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Transcriptomic analysis of generative and vegetative organs of male and female poplar plants

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Motivation and Aim: Poplars (genus *Populus*) belong to dioecious species, in which male and female flowers are formed on separate plants. In recent years, large-scale studies of the genus *Populus* species were performed, and genes that play a key role in sex determination were identified. However, the data on the number of these genes diverge in the works of different authors. It is also not clear which pathways are involved in the development of male and female flowers. Moreover, contradictory data have been obtained on the differences between male and female plants, which are not limited to generative organs. This work aims at identifying the key mechanisms involved in sex determination of *Populus* species and the development of generative and vegetative organs of male and female poplar plants.

Methods and Algorithms: A set of generative and vegetative organs at 7 time points of two male and two female *Populus* × *sibirica* plants (total 56 samples) was collected in Moscow. Harvested generative and vegetative organs were immediately frozen in liquid nitrogen and then stored at -70 °C until RNA was extracted. Using the MagNA Lyser system (Roche, Switzerland), we homogenized 0.2 g of each *P.* × *sibirica* leaf, flower, and catkin axis samples during 90 s at 700 rpm in 600 µl of RNA Lysis Buffer from the Quick-RNA Miniprep Kit (Zymo Research, USA) and in the presence of Solid-glass beads (Sigma-Aldrich, USA) being 2 mm in diameter and having a mass of 0.14 g. Then, the homogenate was cooled. Total RNA was extracted using the Quick-RNA Miniprep Kit (Zymo Research) according to the standard protocol of the manufacturer, including the step of treatment by DNase I. The quality and concentration of the RNA were evaluated using a 2100 Bioanalyzer instrument (Agilent Technologies, USA) and Qubit 4 fluorometer (Thermo Fischer Scientific, USA) respectively. cDNA libraries for the high-throughput sequencing were prepared with the RNeasy Plant Mini Kit (Qiagen, USA) for the isolation of mRNA fraction from 1 µg of the total RNA and the QIAseq Stranded Total RNA Library Kit (Qiagen) for the subsequent RNA fragmentation and adapter ligation according to the manufacturer's protocols. The quality of the cDNA libraries was evaluated using a 2100 Bioanalyzer instrument (Agilent Technologies), and concentration – using a Qubit 4 fluorometer (Thermo Fischer Scientific). The cDNA libraries were sequenced on NextSeq500 (Illumina, USA) with a read length of 86 nucleotides.

Results: On the Illumina platform, we performed transcriptome sequencing of 56 cDNA libraries: two male and two female *P.* × *sibirica* plants, generative and vegetative organs, 7 stages of development: the end of June-beginning of July (the next-year bud begins to

form), the end of July-beginning of August (flower bud has already appeared, but there is no visible difference between male and female inflorescences), end of August-beginning of September (differences between male and female inflorescences are visible), end of September-beginning of October (rudiments of gynoecium and stamens become visible), end of October-beginning of November (carpels and stamens are formed, but have a small size), end of March-beginning of April (generative organs slightly increase in size), end of April-beginning of May (flowers are fully formed). The collection of plant material during these time points covers the main development stages of generative and vegetative poplar buds. We carried out a bioinformatics analysis of the obtained data and identified genes that are expressed at different development stages in the generative and vegetative organs of male and female *P.* × *sibirica* plants, as well as genes with sex-differential expression. Expression profiles were more similar for vegetative buds of male and female plants than for generative ones.

Conclusion: We obtained the expression profiles for the main development stages of generative and vegetative buds of male and female *P.* × *sibirica* plants and identified differentially expressed genes. The comparison of our results with the results of studies on other species of the genus *Populus* will allow establishment of the gene pathways involved in the development of generative and vegetative organs in male and female poplars.

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