Cloning and Expression of *Omission of Second Division-like* Genes from Carnation

Xuhong Zhou, Xijun Mo, Yalian Jiang, Hao Zhang, Rongpei Yu, Lihua Wang, Jihua Wang¹ and Suping Qu¹

Flower Research Institute, Yunnan Academy of Agricultural Science, Kunming 650205, China; and National Engineering Research Center for Ornamental Horticulture, Kunming 650205, China

ADDITIONAL INDEX WORDS. *Dianthus caryophyllus*, *OSD1*, meiosis, 2n pollen, mechanism, polyploidization

ABSTRACT. The *omission of second division* gene (*OSD1*) gene plays a fundamental role in meiosis and is associated with 2n gamete formation in *Arabidopsis thaliana*. The objective of this work was to unravel the mechanisms leading to 2n pollen production, and isolate and analyze the expression patterns of *OSD-like* (*OSDL*) genes in carnation (*Dianthus caryophyllus*). We found an absence of the second meiotic division caused the formation of 2n pollen. Three homoeologous genes were cloned and labeled as *OSDLa*, *OSDLb*, and *OSDLc* in a diploid carnation. The cDNAs were 1180 bp for *OSDLa*, 1288 bp for *OSDLb*, and 971 bp for *OSDLc*. A strong similarity was found between the amino sequences of *OSDLb* and *OSDLc*. An evident feature of *OSDLs* proteins is the presence of D-box and MR-tail domains; however, the GxEN/KEN-box domain, which is distinct among the other plant proteins was absent. Quantitative real time polymerase chain reaction (qRT-PCR) analysis showed that *OSDL* genes maintain continuous expression in buds and other tissues. *OSDLa* has the highest expression in buds of 1.1–1.2 cm long (stage 2), and *OSDLb* has a high level of expression in buds of 0.9–1.0 cm long (stage 1) and stage 2 buds and ovary tissues in three carnation cultivars. The expression level of *OSDLc* was highest in ovaries. These expression patterns strongly suggest that *OSDLs* in carnation involve male meiosis and ovary development. These findings can have potential applications in fundamental polyploidization research and plant breeding programs in carnation.

Meiosis is a specialized cell division essential for sexual reproduction. It involves a single DNA replication event followed by two rounds of chromosome segregation- meiosis I (M I) and meiosis II (M II) – to produce cells with half the chromosome number of the mother cell. Thus, to generate haploid spores, a meiocyte must enter M I after prophase, pass through the M I to M II transition, and complete M II. Errors in transitions are not uncommon and may lead to the production of viable, 2n gametes, as have been described in a range of plants (Bretagnolle and Thompson, 1995; Ramanna and Jacobsen, 2003). Unreduced gamete is now believed to be the major route for polyploidization through sexual reproduction. Polyploidy has played a key role in the evolution of many fungal, plant, invertebrate, and vertebrate lineages, and is particularly frequent in plants (Otto, 2007; Otto and Whitton, 2000). 2n gametes are also an important tool for plant breeding (Ramanna and Jacobsen, 2003). However, despite the evolutionary and agricultural significance of sexual polyploidization in plants, the underlying genes have not been fully characterized.

Unreduced gamete formation derived via abnormal meiotic cell division is an important phenomenon for polyploid breeding (Ramanna and Jacobsen, 2003). More than 10 abnormal mechanisms leading to 2n gamete formation have been reported to date (Bretagnolle and Thompson, 1995). Within the same species, several abnormal cytogenetic events can lead to the formation of the 2n gametes (Gómez-Rodríguez et al., 2012;

Teoh, 1984). Teoh reported four mechanisms that resulted in 2n gamete formation in orchid species belonging to the Arethuseae tribe (Teoh, 1984): 1) premeiotic disturbances, 2) faulty anaphase disjunction, 3) failure to participate in M I or M II, and 4) co-orientation of the two spindles during metaphase II. In *Agave tequilana* and *Agave angustifolia*, two different mechanisms led to 2n gamete formation: failure of cytokinesis in M II and abnormal spindles (Gómez-Rodríguez et al., 2012). Four mechanisms of 2n pollen formation observed in diploid potato (*Solanum tuberosum*) are termed parallel spindles, premature cytokinesis 1, premature cytokinesis 2 (Mok and Peloquin, 1975), and desynapsis (Ramanna, 1983; Veilleux, 1985).

Entry into either mitosis or meiosis requires high cyclin-dependent kinase (CDK) activity, which is achieved by cyclins of the A and B groups in *A. thaliana* (Francis, 2007; Inzé and De Veylder, 2006; Menges et al., 2005). Anaphase progression is regulated by a gradual decline in CDK activity, and mitotic program exit requires low CDK activity (de Gramont and Cohen-Fix, 2005). CDK activity is regulated by the anaphase promoting complex/cyclosome (APC/C), a conserved multi-subunit E3 ubiquitin ligase, during mitosis and meiosis. Depletion of CDK activity during meiosis needs to be tightly coordinated, so that the remaining CDK level at the end of M I enables exit from M I, but still allows initiation of M II (Furuno et al., 1994; Fitcher, 2008; Iwabuchi et al., 2000; Marston and Amon, 2004; Stern, 2003). Relatively small modifications of the cell cycle during M I or M II may offer the potential to create diploid gametes (Brownfield and Köhler, 2011).

A gene involved in the M I to M II transition is the *A. thaliana* *OSD1/GIG1* (*gigas cell 1*) gene. It has been proposed that *OSD1* may inhibit the activity of the APC, which would promote CDK activity (d'Erfurth et al., 2009). Thus, because of the loss of *OSD1*, a moderate decrease in CDK activity may

Received for publication 14 Mar. 2017. Accepted for publication 24 Aug. 2017. This work was supported by grants from the National Engineering Research Center for Ornamental Horticulture (grant number 2012FU125X10), Chinese Natural Science Foundation (grant number 31460530), Yunnan Foundation Research Projects for Application (grant number 2014FA044), Yunnan Young Academic and Technical Leader Training (grant number 2015HB077), and Yunnan Science and Technology Leader Training (grant number 2016HA005).
¹Corresponding authors. E-mail: wjh0505@gmail.com or qsp2002@sohu.com.

prevent entry into M II without impairing the prophase to M I transition. As *osd1* mutants fail to enter the second division in both male and female meiosis, functional 2n gametes and tetraploid progeny are produced (d'Erfurth et al., 2009). Male meiocytes lacking both *CYCA1;2/TAM* and *OSD1* fail to enter the first meiotic division (d'Erfurth et al., 2010). Loss of both *CYCA1;2/TAM*, and *OSD1* may cause a loss of *CDK* activity sufficient to impair entry into M I. These differences highlight the essential and tight regulation of *CDK* activity required during meiosis (Brownfield and Köhler, 2011).

The *A. thaliana* *OSD1* has the same three putative cell-cycle-related domains. These three domains are very well conserved over *OSD1* homologues (d'Erfurth et al., 2009). Two of these domains are putative *APC/C* degradation motifs: a D-box (residues 104–110, RxxLxx) and a GxEN/KEN-box (residues 80–83, GxEN in eudicotyledon and KEN in monocotyledon *OSD1* homologues) (Cromer et al., 2012). *OSD1* also has a C-terminal MR-tail in common (the two last amino acid of the protein are a methionine and an arginine) (Cromer et al., 2012). Both the interactions with *APC/C* and in-plant meiotic function of *OSD1* are dependent on its D-box domain and its MR tail. Remarkably, the *OSD1* GxEN-box is not required for *OSD1* function, but its mutation allows the *OSD1* protein mutated in its D-box to fulfill its function (Cromer et al., 2012).

Interestingly, *OSD1* has a paralogue, *uv-insensitive4* (*UVI4*), which negatively regulates endoreplication onset in *A. thaliana* that play roles in the mitotic cell cycle through *APC/C* regulation (Heyman et al., 2011; Pesin and Orr-Weaver, 2008). However, *OSD1* also has a somatic function as revealed by the mitotic phenotype of the single *osd1* mutants which trigger ectopic endomitosis (Iwata et al., 2011). Iwata et al. (2011) showed that *GIG1/OSD1* and *UVI4* encode novel plant-specific inhibitors of *APC/C* ubiquitin ligase.

Carnation is a major floricultural crop in China and worldwide. It is a member of the Caryophyllaceae. Most carnation cultivars can produce 2n gametes, and the frequency of 2n pollen produced ranged from 0% to 4.17% (Zhou et al., 2012, 2015). Spindle abnormalities during metaphase II have been considered to be the major cytological mechanism involved in the formation of 2n pollen in carnation (Zhou et al., 2015). In this study, we describe another abnormality mechanism and found some cells were normal in the first meiotic division; however, a failure occurred in the second meiotic division, which generated dyads during microsporogenesis. The carnation *OSD1* gene may be involved in the formation of 2n gametes. The role of carnation *OSDL* in the formation of unreduced gametes is described herein through the isolation, characterization, and analysis of expression patterns.

Materials and Methods

PLANT MATERIALS. The experiments were carried out in four diploid carnation (2n = 30) cultivars: Promesa, Guernse Yellow, Nogalte, and YunhongErhao, which displayed

high pollen viability. The frequency of dyad production in 'Nogalte' in June 2013 was 3.3%, and the frequency of 2n pollen was 1.63% (Zhou et al., 2015). The percentage of 2n pollen in 'Promesa', 'Guernse Yellow', and 'YunhongErhao' was 0.06%, 3.09%, and 1.72%, respectively (Zhou et al., 2015). They were cultivated under plastic in an ambient light and temperature regimen in Sept. 2012 at Jinning district, in Kunming, Yunnan, China. During the flowering period (June 2013), the daily temperature averaged 22.84 ± 0.02 °C, and the photoperiod averaged 13 h.

CYTOLOGY. 'Nogalte' was used for cytological analysis of male meiosis. Observation of the final male meiotic products and chromosome spreads were carried out as previously described (Zhou et al., 2015), observed under a light microscope (E800; Nikon, Tokyo, Japan) at $\times 1000$.

Three carnation cultivars Promesa, Guernse Yellow, and YunhongErhao were sampled in June 2013. Buds were classified into eight stages (1–8) in accordance with the methods described by Zhou et al. (2015). Stage 1 and stage 2 buds usually have developed microspore mother cells (bud size 0.9–1.0 and 1.1–1.2 cm, respectively); stage 3 buds contain anthers that have generally entered meiosis (bud size 1.3–1.4 cm); stage 4 buds have developed tetrads (bud size 1.5–1.6 cm); and stages 5–8 have further progressed to the mature pollen stages (bud size 1.7–2.4 cm).

CARNATION *OSDL* CLONING. An *A. thaliana* *OSD1* cDNA sequence was retrieved from the GenBank database (Benson et al., 1997). A homology search was conducted with BLASTX on the DNA Data Bank of Japan (DDBJ) website (Tanase et al., 2012). Accession numbers in *D. caryophyllus* FX309965, FX311539, FX311540, FX315550, and FX332100 resulted from this search (Tanase et al., 2012). Fragments of accession numbers FX311540 and FX311539 could be assembled into a 1262-bp segment with a polyA; FX315550 and FX332100 could be assembled into 758-bp segment with a polyA; and FX309965 was 681-bp long. These three putative homologous genes in *D. caryophyllus* were named *OSDLa*, *OSDLb*, and *OSDLc*, respectively. Total RNA from prebolting buds of 'Promesa' was extracted with TRIZOL Reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Total

Fig. 1. Abnormal meiosis lacking meiosis II in carnation: (A) a wild-type tetrad; (B) a dyad; (C–H) male abnormal meiosis is indistinguishable from wild type until telophase I, but no figures characteristic of a second division were observed; (C) pachytene; (D) diakinesis; (E) metaphase I; (F) anaphase I; (G) telophase I; (H) an incipient dyad; bars = 10 μ m.

RNA was reverse transcribed to the first-strand cDNA with an oligo (dT) primer designed with an adaptor sequence according to the protocol of the SMART RACE cDNA Amplification Kit (Clontech, Mountain View, CA). To isolate the 5'-ends of the carnation *OSDLa*, *OSDLb*, and *OSDLc* cDNA, gene-specific primers, OSDL1a-5Ra-364R, OSDL1b-5Ra-189R, and OSDL1c-5R-468R (Supplemental Table 1) were used in combination with a universal primer mix (UPM) for 5'-rapid amplification of cDNA ends (RACE), respectively. The carnation *OSDLa* and *OSDLb* cDNA were first amplified. Initial 5'-RACE products of *OSDLc* gene were then used for nested polymerase chain reaction (PCR) amplifications by using

a gene-specific, nested primer [OSDL1c-328R (Supplemental Table 1)], in combination with a nested universal primer (NUP). To isolate the 3'-ends of the carnation *OSDLa*, *OSDLb*, and *OSDLc* cDNA, gene-specific primers, OSDL1a-82-length-F, OSDL1b-138-length-F, and OSDL1c-69-length-F (Supplemental Table 1) were used, respectively. The initial 3'-RACE products of *OSDLa*, *OSDLb*, and *OSDLc* were then used for nested PCR amplifications by using gene-specific nested primers, OSDL1a-103-length-F, OSDL1b-147-length-F, and OSDL1c-113-length-F (Supplemental Table 1). Both the UPM and NUP were supplied with the SMART RACE cDNA amplification kit (Clontech). All RACE reactions were carried

Fig. 2. Alignment of protein carnation omission of second division-like (*OSDL*) sequences obtained from the National Center for Biotechnology Information online Web tool. The conserved D-box and MR tail motifs are indicated. AL = *Arabidopsis lyrata* ssp. *lyrata*, AT = *A. thaliana*, BN = *Brassica napus*, BR = *Brassica rapa*, BV = *Beta vulgaris* ssp. *vulgaris*, CC = *Citrus clementina*, CM = *Cucumis melo*, CR = *Capsella rubella*, CaS = *Camelina sativa*, CiS = *Citrus sinensis*, DC = *Dianthus caryophyllus*, EG = *Eucalyptus grandis*, ES = *Eutrema salsugineum*, FV = *Fragaria vesca*, GM = *Glycine max*, GR = *Gossypium raimondii*, MD = *Malus domestica*, NS = *Nicotiana sylvestris*, NT = *Nicotiana tomentosiformis*, PB = *Pyrus bretschneideri*, PE = *Populus euphratica*, PM = *Prunus mume*, PP = *Prunus persica*, PT = *Populus trichocarpa*, SL = *Solanum lycopersicum*, SO = *Spinacia oleracea*, SP = *Solanum pennellii*, ST = *Solanum tuberosum*, TH = *Tarenaya hassleriana*, VV = *Vitis vinifera*.

out according to the manufacturer's instructions with Advantage 2 polymerase (Clontech). RACE products were subcloned into a pMD18-T vector (TaKaRa, Kyoto, Japan) and checked for the correct orientation by DNA sequence analysis. Sequencing and phylogenetic analysis were carried out as previously described (Zhou et al., 2015). Theoretical molecular weights of each ribosomal protein were calculated using the Compute pI/Mw tool (Gasteiger et al., 2003).

PHYLOGENETIC ANALYSIS. After obtaining the carnation *OSDLs* amino acid sequences, a BLAST search for homology was performed at the National Center for Biotechnology Information [NCBI (Benson et al., 1997)]. A phylogenetic tree was constructed with MEGA 5.1 (Tamura et al., 2011) based on the converted result from BIOEDIT alignments. The construction of a phylogenetic tree was performed using maximum

likelihood. The robustness of clustering was evaluated by bootstrap on 1000 replications.

qRT-PCR ANALYSIS. *OSDL* expression profiles in various organs (roots, stems, leaves, and ovaries) and developmental stages of buds were investigated using qRT-PCR in carnation 'Promesa', 'Guernse Yellow', and 'Yunhong Erhao'. Total RNA was isolated and reverse transcribed to the first-strand cDNAs following the manual of the PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa). The *GAPDH* gene (*GAPDH* 579-F and *GAPDH* 788-R) was used as an internal control in all qRT-PCR reactions (Supplemental Table 1). Real time polymerase chain reaction (RT-PCR) was carried out on 50 ng of cDNA with the *OSDL* 1a-422F and *OSDL* 1a-656R primers for *OSDL*a, the *OSDL* 1b-99-F, and *OSDL* 1b-223-R primers for *OSDL*b, and the *OSDL* 1c-133F and *OSDL* 1c-270-R primers

Fig. 2. (Continued)

for *OSDLc* (Supplemental Table 1). Gene expression in different tissues was characterized by using the Faststart SYBR Green Master Mix kit (Roche, Mannheim, Germany) following the manufacturer's instructions. Each result reported herein resulted from three biological replicates. For each sample, quantification was made in triplicate (technical replicates). Three plants for each cultivars with different sizes of buds were selected which were grown and arranged in the same growing area. Values are means $\pm$ SE of three replicates. Different letters indicate significant differences ($P < 0.05$). Data were subjected to analysis of variance with the least

significant difference method by statistical software package SPSS 18 (IBM Corp., Armonk, NY).

Results

THE CYTOLOGICAL MECHANISM LEADING TO 2N POLLEN PRODUCTION AND 2N FREQUENCY. To unravel the mechanisms leading to 2n pollen production, we investigated chromosome behavior during meiosis. During microsporogenesis in 'Nogalte', subsequent reductional and equational divisions normally result in the formation of a group of four haploid

MR tail

AL ARALYDRAFT	VTPGPSVGKRPSC--PSTAK--VHKMLLDITK--EIAEE--EAGFITPEKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	249
AT POLYCHOME	VTPGPSIGKRPWP--PSTAK--VHQILLDITR--ENTGE--EDALTPEKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	259
AT UV-B-insensitive 4-like	VTPGPSVGKRPSC--PSTAK--VHKMLLDITK--EIAEE--EAGFITPEKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	243
BN GIGAS CELL1-like	MTAPAVGLKRPSC--PSTAK--VHKMLLDITK--EISEEGEAGFITPEKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	249
BR GIGAS CELL1	VTPAPVGLKRPSC--PSTAK--VHKMLLDITK--EISEKDEEAGFITPEKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	247
BV GIGAS CELL1-like	STP--PQA--CKFKD-----DNASKGKDI-----EFETPQKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	222
BV POLYCHOME-like	QTPLEPTISVKPQS--PAMQVVKITPTNADNNEKGS--DFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	241
CaS GIGAS CELL1-like	ETPGPSVGKRPSC--PSTAR--VHKMLLDITK--EIAEE--EAGFITPEKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	258
CaS GIGAS CELL1-like	ETPGPSLGKRPSC--PSTAR--VHKMLLDITK--EIAEE--EAGFITPEKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	259
CaS POLYCHOME	VTPGPSVGKRPWP--PSTAK--VHQILLDITR--ENTGE--EDALTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	260
CaS POLYCHOME-like	VTPGPSVGKRPWP--PSTAK--VHQILLDITR--ENTGE--EDALTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	256
CC CICLE	MTPTPTLSVKAOPP--SIGKVPKILLDITN--QAGAE--LESALTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	253
CiS POLYCHOME-like	MTPTPTLSVKAOPP--SIGKVPKILLDITN--QAGAE--LESALTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	253
CM POLYCHOME	LTPRP-----ITVGVKPKILRGIAN--ENTVG--AEFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	249
CR CARUB	VTPGPSLGKRPSC--PSTAK--VHKMLLDITK--EIAEE--EAGFITPEKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	251
CR CARUB	VTPGPSVGKRPWP--PSTAK--VHQILLDITR--ENTGE--EDALTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	258
CuS POLYCHOME	LTPRP-----ITVGVKPKILRGIAN--ENTVG--AEFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	249
DC OSDLa	PTPTPTIPVKILDS-----DVKEIAGDS-----ESMTPEKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	223
DC OSDLb	STPTPTLAAPKLS--PTTQLVRIKAGNADWSVDS--DFVTPQKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	237
DC OSDLc	STPTPTLVKPRLS--PAAQLSIMKAITAECSDNF--DSLTPQKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	77
EG POLYCHOME	YTPGPAPVBPCEFI--SVGVKPKILLDITN--QTSVGD--SEFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	260
ES EUTSA	VTPGPSVGKRPWP--PSAAK--VHKMLLDITR--ENTGE--EESLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	248
ES EUTSA	VTPGPVGVKRPSC--PSTAK--VHKMLLDITN--EYSEED--EAGFITPEKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	250
FV POLYCHOME-like	ATPSTIKRPP--RPP--SVLKVVKILLEVAN--QPDEG--EFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	245
FV POLYCHOME-like	VTPSTIKRPPCAPP--SVLKVVKILLEVAN--QPDEG--EFVTPQKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	243
GM POLYCHOME-like	--SPKSVG--VKLRTP--FGSKVPKILDLIS--ELPEDDE--SEFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	215
GR POLYCHOME	--TPASTTRUKPQP--SVRNYSKILLNVTNQPESS--EELTPQKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	257
MD POLYCHOME-like	TPYSAVRTKHCLPP--SVGVKPKILLDITN--QPSSEG--EFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	253
MD POLYCHOME-like	TPYSALRTKRRLPP--PVXVKIKILRDYSN--QPSSEG--EFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	253
NS POLYCHOME-like isoform X1	MTPTPTIRSRIYPK--SVGVKPKILLDITN--QNDSDG--IDLTPQKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	253
NT POLYCHOME-like isoform X1	MTPTPTIRSRIYPK--SVGVKPKILLDITN--QNDSDG--LDLTPQKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	263
NT POLYCHOME-like isoform X2	MTPTPTIRSRIYPK--SVGVKPKILLDITN--QNDSDG--LDLTPQKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	257
PB POLYCHOME-like	TPYSAVRTKCLPP--SVGVKPKILLDITN--QPSSEG--EFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	253
PE POLYCHOME-like	MSPRPTTEVKGCS--TIGKVPKILQHTN--QASGD--PDSLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	248
PE POLYCHOME isoform X1	MSPRPTPAFKGPP--TIGKVPKILQHTN--QASGD--PESLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	247
PE POLYCHOME isoform X2	MSPRPTPAFKGPP--TIGKVPKILQHTN--QASGD--PESLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	245
PM POLYCHOME	TPYSAVRTKQCP--SVGVKPKILREATNQTAE--EFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	262
PP PRUPE	---AVRTKQCP--SIGRVKIKILREATNQTAE--EFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	248
PT POPTR_0016s05070g	MSPRPTPAFKGPP--TIGKVPKILQHTN--QASGD--PELTPQKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	247
PT POPTR_0006s05660g	MSPRPTPAFKGCS--TIGKVPKILQHTN--QASGD--PDSLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	248
SL POLYCHOME	ITPRPKTRSRHYTR--SVGVKPKILLDITN--QTSSED--AEFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	255
SO SOVF_097620	SKSPES--CEVD-----DNADRKGKEV-----AEFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	222
SO SOVF_038130	QTPLEPTISVKPQS--PATKVMITPTNIDNEMSS--DFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	235
SP POLYCHOME	ITPRPKTRSRHYTR--SVGVKPKILLDITN--QTSSED--AEFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	255
ST POLYCHOME	ITPRPKTRSRHYTR--SVGVKPKILLDITN--QTSSED--SEFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	255
TH POLYCHOME-like	VTPAPVGLKRPSC--PSTAR--VHKMLLDITK--EHCGG--EESLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	246
TH POLYCHOME-like	VTPAPVGLKRPSC--PSTAR--VHKMLLDITK--EHCGG--EAEFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	256
VV POLYCHOME	ISPSPTSGMKLYPK--AVGVKPKILLDITN--QTGGG--SDFLTPOKKLLNSIDKVEKIVMAEIQKLKSTPAKREEREKRVRTLMS	IR	266

Fig. 2. (Continued)

spores, called a tetrad (Fig. 1A). However, a low frequency of dyads (Fig. 1B) was observed, confirming that the diploid microspores are produced by a defect in meiotic chromosome segregation. Following chromosome condensation and crossover formation in pachytene and diakinesis (Fig. 1C and D), the metaphase I spindle aligns the bivalents at the equatorial plane (Fig. 1E) and subsequently, segregates the homologs toward the poles at anaphase I (Fig. 1F). Chromosomes decondensed at telophase I at the end of M I (Fig. 1G) and formed a dyad (Fig. 1H), strongly suggesting that dyad production is due to an absence of the second meiotic division.

CLONING AND CHARACTERIZATION OF *OSDL* GENES IN CARNATION. *OSDL* is involved in the entry into both M I and M II in *A. thaliana*. *osdl* mutants produce high levels of functional 2n gametes in the absence of a second meiotic division (d'Erfurth et al., 2009). Three different *OSDL* cDNAs were isolated from prebolting buds of *D. caryophyllus* 'Nogalte'. After the composite cDNAs were obtained, the nucleotide sequences for the open reading frames (ORFs) of all these cDNAs were confirmed by cloning the complete (full-length) cDNAs by using PCR and sequencing. The three cDNAs were designated *OSDLa*, *OSDLb*, and *OSDLc*, and deposited in the NCBI database under accession numbers KX622764, KX622765, and KX622766, respectively.

ORFs were analyzed using the NCBI ORF Finder (Benson et al., 2004). The structures of the three *OSDL* cDNAs are summarized in Supplemental Figs. 1–3, which include 5'-flanking sequences, ORFs, and 3'-flanking sequences. The cDNAs were 1180 bp long for *OSDLa*, 1288 bp long for *OSDLb*, and 971 bp long for *OSDLc*. The predicted proteins consisted of 223 amino acids plus a 171-bp untranslated 5' region and a 337-bp untranslated 3' region for *OSDLa*, 237 amino acids plus a 130-bp untranslated 5' region and a 444-bp untranslated 3' region for *OSDLb*, and 177 amino acids plus a 2-bp untranslated 5' region and a 435-bp

Fig. 3. Phylogenetic tree of plant omission of second division-like (*OSDL*) proteins. Maximum likelihood phylogenetic tree of *OSDL* predicted proteins from 30 plant species: AL = *Arabidopsis lyrata* ssp. *lyrata*, AT = *Arabidopsis thaliana*, BN = *Brassica napus*, BR = *Brassica rapa*, BV = *Beta vulgaris* ssp. *vulgaris*, CC = *Citrus clementina*, CM = *Cucumis melo*, CR = *Capsella rubella*, CaS = *Camelina sativa*, CiS = *Citrus sinensis*, DC = *Dianthus caryophyllus*, EG = *Eucalyptus grandis*, ES = *Eutrema salsugineum*, FV = *Fragaria vesca*, GM = *Glycine max*, GR = *Gossypium raimondii*, MD = *Malus domestica*, NS = *Nicotiana sylvestris*, NT = *Nicotiana tomentosiformis*, PB = *Pyrus bretschneideri*, PE = *Populus euphratica*, PM = *Prunus mume*, PP = *Prunus persica*, PT = *Populus trichocarpa*, SL = *Solanum lycopersicum*, SO = *Spinacia oleracea*, SP = *Solanum pennellii*, ST = *Solanum tuberosum*, TH = *Tarenaya hassleriana*, VV = *Vitis vinifera*.

untranslated 3' region for *OSDLc*, corresponding to calculated molecular masses of 25.34, 25.95, and 19.95 kDa, respectively. Carnation *OSDLs* have two putative cell-cycle-related domains which are putative APC/C degradation motifs: a D-box (RxxLxx, residues 104–109 for *OSDLa*, residues 111–116 for *OSDLb*, and residues 48–53 for *OSDLc*) and a C-terminal MR-tail (Fig. 2). However, carnation *OSDLs* do not have a GxEN/KEN-box motif which is otherwise very well conserved across *OSDL* homologues in plants (Cromer et al., 2012; Heyman et al., 2011).

The amino sequences of *OSDLa* have 47% and 48% similarity to *OSDLb* and *OSDLc*, respectively, and *OSDLb* has 55% similarity to *OSDLc*. A phylogenetic analysis was conducted by comparing the carnation *OSDLs* with 30 homeologous protein sequences from different plant species (Fig. 3). The three homologues of carnation *OSDLs* clustered on the same basal branch. A strong similarity was found between the amino acid sequences of *OSDLb* and *OSDLc*, which clustered together as sisters.

COMPARISON OF TRANSCRIPT LEVELS OF *OSDLs* AMONG DIFFERENT TISSUES AND DIFFERENT DEVELOPMENTAL STAGES OF FLOWER BUDS. To test whether *OSDLs* were only expressed in the buds or also in other organs, we analyzed expression patterns. qRT-PCR analyses were performed to reveal expression levels in roots, leaves, stem, ovary, and buds. Expression analysis revealed that *OSDLs* are expressed in many organs, including roots, leaves, stem, ovary, and buds (Fig. 4). Interestingly, *OSDLa* shows the highest expression in stage 2 buds in three carnation cultivars (Fig. 4A–C). The expression level of *OSDLa* is significantly higher in stage 2 of buds in ‘Promesa’ (Fig. 4A) and in ‘YunhongErhao’ (Fig. 4C) than other stages of buds and tissues, and significantly higher in stage 1–3 of buds in ‘Guernse Yellow’ (Fig. 4B) than other stages of buds and tissues. *OSDLb* has a high level of expression in stage 1 buds and ovaries of ‘Promesa’ (Fig. 4D) and ‘YunhongErhao’ (Fig. 4F), and a high level of expression in stage 2 buds and ovaries of ‘Guernse Yellow’ (Fig. 4E). We also found that the expression level of *OSDLc* in ovaries is higher than in other tissues (Fig. 4G–I).

Discussion

We cloned and characterized *OSDL* genes in a diploid carnation and found three homeologous genes that we called *OSDLa*, *OSDLb*, and *OSDLc*, which differ from those of *A. thaliana*. *OSDLa* and *OSDLb* genes

Fig. 4. Expression analysis of the *OSDL*-like gene in different bud stages and tissues of carnation. *OSDLa* gene expression analysis in carnation cultivars (A) Promesa, (B) Guernse Yellow, and (C) YunhongErhao; *OSDLb* gene expression analysis in carnation cultivars (D) Promesa, (E) Guernse Yellow, and (F) YunhongErhao; *OSDLc* gene expression analysis in carnation cultivars (G) Promesa, (H) Guernse Yellow, and (I) YunhongErhao. Values are means $\pm$ SE of three replicates. Different letters indicate significant differences ($P < 0.05$). Data were subjected to analysis of variance with the least significant difference method by statistical software package SPSS 18 (IBM Corp.).

have longer sequences than does the *OSDLc* gene. The evident feature of these *OSDLs* proteins is the presence of D-box and MR-tail domains; however, the GxEN/KEN-box domain is lacking (Heyman et al., 2011). Thus, our results suggest that the GxEN/KEN-box might not be necessary for carnation *OSDL* gene function.

Loss of *OSD1* function was originally shown to cause defects in the second mitotic division during the meiotic cell cycle, thus leading to the production of diploid male and female gametes in *A. thaliana* (d'Erfurth et al., 2009), suggesting that *OSD1* gene was expressed in buds. Bao and Hua (2014) found that megasporocytes cannot complete mitosis to develop into a functional female gametophyte in *osd1 uvi4* genotypes, and indicating the *OSD1* and *UVI4* together are essential for zygote development as well. *UVI4* is expressed during the mitotic cell cycle, peaking at the G1-to-S transition, whereas the expression of *OSD1* peaks at the G2-to-M transition (Heyman et al., 2011). *UVI4* plays an important role in determining the meristem size in roots and the cell number and size in leaves (Heyman et al., 2011). The level of *OSD1* is critical in controlling ploidy levels in leaf cells, most likely through regulating endoreduplication (Bao and Hua, 2014). From the expression patterns we observed in carnation, *OSDL* genes maintain continuous expression within buds and many other tissues, suggesting a role in somatic growth. *OSDLa* was most highly expressed in stage 2 buds of 'Promesa', 'Guernse Yellow', and 'YunhongErhao'. This suggests that *OSDLa* may be involved in male meiosis. *OSDLb* has a high level of expression in both stage 1 and stage 2 buds and in ovary tissues in carnation cultivars. *OSDLb* may be associated with both pollen and ovary development. The expression level of *OSDLc* in ovaries is highest compared with other tissues, suggesting *OSDLc* probably functions in female gametogenesis, meiotic progression, or both. However, these hypotheses regarding the function of *OSDLs* in carnation have not been confirmed. In future studies, we plan to focus on the function and the regulation network of *OSDLs*.

Conclusion

Diploid gamete formation occurred in carnation. Analysis of chromosome behavior at meiosis indicated that 2n male gamete formation is probably attributable to the absence of the second meiotic division. We cloned *OSDL* genes in carnation for the first time by using RACE technology and have identified three homoeologous genes, which can be distinguished from *OSD1* in *A. thaliana*. The evident feature of these *OSDLs* proteins is the absence of the GxEN/KEN-box domain, suggesting that the GxEN/KEN-box is probably not necessary for gene function in carnation. Our analyses of expression levels of *OSDL* indicated that *OSDLs* in carnation may play roles in male meiosis and ovary development. These findings have potential applications in fundamental polyploidization research and carnation breeding programs.

Literature Cited

Bao, Z. and J. Hua. 2014. Interaction of CPR5 with cell cycle regulators UVI4 and OSD1 in *Arabidopsis*. *PLoS One* 9:e100347.
 Benson, D.A., K.M. Ilene, D.J. Lipman, O. James, and D.L. Wheeler. 1997. GenBank. *Nucleic Acids Res.* 25:1–6.
 Benson, D.A., I. Karschmizrach, D.J. Lipman, J. Ostell, and D.L. Wheeler. 2004. GenBank: Update. *Nucl. Acids Res.* 32:D23–D26.

Bretagnolle, F. and J. Thompson. 1995. Gametes with the somatic chromosome number: Mechanisms of their formation and role in the evolution of autopolyploid plants. *New Phytol.* 129:1–22.
 Brownfield, L. and C. Köhler. 2011. Unreduced gamete formation in plants: Mechanisms and prospects. *J. Expt. Bot.* 62:1659–1668.
 Cromer, L., J. Heyman, S. Touati, H. Harashima, E. Araou, C. Girard, C. Horlow, K. Wassmann, A. Schnittger, and L. De Veylder. 2012. OSD1 promotes meiotic progression via APC/C inhibition and forms a regulatory network with TDM and CYCA1; 2/TAM. *PLoS Genet.* 8:e1002865.
 d'Erfurth, I., L. Cromer, S. Jolivet, C. Girard, C. Horlow, Y. Sun, J.P.C. To, L.E. Berchowitz, G.P. Copenhagen, and R. Mercier. 2010. The CYCLIN-A CYCA1;2/TAM is required for the meiosis I to meiosis II transition and cooperates with OSD1 for the prophase to first meiotic division transition. *PLoS Genet.* 6:e1000989.
 d'Erfurth, I., S. Jolivet, N. Froger, O. Catrice, M. Novatchkova, and R. Mercier. 2009. Turning meiosis into mitosis. *PLoS Biol.* 7:e1000124.
 de Gramont, A. and O. Cohen-Fix. 2005. The many phases of anaphase. *Trends Biochem. Sci.* 30:559–568.
 Francis, D. 2007. The plant cell cycle – 15 years on. *New Phytol.* 174:261–278.
 Furuno, N., M. Nishizawa, K. Okazaki, H. Tanaka, J. Iwashita, N. Nakajo, Y. Ogawa, and N. Sagata. 1994. Suppression of DNA replication via Mos function during meiotic divisions in *Xenopus* oocytes. *EMBO J.* 13:2399–2410.
 Fletcher, B. 2008. Cyclins in meiosis: Lost in translation. *Dev. Cell* 14:644–645.
 Gasteiger, E., A. Gattiker, C. Hoogland, I. Ivanyi, R.D. Appel, and A. Bairoch. 2003. ExPASy: The proteomics server for in-depth protein knowledge and analysis. *Nucl. Acids Res.* 31:3784–3788.
 Gómez-Rodríguez, V.M., B. Rodríguez-Garay, and R. Barba-Gonzalez. 2012. Meiotic restitution mechanisms involved in the formation of 2n pollen in *Agave tequilana* Weber and *Agave angustifolia* Haw. *Springerplus* 1(17):1–7.
 Heyman, J., H. Van den Daele, K. De Wit, V. Boudolf, B. Berckmans, A. Verkest, C.L.A. Kamei, G. De Jaeger, C. Koncz, and L. De Veylder. 2011. *Arabidopsis ultraviolet-b-insensitive4* maintains cell division activity by temporal inhibition of the anaphase-promoting complex/cyclosome. *Plant Cell* 23:4394–4410.
 Inzé, D. and L. De Veylder. 2006. Cell cycle regulation in plant development. *Annu. Rev. Genet.* 40:77–105.
 Iwabuchi, M., K. Ohsumi, T.M. Yamamoto, W. Sawada, and T. Kishimoto. 2000. Residual Cdc2 activity remaining at meiosis I exit is essential for meiotic M–M transition in *Xenopus* oocyte extracts. *EMBO J.* 19:4513–4523.
 Iwata, E., S. Ikeda, S. Matsunaga, M. Kurata, Y. Yoshioka, M.C. Criqui, P. Genschik, and M. Ito. 2011. GIGAS CELL1, a novel negative regulator of the anaphase-promoting complex/cyclosome, is required for proper mitotic progression and cell fate determination in *Arabidopsis*. *Plant Cell* 23:4382–4393.
 Marston, A.L. and A. Amon. 2004. Meiosis: Cell-cycle controls shuffle and deal. *Nat. Rev. Mol. Cell Biol.* 5:983–997.
 Menges, M., S.M. De Jager, W. Gruissem, and J.A. Murray. 2005. Global analysis of the core cell cycle regulators of *Arabidopsis* identifies novel genes, reveals multiple and highly specific profiles of expression and provides a coherent model for plant cell cycle control. *Plant J.* 41:546–566.
 Mok, D. and S. Peloquin. 1975. Three mechanisms of 2n pollen formation in diploid potatoes. *Can. J. Genet. Cytol.* 17:217–225.
 Otto, S.P. 2007. The evolutionary consequences of polyploidy. *Cell* 131:452–462.
 Otto, S.P. and J. Whitton. 2000. Polyploid incidence and evolution. *Annu. Rev. Genet.* 34:401–437.
 Pesin, J.A. and T.L. Orr-Weaver. 2008. Regulation of APC/C activators in mitosis and meiosis. *Annu. Rev. Cell Dev. Biol.* 24:475–499.

- Ramanna, M. and E. Jacobsen. 2003. Relevance of sexual polyploidization for crop improvement – A review. *Euphytica* 133:3–8.
- Ramanna, M.S. 1983. First division restitution gametes through fertile desynaptic mutants of potato. *Euphytica* 32:337–350.
- Stern, B.M. 2003. FEARless in meiosis. *Mol. Cell* 11:1123–1125.
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28:2731–2739.
- Tanase, K., C. Nishitani, H. Hirakawa, S. Isobe, S. Tabata, A. Ohmiya, and T. Onozaki. 2012. Transcriptome analysis of carnation (*Dianthus caryophyllus* L.) based on next-generation sequencing technology. *BMC Genomics* 13:1–11.
- Teoh, S. 1984. Polyploid spore formation in diploid orchid species. *Genetica* 63:53–59.
- Veilleux, R. 1985. Diploid and polyploid gametes in crop plants: Mechanisms of formation and utilization in plant breeding. *Plant Breed. Rev.* 3:253–288.
- Zhou, X., M. Gui, J. Wang, M. Chen, J. Long, Z. Lu, J. Song, and X. Mo. 2012. Pollen tube growth after crossing between different ploidies *Dianthus caryophyllus* by fluorescence microscopy and seed setting. *Acta Bot. Boreali-Occidentalis Sin.* 32:67–74.
- Zhou, X., X. Mo, M. Gui, X. Wu, Y. Jiang, L. Ma, Z. Shi, Y. Luo, and W. Tang. 2015. Cytological, molecular mechanisms and temperature stress regulating production of diploid male gametes in *Dianthus caryophyllus* L. *Plant Physiol. Biochem.* 97:255–263.

nt		aa
1	acatggggacaccattataaaactcctttcttttcttcccccttttaatcgaccattttat	
61	tattaatctcttttattaatcactttcctctcctaattccccccatttattctgtttatt	
121	cactcagaaatttgatctggttgaaaaaccggtagaatttgtagcagagtaatgccggtg	3
		M P V
181	tccagggatagattgtcgagaccggtagacatatcgagcctactaccaaatgcacagcgc	23
	S R D R L S R P V D I S S L L P N A Q R	
241	cgagttaatctttagtagatgaaccagggttgcgacttaggggactatcgctcgctaaat	43
	R V N L V V D E P G L R L R G L S S L N	
301	gcgtccatcagtaaacctagtggttagcgctagcaggctgcgcagacgcagcagtcgaat	63
	A S I S K P S G S A S R L R R R S S Q Y	
361	agagcgcgattgagacaacaacggtagcttggctaggttgcctcctgaccaagaaaaatcgg	83
	R A R L R Q Q R Y L A R L P P D Q E N R	
421	acacctgtgggtttacggaatgtacaagtagacggagtgctttaccttcttggcaccgc	103
	T P V G L R N V Q G R R S V L P S W H P	
481	cgaacgcctcttagggatattactgccattgttagggcaatagagaggagaagagccgaa	123
	R T P L R D I T A I A R A I E R R R A E	
541	cttcaagaacagagacgagaaacatctgtacctgaatcaagtgtgcacatcgagttctcaa	143
	L Q E Q R R E T S V P E S S A A S S S Q	
601	cctgagaacgaaaccaataccgactccaacacccagattcctgtaaaaatttttagac	163
	P E N E T N I P T P T P T I P V K I L D	
661	agcgatgtaaaagacgagattgcgggagactcagaatccatgaacccagagaagcgactt	183
	S D V K D E I A G D S E S M T P E K R L	
721	ctaaactcaatcgatacagtgagacagggttgggttagaggagcaagaaaaagttgagaaa	203
	L N S I D T V R Q V W V E E Q E K V E K	
781	actccagccgctaaaaaggctgaaagagaccgaaagggttcgtacattgatgtcgatgcga	223
	T P A A K K A E R D R K V R T L M S M R	
841	tgatttaatacattatattggcttgggtgtatgcttggtgcgctccatttcaaatgagat	224
	*	
901	taggccgggtttgagaatggccagtcgtctggcttgaaattcgtgcctgacgttttttaggc	
961	gtaggtgcacagctgagctttgccactgctggttttgcgatagtaactgttagcctct	
1021	gctaacgtgtccgttaattgtttttttctttccggtgtaaatagggttacgaggccatc	
1081	agggcatttttatgctaaccgggttttgaaacccggttgcgagttccatacatctcttaa	
1141	agtatcatcaatatattccagattttgtcaaaaaaaaaa	

Supplemental Fig. 1. Open reading frame and presumed amino acid sequences of *OSDL* in carnation.

nt		aa
1	acatgggggcccctcatttttctcctcactctctttctctctctaaaatgttgatccccct	
62	taacttttctctctctaaaaacttgtataaatgttgacttttttgggtggattttgaagaatc	
122	tagagaaagatgcctgtaccagtgtcaagagatagggttcctaggccagttgacatttct	17
	M P V P V S R D R L P R P V D I S	
182	gcccttcttggcagaacgactcgctcgtgtaaatttatagttgacgagcctgggttgagg	37
	A L L G R T T R R V N L I V D E P G L R	
242	cgggtggggctatcaggggcacgggttgggcaatgtgtcgagaaatgggtgggtgggttct	57
	R V G L S G H G L G N V S R N G G G G S	
302	tcagcagggagttccaattggagttcagagaaacgggttatgggtggaggtggcaatcagcag	77
	S A G S P I G V Q R T G Y G G G G N Q Q	
362	aggtcgatgggtggtagtcgggatccaagaacgcgggggactgttgctgctcgacgtgat	97
	R S M V V S R D P R T P G T V A A R R D	
422	gggtggccgaggaggaggtcagttgccttcttggcacccaagaacctcttagggacatt	117
	G G R G R S Q L P S W H P R T P L R D I	
482	acagacattatgaggggccatagaaaggagaagagcagaacttgcttgaacgaagacgca	137
	T D I M R A I E R R R A E L G L N E D A	
542	gatgtgcagcaagaacataacaatgcgcagactgagcaagatatcggttacctcgacaccc	157
	D V Q Q E H N N A Q T E Q D I G T S T P	
602	attcccaccccttgctgccaaagccgaaactctcaaccaaccactcagctggtaagaattaag	177
	I P T L A A K P K L S P T T Q L V R I K	
662	gcaggcaatgctgactggagtggttagacagctctgatttcgtaacgcgcgagaagaagcgt	197
	A G N A D W S V D S S D F V T P Q K K L	
722	ctgaactcgattgaaaaggtaggggaagtgtggctggaaagtgcgcaaactggagagg	217
	L N S I E K V R E V W L E S Q R K L E R	
782	acacctgccgcaaagagagctgagagggaaaaacaagggttcgggtactgtgtcgatgaga	237
	T P A A K R A E R E N K V R V L L S M R	
842	taatctactgattatcgaagggttcagagatcagagagtgaatgagttgagaagaggaa	257
	*	
902	ggtaacggccacaggagatcatcgtctgatgatcccatatcagtaaactacttacctata	
962	caaaaagctatgctttagctaaattgatcgagatccttgggctcactagtcactactca	
1022	ctagtagtagacttagtagctaatagttcagcacttacgttagctatgctatagctgtagg	
1082	aattgggttaggtaaatactagaagtagctggtatagatggtcagcctctgctgacggtttg	
1142	ccgttttgggtccttgatgctgttttaagtgatgacaagcaatacttgtgtacttcttta	
1202	gcattggatttgtacatatgattgaagtttgattctgagtaatgatcagtcagtggaaga	
1262	cagacttttagaaaaaaaaaaaaaaaaa	

Supplemental Fig. 2. Open reading frame and presumed amino acid sequences of *OSDLb* in carnation.

nt		aa
1	acatgggggtggaaggtggagaaggagtcagagactggttaatggtcgaggccacgaccgt	20
	M G V E G G E G V Q R L V N G R G H D R	
63	cagacgacagcgggtgactgctcgacgtggtgatggtgatgacggtcgagggaaacaggsggt	40
	Q T T A V T A R R G D G D D G R G N R G	
123	cagttgccttcttgggtacccgagaacccctcttcgggacatcacagacatcatgaggcg	60
	Q L P S W Y P R T P L R D I T D I M R A	
183	atcgagagaaggagagcagaactggggctgaaccaagactcggaaactccagaagcacac	80
	I E R R R A E L G L N Q D S E T P E A H	
243	gacaacgctgcaaatcctgaagttgaccgcgaacttgtgtctcgacacccatgcccacg	100
	D N A A N P E V D R E L V V S T P M P T	
303	atcgtagtcaagccacgactgtcaccagcagctcagctctctatcatgaaggccattact	120
	I V V K P R L S P A A Q L S I M K A I T	
363	gctgaatgtagtgtcgataactttgattccttaaccccgagaaagcagcttctgaactcc	140
	A E C S V D N F D S L T P Q K Q L L N S	
423	attgaaaaggtaaggcaagtcctgggtacaccagcggctaaaactggagaagacgccttgt	160
	I E K V R Q V W V H Q R L K L E K T P C	
483	gctaggagagtcgagagggaaaacaagatgcgggttttaatgtcaatgcgatagtcacct	177
	A R R V E R E N K M R V L M S M R *	
543	gctgtttacctcgtgaagaagtacaactaccgataaaaagataagtgagttgagatgagaa	
603	gagccacaagagatcatcgtctgatgatctcataccgctattacttcagctatgcttgt	
663	agctaatacttcgggattttgttaagtaactactcagacagtatttgtttagctatgctt	
723	tagctatccatactacaagcacgacagaatcgtcgactctgaaatgctgatacttttagt	
783	gtgtagatagtgcaaaactcaaagacttaaaaaaggagattgtttcttattttgttatata	
843	ggtaacttaatagaatacagatctaattgttggtgattaatgaaattcaagtcagtactc	
903	cttgtgttattccaattgtggaattaaagtaagttaatgaaatcataaattgaatgcaaa	
963	aaaaaaaaa	

Supplemental Fig. 3. Open reading frame and presumed amino acid sequences of *OSDLC* in carnation.

Supplemental Table 1. Primers used in this study [forward primer (FP), reverse primer (RP)].

Name	Primer sequence 5' to 3'
OSDL1a-5Ra-364R	RP CCTAGCCAAGTACCGTTGTTGTCTCA
OSDL1a-82-length-F	FP ACTTTCCTCTCCTAATTCC
OSDL1a-103-length-F	FP CCATTTATTCTGTTTATTCCTCA
OSDL1b-5Ra-189R	RP GCTTCTTCTGCGGCGTTACGA
OSDL1b-138-length-F	FP ATGCCTGTACCAGTGTCAAG
OSDL1b-147-length-F	FP ATGCCTGTACCAGTGTCAAGAGATAGGT
OSDL1c-5R-468R	RP CTGTCGTGCTTGTAGTATGG
OSDL1c-328R	RP GTGGCTCTTCTCATCTCAA
OSDL1c-69-length-F	FP ACAGCGGTGACTGCTCGACGTG
OSDL1c-113-length-F	FP GAACAGGGGTCAGTTGCCTTCTTGTA
GAPDH 579-F	FP CAGTGGGCACACGGAAGC
GAPDH 788-R	RP TGGCATCGTTGAGGGTCT
OSDL1a-422F	FP CGGAGTGTCTTACCTTCTT
OSDL1a-656R	RP CTCTCGCAATCTCGTCTT
OSDL1b-99-F	FP CTCTCACCAACCACTCAG
OSDL1b-223-R	RP CACACTTCCCTCACCTTT
OSDL1c-133F	FP CTGAACTCCATTGAAAAGGTAAGGC
OSDL1c-270-R	RP GTAAACAGCAGGGGACTATCGCAT