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RESEARCH ARTICLE

Genome-wide identification of the BURP domain-containing genes in *Phaseolus vulgaris*

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Abstract Plant-specific BURP domain-containing proteins have an essential role in the plant's development and stress responses. Although BURP domain-containing proteins have been identified in several plant species, genome-wide analysis of the BURP gene family has not been investigated in the common bean. In the present study, we identified 11 BURP family members in the common bean (*Phaseolus vulgaris*) genome with a comprehensive in silico analysis. Pairwise alignment and phylogenetic analyses grouped *PvBURP* members into four subfamilies [RD-22 like (3), PG1β-like (4), BNM2-like (3), and USP-like (1)] according to their amino acid motifs, protein domains and intron–exon structure. The physical and biochemical characteristics of amino acids, motif and intron–exon structure, and *cis*-regulatory elements of BURPs members were determined. Promoter regions of BURP members included stress, light, and hormone response-related cis-elements. Therefore, expression profiles of *PvBURP* genes were identified with in silico tools and qRT-PCR analyses under stress (salt and drought) and hormone treatment (ABA, IAA) in the current study. While significant activity changes were not observed in BURP genes in RNA-seq data sets related to salt stress, it was determined that some BURP genes were expressed differently in those with drought stress. We identified 12 different miRNA, including miRNA395, miRNA156, miRNA169, miRNA171, miRNA319, and miRNA390, targeting the nine *PvBURP* genes using two different in silico tools based on perfect or near-perfect complementarity to their targets. Here we present the first study to identify and characterize the *BURP* genes in common bean using whole-genome analysis, and the findings may serve as a reference for future functional research in common bean.

Keywords ABA · Abiotic stress · BURP · miRNA · IAA

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important foods used for direct human consumption providing essential proteins, complex carbohydrates, dietary fibers, minerals (Fe, Zn), and vitamins (Hayat et al. 2014). It also has disease-preventing and health-promoting effects on humans due to the existence of many phytochemicals such as polyphenolic compounds, fibers, lectins, and flavonoids in its seeds (McClean and Raatz 2017). As a legume species, it enhances soil fertility by fixing atmospheric nitrogen. All these qualities make common bean a good food of choice for more than 300 million people living in lower-income counties found in Asia, Eastern Africa, and Latin America (Cortés et al. 2013).

Besides its high demand and production, this crop is threatened by a series of abiotic stress. Common bean is known to be more susceptible to water shortages during the flowering and grain-filling stages. Even moderate levels of water deficit were reported to cause a reduction in common bean biomass, seed yield, and nitrogen fixation (Fageria et al. 2010). Common bean is primarily grown in drought-prone areas, and prolonged water deficiency is reported to create a global and endemic threat for most bean

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production areas (Caldas et al. 2016). Global warming-dependent climatic stresses became more widespread and intense in recent years. Effects of these stresses on the common bean production are most frequently handled in terms of water shortage and drought-dependent salinity (Pareek et al. 2020). Higher temperatures, combined with lower rainfall, are started to exacerbate evapotranspiration and drought in especially bean-producing areas of Asia, Latin America, and southern Africa (Darkwa et al. 2016). It is known that the accumulation of salts and ions in upper soil layers leads to osmotic stress and ion toxicity in plants during moderate or severe drought conditions. Therefore, global warming is estimated to change the adaptive altitudinal range of bean genotypes, accelerate the decomposition of soil organic matter (mineralization) and ion accumulation (salinization), combining the abiotic stresses even more acute in the near future. These estimates were verified with some studies that 73% and 40% of common bean production areas had been reported to be affected by drought and metal toxicity, respectively (Al Hassan et al. 2016; Arteaga et al. 2020; Beebe et al. 2009; Celmeli et al. 2018; Dipp et al. 2017; dos Santos Neto et al. 2020; Fageria et al. 2010; Lizana et al. 2006). Common bean can cope with these stress factors by activating tolerance genes and changing their cellular, biochemical and molecular mechanisms. Therefore, identifying the genes and transcription factors responsible for stress tolerance is highly important to develop resistant common bean cultivars and maintain their productivity.

Increasing evidence indicates that a gene family encoding BURP domain-containing proteins has important functions in plant development, metabolism, and stress tolerance. Members of the BURP protein family are distinguished from other proteins by the presence of a conserved amino acid motif located at the N-terminus. The *BURP* gene family derives its name from the four members of its family; the microsporogenesis-specific protein (BNM2) of *Brassica napus* (Boutilier et al. 1994), the unknown seed protein (USP) of *Vicia faba* (Bassüner et al. 1988), the responsive to dehydration 22 (RD22) in *Arabidopsis thaliana* (Yamaguchi-Shinozaki et al. 1993) and the non-catalytic β-subunit of the polygalacturonase isozyme 1 (PG1β) in *Lycopersicon esculentum* (Hattori et al. 1998; Zheng et al. 1992). The structure of BURP domain-containing proteins has three conserved modules: a conserved region containing four repeats of cysteine-histidine motifs following a single phenylalanine-glycine residue at the C-terminal region, a member-specific variable internal region, and a signal peptide at the hydrophobic N-terminal domain (Hattori et al. 1998). Depending on the variable region, the BURP domain-containing proteins are classified into seven subfamilies; BNM2-like, USP-like, RD22- like, PG1β-like, BURPV, BURPVII, and BURPVIII (Granger et al. 2002).

Several gene families encoding BURP domain-containing proteins have been identified and are found to be unique to plant species. Expression of these genes was associated with significant developmental and tolerance metabolisms in plants. For example, BNM2 expression was first linked to microspore embryogenesis in *Brassica napus L.* (Boutilier et al. 1994) and then realized to be related to seed formation due to its localization in seed protein storage vacuoles (Teerawanichpan et al. 2009). Another BURP domain-containing protein in *Vicia faba L.* called *VfUSP* is reportedly involved in regulating the early development of zygotic embryogenesis. In *Panicum maximum*, *ASG1* was found to control the formation of apospory initial cells (Bassüner et al. 1988; Chen et al. 1999). A BURP protein in soybean (SCB1) was reported to be functional in the differentiation of seed coat parenchyma cells (Batchelor et al. 2002). In cotton, the BURP domain-containing proteins are expressed during fiber initiation, development, and elongation stages (Lee et al. 2007).

In addition to their contribution to plant development, some BURP domain-containing proteins (RD22-like and BNM2-like subfamilies) have been reported to show co-expression with stress conditions. Especially, the RD22 gene exhibited a strong molecular link between abscisic acid (ABA) and abiotic stress responses in plants. For instance, RD22-like genes of *Arabidopsis* and *Vitis vinifera* were significantly expressed in response to salt, drought, and ABA stress (Abe et al. 1997; Matus et al. 2014; Yamaguchi-Shinozaki et al. 1993). Similarly, RD22-like genes in *Gossypium hirsutum* (GhBURPs) were induced by ABA and salicylic acid exposure (Sun et al. 2019). Xun et al. (2019) identified some BURP domain-containing genes in soybean and indicated their upregulation in response to ABA exposure and soybean mosaic virus infection.

Some reports indicate the essential roles of BURP domain-containing proteins in metal toxicity tolerance in plants. *SALI3-2*, a BURP gene in soybean, was found to be induced by excess cadmium and copper exposure. This gene was estimated to have sequestering effects on metal ions in the soybean roots (Tang et al. 2014). Similarly, two other BURP genes, *Sali5-4a* and *Sali3–2*, found in soybean, were overexpressed by aluminum stress and *Sali3–2* was also involved in the salt tolerance (Tang et al. 2014). In poplar, 18 BURP family genes, named *PtBURPs*, were identified and characterized according to their physical positions on the *P. trichocarpa* chromosomes (Shao et al. 2011). Genome-wide expression analysis of poplar verified the essential roles of *PtBURPs* on metal detoxification and drought tolerance (Yıldırım and Kaya 2017).

These studies represented that BURP domain-containing family genes had a significant role in plant development, abiotic stress response, and phytohormone signaling pathways. Although these plant-specific genes have been characterized in several plant species, a genome-wide analysis of *BURP* genes in common bean has not been reported to this date. The whole-genome sequence of *P. vulgaris* was released in 2014 (Schmutz et al. 2014), and now it is possible to analyze the entire family of common bean BURP domain-containing proteins. In the current study, 11 putative BURP genes were characterized with phylogenetic analysis, structural analysis, and expression profile analysis. The transcript levels of all 11 genes were determined under drought and salt stress as well as ABA and IAA hormone treatments. This study is the first report on genome-wide identification of *PvBURP* genes in common bean and characterization of their function on development and stress response of the common bean.

Material and methods

Identification of BURP members in common bean

We used two complementary methods to identify the genes encoding the BURP domain-containing proteins in common bean genome. In the first step, the Hidden Markov Model (HMM) profile of the BURP domain (PF03181) was obtained from Pfam30.0 (Finn et al. 2016). This profile information was used as a query to identify candidate BURPs from the bean genome using HMMER3.0 (Finn et al. 2015). In the second step, we made a keyword search in the Phytozome v12 database (https://phytozome.jgi.doe.gov/pz/portal.html) to find out other candidate *BURP* genes that may be overlooked from the first step. The presence of the BURP domain in a potential gene detected in *Phaseolus vulgaris* was further confirmed in Pfam (http://pfam.xfam.org/) and SMART databases (http://smart.embl-heidelberg.de/). We removed protein sequences that do not contain the BURP domain or have an uncertain domain belonging to other protein families. Finally, these remaining protein sequences were considered as members of the BURP family in *P.vulgaris* and used in subsequent analysis. Chromosome locations and protein-coding sequences (CDS) of all candidate *PvBURP* genes were downloaded from the Phytozome v12 database (https://phytozome.jgi.doe.gov/pz/portal.html#). We also determined the molecular and physicochemical properties of *PvBURP* genes by calculating the Mw, pI, instability index, and GRAVY using the ProtParam tool on the ExPASy server (https://web.expasy.org/protparam/). The PROSOII tool was used to predict the solubility of candidate BURP proteins based on their sequences (Smialowski et al. 2012).

Phylogenetic analysis

The amino acid sequences of BURPs from *Arabidopsis thaliana, Brachypodium distachyon, Cucumis sativus, Glycine max, Medicago truncatula, Oryza sativa, Sorghum bicolor,* and *Zea mays* were retrieved from the Phytozome v12 database to evaluate the expansion of BURP encoding genes within different species. The genes of BURPs were named based on numbering and their sequence homology with *A. thaliana* ortholog genes. Multiple sequence alignment was carried out by ClustalW 2.0 program. The neighbor-joining method-based phylogenetic tree was constructed with the bootstrap test (1000 replicates) and Jones-Taylor-Thornton (JTT) model in MEGAX. Phylogenetic trees were visualized with ITOL v3 (http://itol.embl.de/).

Sequence analysis of *PvBURP* genes

The intron/exon, motif, and domain structure of PvBURPs were shown using Gene Structure View in TBtools software (https://github.com/CJ-Chen/TBtools) (Chen et al. 2018). Genomic DNA sequences and CDS of *PvBURP* genes were downloaded from the Phytozome v12 database (https://phytozome.jgi.doe.gov/pz/portal.html#) to determine their intron–exon structures. Conserved motifs in *PvBURP* proteins were analyzed by using the MEME suite (http://meme-suite.org/tools/meme) tool. Conserved motifs were analyzed by choosing 15 motifs and repeating any number of motifs. NCBI Batch-CD Search tool was used to show potential BURP domains (Lu et al. 2020). We visualized the distribution of *PvBURP* genes on chromosomes using TBtools software (https://github.com/CJ-Chen/TBtools). Locations of genes on chromosomes were determined with Gene on Genome from Fasta application using Blast. Potential cis-acting regulatory DNA elements (*cis*-elements) in the promotor sequences of *PvBURP* genes were analyzed using the place database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) with 2000 bp of upstream region of the predicted CDS. Putative miRNAs targeting *PvBURP* genes were estimated using psRNATarget server based on miRNA-target complementary match patterns (Dai et al. 2018). *Arabidopsis thaliana, Glycine max, Populus trichocarpa, Vitis vinifera,* and *Brachypodium distachyon* were selected for miRNA analysis. CLC Genomics Workbench software was used to assess the positions of conserved BURP domains and potential signal peptides of these proteins. In this study, the sequence of all candidate miRNA identified in *Phaseolus vulgaris* genome was retrieved from Plant miRNA Encyclopedia (PmiREN, http://www.pmiren.com/) (Guo et al. 2019). Potential miRNAs targeting the *PvBURP* genes are determined using the web-based psRNA Target Server

(http://plantgrn.noble.org/psRNATarget) and Miranda with their default parameters (Enright et al. 2003).

Plant Genome Duplication Database (Lee et al. 2013) and BLASTP were used to identify gene duplications. Duplicated *PvBURP* genes found within the same chromosome were accepted as tandem duplication. For segmental duplications, the BLASTP search was performed against all identified peptide sequences of *PvBURPs* in the common bean. As potential anchors, the top five matches were taken into consideration according to their E-value ($\leq$ 1e−05), and then, MCScan was utilized to determine their collinear blocks (Wang et al. 2012). In evolutionary analysis, ratio of the nonsynonymous mutation rate to the synonymous mutation rate (Ka/Ks) was calculated using TBtools software. First, BLAST analysis of *PvBURP* protein sequences was performed in the Phytozome v12 database. Sequences with a similarity of over 60% as a result of BLAST were obtained. At the end of this BLAST process, a tab-delimited text file was created, and Ka/Ks calculation was then performed in TBtools software.

Prediction of 3D protein homology, transmembrane helix, and sub-cellular localization

Subcellular localization of *PvBURPs* was predicted with the Plant-mPLoc server (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/) (Chou and Shen 2008). The Phyre2 server (Protein Homology / Analogy Recognition Engine) was used to estimate BURP proteins' 3D structure (Kelley et al. 2015). All *PvBURP* protein sequences have been downloaded from Phytozome v12 database (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris). Then, *PvBURPs* protein sequences were analyzed in the Phyre2 server with an "intensive" mode to define the 3D structure. Trans-membrane helical domains were predicted using and TMHMM (http://www.cbs.dtu.dk/services/TMHMM/) (Krogh et al. 2001).

Plant material and stress treatments

A Turkish common bean genotype named 'İspir' was used in the study for gene expression analysis of *PvBURPs* under various stress conditions. İspir seeds were firstly surface sterilized in a 5% sodium hypochlorite solution and then planted in pots filled with vermiculite. Plants were grown in a fully-controlled growth chamber at 24 °C supplemented with 16 h light and 8 h dark photoperiod. After the plants were grown for 4 weeks, they were subjected to drought, salinity, and hormone treatments by applying polyethylene glycol 6000 (PEG), NaCl, ABA, and IAA. Salt and drought stress was subjected to the plants by adding 200 mM NaCl and PEG (20%) into the Hoagland's solution, respectively. Hormone treatment was achieved by spraying with 100 µM ABA and 100 µM IAA to the leaves. Stress and hormone-treated leaves and roots samples were collected at 6, 12, 24, 48, 60th hour and seventh day after stress treatment and stored at − 80 °C until their usage in RNA isolation.

RNA isolation and gene expression analysis

We isolated total RNA with the RNeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol. RNA quality was checked on NanoDrop ™ 2000/2000c spectrophotometer and on a 1.5% (w/v) agarose gel. The first strand of cDNA was synthesized with the iScript ™ cDNA Synthesis Kit. Tissue, hormone, and stress-related expression of 11 *PvBURP* genes were measured with qRT-PCR analysis performed on the Agilent Mx3000P device with Solis BioDyne 5 × HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX). qRT-PCR conditions were carried out at 95 °C for 15 min, at 95 °C for 15 s, at 60 °C for 20 s, and at 72 °C for 20 s. Relative expression was calculated according to the $2^{-\Delta\Delta Ct}$ method. Primers of 11 *PvBURP* genes used in this study are shown in Table S1.

In silico expression analysis

The expression profiles of *PvBURP* genes were also determined using eight RNA-seq data sets that were previously obtained from different genotypes in response to salt drought and pathogen stress. To evaluate the expression profiles of *PvBURPs* in different tissues under different conditions, the raw RNA-seq data sets were downloaded from NCBI Sequence Read Archive (SRA) under the accession number; PRJNA327176 and PRJNA508605 for drought treatment; PRJNA656794, PRJNA558376, PRJNA574280, PRJNA691982 for salt stress and PRJNA574280 for pathogen infection. The transcriptional analysis of downloaded files was done via CyVerse (https://www.cyverse.org/) and Galaxy (usegalaxy.eu), including virtual bioinformatics tools. The mapping of reads was done with the HISAT2 tool. Transcript assembly and differential expression analyses were performed with Stringtie 1.3.3 and Ballgown, respectively. The genes having fold change value $\log2 > 1$ and a p value < 0.05 were accepted as differentially expressed genes (DEGs). Heatmap based on the $\log_2$ FC was prepared using TBTools. For this analysis, eight different comparisons were made. To determine the expression level of BURPs under salt stress, the first comparison was carried out with data previously obtained from salt-treated leaf explants of T43 (sensitive) and Ispir (resistant) common bean genotypes (PRJNA656794). The data belonging to root samples of the same genotypes were used for the second comparison. The third expression analysis was

made by comparing the data obtained from the salt-tolerant Ispir's root tissues grown under both control and salt stress conditions (PRJNA656794). The transcription data obtained by applying salt stress to the salt-tolerant and the sensitive genotype at the bud stage were used for the fourth comparison to estimate the expression level of *PvBURPS* (PRJNA558376). In the fifth expression analysis, the data obtained by applying salt stress to the salt-tolerant bean genotype in the sprout stage were used (PRJNA691982). The sixth and seventh expression analyses were made to find the response of *PvBURP* genes under drought stress. The data used for these comparisons were obtained by RNA sequencing of drought-tolerant Pinto Saltillo (PRJNA508605) and Perola (PRJNA327176) genotypes grown under control and drought stress. The last comparison was made with data obtained from a library prepared with a common bean plant infected by the fungal pathogen *Sclerotinia sclerotiorum* (strain 1980) (PRJNA574280). Tissue-specific expression patterns of BURPs were retrieved from Phytozome v12.

Results

Genome-wide identification of *BURP* genes from the common bean genome

To identify the BURP family members in the common bean, we used different approaches included in the HMM search. After manually removing sequences containing a missing BURP domain, 11 putative *PvBURP* genes named *PvBURP1-PvBURP11* were identified, depending on their chromosomal location (Table 1). We have found that *PvBURP* genes in the common bean genome vary widely in their length, MW, and pI value. In this context, gene lengths ranged from 1339 (*PvBURP8*) to 3379 bp (*PvBURP7*), MWs from 29.88 (*PvBURP11*) to 69.51 kDa (*PvBURP8*), and pI values from 5.75 (*PvBURP6*) to 9.03 (*PvBURP8*). The prediction of subcellular localization made by mGOASVM indicated that PvBURP proteins are active in golgi, chloroplast and cell walls. According to the instability index (II), most of the PvBURP proteins (8 out of 11) were determined to be stable in a test tube. Protein solubility prediction of *PvBURPs* based on amino acid sequence indicated that approximately 91% of those are insoluble in *Escherichia coli*. When the amino acid composition of these 11 BURP proteins is examined, it is seen that the most abundant amino acid is Ser (S).

All genes encoding BURP domain-containing proteins have been successfully inserted into chromosomes and shown in Fig. 1. The chromosomal localizations indicated that one *PvBURP* gene was found on chromosomes 2 and 3 while two genes on chromosomes 8 and 11 and 5 genes on chromosome 9. There were no *PvBURP* genes found on other chromosomes. There is no common point regarding the positions of BURP genes on chromosomes. Few were seen located on the upper arm, some on the lower arm, and rest in the middle position.

Phylogenetic analysis of the BURP family

In this study, PvBURPs were divided into four groups according to the method of Hattori et al. (1998); BNM2A (*PvBURP5*, *PvBURP6*, and *PvBURP10*), USP (*PvBURP11*), RD22 (*PvBURP3*, *PvBURP7*, *PvBURP9*), and PG1β (*PvBURP1*, *PvBURP2*, *PvBURP4*, and *PvBURP8*) (Fig. 2). Alignment of BURP domains revealed that a total of 21 consensus amino acids (3F, 39P, 40F, 71C, 78G, 81K, 83C, 86S, 88E, 93F, 99G, 139C, 140H, 150Y, 151C, 152H, 180C, 181H, 183D, 184T, and 197L) were wholly conserved among the 11 proteins in the common bean BURP family members (Fig. 3). Additionally, this analysis of the BURP family members showed to have many extremely conserved amino acid sites and 4 CH motifs, indicating that these amino acids were essential for the basic functionality of these members of the BURP family. Members of the BURP family are generally characterized by the amino acid sequence located at the C-terminus, summarized as $CHX_3YX_6CHX_{23\text{-}28}$-$CHXDX_{18\text{-}23}CHX_8W$. However, *PvBURP11*, *PvBURP1*, *PvBURP2*, and *PvBURP4* members, whose sixth amino acid is F, do not follow this rule (Fig. 3). We constructed a phylogenetic tree to investigate the evolutionary relationship of the BURP family in different species. For this purpose, members of the BURP family were identified in *Arabidopsis thaliana* (5 genes), *Brachypodium distachyon* (14 genes), *Cucumis sativus* (7 genes), *Glycine max* (26 genes), *Medicago truncatula* (52 genes), *Oryza sativa* (18 genes), *Sorghum bicolor* (11 genes), and *Zea mays* (10 genes) following the same workflow used in the identification of *PvBURP* genes. According to the phylogenetic tree, all BURPs were grouped into the five sub-families. These are. BNM2A, USP, RD22, PG1β, and BURP V. None of the BURP family members in *Phaseolus vulgaris, Glycine max, Arabidopsis thaliana,* and *Cucumis sativus* are in the BURP V group (Fig. 4).

Analysis of gene structure, gene duplication, and conserved motifs of the *PvBURP*s

The analysis results of PvBURP proteins for identifying signal peptides found in the N-terminus revealed that all PvBURP proteins, excluding *PvBURP7* and *PvBURP9*, have one signal peptide (Table S2). We also studied the exon/intron structure within the PvBURP family members to obtain more knowledge of the common bean BURPs'

Table 1 Molecular and physicochemical properties of *PvBURP* genes

Gen ID	Phytozome Identfier	Chromosomes	Start and end positions (bp)	Length (bp)	CDS (bp)	Protein length (aa)	NCBI Accession number
PvBURP1	Phvul.002G071500	Chr02	9480419–9483312	2894	1881	626	XM_007157400.1
PvBURP2	Phvul.003G052000	Chr03	6643545–6646040	2496	1875	624	XM_007153571.1
PvBURP3	Phvul.008G158600	Chr08	43164301–43167172	2872	1023	340	XM_007140936.1
PvBURP4	Phvul.008G210500	Chr08	55885018–55887616	2599	1836	611	XM_007141548.1
PvBURP5	Phvul.009G024400	Chr09	5998841–6000194	1354	939	312	XM_007136113.1
PvBURP6	Phvul.009G024700	Chr09	6024647–6026346	1700	1110	369	XM_007136116.1
PvBURP7	Phvul.009G105300	Chr09	16335114–16338492	3379	1110	369	XM_007137102.1
PvBURP8	Phvul.009G179500	Chr09	26920688–26923132	2445	1884	627	XM_007138028.1
PvBURP9	Phvul.009G180600	Chr09	27156514–27159612	3099	1341	446	XM_007138042.1
PvBURP10	Phvul.011G046300	Chr11	4156692–4158030	1339	903	300	XM_007131785.1
PvBURP11	Phvul.011G182200	Chr11	49491661–49493447	1787	798	265	XM_007133419.1

Gen ID	pI	Molecular weight (Da)	Instability index	Stable or unstable	GRAVY	Solubility/Score	Subcellular location
PvBURP1	8.34	68435.07	29.21	Stable	− 0.499	Insoluble/0.353	Cell wall
PvBURP2	6.44	68108.46	31.23	Stable	− 0.500	Insoluble/0.312	Cell wall
PvBURP3	8.12	36925.07	32.18	Stable	− 0.266	Insoluble/0.336	Cell wall/Chloroplast
PvBURP4	8.52	67265.46	25.42	Stable	− 0.562	Insoluble/0.354	Cell wall
PvBURP5	6.99	35203.58	49.69	Unstable	− 0.196	Insoluble/0.224	Cell wall
PvBURP6	5.75	42198.78	34.37	Stable	− 0.588	Soluble/0.526	Nucleus
PvBURP7	8.56	40277.16	31.17	Stable	− 0.107	Insoluble/0.301	Cell wall
PvBURP8	9.03	69512.12	34.71	Stable	− 0.549	Insoluble/0.330	Cell wall
PvBURP9	6.23	51213.07	55.90	Unstable	− 0.389	Insoluble/0.336	Cell wall/Chloroplast
PvBURP10	6.12	34038.78	46.29	Unstable	− 0.331	Insoluble/0.412	Cell wall
PvBURP11	6.83	29885.33	39.92	Stable	− 0.268	Insoluble/0.351	Golgi

structural diversity. This structural analysis revealed that none of the BURP genes were without introns. Additionally, just one intron was present in seven of the BURP genes, while the others had two introns. The organization and number of introns of *PVBURP* genes included various patterns and distributions in the distinct subfamily. Accordingly, while two relatively long introns were found in the BURP genes belonging to the RD22 and USP subfamilies, members of the other sub-families had a short and single intron. Another structural difference observed between proteins in subfamilies was the motifs. All members of *PvBURPs* have motif 1, 2, and 3 (Figure S1). Unlike other BURPs, those belonging to RD22 and BNM2 sub-family have motifs numbered 1, 2, 3, 5, and 11. Members of the PG1β sub-family with motifs numbered 1, 2, 3, 4, 6, 7, 8, 9, 12, 13, 14, and 15 were the most motif-bearing PvBURPs. The BURP member in the common bean with the least motifs is *PvBURP11*, annotated as USP (Fig. 2). Similar motifs and motif layouts were identified in closely related BURPs. Considering only the BURP domain located at the C terminal, it can be seen that Motif 5 belongs only to RD22 and BNM2 subfamily.

We have also analyzed the gene duplication events in *PvBURP* genes. According to the analyses conducted with the plant genome duplication database and BLAST-P, we only identified segmentally duplicated *PvBURP* genes. Among the eleven genes in the PvBURP family, we identified four gene pairs (*PvBURP1* /*PvBURP2*, *PvBURP1*/*PvBURP8*, *PvBURP2*/*PvBURP8*, and *PvBURP5*/*PvBURP10*) that had been duplicated in the evolution of the PvBURP family. Considering that synonymous silent substitutions per site (Ks) occur at a constant rate over time, it is possible to estimate the dates of large-scale duplications (Maher et al. 2006). The Ka, Ks, and the Ka/Ks ratios were calculated for each duplicated *PvBURP* gene pair to explore duplicated BURP genes' possible fate. If the Ka/Ks ratio is lower than one, it indicates functional constraints with the negative or purifying selection of the genes; if this ratio equals to zero, then it indicates neutral selection. A ratio higher than one shows accelerated evolution with positive selection (Juretic et al. 2005). Our research found that Ka/Ks ratios from four PvBURP duplicated gene pairs were less than 0.3 (Table 2). This finding indicates that the family of *PvBURP*

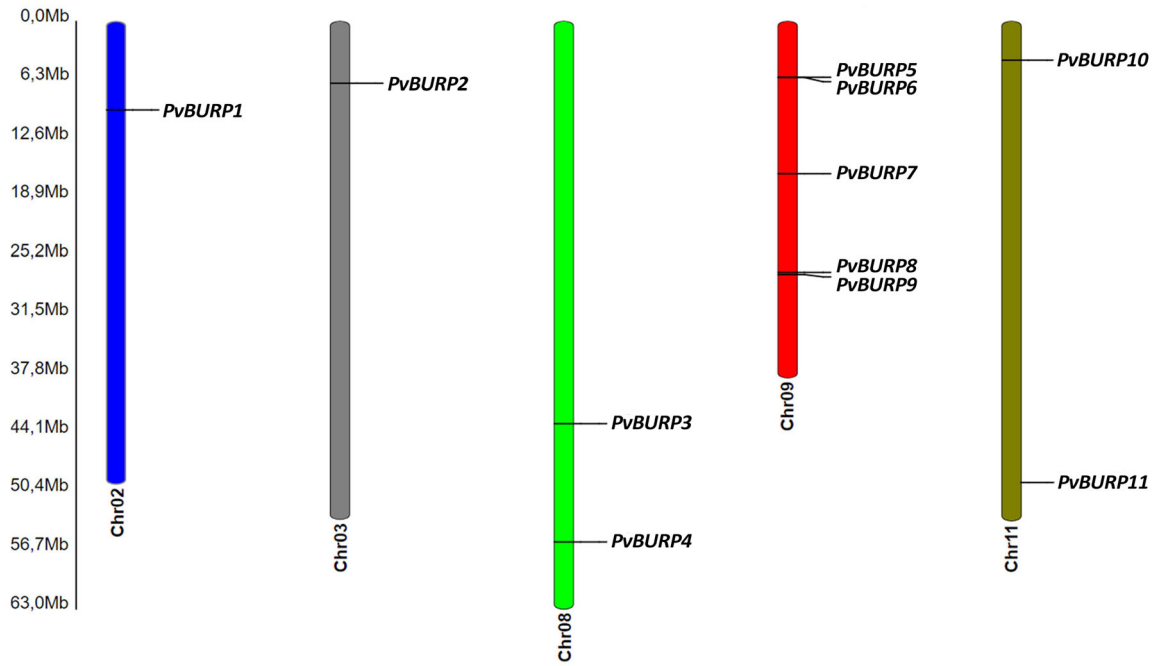

Fig. 1 Chromosomal location of common bean BURP domain-containing genes. The genes are located over the five linkage groups: Chr02, Chr03, Chr08, Chr09 and Chr11. The chromosome number is indicated at the bottom of each chromosome. The scale is in megabases (Mb)

genes has mainly evolved under strong purifying selection pressure, with a few functional variations following duplication. It was estimated that the duplication that created the four segmentally duplicated gene pairs occurred between 5.09 and 13.76 million years ago (Mya) (Table 2).

Analysis of cis-elements in putative *PvBURP* promoter regions

As recommended in all previous genome-wide studies, *BURP* genes have an important functional role in plants against abiotic stresses. We identified stress, light, and hormone response-related *cis*-elements in the promoter sequences of *PvBURPs* covering the upstream region of 2000 nucleotides from the gene start codon to fully understand and clarify the potential regulatory role of *PvBURPs* under various stresses. In this context, we identified 61 different cis-regulatory elements in the putative promoter regions. Ten types of elements related to plant hormones have been described. These are AuxRR-core (auxin), TGA-element (auxin), P-box (gibberellin), TATC-box (gibberellin), GARE-motif (gibberellin), CGTCA-motif (MeJA), TGACG-motif (MeJA), ERE (ethylene), TCA-element (SA), and ABRE (ABA). In these putative promoter regions, eight types of cis-regulatory elements were found to be related to various stress responses. Their names and potential functions are as follows; WUN (a wounding-responsive element), GC-motif (anoxic specific inducibility), W-box (defense and stress responsiveness), GT1 (drought), MBS (drought), TC-rich repeats (defense and stress responsiveness), and LTR (cold stress), ARE (anaerobic responsive elements). Another group of *cis*-elements found in the putative promoter regions of *PvBURP* genes those related to light response are Box4, G-box, I-box, Sp1, GT1-motif, TCT-motif, AT1-motif, MRE, L-box, AE-box, Gap-box, GATA-motif, chs-CMA1a, 3-AF1 binding site, Box3, GA-motif, chs-CMA2a, ATC-motif. The existence of CAAAGATATC-motif showed that most *PvBURPs* also have potential roles in regulating the circadian cycle in plants. (Fig. 5, Table S3). Likewise, 5UTR Py-rich (TTCTTCTAT) stretch found in the putative promoter region in *PvBURP6*, *PvBURP8,* and *PvBURP11* provide them high transcription level.

Identification of miRNAs targeting the genes plays a vital role in understanding both miRNAs and their target's functions. We identified 12 different miRNA targeting the nine *PvBURP* genes using two different in silico tools based on perfect or near perfect complementarity to their targets. We were unable to find a miRNA corresponding to the *PvBURP5* and *PvBURP6* during this study. The genes most targeted by the miRNAs in this analysis were *PvBURP2* and *PvBURP8*. The most significant miRNA within this group is miRNA395, as it targets the highest number of genes: *PvBURP9, PvBURP2, PvBURP11,* and *PvBURP8* (Table 3). The other important miRNAs targeting *PvBURP* genes were miRNA156, miRNA169, miRNA171, miRNA319, and miRNA390.

Prediction of protein structure and subcellular localization

To estimate 3D structures of PvBURPs protein on the basis of homology modeling principles, Phyre2, a web-based bioinformatic server, was utilized (Figure S2). Although beta-sheets are very common in all PvBURP proteins, this situation is quite striking in PvBURP7 protein, containing only 10% alpha-helix (Table S4). The protein with the highest alpha-helix structure was estimated to be PvBURP3 (45.5%). Identifying where transmembrane (TM) segments are located can help narrow the potential conformations of the tertiary structures for the given protein and predict its function (Krogh et al. 2001). This analysis showed that there is one transmembrane helix in 6 PvBURP proteins (PvBURP1, PvBURP2, PvBURP3, PvBURP5, PvBURP6, and PvBURP7) (Figure S3). The most extended transmembrane helix consisting of 22 residues was observed in PvBURP3 and PvBURP7. The sizes of these segments in other proteins vary between 17 and 19 residues. When PvBURP proteins are compared structurally, another remarkable finding is the existence of signal peptides. In silico analysis indicated that all proteins except PvBURP7 and PvBURP9 contain Sec signal peptide (Sec / SPI) at the N-terminal (Table S2). Cell-PLoc2.0 predictions have shown that most PvBURPs except PvBURP7, active in the nucleus, were functional within the cell's membrane (Table 1).

Stress-responsive expression profiles of *BURP*s

To evaluate the expression pattern of BURPs in different stress conditions, available RNA-seq data sets were retrieved from the NCBI SRA database. In this context, six BioProject was analyzed conducting in common bean under drought, salt, and pathogen stress. As shown in Fig. 6 prepared based on log2 fold change, *BURP* genes' expression differs according to stressors. It was observed that in all experiments, at least one *BURP* gene was expressed differentially. The data set in which the least number of *BURP* genes were differentially expressed were first and fourth. In the first data set, the expression levels of *BURP* genes in salt-treated leaves of the T43 (salt-sensitive) and ISPIR (salt resistant) genotype were studied. In

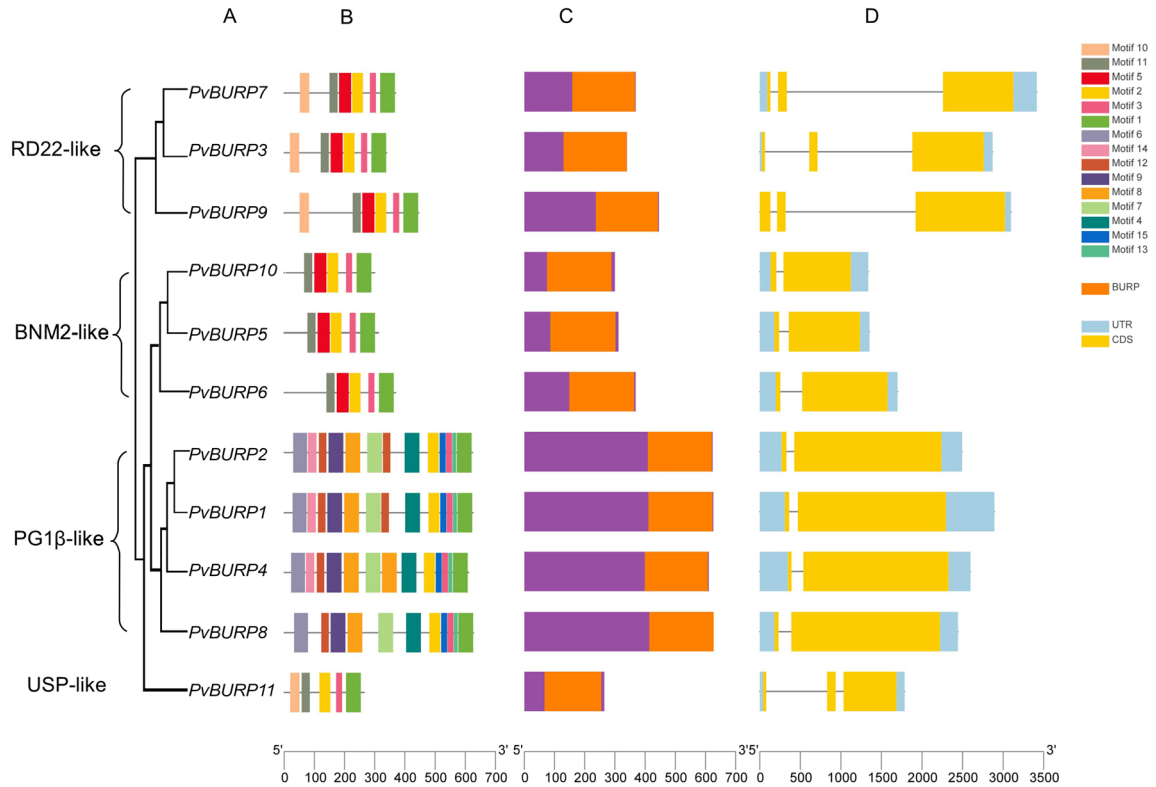

Fig. 2 Phylogenetic relationships, gene structure, conserved motifs and conserved domains in *BURP* genes from *P. vulgaris*. **A** Phylogenetic tree was constructed using the MEGA X software based on the full-length sequences *of P. vulgaris BURP* genes. PvBURP members are divided into four subfamilies RD-22 like, PG1β-like, BNM2-like and USP-like. **B** Conserved motifs were identified by MEME Suite and displayed in different colored boxes. **C** Distributions of the conserved domains in BURP proteins. Orange color boxes indicate cation BURP domains **D** Exon–intron structure of *P. vulgaris BURP* genes. Blue boxes indicated untranslated 5′- and 3′-regions; yellow boxes indicate exons

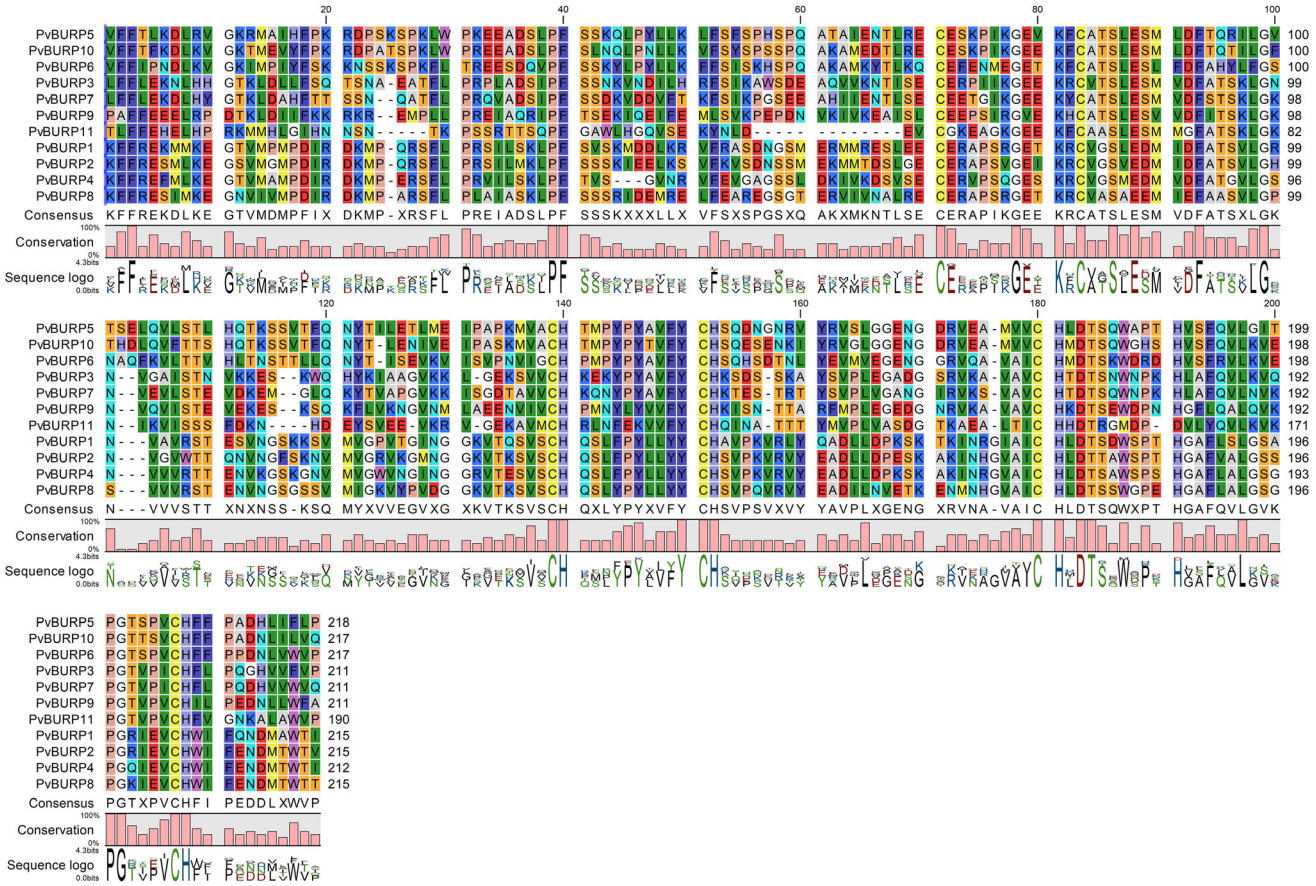

Fig. 3 Multiple alignment of amino acid sequences of BURP proteins from *P. vulgaris*. BURP family members represent these to have many extremely conserved amino acid sites and 4 CH motifs

this first comparison, *PvBURP6* was significantly up-regulated with salt stress ($log_2FC = 1.58$), while *PvBURP4* was down-regulated ($log_2FC = 1.50$) with the same stressor. In the second data set obtained by comparing the data retrieved from the roots of T43 and Ispir genotypes under salt stress, only the *PvBURP10* gene was significantly induced in both genotypes ($log_2FC = 1.94$).

Additionally, *PvBURP5*, *PvBURP7, PvBURP5,* and *PvBURP11* were differentially up-regulated in the salt-treated Ispir roots (third data set) compared to control conditions. In the fourth data set, when the salt-stressed sensitive and resistant genotypes were compared, it was found that only two genes, *PvBURP7* (log_2 FC = − 1.35) and *PvBURP10* (log_2 FC = − 1.96), differentially suppressed with salt stress. In the last data set (fifth data set), four genes (*PvBURP2, PvBURP4, PvBURP9, and PvBURP11)* were differentially up-regulated and *PvBURP10* was down-regulated in response to salt stress. In the sixth and seventh data sets expression level of *PvBURP* genes under drought indicated similar regulations to salt stress. All these in silico gene expression analyses indicated that *PvBURP1, PvBURP2, PvBURP3, and PvBURP10* were differentially up-regulated in both data sets, while the *PvBURP11* gene was down-regulated. When compared with other stress conditions, it was observed that more *PvBURP* genes were expressed differentially in drought stress applications. In this context, the data set having the highest number of BURP genes (9 genes) with different expression levels was the seventh. In this study, drought stress was applied when the plants reached the true three leaves stage, and after two weeks of stress application, all aboveground organs were collected for RNA isolation. During the in silico expression analysis of these data, expression profiles of control and drought-treated Pinto Saltillo plants were compared. According to this comparison, *PvBURP1*, *PvBURP2*, *PvBURP3*, *PvBURP4*, *PvBURP8*, *PvBURP9,* and *PvBURP10* were found to be differentially up-regulated (Fig. 6 and Table S5). However, *PvBURP7* and *PvBURP11* genes were found to be down-regulated. The maximum log2 fold change value with a

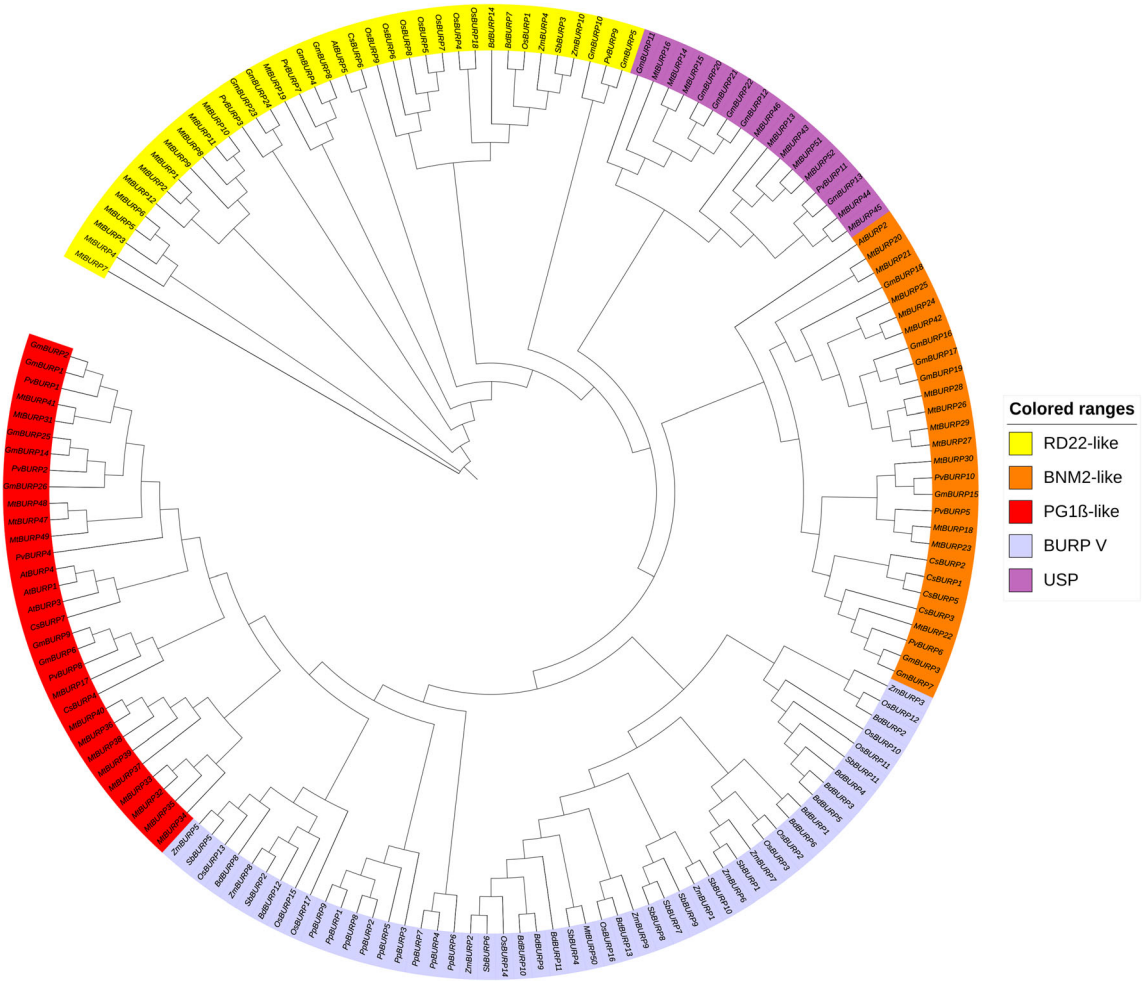

Fig. 4 Phylogenetic relationship of *BURP* genes in *P. vulgaris* and other plant species. *BURP* genes are divided into five subfamilies named RD-22 like, PG1β-like, BNM2-like, USP-like, and BURP V. Each subfamily is shown in different colors. The tree was generated using the MEGA X software by the neighbor-joining method based on the Jones-Taylor-Thornton (JTT) model with bootstrap of 1000 replicates and visualized with ITOL v3

− 12.3 was calculated for the *PvBURP11* gene using the RNA-seq data obtained from a library (eighth data set) prepared with a common bean plant infected by the fungal pathogen *Sclerotinia sclerotiorum* (strain 1980) (PRJNA574280) (Table S5). In addition to this differentially down-regulated gene, *PvBURP2* and *PvBURP7* were also differentially up-regulated in this library. Tissue-specific expression profiles of *PvBURPs* were retrieved from Phytozome v12 (Fig. 7). In the light of this data, we observed that *PvBURP1, PvBURP2, and PvBURP7* were differentially up-regulated in almost all tissues, including flower, flower buds, leaves, young trifoliates, young pods,

Table 2 Ka/Ks ratios of *PvBURP* genes

Group	Gene 1	Gene 2	Identity (%)	Ka	Ks	Ka/Ks	Purifying selection	Effective Length	Mya
PG1β	*PvBURP1*	*PvBURP2*	65.15	0.267357054	1.68629	0.158547497	Yes	1851	12.97146
PG1β	*PvBURP1*	*PvBURP8*	51	0.438637093	1.464318	0.299550437	Yes	1833	11.26398
PG1β	*PvBURP2*	*PvBURP8*	51.75	0.426944018	1.789431	0.238592104	Yes	1833	13.76485
BNM2	*PvBURP5*	*PvBURP10*	64.95	0.202641686	0.662749	0.305759265	Yes	876	5.09807

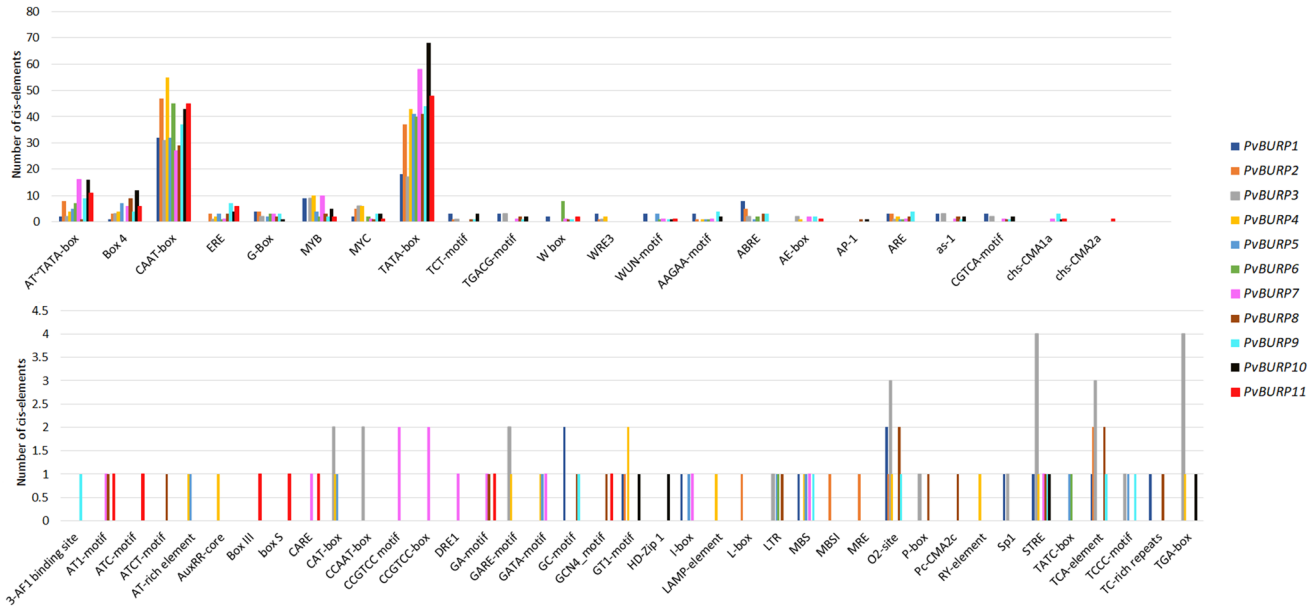

Fig. 5 Stress, light, and hormone response-related cis-elements in the promoter sequences of *PvBURPs* covering the upstream region of 2000 nucleotides from the gene start codon. *PvBURPs* are represented by different colors. The families of cis-elements were identified using place database

stem, root, and nodules. However, the expression levels of *PvBURP8* and *PvBURP4* did not change significantly between tissues of the common bean. The expression levels of other *PvBURP* genes increased in some tissues but decreased in some other tissues. For instance, while the *PvBURP10* gene increased significantly in the tissue of flowers, flower buds, and nodules, it decreased in the tissue of leaves, young three leaves, young pods, and green mature pods (Fig. 7). Likewise, while less *PvBURP11* activity was observed in leaves and young leaves, it was seen that this gene was expressed at a higher rate in nodule and root tissue. It was determined that *PvBURP3* and *PvBURP9* genes' activity did not change in other tissues, but expression levels were increased in green mature pods, nodules, and root tissues. It was seen that the *PvBURP5* gene is less expressed in other aboveground organs other than the stem but has a significantly increased activity in the root tissue (Fig. 7).

Determination of expression pattern of BURPs under various stress conditions

The expression levels of the *BURP* genes in roots and leaves of common bean plants subjected to salt, drought, IAA, and ABA stress were determined by quantitative real-time PCR (Fig. 8). Under salt stress conditions, it was observed that *PvBURP3, 4, 5, 6, 7, 8, 9, 10*, and *PvBURP11* were most significantly up-regulated at seven days in the leaf tissue. These all were up-regulated at different levels, but it was clearly seen that *PvBURP5, 10, 11* represent a significant correlation at the highest response level in leaf tissue and only at seven days. Although no clear correlation appears in root tissue, it was understood that *PvBURP1* at 12 h, *PvBURP2* at six hours and seven days, and *PvBURP3* at 24 h were up-regulated in the root tissue (Fig. 8).

Under PEG-mediated drought stress condition, almost all *BURPs* in that experiment showed different positive responses at different time points in leaf tissue, but the significant ones that show up-regulation were; *PvBURP3* at 12 h, *PvBURP4*, and *PvBURP10* at 24 h, and *PvBURP4, 5, 7, 8, 10* and *11* at seven days. One of the clear positive correlations between *BURP4* and *BURP10* appear at 24 h in leaf and seven days both in root and leaf tissues. The second correlation at seven days in the leaf tissue between *BURP4, 5, 7, 8, 10,* and *11* looks similar to *PvBURPs* under salt treatment except for *PvBURP3*, *6, 9, 10* was no significant up-regulation under PEG condition. *PvBUPR3* at 12 h, *PvBURP4* and *PvBURP9* at four days, and *PvBURP2*, 3, 4, 7, 8, 9, 10 at seven days exhibited up-regulation in the root tissue (Fig. 8).

After ABA treatment, some BURP members represented positive responses at different time-point in the leaf tissue like *PvBURP1, 3, 8, 9* at 12 h, *PvBURP2*, 4, 7, 9 at 24 h, and *PvBURP3*, 7, 9, 11 at 60 h. The exciting correlation results have appeared from *PvBURP9*, and *PvBURP11* respond at the highest level at 24 h and 60 h to ABA only in leaf tissue. The *PvBURP1*, 3, 4, 7 at 12 h, *PvBURP7* at 24 h, and *PvBURP3* at 60 h showed up-regulation in root tissue. The general idea about the response of BURPs to

Table 3 Prediction of potential miRNAs targeting *PvBURPs*

miRNA_Acc	Target_Acc	Expectation	Unpaired energy	miRNA start	miRNA end	Target start	Target end
Pvu-miR156	*PvBURP3*	5.5	− 1	1	21	423	443
Pvu-miR169	*PvBURP10*	5.5	− 1	1	21	498	518
Pvu-miR171	*PvBURP8*	5	− 1	1	21	571	591
Pvu-miR171	*PvBURP4*	5.5	− 1	1	21	1333	1354
Pvu-miR319	*PvBURP2*	6	− 1	1	21	1141	1161
Pvu-miR390	*PvBURP8*	5	− 1	1	21	717	737
Pvu-miR390	*PvBURP10*	6	− 1	1	21	286	306
Pvu-miR395	*PvBURP11*	4	− 1	1	21	91	111
Pvu-miR395	*PvBURP9*	5.5	− 1	1	21	1251	1271
Pvu-miR395	*PvBURP2*	6	− 1	1	21	98	118
Pvu-miR395	*PvBURP11*	4	− 1	1	21	91	111
Pvu-miR395	*PvBURP8*	5.5	− 1	1	21	585	605
Pvu-miR397	*PvBURP2*	5.5	− 1	1	21	54	74
Pvu-miR397	*PvBURP7*	6	− 1	1	21	21	41
Pvu-miR408	*PvBURP3*	6	− 1	1	21	284	303
Pvu-miR482	*PvBURP2*	6	− 1	1	22	1463	1484
Pvu-miR1514	*PvBURP1*	6	− 1	1	22	1340	1361
Pvu-miR4416	*PvBURP4*	4.5	− 1	1	21	924	944
Pvu-miR4415	*PvBURP8*	4	− 1	1	21	1533	1553

miRNA_Acc	miRNA_aligned_fragment	Alignment	Target_aligned_fragment	Inhibition
Pvu-miR156	UGACAGAAGAGAGAGAGCACA	:::::.::::::::::	AUUGGACUUGCUCUUCUCUCA	Cleavage
Pvu-miR169	CAGCCAAGGGUGAUUUGCCGG	:::::::..::::::::	CACUCAAACCAUUCUUGGCUU	Cleavage
Pvu-miR171	UGAUUGAGCCGUGCCAAUAUC	:.:::::.::::::::::	GGUUCUGCCGCGGCUCAAUCC	Cleavage
Pvu-miR171	UUGAGCC-GCGCCAAUAUCACU	:::.::::.:::::.::::	UUUGAGGUUGGUGCCGGUUCAA	Cleavage
Pvu-miR319	UGGACUGAAGGGAGCUCCUUC	::.:::.:.::::::.:.:	GAGGGUGUUUCCUUCGCUUCU	Cleavage
Pvu-miR390	AAGCUCAGGAGGGAUAGCACC	::::::::.::::::::	UGCGCAAUCCUUCAUGAGCUA	Cleavage
Pvu-miR390	AAGCUCAGGAGGGAUAGCACC	:::::.:::::::.:	CCUGCAACCUCUCCUAAGUUG	Cleavage
Pvu-miR395	UGAAGUGUUUGGGGGAACUCU	:::.:.:::::::::::	UCUGUUUUUCCAAACACUACA	Cleavage
Pvu-miR395	UGAAGUGUUUGGGGGAACUCU	:.::.:.:::.:.:::::	CGGCUUUUUACAAGCGCUUCA	Cleavage
Pvu-miR395	CUGAAGUGUUUGGGGGAACUC	:::.:::::.:::::	CGUUUACUCCAAAGGCUUCUG	Cleavage
Pvu-miR395	UGAAGUGUUUGGGGGAACUUU	:::.:.:::::::::::	UCUGUUUUUCCAAACACUACA	Cleavage
Pvu-miR395	UUGAAGUGUUUGGAGGAACUC	:::::::.::::::::	UCAAUCCUCCAGCGACUUCAA	Cleavage
Pvu-miR397	UCAUUGAGUGCAGCGUUGAUG	:::.:.:::.::.::	ACUCACUGUUGCUUUCGGUGG	Cleavage
Pvu-miR397	UCAUUGAGUGCAGCGUUGAUG	:::..:::.::::::::	CAUUUUUGCUUUACUCAAUGU	Translation
Pvu-miR408	AUGCACUGCCUCUUCCCUGGC	::::::::::.:::::::	CAAAGGGAA-AGCCGGUGCAU	Cleavage
Pvu-miR482	UCUUCCCUACACCUCCCAUACC	::.:::.:::::.:::.::	GUUGUGUGGGGUCUGUGGAGGA	Translation
Pvu-miR1514	UUCAUUUUGAAAAUAGGCAUUG	:.:.:.:::::::.:::.:	CGUUUUCCGUUUCCAAGAUGGA	Translation
Pvu-miR4416	UACGGGUCGCUCUCACCUAGG	:::::.:::.:::.:::	CAACGGUGGGAGUGACUCGUU	Cleavage
Pvu-miR4415	UUGAUUCUCAUCACAACAUGU	.:::::::.:::::.:::	CAGUGUUGUGGUGAGGAGCAC	Cleavage

ABA was that BURPs were much more active in leaf tissue than the root tissue under ABA treatment (Fig. 8).

Under IAA treatment, almost all *PvBURPs* except for *PvBURP1* and *PvBURP4* are highly active at 60 h, and *PvBURP8* is found highly active at all time-point except for six hours in leaf tissue. Moreover, only *PvBURP7* shows up-regulation at 48 h. In the root tissue, *PvBURPs* are much less active compared to the result in leaf tissue.

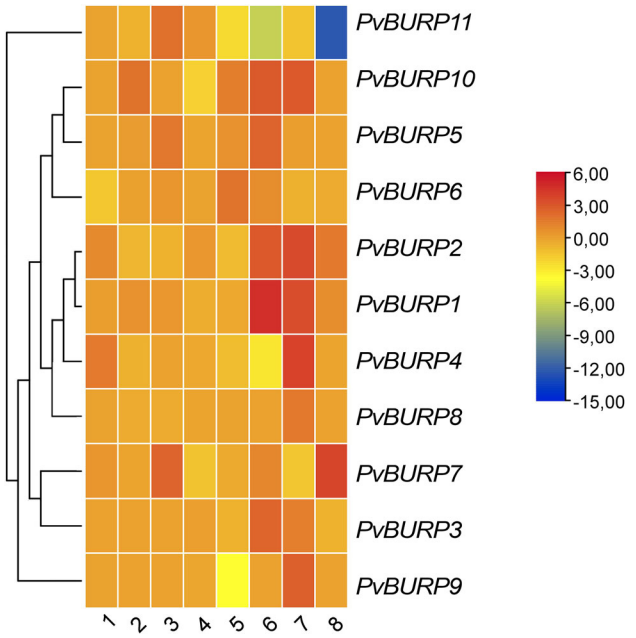

Fig. 6 A Heat map illustrating *BURP* expression in various RNA-Seq datasets. Red, positive log fold-change (log FC) indicates higher expression in the first genotypes compared with the second; blue, negative log FC. The raw RNA-seq datasets were downloaded from NCBI Sequence Read Archive (SRA) under the accession number; 1 and 2 represent salt-treated leaf explants of T43 (sensitive) and Ispir (resistant) common bean genotypes (PRJNA656794), 3 represents salt-tolerant Ispir's root tissues grown under both control and salt stress conditions (PRJNA656794), 4 indicates salt stress to the salt-tolerant and the sensitive genotype at the bud stage (PRJNA558376), 5 represents salt stress to the salt-tolerant bean genotype in the sprout stage (PRJNA691982), 6 and 7 represent RNA sequencing of drought-tolerant *Pinto saltillo* (PRJNA508605) and *Perola* (PRJNA327176) genotypes grown under control and drought stress and 8 indicates a common bean plant infected by the fungal pathogen *Sclerotinia sclerotiorum* (strain 1980) (PRJNA574280). The transcriptional analysis of downloaded files was done via CyVerse and Galaxy (usegalaxy.eu) with in silico tools. The mapping of reads was done with the HISAT2 tool. Transcript assembly and differential expression analyses were performed with Stringtie 1.3.3 and Ballgown, respectively. The genes having fold change value log2 > 1 and a p value < 0.05 were accepted as differentially expressed genes (DEGs). Heatmap based on the log2FC was prepared using TBTools

Only *PvBURP3* and *PvBURP7* at 12 h and *PvBURP2* at 60 h represent significant up-regulation in root tissue (Fig. 8).

Overall, as it is evident from these results, *BURP*s are not regulated at the onset of the stress response in both leaf and root tissues. There is a robust correlation indicating the up-regulation of *BURP*s towards the end of all stress conditions in leaf tissue. Moreover, *BURP*s are much more active in leaf tissue than root tissue for all conditions.

Discussion

There are studies in the literature which aims to understand the roles of *BURP* genes in development and stress responses in plants, but it mainly covers the RD22 and USP-like subfamily only (Bassüner et al. 1988; Batchelor et al. 2002; Harshavardhan et al. 2014; Hattori et al. 1998; Yamaguchi-Shinozaki et al. 1993). In parallel with the development of new generation sequencing systems, although a considerable increase has been observed in the genome-wide analysis of different genes and transcription factors, the same cannot be said for *BURP*s. Until now, genome-wide analysis studies of *BURP* genes have been carried out on very few plants, including rice, maize, grapevine, soybean, cotton, sorghum, and poplar (Ding et al. 2009; Gan et al. 2011; Matus et al. 2014; Shao et al. 2011; Sun et al. 2019; Xu et al. 2010). When these previous studies were examined, it was found that the richest genome in terms of *BURP* genes was in cotton with 30 genes, and the least found was in sorghum with 11 genes. Consistent with previous studies, we also found 11 putative *BURP* genes distributed to the five chromosomes in the common bean genome using various in-silico tools. We observed that most of the BURP encoding genes, 5 out of 11, were located on chromosome 9. During the evolution and expansion of gene families in plants, gene duplication such as segmental and tandem play a key role (Cannon et al. 2004). Duplication events allow the quantity of genetic material to evolve during evolution and natural selection. When a gene duplication event takes place, some duplicated genes keep their functions, while others show partial or complete divergence from another (Pickett and Meeks-Wagner 1995). It can be speculated that the evolutionary origin of the *BURP* gene family in the common bean genome by searching the location of these genes in the genome. A gene cluster, located on chromosome 9, consists of five genes (*PvBURP5–9*), of which three genes (*PvBURP5*, *PvBURP6*, and *PvBURP8*) share common parent genes. Five out of 11 BURP genes (*PvBURP1*, *PvBURP2*, *PvBURP8*, *PvBURP5*, and *PvBURP10*) were found located in duplicated regions (Table 2). The analysis of gene duplication events indicated that segmental gene duplication played a significant role in forming the *PvBURP* gene family. The same is reported for poplar (Shao et al. 2011), grapevine (Matus et al. 2014), and soybean (Xu et al. 2010) alike. Another similarity of our study with previous studies was related to the distribution of BURP genes on chromosomes. When previous studies carried out in poplar, grapevine, sorghum, and maize were examined, it was seen that *BURP* genes were unevenly distributed to the chromosomes, such as seven genes in the seventh chromosome at poplar, 13 genes in the fourth

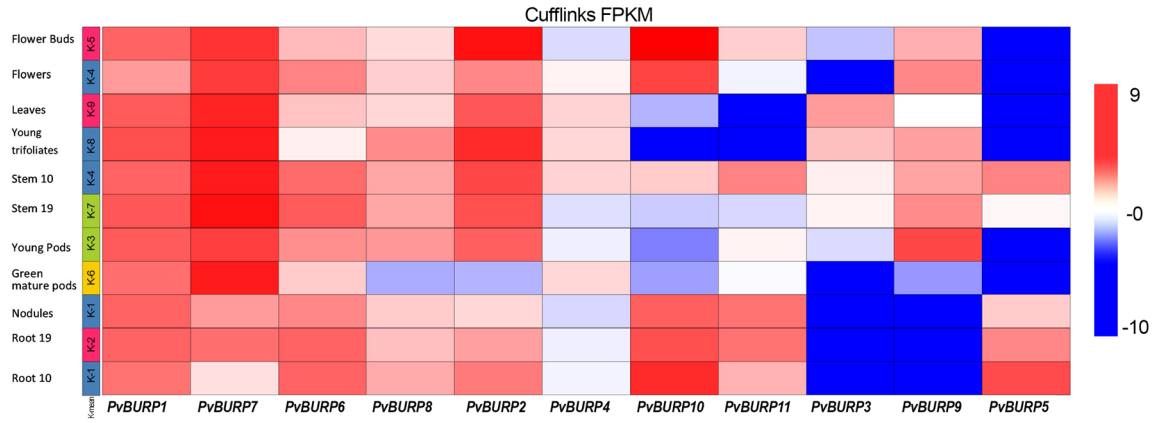

Fig. 7 Hierarchical clustering of FPKM values of *PvBURP* genes in different tissues. Red to blue frames represent positive to negative expressions. The raw data was normalized and retrieved from the Phytozome v12

chromosome at grapevine, four genes in the eighth chromosome at sorghum and six genes in the seventh chromosome at maize. This data suggests that, even though the number of *BURP* genes between species is similar, the BURP superfamily may have undergone a specific expansion of a particular group within these chromosomes (Matus et al. 2014).

To gain insight into the relationships between the common bean *BURP* genes, we first constructed a phylogenetic tree including the 11 common bean proteins identified in this work based on their deduced amino acid sequences. As reported in previous studies, the common bean BURP proteins are subdivided into four different subfamilies, including USP-like, BNM2- like, PG1β-like and RD22-like. We identified at least one member for each subfamily. However, the classification of the *BURP* genes varies in different plant species. For instance, Xu et al. (2010) reported that there were five subfamilies, including *BURP V*, in addition to the previously mentioned subfamilies in the soybean genome. We confirmed this phylogenetic relationship using the motif patterns retrieved from MEME Suite. As in our study, Ding et al. (2009) found that PG1βs show a range of unique motif patterns with a minimal degree of divergence amongst species. For this reason, these sequences were not included in the further analysis. We observed that USP-like, BNM2-like, and PG1β-like members were all detected in dicotyledons only, whereas RD22-like members were identified in both dicotyledon and monocotyledon plants, based on the phylogenetic relationship between PvBURPs and other BURP proteins identified in other analyzed plants. Additionally, the BURPV subfamily consists of the members belong to the monocotyledons, indicating that these genes might evolve separately and perform different functions between monocots and dicots (Ding et al. 2009; Gan et al. 2011; Sun et al. 2019). Our findings related to the classification of BURPs is consistent with previous studies, as no Arabidopsis gene was present in the USP subfamily (Matus et al. 2014). Actually, it was seen that this subfamily included BURP genes belonging to barrelclover, soybean, and bean plants; that is, it contained only the leguminous family.

In previous studies, it has been shown that at least one of the *BURP* genes in different plants, including maize and rice, is without introns and there are BURP members, which generally carry 2 or 3 introns (Ding et al. 2009; Gan et al. 2011). In contrast, in the present study, we found that there was no intronless *PvBURP* gene and the maximum number of introns in the *PvBURP* genes was two. The intron–exon distribution pattern can be used as an independent criterion for testing the reliability of the phylogenetic analysis (Du et al. 2012). The intron–exon pattern of *PvBURP* genes of a particular subfamily displayed remarkable consistency with the location of the intron almost fully conserved, which highlighted how related the individual members of the subfamily are.

miRNA can play various roles in plant growth and development, reproductive processes, response to different stresses, and cellular signaling (Zhao et al. 2015). Predicting the miRNAs that target genes allows us to obtain information about the possible functions of those genes. The best example of this assumption is miRNA156. Because miRNA156, one of the first discovered miRNAs, plays a role in regulating many developmental events in plants (Xing et al. 2010). The most important micro-RNA found in our study was mirRNA395, which is involved in sulfur metabolism. Sulfur actively participates in numerous biological processes and plays a vital role in plant development (Ai et al. 2016). Since micro RNAs such as miRNA169, mirNA171, and miRNA319, which are among the other miRNAs found in our study, play a key role in the response of plants to various stresses and the formation of

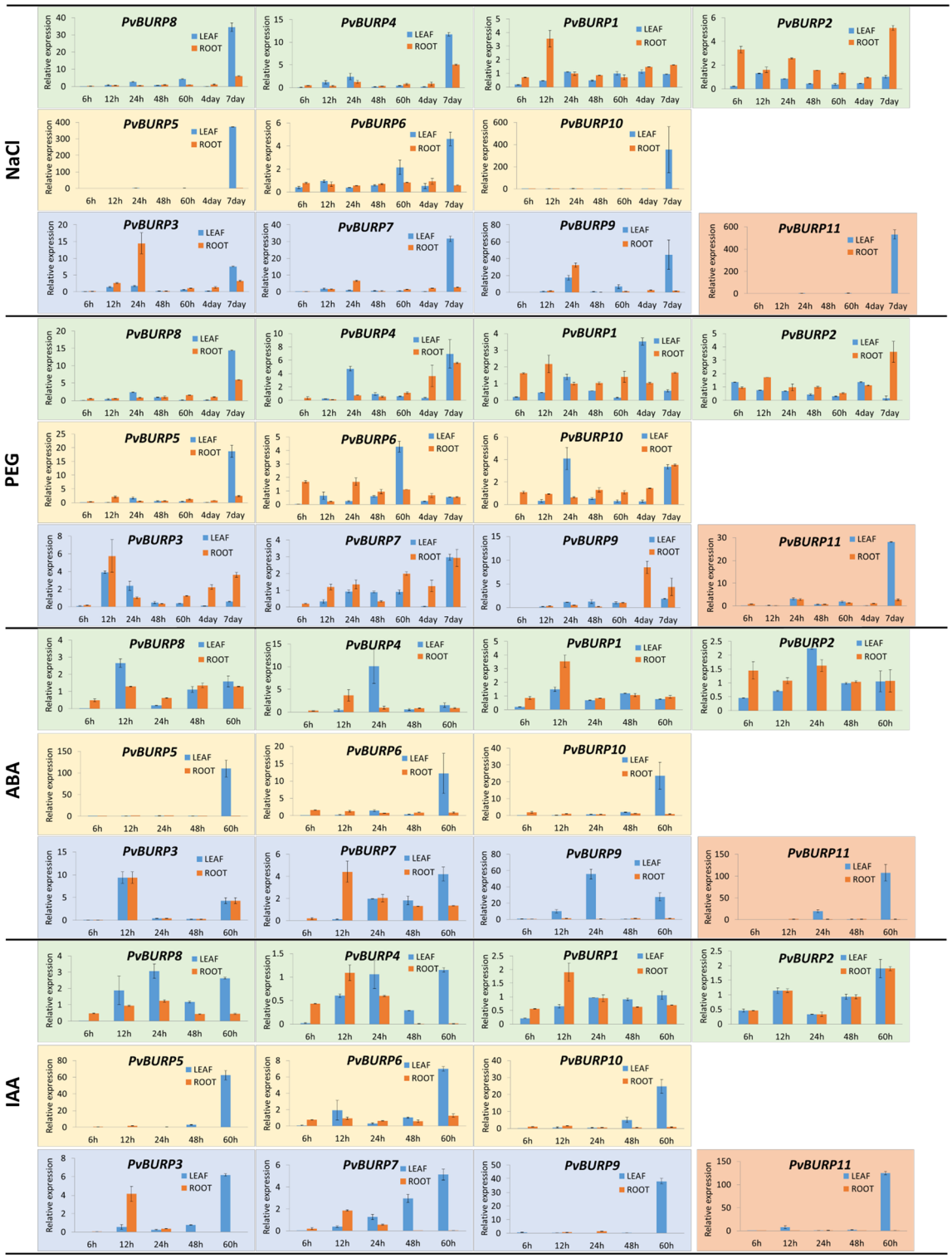

Fig. 8 qRT-PCR analysis of *PvBURP* genes in roots and leaves of common bean plants under salt, drought, IAA and ABA treatments. Expression levels were measured at different time points. Relative expression was calculated according to the $2^{-\Delta\Delta Ct}$ method

plant organs, this confirms the previously defined functions of the *BURP* genes they target.

Another proof that *BURP* genes have roles in response to various stress factors and regulating many different developmental processes in plants is the different *cis*-elements found in the promoter regions of *PvBURPs*. Since they regulate a variety of stress responses, cis-acting regulatory elements are essential transcriptional gene

regulatory units (Sheshadri et al. 2016). In the present study, we identified ten types of plant hormone-related and eight types of stress response-related cis-acting regulatory elements in the promoter region of *PvBURP* genes inconsistent with their potential functions. Some of the cis-acting regulatory elements identified in this study are not frequently observed in other plants. These are GARE and GT1 motifs, thymine- and cytosine-rich repeats. GARE element, located between 139 and 145 bp downstream of the TSS, is involved in the gibberellin response. One of the light response elements is the G1 motif (ATGGTGGTTGG), which can be located 168–178 bp downstream of the TSS. (Biłas et al. 2016).

Gene expression patterns are considered to be important clues used to make inferences about the functions of those genes (Kavas et al. 2016). To determine the potential functions of *PvBURP* genes, RNA-seq data belong to different tissues exposed to various stresses were evaluated. The RNA-seq data from Perola and Pinto Saltillo genotypes have been analyzed to determine expression profiles of *PvBURP* genes under drought stress. It was reported that the total RNAs from drought tolerant Pinto Saltillo were isolated from all aboveground tissues after two weeks of stress treatment to the 45 days-old plants (Gregorio Jorge et al. 2020). However, in the other drought-tolerant Perola genotype, drought stress was applied to the two-week-old plants for just 150 min (Pereira et al. 2020). Although the age of plants when stress treatment was started and the duration of stress were different in these two experiments, the expression pattern of *BURP* genes was generally consistent in both genotypes. In this context, the expression level of members of RD22 (*PvBURP3*), BNM2 (*PvBURP10*), and PG1β (*PvBURP1* and *PvBURP2*) were significantly increased in both treatments. As a similar result, the increase in the expression of some *BURP* genes in response to drought stress and the decrease in some of them were observed in the genome-wide analysis carried out in the *Medicago* plants (Li et al. 2016). A similar change in expression of *BURP* genes occurred under salt stress. As a result of the comparison of the transcriptome data obtained from the roots of the salt-tolerant Ispir and sensitive T43 genotypes exposed to salt stress, it was determined that only the *PvBURP10* gene was expressed differently. However, in the same study conducted with the roots of the ISPIR genotype exposed and not exposed to salt stress, more genes (*PvBURP11*, *PvBURP7*, and *PvBURP5*) were found to be up-regulated. Banzai et al. (2002) cloned and evaluated the expression of *BgBDC* genes, RD22 homolog in *Bruguiera gymnorrhiza.* They reported that the expression of these genes could be changed between different leaves depending on ABA levels under salt stress. A similar result to previous studies was that the USP-like gene, *PvBURP11*, is down-regulated under drought stress. In their study, Harshavardhan et al. (2014) found that plants with the mutant *AtUSPL1* gene have higher drought tolerance than wild-type plants. We also determined the expression pattern of *PvBURPs* under IAA, ABA, salt, and drought stress conditions with qRT-PCR. We observed that *PvBURPs* were not regulated at the onset of the stress response in both leaf and root tissues. There is a solid correlation indicating the upregulation of *PvBURPs* towards the end of all stress conditions in leaf tissue. Moreover, *PvBURPs* are much more active in leaf tissue than root tissue for all conditions.

In conclusion, for the first time, a complete analysis of the BURP family in common bean was analyzed, and the relationship between this family and drought, salt, IAA, and ABA stress response was evaluated. Additionally, we showed gene structures, chromosomal locations and sequence homologies of these genes. This study provided a useful resource for future studies on the structure and function of BURP proteins in the regulation of drought, salt, IAA, and ABA stress response in plants.

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Declarations

Conflict of interest All authors declare that they have no conflict of interest.

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