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Original Research Article

Genome-wide Comprehensive Analysis and Abiotic Stress Responsive Expression Profiles of MCM Gene Family in Wheat

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A B S T R A C T

The Minichromosome maintenance (MCM) complex proteins are associated with helicase activity and are conserved eukaryotic replicative factors essential for the DNA replication at its initiation and elongation steps, and act as a licensing factor. In wheat these proteins have not been studied, therefore, in the present study, we identified 20 putative MCM genes from wheat genome using computational approach. We have also performed chromosomal localization study, motif analysis, phylogenetic analysis and *in silico* expression analysis of the MCM gene in wheat. These MCM proteins are present on wheat chromosomal arm 1AS, 1AL, 1BS, 1DL, 1DS, 3AL, 3B, 3DL, 5AS, 5BS, 5DS, 6AL, 6BL, 6DL, 7AS, 7BS, 7DS except group 2 chromosomes and group 4 chromosomes of wheat. Maximum number of MCM protein (3MCM protein) present on chromosomal arm 1AL, 2 MCM proteins are present on chromosomal arm 1BL and only one MCM protein each were present on rest of the wheat chromosome. The length of proteins encoded by these MCM proteins ranged from 561 to 3071 aa. The isoelectric point ranged from 4.86 to 6.28 and their molecular mass ranged from 62.47 to 108.04 kDa. Conserved domain of all the MCM protein contained a zinc finger region, Walker A, Walker B domains, and an Arg finger motif. Homologs of MCM genes have been also found in Arabidopsis. Earlier we have reported that overexpression of single subunit of pea MCM6 involved in providing stress tolerance to transgenic tobacco plants for salinity without affecting yield. *In silico* expression analysis of MCM genes revealed that expression of TaMCM5-6AL and TaMCM7-5BS were regulated under abiotic stresses. Up to our knowledge, this is the first report for genome-wide analysis of MCM genes in wheat. This work provides a foundation for further characterization of MCM genes at functional and molecular level in wheat.

Keywords

MCM gene family, abiotic stress, wheat chromosomes, *in silico* expression analysis

Introduction

DNA replication is essential for development, growth and proliferation of living cells in plants, animals and other eukaryotes (Sanchez *et al.*, 2012). In eukaryote genome, multiple chromosomes are present and replication of DNA initiates at a very large number of origins to make sure that entire genome replicated. In order to maintain the stability and integrity of genome and, the process of replication should be strictly regulated to ensure that in S phase of single cell cycle, replication of DNA is completely and accurately taken place only once (Tuteja *et al.*, 2011). For

initiation of DNA replication, origin recognition complex (ORC) and Cdc6 binds to replication origins. ORC-Cdc6 plays important role in recognition of origin and a scaffold for assembling the 12-subunit MCM2-7double hexamer, which is known as pre replication complex (pre-RC) (Evrin *et al.,* 2009; Remus *et al.,* 2009). This is known as licenced state. For the ordered assembly of many replication factors including ORC, Pre-RC formation is needed (Bell, 2002; Stillman, 1994), Cdc6/CDC18 (Coleman *et al.,* 1996), Cdt1 (Nishitani *et al.,* 2000, 2001), and MCM proteins (MCM2–7). In the G1 phase, this activates replication fork formation (Tuteja *et al.,* 2011; Ravoitytė and Wellinger, 2017). Large number of factors including, CDT1, MCM(2-7), ORC, CDC45, CDC6 and GINS proteins involved in regulation of MCM(2-7) helicase complex which forms the replication fork (Dang *et al.,* 2011a; Herridge *et al.,* 2014). As the replication fork formed, the MCM (2-7) helicase complex removed and this is unlicensed state of the MCM (2-7) complex (Tuteja *et al.,* 2011; Ravoitytė and Wellinger, 2017). In addition to MCM (2-7), MCM8, MCM9 and MCM10 also play important role during replication process and act as co-regulators of MCM (2-7) hexamer (Shultz *et al.,* 2007). MCM8 may be involved in recruitment of CDC6 (Celldivision cycle protein 6), while role of MCM9 has not clearly mentioned in plants (Shultz *et al.,* 2007; Tuteja *et al.,* 2011; Baxley and Bielinsky, 2017; Shanmugam *et al.,* 2017).

There are various exogenous and endogenous factors that can slow down the formation of replication fork and this is known as replication stress (Burhans and Weinberger, 2007; Magdalou *et al.,* 2014). Chemical agents, UV light and heavy metals includes major exogenous factors and major endogenous factors includes alterations in pools of dNTP precursors required for DNA synthesis, alteration in the expression of proteins required for synthesis of dNTPs or other components of DNA synthesis, hyper-DNA replication caused by the activation of origins more than once per S phase, DNA damage lesions that block replication forks, decreased frequency for initiation of DNA replication occurs at origins of replication (Burhans and Weinberger, 2007). In earlier reports in Arabidopsis and rice, it has been reported that genome stability and integrity were affected by replication stress which ultimately leads to death of cells (Schuermann *et al.,* 2009; Cools *et al.,* 2010; Kwon *et al.,* 2013). In a recent study, MCM genes are shown to involved in regulating abiotic stresses including salt and cold in *Brassica oleracea* and *Brassica rapa* (Shanmugam *et al.,* 2017) and in pea (Dang *et al.,* 2011a; Dang *et al.,* 2011b).

The MCM proteins in eukaryotes, are conserved from yeast to mammals and have six subunits MCM2–7 that participate in DNA replication (Forsburg, 2004). Ishimi (1997), provide the first evidence and shown that purified MCM 4, 6, 7 complexes have 3’ 5’ DNA unwinding capacity. Labib and Diffley (2001), enquired whether MCM 2–7 is the eukaryotic DNA replication fork helicase. MCM2–7 proteins are categorized in superfamily 6 (SF6) helicases where each subunit of a hexameric helicase contains an AAA + domain. The ATPase sites are generated in between two adjacent subunits in these helicases where one provides the walker A and walker B motif for ATP binding and the other partner provides the arginine finger motif for ATP hydrolysis (Lyubimov *et al.,* 2011; Aparicio *et al.,* 1997).

The minichromosome maintenance (MCM) genes were first identified in the yeast *Saccharomyces* cerevisiae (Maine *et al.,*

1984). The PROLIFERA MCM7 is the first MCM protein identified from *Arabidopsis thaliana* and this is a MCM2/3/5 homolog (Springer *et al.,* 1995). MCM proteins have been found in different plant species including Arabidopsis (Springer *et al.,* 1995, 2000; Masuda *et al.,* 2004; Dresselhaus *et al.,* 2006; Shultz *et al.,* 2007; Ni *et al.,* 2009; Rizvi *et al.,* 2016), maize (Sabelli *et al.,* 1996 ; Bastida and Puigdomenech, 2002; Dresselhaus *et al.,* 2006), rice (Shultz *et al.,* 2007), tobacco (Dambrauskas *et al.,* 2003; Chaudhry *et al.,* 2012), pea (Tran *et al.,* 2010; Dang *et al.,* 2011a), soyabean (Leisner *et al.,* 2017).

Dang *et al.,* (2011) firstly reported that overexpression of single subunit of pea MCM6 involve in providing stress tolerance to transgenic plants for salinity without affecting yield (Dang *et al.,* 2011). Recently characterization of MCM genes and their expression analysis have been reported at genome-wide level in different stress conditions in *Brassica oleracea* and *Brassica rapa* (Shanmugam *et al.,* 2017).

Wheat is one of the most important cereal crops in the world. It provides 20% of the calories and protein consumed by human (Brenchley *et al.,* 2012). In the present study, using bioinformatics approach we identified the MCM gene family in wheat, their motif analysis, chromosomal localization, *in silico* expression analysis and phylogenetic analysis with Arabidopsis, rice, maize and pea. Up to our knowledge, this is the first study where genome-wide MCM genes have been identified in wheat using *in silico* approach. Our study will provide a foundation for further functional analysis of the wheat MCM genes, and will contribute to better understanding the molecular mechanism of MCM genes involving in regulating growth, development as well as stress processes in wheat.

Materials and Methods

Identification of MCM Genes in wheat

For identification of MCM genes in wheat, we utilized an available database Ensembl Plants (http://plants.ensembl.org/Triticum_aestivum/) by using keyword `helicase'. All sequences were downloaded and searched for specific conserved domains and motifs of MCM gene using NCBI CD search tool. Using the sequence IDs of the MCM proteins in wheat, amino acid sequences were isolated from the Ensembl Plants database v 37.0. All protein sequences were used as query sequence to search other similar sequences in the Ensembl Plants database to find all the probable MCM genes in wheat genome. The chromosomal locations, number of amino acids and uniprot id of the MCM genes were retrieved from the Ensembl Plants database.

Identification of homologs for wheat helicase proteins in Arabidopsis

TBLASTN was performed in EnsemblPlants db for searching homologs of wheat MCMC protein in Arabidopsis (http://plants.ensembl.org/Arabidopsis_thaliana/Tools/Blast?db=core).

Motif analysis and phylogenetic analysis

A Neighbor-joining phylogenetic tree of the MCM proteins were constructed with MEGA6.0 using default parameters and bootstrap 1000 (Tamura *et al.,* 2013). For conducting phylogenetic analysis, MCM proteins from *Zea mays*, *Oryza sativa*, and *Pisum sativum*, *Arabidopsis thaliana* were used (Herridge *et al.,* 2014; Dang *et al.,* 2011a; Shanmugam *et al.,* 2017). Sequence id for each of the sequences used for phylogenetic study are given in Table 1.

Alignment of the MCM protein sequences were performed by CLUSTALW (http:// www.genome. jp/ tools/clustalw/) and neighbor joining with 1000 bootstrap was used to construct a phylogenetic tree. The phylogenetic analysis was conducted using MEGA6.0. For MCM motif detection in MCM protein, NCBI CD search, alignment by Custal Omega (https://www. ebi. ac.uk/ Tools /msa/clustalo/) were used and finally the sequences were verified manually.

Expression analysis

In silico expression analysis of MCM gene of wheat were carried out by using micro array database of Genevestigator (https://www.genevestigator. com/gv ; Hruz *et al.,* 2008) with the *Triticum aestivum* (TA_AFFY_WHEAT-0).

Results and Discussion

MCM gene family in wheat

A total of 20 MCM proteins are encoded by the wheat genome and of the entire enlisted MCM protein, only 12 are encoded by single loci and remaining were redundant. These include DNA replication licensing factor MCM2, MCM3, MCM4, MCM5, MCM6, MCM7, probable MCM8 and probable MCM9.

These MCM proteins are present on wheat chromosomal arm 1AS, 1AL, 1BS, 1DL, 1DS, 3AL, 3B, 3DL, 5AS, 5BS, 5DS, 6AL, 6BL, 6DL, 7AS, 7BS,7DS except chromosome 2 and 4. Maximum number of MCM protein i.e. 3 (MCM2, MCM3, MCM8) present on chromosomal arm 1AL, 2 MCM proteins are present on chromosomal arm 1BL and only one MCM protein each were present on rest of the wheat chromosome (Table 2). The length of proteins encoded by these MCM genes ranged from 561 to 3071 amino acid. The isoelectric point ranged from 4.86 to 8.77 and their molecular mass ranged from 62.47 to 108.04 kDa (Table 3).

Homolog of MCM genes have been also searched in Arabidopsis using TBLASTN at EnsemblPlants db. These include 8 unique MCM proteins in Arabidopsis. The length of proteins encoded by these MCM genes ranged from 570 to 936 amin acid. The isoelectric point ranged from 4.76 to 8.12 and their molecular mass ranged from 63.52 to 105.58 kDa (Table 2 and Table 4). As there is considerable sequence homology among MCM2-7, it gives an idea that these MCM proteins may be derived by gene duplication from a single, archaeal-like, ancestral MCM (Tye 1999). However, in plant how regulation of DNA replication by MCM genes is not very much clear (Dang *et al.,* 2011a; Tuteja *et al.,* 2011; Costas *et al.,* 2011; Lee *et al.,* 2010).

MCM motifs and phylogenetic analysis

MCM proteins belong to the AAA^+ (ATPases Associated with various cellular Activities) super family (Ogura and Wilkinson 2001) and these are present at N-terminal of MCM domain. Adjacent to this, walker A, walker B and arginine finger present which are conserved motifs and these have nucleotide binding properties, which is involved in initiating the helicase activity of MCM genes (Singleton *et al.,* 2007; Sun *et al.,* 2014; Rizvi *et al.,* 2016).

Motif structure analysis of MCM genes in wheat showed that all the 20 MCM protein have the conserved MCM motif for proper function during DNA replication in these proteins. These includes a zinc finger region, Walker A and Walker B domains and an Arg finger motif (Figure 1; Forsburg, 2004; Maiorano *et al.,* 2006).

Table.1 Reference sequences used in phylogenetic tree analysis

Name	NCBI Reference Sequence ids
OsMCM2	NP_001067910.1
ZmMCM2	AFW60764.1
PsMCM2	ACN78877.2
OsMCM3	NP_001055835.1
ZmMCM3	NP_001106065.1
PsMCM3	AAN73053.2
OsMCM4	EEE54765.1
ZmMCM4	NP_001147978.1
PsMCM4	ABY81650.1
OsMCM5	NP_001048396.1
ZmMCM5	XP_008681294.1
PsMCM5	ACD87452.2
OsMCM6	NP_001054989.1
PsMCM6	AAN73052.2
ZmMCM6	NP_001105289.1
OsMCM7	NP_001067020.1
ZmMCM7	NP_001105524.1
PsMCM7	AAQ72567.1
ZmMCM8	XP_008656376.1
OsMCM9	NP_001057158.1

Table.2 A list of 20 MCM gene from wheat genome along with their corresponding homologs in Arabidopsis

S.No	Gene id (Wheat)	Gene symbol (Wheat)	Description	Gene id (Arabidopsis)	Gene symbol (Arabidopsis)
1	TRIAE_CS42_1DS_TGACv1_080203_AA0243110	TaMCM6-1DS	DNA replication licensing factor MCM6	AT5G44635	At MCM6
2	TRIAE_CS42_1AL_TGACv1_000388_AA0010880	TaMCM3-1AL	DNA replication licensing factor MCM3	AT5G46280	AtMCM3
3	TRIAE_CS42_1AL_TGACv1_001932_AA0036950	TaMCM8-1AL	Probable DNA helicase MCM8	AT3G09660	AtMCM8
4	TRIAE_CS42_1AL_TGACv1_002646_AA0044220	TaMCM2-1AL	DNA replication licensing factor MCM2	AT1G44900	AtMCM2
5	TRIAE_CS42_1AS_TGACv1_019881_AA0072380	TaMCM6-1AS	DNA replication licensing factor MCM6	AT5G44635	At MCM6
6	TRIAE_CS42_1BL_TGACv1_030496_AA0092330	TaMCM2-1BL	DNA replication licensing factor MCM2	AT1G44900	AtMCM2
7	TRIAE_CS42_1BL_TGACv1_032599_AA0131840	TaMCM3-1BL	DNA replication licensing factor MCM3	AT5G46280	AtMCM3
8	TRIAE_CS42_1DL_TGACv1_061701_AA0201930	TaMCM3-1DL	DNA replication licensing factor MCM3	AT5G46280	AtMCM3
9	TRIAE_CS42_3AL_TGACv1_195295_AA0647460	TaMCM4-3AL	DNA replication licensing factor *MCM*4	AT2G16440	AtMCM4
10	TRIAE_CS42_3B_TGACv1_224285_AA0794840	TaMCM4-3B	DNA replication licensing factor MCM4	AT2G16440	AtMCM4
11	TRIAE_CS42_3DL_TGACv1_252076_AA0887540	TaMCM4-3DL	DNA replication licensing factor MCM4	AT2G16440	AtMCM4
12	TRIAE_CS42_5AS_TGACv1_393478_AA1272980	TaMCM7-5AS	DNA replication licensing factor MCM7	AT4G02060	AtMCM7
13	TRIAE_CS42_5BS_TGACv1_423792_AA1383390	TaMCM7-5BS	DNA replication licensing factor MCM7	AT4G02060	AtMCM7
14	TRIAE_CS42_5DS_TGACv1_457219_AA1483870	TaMCM7-5DS	DNA replication licensing factor MCM7	AT4G02060	AtMCM7
15	TRIAE_CS42_6AL_TGACv1_473429_AA1531030	TaMCM5-6AL	DNA replication licensing factor MCM5	AT2G07690	AtMCM5
16	TRIAE_CS42_6BL_TGACv1_499834_AA1592800	TaMCM5-6BL	DNA replication licensing factor MCM5	AT2G07690	AtMCM5
17	TRIAE_CS42_6DL_TGACv1_527835_AA1709090	TaMCM5-6DL	DNA replication licensing factor *MCM*5	AT2G07690	AtMCM5
18	TRIAE_CS42_7AS_TGACv1_571397_AA1847340	TaMCM9-7AS	Probable DNA helicase MCM9	AT2G14050	AtMCM9
19	TRIAE_CS42_7BS_TGACv1_592567_AA1940470	TaMCM9-7BS	Probable DNA helicase *MCM*9	AT2G14050	AtMCM9
20	TRIAE_CS42_7DS_TGACv1_625005_AA2064200	TaMCM9-7DS	Probable DNA helicase MCM9	AT2G14050	AtMCM9

Fig.1 Conserved MCM domain of 20 MCM protein in wheat by searched by NCBI CD search and Clustal Omega alignment. The results were further checked manually to confirm that all the domains are present and then used in the figures

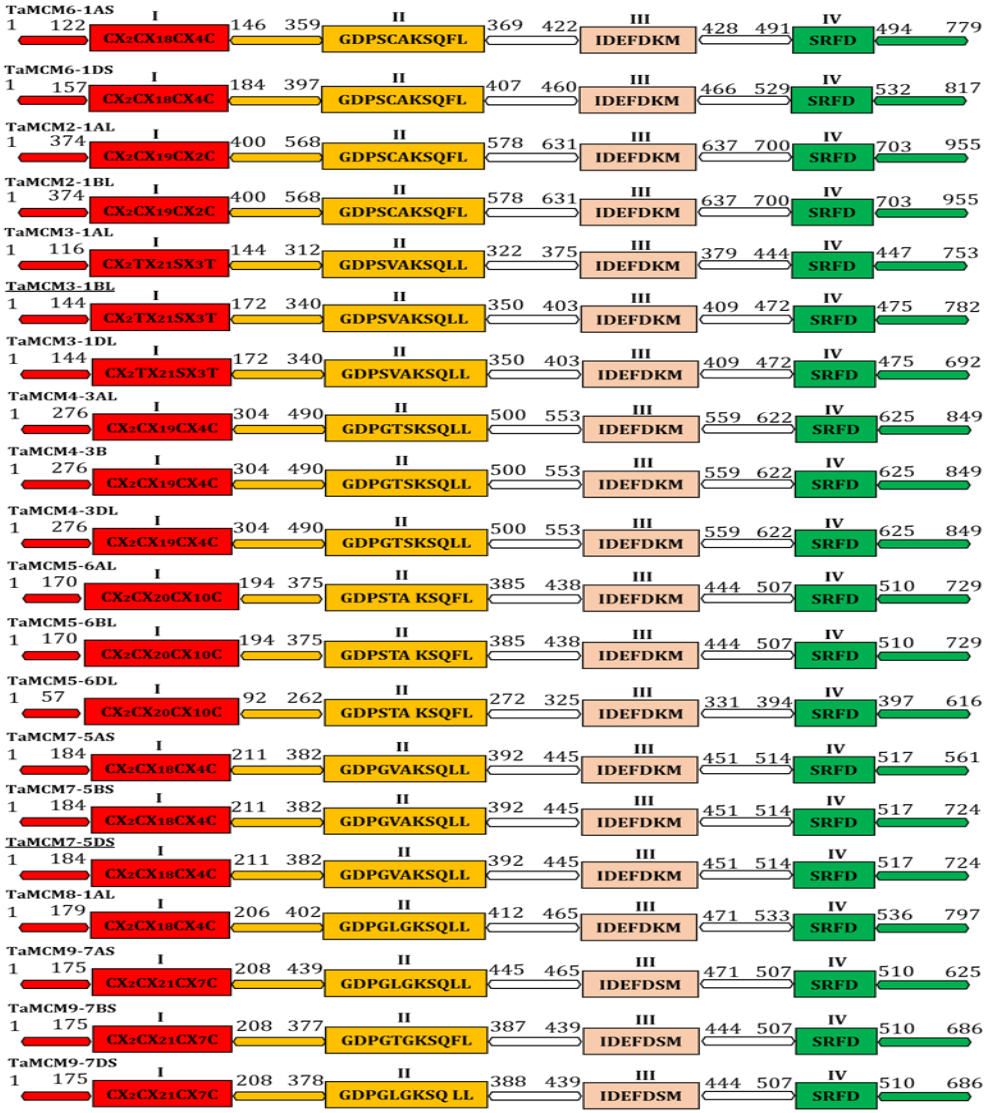
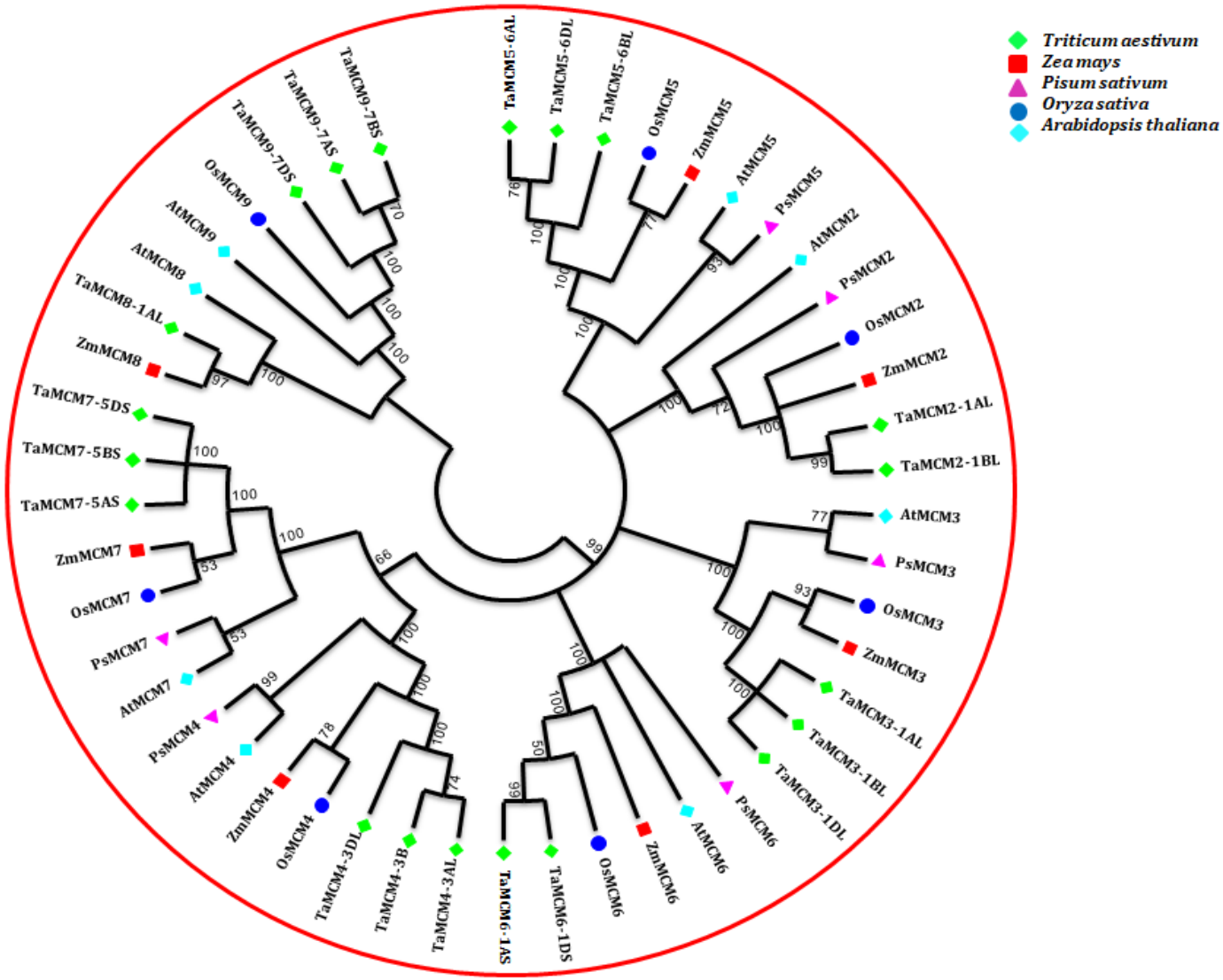


Fig.2 Phylogenetic tree constructed by neighbourhood joining method analysis of MCM proteins. At-*A.thaliana*, Os- *Oryza sativa*, Zm- *Zea mays*, Ps- *Pisum sativam*. Ta-*T.aestivum*. Accession number of respective genes given as Table 1



We have identified potential zinc-finger motifs in all the MCM proteins that might be involved in protein- protein interactions in the N-terminal regions MCM proteins. The zinc-finger motif $CX_2CX_{19}CX_2C$ and $CX_2TX_{21}SX_3T$ was found in TaMCM2-1AL, TaMCM2-1AL and TaMCM 3-1AL, TaMCM 3-1BL, TaMCM 3-1DL protein sequences respectively while $CX_2CX_{19}CX_4C$ and $CX_2CX_{20}CX_{10}C$ was present in TaMCM 4-3AL, TaMCM 4-3B, TaMCM 4-3DL and TaMCM 5-6AL, TaMCM 5-6BL, TaMCM 5-6DL protein sequences respectively. In all MCM6 and MCM7 protein $CX2CX_{18}CX_4C$ and $CX_2CX_{18}CX_4C$ were found in zinc-finger motif. Similarly $CX_2CX_{18}CX_4C$ and $CX_2CX_{21}CX_7C$ were present in zinc finger motif of MCM8 and MCM9 protein (Dresselhaus *et al.,* 2006; Figure 1).

In the present study also, we found GKS sequence after 14 residues from Walker A motif of MCM8 and MCM9 protein as compared to MCM2-7 (Figure 1). Earlier it has been reported that only plant MCM6 classes (*P. sativum*, *A. thaliana* and *Z. mays*) contain an extra classic GKS motif occurring 14 residues after Walker A motif (Tran *et al.,* 2010). TaMCM8-1AL, TaMCM9-7AS, TaMCM9-7BS and TaMCM9-7DS contained GKS (in walker A motif) in place of A/SKS found in MCM2-7 complex (Figure 1). Earlier report also provide evidence for the presence of the A/SKS sequence in MCM2-7, in both plants and animals while the MCM8 and MCM9 proteins contain a classic GKS sequence in the Walker A motif (Maiorano *et al.,* 2006). TaMCM9-7AS, TaMCM9-7BS and TaMCM9-7DS possess IDEF as conserved sequence on walker B motif as compared to MCM2-8 protein. Similar report for conserved sequence of walker B sequence of MCM9 has been reported earlier (Neuwald *et al.,* 1999; Shultz *et al.,* 2007).

The MCM proteins were arranged in eight groups for pea, maize, rice, wheat and Arabidopsis in the phylogenetic tree. MCM2 and MCM5was closely related; MCM3 was closely related to MCM6; MCM4 was closely related to MCM7; MCM8 was closely related 9 (Figure 2).The phylogenetic tree revealed that MCM genes were highly conserved during course of evolution among different plant species. Protein present in same clade shared comman function. The high sequence similarity with known MCM genes in maize, rice, pea Brassica species and Arabidopsis to wheat MCM protein may suggest a similar role of MCM genes in wheat during growth and development under abiotic stress (Figure 2).

In a study it has been reported that in transgenic tobacco over expression of MCM6 improved plant growth in high-salinity conditions (Tuteja *et al.,* 2011; Dang *et al.,* 2011a).Various studies reported that during replicative stress, the MCM (2-7) protein complex is up regulated with variation at the subunit level for regulation of DNA replication (Crevel *et al.,* 2011; Tuteja *et al.,* 2011; Herridge *et al.,* 2014; Rizvi *et al.,* 2016). During salt stress, MCM5 and MCM7 genes involved in providing resistance to salt stress (Shultz *et al.,* 2009). In other studies, MCM (2-7) helicase complex genes involved in salt stress tolerance in pea and *Brassica* (Shultz *et al.,* 2009; Dang *et al.,* 2011a).

Expression analysis

The expression profile of the 20 MCM genes were analysed using microarray data of Genevestigator as mentioned in material method section. Here, probe ids of only 2 MCM genes (TaMCM7-5BS and TaMCM5-6AL) of the 20 predicted MCM genes were found and we were unable to find probe ids

of rest of the MCM genes of wheat. The scatter plot diagram for all the five abiotic stress conditions (dehydration, salt, drought and cold) were drawn by using "Genevestigator" microarray database. The expression of both the TaMCM7-5BS and TaMCM5-6AL genes were up regulated in dehydration stress in root during seedling growth stage of development (Figure 3). On the other hand expression of the above mentioned two genes were down regulated in cold, drought as well as in salt stress. Shultz *et al.,* (2009), in *A. thaliana*, reported MCM5 and MCM7 genes involved in salt stress. Similarly MCM (2-7) helicase complex genes in pea and *Brassica* may involve regulating salt stress (Shultz *et al.,* 2009; Dang *et al.,* 2011a). In a recent study by Shanmugam *et al.,* (2017), BrMCM5 in *Brassica rapa* were up regulated in cold stress and expression of BoMCM5 was down regulated during cold stress treatment. In salt stress treatment between 3 h and 6 h of salt stress, expression of BrMCM5 genes was down regulated. On the other hand in the same study, expression of BoMCM5 was up regulated by 3.3 and 3.5 at 1 h and 48 h of salt treatment. In transgenic tobacco over expression of MCM6 improved plant growth in high-salinity conditions (Dang *et al.,* 2011a; Tuteja *et al.,* 2011). Although the accurate mechanisms have not been detected, it has been proposed that stress-related proteins or transcription factors can interact with subunits of MCM complex (Dang *et al.,* 2011a; Tuteja *et al.,* 2011). BoMCM7_in *Brassica oleracea* had the maximum level of expression at 1 h from the commencing of cold treatment. Expression of BoMCM7 was upregulated in salt stress treatment while in the same study the expression of BrMCM7 genes not changed in the salt stress. Differential expression of MCM among different crops species indicated that MCM has species-specific regulation.

The present study is the first report of genome-wide analysis of MCM gene family in wheat. Here we identified 20 MCM genes in wheat. Here chromosomal localization, motif analysis, phylogenetic analysis and *in silico* expression analysis have been conducted. It has been found that MCM2-7, MCM8 and MCM9 genes are present in what genome. All the MCM protein possess all four MCM motifs including zinc finger domain, walker A, walker B and Arginine motif. These genes can be used as candidate gene for expression analysis and functional characterization to overcome replication stress and other abiotic stresses to maintain genome integrity and stability.

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