Cloning and expression analysis of DnMSI1 gene in orchid species Dendrobium nobile Lindl

Baolu Cui#, Min Huang#, Chongdai Guo†, Ruihong Li†, and Yuqi Wang##

*School of Environmental Science and Engineering, Guangzhou University, Guangzhou, Guangdong, China; †School of Biological Science and Agriculture, Qiannan Normal University, Duyun, Guizhou, China

ABSTRACT
WD40 repeat proteins, the homologs of yeast MSI1, are conserved in plants, participating in protein complexes and playing fundamental functions in plant development. Although several MSI1-like proteins have been cloned and characterized in plants, the roles of MSI1-like proteins in the biennial ornamental plant, Dendrobium nobile Lindl, are still unclear. Here, we report the cloning of the DnMSI1 gene from Dendrobium nobile Lindl with RACE technology. We found that DnMSI1 expression was induced by GA3 and TDZ but inhibited by ABA, PP333, and drought and salt stress. Furthermore, DnMSI1 over-expression in Arabidopsis resulted in decreased tolerance to NaCl stress. These results suggest that DnMSI1 plays negative regulation roles in regulating salinity-stress resistance in Dendrobium nobile Lindl.

Introduction
MSI1-like proteins of the WD40 family are prevalent in all eukaryotes. They play fundamental roles in plant development, including determining cell fate and regulating cell cycle, signal transduction, transcription, and cytoskeletal organization.1–3 MSI1 proteins are subunits of several protein complexes. For instance, Arabidopsis MSI1 (AtMSI1) is a subunit of Chromatin Assembly Complex CAF-13,4 and PRC2 (Polycomb repressive complex 2)-like complexes, which interact with the Retinoblastoma-related protein RBR15,6 and participate in various aspects of chromatin assembly and dynamics.2 Reduced expression of MSI1 leads to pleiotropic phenotypes, reflecting the complexity of MSI1 protein functions. AtMSI1 is the part of FIS (Fertilization Independent Seed) and CLF (CURLY-LEAF) complex, playing a critical role in seed development. In Arabidopsis, when a mutant allele msi1 is inherited from the mother, the seeds are aborted regardless of the paternal contribution.7–9 Furthermore, heterozygous Atmsi1 mutants also show a high penetrance of fertilization-independent defects in seed development.10

AtMSI1 is also a subunit of the CUL4-DDB1A-MSI1 protein complex, essential for seed development.11,10 Reducing AtMSI1 levels by co-suppression to about 5% of the wild-type levels causes severe defects in vegetative and reproductive development, leading to sterility.1 Furthermore, transgenic AtMSI1 antisense plants resulted in late flowering and increased trichome branching, suggesting that AtMSI1 regulates flowering time and trichome development.6,11,12

In addition, Alexandre et al. (2009) revealed a new role of AtMSI1 in regulating drought-stress responses as indicated by increased drought tolerance of the msi1-cs mutant plants because of enhanced expression of osmotic stress-responsive genes and accumulation of free proline.13 Therefore, identifying stress-inducible and MSI1-regulated genes will help us better understand how chromatin modifications affect plant responses to stress.

Presently, plant MSI1-like genes have been cloned from Arabidopsis thaliana, Nicotiana tabacum, Hieracium caespitosum, Triticum aestivum, Chlamydomonas reinhardtii, Zea mays, Glycine max, Hieracium pilosella, Oryza Sativa, etc. Still, there is no report about MSI1 from Dendrobium nobile, an important economic orchid in flower markets worldwide with high ornamental and medicinal values. Studies of gene regulation by chromatin modifications in Dendrobium nobile may provide guidelines to control their growth status in the future. In this study, we reported the isolation of an MSI1 gene from Dendrobium nobile using a functional genomics approach and the analysis of its expression and interactions with plant hormones and growth regulators.

Materials and methods
Plant materials and growth conditions
D. nobile plants were grown in moss media in a greenhouse with temperature maintained at 22°C and 18°C during day and night. The relative air humidity and light period were 75% and 12:12 (day: night), respectively. For treatment experiments, plants with finished floral bud differentiation were selected and treated with 1 mg/L Gibberellin A3(GA3), 1 mg/L Thidiazuron (TDZ), 1 mg/L Abscisic Acid (ABA), 1 mg/L Paclobutrazol(PP333), drought, 10 μM CdCl2, 10 μM NiCl2, and 10 mM NaCl, the distilled water as a negative control. After the treatment, plants were transferred to a growth

CONTACT Yuqi Wang yqwang@gzhu.edu.cn School of Environmental Science and Engineering, Guangzhou University, Guangzhou 510006, China
#Contribute equally to this work
*These authors contributed equally to this work
© 2022 The Author(s). Published with license by Taylor & Francis Group, LLC. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
chamber with the same growing conditions. The roots, stems, leaves and buds were collected on 5d for RNA isolation and expression patterns analysis. After different treatments of 0d, 5d, 10d, 15d, 20d, 25d, and 30d, the buds were collected separately and frozen in liquid nitrogen for further analyses. Each treatment contains 6 plants with three biological repeats.

The wild type (Col) Arabidopsis thaliana were acquired from the Arabidopsis Biological Resource Center (ABRC) (https://abrc.osu.edu/). Homozygosity of each DnMSI1-overexpression line (AtOE) was confirmed by PCR-based sequencing. Wild-type (Col-0) and AtOE seeds were surface-sterilized and cold stratified in the dark at 4 °C for 3 days before sown on 250 μm polypropylene meshes floating on hydroponic growth solutions supplemented without or with NaCl in Magenta boxes. The hydroponic solution consisted of the following macronutrients in mM: MgCl₂, 3.0; (NH₄)₂SO₄, 0.25; Ca(NO₃)₂, 1.0; KCl, 2.0; CaCl₂, 2.75; KH₂PO₄, 0.18; and the following micronutrients in μM: H₂BO₃, 5.0; MnSO₄, 1.0; CuSO₄, 0.05; ZnSO₄, 0.2; Na₂MoO₄, 0.02; CoCl₂, 0.001. Plants were grown in a plant growth chamber with 16/8 h day/night at 22°C.

Relative root growth (RRG%) of Arabidopsis was calculated as the percentage of root growth of individual plants under NaCl treatment over the average root growth under control (−NaCl) condition. In detail, ~30–40 seeds (technical replicates) of the WT or the DnMSI1-overexpressing lines were germinated in a hydroponic solution in a Magenta box supplemented with or without 30–300 mM NaCl. Three biological replicates (Magenta boxes) were conducted for each treatment and line. Primary root lengths of 10 randomly selected 7-d-old seedlings from a biological replicate were measured manually. The means of the primary root length of three biological replicates were calculated for the control of each line. The RRG% of a randomly selected 7-d-old seedling under treatment condition was calculated as the individual primary root length divided by the mean root length of the same line of the control condition. Ten seedlings from each biological replicate were randomly selected for RRG% calculation. The presented RRG% data were the means of three biological replicates.

**RNA isolation and cDNA synthesis**

Total RNA was isolated using the TRIzol method.⁴ RNA quality and concentration were determined using ethidium bromide (EB)-stained agarose gel electrophoresis and a UV spectrophotometer. The first strand of cDNA was synthesized using a Prime Script RT-PCR Kit (TaKaRa, Dalian, China) with Oligo d(T)₁₈ as a primer.

**Isolation of DnMSI1 gene in D. nobile Lindl**

Total RNAs isolated from frozen D. nobile Lindl buds were used as templates for reverse transcription polymerase chain reaction (RT-PCR). The product (the first-strand cDNA) was subjected to PCR amplification by forward primer DnMSI1f1: (5′-CACCTATTAGTGTTCTGCAC-3′) and reverse primer DnMSI1r1: (5′-CTCCTTCTGACATCATCCCTAGGTG-3′), which were designed with the conserved motifs of Nicotiana tabacum MS11 (multicopy suppressor of IRA1) gene (ABY84675.1). The 3′ and 5′ end of DnMSI1 was isolated with a 3′ and 5′ rapid amplification of cDNAs ends (RACE) cDNA Amplification Kit (TaKaRa, Shiga, Japan), respectively. In addition, gene-specific primers of DnMSI1f2 (5′-GTAGCGACAGGTTCACACTGA-3′), and DnMSI1r3 (5′-ATACCTGCGTCTTTGCGC-3′) were used for 3′ RACE, while DnMSI1r2 (5′-TTGATCATCACCAACAGAGG-3′) and DnMSI1r3 (5′-GCATATTGAGCATGCTGAC-3′) for 5′ RACE. The amplified fragment was recovered from agarose electrophoresis with an Agarose Gel DNA Extraction Kit (RealTms, China) and cloned into the pMD18-T vector (TaKaRa, Dalian, China). The full-length DnMSI1 cDNAs were obtained by PCR amplification using the forward primer: 5′-ATGGCCAAGGATGAAGATGAC-3′ and reverse primer: 5′-TAAAGAGGCTTGTGAAGGCTCG-3′.

**Sequence analysis**

WD40 protein sequences were downloaded from GeneBank for comparison. Sequence alignment was performed using DNAssist Package version 2 and CLUSTAL W (http://www.igbmc.u-strasbg.fr/BioInfo/ClustalX) Sequence relatedness was analyzed using CLUSTAL W and the neighbor-joining method, and the rooted tree was visualized using TreeDraw (http://taxonomy.zoology.gla.ac.uk/rod/treeview.htm).

**Quantitative real-time RT-PCR analysis**

Quantitative real-time RT-qPCR was carried out using SYBR Premix Ex Taq Kit (TaKaRa, Shiga, Japan). The amplification conditions were 95°C for 30 s, followed by 40 cycles of amplification (95 °C for 5 s, 60°C for 20 s) and plate reading after each cycle. Primer pairs used for quantitative real-time RT-qPCR detection are as follows: forward primer DnMSI1Rf 5′-TGGAGTCTCCAAAAGATGAGC-3′ and reverse primer DnMSI1Rr: 5′-ATCCTTGTACTGTGCTGCC-3′. In addition, the UBQ (Ubiquitin) was used for normalization with forward primer (5′-CGGCGTCACCTCTCATCCC-3′) and reverse primer (5′-GTAGGCTAGCGACGGCGTGG-3′). As for detection of DnMSI1 transcript levels in wild-type (WT) and DnMSI1 overexpression Arabidopsis lines (AtOE lines), the Arabidopsis tubulin-2 gene was used for calibration with forward primer (5′-ACCATTTCCAAGCCACACTACT-3′) and reverse primer (5′-ACGGTAAAGCTGCTGGT-3′). All experiments were replicated at least three times with similar results.

**Transgenic DnMSI1 constructs and genetic transformation**

To generate the constitutive expression constructs p35S::DnMSI1 for use in Arabidopsis thaliana, the DnMSI1 coding sequence was PCR-amplified from cDNA using the forward primer: 5′-CTG TCTAGAATTGCGCAAGTGAAGATGAC-3′ and reverse primer: 5′-CTGGAGCTCCTAAGAGGCTTGTGAAGGCTCG-3′ (the underlined sequences are restriction enzyme sites for Xba I and Sac I, respectively) and then cloned into the pCAMBIA1300 transformation vector (Takara Biotechnology Co., Ltd., Dalian, China). The resultant 35S promoter:DnMSI1 construct was then transformed into Agrobacterium tumefaciens
strain GV3101, followed by stable transformation into Arabidopsis Col-0, using the floral dip method. Homozygous T3 progenies with DnMSI1 overexpression (AtOE line) were used for subsequent analysis.

Results

Cloning and sequence comparison of DnMSI1

MSI1-like proteins are WD40 repeat proteins that carry one or more WDxR motif(s). The WD domain folds into a β-propeller structure, providing a platform for the protein–protein or protein–DNA interaction and assembly of several proteins.\(^5\) In this study, the initial 511bp fragment of DnMSI1 was amplified from D. nobile cDNAs, using a primer set of DnMSI1f1 and DnMSI1r1, which were designed with the conserved motifs of Nicotiana tabacum MSI1(multicopy suppressor of IRA1) gene (ABY84675.1). Then the 3′ and 5′ end of DnMSI1 was isolated with a 3′ and 5′ rapid amplification of cDNAs ends (RACE). The full-length of DnMSI1 cDNA was obtained by RT-PCR. Sequence analysis revealed that DnMSI1 encodes a 424 amino acid polypeptide with theoretical Mr of 48.1kD and pI of 4.58, containing four WD40 repeat sequences (Figure 1) according to the WD40 repeat detection method.\(^15\)

The deduced protein sequence shows high similarity with MSI1 from the other plants with 97, 89, 86, 82, and 81% identity to Dendrobium catenatum DcMSI1, Arabidopsis thaliana AtMSI1, Nicotiana tabacum NtMSI1, Zea mays ZmMSI1, and Oryza sativa OsMSI1, respectively. To investigate the phylogenetic relationship of the MSI1 proteins, a phylogenetic tree was constructed for several other MSI1 orthologs. The results revealed that DnMSI1 is closely related to DcMSI1 of Dendrobium catenatum (Figure 2).

Expression profile of DnMSI1 transcript in D. nobile Lindl

Semi-quantitative RT-PCR analysis showed that the transcript of DnMSI1 was detected in buds, leaves, and stems, but hardly detected in roots. DnMSI1 expression is more robust in buds than in stems and leaves (Figure 3). To determine whether abiotic stress regulated DnMSI1 transcription, quantitative RT-PCR was performed. Compared to the control condition, the levels of DnMSI1 expression in buds were decreased 25, 19, 22, and 59% by drought, Cd, Ni, and salt stress, respectively (Figure 4). The results suggested that DnMSI1 is negatively regulated by abiotic stresses, including drought, salt, and heavy metal stresses.

Interactions of DnMSI1 protein with plant hormones and regulators and their roles in regulating D. nobile development were further investigated. Four types of hormones or plant regulators, i.e., GA3, thidiazuron (TDZ), ABA, and PP333, were selected, and DnMSI1 expression in response to the hormone and growth regulator treatment was analyzed at different developmental stages. The level of DnMSI1 transcripts was up-regulated post GA3 and TDZ treatment and peaked at 10d or 15d, respectively (Figures 5, 6). In contrast, ABA and PP333 treatment down-regulated DnMSI1 expression after 5-d treatment compared to normal condition (Figures 7, 8). These results demonstrated that DnMSI1 responds differently to plant growth regulators or hormones.

Constitutive DnMSI1 expression in A. thaliana decreases tolerance to salinity

To explore the effect of DnMSI1 expression on plant responses to salinity stress, DnMSI1 was constitutively expressed in Arabidopsis thaliana (AtOE lines). Quantitative RT-PCR analysis showed that the DnMSI1 transcript levels are significantly higher in AtOE lines than that in wild-type (WT) Arabidopsis, when calibrated with the internal reference gene (Figure 9b). In addition, the DnMSI1 expression is decreased by NaCl in AtOE lines, but its transcript levels are still much higher than that in WT Arabidopsis (Figure 9b).

The responses to different NaCl concentrations were further compared between two independent DnMSI1 AtOE lines and the WT control line. Under the standard condition without stresses (CK), no phenotypic differences were observed among the lines (Figure 9a), which indicated that over-expression of DnMSI1 in Arabidopsis has no obvious effect on the growth of Arabidopsis seedlings at standard condition. However, the two independent DnMSI1-overexpression Arabidopsis (AtOE) lines were comparably more sensitive to NaCl stress than the WT (Figure 9a). At 180 mM NaCl, the root growth of WT was inhibited by 43%, whereas the AtOE lines by 58 and 65%, respectively (Figure 9c). These findings indicated that the DnMSI1 over-expression transgene notably decreases the Arabidopsis plants’ tolerance to NaCl stress, and DnMSI1 plays a negative regulatory role in plant response to salinity stress.

Discussion

MSI1-like proteins play essential roles in plant development and various aspects of chromatin assembly. MSI1 functions are conserved across plant species. Altered levels of MSI1 expression result in pleiotropic phenotypes, reflecting the complexity of MSI1 protein functions.\(^10\) In this study, we identified an MSI1 homolog in orchid D. nobile Lindl, and the DnMSI1 protein has four WD40 characteristic domains (Figure 1) and close relation to DcMSI1 of Dendrobium catenatum (Figure 2).

Like plant hormones or plant growth regulators, MSI1 is required for regulating plant development throughout plant life.\(^16,17\) GAs play pivotal roles in many developmental processes in plants, including pollen maturation, seed germination, leaf expansion, stem elongation, flowering induction, and trichome development.\(^16,17\) DnMSI1 expression was enhanced
| Species | Accession    | Description                |
|---------|--------------|----------------------------|
| HcMSI1  | ABZ85631.1   | Hieracium caespitosum      |
| OtMSI1  | XP_003082166.1 | Ostreococcus tauri          |
| TaMSI1  | ABB92268.1   | Triticum aestivum           |
| ZmMSI1  | NP_200631.1  | Arabidopsis thaliana        |
| AtMSI1  | XP_001696907.1 | Chlamydomonas reinhardtii  |
| CrMSI1  | NP_001105556.1 | Zea mays                   |
| GmMSI1  | ABW23439.1   | Glycine max                 |
| HpMSI1  | ABZ85629.1   | Hieracium pilosella         |
| NtMSI1  | XP_015632366.1 | Oryza Sativa               |
| OsMSI1  | XP_015362366.1 | Dendrobium nobile           |

**Figure 1.** Sequence alignment of DnMSI1 with other closely related MS1 (Multicopy suppressor of IRA1) proteins. Shaded in purple are amino acids positions identical in all sequences, conserved GH and WD amino acid in WD40 repeat are highlighted in yellow. Squares in different colors indicate the WD Domains.
by plant hormone Gibberellin (GA$_3$), reaching the maximum level 10 days after the treatment. Then, DnMSI1 expression levels in the buds gradually decreased but were still higher than the control condition 30 days after the treatment. Moreover, DnMSI1 expression was promoted by TDZ (thidiazuron), a cytokinin-like plant regulator. However, the expression peak was delayed compared with that of GA$_3$-treatment. These results suggest that TDZ regulates DnMSI1 expression differently from GA.

By contrast, the expression of DnMSI1 was decreased after applying the plant growth inhibitor, Paclobutrazol (PP333). It has been well documented that PP333 can inhibit the biosynthesis of plant gibberellin (GA) to delays plant growth and development.$^{16}$ So, GA could up-regulate the expression of DnMSI1 directly in D.nobile Lindl, and PP333 down-regulate DnMSI1 expression through inhibition of GA biosynthesis.

As a plant growth retardant, Abscisic acid (ABA) regulates diverse plant growth and development processes under stress conditions. It plays a crucial role in abiotic stress tolerance by mediating genetic and epigenetic processes in plant stress responses.$^{18}$ In this study, we found that DnMSI1 expression was suppressed by exogenously applied ABA and by salt, drought, and heavy metal (Cd, Ni) stresses. The Arabidopsis MSI1 suppresses the expression of ABA-responsive genes, specifically salt and osmotic stress-related genes, and plays negative roles in regulating drought stress responses.$^{13}$ MSI1 is involved
in chromatin dynamics and inheritance of epigenetic states during mitosis. 19 When over-expressing DnMSI1 in Arabidopsis, the transgenic plant lines exhibited obvious sensitivities to NaCl stress. These results suggest that an epigenetic response mechanism could be triggered through ABA-regulated and MSI1-mediated chromatin assembly and dynamics in response to

---

**Figure 2.** Phylogenetic tree of plant MSI1 proteins from various species. On the scale, the bar 0.1 is equal to 10% sequence divergence. VuMSI1 (Vigna unguiculata, XP_017442812), GmMSI1 (Glycine max, ABW23430.1), OsMSI1 (Oryza sativa, XP_023886175.1), PaMSI1 (Populus alba, XP_034908184.1), RcMSI1 (Ricinus communis, XP_002526518.1), HpMSI1 (Hieracium pilosella, ABZ85629.1), PoMSI1 (Pilosella officinarum, ABZ85626.1), HcMSI1 (Hieracium caespitosum, ABZ85631.1), CaMSI1 (Coffea arabica, XP_027113084.1), NtMSI1 (Nicotiana tabacum, ABY84675.1), CsMSI1 (Cucumis sativus, XP_004133950.1), McMSI1 (Momordica charantia, XP_022147047.1), MsMSI1 (Malus domestica musashi, XP_028961311.1), PbMSI1 (Pyrus X britschneideri, XP_009376858.1), AtMSI1 (Arabidopsis thaliana, NP_200631.1), DnMSI1 (Dendrobium nobile), DcMSI1 (Dendrobium catenatum, XP_020679078.1), PdMSI1 (Phoei X dactylyfera, XP_008794440), EgMSI1 (Elsies guineensis, XP_010923692), AcMSI1 (Ananas comosus, XP_020092943.1), SjMSI1 (Setaria italic, XP_004982341.1), SwMSI1 (Setaria viridis, XP_024575367.1), ZmMSI1 (Zea mays, NP_001105556.1), ObMSI1 (Oryza brachyantha, XP_006650332.1), TaMsi1 (Triticum aestivum, ABB892268.1), Cmsi1 (Chlamydomonas reinhardtii, XP_001696907.1), Otsi1 (Ostreococcus tauri, XP_003082166.1), OsMSI1 (Oryza Sativa, XP_015632366.1), PeMSI1 (Phalaenopsis equestris, XP_020572556.1).

---

**Figure 3.** Expression profiles of DnMSI1. Semi-quantitative RT-PCR analysis of the expression level of DnMSI1 in different tissues under normal conditions. The D. nobile ubiquitin gene was amplified as an internal control.

---

**Figure 4.** The expression level of DnMSI1 after drought, Cd, Ni, and NaCl treatment. Distilled water as a negative control. The D. nobile ubiquitin gene was amplified as an internal control for normalization of DnMSI1 mRNA levels. Data are means ± SD (n = 3). Different letters above the columns indicate statistically significant differences at P < .05 by Tukey’s test.
Figure 5. Time-dependent expression pattern of DnMSI1 in buds by GA$_3$, distilled water as a negative control. D. nobile ubiquitin gene was amplified as an internal control for normalization of DnMSI1 mRNA levels. Data are means ± SD (n = 3). Different letters above the columns indicate statistically significant differences at P < .05 by Tukey’s test.

Figure 6. Time-dependent expression pattern of DnMSI1 in TDZ-treated buds. Distilled water as a negative control. D. nobile ubiquitin gene was amplified as an internal control for normalization of DnMSI1 mRNA levels. Data are means ± SD (n = 3). Different letters above the columns indicate statistically significant differences at P < .05 by Tukey’s test.

Figure 7. Time-dependent expression pattern of DnMSI1 in buds by ABA, distilled water as a negative control. D. nobile ubiquitin gene was amplified as an internal control for normalization of DnMSI1 mRNA levels. Data are means ± SD (n = 3). Different letters above the columns indicate statistically significant differences at P < .05 by Tukey’s test.

Figure 8. Time-dependent expression pattern of DnMSI1 in buds by PP333, distilled water as a negative control. D. nobile ubiquitin gene was amplified as an internal control for normalization of DnMSI1 mRNA levels. Data are means ± SD (n = 3). Different letters above the columns indicate statistically significant differences at P < .05 by Tukey’s test.

Figure 9. DnMSI1 over-expression Arabidopsis lines are more sensitive to NaCl stress. (a) NaCl sensitivity of WT and two AtOE lines. Seedlings were treated with 0 (CK) or 100 mM NaCl for 3 d. (b) DnMSI1 relative expression in wild type Arabidopsis (WT) and DnMSI1 over-expression transgenic lines (AtOE-2 and AtOE-7). Data are means ± SD (n = 3). Different letters above the columns indicate statistically significant differences at P < .05 by Tukey’s test. (c) Relative root growth (RGR%) of individual lines. Data are means ± SD (n = 10).
abiotic stresses, leading to negative regulation of salinity stress responses in the plant.

Acknowledgments

The authors would like to thank Jiping Liu for thoughtful discussions, critical reading, and substantial editing of the manuscript. They additionally thank X. Lin for technical assistance with cloning the gene.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by grants from the Guangzhou Science and Technology Project [202102010385] and Rural Revitalization Strategy Project from the Department of Science and Technology of Guangdong Province [620096–3] to Y.W.

ORCID

Yuqi Wang http://orcid.org/0000-0001-5943-280X

Availability of Data and Material

The data supporting this study’s findings are available from the corresponding author, Y.W., upon reasonable request.

Author’s contributions

Y.W. conceived, projected, guided the experiments, and wrote the article; B. C., M. H., C.G., and R.L. performed the experiments and analyzed the data. All authors have read and approved the final manuscript.

References

1. Hennig L, Taranto P, Walser M, Schönrock N, Gruissem W. Arabidopsis MSI1 is required for epigenetic maintenance of reproductive development. Development. 2003;130(12):2555–2565. doi:10.1242/dev.00470.

2. Hennig L, Bouveret R, Gruissem W. MSI1-like proteins: an escort service for chromatin assembly and remodeling complexes. Trends Cell Biol. 2005;15(6):295–302. doi:10.1016/j.tcb.2005.04.004.

3. Kaya H, Shibahara K, Taoka K, Iwabuchi M, Stillman B, Araki T. FASCIATA genes for chromatin assembly factor-1 in Arabidopsis maintain the cellular organization of apical meristems. Cell. 2001;104(1):131–142. doi:10.1016/s0092-8674(01)00197-0.

4. Exner V, Taranto P, Schönrock N, Gruissem W, Hennig L. Chromatin assembly factor CAF-1 is required for cellular differentiation during plant development. Development. 2006;133(21):4163–4172. doi:10.1242/dev.02599.

5. Jain BP, Pandey S. WD40 repeat proteins: signalling scaffold with diverse functions. The Protein Journal. 2018;37(5):391–406. doi:10.1007/s10930-018-9785-7.

6. Jullien PE, Mosquina A, Ingouff M, Sakata T, Ohad N, Berger F, Scott R. Retinoblastoma and its binding partner MS11 control imprinting in Arabidopsis. PLoS Biol. 2008;6(8):e194. doi:10.1371/journal.pbio.0060194.

7. Guittton AE, Page DR, Chambrier P, Lionnet C, Faure JE, Grossniklaus U, Berger F. Identification of new members of fertilisation independent seed polycomb group pathway involved in the control of seed development in Arabidopsis thaliana. Development. 2004;131(12):2971–2981. doi:10.1242/dev.011168.

8. Guittton AE, Berger F. Loss of function of MULTICOPY SUPPRESSOR OF IRA 1 produces nonviable parthenogenetic embryos in Arabidopsis. Current Biology. 2005;15(8):750–754. doi:10.1101/pdb.prot5439.

9. Leroy O, Hennig L, Breuninger H, Laux T, Köhler C. Polycomb group proteins function in the female gametophyte to determine seed development in plants. Development. 2007;134(20):3639–3648. doi:10.1242/dev.009027.

10. Dumbliauskas E, Lechner E, Jaciubek M, Berr A, Pazhouhandeh M, Alioua M, Cognat V, Brukhin V, Koncz C, Grossniklaus U, et al. The Arabidopsis CU14-DBD1 complex interacts with MSI1 and is required to maintain MEDEA parental imprinting. The EMBO Journal. 2011;30(4):731–743. doi:10.1038/emboj.2010.359.

11. Bouveret R, Schönrock N, Gruissem W, Hennig L. Regulation of flowering time by Arabidopsis MSI1. Development. 2006;133(9):1693–1702. doi:10.1242/dev.02340.

12. Exner V, Gruissem W, Hennig L. Control of trichome branching by chromatin assembly factor-1. BMC Plant Biol. 2008;8(1):54. doi:10.1186/1471-2229-8-54.

13. Alexandre C, Möller-Steinbach Y, Schönrock N, Gruissem W, Hennig L. Arabidopsis MSI1 is required for negative regulation of the response to drought stress. Mol Plant. 2009;2(4):675–687. doi:10.1093/mpsp012.

14. Rio DC, Ares M Jr, Hannon GJ, Nilsen TW. Purification of RNA using TRIzol (TRI reagent). Cold Spring Harb Protoc. 2010;6:2010(6):db.prot5439. doi:10.1101/pdb.prot5439.

15. Wang Y, Jiang F, Zhuo Z, Wu XI, Wu YD, Tosatto SCE. A method for WD40 repeat detection and secondary structure prediction. PLoS One. 2013;8(6):e65705. doi:10.1371/journal.pone.0065705.

16. Yamaguchi S. Gibberellin metabolism and its regulation. Annu Rev Plant Biol. 2008;59(1):225–251. doi:10.1146/annurev.arplant.59.032607.092804.

17. Achar P, Genschik P. Releasing the brakes of plant growth: how GAs shut down DELLA proteins. J Exp Bot. 2009;60(4):1085–1092. doi:10.1093/jxb/ern301.

18. Chinnusamy V, Gong Z, Zhu JK. Abscisic acid-mediated epigenetic processes in plant development and stress responses. J Integr Plant Biol. 2008;50(10):1187–1195. doi:10.1111/j.1744-7909.2008.00727.x.

19. Köhler C, Hennig L, Bouveret R, Gheyselinck J, Grossniklaus U, Gruissem W. Arabidopsis MSI1 is a component of the MEA/FIE Polycomb group complex and required for seed development. The EMBO Journal. 2003;22(18):4804–4814. doi:10.1093/emboj/cdg444.