Genomic Exploration of Challenging-to-Culture Blueberry Varieties

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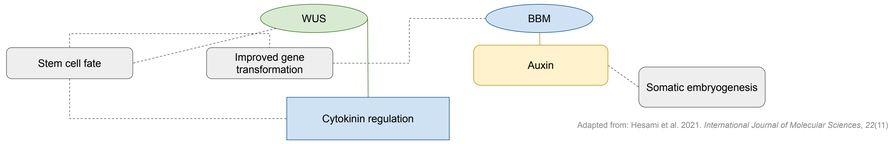
Background

- **Current Issues:** Despite progress, blueberry (*Vaccinium spp.*) regeneration and transformation processes remain inefficient, limiting the production of plants with desired traits through traditional breeding methods.
- **Industry Impact:** This bottleneck hinders gene editing and slows the development of enhanced blueberry varieties, reducing the industry's ability to meet growing demand.
- ***WUSHEL* (*WUS*) and *BABY BOOM* (*BBM*):** Recent investigations have shown these genes can overcome obstacles encountered during regeneration and transformation processes.

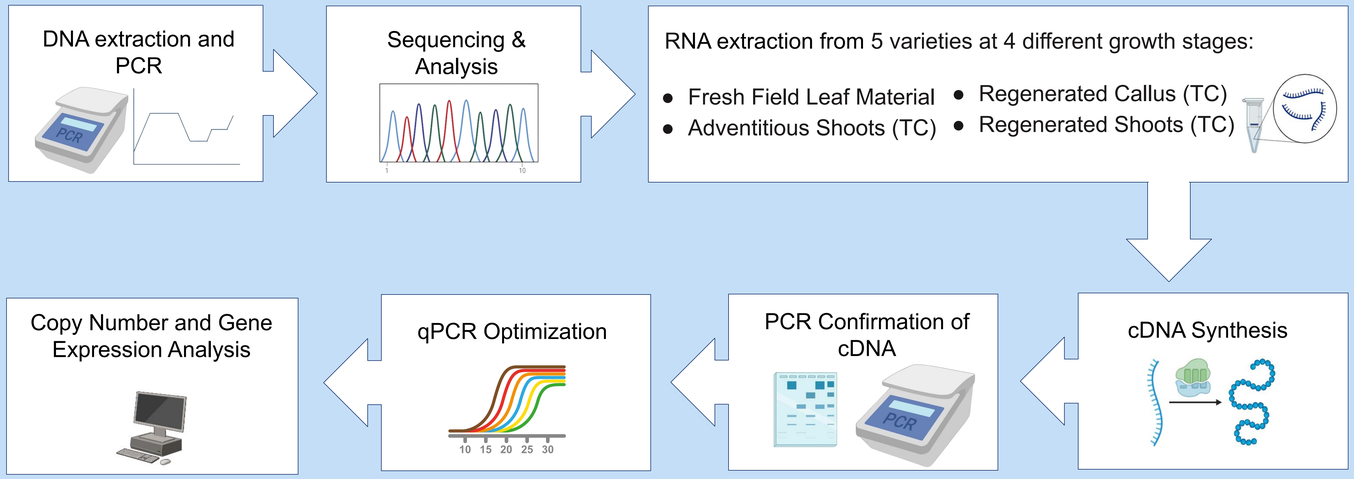


Adapted from: Hesami et al. 2021. *International Journal of Molecular Sciences*, 22(11)

Figure 1. Schematic view of morphogenic genes, *WUS* and *BBM*, demonstrating their roles in plant growth and development as well as in vitro plant regeneration.

- Ectopic expression of *AtBBM* and *AtWUS*, has demonstrated success in enhancing regeneration and transformation frequencies in various species such as melon, cotton, and tobacco.
- **Objective:** Our research seeks to determine if the beneficial effects of exogenous *BBM* and *WUS* genes seen in other species can be applied to blueberries. Before attempting transformations, we are focusing on understanding the native BBM and WUS genes, especially in varieties that are difficult to culture. This understanding is essential for enhancing regeneration with exogenous genes.

Methods



Results

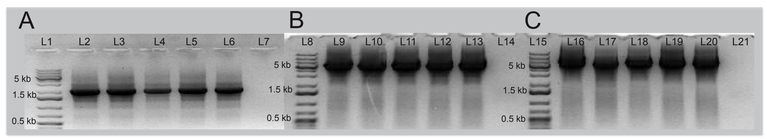


Figure 2 A-C. Gel electrophoresis visual confirmation of **(A)** *WUS*, **(B)** *BBM1*, and **(C)** *BBM2*. L1, L8, and L15: 1 kb Ladder; L7, L14, and L21: Water control; L2, L9, and L16: 'Pinnacle'; L3, L10, and L17: 'Legacy'; L4, L11, and L18: 'O'Neal'; L5, L12, and L19: 'NC4499'; L6, L13, and L20: 'NC5271'. 1% agarose gel.

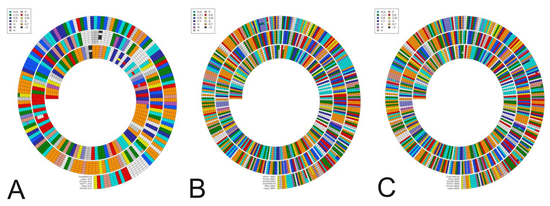


Figure 3 A-C. (A) WUS, **(B)** BBM1, and **(C)** BBM2 protein sequences from *V. corymbosum* cvs. 'O'Neal', 'NC4499', 'NC5271', 'Pinnacle', and 'Legacy' aligned with reference genome.

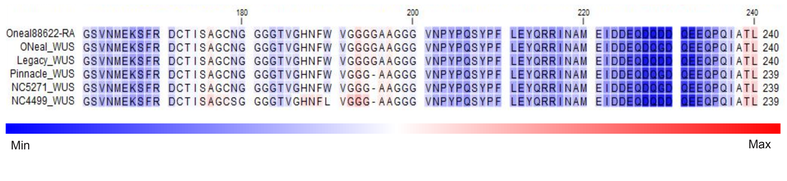


Figure 4. MSA analysis of the WUS protein region 191-200 reveals a significant three-nucleotide deletion in challenging blueberry varieties. This deletion is associated with increased hydrophobicity, as visualized using the Kyte-Doolittle scale.

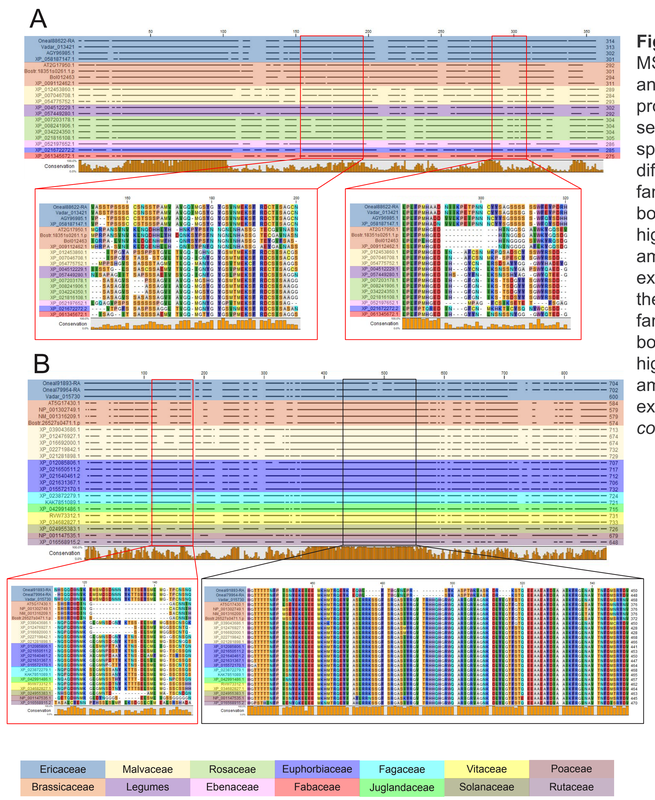


Figure 5 A-B. MSA of **(A)** WUS and **(B)** BBM protein sequences from species of 14 different plant families. Red boxes indicate high homology among species, except those in the Brassicaceae family. Black boxes indicate high homology among species, except *V. corymbosum*.

Discussion

- **Gene Presence:** *WUS* and two *BBM* gene variants were confirmed in three difficult-to-propagate blueberry varieties and two easily cultivated varieties via PCR (Fig. 2A-C).
- **Sequencing Findings:** Sanger sequencing revealed nsSNPs, including a significant three-nucleotide deletion in *WUS* sequences of challenging varieties (Fig. 3A). This deletion is associated with increased hydrophobicity which could affect protein folding (Fig. 4).
- **Conserved Regions:** MSAs revealed conserved regions crucial to protein function (Fig. 5 A-B). A 50 amino acid sequence in *WUS* showed high homology across species, except in Brassicaceae. Similarly, conserved regions in *BBM* displayed a 10 amino acid gap in Brassicaceae. These conserved regions suggest critical functional roles, potentially explaining why Brassicaceae genes might enhance regeneration and transformation in other species.

Future Work

- Further protein analysis is needed to determine the functional significance of the three-nucleotide deletion in the WUS gene in challenging blueberry varieties.
- We will continue to investigate gene expression using qPCR to gain a deeper understanding.
- Following this, our team will explore the potential benefits of expressing *Arabidopsis thaliana* and *Boechera stricta WUS* and *BBM* genes in blueberry plants to address the regeneration and transformation challenges in difficult-to-propagate varieties.