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Genome-wide identification, abiotic stress, and expression analysis of PYL family in Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) during grain development

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Abstract

Background Abscisic acid (ABA) is a plant hormone that plays an important role in plant resistance to drought, salinity, cold, and pathogens. It is also important for regulating plant growth and development. Pyrabactin resistance/pyr1-like/regulatory components of the ABA receptor (PYL/RCAR) are ABA receptor proteins in plants and the core of ABA signal transduction pathways in plant regulatory factors. At present, there are no reports on the PYL family of Tartary buckwheat.

Results In this study, 19 paralogous form *PYL* genes in buckwheat were identified at the whole-genome level and named *FtPYL1-FtPYL19* according to their positions on chromosomes. We further analyzed the gene structure, conserved motifs, *cis*-acting elements, gene duplication, phylogenetic relationships, and expression patterns under different stress treatments and during grain development of the 19 paralogous form *PYL* genes in Tartary buckwheat. The *FtPYL* gene exhibits a single exonic gene structure for about 68.4% of the duplicated forms from the total paralogous forms. The remaining subfamilies, such as I and II, contain three exons and two exons (e.g., *FtPYL19*), respectively. Nineteen *FtPYL* genes were evenly distributed across the eight chromosomes, with at least one *FtPYL* gene on each chromosome. In the *FtPYL* gene family, there was one tandem repeat event and five gene duplication events. We investigated the gene expression levels of *FtPYL* gene under four abiotic stresses and different stages of grain development. Under drought stress (PEG6000), the relative expression levels of *FtPYL14* and *FtPYL15* increased by fourfold. Under high temperature stress (38℃), the relative expression level of *FtPYL16* dropped to 0.12, and that of *FtPYL17* fell to 0.22. At different stages of grain development, the gene expression level of *FtPY15* is extremely high at 19 D. The relative expression level of *FtPYL7* in roots and stems reaches up to approximately 450, and the relative expression level of *FtPYL10* in 13 D also reaches up to 248.

Summary In this study, the *PYL* gene family of Tartary buckwheat was identified and analyzed based on the whole genome, and 19 paralogous form *FtPYL* genes of Tartary buckwheat were bioinformatically analyzed. The expression patterns of 19 paralogous form *FtPYL* genes in Tartary buckwheat cultivars under different stress treatments and during grain development were analyzed. It was found that the *FtPYL* gene played an important role in grain development.

Keywords Tartary buckwheat, *PYL* gene family, Evolution analysis, Gene expression level

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Background

Abscisic acid (ABA) is an important hormone in plants that helps them resist adverse environmental conditions and regulate growth and development. During plant growth and development, ABA is involved in many plant life activities, such as seed dormancy and germination, plant organ morphogenesis, grain maturation, leaf senescence, leaf shedding, response to biotic and abiotic stresses, and leaf stomatal regulation [1–4]. For example, after the synthesis of ABA in plant roots, it is transported upward through the xylem to the leaves, where it regulates the potassium, chloride, and malate ions in leaf cells, thereby changing the turgor pressure of cells and inducing the opening and closing of leaf stomata to protect plants [5–7].

The ABA Receptor pyrabactin resistance 1/PYR1-like/regulatory component of the ABA receptor (PYR/PYL/RCAR, hereafter referred to as PYL) is a core component of the ABA signal transduction regulatory network. In plants, ABA is sensed and combined with PYL [8–10]. When ABA is sensed and bound by PYL, the resultant conjugation inhibits the activity of protein phosphatase 2C (PP2C) in plants, thereby releasing the serine/threonine protein kinase SRK2 (SnRK2) [8]. However, SnRK2 activates serine residues in the ring without inhibiting PP2C, and self-phosphorylation activates SnRK2 kinase activity [5], and the activated SnRK2 kinase will then activate downstream ABA response genes [11–13].

PYL genes have been identified as core regulators of the ABA signal transduction network in many plants, including *Arabidopsis* [14–17], rice [18–21], tomato [22], maize [23, 24], wheat [25], tobacco [26], cucumber [27], sweet potato [28] and rapeseed (*Brassica napus*) [29]. According to research findings, when plants are subjected to abiotic stresses, such as drought, high temperature, low temperature, and salt, they accumulate ABA in their bodies through a series of signal transduction pathways [2, 30], thus prompting plants to make a series of defense responses and improve plant resistance [31]. For example, in *Arabidopsis thaliana*, overexpression of *AtPYL4* was found to improve drought tolerance [32]. In rice, *OsPYL5* overexpression can improve drought and salt tolerance in rice [21]. Through transgenic technology, grape *VaPYL9* was overexpressed in tomatoes, the antioxidant oxidase activity and proline content of transgenic tomatoes were higher, and their cold resistance was significantly improved compared to those of the wild-type [33]. In maize, three transgenic *Arabidopsis thaliana* lines, *ZmPYL8*, *ZmPYL9* and *ZmPYL12*, showed strong drought resistance [34]. In terms of growth and development, two genes, *AtPYL6* and *AtPYL13* of the *Arabidopsis* family, were found to inhibit seed germination [35], whereas PYL8 played a key role in mediating ABA-controlled root growth [15]. In rice, OsPYL/RCAR5 has involved a certain role in seed germination and seedling growth [19]. In conclusion, several studies have shown that the *PYL* gene family plays important roles in plant abiotic stress, growth, and development.

Tartary buckwheat a genus of *Fagopyaceae* and *Fagopyrum Mill*, is a healthy plant food rich in a variety of bioactive compounds and low in calories [36]. Tartary buckwheat has strong resistance to adverse environments and abiotic stresses, such as drought, saline-alkali stress, cold, and heat, and is often grown and planted under extreme environmental conditions [36, 37]. This indicates that Tartary buckwheat has abundant resistance genes; therefore, identifying and studying these genes is of great significance for new varieties with high resistance. Although the *PYL* gene family has been identified in many species and studied in-depth, no reports on the *PYL* gene family in Tartary buckwheat have been published to date. In this study, we identified and analyzed the *PYL* gene family of Tartary buckwheat based on the whole genome, including the physical and chemical information of genes, chromosome localization and distribution, gene structure analysis, promoter *cis*-acting elements, phylogenetic relationships, gene duplication, and collinearity analysis. In addition, based on the research of PYL family in abiotic stress response, we analyzed its expression patterns under four major abiotic stresses (drought, salt, high temperature and low temperature) during grain development.

Result

Identification, basic information analysis and phylogenetic analysis of Tartary buckwheat PYL

Based on the whole Tartary buckwheat genome, possible *PYL* genes of Tartary buckwheat were obtained using two BLAST methods. After domain identification and removal of duplicate genes, 19 paralogous form *PYL* genes were obtained from Tartary buckwheat. According to the positional relationship of 19 paralogous form *PYL* genes in eight Tartary buckwheat chromosomes, the 19 paralogous form *PYL* genes were named *FtPYL1-FtPYL19*. Simultaneously, we analyzed the amino acid sequence length, protein molecular weight, protein isoelectric point, subcellular localization prediction, gene exon number, protein hydrophilicity and other biological information of 19 Tartary buckwheat paralogous form *PYL* genes (Additional file Table S1). To better analyze the evolutionary relationships of the *FtPYL* gene family, we added 19 buckwheat paralogous form PYL sequences, 14 *Arabidopsis* paralogous form PYL sequences, and 13 rice paralogous form PYL sequences to construct a phylogenetic tree with a bootstrap value of 1000 using Neighbor-Joining (NJ) (Table 1). According to the PYL family classification of *Arabidopsis* and rice [18], the 19

Table 1 AtPYL and OsPYL protein sequence for phylogeny

Gene	Gene ID	Group	Sequence Type	Sequences
AtPYR1	AT4G17870	SubfamilyIII	pep	MPSELTPEERSELKNSIAEFHTYQLDPGSCSSLHAQRIHAPPELVWSIVRRFDKPQTYKHFIKSCSVEQN-FEMRVGCTRDVIVISGLPANTSTERLDILDDERRVTGFSIIGGEHRLTNYKSVTTVHRFEKENRIWTVVLESY-VVDMPEGNSEDDTRMFADTVVKLNLQKLATVAEAMARNSGDGSGSQVT
AtPYL1	AT5G46790	SubfamilyIII	pep	MANSESSSSPVNEEENSQRISTLHHQTMPSDLTQDEFTQLSQSIAEFHTYQLGNGRCSSLLAQRIHAP-PETVWSVVRRFDRPQIYKHFIKSCNVSEDFEMRVGCTRDVNVISGLPANTSRERLDLLDDDRRVTGF-SITGGEHRLRNYKSVTTVHRFEKEEEEERIWTVVLESYVVDVPEGNSEEDTRLFADTVIRLNLQKLASITEAM-NRNNNNNNSSQVR
AtPYL2	AT2G26040	SubfamilyIII	pep	MSSSPAVKGLTDEEQKTLEPVIKTYHQFEPDPTTCTSLITQRIHAPASVVWPLIRRFDNPERYKHFVKRCRL-ISGDGDVGSVREVTVISGLPASTSTERLEFVDDDHRVLSFRVVGGEHRLKNYKSVTSVNEFLNQDSGKVYT-VVLESYTVDIPEGNTEEDTKMFVDTVVKLNLQKLGVAATSAPMHDDE
AtPYL3	AT1G73000	SubfamilyIII	pep	MNLAPIHDPSSSSTTTTSSSTPYGLTKDEFSTLDSIIRTHHTFPRSPNTCTSLIAHRVDAPAHAIWRFVRD-FANPNKYKHFIKSCTIRVNGNGIKEIKVGTIREVSVVSGLPASTSVEILEVLDEEKRILSFRVLGGEHRLN-NYRSVTSVNEFVVLEKDKKKRVYSVVLESYIVDIPQGNTEEDTRMFVDTVVKSNLQNLAVISTASPT
AtPYL4	AT2G38310	SubfamilyII	pep	MLAVHRPSSAVSDGDSVQIPMMIASFQKRFPSLSRDSTAARFHTHEVGPNQCCSAVIQEISAPISTVWSV-VRRFDNPQAYKHFLKSCSVIGGDGDNVGSLRQVHVVSGLPAASSTERLDILDDERHVISFSVVGGDHRLS-NYRSVTTLHPSPISGTVVVESYVVDVPPGNTKEETCDFVDVIVRCNLQSLAKIAENTAAESKKKMSL
AtPYL5	AT5G05440	SubfamilyII	pep	MRSPVQLQHGSDATNGFHTLQPHDQTDGPIKRVCLTRGMHVPEHVAMHHTHDVGPDQCCSSVVQMI-HAPPESVWALVRRFDNPKVYKNFIRQCRIVQGDGLHVGDLREVMVVSGLPAVSSTERLEILDEERHVISFSV-VGGDHRLKNYRSVTTLHASDDEGTVVVESYIVDVPPGNTEEETLSFVDTIVRCNLQSLARSTNRQ
AtPYL6	AT2G40330	SubfamilyII	pep	MPTSIQFQRSSTAAEAANATVRNYPHHHQKQVQKVSLTRGMADVPEHVELSHTHVVGPSQCFSV-VVQDVEAPVSTVWSILSRFEHPQAYKHFVKSCHVVIGDGREVGSVREVRVVSGLPAAFSLERLEIMD-DDRHVISFSVVGGDHRLMNYKSVTTVHESEEDSDGKKRTRVVESYVVDVPAGNDKEETCSFADTIVRCN-LQSLAKLAENTSKFS
AtPYL7	AT4G01026	SubfamilyI	pep	MEMIGGDDTDTEMYGALVTAQSLRLRHLHHCRENQCTSVLVKYIQAPVHLVWSLVRRFDQPQKYKPFIS-RCTVNGDPEIGCLREVNVKSGLPATTSTERLEQLDDEEHILGINIIGGDHRLKNYSSILTVHPEMIDGRSGT-MVMESFVVDVPQGNTKDDTCYFVESLIKCNLKSLACVSERLAAQDITNSIATFCNASNGYREKNHTETNL
AtPYL8	AT5G53160	SubfamilyI	pep	MEANGIENLTNPNQEREFIRRHHKHELVDNQCSSTLVKHINAPVHIVWSLVRRFDQPQKYKPFISRCV-VKGNMEIGTVREVDVKSGLPATRSTERLELLDDNEHILSIRIVGGDHRLKNYSSIISLHPETIEGRIGTLVIESFV-VDVPEGNTKDETCYFVEALIKCNLKSLADISERLAVQDTTESRV
AtPYL9	AT1G01360	SubfamilyI	pep	MMDGVEGGTAMYGGLETVQYVRTHHQHLCRENQCTSALVKHIKAPLHLVWSLVRRFDQPQKYKPFVS-RCTVIGDPEIGSLREVNVKSGLPATTSTERLELLDDEEHILGIKIIGGDHRLKNYSSILTVHPEIIEGRAGTM-VIESFVVDVPQGNTKDETCYFVEALIRCNLKSLADVSERLASQDITQ
AtPYL10	AT4G27920	SubfamilyI	pep	MNGDETKKVESEYIKKHHRHELVESQCSSTLVKHIKAPLHLVWSIVRRFDEPQKYKPFISRCVVQGK-KLEVGSVREVDLKSGLPATKSTEVLEILDDNEHILGIRIVGGDHRLKNYSSTISLHSETIDGKTGTLAIESFVVD-VPEGNTKEETCFFVEALIQCNLNSLADVTERLQAESMEKKI
AtPYL11	AT5G45860	SubfamilyII	pep	METSQKYHTCGSTLVQTIDAPLSLVWSILRRFDNPQAYKQFVKTCNLSSGDGGEGSVREVTVVSGL-PAEFSRERLDELDDESHVMMISIIGGDHRLVNYRSKTMAFVAADTEEKTVVVESYVVDVPEGNSEEETTS-FADTIVGFNLKSLAKLSERVAHLKL
AtPYL12	AT5G45870	SubfamilyII	pep	MKTSQEQHVCGSTVVQTINAPLPLVWSILRRFDNPKTFKHFVKTCKLRSGDGGEGSVREVTVVSDL-PASFSLERLDELDDESHVMVISIIGGDHRLVNYQSKTTVFVAAEEEKTVVVESYVVDVPEGNTEEETTL-FADTIVGCNLRSLAKLSEKMMELT
AtPYL13	AT4g18620	SubfamilyII	pep	MESSKQKRCRSSVVETIEAPLPLVWSILRSFDKPQAYQRFVKSCTMRSGGGGGKGGEGKGSVRDVTLVS-GFPADFSTERLEELDDESHVMVVSIIGGNHRLVNYKSKTKVVASPEDMAKKTVVVESYVVDVPEGTSEED-TIFFVDNIIRYNLTSLAKLTKKMMK
OsPYL1	Os10g42280	SubfamilyIII	pep	MEQQEEVPPPPAGLGLTAEEYAQVRATVEAHHRYAVGPGQCSSLLAQRIHAPPAAVWAVVRRFDCPQVY-KHFIRSCVLRPDPHHDDNGNDLRPGRLREVSVISGLPASTSTERLDLLDDAHRVFGFTITGGEHRLRNYRS-VTTVSQLDEICTLVLESYIVDVPDGNTEDDTRLFADTVIRLNLQKLKSVSEANANAAAAAAAPPPPPAAAE
OsPYL2	Os06g36670	SubfamilyIII	pep	MEAHVERALREGLTEEERAALEPAVMAHHTFPPSTTTATTAAATCTSLVTQRVAAPVRAVWPIVRSFGN-PQRYKHFVRTCALAAGDGASVGSVREVTVVSGLPASTSTERLEMLDDDRHIISFRVVGGQHRLRNYRS-VTSVTEFQPPAAGPAPAPPYCVVVESYVVDVPDGNTAEDTRMFTDTVVKLNLQKLAAVAEDSSSASRRRD
OsPYL3	Os02g13330	SubfamilyIII	pep	MEPHMERALREAVASEAERRELEGVVRAHHTFPAAERAAGPGRRPTCTSLVAQRVDAPLAAVWPIVRG-FANPQRYKHFIKSCELAAGDGATVGSVREVAVVSGLPASTSTERLEILDDDRHVLSFRVVGGDHRLRNYRS-VTSVTEFSSPSSPPSPPRPYCVVVESYVVDVPEGNTEEDTRMFTDTVVKLNLQKLAAVATSSSPPAAGNHH
OsPYL4	Os01g61210	SubfamilyII	pep	MPYAAVRPSPPPQLSRPIGSGAGGGKACPAVPCEVARYHEHAVGAGQCCSTVVQAIAAPADAVWSVVR-RFDRPQAYKKFIKSCRLVDGDGGEVGSVREVRVVSGLPATSSRERLEVLDDDRRVLSFRIVGGEHRLANYRS-VTTVHEAAAPAMAVVVESYVVDVPPGNTWEETRVFVDTIVRCNLQSLARTVERLAPEAPRANGSIDHA
OsPYL5	Os05g39580	SubfamilyII	pep	MMPYTAPRPSPPQHSRIGGCGGGGVLKAAGAAGHAASCVAVPAEVARHHEHAAGVGQCCSAV-VQAIAAPVDAVWSVVRRFDRPQAYKHFIRSCRLLDGDGDGGAVAVGSVREVRVVSGLPATSSRERLEILD-DERRVLSFRVVGGEHRLSNYRSVTTVHETAAGAAAAVVVESYVVDVPHGNTADETRMFVDTIVRCN-LQSLARTAEQLALAAPRAA

Table 1 (continued)

Gene	Gene ID	Group	Sequence Type	Sequences
OsPYL6	Os03g18600	SubfamilyII	pep	MPCIPASSPGIPHQHQHQHHRALAGVGMAVGCAAEAAVAAAGVAGTRCGAHDGEVPMEVARH-HEHAEPGSGRCCSAVVQHVAAPAPAVWSVVRRFDQPQAYKRFVRSCALLAGDGGVGTLREVRV-VSGLPAASSRERLEILDDESHVLSFRVVGGEHRLKNYLSVTTVHPSPSAPTAATVVVESYVVDVPPGNT-PEDTRVFVDTIVKCNLQSLANTAEKLAAGARAAGS
OsPYL7	Os06g33480	Subfamilyl	pep	MNSGAGGAGGAAVGRMPAGSLQWAQWRLADERCELREEEMEYMRRFHRHEIGSNQCNSFIAKHVRA-PLQNVWSLVRRFDQPQIYKPFVRKCVMRGNVETGSVREIIVQSGLPATRSIERLEFLDDNEYILRVKFIGGD-HMLKKCGP
OsPYL8	Os06g33640	Subfamilyl	pep	MNGAGGAGGAAAGKLPMVSHRQVQWRLADERCELREEEMEYIRQFHRHEPSSNQCTSFVAKHIKA-PLQTVWSLVRRFDQPQLFKPFVRKCVMRENIIATGCVREVNVQSGLPATRSTERLELLDDNEHILKVKFIG-GDHMLKNYSSILTIHSEVIDGQLGTLVVESFVVDIPEGNTKDDICYFIENILRCNLMTLADVSEERLANP
OsPYL9	Os06g36690	Subfamilyl	pep	MNGVGGAGGAAAGKLPMVSHRRVQWRLADERCELREEEMEYIRRFHRHEPSSNQCTSFAAKHIKAPL-HTVWSLVRRFDQPQLFKPFVRNCVMRENIIATGCIREVNVQSGLPATRSTERLELLDDNEHILKVKFIGGD-HMLKNYSSILTIHSEVIDGQLGTLVVESFIVDVLEGNTKDDISYFIENVLRCNLRTLADVSEERLANP
OsPYL10	Os02g15640	Subfamilyl	pep	MVEVGGGAAEAAAGRRWRLADERCDLRAAETEYVRRFHRHEPRDHQCSSAVAKHIKAPVHLVWSLVR-RFDQPQLFKPFVSRCEMKGNIEIGSVREVNVKSGLPATRSTERLELLDDNEHILSVRFVGGDHRLKNYSSILT-VHPEVIDGRPGTLVIESFVVDVPEGNTKDETCYFVEALLKCNLKSLAEVSERLVVKDQTEPLDR
OsPYL11	Os05g12260	Subfamilyl	pep	MVGLVGGGGWRVGDDAAGGGGGGAVAAGAAAAAEAEHMRRLHSHAPGEHQCSSALVKHIKA-PVHLVWSLVRSFDQPQRYKPFVSRCVVRGGDLEIGSVREVNVKTGLPATTSTERLELLDDDEHILSVKFVG-GDHRLRNYSSIITVHPESIDGRPGTLVIESFVVDVPDGNTKDETCYFVEAVIKCNLTSLAEVSERLAVQSPT-SPLEQ
OsPYL12	Os02g15620	Subfamilyl	pep	MRGSTSLAVGCVREVDFKSGFPAKSSVERLEILDDKEHVFGVRIIGGDHRLKNYSSVLTAKPEVIDGE-PATLVSESFVVDVPEGNTADETRHFVEFLIRCNLRSLAMVSQRLLLAQGDLAEPPAQ
OsPYL13	Os06g33490	Subfamilyl	pep	MNGCTGGAGGVAAGRLPAVSLQQAQWKLVDERCELREEEMEYVRWFHRYELVATGATPSLPNTSGCP-SKLGLPSTRRIERLGFPDDNDHTLRVKFIGGDHMLKDYSSTLIIHLEVIDGQLVTLVIESFVVDILEGNTKDE-ISYFIENLLKFNLRTLRV

paralogous form *PYL* genes in Tartary buckwheat were divided into three subgroups (Subfamily I, Subfamily II, and Subfamily III, Fig. 1). Among the three subgroups, subfamily I had the fewest members with only two *FtPYL* genes, subfamily III had the most members with 12 *FtPYL* genes, and subfamily II had five *FtPYL* genes. In subgroup I, the *FtPYL* gene was more closely related to the rice *PYL* gene, whereas in subgroup II, the *FtPYL* gene was more closely related to the *Arabidopsis PYL* gene. In subgroup II, the *FtPYL* gene was more similar to the *Arabidopsis PYL* gene. The *FtPYL* gene was similar to the *PYL* gene of *Arabidopsis thaliana*, but the five genes, *FtPYL1*, *FtPYL9*, *FtPYL13*, *FtPYL16* and *FtPYL17* may be unique to Tartary buckwheat after evolution.

Analysis of gene structure, motif composition and *cis*-acting elements of *FtPYL* gene family

The full-length protein sequences of the 19 paralogous form *FtPYL* genes were constructed into a phylogenetic tree with a bootstrap value of 1000, and their gene structures, motif compositions, and *cis*-acting elements were analyzed (Fig. 2). Using a phylogenetic tree, we found (Fig. 2A) that *FtPYL* genes of the same subgroup converged in the phylogenetic tree and were closer in evolution. In terms of gene structure (Fig. 2B), the gene structures of the same subgroup were similar, such as *FtPYL10* and *FtPYL11* of subgroup subfamily I. The same is true for subfamilies II and III, but *FtPYL1* and *FtPYL9* may be endemic to Tartary buckwheat. The *PYL* gene of Tartary buckwheat with only one exon accounted for 68.4% of the total gene number, of which subfamily I contained three exons, subfamily II had two exons in *FtPYL19*, and the others contained only one exon. However, the exon number of subfamily III varied greatly, indicating that subfamily III may vary greatly and is evolving in a direction different from that of the other members of subfamily III. To explore the 19 paralogous form FtPYL protein-conserved motifs, we used the MEME website (https://meme-suite.org/meme/tools/MEME) for conserved base sequence analysis (Fig. 2C, Additional file Table S2). We found that subfamilies I and II had similar motifs, whereas subfamily III showed some differences in the conserved motifs. In subgroup III, we found that the mods of *FtPYL1* and *FtPYL9* belonged to the same class; *FtPYL13*, *FtPYL16*, and *FtPYL17* belonged to the same class; and the rest belonged to subfamilies I and II and could be classified into the same class. These results indicate that compared with other genes in subfamily III, *FtPYL1*, *FtPYL9*, *FtPYL13*, *FtPYL16* and *FtPYL17* are evolving in different directions, which may be related to the strong stress resistance of Tartary buckwheat.

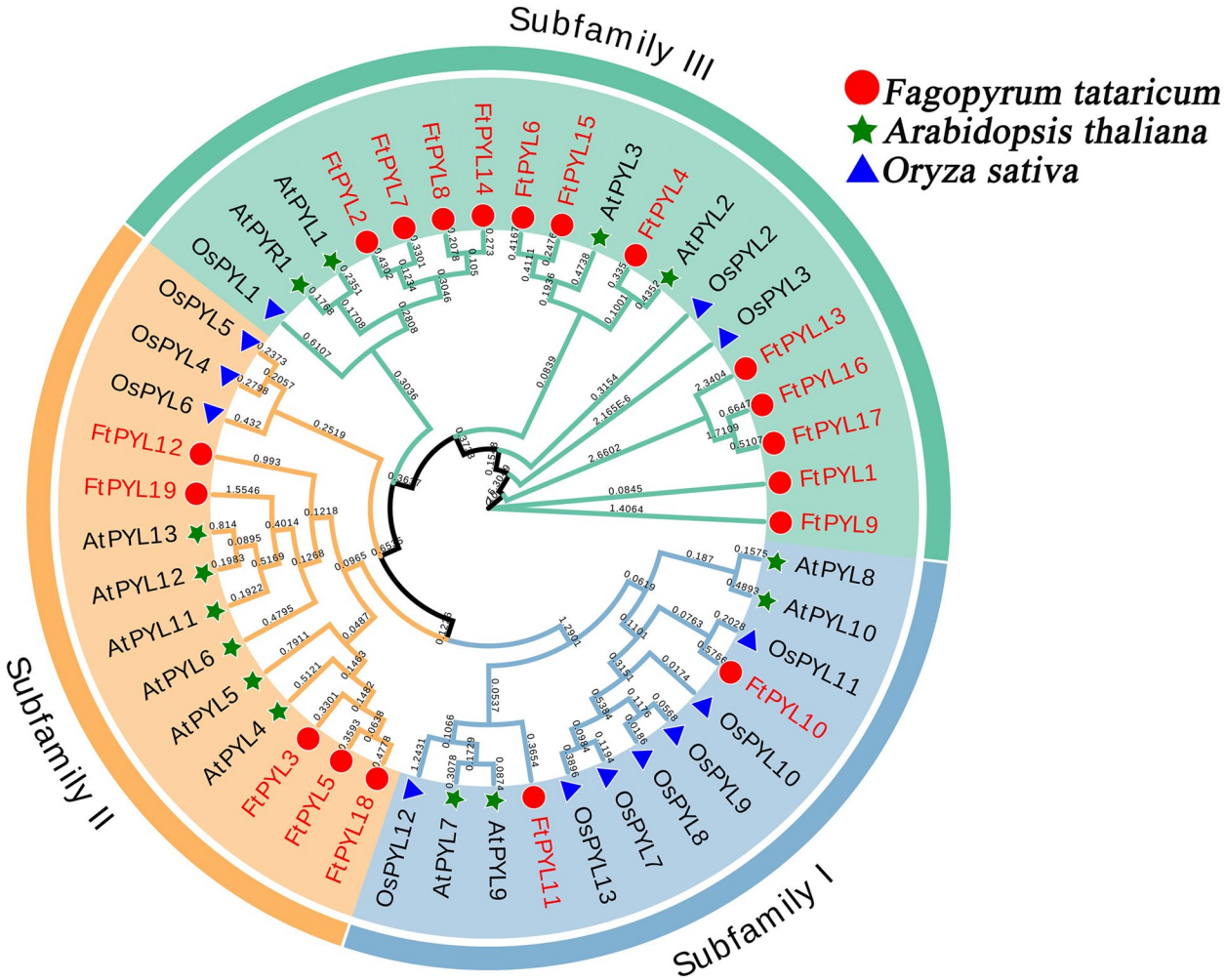

Fig. 1 Phylogenetic tree of PYL proteins in buckwheat, *Arabidopsis*, and rice. Red circles represent Tartary buckwheat, green asterisks represent *Arabidopsis*, and blue triangles represent rice. In phylogenetic trees, Tartary buckwheat PYL protein is red font, and different subgroups correspond to different regional colors

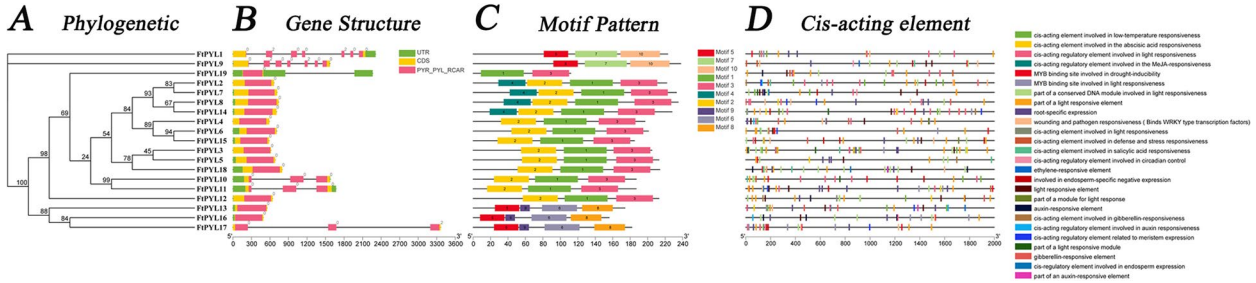

Fig. 2 Phylogenetic relationships, gene structure, motif distribution and *cis*-acting elements of 19 PYL proteins in Tartary buckwheat. **A** A phylogenetic tree of Tartary buckwheat PYL protein with 1000 replicates per node. **B** Buckwheat 19 *PYL* gene genetic structure diagram, respectively UTR (untranslated), CDS (coding sequence), structural domain (PYR_PYL_RCAR) and introns (Number indicates the phase of the corresponding intron.). **C** Amino acid conserved Motif 1–10 in 19 Tartary buckwheat PYL proteins, with different color blocks corresponding to different conserved motifs, and black lines indicating the relative length of corresponding proteins. **D** The 2000 bp promoter sequences of 19 Tartary buckwheat *PYL* genes were *cis*-acting elements, with different color blocks corresponding to different *cis*-acting elements

To better understand the possible functions of the *FtPYL* genes, *cis*-acting element analysis was performed on the upstream 2 kb promoter region of these 19 paralogous form *FtPYL* genes (Fig. 2D, Additional file Table S2). We found that the *FtPYL* gene has a variety of important *cis*-acting elements. Examples include *cis*-acting elements associated with plant hormones such as MeJA (TGACG-motif, CGTCA-motif), ABRE, salicylic acid (TCA-element), and gibberellin (P-box, GARE-motif). We also found a variety of *cis*-acting elements related to stress resistance and growth, such as *cis*-acting elements related to low-temperature reactions and drought induction, *cis*-regulatory elements related to endosperm expression, *cis*-regulatory elements related to flavonoid biosynthesis genes, endosperm expression, and seed-specific regulatory elements involved in various growth- and development-related regulatory elements. These results indicated that the *PYL* gene family plays an important role in the growth and development of Tartary buckwheat.

Chromosome distribution and gene duplication of PYL gene in Tartary buckwheat

Using the Tartary buckwheat genome and genome annotation information, we determined the location of 19 paralogous form *FtPYL* genes on the Tartary buckwheat chromosomes (Fig. 3A). Nineteen paralogous form *FtPYL* genes were evenly distributed across the eight chromosomes, with at least one *FtPYL* gene on each chromosome. Among these, only one *FtPYL* gene (~5.26%) was detected in the Ft6. Four *FtPYL* genes (~21.05%) were identified in Ft5. There were two chromosomes on Ft1, Ft2, Ft3, and Ft8 (~10.53%) and three chromosomes on Ft4 and Ft7 (~15.79%). In the Tartary buckwheat PYL family, one tandem repeat event was found in Ft7, involving two genes, *FtPYL16* and *FtPYL17*. The sequences of *FtPYL16* and *FtPYL17* are highly similar. No tandem repeat events were found on the other seven chromosomes (Fig. 3A, Additional file Table S3). Five fragment duplication events were found in the Tartary buckwheat PYL family, including Ft1, Ft3, Ft4, Ft5, Ft7, and Ft8. There is a segmental duplication event on chromosome Ft4 (FtPYL7 and FtPYL8), while other segmental duplication events occur between two chromosomes (Fig. 3B, Additional file Table S3). When the gene fragments were replicated, they all belonged to the same subgroup. For example, *FtPYL5* and *PYL18* are members of subgroup II, whereas the others are members of subgroup III. The gene structure, phylogeny, and conserved motifs of these genes were very similar, which supported the grouping of the Tartary buckwheat PYL family. Tandem repeats and gene duplication events play indispensable roles in the generation of new functions and gene amplification.

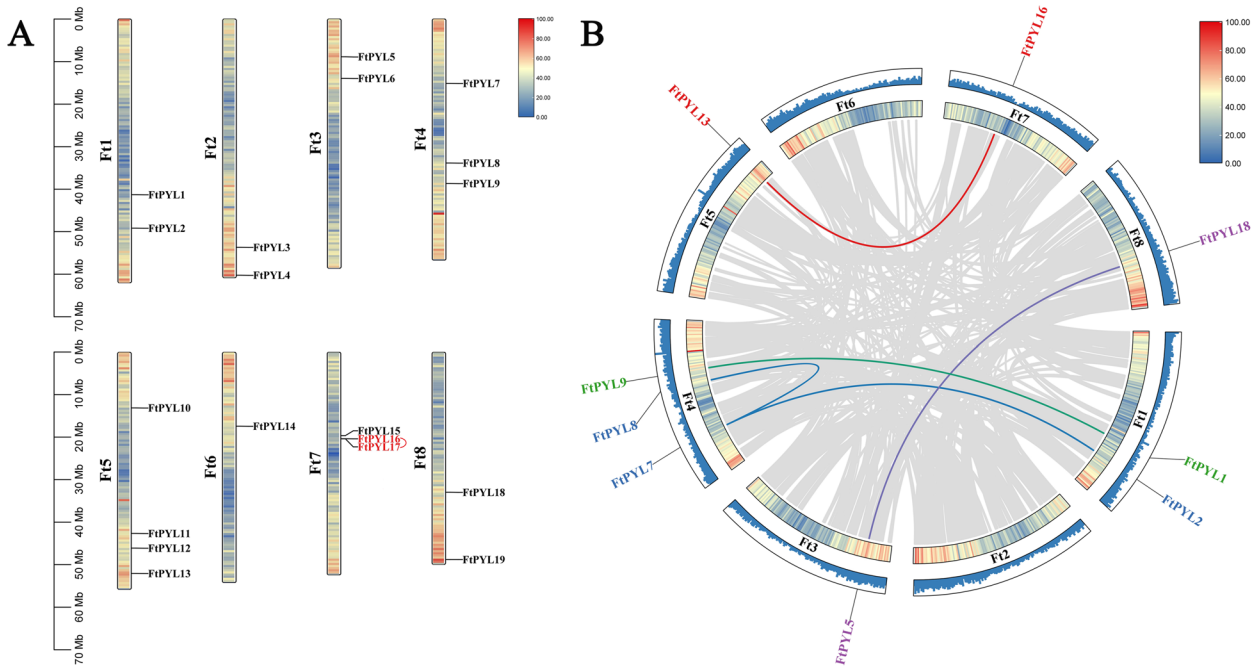

Fig. 3 **A** Distribution of 19 *PYL* genes in Tartary buckwheat on chromosomes, where the chromosome is gene density (Bin Size = 500,000), and the number of genes contained in this length interval is from less to more from blue to red. **B** Distribution and gene duplication relationship of *PYL* gene pairs in Tartary buckwheat on chromosomes. Gray lines represent gene pairs between different chromosomes, and different color lines represent different *FtPYL* gene pairs. From the inside out, the first is the chromosome, the chromosome information is consistent with (A), and the second outer circle is the chromosome density (Bin Size = 500,000)

In the Tartary buckwheat PYL family, there was one tandem repeat event and five gene duplication events. These results indicate that both tandem repeat events and gene duplication events play a role in the generation of new functions and gene expansion in the Tartary buckwheat PYL family and that the contribution of gene duplication events is greater than that of tandem repeat events.

Evolutionary analysis of the PYL family of Tartary buckwheat and the PYL family of different species

To better understand the evolutionary relationships of the PYL family in different species, we included six species of the Tartary buckwheat PYL family (three dicotyledonous plants, *Arabidopsis thaliana*, *Cucumis sativus*, and *Solanum lycopersicum*; three monocotyledonous plants, *Oryza sativa*, *Zea mays*, and *Triticum aestivum* constructed collinearity maps and phylogenetic trees (Fig. 4, Additional file Table S4). In the analysis of *FtPYL* gene and homologous genes of the six species (Fig. 4A, Additional file Table S4), the homologous logarithms were as follows: 14 pairs (*Arabidopsis thaliana*), 19 pairs (*Cucumis sativus*), 15 pairs (*Solanum lycopersicum*), 2 pairs (*Oryza sativa* and *Zea mays*), and 0 pairs (*Triticum aestivum*). Compared to monocotyledonous plants, *FtPYL* is more homologous to dicotyledonous plants, and there is no homologous gene pair with *Triticum aestivum*. The presence of homologous genes between *FtPYL2*, *FtPYL1*, *FtPYL11* and *FtPYL12* and the three dicotyledonous plants suggests that they may be more ancient, originated from ancient dicotyledonous ancestors, and remain highly conserved. These results suggest that *FtPYL* may have originated from dicotyledonous plants after monocotyledonous and dicotyledonous differentiation.

We combined Tartary buckwheat PYL proteins with PYL proteins from six species to construct phylogenetic trees. The conserved protein motifs of the seven species were analyzed using the MEME website (https://meme-suite.org/meme/tools/MEME) (Fig. 4B, Additional file Table S5). Phylogenetic trees revealed that only *FtPYL10* was polymerized with monocotyledonous *PYL*, *FtPYL1* was polymerized with *FtPYL9* independently, and the other *FtPYLs* were polymerized with dicotyledonous *PYL*. In the analysis of protein-conserved motifs, motifs 1, 2, 3, and 5 were present in most PYL proteins, and the phylogenetic trees corresponding to similar protein-conserved motifs also converged, but it was found that the composition of the FtPYL1 and FtPYL9 motifs was quite different from that of other conserved motifs. These results also suggest that FtPYL proteins may have originated from the ancestors of dicotyledonous plants after monocotyledonous and dicotyledonous differentiation and that *FtPYL1* and *FtPYL9* may be unique *PYL* genes that evolved from Tartary buckwheat.

Expression patterns of *FtPYL* genes under four abiotic stresses

To understand and study the physiological function of the *FtPYL* gene in Tartary buckwheat seedlings subjected

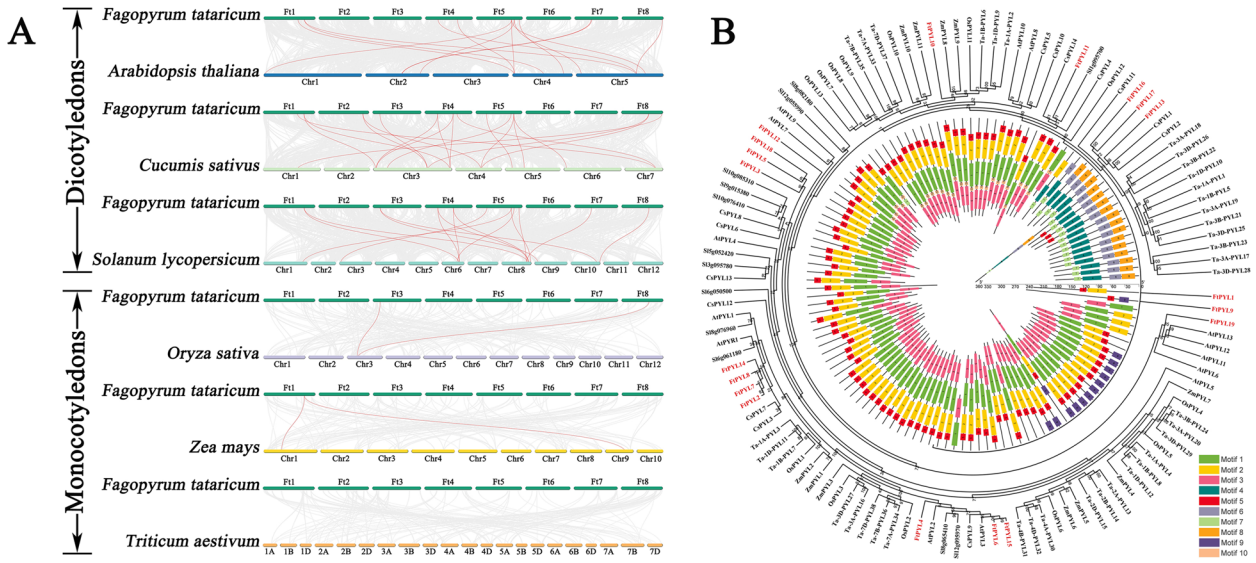

Fig. 4 **A** Collinearity analysis of Tartary buckwheat and six plants (*Arabidopsis thaliana*, *Cucumis sativus*, *Solanum lycopersicum*, *Oryza sativa*, *Zea mays*, and *Triticum aestivum*). The red lines represent Tartary buckwheat *PYL* gene pairs with the plant gene, and the gray lines represent collinear blocks in the Tartary buckwheat genome. **B** Tartary buckwheat and six plants (*Arabidopsis thaliana*, *Cucumis sativus*, *Solanum lycopersicum*, *Oryza sativa*, *Zea mays*, and *Triticum aestivum*) the phylogenetic tree and conserved motif composition of *Triticum* aestivum PYL protein. PYL of Tartary buckwheat is marked red, and different module colors represent different conserved motif

to abiotic stress, qRT-PCR was used to detect the gene expression of buckwheat seedlings under four abiotic stresses: low temperature (4℃), high temperature (38℃), salt stress (NaCl) and drought stress (PEG6000) (Fig. 5A). We found that some genes were significantly expressed or inhibited under abiotic stress and that the expression of many genes was positively or negatively correlated under abiotic stress. For example, the *FtPYL14* gene was significantly expressed under high temperature, salt, and drought stress, and its expression increased gradually with increasing treatment time under salt and drought stress conditions. Under high temperature stress (38℃), the relative expression level of FtPYL13 ultimately increased to 4.3. Under salt stress (NaCl), the relative expression level of *FtPYL14* ultimately increased to 4.9. The expression of some *FtPYL* genes gradually decreased with increasing treatment time. Examples include *FtPYL17* under cold stress; *FtPYL11*, *FtPYL16* and *FtPYL17* under heat stress; and *FtPYL17* under salt stress. Under cold stress (4℃), the relative expression level of *FtPYL17* ultimately decreased to 0.08. Under high temperature stress (38℃), the relative expression level of *FtPYL16* decreased to 0.12, and that of *FtPYL17* ultimately decreased to 0.22. Interestingly, under drought stress, the gene expression of *FtPYL17* gradually increased with increasing treatment time, which was the opposite of the situation under the other three stresses. It was also found that the gene expression of *FtPYL1* changed only under salt stress but hardly under cold, heat, or drought stress.

The correlation between *FtPYL* expression and stress was better understood using gene heat maps (Fig. 5B, C). In the correlation analysis of gene expression under abiotic stress, *FtPYL* showed obvious positive

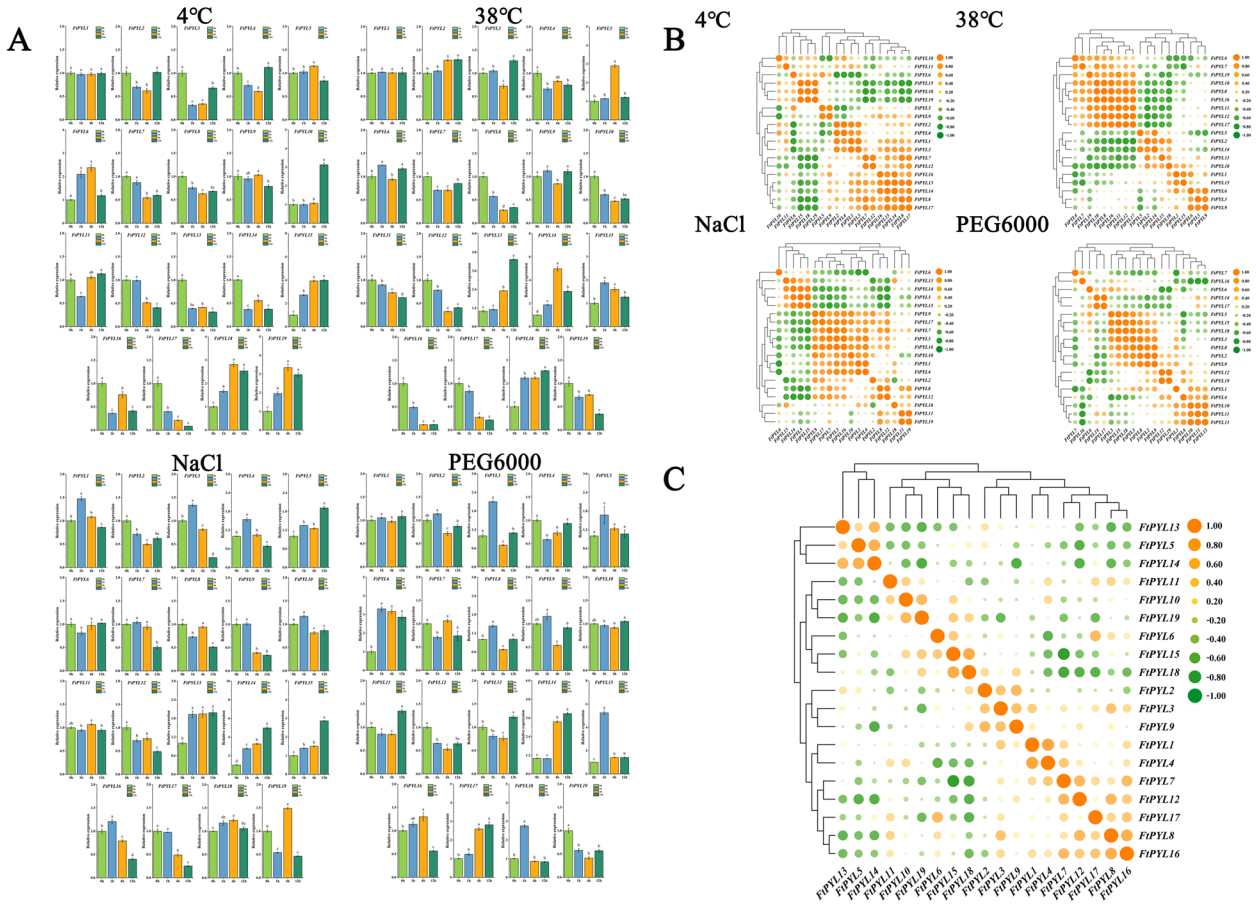

Fig. 5 **A** qRT-PCR was used to detect the expression level of *PYL* gene in Tartary buckwheat seedlings under eight abiotic stresses, and then the expression level of Tartary buckwheat *PYL* gene was normalized as the expression level of internal reference gene *FtH3*, and its relative expression level was displayed at 0 h, 1 h, 4 h and 12 h. Lower case letters above the column indicate significant differences between treatments (α = 0.05, LSD). **B** Correlation of Tartary buckwheat *PYL* gene expression under abiotic stress. The larger the marked circle on the right, the higher the correlation, the positive correlation in orange and negative correlation in green. **C** Correlation of Tartary buckwheat *PYL* gene expression under four abiotic stresses. The larger the marked circle on the right, the higher the correlation, the positive correlation in orange and negative correlation in green

and negative correlations. For example, under heat stress, there were clear positively correlated areas (*FtPYL4*, *FtPYL7*, *FtPYL8*, *FtPYL10*, *FtPYL11*, *FtPYL12*, *FtPYL16*, *FtPYL17* and *FtPYL19*) and negatively correlated areas (*FtPYL5*, *FtPYL15*, *FtPYL13*, *FtPYL14*, and *FtPYL18*) with *FtPYL8*, *FtPYL10*, *FtPYL11*, *FtPYL12*, *FtPYL16* and *FtPYL17*). Under a single abiotic stress condition, the expression of most *FtPYL* was either positively or negatively correlated. In the correlation analysis of *FtPYL* expression under the four abiotic stresses (Fig. 5C), no obvious positive or negative correlation regions were found. However, there were still a few genes with positive or negative correlations. For example, *FtPYL7* was significantly negatively correlated with *FtPYL15* and *FtPYL18*, while *FtPYL16* had a significantly positively correlated with *FtPYL7*, *FtPYL8*, *FtPYL12* and *FtPYL17*.

Expression patterns of *FtPYL* gene during grain development

Tartary buckwheat grain is a primary source of value. Therefore, qRT-PCR was used to detect gene expression levels in the roots, stems, leaves, and flowers of Tartary buckwheat at the flowering stage, and in grain tissues on days 13 (13D), 19 (19D), and 25 (25D) after flowering (Fig. 6A). It was found that *FtPYL* gene was significantly upregulated during the development of Tartary buckwheat grain. For example, *FtPYL7* was highly expressed in the roots and stems, whereas *FtPYL10* and *FtPYL15* were highly expressed 13D and 19D after flowering. The relative expression level of *FtPYL7* in roots and stems is as high as approximately 450, and the relative expression level of *FtPYL10* in 13 D is also as high as 248. The relative expression level of *FtPYL15* reached 380 at 19 D, and 207 at 13 D. The expression of most *FtPYL* in grain tissues decreased gradually, but some *FtPYL* expression increased gradually. For example, *FtPYL1*, *FtPYL4*, *FtPYL6*, *FtPYL7*, *FtPYL8*, *FtPYL9*, *FtPYL10*, *FtPYL11*, *FtPYL12*, *FtPYL13*, *FtPYL14* and *FtPYL17* were all gradually reduced in grain tissues. However, *FtPYL19* levels gradually increased. In grain tissues, the expression of genes increased and then decreased and then increased, such as *FtPYL3* and *FtPYL15*.

To better understand whether there was a correlation between *FtPYL* gene expression and grain development, we conducted a correlation analysis (Fig. 6B). Most *FtPYL* genes were positively correlated during grain development; for example, a positive correlation area was formed among *FtPYL2*, *FtPYL5*, *FtPYL6*, *FtPYL7*, *FtPYL8*, *FtPYL9*, *FtPYL11*, *FtPYL12*, *FtPYL13* and *FtPYL14*. It was also found that *FtPYL4* and *FtPYL16* were negatively correlated with *FtPYL* exception *FtPYL1* and *FtPYL14*.

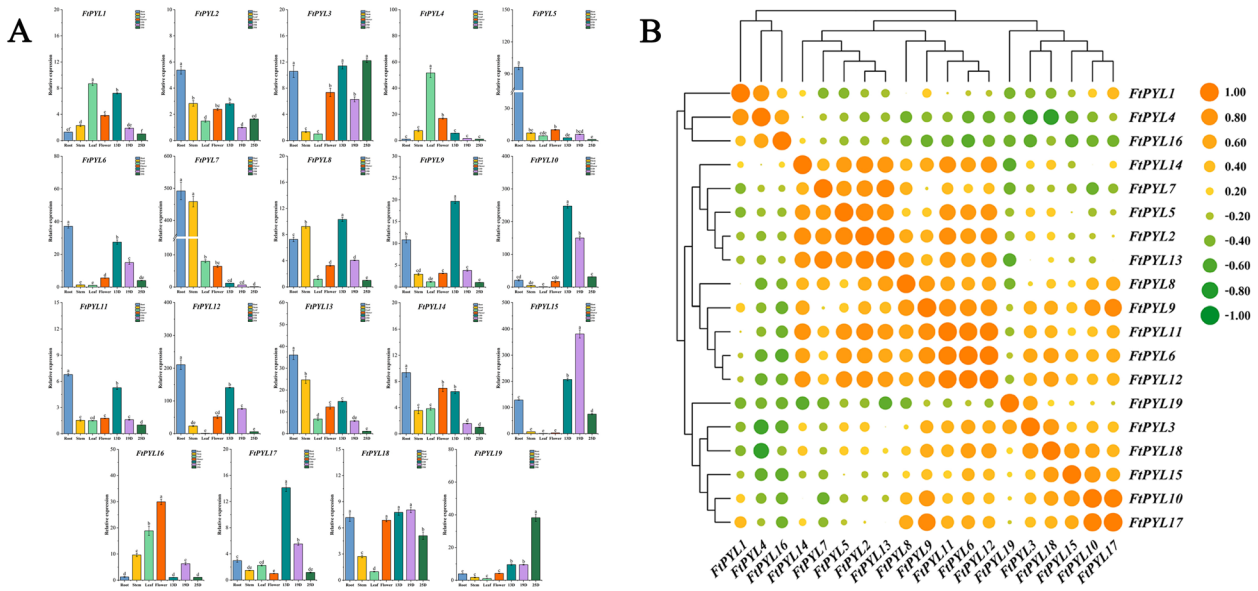

Fig. 6 **A** The expression levels of *FtPYL* gene in the root, stem, leaves, flowers, grain tissues at 13 days (13 D), 19 days (19 D), and 25 days (25 D) during grain development was detected using qRT-PCR technology, and then the expression level of *FtPYL* gene was normalized to the expression level of internal reference gene *FtH3*. Relative expression levels were displayed in roots, stems, leaves, flowers, 13 D, 19 D, and 25 D, with the lower-case letter above the column indicating significant differences between treatments (α = 0.05, LSD). **B** Correlation of *PYL* gene expression in Tartary buckwheat during grain development. The larger the marked circle on the right, the higher the correlation, the positive correlation in orange and negative correlation in green

Discussion

Evolutionary analysis of Tartary buckwheat PYL family

ABA is an important hormone that regulates growth and development, resists adverse environmental factors in plants, plays an important role in abiotic stress, and mediates the germination and maturation of seeds [1, 4, 38–40]. In this study, the PYL family of Tartary buckwheat was systematically analyzed, and the functions of 19 paralogous form *FtPYL* genes were preliminarily investigated. Analysis of the physical and chemical properties of the 19 paralogous form *FtPYL* genes revealed that the physical and chemical properties of *FtPYL19* were significantly different from those of the other 18 *FtPYL* genes. The protein length (111), molecular weight (approximately 12.19 KDa), theoretical isoelectric point (4.37), and instability index (29.61) of *FtPYL19* were the lowest, whereas its Aliphatic Index (108.65) was the highest. It was also found that among the 19 paralogous form FtPYL proteins, except FtPYL19 protein, the other 18 paralogous form FtPYL proteins were hydrophilic proteins. However, *FtPYL3*, *FtPYL5*, *FtPYL12*, and *FtPYL18*, belonging to subfamily II, have similar physical and chemical properties, gene structures, and conserved motifs. This suggests that *FtPYL19* may have different biological functions than the other members of subfamily II. Gene amplification is an extremely important driving force in the evolution of a species' genome, which can enable the emergence of new functions and the differentiation of species genes, accelerate the evolution of species, and help species resist adverse factors [41]. Gene duplication events are important pathways for gene family expansion, including tandem duplication (TD), whole genome duplication (WGD), proximal duplication (PD), transposition duplication (TRD) and decentralized duplication (DSD) [42, 43]. Among these, WGD is an important mode of genetic evolution in eukaryotes and can produce a large number of duplicate genes [43, 44]. One tandem repeat event and five fragment duplication events were observed during the contraction and expansion of the Tartary buckwheat PYLs (Additional file Table S3). Both tandem repetition and fragment duplication affected the expansion of the Tartary buckwheat PYL family, and fragment duplication contributed more than tandem repetition. The *FtPYL* genes generated by gene duplication events were similar in gene structure, conserved motifs were the same, and all belonged to the same subgroup. This indicates that *FtPYL* genes produced by gene duplication events may have similar biological functions; however, the retention of these *FtPYL* gene copies is somewhat biased, and whether they evolve in different directions and produce different biological functions is worth studying.

In the study of the evolution of the family of buckwheat PYL, we chose three monocotyledons (*Oryza sativa*, *Zea mays* and *Triticum aestivum*) and three dicotyledonous plants (*Arabidopsis thaliana*, *Cucumis sativus* and *Solanum lycopersicum*) PYL families with buckwheat PYL evolution analyses (Fig. 4). Phylogenetic trees revealed that Tartary buckwheat PYL was more commonly aggregated with selected dicotyledonous plants and that these aggregated PYL protein-conserved motifs were also highly similar. In addition, collinearity analysis found that there were more homologous genes between Tartary buckwheat PYL and dicotyledonous plants, but very few homologous genes with monocotyledonous plants. Therefore, we believe that the PYL family of Tartary buckwheat existed in the ancestors of monocotyledonous and dicotyledonous plants before differentiation, but expansion occurred mainly in the ancestors of dicotyledonous plants after differentiation of monocotyledonous and dicotyledons. Interestingly, we found that *FtPYL1* and *FtPYL9* of subgroup subfamily III were different from the other members in the phylogenetic tree, gene structure, and conserved motifs and that the number of introns was significantly different. In subfamily III, except for *FtPYL1*, *FtPYL17* and *FtPYL9*, all other *FtPYL* genes had only one exon, and *FtPYL17* had only three exons, which was much fewer than the seven exons of *FtPYL1* and *FtPYL9*. Some scholars believe that introns play an extremely important role in the process of protein translation, and a variety of exon combinations are generated in organisms through alternative splicing, thus translating a variety of proteins and improving the complexity of proteins [45, 46]. Introns have also been found to function independently of coding genes [47]. Therefore, *FtPYL1* and *FtPYL9* may possess distinct functions. In the ancient ancestors of organisms, their genes contained a large number of introns, and during evolution, a large number of introns of these genes were lost, which is the early intron hypothesis [48–50]. We hypothesized that *FtPYL1* and *FtPYL9* may be involved in the evolutionary differentiation of Tartary buckwheat; the degree of differentiation is lower and the *FtPYL* genes are more ancient than the other *PYL* genes.

Response of Tartary buckwheat PYL family to abiotic stress and spatiotemporal expression during grain development

In the studies of various plants, PYL was found to be expressed in various plant tissues, such as roots, flowers, grains and seeds [18, 23, 26]. In rice, the members of the PYL family *OsPYL1* are expressed in the roots, *OsPYL5* in leaves, and *OsPYL7/8* in embryos [20]. In tomatoes, significant changes were detected in the transcript levels of *SlPYL1*, *SlPYL2*, *SlPYL3* and *SlPYL6* during grain development and ripening [39]. We examined

19 paralogous form *FtPYL* expression patterns in Tartary buckwheat under four abiotic stresses (Fig. 5). We found that *FtPYL* was either highly expressed or inhibited under abiotic stress conditions. Interestingly, the expression of the *FtPYL* gene was completely reversed under different stress conditions. For example, the expression of *FtPYL13* was inhibited under low temperature (4℃) stress but was highly expressed under high temperature (38℃) stress. However, *FtPYL19* was highly expressed under low temperature (4℃) and inhibited under high temperature (38℃). At the same time, it was also found that the expression of some *FtPYL* genes increased first and then decreased or decreased first and then increased under abiotic stress. For example, under drought stress (PEG6000), the gene expression levels of *FtPYL15* and *FtPYL18* increased rapidly and then decreased rapidly to approximately pre-stress levels. Under low-temperature stress, the expressions of *FtPYL4* and *FtPYL11* decreased rapidly and then increased. This indicates that these *FtPYL* genes may be fast response genes, which can respond quickly in a very short time, and help Tartary buckwheat alleviate the damage caused by harsh environment in the short term.

We examined the gene expression levels in the roots, stems, leaves, and flowers of Tartary buckwheat at the flowering stage and in grain tissues on day 13 (13D), day 19 (19D) and day 25 (25D) after flowering (Fig. 6). Some *FtPYL* genes were highly expressed during grain development and may play an important role in the grain development of Tartary buckwheat. For example, *FtPYL5*, *FtPYL6*, *FtPYL7*, *FtPYL12* and *FtPYL15* were highly expressed in the roots; *FtPYL7* was highly expressed in the stem; and *FtPYL10* and *FtPYL15* were highly expressed on days 13D and 19D. Abscisic acid has multiple synthesis sites, of which the root is an important synthesis site [51]. After the root synthesis of abscisic acid, ABA is introduced into the grain through the plant stem, forming a complete pathway from the synthesis site (root) to the action site (grain) [52]. Therefore, we speculated that a large amount of ABA was synthesized in the roots of Tartary buckwheat during grain development, and thus, high expression of *FtPYL* genes was detected in the roots. The PYL family plays an important role in grain ripening during grain development. In tomatoes, mutant plants of *SlPYL9* have a later grain maturation [53]. During grain development and ripening, the transcription levels of the five *MnPYL* genes in mulberry are high [54]. High expression of *FtPYL* was also detected during grain development in Tartary buckwheat (13D, 19D and 25D). We speculate that these highly expressed *FtPYL* genes can help in the development of buckwheat grains, so that Tartary buckwheat grains in the embryogenetic stage accumulate proteins and other storage substances so that the grains can develop and mature normally. ABA is also essential for the biosynthesis of flavonoids and polyphenols [52], and Tartary buckwheat is a flavonoid-rich plant. Whether *FtPYL* has this function warrants further research.

Conclusion

In this study, we first identified the PYL family of Tartary buckwheat on a whole-gene basis and identified 19 paralogous form *FtPYL* genes. We analyzed the physicochemical information, gene structure, gene duplication, and evolutionary relationships of the nine *FtPYL* genes. We analyzed gene expression patterns under four abiotic stresses and during grain development using qRT-PCR. Based on this information, the functions of the Tartary buckwheat PYL family were discussed, analyzed, and hypothesized. It is speculated that *FtPYL* may play an important role in abiotic stress and grain development in Tartary buckwheat. Based on the above information, we preliminarily screened some key candidate genes providing a theoretical basis for further exploring the biological function of Tartary buckwheat *FtPYLs* and increasing the yield of Tartary buckwheat.

Materials and methods

Tartary buckwheat materials and abiotic stress

The Tartary buckwheat material used in this study was "CHUANQIAO". The plants were planted in an artificial climate chamber at the College of Agriculture, Guizhou University, China. After waiting for growth until the 20th day, four kinds of abiotic stress (salt: 5% NaCl, drought: 30% PEG6000, heat: 38℃, cold: 4℃) were applied. Samples of whole Tartary buckwheat plants with similar morphological characteristics were collected at 0, 1, 4, and 12 h after the treatment (five replicates). The samples were immediately stored at -80℃ for later use. In addition, plant samples of roots, stems and leaves were taken (5 replicates), and the samples were immediately stored at -80℃ for later use. In addition, wait for the material to grow to the flowering stage, take the plant samples of the roots, stems, leaves and flowers of Tartary buckwheat with good growth state and similar morphology (5 replicates), and immediately store the samples at -80℃ for use. Grain samples were taken at 13, 19 and 25 days after flowering and pollination (5 replicates) and stored at -80℃ immediately for later use.

Genome-wide identification and analysis of *FtPYL* gene in Tartary buckwheat

We downloaded the entire Tartary buckwheat genome sequence information from the MKBbase website (http://www.mbkbase.org/Pinku1/). Possible Tartary buckwheat PYL proteins (score ≥ 100, e ≤ 1e − 10) were identified

Table 2 Primer sequences of 19 selected FtPYL genes and reference genes for qPCR

Gene	F/R	Sequence	Length	Tm(℃)	GC/%	Hairpin	Dimer	False Priming	Cross Dimer
FtPYL1	Sequence(5′to 3′)F	ACGGTGAAGAAAGAAGTTGAT	21	53.3	38.1	None	None	None	None
FtPYL1	Sequence(5′to 3′)R	TTGACAGAAGAAATGAAGGGC	21	57.0	42.9	None	None	None	
FtPYL2	Sequence(5′to 3′)F	GTGGACGCAAAAGCATCAGA	20	59.3	50.0	None	None	None	None
FtPYL2	Sequence(5′to 3′)R	CGCAAGTAGCGAAGAGCATT	20	58.5	50.0	None	None	None	
FtPYL3	Sequence(5′to 3′)F	TCAGTTTTAGTGTTGTCGG	19	48.5	42.1	None	None	None	None
FtPYL3	Sequence(5′to 3′)R	GTCTCCTCGTTTGTATTCC	19	48.8	47.4	None	None	None	
FtPYL4	Sequence(5′to 3′)F	CACTTCCCTCATCACACAACG	21	58.5	52.4	None	None	None	None
FtPYL4	Sequence(5′to 3′)R	TCCCGACACAACTGAAACCTC	21	59.7	52.4	None	None	None	
FtPYL5	Sequence(5′to 3′)F	ACAGTCCTCAATCTTACAAACA	22	51.7	36.4	None	None	None	None
FtPYL5	Sequence(5′to 3′)R	ATCTCCACCTACAACGCTA	19	50.1	47.4	None	None	None	
FtPYL6	Sequence(5′to 3′)F	ACAGTCTCTAACACCACAGGAAC	23	55.2	47.8	None	None	None	None
FtPYL6	Sequence(5′to 3′)R	TGTCAAAAGCACGAACATAAGG	22	58.3	40.9	None	None	None	
FtPYL7	Sequence(5′to 3′)F	TCATCAAAAGTTGTAGTGT	19	41.8	31.6	None	None	None	None
FtPYL7	Sequence(5′to 3′)R	GAATCCAGTGACGAAGTT	18	46.3	44.4	None	None	None	
FtPYL8	Sequence(5′to 3′)F	TTGCTCGGAAGAAGTCGGGA	20	62.9	55.0	None	None	None	None
FtPYL8	Sequence(5′to 3′)R	CTCATAGGTGTGAAACTCGGCG	22	62.3	54.5	None	None	None	
FtPYL9	Sequence(5′to 3′)F	ACACTTGTTCAAATACCATC	20	46.8	35.0	None	None	None	None
FtPYL9	Sequence(5′to 3′)R	GTCTGAGTAGCATTCATAGG	20	46.8	45.0	None	None	None	
FtPYL10	Sequence(5′to 3′)F	GGGCTTCCAGCAACCACTA	19	58.3	57.9	None	None	None	None
FtPYL10	Sequence(5′to 3′)R	GTCCCTGACCTTCCTCCTA	19	53.4	57.9	None	None	None	
FtPYL11	Sequence(5′to 3′)F	TGACATCGTATGGTCTCTGGT	21	54.9	47.6	None	None	None	None
FtPYL11	Sequence(5′to 3′)R	CCTGATTTCACATTCACTTCTCTT	24	57.4	37.5	None	None	None	
FtPYL12	Sequence(5′to 3′)F	AGTATCTTCCGCTGTCGTCC	20	56.7	55.0	None	None	None	None
FtPYL12	Sequence(5′to 3′)R	GGTTTGTAGAGTAAAGGGTTG	21	51.4	42.9	None	None	None	
FtPYL13	Sequence(5′to 3′)F	CCACTCCTCTCCGATTTCTG	20	56.9	55.0	None	None	None	None
FtPYL13	Sequence(5′to 3′)R	AACCCACTTAGTCACCGTCT	20	53.3	50.0	None	None	None	
FtPYL14	Sequence(5′to 3′)F	AAGGAAGGATTCACGGTCG	19	57.2	52.6	None	None	None	None
FtPYL14	Sequence(5′to 3′)R	TCGCTCTGTGCTTGTTGCTG	20	60.6	55.0	None	None	None	
FtPYL15	Sequence(5′to 3′)F	ACCCGCAAACCTACAAACACT	21	58.9	47.6	None	None	None	None
FtPYL15	Sequence(5′to 3′)R	ACCCGAAAACTAATAACCCGAT	22	59.2	40.9	None	None	None	
FtPYL16	Sequence(5′to 3′)F	GTTGTGTGGTTGAGTGGTTGT	21	55.1	47.6	None	None	None	None
FtPYL16	Sequence(5′to 3′)R	ACGAAGCGTGTTTTGAAGAC	20	55.4	45.0	None	None	None	
FtPYL17	Sequence(5′to 3′)F	TGGCAAGGAAAAGTGACAGCA	21	61.6	47.6	None	None	None	None
FtPYL17	Sequence(5′to 3′)R	GTGAAAGATGAGCCTACGCAGT	22	58.9	50.0	None	None	None	
FtPYL18	Sequence(5′to 3′)F	AGTGCTGCTCGCTCGTCATT	20	60.8	55.0	None	None	None	None
FtPYL18	Sequence(5′to 3′)R	AGTGTTTGTAGGCTTGTGGATT	22	56.0	40.9	None	None	None	
FtPYL19	Sequence(5′to 3′)F	CGATGAACTTGATGATGAG	19	48.1	42.1	None	None	None	None
FtPYL19	Sequence(5′to 3′)R	CAACTTCCGTCTTATGTCC	19	49.8	47.4	None	None	None	
FtH3	Sequence(5′to 3′)F	GAAATTCGCAAGTACCAGAAGAG	23	-	-	-	-	-	-
FtH3	Sequence(5′to 3′)R	CCAACAAGGTATGCCTCAGC	20	-	-	-	-	-	-

from the PYL amino acid sequences of *Arabidopsis* and rice by the BLASTp method [55, 56]. Then, from the Pfam database (http://www.pfam.sanger.ac.uk/) protein family in the structure of PYL domain (PF10604) consistent with the Hidden Markov Model (HMM), The HMMER3.3.2 software (default parameter) (http://www.HMMER.org/) was used to search for PYL proteins. All possible buckwheat *PYL* genes through SMART (http://smart.emblheidelberg.de/) and CD—Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml with the PYR_PYL_RCAR domain structure and finally confirmed all *FtPYL* genes. Using the WoLF PSORT website (https://psort.hgc.jp/), the subcellular localization was obtained from the ExPasy website (http://web.expasy.org/protparam/)

to obtain the MW and PI, The Grand Average of Hydropathicity and Instability index (II) were analyzed using TBtools software (https://www.github.com/CJ-Chen/TBtools).

RNA extraction, cDNA reverse transcription, and qRT-PCR analysis of plant materials

Total RNA was extracted using a TaKaRa Bio. The concentration and purity of total RNA were determined using a spectrophotometer, and the RNA was reverse-transcribed into cDNA using the Hiscript II Q RT Supermix for the qPCR kit. qRT-PCR primers for the 19 paralogous form FtPYL genes were designed using Premier software (version 5.0; Table 2). *FtH3* is an internal control gene that is stably expressed at every growth stage in Tartary buckwheat [57]. qRT-PCR was performed using the SYBR premix Ex Taq II (TaKaRa) and repeated at least three times. The $2^{-(\Delta\Delta Ct)}$ calculation method was used to calculate the relative gene expression [57].

Classification and phylogeny of the FtPYL family

According to the PYL family classification of *Arabidopsis* and rice, the 19 paralogous form *FtPYL* genes were classified into different subgroups. MEGA11 was used to construct a phylogenetic tree with a bootstrap value of 1000 and other default parameters by adding AtPYL, OsPYL, and FtPYL proteins using the neighbor-joining (NJ) method. Six species (*Arabidopsis thaliana*, *Cucumis sativus*, *Solanum lycopersicum*, *Oryza sativa*, *Zea mays* and *Triticum aestivum*) were constructed using this method.

FtPYL gene structure and *cis*-acting elements

The *FtPYL* gene was compared with the Tartary buckwheat genome using TBtools (https://www.github.com/CJ-Chen/TBtools), and a gene map of *FtPYL* was constructed. PlantCare website (http://bioinformatics.psb.ugent) be/webtools/ plantcare/html/) to predict the 19 paralogous form *FtPYL* genes upstream 2000 bp of the *cis*-acting elements. The 10 most conserved motifs among the 19 paralogous form FtPYL proteins were analyzed using the MEME website (https://meme-suite.org/meme/tools/MEME).

Chromosome distribution and gene duplication of *FtPYL* gene

Based on the genome and gene annotation information of Tartary buckwheat, we extracted the location and physical information of 19 paralogous form *FtPYL* genes and gene density information of the chromosomes and mapped them. We used a multicollinearity scanning toolkit (MCScanX) to analyze *FtPYL* gene duplication events (default parameters), and TBtools tools (https://www.github.com/CJ-Chen/TBtools) for homology mapping.

Statistical analysis

We performed an analysis of variance (ANOVA) on the qRT-PCR data using JMP6.0 and compared the data with LSD (least significant difference method (LSD) at a significance level was 0.05 ($p < 0.05$). Origin software was used to map the expression histograms of the 19 *FtPYL* genes under abiotic stress and during grain development.

Abbreviations

qRT-PCR	Quantitative real-time polymerase chain reaction
PYL	Pyrabactin resistance/pyr1-like/regulatory components of ABA receptor
NLS	Nuclear localization signal
PEG	Polyethylene glycol
HMM	Hidden Markov Model
MW	Molecular weight
pI	Isoelectric point
ANOVA	Analysis of variance
LSD	Least Significant Difference

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05447-0.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.

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Authors' contributions

G.X. and A.H. conceived and designed the studies; H.Y., L.S., H.L., and C.W. conducted the experiments. G.X. and A.H. analyzed the data and wrote the manuscript, while J.R. supervised the research and revised the manuscript. All the authors have reviewed and approved the final version of the manuscript.

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Availability of data and materials

Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate

Plant materials are widely used worldwide, and no license is required to collect plant samples. The plant materials were maintained by the institutional guidelines of the College of Agriculture, Guizhou University. These methods were performed by the relevant guidelines and regulations. This study did not include any human participants or animal experiments conducted by the authors.

Consent for publication

Not Applicable.

Competing interests
The authors declare no competing interests.

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