Deciphering the function and regulation of *VviEPFL9* paralogs to modulate stomatal density in grapevine through New Genomic Techniques

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Stomata are microscopic pores mainly located in leaf epidermis, allowing gas exchanges between plants and atmosphere. Stomatal initiation relies on the transcription factor SPEECHLESS which is mainly regulated by the MAP kinase cascade, in turn controlled by small signaling peptides, the Epidermal Patterning Factors (EPF and EPF-Like), namely EPF1, EPF2 and EPFL9. While EPF1 and EPF2 induce the inhibition of SPEECHLESS, their antagonist, EPFL9, stabilizes it, leading to stomatal formation. In grapevine, there are two paralogs for EPFL9, *VviEPFL9*-1 and *VviEPFL9*-2. Despite their structural similarity, it remains unclear whether they are differentially regulated and have distinct roles. In our study we showed that while *VviEPFL9*-1 is expressed only in the apex, *VviEPFL9*-2 is expressed both in the apex and in mature leaves along the plant axis and is significantly repressed by ABA. To support experimental data, an *in-silico* transcriptomic analysis has been carried out using publicly available datasets. In addition, both genes were functionally characterized using knock-out (KO) mutants generated via CRISPR/Cas9. Leaf stomatal density and gas exchange parameters were significantly different between 'Sugraone' WT plants and *VviEPFL9-2* KO lines, whereas the differences were negligible between WT and *VviEPFL9-1* KO lines. Moreover, a water stress experiment was carried out to deeply compare the physiology of edited lines and WT plants under drought conditions. Our results suggest that *VviEPFL9* paralogs have distinct roles in determining stomatal plasticity during leaf growth, and that *VviEPFL9-2* may be considered a key target to increase grapevine resilience to water deficiency.

Keywords: *Vitis vinifera*, Epidermal Patterning Factors, CRISPR/Cas9, gas exchange, water stress.