

The Pawpaw Research Program at the Horticulture Department of the University of Florence

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SUMMARY. The Horticulture Department of the University of Florence has been studying the pawpaw (*Asimina triloba*) since 1990 through collaboration with Italian fruit grower Domenico Montanari, who currently hosts the largest pawpaw orchard and variety collection in Italy. Research efforts are focused mainly on the development of new varieties and selections with desirable ornamental characteristics. New variety selection is based on high tree productivity, large fruit size, good organoleptic quality and improved fruit postharvest handling. The breeding program has resulted in 3,000 seedlings that are currently being evaluated in Tuscany, Italy. In addition to breeding efforts, 15 commercially available varieties were evaluated in replicated trials in 2000 and 2001, with selections characterized for vegetative, reproductive and pomological traits. In 2000 and 2001, the varieties 'Prima 1216' and 'Prolific' had high yields and should be suitable for production in Italian fruit-growing areas. Experiments to establish explants of pawpaw in culture were also performed testing a range of genotypes, disinfestation procedures, and methods to induce callus culture. Protocols for the propagation of pawpaw in vitro would offer many advantages for mass multiplication of desirable plants; however, the propagation of pawpaw in vitro has so far been met with limited success.

The north american pawpaw is receiving increasing attention for its potential as a landscape tree, fruit crop, and as a source of important secondary products with anti-tumor and pesticidal properties (Bellini and Montanari, 1992a, 2000; Layne, 1996; McLaughlin, 1997; Pomper et al. 1999). Due to attractive tree form and fall color, as well as cold hardiness for the Italian climate, pawpaws are suitable for ornamental and biodiversification efforts in parks and gardens in Italy. Furthermore, the species has a very low susceptibility to pests and disease (Peterson, 1991) and produces fruit with a unique flavor and a high nutrient content (Peterson et al., 1982), thus also representing an alternative to traditional fruit crops for organic fruit production (Bellini and Montanari, 1992a, 2000).

In Italy, experimentation with pawpaw began in 1983 in Faenza, Italy at the farm of Domenico Montanari, which now hosts the largest Italian pawpaw orchard and collection. Initially, Montanari evaluated a range of American varieties under Italian climatic conditions (Bellini and Montanari, 1992b).

Breeding and selection efforts were initiated with the development of a seedling population of more than 1,000 individuals obtained from seed coming from the U.S., from open pollination of the variety 'Sunflower', and from a number of seedling selections.

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One selection, 'Montanari 1216' (now named 'Prima 1216'), had displayed cold hardiness, self-compatibility, and very large fruit size; a patent is currently pending on that selection (Bellini and Montanari, 2000).

Breeding Program at the Horticulture Department of the University of Florence

Our collaboration with Domenico Montanari began in the early 1990s and is still in progress, with a primary focus on the development of new pawpaw varieties and evaluation of commercially available varieties for suitability for Italian fruit-growing areas. Traits that will be selected for are high productivity, large fruit size, good organoleptic quality, improved fruit storability and resistance to post harvest handling. Storage and handling represent significant obstacles to the development of a large-scale commercial production of pawpaws. Also, interesting genotypes having double aptitude in ornamental value and fruit production will be identified.

Parents used in the breeding program were chosen within the first seedling population on the basis of agronomic, biological and pomological characteristics. In 1992, seed was obtained from a number of open pollinated isolated genotypes, and more than 2,800 seedlings were produced. A separate, parallel study was conducted with a wider genetic pool, to study the

distribution of the progenies in relation to their respective female parents. In this study, 20 parental genotypes were selected on the basis of pomological traits, such as fruit shape (round to oval), size, homogeneity of both fruit shape and size within the tree and flowering form. From this work a total number of 430 seedlings were obtained. A seedling orchard was established in autumn 1995, after plantlets had been transplanted twice in containers of increasing size. These 2-year-old seedlings were planted out at the experimental farm Monna Giovannella at the University of Florence, in a slightly sloping field of about 2 ha (4.9 acres) and have been left unpruned. The seedlings are still in the juvenile phase, and are currently being evaluated for tree growth habit and vigor. Upon fruiting, seedlings will be selected based on fruit characteristics, ripening season, storage quality, as well as for yield and year-to-year consistency. We expect these trials to provide valuable information and genetic material for future work in pawpaw genetic improvement at the University of Florence.

Characterization of some pawpaw varieties cultivated in Italy

An evaluation of 15 pawpaw varieties commercially available in Italy was performed to examine adaptability of these varieties to the Italian climate. An orchard was established in 1990 in an experimental orchard located at Faenza, Italy with planting distances of 4.5 ×

2 m (14.76 × 6.6 ft). Three replicate trees were planted for each variety. The pawpaw accessions included 14 varieties of American origin ('Sunflower', 'Overleese', 'Davis', 'Prolific', 'Sweet Alice', 'Maryfoos Johnson's', 'Mango', 'Taytwo', 'Taylor', 'Rebecca's Gold', 'NCI', 'Ithaca', 'Wells' and 'Wilson') and one of Italian origin ('Prima 1216'). Vegetative, reproductive and pomological characteristics were evaluated during the 2000 and 2001 seasons. Traits recorded for the vegetative characterization of trees included height and volumetric index of trees. Reproductive records included flowering time, ripening time, fruit weight, fruit soluble solids content, and finally tree yield. Data were analyzed by analysis of variance and means separation was performed by Duncan's multiple range test.

Analysis of variance showed a high variability among the varieties for many of the characters examined. There were significant differences among varieties in tree height and tree volumetric growth, with the variety Ithaca having the greatest height measurements both in 2000 and 2001 (Tables 1 and 2). In terms of flowering, there were significant differences among varieties in bloom time, with the varieties 'Overleese', 'Ithaca', 'Mango', and 'Sunflower' having the earliest bloom period in 2000 and 2001. The varieties Prima 1216, 'Sunflower' and 'Davis' bore the largest fruit [average weight of 202, 216, and 246 g (7.1, 7.6, and 8.7 oz), respectively] in both

Table 1. Vegetative and reproductive characteristics of 15 pawpaw varieties evaluated in 2000 in Faenza, Italy.

Variety	Tree ht (cm) ^z	Tree volumetric index ^y	Flowering time (d) ^x	Ripening time (d) ^v	Fruit wt (g) ^w	Soluble solids (%)	Tree yield (kg)
Davis	290.0 bc	1.15 b	110 bc	248 bcd	216.3 a	21.6 cef	3.96 cde
Ithaca	440.0 a	1.60 ab	106 cd	252 ab	123.9 de	24.2 abc	10.08 b
Mango	415.5 ab	1.78 ab	106 cd	246 cde	196.1 abc	24.8 ab	10.73 b
Maryfoos Johnson	258.6 c	1.83 ab	110 bc	253 ab	198.0 ab	22.3 bcd	2.24 de
NC-1	385.0 ab	1.55 ab	112 b	251 abc	164.6 abcd	24.9 ab	7.54 bc
Overleese	360.0 abc	1.39 ab	104 d	242 e	138.3 bcd	20.7 de	4.35 cde
Prima 1216	382.0 ab	1.41 ab	110 bc	250 abc	209.5 a	18.9 e	15.70 a
Prolific	310.0 bc	1.77 ab	112 b	255 a	113.5 de	24.9 ab	11.22 b
Rebecca's Gold	330.5 abc	1.20 b	116 a	246 cde	64.9 e	21.4 cde	2.46 de
Sunflower	320.0 bc	1.18 b	106 cd	250 abc	152.0 abcd	20.6 de	6.59 bcd
Sweet Alice	250.0 c	1.36 ab	108 bcd	253 ab	173.9 abcd	21.2 cde	1.71 e
Taylor	383.0 ab	1.74 ab	111 b	252 ab	106.2 de	22.0 bcde	3.96 cde
Taytwo	380.0 ab	1.56 ab	116 a	244 de	127.2 cde	26.5 a	0.81 e
Wells	257.0 c	1.97 a	109 bc	---	---	---	---
Wilson	370.0 abcd	1.51 ab	111 b	246 cde	102.7 de	19.9 de	7.46 b
P ^a	0.0000	0.0070	0.0000	0.0002	0.0000	0.0000	0.0000

^aBased on three replicate trees for each variety; trees were planted in 1990; 1.0 cm = 0.39 inches; 1.00 kg = 2.205 lb; 1 g = 0.035 oz.

^yTree volumetric index was calculated by $2H / \text{longitudinal } D + \text{transversal } D$; where H = maximum crown height, longitudinally and transversally, D = longitudinal and transversal crown diameter measured at the maximum extent.

^xFlowering time was evaluated at the peak of bloom judged by visual observation and expressed in days from 1 Jan.

^vRipening time was expressed in days from 1 Jan.

^wFruit weight based on the weights of 20 fruit.

^aData were analyzed by analysis of variance and means separation was performed by Duncan's multiple range test with a $P < 0.05$.

Table 2. Vegetative and reproductive characteristics of 15 pawpaw varieties evaluated in 2001 in Faenza, Italy.

Variety	Tree ht (cm) ^z	Tree volumetric index ^y	Flowering time (d) ^x	Ripening time (d) ^v	Fruit wt (g) ^w	Soluble solids (%)	Tree yield (kg)
Davis	310.0 cd	1.16 bc	97 bc	248 bcd	277.0 ab	19.4 ef	6.24 bcde
Ithaca	455.0 a	1.67 abc	92 d	252 ab	134.4 cd	19.8 def	11.35 ab
Mango	430.0 ab	1.77 ab	92 d	246 cde	170.8 bc	18.8 f	8.02 bc
Maryfoos Johnson	270.0 d	1.55 abc	98 b	253 ab	165.8 c	22.7 ab	1.66 de
NC-1	395.0 abc	1.57 abc	99 ab	251 abc	191.2 abc	22.8 ab	8.60 b
Overleese	366.7 abcd	1.48 abc	91 d	242 e	206.8 abc	19.7 def	6.68 bcde
Prima 1216	395.0 abc	1.44 bc	96 bc	250 abc	195.0 abc	19.2 ef	14.37 a
Prolific	330.0 bcd	1.78 ab	97 bc	255 a	148.9 cd	21.5 bc	14.30 a
Rebecca's Gold	351.7 abcd	1.23 bc	102 a	246 cde	62.4 d	24.0 a	2.34 cde
Sunflower	331.7 bcd	1.08 c	92 d	250 abc	280.8 a	22.3 b	11.98 ab
Sweet Alice	256.7 d	1.35 bc	94 cd	253 ab	191.0 abc	20.5 cde	1.91 de
Taylor	393.3 abc	1.75 ab	97 bc	252 ab	174.8 abc	24.0 a	6.36 bcde
Taytwo	390.0 abc	1.58 abc	102 a	244 de	197.3 abc	21.4 bcd	1.26 e
Wells	283.3 cd	2.11 a	94 cd	---	---	---	---
Wilson	370.0 abcd	1.52 abc	97 bc	246 cde	97.2 cd	20.1 cdef	7.11 bcd
P ^a	0.0050	0.0190	0.0000	0.0005	0.0000	0.0000	0.0000

^zBased on three replicate trees for each variety; trees were planted in 1990; 1 cm = 0.39 inches; 1.00 kg = 2.205 lb.

^yTree volumetric index was calculated by $2H / \text{longitudinal } D + \text{transversal } D$; where H = maximum crown height, longitudinally and transversally, D = longitudinal and transversal crown diameter measured at the maximum extent.

^xFlowering time was evaluated at the peak of bloom judged by visual observation and expressed in days from 1 Jan.

^vRipening time was expressed in days from 1 Jan.

^wFruit weight based on the weights of 20 fruit; 1.0 g = 0.035 oz.

^aData were analyzed by analysis of variance and means separation was performed by Duncan's multiple range test with a $P < 0.05$.

2000 and 2001, while 'Rebecca's Gold' produced the smallest fruit (64 g) (2.3 oz). Fruit soluble solids range from 19 to 26.5% soluble solids in the varieties examined in both 2000 and 2001. Tree yield was greatest for the variety Prima 1216 in 2000 and for the varieties Prima 1216 and Prolific in 2001.

In vitro culture

Vegetative propagation of pawpaw by conventional methods is difficult and micropropagation would be an alternative method for the production of many uniform plants of a selected superior genotype. Treatments were employed using various concentrations of sodium hypochlorite to disinfect explants to be used for callus induction. Quiescent buds and internodes were collected from 10-year-old trees of the varieties 'Davis', 'Sunflower', 'Overleese', 'Prima 1216', and 'Prolific' that were grown at the experimental farm of Domenico Montanari at Faenza, Italy. Explants were washed under tap water and then subjected to five disinfection treatments consisting of immersion for 2 or 5 min in 1.0%, 2.0%, 2.5%, 3.5%, and 5.0% sodium hypochlorite. All treated explants were rinsed two times with sterile distilled water and then placed in petri dishes containing 10 mL (0.34 fl oz) of MS basal medium (Murashige and Skoog, 1962) supplemented with 2% sucrose and enriched with zeatine (5 μM), only in the case of internodes. Two different solidification agents were used

with or without 0.3% activated charcoal, 0.7% Difco-Bacto agar (Sigma-Aldrich Chemie GmbH, Munich, Germany), and 0.3% Gelgrow (ICN Biomedicals Inc., Irvine, Calif.). The pH of the medium was adjusted to 5.8 ± 0.1 with 0.1 N sodium hydroxide before autoclaving. Cultures were incubated at 25 °C (77.0 °F) under a photoperiod of 16 h light (cool white fluorescent lamps, 35 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and 8 h dark. Cultures (15 explants per treatment) were subcultured every 20 d.

Most cultures were contaminated with fungal and bacterial organisms, which appeared on the medium surface within a few days. Explants that were free of microbial growth were found only with the buds of variety 'Davis' that were disinfecting with 5% sodium hypochlorite for 2 min. Induction of callus on the 'Davis' explants began 15 d after being placed on the medium where Gelgrow had been added, but that contained no activated charcoal. Callus occurred directly at the cut surface of the explants. However, explants that produced callus exhibited browning and did not survive after the second subculture. The few cultures that had viable callus exhibited poor growth. Alternative surface disinfection treatments with various chemical agents, concentrations and times, as well as alternative explant sources are currently being examined with the hope of increasing the number of aseptic explants.

In conclusion, over 3,000 pawpaw seedlings are under long-term evaluation for fruit appearance, production,

quality, postharvest handling, as well as tree ornamental potential for Italy and the Mediterranean region. Evaluation of commercially available varieties has already identified selections that perform well in the region of Emilia Romagna (e.g., 'sel. 938', 'sel. 1047', and 'sel. 1068'). Protocols for the propagation of pawpaw in vitro would offer many advantages for mass multiplication of desirable plants; however, the propagation of pawpaw in vitro has so far been met with limited success.

Literature cited

- Bellini, E. and D. Montanari. 1992a. La coltura dell'*Asimina triloba* annonacea per i climi temperati. L'Informatore Agrario 38:59-72.
- Bellini, E. and D. Montanari. 1992b. La coltura dell'*Asimina* in Italia. Esperienze maturate a Faenza. L'Informatore Agrario 45:51-64.
- Bellini, E. and D. Montanari. 2000. *Asimina triloba*, una realtà per la frutticoltura amatoriale italiana. Frutticoltura 1:54-61.
- Layne, D.R. 1996. The pawpaw [*Asimina triloba* (L.) Dunal.]: A new fruit crop for Kentucky and the United States. HortScience 31:777-784.
- McLaughlin, J.L. 1997. Anticancer and pesticidal components of pawpaw (*Asimina triloba*). Annu. Rpt. N. Nut Growers Assn. 88:97-106.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 51:473-497.
- Peterson, R.N., J.P. Cherry, and J.G. Simmons. 1982. Composition of pawpaw (*Asimina triloba*) fruit. Annu. Rpt. N. Nut Growers Assn. 73:97-106.
- Peterson, R.N. 1991. Pawpaw (*Asimina*). Acta Hort. 290:567-600.
- Pomper, K.W., D.R. Layne, and R.N. Peterson. 1999. The pawpaw regional variety trial, p. 353-357. In: J. Janick (ed.). Perspectives on new crops and new uses. ASHS Press, Alex., Va.