

Effects of Cold Treatments on Methylation Profiles and Reproduction Mode of Diploid and Tetraploid Plants of *Ranunculus kuepferi* (Ranunculaceae)

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Abstract : Environmental influence can alter the conditions for plant development and can trigger changes in epigenetic variation. Thus, the exposure to abiotic environmental stress can lead to different DNA methylation profiles and may have evolutionary consequences for adaptation. Epigenetic control mechanisms may further influence mode of reproduction. The alpine species *R. kuepferi* has diploid and tetraploid cytotypes, that are mostly sexual and facultative apomicts, respectively. Hence, it is a suitable model system for studying the correlations of mode of reproduction, ploidy, and environmental stress. Diploid and tetraploid individuals were placed in two climate chambers and treated with low (+7°C day/+2°C night, -1°C cold shocks for three nights per week) and warm (control) temperatures (+15°C day/+10°C night). Subsequently, methylation sensitive-Amplified Fragment-Length Polymorphism (AFPL) markers were used to screen genome-wide methylation alterations triggered by stress treatments. The dataset was analyzed for four groups regarding treatment (cold/warm) and ploidy level (diploid/tetraploid), and also separately for full methylated, hemi-methylated and unmethylated sites. Patterns of epigenetic variation suggested that diploids differed significantly in their profiles from tetraploids independent from treatment, while treatments did not differ significantly within cytotypes. Furthermore, diploids are more differentiated than the tetraploids in overall methylation profiles of both treatments. This observation is in accordance with the increased frequency of apomictic seed formation in diploids and maintenance of facultative apomixis in tetraploids during the experiment. Global analysis of molecular variance showed higher epigenetic variation within groups than among them, while locus-by-locus analysis of molecular variance showed a high number (54.7%) of significantly differentiated un-methylated loci. To summarise, epigenetic variation seems to depend on ploidy level, and in diploids may be correlated to changes in mode of reproduction. However, further studies are needed to elucidate the mechanism and possible functional significance of these correlations.

Keywords : apomixis, cold stress, DNA methylation, *Ranunculus kuepferi*

Conference Title : ICPGG 2018 : International Conference on Plant Genetics and Genomics

Conference Location : Zurich, Switzerland

Conference Dates : September 13-14, 2018