

IDENTIFICATION AND EXPRESSION ANALYSIS OF CLASS C AND D MADS-BOX GENES IN *ORCHIS ITALICA* (ORCHIDACEAE)

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Two MADS-box cDNA, called *OitaAG* and *OitaSTK*, respectively, were identified in inflorescence tissue of the *Orchis italica* (Orchidaceae) during a study of genes involved in flower development in orchids. Based on nucleotide and deduced amino acid sequence analysis, *OitaAG* resulted homolog of the class C MADS-box gene *AGAMOUS* and *OitaSTK* homolog of the class D MADS-box gene *SEEDSTICK* of *Arabidopsis thaliana*, where class C genes are involved in petal and stamen development and class D genes drive the ovary formation. The orchid flower includes an external whorl of three sepaloid petals (outer tepals), two lateral inner tepals, a median inner tepal (lip) and the reproductive whorl in which male and female reproductive tissue are fused (column). Pollinia and ovary are located at the top and at the base of the column, respectively. In orchids, the ovary development is triggered by pollination.

In order to analyze the quantitative relative expression pattern of the *OitaAG* and *OitaSTK* genes during the development of the flower tissues, Real Time RT-PCR experiments were conducted on the different floral tissues of *O. italica* dissected from immature and mature inflorescence. In addition, the expression of both genes was checked in immature and mature ovary, before and after manual pollination. The quantitative relative expression of the *OitaAG* and *OitaSTK* genes in the different tissues were calculated applying the $\Delta\Delta C_T$ approach, using the actin *OitaAct* as the endogenous control gene and leaf cDNA as the reference sample.

In the immature inflorescence, *OitaAG* is expressed in outer and inner tepals and only a weak expression is detectable in lip and column. In the mature inflorescence, inner tepals and lip show a significant increment of the *OitaAG* expression, whereas its levels in outer tepals and column remain approximately constant. In addition, a high amount of *OitaAG* mRNA is detectable in mature ovary before pollination, whereas after pollination its expression slowly decreases.

The *OitaSTK* gene is expressed in outer and inner tepals and in lip dissected from immature inflorescence; a strong increment of its expression is detectable in lip tissue of the mature inflorescence. The highest amount of *OitaSTK* mRNA is detectable in the mature ovary before pollination. After pollination, the expression of *OitaSTK* is still high, even though less abundant when compared to the mature ovary before pollination.

The expression patterns of the *OitaAG* and *OitaSTK* genes of *O. italica* do not fully overlap to those of the class C and D MADS-box genes, respectively, described in *Arabidopsis* and other model organisms, leading to hypothesize possible different roles of these genes during the development of the orchid flower.