Cucurbit Genetics Cooperative

2020

Report 43

Cucurbit Genetics Cooperative Report

43

2020

USDA, ARS, U.S. Vegetable Laboratory
2700 Savannah Highway
Charleston, SC 29414
843-402-5326

https://cucurbit.info/

ISSN 2689-1719

On the front cover:

"Melon" (Plate 231 bis.) in *Flore Médicale* Volume 4 by François Pierre Chaumeton (1775-1819). Published by Imprimerie de C.L.F. Panckoucke, Paris, 1830. Image obtained from the Biodiversity Heritage Library (https://www.biodiversitylibrary.org/page/9503681#page/293/mode/1up). Original holding Institution is Missouri Botanical Garden, Peter H. Raven Library.

The **Cucurbit Genetics Cooperative** (CGC) was organized in 1977 to develop and advance the genetics of economically important cucurbits. Membership to CGC is voluntary and open to all individuals who have an interest in cucurbit genetics and breeding. There are no annual dues. An informal membership list is maintained. If you are interested in becoming a CGC member and receiving email communications concerning CGC, including calls for new contributions to the CGC Report, please contact
CGC Chair Amnon Levi at amnon.levi@ars.usda.gov.

CGC Reports are issued on an annual or biennial basis. We welcome contributions from the global cucurbit genetics community. Contact the CGC Chair for information concerning how to submit a manuscript.
CGC Reports and other information can be found at:

https://cucurbit.info/

ISSN 2689-1719

Contents

Research Reports

Melon (*Cucumis melo*)

Development of Differential Hosts to Identify Commercially Relevant Races of Melon *Podosphaera xanthii* Against Which Vegetable Seed Companies Make Claims of Resistance

Valerie Grimault and Sandrine Houdault
Groupe d'Étude et de contrôle des Variétés et des Semences (GEVES), 25 Rue Georges Morel, 49070 Beaucouzé, France

Phyllis Himmel
Seed Biotechnology Center, Department of Plant Sciences, University of California at Davis, Davis, CA
Email: pthimmel@ucdavis.edu

Introduction

Cucurbit powdery mildew (CPM) affects the yield and quality of melon worldwide. Races of *Podosphaera xanthii* (Px) and *Golovinomyces cichoracearum* induce identical symptoms of this disease (Pitrat and Bescombes 2008). There are more than 30 reported sources of resistance in melon to the more than 20 known races of Px (Alvarez et al. 2000, Bertrand 2002, McCreight et al. 2012) and these sources can vary in their responses to commonly occurring races of this pathogen (McCreight 2006). Vegetable seed companies are using these sources of resistance to develop commercial melon varieties with resistance to Px. The International Seed Federation Disease Resistance Terminology Working Group (ISF DRT WG) aims to facilitate the consistent naming of plant pathogen races and strains (Vincent et al. 2019) and in 2017 began discussions on how to support and validate the melon CPM resistance claims.

In June 2018, the ISF DRT WG met with CPM experts to discuss melon-relevant Px races and known melon host differentials that can identify and differentiate races of CPM (Lebeda et al. 2016). The aim of the ISF DRT WG was to build on presented information to develop a manageable subset of differentiating melon hosts, assemble commercially relevant Px races and develop protocols that would lend themselves to routine disease resistance testing in order to support commercial claims of resistance. A more detailed evaluation of these differential responses could facilitate development of a core group of differentiating melon hosts with a focus on major resistance genes. This initial study is a crucial step towards understanding similarities and differences between races of Px on a global scale.

Materials and Methods

A comparative ring test was organized by Sandrine Houdault, of GEVES. Fourteen laboratories based in the European Union (EU) and United States (US) participated. Candidate Px isolates were selected based upon data presented by CPM experts during the June 2018 meeting. Characterized isolates had been collected from commercial melon growing areas in the EU and US, were increased and stored for use in the ring test. Isolates of US races SD and Uber were sent to Sandrine for distribution to EU partners. US partners were not able to import and test the EU isolates and instead exchanged local isolates with each other. Candidate melon differentials were also selected based on data presented by CPM experts during the June 2018 meeting: presence of major resistance gene(s), availability, ability to increase seeds, unique responses to Px races, capacity to differentiate races and consistent results between labs. Tested candidate hosts and isolates are included in Table 1. Seeds and isolates were issued a unique code and distributed to all partners in Fall 2019. A total of 25 isolates and 15 candidate differentials were tested. Each isolate was tested by two labs. All partners used the same inoculation protocol and disease rating scale based upon an established CPVO protocol (https://cpvo.europa.eu/sites/default/files/documents/cucumis_melo_2.1.pdf). Melon seedlings were sown in a greenhouse or a growth room and inoculated at the 4-leaf stage by direct deposit of conidia from infected leaves. Twelve plants per candidate host were tested. Inoculated plants were incubated under 14 h, 20°C day and 10 h, 24°C night conditions. Evaluations began when sporulation developed on the susceptible control (approximately 8 to 14 days post-inoculation). A 1-9 rating scale was used to evaluate disease severity (Fig. 1) and interpret the level of resistance. Data were analyzed using a weighted mean with visual assessments of the extent of symptom development.

Results and Discussion

The candidate differentials generally responded as expected in this test, but there were exceptions as variations from expected variety responses to specific races of Px were still observed in these tests. RIL 1 and RIL 4 were derived from the same source, yet differences were observed in responses to the tested Px races. PI 313970 is reported to be highly resistant to all tested races of Px (McCreight and Coffey 2011), yet intermediate resistant responses were observed in this host. Unexpected responses were difficult to interpret for Px isolates Mel 2381-18-27, Mon 19-04, Matref Px: 3-5 and D SRY 18-0105-1. Low sporulation was observed on 'PMR 5' by the Matref Px: 3 isolate whereas this line usually develops necrosis. Three isolates were dropped due to unreadable responses and 22 isolates were selected for a second ring test. The selected candidate differentials and isolates showed a range of reactions from susceptible and virulent to resistant and avirulent, respectively, and represent the diversity of commercially relevant Px isolates in the main melon growing areas.

Variation in the responses have been reported and can be attributed to genetic variation for virulence in the pathogen (Alvarez et al. 2000, McCreight 2006). Slight differences in rating evaluation and resistance interpretation can also contribute to observed variation in response to inoculation. In comparative testing, unaccounted differences in testing environment introduces other variables that can result in differences in host responses. This comparative ring test will be repeated in early 2021.

Acknowledgement

The authors are grateful for the participation in and contributions to these comparative ring tests by members of the ISF DRT WG.

Literature Cited

1. Alvarez, J. M., M. L. Gomez-Guillamon, N. A. Torres, I. Canovas, E. Floris. 2000. Virulence differences between two Spanish isolates of *Sphaerotheca fuliginea* race 2 on melon. Acta Hort. 510:67-69. https://doi.org/10.17660/ActaHortic.2000.510.10
2. Bertrand, F. 2002. AR Hale's Best Jumbo, a new differential melon variety for *Sphaerotheca fuliginea* races in leaf disk test, p. 234-23. In: D.N. Maynard (ed.). Cucurbitaceae 2002 ASHS Press, Alexandria, Va.
3. Lebeda, A., E. Kristkova, B. Sedlakova, J.D. McCreight. 2016. Initiative for international cooperation of researchers and breeders related to determination and denomination of cucurbit powdery mildew races, p. 148-152. In: E.U. Kozik and H.S. Paris (eds.) Proceedings of the XIth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae, Warsaw, Poland. DOI 10.5281/zenodo.1255670
4. McCreight, J. D., M. D. Coffey. 2011. Inheritance of resistance in melon PI 313970 to cucurbit powdery mildew incited by *Podosphaera xanthii* race S. HortScience 46:838-840. https://doi.org/10.21273/HORTSCI.46.6.838
5. McCreight, J.D., M.D. Coffey, B. Sedlakova, and A. Lebeda. 2012. Cucurbit powdery mildew of melon incited by *Podosphaera xanthii*: Global and western U.S. perspectives, p. 181–189. In: N. Sari, I. Solmaz, and V. Aras (eds.). Cucurbitaceae 2012, Proceedings of the Xth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae, Antalya (Turkey), October 15-18th, 2012. https://zenodo.org/record/1255624#.YFNPkC2cZQI
6. McCreight, J. D. 2006. Melon-powdery mildew interactions reveal variation in melon cultigens and *Podosphaera xanthii* races I and 2 in melon PI 313970. J. Amer. Soc. Hort. Sci. 131:59-65 https://doi.org/10.21273/JASHS.131.1.59
7. Pitrat, M., D. Besombes. 2008. Inheritance of *Podosphaera xanthii* resistance in melon line '90625', p. 135-142. In: M. Pitrat (ed.) Proceedings of the IXth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae. https://toc.library.ethz.ch/objects/pdf/e01_978-2-7380-1251-7_01.pdf
8. Vincent, T., P.T. Himmel, P. Brown, S. Ruthner, V. Grimault. 2019. ISF Guidelines on the nomination of novel plant pest races. Abstr. Phytopathology 109-10-S2.110. https://doi.org/10.1094/PHYTO-109-10-S2.1

Table 1. Summary of results of a ring test of 22 *Podosphaera xanthii* isolates on 15 melon candidate differential lines in 14 laboratories in the European Union and United States; each isolate was tested by two laboratories.

Isolates	Characterized race	Test Code	Vedrantais	Ames 31282	PMR45	WMR29	Edisto47	PMR5	Arago	RIL 1 = RIL N°14	Durango	PI482420	PI124112	RIL 4 = RIL N°44	Arum	SVI105	PI313970
RZ_ID470	Px: 3-5	V	S	S	S	S	S	S	S	S	S	S	S	IR	S	IR	IR
Venturia		M	S	S	S	S	S	S	S	IR/S	IR/S	S	S	IR	S	IR	IR
Mon 19-6		T	S	S	S	S	S	S	S	S	S	IR	S	S	IR/R	IR	IR
RZ_ID4578		S	S	S	S	S	S	IR/S	S	IR/S	S	R	S	S	IR/R	IR/R	IR
Mon 18-1	Px: 3-5	X	S	S	S	S	S	IR/S	S	S	S	IR/S	S	IR/S	IR/R	IR/S	IR
RZ_ID873	Px: 3-5	Q	S	S	S	IR/S	IR	S	S	S	S	S	R	S	R	R	R
SRY 18.0109.2		N	S	S	S	IR/S	IR	S	S	S	S	S	IR/R	S	IR/R	IR	IR/R
RZ_ID1330		W	S	S	S	S	S	IR/S	S	S	S	IR/S	R	R	R	R	R
Envero		G	S	S	S	S	S	IR/S	S	IR	IR	S	IR	IR	IR/S	IR/R	IR/R
Uber race	Uber	R	S	S	S	S	S	S	IR	IR/R	IR	IR/R	S	IR/R	R	R	IR
Mel2381-18-27		C	S	S	S	S	S	IR	R/S	S	S	IR/S	IR	IR/S	IR/R	R	IR/R
CM18043		A	S	S	S	S	S	S	IR/S	IR/R	IR/R	IR/R	IR/R	R	R	R	R
Px:5 Matref	Px: 5	Y	S	S	S	S	S	R	IR	S	IR	R	R	IR	R	R	IR
Px: 3-5 Matref	Px: 5	J	S	S	S	S	S	IR	IR/R	R/IR/S	IR/R	IR/R	R	IR/R	R	R	R
Mon19-04	Px: 3-5	E	S	S	S	S	IR	S	IR	IR	IR	IR	R	R	R	R	R
Race 2 US	Px: 2 US	2 US	S	S	IR	IR	S	R	R	R	S	IR	IR	S	R	IR	IR
Px:2 Matref	Px: 2	I	S	S	S	R	R	R	IR/R	IR/R	IR/R	R	R	R	R	R	R
Px:3 Matref	Px: 3	P	S	S	S	R	R	IR	IR	IR	IR	IR/R	R	IR	R	R	R
RZ_ID1296		F	S	S	R	R	R	R	R	S	R	IR/S	R	IR/R	R	R	R
Px:1 Matref	Px: 1	U	S	S	R	R	R	R	R	IR	R	R	R	R	R	R	R
SalGH2018	SD	H	S	R	R	R	IR/R	IR/R	R	R	R	R	IR/R	S	R	R	R
SRY 18-0105-1	Px: 1	D	S	S	IR/R	R	R	R	R	IR/R	R	R	R	R	R	R	R

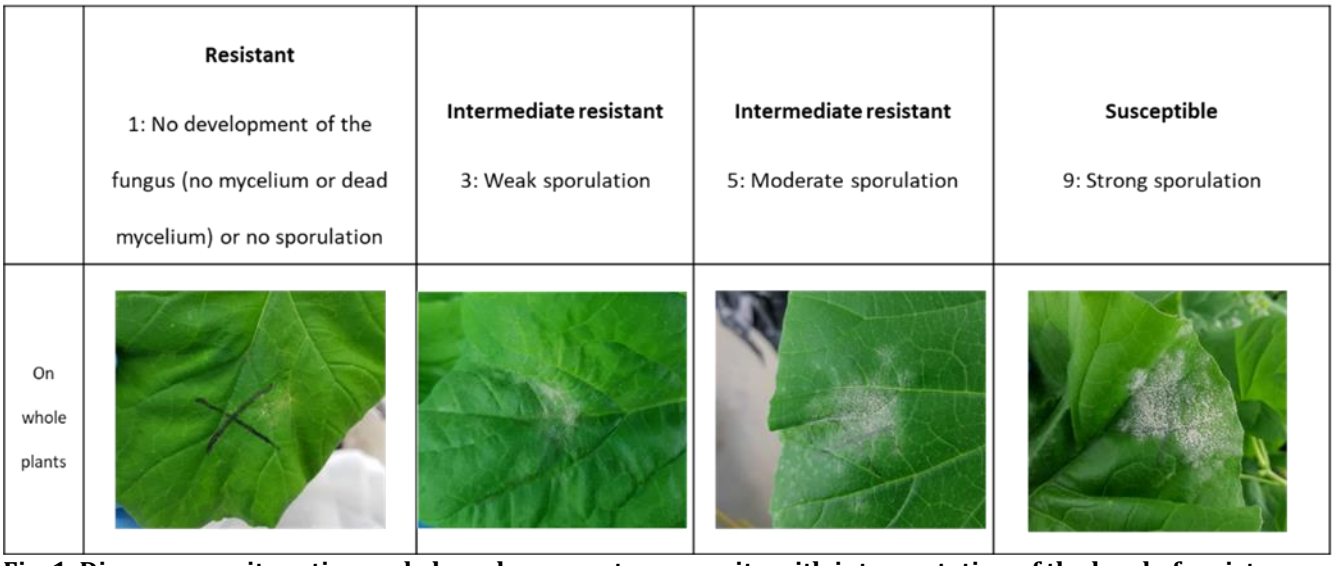


Fig. 1. Disease severity rating scale based on symptom severity with interpretation of the level of resistance

Genetic Control of the Resistance to *Zucchini yellow mosaic virus* Derived from Melon Accession IC 274006

María López-Martín*, Carmelo López, Alicia Sifres, Belén Picó, Ana Pérez-de-Castro
Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV-UPV), Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain
**Email: malom11m@posgrado.upv.es*

Narinder P.S. Dhillon
World Vegetable Center, East and Southeast Asia, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand

Introduction

Viral infections are one of the principal threats for the growth of melon (*Cucumis melo* L.) because most cultivated varieties are susceptible to several viruses. Zucchini yellow mosaic virus (ZYMV) is a potyvirus transmitted in a non-persistent manner by aphids, which causes leaf distortion, mosaic, yellowing and as a consequence reduces yield production and fruit quality (Lisa *et al.,* 1981). ZYMV was first described in Italy and nowadays it has a worldwide distribution, being one of the viruses causing some of the most important economic losses in melon (Martín-Hernández and Picó, 2021).

As insecticide application alone is not efficient for control of viruses transmitted in a non-persistent manner (Fereres, 2000), the introgression of resistance genes into commercial breeding lines is the most effective, economic, stable and environmentally respectful way to control these pathogens (Gadhave *et al.,* 2020). Availability of resistance sources is necessary to achive this objective.

The Indian accession PI 414723 has been reported as resistant to ZYMV (Pitrat and Lecoq, 1984), watermelon mosaic virus (WMV) (Gilbert et al., 1994), papaya ringspot virus (PRSV) (Pitrat and Lecoq, 1984), cucurbit aphid-borne yellows virus (CABYV) (Dogimont et al., 1996) and tomato leaf curl New Delhi virus (ToLCNDV) (López et al., 2015). The resistance to ZYMV conferred by PI 414723 has been described as either monogenic (gene *Zym*) and dominant (Pitrat and Lecoq, 1984) or oligogenic, with three complementary dominant genes: *Zym-1, Zym-2* and *Zym-3* (Danin-Poleg *et al.*, 1997). The dominant gene *Zym* has been mapped to chromosome 2 (Périn *et al*., 2002). In any case, completely resistant cultivars against ZYMV are not commercially available. Broadening the genetic base of the resistance will increase its durability in the event of appearance of new isolates that overcome the resistances available. In fact, resistance in PI 414723 has been reported as isolate-dependent (Lecoq *et al.,* 2002). Other resistance sources to ZYMV, such as IC 274014 and IC 274007 (Dhillon *et al*., 2007) or IC 274006 (Sanchís, 2018), have been described within germplasm from India. Other studies previously reported accession IC 274006 as susceptible to ZYMV and segregating for resistance to PRSV (Dhillon *et al.,* 2007). The mechanisms underlying these resistances have not been studied.

The development of resistant cultivars is especially important for organic agriculture, where the incidence of ZYMV seems to be higher (Pérez-de-Castro *et al*., 2019) and for the recovery of traditional landraces that have been displaced by elite cultivars. This is the case of the Valencian landrace 'Meló d´Or' (BGV016451), which has high organoleptic quality, but is susceptible to a wide range of pathogens, among them ZYMV.

In this work, the genetics of the resistance to ZYMV derived from melon accession IC 274006 was studied for the first time in a cross of this resistant line with the susceptible landrace BGV016451. We have also begun the genotyping of this population to identify the putative genomic regions associated with the resistance derived from this source.

Materials and Methods

In previous studies developed by the research group, the Indian melon accession IC 274006 was identified as resistant after mechanical inoculation with ZYMV (isolate ZYMV courgette, provided by GEVES-SNES). One resistant plant was selected and crossed with the Spanish susceptible melon cultivar 'Meló d´Or' (BGV016451). The F_1 generation was used to construct the whole family: F_2, $BC_{1\text{-IC}}$ and $BC_{1\text{-BGV016451}}$ (backcrosses to IC 274006 and BGV016451 respectively). Both parents (10 plants each), their F_1 (19 plants), and the segregant populations F_2 (99 plants), $BC_{1\text{-IC}}$ (78 plants) and $BC_{1\text{-BGV016451}}$ (90 plants) populations were mechanically inoculated with ZYMV. Symptoms were visually scored

according to a scale from 0 (no symptoms) to 4 (yellowing and severe mosaic symptoms) at 15-, 22- and 30-days post-inoculation (dpi). The virus infection in each plant was tested through tissue printing followed by molecular hybridization using an RNA probe specific for ZYMV, corresponding to the sequence of the viral capsid gene.

A total of 26 susceptible and 24 resistant F_2 plants were genotyped with an existing set of 124 SNPs markers evenly distributed throughout the genome and implemented for their use in the Agena Bioscience platform (Epigenetic and Genotyping unit of the University of Valencia, Unitat Central d´Investigació en Medicina (UCIM), Spain). Additionally, three IC 274006 plants as well as one BGV016451 and two F_1 plants were included in this genotyping.

Results and Discussion

All the plants of the cultivar BGV016451 were susceptible when inoculated with ZYMV (Figure 1), and it was possible to detect viral accumulation since 15 dpi. Furthermore, a high mortality rate was detected in this variety due to the viral infection. A variable response to ZYMV was observed within the accession IC 274006, as 50 % of the plants showed symptoms from 15 dpi; virus was detected in symptomatic plants of this accession. This variability observed in IC-274006 could explain that the accession was reported as susceptible to ZYMV in a previous study (Dhillon *et al*., 2007). In fact, segregation was observed in this same study for resistance to another potyvirus, PRSV (Dhillon *et al*., 2007). In any case, the offspring populations used in the work presented here were obtained from a resistant IC 274006 plant.

In the F_1 generation, 84.21 % of the plants were susceptible, which suggested recessive genetic control of the resistance. This type of control of the resistance is the most common for viruses (Truniger and Aranda, 2009) and it is usually associated with the inhibition of virus multiplication and/or movement. For example, several recessive mutations in the translation initiation factors eIF4E and eIF(iso)4E confer resistance to potyvirus infection in several hosts (Robaglia and Caranta, 2006). The fact that 15.79% of the plants in the F_1 generation were resistant suggested an incomplete penetrance of the resistance gene (Table 1).

In the F_2 population, 72 plants showed moderate to very severe symptoms (scores 2-4) with high viral accumulation, so they were considered susceptible (Figure 1, Table 1). The rest of the plants showed light or an absence of symptoms (scores 0-1) and viral accumulation was low or undetectable; therefore, these plants were classified as resistant. $BC_{1\text{-IC}}$ also showed segregation of symptoms as 37 plants were found to be resistant (scores 0-1) and 41 susceptible (scores 2-4). The $BC_{1\text{-BGV016451}}$ population also segregated for symptom severity with 12 resistant (scores 0-1) and 78 susceptible (scores 2-4) plants, supporting incomplete penetrance. In both BC generations, viral accumulation also supported the visual evaluation of each plant.

Considering the incomplete penetrance observed in the F_1 generation, measured as the percentage of resistant plants in heterozygotes for the resistant gene (p=0.15789), the segregation observed in F_2 and both BC generations fit the expected ratio for a single recessive gene (Table 1).

To determine the contribution of different genomic regions to ZYMV resistance, 24 resistant and 22 susceptible F_2 plants were genotyped, along with both parents and their F_1 offspring. The results indicated that a genomic region in chromosome 5 could be related to the resistance, because a significant difference was found in the phenotypic response between the different genotypes for one of the SNPs located in this region (*p-value* = 0.00194). The highest concentration of resistance genes in the melon genome is located in chromosome 5 (González *et al.*, 2013), in a region including that proposed here as associated to IC 274006-derived resistance to ZYMV. Further work will be carried out, including quantification of virus titer in resistant and susceptible plants as well as a wider genotyping, to confirm these results.

Conclusion

The resistance to ZYMV derived from IC 274006 could be used in combination with resistance derived from other sources, to achieve a more durable resistance against ZYMV and increase its level. The molecular markers identified here and the generations available will be useful to continue the breeding program for the introgression of the resistance derived from this source in the 'Meló d'Or' genetic background and in other commercial types.

Acknowledgements

This research was funded by the Spanish Ministerio de Ciencia, Innovación y Universidades, grants number AGL2017-85563-C2 (1-R) and RTA2017-00061-C03-03 (INIA), and by the Conselleria d'Educació, Investigació, Cultura i Esports de la Generalitat Valenciana, grant number PROMETEO/2017/078 (to promote excellence groups, cofinanced with FEDER funds). M.L. is a recipient of a predoctoral fellowship (PRE2018-083466) of the Spanish Ministerio de Ciencia, Innovación y Universidades. The authors would like to thank M. García for her help in the data evaluation. Authors also thank the UPV Genebank for providing seeds of Spanish traditional melons.

Literature Cited

1. Danin-Poleg, Y., Paris, H. S., Cohen, S., Rabinowitch, H. D., and Karchi, Z. (1997). Oligogenic inheritance of resistance to *Zucchini Yellow Mosaic Virus* in melons. Euphytica 93(3): 331–337.
2. Dhillon, N. P. S., Ranjana, R., Singh, K., Eduardo, I., Monforte, A. J., Pitrat, M., … Singh, P. P. (2007). Diversity among landraces of Indian snapmelon (*Cucumis melo* var. momordica). Genetic Resources and Crop Evolution 54(6): 1267–1283.
3. Dogimont, C., Slama, S., and Martin, J. (1996). Sources of Resistance to *Cucurbit Aphid-borne Yellows Luteovirus* in a Melon Germ Plasm Collection. Plant Disease 80(12):1379-1382.
4. Fereres, A. (2000). Barrier crops as a cultural control measure of non-persistently transmitted aphid-borne viruses. Virus Research 71(1–2):221–231.
5. Gadhave, K. R., Gautam, S., Rasmussen, D. A., and Srinivasan, R. (2020). Aphid transmission of potyvirus: The largest plant-infecting RNA virus genus. Viruses 12(7):1–22.
6. Gilbert, R., Kyle, M., Munger, H., and Gray, S. (1994). Inheritance of resistance to *Watermelon Mosaic Virus* in *Cucumis melo* L. HortScience 29:107-110.
7. González, V. M., Aventín, N., Centeno, E., and Puigdomènech, P. (2013). High presence/absence gene variability in defense-related gene clusters of *Cucumis melo*. BMC Genomics 14(1):782.
8. Lecoq, H., Desbiez, C., Wipf-Scheibel, C., Girard, M., and Pitrat, M. (2002). Durability of *Zucchini yellow mosaic viru*s resistances in cucurbits. Cucurbitaceae, 294–300. Maynard DN. (editor)
9. Lisa, V., Boccardo, G., D´Agostino, G., Dellavalle, G., and D´Aquilio, M. (1981). Characterization of a Potyvirus That Causes Zucchini Yellow Mosaic. Phytopathology 71:667–672.
10. López, C., Ferriol, M., and Picó, M. B. (2015). Mechanical transmission of *Tomato leaf curl New Delhi virus* to cucurbit germplasm: selection of tolerance sources in *Cucumis melo*. Euphytica 204(3):679–691.
11. Martín-Hernández, A. M., and Picó, B. (2021). Natural Resistances to Viruses in Cucurbits. Agronomy 11(1):23.
12. Pérez-de-Castro, A., Martínez de Alba, A. E., Sáez, C., Flores-León, A., Sifrés, A., Gómez-Guillamón, M. L., … Picó, B. (2019). Incidence and genetic diversity of cucurbit viruses in Spain. Acta Horticulturae.
13. Périn, C., Hagen, L., De Conto, V., Katzir, N., Danin-Poleg, Y., Portnoy, V., … Pitrat, M. (2002). A reference map of *Cucumis melo* based on two recombinant inbred line populations. Theoretical and Applied Genetics 104(6):1017–1034.
14. Pitrat, M., and Lecoq, H. (1984). Inheritance of *Zucchini yellow mosaic virus* resistance in *Cucumis melo* L. Euphytica 33(1):57–61.
15. Robaglia, C., and Caranta, C. (2006). Translation initiation factors: A weak link in plant RNA virus infection. *Trends in* Plant Science 11:40–45.
16. Sanchís, C. (2018). Búsqueda de fuentes de resistencia a los virus ZYMV y WMV en los géneros *Cucurbita* y *Cucumis.* Universitat Politècnica de Valènci*a.*
17. Truniger, V., and Aranda, M. A. (2009). Recessive resistance to plant viruses. *Advances in Virus Research* 75:119–159.

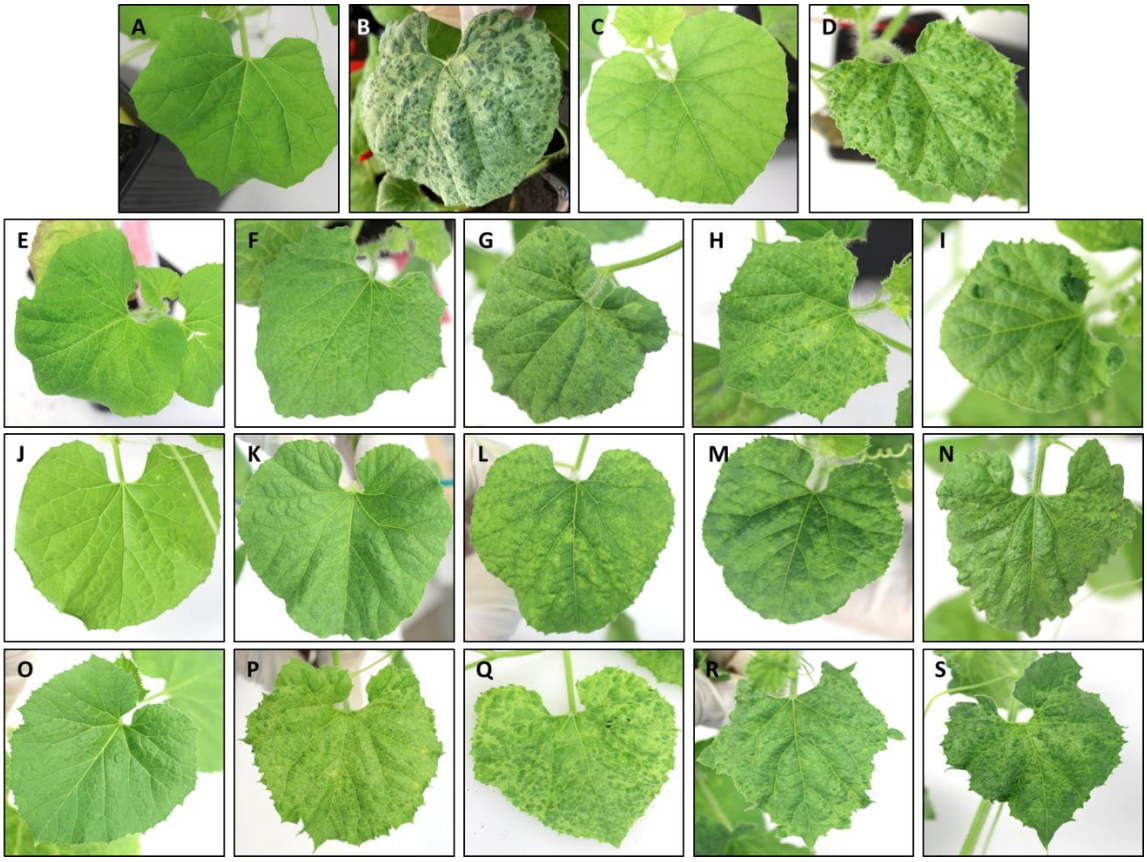


Figure 1. Absence of symptoms in the resistant accession IC 274006 (A) and severe mosaic symptoms caused by *Zucchini mosaic virus* (ZYMV) in the susceptible cultivar 'Meló d´Or' BGV016451 (B). Diversity of symptoms caused in the F_1 (C-D), F_2 (E-I), $BC_{1\text{-IC}}$ (J-N) and $BC_{1\text{- BGV016451}}$ (O-S) generations.

Table 1. Segregation of resistant/susceptible plants in F_1, F_2, $BC_{1\text{-IC}}$ and $BC_{1\text{-BGV016451}}$ offspring derived from the cross IC 274006 x BGV016451. R: resistant; S: susceptible.

Progeny	Phenotype	Frequency	Observed segregation	Expected segregation	X^2 test *
F_1	R	p	3	3	--
	S	1-p	16	16	
F_2	R	0.25 + 0.5p	27	32.57	1.417 (0.233)
	S	0.25 + 0.5(1-p)	72	66.43	
$BC_{1\text{-IC}}$	R	0.5 + 0.5p	37	45.16	3.5 (0.061)
	S	0.5(1-p)	41	32.84	
$BC_{1\text{- BGV016451}}$	R	0.5p	12	7.11	3.661 (0.056)
	S	0.5 + 0.5(1-p)	78	82.89	

* chi-square value calculated for a recessive monogenic expected ratio (probability for the chi-square value with one degree of freedom)

Resistance to *Tomato leaf curl New Delhi virus* Derived from WM-7: Genetic Control and Introgression into Traditional Melon Backgrounds

Clara Pérez Moro, Cristina Sáez, Alejandro Flores-León, Alicia Sifres, Carmelo López, Belén Picó*, Ana Pérez-de-Castro
Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV-UPV), Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain
**mpicosi@btc.upv.es*

Narinder P.S. Dhillon
World Vegetable Center, East and Southeast Asia, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand

Introduction

Melon (*Cucumis melo* L.) is one of the most important crops of the *Cucurbitaceae* family. The world production of this vegetable was more than 27 million tons in 2019 (FAOSTAT, 2021). Spain is one of the ten countries with the highest production and the first producer and exporter in the European Union. The most important melon types cultivated in Spain belong to the *ibericus* group, which includes 'Piel de Sapo', 'Amarillo', 'Tendral', 'Rochet' and 'Blanco' melons. Landraces of snake melon (*flexuosus* group), highly appreciated in other countries, are also cultivated in some eastern coastal regions of Spain, under the name of 'Alficoz'.

Currently one of the major production constraints of cucurbits is the increase in viral diseases due to globalization and climate change, which facilitate their expansion. One of these viral diseases is caused by the whitefly-transmitted geminivirus *Tomato leaf curl New Delhi virus* (ToLCNDV). Plants affected with this disease show vein clearing, yellow mottling, crinkling, puckering, and upward or downward curling of leaves, besides sterility and poor fruit setting (Jyothsna et al. 2013). The first detection of ToLCNDV was in 1995 on tomato (*Solanum lycopersicum* L.) in India (Srivastava et al. 1995). Soon, the host range and the expansion area were extended. The first report of ToLCNDV in Spain was in 2012 in zucchini (*Cucurbita pepo* L.) in Murcia (Juárez et al. 2014).

The development of resistant varieties is one of the best long-term safe and sustainable approaches to manage viral diseases. In previous works of the research group, López et al. (2015) identified resistance to ToLCNDV in five melon genotypes from India; three of them belonged to the *momordica* group and two were wild *agrestis.* Resistance derived from the wild *agrestis* WM-7 has been reported to be controlled by one major dominant *locus* in chromosome 11 and two additional regions in chromosomes 2 and 12 (Sáez et al. 2017). The breeding program for the introgression of this resistance into traditional sweet melon and snake melon genetic backgrounds has been initiated.

The aim of this work was to further study the genetic control of the resistance derived from WM-7, by fine mapping the major *locus* on chromosome 11 and by analyzing the effect of the chromosome 12 region on the resistance. The plant materials developed, and the molecular markers identified will be useful in the breeding program for the introgression of resistance to ToLCNDV in different traditional genetic backgrounds.

Material and Methods

Plant material: The resistance source WM-7 was crossed with seven homozygous lines derived from melon landraces: two 'Piel de Sapo' (11PS-BGV013188 and 03PS-BGV016356), two 'Blanco' (29BL-BGV015753 and 32BL-BGV016453), one 'Amarillo' (22AM-GO-BGV016451), one 'Rochet' (02RC-BGV003718) and one 'Alficoz' (05AL-BGV004853). These F_1 generations were backcrossed to each of the landraces to obtain the backcross generations (BC_1), except for the F_1 with 02RC, which was selfed to produce the F_2 generation (Table 1).

Between 20 and 40 plants of each generation (Table 1) were evaluated for resistance to ToLCNDV. The rest of the BC_1 and F_2 plants (50 per genotype) were used to progress in the introgression of the resistance into the traditional genetic backgrounds. These latter plants were genotyped with a set of markers located on the candidate regions of the resistance to ToLCNDV. Selected genotypes were selfed to obtain the corresponding either BC_1S_1 or F_3 generations. These generations were evaluated for resistance to ToLCNDV.

Inoculation and disease assessment: Inoculation was carried out as described in Lopez et al. (2015). In brief, a ToLCNDV-infectious clone was agroinoculated by injection into petioles of MU-CU-16 zucchini plants. Fifteen days later, leaf tissue from the symptomatic MU-CU-16 plants was mashed with inoculation buffer and used to mechanically inoculate the melon plants, at the two true-leaf stage. One cotyledon and the bigger true-leaf were dusted with carborundum 600 mesh and then were rubbed with a cotton-swab impregnated with the homogenate inoculum. All the plants were reinoculated 10 days later.

Disease assessment was carried out by visual scoring of symptom at 15- and 30-days post inoculation (dpi), following the scale described in López et al. (2015), which ranged from 0 (asymptomatic plants) to 4 (severe symptoms). Plants with no or mild symptoms (0 to 1 in the scale) were considered resistant and plants with moderate to severe symptoms (2 to 4 in the scale) were considered susceptible. Quantitative PCR was carried out as previously described (Sáez et al. 2017) to quantify the amount of virus in DNA isolated from the apical leaves using the CTAB method (Doyle and Doyle 1990).

SNP Genotyping: Fifty plants of each of the BC_1 and F_2 plants used in the backcrossing program were genotyped with a panel of 23 SNPs, covering the three genomic regions associated with resistance in chromosomes 2, 11, and 12 (Sáez et al. 2017). DNA was isolated using the CTAB method (Doyle and Doyle, 1990) and the genotyping was done using the Agena Bioscience platform ('Epigenetic and Genotyping unit of the University of Valencia, Unitat Central d´Investigació en Medicina (UCIM), Spain').

Results and Discussion

Six BC_1 and one F_2 generations derived from the resistance source WM-7 in different traditional genetic backgrounds were evaluated for resistance to ToLCNDV. Segregation was observed in all progenies (Table 1). Plants asymptomatic or showing slight symptoms, with lower virus titer, were classified as resistant, while those displaying moderate to severe symptoms and with high viral accumulation were considered susceptible (Figure 1). Segregation fitted the expected ratio of resistant and susceptible plants for a single dominant gene, except for two BC_1 generations. These two BC_1 progenies had an excess of resistant plants, which could correspond to escapes or to late infections. Sáez et al. (2017) also obtained segregations compatible with a monogenic dominant model in F_2 and BC generations derived from the cross WM-7 x Piñonet Piel de Sapo.

Fifty plants of each of the BC_1 and F_2 generations were genotyped with the ToLCNDV set of SNPs, which included markers in the candidate regions in chromosomes 2, 11 and 12. A selection of certain genotypes was carried out to obtain the selfing progenies (Table 2). Recombinants in chromosome 11 were selected (F_3 derived from 02RC genetic background, and BC_1S_1 derived from 05AL genetic background), with the purpose of narrowing the candidate interval (Table 2). Besides that, plants that only included the candidate region in chromosome 12 in heterozygote state were chosen (BC_1S_1 derived from the genetic backgrounds of 29BL, 32BL, 22AM-GO and 05AL), in order to better analyze the effect of this region on resistance (Table 2).

One of the F_2 plants derived from 02RC genetic background was homozygous for the WM-7 alleles in the candidate region of chromosome 11 between markers SNPCmND7 and SNPCmND16bis and heterozygous below this marker (Table 2). All the selfing descendants of this plant remained asymptomatic when inoculated with ToLCNDV. Virus titer in these plants was also significantly lower than that detected in susceptible plants at 15 and 30 dpi. These results allowed delimiting the candidate region over SNP SNPCmND17 (at position 30,410,537 bp). The selfing progeny from the other F_2 from 02RC, which was heterozygote between markers SNPCmND7 and SNPCmND13bis and homozygous for the allele of 02RC below this marker, segregated for resistance, thus allowing the narrowing of the candidate region, over marker SNPCmND15 (at position 30,377,414). The BC_1 plant derived from 05AL did not carry this region, so the segregation for resistance observed in BC_1S_1 progeny must be explained by the region of chromosome 12 or other regions of the genome. The candidate region proposed here would then expand between markers SNPCmND7 and SNPCmND15 (positions 30,249,798-30,377,414 bp), that is around 130 kb. This interval is included in the one previously proposed, between 30,221,970 and 30,708,662 bp, by Sáez et al. (2017).

Progenies from plants heterozygous in the candidate region in chromosome 12 (derived from 29BL, 32BL, 22AM-GO, and 05AL) showed variable percentages of susceptible plants, from near 100% in progeny from 29BL to 58% in progeny from 22AM-GO. These progenies were not carriers of the regions associated to resistance in chromosomes 2 and 11 (Table 2), so the segregation in resistance must come from the candidate region of chromosome 12. However, the fact that the different families varied in the segregation for resistance suggested that other regions of the genome, or the different traditional genetic backgrounds, would have an effect on resistance conferred by the *locus* on chromosome 12.

Conclusions

Analysis of selected progenies recombinant in the region of chromosome 11 associated with resistance to ToLCNDV has allowed the narrowing of the candidate interval to

approximately 130 kb. The markers available in this region, along with the backcross progenies generated, would be useful in the breeding program for the introgression of this resistance into traditional genetic backgrounds. Future work will focus on the characterization of resistance coming from other genomic regions.

Acknowledgements

This research was funded by the Spanish Ministerio de Ciencia, Innovación y Universidades, grants number AGL2017-85563-C2 (1-R) and RTA2017-00061-C03-03 (INIA), and by the Conselleria d'Educació, Investigació, Cultura i Esports de la Generalitat Valenciana, grant number PROMETEO/2017/078 (to promote excellence groups, cofinanced with FEDER funds). C.P.M. and A.F.L. are recipient of predoctoral fellowships (Programa de Ayudas de Investigación y Desarrollo (PAID-01-19) of the Universitat Politècnica de València and project Prometeo 2017/078 "Selección de Variedades Tradicionales y Desarrollo de Nuevas Variedades de Cucurbitáceas Adaptadas a la Producción Ecológica" of the Conselleria d'Educació, Investigació, Cultura i Esports de la Generalitat Valenciana, respectively) The authors would like to thank the COMAV GenBank for kindly providing the seed of the traditional landraces used as parentals.

Literature Cited

1. Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. Focus, 12(13), 39-40.
2. FAOSTAT 2021 Online Database (available at http://faostat.fao.org/, accessed January 2021)
3. Juárez M, Tovar R, Fiallo-Olivé E, Aranda MA, Gosálvez B, Castillo P, Moriones E, Navas-Castillo J. (2014). First detection of Tomato leaf curl New Delhi virus infecting zucchini in Spain. Plant Dis. 98(6):857. doi: 10.1094/PDIS-10-13-1050-PDN.
4. Jyothsna, P., Haq, Q. M. I., Singh, P., Sumiya, K. V., Praveen, S., Rawat, R., ... & Malathi, V. G. (2013). Infection of tomato leaf curl New Delhi virus (ToLCNDV), a bipartite begomovirus with betasatellites, results in enhanced level of helper virus components and antagonistic interaction between DNA B and betasatellites. Applied Microbiology and Biotechnology, 97(12), 5457-5471.
5. López, C., Ferriol, M., & Picó, M. B. (2015). Mechanical transmission of Tomato leaf curl New Delhi virus to cucurbit germplasm: selection of tolerance sources in *Cucumis melo*. Euphytica, 204(3), 679-691.
6. Sáez, C., Esteras, C., Martínez, C., Ferriol, M., Dhillon, N. P. S., López, C., & Picó, B. (2017). Resistance to tomato leaf curl New Delhi virus in melon is controlled by a major QTL located in chromosome 11. Plant Cell Reports, 36(10), 1571-1584.
7. Srivastava, K. M., Hallan, V., Raizada, R. K., Chandra, G., Singh, B. P., & Sane, P. V. (1995). Molecular cloning of Indian tomato leaf curl vims genome following a simple method of concentrating the supercoiled replicative form of viral DNA. Journal of Virological Methods, 51(2-3), 297-304.

Table 1. Segregation of resistant/susceptible plants in BC_1 and F_2 progenies (derived from the cross of WM-7 with different landraces derived homozygous lines) 30 days after mechanical inoculation with ToLCNDV.

		Symptoms segregation		Expected proportion	χ^2 test
Generation	Background	Resistant	Susceptible	R:S	
BC_1	11PS	22	17	1:1	0.64 (p=0.42)
	03PS	26	11	1:1	6.08 (p=0.01)
	29BL	23	13	1:1	2.78 (p=0.10)
	32BL	15	22	1:1	1.32 (p=0.25)
	22AM-GO	19	17	1:1	0.11 (p=0.74)
	05AL	26	13	1:1	4.33 (p=0.04)
F_2	02RC	27	3	3:1	3.60 (p=0.06)

* χ^2 value calculated for a dominant monogenic expected ratio (probability for the χ^2 value with one degree of freedom). p=probability of finding a value higher or equal to the χ^2.

Table 2. Genotype for the BC_1 and F_2 plants selected to evaluate their descendants. B: homozygous for 'WM-7' allele; A: homozygous for the allele of the susceptible parent; H: heterozygous.

			Generation of the progenies and genetic background					
			F_3	F_3	BC_1S_1	BC_1S_1	BC_1S_1	BC_1S_1
Markers	Position (bp)[1]	Chr[2]	02RC	02RC	29BL	32BL	22AM-GO	05AL
SNPCmND1	23,984,244	**2**	B	B	A	A	A	A
SNPCmND2	25,292,039	**2**	B	B	A	A	A	A
SNPCmND3	25,448,713	**2**	B	B	A	A	A	A
SNPCmND4	25,611,353	**2**	B	B	A	A	A	A
SNPCmND5bis	25,904,726	**2**	B	B	A	A	A	A
SNPCmND6	26,504,936	**2**	B	B	A	A	A	A
SNPCmND7	30,249,798	**11**	B	H	A	A	A	A
SNPCmND9	30,276,355	**11**	B	H	A	A	A	A
SNPCmND11	30,280,637	**11**	B	H	A	A	A	A
SNPCmND13bis	30,347,864	**11**	B	H	A	A	A	A
SNPCmND15	30,377,414	**11**	B	A	A	A	A	A
SNPCmND14	30,395,841	**11**	B	A	A	A	A	A
SNPCmND16bis	30,403,863	**11**	B	A	A	A	A	A
SNPCmND17	30,410,537	**11**	H	A	A	A	A	A
SNPCmND19	30,441,822	**11**	H	A	A	A	A	H
SNPCmND20	30,458,338	**11**	H	A	A	A	A	H
SNPCmND22	30,472,366	**11**	H	A	A	A	A	H
SNPCmND23	30,482,002	**11**	H	A	A	A	A	A
SNPCmND25	30,537,323	**11**	H	A	A	A	A	H
SNPCmND26bis	10,175,361	**12**	A	A	H	H	H	H
SNPCmND27	11,965,753	**12**	A	A	H	H	H	A
SNPCmND29	14,425,696	**12**	A	A	H	H	H	A
SNPCmND30	15,368,097	**12**	A	A	A	A	A	H
Susceptible (%)[3]			0	71	94	53	58	73

[1] Physical position in version v4 of the melon genome (available at https://www.melonomics.net/)
[2] Chromosome
[3] Percentage of susceptible plants in the descendants mechanically inoculated with ToLCNDV.

Figure 1. Symptoms after mechanical inoculation with ToLCNDV. From left to right: asymptomatic, slight, moderate and severe symptoms.

Cucumis melo is among the Few Species Independently Domesticated Three Times and on Two Continents

Hanno Schaefer
Technical University of Munich, Department of Life Science Systems, D-85354 Freising, Germany
Email: hanno.schaefer@tum.de

Susanne S. Renner
Washington University, Department of Biology, Saint Louis, MO 63130, U.S.A.
Email: srenner@wustl.edu

Introduction

Melon is among the commercially most important fruit crops and has therefore received a lot of attention from breeders, especially in the U.S. and China. As a result, there are numerous commercially important varieties, all belonging to one species, *Cucumis melo*. Until recently, it was unclear whether melon, which occurs wild in Africa, Asia, and Australia, was initially domesticated in Africa or Asia or even on both continents. The high diversity of landraces in India and East Asia supports the idea of an Asian domestication center, but the occurrence of wild populations in northeast Africa suggest that the species may also have been domesticated there (Kirkbride, 1993).

The question of where and how often *C. melo* was domesticated has now unequivocally been answered in two studies (Endl et al., 2018; Zhao et al., 2019). The first of these used molecular-phylogenetic data from a sample of 90% of all currently accepted 65 species of *Cucumis* (www.cucurbit.de) and dense geographic sampling of *C. melo* itself. Importantly, the sampling included field-collected plants from wild and feral populations in Africa, Iran, India, Australia, North America, and Indian Ocean islands. The results of this uniquely complete sampling revealed that melon was domesticated once in Africa and once in Asia. This is clear from an early split in the phylogenetic tree, with the African wild populations in one clade (marked in red in Fig. 1) and all other melon accessions in a second clade (marked in blue and green in Fig. 1). The African landraces are commonly referred to as "African *agrestis*" melons and formally described as *C. melo* ssp. *meloides*. All remaining melon cultivars from Europe, India and East Asia group with *C. melo* ssp. *melo* forma *agrestis* plants from India and Asia Minor, the "Asian *agrestis*" melons. In other words, the Asian form *agrestis* is the ancestor of most of our modern market melon cultivars, whereas the African subspecies *meloides* is the ancestor of an economically less important, but genetically diverse and probably still underexploited group of African cultivars, including "Tibish", "Fadasi" and presumably also “Seinat”. The Australian wild populations represent another genetically distinct clade, but have never been domesticated (Endl et al., 2018).

The other study analyzed genomic variation in 1,175 resequenced accessions of *C. melo* that represent the global diversity of the species (Zhao et al. including H. Schaefer, 2019). The results support the two domestication events in Africa and Asia but suggest a third independent domestication event in China. The inferred phylogeny (their Fig. 1) shows again an early split between African *C. melo* ssp. *meloides* (their "WAF & CAF" clades) and Asian *C. melo* ssp. *melo*. However, the single-nucleotide polymorphism (SNP) data clearly show that the Asian clade further splits into two clades, each comprising wild and domesticated forms (their "melo" and "agrestis" clades), one involving Indian accessions, the other Chinese accessions.

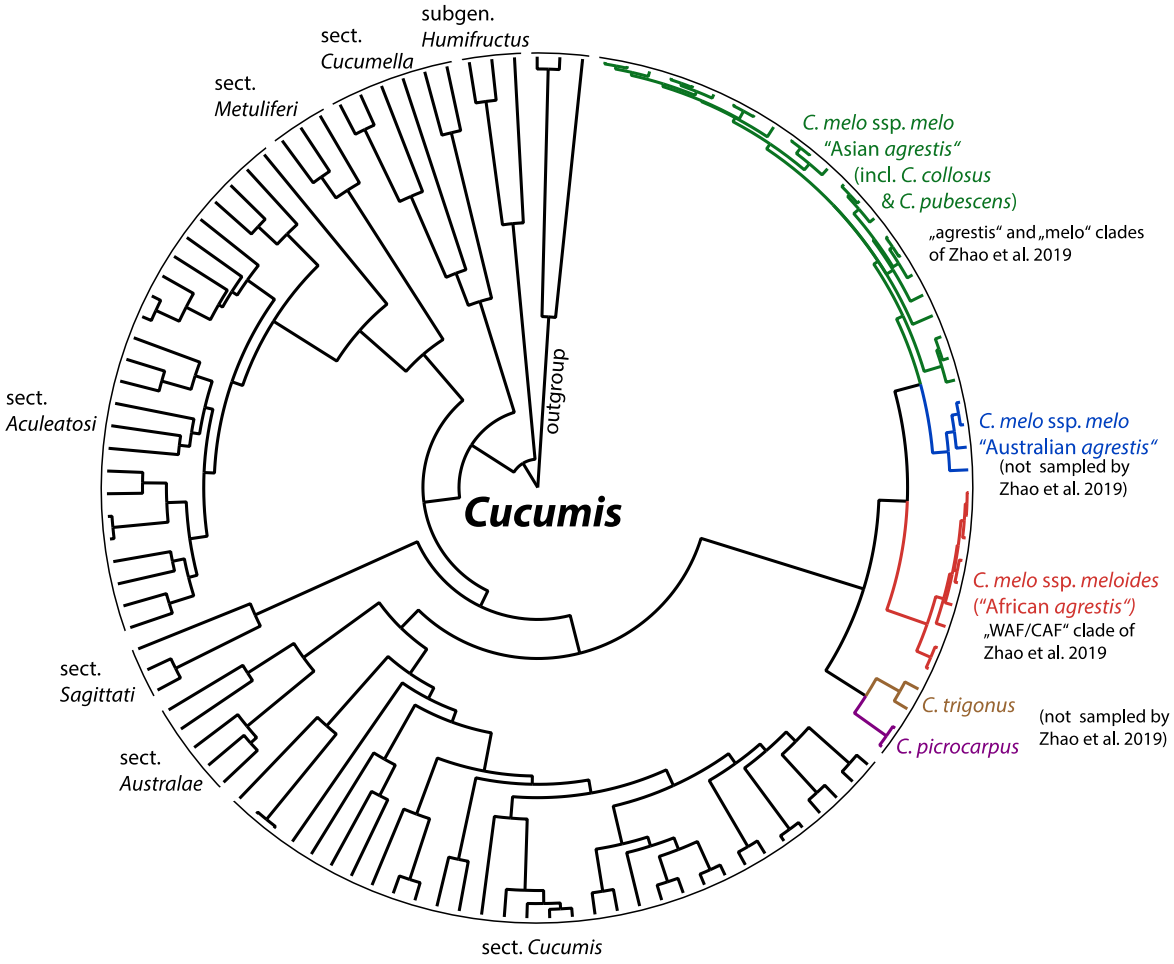
The DNA-based inference of three independent domestication events fits beautifully with archaeobotanical inferences based on fossil melon seeds. The oldest seeds assigned to *C. melo* come from China and date to 4,600 BC (Fuller, 2012), while the oldest African melon seeds are from a site dated to 3,700–3,500 BC in Lower Egypt (see overview in Sabato *et al.*, 2019). Seed remains of *C. melo* from India remain doubtfully identified, but melon cultivars appear to have been present in the Indus, and the Yangzte valleys by the third millennium BC (Fuller, 2006, 2012). Archaeobotanists and economic botanists have therefore suggested one domestication of *C. melo* in the Near East (Egypt?), one in Eastern China (Walters, 1989; Fuller, 2012), and one in India (Fuller, 2006).

It is worth noting that one of the fathers of modern plant breeding, Charles Naudin (1815 – 1899), already in 1859 suggested that the wild populations of melon in India and in Africa were domesticated independently. There is now an urgent need for inventory and storage of traditional African landraces in germplasm collections before they are entirely replaced by modern cultivars of Asian origin.

Literature Cited

1. Endl J, Achigan-Dako EG, Pandey AK, Monforte AJ, Pico B, Schaefer H. 2018. Repeated domestication of melon (*Cucumis melo*) in Africa and Asia and a new close relative from India. American J. Botany 105: 1662–1671.
2. Fuller, D. 2006. Agricultural origins and frontiers in South Asia: A working synthesis. J. World Prehist. 20: 1–86.
3. Fuller, D. 2012. New archaeobotanical information on plant domestication from macro-remains: Tracking the evolution of domestication syndrome traits. Pp. 110 – 135 in Biodiversity in Agriculture: Domestication, Evolution, and Sustainability, edited by P. Gepts, T.R. Famula, R.L. Bettinger et al. Cambridge University Press, UK.
4. Kirkbride J. H. Jr. 1993. Biosystematic monograph of the genus *Cucumis* (Cucurbitaceae). Parkway Publishers, Boone, North Carolina.
5. Naudin C. 1859. Essais d'une monographie des espèces et des variétés du genre *Cucumis*. Annales des Sciences Naturelles; Botanique 4: 5–87.
6. Sabato, D., Esteras, C., Grillo, O., Peña-Chocarro, L., Leida, C., Ucchesu, M., Usai, A., Bacchetta, G., Picó, B. 2019. Molecular and morphological characterisation of the oldest *Cucumis melo* L. seeds found in the Western Mediterranean Basin. Arachaeological and Anthropological Sciences 11: 789–810.
7. Walters TW. 1989. Historical overview of domesticated plants in China with special emphasis on the Cucurbitaceae. Economic Botany 43: 297–313.
8. Zhao, G., Lian, Q., ..., Schaefer, H., Fan, W., Bendahmane, A., Fei, Z., Pitrat, M., Gabaldon, T., Lin, T., Garcia-Mas, J., Xu, Y. and S. Huang. 2019. A comprehensive genome variation map of melon identifies multiple domestication events and loci influencing agronomic traits. *Nature Genetics* 51: 1607–1615

Figure 1. Summary phylogeny of *Cucumis* modified from Endl et al. (2018).



Grafting to Interspecific Rootstocks Increases Fruit Size and Yield of Cantaloupe, *Cucumis melo*

Caterina C. Roman[1], J. Brent Loy and Andrew B. Ogden[2]
Department of Agriculture, Nutrition and Food Systems, University of New Hampshire, Durham, NH
[1]Email: ccr1020@wildcats.unh.edu,
[2]Email: andrew.ogden@unh.edu

Introduction

Grafting of the Cucurbitaceae crop family originated in Japan during the 1920s (Edelstein 2017). Growers began grafting cantaloupe melons (*Cucumis melo*) in 1931 for potential yield increases and disease resistance. Since then, grafting of greenhouse melons has become a standard growing practice in many countries. Documented benefits of grafting include enhanced plant vigor, disease resistance, low temperature tolerance and improved nutrient and water absorption (Davis et al. 2008). The adoption of melon grafting has been slow in the United States. This is likely due to cantaloupe crops being primarily field grown in Southeastern US states where melon growing conditions are ideal. Conversely, farmers in New England have had limited success growing this potentially high value crop primarily due to the abundance of soil-borne pathogens and cooler temperatures (Ohletz & Loy, 2020).

While much research has been conducted on the usefulness of grafting melons within greenhouse systems (Rouphael, C. 2012), few studies have focused on field conditions of the Northeast United States. Grafted plants require additional labor, knowledge, cost, and more intensive care of individual plants than are required for non-grafted plants. Yet, the practice may be worthwhile due to the potentially dramatic yield increases within less optimal growing conditions.

Under sub-optimal melon growing conditions, grafting has been shown to boost productivity (Okimura et al. 1986; Yetisir and Sari 2004). The mechanism by which the interspecific rootstocks promote growth of the melon scion remains unclear. Researchers in the Loy lab had observed increased vegetative growth of grafted plants but had not previously quantified this effect. Other researchers have documented physiological effects of grafting including increased nutrient absorption under abiotic stressors such as soil salinity and low temperatures (Pulgar et al., 2000; Nie and Chen, 2000) as well as enhanced resistance to soil borne pathogens (Davis et al. 2008). Ohletz and Loy (2020) have found that the rootstock used, *Cucurbita maxima x Cucurbita moschata,* is able to withstand a wider range of temperatures than non-grafted plants. Additionally, grafting may increase cytokinin production, a plant hormone synthesized primarily in the roots known to influence vegetative growth (Kato and Lou, 1989). Interspecific rootstocks are likely to produce a larger more robust root system which can support a larger plant canopy than non-grafted melon plants (Bertucci et al., 2018). Melon scions grafted to interspecific rootstocks with a larger, more robust plant canopy may be able to sustain a larger fruit load than non-grafted plants.

More knowledge on the effects of grafting is essential before farmers adopt this potentially beneficial practice. Growers need a better understanding of the costs vs. benefits of grafting and how grafting may affect their yields, fruit size, and fruit quality. The objectives of this research study were to compare vegetative growth between grafted and non-grafted melon plants and to quantify the effects of grafting on yield, fruit size, and soluble solids content among three commercial varieties of cantaloupe.

Materials and Methods

Plant Materials. The interspecific rootstock used was an unreleased F1 rootstock *Cucurbita maxima x Cucurbita moschata*, named IS 1349, created through Dr. Brent Loy's Cucurbit breeding program at the University of New Hampshire and the New Hampshire Agriculture Experiment Station. Three F1 scion cultivars were compared: 'True Love' (High Mowing Seed Company, Wolcott VT), 'Sugar Rush' and 'Sugar Cube' (Seneca Vegetable Research, Geneva NY). Previous researchers in the Loy lab confirmed the grafting compatibility of rootstock and scion cultivars. The scion seeds were sown on 5 May 2019. Both the rootstock and non-grafted plants were sown on 7 May 2019. All seeds were planted in 50-cell plug trays containing soilless growing media (Pro-Mix BX). The seeds were put in a Conviron PGR15 growing chamber to promote germination and kept under a 16-hour photoperiod at 25°C during the lighted period and 22°C while dark.

Grafting technique. Grafting was performed at the Macfarlane Research Greenhouses in Durham, NH on 15 May 2019, using the One Cotyledon Grafting (OCG) method. Grafting was performed after both the scion seedlings and

rootstock seedlings displayed their first true leaf. The OCG method was achieved by using a razor blade to remove one of the rootstock's cotyledons with a 45° cut, while also removing its apical and lateral meristematic tissue. The scion seedlings were cut approximately 1.5 cm below the cotyledon (Ohletz & Loy, 2020). The rootstock and scion were then fused using plastic grafting clips. The grafted seedlings were placed in a Conviron PGR15 growing chamber to heal. The seedlings were kept under a 16-hour photoperiod with florescent lighting at ~250 µmol $m^{-2}s^{-1}$ PPF. The chamber temperature was set to 25°C during the lighted period and 22°C while dark. Clear dome lids were put over the trays and hand misted as needed in order to maintain a relative humidity of ~95%. After three days, the clear lids were removed for two hours at a time to begin plant acclimation. Four days after grafting, the plants were removed from the healing chamber and kept in a temperature-controlled greenhouse (25°C day and 22°C night) with natural sunlight for two weeks until transplanting.

Experimental site description. The field experiment took place at Kingman Horticultural Research Farm in Madbury, New Hampshire from June 2019 to September 2019. All plants were transplanted on 3 June 2019, three weeks after grafting. The soil type of the research plot is Charlton fine sandy loam. Nitrogen and potassium were applied to the field at a rate of 100.9 kg/ha during bed preparations. Soil tests showed that phosphorous levels were already sufficient. Plants were grown in raised black plastic covered beds (0.61 m width x 24.4 m long x 0.15 m high) with drip irrigation. Standard cultivation practices were used to control pests and disease.

Data collection. The number of leaves, both on the main stem and in total, as well as the number of lateral branches on every plant were counted weekly and recorded throughout the month of June. Fertilized flowers were tagged with their pollination dates. Ripe fruits were harvested from the research plot three times per week, when their stems easily slipped from the fruit. Each fruit was then weighed. The soluble solids content (SSC) for every harvested melon was recorded by taking two core samples and squeezing a small sample of the fruit's juice on a refractometer (RHB-32 Handheld Refractometer, Westover Scientific) then averaging the two samples. Observations on netting and any superficial quality issues were also recorded.

Experimental Design and Analysis. The field study was organized in a split plot design with four replicates of grafted vs. non-grafted as the main plot and variety as sub-plots. This experimental design was chosen to limit competition effects of the more vigorous grafted plants. Each block included either 7 grafted plants or 9 non-grafted plants. Within each block there was 0.5 m spacing between individual non-grafted plants and 0.75 m spacing between individual grafted plants. The additional space provided to grafted plant was due to previous findings that grafting may increase vegetative vigor (Tarchoum et al. 2005; Yang et al. 2006). A spacing of 2.74 m was used in between replicate blocks. Guard rows were placed at both outer rows of the field to limit bias from additional space and sunlight. Data were analyzed using the statistical software, JMP Pro 13 (SAS Institute, Cary NC). Analyses of variance were conducted to reveal the impacts of grafting and scion variety on yield, fruit size, SSC, and vegetative growth parameters. Interactions between the cultivars and grafting were also analyzed and wherever a P value <0.05 was obtained, Tukey's HSD was performed to sort out treatment effects.

Results and Discussion

Vegetative growth. Grafting increased vegetative growth in all three scion varieties. Two weeks after transplanting on June 18, all three varieties had approximately 90% more leaves on the grafted plants compared to non-grafted (Table 3). This was likely due to the interspecific rootstock having a larger, more vigorous root system which was able to uptake more water and nutrients, however, it could also be due to the increased space provided to grafted plants. Grafted plants of both 'Sugar Rush' and 'Sugar Cube' produced higher numbers of lateral branches than their non-grafted counterparts throughout the month of June. Grafted 'True Love' plants displayed more lateral branches than non-grafted plants on June 18, however, by June 28 the two did not differ (Table 3). This may have been caused by the grafted rootstock's improved tolerance of the cool soil temperatures experienced during early June. This finding helped confirm previous field observations of the larger, more robust canopy observed on grafted melon plants compared to non-grafted plants.

Fruit Size. Variety and grafting interacted to affect fruit size (Table 2). While the variety played a role in fruit size, grafting increased the average weight of all three varieties. True Love had the largest sized fruit while Sugar Cube had the smallest and Sugar Rush was in the middle. The weight per fruit when grafted increased on average by 40.9% in True Love melons, 25.8% in Sugar Cube melons and 17.6% in Sugar Rush melons (Table 1). Research conducted by Lee and Oda (2003), Condoruso et al. (2012) and Verzara et al. (2014) noted that grafting may affect fruit size differently among varying melon cultivars. These findings are important for growers seeking to obtain melons in a finely defined size class.

Yield. Grafting increased yields in all three scion melon varieties. Sugar Cube yields had an impressive 123% increase in total yield. The increase in yield was likely enabled by the enhanced photosynthetic capacity of the larger plant canopy observed on the grafted plants. The plot yields were converted

into yield in kilograms per hectare. On average, non-grafted Sugar Cube plants produced 4,889 kg/ha, whereas grafted plants produced 10,919 kg/ha. In Sugar Rush, non-grafted plants yielded 6,519 kg/ha compared to 8,904 kg/ha when grafted. Non-grafted True Love plants produced 6,845 kg/ha whereas grafted plants yielded 9,052 kg/ha (Table 1). These yield increases are even more impressive since grafting blocks contained two less plants than non-grafted blocks. Reducing the number of plants per hectare is a way for growers to offset the increased costs of growing grafted melon plants.

Fruit Quality. Soluble solids content (SSC) was used as a measure of fruit quality, due to its high correlation with the approximate sugar level of fruit flesh. The commercial standard for SSC within cantaloupes is a minimum of 9 ºBrix to be marketable, and values of 11 ºBrix and higher are considered gourmet (Suslow, 1997). SSC differed among the three melon scion varieties. (Table 1). True Love had the lowest level of soluble solids at 11 ºBrix whereas Sugar Rush had an average of 13 ºBrix and Sugar Cube with 14.8 ºBrix in non-grafted melons and 13.3 ºBrix in grafted melons. True Love melons had significantly lower SSC levels than Sugar Cube and Sugar Rush, however, the majority of the fruit harvested still had higher than 10 ºBrix. Both grafting and variety interacted to affect SSC. Grafting decreased SSC in Sugar Cube melons but did not affect it in True Love or Sugar Rush. The decrease in SSC was most likely caused by the more than doubled fruit load of the grafted plants. Despite this slight decrease, SSC levels of the grafted Sugar Cube melons still exceeded market standards.

Other studies have found changes in fruit flesh including decrease in firmness, vitrescence, and changes in taste as a result of grafting (Németh et al., 2020). Some grafted Sugar Cube melons displayed slight vitrescence of the fruit flesh whereas none of the non-grafted melon exhibited this trait. This was likely due to the variety Sugar Cube having a lower flesh firmness than the other two cultivars used. Adding calcium to the soil may help minimize this problem (Johnstone et al., 2008). Other studies have noted undesirable changes in the taste of melons when grafted (Rouphael et al., 2010), however, no taste changes were observed in this experiment.

Conclusion

The application of grafting to melon cultivation within the Northeast region of the United States is promising for disease and cold tolerance as well as increased yields. Grafting increased the vegetative growth in three commercial varieties of melon. Grafting also increased yields and fruit size, though in our study, the additional plant spacing used with grafted plants could have impacted this. Additional work is needed to verify this effect. No major changes in quality of the three varieties were observed. Additional studies are needed to focus on the potential season expansion, disease mitigation and fruit quality effects for different varieties.

Acknowledgements

This study was funded by the Weeks Day Fellowship grant and supported by the New Hampshire Agricultural Experimental Station. We are grateful to Dr. Becky Sideman for reviewing our manuscript. Special thanks and acknowledgement to the late Dr. Brent Loy for creating the interspecific rootstock and directing this research prior to his passing.

Literature Cited

1. Bertucci, S. 2018. Comparison of Root System Morphology of Cucurbit Rootstocks for Use in Watermelon Grafting. HortTechnology 28(5):629–636. https://doi.org/10.21273/HORTTECH04098-18
2. Condurso, C., Verzera, A., Dima, G., Tripodi, G., Crinó, P., Paratore, A., and Romano, D. 2012. Effects of different rootstocks on aroma volatile compounds and carotenoid content of melon fruits. Scientia Horticulturae 148: 9-16.
3. Davis, Angela R., Perkins-Veazie, Penelope, Sakata, Yoshiteru, López-Galarza, Salvador, Maroto, Jose Vicente, Lee, Sang-Gyu, Huh, Yun-Chan, Sun, Zhanyong, Miguel, Alfredo, King, Stephen R., Cohen, Roni and Lee, Jung-Myung. 2008. 'Cucurbit Grafting', Critical Reviews in Plant Sciences 27: 1, 50-74.
4. Edelstein, C. 2017. Performance of interspecific Cucurbita rootstocks compared to their parental lines. Scientia Horticulturae 216:45–50. https://doi.org/10.1016/j.scienta.2016.12.031
5. Johnstone, P.R., Hartz, T.K., and May, D.M. 2008. Calcium fertigation ineffective at increasing fruit yield and quality of muskmelon and honeydew melons in California. HortTechnology 18: 685–689.
6. Kato, T. and Lou, H. (1989). Effect of rootstock on the yield, mineral nutrition, and hormone level in xylem sap in eggplant. J. Japan Soc. Hort. Sci. 58: 345–352.
7. Lee, J.M. and Oda, M. 2003. Grafting of herbaceous vegetable and ornamental crops. In: Janick, J. (Ed.), Horticultural reviews, Vol. 28, John Wiley & Sons, New York, NY, pp. 61–124.
8. Németh, Dzsenifer, Gábor Balázs, Zsanett Bodor, John-Lewis Zinia Zaukuu, Zoltán Kovács, and Noémi Kappel. 2020. "Food quality attributes of melon (Cucumis melo L.) influenced by grafting". Progress in Agricultural Engineering Sciences Progress 16.S1:53-66. https://doi.org/10.1556/446.2020.10006
9. Nie, L. C. and Chen, G. L. 2000. Study on growth trends and physiological characteristics of grafted watermelon

seedlings, Acta Agriculturae Boreali- Occidentalis Sinica 9: 100–103.

10. Ohletz, J. L., & Loy, J. B. (2020). Grafting Melons Increases Yield, Extends the Harvest Season, and Prevents Sudden Wilt in New England. HortTechnology 31(1):101–114. https://doi.org/10.21273/horttech04669-20
11. Okimura, M., Matsuo, S., Arai, K., and Okitsu, S. (1986). Influence of soil temperature on the growth of fruit vegetable grafted on different stocks. Bull. Veg. & Ornam. Crops Res. Stn. Japan, Ser. C. 9:43–58. (in Japanese with English summary)
12. Pulgar, G., Villora, G., Moreno, D. A., and Romero, L. (2000). Improving the mineral nutrition in grafted watermelon plants: Nitrogen metabolism. Biologia Plant 43(4):607-609
13. Rouphael, Y., Schwarz, D., Krumbein, A., & Colla, G. (2010). Impact of grafting on product quality of fruit vegetables. Scientia Horticulturae 127(2):172–179. https://doi.org/10.1016/j.scienta.2010.09.001
14. Rouphael, C. (2012). Improving melon and cucumber photosynthetic activity, mineral composition, and growth performance under salinity stress by grafting onto Cucubita hybrid rootstocks. Photosynthetica 50(2):180-188. https://doi.org/10.1007/s11099-012-002-1
15. Suslow, T., Cantwell, M., and Mitchell J. (1997) Cantaloupe: Recommendations for Maintaining Postharvest Quality. http://postharvest.ucdavis.edu/Commodity_Resources/Fact_Sheets/Datastores/Fruit_English/?uid=14&ds=798
16. Tarchoun, N., Boughalleb, N., and El Mbakri, A. (2005). Agronomic evaluation of nine cucurbit rootstocks and watermelon grafted (Citrullus lanatus T.) Revue de l'INAT 20:125–140.
17. Verzera, A., Dima, G., Tripodi, G., Condurso, C., Crinó, P., and Romano, D. (2014). Aroma and sensory quality of honeydew melon fruits (Cucumis melo L. subsp. melo var. indorus H.) in relation to different rootstocks. Scientia Horticulturae 169:118–124.
18. Yang, L. F., Zhu, Y. L., Hu, C. M., Liu, Z. L., and Zhang, G. W. (2006). Effects of NaCl stress on the contents of the substances regulating membrane lipid oxidation and osmosis and photosynthetic characteristics of grafted cucumber, *Acta Botanica* Boreali-occidentalia Sinica 26:1195–1200.
19. Yetisir, H. and Sari, N. (2004). Effect of hypocotyls morphology on survival rate and growth of watermelon seedlings grafted on rootstocks with different emergence performance at various temperatures. J. Agric. Turk. 28:231– 237.

Table 1. Average weight per fruit (kg), yield per 3.25m² plot (kg)*, yield per hectares (kg) and soluble solids content (ºBrix) of three commercial varieties when grafted to *Cucurbita maxima x Cucurbita moschata* vs. non-grafted. Plants were grown and evaluated at Kingman Research Farm in Madbury, NH during the summer of 2019.

Variety and Treatment	Average weight per fruit (kg)	Average yield per plot (kg)	Average yield per hectares (kg)	Average soluble solids content (°Brix)
Sugar Cube- GR[z]	1.22 c[y]	73.7 a	10,919	13.3 b
Sugar Cube- NG	0.97 d	33.0 c	4,889	14.8 a
Sugar Rush- GR	1.60 b	60.1 ab	8,904	13.3 b
Sugar Rush- NG	1.36 c	44.0 c	6,519	13.8 b
True Love- GR	2.55 a	61.1 a	9,052	11.0 c
True Love- NG	1.81 b	46.2 bc	6,845	11.1 c

*all plots were identical in size; grafted plots contained 7 plants whereas non-grafted plants contained 9.
[z] GR symbolizes grafted and NG non-grafted.
[y] Values within the same columns that share a letter in common do not differ at $p<0.05$ according to Tukey's HSD.

Table 2. ANOVA p-values from data analysis of fruit size (kg), yield per plot (kg) and soluble solids content (SSC). P-values <0.05 are significant. There were no significant differences between replicates. P values for the error term Grafting*rep were all > 0.05.

	Fruit size	Yield per plot	SSC
Variety	<0.0001	0.7448	<0.0001
Grafting vs. non-grafting	0.0013	0.0003	0.0259
Variety * grafting vs. non-grafting	0.0002	0.0296	0.0087

Table 3. Average number of leaves and laterals per plant for three commercial varieties when grafted to *Cucurbita maxima x Cucurbita moschata* vs. non-grafted on two separate dates, June 18th and 28th, 2019. Plants were grown and observed at Kingman Research Farm in Madbury, NH during the summer of 2019.

Variety and treatment	Leaves per plant		Laterals per plant	
	6/18	6/28	6/18	6/28
Sugar Cube- GR[z]	9.53 a[y]	21.83 c	3.03 a	3.81 c
Sugar Cube- NG	5.05 b	11.80 d	1.17 b	2.27 e
Sugar Rush- GR	9.67 a	20.65 c	2.91 a	4.13 c
Sugar Rush- NG	5.10 b	12.60 d	1.59 b	3.07 cd
True Love- GR	9.52 a	20.58 c	2.84 a	3.55 cd
True Love- NG	5.04 b	12.15 d	1.59 b	2.66 de

[z] GR symbolizes grafted and NG non-grafted.
[y] Within a column, values that share a letter in common do not differ at p<0.05 according to Tukey's HSD.

Table 4. ANOVA p-values from JMP data analysis of average number of leaves and laterals on 18 June 2019 and 28 June 2019. P-values <0.05 are significant. There were no significant differences between replicates. P values for the error term Grafting*rep were all > 0.05.

	Leaves June 18	Leaves June 28	Laterals June 18	Laterals June 28
Variety	0.9529	0.8153	0.7345	0.0079
Grafting vs. non-grafting	<0.0001	0.0001	0.0151	0.0215
Variety * grafting vs. non-grafting	0.9894	0.3587	0.2757	0.1781