

## MYC2 regulates stomatal density and water use efficiency via targeting EPF2/EPFL4/EPFL9 in poplar

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**Abstract:** Although polyploid plants have lower stomatal density than their diploid counterparts, the molecular mechanisms underlying this difference remain elusive. Here, we constructed a network based on the triploid poplar transcriptome data and TGMI algorithm and found that PpnMYC2 was related to stomatal development-related genes PpnEPF2, PpnEPFL4, and PpnEPFL9. The interactions between PpnMYC2 and PagJAZs were experimentally validated. PpnMYC2-overexpressing poplar and *Arabidopsis thaliana* had reduced stomatal density. Poplar overexpressing PpnMYC2 had higher water use efficiency and drought resistance. RNA-sequencing data of poplars overexpressing PpnMYC2 showed that PpnMYC2 promotes expression of stomatal density inhibitors PagEPF2 and PagEPFL4 and inhibits expression of the stomatal density positive regulator PagEPFL9. Yeast one-hybrid system, electrophoretic mobility shift assay, ChIP-qPCR and dual-luciferase assay were employed to substantiate that PpnMYC2 directly regulated PagEPF2, PagEPFL4, and PagEPFL9. PpnMYC2, PpnEPF2 and PpnEPFL4 were significantly upregulated, whereas PpnEPFL9 was downregulated during stomatal formation in triploid poplar. Our results are of great significance for revealing the regulation mechanism of plant stomatal occurrence and polyploid stomatal density, as well as reducing stomatal density and improving plant water use efficiency by overexpressing MYC2.