



ALLELIC VARIATION IN *CISUN25-26-27a* ASSOCIATED WITH WATERMELON FRUIT SHAPE

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Abstract

Watermelon (*Citrullus lanatus*) is an economically important horticultural crop, with US production valued at over \$574 million in 2020. Watermelon possesses a diverse phenotype for fruit size. This trait is essential for consumer preference and shipping logistics, making fruit size a key selection trait in watermelon breeding. Only one candidate gene, *CISUN25-26-27a* (*Cl*011257), has been identified as a significant contributor to fruit shape. *CISUN25-26-27a* is a member of the SUN gene family, which has been extensively studied in relation to fruit morphology in tomatoes. Currently, three alleles of *CISUN25-26-27a* are known to be associated with fruit shape variation in watermelon: the wild-type, a SNP, and a 159bp deletion in the 3rd exon resulting in variations in fruit shape. This study aims to identify additional allelic variation for *CISUN25-26-27a* in the wider *Citrullus* gene pool and determine the effect of such novel alleles on fruit shape. This study has identified and sequenced four novel alleles with nonsynonymous SNPs in the coding region of *CISUN25-26-27a* across three *Citrullus* species. To determine their effect on fruit shape, the introgression of the novel alleles into a common genetic background with the assistance of KASP marker assays is being carried out. The resulting NILs will be phenotyped in the field to determine the effect of the alleles on fruit shape. The results of this study will contribute to understanding the genetic mechanisms contributing to watermelon fruit shape.

Introduction

- Watermelon possesses phenotypic diversity in fruit shape, which has a major impact on consumer preference.
- CISUN25-26-27a* in chromosome 3 has been identified as influencing fruit shape and had three previously known alleles (Fig.1) ¹.

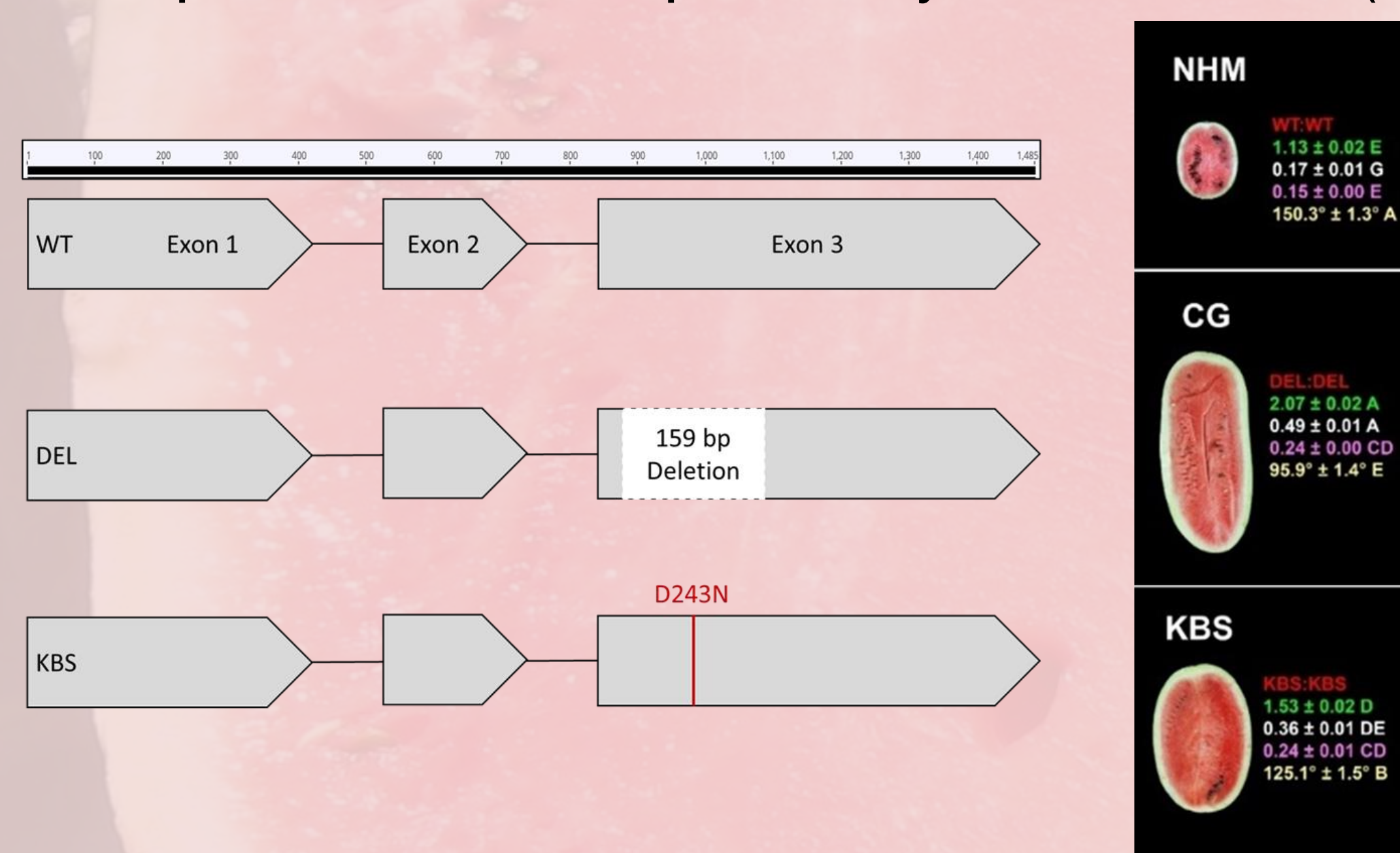


Fig. 1. Known allelic variations on *CISUN25-26-27a* (*Cl*011257). Elongated fruit is associated with the 159 bp deletion on the 3rd exon, blocky fruit is associated with the SNP (KBS allele), and round fruit is associated with the wild type¹ (NHM allele).

Materials and Methods

- Sequenced Read Archive (SRA) reads for 400 *Citrullus* accessions¹ were aligned to the 97103_V2 watermelon reference genome and called with SAMtools ^{3&4}.
- Seed from genotypes with novel alleles were obtained from the USDA germplasm collection.
- CISUN25-26-27a* was sequenced in each of the genotypes to confirm novel alleles.

Current and Future Research

- We have developed KASP markers to use marker-assisted backcrossing for the introgression of the novel alleles into a common genetic background.
- The introgression is currently at BC₂ and BC₃ for the different alleles.
- The resulting NILs will be genotyped in the field for the fruit shape association studies.

References

- Legendre et al. (2020). Molecular Breeding, 40:1-13.
- Guo et al. (2019). Resequencing of 414 cultivated and wild watermelon accessions identifies selection for fruit quality traits. Nature genetics:1-8.
- Li et al. (2009a). Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754-1760.
- Li et al. (2009b). The sequence alignment/map format and SAMtools. Bioinformatics 25:2078-2079.

Results

- 12 nonsynonymous SNPs were identified on exons 2 and 3 of the gene (Fig. 2).
- Four novel alleles were identified in *C. amarus* and *C. colocynthis* (Fig. 2).
- Allele 4 has five PIs with different shapes in different *Citrullus* species (fig. 3).
- All SNPs were confirmed through sequencing the individual PIs.

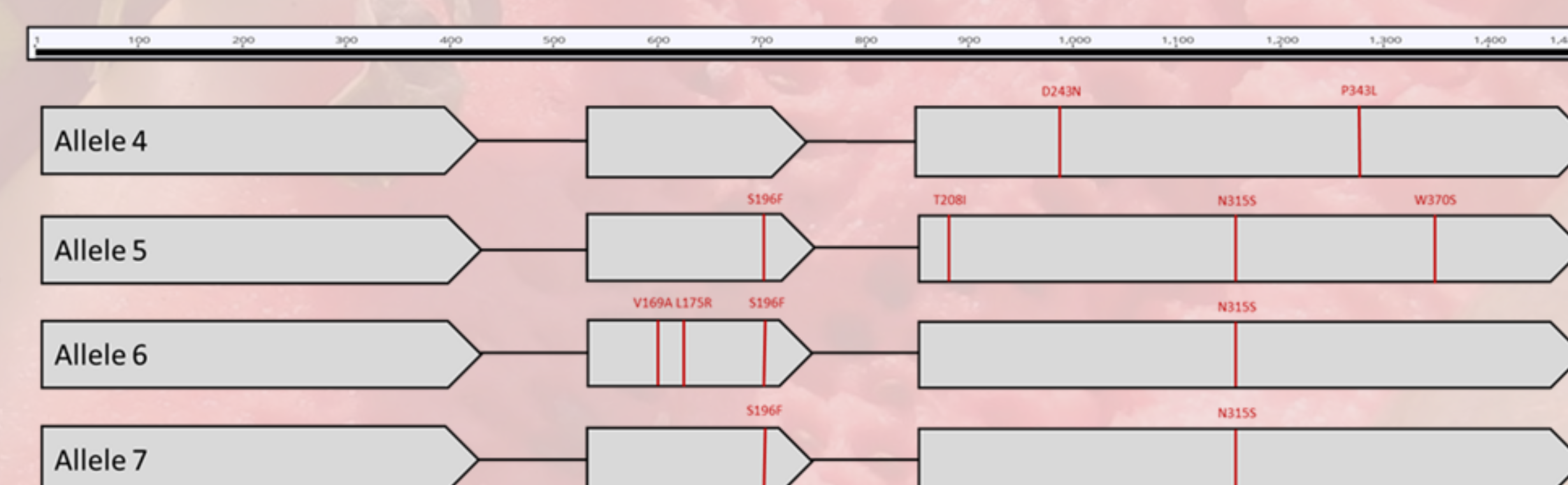


Fig. 2. Three known alleles and the Novel allelic variations on *CISUN25-26-27a* (*Cl*011257).

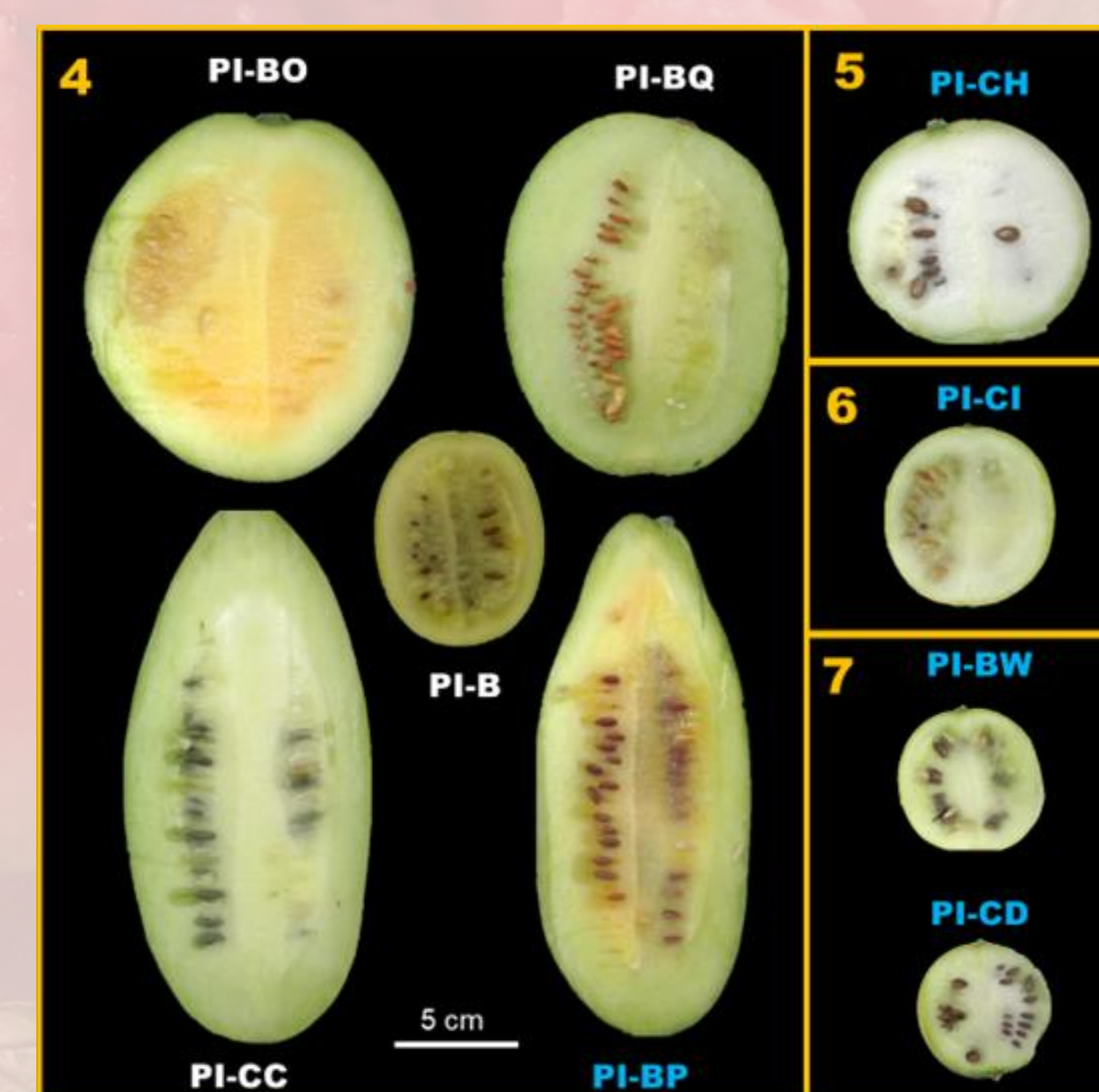


Fig. 3. The different PIs having the novel alleles, allele Four(4) had Five PIs. Four were *C. amarus* (white) and one *C. colocynthis* (blue). Allele Five(5), Six(6), and Seven(7) were all round, and all were *C. colocynthis*.

Conclusions

- Four novel alleles of *CISUN25-26-27a* (*Cl*011257) with nonsynonymous SNPs were identified and confirmed by sequencing.

Acknowledgments

I thank the Department of Horticulture, members of the McGregor lab, CucCap2: National Institute of Food and Agriculture, U.S. Department of Agriculture, Award number: 2020-51181-32139 & National Research Foundation for funding this research -Award Number 204842.

