

## New biotechnological approaches for a comprehensive characterization of AGL11 and its molecular mechanism underlying seedlessness trait in table grape.

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### Abstract

In table grapes seedlessness is a crucial breeding target, mainly results from stenospermocarpy, linked to the Thompson Seedless variety. Several studies investigated the genetic control of seedlessness identifying *AGL11*, a MADS-box transcription factor, as a crucial gene.

We performed a deep investigation of the whole *AGL11* gene sequence in a collection of grapevine varieties revealing three different promoter-CDS combinations. By investigating the expression of the three *AGL11* alleles and evaluating their ability to activate the promoter region, we show that *AGL11* regulates its transcription in a specific promoter-CDS manner. By a multi-*AGL11* co-expression analysis we identified a *methyl jasmonate esterase*, an *indole-3-acetate beta-glucosyltransferase*, and an *isoflavone reductase* as top *AGL11* candidate targets. *In vivo* experiments further confirmed *AGL11* role in regulating these genes, demonstrating its significant influence in seed development and thus in seedlessness trait.

The overall data allowed us to propose a novel regulatory mechanism correlating *AGL11* haplotype assortment and seedlessness class, suggesting potential applications in grapevine breeding for seedlessness and fruit size optimization.

With the aim to validate *in planta* the proposed regulatory mechanism, we are working to stable transform 'microvine' plants for producing a truncated or inactivated *AGL11* protein. We first demonstrated the ability of embryogenic calli, obtained from 'microvine' anthers collection and culture, to regenerate embryos capable of germinating and sprouting into a new plant. The use of 'microvine' as a model system offers promising outcomes for functional gene characterization, benefiting viticulture genetic improvement and seedless table grape cultivation.

**Keywords:** Table grape, Seedlessness, *AGL11*, Regulatory mechanism, Microvine