Cucurbit Genetics Cooperative

2019

Report 42

Cucurbit Genetics Cooperative Report

42

2019

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: *Momordica balfamina* L. Plate 45 in *Icones Plantarum Medicinalium: Centuria I*, by Johannes Zorn (1739-1799). Published by Raspischen Buchhandlung, Nuerenberg,1779. Image from the Biodiversity Heritage Library (biodiversitylibrary.org).

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

Resistance Response of *Citrullus* Genotypes to *Stagonosporopsis* spp. Isolates Causing Gummy Stem Blight

Winnie Gimode, Marin Lonnee and Cecilia McGregor*
University of Georgia, Athens, GA 30602, USA
**cmcgre1@uga.edu*

Introduction

Gummy Stem Blight (GSB) is a major fungal disease affecting watermelon (*Citrullus lanatus*) and other cucurbits (Sherbakoff 1917; Chiu and Walker 1949; Sherf and MacNab 1986). The disease is also commonly known as black rot, when infection occurs on fruit (Chiu and Walker 1949; Maynard and Hopkins 1999). It is a serious problem for cucurbit growers, especially in tropical, subtropical and some temperate areas, where the warm and humid conditions are conducive for disease development (Robinson and Decker-Walters 1997). In the southeastern United States (US), GSB was identified as the second most important research priority in watermelon after fusarium wilt (Kousik et al. 2016).

GSB was previously thought to be caused by a single pathogen: *Stagonosporopsis cucurbitacearum* (syn. *Didymella bryoniae*). However, it was recently determined that GSB is caused by three species of the genus *Stagonosporopsis: S. cucurbitacearum, S. citrulli* and *S. caricae* (Stewart et al. 2015). Morphologically, the species appear similar, but they differ genetically. Among the three *Stagonosporopsis* species*, S. citrulli* was found to be the most widely distributed worldwide (Brewer et al. 2015; Stewart et al. 2015). The study by Stewart et al. (2015) established that most of the isolates obtained from different hosts in North and South America, Europe, north Africa, and Asia were *S. citrulli*. *S. caricae* isolates, some of which were obtained from *Carica papaya*, were found in samples from North and South America, Asia, and southeast Asia, while *S. cucurbitacearum* were specifically from temperate regions in North America, Europe, Asia, and New Zealand. Within the US, *S. citrulli* was the most abundant, especially in the southeast US, while *S. cucurbitacearum* isolates were more common in northeast US (Stewart et al. 2015).

Several studies have shown that the different pathogen species exhibit variation in fungicide sensitivity (Brewer et al. 2015; Li et al. 2016; Li et al. 2019; Newark et al. 2019). For example, tebuconazole resistance was reported in *S. caricae* isolates, whereas *S. citrulli* and *S. cucurbitacearum* isolates were shown to be sensitive to this fungicide (Li et al. 2016). A subsequent study reported sensitivity to boscalid and fluopyram among all *S. caricae* isolates but varied sensitivity among *S. citrulli* isolates to boscalid (Li et al. 2019). Resistance to thiophanate-methyl has also been detected among *S. citrulli* isolates from East China while most isolates from Florida remained sensitive to this fungicide (Newark et al. 2019). This differential sensitivity poses a major challenge in management of GSB, especially because current management efforts rely heavily on fungicide applications, since no commercial watermelon cultivars currently possess genetic resistance to the GSB in the field.

Cultivated watermelon has a very narrow genetic base as a result of domestication that led to loss of some traits while selecting for desirable fruit quality (Levi et al. 2017; Guo et al. 2019). Other *Citrullus* species have been used as a major source of disease resistance traits for various diseases in watermelon (Boyhan et al. 1994; Guner 2005; Thies and Levi 2007; Tetteh et al. 2010; Wechter et al. 2012; Levi et al. 2017). *Citrullus* germplasm resistant to GSB have been identified as early as 1962 (Sowell and Pointer 1962) and efforts to introgress this resistance into commercial cultivars has been attempted, though unsuccessful (Norton 1979; Norton et al. 1986; Norton et al. 1993; Sumner and Hall 1993; Norton et al. 1995; Song et al. 2002). Plant Introduction (PI) 189225 was initially identified as the most resistant accession evaluated from the USDA-ARS watermelon germplasm collection (Sowell and Pointer 1962). PI 271778, was later identified as an additional source of resistance (Sowell 1975; Norton 1979). Both PI 189225 and PI 271778 are wild accessions of *C. amarus,* a close relative of watermelon (Chomicki and Renner 2015; Renner et al. 2017). Crosses between elite cultivars and resistant PIs were made to produce two lines: 'AU-Jubilant' ('Jubilee' x PI 271778) and 'AU-Producer' ('Crimson Sweet' x PI 189225) (Norton et al., 1986). These cultivars have however not shown resistance to GSB in the field (Song et al. 2002).

Gusmini et al. (2005) identified a further ten PIs that displayed significant levels of resistance to GSB under both field and greenhouse conditions. These accessions consisted of genotypes from both *C. amarus* and *C. lanatus* species and included PI 164248, PI 244019, PI 254744, PI 271771, PI 279461, PI 296332, PI 482276, PI 482379, PI 490383 and PI 526233 (Gusmini et al. 2005). Despite all the resistant sources described, breeding efforts for GSB resistant watermelon cultivars have been unsuccessful. With the discovery that GSB can be caused by three different *Stagonosposopsis* species, the question arises whether differential host resistance to the species might be partially responsible for the lack of success in resistance breeding efforts. To date, no studies have examined the effect of different *Stagonosporopsis* species on putative resistant *Citrullus* genotypes. It is vital to establish whether the three species have similar host responses. Understanding the level and breadth of resistance found in *Citrullus* genotypes will be essential in determining the appropriate sources of resistance to use in breeding efforts. The objective of this study was therefore to evaluate the level of resistance of 12 different *Citrullus* genotypes to six isolates from three different *Stagonosporopsis* species.

Materials and Methods

Seeds of 12 different *Citrullu*s genotypes that included both wild and elite genotypes were sown in the greenhouse in 48-cell seedling trays, approximately 2 weeks prior to the screen. The genotypes consisted of cultivars and PIs belonging to *C. amarus, C. mucosospermus* and *C. lanatus* species. They included: 'AU-Producer' (AUP), 'Crimson Sweet' (CS), 'Mickylee' (MICK), 'Sugar Baby' (SB), PI 189225, PI 244019, PI 279461, PI 482276, PI 482379, PI 549160, PI 560023 and PI 593359 (Table 1). The panel of genotypes used in this study were specifically chosen to represent a broad genetic background for watermelon. Moreover, five of the PI used in this study (PI 189225, PI 244019, PI 279461, PI 482276, PI 482379) were chosen because they had been previously described as resistant to GSB (Sowell and Pointer 1962; Gusmini et al. 2005)

The *C. lanatus* genotypes used in the study were from North America (CS, AUP, SB and MICK), Asia (Japan: PI 279461; China: PI 593359) and Africa (PI 549160). Many modern watermelon cultivars are related to CS, which is a parent of AUP (Norton et al. 1986; Wehner and Barrett 2002). SB, which is genetically distant from other North American cultivars, is an ancestral parent of MICK (Wehner and Barrett 2002). PI 549160 is a wild *C. lanatus* from northeast Africa, which is a center of domestication for watermelon (Renner et al. 2017). PI 560023 was the only *C. mucosospermus* (egusi) species used in this study. Egusi watermelon are utilized in West Africa for their edible seeds. The *C. amarus* species included PI 244019, PI 482379, PI 482276 and PI 189225 which are from South Africa, Zimbabwe, Zaire and Zimbabwe, respectively.

Six *Stagonosporopsis* isolates, provided by Marin Brewer (University of Georgia, Department of Pathology), were grown (16h/8h light/dark cycle) on potato dextrose agar (PDA) (Becton, Dickinson and Company, NJ, USA) for 2 weeks. Approximately 1 cm^2 agar plugs were then sub cultured on quarter-strength PDA (qPDA) where they were grown for another 2 weeks. The isolates included *S. citrulli:* 12178A and AcSq5, *S. cucurbitacearum:* RT2 and GSB26 and *S. caricae:* GA8007H and RG3 (Stewart et al., 2015; M. Brewer, *personal communication*) (Table 2).

Three independent screens were performed in a growth chamber. During each screen, seven trays (6 isolates and 1 control) were sown, with four seeds of each genotype (12 genotypes total) per tray. On the day of inoculation, qPDA cultures were flooded with 10 ml of 0.1% tween20 and gently scraped with a sterile spatula to release spores. The inoculum was filtered through 2 layers of sterile cheese cloth and spore concentration was determined using a hemacytometer (Hausser Scientific, PA, USA). Spore concentrations were then adjusted to 5 x 10^5 spores/ml using 0.1% tween20 solution.

At the 2nd true leaf stage, seedling trays were placed in plastic tubs and each tray was sprayed with freshly made inoculum from one isolate using an airbrush sprayer (Master Airbrush Model E91) for 60 seconds. The control tray was sprayed with a mock inoculation consisting of 0.1% tween20 solution. The tubs were then sealed in a transparent, plastic bag to promote high relative humidity of approximately 95% which was measured using a data logger (Lascar Electronics UK). The tubs were placed in a growth chamber set to 26 °C day and 23 °C night with a 12h/12h light/dark cycle. On the 3rd day post-inoculation (dpi), the trays were removed from the tubs and disease severity data was collected 7dpi. Disease symptoms were evaluated on a scale of 0 to 9 as described by (Lou et al. 2013), where 0 = no disease; 1 to 2 = mild trace of infection with less than 10% of leaves covered with lesions; 3 to 4 = 10 to 20% of leaves covered with lesions, 5 to 6 = 21 to 50 % of the leaves covered with small lesions; 7 to 8 = wilting plant and more than 50 % of the leaves covered with lesions; and 9 = dead plant.

Statistical analyses were conducted using a fitted mixed linear model in R, whereby genotype, isolate and their interaction were the fixed effects while screen was treated as a random effect. Post hoc comparisons among groups after fitting the model were done using emmeans to obtain treatment values and significance levels after taking into account other terms in the model. Hierarchical cluster analysis

was performed for both the isolates and the genotypes using JMP® Pro 14.1.

Results and Discussion

No lesions were observed on mock inoculated plants in any of the screens. One of the isolates, *S. citrulli* AcSq5 had slightly lower spore concentration (4.34 x 10^5 spores/ml) in the first screen. In the subsequent screens, spores were not observed and therefore data from only one replication was included in the analysis for this isolate. In the treated trays, similar trends were observed in the three screens with *S. citrulli* 12178A exhibiting higher aggressiveness than the other isolates, with most of the seedlings dead by 7dpi (data not shown). Results of the ANOVA indicated a significant difference between the watermelon genotypes used ($P < 0.001$) as well as the isolates ($P < 0.001$), but no significant genotype × isolate interaction (Table 3).

S. citrulli 12178A and *S. caricae* RG3 were significantly the most aggressive of the isolates, followed by *S. cucurbitacearum* RT2 (Fig. 1). The least aggressive isolate was *S. cucurbitacearum* GSB26, however it was not significantly different from *S. caricae* GA8007H and *S. citrulli* AcSq5. Based on the hierarchical cluster analysis the isolates formed two major clusters, with *S. citrulli* 12178A and *S. caricae* RG3 diverged from the four other isolates (Fig. 1). These results indicate that the level of aggressiveness was not species-dependent and that certain isolates within a species could be more aggressive than others.

The watermelon genotypes exhibited a wide distribution of resistance levels to the different isolates of *Stagonosporopsis* (Fig. 1) as would be expected from our choice of genotypes. The genotypes separated into two major clades in the hierarchical cluster analysis, with the *C. amarus* genotypes forming one clade and all the *C. lanatus* and the *C. mucosospermus* (PI 560023) genotypes in the other clade. Among the genotypes, PI 189225 and PI 482276 were generally more resistant than the other genotypes and they clustered together. These two lines had been previously described as resistant to GSB (Norton et al. 1993; Gusmini et al. 2005) and this study confirms their broad resistance to GSB isolates. The other two *C. amarus* lines, PI 482379 and PI 244019 also displayed intermediate resistance to most of the isolates, however the latter was more susceptible to *S. cucurbitacearum* RT2.

AUP had the highest disease severity score overall (7.51) followed by SB (7.26), CS (7.09) and PI 279461 (6.63) (Fig. 1). AUP and CS clustered together in the hierarchical clade. It is worth noting that AUP, which was formerly described as resistant to GSB (Norton et al. 1986) but demonstrated to be susceptible in the field (Song et al. 2002), only showed resistance to *S. cucurbitacearum* GSB26, the least severe of the isolates tested (Fig. 1). PI 279461 was among the most resistant lines described by Gusmini et al. (2005) but displayed high disease severity in the present study. Similar to AUP, it seemed slightly more resistant to the least aggressive *S. cucurbitacearum* GSB26. It is tempting to speculate that an isolate similar to *S. cucurbitacearum* GSB26 was used in these studies for phenotyping, but the current study does not allow us to determine that with any certainty. AUP however displayed very high susceptibility to all other isolates, confirming the susceptibility of this cultivar to GSB. The elite cultivars were generally susceptible to the various isolates (Fig. 1). PI 189225 (2.89) and PI 482276 (2.83) were more resistant than the other genotypes across isolates. The results observed on these genotypes confirm the resistance of these two *C. amarus* genotypes against GSB as previously described by Norton et al., (1993) and Gusmini et al., (2005).

This study confirms that some *Stagonosporopsis* isolates are more aggressive than others, but with the isolates tested in this study, there is no pattern of aggressiveness within species. The two most aggressive isolates (12178A and RG3), which were *S. citrulli* and *S. caricae*, respectively, were originally obtained from *C. lanatus* hosts, therefore it could be argued that there could be some host specificity. However, RT2, which also displayed high aggressiveness, was obtained from *Cucurbita moschata*, while GA8007H which displayed lower aggressiveness was isolated from watermelon (Stewart et al., 2015). One limitation of this study was that one isolate (AcSq5) only had one replication due to low sporulation.

Our results could explain the inconsistency that has been observed with GSB phenotyping in different research programs and why efforts to introgress GSB resistance into commercial cultivars have been complex and unsuccessful. It is possible that different *Stagonosporopsis* isolates with varying levels of aggressiveness are used for phenotyping, especially considering the pathogen in the screens is only referred to as *Didymella bryoniae*. It is also highly likely that a mixture of isolates exists in the field (Brewer et al., 2015). This further complicates the breeding process for GSB resistance. From the results of this study, it should be noted that phenotyping using a less aggressive isolate may confer resistance to the specific isolate, but when the genotype is challenged with a more aggressive isolate present in the field, it may not survive. Results from Gusmini et al. (2017) also displayed large environmental effects associated with GSB, which would impact the severity of symptoms observed in the field.

It is still unknown whether the same resistant loci in *Citrullus* genotypes confer broad resistance against different *Stagonosporopsis* isolates. Utilization of highly aggressive

Stagonosporopsis isolates during GSB resistance breeding provides a greater likelihood of obtaining field-level resistance to natural GSB epidemics. Knowledge of the effect of different *Stagonosporopsis* isolates on *Citrullus* genotypes may inform breeders on the appropriate resistance sources and pathogen isolates to utilize for breeding. These results can inform watermelon breeders in developing strategies for phenotyping and resistance loci deployment when breeding for GSB resistance.

Acknowledgement

The authors thank Marin Brewer, Department of Pathology University of Georgia, USA, for providing the *Stagonosporopsis* isolates used in this study.

Literature Cited

1. Boyhan G, Norton J, Abrahams B (1994) Screening for resistance to anthracnose (race 2), gummy stem blight, and root knot nematode in watermelon germplasm. Cucurbit Genet Coop Rep 17:106-110.
2. Brewer MT, Rath M, Li H-X (2015) Genetic diversity and population structure of cucurbit gummy stem blight fungi based on microsatellite markers. Phytopathology 105 (6):815-824.
3. Chiu W, Walker J (1949) Physiology and pathogenicity of the cucurbit black-rot fungus. J Agric Res 78:589-615.
4. Chomicki G, Renner SS (2015) Watermelon origin solved with molecular phylogenetics including Linnaean material: another example of museomics. New Phytol 205 (2):526-532.
5. Guner N (2005) Papaya ringspot virus watermelon strain and Zucchini yellow mosaic virus resistance in watermelon. PhD Diss, Department of Horticultural Sciences, North Carolina State Univ, Raleigh, NC.
6. Guo S, Zhao S, Sun H, Wang X, Wu S, Lin T, Ren Y, Gao L, Deng Y, Zhang J, Lu X, Zhang H, Shang J, Gong G, Wen C, He N, Tian S, Li M, Liu J, Wang Y, Zhu Y, Jarret R, Levi A, Zhang X, Huang S, Fei Z, Liu W, Xu Y (2019) Resequencing of 414 cultivated and wild watermelon accessions identifies selection for fruit quality traits. Nat Genet 51:1616-1623.
7. Gusmini G, Rivera-Burgos LA, Wehner TC (2017) Inheritance of resistance to gummy stem blight in watermelon. HortScience 52 (11):1477-1482.
8. Gusmini G, Song R, Wehner TC (2005) New sources of resistance to gummy stem blight in watermelon. Crop Sci 45 (2):582-588.
9. Kousik CS, Brusca J, Turechek WW (2016) Diseases and disease management strategies take top research priority in the Watermelon Research and Development Group members survey (2014 to 2015). Plant Health Prog 17 (1):53-58.
10. Levi A, Jarret R, Kousik S, Wechter WP, Nimmakayala P, Reddy UK (2017) Genetic resources of watermelon. In: Grumet RK, N., Garcia-Mas J. (ed) Genetics and Genomics of Cucurbitaceae. Springer, pp 87-110.
11. Li H-X, Stevenson KL, Brewer MT (2016) Differences in sensitivity to a triazole fungicide among *Stagonosporopsis* species causing gummy stem blight of cucurbits. Plant Dis 100 (10):2106-2112.
12. Li HX, Nuckols TA, Harris D, Stevenson KL, Brewer MT (2019) Differences in fungicide resistance profiles and multiple resistance to a quinone-outside inhibitor (QoI), two succinate dehydrogenase inhibitors (SDHI), and a demethylation inhibitor (DMI) for two *Stagonosporopsis* species causing gummy stem blight of cucurbits. Pest Manage Sci 75 (11):3093-3101.
13. Lou L, Wang H, Qian C, Liu J, Bai Y, Chen J (2013) Genetic mapping of gummy stem blight (*Didymella bryoniae*) resistance genes in *Cucumis sativus-hystrix* introgression lines. Euphytica 192 (3):359-369.
14. Maynard DN, Hopkins DL (1999) Watermelon fruit disorders. HortTechnology 9 (2):155-161.
15. Newark MJ, Li P, Yang X-P, Paret ML, Dufault NS (2019) Comparing *Stagonosporopsis* spp. fungicide resistance profiles in Florida and East China cucurbit production systems. Plant Dis:PDIS-02-19-0370-RE.
16. Norton J, Boyhan G, Smith D, Abrahams B (1993) 'AU-Golden Producer' watermelon. HortScience 28 (6):681-682.
17. Norton J, Boyhan G, Smith D, Abrahams B (1995) 'AU-Sweet Scarlet' watermelon. HortScience 30 (2):393-394.
18. Norton J, Cosper R, Smith D, Rymal K (1986) 'AU-Jubilant' and 'AU-Producer' watermelons. HortScience 21 (6):1460-1461.
19. Norton JD (1979) Inheritance of resistance to gummy stem blight in watermelon. HortScience 14:630-632.
20. Renner SS, Sousa A, Chomicki G (2017) Chromosome numbers, Sudanese wild forms, and classification of the watermelon genus *Citrullus*, with 50 names allocated to seven biological species. Taxon 66 (6):1393-1405.
21. Robinson RW, Decker-Walters D (1997) Cucurbits. CAB International, New York.
22. Sherbakoff, C.C. (1917) Some important diseases of truck crops in Florida. Florida Agriculture Experimental Station Bulletin 139:269-273.
23. Sherf AF, MacNab AA (1986) Vegetable diseases and their control. John Wiley & Sons, New York.
24. Song R, Gusmini G, Wehner TC Screening the watermelon germplasm collection for resistance to gummy stem

blight. In: XXVI International Horticultural Congress: Advances in Vegetable Breeding 637, 2002. pp 63-68.
25. Sowell G (1975) Additional source of resistance to gummy stem blight in watermelon. Plant Disease Reporter 59:413-415.
26. Sowell G, Pointer GR (1962) Gummy stem blight resistance of introduced watermelons. Plant Disease Reporter 46:883-885.
27. Stewart JE, Turner AN, Brewer MT (2015) Evolutionary history and variation in host range of three *Stagonosporopsis* species causing gummy stem blight of cucurbits. Fungal Biology 119 (5):370-382.
28. Sumner D, Hall M (1993) Resistance of watermelon cultivars to Fusarium wilt and gummy stem blight. Biol Cult Tests 8:36.
29. Tetteh AY, Wehner TC, Davis AR (2010) Identifying resistance to powdery mildew race 2W in the USDA-ARS watermelon germplasm collection. Crop Sci 50 (3):933-939.
30. Thies JA, Levi A (2007) Characterization of watermelon (*Citrullus lanatus* var. *citroides*) germplasm for resistance to root-knot nematodes. HortScience 42 (7):1530-1533.
31. Wechter WP, Kousik C, McMillan M, Levi A (2012) Identification of resistance to *Fusarium oxysporum* f. sp. *niveum* race 2 in *Citrullus lanatus* var. *citroides* plant introductions. HortScience 47 (3):334-338.
32. Wehner T, Barrett C (2002) Vegetable cultivar descriptions for North America list 26 2002. 37 (1):15-78.

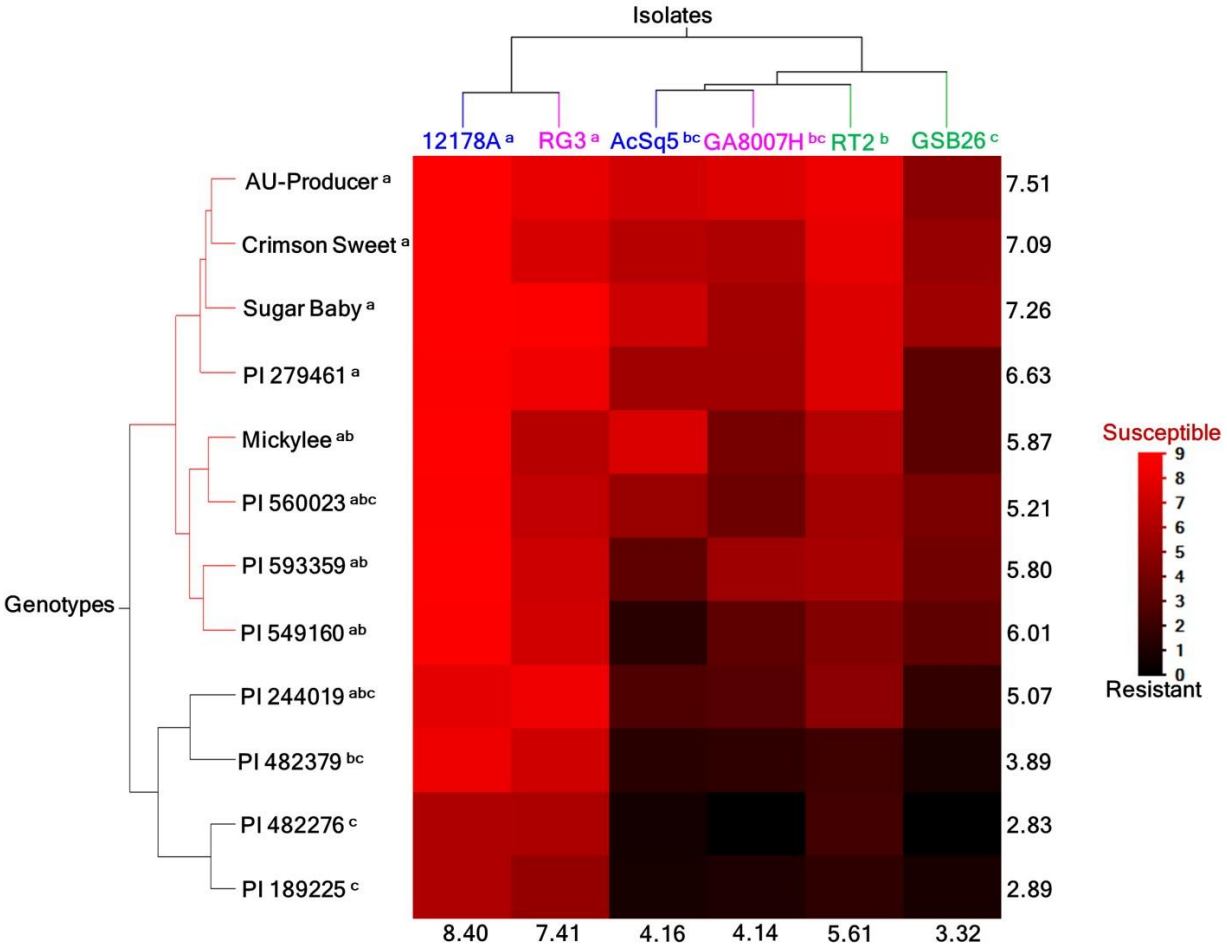

Fig. 1 Heat map displaying the disease severity of different *Citrullus* genotypes (x-axis) against various *Stagonosporopsis* spp. isolates (y-axis). The isolates are *S. citrulli* (blue), *S. caricae* (pink) and *S. cucurbitacearum* (green). On the right and bottom are the mean severity scores for each genotype and isolate, respectively. Levels not connected by the same letter (superscript) are significantly different.

Table 1. Seed sources for *Citrullus* genotypes used in this study.

Genotype	Seed source	Species
'AU-Producer' (AUP)	Hollar Seeds	*C. lanatus*
'Crimson Sweet' (CS)	Seedway Seeds	*C. lanatus*
'Sugar Baby' (SB)	Reimer Seeds	*C. lanatus*
'Mickylee' (MICK)	Hollar Seeds	*C. lanatus*
PI 279461	USDA-ARS, Griffin, GA	*C. lanatus*
PI 593359	USDA-ARS, Griffin, GA	*C. lanatus*
PI 549160	USDA-ARS, Griffin, GA	*C. lanatus*
PI 560023	USDA-ARS, Griffin, GA	*C. mucosospermus*
PI 244019	USDA-ARS, Griffin, GA	*C. amarus*
PI 482379	USDA-ARS, Griffin, GA	*C. amarus*
PI 482276	USDA-ARS, Griffin, GA	*C. amarus*
PI 189225	USDA-ARS, Griffin, GA	*C. amarus*

Table 2. Sources of isolates used in this study (Stewart et al., 2015, M. Brewer, *personal communication*).

Isolate name	Original host species	State of origin	*Stagonosporopsis* spp.
12178A	*Citrullus lanatus* (watermelon)	Georgia	*S. citrulli*
AcSq5	*Cucurbita pepo* (acorn squash)	North Carolina	*S. citrulli*
RG3	*Citrullus lanatus* (watermelon)	California	*S. caricae*
GA8007H	*Citrullus lanatus* (watermelon)	Georgia	*S. caricae*
RT2	*Cucurbita moschata* (butternut squash)	Michigan	*S. cucurbitacearum*
GSB26	*Cucumis melo* (muskmelon)	New York	*S. cucurbitacearum*

Table 3. Analysis of variance for mean disease severity scores of 12 watermelon genotypes inoculated with six *Stagonosporopsis* species isolates.

Source of variation	Sum Sq	Mean Sq	DF	F value	Pr (>F)
Genotype***	422.99	38.45	11	7.14	3.644×10^{-9}
Isolate***	671.30	134.26	5	24.92	$< 2.2\times10^{-16}$
Genotype × Isolate^{NS}	142.04	2.58	55	0.48	9.986×10^{-1}

A New Watermelon Variety 'Longshengjiafeng' with High Quality and Resistance to Fusarium Wilt

X.Q. Wang*, Y.H. Jia, W. Yan, Y. K. Fu, W.J. YE, H.B. You
Horticulture Branch of Heilongjiang Academy of Agricultural Sciences, Heilongjiang, Harbin 150069, China
**xiqingwang100@163.com*

Abstract

Watermelon 'Longshengjiafeng' is a new hybrid with high fruit quality and resistance to Fusarium wilt. The fruit is oval shaped with a durable rind (1.0 cm thick), green skin and dark green stripes. The flesh is red and crisp, with Brix 13.2%. Fruit matures 95 days from sowing and 32 days post flowering. Average fruit weight is 12.0 kg, and the yield is about 60 ton/ha. The plant has high levels of resistance to Fusarium wilt race 1. This hybrid is suitable for production in the open field and plastic house in Northeast China.

Breeding Objectives

Northeast China is well known for organic production and high fruit quality watermelon. Fusarium wilt is a major soil-borne disease for watermelon production in Northeast China. A rotation of 7-8 years is needed for watermelon production (Liang Li et al., 2014). The availability of land in this area makes long rotation very challenging. Use of resistant varieties would allow cropping with shorter rotation time. The demanding of high quality by the market and consumers is ever increasing in recent years. Based on the urgent needs of producers and consumers our group has been focusing on development of high-quality large fruit watermelon varieties with high level resistance to Fusarium wilt.

Breeding Process

'Longshengjiafeng' is a new hybrid watermelon with large fruit size. It was bred by crossing two inbred lines 'K10197FR' and 'KJ1028'. Female parent KJ1028 is an inbred line derived from an F2 selection from the cross between Jingxin 1 and Calhoun Gray followed by 7 generations of self-pollination. This inbred line produces round fruit with red flesh, striped skin and wax on skin. It is resistant to Fusarium wilt race 1 based on phenotyping and genotyping. Male parent K10197FR is an inbred line derived from a cross between K10197 and Calhoun Gray followed by 4 generations of backcrossing and 4 generations of self-pollination. This inbred line produces oval shaped fruit with red flesh, stripped skin and wax on skin. It is resistant to Fusarium wilt race 1 based on phenotyping and genotyping.

In our trials the average yield of 'Longshengjiafeng' was 60.1 ton/ha, 10.1% higher than check variety 'Angdatianwang' (54.6 ton/ha), and the Brix was 13.2%, 21.0% higher than check 'Angdatianwang' (11.2%). During our Fusarium wilt test with sample size of 30 plants each, the disease incidences at seedling stage (Figure 2) for 'Longshengjiafeng', '8424' (susceptible control) and 'Xinong 8' (resistant control) were 3.62%, 83.51% and 8.97%, respectively. The disease severity indexes for 'Longshengjiafeng', '8424' and 'Xinong 8' were 1.45, 69.98 and 4.10, respectively. The Fusarium wilt resistance of 'Longshengjiafeng' reached high level. 'Longshengjiafeng' also performed well in disease nursery at late development stage (Figure 3). In the regional trials, 'Longshengjiafeng' demonstrated stable growth, high yield, high fruit quality and high level resistance to Fusarium wilt.

Variety Characteristics

The growth period of this variety is about 95 days, and the fruit development period is about 32 days. The fruit (Figure 1) has an oval shape and durable rind with green skin and dark green strips. The flesh is red and crisp with Brix 13.2%. The tough rind makes it suitable for long distance transportation and long shelf-life. The variety is highly resistant to Fusarium wilt. The average fruit weight is 12 Kg and average yield is 60 ton/ha.

Cultivation Technique

This hybrid is suitable for watermelon production in the open field and plastic house in Northeast China. The seedlings, 25 days post germination, are transplanted into soil covered with plastic film at spacing 0.55-0.66 m between plants and 2.00 m between rows, i.e. 500-600 plants per 667 m^2. Two tons of manure compost, 25 kg of ammonium phosphate, 40 kg of potassium sulfate and 10 kg of urea are recommended for every 667m^2. Plants are pruned to three or four branches per plant. The second or third female flowers on the plant are pollinated for fruit set.

Acknowledgements

This work is supported by The National Key Research and Development Program of China（2018YFD0100704-4), and Earmarked Fund for China Agricultural Research System (CARS-25).

Literature Cited

1. Liang Li, 2014, Overview of Watermelon and Melon Production in Inner Mongolia Autonomous Region [J]. Chinese Vegetables 27:177-178.

Figure 1. Fruit of watermelon 'Longshengjiafeng'.

Figure 2. Fusarium wilt test at the seedling stage. Left: resistant control 'Xinong 8'; Middle: 'Longshengjiafeng'; Right: susceptible control '8424'.

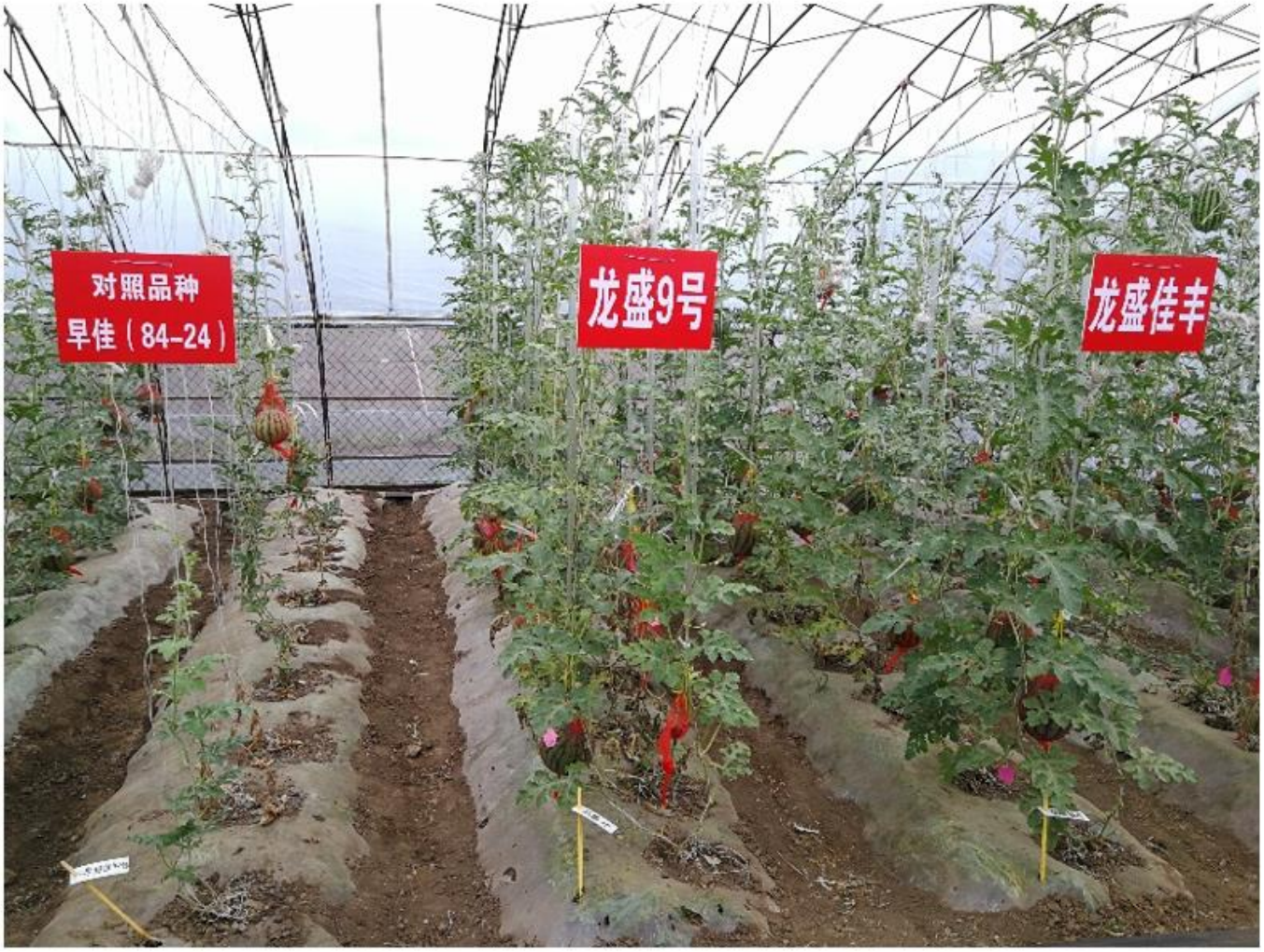

Figure 3. Performance of 'Longshengjiafeng' in disease nursery with 10 year mono cropping of watermelon. Left: susceptible control '8424'; Middle: resistant control 'Longsheng 9'; Right: 'Longshengjiafeng'.

How Russian Breeders Discovered *Citrullus mucosospermus* and *Citrullus lanatus* var. *cordophanus*, the Likely Closest Relatives of Domesticated Watermelon

Susanne S. Renner
Institute of Systematic Botany and Mycology, Department of Biology, University of Munich (LMU), Munich, Germany
renner@lrz.uni-muenchen.de

Watermelon is among the commercially most important fruit crops and has therefore received a lot of attention from breeders, especially in the US and China. Probably the most successful modern work on wild watermelons, however, was carried out by Russian breeders connected to the Vavilov institute in St. Petersburg and breeding stations near Astrakhan and Tashkent. Thus, it was Russians who discovered both current contenders for the position as closest relatives or progenitors of domesticated watermelon, *Citrullus mucosospermus* and *Citrullus lanatus* var. *cordophanus*, although it is only with molecular-genomic methods that the importance of these discoveries is becoming clear.

Between 1959 and 1964, the Armenian botanist David Ter-Avanesyan (1909-1979), whose life and travels are described on a website linked to the Vavilov institute (http://www.rasl.ru/science/14_Exhibitions/ter.php), travelled to the Kordofan region of Sudan to collect seeds of economically important plants. The seeds he brought back were grown out and are still being maintained at the Vavilov Institute (personal communication, Larisa Bagmet, Curator of the herbarium of the Vavilov Institute, 30 March 2017). His Sudanese watermelons, which he described as '*C. lanatus* subspecies *cordophanus*', have striped fruits with white non-bitter pulp and beige seeds, all illustrated by B/W photos (Ter-Avanesyn, 1966). He considered these plants to represent the progenitors of cultivated watermelon.

The next discovery came from Ter-Avanesyan's colleague, Tatjana Borisovna Fursa (12 May 1928-), now retired from the Vavilov Institute. It was she who first clarified the close relationships among sweet watermelon, subsp. or var. *vulgaris* (Schrad.) Fursa, egusi melon, *C. mucosospermus* (Fursa) Fursa, and Ter-Avanesyan's Kordofan melon, in an immunochemical analysis of seed proteins from all then-known species of *Citrullus* (Fursa and Gavrilyuk, 1990). This revealed that *cordophanus* is closer to the dessert watermelon than are *mucosospermus* and the South African watermelons *C. amarus* and *C. ecirrhosus*, a finding since borne out by molecular data (Chomicki and Renner, 2015; Guo et al., 2019; Wu et al., 2019). The egusi melons, which Fursa (1983) described as new to science, had been discovered by a Russian collector, N.P. Oltarshevskyi (Oltarshevskyi) in Ghana in 1957 (type material is in the herbarium of the Vavilov institute). Egusi melons are of great interest for their seeds, which lack a hard seed coat and can be eaten raw; the fruit pulp, on the other hand, is too bitter for human consumption (Achigan-Dako et al., 2015). Fursa (1972: 38) also validated Ter-Avanesyan's name 'cordophanus' by preparing and then citing a type specimen (Fursa, 1972: 38). She grew the specimen near Tashkent from some of his Sudanese seeds and formally named the material *Citrullus lanatus* subsp. *vulgaris* var. *cordophanus* (Ter-Avan.) Fursa.

Unfortunately, Fursa's insights and her protein-based phylogeny (Fursa and Gavrilyiuk, 1990), from which she inferred that "*Citrullus cordophanus* seems to be the nearest ancestor of the cultivated water-melon," were not widely read by Western or Chinese breeders. To remedy this, a recent paper on the taxonomy of *Citrullus* was dedicated to her and includes a color photo provided by her daughter that shows Tatjana in Astrakhan in 1977, holding one of watermelons (Renner et al. 2017).

Literature Cited

1. Achigan-Dako EG, Avohou ES, Linsoussi C, Ahanchede A, Vodouhe RS, Blattner FR. 2015. Phenetic characterization of egusi-type (*C. mucosospermus*). Genet Resour Crop Evol 62: 1159–79.
2. Chomicki, G., and S. S. Renner. 2015. Watermelon origin solved with molecular phylogenetics including Linnaean material: Another example of museomics. New Phytologist 205(2): 526-532.
3. Fursa TB. 1983. Novyi vid arbuza *Citrullus mucosospermus* (Fursa) Fursa. (A new species of watermelon *Citrullus mucosospermus* (Fursa) Fursa.) *Trudy po prikladnoi botanike, genetike i selektsii* 81: 108–112.

4. Fursa TB. 1972. K sistematike roda *Citrullus* Schrad. (On the taxonomy of genus *Citrullus* Schrad.). Bot. Zhurn. 57: 31–41.
5. Fursa TB, Gavrilyuk IP. 1990. Phylogenetic relations of the genus *Citrullus* Schrad. based on the immunochemical analysis of seed proteins (in Russian with English summary). Sbornik Nauchnykh Trudov po Prikladnoi Botanike, Genetike y Selektsii 133: 19–26.
6. Guo, S., S. Zhao, H. Sun, X. Wang, S. Wu, T. Lin, Y. Ren, and 21 others. 2019. Resequencing of 414 cultivated and wild watermelon accessions identifies selection for fruit quality traits. Nature Genetics 51: 1616–1623.
7. Renner, S. S., A. Sousa, and G. Chomicki. 2017. Chromosome numbers, Sudanese wild forms, and classification of the watermelon genus *Citrullus*, with 50 names allocated to seven biological species. Taxon 66(6): 1393-1405.
8. Ter-Avanesyan DV. 1966. Arbuz Kordofanskyi *Citrullus lanatus* Mansf. ssp. *cordophanus* Ter-Avan. Bot. Zhurnal 51: 423–426.
9. Wu, S., X. Wang, U. Reddy, H. Sun, K. Bao, L. Gao, L. Mao and 26 others. 2019. Genome of 'Charleston Gray', the principal American watermelon cultivar, and genetic characterization of 1,365 accessions in the U.S. National Plant Germplasm System watermelon collection. Plant Biotechnology Journal 17: 2246–2258.

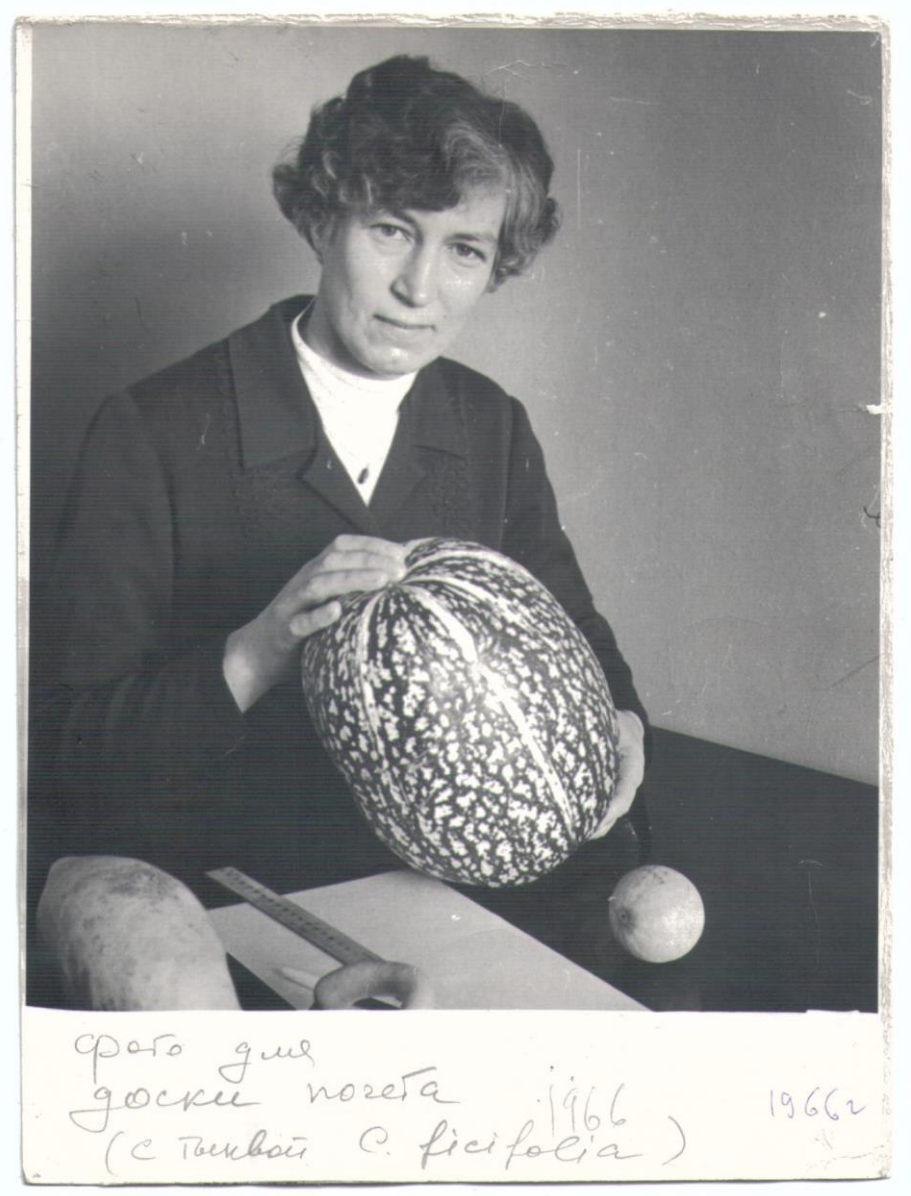

Figure 1. Photograph taken in 1966 of Tatjana Borisovna Fursa holding a fruit of *Cucurbita ficifolia.*

Figure 2. Photograph taken in 1977 in Astrakhan, Russia, of Tatjana Borisovna Fursa holding a fruit of *Citrullus* sp.

Optimum Experiment Size for Screening Watermelon Cultigens for Fruit Resistance to Bacterial Fruit Blotch

James Daley* and Todd C. Wehner
Department of Horticultural Science, North Carolina State University, Raleigh NC 27695-7609
**jamd6133@outlook.com*

Introduction

Bacterial fruit blotch (BFB) is a serious seed-borne disease caused by *Acidovorax citrulli* (13) that leads to significant watermelon (*Citrullus lanatus*) production losses (6, 8, 12). Although there are no watermelon cultivars with resistance to BFB (1), numerous studies have identified resistance sources (1, 2, 3, 4, 5, 6, 7, 10, 14). In one such study, watermelon cultigens (plant introductions and cultivars) were screened for fruit resistance to BFB under field conditions (3). Unfortunately, the most resistant cultigens were plant introductions with undesirable horticultural traits (3).

Although fruit resistance screening may be simplified as we better understand the underlying mechanisms, introgression of resistance into elite cultivars and confirmation of resistance in other sources will likely require using the original screening method, which is labor intensive and significantly affected by environmental variation (3). However, in addition to method descriptions and observations, prior empirical variance component estimates provide an opportunity to optimize future resistance screening under similar conditions by adjusting the resource allocations to further mitigate extraneous variance (3).

In this article, we calculated simulated estimates of the variance of a cultigen mean ($\sigma^2_{Cul\ M\ sim}$) (15) and broad-sense heritability ($H^2_{B\ sim}$) using simulated allocation scenarios and experimentally derived variance component estimates to predict the efficiency of hypothetical fruit resistance screenings and provide a framework for optimizing future experiments.

Methods

In order to demonstrate optimization of future experiments, average variance component estimates (3) and scenario-dependent values for years, blocks per year (blocks), and replications per block (replications) were used to calculate $H^2_{B\ sim}$ and $\sigma^2_{Cul\ M\ sim}$ (Table 1) estimates over 10 resource allocation scenarios.

Results and Discussion

In the optimization scenarios, the greatest gains were achieved by increasing allocations in descending order: years > blocks > replications, and scenarios that maximized blocks over years had the fastest results over time (Table 2). The optimum scenario would minimize $\sigma^2_{Cul\ M\ sim}$, increasing the power to discriminate genotypes (15), and maximize $H^2_{B\ sim}$, improving genetic gain (utilizing narrow-sense heritability) (9), over the shortest time. Ultimately, the preferred metric depends on the research goals. Scenario 2 had the maximum number of years and the best $H^2_{B\ sim}$, 0.46, and $\sigma^2_{Cul\ M\ sim}$0.54, and scenario 4, which maximized replications, had the worst $H^2_{B\ sim}$, 0.25, and $\sigma^2_{Cul\ M\ sim}$1.39. Scenario 3 maximized blocks and had the most favorable $H^2_{B\ sim}$, 0.40, and $\sigma^2_{Cul\ M\ sim}$, 0.69, in the shortest time. The respective range of $H^2_{B\ Sim}$ and $\sigma^2_{Cul\ M\ sim}$ among the more balanced scenarios, 5-10, was 0.31 to 0.45 and 0.99 to 0.56. The $H^2_{B\ sim}$ and $\sigma^2_{Cul\ M\ sim}$ of the completely balanced scenario, 1, was 0.39 and 0.70, respectively. Simulated broad-sense heritability and $\sigma^2_{Cul\ M\ sim}$were strongly negatively correlated, Pearson's correlation coefficient, $r(8) = -.97$, $p < 0.001$ (11).

As variance components are further partitioned by adding more replications, blocks, and years, gains are diminishing, i.e., more expensive. In reality, adding years would likely be more expensive than adding blocks, which would be more expensive than adding replications. However, probably the heaviest toll would result from opportunity costs from long experiments that delay breeding decisions and research results (15). For example, the impractically long scenario 2 had the best metrics but would be the most time-expensive, incurring unclear costs beyond what it took to conduct the experiment. A more in-depth demonstration of cost analysis and allocation compromises to balance gains for multiple traits is given in a similar optimization by Swallow and Wehner (15). Customized to resources and experimental objectives, expanded optimization scenarios could be used to evaluate the effects of various allocations beyond the 10 scenarios provided here.

When evaluating idealized scenarios, researchers need to consider the severity of missing data on their outcomes. The simulation calculations for 3 years, 3 experiments, and 1 replication (not shown) that matched the resource allocation for the actual screening experiments (3), projected 0.47 $H^2_{B\,sim}$ and 0.52 $\sigma^2_{Cul\ M\ sim}$. By using 2 years, 4 blocks, and 1 replication or 1 year, 8 blocks, and 1 replication similar metrics may have been achieved and time and resources saved. However, these simulated results are better than the realized average of 0.343 $H^2_{B\,sim}$ and 0.868 $\sigma^2_{Cul\ M\ sim}$ for the actual dataset that included the screening experiments and additional testing (3). This discrepancy was because of unexpected missing data that led to average harmonic means of 2.6 years, 4.8 blocks, and 5.2 replications (3), whereas, with no missing data, each cultigen would have been replicated at least 9 times over 9 blocks and 3 years just for the screening experiments. Considering the equations for $H^2_{B\,sim}$ and $\sigma^2_{Cul\ M\ sim}$, missing data adversely affects the metrics by decreasing the denominators for the cultigen interaction variances. In order to mitigate projected attrition and achieve their objectives while remaining within budget for time and resources, researchers must either conduct their experiments more efficiently or over-allocate resources, and increase costs, to compensate for missing data.

Post hoc optimization scenarios can be used to guide future experiments. Of course, by using prior estimates to predict future experimental outcomes, as presented here, we are making the assumptions that our conditions are typical, future variance component estimates will be the same, and the variance components can be infinitely partitioned. Indeed, the value of using prior data to predict outcomes will only be known following actual testing and post hoc analysis. Naturally, this additional data can then be used to further refine future experiments. While inherently flawed, using empirical variance component estimates to shape future experimental design outcomes is preferable to designing experiments based merely on resources and observations.

We simulated alternative resource allocation scenarios and calculated $H^2_{B\,sim}$ and $\sigma^2_{Cul\ M\ sim}$ in order to identify optimized testing conditions for screening for resistance to BFB in watermelon fruit and to illustrate a simple exercise to optimize useful metrics based on prior data. Scenarios 1, 3, 7, and 9 had favorable $H^2_{B\,sim}$ and $\sigma^2_{Cul\ M\ sim}$ that could be attained by running screening experiments for 2 years or less. These scenarios provide a guide for researchers and breeders to design more efficient experiments and trials based on available resources and variance component estimates.

Literature Cited

1. Branham, S. E., A. Levi, M.L. Katawczik, and W.P. Wechter. 2019. QTL mapping of resistance to bacterial fruit blotch in *Citrullus amarus*. *Theor Appl Genet*, 132, 1463-1471. https://doi.org/10.1007/s00122-019-03292-6.
2. Carvalho, F. C., L.A. Santos, R.C. Dias, R.L. Mariano, and B.S. Elineide. 2013. Selection of watermelon genotypes for resistance to bacterial fruit blotch. *Euphytica*, 190, 169-180. https://doi.org/10.1007/s10681-012-0766-1.
3. Daley, J. and T.C. Wehner. (In press). Screening for Resistance to Bacterial Fruit Blotch in Watermelon Fruit. *Crop Science.*
4. Goth, R.W. and R.E. Webb. 1981. Resistance of commercial watermelon (*Citrullus lanatus*) to *Pseudomonas pseudoalcaligenes* subsp. *citrulli*. *Plant Disease*, 65, 671-672.
5. Hopkins, D.L. and A. Levi. 2008. Progress in the Development of Crimson Sweet-type Watermelon Breeding Lines with Resistance to *Acidovorax avenae* Subsp. c*itrulli*. In M. Pitrat (Ed.), *Proceedings of the IXth Eucarpia Meeting on Genetics and Breeding of Cucurbitaceae*, 157-162. Avignon, France: INRA.
6. Hopkins, D.L. and C.M. Thompson. 2002. Evaluation of *Citrullus* sp. Germ Plasm for Resistance to *Acidovorax avenae* subsp. *citrulli*. *Plant Disease*, 86, 61-64. https://doi.org/10.1094/PDIS.2002.86.1.61.
7. Hopkins, D.L., C.M. Thompson, and G.W. Elmstrom. 1993. Resistance of watermelon seedlings and fruit to the fruit blotch bacterium. *HortScience*, 28, 122-123. https://doi.org/10.21273/HORTSCI.28.2.122.
8. Latin, R.X. and D.L. Hopkins, D. L. 1995. Bacterial fruit blotch of watermelon - the hypothetical exam question becomes reality. *Plant Disease*, 79, 761-765.
9. Lush, J. L. 1945. *Animal breeding plans*. Iowa State University Press, Ames.
10. Ma, S. and T.C. Wehner. 2015. Flowering stage resistance to bacterial fruit blotch in the watermelon germplasm collection. *Crop Science*, 55, 727-736. https://doi.org/10.2135/cropsci2014.01.0071.
11. R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
12. Schaad, N.W., E. Postnikova, and P. Randhawa. 2003. Emergence of *Acidovorax avenae* subsp. *citrulli* as a crop threatening disease of watermelon and melon. In: N.S. Iacobellis, et al. (Eds.), *Pseudomonas syringae* and related pathogens, 573-581. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-0133-4_63.
13. Schaad, N. W., E. Postnikova, A. Sechler, L.E. Claflin, A.K. Vidaver, J.B. Jones, I. Agarkova, A. Ignatov, E. Dickstein, and B.A. Ramundo. 2008. Reclassification of subspecies of *Acidovorax avenae* as *A. avenae* (Manns 1905) emend., *A. cattleyae* (Pavarino, 1911) comb. nov., *A. citrulli* Schaad et

al., 1978) comb. nov., and proposal of *A. oryzae* sp nov. *Systematic and Applied Microbiology*, 31, 434-446. https://doi.org/10.1016/j.syapm.2008.09.003.

14. Sowell, G. and N.W. Schaad. 1979. *Pseudomonas pseudoalcaligenes* subsp. *citrulli* on watermelon: seed transmission and resistance of plant introductions. *Plant Disease Reporter*, 63, 437-441.
15. Swallow, W.H. and T.C. Wehner. 1989. Optimum allocation of plots to years, seasons, locations, and replications, and its application to once-over-harvest cucumber trials. *Euphytica*, *43*, 59-68. https://doi.org/10.1007/BF00037897.

Table 1. Equations for simulated broad-sense heritability ($H^2_{B\ Sim}$) and variance of a cultigen mean ($\sigma^2_{Cul\ M\ sim}$).

Equation 1: $$H^2_{B\ sim} = \frac{\sigma^2_{Cultigen}}{\sigma^2_{Cultigen} + \frac{\sigma^2_{Cultigen \times Year}}{\text{Years}} + \frac{\sigma^2_{Block(Year) \times Cultigen}}{\text{Years X Blocks}} + \frac{\sigma^2_{Error}}{\text{Years} \times \text{Blocks} \times \text{Replications}}}$$

Equation 2: $$\sigma^2_{Cul\ M\ sim} = \frac{\sigma^2_{Cultigen \times Year}}{\text{Years}} + \frac{\sigma^2_{Block(Year) \times Cultigen}}{\text{Years X Blocks}} + \frac{\sigma^2_{Error}}{\text{Years} \times \text{Blocks} \times \text{Replications}}$$

Note. Average variance component estimates (3):
$\sigma^2_{Cultigen} = 0.454$; $\sigma^2_{Cultigen \times Year} = 0.172$; $\sigma^2_{Block(Year) \times cultigen} = 0.804$; $\sigma^2_{Error} = 3.320$. Blocks refer to blocks per year; replications refer to replications per block.

Table 2. Simulated broad-sense heritability ($H^2_{B\ Sim}$) and variance of a cultigen mean ($\sigma^2_{Cul\ M\ sim}$) optimization scenarios using different allocations of eight plots over years, blocks per year (blocks), and replications per block (replications).

	Allocations			Estimates	
Scenario	Years	Blocks	Replications	$H^2_{B\ Sim}$[z]	$\sigma^2_{Cul\ M\ sim}$[y]
1	2	2	2	0.39	0.70
2	8	1	1	0.46	0.54
3	1	8	1	0.40	0.69
4	1	1	8	0.25	1.39
5	4	2	1	0.45	0.56
6	4	1	2	0.41	0.66
7	2	4	1	0.43	0.60
8	2	1	4	0.33	0.90
9	1	4	2	0.37	0.79
10	1	2	4	0.31	0.99
$H^2_{B\ Sim}$ and $\sigma^2_{Cul\ M\ sim}$ Pearson's correlation coefficient: -.97 ($p < .001$)					

[z]Table 1, equation 1.
[y]Table 1, equation 2.

Studies on Sporophytic Development of Cucumber Microspores

Lyle T. Wallace and Michael J. Havey*
Agricultural Research Service, U.S. Dept. of Agriculture, Vegetable Crops Unit, Department of Horticulture, University of Wisconsin, Madison, Wisconsin 53706
**michael.havey@usda.gov*

Abstract

Direct embryogenesis from cucumber microspores offers the possibilities of highly efficient inbred production and a unique model system for mitochondrial genetics; however, a reliable method of microspore embryogenesis is lacking. We evaluated previously published and new experimental approaches for microspore embryogenesis of cucumber. Plants must be grown in highly controlled environmental conditions within a narrow temperature range to synchronize microspore development. Buds between 5 and 7 mm had a majority of microspores in the late uninucleate stage of development. Isolated buds were treated by cold and heat prior to microspore isolation, and isolated microspores were cultured in the presence of a histone deacetylase inhibitor. Cultured microspores showed changes in the sizes and shapes, potentially an indication of sporophytic development, but did not develop further. A maltose-based culture medium was found to extend the vitality of cucumber microspores in culture. These results and observations should be of interest to researchers trying to develop microspore embryogenesis in cucumber.

Introduction

In vitro culturing and embryogenesis of cucumber microspores offer the possibility to develop large numbers of doubled haploids (DH) for genetic studies and hybrid production. Presently cucumber haploids are produced by culturing female flowers, which is labor intensive with relatively low efficiency at 7 to 11 embryos per 100 ovaries plated (Gałązka et al., 2013). Male flower buds of cucumber produce thousands of microspores and even at a relatively low rate of multicellular development reported at 0.54% to 16.5% could generate hundreds to thousands of embryos (Chen et al. 2018). In comparison, the use of irradiated pollen with embryo rescue typically resulted in 1 to 16 embryos per fruit (Gałązka et al., 2013). Anther culture yielded 1.9 to 2.6 embryos per anther (Gałązka et al., 2013), and even though anthers are more numerous and easier to harvest than ovaries or fruits, this approach yielded fewer embryos per anther than could potentially be achieved with microspores. Moreover, reports of successfully development of cucumber haploids from anther culture may have been insufficiently scrutinized for contaminating sporophytic tissue that may have inflated the success rate (Asadi et al., 2018).

Microspore embryogenesis would also have great value as a tool for transformation of the mitochondrial genome (Havey et al., 2002). Transformation of the organellar genomes would be useful for basic and applied research, for example allowing for the rapid development of novel cytoplasmic male sterile lines for breeding and hybrid seed production, protein production, and stress tolerance (Havey et al., 2002; Laluk et al., 2011). Cucumber microspores offer unique advantages for mitochondrial transformation such as relatively few, large mitochondria that provide larger targets for ballistic particles and paternal transmission of mitochondria to progenies (Havey et al., 2002).

In vitro culture of cucumber microspores has been reported by three research groups. Suprunova and Shmykova (2008) harvested flower buds longer than 6 mm with microspores in the late uninucleate stage and gently macerated the buds in NLN medium supplemented with 10% sucrose. Microspores were filtered through a nylon screen and washed three times with NLN medium followed by centrifugation at 100 xg for 3 minutes. Microspores were cultured in the dark at 22° C for two to four weeks in NLN medium supplemented with 10% sucrose and 2.0 ppm 2,4-dichlorophenoxyacetic acid (2,4-D). Only callus tissue was observed with no further development. Zhan et al. (2009) isolated microspores at the late uninucleate stage from buds that had been pretreated for 2 to 4 days at 4° C. Buds were macerated in B5 medium supplemented with 13% sucrose, the liquid was filtered through a nylon screen, and washed three times with B5 medium with centrifugations at 600 rpm for 3 minutes. The microspores were resuspended at a density of 1 x 10^5 cells/ml in a 2 ml volume of NLN medium with 0.5 ppm 2,4-D and 0.2 ppm 6-benzyladenine (BA) and plated on a 60 mm petri dish. After two days of culture in the dark, some cells were observed to darken and enlarge. After two more

days of culture, cell division was observed and after more than 20 days in culture, plantlets were obtained. Heat treatment of isolated microspores at 33° C for two days did not increase embryogenesis. Finally, Chen et al. (2018) described numerous approaches for microspore embryogenesis in a patent application with apparently high degrees of success. Buds were harvested between 3 and 6 mm at the late uninucleate stage of development. Whole plants were treated at 4° C to 16° C in a growth chamber for one day before harvesting buds, or harvested buds were treated at 0° C to 8° C for one to four days before isolating microspores. Buds were macerated in M404 medium supplemented with 90 g/L of maltose. The liquid was filtered through a 100 micron mesh and then through a 65 to 80 micron mesh. Microspores were washed and spun again for three repetitions and then resuspended at a density of 30,000 to 150,000 cells/ml in a total volume of 4 to 6 ml and cultured in a 60 x 50 mm dish. This final culture medium was M404 supplemented with 3% to 17% w/v of either sucrose or maltose, 0.05 ppm to 2.0 ppm of BA, 0.05 ppm to 2.0 ppm of 2,4-D, 0.5 ppm to 50.0 ppm of phenylacetic acid and 0.5 µM to 40 µM of suberoylanilide hydroxamic acid (SAHA) or 0.001 µM to 1.0 µM of trichostatin A (TSA), although Tables 6 and 9 of the patent strongly suggested that the primary medium used contained 0.5 ppm 2,4-D, 0.2 ppm BA, and 10 µM SAHA. Cultures were subjected to a heat shock of 28° to 35° for 24 to 72 hours and then cultured in the dark at 25° C. Putrescine was added at day seven to promote embryo development. Globular embryos were observed at 20 to 40 days and plantlets were produced shortly thereafter. Numerous accessions were tested and all produced embryos at rates varying from 0.54% to 17.3% of microspores.

Although these studies appear promising for microspore embryogenesis of cucumber, there are no reports of experimental replication of the approaches. The initial report by Suprunova and Shmykova (2008) was found in a conference poster and never published, to our knowledge, in a peer-reviewed journal. The research published by Zhan et al. (2009) has not been repeated by other researchers. Finally, the patent application on cucumber microspore embryogenesis by Chen et al. (2018) has ambiguous language regarding specific details of their protocol. To better understand progress towards embryogenesis of cucumber microspores, new experiments are needed to replicate previous research and to further refine techniques to maximize successful application of this important technology.

Materials and Methods

Cucumber seeds were germinated in vermiculite on a hot pad at 27° C, seedlings were transplanted to a four-inch pot, and then transplanted again when true leaves were present into #1200 11-liter pots in Sungro medium. Temperatures were between 21° C and 24° C during the day and 16° C to 17° C at night with a 16-hour days with high intensity lighting. A closed heating and cooling system maintained environmental conditions. Temperatures were continuously recorded at bench level with a sun shaded probe using an Elitech Temperature and Humidity Data Logger. Several measurements at different times of day were taken with an Apogee Quantum Flux light meter (Model MQ-200) that measured light levels between 200 and 900 µmol/m^2/s. Plants were fertilized every other day at a concentration of 400 ppm of elemental nitrogen (Peters Professional 20-10-20) containing the following micronutrients: 0.15% water soluble magnesium, 0.025% boron, 0.025% chelated copper, 0.1% chelated iron, 0.05% chelated manganese, 0.01% molybdenum, and 0.05% chelated zinc. Deionized water was used for mixing the fertilizer and for watering. Plants were five to eight weeks old when buds were harvested for microspores. Buds from four-week-old plants were isolated on several occasions but microspore production was unacceptably low. No powdery mildew growth was observed at any time on plants.

Three cucumber accessions were used for microspore isolation. USDA Plant Introduction (PI) 518848 was selected because it showed the highest embryogenesis in the patent of Chen et al. (2018). 'Poinsett 76' was also selected because it is closely related to the 'Pointsett 97' reported to be highly embryogenic by Zhan et al. (2009) and moderately embryogenic by Chen et al. (2018). Finally, inbred B was selected from the Polish cultivar 'Borszczagowski' and is highly amenable for plant regeneration from cell cultures (Burza and Malepszy, 1995). Male flower buds were harvested, carefully sized using a digital caliper, and placed on a moist towel until counting and selection was completed (Fig. 1). Cold treatments of harvested buds were in a sealed tube or petri dish with a moistened towel added for humidity. Buds were surface sterilized by treating with 70% ethanol for 30 seconds followed by two washes in sterile water. Buds were then treated with freshly made 1% sodium hypochlorite with 0.01% Tween20 for 10 minutes followed by four washes in sterile water. There was then one last wash in a wash and grinding buffer consisting of Murashige & Skoog modified basal medium with Gamborg vitamins (M404 medium, Phytotechnology Laboratories, Shawnee Mission, KS) supplemented with 10% w/v D-maltose at pH of 5.8. Surface sterilization and washes were conducted in 50 ml conical tubes within a transfer cabinet. Buds were then ground in 0.5 ml of the wash & grinding buffer in a 30 ml sterile glass beaker with a sterile glass stirring rod. Buds were gently but thoroughly macerated to release microspores, and

examination under a microscope showed that the vast majority of microspores were intact (Fig.2).

Liquid from tubes was removed using a pipette tip leaving behind fiber and tissue, and then filtered through a sterile 100 µm cell strainer. The liquid was centrifuged 100 x g for 3 minutes in multiple 1.5 ml tubes. After this first centrifugation, tubes were gently tapped to help collect together microspres that were spread along the side of the tube, and then the liquid was centrifuged again at 100 x g for 3 minutes. The supernatant and any green debris were removed with a pipette and the white pellet was resuspended in 0.5 ml of wash and grinding buffer. Two more washes were conducted to remove as much of any green material as possible leaving a white pellet and a clear supernatant. After the three washes, the pellet was resuspended in M404 medium containing 13% w/v D-maltose, 0.5 ppm 2,4-D, 0.2 ppm BA, and 10 µM SAHA at pH of 5.8. Microspore density was adjusted to 30,000 to 150,000 per ml. Two to five mL of resuspended cells were placed in sterile glass jars (approximately 5 cm in diameter and 6.5 cm in height with a volume of ~130 mL) and sealed with parafilm to prevent evaporation. Jars were placed in the dark at 25° C.

SAHA (commercial name 'Vorinostat' (Selleckchem, Houston, TX)) is potentially useful for reprogramming of microspores from gametophytic to sporophytic development, and was prepared at a concentration of 10 mM in DMSO. Because of the relatively short half-life of SAHA in water, it was added to the medium less than a half hour before the cells were added. To avoid multiple freeze-thaw cycles, the SAHA was aliquoted into small tubes, stored at -20° C, and discarded after each thawing. All other medium components were purchased from PhytoTechnology Laboratories (Shawnee Mission, KS).

Numerous combinations of medium components and concentrations were tested. Filter sterilized maltose and sucrose were tested at 10% and 13% (w/v) in the culture (Suprunova and Shmykova, 2008; Zhan et al., 2009; Scott et al. 1995). Sucrose was also tested as an autoclaved carbon source, which can break down sucrose to produce different composition of sugars. A continuous 25 rpm agitation of isolated microspores in the culture medium was tested based on the reports of improvements in microspore culture with agitation by Yang et al. (2013). Three different culture vessels were tested – 35 mm by 10 mm plastic Falcon Easy Grip petri dish, 100 mm by 15 mm plastic Fisher petri dish, and 50 mm by 65 mm glass jars, based on research by Park et al. (2009) that showed improved embryogenesis of pepper microspores with the utilization of a larger culture vessel, and research by Hoekstra et al. (1992) that showed improvements to microspore culture from exogenous oxygen addition to the culture vessel suggesting that aeration may be important for microspore culture. Different bud treatments were attempted, including 4° C for one to three days and 8° C for one to three days based on a range of temperatures suggested by Chen et al. (2018). Heat treatments of isolated microspores at 30° C for one to three days and 31° C, 32° C, and 33° C were evaluated based on Chen et al. (2018).

Microspores were stained with 1% acetocarmine or 0.01% methylene blue to reveal vacuole development and position of the nucleus. Acetocarmine stains were observed immediately with no washes. Methylene blue stains were observed after incubation for 10 minutes and three washes with M404 medium containing 10% w/v maltose to reduce background staining. Vitality of microspores was estimated using tetrazolium dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), fluorescein diacetate, propidium iodide, DAPI, and a custom stain from Peterson et al. (2010) developed as an alternative to Alexander's stain. The most effective vitality stain was 0.8% tetrazolium dye MTT, which is converted by native enzymes in the cell into dark purple formazan, thus indicating vitality. The MTT stain was diluted to 0.8% in a 5% sucrose solution. All vitality experiments were conducted with MTT. Fluorescein diacetate, propidium iodide, DAPI, and the custom stain were also tested, but not utilized extensively.

Microbial contamination of microspores was assayed by streaking culture liquid onto LB plates containing 1.5% agar and 3% glucose at pH 6.8. Strain LE392 (Promega, Fitchburg WI) of *E. coli* and common baker's yeast were used as positive controls for microbial growth on plates.

Results

Environmental conditions were essential for synchronized production of viable microspores, and strict maintenance of temperatures between 21° C and 24° C during the day and 16° C to 17° C at night with a 16-hour day with high intensity lighting was necessary. Buds from 3 to 6 mm in length were initially selected based on the optimal size for microspores in the late uninucleate stage reported by Chen et al. (2018), although this contradicted Suprunova and Shmykova (2008) who chose buds larger than 6 mm for maximizing late uninucleate stage microspores. Buds in the 3 to 4 mm range had large numbers of tetrads and were discarded. The optimal bud size was 5 to 7 mm which contained mostly vacuolated microspores roughly 50 µm in diameter, and was used for all further experiments.

Cold treatments of isolated buds or entire plants at temperatures ranging from 0° C to 8° C for four days were tested and no cell division or embryogenesis of isolated microspores was observed. Heat shock of the isolated microspores at 28° to 35° for 24 to 72 hours produced no evidence of cell division or embryos.

After one to three days in the dark at 25° C in the M404 medium containing 10% w/v maltose, 0.5 ppm 2,4-D, and 0.2 ppm BA, up to 20% of microspores were observed to enlarge from approximately 40 to 50 microns in diameter, darken in color, and develop a more rounded shape (Fig. 3). These morphological changes and positive MTT staining indicated that the microspores were likely viable after isolation. Plating of the culture medium containing microspores onto LB plates revealed no contaminating microbes.

Despite these cellular changes, no evidence of cell division was observed. Modifications to the protocol included 10% sucrose, 13% sucrose, 10% autoclaved sucrose, 25 rpm agitation, and larger vessel size with a greater trapped air volume, all of which did not result in cell division or embryogenesis. Pretreatments of harvested bud included 4° C or 8° C for one, two, or three days before isolating microspores, and did not induce cell division or embryogenesis. Similarly, heat treatments of the freshly isolated microspores varied from 30° C to 33° C and lasted one to three days, but also resulted in no cell division or embryogenesis.

To determine if the carbon source has an effect on vitality, even if it did not induce cell division, microspores were isolated from 7-week old plants without heat shock and then were divided into either a 10% w/v maltose culture medium or a 10% w/v sucrose culture medium. Microspores were stained with 0.8% MTT on days 0, 1, 2, and 3 after isolation and observed at 40x magnification. MTT staining was scored for several hundred microspores from each time point. In the maltose medium, microspore vitality fell from 15% on day 0 to 7.3% on day 1, 2% on day 2, and less than 1% on day 3. In the sucrose medium, microspore vitality fell from 13% on day 0 to 4.6% on day 1 and no living cells were detectable by MTT staining on day 2.

Discussion

The morphological changes observed for some microspores appear to be consistent with a switch from gametophytic to sporophytic development. Previous researchers have observed that sporophytic development of cucumber microspores appears to show a darkening of the cytoplasm and enlargement of the microspores with a more rounded shape (Suprunova and Shmykova, 2008). Other research has shown that microspores will expand and darken after two day in culture and the vacuoles will disappear in embryogenic microspores (Zhan et al., 2009). Microspores from *Brassica napus* have been carefully observed during sporophytic development and show enlarged size and cytoplasmic changes (Telmer et al., 1993). Maturation of microspores to pollen will also result in enlargement, although no divisions into vegetative and generative cells was observed and at no point did an opaque exine form (Dupuis and Pace, 1993; Telmer et al., 1993). For numerous plants, microspore embryogenesis requires stress treatments or depleted cultures, and non-stressful, enriched conditions lead to pollen development (Dupuis and Pace, 1993; Touraev et al., 1996).

The original experiments on cucumber microspore embryogenesis utilized sucrose as a carbon source (Suprunova and Shmykova, 2008; Zhan et al., 2009). Chen et al. (2018) did not clearly state the carbon source utilized but suggested a broad range of concentrations of either sucrose or maltose. The evidence presented here indicates that maltose is superior to sucrose for maintaining the vitality of cells and presumably for inducing embryogenesis. The rapid death of microspores in a medium with sucrose may be due to hypoxic conditions and ethanol production, both of which are lethal to microspores. Maltose is metabolized more slowly and may avoid hypoxic conditions and resulting in less ethanol production with a more stable osmotic balance (Scott et al., 1995).

In comparing previous experiments on cucumber microspore embryogenesis to these experiments, all the main parameters were tested but did not yield the expected result. Previous experiments had apparent success with 10% sucrose, 13% sucrose, and a range of values from 3% to 17% of sucrose or maltose (Suprunova and Shmykova, 2008; Zhan et al., 2009; Chen et al., 2018). Carbon sources of 10% sucrose, 13% sucrose, and 10% maltose were tested and yielded no embryos. Previous experiments had apparent success with either 2.0 ppm 2,4-D or 0.5 ppm 2,4-D and 0.2 ppm BA (Suprunova and Shmykova, 2008; Zhan et al., 2009; Chen et al., 2018). Plant growth regulators at 0.5 ppm 2,4-D and 0.2 ppm BA were tested and yielded no cell division or embryos. Previous experiments had utilized cold treatments of one to four days of either the buds or the entire plant at temperatures ranging from 0° C to 12° C, although most experiments were conducted at 4° C (Zhan et al., 2009; Chen et al., 2018). Cold treatments of both the buds and whole plants were tested at 4° C and 8° C with no cell division or embryogenesis. Heat shock treatments of 28° to 35° for 24 to 72 hours of the isolated microspores were reported by previous researchers (Zhan et al., 2009; Chen et al., 2018). Heat shock treatments of 28° to 35° for 24 to 72 hours were tested and yielded no cell division or embryos. One previous researcher utilized 10 µM SAHA, a histone deacetylase inhibitor, as a key reagent for reprogramming of microspores to the sporophytic path (Chen et al., 2018). Ten µM SAHA was utilized and resulted in no cell division or embryogenesis. In conclusion, we were not able to repeat the results of previous researchers who reported successful embryogenesis of cucumber microspores, and hope that our observations will be useful for future researchers pursuing this area of research.

Literature Cited

1. Asadi, A., A. Zebarjadi, M.R. Abdollahi, J.M. Seguı´-Simarro. 2018. Assessment of different anther culture approaches to produce doubled haploids in cucumber (*Cucumis sativus* L.). Euphytica 214:216.
2. Burza, W., and S. Malepszy. 1995. Direct plant regeneration from leaf explants in cucumber (*Cucumis sativus* L.) is free of stable genetic variation. Plant Breeding 114: 341-345.
3. Chen, J., E. Vanek, M. Pieper. 2018. Method for producing haploid, dihaploid, and doubled haploid plants by isolated microspore culture. Vilmorin & Cie , Paris, France. Patent no US 2018 / 0213736 A1.
4. Dupuis, I., and G.M. Pace. 1993. Factors affecting in vitro maturation of isolated maize microspores. Plant Cell Reports 12:564-568.
5. Gałązka, J., and K. Niemirowicz-Szczytt. 2013. Review of research on haploid production in cucumber and other cucurbits. Folia Horticulturae 25(1): 67-78.
6. Havey, M.J. J.W. Lilly, B. Bohanec, G. Bartoszewski, and S. Malepszy. 2002. Cucumber: A Model angiosperm for mitochondrial transformation? J. Appl. Genet. 43(1): 1-17.
7. Hoekstra, S., M.H. van Zijderveld, J.D. Louwerse, F. Heidekamp, and F. van der Mark. 1992. Anther and microspore culture of *Hordeum vulgare* L. cv. Igri. Plant Science 86: 89-96.
8. Laluk, K., S. AbuQamar, and T. Mengiste. 2011. The Arabidopsis Mitochondria-Localized Pentatricopeptide Repeat Protein PGN Functions in Defense against Necrotrophic Fungi and Abiotic Stress Tolerance. Plant Physiology 156:2053–2068.
9. Park, E.-J., J.-A. Kim, and M. Kim. 2009. Influence of pretreatment medium, fresh medium addition, and culture plate size on the production of embryos in isolated microspore culture of hot pepper (*Capsicum annuum* L.). J. Plant Biotechnol. 36(2): 184-192.
10. Peterson, R., J.P. Slovin, and C. Chen. 2010. A simplified method for differential staining of aborted and non-aborted pollen grains. International Journal of Plant Biology 1:e13.
11. Scott, P., R.L. Lyne, T. ap Rees. 1995. Metabolism of maltose and sucrose by microspores isolated from barley (*Hordeum vulgare* L.). Planta 197:435-441.
12. Suprunova, T., and N. Shmykova. 2008. In vitro induction of haploid plants in unpollinated ovules, anther and microspore culture of Cucumis sativus. In: Cucurbitaceae 2008. M. Pitrat (ed.). Proc. IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae. 21-24 May, Avignon, France.
13. Telmer, C.A., W. Newcomb, and D.H. Simmonds. 1993. Microspore development in *Brassica napus* and the effect of high temperature on division in vivo and in vitro. Protoplasma 172:154-165.
14. Touraev, A., A. Iiham, O. Vicente, and E. Heberle-Bors. 1996. Stress-induced microspore embryogenesis in tobacco: an optimized system for molecular studies. Plant Cell Reports 15:561-565.
15. Yang, S., X. Liu, Y. Fu, X. Zhang, Y. Li, Z. Liu, and H. Feng. 2013. The effect of culture shaking on microspore embryogenesis and embryonic development in Pakchoi (*Brassica rapa* L. ssp. *chinensis*). Scientia Horticulturae 152: 70–73.
16. Zhan, Y., J. Chen, A. Abbas Malik. 2009. Embryoid induction and plant regeneration of cucumber (*Cucumis sativus* L.) through microspore culture. Acta Hort. Sinica 36(2): 221-226.

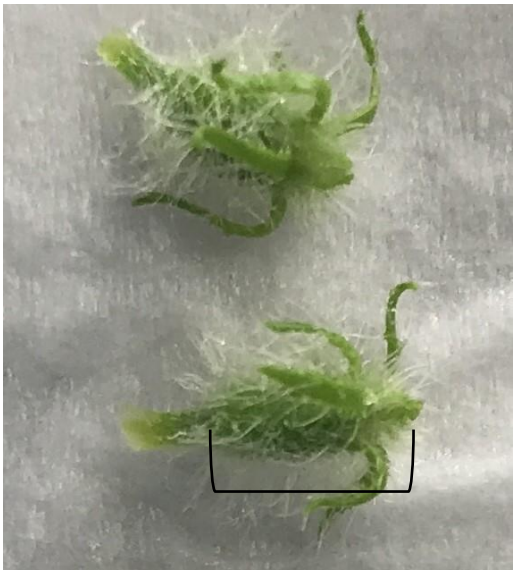

Figure 1. Buds with 5 mm size; bracket indicates where measurement was taken.

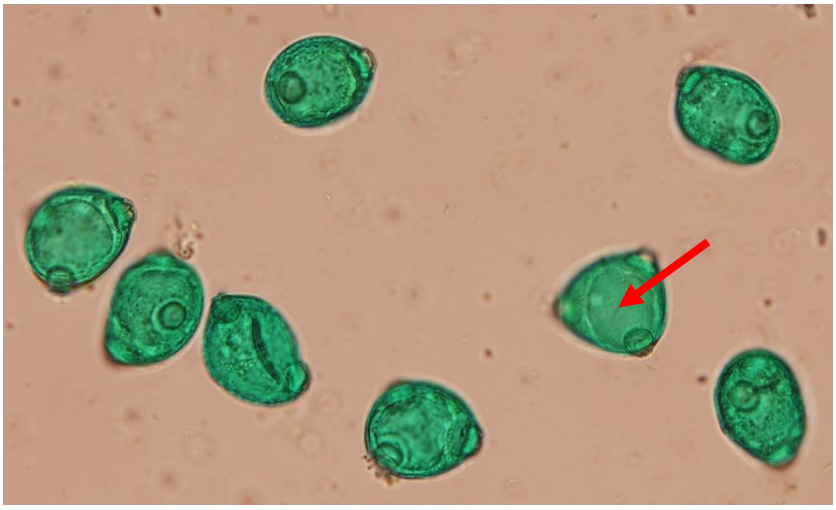

Figure 2. Harvested microspores stained with 1% methylene blue at 400x magnification. Arrow indicates a vacuole typically seen at the late uninucleate stage.

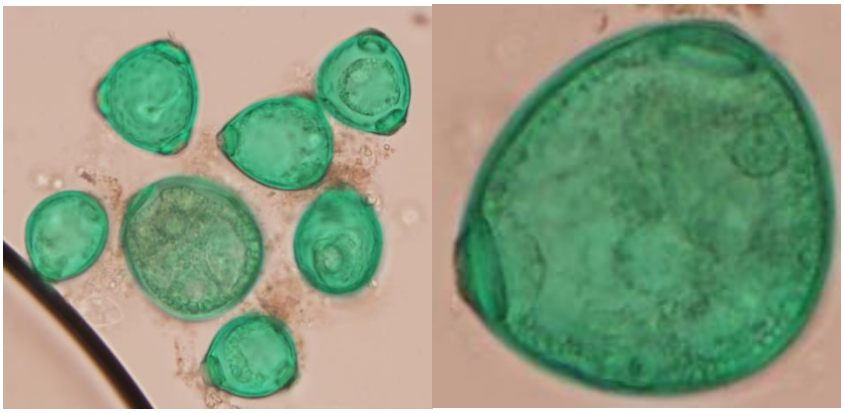

Figure 3. Methylene blue stained microspores 4 days post isolation at 400x magnification. Shown on the left are seven microspores with one darkened and enlarged microspore in the center of the image. Shown on the right is increased magnification of the darkened and enlarged microspore.

First Report of *Podosphaera xanthii* Melon Race N2 Causing Powdery Mildew on Cucumber in China

Xiaoni Li, Fanhui Kong, Li Gong, Yun Deng*
Institute of Advanced Agricultural Sciences, Peking University, Weifang, Shandong 261325, China
**yun.deng@pku-iaas.edu.cn*

Abstract

Powdery mildew is one of the major diseases affecting yield and quality of cucurbit crops. As part of our disease resistance breeding efforts, we collected powdery mildew from cucumber plants organically grown in a plastic greenhouse located in Xiashan, Weifang, Shandong province of China, N36.495136, E119.444028. Morphological characteristics of conidia were observed under the microscope to confirm species identity, and the race was determined using common melon powdery mildew race differentials. Based on the reactions of the melon powdery mildew differentials this isolate, XS2019, is *Podosphaera xanthii* race N2. In addition, reactions of nine other melon accessions were observed. When the isolate was inoculated on watermelon and squash, we observed typical powdery mildew symptoms in addition to the symptoms on melon differentials. Therefore, the N2 race is pathogenic to all major cucurbit crops grown in China.

Introduction

China is the leading producer and consumer of cucurbit crops in the world. Powdery mildew (PM) often causes significant yield and quality loss for cucurbit production in China, like the rest of the world. PM is probably the most widespread and easily recognizable disease of cucurbits. *Podosphaera xanthii* and *Golovinomyces cichoracearum* cause powdery mildew, and *P. xanthii* is the predominant pathogen (Davis et al., 2007; Tetteh et al., 2007; Zhang et al., 2011; Kim et al., 2013). This disease becomes more difficult to manage in plastic house production in late fall and winter season in China. It causes especially severe damage to watermelon grafted on interspecific squash rootstocks, which are becoming more popular with the advantages of combating soil borne pathogens, providing cold tolerance, and increasing yield (Tirupathamma et al., 2019). PM may affect rootstock and watermelon seedlings before and after grafting during the healing phase, when the relative humidity is almost 100% in plastic house or misting chamber. Fungicide application is an effective method to control powdery mildew disease, however, the development of resistant variety is the most suitable alternative considering the environment impact and emergence of fungicide-resistant strains.

Severe PM symptoms were observed on cucumber (*Cucumis sativus* L.) plants grown in an organic vegetable production farm in Xiashan, Weifang, Shandong province of China, N36.495136, E119.444028. The white sporulation covered the chlorotic angular lesions all over the entire leaf. Infected leaves were collected for pathogen identification and characterization of this PM isolate, XS2019.

Materials and Methods

Pathogenicity Test on Watermelon and Summer Squash: The spores of PM isolate XS2019 were inoculated on watermelon hybrid 'XiaWei105' and summer squash hybrid 'XiaWei8' seedlings grown in pots in a growth room at 23°C and 60% RH. Three non-inoculated seedlings were used as controls. PM symptoms were evaluated 10-days after inoculation.

Race Identification: Seedlings of 14 melon (*Cucumis melo* L.) accessions 'Iran H', 'Top Mark', 'Védrantais', 'PMR 45', 'PMR 5', WMR 29, 'Edisto 47', PI 414723, MR-1, PI 124111, PI 124112, 'PMR 6', 'Nantais Oblong', and AR 5 were used as differential hosts to identify the race of the isolate. PM spores were collected from 'XiaWei105' watermelon leaves 10-days after inoculation for inoculation of the 3rd true leaf of the melon differentials using a small brush. Powdery mildew was

evaluated 10-days after inoculation. Inoculated plants were grown in pots placed in a growth room with 23°C and 60% RH before and after inoculation.

In a second test, seedlings of the 14 melon differentials were transplanted into a plastic greenhouse near the organic vegetable production farm on 28 April 2020 subject to natural PM infection that was evaluated on June 28, 2020, 61 days post-transplanting.

Result and Discussion

Pathogenicity to Cucurbit Crops: The PM isolate XS2019 caused severe symptoms on watermelon plants with white mycelium and chains of conidia covering the entire leaf surface and leaves gradually became more necrotic. The isolate also infected summer squash seedlings and melon differential hosts 'Iran-H', 'Top Mark', 'Védrantais', 'PMR 45', PI 414723, and 'Nantais Oblong', whereas control plants remained symptomless (data not shown). Therefore, this isolate is pathogenic to all major cucurbit crops grown in China, cucumber, watermelon, squash and melon.

Morphological Characteristics: Microscopic observation of conidia in 3% KOH showed that they were ovoid to barrel-shaped, and contained fibrosin bodies commonly found in *P. xanthii* (Braun and Cook, 2012). Therefore, isolate XS2019 is identified as *P. xanthii*.

Race Identification: 'Iran H', 'Top Mark', 'Védrantais', 'PMR 45', PI 414723, and 'Nantais Oblong' were rated as susceptible based on presence of abundant conidia 10-days after inoculation (Figure 1 and Table 1). The other eight accession were rated as resistant based on absence of mycelia and conidia (Figure 1 and Table 1). Small chlorotic angular lesions were, however, observed on WMR 29, PI 124112, 'PMR 5' and 'PMR 6' (Figure 1). PI 124111 exhibited some susceptibility on hypocotyls and cotyledons, but true leaves were resistant.

Reactions of the 14 melon differentials in the plastic greenhouse were consistent with the seedling test conducted in the growth room (Figure 2). Based on the reaction pattern of 'PMR 5', 'PMR 6', WMR 29, 'Edisto 47', and PI 414723, we conclude that isolate XS2019 is *P. xanthii* race N2 (Hosoya et al., 2000; McCreight et al., 2012). This is different from *P. xanthii* race 1, race pxCh1, race 2F, race 5 and race 7 that were previously reported in China (Bao et al., 2008; Liu et al. 2010b; Ma et al., 2011; Zhang et al., 2011; Liu et al., 2010a; Su et al., 2013). To our knowledge, this is the first report of powdery mildew caused by *P. xanthii* melon race N2 on cucumber and other cucurbit crops in China. 'Iran H' was considered susceptible to all melon races reported to date but its reaction to race N2 had not previously been reported. 'Védrantais' and 'Top Mark' are resistant to melon race 0 (Pitrat et al., 1998), but otherwise considered susceptible to all other reported races (McCreight et al., 2012), likewise their reactions to race N2 had not previously been reported.

Literature Cited

1. Bao, H., Y. Xu, Y. Du, J. Li, and Y Feng. 2008. Identification of Physiological Races of Powdery Mildew on Cucurbits in Sanya, Hainan.J Changjiang Veg 1:49-51 (in Chinese)
2. Braun, U., and R. T. A. Cook. 2012. Taxonomic manual of the Erysiphales (Powdery Mildews). CBS Biodiversity Series No. 11. CBS, Utrecht, The Netherlands
3. Davis, A.R., A. Levi, A. Tetteh, T. Wehner, V. Russo, and M. Pitrat. 2007. Evaluation of watermelon and related species for resistance to race 1W powdery mildew. J Amer Soc Hort Sci 132:790-795
4. Hosoya, K., M. Kuzuya, T. Murakami, K. Kato, K. Narisawa, and H. Ezura. 2000. Impact of resistant melon cultivars on *Sphaerotheca fuliginea*. Plant Breeding 119:286-288.
5. Kim, K. H., S. G. Ahn, J. H. Hwang, Y. M. Choi, H.S. Moon, and Y. H. Park. 2013. Inheritance of resistance to powdery mildew in the watermelon and development of a molecular marker for selecting resistant plants. Hortic Environ Biotech 54:134-140.
6. Liu, D., H. Cheng, W. Kong, Y. Su, Y. Xu, and Q. He. 2010a. Identification of physiological race of powdery mildew on melon in Gansu province. Chin Vege 6:28-32 (in Chinese)
7. Liu, L., Y. Chen, Z. Su, H. Zhang, and W. Zhu. 2010b. A sequence-amplified characterized region marker for a single, dominant gene in melon PI 134198 that confers resistance to a unique race of *Podosphaera xanthii* in China. HortScience 45:1407–1410.
8. Ma, H., Z. Wei, Y. Zu, and F. Luan. 2011. Physiological races identification of powdery mildew on main cucurbits in Heilongjiang Province during 2009-2010.Acta Phyt Sini 3:287-288 (in Chinese)

9. 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.
10. Pitrat, M., C. Dogimont, and M. Bardin. 1998. Resistance to fungal diseases of foliage in melon, p. 167–173. In: J.D. McCreight (ed.). Cucurbitaceae '98: Evaluation and enhancement of cucurbit germplasm. ASHS Press, Alexandria, Va.
11. Su, R., H. Wang, Q. Gao, S. Jia, and J. Zheng. 2013. Identification of physiological race of powdery mildew on seed purpose watermelon and melon in part of Xinjiang Uygur Autonomous Region. Chin Vege 10:90-94 (in Chinese)
12. Tetteh, A., T. Wehner, and A. Davis. 2007. Screening the watermelon germplasm collection for resistance to powdery mildew race 2. HortScience 42:896-896
13. Tirupathamma, T. L., C. V. Ramana, L .N. Naidu, and K. Sasikala. 2019. Vegetable grafting: a multiple crop improvement methodology. Curr J Appl Sci and Tech 33:1-10
14. Zhang, H., S. Guo, G. Gong, Y. Ren, A. Davis, and Y. Xu. 2011. Sources of Resistance to Race 2WF Powdery Mildew in U.S. Watermelon Plant Introductions. HortScience 46:1349-1352

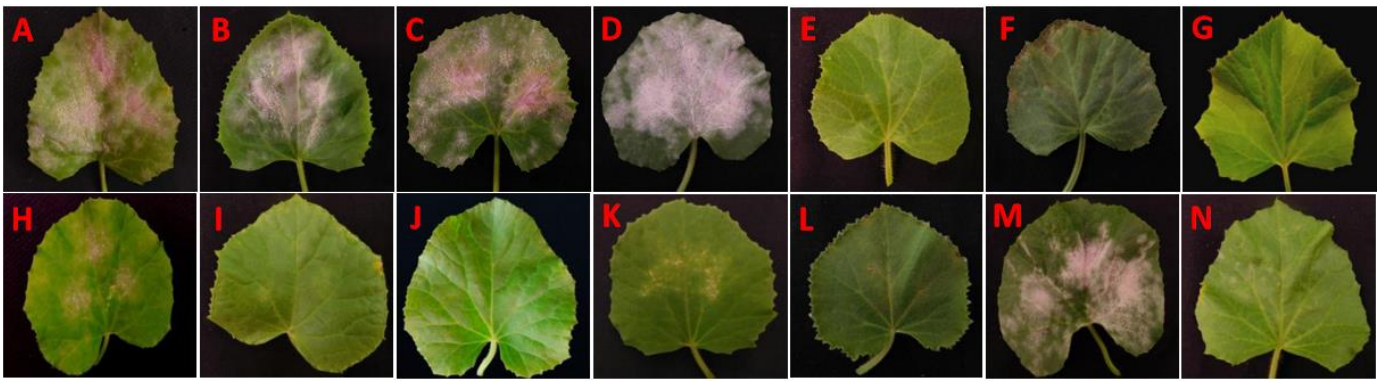

Figure 1: Reactions of 14 melon powdery mildew race differential host seedlings, grown in growth room, to cucurbit powdery mildew isolate XS2019. A, Iran H; B, Top Mark; C, Védrantais; D, PMR 45; E, PMR 5; F, WMR 29; G, Edisto 47; H, PI 414723; I, MR-1; J, PI 124111; K, PI 124112; L, PMR 6; M, Nantais Oblong; N, AR 5.

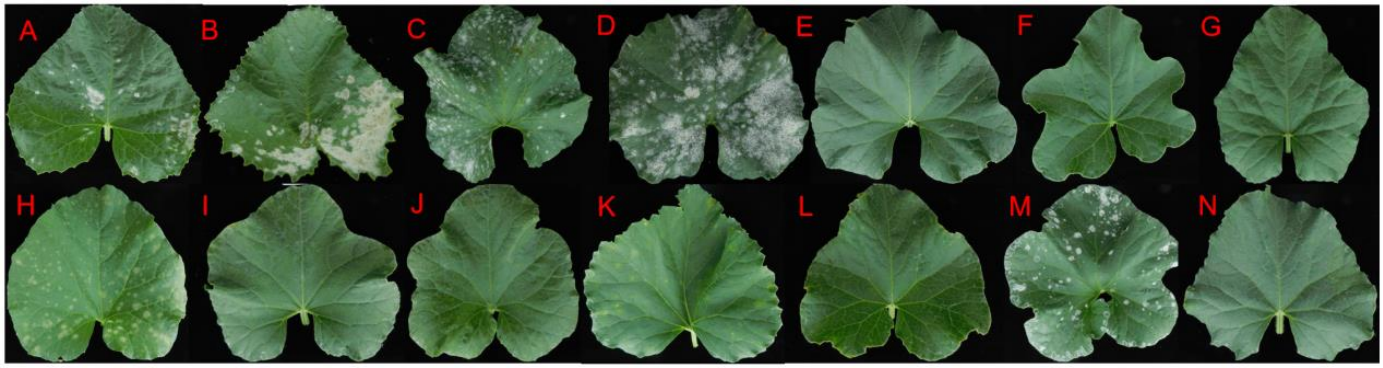

Figure 2: Reactions of 14 melon powdery mildew race differentials to cucurbit powdery mildew isolate XS2019 in a naturally infected greenhouse test. Capital letters A-N represent melon differentials as labeled in Figure 1.

Table 1. Reactions of melon differential hosts to powdery mildew isolate XS2019 in inoculated growth room (seedling) and naturally infected greenhouse (61 days post-transplanting) tests.

Differential host	Seedling	Greenhouse
Iran H	S	S
Top Mark	S	S
Védrantais	S	S
PMR 45	S	S
PMR 5	R	R
PMR 6	R	R
WMR 29	R	R
Edisto 47	R	R
PI 414723	S	S
MR-1	R	R
PI 124111	R	R
PI 124112	R	R
Nantais Oblong	S	S
AR 5	R	R

S = Susceptible; R = resistant

Suppression of *Cucurbit chlorotic yellows virus* Accumulation in Melon Breeding Line MR-1 under Natural Infection in Imperial Valley, California

Prabin Tamang, William M. Wintermantel, and James D. McCreight*
USDA-ARS, Crop Improvement and Protection Research Unit, Salinas, CA.
**jimmccreight@usda.gov*

Introduction

Cucurbit chlorotic yellows virus (CCYV) infection of melon was observed for the first time in the New World in Imperial Valley, California in 2018 (Wintermantel et al., 2019). CCYV was first reported in Japan (Okuda et al., 2010) followed by reports of its presence in China (Gu et al., 2011; Zeng et al., 2011), Taiwan (Huang et al., 2011), Sudan (Hamed et al., 2011), Lebanon (Abrahamian et al., 2012), Iran (Bananej et al., 2013), and Greece (Orfanidou et al., 2014).

CCYV-susceptible melons react to infection by the virus with foliar symptoms and disease development nearly identical those induced by *Cucurbit yellow stunting disorder virus* (CYSDV) (Kuo et al., 2007; Wintermantel et al., 2017). Resistance breeding for control of CYSDV has been ongoing in Imperial Valley, California since 2006 (McCreight and Wintermantel, 2008) with resistance to CYSDV conditioned by a single recessive gene in PI 313970 (McCreight and Wintermantel, 2011) and TGR 1551 (= PI 482420) (McCreight et al., 2017), but selection under naturally-infected conditions was confounded unbeknownst to us by CCYV beginning in Fall 2014. CCYV and CYSDV have been co-infecting melons in Imperial Valley since 2014; however, due to similarity in symptoms on melon plants infected by these viruses, the presence of CCYV was not determined until 2018 (Wintermantel et al., 2019).

Five potential sources of resistance to CCYV in melon were found among 51 lines in controlled inoculation tests: JP 138332, JP 215154, JP 21515, JP 216751, and JP 91204, a.k.a. MR-1 (Okuda et al., 2013). These five lines exhibited no or only faint symptoms in response to CCYV inoculation. JP 138332 was, however, the only one of the five putative CCYV-resistant lines with a detectable virus titer significantly lower than that of 'Earl's Seine'. Okuda et al. (2013) also noted susceptibility of TGR 1551 (= PI 482420) to CCYV including CCYV-induced yellowing symptom expression in a preliminary study. We observed CCYV-induced yellowing of PI 313970 and TGR 1551, lines exhibiting resistance to CYSDV, before we detected CCYV in Imperial Valley in 2018 (Wintermantel et al., 2019). Our stocks of MR-1 exhibited CYSDV-induced yellowing symptoms in numerous field tests in Imperial Valley beginning in Fall 2006 (McCreight and Wintermantel, 2008) and every year thereafter (McCreight and Wintermantel, unpublished). Here we report the reaction of MR-1 to CCYV in a field test naturally co-infected with CYSDV and CCYV as measured by virus copy number and foliar yellowing.

Materials and Methods

Four melon lines were planted at the University of California Desert Research and Extension Center, Holtville: 'Ananas Yoqne'am' (AY), 'Top Mark' (TM) and PI 313970 (PI), and MR-1. AY is an Israeli melon and a member of the Ameri Group (Burger et al., 2010). TM is a western U.S. shipper type cantaloupe susceptible to CYSDV and CCYV (unpublished data). PI, a land race from India, is resistant to CYSDV (McCreight and Wintermantel, 2011) and susceptible to CCYV (unpublished data). MR-1 was derived from PI 124111 for uniform reaction to downy mildew caused by two races of *Pseudoperonopsora cubensis* and races 1, 2 and 3 of powdery mildew caused by *Podosphaera xanthii* (Thomas, 1986). The test was planted and watered with subsurface drip irrigation on 15 August 2019. Seeds were sown in standard western U.S. melon beds, on 2 m centers and two hills spaced 1.5 m apart within 3 m-long plots. Plants were sampled for virus content and rated for virus yellowing ca. 40 and 70 days post-planting (dpp). Imidacloprid was applied after emergence in order to prevent the plants from being damaged by whitefly feeding (Wisler et al., 1998).

Yellowing leaves were collected from two plants of each of the four lines and stored in a cold room until sampled for RNA extraction. Approximately 100 mg of leaf tissue from each plant was collected, lyophilized, and ground to a fine powder. Total RNA was extracted from each sample using the MagMax Plant RNA extraction kit (Thermo Fisher Scientific) in a KingFisher Flex Magnetic particle processor, and cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad) following the manufacturer's protocol. The CYSDV and CCYV-

specific primers and probes were designed targeting the RNA dependent RNA polymerase (RdRp) gene on RNA1 of each virus (Table 1). The CYSDV probe was labeled at the 5' end with HEX, CCYV with FAM and ADP with CALFluor610. The melon ADP gene was used as an internal control for RT-qPCR. All primers were subjected to NCBI BLASTn to verify specificity and were validated against both viruses and healthy melon prior to use in these studies.

RT-qPCR was conducted in a CFX96 Real-time system thermal cycler (Bio-Rad) with cycling parameters: denaturation at 95° for 3 min followed by 40 cycles of 95° for 10 s and 60° for 1 min. Each single RT-qPCR reaction contained 5 µl of 5x PerfecTa Multiplex qPCR supermix (Quantbio), 0.6 µl forward primers, reverse primers and the respective probes for CYSDV, CCYV and ADP, 13.6 µl nuclease free water and 1 µl cDNA for a total reaction of 25 µl in a 96-well PCR plate. Two technical replications for each sample were included in RT-qPCR to minimize the error. A non-template control using nuclease-free water was used for each run. The data were analyzed using Bio-Rad CFX manager software V3.1. The quantification cycle value (Ct) was determined at the default settings. A 5-fold dilution series of CYSDV and CCYV was generated with known cDNA concentration and run in each plate to construct standard curves by plotting log value of cDNA against Ct number. The amplification efficiency (*e*) and the coefficient of determination (R^2) of the primers used in qPCR were calculated automatically by Bio-Rad CFX manager software according to the Ct value generated for the serial dilutions against the corresponding $\log_{10}$ amount of the cDNA template. Mean relative virus copy number in the sample was calculated as follows. Virus copy number/ µl= [cDNA concentration (g/µl)/ (PCR product in bp x 660)] x 6.022 x 10^{23}.

Results and Discussion

MR-1 and TM exhibited extensive virus yellowing symptoms 41 dpp compared with intermediate symptoms on AY and PI, but by 70 dpp all four lines exhibited extensive yellowing (Table 2). PI is resistant, not immune, to CYSDV (McCreight and Wintermantel, 2011). The titer of CYSDV in PI was 0.023 % of the level in TM, whereas the CYSDV titer in MR-1 was 527 % higher than in TM. Extensive yellowing exhibited by PI was, therefore, likely due to CCYV infection. The CCYV titer in PI was 136 % of that in TM (Table 2), whereas the CCYV titers in MR-1 and AY were 1 % and 2 % that of TM, respectively. The extensive yellowing of MR-1 and AY may have been largely due to CYSDV infection, if their CCYV titers are indicative of resistance. Resistance to CYSDV in PI resulted in much greater reduction of CYSDV titer relative to TM than the putative resistance to CCYV did in MR-1 and, possible resistance in AY relative to TM. Interestingly, the lower titer of CCYV in MR-1 contradicts the results of Okuda et al. (2013) who found that although MR-1 exhibited mild symptoms in response to inoculation with CCYV, virus titer was comparable to that in the CCYV-susceptible 'Earl's Seine'.

The different reactions of MR-1 could have been due to CCYV strain differences, although this is not likely, since the genomes of CCYV isolates from Japan and California are 99% identical to each other (Wintermantel unpublished). Another possibility would be differences in the MR-1 sources between the two sets of experiments. Different test conditions may have played an important role, too. Okuda et al. (2013) exposed the test plants to 4-days of feeding by 20-30 viruliferous *B. tabaci* Q biotype whiteflies per plant. The plants in the Imperial Valley test were subjected to much higher numbers (often > 100 adults per leaf) of *B. tabaci* MED biotype whiteflies feeding continuously from time of emergence through the second leaf sample collection, with mean daily maximum temperatures of 42, 37 and 31°C in August, September and October, respectively. A more likely explanation may be that co-infection of CCYV with CYSDV in the Imperial Valley field test influenced CCYV titer in test plants. Co-infection of CCYV and CYSDV often resulted in reduced titers of both viruses, especially CCYV in cucumber plants (Abrahamian et al., 2013; Orfanidou et al., 2020). In this regard it is interesting to note the CCYV:CYSDV ratio within each line. By this measure, the CCYV titer was greater than the CYSDV titer in all four lines, ranging from 7x for MR-1 to 1.8E+07x for PI in this test (Table 1). CYSDV infection may have resulted in extensive virus yellows symptoms and perhaps suppression of CCYV multiplication in MR-1 and the other lines relative to TM, although CCYV titers still exceeded those of CYSDV within each entry.

Acknowledgements

This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2015-51181-24285.

Literature Cited

1. Abrahamian, P.E., H. Sobh, and Y. Abou-Jawdah. 2012. First Report of *Cucurbit chlorotic yellows virus* on cucumber in Lebanon. Plant Dis. 96:1704.
2. Abrahamian, P.E., R. Seblani, H. Sobh, and Y. Abou-Jawdah. 2013. Detection and quantitation of two cucurbit criniviruses in mixed infection by real-time RT-PCR. Journal of Virology Methods 193:320–326.
3. Bananej, K., W. Menzel, N. Kianfar, A. Vahdat, and S. Winter. 2013. First report of *Cucurbit chlorotic yellows*

virus infecting cucumber, melon and squash in Iran. Plant Dis. 97:1005.

4. Burger, Y., H.S. Paris, R. Cohen, N. Katzir, Y. Tadmor, E. Lewinsohn, and A.A. Schaffer. 2010. Genetic diversity of *Cucumis melo*, p. 165–198. In: J. Janick (ed.). Hort. Rev. Wiley-Blackwell, New York.
5. Gu, Q.S., Y.H. Liu, Y.H. Wang, and W.G. Huangfu. 2011. First report of *Cucurbit chlorotic yellows virus* in cucumber, melon, and watermelon in China. Plant Dis. 95:73.
6. Hamed, K., W. Menzel, G. Dafalla, A.M.A. Gadelseed, and S. Winter. 2011. First report of *Cucurbit chlorotic yellows virus* infecting muskmelon and cucumber in Sudan. Plant Dis. 95:1321.
7. Huang, L.-H., H.-H. Tseng, J.-T. Li, and T.-C. Chen. 2011. First report of *Cucurbit chlorotic yellows virus infecting* cucurbits in Taiwan. Plant Dis. 94:1168.
8. Kuo, Y.-W., M.R. Rojas, R.L. Gilbertson, and W.M. Wintermantel. 2007. First report of *Cucurbit yellow stunting disorder virus* in California and Arizona, in association with *Cucurbit leaf crumple virus* and *Squash leaf curl virus*. Plant Dis. 91:330.
9. McCreight, J.D. and W.M. Wintermantel. 2008. Potential new sources of genetic resistance in melon to *Cucurbit yellow stunting disorder virus*, p. 173–179. In: M. Pitrat (ed.). Cucurbitaceae 2008, Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae, May 21-24th, 2008. INRA, Avignon (France).
10. McCreight, J.D. and W.M. Wintermantel. 2011. Genetic resistance in melon PI 313970 to *Cucurbit yellow stunting disorder virus*. HortScience 46:1582–1587.
11. McCreight, J.D., W.M. Wintermantel, E.T. Natwick, J.W. Sinclair, K.M. Crosby, and M.L. Gómez-Guillamón. 2017. Recessive resistance to *Cucurbit yellow stunting disorder virus* in melon TGR 1551. Acta Hort. 1151:101–107.
12. Okuda, M., S. Okazaki, S. Yamasaki, S. Okuda, and M. Sugiyama. 2010. Host range and complete genome sequence of *Cucurbit chlorotic yellows virus*, a new member of the genus Crinivirus. Phytopathology:560–566.
13. Okuda, S., M. Okuda, M. Sugiyama, Y. Sakata, M. Takeshita, and H. Iwai. 2013. Resistance in melon to *Cucurbit chlorotic yellows virus*, a whitefly-transmitted crinivirus. European J. Plant Pathol. 135:313–321.
14. Orfanidou, C., A. Katsiani, L. Papayiannis, Katis Nikolaos I., and V.I. Maliogka. 2020. Interplay of *Cucurbit yellow stunting disorder virus* with cucurbit chlorotic yellows virus and transmission dynamics by *Bemisia tabaci* MED. Plant Dis. https://doi.org/10.1094/PDIS-03-20-0621-RE
15. Orfanidou, C.G., V.I. Maliogka, and N.I. Katis. 2014. First report of Cucurbit chlorotic yellows virsu in cucumber, melon, and watermelon in Greece. Plant Disease 98:1446.
16. Thomas, C.E. 1986. Downy and powdery mildew resistant muskmelon breeding line MR-1. HortScience 21:329.
17. Wintermantel, W.M., R.L. Gilbertson, E.T. Natwick, and J.D. McCreight. 2017. Emergence and epidemiology of *Cucurbit yellow stunting disorder virus* in the American desert southwest, an development of host pant resistance in melon. Virus Research 241:213–219.
18. Wintermantel, W.M., L.L.J. Hladky, P. Fashing, K. Ando, and J.D. McCreight. 2019. First report of *Cucurbit chlorotic yellows virus* infecting melon in the New World. Plant Dis. 103:778. https://doi.org/10.1094/PDIS-08-18-1390-PDN
19. Wisler, G.C., J.E. Duffus, H.Y. Liu, and R.H. Li. 1998. Ecology and epidemiology of whitefly transmitted closteroviruses. Plant Dis. 82:270–280.
20. Zeng, R., F.M. Dai, W.J. Chen, and J.P. Lu. 2011. First Report of *Cucurbit chlorotic yellows virus* infecting melon in China. Plant Dis. 95:354.

Table 1. CYSDV and CCYV-specific primers and probes for multiples qPCR assay.

Virus	Forward primer	Reverse Primers	Probes
CYSDV	TGATGACGGGAAGGTTAGAGT	CTTCGGATCGGGTTGGACA	HEX-TGCCAGATGCACAGAGGATGTTCG-BHQ1
CCYV	ACGGTGGGAGAGTTAGAGTGA	CTCTTCGTCTGATTGGTGTGGATA	FAM-CACCAGACGCGCAGAGGATGTTC-BHQ1
ADP	GTGGTGGATAGCAATGACAGAGA	CCTCAGCTCGTCCTCATTCAAC	CALFluor610-TCGAAGCTAGGGATGAGCTGCAC-BHQ2

Table 2. Mean titer (copies/µl) of CYSDV and CCYV and virus yellows symptoms in a naturally-infected field test in Imperial Valley, California, Fall, 2019.

	CYSDV			CCYV		Yellowing (dpp)[z]	
Line	copies/µl	R_{TM}[y]	CCYV: CYSDV	copies/µl	R_{TM}	41	70
Ananas Yoqne'am	8.1E+10	2.12264	3.0E+01	2.4E+12	0.02	4.5	8.0
MR-1	2.0E+11	5.26730	7.1E+00	1.4E+12	0.01	7.0	8.5
PI 313970	8.8E+06	0.00023	1.8E+07	1. 6E+14	1.36	4.0	7.0
Top Mark	3.8E+10	1.00000	3.0E+03	1.1E+14	1.00	7.5	6.5

[z]Virus yellowing rated on a 1 (asymptomatic) to 10 (100 % yellowed leaves) visual scale; dpp = days post planting.
[y]Virus copies/µl relative to 'Top Mark'

A Single Dominant Gene, *Ef*, Confers Early Flowering in Acorn Squash (*Cucurbita pepo* subsp. *ovifera*)

Andrew B. Ogden*, J. Brent Loy and Rebecca G. Sideman
University of New Hampshire, Spaulding Hall Room G44, University of New Hampshire
**Andrew.ogden@unh.edu*

Introduction

Cucurbita species are monoecious and produce staminate flowers on basal nodes close to the crown of the plant and pistillate flowers on more distal nodes of the main stem and lateral branches. However, there is considerable variability in flowering patterns in terms of node number of first flowers reaching anthesis and the time course for initiation of flower buds. For example, Hassan et al. (2016) reported that first female flowering among summer squash and acorn squash varieties occurred at nodes ranging from 7 to 33. Early and prolific flowering is characteristic of yellow summer squash and zucchini, *Cucurbita pepo* subsp. *ovifera* and *Cucurbita pepo* subsp. *pepo* respectively (Montero-Pau et al., 2017), whereby fruit sinks are continually removed, allowing continuous fruit set over an extended period. Early flowering in winter squash may be desirable in cultigens grown in short growing seasons like New England as it allows for full fruit maturation. Also, early flowering cultigens have proven useful as rootstocks for inducing early flowering in late flowering cultigens of squash (Ogden and Loy, 2018). On the other hand, excessively early flowering may result in nutrient sink competition between developing fruits and newly developing leaves, thereby suppressing development of the leaf canopy and resulting in a deficiency of photosynthate to support the developing fruit. Developing fruits and seeds may act as dominant sinks thereby limiting further vegetative development. This concept is well established in a variety of crops including members of the Cucurbitaceae family (Delesalle & Mooreside, 2020; El-Keblawy, 2020; Wardlaw, 1968).

Genomics research has revealed numerous quantitative trait loci (QTL) regulating flowering time in related crop species (Lu et al., 2014; Mcgregor et al., 2014). In cucumber, for example, Lu et al. (2014) identified a major QTL for earlier flowering. They speculated that early flowering in cucumber is caused by a single dominant gene designated *Ef1.1.* Also in Cucurbitaceae, a major QTL in watermelon regulating flowering time was recently discovered (McGregor, et. al, 2014). In squash, *C. pepo* subsp. *pepo*, SNP analyses conducted by Montero-Pau (2017) revealed at least two QTL regulating flowering time. This finding remains tentative and does not support that team's previous identification of a single major QTL regulating flowering time (Esteras et al., 2012). Despite advancement in the genomics field, we are not aware of any classical studies on inheritance of genes controlling flowering time in squash reported to date.

A better understanding of the genetics controlling flowering time in winter squash will contribute to plant breeders' ability to improve varieties of summer and winter squash and may provide insights into the genetic control of substances affecting photoperiodic and late flowering patterns. Season extension by using a mixture of early, mid, and late season cultivars is a popular practice among many vegetable growers and breeders must respond to this demand. This research could also provide germplasm to identify molecular markers for early flowering and better understand the genes involved in regulation of flowering time in squash.

At the University of New Hampshire, Dr. Brent Loy identified and generated two highly inbred lines of acorn squash which display early and late flowering. Because the two breeding lines display large differences in flowering time, they appeared to be good choices for an inheritance study on flowering time in acorn squash. Through examination of F_1, F_2, and backcross populations derived from two breeding lines with distinct flowering times, we sought to elucidate the genetic control behind early flowering.

Materials and Methods

Experimental site description. Experiments took place at the Kingman Horticultural Research Farm in Madbury, New Hampshire between the months of June to August during 2017, 2018, and 2019. All plants were grown on raised black plastic covered beds, 0.81 meters in width and 0.15 meters in height and fertilized with a pre-plant granular fertilizer with 90 kg/ha N and K. Standard pest and disease management methods were employed to control any pest or pathogen problems. Weed control between beds was provided with mechanical and manual cultivation.

Plant materials and data collected. The two parent lines, NH27-15-5-10 (hereafter P1) and NH8-17-12-7 (hereafter P2), along with their F1, F2 (2018 only), and backcross populations (2018 and 2019 only) were all seeded in 50 cell plug trays at the Macfarlane greenhouse in Durham, New

Hampshire and after germination, fertilized by hand watering fertigated water at a constant feed rate of 100 ppm-N with the fertilizer 17-4-17 (N-P_2O_5-K_20). P1 flowers early and initiates pistillate flowers as early as nodes three or four, whereas P2 often flowers 12 to 16 days later and initiates pistillate flowers at node 12 or later. Both parent lines have a bush growth habit and produce fruit with high starch content in the fruit mesocarp tissue. Seedlings were transplanted into the field at the 1 to 2 leaf stage. Plants were grown at a spacing of 0.6 meters between plants and 2.7 meters between raised beds. Upon reaching the 6-7 leaf stage, a plastic tag was placed around the petiole of the leaf at the 5th node from the cotyledons to facilitate counting of nodes. Daily observations of the plants enabled noting the date and the node number at which the first male and female flowers reached anthesis. Dates of flowering were converted to days to first male and female anthesis from transplanting.

Statistical design and analysis. Each year, a randomized complete block design was employed. A block was represented by a single row and there were eight rows. Treatments consisted of the genotypes, and each treatment was assigned randomly within each row and replicated once. For 2017, each row consisted of 5 F_1 plants, 5 plants of each parent, 10 backcrosses to the early parent (BCP1), and 10 backcrosses to the late parent (BCP2), for a total of 35 plants per block. For 2018, each row contained 4 F_1 plants, 4 plants of each parent (P1 and P2), 10 backcrosses to the early parent (BCP1), 10 reciprocal backcrosses to the late parent (BCP2R), and 24 F_2 plants for a total of 56 plants per row. In 2019, each row contained 4 F_1 plants, 4 plants of each parent, 4 backcrosses to the early parent (BCP1), 12 backcrosses to the late parent (BCP2), and 12 reciprocal backcrosses to the late parent (BCP2R) plants for a total of 40 plants per row.

Two-way ANOVA analyses were conducted to detect differences in flowering time as affected by genotype. Flowering time was represented as both days to first male and female flower reaching anthesis and node number of first male and female flower which reached anthesis. Each row contained a randomized complete block to enable assignment of some phenotypic variation to variability in field conditions. Frequency distributions were calculated for each genotype separately by year and compared. To test the single dominant gene model, χ^2 analysis was employed by categorizing each plant as either early or late. Because the female flower gives rise to the fruit, earliness was determined by the first female flower only. During 2017 and 2018, earliness was defined as having a female flower which reached anthesis at node number 12 or less and this flower reached anthesis in 37 days or less after transplant. In 2019, earliness was defined as having a female flower which reached anthesis at node number 15 or less and reached anthesis in 37 days or less after transplant. A later planting date and prolonged period in the greenhouse in 2019 likely caused more rapid shoot elongation than in 2017 and 2018. After classification based on female flowering patterns, male flowering was also compared between the classified segregants. We tested the hypothesis that early flowering was caused by a single dominant gene. Thus, the expected ratios were that all F_1 plants and all backcrosses to the early parent, BCP1, would be early flowering while backcrosses to the late parent, BCP2, would segregate at ratio of 1:1 early:late flowering and the F_2 population would segregate at a ratio of 3:1 for early:late flowering.

Results and Discussion

Flowering phenology, in general, among all genotypes occurred similarly to previous researchers' descriptions with male flowering beginning earlier and at lower node numbers than female flowering (Loy, 2004). Table 1 illustrates these differences. Both node number and days to flower are presented because initiation of flowering is both temporal and morphological and the two variables are highly correlated (data not shown). Variation in male flowering time was limited compared to female flowering across all genotypes. Male flowering initiated at nodes 1-5 between 24 and 30 days after transplant date. Female flowering initiated at nodes 9-22 between 29 and 46 days after planting. This greater variation in female flowering patterns was the justification for the use of female flowering time as an indicator of earliness of flowering.

Although use of first female flowering as an indicator of earliness captured clear differences in flowering time among the different populations grown, this method was not without its limitations. Flowering time, while genetically controlled, is also highly influenced by other factors such as transplant vigor, field fertility and soil quality levels, pest and disease pressure, and environmental variables such as temperature, photoperiod, and light intensity. Use of first female flowering resulted in misclassification of some late flowering plants as early. This phenomenon was observed during the 2018 season when 29% of the late flowering parent (P2) plants were classified as early flowering (Table 1), based on a single flower reaching anthesis early. However, these precocious flowers did not typically set fruits and the subsequent flowering of these plants was late. In the future, days to two fruits set could be a possible way to eliminate this problem. Further, the P1 and P2 lines were not isogenic, and thus, it is possible that segregation of modifying genes affected flowering time to a minor extent.

There were clear differences between the flowering patterns of the two parent lines (Table 1). Most P1 plants initiated male and female flowering at earlier dates and at lower node numbers than P2. In 2017 and 2019, P1 male and female flowers initiated earlier and at lower node numbers than P2. In 2018, the node number of first male anthesis did not differ between P1 and P2 but days to male and female anthesis and node number of first female anthesis were all greater in P2.

Female flowering in the F_1 generation occurred simultaneously and at similar node numbers as P1 during 2017 and 2018. During 2019, F1 flowering was delayed compared to P1. In all three seasons, F1 progeny produced female flowers earlier and at lower node numbers than P2. Male flowering in F1 progeny was intermediate between P1 and P2 during 2017 while in 2019, F1 male flowering occurred simultaneously with P1, both earlier and at lower node numbers than P2. In 2018, F1 progeny produced male flowers at similar node numbers as P1 and P2 while days to male anthesis was delayed slightly (1 day) compared to P1 and more closely resembled P2.

Frequency distributions for female flowering patterns among parental genotypes for years 2017, 2018, and 2019 are depicted in Figures 1, 2, and 3, respectively. The clear difference between parent lines in the two response variables, days to first female flower reaching anthesis and node number of first female flower tor each anthesis, is shown in all figures. Frequency distributions of F_1 progeny also closely resembled those of the early flowering parent, P1, indicative of dominant gene action. In 2019 only, female flowering in the F_1 progeny occurred at an intermediate state between the two parents for both response variables. This is suggestive that partial dominance of the early flowering trait can occur under some environmental conditions.

Expected ratios for a single dominant gene conferring early flowering were met in both parent lines, F_1 progeny, and backcrosses to the early parent, BCP1, during all three years. Progeny of the F_1 backcrossed to each of the two parent lines initiated female flowering in two distinct patterns as depicted in Figures 1, 2, and 3. The backcross to P1, BCP1, flowered uniformly early during all three seasons. Also, in all three seasons, the backcross to the late parent P2, BCP2, segregated into two flowering groups, late and early. Individuals within the F_2 and backcross populations to the late parent, BCP2 and BCP2R, were classified as either late or early flowering based on previously described parameters. Only female flowering was used to classify plants as early or late because it was the most consistent flowering variable among genotypes across years. Early flowering plants showed both earlier male and female flowering although the difference was more pronounced in the female flowering pattern.

In 2017 and 2019, backcrosses to the late parent, BCP2 segregated according to expected ratios. In 2018 there was a lack of late flowering segregants among the BCP2 population and the F_2 population (Figure 3) and data failed to confirm the hypothesis that early flowering is caused by a single dominant gene during that season (Table 2). ANOVA analysis from 2018 revealed that P1 flowered earlier that season than the other two years, indicating a possible influence from environmental factors. Also, in 2018, 29% of the late flowering parent, P2, produced a single early female flower as mentioned previously. If a similar phenomenon occurred in the segregating populations, this could help to explain the large number of early flowering segregants observed that year. The frequency distributions for those two populations during 2018 (Figure 2) reveal that although there was a higher proportion of early flowering plants, segregation into two distinct groups of early and late flowering did still occur.

The hypothesis that cytoplasmic factors could have contributed to the observed results was tested in 2019. Data from reciprocal backcrosses to the late parent (P2) were compared during 2019 using t tests and revealed that female flowering patterns did not differ between the two populations (data not shown). This reduces the probability that significant cytoplasmic factors affected the observed female flowering patterns.

Based on the preceding analyses of variance, frequency distributions, and inheritance data, we propose the naming of a new gene for acorn squash, *Cucurbita pepo* subsp. *ovifera,* as *Ef* for early flowering. Genotypes of acorn squash carrying the *Ef* allele typically produce mature female flowers approximately 14-18 days earlier and at node numbers approximately 7-11 nodes lower than genotypes homozygous for the recessive allele, *ef*. Male flowers of genotypes carrying the *Ef* allele may mature slightly earlier and at lower node numbers than genotypes homozygous for *ef* but the difference is small, as acorn squash tends to produce male flowers at low node numbers and often earlier than female flowers.

This finding should be useful for breeders aiming to breed acorn squash with varied maturation times. Future studies could include genomics research to map *Ef* on existing genetic linkage maps of *C. pepo.* The clear segregation into early and late flowering groups could enable approaches such as bulk segregant analysis for mapping purposes, and later, metabolomics research to help identify specific substances associated with the early versus late flowering responses.

Literature Cited

1. Delesalle, V.A., Mooreside, P.D. 1995. Estimating the costs of allocation to male and female functions in a monoecious cucurbit, *Lagenaria siceraria*. Oecologia. 102:9-16.
2. El-Keblawy, A.A., Lovett-Doust, J. 1996. Resource re-allocation following fruit removal in cucurbits: patterns in two varieties of squash. New Phytologist. 133: 583-593.
3. Esteras, C., Gómez, P., Monforte, A.J., Blanca, J., Vicente-Dólera, N., Roig, C., Nuez, F., Picó, B. 2012. High-throughput SNP genotyping in *Cucurbita pepo* for map construction and quantitative trait loci mapping. BMC Genomics 13:80.
4. Hassan, A.A., Abdel-Ati, K.E.A., Mohamed, M.I.A. 2016. Squash germplasm evaluation for some vegetative growth, flowering, and yield characters. Middle East J. Agric. Res. 5: 109-116.
5. Loy, J.B. 2004. Morpho-physiological aspects of productivity and quality in squash and pumpkins (*Cucurbita spp.*). Crit. Rev. Plant Sci. 23: 337-363.
6. Lu, H., Lin, T., Klein, J., Wang, S. 2014. QTL-seq identifies an early flowering QTL located near *Flowering Locus T* in cucumber. Theor. Appl. Genetics. (doi:10.1007/s00122-014-2313-z)
7. McGregor, C.E., Sciences, M.P., Abdel-Haleem, H. 2014. Flowering time in watermelon is associated with a major quantitative trait locus on chromosome 3. J. Amer. Soc. Hort. Sci. 139: 48–53.
8. Montero-Pau, J., Blanca, J., Esteras, C., Martínez-Pérez, E.M., Gómez, P., Monforte, A.J. 2017. An SNP-based saturated genetic map and QTL analysis of fruit-related traits in zucchini using genotyping-by-sequencing. BMC Genomics. 18:94. (doi:10.1186/s12864-016-3439-y)
9. Ogden, A.B., Loy, J.B. 2018. Use of grafting to promote flowering in late and short-day flowering cultigens of squash. Cucurbitaceae Conference Abstracts. p. 78. http://cucurbit2018.ucdavis.edu/wp-content/uploads/2018/10/2018_Cucurbits_Abstracts_Book.pdf
10. Wardlaw, I.F. 1968. The control and pattern of movement of carbohydrates in plants. Botanical Review. 34: 79-105.

Table 1. Node number and days to male and female anthesis among parental lines and F1 hybrids of acorn squash, *Cucurbita pepo*. subsp. *ovifera,* grown and evaluated in field experiments conducted at the Kingman Research Farm in Madbury, NH.

Genotype[z]	Node number first male anthesis	Days after transplant to male anthesis	Node number first female anthesis	Days after transplant to female anthesis
2017				
P1	1.7 a[y]	24.7 a	10.4 a	32.6 a
P2	5.3 c	32.8 c	17.7 b	47.9 b
F_1	3.0 b	28.7 b	11.3 a	32.6 a
2018				
P1	1.5 a	26.3 a	4.3 a	24.4 a
P2	1.7 a	27.5 b	15.3 b	42.5 b
F_1	1.5 a	27.2 b	4.7 a	25.1 a
2019				
P1	2.2 a	22.6 a	12.5 a	29.2 a
P2	5.4 c	29.5 c	22.2 c	43.6 c
F_1	3.2 b	23.6 b	15.0 b	33.1 b

[z] P1 is an early flowering inbred bush breeding line designated NH27-10-5-10; P2 is a late flowering inbred bush breeding line designated NH8-17-12-7; the F1 is the cross of P1 x P2.
[y] Within a column, means followed by the same letter are not significantly different at $P \leq 0.05$ using Tukey's honestly significant difference test.

Table 2. Inheritance of the early flowering gene (*Ef*) in acorn squash, *Cucurbita pepo* subsp. *ovifera* grown at Kingman Farm in Madbury, New Hampshire during 2017, 2018 and 2019.

		2017		2018		2019	
Genotype	Expected ratio (early:late)	Number of plants (early:late)	χ^2 (p)	Number of plants (early:late)	χ^2 (p)	Number of plants (early:late)	χ^2 (p)
P1 (early)	1:0	32:0	1	30:0	1	24:0	1
P2 (late)	0:1	0:29	1	9:22	0.11	1:24	0.84
F_1	1:0	27:5	0.38	31:1	0.86	20:1	0.83
BCP1	1:0	74:5	0.57	76:0	1.00	32:0	1.00
BCP2	1:1	43:37	0.5	NA	NA	44:37	0.44
BCP2 R	1:1	NA	NA	59:16	<0.0001	50:33	0.06
F_2	3:1	NA	NA	160:24	<0.0001	NA	NA

[z] All genotypes are acorn squash breeding lines and offspring generated at the Loy cucurbit breeding laboratory at the University of New Hampshire. P1 is an early flowering line designated NH 8-27-15-5-10 while P2 is a late flowering line designated NH 8-17-12-7. F_1 is the cross of P1 X P2. BCP1 is P1 X (P1 X P2) and BCP2 is P2 X (P1 X P2). BCP2R is the reciprocal backcross (P1 X P2) X P2 and F2 is the F1 cross P1 X P2 which was then self-pollinated. F_2 population was grown only in 2018 and BCP2R population was grown in 2018 and 2019.

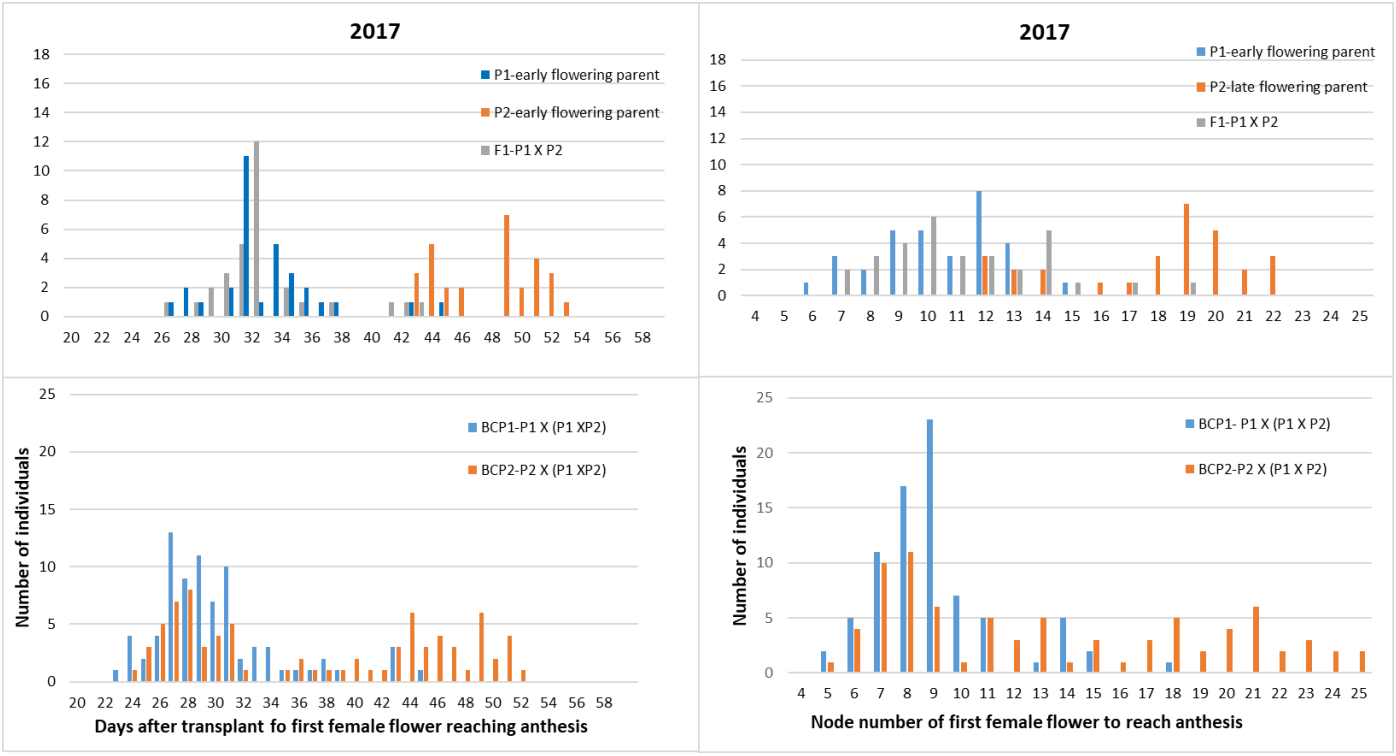

Figure 1. Frequency distributions of days after transplant to first female flower and node number of first female flower among acorn squash, *Cucurbita pepo* subsp. *ovifera,* breeding lines and F1 offspring. P1 is an early flowering line designated NH 8-27-15-10 while P2 is a late flowering line designated NH 8-17-12-7. F1 is the cross of P1 X P2. All backcross generations are derived from crossing the F1 with each of the two parent lines. All plants were grown under field conditions at Kingman Farm at the University of New Hampshire and the New Hampshire Agriculture Experiment Station in Madbury NH during 2017.

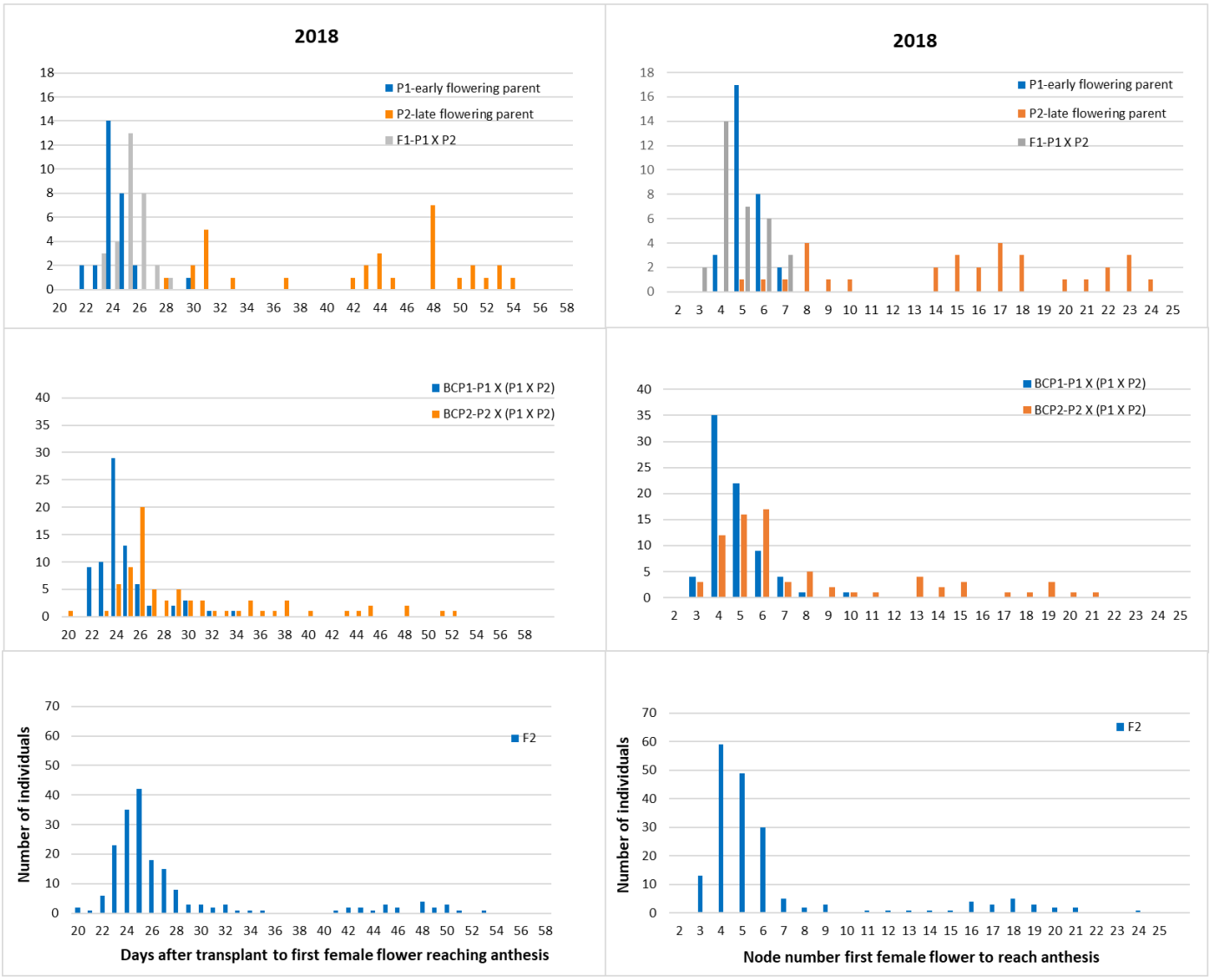

Figure 2. Frequency distributions of days after transplant to first female flower and node number of first female flower among acorn squash, *Cucurbita pepo* subsp. *ovifera,* breeding lines and F1 offspring. P1 is an early flowering line designated NH 8-27-15-10 while P2 is a late flowering line designated NH 8-17-12-7. F1 is the cross of P1 X P2. All backcross generations are derived from crossing the F1 with each of the two parent lines. The F2 is a self-pollinated selection derived from the F1 cross of P1 x P2. All plants were grown under field conditions at Kingman Farm at the University of New Hampshire and the New Hampshire Agriculture Experiment Station in Madbury NH during 2018.

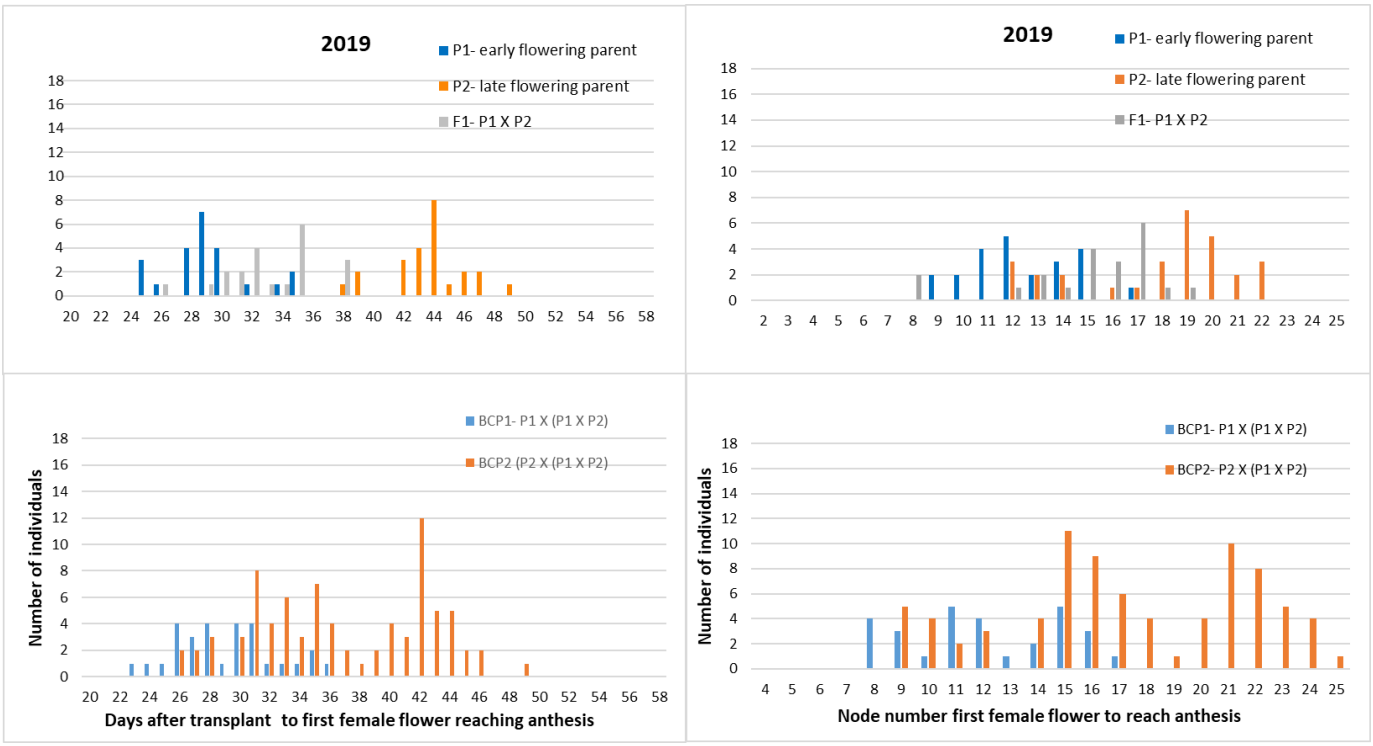

Figure 3. Frequency distributions of days after transplant to first female flower and node number of first female flower among acorn squash, *Cucurbita pepo* subsp. *ovifera,* breeding lines and F1 offspring. P1 is an early flowering line designated NH 8-27-15-10 while P2 is a late flowering line designated NH 8-17-12-7. F1 is the cross of P1 X P2. All backcross generations are derived from crossing the F1 with each of the two parent lines. All plants were grown under field conditions at Kingman Farm at the University of New Hampshire and the New Hampshire Agriculture Experiment Station in Madbury NH during 2019

Non-transmission of ZYMV and PRSV through Resistant *Cucurbita moschata* Genotypes 'Nigerian Local' and 'Menina'

Magdiel A. Miranda-Vélez and Linda Wessel-Beaver*
Department of Agroenvironmental Science, University of Puerto Rico, Mayagüez, Puerto Rico, USA

Jose Carlos V. Rodrigues
Center for Excellence in Quarantine & Invasive Species, Agricultural Experiment Station, University of Puerto Rico at Mayagüez, Río Piedras, Puerto Rico, USA

**lindawessel.beaver@upr.edu*

Introduction

Surveys conducted in 2001 and 2002 (Paz-Carrasco and Wessel-Beaver, 2002) and in 2006 to 2011 (Rodrigues et al., 2012) found a high incidence of *Zucchini yellow mosaic virus* (ZYMV) and *Papaya ringspot virus* (PRSV) in cucurbits in Puerto Rico. Severe virus and virus vector outbreaks are associated with low yields and limitations to growing cucurbits in Puerto Rico. Overlapping of susceptible cucurbit crops and continuous growing of cucurbit crops throughout the year makes Puerto Rico, an ecologically diverse island with an abundance of alternative hosts and large, year-round populations of insect vectors, an excellent and dynamic environment for plant viruses (Rodrigues et al., 2012). For these reasons, it is very difficult to develop strategies of control that do not consider the use of genetic resistance.

'Nigerian Local' and 'Menina' are two well-known sources of resistance to PRSV and ZYMV in *Cucurbita moschata*. At least five loci are thought to be involved in controlling resistance to ZYMV, and the genes involve vary depending on the source of resistance (Pachner et al., 2011). 'Nigerian Local' carries two dominant genes for resistance (*Zym-0* and *Zym-4*), while resistance in 'Menina' is conferred by *Zym-1*. The inheritance of resistance to PRSV has been less well studied. 'Nigerian Local' appears to carry at least two genes for resistance to PRSV (McPhail-Medina et al., 2012).

Whether the resistance to ZYMV and PRSV in 'Nigerian Local' and 'Menina' is related to impeded replication and/or translocation of the virus in the plant, or some other mechanism, is not known. If resistance is primarily a matter of either reduced replication or minimal translocation of the virus within the plant, we wondered if resistant genotypes could still serve as a sufficiently large reservoir of virus to enable them to infect susceptible genotypes. For resistant genotypes to serve as an effective source of resistance, the answer to this question should be "no." Therefore, the objective of this work was to determine if plants of 'Nigerian Local' and 'Menina', mechanically inoculated with ZYMV and PRSV, produce sufficient virus titer to enable those plants to infect susceptible genotypes of *C. moschata*.

Materials and Methods

Cotyledons of six-days-old plants of resistant genotypes 'Menina' and 'Nigerian Local' were mechanically inoculated with either PRSV or ZYMV. Eighteen days post-inoculation, tissue from these plants (three plants each of 'Menina' inoculated with PRSV, 'Menina' inoculated with ZYMV, 'Nigerian Local' inoculated with PRSV, and 'Nigerian Local' inoculated with ZYMV) was used as inoculum to mechanically inoculate cotyledons of six-day-old plants of highly susceptible *C. moschata* genotypes 'Waltham' and 'Mos166'. For each of the four genotype-virus combinations, five plants were inoculated. At 20 days post-inoculation, the fourth leaf of each inoculated plant was tested with ELISA for the virus used in the inoculation. Readings of <0.400 were consider negative for virus. Data was analyzed as a factorial arrangement (2 genotypes x 2 inoculation treatments) in a one-way analysis of variance with four or five replications (a few plants died during the experiment). Means were compared with Fisher's Least Significant Difference test at the 0.05 level of probability. Plants were evaluated for the presence of any virus symptoms until 25 days post-inoculation.

Results and Discussion

No virus symptoms were observed on either the inoculum source plants ('Menina' and 'Nigerian Local' inoculated with each virus) nor the test plants ('Waltham' and 'Mos166'). Source plants had weakly positive ELISA readings in some cases. 'Menina' source plants inoculated with PRSV or ZYMV

had average ELISA readings of 0.374 and 0.671, respectively, on tissue sampled from the fourth leaf. 'Nigerian Local' source plants inoculated with PRSV or ZYMV had average ELISA readings of 0.462 and 0.360, respectively. Susceptible genotypes 'Waltham' and 'Mos166' had negative ELISA readings when inoculated with fresh inoculum from resistant genotypes 'Menina' and 'Nigerian Local' that had been previously inoculated with either PRSV (Table 1) or ZYMV (Table 2).

In this experiment resistant genotypes 'Menina' and 'Nigerian Local' did not have the capacity to transmit PRSV or ZYMV to susceptible genotypes 'Waltham' and 'Mos166'. 'Menina' and 'Nigerian Local', when mechanically inoculated with PRSV and ZYMV, are not suitable hosts for replication of these two viruses. While this experiment was conducted in the greenhouse, we expect that vector-infected plants of 'Menina' or 'Nigerian Local', or genotypes that carry the same genes for resistance, would also be unable to serve as sources of ZYMV or PRSV inoculum in the field. This research supports of use of 'Nigerian Local' and 'Menina' as excellent sources of resistance to ZYMV and PRSV in *C. moschata*.

Acknowledgement

This work was supported by USDA National Institute of Food and Agriculture, Specialty Crop Research Initiative project 2015-51181-24285, and the Puerto Rico Agricultural Experiment Station. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA or NIFA. This paper is a portion of a thesis submitted by the first author in partial fulfillment of the requirements in a master's degree program.

Literature Cited

1. McPhail-Medina, R., Wessel-Beaver, L., Rodrigues, J.C.V. 2012. Inheritance of resistance to Papaya ringspot virus in tropical pumpkin is controlled by at least two genes. *In:* (Sari, Solmaz and Aras, eds.) Proceedings of the Xth EUCARPIA meeting on genetics and breeding of Cucurbitaceae, pp. 697-701.
2. Pachner, M., H.S. Paris, and T. Lelley. 2011. Genes for resistance to zucchini yellow mosaic in tropical pumpkin. Journal of Heredity. 102(3):330–335. http://doi.org/10.1093/jhered/esr006.
3. Paz-Carrasco, L. and L. Wessel-Beaver. 2002. Survey of cucurbit viruses in Puerto Rico. D. N. Maynard (ed.), Cucurbitaceae 2002: 256-264. American Society for Horticultural Science Press. Alexandria, VA.
4. Rodrigues, J.C.V., I. Quintero-Lopez and L. Wessel-Beaver. 2012. Potyviridae as a major challenge to growing cucurbits in Puerto Rico. *In:* (Sari, Solmaz and Aras, eds.) Proceedings of the Xth EUCARPIA meeting on genetics and breeding of Cucurbitaceae, pp. 786-790.

Table 1. Mean *Papaya ringspot virus* (PRSV) ELISA readings (A_{405nm}) of susceptible genotypes 'Waltham' and 'Mos166' inoculated with sap from plants of 'Menina' and 'Nigerian Local' inoculated with PRSV

Inoculum Source	Tested Genotype	PRSV ELISA Reading
Menina	Waltham	0.310 a
	Mos166	0.275 a
Nigerian Local	Waltham	0.339 a
	Mos166	0.259 a
Mean		0.296
Genotype F-test		0.0723
Source of inoculum F-test		0.8182
Interaction F-test		0.4461
LSD (0.05)		0.090

The fourth leaf was sample at 20 days after inoculation.
LSD=Least Significant Difference at α=0.05 for the inoculum source x tested genotype combination of treatments.
Within a column, means followed by a common letter are not significantly different according to Fisher's Least Significant Difference (α=0.05).

Table 2. Mean *Zucchini yellow mosaic virus* (ZYMV) ELISA readings (A_{405nm}) of susceptible genotypes 'Waltham' and 'Mos 166' inoculated with sap from plants of 'Menina' and 'Nigerian Local' inoculated with ZYMV

Inoculum Source	Tested Genotype	ZYMV ELISA Reading
Menina	Waltham	0.226 a
	Mos166	0.276 a
Nigerian Local	Waltham	0.245 a
	Mos166	0.254 a
Mean		0.250
Interaction F-test		0.4606
LSD (0.05)		0.087
Genotype F-test		0.2974
Source of inoculum F-test		0.9559

The fourth leaf was sampled at 20 days after inoculation.

LSD= Least Significant Difference at α=0.05 for the inoculum source x tested genotype combination of treatments.

Within a column, means followed by a common letter are not significantly different according to Fisher's Least Significant Difference (α=0.05).

In memoriam
Jack Ernest Staub, Plant Breeder (1948-2019)

Dr. Jack Ernest Staub, husband, father, friend, educator, mentor, and plant breeder, died unexpectedly on July 8, 2019 at age 70. He spent his last days with his wife Frances at their Big Fork, Montana log home. Jack will be remembered by many beloved colleagues around the world.

Jack was born in 1948 in Denver, Colorado before moving to Bountiful, Utah where he spent his youth. He earned his B.S. in botany (chemistry minor) and M.S. in botany (cytogenetics) from Utah State University, where he lettered in wrestling, which is telling of his tenacious spirit. Somewhere along the line he mastered the banjo. His sister, Sonnya, was a childhood friend of his future wife Frances Cook, whom Jack did not know until their fateful meeting at The Pennsylvania State University, where she was studying for a M.A. in theater and Jack was working on his Ph.D. in Horticulture and Genetics. Kodie Ann entered their lives in 1990.

Jack's career path took a number of turns following his M.S. studies, first as a staff member, Toxicology Department, Sandoz, Ltd., Basel, Switzerland (1973-1974), then returning, to be Head, Cytogenetics Laboratory, Microbiological Reset Corporation, Bountiful, Utah (1974-1975), and finally Research Associate, Pharmacology Department, Medical College of Virginia, Richmond, Virginia (1975-1976).

In 1980 Jack completed his Ph.D. at The Pennsylvania State University. From 1981 to 1983 he served as Project Associate and Adjunct Assistant Professor of Horticulture, University of Wisconsin-Madison as part of Clint Peterson's pickling cucumber team in the USDA-ARS Vegetable Crops Research Unit and began his association with long-time colleague Phil Simon, ARS carrot and onion breeder and geneticist and, later, Mike Havey, ARS cucumber and onion breeder and geneticist. Jack became a USDA-ARS Research Horticulturist at Madison two years later, focusing on cucumber improvement until the mid-1990s when he began to work also with melon (*Cucumis melo*) and then squash (*Cucurbita*).

Jack's research in the ARS Vegetable Crops Research Unit, Madison involved the development of multiple disease resistant, high fruit number cucurbit germplasm that is basic to the needs of private industry and the consuming public. The research included: 1) the collection, identification, description, and application of unique germplasm in *Cucumis* species; 2) investigations of the genetic, biochemical, and physiological nature of cucumber, melon, and squash (*Cucurbita pepo* L.), and; 3) the modification of cultural practices which exploit the genetic potential of cucumber. His scientific interests in the genetic relationships among wild melon and squash and their commercial counterparts, as well as the relationships among melon market classes were initiated because they were not well defined and improvement for yield and quality in U.S. processing cucumber and Western Shipping melon had plateaued around 1990. The widespread debate concerning the potential use of molecular marker technologies for the use of marker/trait associations for plant improvement spurred his activities in mapping, quantitative trait loci (QTL) analysis, and marker assisted selection (MAS). Jack received tenure as an Associate Professor, University of Wisconsin-Madison in 1989 and Full Professor in 1993 in the Department of Horticulture, where he trained 21 graduate students, and mentored six postdocs and seven visiting scientists. As a research mentor, Jack was an ardent supporter of minority recruitment, especially through the Society for the Advancement of Native Americans and Chicanos in Science (SACSNAS), where he regularly convened professional development courses and trained graduate students that are leaders in science today.

Jack relished his germplasm related activities beginning with a 5-week cucumber and melon germplasm collection expedition in 1992 to the states of Rajasthan, Madhya Pradesh, and Uttar Pradesh, India (Figure 1). This cooperative project under the auspices of Indo-NBPGR and USAID Cooperative Agreement and coordinated by USDA, OICD (October-November 1992 increased the U.S. National cucumber and melon germplasm collection each by 20%. Before this time only 10% of each U.S. collection was from the country of origin or diversity where the greatest source of genetic variation occurs. This was followed by three trips in 2000 related to geneflow of melon. First, a 2-week squash and melon germplasm collection expedition in Mississippi, Arkansas, and Louisiana funded by a grant obtained through the U.S. National Research Initiative that resulted in the description of *Cucumis melo* ssp. *agrestis* var. *texanus*, a potential source of traits for melon. Second, a 2-week melon germplasm collection expedition in Arizona, California, and Mexico funded by a grant obtained through Agritope.

One of his more interesting germplasm projects focused on taxonomic relationships of a rare *Cucumis* species (*C. hystrix* Chakr.) with cucumber that led to their interspecific hybridization, a system for micropropagation for hybrid recovery, reproductive and cytogenetic characterization of interspecific hybrids from *Cucumis hystrix*, backcross introgression of the *Cucumis hystrix* Chakr. genome to cucumber to increase genetic diversity in U.S. processing

cucumber, and release of a *Cucumis hystrix*-derived U.S. processing cucumber inbred backcross line population.

Jack implemented and developed marker systems in *Cucumis* that led to: 1) the genomic characterization and moderately saturated maps in cucumber and melon; 2) characterization of evolutionary relationships in *Cucumis* and *Cucurbita* species; 3) construction of genetic stocks to provide the first estimates of linkage marker/trait relationships (morphological traits, disease resistance loci and yield components (QTL)) in cucumber, and linkage analysis in melon; 4) development of methodologies for germplasm diversity assessment; 5) the use of molecular markers for plant variety protection; 6) development and release of genetic stocks that broadens the genetic base of cucumber; and 7) the successful application of marker-assisted multi-trait selection in cucumber, and made comprehensive efficacy comparisons between marker-assisted and phenotypic selection in a vegetable crop species. Jack was the first to characterize cucumber plant response under soil water deficits and chilling temperatures, defined physiological differences in cucumber varieties to water stress to characterize a calcium-related cucumber fruit disorder, and provided irrigation management information that improved water use efficiency through a systems management approach.

Jack yearned to return to his native west and in 2007 moved with Francy and Kodie to Logan, Utah where he was Supervisory Plant Physiologist and Research Leader of the ARS Forage and Range Research Laboratory (FRRL) and Location Coordinator through his retirement in 2017. Jack was responsible at Logan for the genetics and physiology of fine-leafed *Festuca* species to develop improved selection methodologies and strategies. He was also responsible for planning, conducting, and reporting research in scientific and customer meetings, and establishing domestic and international cooperative research linkages related to Pasture, Forage, Turf, and Rangeland Systems. His creative leadership of FRRL resulted in the strategic expansion of FRRL research in emerging areas of importance, and his scientific accomplishments led to the establishment of research on plant endophytes to enhance abiotic stress tolerance and the initiation of fine-fescue germplasm development for use in greenstrips for wildfire control and low-input turf, ornamental, and rangeland settings.

Jack was elected Fellow of the American Society for Horticultural Science (ASHS, 2001), the Heilongjiang Academy of Agricultural Sciences, China (2007), and was recognized for his contributions to cucurbit breeding with the Lifetime Achievement Award at Cucurbitaceae 2018 (Figure 2). Posthumously he received in 2019 the Award of Excellence from the ASHS Vegetable Breeding Professional Interest Group. Jack served as associate editor of four international journals (HortScience, Cucurbit Genetics Cooperative Report, Journal of Crop Production/Journal of New Seeds, Plant Breeding), and was organizing chairman for 17 national workshops or symposia. He authored 163 manuscripts in refereed journals, 27 published proceedings, 19 germplasm releases, 11 book chapters, two books, 71 technical reports, and made 35 presentations at national horticulture and rangeland meetings or workshops, including 17 at international meetings, of which 11 were invited papers at symposia/congresses or at international technical workshops. He attracted more than $2.5 million in grants from stakeholders, the cucumber and melon seed industry and U.S. cucumber processing companies, USDA-NRI, USAID/FAO/OAS, BARD, and USDA-ARS.

For all his widely ranging research interests, Jack was a passionate friend and colleague. He was known by many for his "interviews," whereby, upon meeting you for the first (and any time thereafter), he would query your background, how you got to be where you were, and among other things, your 5-year plan. Your walk in life did not matter, a scientist, a waiter in a hotel restaurant in Hyderabad, India, the Police Chief in Ratlam, India, or a farmer at a roadside fruit stand along the coast of Oregon, Jack loved to meet and know everyone. The biographical sketch at his memorial service in Logan, Utah in 2019 included one of his many poems, 'I Don't Expect.' A line in the second stanza revealed Jack's heart and soul: "…Because I don't consider you a shadow that follows in the sun. And then vanished in the dark when day is done."

(submitted by Dr. Jim McCreight, USDA-ARS, Salinas California, one of Jack's many friends, professional and personal.)

Figure 1. Dr. Jack Staub (right) and host scientist Dr. M.N. Koppar (left, National Bureau of Plant Genetic Resources, New Delhi) on a cucumber and melon germplasm collecting trip in November 1992. The photograph was taken en route from Kajuraho to Bhopal in the west central area of the state of Madhya Pradesh, India. Two wild melon plants are seen near the creek bed.

Figure 2. Dr. Jack Staub congratulated by colleagues after receiving the Lifetime Achievement Award at Cucurbitaceae 2018, Davis California. Left to right: Rebecca Grumet, Jim McCreight, Jack Staub, Linda Wessel-Beaver, Michael Havey, Amnon Levi.

In memoriam
J. Brent Loy, Plant Breeder (1941-2020)

Dr. James Brent Loy, husband, father, grandfather, friend, mentor, and plant breeder died on July 24, 2020 at age 79. He spent his last days with his family at their New Hampshire home.

Born in 1941, Brent's love of plants and agriculture began as a child in Bountiful, Utah, where he planted and tended his own vegetable gardens from a young age and, as a teenager, worked on a local truck farm before and after school. Brent received his bachelor's degree in horticulture from Oklahoma State University and master's and doctoral degrees in genetics and horticulture from Colorado State University before accepting a professorship at the University of New Hampshire in 1967, where he taught classes in plant genetics and reproduction and vegetable production and mentored many graduate and undergraduate students.

As a researcher, Brent contributed much to our knowledge of cucurbit genetics, physiology, and agronomy, but he considered himself, foremost, a plant breeder. In addition to his deep scientific and practical knowledge, he possessed an intuitive sense for crops and of traits valuable to growers and consumers. His patient and keen eye perceived subtle differences in often-overlooked traits that contributed to the performance of his varieties and, also, gave many of his varieties distinct aesthetic qualities.

During his five decades at UNH, Brent built a rich and diverse cucurbit breeding program that is now the longest lasting in North America. Based at the Woodman and Kingman Research Farms in Durham and Madbury, NH, he developed over 100 varieties of assorted melons, ornamental pumpkins and gourds, winter and summer squashes, interspecific *Cucurbita* hybrids, and hull-less seeded pumpkins. He always sought to improve eating and nutritional quality, agronomic performance, and aesthetic appearance in his crops. In 1981, he released his first variety, 'Autumn Pride', a bush *C. maxima* processing squash that exemplified his work in utilizing the bush trait in a range of squash types and species. Among his other unique breeding achievements are glabrous Slick Pik® yellow summer squash varieties, dioecious cantaloupes, high-eating quality acorn squash varieties like 'Honey Bear' and 'Sugar Bush', and high-yielding hull-less seeded pumpkins. His collaborations with seed companies resulted in the commercialization of dozens of these varieties that can be purchased from most retail seed catalogues and are grown in virtually all market farms in New England, and even throughout the US and beyond.

Brent was passionate about the importance of land grant universities and took his role as a public servant very seriously. He believed strongly in the scientific value of public plant breeding research and worked closely with the UNH Office of Intellectual Property to ensure that intellectual property rights of germplasm extended to breeders, especially since royalties generated from released material were vital in maintaining breeding programs. In fact, Brent's varieties have generated 29% of all of UNH's royalty earnings since 1999. In appreciation of his service, UNH awarded Brent their inaugural Innovator of the Year Award, which is now named after him.

Beyond plant breeding, Brent worked to educate growers and consumers about cucurbit eating quality, particularly in winter squash. He had a knack for communicating the practical applications of scientific findings, and he frequently spoke at growers' conferences about proper management and harvest strategies for achieving optimal eating quality. His broad interest in vegetable production and, specifically, in improving cultural conditions for melon production in New England led to work with plastic mulches and the development of infrared transmitting mulches.

Brent always possessed humility and never sought recognition, but he received many awards during his career, including the 2009 All-America Selections Breeders' Cup Trophy, the 2015 American Society for Horticultural Science Vegetable Breeding Working Group Award of Excellence, the 2000 Pioneer Award of the American Society of Plasticulture, and most recently, the Eastern States Exposition's 2020 New England Fellowship of Agricultural Adventurers Award. Each time he received some honor, Brent only remarked that he was glad to have contributed to agriculture. He valued his association with research and academic groups very much and had great respect for his colleagues. His attitude was not one of competition but collaboration, and he assumed the best intentions in everyone with whom he worked. He was endlessly generous with his knowledge—anyone who had an extended conversation with him learned something about breeding cucurbits.

Brent's persistent curiosity, creativity, and almost childlike wonder, even after a life of expertise, were inspirational. He became completely absorbed in observing his plants in the field or greenhouse and never ceased initiating novel and creative breeding projects, his most recent pursuits involving interspecific squash crosses both for F1 hybrid melon

rootstocks and for introgressing valuable traits among species.

As a friend, Brent was very supportive and laughed easily. He loved to chat, and he spoke of only two things: plant breeding and family. His unconditional love and support for his wife Sarah and their three children, Reed, Laura and Jamie, is evident in the success they have all had in their diverse endeavors. This familial love and loyalty might have been Brent's greatest attribute.

Brent's huge wealth of knowledge and experience will not be easily replaced. His family, friends and colleagues will miss him dearly.

(submitted by Dr. Loy's former field technician and M.S. student Jake Uretsky. Dr. Uretsky is a melon breeder with Sakata Seed America.)

Figure 1. Dr. Brent Loy in 2007 at the Woodman Research Farm in Durham, New Hampshire, USA, in front of plots of egg and spoon gourds (*Cucurbita pepo*). Materials like these were used as a source of genes in Dr. Loy's research on inheritance of fruit color patterns reported in CGC Report 35-36. (photograph courtesy of the University of New Hampshire)