Flowcytometric analysis of ploidy in *Physcomitrella patens*

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The current production of a saturated mutant collection is based on PEG-mediated protoplast transformation and after regeneration and selection on antibiotic-containing medium the ploidy level of each stable transformant is determined by flowcytometry. The ploidy level of 45,000 transgenic *Physcomitrella* plants has been tested so far and 13.2 +/- 7.8 % were polyploid (2n = 13.0 % and 4n = 0.2 %), probably due to protoplast fusion during the transformation procedure. The percentage of polyploid plants differs a lot between single transformations; however no correlation between preculture or the transformation procedure and the number of polyploid mutants regenerated afterwards could be detected so far. In two experiments we compared two ways of mixing protoplasts and DNA during the transformation by either carefully rotating the glass tubes in our hands or using a vortexer. However the number of polyploid transformants coming out of 2. selection were in the same range.

After quality control (identification of stable transformants by PCR or third selection and determination of ploidy level) moss plants are characterized phenotypically. To check whether variation in moss phenotype could be induced not only by gene knockout but also by changes of the ploidy level, ploidy and phenotype of 418 haploid and 80 diploid untransformed regenerated moss plants were correlated. While most of the haploid regenerants looked like wildtype (> 90 %), around 75 % of the diploid plants showed a phenotype deviating from wildtype. More than 90 % of the haploid plants showed normal growth on Knop medium, compared to only 20 % of the polyploid plants. Changes in the ploidy level did rarely affect the cell form of the gametophores and had no effect on the color of the plant, but many diploid plants showed a reduced number of gametophores (> 90 %) and changes in the form of the gametophores, like double tips (> 20 %) or cell outgrowth (> 5 %). Phenotypic deviations in more than one characteristic strongly indicated polyploidization.

Correlation coefficients for ploidy level and different phenotypic characteristics were between 0.3 and 0.7 for the 500 plants that had been regenerated after mock-transformation. Correlation coefficients higher than 0.5 were found for the features leaf shape, growth on minimam medium, coverage of the protonema with gametophores, and multiple phenotypic deviations from wildtype. We calculated the correlation coefficient for the first 10,000 stable transformands of our mutant collection as well and they were in the same range (0.15-0.7). Correlation lower than 0.5 were calculated for the features growth on full medium, color, and cell shape. Plant structure, coverage with gametophores, leaf shape, growth on minimal medium and multiple deviations were highly correlated (>0.5) with the ploidy level of the mutants.

Although specific phenotypic deviations indicate changes in the ploidy level of plants, identification of polyploid plants (either mutants or regenerants after protoplast isolation) by phenotypic assessment is not possible. Flowcytometry allows unequivocal determination of ploidy level of Physcomitrella plants. In our project around 200-350 plants are tested daily.

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