Expression of flowering genes in *in vitro* propagated silver birch clones with differing flowering

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Silver birch (*Betula pendula* Roth.) is an ecologically and economically important deciduous tree species in Northern Europe. Vegetatively propagated *in vitro* silver birch material is used for breeding and for the development of seed plantations. However, reduced flowering and seed formation is often observed, which delays breeding activities and hinders seed production in nurseries. This is probably related to the long juvenile phase in trees, which can last 10–20 years. Birch trees in nature start to flower at the age of 10–15 years producing unisexual flowers that develop on the same tree but on separate inflorescences (catkins).

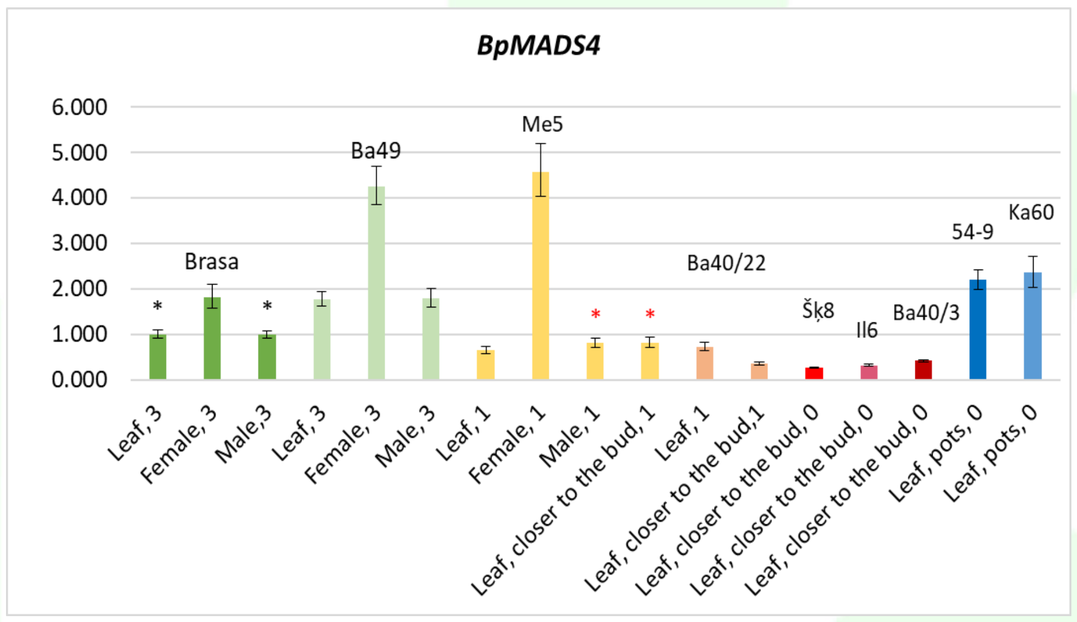

Figure 1. *BpMADS4* gene expression between clones and sample types. * indicates samples with statistically non-significant differences in expression (p-value > 0.05).

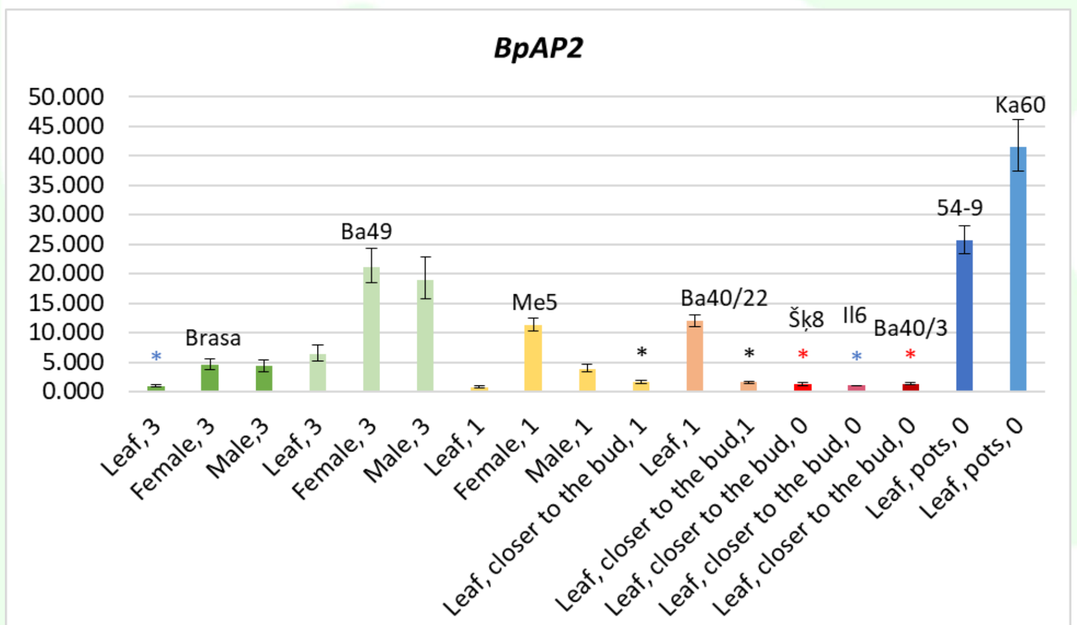

Figure 2. *BpAP2* gene expression between clones and sample types. * of the same colour indicate samples with statistically non-significant differences in expression (p-value > 0.05).

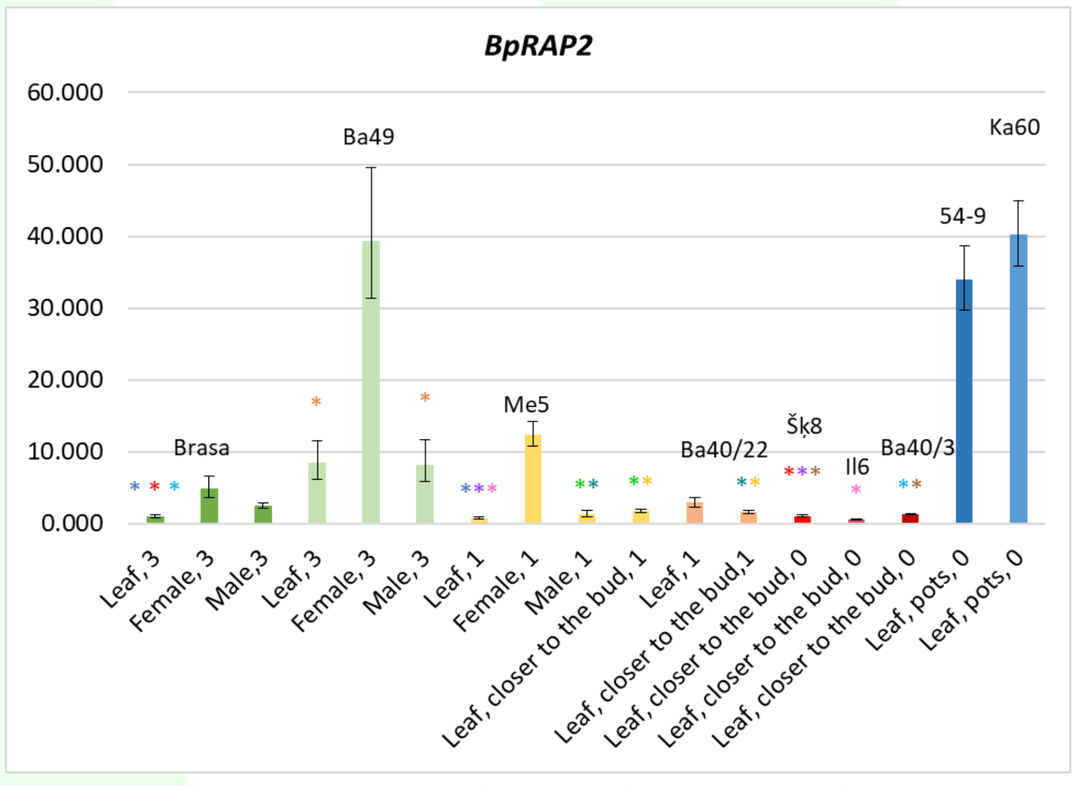

Figure 3. *BpRAP2* gene expression between clones and sample types. * of the same colour indicate samples with statistically non-significant differences in expression (p-value > 0.05).

FUTURE RESEARCH DIRECTIONS

A larger number of clones with differing flowering that have been collected in 2024 to further elucidate the role of these genes in the transition from the juvenile phase and initiation of flowering will be analyzed. 30 birch clones before and after experimental treatment to promote flowering(in collaboration with the plant physiology laboratory) will be analyzed.

The *BpMADS4* gene

BpMADS4 is one of the major genes controlling initiation of flowering and inflorescence development in birch and in the transition from the vegetative to the reproductive phase. Overexpression of *BpMADS4* can cause very early flowering, and studies suggest that the expression of the *BpMADS4* gene alone is capable of turning the vegetative apical meristems into inflorescence meristems, but suppression of the gene inhibits inflorescence formation.

AIMS and MATERIALS

The aim of this study was to assess expression of juvenility (*BpAP2*, *BpRAP2*) and maturity (*BpSPL1, BpSPL9*) related target genes, as well the flowering associated gene *BpMADS4* in 12-year-old silver birch trees with differing flowering. Several birch clones growing at the Norupe nursery were analysed, some of which regularly flowered (Brasa, Ba49), while others had reduced flowering (Me5, Ba40/22), or did not flower at all (Šķ8, Il6, Ba40/3). In addition, three-year-old non-flowering birch trees (54-9, Ka60), which were planted in pots after microclonal propagation were analysed. Samples were collected from leaves, male and female catkins.

RESULTS

Gene expression data were obtained for three genes – *BpMADS4*, *BpAP2* and *BpRAP2.* Numbers after sample type indicate regularly flowering (3), poorly (1) or non flowering (0) clones (Figure 1-4). The highest gene expression of all three genes, both within clones and between clones, was observed in female flowers (Figure 1-3). In the case of the *BpMADS4* gene, expression was similar between male flowers and leaves, while in the case of *BpAP2*, higher gene expression was observed in male flowers than in leaves, but in the case of *BpRAP2*, gene expression differed between clones.

When analysing the data obtained from leaf samples, it was observed that the lowest *BpMADS4* gene expression was observed in those clones that were not flowering at the time of sample collection (Figure 4). High expression of the juvenility related *BpAP2* and *BpRAP2* genes was observed in male and female inflorescences, which was unexpected, as flowering indicates that the tree has entered the mature phase. However, this could be related to the tissues sampled (young flower buds) rather than with the maturity of the tree. However, in the case of the 3-year-old birch clones, a correlation was found between expression of the *BpAP2* and *BpRAP2* genes and juvenility – lower *BpAP2* and *BpRAP2* gene expression was observed in the clone that was not flowering at the sample collection time, but flowered later (54-9). Higher *BpAP2* and *BpRAP2* expression was observed in the other clone that did not flower at all in the 2024 season (Ka60).

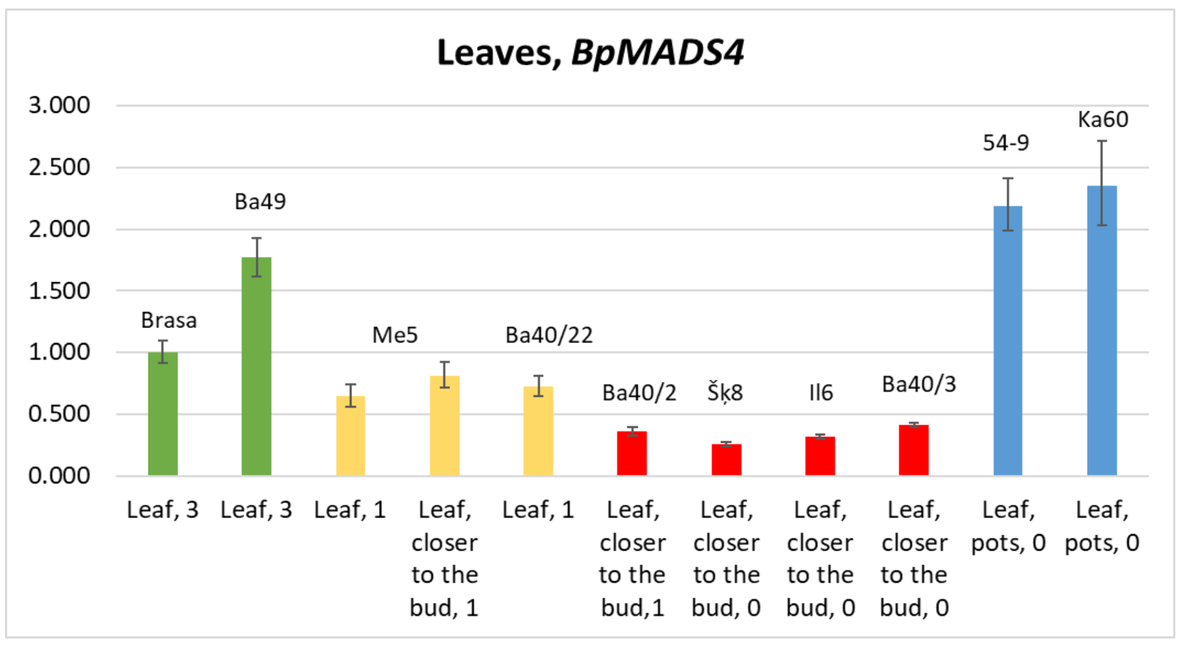

Figure 4. *BpMADS4* gene expression between clones in leaves.

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