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E-mail: jzus_b@zju.edu.cn

Correspondence

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Tartary buckwheat database (TBD): an integrative platform for gene analysis of and biological information on Tartary buckwheat

Moyang LIU[1,2], Wenjun SUN[1], Zhaotang MA[3], Yuan HU[1], Hui CHEN[1]✉

[1]*College of Life Science, Sichuan Agricultural University, Ya'an 625014, China*
[2]*Joint Center for Single Cell Biology, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, China*
[3]*State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China, Key Laboratory of Major Crop Diseases and Rice Research Institute, Sichuan Agricultural University, Chengdu 611130, China*

Rice, wheat, corn, and potatoes are four crops that provide a daily source of nutrition for humans, but there are many problems that have been found with these crops. First, they lack amino acids and minerals which are necessary for balanced nutrition, and they also are grown very widely and as monocultures, which increases the risk of the human food system being destroyed by climate change. Thus, by introducing coarse cereals with good characteristics, we can enrich human food resources, realize agricultural diversification, improve dietary structure, and mitigate risks. Tartary buckwheat (*Fagopyrum tataricum*) is a widely cultivated edible and medicinal crop with unique nutritional and excellent economic value. It contains flavonoids, such as rutin and quercetin, which are not found in cereal crops. Rutin is a major flavonoid that can enhance blood flow and aid in the use of vitamin C and the production of collagen. In addition, such antioxidants have been shown to effectively reduce cholesterol levels, blood clots, and hypertension, particularly for the prevention of inflammatory liver injury (Middleton et al., 2000; Lee et al., 2013; Suzuki et al., 2014; Huang et al., 2016; Nishimura et al., 2016). Meanwhile, Tartary buckwheat can tolerate poor climate and acidic soils containing high amounts of aluminum, which is toxic to other crops (Wang et al., 2015). The self-pollination of Tartary buckwheat has resulted in a decrease in genomic heterozygosity, which is valuable for breeding and a stable production trait (Wang and Campbell, 2007). Therefore, Tartary buckwheat is an important minor crop, which is expected to become the target of many breeding efforts in the future.

Due to the publication of the genome data for Tartary buckwheat, the pace of identification of key genes related to its development has accelerated (Zhang et al., 2017). Multiple transcription factors (TFs) involved in growth and development have been identified. For example, the auxin response factor (ARF) gene family of the Tartary buckwheat genome has been comprehensively and systematically analyzed (Liu et al., 2018c), and *FtARF2*, which potentially regulates fruit size, has been further studied (Liu et al., 2018b). By systematic identification of MADS-box family members, the potential genes that regulate fruit cracking were also excavated (Liu et al., 2019h). Furthermore, various TF families, including APETALA2/ethylene-responsive factor (AP2/ERF) (Liu et al., 2019d), basic helix-loop-helix (bHLH) (Sun et al., 2020a), basic leucine zipper (bZIP) (Liu et al., 2019b), GRAS (Liu et al., 2019g), heat shock transcription factor (HSF) (Liu et al., 2019c), NAM, ATAF1/2, and CUC2 (NAC) (Liu et al., 2019a), squamosa promoter-binding protein-like (SPL) (Liu et al., 2019f), trihelix (Ma et al., 2019), WRKY (Sun et al., 2020b), and zinc finger-homeodomain (ZF-HD) (Liu et al., 2019e), have been identified from the genome to excavate the genes that might improve Tartary buckwheat quality. The genes involved in regulating the synthesis of flavonoids in Tartary buckwheat were also found, including *FtMYB13*, *FtMYB14*, *FtMYB15*,

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✉ Hui CHEN, chenhui@sicau.edu.cn
Hui CHEN, https://orcid.org/0000-0001-9185-1764

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and *FtMYB16* (Zhou et al., 2017). The construction of plant databases can effectively preserve and utilize large-scale datasets. One example is the maize database, which provides the expression of development-related genes and thus lays the foundation for improving maize quality (Guillaumie et al., 2007). Despite the many open species databases, comprehensive information databases about some crops with important agricultural significance and development potential are very limited (Gayali et al., 2016). Until now, there has been no available database to save, analyze, or utilize the important dataset of Tartary buckwheat. To promote the usage and application of identified TF families in Tartary buckwheat, we developed Tartary buckwheat database (TBD) (http://tbd.sicau.edu.cn) as a highly integrated information platform with online query tools. TBD will provide comprehensive information and a query platform for researchers interested in understanding and improving Tartary buckwheat crop quality.

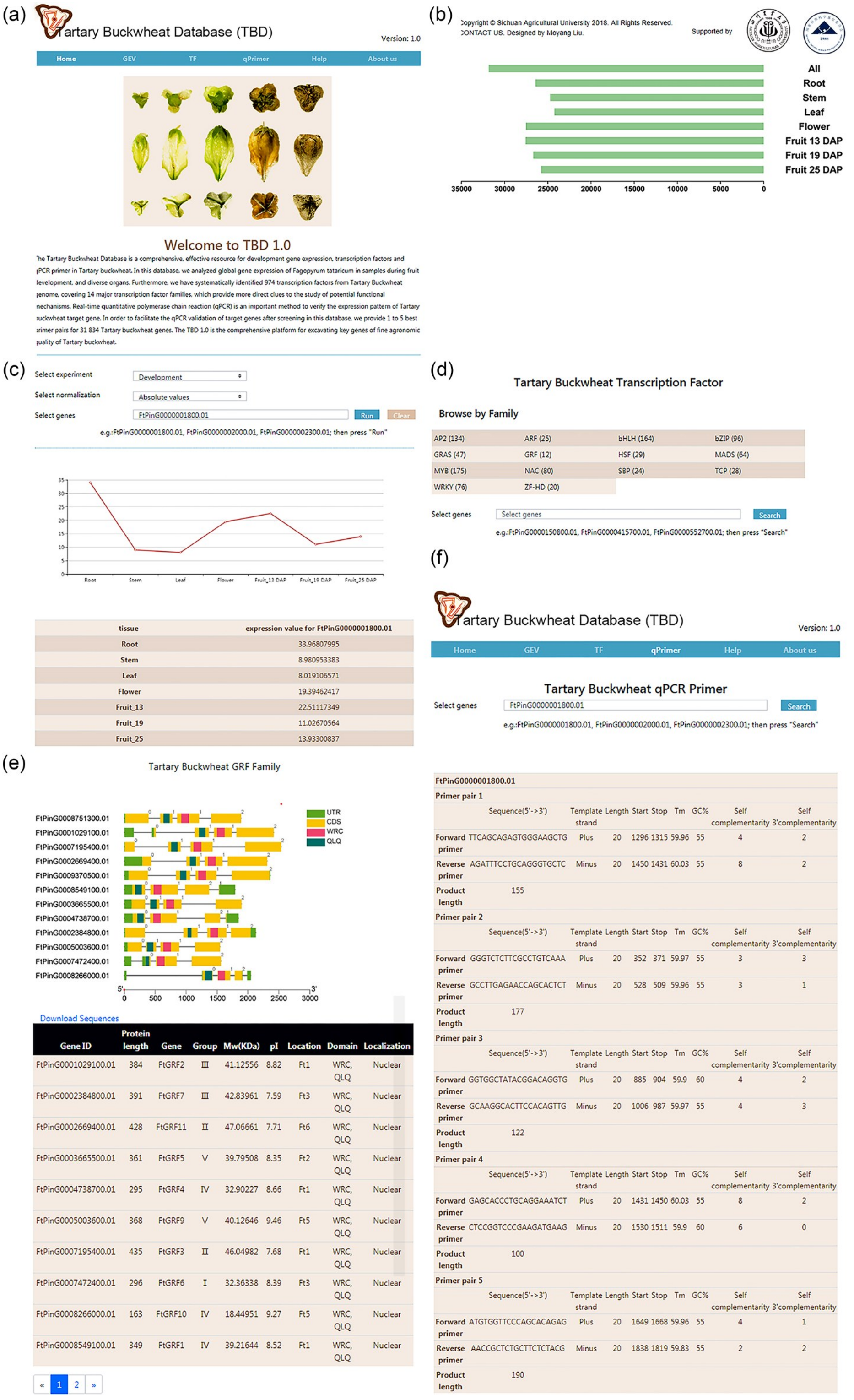
To facilitate queries for gene information, we provide basic retrieval forms for Tartary buckwheat gene expression, TFs, and primers, and more. Users simply need to input a gene ID to retrieve specific gene information. Each gene has a detailed information page which is divided into several subsections for gene expression value (GEV), TF, and quantitative real-time polymerase chain reaction (qRT-PCR) primer (qPrimer). The TBD website includes six functional sections, including "Home," "GEV," "TF," "qPrimer," "Help," and "About Us."

On the "Home" page, users can view a presentation, receive news updates, and see the current information in the TBD database (Fig. 1a).

In the "GEV" section, we provide a flexible interface for efficient retrieval of gene expression for all Tartary buckwheat genes. On this page, after one enters the Tartary buckwheat gene accession number and clicks "Run," all visualizations showing the absolute and relative expression of each gene in the roots, stems, flowers, leaves, and fruit (green fruit stage, 13 days after pollination (DAP); discoloration stage, 19 DAP; initial maturity stage, 25 DAP) are provided. The transcriptome data accession number of fruit at different developmental stages is GSE111937 (Liu et al., 2018a), and the transcriptional data accession number of different tissues of Tartary buckwheat (roots, stems, leaves, and flowers) is SRP103493 (Zhang et al., 2017). A total of 31 839 genes were detected, of which 26 426 genes were detected in roots, 24 716 genes were detected in stems, 24 227 genes were detected in leaves, 27 533 genes were detected in flowers, 27 569 genes were detected in early-development fruit (13 DAP), 26 673 genes were detected in mid-development fruit (19 DAP), and 25 767 genes were detected in late-development fruit (25 DAP) (Fig. 1b). Moreover, there were 392 genes expressed only in roots, 28 genes expressed only in stems, 49 genes expressed only in leaves, 504 genes expressed only in flowers, and 288 genes (13 DAP, 153 genes; 19 DAP, 57 genes; 25 DAP, 49 genes) expressed only in fruit (Fig. 1a). For each detected gene, we provide expression images for researchers to analyze, and attach specific values to the images. For example, a search for *FtPinG0000001800.01* will return a detailed expression profile (Fig. 1c). These specifically expressed genes provide candidates for researchers to study specific biological processes. The abundant data provide a rich selection for researchers to discover genes of interest.

In the "TF" section, we provide biological information on 974 TFs from 14 major TF families, including AP2/ERF, ARF, bHLH, bZIP, GRAS, growth-regulating factor (GRF), HSF, MADS, v-myb avian myeloblastosis viral oncogene homolog (MYB), NAC, SPL, TEOSINTE-BRANCHED 1/cycloidea/PCF (TCP), WRKY, and ZF-HD. To assist in the screening of important regulators, we also integrate the gene structure, grouping, and localization of these important TFs, which is advantageous for researchers when mining key genes. In addition, we provide a query function for identifying whether genes of interest to researchers belong to one of the 14 identified TF families (Fig. 1d). By clicking on any TF family, researchers can obtain the intron/exon structural characteristics of all genes in that family. For example, clicking on *GRF* provides structural information for 12 genes (Fig. 1e). Detailed information on protein length, molecular weight (MW), isoelectric point (pI), chromosome location, and localization of the corresponding gene can also be viewed (Fig. 1e).

In "qPrimer," TBD offers a user-friendly interface for conveniently downloading the best or all primer sequences for the target gene. We provide 1–5 pairs of the best primers for each of 31 839 Tartary buckwheat genes (Fig. 1f). For example, when searching for *FtPinG0000001800.01*, researchers will be given five pairs of different primers, and can select primer pairs



Welcome to TBD 1.0

'he Tartary Buckwheat Database is a comprehensive, effective resource for development gene expression, transcription factors and ιPCR primer in Tartary buckwheat. In this database, we analyzed global gene expression of Fagopyrum tataricum in samples during fruit levelopment, and diverse organs. Furthermore, we have systematically identified 974 transcription factors from Tartary Buckwheat jenome, covering 14 major transcription factor families, which provide more direct clues to the study of potential functional nechanisms. Real-time quantitative polymerase chain reaction (qPCR) is an important method to verify the expression pattern of Tartary uckwheat target gene. In order to facilitate the qPCR validation of target genes after screening in this database, we provide 1 to 5 best rimer pairs for 31 834 Tartary buckwheat genes. The TBD 1.0 is the comprehensive platform for excavating key genes of fine agronomic juality of Tartary buckwheat.

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CONTACT US. Designed by Moyang Liu.

Select experiment: Development
Select normalization: Absolute values
Select genes: FtPinG0000001800.01

e.g.:FtPinG0000001800.01, FtPinG0000002000.01, FtPinG0000002300.01; then press "Run"

tissue	expression value for FtPinG0000001800.01
Root	33.96807995
Stem	8.980953383
Leaf	8.019106571
Flower	19.39462417
Fruit_13	22.51117349
Fruit_19	11.02670564
Fruit_25	13.93300837

Tartary Buckwheat Transcription Factor

Browse by Family

AP2 (134)	ARF (25)	bHLH (164)	bZIP (96)
GRAS (47)	GRF (12)	HSF (29)	MADS (64)
MYB (175)	NAC (80)	SBP (24)	TCP (28)
WRKY (76)	ZF-HD (20)		

Select genes: Select genes

e.g.:FtPinG0000150800.01, FtPinG0000415700.01, FtPinG0000552700.01; then press "Search"

Tartary Buckwheat GRF Family

FtPinG0008751300.01
FtPinG0001029100.01
FtPinG0007195400.01
FtPinG0002669400.01
FtPinG0009370500.01
FtPinG0008549100.01
FtPinG0003665500.01
FtPinG0004738700.01
FtPinG0002384800.01
FtPinG0005003600.01
FtPinG0007472400.01
FtPinG0008266000.01

Gene ID	Protein length	Gene	Group	Mw(KDa)	pI	Location	Domain	Localization
FtPinG0001029100.01	384	FtGRF2	Ⅲ	41.12556	8.82	Ft1	WRC, QLQ	Nuclear
FtPinG0002384800.01	391	FtGRF7	Ⅲ	42.83961	7.59	Ft3	WRC, QLQ	Nuclear
FtPinG0002669400.01	428	FtGRF11	Ⅱ	47.06661	7.71	Ft6	WRC, QLQ	Nuclear
FtPinG0003665500.01	361	FtGRF5	V	39.79508	8.35	Ft2	WRC, QLQ	Nuclear
FtPinG0004738700.01	295	FtGRF4	IV	32.90227	8.66	Ft1	WRC, QLQ	Nuclear
FtPinG0005003600.01	368	FtGRF9	V	40.12646	9.46	Ft5	WRC, QLQ	Nuclear
FtPinG0007195400.01	435	FtGRF3	Ⅱ	46.04982	7.68	Ft1	WRC, QLQ	Nuclear
FtPinG0007472400.01	296	FtGRF6	I	32.36338	8.39	Ft3	WRC, QLQ	Nuclear
FtPinG0008266000.01	163	FtGRF10	IV	18.44951	9.27	Ft5	WRC, QLQ	Nuclear
FtPinG0008549100.01	349	FtGRF1	IV	39.21644	8.52	Ft1	WRC, QLQ	Nuclear

Tartary Buckwheat qPCR Primer

Select genes: FtPinG0000001800.01

e.g.:FtPinG0000001800.01, FtPinG0000002000.01, FtPinG0000002300.01; then press "Search"

FtPinG0000001800.01									
Primer pair 1									
	Sequence(5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3'complementarity
Forward primer	TTCAGCAGAGTGGGAAGCTG	Plus	20	1296	1315	59.96	55	4	2
Reverse primer	AGATTTCCTGCAGGGTGCTC	Minus	20	1450	1431	60.03	55	8	2
Product length	155								
Primer pair 2									
	Sequence(5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3'complementarity
Forward primer	GGGTCTCTTCGCCTGTCAAA	Plus	20	352	371	59.97	55	3	3
Reverse primer	GCCTTGAGAACCAGCACTCT	Minus	20	528	509	59.96	55	3	1
Product length	177								
Primer pair 3									
	Sequence(5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3'complementarity
Forward primer	GGTGGCTATACGGACAGGTG	Plus	20	885	904	59.9	60	4	2
Reverse primer	GCAAGGCACTTCCACAGTTG	Minus	20	1006	987	59.97	55	4	3
Product length	122								
Primer pair 4									
	Sequence(5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3'complementarity
Forward primer	GAGCACCCTGCAGGAAATCT	Plus	20	1431	1450	60.03	55	8	2
Reverse primer	CTCCGGTCCCGAAGATGAAG	Minus	20	1530	1511	59.9	60	6	0
Product length	100								
Primer pair 5									
	Sequence(5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3'complementarity
Forward primer	ATGTGGTTCCCAGCACAGAG	Plus	20	1649	1668	59.96	55	4	1
Reverse primer	AACCGCTCTGCTTCTCTACG	Minus	20	1838	1819	59.83	55	2	2
Product length	190								

Fig. 1 Summary and functions of Tartary buckwheat database (TBD). (a) Home page of TBD; (b) Summary of gene expression in five Tartary buckwheat organs in TBD and statistics on the numbers of genes expressed in different tissues and different fruit development stages; (c) An example entry of *FtPinG0000001800.01* in gene expression value (GEV); (d) Transcription factor (TF) page of TBD; (e) An example entry of the growth-regulating factor (GRF) family in TF; (f) An example entry of *FtPinG0000001800.01* in quantitative real-time polymerase chain reaction (qRT-PCR) primer (qPrimer).

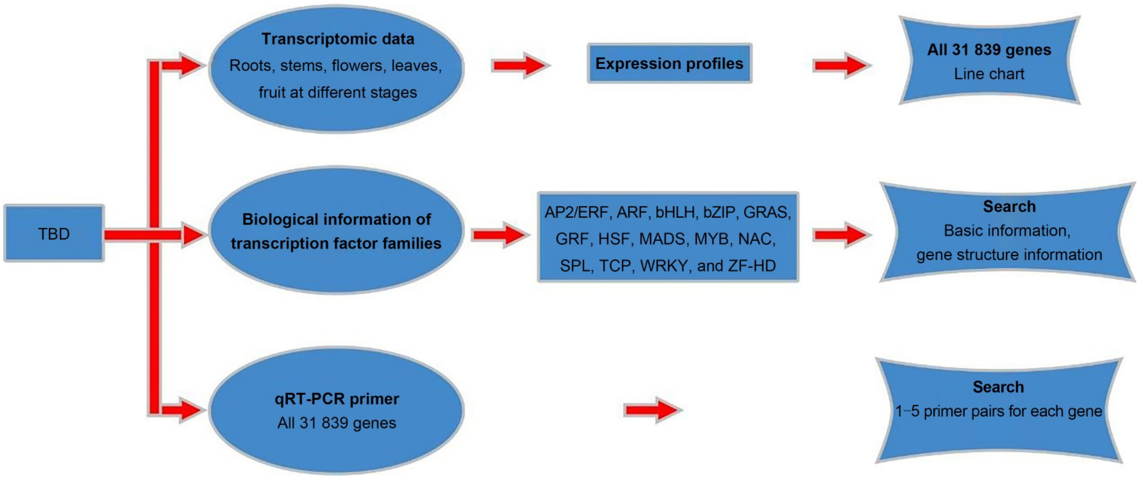

Fig. 2 Data and feature diagram of Tartary buckwheat database (TBD). TBD incorporates the biological information, quantitative real-time polymerase chain reaction (qRT-PCR), and transcriptomic data for Tartary buckwheat. All the data are manually curated and integrated. Various tools are embedded in the webpage to retrieve and analyze the data.

that meet the experimental requirements with regard to parameters such as sequence, melting temperature, and GC content (Fig. 1f).

The "Help" page starts with an overview of TBD. It also details the features of each page of the website to help new users understand the design and layout of the site and become proficient with the database.

"About Us" provides an avenue for users with new discoveries to contact us to submit their data requests. Our database will be updated regularly based on user feedback.

In conclusion, we developed TBD as the central portal for genetic study of this promising crop. TBD stores expression profiles of all Tartary buckwheat genes, qPrimers of all genes, and bioinformatics information of important TF family genes. Since the database is manually integrated, all raw data collected/submitted are processed through our own custom pipeline. The processed data are also correlated to the retrieval analysis module that performs the main functions of TBD, including various query and visualization tasks (Fig. 2). We believe that this database will not only promote research on improving Tartary buckwheat breeding, but also bring increased attention to the genetic improvement of other important crops.

TBD is a platform based on the currently sequenced Tartary buckwheat genome and will be updated in the future. As research progresses, we will continuously update information on the other TF families, while also providing evolutionary and expansion information on corresponding families in multiple species. Meanwhile, we will provide bioinformatics tools such as a Genome Browser and BLAST sequence search in the next version of TBD. Future research is not limited to improving Tartary buckwheat breeding, but will also provide valuable information for other important species.

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Author contributions

Moyang LIU and Hui CHEN designed research. Moyang LIU, Wenjun SUN, and Zhaotang MA performed research. Zhaotang MA, Yuan HU, and Hui CHEN analyzed data. Wenjun SUN and Yuan HU wrote the paper. Hui CHEN, Wenjun SUN, and Moyang LIU revised the manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Moyang LIU, Wenjun SUN, Zhaotang MA, Yuan HU, and Hui CHEN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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