MADS-Box Genes Are Associated with the Petaloidy/Sepaloidy of Stamens in Cytoplasmic Male Sterile *Brassica*

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ABSTRACT MADS-box genes are well known for the ABC model of flower development. In this study, we investigated the expressions of A, B and C functions *Brassica rapa* MADS-box genes in different Ogura cytoplasmic male sterile (CMS) lines of *B. juncea*, *B. oleracea*, and their wild types. We observed two *API*-like (*BjAPI* and *BoCAL1*), three *PISTILLATA*-like (*PI*-like, *BjPI1*, *BoPI1*, and *BoPI2*) and six *AGAMOUS*-like (*AG*-like, *BjAGL1*, *BjAGL2*, *BjAGL3*, *BjAGL4*, *BoAGL1*, and *BoAGL2*) genes to be altered their expressions in the CMS *B. juncea* and *B. oleracea* compared to their wild types. Partial and complete petaloidy in the third whorl (stamen) were observed of two CMS *B. juncea* lines J26 and J27, respectively. Besides, a sepaloidy structure was evident in the third whorl of CMS *B. oleracea* line 25053. Altered expressions of *BjAPI* and *BjPI1* in the fourth whorl (pistil) can be correlated with curved and robust stature of pistils in CMS *B. juncea*. Furthermore, an *in silico* protein interaction analysis revealed that AP-like, *PI*-like, and AG-like proteins are in close association with different MADS-box proteins and LEAFY (LFY), UNUSUAL FLORAL ORGANS (UFO), SEUSS (SEU), LEUNIG (LUG) for different floral organ development. We suggest that expressions of MADS-box genes might be dependent on mitochondrial signaling for cytoplasmic homeosis in CMS *B. juncea* and *B. oleracea*. The expression dataset on A, B, and C functions MADS-box genes of CMS and wild type *B. juncea* and *B. oleracea* presented in this study might be useful for the development of CMS in different *Brassica* species.

Keywords CMS, MADS-box, Petaloidy, Sepaloidy, *Brassica juncea*, *Brassica oleracea*

INTRODUCTION

Development of floral organs is controlled by the homeotic genes in higher plants which have been studied widely in dicotyledonary plants, especially Arabidopsis and Antirrhinum (Theissen and Saedler 1999; Theissen 2001). Normally, a complete flower from dicotyledonary plant has four whorl structures, where sepals are in the first whorl, petals in the second whorl, stamens in the third whorl and carpels inside of flower. Based on the ABC model of floral organ development, involvement of three classes (A, B, and C) of nuclear homeotic genes could explain and predict flower organ families (Davies and Schwarz-Sommer 1994; Ma 1994; Weigel and Meyerowitz 1994). This model has further extended by two other class

D and E genes (Theissen 2001). It is hypothesized that these five classes of genes are associated with their genetic functions through intensive interactions. For specifying of sepals, class-A genes interact with class-E genes. The expressions of class-A, B, and E genes determine petals development and the expressions of class-B, C, and E determine stamens development, respectively. In addition, the combined actions of class-C and E genes transformed into carpels and class-D genes are identified for ovule constructions. The total process is termed as nuclear homeosis (Kramer *et al.* 1998, 2004; Krizek and Fletcher 2005; Hernández-Hernández *et al.* 2007).

Cytoplasmic male sterility (CMS) is a maternally inherited trait where plants fail to produce viable pollen. CMS exists in many plant species and they have been

Received January 25, 2016; Revised February 6, 2016; Accepted February 11, 2016; Published February 28, 2016

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exploited widely for hybrid production. Genetic research gave the evidences that CMS is often related with unusual open reading frames found in mitochondrial genomes, and in many cases, its male sterility can be restored specifically by nuclear-encoded, fertility restorer genes (Wise and Pring 2002; Brown *et al.* 2003; Desloire *et al.* 2003; Koizuka *et al.* 2003).

According to the abortive stage of pollen there are different types of CMS and they exhibit different CMS-related abnormalities in flower morphology (Linke and Börner 2005). Striking alterations in flower morphology were observed in CMS tobacco (Zubko *et al.* 2001), CMS wheat (Murai *et al.* 2002), CMS carrot (Linke *et al.* 2003) and CMS stem mustard (Yang *et al.* 2008).

Any kind of mutations or rearrangements in the transcription of these nuclear homeotic genes can lead to differences in the four whorl structures of a flower. A certain type of flower organ is replaced by others (Coen and Meyerowitz 1991; Mandel *et al.* 1992). There are several reports on mitochondrial mutant and CMS system. The nuclear MADS-box genes, such as *AP3-like*, *GLOBOSA-like* (*GLO-like*), and *DEFICIENS-like* (*DEF-like*), were found to be transcriptionally down-regulated and involved in this kind of alteration (Zubko *et al.* 2001; Murai *et al.* 2002; Linke *et al.* 2003; Teixeira *et al.* 2005). This phenomenon is termed as cytoplasmic homeosis. In cytoplasmic homeosis, rearrangement or replacement of the mitochondrial genome is evident which alters the expressions of nuclear homeotic genes or genes related to homeotic functions (Zubko 2004). Most studies of cytoplasmic homeosis were focused on the CMS systems.

Wild *Brassica* species are the source of a vast cytoplasmic variability for production of alloplasmic male sterile lines, and therefore, they have great importance in case of breeding (Prakash *et al.* 1995; Banga and Banga 1998). This genetic variability of *Brassica* cytoplasm is essential to preserve and enlarge the cytoplasmic diversity in order to afford breeders with new and improved CMS systems and get rid of the possible problem of epidemic diseases (Cardi and Earle 1997). There is report that CMS in *Brassica napus* has been conferred by alien cytoplasm, such as the *Ogura* cytoplasm of radish (Ogura 1968), *B. tournefortii* or the *B. oxyrrhima* cytoplasm (Liu *et al.* 1996;

Banga and Banga 1998) or by endogenous cytoplasm like the *polima* or *nap* cytoplasm from *B. napus* (Li *et al.* 1998; Brown 1999). The *Ogura* cytoplasm also confers male sterility to *B. juncea* (Labana and Banga 1989) and 'CMS *juncea*' promotes CMS in *B. napus* (Pradham *et al.* 1991; Liu *et al.* 1996). And, CMS in *B. oleracea* was developed by the CMS *B. juncea* cytoplasm from *B. rapa* through protoplast fusion (Cardi and Earle 1997).

In this study, we carried out expression analysis of different MADS-box homeotic genes in *Ogura* CMS lines of *B. juncea* and *B. oleracea*. We also made use of their wild types to study the expression of the same set of genes. We identified candidate MADS-box genes which might be responsible for the conversion of homeotic transformations in the selected CMS lines. We speculate nuclear MADS-box transcription factors subject to mitochondrial retrograde regulation are associated with cytoplasmic homeosis in CMS *B. juncea* and *B. oleracea*.

MATERIALS AND METHODS

Plant materials

We selected *B. juncea* and *B. oleracea* *Ogura* CMS lines for the expression analysis of the homeotic genes. Two *B. juncea* *Ogura* CMS lines J26, J27, and one *B. oleracea* rapid cycle *Ogura* CMS line 25053 along with their wild relatives (*B. juncea* J21 and *B. oleracea* 25050) were grown in soil culture media in a green house with a dark/light cycle of 8/16 hours at 25°C at the Department of Horticulture, Sunchon National University, Korea. For the flower organ study, 100 fresh flower buds were harvested and four floral parts sepals, petals, stamens and pistils were separated and frozen immediately in liquid nitrogen, and stored at -80°C for RNA isolation.

Isolation of nucleic acid and reverse transcription

Total RNA was isolated from the every whorl of floral organs using an RNeasy mini kit (Qiagen, Hilden, Germany), after which it was treated with RNase-free DNase (Qiagen) for 15 min at ambient temperature to remove genomic DNA contaminants. Total amount of RNA was determined by ultraviolet spectrophotometry. A reverse transcription

kit Superscript III (Invitrogen, Carlsbad, CA, USA) was used to transcribe total RNA into cDNA.

Identification of MADS-box genes and *in silico* analysis

We selected 20 flower homeotic *B. rapa* MADS-box genes for the expression study in CMS *B. juncea* and *B. oleracea* flower organs based on our previously published dataset (Saha *et al.* 2015). Polymerase chain reaction (PCR) primer information was also collected from our published data. An online tool STRING 10 (<http://string-db.org/>) was used to predict association and interaction of MADS-box proteins with other proteins.

Expression analysis of MADS-box genes

Samples from every whorl of *B. juncea* and *B. oleracea* flowers were collected from CMS lines and their wild types as well. Reverse transcription (RT)-PCR was conducted using an AMV one step RT-PCR kit (Takara Bio Inc., Shiga, Japan). Specific primers for all genes as mentioned in Supplementary Table 1 were used in RT-PCR. *B. rapa* *Actin* (*BrActin*) primer was used as control for RT-PCR expression analysis (Supplementary Table 1, available online only). PCR was conducted using 50 ng cDNA from the plant and flower organs as templates in master mixes composed of 20 pmol each primer, 150 μ M each dNTP, 1.2 U Taq polymerase, 1 $\times$ Taq polymerase buffer and double-distilled H₂O diluted to a total volume of 20 μ l in 0.5-ml PCR tubes. The samples were subjected to the following conditions: pre-denaturing at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 45 seconds, with a final extension for 5 minutes at 72°C.

RESULTS

Flower phenotypes of CMS *B. juncea* and *B. oleracea* and their wild types

An interesting alteration in the morphology of floral organs of CMS *B. juncea* lines (J26 and J27) was observed. Flowers of CMS *B. juncea* reduced in size compared to their wild types (Fig. 1A). Specifically, the size of the

petals was dramatically reduced. Besides, size and shape of the pistils in CMS *B. juncea* were also changed. Pistils in CMS J26 became larger in size and severely curved with a deformed stigma, whereas the pistils in case of CMS J27 showed only larger stature keeping other features intact. CMS *B. juncea* exhibited single and mixed homeotic transformation of stamens. In CMS J26, stamens have been transformed into a mixed structure where both whorl two (stamen) and whorl three (petal) organs were sticking. And, in J27 CMS flowers whorl three (stamen) completely transformed into whorl two i.e., petaloidy (Fig. 1B). Besides, *B. oleracea* 25053 CMS line also exhibited reduced flower size with severely curved pistils compared to its wild type. More importantly, a complete homeotic conversion of the whorl three (stamen) into whorl one (sepaloid) with a green appearance has been observed here.

Selection of MADS-box homeotic genes for expression analysis

Evolutionary relations among different *Brassica* species revealed that they evolved from a common ancestor following ancient genome triplication along with numerical and structural chromosomal changes that occurred 7.9-14.6 million years ago (MYA) (Lysak *et al.* 2005). The core *oleracea* lineage that gave rise to *B. oleracea* and *B. rapa* originated $\approx$ 3 MYA (Arias *et al.* 2014). Besides, *B. juncea* is an amphidiploid, evolved from *B. rapa* and *B. nigra* which contained the intact *Brassica* A genome. Moreover, data from molecular studies suggest that *B. rapa* was the cytoplasmic donor of *B. juncea*. Considering the evolutionary relationship, we were highly optimistic to find constitutive and corresponding expressions of *B. rapa* MADS-box (*BrMADS-box* from here on) homeotic genes in different *B. juncea* and *B. oleracea* lineage.

MADS-box genes are the major components of the well-known ‘ABC’ model where they perform core functions in flower organ development of higher plants (Ma and dePamphilis 2000). We identified and characterized MADS-box genes relating to A, B, and C functions in the *B. rapa* genome (Saha *et al.* 2015). From our published dataset, we identified 20 *BrMADS-box* genes those showed constitutive expression patterns in flowers of *B. rapa*, have been considered here to check their expressions in CMS

Fig. 1. A representation of *Brassica juncea* wild type (J21) and cytoplasmic male sterility (CMS; J26 & J27) phenotypes with expression profiles of A, B, and C class MADS-box genes in the four flower whorls. (A) Showing four flower organs (whorl 1-4; sepal, petal, stamen, and pistil) in wild type *B. juncea* J21 where all the organs showed regular patterns. Besides, CMS J26 phenotypes showed a reduced petal, sticking stamen, and comparatively large curved pistil. And, CMS J27 phenotypes are presented with the smallest petals, petaloid stamen, and a large pistil. (B) Showing comparative expressions of A (*BjAP1*), B (*BjPI1*), and C (*BjAGL1*, *BjAGL2*, *BjAGL3*, and *BjAGL4*) class MADS-box genes in CMS lines and their wild type.

lines of *B. juncea*, *B. oleracea*, and their wild relatives (Supplementary Fig. 1, available online only). All these genes were under the MADS-box family due to their conserved MADS-box domain in the N-terminal regions (Saha *et al.* 2015). We selected two *BrMADS*-box genes from *AGL15*-like subfamily (*BrMADS3* and 5), three from *GGM13*-like (*BrMADS26*, 27, and 28), three under *GLO*-like (*BrMADS29*, 30, and 31), two from *DEF*-like (*BrMADS32* and 33), seven MADS-box genes from *AGAMOUS*-like (*BrMADS53*, 54, 55, 56, 57, 59, and 60) and three genes from *SQUA*-like subfamily (*BrMADS61*, 63, and 70). Based on the expression patterns of these flower expressed genes, we focused on eight MADS-box genes (*BrMADS63*, *BrMADS70*, *BrMADS30*, *BrMADS31*, *BrMADS53*, *BrMADS55*, *BrMADS57*, and *BrMADS59*) from A, B, and C category which had

constitutive expression patterns in different flower organs of *B. juncea* and *B. oleracea* Ogura CMS lines including their wild types (Table 1).

Expression analysis of MADS-box homeotic genes in CMS *B. juncea*

In CMS *B. juncea*, we found homeotic transformation of whorl three (stamen) into other structures (Fig. 1A). To investigate the ABC model of flower development, here we investigated the expressions of A, B, and C functions MADS-box genes in two *B. juncea* CMS lines J26 and J27 and also in their wild type J21 (Table 1). In the wild type *B. juncea* J21, *BjAP1* (*APETALA1*-like under *SQUA*-like subfamily) gene from class A was expressed in sepal, petal and stamen (whorl one, two, and three) (Fig. 1B). From the B class gene, *BjPI1* (*PISTILLATA*-like) showed high

Table 1. List of *MADS*-box genes was used for expression study under ‘ABC’ model of flower development in cytoplasmic male sterility *Brassica juncea* and *B. oleracea*.

Category under ABC model	Name of MADS-box subfamily	<i>B. rapa</i> MADS-box	<i>BrMADS</i> -box homologs in <i>B. juncea</i>	<i>BrMADS</i> -box homologs in <i>B. oleracea</i>
A - Function	<i>APETALA1</i> -like (<i>API</i>)	<i>BrMADS63</i>	<i>BjAPI</i>	
	<i>CAULIFLOWER</i> -like (<i>API</i>)	<i>BrMADS70</i>		<i>BoCAL1</i>
B - Function	<i>PISTILLATA</i> -like	<i>BrMADS30</i>		<i>BoPI1</i>
	<i>PISTILLATA</i> -like	<i>BrMADS31</i>	<i>BjPI1</i>	<i>BoPI2</i>
C - Function	<i>AGAMOUS</i> -like	<i>BrMADS53</i>	<i>BjAGL1</i>	<i>BoAGL1</i>
	<i>AGAMOUS</i> -like	<i>BrMADS55</i>	<i>BjAGL2</i>	
	<i>AGAMOUS</i> -like	<i>BrMADS57</i>	<i>BjAGL3</i>	
	<i>AGAMOUS</i> -like	<i>BrMADS59</i>	<i>BjAGL4</i>	
	<i>AGAMOUS</i> -like			<i>BoAGL2</i>

expression in petals and stamens (whorl two and three) with a very low level expression in the sepals (whorl one). And, under class C, four *AGAMOUS*-like genes (*BjAGL1*, 2, 3, and 4) showed their expressions exclusively in whorl three (stamens) and four (pistils). Expression patterns of these genes in *B. juncea* support the ABC model of flower development. However, their expressions were found to be altered in the CMS *B. juncea* lines. *BjAPI* in CMS J26 was additionally expressed the pistils besides sepals, petals and stamens. In case of *BjPI1*, we found a differential expression patterns in all the flower whorls. Specifically, in J26 CMS line, expressions of *BjPI1* in sepals and petals were altered compared to its wild type. Expression patterns of four *AGAMOUS*-like genes in CMS J26 and J27 were altered dramatically. *BjAGL1* expressed in sepals of J26 and sepals and petals of J27, in addition to stamen and pistil expressions. *BjAGL2* showed expressions mainly in the sepals and stamens of J26. Besides, in CMS J27, *BjAGL2* was expressed in the three whorls except sepals. Two other *AG-like* genes, *BjAGL3* and *BJAGL4*, showed expressions in all the flower whorls of J26 flower, where transcript levels of both these genes were altered compared to wild type J21. In case of J27 CMS, *BjAGL3* did not show any expression in stamens rather it expressed in the sepals and petals beside pistils. But, expression of *BjAGL4* was observed in all the four flower whorls of CMS J27.

Expression analysis of MADS-box homeotic genes CMS *B. oleracea*

In *B. oleracea* CMS 25053, a complete homeotic

conversion of whorl three (stamen) into whorl one (sepaloid) has been observed (Fig. 2A). To understand the reasons behind this alteration, we closely investigated expression patterns of A, B, and C functions MADS-box genes in CMS *B. oleracea* and compared their expressions with wild type 25050. In the wild type *B. oleracea*, *BoCAL1*, which is an A- function MADS-box gene (*CAULIFLOWER*-like/*API*-like), showed expression in the whorl one, two and three. On the other hand, in Ogura CMS 25053, it showed expression with higher transcript abundance in whorl one (sepal) and two (petals) and which has been drastically reduced in whorl three (stamen). Additionally, it expressed at lower level in the whorl four (pistil). From the B- function gene, we found two *PI-like* genes *BoPI1* and *BoPI2* to be expressed constitutively between the CMS line and its wild type. In the wild type *B. oleracea*, *BoPI1* exhibited expressions mainly in the whorl two and three. These expression patterns have been changed dramatically in the CMS line, where the transcript level of *BoPI1* has been increased a bit more in the whorl two (petal). Conversely, expression of this gene in whorl three (stamen) drastically reduced nearly to zero. Furthermore, it expressed in the whorl one (sepal) and whorl four (pistil) of CMS *B. oleracea*. Besides, another *PI-like* B-function gene *BoPI2* in wild type showed very high expressions in whorl one, two and three compared to whorl four. In CMS line, expression of *BoPI2* in the whorl two and four found to be increased, which have been reduced in whorl one and three. Finally, from the C- function *AG-like* gene, we closely compared expression of two genes

Fig. 2. A representation of *Brassica oleracea* wild type (25050) and cytoplasmic male sterility (CMS) 25053 phenotypes with expression profiles of A, B, and C class MADS-box genes in the four flower whorls. (A) Showing four flower organs (whorl 1-4; sepal, petal, stamen, and pistil) in wild type *B. oleracea*, where all the organs showed regular patterns. Besides, CMS 25053 phenotypes showed comparatively larger sepals, sepaloid stamens, and a curved pistil. (B) Showing comparative expressions of A (*BoCAL1*), B (*BoPI1* and *BoPI2*), and C (*BoAGL1* and *BoAGL2*) class MADS-box genes in CMS line and its wild type.

(*BoAGL1* and *BoAGL2*) between the CMS *B. oleracea* and its wild type. *BoAGL1* in wild type showed highest expression in the whorl four besides very low level expressions in the whorl one and three. Interestingly, its whorl three expression in the CMS line surprisingly increased along with greater increment of its transcripts in whorl one and two as well. Another *AG-like* gene *BoAGL2* expressed exclusively in the whorl four of wild *B. oleracea* which has also found to be expressed in the whorl three in addition to the whorl four of CMS *B. oleracea*.

Analysis of interactions among homeotic MADS-box proteins

We constructed a protein association network among A,

B, and C functions BrMADS-box proteins (predicted close homologs in *B. juncea* and *B. oleracea*) and related *Arabidopsis* proteins using online software String 10.0 (Fig. 3). We found our query MADS-box proteins (*BjAP1*, *BoCAL1*, *BjPI1*, *BoPI1*, *BoPI2*, *BjAGL1*, *BjAGL2*, *BjAGL3*, *BjAGL4*, *BoAGL1*, and *BoAGL2*) were in stronger association with other homeotic proteins including A, B and C functions MADS-box proteins. We found that the A function proteins (*BjAP1* and *BoCAL1*) interacted with SHORT VEGETATIVE PHASE (SVP) and AGL24. Besides, three B function proteins *BjPI1*, *BoPI1*, and *BoPI2* (*PI-like*) exhibited stronger association with the AP1/2/3, LEAFY (LFY), and UNUSUAL FLORAL ORGANS (UFO). And, we observed six *AGAMOUS-like*

Fig. 3. Interaction network among predicted *Brassica juncea* and *B. oleracea* homeotic MADS-box proteins (marked in bold letter) and related functional partners based on their close *Arabidopsis* counterpart proteins. Stronger associations are represented by thicker lines.

C function proteins BjAGL1, BjAGL2, BjAGL3, BjAGL4, BoAGL1, and BoAGL2 interacted strongly with AP1/2/3, TRANSPARENT TESTA16 (TT16), PI-like (BjPI1, BoPI1, and PoPI2), SEUSS (SEU), and LEUNIG (LUG) flower organ development related proteins. We speculate these predicted functional partners of A, B, and C functions MADS-box proteins might play important roles in specifying different flower whorls in *B. juncea* and *B. oleracea*.

DISCUSSION

CMS is an outcome of miscoordination between foreign nuclear and cytoplasmic gene products (Aviv and Galun 1980). These alterations are primarily caused by mutations,

rearrangements, and/or recombinations inside the mitochondrial genome (Carlsson and Glimelius 2011). And, changes in mitochondrial genes are associated with the development of male sterility. Normally, homeotic transformation of floral organs can be observed in the male sterile condition (Linke and Börner 2005). Only mitochondrial factors do not control reproductive development in plants. We speculate there might some signaling activities from mitochondria to nucleus could be involved in the homeotic transformation of CMS floral organs and it happens through set of CMS-inducing genes.

In this study, phenotypic variations in floral organization and stamens have been observed in the Ogura CMS lines of *B. juncea* (J26 and J27) and *B. oleracea* (25053). Similar types of variations were also observed in some *Brassica* alloplasmic cytoplasm, e.g., the *Raphnnus*/Ogura (Bannerot

et al. 1974), species cross *B. nigra* (L) Koch/*B. oleracea* L. (Pearson 1972) and *B. napus* with *Arabidopsis* cytoplasm (Leino *et al.* 2003). In higher plants, flower development mechanism has been interpreted through the functioning of ABC model, where mutation and over-expression of these genes might lead to homeotic transformations in the flower organization (Zubko 2004). In this study, we investigated the expressions of *API-like*, *PI-like*, and *AG-like* MADS-box genes where they expressed absolutely in their whorls of floral organ as per ABC model in the wild types of *B. juncea* and *B. oleracea* (Fig. 1, 2). However, their reduced/alterd transcript levels in the CMS lines could possibly involved in the development of petaloid and sepaloid stamens.

According to ABC flower development model, nuclear homeotic genes are expected to be expressed organ-specifically in the individual flower whorls of normal plants. Expression analysis of *API-like* (*BjAPI* and *BoCAL1*), *PI-like* (*BjPII*, *BoPII*, and *BoPI2*) and *AG-like* (*BjAGL1*, *BjAGL2*, *BjAGL3*, *BjAGL4*, *BoAGL1*, and *BoAGL2*) genes in CMS *B. juncea* (J26 and J27) and *B. oleracea* (25053) revealed their ectopic expressions, of which *AP-like* and *AG-like* genes were unexpectedly expressed in pistils and petals, respectively. These types of homeotic conversions have also been evident in CMS *B. juncea* var. *tumida* Tsen et Lee (stem mustard) and *B. napus* (Geddy *et al.* 2004; Yang *et al.* 2008). In *B. juncea* CMS J26 and J27, pistil expressions of *API-like* and *PI-like* genes can be correlated with the phenotypes, where they produced severely curved and robust pistils. Besides, altered expressions of *PI-like* gene in the second whorl might be responsible for the reduced growth of petals. These phenomenons have also been observed in case of *AP3* and *PI* mutant phenotype of *Arabidopsis*, where stamens were fully replaced by carpels and petals were reduced (Bowman *et al.* 1989; Farbos *et al.* 2001). Meur *et al.* (2006) also reported about the modification of stamens into petals in CMS Ogura *B. juncea*. And interestingly, in the case of CMS *B. oleracea* *API-like* and *PI-like* expressions have almost abolished in stamens, instead they showed increased transcript levels in the sepals and petal. Finally, a sepaloid phenotype in the third whorl of this CMS was found (Fig. 2). This phenomenon is very rare

among the features of other CMS in higher plants. In mutant *Petunia*, Van Der Krol and Chua (1993) reported sepaloid stamens with normal stamens in the inner two whorls. In a nut shell, alterations in the functions of A, B, and C class genes in the CMS *B. juncea* and *B. oleracea* might be the underlying causes for the development of aberrant flowers. And, we speculate that two different phenotypes like petaloidy and sepaloidy in *B. juncea* and *B. oleracea* Ogura CMS lines might be due to differential interaction effects between mitochondrial genes and different- A function (*AP-like*) nuclear MADS-box genes.

On the other hand, a strong association was evident among *API*, *LFY* and *UFO* proteins in Sasaki *et al.* (2012) study, which regulates expression of B class genes in *Arabidopsis*. In this study, we conducted an *in silico* protein interaction study using string database which also revealed strong interactions among *API*, *LFY*, and *UFO* proteins (Fig. 3). Moreover, interactions among *AG-like*, *SEU* and *LUG* proteins have been reported in Frank *et al.* (2002) study, where they demonstrated that *SEU* interacts together with *APETALA2* and *LEUNING* to repress *AGAMOUS* expression. In conclusion, CMS *B. juncea* and *B. oleracea* showed partial and complete homeotic transformation of stamens into petaloid and sepaloid structures. We speculate that in the CMS line, expressions of *API-like* (*BjAPI* and *BoCAL1*), *PI-like* (*BjPII*, *BoPII*, and *BoPI2*) and *AG-like* (*BjAGL1*, *BjAGL2*, *BjAGL3*, *BjAGL4*, *BoAGL1*, and *BoAGL2*) genes those encode MADS-box transcription factor might be influenced by mitochondrial genome and thus, a retrograde signalling between mitochondria and nucleus might trigger ectopic expressions of the floral homeotic genes (A, B, and C) in different whorls. We assume that expressions data related to floral organ development of wild and CMS *B. juncea* and *B. oleracea*, presented in this study might be useful for undertaking specific investigation to uncover the mechanism of floral homeotic conversions in different CMS *Brassica*.

ACKNOWLEDGEMENTS

This research was jointly supported by Basic Science Research Program through the National Research Foun-

dition of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A1A2044500) and Golden Seed Project (Center for Horticultural Seed Development, No. 213003-04-3-SB110, 213003-04-3-SB420), Ministry of Agriculture, Food and Rural Affairs (MAFRA), Ministry of Oceans and Fisheries (MOF), Rural Development Administration (RDA) and Korea Forest Service (KFS).

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