MARKER-ASSISTED BREEDING FOR GUMMY STEM BLIGHT **RESISTANCE IN WATERMELON.**

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Introduction

Previously, five QTL associated with GSB resistance were identified from two different *C. amarus* accessions^{1,4,5,6} (Fig 1).

Marker-assisted backcrossing (MABC) and selection allows Introgression of GSB resistant QTL from *C. amarus* into *C. lanatus*.

Objectives

- To develop and validate Kompetitive allelespecific PCR (KASP) assays to select for the target QTL, and fruit quality-related domestication traits in watermelon.
- To introgress GSB resistant QTL from PI 482276 into Crimson Sweet, and from PI 189225 into UGA127 (Breeding line) by performing MABC.



and PI 482276⁴ (Green)



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Method

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The F_{2:n} lines from two populations, derived from CS x PI 482276⁴ and SB x PI 189225¹, were selected as the donors of GSB resistant QTL..

The recurrent parent, C. lanatus was used as the female due to early flowering and fruit maturity.

The QTL from PI 189225 (Fig 1) were selected based on flanking markers for Qgsb5.2¹, Qgsb8.1⁶, and *Qgsb8.2*⁵. One of the markers for *Qgsb8.1* was redesigned.

The QTL from PI 482276 (Fig 1, green) was selected based on markers closest to and flanking the QTL peak, including a candidate gene within *Qgsb7.1*⁴.

Additional markers were designed to check for recombination within *Qgsb7.1*.

 Primers were designed to select for fruit qualityrelated domestication traits in watermelon (Table 1).

Table 1: Fruit quality-related domestication genes.				
Trait	Domestication Gene			
ss of Bitterness	bHLH (basic helix-loop-helix transcription factor) ¹⁰			
Brix	ClAGA2 (alkaline α-galactosidase) ⁷			
Red Flesh	LYCB (lycopene β-cyclase) ⁹			

Gummy stem blight (GSB) is a major disease in cucurbits caused by three different species of Stagonosporopsis: S. cucurbitacearum, S. citrulli, and S. caricae. Cultivated watermelons (C. lanatus) are susceptible to GSB, but resistance has been identified in C. amarus, a crop wild relative (CWR) of watermelon. Five QTL for S. citrulli and S. cucurbitacearum resistance have been identified from two different *C. amarus* accessions. Our objective is to pyramid these small-effect QTL into the *C. lanatus* background. In this study, marker-assisted backcrossing (MABC) was carried out using KASP marker assays flanking the target QTL. C. lanatus genome recovery is being accelerated by selection for known fruit qualityrelated domestication traits, including loss of bitterness (bHLH), Brix (CIAGA2), and red flesh color (LYCB). Once QTL introgression is complete, the lines will be evaluated for GSB resistance and horticultural traits. The breeding lines have potential utility in developing resistant cultivars to help watermelon growers reduce the economic losses associated with gummy stem blight.



Line 262

Fig 2: Fruit phenotype, and genotype for domestication genes of the backcross lines that are homozygous for *Qgsb7.1*, and *Qgsb5.1* in BC_1F_1 (left) and BC_2F_1 (right) generations.

Abstract

Results The QTL from PI 482276 and PI 189225 were introgressed into five lines from CS x PI 482276 and four from SB x PI189225, respectively (Table 2). Some backcross lines have recovered cultivar-type alleles for domestication genes (Fig. 2). PI 482276 CS CS x PI 482276 Homozygous for Cultivar-allele Homozygous for PI-allele Heterozygous $BC_{2}F_{1}$ BC_1F_1 bhlh 🦲 CIAGA Line 9 CIAGA LYCB LYCB 🦲 Brix =7 Brix = 3bHLH CIAGA CIAGA Line 35 LYCB Brix = 7CIAGA Line 62 LYCB Brix = 7Brix = 6bHLH bhlh 🤇 CIAGA Line 177 LYCB Brix = 7 Brix = 7CIAGA

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Table 2: QTL Introgression progress					
	Population	Line	Gen	QTL Introgressed	
		9	BC3	Qgsb7.1, Qgsb5.1	
	CS	35	BC3	Qgsb7.1, Qgsb5.1	
	Х	62	BC3	Qgsb7.1, Qgsb5.1	
	PI 482276	177	BC3	Qgsb7.1, Qgsb5.1	
		262	BC3	Qgsb7.1, Qgsb5.1	
	CD	5	BC2	Qgsb5.2, Qgsb8.1, Qgsb8.2	
	3D V	73	BC2	Qgsb5.2, Qgsb8.1, Qgsb8.2	
		80	BC2	Qgsb5.2, Qgsb8.1, Qgsb8.2	
	FT 103223	140	BC2	Qgsb5.2, Qgsb8.1, Qgsb8.2	

Conclusion

- KASP markers designed for GSB resistant QTL and domestication genes facilitated the selection of desirable alleles.
- Some of the backcross progeny with GSBassociated QTL have recovered cultivar phenotype (Fig 3).
- Selection for the next generations will include background markers.
- Introgressed lines will be phenotyped for GSB resistance in the greenhouse and the field.

References

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Brix = 6

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