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Effect of the Seedlessness (Fs) Gene in Fruit Quality Traits in Mandarin Segregating Populations

Zach Tucker[1], Dario J. Chavez[2], and José X. Chaparro[1]

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Abstract

Xenia and metaxenia effects can be responsible for variation in fruit size, fruit shape, and sugar content in fruit. In the process of developing new mandarin citrus hybrids, the University of Florida Fruit Tree Breeding Program produced four populations segregating for the seedlessness gene *Fs*. The objective of this research was to determine if the presence or absence of seed had xenia-like effects on the mandarin hybrids. The four populations contained a total of 213 trees. The fruit produced by these trees were evaluated by sampling three random fruit and measuring the soluble solids concentration (SSC) of each fruit. Additionally, the fruit were scored for the presence or absence of a fruit neck at the stem end. There were no statistically significant differences between seedless and seeded offspring in the four hybrid populations for fruit weight (g) or SSC (% w/w). The "neck" phenotype also appears to be controlled by a single locus and follows a Mendelian segregation ratio of 3:1 (neck: flush). These results support the use of the seedless gene *Fs* without negative effects on fruit size and sugar concentration in the resulting progeny.

Seedlessness is an important trait in many fresh fruit crops. Consumers desire seedless fruit in a number of fruit crops including grapes, watermelon, and citrus. The seedless trait has been induced in citrus using several techniques, including chromosomal variation, triploidy, self-incompatibility, and mutants affecting seed development (Khan, 2007).

Self-incompatibility coupled with parthenocarpy has been used in citrus to produce seedless cultivars. One such notable example is 'Clementine' mandarin *Citrus reticulata* Blanco.'Clementine' plants must be grown in isolated blocks to minimize the number of seed per fruit (Spiegel-Roy and Goldschmidt, 1996). Another method to produce seedlessness is to apply gibberellins 1-14 days after flowering (DAF) (García-Martínez and García-Papí, 1979). In some hybrids, such as 'Orlando' Tangelo and 'Imperial', the reduction in fruit size is so severe that the fruit is unmarketable (Wallace and Lee, 1999; Wallace et al., 2002).

The effects of seedlessness on other fruit characteristics such as fruit size are due to xenia, or the effect of the pollen source on the seeds of the fruit. In addition, seedlessness could also be due to metaxenia, which refers to the effect that the pollen source may have on any structure outside of the embryo and endosperm. This means any tissues derived entirely from the mother plant (Denney and Martin, 1990). These effects have been shown to occur in several citrus interspecific crosses. 'Ellendale' tangor experienced changes in fruit set, fruit size, and seed count depending on the pollen donor cultivar (Vithanage, 1991). Similar changes occurred in cultivars such as 'Minneola', 'Orlando', 'Page', and 'Robinson' (Futch and Jackson, 1993; Hearn et al., 1968). For example, the use of specific pollinators increases fruit set in Clementines and is associated with greater early ovary growth due to increased size of fertilized ovules (García-Papí and García-Martínez,1984).

[1] Horticultural Sciences Department, University of Florida, Gainesville, FL 32611.
[1] To whom reprint requests should be addressed. Email address: jaguey58@ufl.edu
[2] Department of Horticulture, University of Georgia, Griffin Campus, Griffin, GA 30223.

Another technique for obtaining seedless mandarin hybrids is the creation of triploids, such as 'Tahoe Gold' from University of California Riverside (Chao, 2005). Important problems with many citrus triploids include low fruit set and thorniness (Khan, 2007).

Citrus kinokuni 'Mukaku kishu' is a completely seedless bud sport of the seedy kinokuni mandarin (Nesumi et al., 2001). Seedlessness was produced by female sterility resulting from arrested embryo development. Two genes were responsible for the abortion of the zygote, a *Fs* dominant gene and an *Is* repressor gene. The *Is* repressor gene inhibits the expression of the seedless trait (Yamasaki et al. 2009; Chavez and Chaparro, 2011).

At the Fruit Tree Breeding Program at the University of Florida (Gainesville , FL), *C. kinikuni* 'Mukaku kishu' has been crossed with two advanced breeding lines of seedy citrus. The objective of this research was to understand if there are any xenia-like effects for fruit size and soluble solids content (SSC) between seeded and seedless individuals in the populations.

Materials and Methods

Plant material. In fall 2013, a total of 213 ten year-old F_1 individuals from two breeding populations segregating for genetic seedlessness *Fs* were used in this study. Breeding selections Robinson OP 'GS' and 'G96-01' were used as female parents in crosses with *Citrus kinokuni* 'Mukaku kishu' PI539530 at the Fruit Tree Breeding Program at the University of Florida, Gainesville, FL. Segregating populations were planted, maintained, and grown following standard commercial production practices in Florida.

Phenotypic studies. Populations were evaluated for a period of 3-4 fruiting seasons to confirm presence or absence of seeds [as previously reported by Chavez and Chaparro (2011)]. Additional fruit phenotypic characteristics, fruit weight (g), SSC (%), and presence (neck)/absence of a neck (flush) at the fruit stem end, were evaluated in at least three fruit per genotype for one season. Fruit was harvested and evaluated on-site using a handheld refractometer (Cat. no. FS1394621, Thermo Fisher Scientific, Waltham, MA) and a portable OHAUS™ Scout™ Pro Series Electronic Toploading Balances (OAHUS corporation, Parsippany, NJ) to measure SSC and fruit size, respectively.

Data analysis. The Mendelian segregation ratios for seedlessness and the presence/absence of neck in the F_1 progeny were calculated using the Chi-square 'goodness-of-fit' test. Analysis of variance (ANOVA) was performed using SAS's PROC GLM procedure (Statistical Analysis System Version 9.1, SAS Institute, Cary, NC). Means for weight and SSC were compared with Tukey's test (*p-value* <0.05). Correlations between fruit weight and SSC were calculated using the PROC CORR procedure of SAS.

Results

For the Robinson OP 'GS' × *C. kinokuni* segregating population, seedless (*Fsfs*) fruits had higher SSC than seedless/seeded (leaky) fruit and seeded (*fsfs*) fruit were intermediate (Table 1). For the 'G96-01' × *C. kinokuni* family, both seedless and seeded genotypes

Table 1. Fruit weight and soluble solids concentration of Robinson OP 'GS' × *C. kinokuni* segregating population for genetic seedlessness *Fs* as separated by presence (*Fsfs*) or absence (*fsfs*) of seeds.

Phenotype	Genotypes (no)	Fruit (no)	Weight (g)	SSC (%)
Seedless *Fsfs*	82	227	96.9 a[z]	9.1 a
Seeded *fsfs*	84	241	102.8 a	8.9 ab
Seedless/Seeded[y]	12	36	106.4 a	8.8 b

[z] Similar letters within a column indicates means not significantly different, Tukey's test, α=0.05.

[y] Seedless/Seeded represented genotypes that contain one or traces of seeds.

Table 2. Fruit weight and soluble solids concentration of 'G96-01' × *C. kinokuni* segregating population for genetic seedlessness *Fs* as separated by presence (*Fsfs*) or absence (*fsfs*) of seeds.

Phenotype	Genotypes (no)	Fruit (no)	Weight (g)	SSC (%)
Seedless *Fsfs*	18	49	91.4 a[z]	10.0 a
Seeded *fsfs*[y]	17	44	127.6 a	9.9 a

[z] Similar letters within a column indicates means not significantly different, Tukey's test, α=0.05.
[y] Seedless/Seeded genotypes were not included in the analyses because only one was identified in this population.

had similar SCC (Table 2). Seeded/seedless (leaky) individuals were hybrids that presented minute traces of seeds or one/two small seeds in the flesh. The average SSC of the seedless (*Fsfs*) was higher than that of the seedless/seeded (leaky) individuals for the Robinson OP 'GS' ×*C. kinokuni* segregating population (Table 1). There was no difference in fruit weight between the three types.

A histogram showing the distribution of the fruit weight from the Robinson OP 'GS' × *C. kinokuni* 'Kishu' family (Fig. 1) showed that there was little difference between the averages of the seeded (101.0g) and seedless (98.4g) types. Additionally, SSC was similar for the two types in this family (Fig. 2).

The presence or absence of the neck at the stem end of the fruit did not deviate from a Mendelian segregation of 3:1 (neck/no neck; Fig. 3) for both segregating populations (χ^2=0.31). It is difficult to determine the nature of the allelic gene composition for both parents because no additional test crosses were made.

The presence or absence of the enlarged neck in progeny of the Robinson OP 'GS' x *C. kinokuni* 'Mukaku kishu' PI539530 progeny did not affect in the overall fruit size and

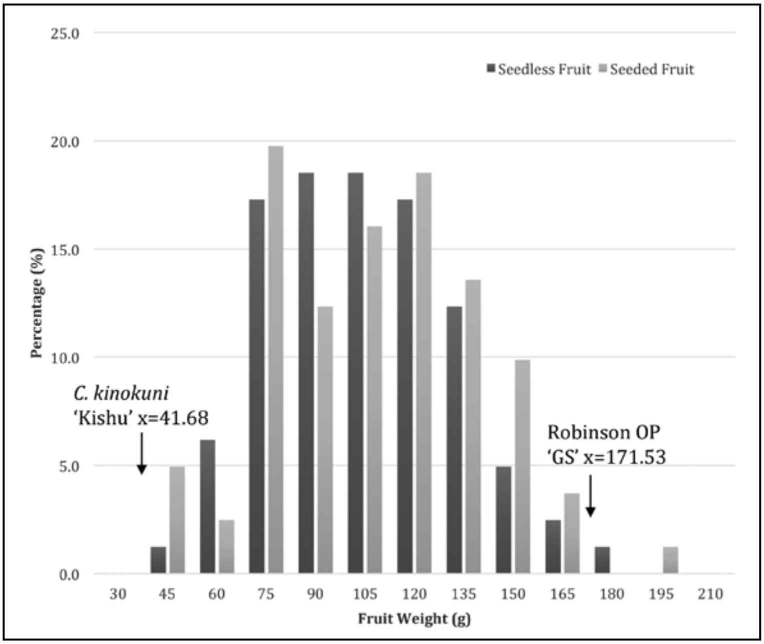

Fig. 1. Fruit size (g) distribution in segregating population between breeding selection Robinson OP 'GS' and *Citrus kinokuni* 'Mukaku kishu' PI539530.

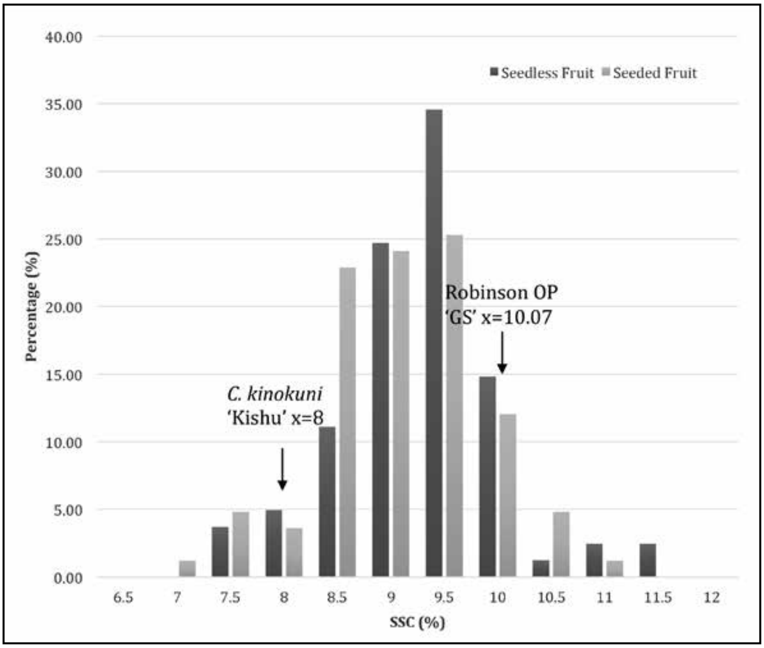

Fig. 2. Soluble solids concentration (%) distribution in segregating population between breeding selection Robinson OP 'GS' and *Citrus kinokuni* 'Mukaku kishu' PI539530.

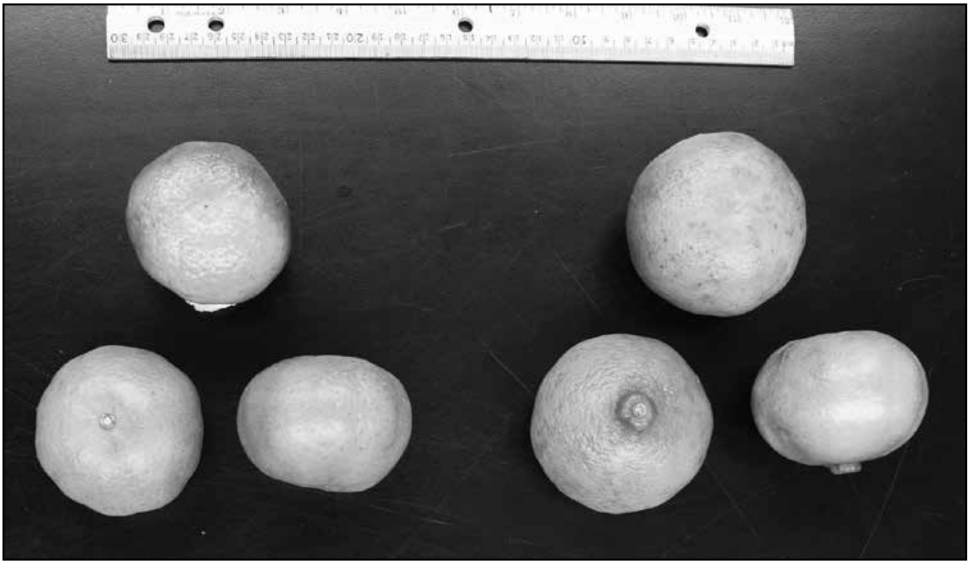

Fig. 3. Absence (left) or presence (right) of a neck at the stem end of fruit from progeny of the segregating population of Robinson OP 'GS' and *C. kinokuni* 'Mukaku kishu' PI539530.

Table 3. Fruit weight and soluble solids concentration of Robinson OP 'GS' × C. kinokuni segregating population for genetic seedlessness Fs as separated by presence or absence of fruit neck.

Phenotype	Genotypes (no)	Fruit (no)	Weight (g)	SSC (%)
Neck	138	396	100.31 a[z]	8.95 a
Flush (no neck)	40	108	100.70 a	9.05 a

[z] Similar letters within a column indicates means not significantly different, Tukey's test, α=0.05.

SSC (Table 3). However, this characteristic was associated with the mean fruit weight in the 'G96-01' ×*C. kinokuni* 'Mukaku kishu' PI539530 segregating population, with fruit from genotypes with enlarged neck having an average weight of 90.7g in comparison with their flush counterparts of 137.9g. Similarly, fruit from genotypes with a neck had a SSC of 9.7 in comparison with fruit with no neck with an average SSC of 10.2. For all the segregating populations SSC was not correlated with fruit weight.

Conclusions

In the pursuit of developing a seedless citrus cultivar with a heritable seedless trait, it is important to identify and understand any affects that this trait may have on fruit size and sugar content. The research presented shows that the four families of F1 breeding populations segregating for the seedless (*Fsfs*) trait, have no significant difference in fruit weight (g) or SSC (%) from their seeded counterparts. In addition, the presence of a neck at the stem segregated in a 3:1 (+/-) fashion among these populations. This trait had no consistent effect on the measured parameters in this study.

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