Flowering Time and Productivity of Interspecific Grafts Between Pepper Species in Contrasting High Tunnel-sheltered and Open-field Production Environments in Costa Rica

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SUMMARY. The production of sweet peppers (*Capsicum annuum*) is often constrained in tropical environments by susceptibility to persistent soil-borne diseases, including bacterial wilt (*Ralstonia solanacearum*). However, the production of sweet peppers in high tunnels using sterile soilless media irrigated with nutrient solution offers the potential to reduce the incidence of bacterial wilt. An additional strategy for disease management is the use of sweet pepper scions grafted onto rootstocks that are resistant to soil-borne pathogens. Two sweet pepper cultivars grown extensively in the tropics, Nathalie and 4212, were used as scions and grafted onto the habanero pepper cultivar Habanero TEC (*Capsicum chinense*) and the aji pepper cultivar Baccatum TEC (*Capsicum baccatum*). Two cultivars related to the two rootstocks were prescreened for susceptibility to two virulent strains of bacterial wilt. Graft combinations were grown in two environments, a high tunnel with automatic nutrient solution irrigation of containers filled with sterile coconut fiber and an open field with known high levels of bacterial wilt inoculum. Self-grafted and nongrafted plants of scions were included as checks. The disease susceptibility screening showed that the area under the disease progress curve was consistently low for 'Habanero TEC' and 'Baccatum TEC' when inoculated with two virulent strains of bacterial wilt, suggesting that habanero pepper cultivars and, to a lesser degree, aji pepper cultivars may be useful as rootstocks in soils with bacterial wilt inoculum. Significant increases in yield, fruit number, and reduced time to flowering were observed in the high tunnel compared with the open-field environment. Individual fruit weight was reduced in the high tunnel compared with the field. Yield, fruit number, fruit weight, and time to flowering were consistent between scions regardless of rootstock. No differences were observed for yield, fruit number, fruit weight, or time to flowering of self-grafted and nongrafted scion checks. In the high tunnel, yield was higher in scions grafted onto 'Habanero TEC' compared with self-grafted and nongrafted checks. In the open field, yield and fruit number were highest on scions grafted onto 'Habanero TEC'. Regardless of graft treatment, high-tunnel production in tropical environments can result in significant increases in yield and fruit number compared with open-field production. No advantage of grafted plants was observed in the high-tunnel production environment. In contrast, in the open-field environment, grafting sweet pepper scions onto pungent habanero rootstocks resulted in a significant increase in yield, fruit number, and fruit size compared with self-grafted and nongrafted checks. The increase was likely attributable to the resistance of habanero pepper cultivars to soil-borne diseases, including bacterial wilt.

Solar-heated, passively ventilated, plastic or mesh-coated high tunnels are being increasingly adopted by growers of tomato (*Solanum lycopersicum*) and sweet pepper (nonpungent *Capsicum annuum*) in temperate and tropical regions worldwide (Carey et al., 2009; Jett, 2017; Ramírez-Vargas, 2019). In temperate environments, high tunnels are advantageous largely because of season extension and protection from adverse

weather, including rain, wind, and hail (Lamont, 2009). Harvest from high-tunnel crops can occur up to 5 weeks ahead of crops grown in open-field conditions, providing a marketing advantage that often increases profitability (Kaiser and Ernst, 2017). However, in tropical environments, high-tunnel structures covered with tight mesh rather than plastic on the walls and equipped with drip irrigation systems are being adopted by

growers for different reasons. The primary advantage of high-tunnel production in the tropics is protection from insects and diseases, and the use of sterile, well-drained, soilless media and drip irrigation provides increased protection from bacterial wilt (*Ralstonia solanacearum*), a soil-borne and water-borne disease common in the lowland tropics (Champoiseau et al., 2009; Ramírez-Vargas and Nienhuis, 2012). The major challenge to high-tunnel production in the tropics is dissipation of heat using structural designs or fans that facilitate ventilation (Ramírez-Vargas and Nienhuis, 2012).

In warm temperate and tropical regions worldwide, one of the most devastating and difficult to manage plant pathogens is the soil-borne and waterborne bacterial wilt species complex. Because of the wide host range, adaptation to diverse ecological niches, and genetic diversity among strains, biovars, and phylotypes, options for sustainable disease management of bacterial wilt are often limited to disease avoidance in high tunnels, crop rotation with nonhost crops, and time out of production (Gutarra et al., 2017; McAvoy et al., 2012; Rahman et al., 2021). Control by soil fumigation is expensive and, in some cases, ineffective, and it can result in environmental externalities (Yagi et al., 1993). Most chili (pungent *C. annuum*) and sweet pepper cultivars are susceptible to bacterial wilt, and this limitation often constrains the production area, especially in tropical and warm temperate environments (Du et al., 2019). However, broad-based tolerance and resistance to a wide range of bacterial wilt strains have been observed for the habanero pepper (*C. chinense*) and aji pepper (*C. baccatum*) (Di Dato et al., 2015; Rossato et al., 2018; Silvar and García-González, 2017). Breeding for durable and broad disease resistance against multiple phylotypes or biovars of bacterial wilt in tomato and sweet pepper has had limited success because of the large genetic variations in pathogenicity among biotypes of the pathogen. Moreover, breeding for disease resistance is complicated by the multigenic inheritance of resistance derived from wild tomato and sweet pepper species (Lebeau et al., 2011). Because of the complex inheritance of resistance derived from habanero and aji pepper cultivars, genetic drag associated with disease resistance genes can result in

undesirable plant growth and fruit quality characteristics (Matsunaga et al., 1998; Mimura et al., 2009). An alternative method for disease management in soils with a high incidence of soil pathogens is to physically combine elite cultivars onto rootstocks with broad resistance by grafting (Gutiérrez-Benites, 2018; King et al., 2010; Lee, 1994; Louws et al., 2010; Rivard and Louws, 2008; Rivard et al., 2010). Interspecific grafting of sweet pepper cultivars onto habanero and aji pepper cultivars provides a potential mechanism for bacterial wilt management and may allow for the expansion of successful production environments, thus bypassing problems associated with genetic drag of resistance genes (Lee et al., 2010; Warschefsky et al., 2016).

Grafting is a technology that has been successfully implemented for soil-borne pathogen management; however, the effects of different rootstocks on the scion have been inconsistent across scions (Mackey et al., 2018; Suchoff et al., 2019). The physiological stress associated with grafting may alter the nutritional and hormonal signaling between scion and rootstock, which could have a significant role during graft establishment and may contribute to changes in the scion's fruit characteristics (Davis et al., 2008; Gutiérrez-Benites, 2018; Wang et al., 2017). Moreover, grafting may induce epigenetic effects by producing mobile signals (siRNAs) that alter the transcription of genes and direct methylation changes in the scion (Bhogale et al., 2014; Goldschmidt, 2014; Springer and Schmitz, 2017; Tsaballa et al., 2021; Zhang et al., 2013). For sweet and chili pepper, changes associated with grafting have been observed in fruit shape,

size, and weight (Chávez-Mendoza et al., 2015; Doñas-Uclés et al., 2015; Sánchez-Chávez et al., 2015; Tsaballa et al., 2013). However, the observed changes are mostly limited to specific rootstock–scion combinations. For example, several studies have shown that grafting contributes to changes in fruit quality traits, including shape, capsaicin content, and plant growth traits in scions of ‘Yatsubusa’ chili pepper grafted onto ‘Spanish Paprika’ sweet pepper (Taller et al., 1998, 1999; Yagishita and Hirata, 1986, 1987; Yagishita et al., 1985, 1990).

Interspecific grafting of sweet pepper cultivars onto disease-resistant habanero and aji rootstocks is currently being investigated as a technology to manage soil pathogens and expand sweet pepper production in tropical environments (Loewen et al., 2021; Vega-Alfaro, 2020). The objectives of this research were 2-fold: 1) to evaluate the disease susceptibility of two sweet pepper cultivars and one each of habanero and aji pepper to two virulent phylotypes of bacterial wilt, and 2) to evaluate the yield and flowering time of interspecific graft combinations using sweet pepper cultivars as scions and pungent habanero and aji cultivars as rootstocks in an open field and a high tunnel with soilless media, which are two contrasting tropical production environments.

Materials and methods

DISEASE SENSITIVITY ASSAYS. The assay was performed in a 90-ft² growth chamber at the University of Wisconsin-Madison in 2018. Air temperature inside the chamber was maintained at 28 °C. The chamber had a 12-h photoperiod. The average photosynthetic photon flux was 335 μmol·m⁻²·s⁻¹

during the light period. Two virulent bacterial wilt strains, UW300 (phylotype I), and UW29 (phylotype II), isolated from sweet or chili pepper host plants were used for inoculations. Strains were cultured in casamino acid peptone glucose or casamino acid peptone glucose plus triphenyl tetrazolium chloride agar. ‘Aji Rico’ aji pepper (PanAmerican Seed, West Chicago, IL), ‘Primero Red’ habanero pepper (PanAmerican Seed), and sweet pepper cultivars Nathalie (Petoseed, Saticoy, CA) and Dulcítico (Universidad de Costa Rica, Alajuela, Costa Rica) were used for soil-soak inoculations. Plants were seeded on 96-cell trays and transplanted 2 weeks later to 4-inch-diameter pots filled with 80 g of soilless media (ProMix HP; Premier Horticulture, Quakertown, PA); they were inoculated 18 d after planting as described by Tans-Kersten et al. (2001). Briefly, 50 mL of bacterial suspension containing 2.83×10^9 cfu/mL of UW300 or 4.33×10^8 cfu/mL of UW29 was poured onto the potting media. Roots were wounded before inoculation by plunging a 3-cm-wide × 20-cm-long stainless-steel spatula into the pot 2 cm away from the plant stem. The concentration and morphology of both strains were confirmed by dilution-plating of a 100-μL inoculum sample. Ten plants from each cultivar were rated for wilting symptoms daily for 14 consecutive days after inoculation using a disease index scale ranging from 0 to 4 [0 = no wilting; 1 = 1% to 25% of the leaves of each plant wilting; 2 = 26% to 50% wilting; 3 = 51% to 75% wilting; 4 = 76% to 100% wilted leaves or dead (Tans-Kersten et al., 2001)]. The area under disease progress curve (AUDPC) was estimated from the disease index scores and time (days) using statistical software

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.574	fl oz	μL	3.3814×10^{-5}
29.5735	fl oz	mL	0.0338
0.3048	ft	m	3.2808
0.0929	ft ²	m ²	10.7639
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
0.4536	lb	kg	2.2046
0.0254	mil(s)	mm	39.3701
28.3495	oz	g	0.0353
7.4892	oz/gal	g·L ⁻¹	0.1335
1	ppm	mg·L ⁻¹	1
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

(R version 3.6.2; R Core Team, Vienna, Austria) and the agricolae package (De Mendiburu and Yassen, 2020) as described by the American Phytopathological Society (2020). The AUDPC was used as a quantitative measure of disease severity over time for comparisons.

PLANT MATERIAL FOR GRAFTING. Sweet pepper cultivars 4212 and Nathalie were used as scions (Table 1). Both produce nonpungent, long-fruited, lamuyo-type peppers that are popular among growers in Costa Rica. The cultivars used as rootstocks were the inbred lines Habanero TEC and Baccatum TEC (Table 1). Both ‘Habanero TEC’ and ‘Baccatum TEC’ were derived by open-field pedigree selection among self-pollinated progeny of ‘Primero Red’ and ‘Aji Rico’, respectively, in soils in San Carlos, Costa Rica, with known high levels of bacterial wilt inoculum (C. Ramírez-Vargas, unpublished data).

GRAFTING PROCEDURE. Seed of each cultivar was sown on 128-cell trays with peatmoss media and grown until the stem at 1 cm below the cotyledon reached ≈ 2.0 mm (30–35 d after seeding). Plants were grafted at the Vivero Rincón de Paz nursery (Sarchí, Costa Rica). Rootstock plants 30 to 35 d old were cut at a 75° angle underneath the cotyledons. A 2.0-mm silicon clip (Centro de Semillas, Ochozogo, Costa Rica) was inserted halfway onto the rootstock. Scion

plants with a similar diameter were cut at the same angle and inserted in the grafting clip until both cuts aligned properly and the scion and rootstock were in full contact. Immediately after grafting, plants were placed in a dark healing chamber for 7 d. The average daily relative humidity and temperature during the 7-d healing period ranged between 85% and 95% and 24 and 28 °C, respectively. Grafted plants were acclimatized for 1 week in a high-tunnel structure with an average daily temperature of 24.6 °C before transplanting.

OPEN-FIELD PRODUCTION ENVIRONMENT. A field located at Tecnológico de Costa Rica, Campus San Carlos, Costa Rica (TEC), with records showing a persistently high incidence of bacterial wilt was selected for the open-field trials. The presence of bacterial wilt in the field environment was confirmed by symptom characterization of infected plant samples from the experimental plot in 2019 by the Plant Pathology Laboratory at TEC. Grafted plants and nongrafted checks were transplanted to 0.80-m-wide $\times$ 0.40-m-high raised beds spaced 1.50 m apart. In-row spacing between plants was 0.30 m. The soil type was a Tropic Dystropepts. The rows were covered using silver-on-black plastic mulch with a thickness of 1.2 to 2.0 mils and a width of 4 ft. Rainfall supplied most of the water to the

field and an irrigation system provided nutrients. Plants were drip-irrigated twice per day (0.26 L/plant per day) with a modified Steiner universal solution (Steiner, 1984) adjusted to an electric conductivity of 2 mS·cm⁻¹ {1.18 g·L⁻¹ calcium nitrate tetrahydrate [Ca (NO₃)₂·4 H₂O], 0.37 g·L⁻¹ magnesium sulfate heptahydrate (MgSO₄·7H₂O), 0.50 g·L⁻¹ potassium nitrate (KNO₃), and 0.27 g·L⁻¹ of monopotassium sulfate (KH₂PO₄)}. Micronutrients were supplied with 16 mg·L⁻¹ of micro-nutrient fertilizer (Microplex; Miller Chemical & Fertilizer, Hanover, PA). Weeds were manually controlled. Air temperature and rainfall were recorded using a weather station data logger (HOBO U30-NRC; Onset Computer Corp. Bourne, MA).

HIGH-TUNNEL PRODUCTION ENVIRONMENT. The high-tunnel production environment was located at TEC and consisted of a mesh structure that enclosed containers filled with soilless media. The high tunnel was 12 m long, 10 m wide, and 5 m high, and it consisted of an arched top covered with low-density ultraviolet-filtering polyethylene, a 0.8-m overhead window across the length of the high tunnel, and vertical walls 3 m high covered with anti-aphid mesh. The ground was covered with a reflective white groundcover. One plant per container was grown in sterilized 10-L containers filled with ≈ 10 L of

Table 1. Cultivar name, species, breeder, days to maturity, and fruit characteristics of sweet pepper scions grafted onto habanero and ají pepper rootstocks including self-grafted and nongrafted checks evaluated in open-field and high-tunnel production environments in San Carlos, Costa Rica, in 2019.

Cultivar	Species	Breeder (yr) ^z	Fruit				
			Maturity (d) ^y	Size (mm) ^x	Color	Type	Pungency
Scions							
4212	<i>Capsicum annuum</i>	Seminis	100	150 × 65	Green to red	Lamuyo	No
Nathalie (PEP 2129)	<i>C. annuum</i>	Petoseed (1980)	105	140 × 65	Green to red	Lamuyo	No
Rootstocks							
Habanero TEC	<i>C. chinense</i>	C. Ramírez-Vargas (2013)	75	45 × 30	Green to red	Habanero	Hot
Baccatum TEC	<i>C. baccatum</i>	C. Ramírez-Vargas (2012)	70	50 × 40	Green to yellow	Ají	Medium

^zSeminis = Seminis Vegetable Seeds, St. Louis, MO; Petoseed = Petoseed Co., Saticoy, CA. ‘Habanero TEC’ and ‘Baccatum TEC’ are inbred lines developed by pedigree selection for uniformity by C. Ramírez-Vargas at the Tecnológico de Costa Rica (San Carlos, Costa Rica) within populations derived by self-pollination from cultivars Habanero Red and Aji Rico, respectively. Original cultivars were obtained from PanAmerican Seed Co. (West Chicago, IL).

^yMaturity is defined as the days from transplant to the stage of development of the fruit that includes all the characteristics for use by consumers. Maturity data for ‘Habanero TEC’ and ‘Baccatum TEC’ were recorded in San Carlos, Costa Rica. Information regarding maturity was obtained from Bosland (2016) and C. Ramírez-Vargas (unpublished data).

^xLength $\times$ width. Fruit size data for ‘Habanero TEC’ and ‘Baccatum TEC’ were recorded in San Carlos, Costa Rica. Information regarding size was obtained from Bosland (2016) and (C. Ramírez-Vargas, unpublished data). 1 mm = 0.0394 inch.

sterilized coconut fiber media (coir). Spacing between rows was 1.50 m, and in-row spacing between plants was 0.40 m. Plants were irrigated daily with the same nutrient solution as that used in the field, and irrigation was scheduled using an irrigation timer (VYR-6045 Rain Pro 4; VYRSA, Burgos, Spain) with a 2-min irrigation time every 30 min from 0600 to 1800 HR (≈ 1 L of nutrient solution per plant per day averaged across all growth stages). Insect, mite, and fungal pests in both environments were controlled based on weekly scouting according to recommended thresholds (Centro Agronómico Tropical de Investigación y Enseñan, 1993).

EXPERIMENT DESIGN AND MEASURED VARIABLES. The experiment was a factorial of two rootstocks and two scions evaluated as a completely randomized design with six (field) and three (high tunnel) replications. The experimental unit comprised six plants in the field and three plants in the high tunnel. The exception was for scions grafted onto ‘Habanero TEC’, which had an experimental unit of three plants that was replicated five times in the field experiment. Field and high-tunnel experiments were planted between 10 and 11 June 2019. The harvest of mature, full-size scion fruit consistent with scion commercial descriptions began 24 Aug. 2019 in the high tunnel and 2 Sept. 2019 in the field environment. Harvests in both production environments ceased when no additional full-size fruit were produced, which occurred 21 Oct. 2019 in the field environment and 4 Nov. 2019 in the high tunnel. Time to flowering was measured as the number of days after transplanting until at least one fully opened flower was observed. Fruit number was the cumulative number of full-size fruit harvested. Fruit weight was calculated by dividing the total weight of fruit by the number of fruit per plant. Values for each variable used for statistical analyses were the means of individual plant measurements from each experimental unit.

STATISTICAL ANALYSIS. Time to flowering and AUDPC were Box-Cox transformed to better approximate normality and fulfill the assumptions for the analysis of variance (ANOVA) and mean separation procedures; however, for clarity, untransformed means

are presented for all variables. A combined ANOVA was performed to investigate whether the growing environments were different. Estimates of each environment were used for a second ANOVA of means to compare scion and rootstock treatments within each environment. The ANOVA, least square means, correlations, and mean separation procedures for all the variables were estimated using statistical software (JMP Pro version 15; SAS Institute, Cary, NC). Pairwise comparisons within each environment were made using the least significant means statement and Tukey’s honestly significant difference for multiple comparisons at an alpha level of 0.05.

Results and discussion

DISEASE SEVERITY OF SWEET, HABANERO, AND AJI PEPPER CULTIVARS INOCULATED WITH BACTERIAL WILT. Sweet pepper cultivars Dulcítico and Nathalie are in the same market class, lamuyo, and share similar fruit characteristics. Both are commonly grown in Costa Rica in open fields with low levels of bacterial wilt inoculum and in drip-irrigated high tunnels (C. Ramírez-Vargas unpublished). ‘Primero Red’ habanero pepper and ‘Aji Rico’ aji

pepper are small-fruited, pungent cultivars with aromatic fruit that are bred for their increased fruit size and early maturity in temperate environments (PanAmerican Seed, 2021). The ranking of the AUDPC of the four cultivars was consistent for the two virulent strains of bacterial wilt, UW29 and UW300, with ‘Primero Red’ and ‘Aji Rico’ having the smallest AUDPC values compared with ‘Dulcítico’ and ‘Nathalie’; however, the values were not always significantly different at the 0.05 level (Fig. 1). The consistent low ranking of AUDPC scores of ‘Primero Red’ and ‘Aji Rico’ for strains and phylogenotypes suggest that ‘Primero Red’ and, to a lesser degree, ‘Aji Rico’ may contain alleles for functional, broad-based resistance to bacterial wilt. This observation is consistent with that of previous studies that have indicated tolerance and resistance to a wide range of bacterial wilt strains in habanero and aji pepper cultivars (Di Dato et al., 2015; Rossato et al., 2018; Silvar and García-González, 2017). The derivative cultivars used for grafting experiments in the current study, Habanero TEC and Baccatum TEC, were developed by open-field pedigree selection among self-pollinated progeny of Primero Red and Aji Rico, respectively, in soils in San

Fig. 1. Bacterial wilt severity measured as the area under disease progress curve (AUDPC) of four pepper cultivars with the potential to be used as rootstocks (Primero Red and Aji Rico) and scions (Nathalie and Dulcítico). Cultivars were soil-soaked inoculated with two bacterial wilt strains from the University of Wisconsin-Madison bacterial wilt collection, UW300 (Phylotype I) (dark bars) and UW29 (Phylotype II) (light bars). Mean AUDPC values represent each the mean of 10 replications. Bars not sharing a letter within each phylotype are significantly different at $P \leq 0.05$.

Carlos, Costa Rica, known to have high levels of bacterial wilt inoculum.

EFFECTS OF CONTRASTING ENVIRONMENTS ON SWEET PEPPER PRODUCTION. The open-field air temperatures in San Carlos, Costa Rica, were favorable for plant growth, averaging 25.2 °C and ranging from 18.2 to 34.3 °C, with a total of 1409 mm of rainfall over the course of the experiment. Sweet pepper plants growing in the field exhibited symptoms of bacterial infection. Bacterial infection was validated by morphological tests using dilution-plating of inoculum samples and characterization of colony growth in triphenyl tetrazolium chloride media performed by the Plant Pathology Laboratory at TEC. The air temperatures in the high tunnel were higher than those in the open field, averaging 28.2 °C and ranging from 18.4 to 49.6 °C. Bacterial wilt inoculum was not detected in the growing media or irrigation water in assays conducted by the Plant Pathology Laboratory at TEC. Significant differences between production environments were observed for yield, fruit number, fruit weight, and time to flowering (Table 2). The optimal temperature for sweet pepper ranges between 20 and 25 °C; temperatures above 32 °C can result in yield reduction because of heat stress and flower abortion (Cochran, 1932; Saha et al., 2010). High-tunnel air temperatures in the current experiment were greater than optimal; nevertheless, the yield

and fruit number per plant were four-times higher compared with those in the open-field environment (Fig. 2). The high temperature stress and flower abortion expected in the high-tunnel environment may have been mitigated by frequent irrigation from the automatic drip irrigation system. By preventing excessive water stress, plants may have maintained a high rate of evapotranspiration that kept leaves substantially cooler than ambient air temperature. The accelerated growth rate, extended harvest season, and reduction in disease incidence may have contributed to the increase in yield and fruit number in the high tunnel compared with the open-field production environment.

Plants in the high tunnel produced fruit for 28 d longer than those in the field, thus contributing to significantly higher total yield and fruit number in the high tunnel compared with that in the field. Time to flowering was reduced by more than 3 d in the high tunnel compared with the open field, likely because of higher average air temperatures (Table 2). This result is consistent with that of reports of an increased rate of accumulation of growing degree days in high tunnels that accelerate growth, development, and ripening of solanaceous crops (Solanaceae) (Both et al., 2007). The weight of individual mature fruit was almost 10 g less in the high tunnel compared with the field (Table 2), an effect that may have been associated

with high temperature stress experienced by fruit. A reduction in individual fruit weight was previously observed for sweet pepper grown in growth chambers at higher day/night temperatures (29/23 °C) compared with lower day/night temperatures (24/18 °C) (Saha et al., 2010). Fruit size depends on the number of cell divisions that take place after fertilization, the number of successful fertilizations within the ovary, and the extent of cell enlargement (Gillaspy et al., 1993). Air temperatures above 30 °C have been reported to reduce chili pepper pollen viability, likely reducing the rate of ovule fertilization and, subsequently, fruit size (Erickson and Markhart, 2002).

EFFECTS OF GRAFTING ON SWEET PEPPER PRODUCTION IN CONTRASTING ENVIRONMENTS. The two scions, ‘Nathalie’ and ‘4212’, although developed and marketed independently, share similar fruit and plant characteristics and are widely used in both open-field and high tunnel tropical production environments in Costa Rica (Table 1). Flowering time and fruit characteristics of ‘Nathalie’ and ‘4212’ scions were consistent with cultivar descriptions, regardless of the rootstock treatment (Tables 1 and 2, Fig. 2). Moreover, no rootstock × scion interactions were observed for yield, fruit number, individual fruit weight, or time to flowering, indicating that the ranking of the scions was consistent regardless of rootstock, and no

Table 2. Analysis of variance for factorial combinations of two sweet pepper scions grafted onto habanero and ají pepper rootstocks including self-grafted and nongrafted checks evaluated in open-field and high-tunnel production environments in San Carlos, Costa Rica, in 2019.

Source		df	Mean squares			
			Yield (kg/plant) ^z	Fruit (no./plant)	Fruit wt (g) ^z	Time to flowering (d)
Production environment (PE)		1	81.30***	14,221.2***	1367.3***	656.9***
Replication		7	0.91	13.3	157.8	1.2
Scion (S)		1	0.23	7.2	0.2	7.2*
Rootstock (R)		3	1.00*	71.8**	213.1*	2.9*
S × R		3	0.10	7.4	64.8	0.2
S × PE		1	0.00	13.8	244.5	2.8
R × PE		3	0.08	33.3	241.9 *	0.4
R × S × PE		3	0.84	30.0	132.5	0.6
Pooled error		47	0.11	17.8	92.9	1.1
Means for the production environment						
PE	Field		0.57	6.4	90.2	35.9
	High tunnel		2.05	25.9	80.9	32.5

^z1 kg = 2.2046 lb; 1 g = 0.0353 oz.

^yTime to flowering was Box-Cox transformed. Nontransformed data are shown.

*, **, ***Significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Fig. 2. Main effect of scions within each production environment for (A) yield, (B) fruit number, (C) fruit weight, and (D) time to flowering of scions ‘4212’ and ‘Nathalie’ sweet pepper grafted onto ‘Baccatum TEC’ aji pepper and ‘Habanero TEC’ habanero pepper rootstocks evaluated under two contrasting tropical production environments, open field (dark bars) and high tunnel (light bars), at Tecnológico de Costa Rica, San Carlos, Costa Rica, in 2019. Means not sharing a letter within each production environment are significantly different. The mean separation procedure was based on Tukey’s honestly significant difference test calculated within each production environment. *** indicates significant differences at a level of <0.001 between the field and high-tunnel production environments. 1 kg = 2.2046 lb; 1 g = 0.0353 oz.

exceptional rootstock–scion combinations were observed (Table 2). Although the effects of production environment were significant for all variables, within environments, the only difference in main effects observed between scions was an increase in time to flowering of 1.1 d for ‘Nathalie’ compared with ‘4212’ in the high tunnel (Fig. 2D).

Selecting the appropriate check to contrast rootstock–scion combinations in grafting experiments (i.e., self-grafted vs. nongrafted) is an experimental design conundrum that depends on the hypothesis being tested. Self-grafted plants are the appropriate check for experimental settings in which both scion and rootstock have experienced similar seedling environments, including graft wounding and time to healing, or when the interest is evaluating the physiological effects of grafting. The observed

differences in this scenario could be attributed to the different rootstock or grafting treatments. However, a grower might instead be interested in comparing the horticultural performance of a nongrafted cultivar of their preference with one or more cultivars used as scions grafted onto one or more rootstocks. In that scenario, nongrafted plants are the suitable check. In the present experiment, both checks were included, and differences between self-grafted and nongrafted checks were not detected in any of the measured variables (Fig. 3).

In the open-field environment, the main effects for yield and fruit number of scions grafted onto ‘Habanero TEC’ were significantly higher compared with nongrafted checks (Fig. 3A and C). In the high tunnel, yield and fruit number were also higher than self-grafted and nongrafted checks, but

they were only significant for yield (Fig. 3A and C). In a parallel experiment, no differences were found in yield or fruit number of scions grafted onto the ‘Primero Red’ habanero pepper rootstocks and those evaluated in multiple years in open-field locations in a temperate environment in which there was no detectable bacterial wilt inoculum (Vega-Alfaro, 2020). In high tunnel evaluations, increases in total fruit weight, number of fruits per plant, and fruit weight per square meter were observed in specific rootstock–scion combinations among a factorial set of four commercial cultivars grafted onto five rootstocks with resistance to soil-borne pathogens (all sweet or pungent chili pepper cultivars) (Soltan et al., 2017). The results of the current experiment suggest that habanero pepper rootstocks may provide a barrier against

Fig. 3. Main effect of rootstock within each production environment for (A) yield, (B) fruit weight, (C) fruit number, and (D) time to flowering of sweet pepper scions grafted onto ‘Baccatum TEC’ aji pepper and ‘Habanero TEC’ habanero pepper rootstocks evaluated under two contrasting tropical production environments, open field (dark bars) and high tunnel (light bars), at Tecnológico de Costa Rica, San Carlos, Costa Rica, in 2019. Nongrafted scions and self-grafted scions are included as controls. Means not sharing a letter within each production environment are significantly different. The mean separation procedure was based on Tukey’s honestly significant difference test calculated within each production environment. * indicates significant differences at a level of <0.001 between the field and high tunnel production environments.**

1 kg = 2.2046 lb; 1 g = 0.0353 oz.

soil pathogens and allow for increased production of otherwise susceptible scions. The increase in yield in scions grafted onto habanero pepper rootstocks in the high-tunnel environment may be attributable to the rootstock providing superior root architecture or tolerance to other unidentified biotic and abiotic stresses (Fig. 3A).

Regardless of the scion or rootstock, individual fruit weight was larger in the open field compared with the high-tunnel production environment (Table 2, Fig. 3B). In the high tunnel, no differences among rootstocks were observed for individual fruit weight (Fig. 3B). In the field environment, scions grafted onto ‘Habanero TEC’ had larger individual fruit weights compared with those of the nongrafted plants, but not compared with those of the self-grafted check (Fig. 3B). This differential

response to the environments by the rootstock treatments contributed to the observed rootstock × production environment interaction in fruit weight (Table 2). Increases in the individual fruit weight of grafted sweet pepper cultivars have also been observed in field-grown plants in Almería, Spain, but only for the specific combinations of ‘Jalapeño’ and ‘SCM-334’ rootstocks and the scion ‘Palermo’ (Doñas-Uclés et al., 2015). Reductions in fruit size have been also reported for intraspecific ‘Yatsubusa’ sweet pepper scions grafted onto ‘Spanish Paprika’ chili pepper (Yagishita et al., 1985).

Regardless of the production environment, no significant differences in time to flowering of scions grafted onto either ‘Habanero TEC’ or ‘Baccatum TEC’ were observed (Fig. 3D). Time to flowering within

each production environment was not significantly different between self-grafted and nongrafted checks (Fig. 3D). Because time to flowering was measured after grafting (i.e., from transplanting to first flower), the recovery time from grafting was not included. These results suggest that grafting did not result in a significant delay in transition to reproductive growth, although a 2-week period of recovery and acclimation of the grafted plants was required. In a parallel field-based experiment in a temperate environment where the average air temperature was ≈20 °C, sweet pepper scions grafted onto ‘Aji Rico’ aji pepper had reduced time to flowering compared with self-grafted and nongrafted checks (Vega-Alfaro, 2020). The rootstock ‘Aji Rico’ is an aji pepper bred specifically for early maturity in temperate environments (J. Nienhuis, unpublished

data). The average air temperature in the field and high-tunnel environments in tropical San Carlos, Costa Rica, were much higher, 25 and 28 °C, respectively; therefore, the effects of reduced time to flowering provided by aji pepper rootstocks may depend on specific aji pepper cultivar and the production environment. The maturity characteristics are inherently of the scion, but the rootstocks could significantly impact scion maturity by altering the production and passage of flowering stimuli, phytohormones, siRNAs, and mRNAs, or by regulating the gene expression of flowering loci in the scion (Haroltsen et al., 2012; Pant et al., 2008; Wada and Takeno, 2010). Research of a sweet pepper relative, potato (*Solanum tuberosum*), has shown that flowering and tuber induction signals are graft-transmissible (Martin et al., 2009; Navarro et al., 2011).

Conclusions

Regardless of graft treatment, the results presented here suggest that high-tunnel production using soilless media in tropical environments can result in significant increases in sweet pepper yield and fruit number compared with open-field production. Insect-resistant screening combined with sterile soilless media and automatic drip irrigation may reduce the potential for disease and high-temperature stress and contribute to significantly higher yield and fruit number. In contrast to temperate environments where season extension in high tunnels is attributable to the early-season accumulation of degree days (Ohletz and Loy, 2021), season extension in this study in a hot and humid tropical environment was likely attributable to a significant reduction in insect transmission of disease and a reduction of soil-borne and water-borne disease on scions in the high tunnel compared with the field. The habanero and aji pepper rootstocks used in this study were selected for resistance to soil pathogens and did not provide any observable graft-transmissible disease resistance to the scion. The results of this study indicate that there is no yield, fruit size, or maturity advantage gained from using sweet pepper scions grafted onto either habanero or aji pepper rootstocks in container-grown plants in high tunnels. In contrast, the results suggest that in open-field

production environments, especially those with a high level of soil pathogens, including bacterial wilt, grafting sweet pepper scions onto habanero pepper rootstocks can provide a significant increase in yield, fruit number, and individual fruit size compared with self-grafted and nongrafted checks. The initial high construction cost of a semi-permanent high tunnel equipped with automatic drip irrigation and ventilation to minimize excessive heat stress may preclude adoption of this technology by many growers in the tropics. To promote the adoption of technology such as either high-tunnel production or the use of sweet pepper scions grafted onto rootstocks resistant to soil pathogens in open-field production in the tropics, additional research and outreach efforts are needed to clarify the potential economic benefits and time required for the return on investment for either grafted plants or the construction of high tunnels.

Literature cited

- American Phytopathological Society. 2020. Calculating the area under the disease progress curve to quantify disease progress. 20 May 2020. <<https://www.apsnet.org/edcenter/disimpactnmgmt/topc/EcologyAndEpidemiologyInR/DiseaseProgress/Pages/AUDPC.aspx>>.
- Bosland, P.W. 2016. Vegetable cultivar descriptions for North America – Pepper (M-Z). 3 June 2018. <<https://cucurbit.info/2016/06/pepper-m-z/>>.
- Bhogale, S., A.S. Mahajan, B. Natarajan, M. Rajabhoj, H.V. Thulasiram, and A.K. Banerjee. 2014. MicroRNA156: A potential graft-transmissible microRNA that modulates plant architecture and tuberization in *Solanum tuberosum* ssp. *andigena*. Plant Physiol. 164(2):1011–1027, <https://doi.org/10.1104/pp.113.230714>.
- Both, A.J., E. Reiss, J.F. Sudal, K.E. Holmstrom, C.A. Wyenandt, W.L. Kline, and S.A. Garrison. 2007. Evaluation of a manual energy curtain for tomato production in high tunnels. HortTechnology 17(4):467–472, <https://doi.org/10.21273/HORTTECH.17.4.467>.
- Carey, E.E., L. Jett, W.J. Lamont, T.T. Nennich, M.D. Orzolek, and K.A. Williams. 2009. Horticultural crop production in high tunnels in the United States: A snapshot. HortTechnology 19(1):37–43, <https://doi.org/10.21273/HORTSCI.19.1.37>.
- Centro Agronómico Tropical de Investigación y Enseñanza. 1993. Guía para el manejo integrado de plagas del cultivo de chile dulce. Serie Técnica, Informe Técnico No. 201. CATIE, Turrialba, Costa Rica.
- Champoiseau, P.G., J.B. Jones, and C. Allen. 2009. *Ralstonia solanacearum* race 3 biovar 2 causes tropical losses and temperate anxieties. Plant Health Prog. 10:35–45, <https://doi.org/10.1094/PHP-2009-03.13-01-RV>.
- Chávez-Mendoza, C., E. Sánchez, E. Muñoz-Marquez, J.P. Sida-Arreola, and M.A. Flores-Córdova. 2015. Bioactive compounds and antioxidant activity in different grafted varieties of bell pepper. Antioxidants 4(2):427–446, <https://doi.org/10.3390/antiox4020427>.
- Cochran, H.L. 1932. Factors affecting flowering and fruit setting in the pepper. Proc. Amer. Soc. Hort. Sci. 29:434–437.
- Davis, A.R., P. Perkins-Weazie, R. Hassell, A. Levi, S.R. King, and X. Zhang. 2008. Grafting effects on vegetable quality. HortScience 43(6):1670–1672, <https://doi.org/10.21273/HORTSCI.43.6.1670>.
- De Mendiburu, F. and M. Yassen. 2020. agricolae: Statistical procedures for agricultural research. R package version 1.4.0. 16 Nov. 2020. <<https://myaseen208.github.io/agricolae/>><https://cran.r-project.org/package=agricolae>>.
- Di Dato, F., M. Parisi, T. Cardi, and P. Triodi. 2015. Genetic diversity and assessment of markers linked to resistance and pungency genes in *Capsicum* germplasm. Euphytica 204(1):103–119, <https://doi.org/10.1007/s10681-014-1345-4>.
- Doñas-Uclés, F., D. Pérez-Madrid, C. Amate-Llobregat, E.M. Rodríguez-García, and F. Camacho-Ferre. 2015. Production of pepper cultivar Palermo grafted onto Serano de Morelos 2, Jalapeno, and three commercial rootstocks. HortScience 50(7):1018–1022, <https://doi.org/10.21273/HORTSCI.50.7.1018>.
- Du, H., C. Wen, X. Zhang, X. Xu, J. Yang, B. Chen, and S. Geng. 2019. Identification of a Major QTL (qRRs-10.1) that confers resistance to *Ralstonia solanacearum* in pepper (*Capsicum annuum*) using SLAF-BSA and QTL mapping. Int. J. Mol. Sci. 20(23):5887, <https://doi.org/10.3390/ijms20235887>.
- Erickson, A.N. and A.H. Markhart. 2002. Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annuum* L.) to elevated temperature. Plant Cell Environ. 25:123–130, <https://doi.org/10.1046/j.0016-8025.2001.00807.x>.
- Gillaspy, G., H. Ben-David, and W. Gruissem. 1993. Fruits: A developmental perspective. Plant Cell 5:1439–1451, <https://doi.org/10.1105/tpc.5.10.1439>.

- Goldschmidt, E.E. 2014. Plant grafting: New mechanisms, evolutionary implications. *Front. Plant Sci.* 5:727, <https://doi.org/10.3389/fpls.2014.00727>.
- Gutarra, L., J. Herrera, E. Fernandez, J. Kreuze, and H. Lindqvist-Kreuzer. 2017. Diversity, pathogenicity, and current occurrence of bacterial wilt bacterium *Ralstonia solanacearum* in Perú. *Front. Plant Sci.* 8:1221, <https://doi.org/10.3389/fpls.2017.01221>.
- Gutiérrez-Benites, E.D. 2018. Effects of grafting tropical rootstocks on yield and fruit quality in tomato (*Solanum lycopersicum* L.) scions. MS Thesis, Univ. Wisconsin-Madison.
- Haroltsen, V., M.W. Szczerba, H. Aktas, J. Loipez, M.J. Odias, C.L. Chi-Ham, J. Labavitch, A.B. Bennett, and A.L. Powell. 2012. Mobility of transgenic nucleic acids and proteins within grafted rootstocks for agricultural improvement. *Front. Plant Sci.* 3:39, <https://doi.org/10.3389/fpls.2012.00039>.
- Jett, L.W. 2017. High tunnels, p. 107–116. In: M.D. Orzolek (ed.). *A guide to the manufacture, performance, and potential of plastics in agriculture*. Elsevier, Cambridge, MA.
- Kaiser, C. and M. Ernst. 2017. High tunnel tomatoes. 10 June 2021. <<https://www.plantgrower.org/uploads/6/5/5/4/65545169/hightunneltomatoes.pdf>>.
- King, S.R., A.R. Davis, X. Zhang, and K. Crosby. 2010. Genetics, breeding and selection of rootstocks for Solanaceae and Cucurbitaceae. *Scientia Hort.* 127(2):106–111, <https://doi.org/10.1016/j.scienta.2010.08.001>.
- Lamont, W.J. 2009. Overview of the use of high tunnels worldwide. *HortTechnology* 19(1):25–29, <https://doi.org/10.21273/HORTSCI.19.1.25>.
- Lebeau, A., M. Daunay, A. Frary, A. Palloix, J. Wang, J. Dintinger, F. Chiroleu, E. Wicker, and P. Prior. 2011. Bacterial wilt resistance in tomato, pepper, and eggplant: Genetic resources respond to diverse strains in the *Ralstonia solanacearum* species complex. *Phytopathology* 101(1):154–165, <https://doi.org/10.1094/PHYTO-02-10-0048>.
- Lee, J. 1994. Cultivation of grafted vegetables I. Current status, grafting methods, and benefits. *HortScience* 29(4):235–239.
- Lee, J., C. Kubota, S.J. Tsao, Z. Bie, P.H. Echevarria, L. Morra, and M. Oda. 2010. Current status of vegetable grafting: Diffusion, grafting techniques, automation. *Scientia Hort.* 127(2):93–105, <https://doi.org/10.1016/j.scienta.2010.08.003>.
- Loewen, D.E., E. Pliakoni, and C.L. Rivard. 2021. Evaluating *Capsicum* and *Solanum* rootstocks for fresh-market bell pepper production. *Acta Hort.* 1302:259–264, <https://doi.org/10.17660/ActaHortic.2021.1302.34>.
- Louws, F.J., C.L. Rivard, and C. Kubota. 2010. Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropods and weeds. *Scientia Hort.* 127(2):127–146, <https://doi.org/10.1016/j.scienta.2010.09.023>.
- Mackey, M., A. Kurosky, E.J. Robb, and R.N. Nazar. 2018. A graft mimic strategy for verticillium resistance in tomato. *Mol. Biotechnol.* 60(9):665–669, <https://doi.org/10.1007/s12033-018-0101-8>.
- Martin, A., H. Adam, M. Diaz-Mendoza, M. Zurczak, N.D. González-Schain, and P. Suárez-Loipez. 2009. Graft-transmissible induction of potato tuberization by the microRNA miR172. *Development* 136(17):2873–2881, <https://doi.org/10.1242/dev.031658>.
- Matsunaga, H., T. Sato, and S. Monma. 1998. Inheritance of bacterial wilt resistance in the sweet pepper cv. Mie-Midori, p. 7–11. In: *Proc. 10th EUCARPIA Mtg. Genet. Breed. Capsicum Eggplant*, Avignon, France, 7–11 Sept. 1998.
- McAvoy, T., J.H. Freeman, S.L. Rideout, S.M. Olson, and M.L. Paret. 2012. Evaluation of grafting using hybrid rootstocks for management of bacterial wilt in field tomato production. *HortScience* 47(5):621–625, <https://doi.org/10.21273/HORTSCI.47.5.621>.
- Mimura, Y., M. Yoshikawa, and M. Hirai. 2009. Pepper accession LS2341 is highly resistant to *Ralstonia solanacearum* strains from Japan. *HortScience* 44(7):2038–2040, <https://doi.org/10.21273/HORTSCI.44.7.2038>.
- Navarro, C., J.A. Abelenda, E. Cruz-Oró, C.A. Cuéllar, S. Tamaki, J. Silva, K. Shimamoto, and S. Prat. 2011. Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. *Nature* 478(7367):119–122, <https://doi.org/10.1038/nature10431>.
- Ohletz, J.L. and J.B. Loy. 2021. Grafting melons increases yield, extends the harvest season, and prevents sudden wilt in New England. *HortTechnology* 31(1):101–114, <https://doi.org/10.21273/HORTTECH04669-20>.
- PanAmerican Seed. 2021. Aji Rico pepper. 8 July 2021. <http://www.panamseed.com/plant_info.aspx?phid=048300001036967>.
- Pant, B.D., A. Buhtz, J. Kehr, and W. Scheible. 2008. MicroRNA399 is a long-distance signal for the regulation of plant phosphate homeostasis. *Plant J.* 53(5):731–738, <https://doi.org/10.1111/j.1365-313X.2007.03363.x>.
- Rahman, M., T. Islam, L. Jett, and J. Kotcon. 2021. Biocontrol agent, biofumigation, and grafting with resistant rootstock suppress soil-borne disease and improve yield of tomato in West Virginia. *Crop Prot.* 145:105630, <https://doi.org/10.1016/j.cropro.2021.105630>.
- Ramírez-Vargas, C. 2019. Red de Horticultura Protegida de la Región Huasteca Norte. Tecnológico de Costa Rica. 11 Feb. 2021. <<https://www.tec.ac.cr/proyectos/red-colaboracion-horticultura-protegida-region-huasteca-norte>>.
- Ramírez-Vargas, C. and J. Nienhuis. 2012. Evaluación del crecimiento y productividad del tomate (*Lycopersicon esculentum* Mill) bajo cultivo protegido en tres localidades de Costa Rica. *Revista Tecnología en Marcha* 25:3–15, <https://doi.org/10.18845/tm.v25i1.172>.
- Rivard, C.L. and F.J. Louws. 2008. Grafting to manage soilborne diseases in heirloom tomato production. *HortScience* 43:2104–2111, <https://doi.org/10.21273/HORTSCI.43.7.2104>.
- Rivard, C.L., O. Sydorovych, S. O'Connell, M.M. Peet, and F.J. Louws. 2010. An economic analysis of two grafted tomato transplant production systems in the United States. *HortTechnology* 20:794–803, <https://doi.org/10.21273/HORTTECH.20.4.794>.
- Rossato, M., T.R. Santiago, and C.A. Lopes. 2018. Reaction of *Capsicum* peppers commercialized in the Federal District to bacterial wilt. *Hort. Bras.* 36:173–177, <https://doi.org/10.1590/S0102-053620180204>.
- Saha, S.R., M.M. Hossain, M.M. Rahman, G.C. Kuo, and S. Abdullah. 2010. Effect of high temperature stress on the performance of twelve sweet pepper genotypes. *Bangladesh J. Agr. Res.* 35:525–534, <https://doi.org/10.3329/bjar.v35i3.6459>.
- Sánchez-Chávez, E., A. Torres González, M.A. Flores Córdova, P. Preciado Rangel, and C. Márquez Quiroz. 2015. Uso de portainjerto sobre el rendimiento, calidad del fruto y resistencia a *Phytophthora capsici* Leonian en pimiento morrón. *Nova Sci.* 7:227–244.
- Silvar, C. and C.A. García-González. 2017. Screening old peppers (*Capsicum* spp.) for disease resistance and pungency-related traits. *Scientia Hort.* 218:249–257, <https://doi.org/10.1016/j.scienta.2017.02.037>.
- Soltan, M., F. ElAidy, J. Scheerens, and M. Kleinhenz. 2017. Grafting, scion and

- rootstock effects on survival rate, vegetative growth and fruit yield of high tunnel-grown grafted pepper (*Capsicum annuum* L.) plants. *Adv. Crop. Sci. Tech.* 5(312):2, <https://doi.org/10.4172/2329-8863.1000312>.
- Springer, N.M. and R.J. Schmitz. 2017. Exploiting induced and natural epigenetic variation for crop improvement. *Nat. Rev. Genet.* 18:563–575, <https://doi.org/10.1038/nrg.2017.45>.
- Steiner, A.A. 1984. The universal nutrient solution, p. 633–650. In: 6th Int. Congr. Soilless Culture. Proc. Intl. Soc. Soilless Cult., Lunteren, The Netherlands.
- Suchoff, D.H., F.J. Louws, and C.C. Gunter. 2019. Yield and disease resistance for three bacterial wilt-resistant tomato rootstocks. *HortTechnology* 29(3):330–337, <https://doi.org/10.21273/HORTTECH04318-19>.
- Taller, J., N. Yagishita, and Y. Hirata. 1999. Graft-induced variants as a source of novel characteristics in the breeding of pepper (*Capsicum annuum* L.). *Euphytica* 108(2):73–78, <https://doi.org/10.1023/A:1003681913996>.
- Taller, J., Y. Hirata, N. Yagishita, M. Kita, and S. Ogata. 1998. Graft-induced genetic changes and the inheritance of several characteristics in pepper (*Capsicum annuum* L.). *Theor. Appl. Genet.* 97(5-6):705–713, <https://doi.org/10.1007/s001220050946>.
- Tans-Kersten, J., H. Huang, and C. Allen. 2001. *Ralstonia solanacearum* needs motility for invasive virulence on tomato. *J. Bacteriol.* 183(12):3597–3605, <https://doi.org/10.1128/JB.183.12.3597-3605.2001>.
- Tsaballa, A., A. Xanthopoulou, P. Madeisis, A. Tsiftaris, and I. Nianiou-Obeidat. 2021. Vegetable grafting from a molecular point of view: The involvement of epigenetics in rootstock-scion interactions. *Front. Plant Sci.* 11:2129, <https://doi.org/10.3389/fpls.2020.621999>.
- Tsaballa, A., C. Athanasiadis, K. Pasentsis, I. Ganopoulos, I. Nianiou-Obeidat, and A. Tsiftaris. 2013. Molecular studies of inheritable grafting induced changes in pepper (*Capsicum annuum*) fruit shape. *Scientia Hort.* 149:2–8, <https://doi.org/10.1016/j.scienta.2012.06.018>.
- Vega-Alfaro, A.A. 2020. Fruit yield, maturity and survival rate of interspecific *Capsicum* graft combinations evaluated in tropical and temperate environments. MS Thesis, Univ. Wisconsin-Madison.
- Wada, K.C. and K. Takeno. 2010. Stress-induced flowering. *Plant Signal. Behav.* 5(8):944–947, <https://doi.org/10.4161/psb.5.8.11826>.
- Wang, J., L. Jiang, and R. Wu. 2017. Plant grafting: How genetic exchange promotes vascular reconnection. *New Phytol.* 214(1):56–65, <https://doi.org/10.1111/nph.14383>.
- Warschewsky, E.J., L.L. Klein, M.H. Frank, D.H. Chitwood, J.P. Londo, E.J. von Wettberg, and A.J. Miller. 2016. Rootstocks: Diversity, domestication, and impacts on shoot phenotypes. *Trends Plant Sci.* 21(5):418–437, <https://doi.org/10.1016/j.tplants.2015.11.008>.
- Yagi, K., J. Williams, N.Y. Wang, and R.J. Cicerone. 1993. Agricultural soil fumigation as a source of atmospheric methyl bromide. *Proc. Natl. Acad. Sci. USA* 90(18):8420–8423, <https://doi.org/10.1073/pnas.90.18.8420>.
- Yagishita, N. and Y. Hirata. 1986. Genetic nature of bushy plant type in the variant strain induced by grafting in *Capsicum annuum* L. *Euphytica* 35(1):17–23, <https://doi.org/10.1007/BF00028536>.
- Yagishita, N. and Y. Hirata. 1987. Graft-induced change in fruit shape in *Capsicum annuum* L. genetic analysis by crossing. *Euphytica* 36(3):809–814, <https://doi.org/10.1007/BF00051864>.
- Yagishita, N., Y. Hirata, H. Mizukami, H. Ohashi, and K. Yamashita. 1990. Genetic nature of low capsaicin content in the variant strains induced by grafting in *Capsicum annuum* L. *Euphytica* 46:249–252, <https://doi.org/10.1007/BF00027224>.
- Yagishita, N., Y. Hirata, K. Okochi, K. Mimura, H. Mizukami, and H. Ohashi. 1985. Characterization of graft-induced change in capsaicin content of *Capsicum annuum* L. *Euphytica* 34:297–301, <https://doi.org/10.1007/BF00022922>.
- Zhang, Y., M. Fischer, V. Colot, and O. Bossdorf. 2013. Epigenetic variation creates potential for evolution of plant phenotypic plasticity. *New Phytol.* 197(1):314–322, <https://doi.org/10.1111/nph.12010>.