Sequencing of the SbGl15 (Sb10g025053) gene sequence controlling glossiness in juvenile leaves of *Sorghum bicolor*

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ABSTRACT

Glossy15 is a gene found within the *Zea mays* genome and codes for an Apetala2-like transcription factor. This gene is involved in cuticle wax formation on the leaves of juvenile plants. A *Sorghum bicolor* gene with 69.9% similarity to Glossy15 has been identified (*Sorbidraft_10g025053).* We are sequencing the entire *Sorbidraft_10g025053* gene, which includes the promoter as well as all exons and introns, in 9 sorghum lines that differ for glossiness. The expected results are that sequence differences (SNP's, InDels, or Frameshifts) will be present between the glossy and non-glossy lines.

BACKGROUND

The Sequence of the phenotype for Glossiness

- *Glossy15 (gl15)* is a gene that regulates the presence of juvenile epidermal wax after the growth of the second leaf within *Zea mays.*
- *Sorbidraft_10g025053* is a hypothetical protein sequence in the *Sorghum bicolor* genome that is 69.9% similar in DNA sequence to *gl15.*
- *Zea mays* and *Sorghum bicolor* are very closely related species because they are both members of the grass family *Poaceae* and have genomic similarity.
- The phenotype for glossiness is expressed within *Sorghum bicolor* but no gene controlling this trait has been cloned or sequenced.
- Based on the *Sorghum bicolor* reference genome, *Sorbidraft_10g025053* is 4707 basepairs long with 11 exons
- The *Sorbidraft_10g025053* contains two AP2 domains within its gene sequence

OBJECTIVES

- Design overlapping primer pairs to amplify the entire *Sorbidraft_10g025053* gene sequence including the promoter and all exons and introns.
- Amplify and sequence the products of these primers in 9 *S. bicolor* lines (4 glossy, 4 non-glossy and 1 wild outgroup) using Polymerase Chain Reaction (PCR) and gel electrophoresis.
- To learn to use genetic tools, protocols, and software to find sequences within a genome.

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PRIMER DESIGN

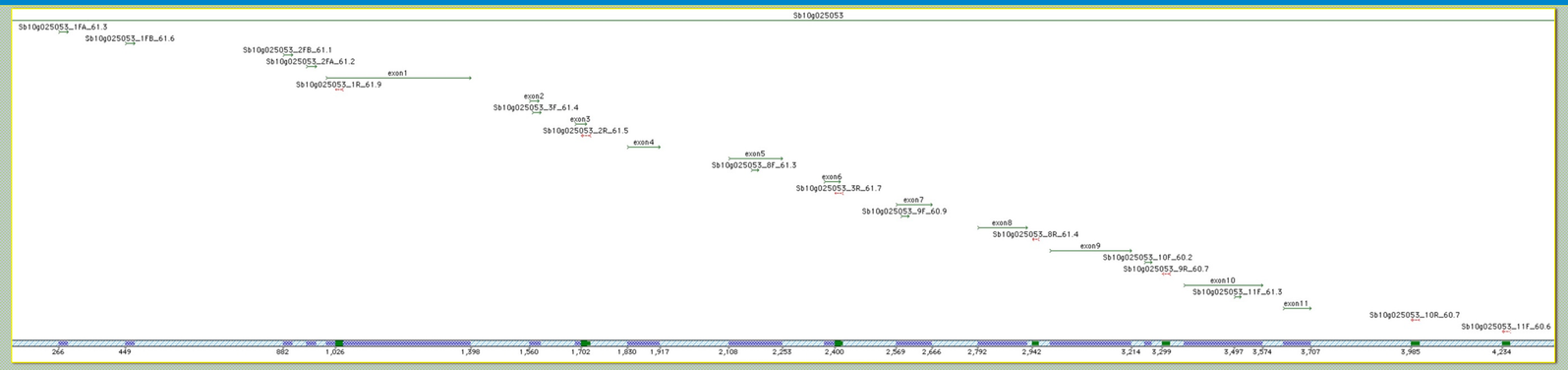

Figure 1. Sequencher diagram of *Sorbidraft_10g025053* gene. The 11 exons are shown as large green forward arrows and the 16 primers are shown as small green arrows for forward primers and small red arrows for reverse primers.

METHODS

- Sixteen primers were designed for the *Sorbidraft_10g025053* gene using the computer program Sequencher and ordered through IDT.
- PCR was performed on genomic DNA of 9 *S. bicolor* lines using Redtaq DNA polymerase in a 25 uL reaction and a melting temperature of 58°C.
- Gel electrophoresis using a 1% agarose gel and TBE buffer was performed to visualize the amplification products.

Table 1. Primer sequences found within *Sorbidraft_10g025053* gene R (Reverse) and F (Forward), colored primers were tested against glossy and non-glossy varieties

Primer sequence	Tm	Nucleotide Sequence
Sb10g025053_1R	61.9	GTCGACGCCGCAGTAGTGGA
Sb10g025053_1FA	61.3	GTGCTGCAAGATTCGATGTGACGGA
Sb10g025053_1FB	61.6	ACGTACGATTGTGCTGTGTACTCGGT
Sb10g025053_2FA	61.2	GTGCGTGATCTTTATCTACGCACCGG
Sb10g025053_2R	61.5	ATCTATGAACCTTGCAGCAGCCTGC
Sb10g025053_2FB	61.1	AGAGTTGCAATGATGCATTCCATCGCA
Sb10g025053_3F	61.4	GGGAAGCAGGTGTACTTGGGTGAG
Sb10g025053_3R	61.7	CACTTACTGGGCAGCCTCCGT
Sb10g025053_6F	60.7	GGATTTGACACAGCGCAGGCT
Sb10g025053_6R	61.2	CAGAGTAGGGCAGCGCAGC
Sb10g025053_7F	61.8	TCTTGCCATGGCGGCATTCTCA
Sb10g025053_7R	60.9	CCGTAGCACTTGATGGCAGCC
Sb10g025053_8F	61.3	TCAGGGGGAGCTCCAGGTTC
Sb10g025053_8R	61.4	GGCTCAGCGTCGGCTACG
Sb10g025053_9F	60.9	GGCTGCCATCAAGTGCTACGG
Sb10g025053_9R	60.7	GGAAACCGAACCAACGATCGCTG
Sb10g025053_10F	60.2	CGATGCACCAGTGATCCTCCG
Sb10g025053_10R	60.7	GCCGTGTCATCCACAATCCACC
Sb10g025053_11F	61.3	CGACGACGAAGACGCCAGC
Sb10g025053_11F	60.6	GGGTTACCACCCAGAGGTACTCC

PCR/ELECTROPHORESIS METHODS

Table 2. All wells contained a forward primer 1FB and a reverse primer 1R

Wells	Species	Phenotype
1	SC0389	Non-Glossy
2	SC0371	Glossy
3	SC1021	Non-Glossy
4	SC1022	Glossy
5	SC0389	Non-Glossy
6	SC0558	Glossy
7	SC0557	Non-Glossy
8	Propin-quum	N/A Wild
9	BT x 623	Glossy

Figure 2. UV photograph of gel plates loaded with species of *Sorghum bicolor* with 1Fb/R primers whose PCR amplification was set at Tm 58° C

CONCLUSIONS/FUTURE DIRECTIONS/ EXPECTED RESULTS

The future direction is to amplify all the primers with all nine species of *Sorghum bicolor* to find noticeable differences with glossy and non-glossy species.

- Expected results are to find differences in sequence between the glossy and non-glossy lines of *Sorghum bicolor* using all sixteen designed primers.
- Expecting a result on most *Sorghum bicolor* lines to amplify with a primer, especially BTx623, because this specific line was what the primers were designed against

In conclusion, further research is required to find which primer, at the prefect Tm will amplify under PCR for discovering sequence differences between glossy and non-glossy species of *Sorghum bicolor.*