Enhancing Leaf Biomass in Alfalfa (*Medicago sativa* L.) by Overexpressing Growth-Regulating Factor (GRF) & GRF-Interacting Factor (GIF)

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Alfalfa (*Medicago sativa* L.) is a perennial legume valued for its high yield potential. It is primarily used as feed for dairy cattle and livestock, as it has a high nutritive value due to its digestibility and crude protein content. However, on-farm alfalfa yield has been stagnant in recent years, and despite the adoption of improved breeding practices and genomic methods, this trend does not appear to have changed substantially. We propose 'redesigning' alfalfa by modifying the structure of the crop to increase yield while still maintaining forage quality.

One gene of interest is *GROWTH-REGULATING FACTOR (GRF)* along with its cofactor *GRF-INTERACTING FACTOR (GIF)*. GRF is a family of transcriptional activators linked to plant development processes, namely leaf size and cell expansion (Kim et al., 2003; van der Knaap et al., 2000). GRFs are characterized by two conserved domains at the N-terminus: the WRC and QLQ domains (van der Knaap et al., 2000). Through the QLQ domain, GRFs interact with GIFs to form a functional transcriptional activation complex (Kim & Kende, 2004). In *Arabidopsis thaliana*, *GRF3* and *GIF1* overexpression resulted in increased leaf size and delayed senescence (Debernardi et al., 2014). *GRF-GIF* overexpression has also been linked to enhanced regeneration in wheat, rice, and soybean (Debernardi et al., 2020; Zhao et al., 2024).

Given *GRF-GIF*'s role in leaf development, where overexpression has been shown to increase leaf size and enhance regeneration efficiency, we selected it as an initial candidate to improve biomass yield and yield components in alfalfa. We inserted a soybean *GRF4-GIF1* construct into a non-dormant alfalfa genotype derived from the cultivar 'Highline' via *Agrobacterium*-mediated transformation; an empty vector transgenic control was included for comparison. Seven independent *GRF-GIF* events and three empty vector control events were obtained and clonally propagated for evaluation in the greenhouse. To characterize the phenotype and morphology of the transgenic plants, biomass, leaf:stem ratio, and leaf area are being evaluated. Preliminary data suggest that plants containing *GRF-GIF* have an increased leaf:stem biomass ratio compared to empty vector controls. We are continuing to evaluate biomass yield and yield components over multiple regrowth cycles. We will discuss possible avenues for further redesign of the alfalfa plant.

References

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