Pixie, a dwarf grapevine for teaching and research

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Introduction:
Pinot Meunier is a periclinal L1/L2 chimera in which the L1 layer has a semidominant mutation that reduces sensitivity to gibberellic acid (Boss and Thomas 2002). Attempts to separate the layers of Pinot Meunier through fragmented shoot tip culture resulted in dwarf plants with short internodes (Skene and Barlass 1983), but these plants did not exhibit inflorescences in place of tendrils as observed by Boss and Thomas (2002), who used somatic embryogenesis to produce plants derived from a single cell of the L1 layer. When vines derived from the L1 layer are grown, they are dwarves with short internodes and preferentially produce tendrils instead of flowers. We sought to make this vine available for viticulture and grapevine biology research and teaching in the United States.

Materials and Methods:
Unopened flower buds of Pinot Meunier vines were collected from the vineyards of Foundation Plant Services and the Department of Viticulture and Enology, University of California, Davis, during the spring bloom season, 2004. Flowers were disinfested and anthers were explanted on medium to induce embryogenic callus cultures. Embryos were collected and grown into plants in tissue culture. Small tissue cultured plants were acclimated to soil in a growth chamber and then transferred to a greenhouse. All tissue culture procedures were conducted at The Ralph M. Parsons Foundation Plant Transformation Facility, University of California, Davis. Green growing cuttings were propagated in a mist bed in the greenhouse at the USDA ARS Grape Genetics Research Unit. Plants were grown in 2:1 volume:volume perlite:Cornell peat-Lite mix and fertigated with Miracle-Gro Excel brand 21-5-20 All Purpose water soluble fertilizer. Throughout the year they were grown under artificial lights to provide 24 hour illumination. Pollen was collected from greenhouse grown plants and used to pollinate clusters of other varieties grown in a vineyard. Open pollinated seeds from greenhouse grown plants and controlled hybridization seeds were collected when seeds were brown and hard. The seeds were treated with 1.5% hydrogen peroxide for 24 hrs, 1000 ppm GA3 for 24 hours, followed by three months moist stratification, then 5000 ppm GA3 for 24 hours before plating in an incubator at 29.3 °C. Germinated seeds were transferred to potting mix and treated as mature plants.

Results and Discussion:
When the young Pixie plants were first removed from tissue culture and established in potting mix, a dwarf phenotype with extremely short internodes was observed immediately. However, the small plants did not immediately begin making inflorescences, instead producing at first small bifiurcate tendrils, followed by tendriloid inflorescences. Because of the developmental relationship of tendrils and inflorescences (tendrils being evolutionarily derived from inflorescences), tendrils and inflorescences in grapevine occupy the two ends of a continuum. A tendriloid inflorescence is predominantly a tendril, but bears a few flowers, sometimes rudimentary or abortive, sometimes fully formed and capable of producing a berry which completely matures. The earliest inflorescences on Pixie vines and their seedlings were of this type. As Pixie vines grew, larger clusters developed that did not show tendriloid characters. Maximum cluster size was approximately 10 cm.

Pixie vines are simple to cultivate in a greenhouse, although we have not attempted to grow them outdoors on station in Geneva, New York (where the colony is primarily held) due to winter conditions. High light conditions promote growth; in Geneva we have provided artificial illumination during the day in the winter (in addition to nighttime supplemental lighting). In our experience vines that are allowed to set all of their clusters tend to set a lot of fruit, followed by a slowing of growth and reduction in flowering.

Active cluster thinning helps maintain active growth and flowering. As reported by Boss and Thomas (2002), the dwarf and continuous flowering characters are inherited as a semidominant allele. We found that open pollinated seeds collected from greenhouse grown Pixie vines and seeds collected from controlled hybridizations in which Pixie was the pollen parent germinated and grew easily following a simple seed stratification protocol (3 months at 4 °C), although no offspring demonstrating the mutant phenotype were recovered. We suspect that this is due to the increased need for GA treatment in order to stimulate germination of embryos carrying the mutant allele. We modified the seed germination protocol to incorporate additional GA treatment; now we have recovered dwarf seedlings from Pixie and Pixie hybrids that are showing flowers.

Because of their small size and continuous flowering habit, Pixie vines may be useful in viticulture and grapevine biology research and teaching. The semidominant character of the dwarfing phenotype suggests that there is an opportunity for using Pixie in accelerating backcrossing or pseudobackcrossing following the method of Ryder (1985). Dwarf plants with rapid flowering (which carry the mutant allele) are used to advance generations in crosses with wild type plants. In the final generation the dwarf plants would be avoided by using a seed treatment protocol that is below the germination induction threshold for embryos with the mutant allele. In a greenhouse or growth chamber Pixie vines can be cultivated to produce clusters of any age at any time of year. This provides an opportunity to use Pixie vines to teach grape growth, development, and morphology without regard to seasons. Plant pathologists have expressed interest in using Pixie vines to investigate the interaction of grapevines with fungi, oomycetes, and other disease causing organisms and physiologists have already acquired Pixie vines for research. It is known that plant growth regulators play a role in signaling in grapevine/pathogen interactions and in many aspects of berry development, so consideration of the GAI mutation is important in the development of Pixie as a model system.

The Pixie grape variety was released in August 2006 by the USDA Agricultural Research Service without any intellectual property restrictions. Plant material, including cuttings, plants, pollen, and seeds, are available upon request to the corresponding author. David Tricoli and and staff of The Ralph M. Parsons Plant Transformation Facility maintain Pixie germplasm in several forms and are investigating Pixie transformation and other aspects of genetic improvement. The USDA ARS National Clonal Germplasm Repository, Davis, California, a part of the National Plant Germplasm System, holds Pixie as accession DVIT 3321.

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