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‘HoneySweet’ (C5), the First Genetically Engineered *Plum pox virus*–resistant Plum (*Prunus domestica* L.) Cultivar

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Origin

‘HoneySweet’ originated as a seedling from the open pollination of ‘Bluebyrd’ plum (Scorza and Fogle, 1999). The pollen parent

of ‘HoneySweet’ is unknown. ‘HoneySweet’ was originally selected in vitro as a regenerated shoot from a hypocotyl slice that had been transfected with *Agrobacterium tumefaciens* EHA 101 carrying the plasmid

pGA482GG/PPV-CP-33 (Scorza et al., 1994). The regenerated, transgenic shoot, coded as C5, along with other transgenic shoots, was rooted in vitro and transferred to a greenhouse. Following greenhouse testing using graft and aphid inoculations with the M and D strains of *Plum pox virus* (PPV), C5 (later patented as ‘HoneySweet’), was asexually propagated by bud grafting on to standard rootstocks, including *Prunus persica* (GF305 peach seedlings), *Prunus domestica* (European plum seedlings), *Prunus myrobalan*, and GF 8-1 (*Prunus cerasifera* × *P. munsoniana*). An overview of the development of ‘HoneySweet’ plum and molecular characterization can be found in Scorza et al. (2013a).

Description

‘HoneySweet’ has been evaluated in Skierniewice, Poland, Llíria-Valencia, Spain, Bistrita, Romania (Fig. 1), Prague, Czech Republic (Table 1), and Kearneysville, WV. ‘HoneySweet’ trees have an upright growth habit and moderate level of fruiting-spur development. Flowering in Kearneysville, WV, occurs in late March to mid-April or ≈5–10 d before ‘Stanley’ depending on environmental conditions. In Kearneysville, WV, and in the European test plots, depending on environmental conditions, ‘HoneySweet’ fruit ripen in early- to mid-August or ≈10–20 d before the ripening of ‘Stanley’ plum. ‘HoneySweet’ fruit evaluated in Kearneysville, WV, are medium to large in size with an average weight of 60 g (≈2 oz) and average dimensions of 43–45 mm (≈1.75”) in diameter and 52 mm (≈2”) in length. Flesh is yellow, firm, juicy, and sweet (20–22 °Brix in U.S. and Spain tests) with a pleasing flavor balance. The skin is a deep purple with a waxy overcoat. The stone is mostly free with only a small area of adhesion to the flesh. Additional analyses of ‘HoneySweet’ fruit composition can be found in Ravelonandro et al. (2013) and Sochor et al. (2015).

Myrobalan (*P. cerasifera*) rootstocks are not recommended for ‘HoneySweet’ due to overgrowth of the ‘HoneySweet’ scion at the graft union. ‘HoneySweet’ trees grafted on to GF 8-1 rootstocks observed for over a decade of field testing have performed well and without signs of overgrowth. Limited observations suggest that peach (*P. persica*) is also a suitable rootstock for ‘HoneySweet’.

A detailed description of ‘HoneySweet’ may be found in the ‘HoneySweet’ plant patent (US PP15154 P2).

‘HoneySweet’ is self-incompatible. It has fruited consistently and abundantly in mixed plantings of *P. domestica* plum varieties. When ‘HoneySweet’ was used as a female parent in limited controlled crosses, it was shown to be compatible with *P. domestica* plums ‘Castelton’, ‘Jojo’, ‘LongJohn’, ‘Victory’, ‘Vision’, and ‘C11’ [U.S. National Plant Germplasm System (NPGS), Davis, CA, accession DPRU 2266] ‘Anna Spath’, ‘Cacanska Lepotica’, ‘Cacanska Rana’, and ‘Reine Claude de Bavay’. Fruit were not produced from limited controlled crosses

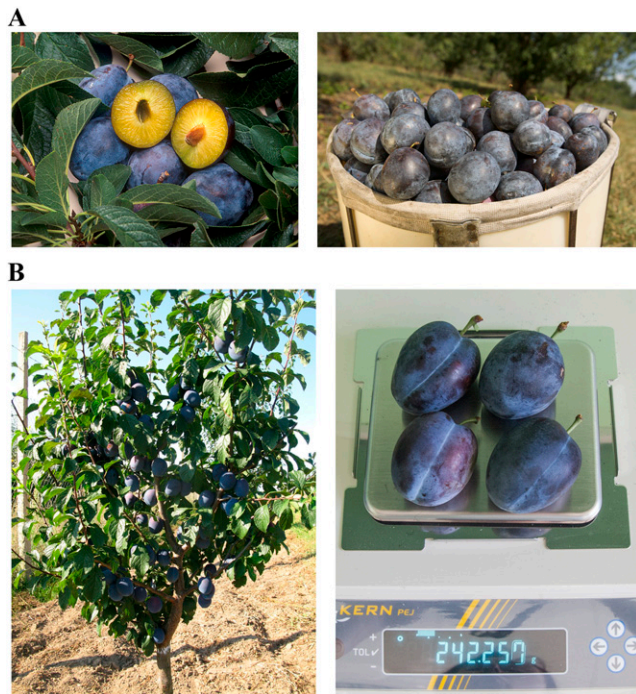


Fig. 1. (A) 'HoneySweet' plum (left); 'HoneySweet' plum harvest at the U.S. Department of Agriculture–Agricultural Research Service, Kearneysville, WV (right). (B) Three-year old 'HoneySweet' tree in Bistrita, Romania (left), with a weighed sample of 'HoneySweet' fruit (right). Photo credits: Scott Bauer, Peggy Greb, and Ioan Zagrai.

with pollen of 'President', 'Stanley', 'Improved French', and 'Sutter'. Since, as a *P. domestica* species, 'HoneySweet' is a hexaploid, it is sexually incompatible with most other *Prunus* species including peach and nectarine, 'Japanese' plum (*Prunus salicina*), apricot (*Prunus armeniaca*), sweet (*Prunus avium*) and sour (*Prunus cerasus*) cherry, and almond (*Prunus dulcis*).

'HoneySweet' is heterozygous for the PPV-CP insert; therefore $\approx 50\%$ of the male and female gametes leading to pollen and egg development will contain the PPV-resistance transgene. When used as a parent in conventional hybridization, 'HoneySweet' transfers the PPV-CP transgene insert to progeny as a single dominant locus and, as predicted by Mendelian genetics, $\approx 50\%$ of the progeny carry the transgene insert. These are resistant to PPV (Ravelonandro et al., 2002; Scorza et al., 1998), making 'HoneySweet' a useful source of resistance for developing additional PPV-resistant cultivars. 'HoneySweet' is also

highly resistant to black knot disease caused by the fungus *Apiosporina morbosa*. It appears to have inherited this resistance from 'Bluebyrd', its seed parent, which is also highly resistant to black knot (Scorza and Demuth, 2015).

The expression of the *uidA* (GUS) gene in the PPV-CP cassette allows for the rapid identification of seedlings carrying the PPV-CP insert through a simple and rapid histochemical assay (Jefferson, 1987). Polymerase chain reaction (PCR) can be used to confirm the presence of the hairpin-PPV-CP insert using specific primers for the hairpin insert [C5 Forward-GTGCATTGCGAAGCAAC and 35S Reverse-CGCAATGATGGCATTGTAGG resulting in a 675-base amplicon using GoTaq polymerase (Promega, Madison, WI) and an amplification program consisting of 2 min at 95.0 °C, 30 cycles of 1 min each at 95.0°, 59.0°, and 72.0°, followed by a 10-min extension at 72.0 °C, or for real-time PCR: primer 1-GGTAGTCCCCTGAATCAAAGGC, primer 2-TTATTCAACGCCA GTCCTGTCCC, and probe-56-FAM/TGGCAAGGA/ZEN/AATGTGCGAGTTC TGT/31ABkFQ (IDT, Coralville, IA) following the protocol in Scorza et al. (2013b).

PPV Resistance

Sharka disease caused by PPV affects most stone fruit species, cultivated and wild (Damsteeg et al., 2007). Symptoms of Sharka disease are described in Levy et al. (2000). Although resistance to PPV has been pursued ever since the disease was first identified, there are few reports of high-level

resistance in commercial *Prunus* species and new sources of high-level resistance to PPV are needed.

Initial 'HoneySweet' PPV-resistance tests were greenhouse-based (Ravelonandro et al., 1997). Although no PPV field inoculations were performed in the United States due to the quarantine status of PPV, through nearly 20 years of field testing in Europe, under heavy natural aphid-vectored infection pressure, of the 99 trees of 'HoneySweet' that have been evaluated, none have become infected with PPV through natural aphid transmission, whereas control trees have become rapidly and severely infected.

When graft inoculated with PPV-infected budwood or when vegetative "root suckers" from susceptible rootstocks have become infected, 'HoneySweet' trees have shown scarce and mild symptoms in the basal leaves near the source of infection. Systemic infection has generally not been detected, but when detected, it has been limited with mild symptoms that have diminished over multiple growing seasons (Polak et al., 2012). 'HoneySweet' trees have been shown to be resistant to all major strains of PPV (Ravelonandro et al., 2014). When grafts were inoculated with PPV in combination with *Prunus necrotic ringspot virus*, *Apple chlorotic ringspot virus*, and *Prune dwarf virus*, 'HoneySweet' has remained resistant to PPV (Polak et al., 2013; Ravelonandro et al., 2013; Zagrai et al., 2008). Details of PPV resistance in field trials of 'HoneySweet' can be found in Hily et al. (2004), Malinowski et al. (2006), Polak et al. (2008), Zagrai et al. (2011), and Capote et al. (2008).

PPV is not currently affecting the U.S. plum production; however, this virus remains as a significant threat. The U.S. Department of Agriculture–Agricultural Research Service (USDA–ARS) release of 'HoneySweet' makes this cultivar available for cultivation in the United States and provides a genetic resource for breeding additional PPV-resistant varieties.

Coexistence

Considering coexistence of 'HoneySweet' with conventional or organic plum production, pollen-mediated gene flow in *P. domestica* has been found to be very low. Furthermore, unlike crops where gene flow manifests in seeds which are the products of commerce such as soybean and maize, plum seeds are not consumed and are rarely used for planting purposes. Pollen-mediated gene flow from a plot of genetically engineered (GE) *P. domestica* plums, including 'HoneySweet', was evaluated for 11 years (Scorza et al., 2013b). Under the study conditions, gene flow measured as the number of GUS-positive seeds detected in conventional plum fruits occurred in only 4 of 11 years and then in only 0.31% of the 12,116 seeds analyzed. Spatial modeling indicated that the low levels of gene flow decreased dramatically to zero or near zero at distances over 400 m from the GE plot. In this same study, no GE plum trees were found in fields or forested areas adjacent to the GE plum plot,

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Table 1. Comparisons of 'HoneySweet' fruit characteristics in 2011, 2012, and 2014, Prague, Czech Republic.

Cultivar	Characteristics						
	Fruit wt (g)	Fruit length (mm)	Fruit width (mm)	Fruit thickness (mm)	Flesh thickness (mm)	% Soluble solids	% Fruit wt in stone
2011							
HoneySweet	56.2 ± 1.98	46.6 ± 0.64	39.7 ± 0.65	37.6 ± 0.66	11.5 ± 0.31	16.9 ± 0.27	No data
JoJo	23.9 ± 0.33	39.6 ± 0.25	29.2 ± 0.18	29.7 ± 0.18	6.4 ± 0.21	12.1 ± 0.36	No data
Stanley	36.6 ± 0.75	47.1 ± 0.38	34.4 ± 0.34	33.8 ± 0.29	9.8 ± 0.39	15.3 ± 0.61	No data
<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	—
2012							
HoneySweet	48.0 ± 2.61	50.3 ± 1.02	40.7 ± 0.89	39.9 ± 1.50	14.4 ± 0.57	14.2 ± 0.73	4.9 ± 0.34
JoJo	32.9 ± 0.95	47.4 ± 0.62	34.1 ± 0.39	34.1 ± 0.35	10.9 ± 0.31	23.4 ± 0.60	4.9 ± 0.15
Stanley	31.3 ± 0.68	48.1 ± 0.34	34.8 ± 0.37	33.5 ± 0.23	10.9 ± 0.33	16.8 ± 0.24	5.6 ± 0.15
<i>P</i> value	<0.0001	0.0430	<0.0001	<0.0001	<0.0001	<0.0001	0.0032
2014							
HoneySweet	60.7 ± 3.04	46.2 ± 0.90	43.6 ± 0.74	41.5 ± 0.97	11.2 ± 0.33	16.1 ± 0.51	3.5 ± 0.12
JoJo	46.4 ± 1.39	50.5 ± 0.71	38.1 ± 0.74	35.7 ± 0.35	7.6 ± 0.20	18.9 ± 0.54	4.3 ± 0.11
Stanley	36.0 ± 2.11	50.1 ± 0.61	36.0 ± 0.47	30.1 ± 0.48	6.3 ± 0.19	19.2 ± 0.47	5.1 ± 0.09
<i>P</i> value	<0.0001	0.0003	<0.0001	<0.0001	<0.0001	0.0002	<0.0001

Multivariate analysis of variance for cultivar differences/year: 2011 Wilk's lambda = 0.01633, *F* = 39.58; 2012 Wilk's lambda = 0.06316, *F* = 13.81; 2014 Wilk's lambda = 0.02529, *F* = 20.19. Values are means ± SE.

indicating that over the 11 years of the study, there was no seed-based gene flow from GE plum trees. These results support the feasibility of coexistence of GE and non-GE plum orchards.

Availability

'HoneySweet' was deregulated by the U.S. Animal and Plant Health Inspection Service in 2007, was reviewed by the U.S. Food and Drug Administration in 2009, and was registered by the Environmental Protection Agency in 2011 (Scorza et al., 2013a). 'HoneySweet' was awarded a U.S. plant patent in 2004, but is being made freely available for fruit production and for use as a source of PPV resistance for developing new PPV-resistant *P. domestica* plum varieties in the United States. Currently, 'HoneySweet' plum has not received approval for cultivation in the EU or other locations outside of the United States. 'HoneySweet' is being made freely available to requestors outside of the United States contingent upon the certification that appropriate foreign regulatory approvals have been obtained by the requestor.

The Agricultural Research Service has no trees of 'HoneySweet' for distribution. A limited amount of heat-treated budwood of 'HoneySweet' is available from Clean Plant Center Northwest—Fruit Trees (CPCNW—FT) (<http://cpcnw.wsu.edu/fruit-trees/bud-buying>). Genetic material of this release will be deposited in the NPGS where it will be available for research purposes, including development and commercialization of new cultivars.

The recipient of genetic material from CPCNW—FT or NPGS of 'HoneySweet', including but not limited to, buds, pollen, and seeds is subject to the conditions stated in the USDA—ARS 'HoneySweet' Cultivar Release Notice. The 'HoneySweet' release notice and additional information on 'HoneySweet' and PPV can be found at <http://www.ars.usda.gov/is/br/plumpox/>.

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