

THE *ARABIDOPSIS* MOB1-LIKE GENE IS INVOLVED IN BOTH VEGETATIVE GROWTH AND REPRODUCTIVE BEHAVIOUR

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The MOB family includes a group of cell cycle-associated, non-catalytic proteins highly conserved in eukaryotes, whose founding members are implicated in mitotic exit and co-ordination of cell cycle progression with cell polarity and morphogenesis. Two distinct Mob proteins, Mob1 and Mob2, are known in fungi, while an expansion in metazoans gives rise to six in human, four in *Drosophila*, and four in *C. elegans*. For what concerning plants, alfalfa Mob1-like genes were shown to be specifically expressed in degenerating megaspores of normal ovules and in enlarged megaspore mother cells and embryo sacs of apomeiotic ovules. Gene products were also found in microspore tetrads at the beginning of pollen development as well as in tapetum cells of anthers undergoing programmed cell death to allow pollen dispersal at maturity. Present research deals with the elucidation of the role of Mob1-like genes in order to gain further insights on their function in plants. Functional analysis of Mob1 genes of *Arabidopsis thaliana* (loci At5g45550 and At4g19050) was attempted by using RNA-interfered Mob1 mutants. Silenced single-insertion homozygous lines were investigated on the basis of plant morphological traits and cytohistological observations of female meiosis and gametogenesis. Both temporal and spatial gene expression patterns of AtMob1-like genes were also analyzed by means of Real-Time PCR with member-specific primers and immuno-localization within ovules using polyclonal antibodies against MOB1 proteins.

Analysis of gene expression in the plant organs revealed the presence of Mob1-specific transcripts in all analyzed samples, even if a stronger expression was detected in flowers and siliques. It is worth mentioning that AtRNAi lines were characterized by a marked decrease of Mob1 gene expression within flowers at different developmental stages. On the whole, our data support an altered growth habit and a strongly reduced seed set in the Mob1-interfered plants. In particular, a faster development of plants along with thinner shoots and smaller flowers and siliques were observed. Moreover ovules were shown to contain binucleated megaspores and non-polarized embryo sacs. To confirm the possibility of those unreduced megaspores to proceed throughout gametogenesis, leading to the formation of unreduced functional egg cells, a FCSS analysis of

RNAi lines was attempted. Similarly, image densitometry and pollen cytometry was performed to assay possible variation for the ploidy of pollen grains. Sub-cellular localization of MOB1 proteins within somatic and reproductive organs was also attempted by means of Arabidopsis lines characterized for the production of the GFP::MOB1 fusion protein.

Overall results in terms of gamete ploidy along with transcript expression and protein localization patterns in the Arabidopsis RNAi Mob1 lines are reported and critically discussed.