Does DNA Methylation Pattern Mark Generative Development in Winter Rape?

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In this paper we report on changes in DNA methylation pattern in rape apices and leaves during transition from vegetative to reproductive stage due to grafting and/or vernalization. Grafted plants of winter rape (*Brassica napus* L., var. "Górczański") (stock from vernalized, scion from non-vernalized plants) were used together with vernalized non-grafted plants. In addition, methylation status was determined also in spring rape (var. "Młochowski") grown under normal and low temperature. The methylation-sensitive amplification polymorphism (MSAP) method with *Eco*RI/*Msp*I and *Eco*RII/*Hpa*II restriction enzymes was employed. The majority (ca. 68%) of analyzed loci (566 in winter and 551 in spring rape) were monomorphic, *i.e.* did not undergo methylation. Both cultivars showed a similar degree of methylation. 188 loci in winter and 176 in spring cultivars expressed changes in the methylation pattern. All differentially amplified fragments resulted from either full methylation of an internal cytosine or from hemi-methylation of an external cytosine. A pair-wise comparison showed that a similar number of loci underwent development-related methylation changes in apices of the winter and spring rape. The majority (80%) of changes were demethylation events in generative (vernalized) apices of the winter cultivar. However, an increased number of demethylated loci was detected in vernalized apices in comparison with generative, non-vernalized ones. In apices of vegetative and generative grafted plants the same number of demethylation events was observed. Overall, 10 MSAP loci were detected that expressed methylation changes in vernalized apices only; among them 7 loci underwent demethylation after vernalization and remained methylated in both vegetative and generative non-vernalized stage. Only 1 locus was demethylated in generative non-vernalized apices. Thus, most of demethylation events can be ascribed to vernalization and not to the generative stage. In leaves of winter rape methylation and demethylation events occurred with similar frequency, while in the spring cultivar more demethylation events were detected. The results show that during vernalization and transition to the generative stage different sets of genes are activated.

Key words: DNA Methylation, Rape, Vernalization