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Research article

Genome-wide comparison and identification of myosin gene family in *Arabidopsis thaliana* and *Helianthus annuus*

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ABSTRACT

Myosins are essential components of organelle trafficking in all the eukaryotic cells. Myosin driven movement plays a vital role in the development of pollen tubes, root hairs and root tips of flowering plants. The present research characterized the *myosin* genes in *Arabidopsis thaliana* and *Helianthus annuus* by using different computational tools. We discovered a total of 50 *myosin* genes and their splice variants in both pant species. Phylogenetic analysis indicated that myosin genes were divided into four subclasses. Chromosomal location revealed that *myosin* genes were located on all five chromosomes in *A. thaliana*, whereas they were present on nine chromosomes in *H. annuus*. Conserved motifs showed that conserved regions were closely similar within subgroups. Gene structure analysis showed that *Atmyosin2.2* and *Atmyosin2.*3 had the highest number of introns/exons. Gene ontology analysis indicated that *myosin* genes were involved in vesicle transport along actin filament and cytoskeleton trafficking. Expression analysis showed that expression of myosin genes was higher during the flowering stage as compared to the seedling and budding stages. Tissue specific expression indicated that *HanMYOSIN11.2, HanMYOSIN16.2* were highly expressed in stamen, whereas *HanMYOSIN 2.2, HanMYOSIN 12.1* and *HanMYOSIN 17.1* showed higher expression in nectary. This study enhance our understanding the function of myosins in plant development, and forms the basis for future research about the comparative genomics of plant myosin in other crop plants.

1. Introduction

The movement of organelles and other cellular components within a cell is termed as cytoplasmic streaming (Wang et al., 2022). Cytoplasmic streaming assists the plants in nutrient delivery, chloroplast movement, metabolites, and other materials throughout the cell (Nebenführ, 2020; Nebenführ and Dixit, 2018a). Various biological processes of plants, such as pollen tubes, cell growth, and root hair development, are associated with cytoplasmic streaming. Organelles movement along the cellular components depends on actin filaments or microtubules (Ouyang et al.,

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Table 1. Physio chemical properties of *A. thaliana* myosin proteins.

Transcript ID	Common Name	Chr.	Strand	Protein length	pI	M.W.
AT3G19960.1	AtMYOSIN1.1	3	Forward	1166	8.16	131090.98
AT3G19960.2	AtMYOSIN1.2	3	Forward	1176	8.41	132454.48
AT5G43900.1	AtMYOSIN2.1	5	Reverse	1505	7.69	169930.72
AT5G43900.2	AtMYOSIN2.2	5	Reverse	1562	7.93	176522.26
AT5G43900.3	AtMYOSIN2.3	5	Reverse	1565	7.87	176863.67
AT1G17580.1	AtMYOSIN5.1	1	Forward	1520	8.39	172907.86
AT1G04600.1	AtMYOSIN7.1	1	Forward	1730	5.45	194815.33
AT1G04160.1	AtMYOSIN8.1	1	Forward	1500	7.91	169380.28
AT1G08730.1	AtMYOSIN9.1	1	Forward	1538	9.39	174625.59
AT1G54560.1	AtMYOSIN11.1	1	Reverse	1529	9.36	173610.11
AT2G31900.1	AtMYOSIN12.1	2	Reverse	1556	8.36	176970.04
AT2G31900.2	AtMYOSIN12.2	2	Reverse	1557	8.26	177,098.21
AT2G31900.3	AtMYOSIN12.3	2	Reverse	1299	8.47	148580.84
AT2G31900.4	AtMYOSIN12.4	2	Reverse	1556	8.2	176970.04
AT2G31900.5	AtMYOSIN12.5	2	Reverse	1442	8.57	164557.9
AT2G20290.1	AtMYOSIN13.1	2	Reverse	1493	7.42	169072.88
AT4G28710.1	AtMYOSIN14.1	4	Forward	1516	7.46	172154.64
AT4G33200.1	AtMYOSIN15.1	4	Reverse	1522	8.89	173362.81
AT4G33200.2	AtMYOSIN15.2	4	Reverse	1492	8.8	169,942.95
AT4G33200.3	AtMYOSIN15.3	4	Reverse	1503	8.83	171102.13
AT5G54280.1	AtMYOSIN16.1	5	Forward	1030	8.83	117255.79
AT5G54280.2	AtMYOSIN16.2	5	Forward	1220	8.86	138561.82
AT5G20490.1	AtMYOSIN17.1	5	Reverse	1530	8.4	173245.97
AT5G20490.2	AtMYOSIN17.2	5	Reverse	1531	8.4	173377.16

Chr., chromosome; M.W, molecular weight pI, isoelectric point.

Table 2. Physio chemical properties of *H. annuus* myosin proteins.

Transcript ID	Common Name	Chr.	Strand	Protein length	PI	M.W
HannXRQ_Chr16g0526111	HanMYOSIN1.1	13	Forward	1104	7.56	130875
HannXRQ_Chr13g0407311	HanMYOSIN2.1	13	Reverse	1104	8.45	124476
HannXRQ_Chr08g0234651	HanMYOSIN2.2	8	Forward	900	6.66	101268
HannXRQ_Chr08g0234661	HanMYOSIN2.3	8	Forward	1163	8.66	131606
HannXRQ_Chr12g0366221	HanMYOSIN2.4	12	Reverse	1106	8.48	125302
HannXRQ_Chr05g0139901	HanMYOSIN5.1	5	Forward	1523	9.3	173685
HannXRQ_Chr13g0403781	HanMYOSIN6 .1	14	Forward	1474	8.34	168913
HannXRQ_Chr01g0014821	HanMYOSIN6 .2	1	Reverse	1502	8.20	170036
HannXRQ_Chr11g0342761	HanMYOSIN7.1	11	Reverse	1636	5.7	186421
HannXRQ_Chr11g0336841	HanMYOSIN9.1	11	Forward	472	6.19	53013.6
HannXRQ_Chr11g0339751	HanMYOSIN9.2	11	Forward	466	6.3	52374
HannXRQ_Chr04g0113721	HanMYOSIN9.3	4	Forward	1530	9.04	173687
HannXRQ_Chr11g0321791	HanMYOSIN11.1	11	Reverse	1526	9.11	173596
HannXRQ_Chr13g0404551	HanMYOSIN11.2	13	Forward	305	9.28	34918.6
HannXRQ_Chr11g0339771	HanMYOSIN11.3	11	Forward	1378	9.01	158527
HannXRQ_Chr08g0217201	HanMYOSIN11.4	8	Forward	1626	9.21	185002
HannXRQ_Chr07g0204511	HanMYOSIN11.5	7	Reverse	1523	9.19	173685
HannXRQ_Chr08g0217201	HanMYOSIN11.6	8	Reverse	1578	9.21	185002
HannXRQ_Chr04g0117591	HanMYOSIN12.1	4	Forward	1563	7.09	178440
HannXRQ_Chr07g0185821	HanMYOSIN14.1	7	Forward	1434	6.36	162826
HannXRQ_Chr08g0207711	HanMYOSIN14.2	8	Forward	205	10.27	23533.8
HannXRQ_Chr14g0434251	HanMYOSIN14.3	13	Reverse	167	5.76	18314
HannXRQ_Chr09g0261841	HanMYOSIN15.1	9	Forward	1523	9.09	172946
HanXRQ_Chr11g0336851	HanMYOSIN16.1	11	Forward	180	9.47	20773.9
HanXRQ_Chr09g0237941	HanMYOSIN17.1	9	Forward	1531	8.55	173685

Chr., chromosome; M.W, molecular weight pI, isoelectric point.

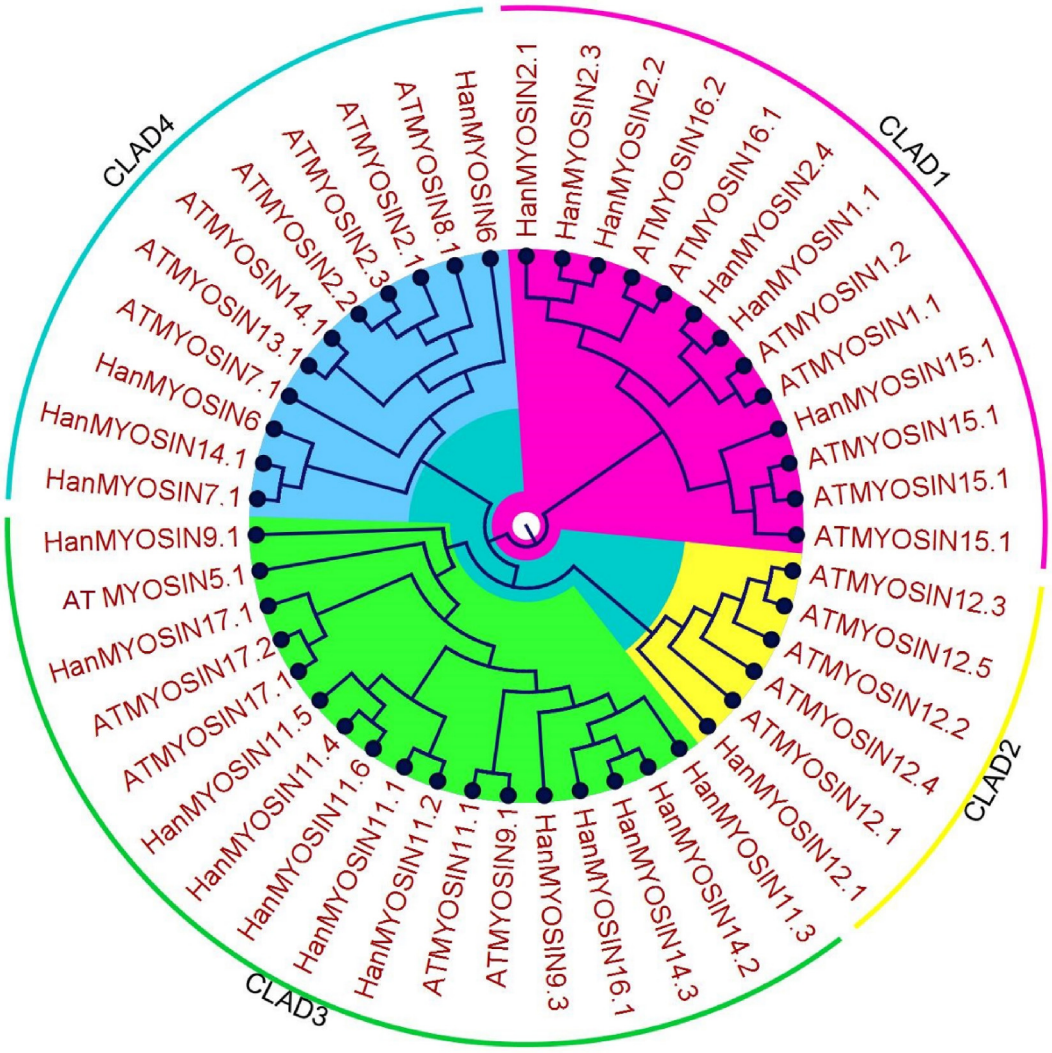

Figure 1. The phylogenetic relationship among *A. thaliana* and *H. annuus* myosin proteins constructed using CLC Sequence Viewer 8.0. The four clads (CLAD1 to CLAD4) were indicated by different colors.

2021). Motor proteins help to move the organelles by binding with specific lipids in the cell membrane (Nebenführ, 2020). In plants, molecular motor proteins comprise three major families: kinesins, dynein, and myosin (Nebenführ and Dixit, 2018a). The first two families play a role as motors on microtubule filaments, while myosin works on actin filaments (Nebenführ and Dixit, 2018a). Plants possess two unique myosin groups, i.e., myosin proteins of class VIII and XI (Ma et al., 2020). Myosin proteins of class VIII are known as slow motor proteins, whereas myosin proteins of XI are the fastest motor proteins. According to their velocity, plant myosin can be categorized into three groups: low-, medium-, and high-velocity groups (Haraguchi et al., 2016, 2018a). In *Arabidopsis*, 13 genes of *myosin XI* (*XI A*, *XI B*, *XI C*, *XI D*, *XI E*, *XI F*, *XI G*, *XI H*, *XI I*, *XI J*, *XI K*, *XI 1*, and *XI 2*) have been identified. Low-velocity group is comprised of only one myosin member, i.e. *XI I*, whereas there are six myosin genes each in the medium – (*XI B*, *XI H*, *XI J*, *XI K*, *XI 1*, *XI 2*) and high- (*XI A*, *XI C*, *XI E*, *XI F* and *XI G*) velocity groups. Further, it has been observed that myosin groups categorized on velocity bases perform specific functions, *i.e.* low-velocity myosin XI I is known as the nuclear membrane. Medium velocity group members generate motive forces for cytoplasmic streaming. High-velocity group myosin proteins were called pollen-specific (Haraguchi et al., 2018a). Myosin proteins in plants are divided into two main classes, Myosin XI and Myosin V, which act as molecular motors to move fluids across the filaments of actin (Haraguchi et al., 2018a). Myosin proteins control the organization of actin, fluidic dynamics and play a key role in the locomotion of vesicles and organelles (Haraguchi et al., 2016).

Eukaryotes have 35 different types of *myosin* genes. However, not all of these genes have been discovered in a single organism. Myosin performs various functions in plants; for example, myosin *XI B* controls pollen tube development in *Oryza sativa* (Jiang et al., 2007). Myosin *XI F* strengthens plant organs through tension generation to make them straight (Ueda et al., 2015). Myosin *XI I* is associated with nuclear shape and movement and is called a nucleocytoplasmic linker (Ueda et al., 2015). Another study proved that Myosin *XI I* generates the tension and regulates organelle velocity; however, it doesn't participate in cytoplasmic streaming (Haraguchi et al., 2016). Myosin *XI K* is involved in remodeling and movement of endoplasmic reticulum (Sparkes, 2010). However, the class *XI* Myosin functional analysis has largely been hindered due to the redundancy of different paralogous genes. Recently, plant myosin have been involved in sugar-induced hypocotyl elongation under dark conditions (Olatunji and Kelley, 2020).

Sunflower (*Helianthus annuus*) is the world's fourth most important oilseed crop, flowing the palm, soybean and rapeseed (Ahmad et al., 2021; Li et al., 2021). Increasing demand for sunflower cooking oil has further boosted the growing trend of this crop (Ahmad et al., 2020). Recent sequencing and release of the *H. annuus* genome have opened new horizons to characterize this crop's various traits (Badouin et al., 2017). Improved breeding for cytoplasmic streaming and characterization of

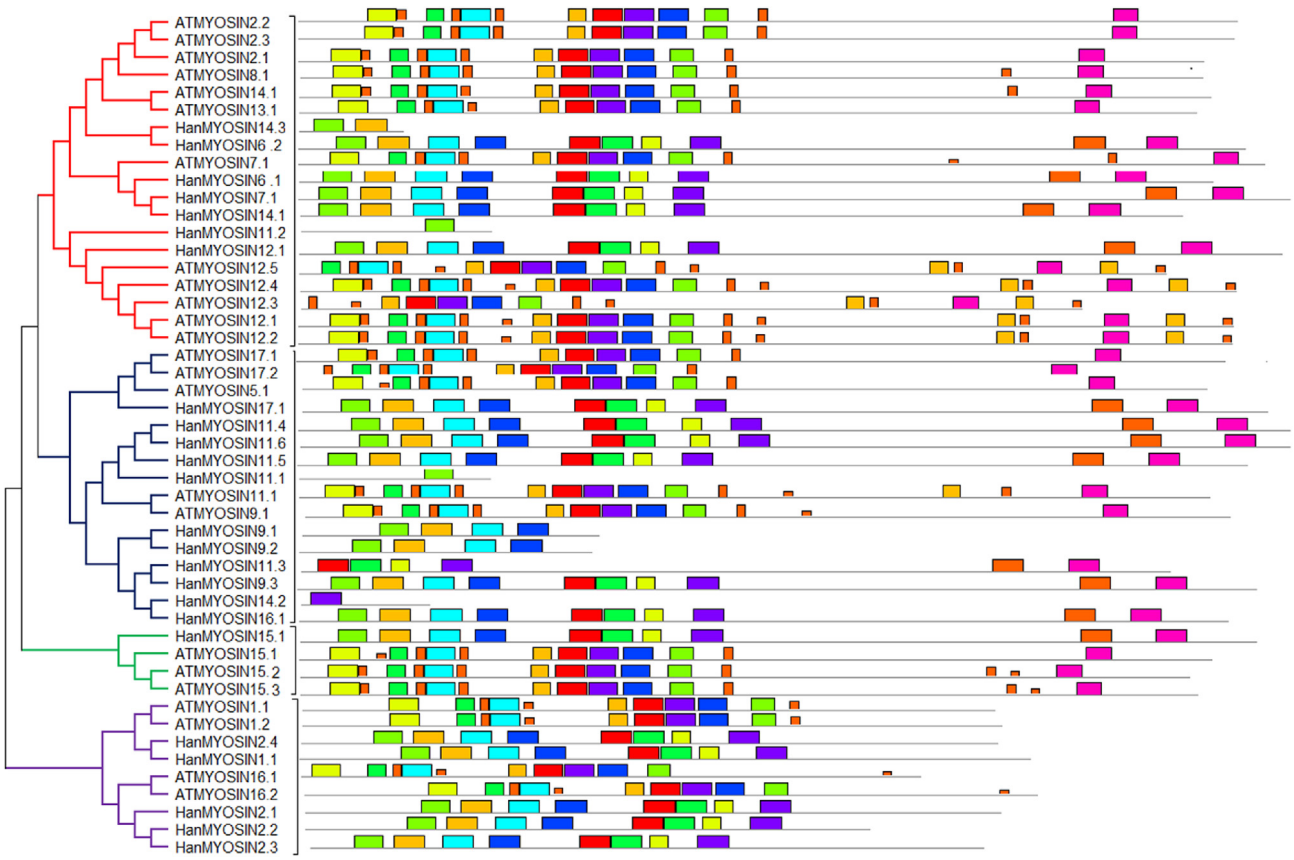

Figure 2. Conserved motif analysis of *A. thaliana* and *H. annuus* myosins. Ten distinct motifs were discovered, and each motif was distinguished with different color.

molecular motor proteins may be useful to enhance sunflower production to meet the cooking oil demand of the globe. Myosin proteins have been identified and characterized in *Arabidopsis thaliana*, *Gossypium hirsutism, Zea maize*, and *Glycine max* (Ma et al., 2020; Olatunji and Kelley, 2020; Wang et al., 2013). Since the release of *H. annuus* genome, the *myosin* gene family has not yet characterized in this specie. This research disparity provoked us to comprehensively explore the myosin family in *H. annuus* by identifying and comparing protein properties, phylogenetic analysis, intron and exon organization, cis-acting elements, chromosomal distribution, and gene duplication. This study also aimed to discover the expression of *myosin* genes in various subcellular components in *H. annuus*.

2. Materials and methods

2.1. Retrieval of protein sequences and characteristics of myosin protein properties

Sequences of myosin proteins of *A. thaliana* and *H. annuus* species were downloaded from Phytozome V.13 (https://phytozome-next.jgi.doe.gov) database. Motif search tool (https://www.genome.jp/tools/motif/) was used to confirm the myosin head domains (PF00063), myosin N-terminal SH3-like domains (PF02736) and IQ calmodulin-binding motifs (PF00612) in these proteins. An Online webtool ProtPram (https://web.expasy.org/protparam/) and plant ensemble (https://plants.ensembl.org/) was used to determine the, gene start and endpoint, DNA strand, number of exons, genomic length, transcriptional length (a.a), molecular weight (M.W) and isoelectric point (P.I) of myosins in *H. annuus* and *A. thaliana* species.

2.2. Sequence alignment, phylogenetic analysis

Fifty full-length amino acid sequences of *A. thaliana* and *H. annuus* myosin proteins were aligned by ClustalX (Thompson et al., 1997). These aligned sequences were used to construct the un-rooted phylogenetic tree at 1000 bootstrap values using CLC sequence viewer software flowing the neighbor-joining methods (Saitou and Nei, 1987).

2.3. Discovery of conserved motifs and gene structure

An online tool MEME suite (https://meme-suite.org/meme/index.html) was used to determine the conserved regions of myosin proteins in *A. thaliana* and *H. annuus* according to the method described by (Bailey et al., 2009). Using the default parameters, maximum number of motifs and maximum sites per motif were fixed 10 and 5 respectively whereas motif width was in rang of 5–50 amino acids. Gene structure display server (GDS 2.0) (http://gsds.gao-lab.org./) was used to display the intron and exon organization of *myosin* genes, using genomic and CDS sequences as input files, and the intron and exon organization was illustrated to the corresponding branch of the tree.

2.4. Chromosomal mapping and gene ontology (GO)

To determine the position of myosin genes on *A. thaliana* and *H. annuus* chromosomes, we used MapGene2Chrom web service (http://mg2c.iask.in/mg2c_v2.0/) using the gene start point, gene endpoint, chromosomal length as input files.

To explore myosin proteins' biological functions and molecular and cellular components, we used SHINAYGO VO.741 (http://bioinformat

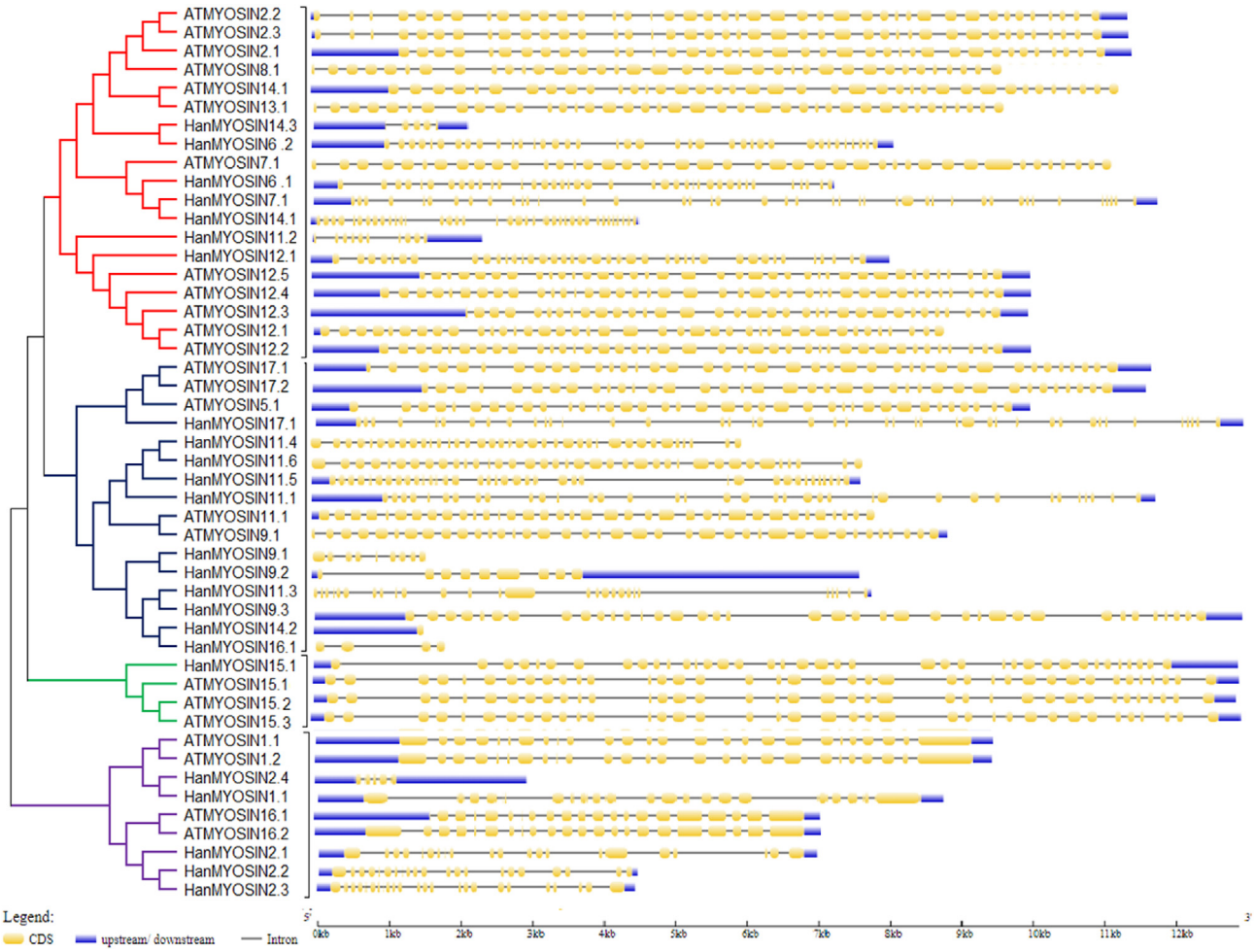

Figure 3. Intron exon organization pattern of *A. thaliana* proteins. The pattern was organized by using GSDS 2.0. Yellow lines show the complementary sequences, whereas black lines represent introns.

ics.sdstate.edu/go/) program with their default parameters and data was presented in a graphical format showing the percentage value of each function/component.

2.5. Synteny and gene duplication analysis

Comparative synteny analysis was carried out using the Circoletto Tool (http://tools.bat.infspire.org/circoletto/) with the goal of visualizing genome conservation among *A. thaliana* and *H. annuus* myosin genes. The coding sequences of duplicated genes were structured in MEGA7 using the Muscle (codon) method. The KaKs Calculator 2.0 tool was used to calculate the synonymous and nonsynonymous substitution rates, with the MYN approach. Furthermore, the divergence time 't = Ks/2λ, where $\lambda = 1.5 \times 10^{-8}$ for dicots.

2.6. Prediction of subcellular location and expression of myosin genes

We used an online tool WoLFSPORT (https://wolfpsort.hgc.jp) to assess the positions of *myosin* genes in various subcellular components of *A. thaliana* and *H. annuus* and a heat map was generated by using TBtool. The expression of *myosin XI* genes in different plant tissues of *A. thaliana* and *H. annuus* at the different growth stages and tissues were predicted using GENEVESTIGATOR V3 (https://genevestigator.com) tool, and results were represented in a heat map.

3. Results

3.1. Physio-chemical properties of A. thaliana and H. annuus myosin proteins

Physio-chemical properties of *A. thaliana* and *H. annuus* myosin proteins are shown in Tables 1 and 2, respectively. Protein AtMYOSIN7.1 showed the highest amino acid length (1730), while AtMYOSIN16.1 exhibited the minimum (1030) number of amino acids. Each protein has an isoelectric point (pI) fixed value at which it will move in an electrical field. The pI value indicates that which protein is basic or acidic in nature. The results in Table 1 showed that almost all myosin proteins are basic in nature having pI value in range of 7.42–9.39 except AtMYOSIN7.1 which is basic possessing pI value 5.45.

3.2. Sequence alignment and phylogenetic tree

Phylogenetic tree of *A. thaliana* and *H. annuus* myosin proteins indicated that myosin proteins were subdivided into 4 clads (Figure 1). The 1st clad (CLAD1) was comprised of 13 proteins. The 2nd clad consisted of six members belonging to myosin12 proteins from both plant species. The 3rd clad was the largest subclass having seventeen myosin members. The 4th clad was possessing 11 myosin members.

By submitting full-length amino acid sequences to MEME Tool, conserved motifs of myosin proteins were defined. Different colors represented each preserved motif. Ten conserved motif sequences were

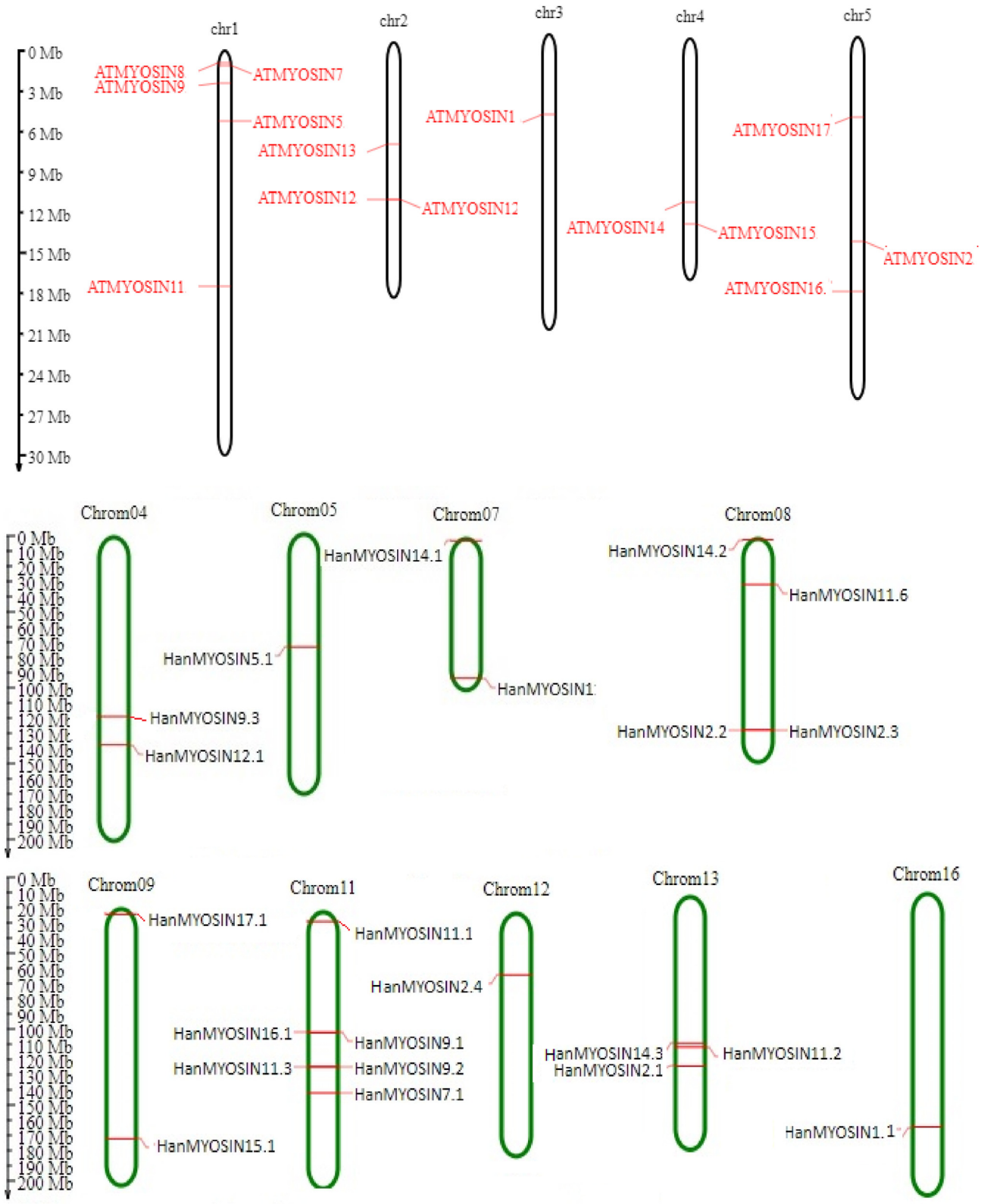

Figure 4. (a) Location of myosin genes on *A. thaliana* chromosomes predicated by TAIR. (b), Location of myosin genes on *H. annuus* chromosomes predicted by MapGene2Chrom web service.

identified in these proteins (Figure 2). It was observed that pattern of conserved motif within sub clads of phylogenetic tree was conserved. AtMYOSIN12.4 showed the maximum number of motifs conserved, while myosin HanMYOSIN11.2 possessed only one conserved motif. Previous findings on preserved motifs have confirmed that proteins falling in the same clad showed similar conserved region patterns. Proteins that share identical motifs within a subgroup are probably likely to have similar roles, which may help predict these proteins' function.

The exon and intron pattern of myosin proteins was created in GSDS.2 using CDS and genomic sequences as input files. These genes were

Figure 5. (a) Biological process, (b) Molecular function, (c) Cellular components: ShinyGO v0.741: Gene Ontology Enrichment Analysis was performed to predict the gene ontology of *A. thaliana* and *H. annuus* myosins.

further depicted with their corresponding genes in the phylogenetic tree. CDS indicates red dots, blue upstream/downstream lines, and black line introns. Difference in the exon-intron structure and pattern between *A. thaliana* and *H. annuus* myosin genes was identified (Figure 3). The highest number of introns and exons were counted in *AtMYOSIN7.1, AtMYOSIN9.1.* The number of introns and exons ranged from 25 to 37. The number of exons in rice ranged from 38 to 52, indicating that the number of exons of these motor proteins in *A. thaliana* and *H. annuus* are

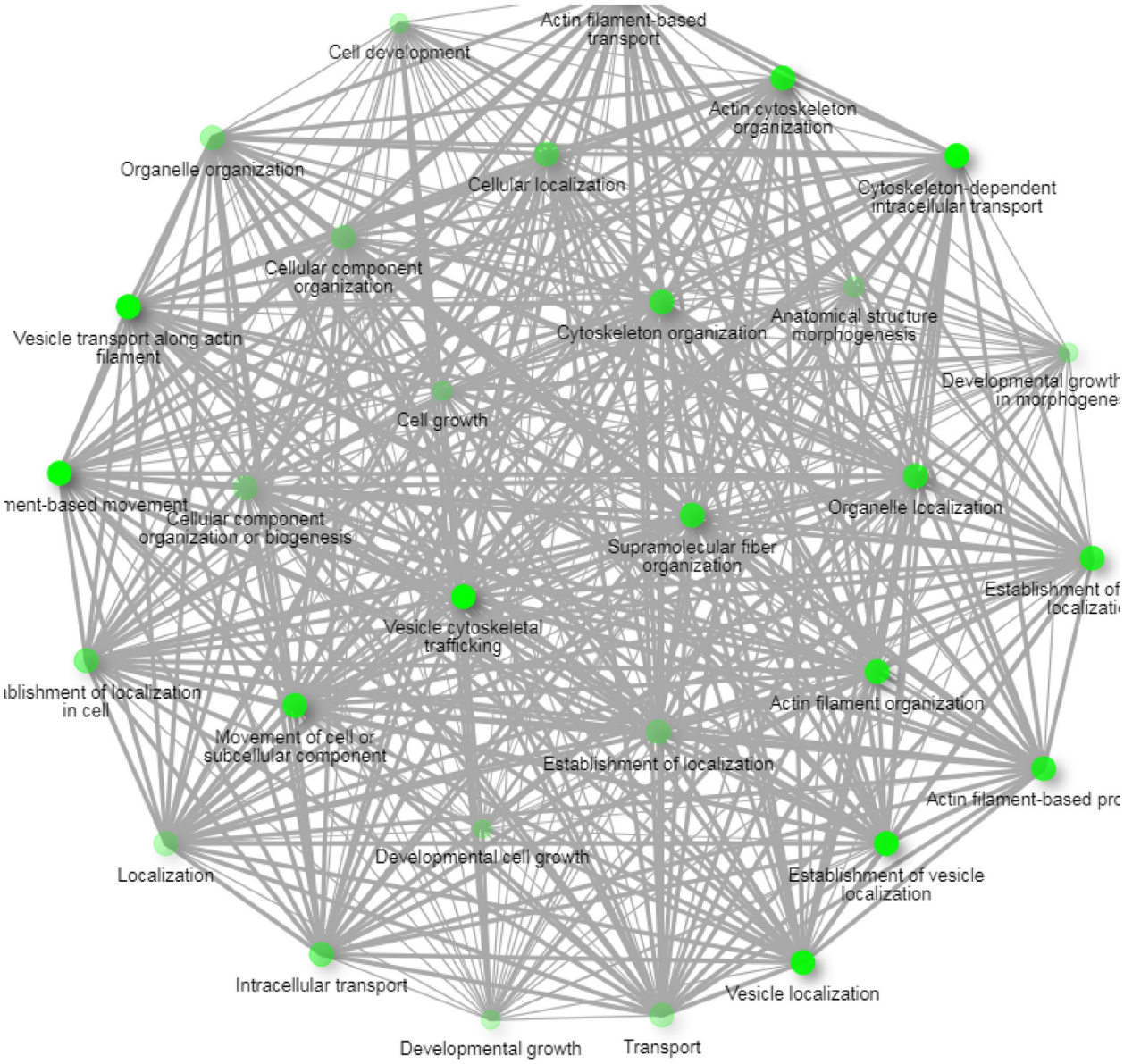

Figure 6. Interaction network among myosin genes in *H. annuus*.

Table 3. Ka/Ks value and time of divergence of myosin genes in *H. annuus*.

Seq_1	Seq_2	Ka	Ks	Ka_Ks	Time (MYA)
HanMYOSIN11.2	HanMYOSIN16.1	1.128058048	1.955175163	0.576960095	65.17251
HanMYOSIN14.2	HanMYOSIN9.1	0.963793624	3.136308564	0.307301914	104.5436
HanMYOSIN14.3	HanMYOSIN11.3	0.876571223	3.578195044	0.244975808	119.2732
HanMYOSIN1.1	HanMYOSIN2.4	3.322480365	2.72824617	1.217808129	90.94154

lower than *O. sativa*. Within the subset, the chromosomal structure of myosin genes was similar. Similarities in the structure of genes within a subclass has been reported by (Azeem et al., 2018; Waqas et al., 2019).

To predict the physical location of *myosin* genes on *A. thaliana* and *H. annuus* chromosomes, non-overlapping Arabidopsis myosin orthologs were used. The results of Figure 4(a) showed that myosin genes were distributed on all five chromosomes of Arabidopsis. Nevertheless, the number of genes is variable. Chromosome 1 carries five genes and chromosomes 2 contains three genes. One gene was present on chromosome 3, while chromones 4 consisted of two gens. Chromosome 5 possessed three genes. In *H. annuus* myosin genes was present on nine different chromosomes with maximum 6 myosin genes present on chromosome 11 (Figure 4(b)).

Gene Ontology analysis revealed that major biological functions of myosin genes were vesicle transportation along actin filaments, vesicle cytoskeleton, actin filament-based movement, cytoskeleton dependent intracellular movement, vesicle localization, movement of cell and subcellular components, organization of actin filament, action cytoskeleton, cell growth, and development (Figure 5(A)). Molecular functions of myosin genes include microfilament motor activities, actin-based ATPs activity, GTP-dependent protein binding, actin filament binding, actin binding and motor activity (Figure 5(B)). Gene ontology of cellular

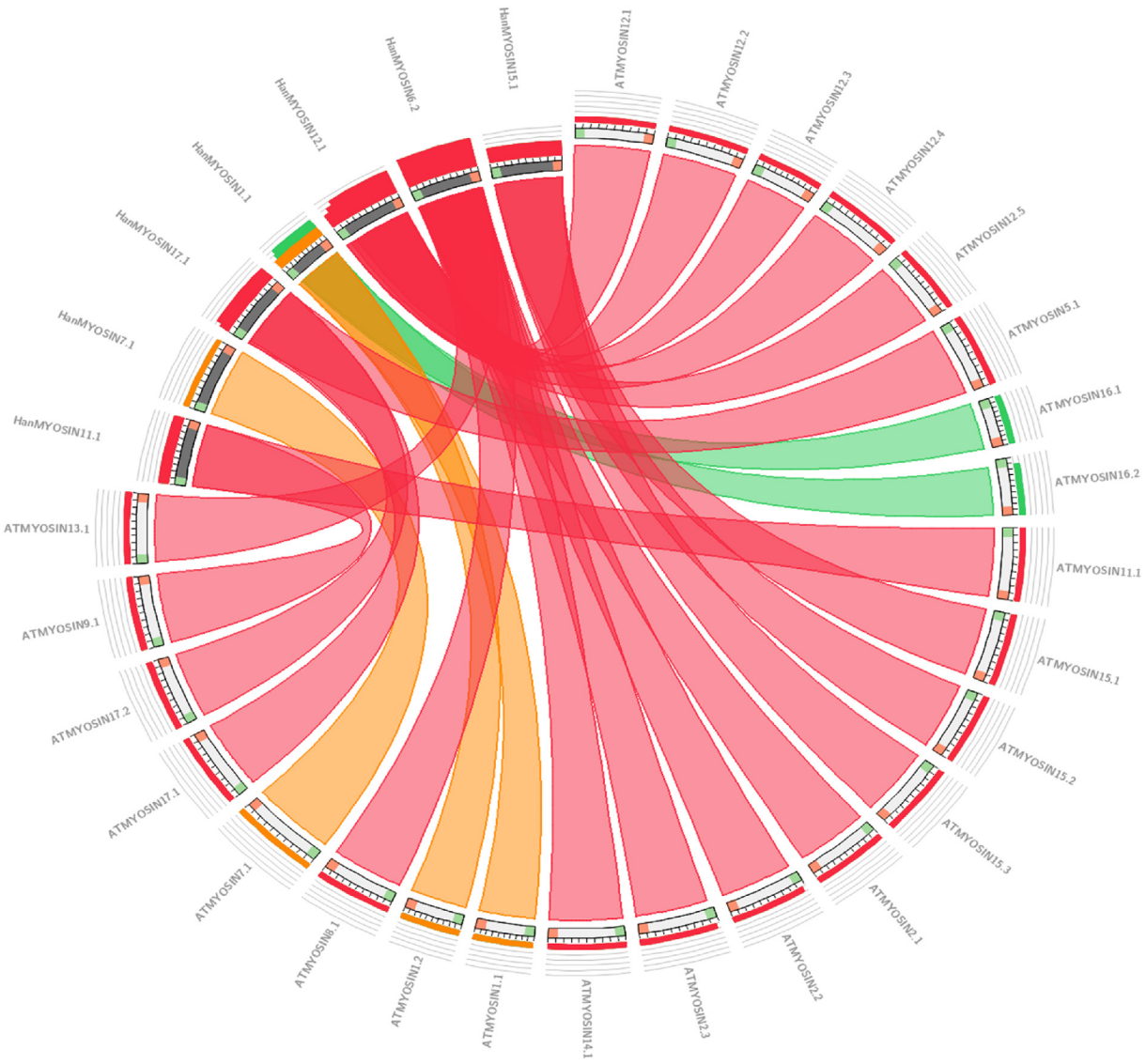

Figure 7. Synteny analysis of myosin genes between *A. thaliana* and *H. annuus.* Colored lines which connect two regions indicate syntenic regions between *A. thaliana* and *H. annuus*.

components indicated that these genes were located in myosin complex, actin cytoskeleton, root hair tips, root tips, cell tips, cell plate, cell vesicle and nuclear membrane (Figure 5(C)).

Interaction analysis among various functions was carried by using online tool of SHINAYGO VO.741. results indicated that functions are interlinked with each other as shown in Figure 6. Myosin genes were generally interacting with actin filament based transport, cell development, actin cytoskeleton organization, organelle organization, cellular localization, cytoskeleton dependent transportation, cellular components organization, vesicle transport along action filament, cell growth, vesicle cytoskeleton trafficking actin filament organization, vesicle localization and intracellular transport.

The molecular evolution rate was calculated by finding the value of Ka/Ks for each pair of duplicated genes. When Ka/Ks > 1, it was supposed that selection on the duplicated genes was positive selection; however, when Ka/Ks < 1, it was expected that selection on the duplicated genes was purifying selection, while neutral selection was assumed when Ka/Ks $= 1$. An increasing amount of purifying selection pressure was applied to the *MYOSIN* genes that were duplicated in our results, indicating that their function will not be significantly altered in further evolutionary process. In our study we estimated deviation time of the duplicated genes pairs. It is predicted that deviation time was 100 million years ago (MYA), if the *MYOSIN* genes had Ks values greater than 0.52. In our results, the Ks value of *HanMYOSIN14.3*, *HanMYOSIN11.3* was observed 3.57, so the duplication time maybe 119.27 million years ago (MYA) (Table 3).

The comparative synteny analysis was performed between *H. annuus*, and *A. thaliana* that shows a remarkable relationship in the function of genes, duplication, triplication, and evolution expression. Gene sequences of *HanMYOSIN1.1*, exhibited synteny with the sequence *AtMYOSIN16.1* and *AtMYOSIN16.2* of *A. thaliana.* Similarly, *AtMYOSIN11.1, AtMYOSIN7.1* were syntenic to *HanMYOSIN11.1* and *HanMYOSIN71* respectively. Two genes of A. thaliana such as *AtMYOSIN1.1, AtMYOSIN1.2* showed synteny with *HanMYOSIN1.1.* The synteny analysis have exposed the duplication and conservation of outward and inward tangling events in the form of ribbons, respectively as shown in Figure 7.

Subcellular analysis enables the researchers to know that these proteins are located at cell membrane, nucleus, or another component. Results showed that myosin genes are located in cytoplasm, chloroplast, nucleus, mitochondria, endoplasmic reticulum, plasma, cell vacuoles, extra cellular locations, and Golgi bodies in both plant species. However,

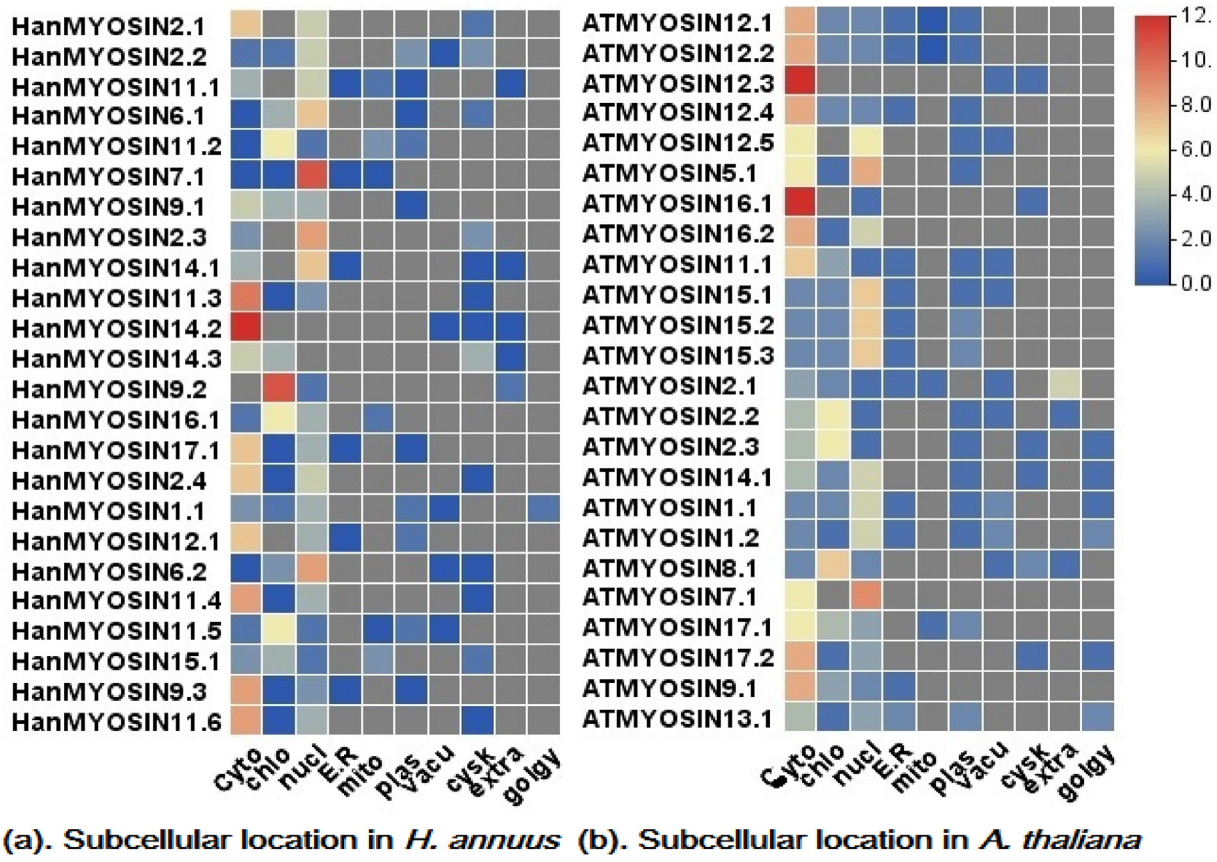

Figure 8. (a) Subcellular locations of myosin genes in *H. annuus*, (b) subcellular locations of myosin genes in *A. thaliana.* Heat map was constructed using TB tool.

their concentration varies among these subcellular locations as shown in Figure 8(a) and (b) for *H. annuus* and *A. thaliana* respectively.

The expression analysis results revealed that expression of *H. annuus* genes varied during three growth stages such as seedling, budding and flowering (Figure 9). *HanMYOSIN5.1, HanMYOSIN14.2* and *HanMYOSIN17.1* showed their expression in all three development stages of *H. annuus* plants. *HanMYOSIN7.1,* and *HanYOSIN11.2* dint showed significant expression during any development stage of *H. annuus*, indicating that this gene can be specific to stress and may express itself during stress conditions. Tissue specific expression analysis indicated that no myosin gene was significantly expressed during ovary and bract however they were expressing in stamen, pollen, flowers, roots and nectary. Expression of genes in these tissues mean that these genes are involved stamen, pollen development similar results were supported by gene ontology analysis.

4. Discussion

Computational research is used to study the complex biological systems through realistic modelling and theoretical exploration. Further computational biology may lead to understanding the fundamentals of genomics and proteomics (Ahmad et al., 2021). Bioinformatics tools are also helpful to discover the interaction among macromolecules, distribution of protein properties, their biological functions (Ahmad et al., 2020) and identification of new capable compounds (Azeem et al., 2018). Myosin proteins belong to a superfamily of molecular motors that binds and hydrolyzes ATP to generate the force and movement along actin filaments (Bechtel and Bollhagen, 2021; Ma et al., 2020). This force is necessary to drive various cellular functions, cell movement, muscular contraction, cytokinesis, membrane trafficking and signal transduction (Steffens et al., 2014). In eukaryotic cells, various intracellular cargos, like cytoskeleton polymers, mRNA, protein complexes and membranous organelles are transported by the energy produced by ATP (Tominaga and Ito, 2015). Whereas twelve genes of this family have been identified in *O. sativa* (Fan et al., 2021; Jiang et al., 2007), and 11 genes have been characterized in *Z. mays* (Huang et al., 2020; Krendel and Mooseker, 2005b). For developing better understanding of myosin XIs, it is essential to study their functions from molecular to tissue level (Haraguchi et al., 2018b). Directional movement of motor proteins is essential for regulating and maintaining various biological phenomena through the generation of motive force (Tominaga and Nakano, 2012; Vale, 2003).

Plentiful information about the biochemical, functional, and structural characteristics of the motor domain of myosin family is available (Nebenführ and Dixit, 2018b; Thompson and Langford, 2002). But phylogenetic and evolutionary processes of myosin family are not fully understood yet and only few studies are available in this dimension (Sebé Pedrós et al., 2014). Some of these studies have focused on the phylogenetic relationships of restricted myosin clades, while others have chosen exemplars of functionally different classes of myosin. Myosin have structurally variable features, such as sequence divergence, gene duplications, length and domain architecture that may explain the ongoing need for a comprehensive phylogenetic analysis of such a prolific family (Allaby and Woodwark, 2004; De Souza et al., 2018). Myosin is usually classified based on their motor domain sequences (Haraguchi et al., 2019; Kijima et al., 2021). As it is well-documented that strongly supported phylogenetic clustering analysis and conserved domain organizations define new classes, a concise nomenclature of multiple members within these classes has not yet been developed. Such a nomenclature should reflect the phylogenetic relationship of different subtypes within classes, and thus must adhere to single gene duplication,

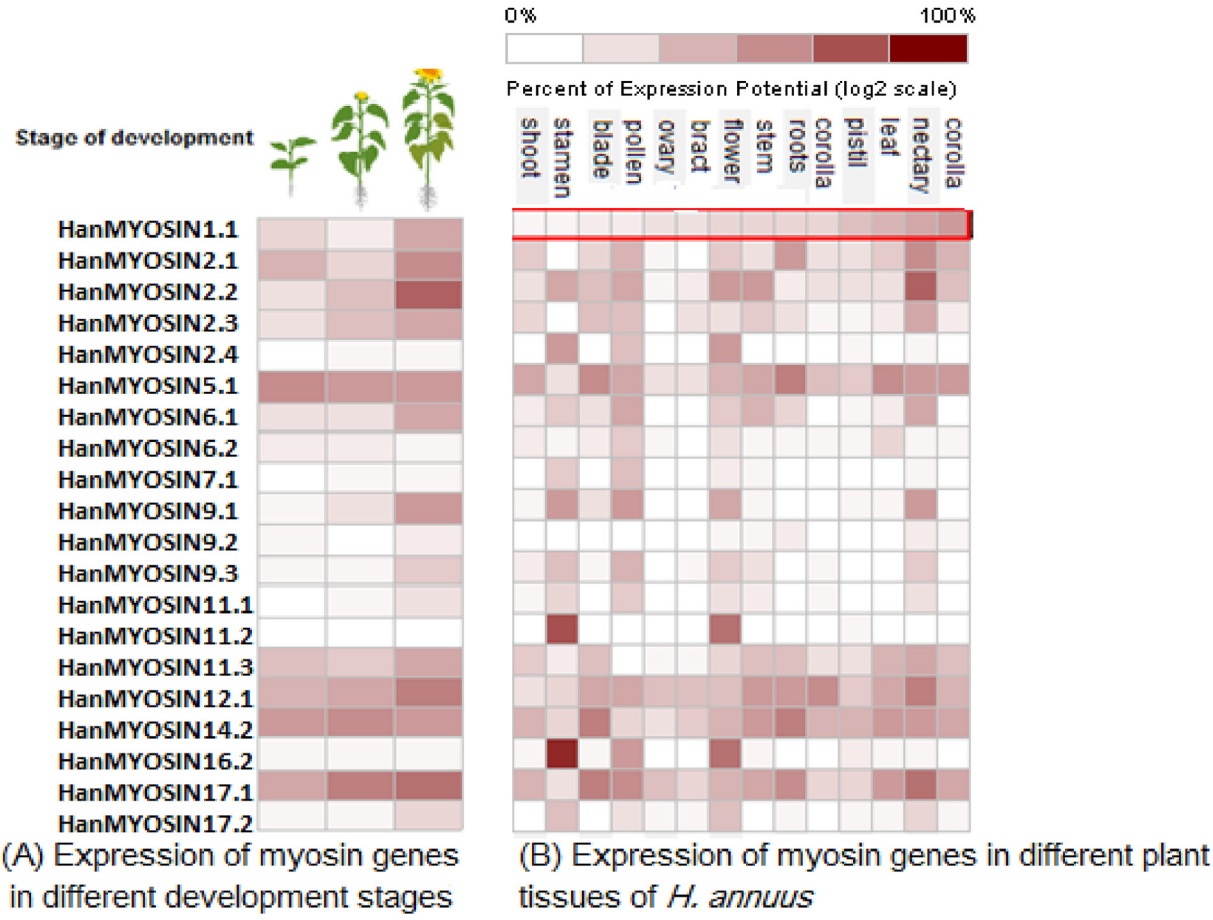

Figure 9. Heat map representation of expression pattern of *H. annuus* genes at different development stages (A) and in different plant tissues.

genomic region and branch specific whole genome that result in orthologs and paralogs (Mühlhausen and Kollmar, 2013; Ryan and Nebenführ, 2018).

We used the amino acid sequences of Arabidopsis myosin and their orthologous and constructed the un-rooted phylogenetic tree to study the evolutionary relationship between Arabidopsis myosin members. These proteins were split into six subgroups by the phylogenetic tree, indicating great divergence between these members. Various researchers supported our results in other crops such as *Nicotiana tobacco* (Amari et al., 2014) and *Gossypium hirsutum* (Ma et al., 2020). Motif analysis showed that most of the motifs were preserved in a group, suggesting that these motifs were of similar evolutionary origin. Expression analysis showed that the expression of myosin gene varies in the *H. annuus* genome during different growth conditions. Many other myosin genes were created by gene duplication, divergence, and the acquisition of extra domains, encoding proteins specialized for specific biological functions enabled by alterations in the mechano-chemical ATPase cycle and acquiring various tails to interact with cargo. Abundant expression of myosin genes in Arabidopsis has been reported during organ growth and development (Krendel and Mooseker, 2005a; Tian et al., 2021). Similarly, myosin genes contribute to the growth of root tips, elongation of root hair and formation of pollen tubes (Tian et al., 2021).

5. Conclusion

The Myosin family in plants works by interacting with filaments in cells for cytoplasmic streaming. We discovered 15 *myosin* genes and their splice variants in *A. thaliana* and *H. annuus*. These myosin proteins perform various functions regarding the assigned task within plant cells. Their motility, velocity and enzymatic activities varies regarding plant species such as it is higher in higher plants than their motility in smaller plants. Plant myosins play significant role in the movement of fluids among organelles. The amino acid length of these proteins ranged from 1170 to 1493, suggesting physicochemical characteristics. There were only three acidic myosin proteins, and the remaining ten were basic. The molecular weights of these proteins ranged from 35,000 to 70,000 DKA. Myosins are non-membranous proteins, as suggested by their subcellular localization. Synteny analysis indicate that Arabidopsis and sunflower myosin genes are syntenic to each other although their time of divergence varies.

Declarations

Author contribution statement

Hafiz Muhammad Ahmad: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Sidra Toor, Nouman Rasool, Madiha Zaynab: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Munazza Ijaz, Saira Azmat, Asmaa M. Abushady, Sajid Fiaz, Hayat Ali Alafari, Madiha Zaynab, Yinglong Chen: Analyzed and interpreted the data.

Dalal S. Alshaya, Sidra Toor, Kotb A. Attia, Sajid Fiaz, Munazza Ijaz: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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