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SHORT COMMUNICATION

Dissecting the role of *EPFL9-2* in grapevine root system architecture through rhizotron-based phenotyping

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ABSTRACT

Epidermal Patterning Factors (EPFs) constitute a family of small, highly conserved peptides that regulate diverse physiological and developmental processes in plants. In grapevine (*Vitis* spp.), *EPFL9-2* has been identified as a positive regulator of stomatal development and a critical determinant of root system architecture (RSA). Unlike aboveground organs, roots present significant challenges for non-invasive phenotyping. This study aimed to establish a root phenotyping system to deeply characterize the RSA of *epfl9-2* knockout (KO) lines of the 'Kober 5BB' (*Vitis berlandieri* x *Vitis riparia*) rootstock.

Two distinct *epfl9-2* KO lines and wild-type (WT) control were cultivated in a greenhouse using a rhizotrons (25 x 50 x 4 cm). Root growth was monitored at three time-points using standardized digital imaging. Furthermore, the RSA was imaged also in grafted plants where an *epfl9-2* KO line and Kober WT were grafted with a 'Syrah' (WT) scion. Images were processed using RhizoVision Explorer software to quantify key parameters, including total area covered by roots, root length density, and lateral root branching angles.

Our results indicate that *epfl9-2* mutants exhibit a more compact RSA compared to the WT, characterized by reduced total length and lateral expansion. Notably, KO lines showed a significant reduction in the gravitropic angle and total root length, while simultaneously displaying increased root diameters and higher density per unit area. Grafting experiments demonstrated that the *epfl9-2* root phenotype persists regardless of the presence of a WT scion, suggesting that these altered root traits are independent of the shoot apparatus. This phenotyping approach provides a robust framework for further evaluating the performance of *epfl9-2* genotypes under simulated environmental stress. Future research will investigate how these specific root traits may impact scion resilience to abiotic stress in grafted grapevine varieties of economic interest.