.dk/services/TargetP-1.1/>); The remaining physical and chemical properties were completed by li Qing et al. (2015).

3.4 Secondary and tertiary structure prediction

SOPMA, a website for protein secondary structure prediction, and Swiss-Model, a website for tertiary structure analysis, were completed by referring to the analysis methods of Li Qing et al. (2015) and Liu Mingyue et al. (2018) respectively.

Authors' contributions

Yang Xinrong is the experimental designer and executor of this study. Yang Xinrong completed the data analysis and the first draft of the paper; Yang Xinrong, Wu Fanhua, Yu Xudong, CAI Zeping, Cao Peina and Luo Jiajia participated in the experimental design and analysis of the experimental results. Wu Fanhua is the initiator and responsible person of the project, guiding data analysis, paper writing and revision. All authors read and approved the final manuscript.

Acknowledgments

This study was funded by Hainan Natural Science Foundation (319MS017).

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