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Vol. 15(15), pp. 580-592, 13 April, 2016
DOI: 10.5897/AJB2015.15061
Article Number: D4716DB58067
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Genome-wide analysis of mechanosensitive channel of small conductance (MscS)-like gene family in common bean

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Received 22 October, 2015; Accepted 18 March, 2016.

Mechanosensitive (MS) ion channels are transmembrane proteins that open and close in response to mechanical forces produced by osmotic pressure, sound, touch and gravity. In plants, MS have an important role in different biological processes like gravity detection, maintenance of plastid shape and size, lateral root emergence, growth of pollen tube, and plant-pathogen interactions. In this study, homologous mechanosensitive channel of small conductance (MscS)-like gene family in common bean was identified. Nine *Phaseolus vulgaris* MscS-like (*PvMSL*) genes were found to be distributed on five chromosomes. A complete overview of *PvMSL* genes in common bean is presented, including gene structures, chromosome locations, phylogeny, protein motifs and expression pattern. Subcellular localization predictions of *PvMSL* family revealed their location to plasma and chloroplast membrane. Phylogenetic analysis of nine *PvMSL* proteins resulted in two main classes. The predicted gene structure, conserved motif, domain and presence of transmembrane regions in each *PvMSL* strongly supported their identity as members of MscS-like gene family. Four duplicate events of *PvMSL* genes were discovered in *P. vulgaris* chromosomes, and tandem and segmental duplication may cause the expansion of *PvMSL* genes. Furthermore, *PvMSL* genes displayed differential expression patterns in tissues and organs. This is the first step towards genome-wide analyses of MSL genes in common bean. Thus, the data obtained in this study provide resources to select candidate genes for future functional analyses that will help understand plant growth, development, and function of MSL gene family in *P. vulgaris*.

Key words: Mechanosensitive, phylogenetic analysis, gene duplication, plant, *in silico.*

INTRODUCTION

Plants perceive and respond to a lot of mechanical stimulli, including touch, gravity, osmotic pressure and stress (Ding and Pickard, 1993; Blancaflor and Masson, 2003; Braam, 2005; Furuichi et al., 2008a; Hamilton et

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al., 2015). Moreover, they respond to signals associated with plant development processes like emergence of lateral roots, growth of pollen tube, damages to the cell wall, and plant-pathogen interactions (Lucas et al., 2013; Appel and Cocroft, 2014; Jayaraman et al., 2014). In many of these cases, the application of a mechanical stimulus results in a quick explosion of the ion flow which can be attributed to mechanosensitive (MS) ions due to the response speed (Fasano et al., 2002; Jaffe at al., 2002; Monshausen and Haswell, 2013).

Some responses are rapid, such as regulation of volume and turgor pressure of guard cells and closing of Venus's flytrap leaf on its prey (Cosgrove and Hedrich, 1991; Haswell and Meyerowitz, 2006; Furuichi et al., 2008b). Other responses to mechanical perturbation are growth-related and more slowly like repeated tactile stimulation of *Arabidopsis* seedlings which causes late flowering and short inflorescences (Braam and Davis, 1990) and *Arabidopsis* roots that alter their course through the soil to avoid a barrier (Massa and Gilroy, 2003). It has recently been shown that at least some mechanical stimuli trigger rapid molecular events that are then transduced into a necessarily slower growth response. In *Arabidopsis*, cytoplasmic alkalinization of root gravi-responsive cells occurs within 2 min of gravity stimulation and touch can elicit immediate Ca^{2+} transients in root cells (Legue et al., 1997; Fasano et al., 2001).

The MS channels, formed by transmembrane proteins, work as mechano-electric transductors where mechanical stimuli are converted into electrical or chemical signals in live cells that perform numerous cellular processes associated with mechanosensory transduction (Kloda and Martinac, 2002). The first MSs were identified during the electrophysiological characterization of the plasmatic membrane of *Escherichia coli* (Martinac et al., 1987; Sukharev et al., 1993; Berrier et al., 1996). The MS channels were classified according to their ion conductance and the main ones are: Mechanosensitive Channels of Large Conductance (MscL) and Mechanosensitive Channels of Small Conductance (MscS) (Edwards et al., 2012; Cox et al., 2015). The MscL present conductance (larger than 2.4 nS), open undermembrane high tension and present rapid kinectic (Perozo, 2006). Each MscL subunit is composed of a polypeptide of 136 amino acids with two transmembrane helices, TM1 and TM2 (Sukharev et al., 1994, 1999). A 3.5 Å crystalline structure in *TbMsLC* ortholog (151-amino acids) in *Mycobacterium tuberculosis* revealed a homo pentamer in an apparently closed state (Chang et al., 1998). On the other hand, MS of small conductance is a 0.8 to 1-nS channel opened by moderate pressure (Perozo, 2006). *EcoMsSC* structure of the channel from *E. coli* (286 amino acids) was originally resolved at 3.9 Å (Bass et al., 2002) and recently at 3.45 Å in an open configuration (Wang et al., 2008).In bacterial systems and animals, MS ion channels mediate the perception of pressure, touch and sound.

Although plants respond to a wide variety of mechanical stimulli, the molecular nature of transmembrane protein mechanical perception in vegetal system has been little studied (Hedrich, 2012). MS ion channels were characterized in *Arabidopsis thaliana* and *Oriza sativa* model plants *(*Haswell and Meyerowitz, 2006; Saddhe and Kumar, 2015). In *Arabidopsis* and rice, ten and five MscS homologs, respectively, were identified and classified into two main classes, Class I and Class II. There has been no study done on *Phaseolus vulgaris* to identify MscS homologs.

Common bean (*P. vulgaris*) is a main socially important crop and is a major source of protein and essential nutrients. Common bean is the most consumed legume in the worldwide (Schmutz et al., 2014). Brazil is the largest producer with an average annual production of 3.5 million tons (MAPA, 2015). However, the grain yield in Brazil is considered low and several factors are related to this, as the adverse effects of climate conditions, as well as the occurrence of pests and diseases (Beebe et al., 2013).

Therefore, knowing the importance of MS ion channels of small conductance in plant development and the important role of MscS genes to overcome different stresses, and considering lack information of MscS in *P. vulgaris,* this study aimed to identify homologous MscS-like (MSL) genes in common bean and analyze their structure, subcellular location, phylogenetic relationship, expression pattern and chromosomal distribution. Our analysis may contribute to select candidate genes for future functional analyses that will aid researchers in understanding plant growth, development and function of MSL gene family in *P. vulgaris.*

MATERIALS AND METHODS

Identification of MSL genes in *P. vulgaris* genome

Protein sequences of MSL genes identified in *A. thaliana* and *O. sativa* model plants from databases of TAIR (http://www.arabidopsis.org/) and TIGR (http://rice.plantbiology.msu.edu/), respectively, were utilized for studies in Phytozome Database of *P. vulgaris* version 11.0 *(*http://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris), using BlastP algorithm (Altschul et al., 1997). The sequences were compared with other sequences deposited in GenBank database, utilizing BlastP and BlastX programs (National Center for Biotechnology Information, NCBI; http://www.ncbi.nlm.nih.gov) to confirm their identity. The initially collected sequences, whose ORFs were incomplete, were excluded from the analyses. The protein sequences were aligned utilizing Clustal Omega algorithm version 2.0.3 (Sievers et al., 2011) and the redundant inputs were removed.

Characteristics of predicted proteins, transmembrane regions and subcellular location of MSL genes in *P. vulgaris*

The physical and chemical characteristics of MSL proteins in bean were calculated by ProtParam tool (http://web.expasy.org/protparam), including the number of amino

Table 1. Comparison of number of members of each MSL class of genes between species *Phaseolus vulgaris, Arabidopsis thaliana, Glycine max, Solanum lycopersicum, Oryza sativa* and *Zea mays*, respectively.

Species	*Phaseolus vulgaris*	*Arabidopsis thaliana* [a]	*Glycine max*[b]	*Solanum lycopersicum*[b]	*Oryza sativa*[b]	*Zea mays* [b]
Class	n	n	n	n	n	n
Class I	4	3	8	4	3	2
Class II	5	7	6	4	3	3
Total	9	10	14	8	6	5
Size Genome (Mb)	580	115	1,115	950	420	2,500

[a]Haswell and Meyerowitz (2006). [b]Saddhe and Kumar (2015).

acids, molecular weight (KDa) and theoretical isoelectric point (PI). The transmembrane (TM) regions with drawing diagrams were predicted utilizing Phyre[2] (http://www.sbg.bio.ic.ac.uk/phyre2/). All sequences of predicted MSL proteins were analyzed *in silico* regarding their subcellular location, using LOCTREE3 (https://www.rostlab.org/services/loctree3) and WoLF PSORT algorithms (http://wolfpsort.org/).

Phylogenetic analysis

Phylogenetic analysis was done by aligning the protein sequences utilizing Clustal Omega algorithm, version 2.0.3 (Sievers et al., 2011). The phylogenetic tree was built by Maximum-Likelihood (ML) method using pair-wise deletion with the help of MEGA program, version 6.06 (Tamura et al., 2013). 1,000 bootstrap replicates were utilized to test analysis confidence.

Analysis of conserved transmembrane motifs

The identification of transmembrane (TM) motif of predicted proteins were examined utilizing MEME (http://meme.sdsc.edu/meme/cgi-bin/meme.cgi) with the following paramteres: sequence repetition: 1, maximum number of found motifs: 1 and ideal size: 70 to 80 amino acids. The resulting motifs were verified in the databases of NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and PROSITE (http: //www.expasy.org) to verify their significance.

Genome structure

Complete sequences of genomic DNA and coding sequences CDS, corresponding to each gene using Gene Structure Display Server 2.0 (GSDS) (http://gsds.cbi.pku.edu.cn/), were used to identify the position of introns and the organization of exon/intron in genes.

Annotation, duplication and synteny of MSL genes in *P. vulgaris* genome

P. vulgaris MSL genes (*PvMSL*) were mapped in bean chromosome according to their genome coordinates. The genes were plotted in chromosomes using MapChart software and data on their physical location are avaialble in Phytozome. Duplications of *PvMSL* genes considered as parameters 50% identity and 80% coverage, adapted from Lopes-Caitar et al. (2013). Plant Genome Duplication Database (PGDD; http://chibba.agtec.uga.edu/duplication/) was used to search for orthologous genes in *P. vulgaris* and *Arabidopsis*. The synteny map was displayed using Circos software (http://circos.ca/) (Krzywinski et al., 2009).

Analysis of gene expression

Illumina RNA-seq datasets were downloaded from Phytozome Database (http://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris). The expression profile *in silico* of common bean *PvMSL* genes were calculated by Cufflinks in FPKM units (expected number of fragments per kilobase of transcript sequence per millions base pairs sequenced) (Mortazavi et al., 2008). FPKM values were log2 transformed and the heatmap was generated with the algorithm CIMMiner (http://discover.nci.nih.gov/cimminer).

RESULTS AND DISCUSSION

Identification and classification of MSL genes in the common bean (*P. vulgaris)* genome

In order to identify *MSL* gene family in common bean genome, the amino acid sequence PF00925 and a Hidden Markov Model profile of MSL protein were used to perform a BLASTP search against the Common Bean databases v1.1 (Phytozome v10.3: http://www.phytozome.net). In this study, a total of nine genes encoding putative proteins from a MS ion channel of small conductance were identified from the whole genome and were named *PvMSL1* to *PvMSL9.* Nine genes encoding MSL family were identified in *P. vulgaris*, while different numbers of genes were identified in other plant species (Table 1). The number of genes in MSL family identified in common bean was similar when compared with tomato (8), smaller than in *Arabidopsis* (10) and soybeans (14), and larger than in rice (6) and maize (5). Generally, the number of MSL genes distribution in dicotyledons is nearly double than in monocotyledons, possibly due to the higher rate of genome duplication events in the former. In *Arabidopsis*, Saddhe and Kumar (2015) attributed this to four different major genome duplication events over 100 to 200 million years ago. Moreover, the number of MSL genes in *P. vulgaris* is not associated with the size of the genome itself. Although *P. vulgaris* has a much larger genome

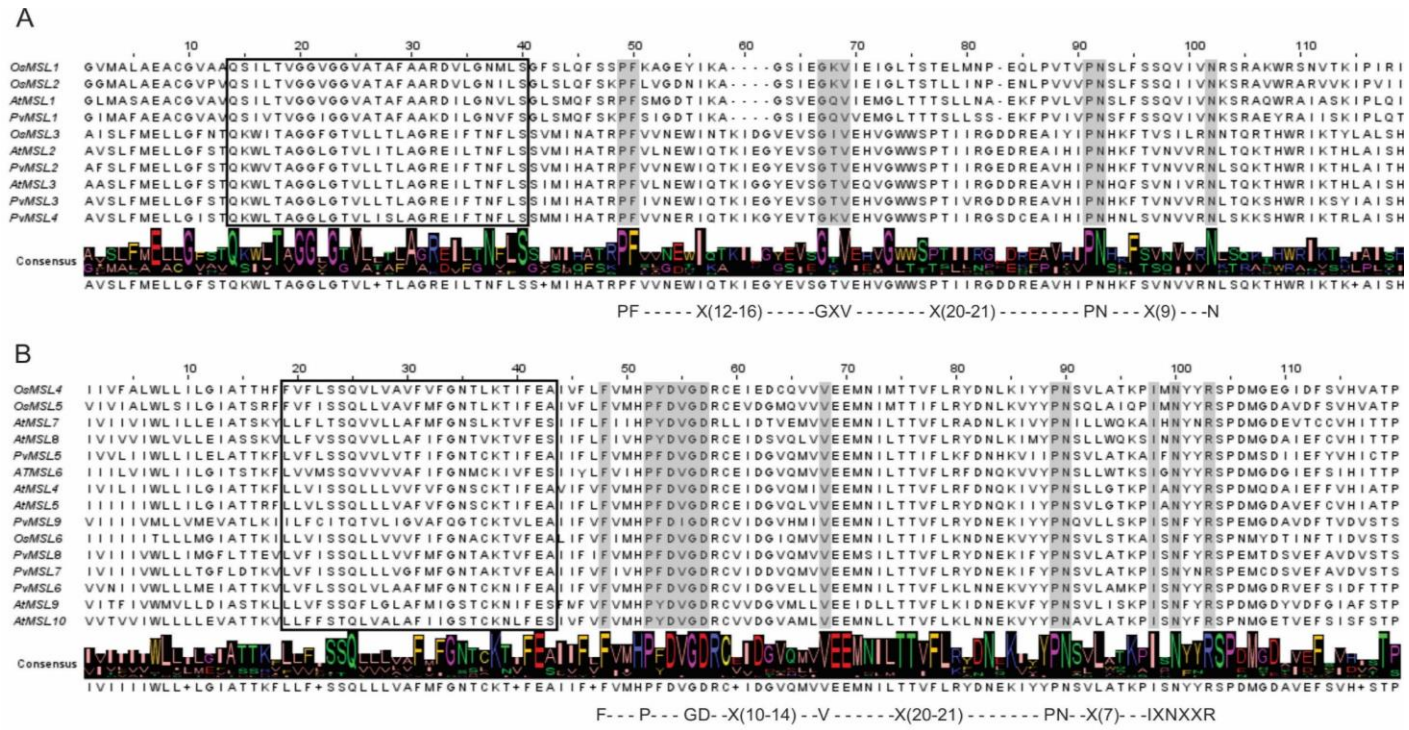

Figure 1. Alignment of MSL family conserved domains. Amino acid sequence of MSL family members from *A. thaliana* (*AtMSL1* to *AtMSL10*), *O. sativa* (*OsMSL1 to OsMSL6*) and *P. vulgaris* (*PvMSL1* to *PvMSL9).* Consensus sequences derived from this analysis is presented at the bottom of each alignment in dark type; the consensus sequence derived by Pivetti et al. (2003) is below and of each alignment in gray type. (A) Class I proteins. The box indicate the experimentally derived MscS TM3 domain. (B) Class II proteins. The box indicate the location of a consensus TM3 domain.

(580 Mb) (Schmutz et al., 2014) than *Arabidopsis* (145 Mb) (Huala et al., 2001), the structure and phylogenic arrangements are quite similar. Indeed, the number of MSL genes in monocotyledons as well as in dicotyledonous seemed to be quite similar, despite the different sizes of the genomes (Table 1). In fact, any irregular distribution of genes in MSL family among different plant species may have evolved from genomic losses or duplication events of a common ancestor, contributing to the expansion of or restriction on the number of these genes. All of the putative MSL proteins were divided into two classes (that is, I and II). The members of Class I resembled prokaryotic MsSC channels, and the ones of Class II form independent plant/fungus lineages (Pivetti et al., 2003; Haswell, 2007). Table 1 summarizes MSL protein classification of common bean genome and other plant species. Classes I and II had four and five members, respectively. According to Pivetti et al. (2003), the eukaryotic members of MscS family can be organized into two main classes based on sequence similarity. Proteins in Class I have transmembrane (TM) domains at the C-terminal region that resemble TM3 MscS which are rich in glycine and alanine residues, though the pattern is not conserved (Figure 1A). Conserved motif PF(X12–16)GXV(X20–21)PN(X9)N was identified at C-terminal for the TM domain in Class I MSL in common bean (*PvMSL1*, *PvMSL2*, *PvMSL*3 and *PvMSL*4). Class I proteins align relatively closely to *E. coli* MsSC and their C-terminal TM domains

Table 2. Physicochemical characteristics of *PvMSL* genes.

PvMSL	ID Phytozome	Nucleotide CDS bp	AA	PI	MW	Subcellular location
PvMSL1	Phvul.010G102400	1623	540	9.03	58.79	Plasmamembrane
PvMSL2	Phvul.002G147200	2094	697	9.34	76.8	Chloroplastmembrane
PvMSL3	Phvul.009G036900	2052	683	9.34	75.59	Chloroplastmembrane
PvMSL4	Phvul.009G037000	1896	631	9.41	70.34	Chloroplastmembrane
PvMSL5	Phvul.007G016600	2754	917	8.32	105.91	Plasmamembrane
PvMSL6	Phvul.009G081300	2295	764	8.95	86.86	Plasmamembrane
PvMSL7	Phvul.009G228800	2250	749	9.32	85.52	Plasmamembrane
PvMSL8	Phvul.009G228700	2232	743	7.59	85.19	Plasmamembrane
PvMSL9	Phvul.003G279800	2103	700	8.87	81.53	Plasmamembrane

PvMSL, *Phaseolus vulgaris* MscS like, ID Phytozome; CDS, coding sequences; bp, base pair; AA, amino acid; MW, molecular weight (KDa); pI, isoelectric point and subcellular localization.

resemble TM3 of MsSC. Similar motifs have been reported in bacterial MscS channel systems (Pivetti et al., 2003; Saddhe and Kumar, 2015). Proteins in Class II showed the presence of consensus sequence F(X3)P(X3)GD(X10–14)V(X20–21)PN(X7)IXNXXR at the C-terminal TM domain in *P. vulgaris* (*PvMSL5*, *PvMSL6*, *PvMSL7, PvMSL8* and *PvMSL*9) (Figure 1B). In Class II, C-terminal TM domain proteins are not glycine- or alanine rich; however, they contain amino acids with larger hydrophobic side chains. Large hydrophobic amino acids are conserved at certain positions within the domain. In agreement with a previous analysis by Pivetti et al. (2003), the conserved motif includes most C-terminal TM domain and surrounding sequence in *E. coli.* Generally, members of MscS family have good conservation throughout the TM3 helix (Pivetti et al., 2003; Miller et al., 2003; Saddhe and Kumar, 2015).

Protein properties and subcellular localizations of *PvMSL* proteins

The parameters used to describe *PvMSL* proteins are listed in Table 2 and included gene locus number, deduced protein length, molecular weight, isoelectric point and subcellular localization. The deduced length of *PvMSL* proteins ranged from a minimum of 540 amino acids (*PvMSL1*) to a maximum of 913 residues (*PvMSL5*), whereas PI ranged from 7.59 (*PvMSL8*) to 9.41 (*PvMSL1*). The molecular weight ranges from 58.79 to 105.91 KDa for *PvMSL1* and *PvMSL5,* respectively. In general, members of Class I showed smaller size amino acids and lower molecular weight, while Class II presented higher isoelectric point than Class I. Most *PvMSL* proteins share similar physical and chemical characteristics within the same class.

All putative *PvMSL* proteins were found in the membrane. Class I *PvMSL* genes (*PvMSL1-PvMSL4*) were localized either in the plasma membrane or chloroplast membrane, whereas Class II *PvMSL* genes (*PvMSL5 - PvMSL9*) were present only in the plasma membrane (Table 2). Consistently with studies in *A. thaliana*, *AtMSL2* and *AtMSL3* genes were localized in the plastid envelope, most likely in the inner membrane, and are observed in foci at the plastid poles (Haswell and Meyerowitz, 2006; Wilson et al., 2011). MSL genes of Class I were reported to be located either in the plastid envelop or mitochondria (Haswell, 2007; Saddhe and Kumer, 2015). Class II genes were found in the plasma membrane. Saddhe and Kumer (2015) showed that all Class II members of *O. sativa* (*OsMSL4 - OsMSL7*) are located in the plasma membrane. *AtMSL9* and *AtMSL10* genes in *A. thaliana* were found in the plasma membrane of root cells (Haswell et al., 2008). Immunofluorescence of algal *Chlamydomonas reinhardtii* similarly revealed a complex localization pattern for *MSC1* genes located in the chloroplast and cytoplasm (Nakayama et al., 2007).

The predicted topology of *PvMSL* genes were illustrated in Figure 2. In Class I, *PvMSL1* and *PvMSL3* proteins demonstrated the presence of five transmembrane (TM) regions, while *PvMSL2* and *PvMSL4* exhibited the presence of six and seven TM regions, respectively. In Class II, the number of TM regions ranged from six or seven. *PvMSL6, PvMSL7* and *PvMSL9* proteins exhibited the presence of six TM, while PvMSL5 and PvMSL8 showed the presence of seven TM regions. In *E. coli*, MscS channel topologies varied from 3 to 11 putative transmembrane segments (TMS) and adopted an N-terminal out and C-terminal in configuration (Miller et al., 2003; Pivetti et al., 2003). Indeed, the results identified in this study were similar to the ones in *Arabidopsis* and Rice Class I members which showed the presence of five TM domains while Class II members exhibited the presence of six TM domains (Haswell, 2007; Saddhe and Kumer, 2015).

Phylogenetic analyses and identification of conserved domains of *PvMSL* family

To clarify the phylogenetic relationships among MSL

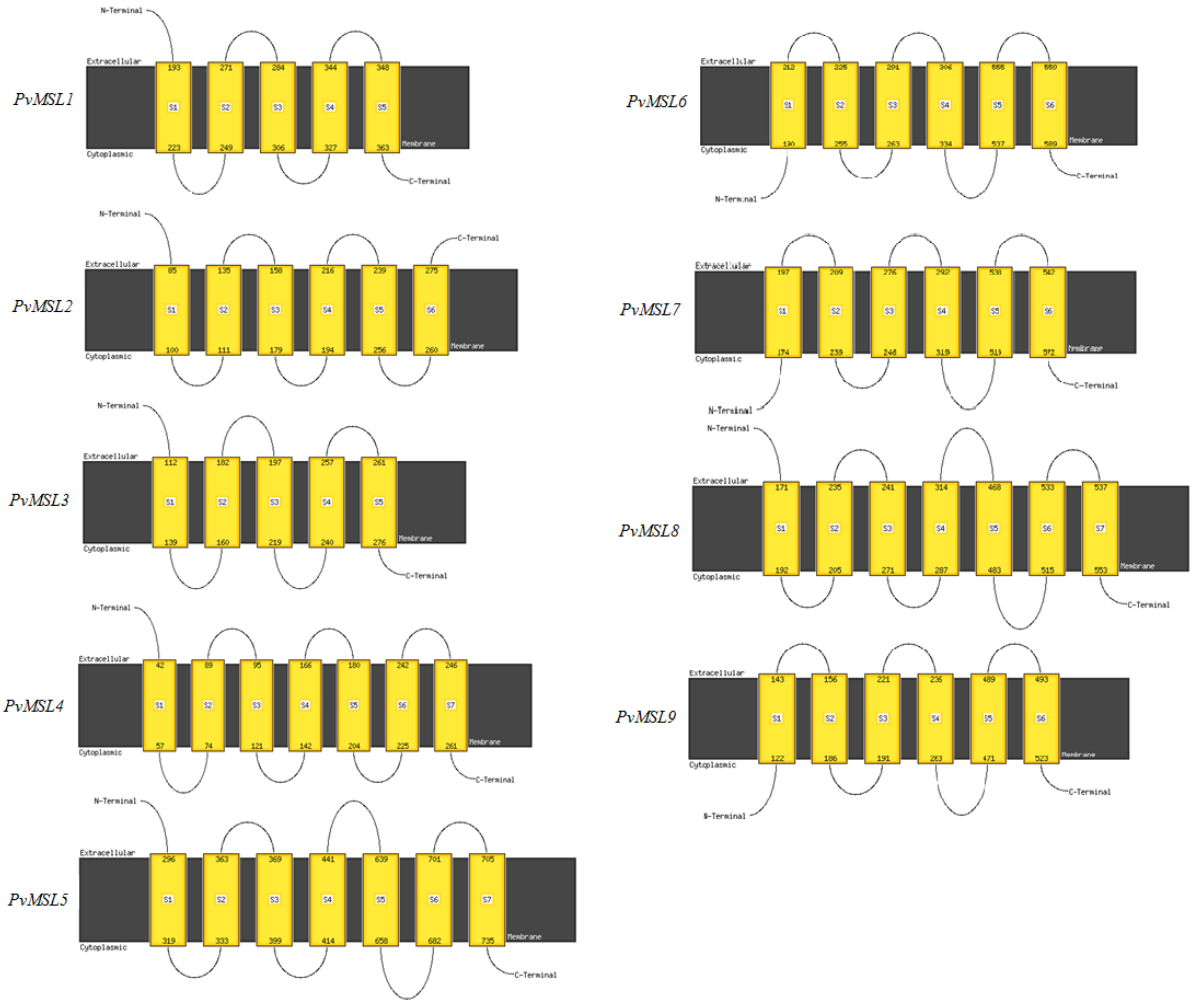

Figure 2. A cartoon of the transmembrane (TM) helix topology summarizing the linear coordinates for the helices and indicating the protein's extra- and intercellular regions. Transmembrane helix region represented by the yellow boxes labeled S1, S2, S3, S4, S5, S6 and S7. Membrane regions were represented by the black boxes. N and C terminal regions were labeled and represented as a single line.

gene family, a phylogenetic tree was constructed based on the alignment of *Arabidopsis*, rice and common bean of MSL proteins sequences (Figure 3). The Maximum-Likelihood (ML) phylogenetic tree divided MSL genes into two classes (Figures 3 and 4), supported by bootstrap values and the occurrence of conserved protein motifs (Figure 4A and B). In *Arabidopsis* and rice, two classes, I and II, have been classified (Haswell and Meyerowitz, 2006; Saddhe and Kumar, 2015) and the corresponding groups in common bean are labeled in Figures 3 and 4A. The Class I and II common bean contains four and five *PvMSL* genes, respectively, according to the classification in *Arabidopsis*. The phylogenetic analysis revealed that common bean MSL genes showed similar phylogenetic relationships with *Arabidopsis* and rice. Among these, Class II constituted the largest clade, containing 15 members and accounting for 60% of total MSL genes, while Class I contained 10 members and accounted for 40% of total MSL genes. Additionally, in rice, a monocotyledon, the number of members within the two classes was the same, while in dicotyledon, *Arabidopsis* and common bean, the number of members was larger, indicating that these genes could have originated from a common ancestor by frequent duplication gene after the split between mono and dicotyledon.

To further reveal the diversification of MSL genes in common bean, putative motifs were predicted using the program MEME, and 10 distinct motifs were identified (Figure 4B). The schematic distribution of 10 motifs among the two gene classes is shown in Figure 4B; these motifs are represented in their relative location within the protein. The identified multilevel consensus sequence for the motifs is shown in Table 3. The 10 motifs identified by MEME were annotated by InterProScan. The superfamily database of structural and functional protein annotations

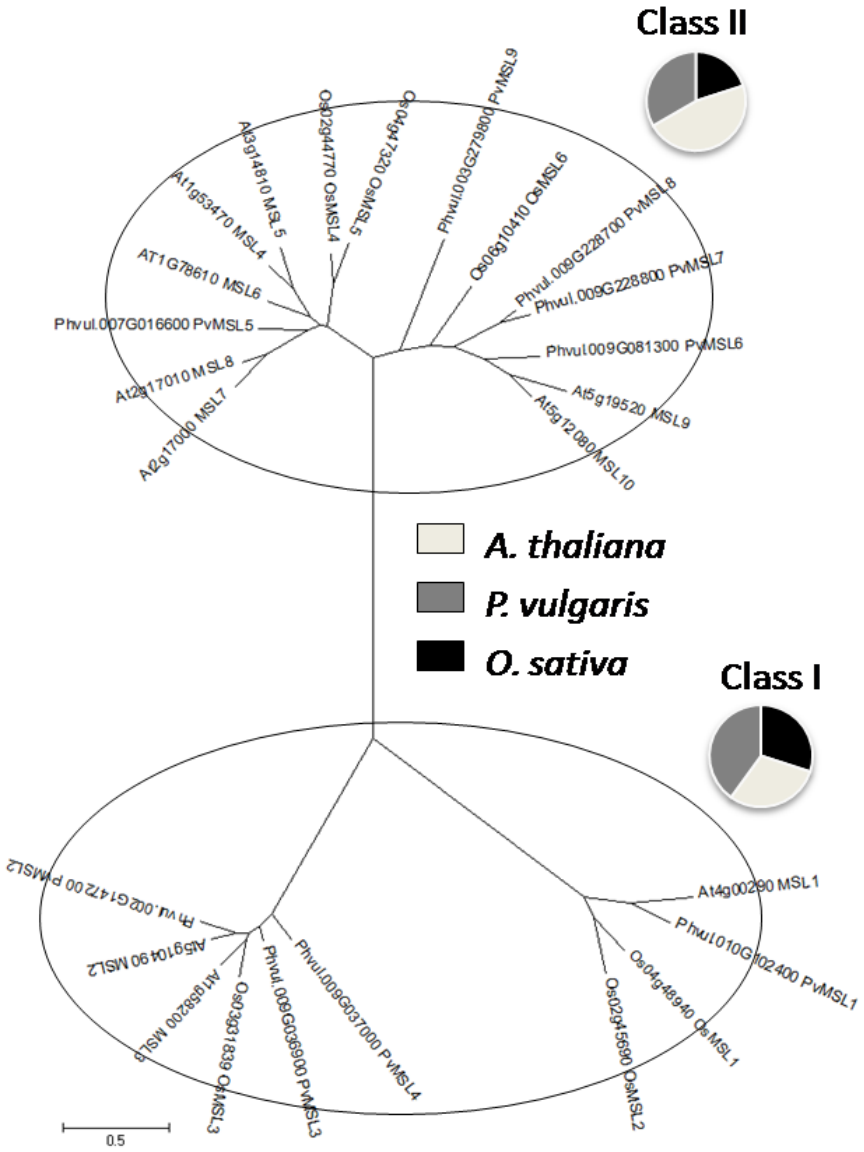

Figure 3. Phylogenetic tree of *A. thaliana, O. sativa,* and *P. vulgaris* MSL genes. The tree was based on 25 MSL sequences (including both N-terminal and C-terminal domains) from three species using the Maximum-Likelihood method.

showed that MS channel and like-Sm (LSM) domains were present in all common bean *PvMSL* protein sequences. The Pfam analysis showed that the MS channel domains were present in all *PvMSL* proteins. Protein information resource (PIR) database showed that common bean MSL, *PvMSL5* to *PvMSL9* have a unique MscS-like plant/fungus domain which is restricted to plant and fungus lineages. Motif 2 is a frequently occurring sequence and is present in most *PvMSL* genes, consisting of MS channel domain. As expected, most of the closely related members in the phylogenetic tree had common motif compositions, suggesting functional similarities among MSL proteins within the same class (Figure 4B). For example, motifs 2, 7 and 9 were specific to Class I, while motifs 3, 4, 5, 7, 8 and 10 were specific to Class II. Motifs3, 4 and 5 showed a casein kinase C phosphorylation site. All members of Class II(*PvMSL5* to *PvMSL9*) were located in plasma membrane, which might be regulated by phosphorylation during protein kinase C. These similarities in motif patterns might be related to similar functions of MSL proteins within the same class.

Structural analyses, genome distribution, duplications and synteny of *PvMSL* genes

It is well known that gene structural diversity is a possible mechanism for the evolution of multigene

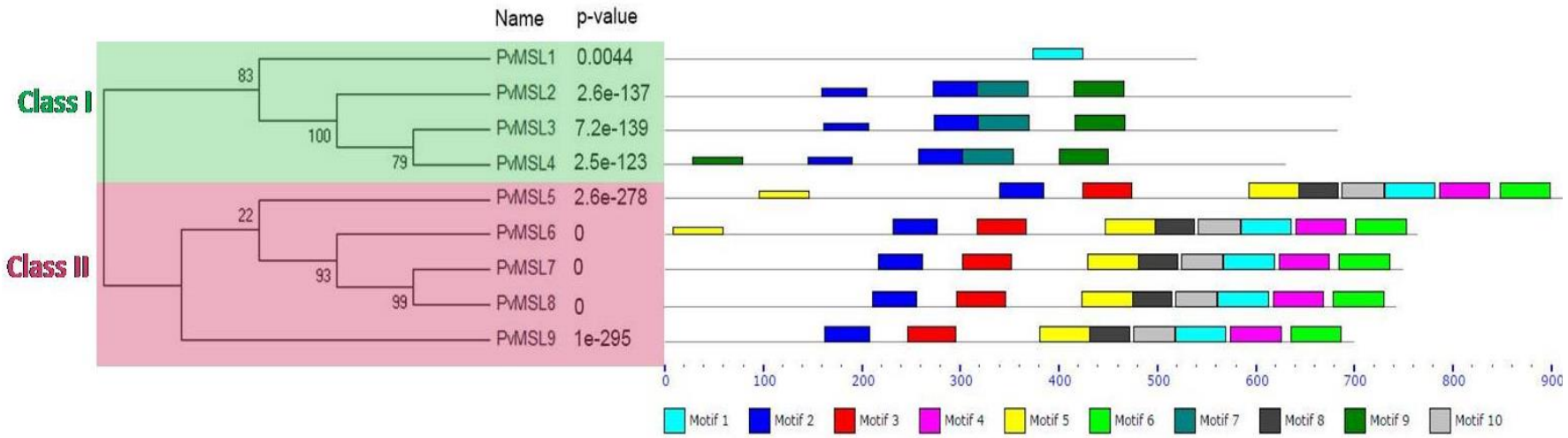

Figure 4. Relationships and schematic representation of motif compositions of 9 members of bean MSL family. Unrooted ML phylogenetic tree was based on nine *PvMSL* protein sequences using MEGA6.06. The motifs identified by MEME software are represented by colored boxes and their consensus sequences are shown in Table 3.

Table 3. Conserved motif compositions of *PvMSL* proteins.

ID[a]	Consensus sequences	E-values	Sites	Width
1	FEAIIFVFVMHPFDVGDRCVIDGVQMVVEEMNILTTVFLRYDNEKIYYPN	5.5e-118	5	50
2	VLVTLCGRLVTTWFINVIMFHIERNFLFNEWVQYFIYGYKKSGQ	9.4e-083	8	44
3	GAAIWLVKTLLIKMLASKFHVTTYFDRIQESIFHQYILQTLSGPPLME	8.1e-067	5	48
4	PISNYYRSPEMCDSVEFAVDVSTPIESIGALKHKIKWYCESKPQHWHPNH	1.8e-065	5	50
5	INSEWEAKAAAYRIFGNVAKPGCKYIEEDDLMRFMKNEEVHNVYPLFEGA	8.6e-069	5	50
6	KMKMAIYVTHTMNFQNYGEKNRRRSELVLELKKIFEELNIKYHLLPQEIH	4.9e-058	5	50
7	EHVGWWSPTIIRGDDREAVHIPNHKFTVNVVRNLSQKSHWRIKTHLAISH	4.6e-046	3	50
8	ETGRITRKSLKNWLIKVYYERRALAHSLNDTKTAVDQLN	2.7e-042	5	39
9	SCFVKTSHFEEYLCVKEAILLDLLRVISHHRARLATPIRTVQKIYSDTD	5.3e-042	3	49
10	IVIIVIIIVWLLIMEFATTKVLVFCSSQLVLVGFMFGNTCK	2.6e-036	5	41

[a]Means motif ID.

families. In order to gain further insight into the structural diversity of MSL genes, the exon/intron structure of each member of *PvMSL* family in common bean was analyzed. A detailed illustration of exon/intron structures is shown in Figure 5. According to their predicted structures, all *PvMSL* genes have introns in their structure and the number of exons varied from 4 to 12. These exon/intron

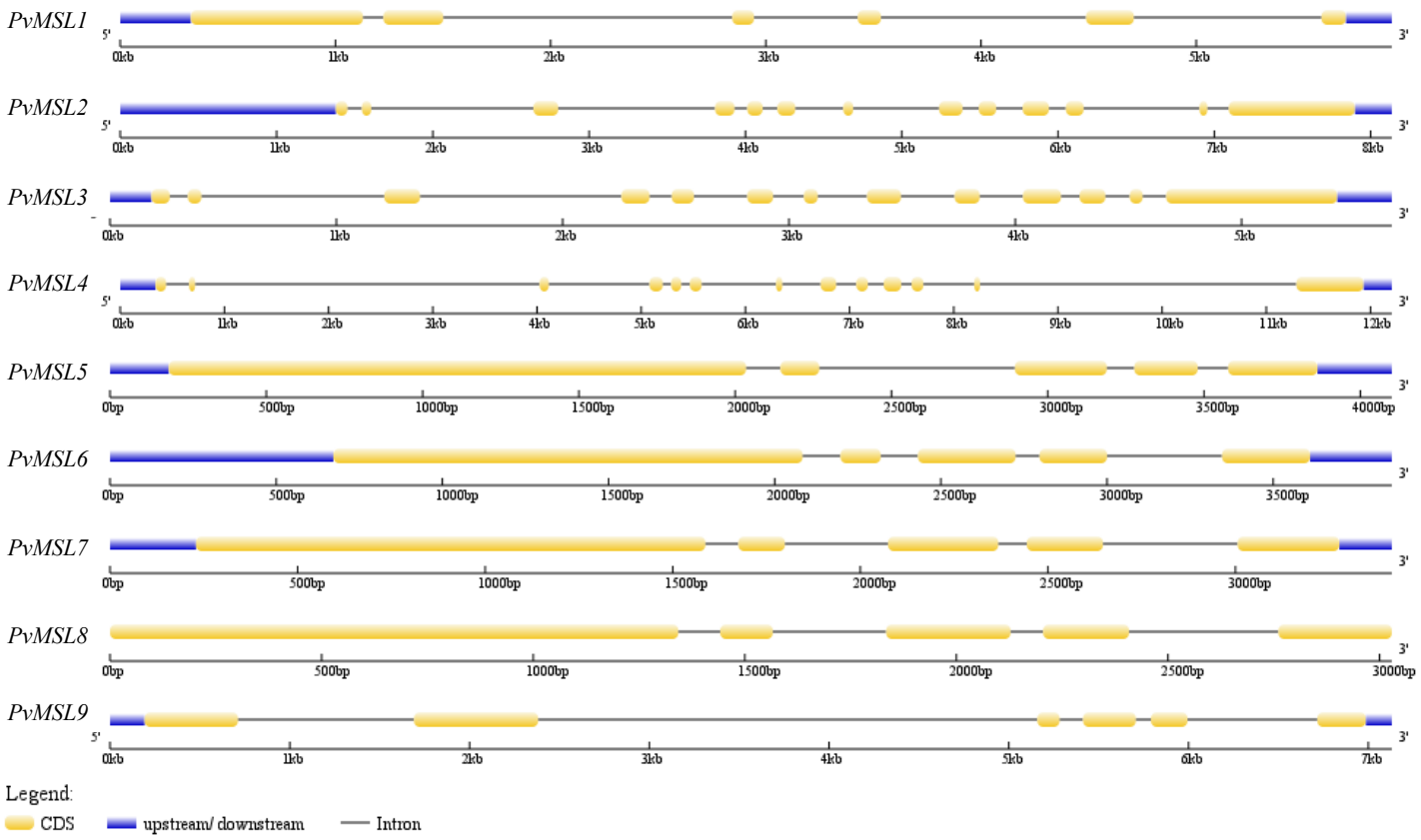

Figure 5. Gene structure schematic diagram for *PvMSL* genes. Exons were demonstrated by filled yellow boxes and introns were demonstrated by black lines. Untranslated region (UTR) was displayed by blue boxes at both ends.

structures are similar to rice (Saddhe and Kumer, 2015). The most closely-related members in the same class generally showed the same exon/intron pattern in which the number, position and length of the intron almost completely conserved within classes (Figure 5). For instance, the gene structure appeared to be more variable in *PvMSL* genes of Class I, where the number of introns ranged from 5 to 12, while the members of Class II contained only four or five introns. Most genes containing introns were clustered into the same class suggesting evolutionary conservation in *P. vulgaris.*

The chromosomal locations of *PvMSLs* were determined based on the information from Phytozome Database of *P. vulgaris.* Genome chromosomal location analyses revealed that *PvMSL* were randomly distributed in 5 out of 11 chromosomes (Figure 6). Among these chromosomes, chromosome 9 contained the largest number of MSL genes, while the other chromosomes contain only one MSL gene. *PvMSL2, PvMSL9*, *PvMSL5* and *PvMSL1* genes were localized in chromosomes 2, 3, 7 and 10, respectively, as shown in Figure 6. Five genes, *PvMSL3, PvMSL4*, *PvMSL6*, *PvMSL7* and *PvMSL8,* are located in chromosome 9. Substantial clustering of *PvMSL* genes was evident in chromosome 9, with high densities of the genes.

Tandem and segmental duplications have been suggested to be two of the main causes for gene family expansion in plants (Cannon et al., 2004). Two or more genes located in the same chromosome are confirmed as a tandem duplication event, while gene duplication between different chromosomes is designated as a segmental duplication event (Liu et al., 2011; Cai et al., 2013). Two pairs of MSL paralogues genes were identified in chromosomes 2, 3 and 9. Segmental duplications with high similarity (55%) were detected between *PvMSL2* in the terminal region of chromosome 2 and *PvMSL3* in the same region of chromosome 9.

PvMSL9 in chromosome 3 also showed a probable (50%) duplicated region shared with *PvMSL8* in chromosome 9, both of which are located in the lower arm of chromosomes. According to Holub (2001), tandem duplications are 200 kb regions in a chromosome that contains two or more genes with high similarity. Therefore, two MSL tandem duplication clusters were identified. Four genes (*PvMSL3, PvMSL4, PvMSL7*, and *PvMSL8*) were arranged in two clusters in 7.7 and 33.7 kb segments in chromosome 9, respectively (Figure 6). This strongly suggested that they might be generated by tandem duplications. The duplication prediction analysis indicated that the evolution of *PvMSL* gene family in

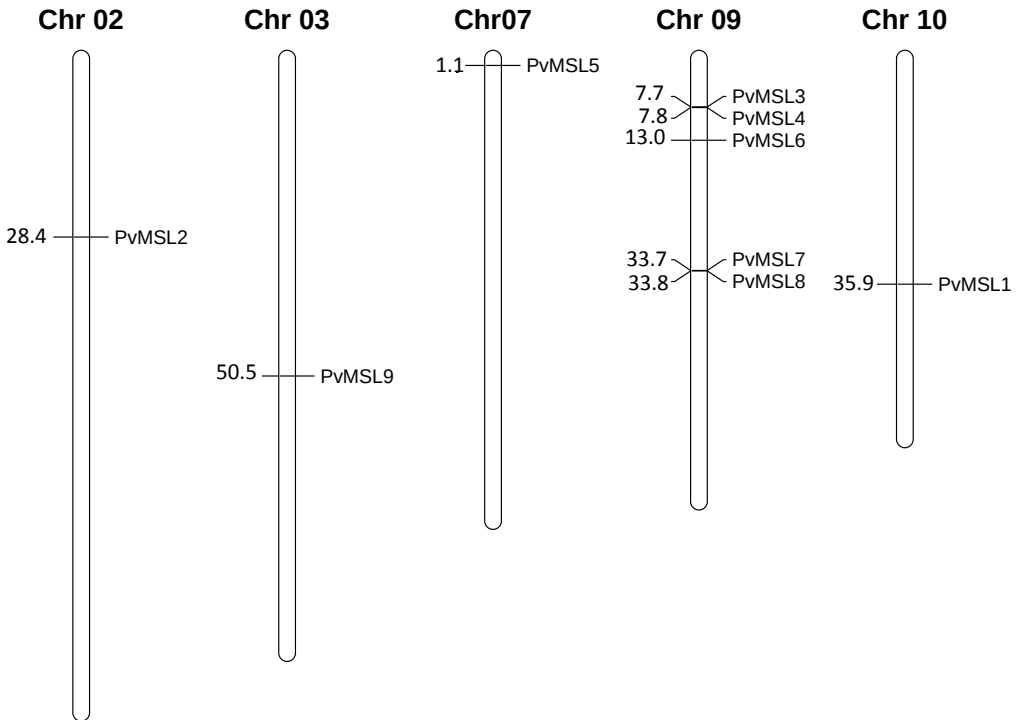

Figure 6. Chromosomal location of *PvMSL* genes. The chromosome number is indicated at the top of each linkage group. The chromosomal positions of putative *PvMSL* were mapped according to genome coordinates.

common bean genome resulted from a total of 4 gene duplications, 2 segmental and 2 tandem duplication events (Figure 6).

In our study, one comparative syntenic map of *P. vulgaris* associated with *A. thaliana* (Figure 7) was built. A total of three pairs of syntenic relations were identified, three *AtMSL* genes (*AtMSL1*, *AtMSL7* and *AtMSL10*) and three *PvMSL* genes (*PvMSL1*, *PvMSL5* and *PvMSL6*) were found to be associated with synteny events. These synteny events suggest that many MSL genes arose before the divergence of *Arabidopsis* and common bean lineages. Thus, this result may indicate that *PvMSL* genes in *P. vulgaris* share similar structure and function with *AtMSL* genes in *Arabidopsis*.

Expression profiles of *PvMSL* genes

It has been noted that MSL gene family exhibits differences among different organisms and different tissues to exert different physiological functions. Thus, gene expression patterns can provide important clues for gene function. To further analyze the tissue specificity of MSL gene family members, the expression profiles of the nine *PvMSL* genes were analyzed as shown in Figure 8. Most of MSL genes showed distinct tissue-specific expression patterns across the nine examined tissues. Some of them were constitutively expressed in almost all tissues and organs, and the expression levels were high, such as those of *PvMSL1*, *PvMSL2, PvMSL3* and *PvMSL6*. The expression levels of *PvMSL1*, *PvMSL2* and *PvMSL3* were relatively higher in leaves and roots, which indicates that they could play a role in the development of plant root and leaves. These expression patterns were similar to *AtMSL9* and *AtMSL10* in *Arabidopsis*, which are expressed at high levels in root and responded to mechanical and gravity stimuli (Haswell, 2007; Kimbrough et al., 2004; Haswell et al., 2008). Likewise, *AtMSL2* and *AtMSL3* were found in leaves and control plastid size, shape, and perhaps division during normal plant development by altering ion flux in response to changes in membrane tension (Haswell and Meyerowitz, 2006). In eukaryotic, MSL genes presented varied distribution and function, in addition to serving as a safety valve (Saddhe and Kumar, 2015). *PvMSL6* and *PvMSL9* were relatively higher expression in nodules and young trifoliates, respectively. *PvMSL4*, *PvMSL7* and *PvMSL8*showed very low expression in almost all tissues and organs of common bean. Detailed analysis of the expression patterns of *PvMSL* showed that some of the genes clustered in the same class of the phylogenetic tree (Figure 4) had similar expression patterns, also indicating the existence of redundancy among *MSL* genes in this class. For example, most *PvMSLs* in Class I were mainly expressed in leaves and roots while all genes in Class II were little expressed in different tissues

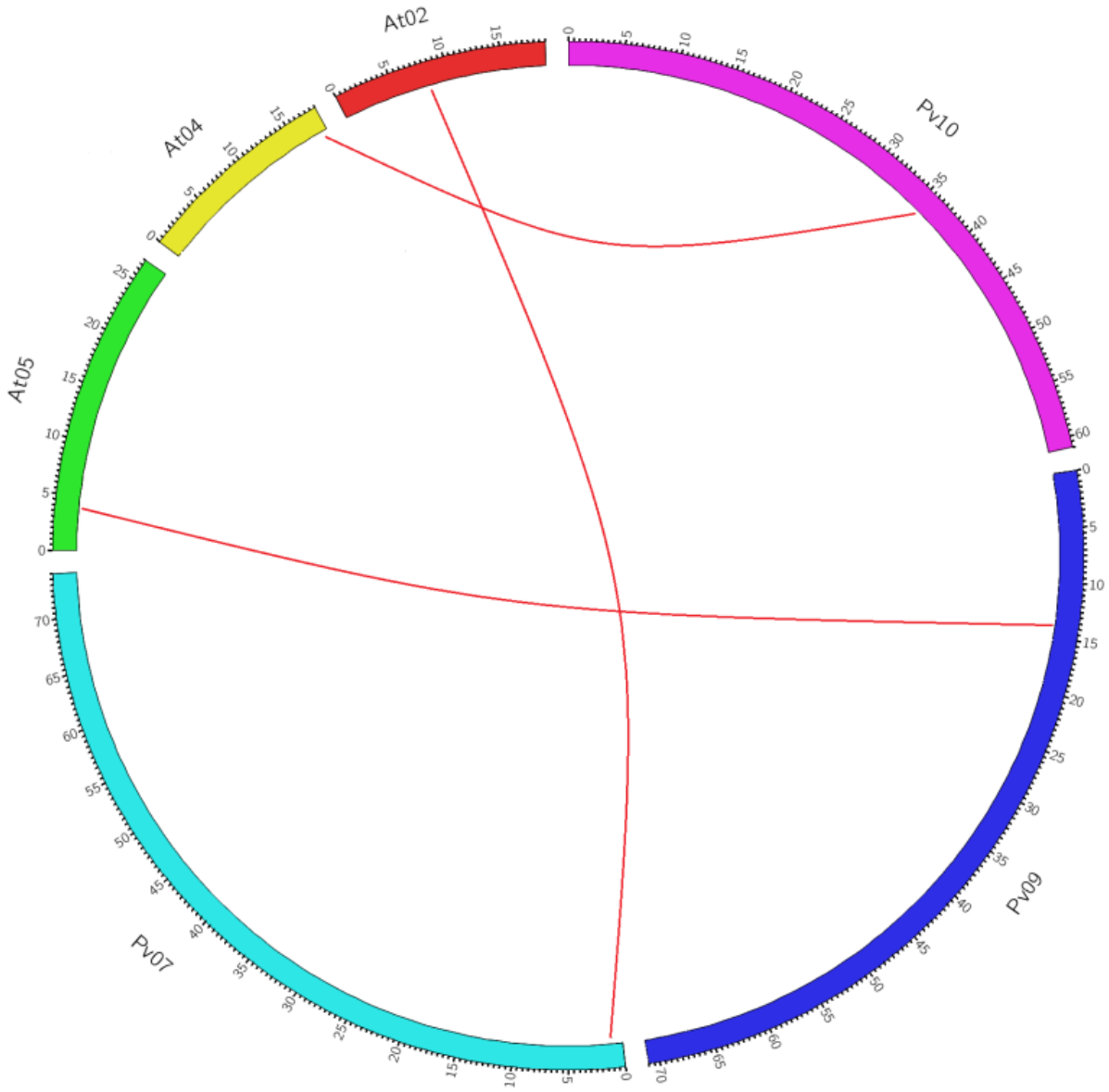

Figure 7. Synteny map showing the orthologous gene positions of MSL genes between *Phaseolus vulgaris* and *Arabidopsis*. *P. vulgaris* (Pv07, Pv09 and Pv10) and *Arabidopsis* chromosomes (At02, At04 and At05) maps were based on the orthologous and demonstrate highly conserved synteny. Each block represents individual chromosome and red lines connecting two chromosomal regions denote syntenic regions of genomes.

and organs of common bean. In addition, paralogous *PvMSL* genes, such as *PvMSL2-PvMSL3* and *PvMSL8-PvMSL9*, revealed distinct expression patterns. These expression profiles suggest a divergence in the biological functions of *PvMSL* genes during plant development.

Conclusion

Based on *in silico* approach, this is the first study that provides some information about MSL family in *P. vulgaris* and has a crucial role in the plant growth and development.

Understanding the genetic bases of MS ion channels provide resources to select candidate genes for future functional analyses of *PvMSL* family in common bean, increasing the possibility to genetically engineer new traits of importance for agriculture and food production.

Conflict of Interests

The authors have not declared any conflict of interests.

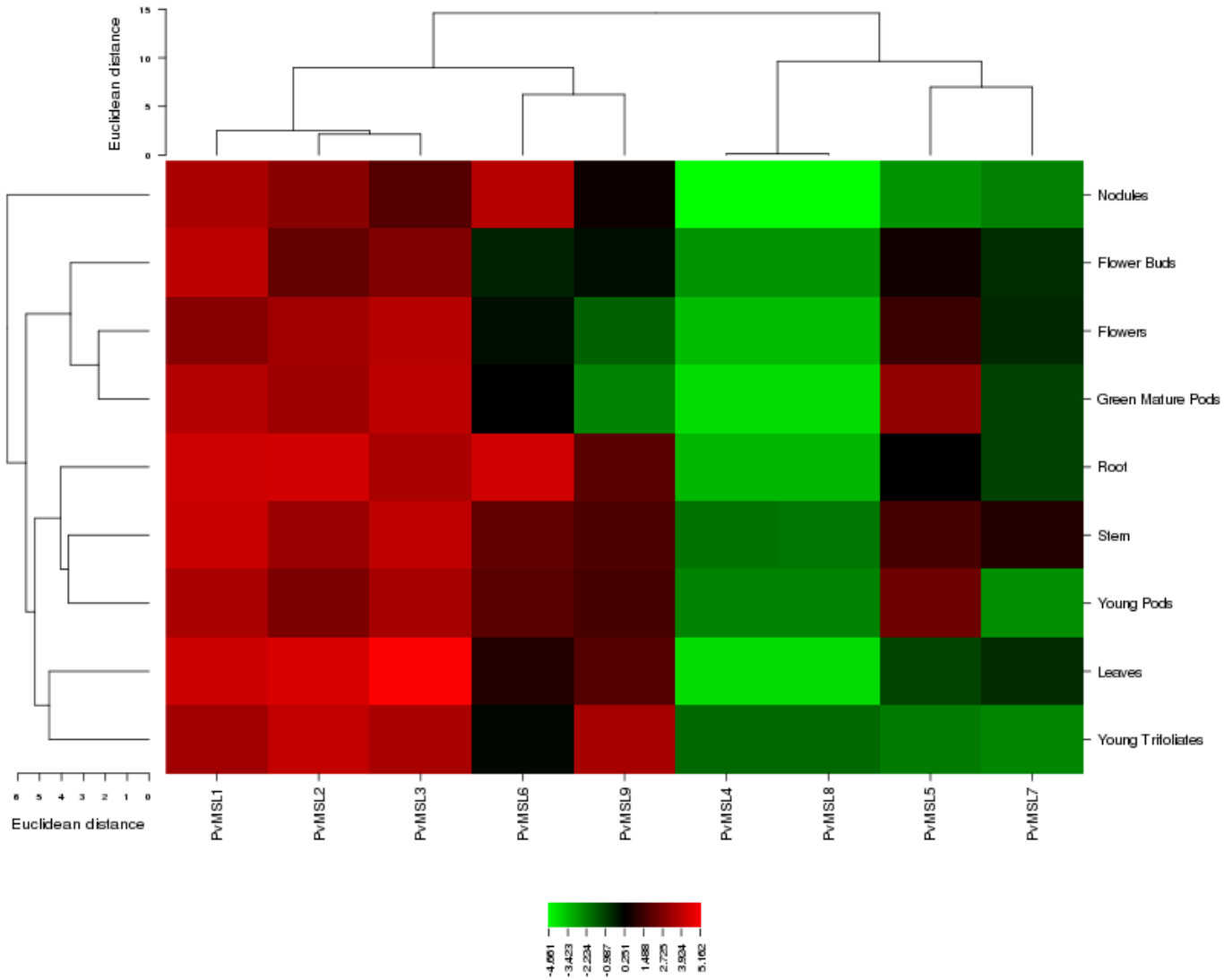

Figure 8. Differential expression patterns of common bean *PvMSL* genes. Heat map showing differential expression profile of common bean *PvMSL* genes inspecifictissues: leaves, roots, nodule, young trifoliates, flower buds, stem, flowers, green mature pods and young pods.

ACKNOWLEDGEMENTS

The authors thank UNIPAR for the financial support. TMI and TC thank CAPES for the fellowship.

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