Cytological Analysis of a Clethra alnifolia ‘Hokie Pink’ × C. pringlei Hybrid

Sandra M. Reed
Floral and Nursery Plants Research Unit, U.S. National Arboretum, Agricultural Research Service, U.S. Department of Agriculture, Tennessee State University Otis L. Floyd Nursery Research Center, 472 Cadillac Lane, McMinnville, TN 37110

Abstract. Clethra alnifolia L., a native deciduous shrub cultivated as an ornamental, was recently hybridized with C. pringlei S. Wats. The purpose of this hybridization was to combine the cold hardiness and adaptability of C. alnifolia with the ornamental foliage of C. pringlei. While most of the C. alnifolia × C. pringlei hybrids more closely resembled C. alnifolia than the paternal species, a ‘Hokie Pink’ × C. pringlei hybrid (NA71586) with foliage that flushes red like C. pringlei was recovered. The objectives of this study were to analyze cytologically the F1 and produce an F2 population from NA71586. Chromosome counts from root tips cells indicated that NA71586 has 32 chromosomes. Since the chromosome number of C. alnifolia is 2n = 32 and that of C. pringlei was found to be 2n = 16, NA71586 appears to have developed following fertilization of a C. alnifolia egg with an unreduced male gamete from C. pringlei. Both ‘Hokie Pink’ and C. pringlei exhibited primarily bivalent pairing in pollen mother cells (PMCs). Over half of the PMCs from NA71586 contained 16 bivalents, indicating substantial homology within the C. alnifolia genome. It was theorized that C. alnifolia is either an autotetraploid that exhibits bivalent pairing or a segmental autotetraploid produced from hybridization of species with similar genomes. More than 700 F2 progeny were obtained from self-pollination of NA71586. Although many of the F2 progeny resembled NA71586, variation in foliage color, size and shape was apparent in the population.

The genus Clethra, which is the sole member of Clethraceae, consists of approximately 70 species of deciduous and evergreen shrubs and small trees (Sleumer, 1967a, 1967b; Hamilton, 1985). The genus is divided into two sections based primarily on geographic distribution and seed characteristics (Sleumer, 1967a). The 25 members of Section Clethra are native to eastern U.S. and eastern Asia, whereas sect. Cuelaria species are found in Mexico, Central and South America, and Madeira. Several Clethra species are cultivated as ornamentals, but the most widely grown member of the genus in the U.S. is C. alnifolia. Commonly known as sweet pepper bush or summersweet, C. alnifolia is found in acid swamps and low, moist woods along the Coastal Plain from Maine to Louisiana (Wilbur and Hespenheide, 1967). It is valued for its dark green pest-resistant foliage, attractive racemes or panicles of fragrant white or pink flowers, and ability to grow under a wide range of environmental conditions. The species is rated hardy in USDA Hardiness Zones 4 to 9 (Dirr, 1998).

Clethra alnifolia was recently hybridized to C. pringlei (Reed, 2001; Reed et al., 2002), which is a Mexican species with glossy, evergreen foliage that flushes a deep reddish-bronze. Because C. pringlei is hardy only to USDA Hardiness Zone 8, it is not commonly used as an ornamental in the U.S. The goal of this hybridization was to combine cold hardiness and adaptability from C. alnifolia with the attractive foliage characteristics of C. pringlei. Pollinations made in 1998 resulted in one ‘Fern Valley Pink’ × C. pringlei and 11 ‘Ruby Spice’ × C. pringlei hybrids (Reed et al., 2002). Comparisons of morphological characteristics and RAPD banding patterns between hybrids and parental species indicated that the hybrids more closely resembled their C. pringlei parent than C. alnifolia; however, individual plants exhibited one or more morphological characteristics from the paternal parent. No plant with the desired foliage characteristics was obtained from F1 populations derived from intercrossing ‘Ruby Spice’ × C. pringlei hybrids (S.M. Reed, unpublished data.)

Pollinations made between C. alnifolia and C. pringlei in 1999 resulted in 19 additional progeny (Reed, 2001). The 17 plants obtained from crosses involving C. alnifolia ‘Hummingbird’ or ‘Ruby Spice’ as the maternal parent were similar in appearance to hybrids obtained the previous year. In contrast, two progeny obtained from ‘Hokie Pink’ × C. pringlei pollinations much more strongly resembled C. pringlei than did the other C. alnifolia × C. pringlei hybrids. Many of the characteristics of these hybrids were either intermediate to the parents or similar to those of C. pringlei (Table 1). While the new foliage of both ‘Hokie Pink’ × C. pringlei hybrids was distinctly redder than that of ‘Hokie Pink’ or other C. alnifolia cultivars, the foliage of one hybrid was much more intensely colored than that of the other. This hybrid, which was previously referred to as 402A (Reed, 2001), was assigned U.S. National Arboretum accession number NA71586. Growth and ornamental characteristics of NA71586 are currently being evaluated in U.S. nurseries at various locations within the C. alnifolia production range. Regardless of its commercial merit, NA71586 potentially represents a valuable source of germplasm for use in a Clethra breeding program. The objectives of this study were to cytologically analyze and produce F2 progeny from NA71586.

Materials and Methods

Plant materials. Clethra alnifolia ‘Hokie Pink’, C. pringlei, and C. alnifolia ‘Hokie Pink’ × C. pringlei NA71586 were grown in 56.8-L containers in full sun and microirrigated using spray stakes. Growing medium consisted of pine bark amended with 6.6 kg·m⁻³ 19N–2.1P–7.4K Osmocote Pro fertilizer (Scotts-Sierra Horticultural Products Co., Maryville, Ohio), 0.6 kg·m⁻³ Micromax (Scotts-Sierra Horticultural Products Co.), 0.6 kg·m⁻³ iron sulfate, and 0.2 kg·m⁻³ Epsom salts. Acetocarmine. Chromosome pairing was determined from cells at diakinesis or metaphase I. Further details are provided in the manuscript.

Mitotic chromosome counts. Root tips were collected from rooted cuttings of NA71586 and placed in 1.2 mL colchicine for 3 h at room temperature, then transferred to Carnoy’s fixative (3:1 95% ethanol : glacial acetic acid) for 24 h, and stored in 70% ethanol at 5 °C for up to 1 week. Root tips were hydrolyzed for 5 min in 5 × HCl at room temperature (21 to 22 ºC), rinsed in distilled water, and soaked in 1% aceticarmine for 30 min. The meristic region of the root tip was squashed in aqueous aceticarmine and chromosomes counted in ten metaphase cells.

Meiotic analysis. Chromosome pairing was studied in pollen mother cells (PMCs) of NA71586 and its parents. Panicles about 3 to 7 cm in length were collected, fixed in Carnoy’s fixative at room temperature for 24 h, then transferred to 70% ethanol and stored at 5 °C for up to 3 months. Anthers were squashed in 1% aceticarmine. Chromosome pairing was determined from cells at diakinesis or metaphase I. Number of lagging chromosomes was recorded in anaphase I, metaphase II, and anaphase II cells. At least 25 cells were analyzed for each meiotic stage examined.

Pollen staining. Three flowers were collected from each taxa on the day of anthesis. Three anthers from each flower were placed on a microscope slide and squashed in a drop
of 1% acetocarmine stain. Specimens were examined using a light microscope and pollen scored as stained or unstained. Three fields of 100 grains each were counted and a mean number of stained grains calculated for each slide. Fisher’s LSD test was used to compare percent stainable pollen among taxa.

Production of F₂ progeny. NA71586 was self-pollinated during summer 2002. Inflorescences were covered with breathable plastic bags (DelStar Technologies, Middletown, Del.) before flower opening. Bags were removed from inflorescences and pollen collected by holding open flowers over a 30-cm plastic petri dish and tapping each flower with a pair of forceps. After pollen was collected from all open flowers, it was applied to stigmas of the same group of flowers using a fine-tipped brush. Self-pollinations were made every second day from the first flower opened until flowering was complete. Bags remained over the inflorescences until 2 weeks after the last pollination was made.

Seed capsules were collected in late October, placed in paper bags and kept at room temperature (21 to 22 °C) until completely dry. Capsules were crushed and seeds separated from debris using a stereomicroscope. Seeds were stored in glassine bags at 5 °C for 2 months before sowing on the surface of a commercial seed propagation mix (Grow Mix #1, Morton’s Horticultural Products, Inc., McMinnville, Tenn.) in shallow (3-cm) seedling flats. Seed trays were placed in a heated greenhouse under mist. Bottom heat was provided during germination. Seedlings were transplanted to individual 5.7 × 4.9 cm cell packs containing Pro Mix BX (Premier Horticulture, Quakertown, Pa.) when the second set of true leaves had developed. Seedlings were later transplanted to square 12.7 cm pots and then to 11.4-L containers using the pine bark media described above. Plants that were very small or that had heavily variegated foliage were kept in the greenhouse under 60% shade, but the remainder of the plants were grown on a gravel lined bed in full sun. In October 2003, general observations on plant phenotype were recorded and plant height measured.

Results

Cytological analysis and pollen staining. Root tip cells of NA71586 contained 32 chromosomes (Fig. 1A). Up to 6 univalents were observed at diakinesis or metaphase I in NA71586, but 60% of the cells contained 16 bivalents (Table 2, Fig. 1B). Lagging chromosomes were observed in 29% to 53% of the PMCs observed at later meiotic stages.
Anaphase II 29 0.7 (0–6)
Metaphase II 40 16 0.9 (0–6)

2 14 II + 4 I
7 15 II + 2 I

Pollen Meiotic observed chromosomes mean (range)

Cells with chromosomes

Diakinesis/metaphase I 15 16 II

Hokie Pink 90.5 a

Diakinesis/metaphase I 23 16 II
Anaphase I 26
Metaphase II 26
Anaphase II 32

C. pringlei 90.8 a

Diakinesis/metaphase I 25 8 II
Anaphase I 34
Metaphase II 27
Anaphase II 28

Hokie Pink × C. pringlei (NA71586) 26.2 b

Diakinesis/metaphase I 15 16 II
Anaphase I 53
Metaphase II 40
Anaphase II 29

Table 2. Percent stainable pollen, chromosome pairing configurations, and lagging chromosomes in Clethra alnifolia ‘Hokie Pink’, C. pringlei and their hybrid.

Mean separation by Fisher’s r test, P ≤ 0.05.

II = bivalent, I = univalent.
formation in NA71586 and the phenotypic similarity between the F₁ and many F₂ plants, many of the F₂ plants are expected to have the same complement of chromosomes as the F₁. The presence of univalents and lagging chromosomes in PMCs of NA71586 should have resulted in gametes with different chromosome complements; however, since only about one-fourth of the pollen grains of NA71586 appeared viable, many of these gametes may not have participated in fertilization. Cytological analysis of the NA71586 F₂ progeny is needed to determine if the phenotypic variation that was found in this population is due strictly to gene segregation or if aneuploid differences in chromosome number are responsible for some of the variability. Continued evaluation of cold hardiness, adaptability and ornamental characteristics of F₂ progeny are needed to determine if plants with the desired combination of parental traits can be obtained without recombination between parental genomes. Fortunately, because C. alnifolia cultivars are vegetatively propagated, interesting and useful variants from F₂ and other advanced populations can be maintained and utilized even in the absence of sexual transmission of the entire chromosome complement.

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