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ELUCIDATING THE ROLE OF VVEPFL9-1 IN GRAPEVINE THROUGH GENE EDITING AND PHYSIOLOGICAL PHENOTYPING

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Global warming will increase the frequency of extreme high?temperature events and consequent severe drought scenarios and thus may constitute a great threat to modern viticulture. These extreme conditions could strongly affect grapevine physiology with a dramatic impact on grape yield and quality. One of the mechanisms that plants can activate in response to environmental stresses relies on the stomatal regulation of transpiration. Stomata are microscopic structures predominantly found in leaf epidermis, formed by two specialized guard cells flanking a central pore which facilitate diffusional gaseous (i.e. the entry of carbon dioxide in the leaf for photosynthesis and the loss of water vapor into the atmosphere). The hormonal peptides of the epidermal patterning factor (EPF and EPF-Like) family are known in cereals and model plants to be responsible for regulating stomatal development during leaf formation and are highly conserved in many species. In particular, the factors EPF1 and EPF2 are negative regulators of stomata while EPFL9 (also known as STOMAGEN) competes with them for the same receptors (ERECTA and Too Many Mouths) and promotes stomatal development. In grapevine we identified two *VvEPFL9* genes homologous to *AtEPFL9* and produced knock-out *Vvepfl9-1* mutants by using the gene editing technology based on the CRISPR/Cas9 system. A specific sgRNA was designed and transformed in a binary vector for the Agrobacterium tumefaciens gene transfer in embryogenic calli. Putative edited lines, regenerated after a selection period of 9 months in medium containing kanamycin, were characterized by the analysis of the editing profile in the target site and the identification T-DNA cassette integration point in the plant genome. Targeted mutation was almost complete in many lines and frequently, within single lines, several kinds of mutation were detected. Key physiological traits were then evaluated on selected edited lines: stomatal anatomical features (density, size), transpiration and gas-exchange. Edited lines showed reduced stomatal density, lowered transpiration and stomatal conductance and enhanced water use efficiency compared to wild-type. Our results indicate that stomata are a key target in view of engineering crop resilience to climate changes in grapevine.