# Cucurbit Genetics Cooperative

2018

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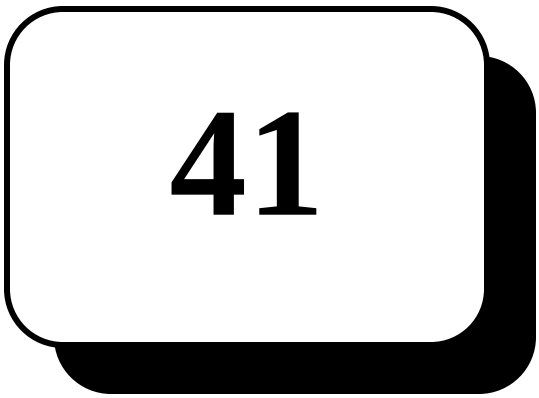


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**On the front cover:** *Cucurbita maxima* L. Francisco Manuel Blanco (1778 – 1845) (O.S.A.) - *Flora de Filipinas. Gran Edición. Atlas II.* 1880 to 1883(?) Image reproduced from the Digital Library of the Royal Botanical Garden of Madrid (CSIC).

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# Contents

# Evaluation of Two Field Inoculation Methods for Testing Resistance to Bacterial Fruit Blotch in Watermelon Fruit

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## Introduction

Bacterial fruit blotch (BFB) caused by *Acidovorax citrulli* is a major seed-borne disease that affects watermelon (*Citrullus lanatus*) seedling and fruit production around the world (5,9). *A. citrulli* can infect all growth stages of the watermelon plant: seeds, seedlings, foliage, flowers, and fruit (5). The scant research on fruit resistance in favor of foliar resistance has three probable explanations: 1- large-scale screening at the fruit stage is resource-intensive (4); 2- foliar resistance may correlate with fruit resistance and, even with susceptible fruit, it may be sufficient for disease control (4); 3- fruit inoculation methods have not been well established. As far as investigated, there have not been any significant evaluations of fruit inoculation methods. An optimized fruit resistance assay would allow researchers to efficiently conduct large-scale screenings, and breed for fruit resistance. Here we report the evaluation of two watermelon fruit inoculation methods under field conditions.

## Materials and Methods

Four group I isolates recovered from the Horticultural Crops Research Station at Clinton, NC in 2016 and stored at -80°C were grown on nutrient agar (NA) (N9405 Nutrient Agar; Sigma-Aldrich, St. Louis, MO) for 48 hrs at 30°C prior to inoculation. The morning of inoculations, bacterial suspensions were created by rinsing *A. citrulli* colonies from the NA plates with sterilized $dH_2O$. The bacterial suspensions from each isolate were diluted with sterilized $dH_2O$ to an OD600 of 0.25 [approximately $10^8$ colony forming units (CFU)/ml] using a spectrophotometer (UV160U; Shimadzu, Columbia, MD). Calibrated isolates were combined in equal portions and serially diluted with sterilized $dH_2O$ to create $10^3$, $10^5$, $10^6$, $10^8$ CFU/ml concentrations. Water controls were created by rinsing unused NA plates with $dH_2O$. Inoculum and water controls were stored at shaded ambient field temperatures (~30°C) immediately prior to use.

In the spring, watermelon cultivars were direct seeded as three-plant-plots into raised beds covered with black plastic mulch at the Horticultural Crops Research Station at Clinton, North Carolina. Rows were 61 m long on 3.1 m centers with 1.2 m spacing between plots and 0.6 m spacing between plants within plots. Plants were spiral trained once just prior to anthesis. Fertilizer and pesticides were applied in accordance with best practices for the area.

We tested 96 plots for each inoculation method consisting of alternating 'Charleston Gray' (CG) (Willhite Seed Inc., Poolville, Texas) and 'Sugar Baby' (SB) (Sakata Seed America, Morgan Hill, CA); each with 10 replications per treatment combination and 16 water control plots. For spray inoculations, immature fruit were sprayed to run off with inoculum and, to decrease the possibility of escapes, resprayed 3 to 4 days later. Exact timing of fruit age in the field was not feasible, so physiological immaturity markers typical of early maturing fruit 1-2 weeks post anthesis were used to gauge fruit readiness: fruit size generally ranging 5 to 13 cm in diameter and, most importantly, a glossy, soft wax cuticle. For fruit injections, we utilized the method described by Burdman et al. (2005) with slight variations; briefly, mature watermelons were identified by the die-back of the primary tendril. At least three watermelon fruit per plot were injected 1 cm deep with 1 ml of bacterial suspension at two equidistant locations along the length of the fruit using a 0.45 mm needle (3ml Sub-Q Syringe with Luer-Lok Tip with BD PrecisionGlide Needle; BD Becton, Dickinson and Co., Franklin Lakes, NJ). Sterilized water-injected fruits were used as negative controls.

Disease severity for both methods was evaluated 21 days post inoculation. Spray inoculations were rated using a 0 to 9 scale based on the percentage of the upper fruit surface showing symptoms and injection inoculations were rated using the scale described by Burdman et al. (2005) and Walcott et al. (2004): 0, no symptoms; 1, small rind surface lesions; 2, large lesions penetrating into the rind; 3, large lesions penetrating into the fruit; 4, partial collapse of the fruit tissue; 5, complete fruit rot.

Statistical analyses were performed separately on the two methods using the R environment (R Development Core Team 2017). Type II analysis of variance was conducted using the 'car' 2.1-3 package (Mendiburu 2016) *Anova()* function to compare the plot disease severity for each cultigen, inoculum treatment, and interaction. A post-hoc

Tukey HSD test was conducted using the 'lsmeans' 2.26-3 package (Lenth 2016) *lsmeans()* and *cld()* functions to compare injection-inoculated inoculum concentrations ($p < 0.05$). Data were visualized using the 'ggplot2' 2.2.1 package (Wickham 2009) *ggplot()* function.

## Results and Discussion

Among the spray-inoculated fruit, all inoculum concentrations produced statistically indistinguishable results, and cultigen was the only significant effect for disease severity ($F[1,55] = 12.65$, $p < 0.001$) with no significant interaction with concentration. Cultigens, over all concentrations combined, indicated that the mean SB disease severity (1.4, SEM = 0.42) was significantly different from the mean CG disease severity (3.5, SEM = 0.44) (Figure 1), matching expectations for these cultivars based on our prior observations. However, cultigens analyzed at each concentration were not significantly different at the $p = 0.05$ level. Spray inoculated water controls were asymptomatic and were excluded from the statistical analysis.

Injection-inoculated watermelons showed no symptoms, significant blotching around the injection sites, or split along the length of the fruit as if the fruit had been sliced open. In addition to the high incidence of splitting, the injection-inoculated fruit exhibited differences compared to the spray inoculation results. First, water controls showed significant damage, likely caused by exogenous surface pathogens being either introduced into the fruit at the time of inoculation or later ingress. Second, post-hoc Tukey HSD tests of the inoculum concentrations and water control showed that the lowest concentration tested, $10^3$ CFU/ml, produced disease severity similar to the water control and significantly less severe than the higher concentrations (Figure 2). All other concentration comparisons were not significantly different from each other. Third, cultigen across all inoculation concentrations combined was significant in the opposite direction ($F[1,82] = 8.05$, $p < 0.01$), with CG having a slightly lower mean disease severity than SB, 3.3 (SEM = 0.17) and 4.1 (SEM = 0.18), respectively (Figure 3). Cultigen by concentration interaction was not significant.

Between the two methods presented here, our data showed that the spray-inoculation method was the most reproducible, differentiated the cultigens according to our expectations, provided the highest consistency, and was relatively easy to administer. In addition, spray inoculation mimics natural infection. We also evaluated other ineffective spray inoculation methods not reported here: fruit spray inoculation under wounded vs unwounded and bagged vs unbagged conditions on 1-2-week-old immature fruit and fruit at anthesis (2). Although further testing would be required, the spray-inoculation method may be extendable to other cucurbits. Also, SB was relatively resistant compared to CG in the spray test, but not in the injection test, suggesting that fruit surface features may contribute important resistance, such as stomatal plugging observed by Frankle and Hopkins (1993). However, this requires further research.

## Acknowlgements

Financial support for this work was provided by the NC Agricultural Research Service and the USDA-ARS Cucurbit Crop Germplasm Committee.

## Literature Cited

1. BURDMAN, S., KOTS, N., KRITZMAN, G. & KOPELOWITZ, J. 2005. Molecular, physiological, and host-range characterization of *Acidovorax avenae* subsp. *citrulli* isolates from watermelon and melon in Israel. *Plant Disease,* 89**,** 1339-1347.
2. DALEY, J. D. 2018. Screening for Resistance to Bacterial Fruit Blotch in Watermelon Fruit (Doctoral Dissertation). Retrieved from http://www.lib.ncsu.edu/resolver/1840.20/35395.
3. FRANKLE, W. G., HOPKINS, D. L. & STALL, R. E. 1993. Ingress of the watermelon fruit blotch bacterium into fruit. Plant Disease, 77, 1090-1092.
4. HOPKINS, D. L. & THOMPSON, C. M. 2002. Evaluation of Citrullus sp. Germ Plasm for Resistance to *Acidovorax avenae* subsp. *citrulli*. *Plant Disease,* 86**,** 61-64.
5. LATIN, R. X. & HOPKINS, D. L. 1995. Bacterial fruit blotch of watermelon - the hypothetical exam question becomes reality. *Plant Disease,* 79**,** 761-765.
6. LENTH, R. V. 2016. Least-Squares Means: The R Package lsmeans. Journal of Statistical Software, 69, 1-33.
7. MENDIBURU, F. D. 2016. agricolae: Statistical Procedures for Agricultural Research. R package version 1.2-4.
8. R DEVELOPMENT CORE TEAM 2017. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
9. SCHAAD, N. W., POSTNIKOVA, E. & RANDHAWA, P. 2003. Emergence of Acidovorax avenae subsp. citrulli as a crop threatening disease of watermelon and melon.
10. WALCOTT, R. R., FESSEHAIE, A. & CASTRO, A. C. 2004. Differences in pathogenicity between two genetically distinct groups of Acidovorax avenae subsp. citrulli on cucurbit hosts. Journal of Phytopathology, 152, 277-285.
11. WICKHAM, H. 2009. ggplot2: Elegant Graphics for Data Analysis, Springer Publishing Company, Incorporated.

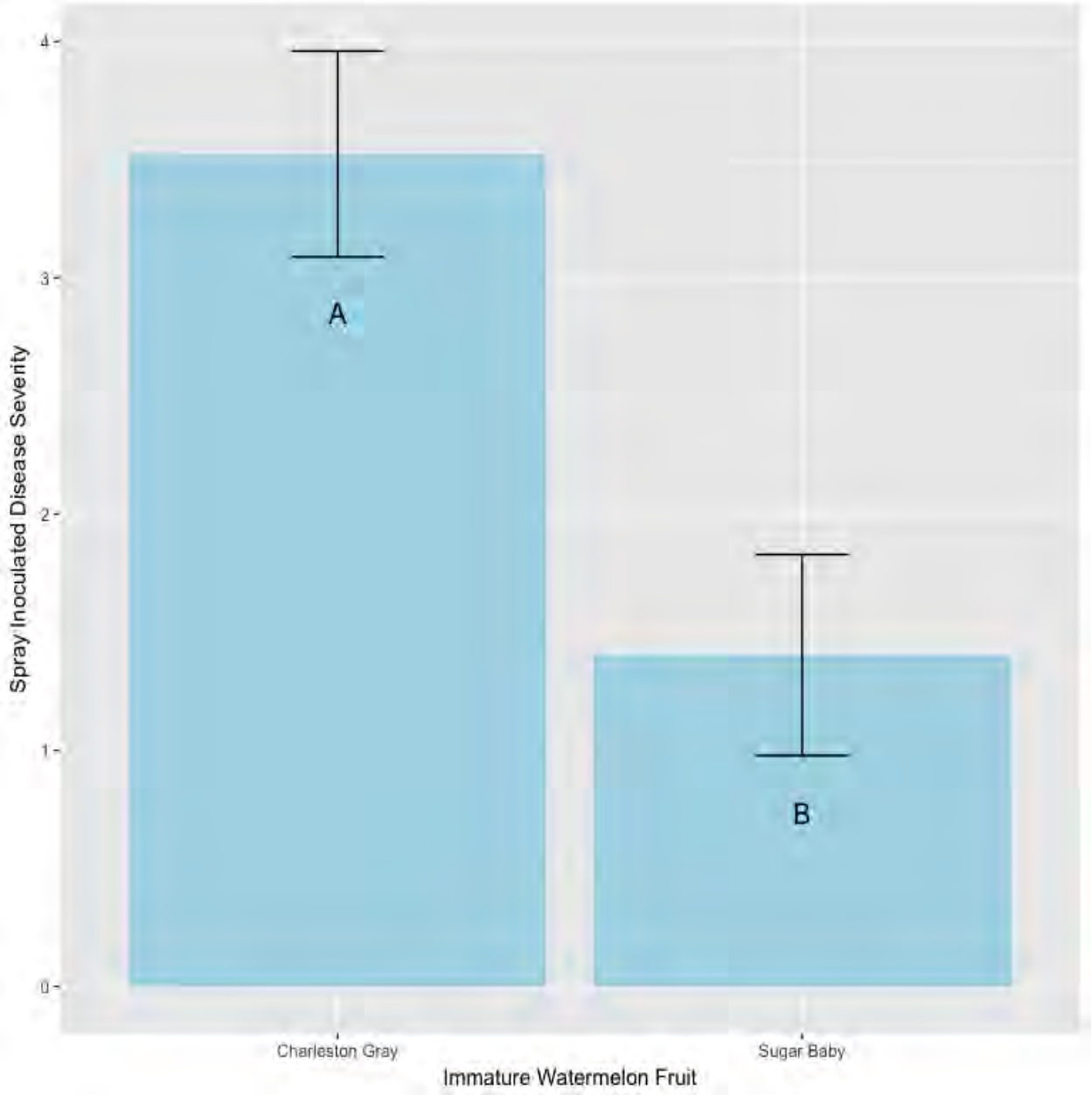


**Figure 1. Predicted spray-inoculated disease severity of immature Charleston Gray (CG) (n = 31, mean = 3.5, SEM = 0.44) and Sugar Baby (SB) (n = 32, mean = 1.4, SEM = 0.42) fruit 21 dpi of all inoculum concentrations combined (~$10^8$ CFU, CG [n=6] and SB [n=8]; ~$10^6$ CFU, CG [n=9] and SB [n=9]; ~$10^5$ CFU, CG [n=8] and SB [n=7]; ~$10^3$ CFU, CG [n=8] and SB [n=7] rated on a 0-9 scale corresponding to percent surface symptoms. Different letters indicate a significant difference according to the type II ANOVA ($F[1, 55] = 12.65$, $p < 0.001$). Error bars represent the SEM.**

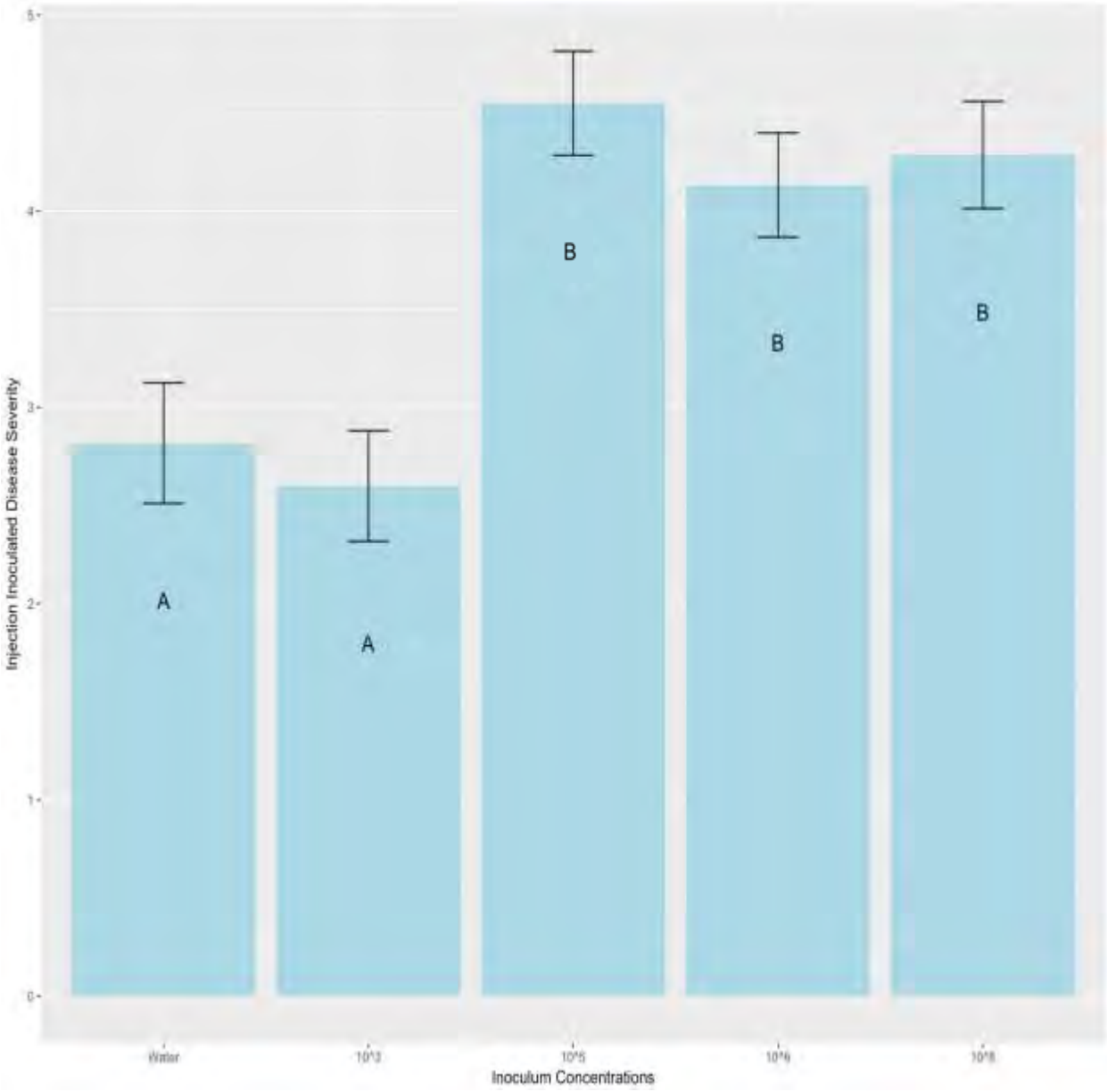


**Figure 2. Predicted injection-inoculated disease severity (mean ± SEM) of immature Charleston Gray (CG) and Sugar Baby (SB) fruit 21 dpi combined across five inoculum concentrations and water: ~$10^8$ CFU [n=19, mean = 4.3, SEM = 0.27]; ~$10^6$ CFU [n = 20, mean = 4.1, SEM = 0.27]; ~$10^5$ CFU [n = 20, mean = 4.6, SEM = 0.27]; ~$10^3$ CFU [n = 18, mean = 2.6, SEM = 0.28]; $dH_2O$ [n = 15, mean = 2.8, SEM = 0.31] rated on a 0-5 scale. Different letters indicate a significant difference according to Tukey HSD test (p = 0.05). Error bars represent the SEM.**

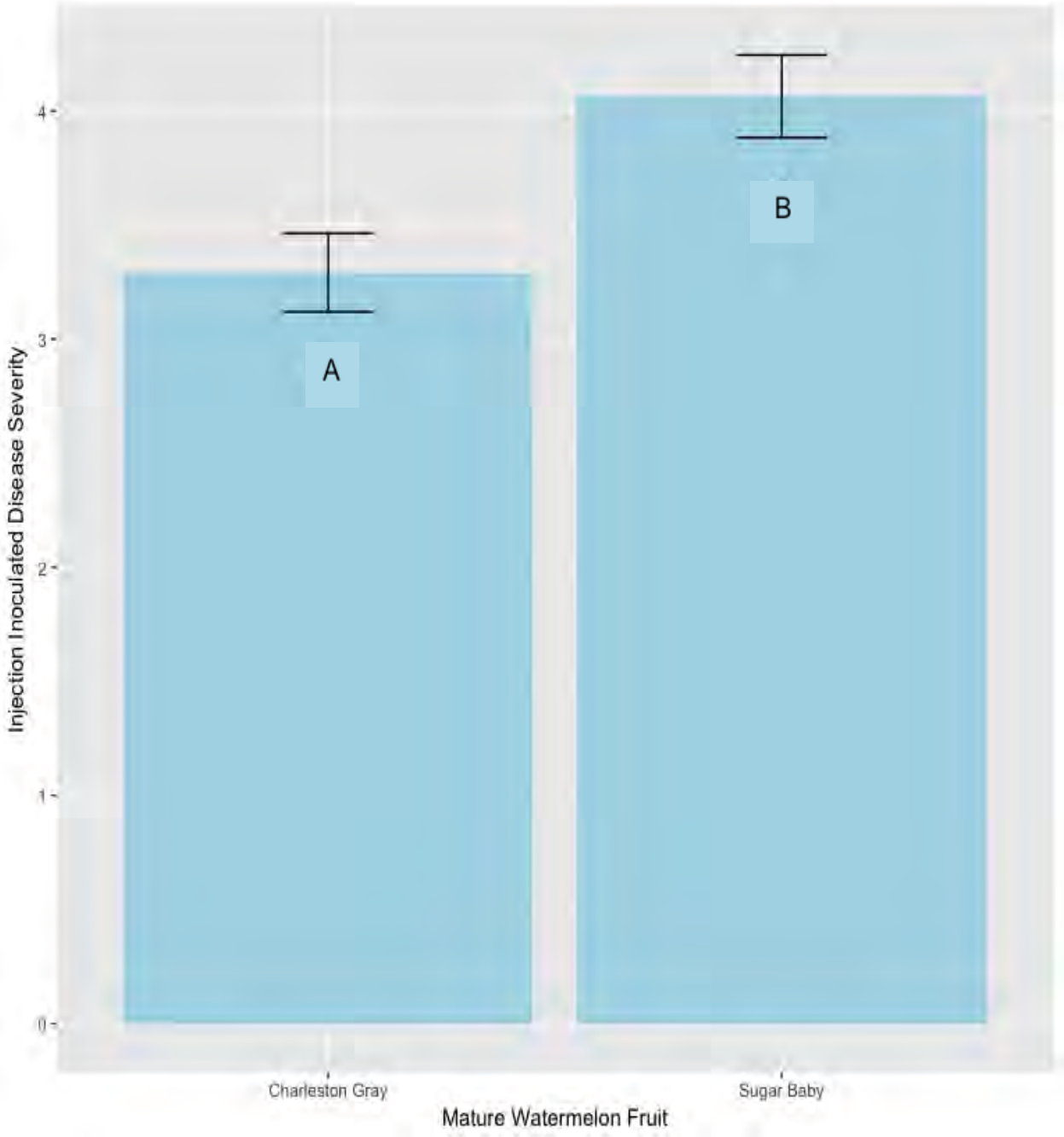


**Figure 3. Predicted injection-inoculated disease severity (mean ± SEM) of immature Charleston Gray (CG) (n = 40, mean = 3.3, SEM = 0.17) and Sugar Baby (SB) (n = 37, mean = 4.1, SEM = 0.18) fruit 21 dpi of all inoculum concentrations combined (~$10^8$ CFU, CG [n=10] and SB [n=9]; ~$10^6$ CFU, CG [n=10] and SB [n=10]; ~$10^5$ CFU, CG [n=10] and SB [n=10]; ~$10^3$ CFU, CG [n=10] and SB [n=8] rated on a 0-5 scale. . Different letters indicate a significant difference according to the type II ANOVA ($F[1, 82] = 8.05$, $p < 0.001$). Error bars represent the SEM.**

# Innovation Research on Germplasm Resources of Watermelon with Resistance to *Fusarium* Wilt

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## Introduction

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* is a destructive soil-borne disease of watermelon. Breeding for resistance is an effective strategy to circumvent this disease. Artificial inoculation technique is mainly used to select plants resistant to *Fusarium* wilt. However, environmental and inoculation conditions may interfere with identification and selection for resistant plants. Molecular markers 7716_fon and InDel1_fon1 are closely linked to *Fusarium oxysporum* f. sp. *niveum* (*Fon*) race 1 resistance gene locus *Fon-1*. These two markers were used in this study for the development of watermelon lines resistant to *Fusarium oxysporum* race 1.

## Materials and Methods

'Calhoun Gray', 'F211' and BW85' were used as the male donor parents of the *Fon-1* locus associated with resistance (Table 1), and the cultivated susceptible watermelon variety was used as the recurrent female parent (Table 2). $BC_4F_2$ plants homozygous for the 7716_fon and InDel1_fon1 markers (Table 3) were selected and self-pollinated in two generations to produce $BC_4F_4$ plants.

Thirty plants chosen from each $BC_4F_4$ lines were tested for *Fon* race 1 resistance. Fungal inoculation was carried out using conidial suspensions of Fon race 1 adjusted to $5\times10^6$ conidia per 1 ml. Watermelon seedlings with both cotyledons open were root-dipped into the conidial suspension for 15 min, and all seedlings were replanted in sterilized soil in plastic pot (6.7×6.7×7.2cm).

Plants were evaluated 15 days after inoculation for symptom severity using a disease index on a scale of 0–5: 0= asymptomatic plants; 1= plants showing initial wilting on one leaf; 2= plants showing continued wilting in more than one leaf; 3= plants with all the leaves wilted; 4= plants with all leaves wilted and stems collapsing; and 5= dead plants.

Disease Index =100×∑（Number of plants in each score × score/( No. of total plants×5)

Disease incidence = {(No. of plants which score≥3)/No. of total plants} ×100%

## Results

The backcross generation foreground selection was carried out using molecular marker detection, and screening in a natural disease nursery. The heterozygous *Fon-1* $BC_1F_1$ plants selected by using the *Fon-1* markers were planted in a natural disease nursery, which had different survival rate. Eight lines expressed high resistance with disease rate under 20.0 %, and seven lines with disease rate of 22.2-50.0% (Figure 1). The surviving $BC_1F_1$ plants were backcrossed with their respective recurrent parents. Eight $BC_4F_4$ selected lines that carried the homozygous resistant genotypes had similar agronomic performance such as fruit shape and weight (Table 4) to the respective recurrent parents, and which expressed higher resistance than recurrent parents (Table 4). Our team has bred several watermelon lines with sufficient fruit quality and high resistance to *Fon* race 1 using the selected lines. Among them, the varieties 'Longshengjiali' expressed higher resistance than 'Xinong No. 8' in indoor seedling with artificial inoculation (Table 4 and Figure 2), and it expressed similar resistance with 'Xinong No. 8' in the natural disease nursery (Figure 3).

## Acknowledgements

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## References

1. Li Na, Wang Jiming, Shang Jianli, Li Nannan, Xu Yongyang, Ma Shuangwu. 2017. Fine-Mapping of QTL and Development of InDel Markers for *Fusarium oxysporum* Race 1 Resistance in Watermelon, Scientia Agricultura Sinica. 50:131-141.

2. Zhang Yi, Zhang Haiying, Guo Shaogui, Ren Yi, zhang Jie, Geng Lihua, Liang Zhihuai, Xu Yong. 2013. Developments of Molecular Markers Tightly Linked to *Fon-1* for Resistance to *Fusarium oxysporum* f. sp. *Niveum* Race 1 in Watermelon, Scientia Agricultura Sinica. 46:2085-2093.

**Table 1. The characteristics of the donor parents.**

| Donor parent | Origin | Type | Fruit shape | Fruit weight (kg) | Molecular Identification (genotype) | Inoculation identification (phenotype) | Disease incidence (%) | Disease Index |
|---|---|---|---|---|---|---|---|---|
| Calhoun Gray | USA | Calhoun Gray | oval | 8.5 | resistant | resistant | 7.14 | 5.71 |
| F211 | USA | Sugarlee | round | 8.2 | resistant | Resistant | 21.43 | 20.00 |
| BW85 | USA | Sugarlee | round | 8.4 | resistant | Resistant | 23.08 | 20.67 |

**Table 2. The characteristics of the recurrent parent.**

| Recurrent parent | Origin | Fruit shape | Fruit weight (kg) | Molecular Identification (genotype) | Inoculation identification (phenotype) | Disease incidence (%) | Disease Index |
|---|---|---|---|---|---|---|---|
| BWM81 | Japan | round | 5 | susceptible | susceptible | 90.00 | 88.00 |
| BW88 | China | round | 7.5 | susceptible | susceptible | 93.33 | 93.33 |
| H35 | China | round | 1.5 | susceptible | susceptible | 90.00 | 90.00 |
| BW40 | China | round | 5 | susceptible | susceptible | 86.67 | 88.67 |
| KW131 | Korea | round | 5 | susceptible | susceptible | 73.33 | 76.67 |
| BWB051 | China | round | 6 | susceptible | susceptible | 93.33 | 86.67 |
| K10197 | Korea | oval | 8 | susceptible | susceptible | 96.67 | 96.00 |

**Table 3. Molecular markers for foreground selection.**

| Markers | Types | Chromosome | genetic distance (cM) | References |
|---|---|---|---|---|
| 7716_fon | CAPS | 1 | 0.8 | Zhang Yi et al., 2013 |
| InDel1_fon1 | Indel | 1 | 0.8 | Li Na et al. ,2017 |

**Table 4. Fruit shape and weight and disease incidence and index of selected $BC_4F_4$ lines and resistant watermelon varieties inoculated with *Fusarium oxysporum* f. sp.*niveum* (FON) race 1.**

| $BC_4F_4$ lines or Varieties | Fruit shape | Fruit weight (kg) | Disease incidence (%) | Disease Index | Donor parents | Recurrent parent |
|---|---|---|---|---|---|---|
| 113 | round | 5 | 89.66 | 82.76 | Calhoun Gray | BWM81 |
| 229 | round | 7.5 | 73.33 | 78.67 | F211 | BW88 |
| 153 | round | 1.5 | 56.67 | 55.33 | BW85 | H35 |
| 266 | round | 5 | 40.00 | 43.33 | BW85 | BW40 |
| 364 | round | 5 | 66.67 | 66.67 | Calhoun Gray | BW40 |
| 179 | round | 5 | 26.67 | 29.33 | Calhoun Gray | KW131 |
| 191 | oval | 8 | 20.00 | 16.67 | Calhoun Gray | K10197 |
| 2911 | oval | 8 | 33.33 | 36.00 | BW85 | K10197 |
| Longshengjiali | oval | 8.7 | 14.81 | 13.33 | | |
| Xinong No. 8 | oval | 8.9 | 40.00 | 40.00 | | |

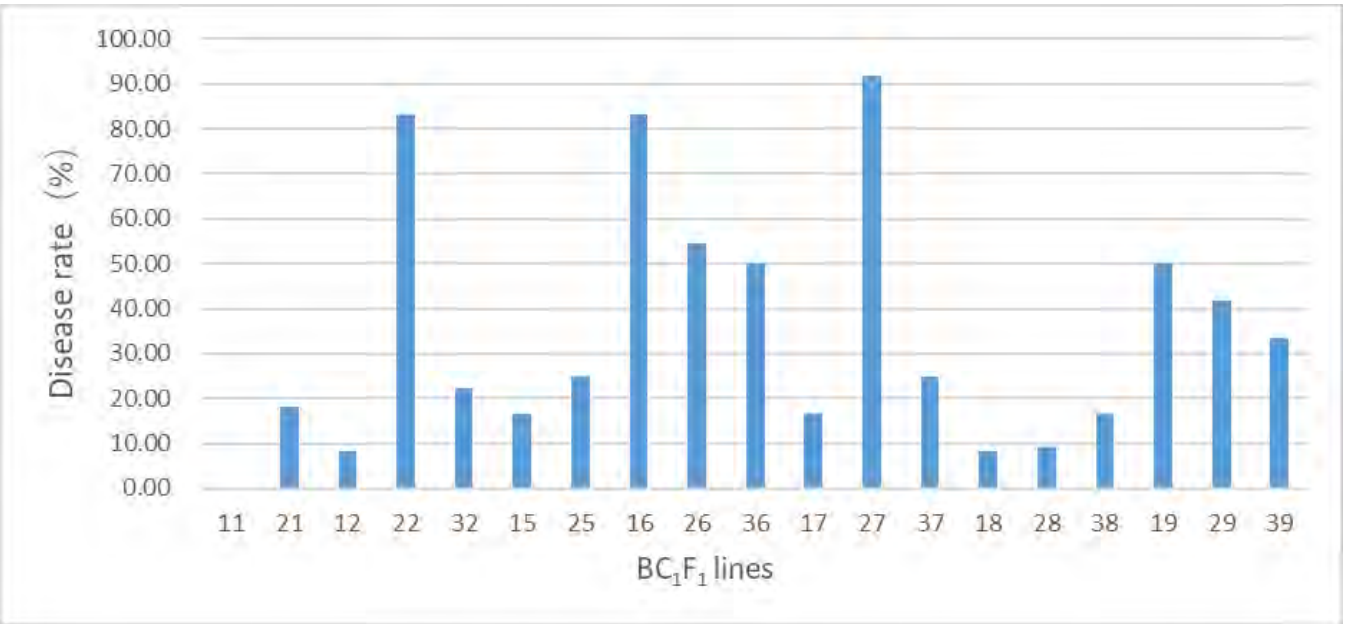


**Figure 1. Disease rate of BC1F1 lines in the natural disease nursery.**


**Figure 2. Disease development in resistant watermelon varieties 15 days after inoculation with *Fusarium oxysporum* f. sp. *niveum* (FON) race 1. A. Longshengjiali. B. Xinong No. 8.**

**Figure 3. Watermelon varieties Xinong No. 8 (left) and Longshengjiali (right) resistant to Fusarium wilt in a natural disease nursery.**

# Segregation and Linkage of 14 Loci in Cucumber

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## Introduction

Cucumber (*Cucumis sativus* L. var. *sativus*, 2n = 2x = 14) gene mutants and their linkage relationships have been studied extensively in the past 25 years. A total of 199 gene mutants have been reported for cucumber, although many are no longer available (Pierce and Wehner, 1990; Weng and Wehner, 2017). These gene mutants were identified and grouped into cotyledon and leaf, flower, fruit, plant architecture, disease and insect resistant, abiotic stress tolerance and miscellaneous mutants (Weng and Wehner, 2017). In the late 80's and early 90's, several genetic maps were constructed using morphological traits, helping to provide a better understanding of genetic structure of the cucumber genome (Fanourakis and Simon, 1987; Kennard *et al*., 1994; Vakalounakis, 1992). Fanourakis and Simon (1987) placed 13 of 15 economically important cucumber traits into three linkage groups. Genetic maps were also constructed using isozymes (Knerr and Staub, 1992), and combinations of isozymes and phenotypic markers (Meglic and Staub, 1996), and molecular markers [restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD)], and phenotypic markers (morphological or disease resistance) (Kennard *et al*., 1994; Serquen *et al*., 1997). In the past two decades, more than ten genetic maps were constructed (e.g., Park *et al*. 2000; Young *et al*., 2000; Bradeen *et al*., 2001; Fazio *et al*., 2003; Sakata *et al*., 2005; Heang *et al*., 2008; Fukino *et al*., 2008; Yuan *et al*., 2008) and the majority of the molecular markers placed on those low-resolution genetic maps were RAPDs, AFLPs (amplified fragment length polymorphism) or SRAPs (Sequence-related Amplified Polymorphism). Recently, the sequencing of the whole cucumber genome (Huang *et al*., 2009) made it possible to use more breeder friendly molecular markers such as microsatellites (simple sequence repeats, SSR) for genetic mapping and marker-assisted selection (MAS) in cucumber. The usefulness of these cucumber microsatellite markers has already been demonstrated in several recent linkage maps (Ren *et al*., 2009; Weng *et al*., 2010; Miao *et al*., 2011; Zhang *et al*., 2012).

The estimated ~~cucumber genome length~~ ranges from ~712 to ~1016 cM (Yuan *et al*., 2008; Miao *et al*., 2011; Yang *et al*., 2012; Zhang *et al*., 2012; Xu *et al*., 2015). The first genetic linkage maps built by Fanourakis and Simon (1987), Knerr and Staub (1992), Vakalounakis (1992), Kennard *et al*. (1994), Meglic and Staub (1996), and Serquen *et al*. (1997) spanned 168, 185, 95, 766, 584 and 628 cM respectively. In 1992, Knerr and Staub assigned 12 of 14 isozyme loci in cucumber to four linkage groups. In 1994, Kennard et al. constructed two genetic linkage maps, a 58-point map using a narrow cross (*C. sativus* var. *sativus* x *C. s.* var. *sativus*) and a 70-point map using a wide cross (*C. sativus* var. *sativus* x *C. s.* var. *hardwickii* (R.) Alef.). They identified ten linkage groups in cucumber using RFLP, RAPD, isozyme, morphological, and disease resistance markers. The narrow and wide crosses spanned a genomic length of 766 cM and 480 cM with an average distance between loci of 21 cM and 8 cM, respectively. Later on, 14 morphological markers were found to be linked to isozyme loci, and were integrated to form a map containing four linkage groups (Meglic and Staub, 1996). In 1997, a map was constructed in a cross of *C. sativus* var. *sativus* (G421 x H-19). It consists of 80 RAPD and 3 morphological marker loci that span ~600 cM over nine linkage groups (Serquen *et al*., 1997). That map was useful for identifying markers linked to femaleness (*F*), determinate plant habit (*de*), littleleaf (*ll*), and quantitative trait loci for yield components. During last decade, consensus and integrated genetic linkage maps built by Park *et al*. (2000), Sakata *et al*. (2005), Heang *et al*. (2008), Fukino *et al*. (2008), Yuan *et al*. (2008), Ren *et al*. (2009) and Weng *et al*. (2010) spanned 816, 533, 570, 626, 1016, 573 and 400 cM, respectively. Weng *et al*. (2010) developed an intra-varietal genetic map in cucumber by placing 182 molecular markers, SSR and SCAR (sequence characterized amplified region), and two phenotypic markers (*ll* for littleleaf and *de* for determinate growth habit) on a genetic linkage map of a RILs population developed from two cucumber inbred lines, Gy7 and H-19. To date, high-density genetic linkage maps were built by Miao *et al*. (2011), Zhang *et al*. (2012), Wei *et al*.

(2014) and Xu *et al*., (2015). These maps consistently showed seven linkage groups and span 712, 749, 891 and 846 cM respectively. In the Miao *et al*. (2011) study, four fruit epidermal feature-related genes, *u*, *d*, *H* and *fr* (no fruit ribbing) were found to be tightly linked loci in Chromosome 5, and the other three (*F*, *bi* and *v-1*(virescent leaf)) were placed in different locations of Chromosome 6. These genetic linkage maps provide an essential framework for genetic analysis, genome assembly and QTL fine mapping in cucumber (Zhang *et al*., 2012 and Xu *et al*., 2015). However, more linkage information between loci controlling economically important traits is needed to merge it into existing linkage groups and provide a more saturated map in cucumber.

Many genes controlling the inheritance of morphological traits (Table 1) have been studied in cucumber (Pierce and Wehner, 1990; Wehner, 1993; Wehner and Staub, 1998a and 1998b), and have been useful in improving cultivars (Peterson, 1975) for seedling vigor (*rc-2*), vine type (*dvl, ll, sp*), sex expression (*df, F*), fruit quality (*B, bi, bi-2, D, ns, ss, Tu, u*) and disease resistance. For instance, close linkage between resistance to scab and fusarium wilt has facilitated the incorporation of both resistances in elite lines by breeders (Vakalounakis, 1993).

Fanourakis and Simon (1987) detected linkage among some of those genes (*ns--ss--Tu--D--u--pm--te*). Meglic and Staub (1996) used isozyme markers to map disease resistance and morphological traits in cucumber. Although some traits (*bi--F--df, cla--dm--cp*; Fanourakis and Simon, 1987) were loosely (>25 cM) linked, some relatively tight (<15 cM) linkages were detected (*Pe--u--Tu--D*, Fanourakis and Simon, 1987; *B*--peroxidase isozyme, *dm*--phosphoglucomutase isozyme, Meglic and Staub, 1996).

The objective of this study was to determine the segregation and linkage relationships among 14 genes controlling morphological traits in cucumber. We were especially interested in detecting and estimating linkage (cM) of four gene mutants: *bl* (Wehner and Staub, 1998; Guner and Wehner, 2004; Weng and Wehner, 2017), *rc-2* (Wehner *et al*., 1998b); *ll* (Wehner *et al*., 1987); and *sp* (Zijlstra and den Nijs, 1986).

## Materials and Methods

*Plant material*. Four inbred lines with contrasting morphological traits were selected to generate three cucumber families. The families were developed from crosses between NCG-089 × WI 2757, NCG-092 × WI 2757, and NCG-093 × WI 2757 at greenhouses in Raleigh, North Carolina (Table 1). For each family, six progenies were obtained ($P_aS_1$, $P_bS_1$, $F_1$, $F_2$, $BC_1P_d$, and $BC_1P_r$), and 45 $S_1$ (of each parent), 54 $F_1$, 162 $F_2$, and 81 $BC_1$ (to each parent) plants evaluated in each family.

*Plant growth.* Seeds were planted in wooden flats containing vermiculite at the greenhouses in early May. After one week and a half (cotyledon stage), seedlings were transplanted to the field at the University of Wisconsin Experiment Station, Hancock, WI. Plants were spaced 0.7 m apart in rows 1.5 m apart. Standard cultural practices for cucumber production in Wisconsin were used in this study.

*Data collection*. Fourteen morphological gene loci controlling economically important traits were evaluated at different growth stages: a) seedling: *bi*, *bi-2*, *sp*, and *rc-2*; b) vegetative: *dvl* and *ll*; c) flowering: *df* and F; and d) fruiting: *B*, *D*, *ns*, *ss*, *u*, and *Tu*. Evaluations were made on seedling traits before transplanting, on vegetative traits 30 to 40 days after transplanting, and on flowering and fruits traits 45 to 70 days after transplanting. The Bitterfree (*bi*) and bitterfree 2 (*bi-2*) traits were evaluated by tasting the cotyledons of single plants, while the mutants *sp* (Zijlstra and den Nijs 1986) and *rc-2* (Wehner *et al*., 1998b) were observed and compared to the parental controls. For the divided leaf (*dvl)* trait, plants were classified as divided if the true leaves were either partly or fully divided, making the leaves appear to be compound. The little leaf (*ll*) trait was evaluated by rating leaf size at the 4th node from the terminal whorl against the parent controls (Wehner *et al*., 1987). Plants were classified for flowering traits as having early (*Df*, ≤ 55 days after transplanting) or delayed (*df*, >55 days after transplanting) flowering habit, and gynoecious (*F*) or monoecious (*f*) sex expression (Della Vecchia and Peterson 1984). Fruits were classified as black (*B*) or white (*b*) spines, dull (*D*) or glossy (*d*) skin, few (*Ns*) or numerous (*ns*) spines, large (*SS*) or small (*ss*) spines, mottled (*U*) or uniform (*u*) color, and tuberculate (*Tu*) or smooth (*tu*) skin (Pierce and Wehner 1990).

*Data analysis.* $F_2$ and $BC_1P_r$ data of each progeny was treated as dominant phenotypic markers??? and analyzed using two different methods described in two different computer programs: SASGENE 1.2 (Liu *et al*., 1997) and R/qtl (Broman *et al*., 2003). SASGENE 1.2 consists of two SAS macros that determined genetic linkage among gene pairs in both coupling and repulsion phases. The first macro, SGENE, tested each of the phenotypic markers for monogenic inheritance. Only phenotypic markers that followed monogenic inheritance were used to detect and estimate linkage between gene pairs. The second macro, LINKAGE, performed the pair gene linkage analysis. Chi-square tested gene pairs for independent assortment in the $F_2$ and $BC_1$ progenies, and recombination frequencies (r) and standard errors (SE) were calculated using the formulae by Sinnott

and Dunn (1939) and Weir (1994). The R/qtl package was also used to determine linkage among pair genes (Broman *et al*., 2003). We used the function "geno.table" in the "qtl" library of R to test for segregation distortion (monogenic inheritance) at all phenotypic markers. Only phenotypic markers that followed monogenic inheritance were used to detect and estimate linkage between gene pairs. Then, with the "est.rf" function we calculated recombination fractions ("r") and logarithm of the odds (LOD) scores to determine linkage between gene pairs. A theoretical LOD score of 3.0 and a maximum distance of 50 cM (test of r = 0.5) were used as linkage threshold to detect linkage between gene pairs (Lander and Botstein, 1986; Broman *et al*., 2003).

## Results and Discussion

The genetic distance between two loci is measured by the probability of recombination frequency between them (Broman *et al*., 2003). This measurement has no dimension and is designated by a lower case letter "r" (Ziegler and Konig, 2005). When two loci are located on different chromosomes, they only have 50% chance of being inherited together from generation to generation, and recombinant and parental types are expected in equal proportions. Thus, recombination frequency "r" can take values from 0.0 to 0.5. When the recombination frequency between loci is zero, the two loci are completely linked and recombinant types are not observed in the progeny. When recombinant frequency is ≥ 0.5, there is free combination between the loci and recombinant types are observed in the progeny (Ziegler and Konig, 2005; Vinod, 2011). In our study we show evidence of linkage between 10 gene-pairs in cucumber bases on estimates of "r" using two powerful statistical software i.e. SASGENE and R/qtl.

*Single locus goodness-of-fit test.* The analysis showed that all 14 traits (Table 1) fitted the single-locus inheritance model in at least one of the progenies (i.e. $F_2$ and/or $BC_1P_r$) of the three families (data not presented). Only phenotypic data that fitted a single-locus inheritance model were used in the gene-pair linkage analysis.

As expected, we also observed that the $F_1$ from the cross of two bitterfree lines, NCG-093 × WI 2757, was bitter indicating that there were two recessive genes (Table 1) controlling the bitterfree trait in cucumber. The second gene for bitterfree contributed by NCG-093 was characterized, and named *bi-2* (Wehner *et al*., 1998a) but later re-named *bl* (Weng and Wehner, 2017).

*Unlinked genes.* More than 100 combinations of gene pairs were segregating for 14 genes evaluated in the three families. There were 33 gene pairs that segregated independently (data not shown) as follows: *B--ns*, *B--u*, *bi--B*, *bi--D*, *bi--df*, *bi--dvl*, *bi--F*, *bi--ns*, *bi--ss*, *bi--Tu*, *bi--u*, *D--ns*, *df--D*, *df--ns*, *df--ss*, *df--Tu*, *df--u*, *dvl--B*, *dvl--F*, *dvl--ns*, *dvl--u*, *F--B*, *F--D*, *F--ns*, *F--Tu*, *F--u*, *ll--D*, *ll--ns*, *ll--Tu*, *ll--u*, *rc-2--D*, *rc-2--df*, and *rc-2--ns*. The SASGENE output file indicated that the $F_2$, and $BC_1P_r$ observed segregation ratios of these 33 gene pairs fitted the expected 9:3:3:1 ($F_2$) and 1:1:1:1 ($BC_1$) ratios indicating that independent assortment occurred. In the case of R/qtl, these 33 gene pairs showed LOD scores lower than 3.0 (Lander and Botstein, 1986; Broman *et al*., 2003).

*Gene pair linkage*. The two computer program methods detected and estimated linkage among 10 gene pairs (Table 2). Although both computer program methods identified similar linkage arrays, the map distances slightly varied in some gene pairs. Both computer program methods identified two new linkage pairs: *bi--ll* and *rc-2--sp*. In the $F_2$ progeny of the family 2 (NCG-092 × WI 2457), *bi* and *ll* genes were found linked at a map distance of 21 cM. In the $F_2$ and $BC_1$ progenies of the family 3 (NCG-093 × WI 2457), *rc-2* and *sp* genes were found linked at a map distance of 19-20 cM and 25 cM respectively (Table 2). Interestingly, we found a new map distance of 27 cM between *ns* and *u* genes that was previously reported by Walters *et al*. (2001). Additionally, seven previously reported linked gene-pairs were confirmed in this study using both computer program methods i.e. *F--df*, *n--ss*, *ns--Tu*, *ss--u*, *Tu--u*, *Tu--D*, and *u--D* (Table 2).

A basic assumption determining the conversion of recombination frequency ("r") to map distance is that the probability of a recombination event (crossover) is proportional to the length of the chromosomal region between loci. Then, the map distance of a chromosomal segment can be defined as the expected number of recombination events taking place in this segment (Lynch and Walsh, 1998). The dimension of map distance is the Morgan (1 M=100cM), and is defined as the probability of one recombination event per meiosis. Then, the recombination frequency, "r", of the 10 gene pairs presented in this study can be expressed in centimorgan (cM) or Morgan map function (Ziegler and Koning, 2005).

In this study, we tested the segregation ratios and detected linkage of 14 loci in cucumber associated to important agronomic traits. Ten genes-pairs were linked with map distance that ranges from 5 to 28 cM. Our finding provides valuable information to breeders for developing elite cucumber cultivars.

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## Literature Cited

Bradeen, J.M., Staub J.E., Wye C., Antonise R. and Peleman, J. 2001. Towards an expanded and integrated linkage map of cucumber (*Cucumis sativus* L.). Genome 44:111-119.

Broman, K.W., Wu H., Sen S. and Churchill G.A. 2003. R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889–890.

Della Vecchia, P.T. and Peterson, C.E. 1984. Inheritance of flowering response in cucumber. J. Amer. Soc. Hort. Sci. 109:76-763.

Fanourakis, N.E. and Simon, P.W. 1987. Analysis of genetic linkage in the cucumber. J. Heredity 78: 238-242.

Fazio, G., Staub, J.E. and Stevens, M.R. 2003. Genetic mapping and QTL analysis of horticultural traits in cucumber (*Cucumis sativus* L.) using recombinant inbred lines. Theor Appl Genet 107:864-874.

Fukino, N., Yoshioka, Y., Kubo, N., Hira, M., Sugiyama, M., Sakata, Y. and Matsumoto, S. 2008. Development of 101 novel SSR markers and construction of an SSR-based genetic linkage map in cucumber (*Cucumis sativus* L.). Breed Sci 58:475-483.

Guner, N. and Wehner, T.C. 2004. The Genes of Watermelon. HortScience 39:1175-1182.

Heang, D., Sato, H., Sassa, H. and Koba, T. 2008. Detection of two QTLs for fruit weight in cucumber (*Cucumis sativus*). Pitrat M (ed): Cucurbitaceae 2008, Proceedings of the IX$^{th}$ EUCARPIA meeting on genetics and breeding of Cucurbitaceae, Avignon (France), May 21-24$^{th}$: 511-514

Huang, S., Li, R., Zhang, Z., Li, L., Gu, X., Fan, W., Lucas, W., Wang, X., Xie, B., et al. 2009. The genome of the cucumber, *Cucumis sativus* L. Nat. Genet. 41:1275-1281.

Kennard, W., Poetter C., Dijkhuizen, A., Meglic, V., Staub, J. E. and Havey, M.J. 1994. Linkage among RFLP, RAPD, Isozyme, disease-resistance, and morphological markers in narrow and wide crosses of cucumber. Theor. Appl. Genet. 89:42-48.

Knerr, L.D. and Staub, J.E. 1992. Inheritance and linkage relationships of isozyme loci in cucumber (*Cucumis sativus* L.) Theor. Appl. Genet. 84:217-224.

Lander, E.S. and Botstein, D. 1986. Mapping complex genetic traits in humans: new method using a complex RFLP linkage map. Cold Spring Harbor Symp. Quant. Biol. 51:49-62.

Liu, J.S., Wehner, T.C. and Donaghy, S.B. 1997. SASGENE: A SAS computer program for genetic analysis of gene segregation and linkage. J. Hered. 88:253-254.

Lynch, M., and Walsh, J.B. 1998. Genetics and Analysis of Quantitative. Traits. Sinauer Associates, Sunderland, MA.

Meglic V. and Staub, J.E. 1996. Inheritance and linkage relationships between allozyme and morphological loci in cucumber. Theor. Appl. Genet. 92:865-872.

Miao, H., Zhang, S., Wang, X., Zhang, Z., Li, M., Mu, S., Cheng, Z., Zhang, R., Huang, S., Xie, B., Fang, Z., Zhang, Z., Weng, Y. and Gu, X. 2011. A linkage map of cultivated cucumber (*Cucumis sativus* L.) with 248 microsatellite marker loci and seven genes for horticulturally important traits. Euphytica 182:167-176.

Park, Y.H., Sensoy, S. and Wye, C. 2000. A genetic map of cucumber composed of RAPDs, RFLPs, AFLPs, and loci conditioning resistance to papaya ring spot and zucchini yellow mosaic viruses. Genome. 43:1003-1010.

Peterson, C.E. 1975. Plant introductions in the improvement of vegetable cultivars. HortScience 10:575-579.

Pierce, L.K. and Wehner, T.C. 1990. Review of genes and linkage groups in cucumber. HortScience 25:605-615.

Ren, Y., Zhang, Z., Liu, J., Staub, J.E., Han, Y., Cheng, Z., Li, X., Lu, J., Miao, H., Kang, H., Xie, B., Gu, X., Wang, X., Du, Y., Jin, W. and Huang, S. 2009. An integrated genetic and cytogenetic map of the cucumber Genome. PLoS ONE 4:5795.

Sakata, Y., Kubo, N., Morishita, M., Kitadani, E., Sugiyama, M. and Hirai, M. 2006. QTL analysis of powdery mildew resistance in cucumber (*Cucumis sativus* L.). Theor. Appl. Genet. 112:243-250.

Serquen, F.C., Bacher, J. and Staub, J. 1997. Mapping and QTL analysis in a narrow cross of cucumber using RAPD Markers. Molecular Breeding 3:257-268.

Sinnott, E.W. and Dunn, L.C. 1939. Principles of genetics, 3rd edition. McGraw-Hill, New York.

Vakalounakis, D.J. 1992. Heart leaf, a recessive leaf shape marker in cucumber: linkage with disease resistance and other traits. J. Heredity 83:217-221.

Vakalounakis, D.J. 1993. Inheritance and genetic linkage of fusarium wilt (*Fusarium oxysporum* f. sp. *cucumerinum* race 1) and scab (*Cladosporium cucumerinum*) resistance genes in cucumber (*Cucumis sativus*). Ann. Appl. Biol. 123:359-365.

Vinod, K.K. 2011. Kosambi and the genetic mapping function. Resonance 16:540–550.

Walters, S.A., Shetty, N.V. and Wehner, T.C. 2001. Segregation and linkage of several genes in cucumber. J. Am. Soc. Hort. Sci. 126:442–450.

Wehner, T.C. 1993. Gene list update for Cucumber. Cucurbit Genetics Coop. Rpt. 16:92-97.

Wehner, T.C. and Staub, J.E. 1998. Gene list for cucumber. Cucurbit Genet. Coop. Rpt. 20:66-88.

Wehner, T.C., Staub, J.E. and Peterson, C.E. 1987. Inheritance of littleleaf and multi-branched plant type in cucumber. Cucurbit Genetics Coop. Rpt. 10:33.

Wehner, T. C., Liu J.S. and Staub, J.E. 1998a. Two-gene interaction and linkage for bitterfree foliage in cucumber. HortScience 123:401-403.

Wehner, T.C., Staub, J.E. and Liu, J.S. 1998b. A recessive gene for revolute cotyledons in cucumber. J. Heredity 89:86-87.

Wei, Q., Wang, Y., Qin, X., Zhang, Y., Zhang, Z., Wang, J., Li, J., Lou, Q. and Chen, J. 2014. An SNP-based saturated genetic map and QTL analysis of fruit-related traits in cucumber using specific-length amplified fragment (SLAF) sequencing. BMC Genomics 15:1158.

Weir, B.S. 1994. Genetic data analysis: methods for discrete population data. Sinauer, USA.

Weng, Y.Q. and Wehner, T.C. 2017. Cucumber Gene List 2017. Cucurbit Genetics Coop.

Weng, Y.Q., Johnson, S., Staub, J.E. and Huang, S.W. 2010. An extended microsatellite genetic map of cucumber, *Cucumis sativus* L. HortScience 45:880-886.

Xu, X., Xu, R., Zhu, B., Yu, T., Qu, W., Lu, L., Xu, Q., Qi, X. and Chen, X. 2015. A high-density genetic map of cucumber derived from Specific Length Amplified Fragment sequencing (SLAF-seq). Front. Plant Sci. 5:768.

Yang, L., Koo D-H., Li, Y., Zhang, X., Luan, F., Havey, M.J., Jiang, J. and Yiqun, W. 2012. Chromosome rearrangements during domestication of cucumber as revealed by high-density genetic mapping and draft genome assembly. The plant Journal 71:895-906.

Young, H.P., Suat, S., Crispin, W., Rudie, A., Johan, P. and Michael, J.H. 2000. A genetic map of cucumber composed of RAPDs, RFLPs, AFLPs, and loci conditioning resistance to papaya ringspot and zucchini yellow mosaic viruses. Genome. 43:1003-1010.

Yuan, X.J., Pan, J.S., Cai, R., Guan, Y., Liu, L.Z., et al. 2008. Genetic mapping and QTL analysis of fruit and flower related traits in cucumber (*Cucumis sativus* L.) using recombinant inbred lines. Euphytica 164:473-491.

Zhang, W-W., Pan, J-S., He, H-L., Zhang, C., Li, Z., Zhao, J-L., Yuan X-J., Zhu L-H., Huang, S-W. and Cai, R. 2012. Construction of a high density integrated genetic maps for cucumber (*Cucumis sativu*s L.). Theoretical applied genetics 124:249-259.

Ziegler, A. and Koning, I.R. 2005. Genetic distance and mapping functions. Encyclopedia of life science. John Wiley & Sons, Ltd.

Zijlstra, S. and den Nijs, A.P.M. 1996. Further results of linkage studies in *Cucumis sativus* L. Cucurbit Genetics Coop. Rpt. 9:55.

**Table 1. Phenotypic description of five cucumber inbred lines for 14 genes used in linkage studies.[z]**

| | Phenotypic classes[z] | | Crosses | | | | | |
|---|---|---|---|---|---|---|---|---|
| Trait[y] | Dominant (+) | Recessive (-) | NCG-089 × WI 2757 | | NCG-092 × WI 2757 | | NCG-093 × WI 2757 | |
| Bitterfree (*bi*) | Bitter | Bitterfree | + | - | + | - | + | - |
| Bitter leaf (*bl*) | Bitter | Bitterfree | + | + | + | + | - | + |
| Black spine (*B*) | Black | White | + | - | - | - | + | - |
| Delayed flowering (*df*) | Early | Delayed | + | - | + | - | + | - |
| Divided leaf (*dvl*) | Single | Divided | - | + | + | + | + | + |
| Dull fruit skin (*D*) | Dull | Glossy | + | - | + | - | + | - |
| Femaleness (*F*) | Gynoecious | Monoecious | - | + | - | + | - | + |
| Littleleaf (*ll*) | Big | Little | + | + | - | + | + | + |
| Numerous spines (*ns*) | Few | Numerous | + | - | + | - | + | - |
| Revolute cotyledon-2 (*rc-2*) | Flat | Revolute | + | + | + | + | - | + |
| Short petiole-1 (*sp*) | Long | Short | + | + | + | + | - | + |
| Small spines (*ss*) | Large | Small | + | - | + | - | + | - |
| Tuberculate fruit (*Tu*) | Tuberculate | Smooth | + | - | + | - | + | - |
| Uniform immature fruit color (*u*) | Mottled | Uniform | + | - | + | - | + | - |

[z] '+' = dominant, '-' = recessive condition of cultivars and lines for both alleles of the genes listed.

[y]In parenthesis, gene designation of the trait.

**Table 2. Number of plants segregating in $F_2$ and $BC_1$ generations, and linkage association between 11 gene pairs in cucumber.[z]**

| Gene pair | Generation | Family[Z] | Linkage phase[Y] | No. plants of 4 classes | | | | $\chi^2$ | SASGENE | | R/qtl | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | A_B_ | A_bb | aaB_ | aabb | | r | SE | r | LOD |
| **New linkages or new linkage values detected** | | | | | | | | | | | | |
| *bi--ll* | $F_2$ | 2 | R | 75 | 36 | 41 | 2 | 14* | 0.21 | 0.06 | 0.21 | 3.48 |
| *rc-2--sp* | $F_2$ | 3 | C | 112 | 8 | 19 | 20 | 36* | 0.19 | 0.03 | 0.20 | 7.33 |
| | $BC_1$ | 3 | C | 36 | 10 | 10 | 23 | 24* | 0.25 | 0.05 | 0.25 | 4.37 |
| *ns--u* | $F_2$ | 2 | C | 88 | 17 | 20 | 24 | 30* | 0.27 | 0.03 | 0.27 | 4.48 |
| **Previously reported linkages confirmed** | | | | | | | | | | | | |
| *F--df* | $F_2$ | 2 | R | 72 | 29 | 49 | 3 | 21* | 0.25 | 0.05 | 0.25 | 3.1 |
| *ns--ss* | $BC_1$ | 3 | C | 24 | 9 | 5 | 40 | 39* | 0.18 | 0.04 | 0.18 | 7.54 |
| *ns--Tu* | $F_2$ | 2 | C | 89 | 11 | 20 | 30 | 58* | 0.21 | 0.03 | 0.21 | 8.17 |
| *ss--u* | $BC_1$ | 3 | C | 22 | 9 | 11 | 36 | 24* | 0.26 | 0.05 | 0.26 | 4.2 |
| *Tu--u* | $F_2$ | 2 | C | 97 | 8 | 2 | 42 | 155* | 0.05 | 0.02 | 0.06 | 25.4 |
| *Tu--D* | $F_2$ | 2 | C | 91 | 18 | 9 | 30 | 63* | 0.18 | 0.03 | 0.19 | 9.99 |
| *u--D* | $F_2$ | 1 | C | 113 | 19 | 4 | 20 | 43* | 0.15 | 0.02 | 0.18 | 8.77 |
| | $F_2$ | 2 | C | 96 | 12 | 9 | 30 | 71* | 0.15 | 0.03 | 0.15 | 12.71 |
| | $BC_1$ | 3 | C | 82 | 13 | 11 | 52 | 88* | 0.15 | 0.05 | 0.23 | 5.18 |

[Z]Family: 1 = NCG-089 × WI 2457; 2 = NCG-092 × WI 2457; 3 = NCG-093 × WI 2457.

[Y]Linkage phase is coupling (C) or repulsion (R). *A_B_* denotes dominant expression for both genes; *A_bb* denotes dominant expression for the first gene and recessive for the second; *aaB_* denotes dominant expression for the first gene and recessive for the second; *aabb* denotes recessive expression for both genes. R/qtl analyses were run with a minimum LOD score of 3.0.

* Shows chi-square values that indicate the recombination frequency (r) is different from independent assortment using SASGENE.

# Initiative for Uniform Cucurbit Powdery Mildew Race Determination and Denomination: Status of Race Differentials

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*Golovinomyces orontii* (Castagne) Heluta (*Go*) and *Podosphaera xanthii* (Castag.) U. Braun & N. Shish. (*Px*) (1) are two fungal obligate ectoparasites from the order Erysiphales causing cucurbit powdery mildew (CPM), a disease on field and greenhouse cucurbit crops worldwide. Highly variable pathogenicity and virulence of both pathogens are manifested by the existence of large number of races. Various independent systems of CPM race determination and denomination are used worldwide, having been based on different cultivars or lines of melon, *Cucumis melo* L. (3). The heterogeneity of these systems and the lack of clear and uniform descriptions of the genetic variation in the virulence of the CPM pathogens on melon limit study of genetics of resistance, resistance breeding, and production.

Establishment of a uniform system for CPM race determination and denomination is based upon three components: (1) a standard set of race differentials; (2) uniform screening methodology; and (3) a uniform code for host-CPM interactions/scores (2, 4). The proposed set of 21 melon differentials (Table 1) was acceptable to commercial melon breeders and pathologists in attendance at Cucurbitaceae 2016, XIth Eucarpia Meeting on Genetics and Breeding of Cucurbitaceae Warsaw, Poland (2). It is now time to increase seed stocks of the differentials, inform the larger melon seed industry and public research community, establish a means for maintaining and distributing the differentials, and establish a cucurbit crop and CPM expert panel as a coordination group, as well as an international CPM race network.

The general principles of a system for CPM race determination follows the guidelines postulated more than 20 years ago (5, 6). The International Bremia Evaluation Board) adopted this approach for the many races of downy mildew incited by *Bremia lactucae* of lettuce, *Lactuca sativa* (http://www.worldseed.org/isf/ibeb.html).

Discussion at Cucurbitaceae 2016 among the authors and representatives of nine melon breeding companies led to the unanimous agreement that the international melon seed industry must now take the lead in implementing the proposed system for uniform cucurbit powdery mildew (CPM) race determination and denomination, as summarized in recent publications (2, 4). The state-of-the-art of CPM research and breeding is not fully developed. For example, many of the 21 melon differential lines possess genes for resistance to two or more CPM races (7). On the pathogen side of the interaction, there is, at present, little knowledge of the genetics or the basis for race differences of either CPM pathogen on melon. Type isolates have not been established for any race of either pathogen, nor have protocols been evaluated for their long-term storage. Nevertheless, it was agreed that use by the international representatives of the melon breeding community of the set of 21 CPM differentials, as proposed by the authors, was the next logical step in facilitating communication within and among researchers, commercial breeders, plant pathologists, extension specialists, and crop consultant communities.

Representatives of the seed companies that participated in the discussions in Warsaw 2016 agreed to obtain the support of their respective boards of directors and the International Seed Federation for this set of CPM race differentials. The other step was at least twofold: increase seed stocks of the 21 melon CPM differentials and verify their morphological uniformity and purity. Melon breeders from three seed companies at the meeting offered to increase the complete

set of differentials using the stocks developed by the first author: Bayer Crop Science (now BASF), Rijk Zwaan, and VoloAgri. Ultimately, Rijk Zwaan took sole responsibility for this task, and from 2017 through February 2019 finished three cycles of seed multiplication and purification based on phenotyping and molecular markers. In the next cycle that is to be completed in 2019, this process will be finished with the goal of 10,000 seeds per differential line; half to remain at Palacky University and half to be deposited in the U.S. Department of Agriculture, National Plant Germplasm System, North Central Regional Plant Introduction Station, Ames, Iowa. The differentials will be freely available as a complete set for researchers, breeders and seed industry, as well as stored in one specific genebank. Interested parties are urged to contact the authors for further details including obtaining the differential lines.

## Literature Cited


1. Braun, U. and R.T.A. Cook, 2012. Taxonomic manual of the Erysiphales (Powdery Mildews). CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.
2. Lebeda, A., E. Křístková, B. Sedláková, and J.D. McCreight, 2016. Initiative for international cooperation of researchers and breeders related to determination and denomination of cucurbit powdery mildew races, p. 148–152. In: E.U. Kozik and H.S. Paris, (eds.). Cucurbitaceae 2016, XIth Eucarpia Meeting on Genetics and Breeding of Cucurbitaceae, Warsaw, Poland. DOI:10.1007/s10658-015-0776-7:
3. Lebeda, A., E. Křístková, B. Sedláková, M.D. Coffey, and J.D. McCreight, 2011. Gaps and perspectives of pathotype and race determination in *Golovinomyces cichoracearum* and *Podosphaera xanthii.* Mycoscience 52:159–164. DOI: 10.1007/s10267-010-0098-8:
4. Lebeda, A., E. Křístková, B. Sedláková, J.D. McCreight, and M.D. Coffey, 2016. Cucurbit powdery mildews: methodology for objective determination and denomination of races*.* European J. Plant Pathol. 144:399–410. DOI: 10.1007/s10658-015-0776-7:
5. Limpert, E. and K. Müller, 1994. Designation of pathotypes of plant pathogens*.* J. Phytopathol. 140:346–358.
6. Limpert, E., B. Clifford, A. Dreiseitl, R. Johnson, K. Müller, A. Roelfs, and C. Wellings, 1994. Systems of designation of pathotypes of plant pathogens*.* J. Phytopathol. 140:359–362.
7. 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.

**Table 1. Triple septet set of melon CPM race differentials by group number, as proposed by Lebeda et al. (2).**

| Group no. | Cultigen | Other designation(s) | Source[z] | Country origin |
|---|---|---|---|---|
| | | | | |
| 1.1 | Iran H | – | INRA | Iran |
| 1.2 | Védrantais | M 319 | INRA | France |
| 1.3 | PI 179901 | Teti | USDA | India |
| 1.4 | PI 234607 | Sweet Melon | USDA | Rep. South Africa |
| 1.5 | AR HBJ | AR Hale's Best Jumbo | USDA | USA |
| 1.6 | PMR 45 | M 321 | USDA | USA |
| 1.7 | PMR 6 | Ames 26810 | USDA | USA |
| 2.1 | WMR 29 | M 322 | USDA | USA |
| 2.2 | Edisto 47 | NSL 34600 | Clemson Univ. | USA |
| 2.3 | PI 414723 | LJ 90234 | USDA | India |
| 2.4 | PMR 5 | Ames 26809 | USDA | USA |
| 2.5 | PI 124112 | Koelz 2564 | USDA | India |
| 2.6 | MR-1 | Ames 8578 | USDA | USA |
| 2.7 | PI 124111 | Koelz 2563 | USDA | India |
| 3.1 | PI 313970 | VIR 5682, PI 315410, 90625 | USDA | India |
| 3.2 | Noy Yizre' el | – | Bar-Ilan Univ. | Israel |
| 3.3 | PI 236355 | – | USDA | England |
| 3.4 | Negro | – | Univ. Zaragoza | Spain |
| 3.5 | Amarillo | – | Univ. Zaragoza | Spain |
| 3.6 | Nantais Oblong | M 320 | INRA | France |
| 3.7 | Ames 31282 | PI 134198 | USDA | China |

[z]INRA = Institut National de la Recherche Agronomique, Montfavet (France); USDA = United States Department of Agriculture, Agricultural Research Service; information on USDA accessions available on the website of the National Genetic Resources Program, Germplasm Resource Information Network (GRIN), http://www.ars-grin.gov/npgs/acc/acc_queries.html

# Development of Melon Cultivars for Organic Farming

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## Introduction

Melon (*Cucumis melo* L.) has been cultivated in Spain since at least Roman times (10), both non-sweet melons (snake-shape, *flexuosus* type), locally known as 'alficoç', and sweet melons. In terms of production, Spain is the first producer of the European Union followed by Italy and France (5). Snake melons were already cultivated in the first century, as stated by Roman author Lucio Junius Moderatus *Collumela* who mentioned the snake-shaped 'cohombro', a *C. melo* belonging to the botanical variety *flexuosus* (L.) Naud. (8), a long snake-shaped non-sweet melon. With the Islamic conquest of the Iberian Peninsula during the Middle Age, sweet casaba type melons were introduced from Central Asia to Europe (12). During centuries, the preference of Spanish farmers and consumers has favoured the selection of sweet melons adapted to diverse agro-climatic conditions. The local farmers cultivated, conserved and exchanged their seeds, carrying the best fruits to the most popular markets (4). A recent classification of melon groups (13), recognizes the sweet melons from Spain and classifies them into a group denominated as *Ibericus*, with sub-group melons like 'Piel de Sapo', 'Rochet', 'Amarillo', 'Blanco' or 'Tendral'. These sub-groups are still cultivated nowadays, although mainly for self-consumption or for local markets, except for 'Piel de Sapo', being one of the most important market class currently.

Organic agriculture refers to the farming system where local resources are maximized, while forgoing the use of agrochemicals and the use of Genetically Modified Organisms (GMOs) (6). Therefore, alternative methods to control pests and diseases must be used. One of these methods is the use of resistant cultivars, as different cultivars have adapted to the pathogens present in their specific agroecosystem (14). Organic foods are widely perceived to be tastier and healthier than conventionally produced products and the production process is less damaging for the environment (7). For this reason, the Valencian government (Conselleria de Educaciò, Investigaciò, Cultura i, Esport, Generalitat Valenciana) has funded a Project (PROMETEO2017/078) in order to select traditional Spanish melon cultivars with suitable features for organic farming conditions.

## Materials and Methods

The experimental assays were carried out in 2 different assay fields, both located in the surrounding area of Valencia, where organic farming is being promoted. The first field was in the area of Moncada (Latitude: 39°33'26.8"N, Longitude: 0°25'06.5"W). This field had no previous history of agricultural use for 20 years. The second field was in the area of La Punta (Latitude: 39°26'41.3"N, Longitude: 0°21'14.9"W). This field had a long history of melon cultivation. For this study, fifty landraces, representing the six classes of sweet melons and the non-sweet 'alficoç' were selected among those maintained at the COMAV GeneBank of the Universitat Politècnica de València. In the assay field of La Punta melons were grafted on different rootstocks, a commercial F1 hybrid of *Cucurbita* 'Cobalt' and an experimental rootstock F1 hybrid of melon (cross between *C. melo* ssp. *agrestis* and ssp. *melo*). Melon plants were grown under organic farming conditions. The plants were transplanted to the fields in mid-April, with nine total plants per cultivar distributed in three different blocks. Plants were planted

on ridges, with black plastic mulch, to control soil humidity, temperature and weeds. In "Moncada", water was supplied by drip irrigation, whereas in "La Punta" field, water was supplied by flood irrigation.

During the growing cycle, the fields were inspected at least once per week. Soil samples were collected to determine soil conductivity. Pests were identified and plants showing symptoms of disease were sampled to determine the causal agent. Root samples were first cleaned using sodium hypochlorite solution at 20% concentration and any excess of sodium hypochlorite was removed using distilled water. Root tissue showing damage was then planted on Petri Dishes with Potato Dextrose Agar (PDA) + Streptomycin (1ppm concentration) medium and grown for 3 days at 30ºC. Fungal colonies were then characterized to determine the growing fungus. The leaf samples were used to determine the virus which caused the symptoms observed, using the method described by (9). Detected viruses were then cloned and sequenced to determine the virus isolate present.

One fruit per plant was characterized for fruit weight in grams (digital scale) and soluble solids content (SSC), measured as ºBrix using drops of juice (with a hand-held "Pocket" refractometer (PAL-α), Atago CO., LTD, Tokyo, Japan). A t-test was used to compare the results for each cultivar for fruit weight and SSC between both assay fields.

## Results

The most common pest found in both fields were aphids, which are vectors to important viruses. The most common viruses in both fields were the Cucumovirus Cucumber Mosaic Virus (CMV) and the Potyvirus Watermelon Mosaic Virus (WMV). 'Blanco' and 'Amarillo' sweet melons were the most sensitive cultivars to powdery mildew, caused by *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff, whereas non-sweet 'alficoç' was quite tolerant to this fungal pathogen. The principal soil pathogenic fungi found were *Fusarium oxysporum* f. sp. *melonis* Snyder & Hansen, *Fusarium solani* (Mart.) Sacc., *Monosporascus cannonballus* Pollack & Uecker and *Macrophomina phaseolina* (Tassi) Goid. Plants grafted onto F1 melon rootstock displayed more resistance against soil pathogens than those grafted onto F1 *Cucurbita* rootstocks. 'Blanco' and 'Amarillo' sweet melon cultivars were especially susceptible to soil pathogens, whereas non-sweet ''alficoç'' presented higher tolerance to these soilborne pathogens. The soil conductivity of La Punta field (0.674±0.081mS/cm) was significantly higher ($P<0.05$) than that of Moncada (0.357±0.015 mS/cm). Figures 1 and 2 show the average fruit weight and flesh SSC of 10 different ungrafted cultivars representative of the different groups for each field. In general, fruits were larger in Moncada (fruit weight ranging from 970g to 1730g) compared with La Punta (from 840g to 1480g), whereas no differences were found in average SSC (ranging from 9.7 to 14.1, and from 9.8 to 13 ºBrix in Moncada and La Punta respectively). The 'alficoç' cultivar yielded smaller non-sweet fruits (240g and 320g, and 3.2 and 3.8 ºBrix). The higher pathogen incidence and soil conductivity may be the cause of fruit size reduction in La Punta field although fungal attack and /or the significant differences in soil conductivity did not significantly affect the SSC. The effect on fruit weight was different for the different landraces. Figure 1 shows the results for ungrafted plants of two 'Piel de Sapo' (03PS and 12Ps), two 'Amarillo' (22AM and 23AM), two 'Blanco' (01BL and 32BL), two 'Rochet' (02RC and 04 RC) and one snake melon (05AL) cultivar. Significant fruit weight losses ($P<0.05$) were observed in 'Amarillo' and 'Blanco' landraces, (between 20-30% fruit weight loss), likely associated to their higher susceptibility to fungi, whereas 'Piel de Sapo' and 'alficoç' cultivars were less affected, and 'Rochet' displayed an intermediate behaviour. Only one 'Blanco' cultivar (01BL) significantly reduced the Brix degree.

## Discussion

Viral diseases are the main factors affecting cucurbits cultivation, being the most widespread and damaging the *Potyviridae* family viruses (11). The detected viruses belong to the family *Potyviridae* in the case of WMV and to the *Bromoviridae* family in the case of CMV. Both these viruses are transmitted by aphids (3). Therefore, in our conditions, the control of viral diseases must be focused on aphid control. One way would be the use of resistant cultivars possessing the gene *Vat* which confers resistance to both the aphids and the viruses it carries. A vast survey of aphids on vat and non-vat plants showed that aphid populations were globally affected by resistant melons, but that a few adapted (virulent) clones could develop on vat-plants, questioning the durability of the *Vat* gene (17). A study on *Vat* resistance in melon on viral epidemics (16) found that *Vat* had a limited impact on WMV epidemics and the reduction on CMV epidemics was irregular. In organic farming, one of the methods employed for the purpose of controlling aphids is the use of parasitoids and predators (14), as natural enemies are mass-reared for release in large numbers to obtain immediate control of pests in crops with a short production cycle (18).The other main biotic limiting factor in our assays were soil pathogenic fungi that cause fruit weight losses, leading to unmarketable fruits in the most sensitive cultivars. Plants grafted onto F1 *Cucurbita* rootstocks, which are usually used to control *Fusarium oxyporum* (2), showed lower resistance to soil pathogenic fungi than F1 melon rootstocks. These results can be due to the presence

of other *Fusarium* species or other soil pathogens, which specially affect *Cucurbita* rootstocks (1). *Fusarium oxysporum* f. sp. *melonis* (Fom) is specific to melon and it causes a vascular wilt which is considered as one of the most severe disease of melon worldwide, with four races of the pathogen based on the resistance genes (15). The resistance to races 0, 1 and 2 is scattered along all melon botanical types, whereas the high levels of resistance to race 1,2 was found only among accessions belonging to *Cucumis melo* ssp. *agrestis* (15). The results showed that the 'Amarillo' and 'Blanco' cultivars are more sensitive to fungi than the 'Piel de Sapo' and 'alficoç'. Therefore, both the use of resistant rootstocks and the knowledge of soil pathogens in the field are of great importance when dealing with soilborne pathogens. The fact that different cultivars display differential response to biotic and abiotic stresses under organic farming, will allow for an optimized breeding process in each case.

## Conclusion

The main biotic factors (pests, viruses and fungi), which limit the organic production of melon in Eastern Spain have been identified. The use of resistant rootstocks to soilborne pathogens, specially melon rootstocks, allowed the cultivation of susceptible melons on non-treated infested soils, where ungrafted plants of the most sensitive landraces ('Blanco' and 'Amarillo' types) were severely affected. Different traditional cultivars of melon, including some 'Piel de Sapo', 'Rochet' and non-sweet 'alficoç', which stand out due to their adaptation have been identified. The knowledge of the different limiting factors, as well as the identification of the cultivars with the greatest potential, will allow the development of melon cultivars adapted to organic farming.

## Acknowledgments

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## Literature Cited

1. Armengol J., C.M. José, M.J. Moya, R. Sales, A. Vicent, J. Garcia-Jiménez. 2000. *Fusarium solani* f. sp*. cucurbitae* race 1, a potential pathogen of grafted watermelon production in Spain. Bulletin OEPP/EPPO Bulletin 30(2):179-183.

2. Bletsos F.A. 2005. Use of grafting and calcium cyanamide as alternatives to methyl bromide soil fumigation and their effects on growth, yield, quality and fusarium wilt control in melon. Journal of Phytopathology 153(3):155-161.

3. Boissot N., A. Schoeny Alexandra, F. Vanlerberghe-Masutti. 2016. *Vat*, an Amazing Gene Conferring Resistance to Aphids and Viruses They Carry: From Molecular Structure to Field Effects. Frontiers in Plant Science 7:1420.

4. Escribano S., A. Lázaro. 2009. Agro-morphological diversity of Spanish traditional melons (*Cucumis melo* L.) of the Madrid provenance. Genetic Resources and Crop Evolution 56(4):481-497.

5. European Statistical Office (Eurostat). 2017. Database agriculture theme. Available at https://ec.europa.eu/eurostat/data/database.

6. Gomeiro T., D. Pimental, M.G. Paoletti. 2011. Environmental impact of different agricultural management practices: conventional vs. organic agriculture. Critical Reviews in Plant Science 30(1-2):95-124.

7. Gracia A., T. de Magistris. 2008. The demand for organic foods in the South of Italy: A discrete choice model. Food Policy 33(5):386-396.

8. Hammer K., T. Gladis. 2014. Notes on infraspecific nomenclature and classifications of cultivated plants in Compositae, Cruciferae, Cucurbitaceae, Gramineae (with a remark on Triticum dicoccon Schrank) and Leguminosae. Genetic Resources and Crop Evolution 61(8):1455-1467.

9. Herranz M.C., J.A. Sanchez-Navarro, F. Aparicio, V. Pallás. 2005. Simultaneous detection of six stone fruit viruses by non-isotopic molecular hybridization using a unique riboprobe or 'polyprobe'. Journal of Virological Methods 124(1-2):49-55.

10. Lázaro A., I.C. Fernández, M.J. Borrero, F. Cabello, A.I. Lopez-Sesé, M.L. Gómez-Guillamón, B. Picó. 2017. Agromorphological genetic diversity of Spanish traditional melons. Genetic Resources and Crop Evolution 64(7):1687-1706.

11. López C., M. Ferriol, B. Picó. 2015. Mechanical transmission of Tomato leaf curl New Delhi virus to cucurbit germplasm: selection of tolerance sources in Cucumis melo. Euphytica 204(3):679-691.

12. Paris H.S., Z. Amar, E. Lev. 2012. Medieval emergence of sweet melons, *Cucumis melo* (Cucurbitaceae). Annals of Botany 110(1):23-33.

13. Pitrat M. 2016. Melon genetic resources: phenotypic diversity and horticultural taxonomy. In : Grumet R., N. Katzir, J. Garcia-Mas. (eds) Genetics and genomics of cucurbitaceae. Plant Genetics and Genomics: Crops and Models, vol 20.

14. Porcuna J.L., M.I. Gaude, P. Castejon, J. Roselló. 2010. Guía de agricultura ecológica de cultivos hortícolas al aire libre. Valencia: FECOAV.

15. Oumouloud A., J.M. Álvarez. 2016. Breeding and Genetics of Resistance to Fusarium Wilt in Melon. In: Al-Khayri J., Jain S., Johnson D. (eds) Advances in Plant

Breeding Strategies: Agronomic, Abiotic and Biotic Stress Traits. Springer, Cham
16. Schoney A., C. Desbiez, P. Millot, C. Wipf-Scheibel, K. Nozeran, P. Gognalons, H. Lecoq, N. Boissot. 2017. Impact of Vat resistance in melon on viral epidemics and genetic structure of virus populations. Virus Research 241: 105-115
17. Thomas S., F. Vanlerberghe-Masutti, P. Mistral, A. Loiseau, N. Boissot. 2016. Insight into the durability of plant resistance to aphids from a demo-genetic study of Aphis gossypii in melon crops. Evolutionary Applications, 9(6):756-768
18. van Lenteren J.C., K. Bolckmans, J. Köhl, W.J. Ravensberg, A. Urbaneja. 2018. Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl 63(1):39-59.

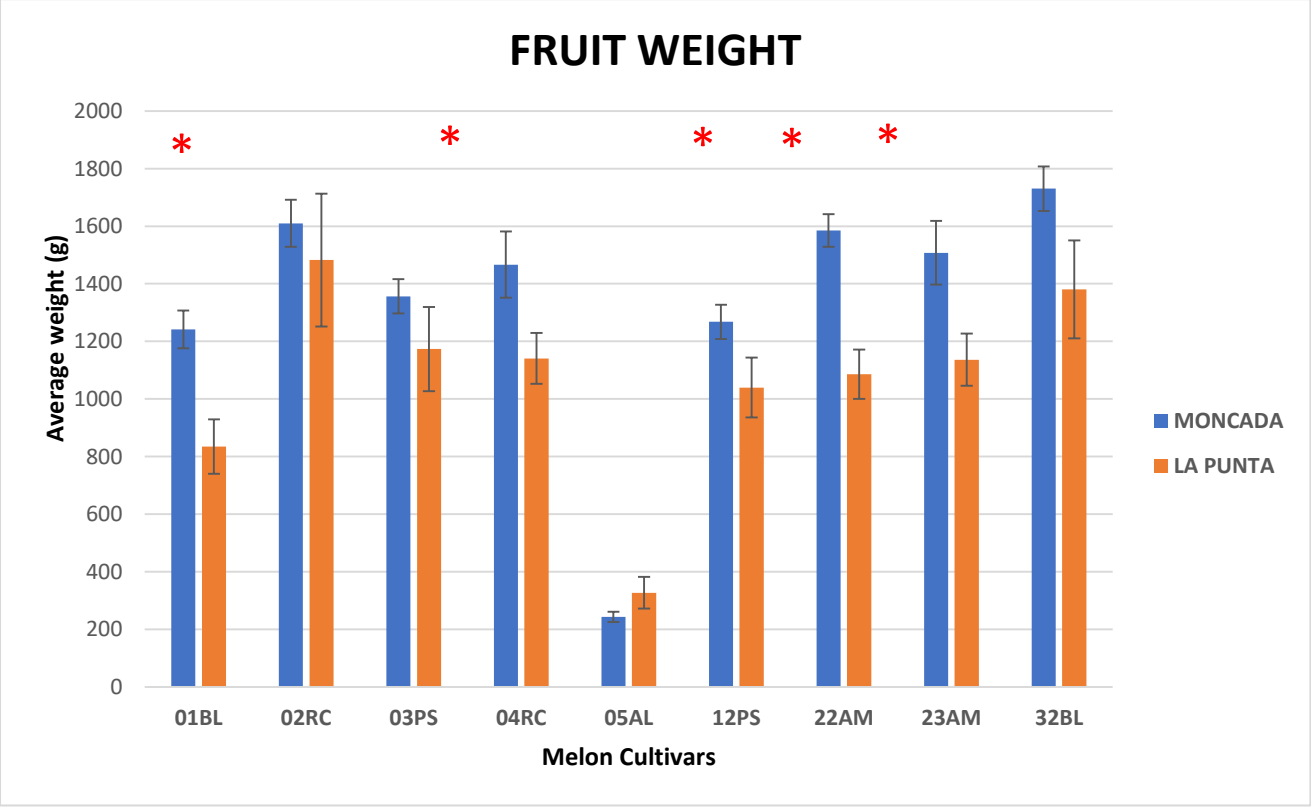


**Figure 1. Graphs showing the average fruit weight for 10 different cultivars including sweet and non-sweet melons. The names of the cultivars indicate if it is sweet 'Blanco' (BL), 'Amarillo' (AM), 'Piel de Sapo' (PS), 'Rochet' (RC) or non-sweet 'Alficoç' (AL). (*) Above the bars represent significant difference (P < 0.05) between the experimental fields.**

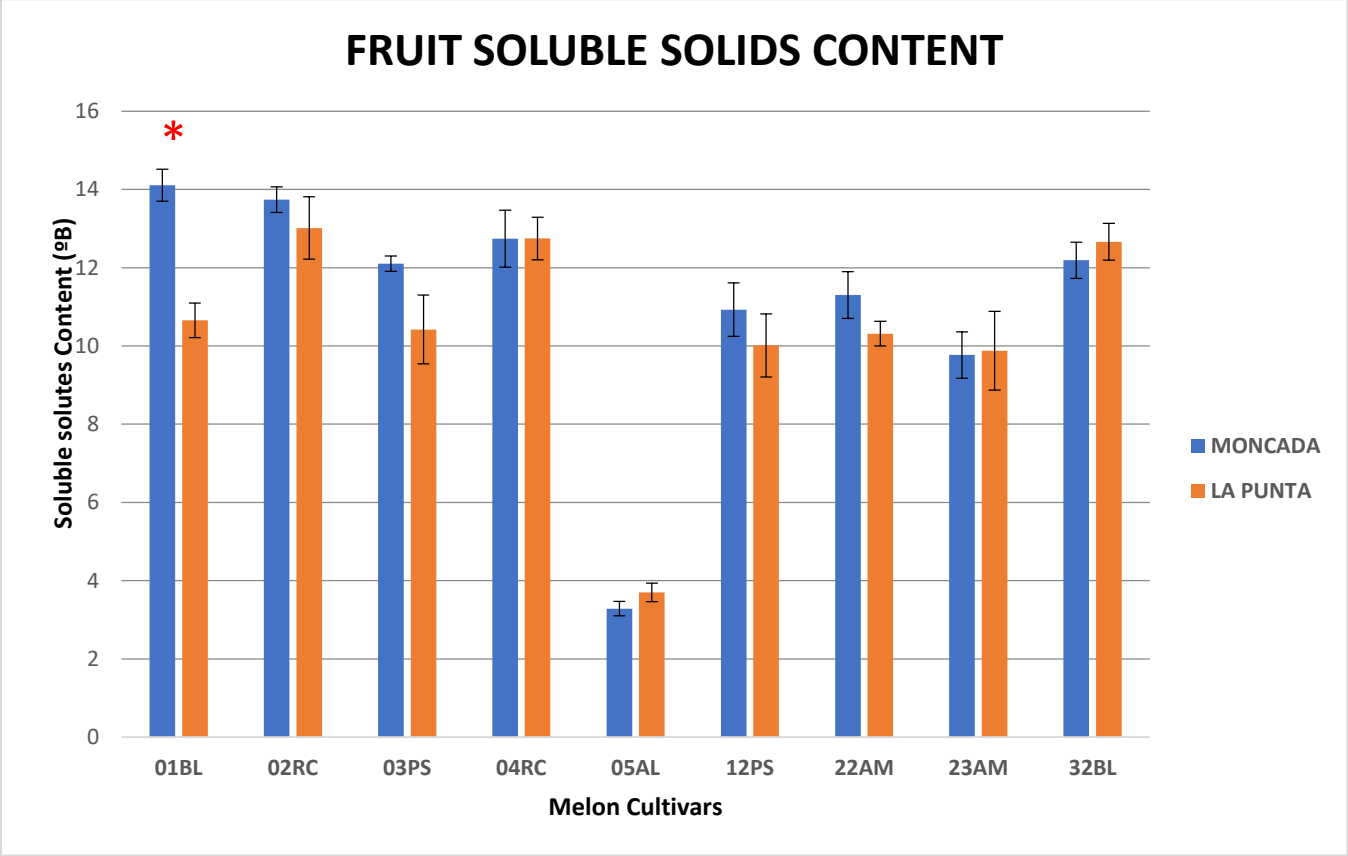


**Figure 2. Graphs showing the average SSC for 10 different cultivars including sweet and non-sweet melons. The names of the cultivars indicate if it is a sweet 'Blanco' (BL), 'Amarillo' (AM), 'Piel de Sapo' (PS),' Rochet' (RC) or non-sweet 'Alficoç' (AL). (*) Above the bars represent significant difference (P < 0.05) between the experimental fields.**

# Performance of Pumpkin Lines Bred by World Vegetable Center in Different Highland Areas of Thailand During Winter season

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## Introduction

The Royal Project Foundation and the Highland Research and Development Institute (a public organization) in Thailand have supported farmers in highland areas to grow vegetables. This could increase employment and income for small-scale farmers. Pumpkin is one of the crops growing in the highland area, with a production of 54,655.33 Kg with a net value of 1,317,562.25 THB (Royal Project Foundation Report, 2017). However, the yield of commercial varieties is not high. Current commercial varieties are also susceptible to virus and their control has a high cost. The World Vegetable Center (Worldveg) has successfully developed new varieties of pumpkin and has increased seed of native landrace varieties. Some varieties are resistant to virus. In over 5 years breeding the new varieties, Worldveg has provided quality seed to many organizations for public use. In addition, seeds produced from Worldveg are open-pollinated varieties, which allow farmers to plant and still be able to collect the seeds for cultivation in the next generation. Hence, testing the varieties from Worldveg in different locations would allow us to find the most suitable pumpkin variety for growing in a particular area, to help farmer produce their own seed, to reduce farming costs, and to carry out sustainable farming in highland areas.

## Materials and Methods

1. Experimental locations
   1.1 Royal Agricultural Station Anghang (1,000 m above sea level area)
   1.2 Royal Agricultural Station Pangda (500 m above sea level area)
   1.3 Royal Park Rajapruek, Muang Chiang Mai (200 m above sea level area)
2. The trials were conducted as a RCBD experiment design with 4 treatments, 3 replications, and 30 plants/replication. Four pumpkin varieties (AVPU1502, AVPU1504, AVPU1505) and a commercial line ('Deliga') were used for this study.
3. Data collected
   3.1 Fruit width
   3.2 Fruit length
   3.3 Seed cavity width
   3.4 Seed cavity length
   3.5 Flesh thickness
   3.6 Number of fruit per plant
   3.7 Average fruit weight
   3.8 Total fruit weight per plant
   3.9 Total soluble solids (TSS) (sweetness)
   3.10 β-carotene
4. Data Analysis: Analysis of variance with mean separation by LSD.

## Results

***Location at 200 m above sea level area***

Fruit width: The commercial line ('Deliga') had the largest fruit width (16.34, $p<0.05$).

Fruit length: AVPU1504 had the longest fruit length (16.47 cm, $p<0.05$)Seed cavity width: 'Deliga' showed the greatest ($p<0.05$) seed cavity width at 10.57 cm.

Seed cavity length: AVPU1504 showed the greatest ($p<0.05$) seed cavity length at 7.19 cm.

Pulp thickness: 'Deliga' showed the greatest ($p <0.05$) pulp thickness at 3.35 cm.

Numer of fruit per plant: AVPU1504 had the greatest ($p <0.05$) number of fruit per plant at 4.67 fruit/plant.

Average fruit weight: 'Deliga' had an average fruit weight (1,154.50 g/fruit) greater than AVPU1504 and AVPU1505 but not significantly different from AVPU1502 at 671.40 g/fruit.

Fruit weight per plant (yield): AVPU1504 yielded more fruit (2.71 kg/plant) than AVPU1505 and 'Deliga', but not more than AVPU 1502 at 2.02 kg/plant.

Total soluble solids (sweetness): Although there were significant differences ($p<0.05$) among lines for TSS, the differences were not large. AVPU1504 was among the lines that showed the highest sweetness (15.93 °Brix),

but it was not significantly different from 'Deliga' and AVPU1505 that showed sweetness at 14.34 and 13.88 °Brix respectively.

β-carotene: AVPU1504 showed the highest ($p<0.05$) content of β-carotene at 6,017.51 µg/100 g.

***Location at 500 m above sea level area***

Fruit width : AVPU1505 showed the highest ($p<0.05$) fruit width at 18.73 cm.

Fruit length: AVPU 1504 showed highest ($p<0.05$) fruit length at 20.10 cm.

Seed cavity width: AVPU1505 showed the highest ($p<0.05$) seed cavity width at 12.44 cm.

Seed cavity length: AVPU1504 showed the highest ($p<0.05$) seed cavity length at 8.35 cm. Pulp thickness: AVPU1505 and 'Deliga' had greater ($p<0.05$) pulp thickness at 3.36 cm and 3.31 cm, respectively, than the other two lines

Number of fruit per plant: AVPU1504 produced the greatest ($p<0.05$) number of fruit per plant at 13.73 fruit/plant.

Average fruit weight: 'Deliga' produced the highest ($p<0.05$) average fruit weight at 1,686.30 g/fruit

Total fruit weight per plant: AVPU1505 and AVPU 1504 had the greatest ($p<0.05$) fruit yield at 11.41 kg/plant and 10.66 kg/plant, respectively.

Total soluble solids (sweetness): There were significant differences ($p<0.05$) among lines, but these differences were small. AVPU1505 was among the lines with the highest sweetness at 15.61 °Brix.

β-carotene: AVPU1504 showed the highest ($p<0.05$) content of β-carotene at 5,692 µg/100 g.

None of the pumpkin varieties could be grown at the location at 1,000 m above sea level.

## Conclusions

At the location 200 m above sea level, lines AVPU1502 and AVPU1504 recorded significantly ($P<0.05$) better fruit weight per plant (2.02 and 2.71 kg/plant) compared to the commercial cultivar (1.31 kg/plant). In the location 2 (500 m above sea level), lines AVPU1504 and AVPU1505 yielded better (10.66 and 11.41 kg/plant) compared to the commercial cultivar (2.3 kg/plant). Beta-carotene content of lines AVPU1504 and AVPU1505 in location 1 (200 m above sea level) were highest (6017 and 4759 µg/100 g fresh weight, respectively) compared to that observed in the commercial cultivar (1213 µg/100 g fresh weight). Similarly in location 2 (500 m above sea level), beta-carotene content of lines AVPU1504 and AVPU1505 were nearly four times higher (5692 and 4283 µg/100 g fresh weight) than recorded in the commercial cultivar (1807 µg/100 g fresh weight). In location 1 (200 m above sea level), total sugars of lines ranged between 12.7 and 15.9 °Brix and in location 2(500 m above sea level), it ranged between 11.7-15.6 °Brix) and it was comparable to the sugar content of the commercial cultivar (14.3 and 14 °Brix, in location 1 and 2, respectively). The pumpkin trial failed in the area 1000 m above the sea level. In conclusion, pumpkin lines developed by the World Vegetable Center recorded better yield and beta-carotene content in trials in highland areas of Thailand compared to the current commercial cultivar grown by local farmers.


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- Royal Agricultural Station Angkhang, Chiang Mai, Thailand
- Royal Agricultural Station Pangda, Chiang Mai, Thailand
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- World Vegetable Center Research & Training Station, Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand


**Figure 1. Characteristics of pumpkins tested at the highland location.**

**Table 1. Data and productivity of pumpkin at 200 MSL (Royal Park Pajapruek) (Date of planting 13 November 2017)**

| Line | Fruit width (cm) | Fruit length (cm) | Seed cavity width (cm) | Seed cavity length (cm) | Flesh thickness (cm) | Number of fruit /plant | Average fruit eight (g) | Total fruit weight/plant (kg) | Total Soluble Solids (°Brix) | β-carotene (µg/100 g)* |
|---|---|---|---|---|---|---|---|---|---|---|
| **AVPU1502** | 9.62c | 12.23b | 6.63c | 6.39b | 2.01b | 3.02b | 671.40ab | 2.02ab | 12.79b | 1,188.20 |
| **AVPU1504** | 8.47c | 16.47a | 5.63d | 7.19a | 1.61c | 4.67a | 588.30b | 2.71a | 15.93a | 6,017.51 |
| **AVPU1505** | 13.63b | 6.66d | 9.59b | 4.03d | 2.19b | 2.27c | 601.80b | 1.31b | 13.88ab | 4,759.07 |
| **Deliga (commercial line)** | 16.34a | 9.92c | 10.57a | 5.57c | 3.35a | 1.27d | 1,154.50a | 1.33b | 14.34ab | 1,213.63 |

*A single reading was taken on a bulk of five samples.

**Table 2. Data and productivity of pumpkin at 500 MSL (Royal Project Pangda Station) (Date of planting 13 November 2017)**

| Line | Fruit width (cm) | Fruit length (cm) | Seed cavity width (cm) | Seed cavity length (cm) | Flesh thickness (cm) | Number of fruit /plant | Average fruit weight (g) | Total fruit weight/plant (kg) | Total Soluble Solids (°Brix) | β-carotene (µg/100 g)* |
|---|---|---|---|---|---|---|---|---|---|---|
| **AVPU1502** | 11.51c | 14.83b | 7.21c | 6.89b | 2.31b | 3.56c | 887.70c | 1.84b | 11.70b | 2,084.57 |
| **AVPU1504** | 10.19d | 20.10a | 6.29d | 8.35a | 2.12b | 13.73a | 778.30c | 10.66a | 13.16b | 5,692.82 |
| **AVPU1505** | 18.73a | 7.16d | 12.44a | 4.56c | 3.36a | 8.40b | 1,358.00b | 11.41a | 15.61a | 4,283.74 |
| **Deliga (commercial line)** | 16.01b | 10.89c | 10.69b | 6.05b | 3.31a | 1.33d | 1,686.30a | 2.30b | 14.07ab | 1,807.63 |

*A single reading was taken on a bulk of five samples.