

Progress of Molecular Floral Development Research in Wheat

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Abstract

Wheat (*Triticum aestivum* L.) is one of the most important crops in the world. Because the wheat floral organs provided the basis for grain formation, study of wheat floral development is important for improving wheat yield potential. In recent years, notable progress has been made in study of molecular floral development in wheat. In this review, we summarized the 'ABC' model and discussed the role of A-, B-, C-, D-, E-class genes in the development and evolution of wheat flowers and inflorescence.

Keywords: Wheat (*Triticum aestivum* L.), Floral development, MADS-box, ABC model

1. Introduction

Wheat (*Triticum aestivum* L.) is the most important food crop in the world and becomes even more important as the global population increases. Because the arable land is very limited, improving the yield is essential. One way to improve wheat yield potential is to increase grain number per spike (Coffman, 1924; Frederic and Bauer, 2000). The grain number of per spike is the ultimate embodiment of the floral differentiation, degeneration and grain-setting. Those physiological processes are closely related to the floral development in wheat. The inflorescence of a wheat plant (spike, ear or head), developed at the tip of the stem, is composed of spikelets. The spikelets are arranged to form two opposite rows along the main axis or rachis. The number of spikelets per spike is determined by the timing of terminal spikelet initiation, which depends on the genotype and the environmental conditions. In this respect, the rachis meristem in wheat is determinate. The spikelet is composed of florets joined at the axis (rachilla) alternately on opposite sides, and encompassed by two small bract leaves called glumes. There are multiple florets (usually six to eight) in each spikelet, of which a few in the apical positions may be sterile due to hypoplasia. In contrast to barley, rice and maize, the rachilla meristem in wheat is classified as indeterminate. A typical floret in wheat consists of four whorls. The first outermost whorl often consists of two leaf-like structures, a lemma and a palea. The lemma bears an awn at its tip in some cultivars, but there are also awnless cultivars such as Chinese Spring. It was recently suggested that the lemma and palea are homologous to dicot sepals in rice and maize (Ambrose et al., 2000; Kang et al., 1998). The second whorl is composed two lodicules. The lodicule is considered to be a modified petal in maize and rice (Ambrose et al., 2000; Kang et al., 1998; Kyozyuka et al., 2000), which swells during anthesis, forcing the lemma and palea apart to facilitate pollination. The third whorl contains three stamens, that is, the male reproductive organs, which produce pollen. Finally, the fourth, innermost whorl contains a pistil, which has a unilocular carpel, is the female part of the flower and consists of the ovary containing the ovule and two filamentous styles, each terminating in a feathery stigma.

Although the structures and functions of lemma, palea, lodicule, stamens and carpels may differ dramatically at maturity, each floral organ starts its development as a little bulge on the floral meristem, a tiny clump of undifferentiated cells. Each cell in the developing floral organ primordium must somehow 'learn' its position within the floret, and differentiate accordingly into an appropriate cell type (Günter, 2001).

2. 'ABC model' and MADS-box gene

Flower development has been the subject of intensive studies about twenty years ago, particularly in two dicot plants, *Arabidopsis* and *Antirrhinum* (Theissen and Saedler, 1999; Theissen, 2001). These studies have provided a general understanding of the development of floral organs in higher plants, explained by the ABC model (Davies and Schwarz-Sommer, 1994; Ma, 1994; Weigel and Meyerowitz, 1994). This model explains how floral organ identity is defined by three classes of homeotic genes, called A, B and C. In this model, each class functions in two adjacent floral whorls, class-A genes specify sepals in the first whorl; A and B, petals in the second whorl; B and C, stamens in the third whorl; and C, carpels in the fourth whorl. The ABC model continues to be revised since it is proposed. When *FLORAL BINDING PROTEIN 11 (FBP11)*, termed D-class gene, is confirmed to determine ovule, ABCD model is suggested (Angenent and Colombo, 1996). Furthermore E-class gene and ABCDE model are proposed based on the fact that *SEPALLATA1 (SEP1)*, *SEPALLATA2 (SEP2)*, *SEPALLATA3 (SEP3)* are proven to be together with A-, B-, C-, D-class genes required for the specification of floral organ identities in *Arabidopsis* (Theissen, 2001). The 'classical ABCDE model', which has been widely accepted until now, was proposed to explain how homeotic genes control organ identity.

The all A-, B-, C-, D-, E-classes gene are homeotic genes, which are translated into proteins. Each protein coded by these genes contains a MADS-box region, which take their name from the *MINICHROMOSOME MAINTENANCE 1 (MCM1)* genes in yeast, *AGAMOUS (AG)* in *Arabidopsis*, *DEFICENS (DEF)* in *Antirrhinum* and *SERUM RESPONSE FACTOR (SRF)* in humans (Riechmann and Meyerowitz, 1997). All MADS-box genes have in common a highly conserved 180-bp-long motif designated MADS-box, which encodes the DNA-binding domain of MADS-domain proteins (Shore and Sharrocks, 1995; West et al., 1998; Alvarez-Buylla et al., 2000). It is believed that these genes are master controlling genes, regulating the action of other genes that will control organ development.

Analysis of the ABCDE genes in monocot species such as rice suggests that the ABCDE model could essentially be extended to monocots except for the role of the class A gene (Kater et al., 2006; Yamaguchi and Hirano, 2006). In wheat, it has reported some ABCDE genes, such as *WAP1 (wheat API)* (Murai et al., 1998; Murai et al., 2002), *WAP3 (wheat APETALA3)* (Murai et al., 1998), *WP11 (wheat PISTILLATA1)* (Hama et al., 2004), *WSEP (wheat SEPALLATA)* (Shitsukawa et al., 2007), et al (Fig.1).

3. AP1-like genes (A-class gene) in wheat

In the core eudicot *Arabidopsis*, the A-class gene *APETALA1 (API)* is required for establishment of the floral meristem and for specification of sepal and petal identity (Mandel et al., 1992). Specification of floral meristem identity in *Arabidopsis* is redundantly regulated by the *API* homologs *CAULIFLOWER (CAL)* and *FRUITFUL (FUL)* (Kempin et al., 1995; Ferrandiz et al., 2000). In rice, some *API*-like genes, such as *OsMADS14*, *OsMADS15*, and *OsMADS18* have been isolated and identified (Moon et al., 1999; Jeon et al., 2000; Kyozuka et al., 2000; Lim et al., 2000). The *AP*-like genes in rice suggest that they are playing a role in specifying floral meristem identity, but are not necessary in sepal and petal identity determination (Kater et al., 2006; Yamaguchi and Hirano, 2006).

The wheat genome contains at least six *API*-like MADS-box genes, *WAP1*, *VRN1*, *TaVRT-1*, *TaAPI-1*, *TaAPI-2* and *TaAPI-3* (Murai et al., 2003; Yan et al., 2003; Danyluk et al., 2003; Paolacci et al., 2007). *WAP1* is 98.8% identical to *TaVRT-1* and has only three amino acid changes in C region. Otherwise, *WAP1* is 98% identical to *VRN-1* and has only five amino acid changes (Murai et al., 2003). *WAP1* was isolated from spring wheat (*T. aestivum*) cv. Norin 26 (Murai et al., 1998; Murai et al., 2002), *TaVRT-1* from winter wheat (*T. aestivum*) cv. Fredrick (Danyluk et al., 2003), and *VRN-1* from *T. monococcum* (Yan et al., 2003). Those results suggesting that the three *API*-like genes should represent the same gene and the difference of sequence is due to varietal polymorphism.

Phylogenetic study clearly indicated that the *WAP1*, *VRN1*, *TaVRT-1*, *TaAPI-1*, *TaAPI-2* and *TaAPI-3* are classified as relatively distant subclass of the monocot *API*-like family (Murai et al., 2003; Paolacci et al., 2007). It will be interesting to determine whether these genes have functions similar to those of the *API*-like genes in endicots because the wheat floret does not have obvious sepals and petals, but instead has a lemma, a palea, and lodicules. But previous studies have suggested that *WAP1*, *VRN1*, *TaVRT-1*, *TaAPI-1*, *TaAPI-2* and *TaAPI-3* are only involved in the phase transition from vegetative to reproductive (Murai et al., 2003; Yan et al., 2003; Danyluk et al., 2003; Paolacci et al., 2007).

As an activator of phase transition, *WAP1* expression starts just before the phase transition and is maintained during the reproductive phase, and is up-regulated by vernalization and long photoperiod. The nulli-tetrasomic analysis indicated that *WAP1* has three homoeologous genes located on chromosome 5A, 5B, and 5D

respectively in allohexaploid wheat (Murai et al., 2003).

VRN1 is ortholog of *WAP1*, and consequently three homoeologous genes of *VRN1* located on group 5 homoeologous chromosomes (Yan et al., 2003). How is *VRN1* gene up-regulated by vernalization? In diploid wheat, Dubcovsky et al. (1998) identified a second gene affecting vernalization response and named it *VRN2*. Contrary to the *VRN1* dominant allele for spring growth habit, *VRN2* allele for winter growth habit is dominant (Tranquilli and Dubcovsky, 2000). The effect of *VRN1* on heading time was significant only when the dominant *VRN2* allele was present, that is *VRN2* is epistatic to *VRN1*. Although the *VRN2* gene has not been cloned, Yan et al. (2003) proposed a model of the vernalization pathway in diploid wheat according to the knowledge of the epistatic interactions between *VRN1* and *VRN2* and the available results. In their model, *VRN2* encodes a repressor of *VRN1* expression, which binds to the promoter region of the *VRN1* gene. As the vernalization process reduces the abundance of the *VRN2* gene product, *VRN1* transcription gradually increased, leading to the competence to flower.

TaVRT-1 (*Triticum aestivum* vegetative to reproductive transition-1) which also identified as *TaVRN1* (Kane et al., 2007) was isolated from winter wheat. Molecular and sequence analyses indicated that this gene encodes a protein homologous to the MADS-box family of transcription factors that comprises certain flowering control proteins in *Arabidopsis*. Mapping studies have localized this gene on the long arms regions of homeologous group 5 chromosomes. The level of expression of *TaVRT-1* is positively associated with the vernalization response and transition from vegetative to reproductive phase (Danyluk et al., 2003).

TaAPI-1 is closely related to the wheat homoeologous genes *TaVRN-1*, (Fu et al., 2005), which are involved in the transition from vegetative to reproductive phase induced by vernalization. *TaAPI-2* was expressed higher in mature leaves, spikes and in the flower vegetative organs, except the lodicules. *TaAPI-3* was expressed in all development stages of spikes and in vegetative organs of the spikelets, but at lower level in the lodicules (Paolacci et al., 2007).

As a whole, the function of *API*-like genes in wheat is still unclear, as compared to other species such as *Arabidopsis* and rice, probably owing to the lack of loss-of-function analysis and to the genetic redundancy of these genes.

4. B-class genes in wheat

As explained by the ABC model, class-B genes are involved in the homeotic transformation of stamens into carpel/pistil-like structures in dicots such as *Arabidopsis* (Goto and Meyerowitz, 1994; Jack et al., 1992) and *Antirrhinum* (Sommer et al., 1990; Tröbner et al., 1992). In monocots, transgenic rice expressing antisense RNA of the class-B gene *OsMADS4* and *OsMADS2* displayed alteration of stamens into a carpel-like organ (Kang et al., 1998), and a maize class-B gene-deficient mutant, *silky1*, showed male sterility due to homeotic conversions of stamens into carpels (Ambrose et al., 2000). Nowadays, there are two *AP3*-like genes (*TaMADS#51* and *TaMADS#82*) and four *PI*-like genes (*WPII*, *WPI2*, *TaPI-1* and *TaPI-2*) in wheat had been isolated and identified (Murai et al., 1998; Hama et al., 2004; Paolacci et al., 2007).

Murai et al. (1998) isolated two wheat *AP3*-type genes, *TaMADS#51* and *TaMADS#82*, which are highly homologous to each other. Bread wheat is a hexaploid with genome constitution AABBDD and each genome originated from three diploid related species (Feldman, 2001). Therefore, the hexaploid wheat genome generally contains triplicated homeologous genes derived from the ancestral diploid species. *TaMADS#51* and *TaMADS#82* are homoeologous genes of the *AP3* ortholog of wheat (*WAP3*), located on chromosomes 7B and 7D, respectively. Northern blot analysis revealed that the expression of *WAP3* was restricted to young spikes at the floral-organ-developing stage, suggesting that *WAP3* functions in floral organ formation (Murai et al., 1998). At the same time, the *WAP3* gene was reduced expression in the young spikes of the alloplasmic wheat line with pistillody compared with the normal line (Murai et al., 2002). This suggests the involvement of *WAP3* in the induction of pistillody in the alloplasmic lines.

The wheat genome contains four *PI*-like genes, namely, *WPII*, *WPI2*, *TaPI-1* and *TaPI-2*. Phylogenetic reconstruction indicated that *WPII* is orthologous to *OsMADS4* and that *WPI2* is probably an ortholog of *OsMADS2* (Murai et al., 2003). Both *OsMADS4* and *OsMADS2* genes were suggested to be *PI* orthologs in rice (Chung et al., 1995), and the function of *OsMADS4* as a class-B gene was proven by the transgenic study (Kang et al., 1998). An in situ hybridization study demonstrated that the *WPII* gene is expressed in primordia of lodicules and stamens in developing florets in wheat. In the alloplasmic wheat line exhibiting pistillody, the *WPII* transcripts were not detected in the primordia of pistil-like stamens, whereas *WPII* was expressed in the lodicules. (Murai et al., 2003). This clearly indicates that the pistillody resulted from a deficiency of *WPII* gene expression in whorl 3. Despite the homeotic change of stamens into pistil-like structures, normal lodicules are

developed in the alloplasmic wheat line (Murai et al., 2002). The *PI*-like class-B gene *WPI1* and *WPI2* showed an expression pattern similar to that of *WAP3* (*TaMADS#51* and *TaMADS#82*). *TaPI-1* and *TaPI-2* are 99.5% identical to *WPI1* and *WPI2*, respectively, and there are only one amino acid changes. So *TaPI-1* and *WPI1*, *TaPI-2* and *WPI2* may be representing the same gene. Those results revealed that down regulation of the class B genes wheat *PISTILLATA* (*WPI*) and wheat *APETALA3* (*WAP3*) induces pistillody, the homeotic transformation of stamens into carpel-like organs (Hama et al., 2004).

5. C-class genes in wheat

C-class genes, such as *Arabidopsis AGAMOUS* (*AG*) and rice *OsMADS58* play important roles in the specification of stamen and carpel identity, the control of floral meristem determinacy, and the negative regulation of A-function gene activity (Coen et al., 1991). The wheat genome contains five C-class genes, *WAG1*, *WAG2*, *TaMS-MADSbox*, *TaAG-1* and *TaAG-2* (Meguro et al., 2003; Hirabayashi et al., 2009; Zhou et al., 2008; Paolacci et al., 2007). The same to the B-class genes in wheat, C-class genes could be associated with the induction of pistillody in the alloplasmic wheats.

Southern blot analysis proved that the wheat genome contains three homoeologous *WAG-1* genes, located on the homoeologous chromosome group 1, 1A, 1B and 1D, and had two different *WAG-1* gene transcripts (about 1.1 kb and 1.3 kb in size) in spikes at the booting to heading stage (Meguro et al., 2003). In the euplasmic and alloplasmic wheat lines, the 1.1 kb *WAG-1* gene transcript accumulates in both reproductive (pistil and stamen) and non-reproductive (palea and lemma) portions of spikes at the heading stage in both lines. However, the 1.3 kb transcript is restricted to pistil-like stamens of the line alloplasmic as well as to pistils of euplasmic line. These results suggest that the product of the 1.3 kb transcript is involved in pistil development, and is associated with the pistillody caused by the nuclear-cytoplasm interaction in alloplasmic wheat lines (Meguro et al., 2003).

Hirabayashi et al. (2009) identified a contig with high sequence similarity to *OsMADS3* in rice (Kang et al., 1995) and *ZMM2* in maize (Theissen et al., 1995), and named *WAG-2*. The phylogenetic tree indicated that the monocot class C gene family separated into two groups, *WAG-1* clade and *WAG-2* clade. The *WAG-1* clade contains barley *HvAG2*, rice *OsMADS58* (Yamaguchi et al., 2006) and maize *ZAG1* (Mena et al., 1996), whereas the *WAG-2* clade consists of barley *HvAG1*, rice *OsMADS3* (Kang et al., 1995) and maize *ZMM2* (Theissen et al., 1995). This indicates that *AG* orthologs were duplicated in each monocot species. The phylogenetic tree also indicates that dicot class C genes, *Arabidopsis AG* and *Antirrhinum PLE*, are more close to *WAG-2* clade than *WAG-1* clade (Hirabayashi et al., 2009). But nowadays, the function about *WAG-2* is unclear.

TaMS-MADSbox gene was isolated from thermo-sensitive male sterility line, and the results of RT-PCR showed that the expression of this gene in male sterile spikes was much higher than that in fertile spikes. It suggests that the expression of *TaMS-MADSbox* is related to the fertility conversion of male-sterile lines. The spikes are male-sterile under high-level expression of *TaMS-MADSbox*, while fertile under the low-level expression (Zhou et al., 2008).

RT-PCR detected the *TaAG-1* and *TaAG-2* with increasing levels during the spike development, in stamens and pistils. The only divergence was the ten times higher expression of *TaAG-2* in stamens than in pistils, whereas the expression of *TaAG-1* was similar in stamens and pistils (Paolacci et al., 2007).

In conclusion, the class C gene and class B gene have the similar functions in wheat, and they can induce pistillody. But there are still many gaps in our knowledge of pistillody in wheat. To understand the molecular mechanism of this phenomenon, we need to obtain other class C genes and class B genes that are expressed in stamen and pistil primordia.

6. D-class genes in wheat

Arabidopsis SEEDSTICK (*STK*) and its *Petunia* orthologs, *FBP7* and *FBP11*, regulate ovule identity and these MADS-box genes are known as D-class genes (Colombo et al., 1995; Pinyopic et al., 2003). In *Arabidopsis*, the D-class gene *STK* and the C-class genes *AG*, *SHP1*, and *SHP2* redundantly regulate the specification of ovule identity (Pinyopic et al., 2003). Until now, only three class D genes in wheat genomes had been isolated, namely, *WSTK*, *TaAG-3* and *TaAG-4* (Yamada et al., 2009; Paolacci et al., 2007). *WSTK* shows high similarity to *OsMADS13*, a class D gene in rice. The *WSTK* was isolated from pistil-like stamen in the alloplasmic wheat. The in situ expression analysis indicated that the *WSTK* was expressed in the primordia of ectopic ovule in the pistil-like stamens as well as in the true pistil, suggesting that the *WSTK* plays a role in determination of ovule identity in the pistil-like stamen, but complete ovule development fails due to aberrant expression of *WBS* gene (Yamada et al., 2009). *TaAG-3* and *TaAG-4* had very divergent expression patterns. *TaAG-4* showed a pattern similar to that of class C genes, but its expression was higher during late spike development, very weak in

stamens, very high in pistils and caryopses; its expression pattern was compatible with its function as class D gene. *TaAG-3* was expressed in glumes, palea, lemma, lodicules, stamens and pistil, suggesting a functional divergence from its orthologs in other species (Paolacci et al., 2007).

7. SEP-LIKE GENES (E-class genes) in wheat

Arabidopsis *SEPALLATA* genes (*SEP1*, *SEP2*, *SEP3*, and *SEP4*) form an integral part of the mechanisms underlying floral organ specification (Pelaz et al., 2000; Honma et al., 2001; Pelaz et al., 2001; Favaro et al., 2003; Ditta et al., 2004). These *SEP*-like genes can form heterochimeric protein complexes with the products of the class A, B, C and D genes that regulate identity acquirement of sepals, petals, stamens, carpels and ovules (Honma et al., 2001; Pelaz et al., 2001; Favaro et al., 2003). The *SEP*-like genes in the angiosperms are divided into two major clades: the *SEP3* clade and the *LOFSEP* clade (Malcomber et al., 2005). The wheat genome contains nine *SEP*-like MADS-box genes: four of these, *WSEP*, *TaMADS1*, *TaSEP-3* and *TaSEP-4* are classified as members of the *SEP3* clade, whereas the others, *WLHS1*, *TaSEP-1*, *TaSEP-2*, *TaSEP-5* and *TaSEP-6* are classified as member of the *LOFSE* (Shitsukawa et al., 2007; Xiang et al., 2006; Paolacci et al., 2007).

The *TaMADS1* transcripts began to accumulate in spikelets, and then, in floret primordial and floral organ primordial in wheat. In the later stage of floret development, the *TaMADS1* transcripts accumulated in four whorls of young floral organs. The ectopic expression of *TaMADS1* in transgenic *Arabidopsis* caused early flowering and altered the development of all floral organs. Further studies demonstrated that the early flowering phenotype in transgenic plants could be correlated with the upregulation of some flowering time genes and flower meristem identity genes. The results suggest that *TaMADS1* could be a putative *SEP*-like gene, and has diverse roles in flower development (Xiang et al., 2006).

WSEP gene was expressed in the inner three whorls (lodicules, stamens, and pistils) at the floral organ differentiation stage. After floral organ identities had been determined, strong expression of *WSEP* was observed in the palea. The expression patterns suggest that *WSEP* genes are not only involved in floral organ differentiation but also in their subsequent development. Furthermore, overexpression of *WSEP* in transgenic *Arabidopsis* plants caused early flowering and terminal flower formation (Shitsukawa et al., 2007). The three wheat homoeologs of *WSEP* showed similar genomic structure and expression profiles. By contrast, the three homoeologs of *WLHS1* showed genetic and epigenetic alterations. The A genome *WLHS1* homoeolog (*WLHS1-A*) had a structural alteration that contained a large novel sequence in place of the K domain sequence. The *WLHS1-A* protein, which lacks a K domain sequence has lost the normal MADS box function. The B and D genome homoeologs, *WLHS1-B* and *WLHS1-D*, respectively, had an intact MADS box gene structure, but *WLHS1-B* was predominantly silenced by cytosinemethylation. Consequently, of the three *WLHS1* homoeologs, only *WLHS1-D* functions in hexaploid wheat (Shitsukawa et al., 2007).

The expression patterns of *TaSEP-1* and *TaSEP-5* were similar, their transcripts were detected at low level in vegetative tissues such as coleoptile, leaf and stem, at moderate level in developing caryopses and at very high level in spikes at heading time. *TaSEP-2* and *TaSEP-6* showed similar expression levels in vegetative tissues and spikes. The expression of *TaSEP-3* and *TaSEP-4* was very similar to that of their respective rice orthologous *OsMADS7* and *OsMADS8* (Pelucchi et al., 2002); their transcription was restricted to spikes and developing caryopses (Paolacci et al., 2007).

The functions of other *SEP*-like genes in wheat are not fully established, partly because of the functional redundancy of these genes. For example, a loss-of-function mutant of *WLHS1-A* does not show any obvious phenotype (Shitsukawa et al., 2007). It has been suggested, however, that the functions of *SEP*-like genes in wheat may have diversified to some degree. That is, the expression patterns of *SEP*-like genes in the wheat flower are variable and the interactions of *SEP*-like proteins with the products of ABCD MADS-box genes are not identical. Establishing the functional diversification of *SEP*-like genes in wheat and deducing the roles of these genes in the morphological divergence of the floret, spikelet, and inflorescence would throw light on the evolution of the grass family.

8. Future prospects

Although several functional studies have identified the development role of some MADS-box genes in wheat, much remains to be studied, as compared to other species such as *Arabidopsis* and rice. This probably owing to the common wheat is a hexaploid species with the genome constitution AABBDD, it is difficultly to use loss-of-function analysis. Analysis of the interactions of MADS-domain proteins with other types of protein including different MADS-domain proteins will help to elucidate the molecular roles of MADS-box genes in wheat.

Isolation and characterization the three pistils (TP) gene in wheat is also an interesting issue. Because the TP mutation posses normal spike morphology but three pistils in a floret, two more than normal plants, which made it have the potentiality to increase the grain number per spike (peng et al., 2003; 2004; 2008). According to the ABCDE model, the pistil is specified by class C and E gene, so it can be easy speculated that the TP gene might be a class C or E gene, but the detail need further research.

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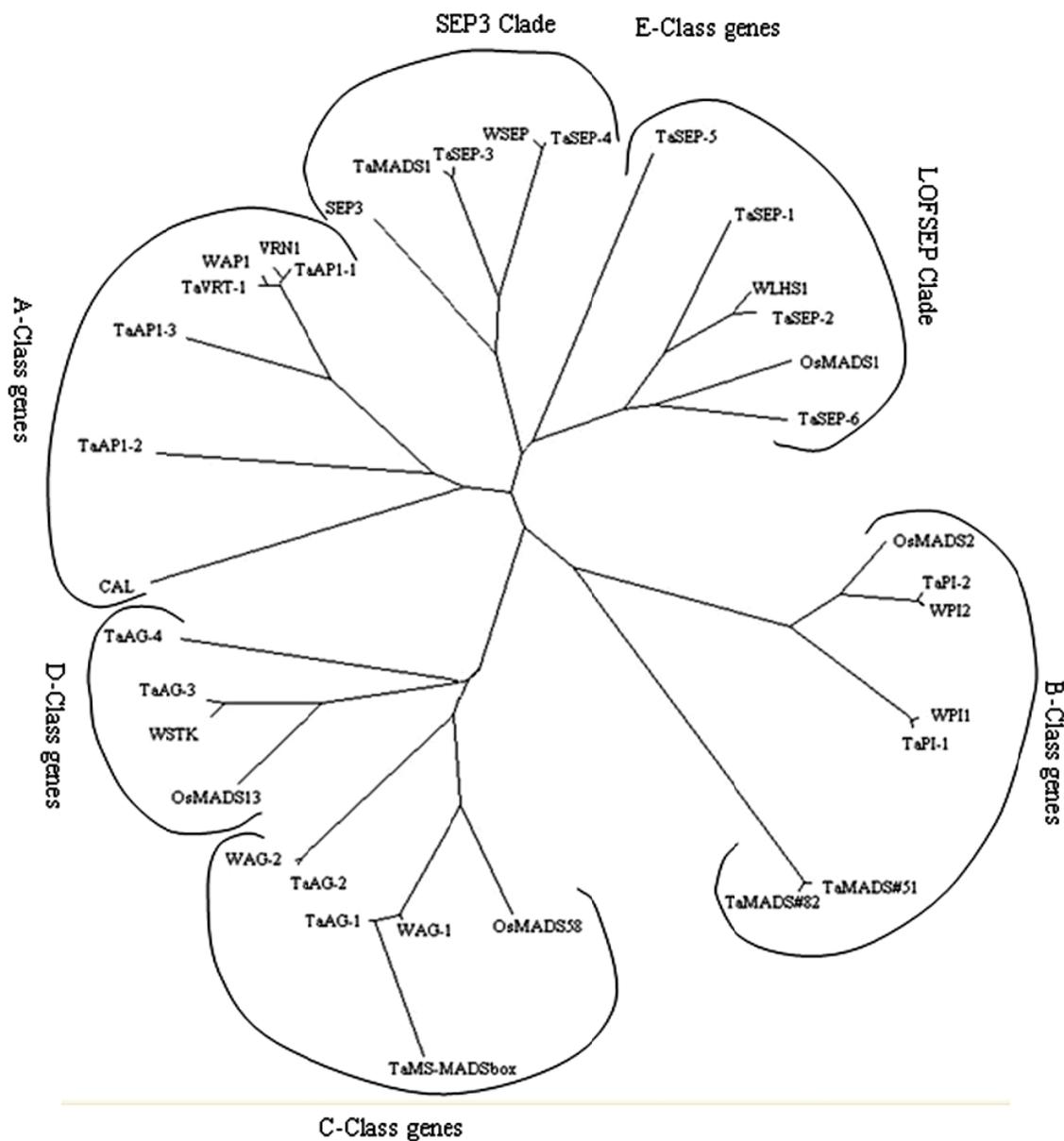


Figure 1. Phylogeny of the major classes of A B C D E genes from wheat, rice and *Arabidopsis*. The phylogenetic tree was constructed using the neighbor-joining method provided by the program of clustalx.