Cytogenetics of Bigleaf Hydrangea

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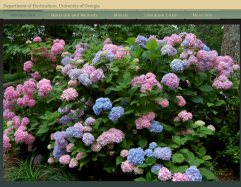

Introduction

Hydrangea macrophylla Serrage, the most popular species in genus *Hydrangea*, is widely used as a bordered plant, which is hardy plant, and are fresh and dried cut flowers (Dailey 1999; Orr, 2004). Flowers are borne in terminal inflorescences, which are of two morphotypes: mophoflorals and sterile flowers (sterile flowers in *macrophylla* ssp. *serrata*), that are identified by the size of flowers, sepals/petals/corolla (McClintock, 1957). Although both morphotypes have been sold as ornamental plants, only bigleaf hydrangea with showy sterile flowers is in large and showy leaves and inflorescences (Dailey, 1999).

Botanic Characteristics

Bigleaf hydrangeas typically ranges from 1 to 6 feet high and grows to 10 feet wide. It forms a rounded shape composed of stems, which are thick, coarse, and hollow. Leaves are 3 to 6 inches long and 3 to 4 inches wide. Changing in color from flat matte green, light green, dark green, to serious dark green depending on exposed conditions. Corymbs are formed by two types of flowers, fertile flowers in the center with sterile showy flowers around them. Sterile flowers are showy and can be white, pink, red, purple, blue, and violet. Blue, and purple. Flowers generally bloom from mid-May to early June in Atlanta, GA and repeat through midsize, when during maturation (Orr, 2004). Color of flower will vary depending on availability of aluminum in the soil. As pH rises (becomes more alkaline), aluminum is less available and flowers will be pink. Flowers will remain blue due to the presence of adequate aluminum (Cebenko et al., 1980).

Ploidy and DNA Content

Although tendency of bigleaf hydrangeas to be sterile has been long recognized, cytogenetic studies have been conducted in cultivated species. A diploid, chromosome number of 2n=2x=36 was first reported in 1967 (Hawcroft-Booth, 1969) and this number was later confirmed by Tuesday (and Tanaka) (1969). The first study that found different ploidy levels within bigleaf hydrangeas was conducted by Tanaka (1971). In this study, 2n=2x=36 was also identified as triploids (2n=3x=54). Unfortunately, cultivars and details of these cultivars were not provided. Zonneveldt (2004) then found new bigleaf plants in 75 years using flow cytometry and reported the results of genome size of 1 pg by cells. The genome size of 1 pg was also found in 2 cultivars of hydrangeas was reported by Jones et al. (2007). Their experiment measurements of total nuclear DNA identified 42 diploids and 18 triploids among 60 tested cultivars. As a result of flow cytometry, Zonneveldt (2004) also found that the genome size of the triploids (35.7 pg) was significantly greater than that of the diploids (26.0 pg), and pollen grains were also larger in the triploids (10.3 pico compared with diploids 8.7 pico). Jones et al. (2007) also measured nuclear DNA content for bigleaf hydrangea. Genome size was found to be 2.0 pg for diploids and 3.0 pg for different triploids cultivars. The 42 diploid cultivars, 21 cultivars ranged from 4.0 to 5.9pg and 6.0 to 7.9pg in diploid and triploid cultivars, respectively.

Karyotype and Nucleolar Organizing Regions

The chromosomes in bigleaf hydrangeas were small (1.3 um) and with a similar size/shape, therefore only total chromosome length and arm ratio were evaluated to construct a karyotype using microscopic identification (Tanaka et al., 1971). The metacentric, four submetacentric, and eight submetacentric chromosome pairs were identified in the diploid cultivar (2n=2x=36). The 18 submetacentric or metacentric chromosomes in the triploid cultivar were found to have satellites and probably nucleolar organizing regions (NOR).

Figure 1. Chromosomes of bigleaf hydrangea (*Hydrangea macrophylla*) cultivar 'Lindamulvey' (2n=2x=36) and ideogram of the diploid cultivar.

NOR in the organ within the nucleus where the nucleolus forms and it is partially made by long repeats of 45S rDNA (Orlando and Ciani, 1989). In the development of fluorescence of silver (FISH) and fluorescence in situ hybridization (FISH), a detailed karyotype was constructed for bigleaf hydrangea 'Forest' (2n=3x=54) by Lee Lanier et al. (2000). After metaphase chromosomes were stained with 4'-diamino-2-phenylindole (DAPI) and 45S rDNA was detected by Biotin-11-dUTP probe, the NOR was found on the short arm of the 5th chromosome in the triplicate of one metacentric, eight submetacentric and four submetacentric chromosomes (Fig. 2). FISH of 45S rDNA with bigleaf hydrangea revealed a signal (three spots on homologous chromosomes) on the NOR of metacentric (Fig. 2), one of the submetacentric chromosomes of the karyotype (Fig. 2) Lee Lanier et al. (2000).

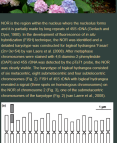

Figure 2. Karyotype of bigleaf hydrangea 'Forest' (2n=3x=54) with FISH of 45S rDNA probe. Arrows indicate the chromosome with the NOR. Bar = 5 µm. Adapted from Lee Lanier et al. (2000).

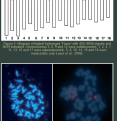

Figure 3. Left: Meiosis of bigleaf hydrangea 'Forest' stained with DAPI. Arrows indicate the lagging chromosomes. Bar = 5 µm. Right: Meiosis of bigleaf hydrangea 'Forest' stained with DAPI. Arrows indicate the lagging chromosomes. Bar = 5 µm. Adapted from Lee Lanier et al. (2000).

Breeding

Infloresceences of bigleaf hydrangea may remain attractive for several months and a few cultivars remaining cool-hued (Dailey 1999). However, they typically provide no vendor interest in the landscape (Dirr et al., 2000). Moreover, most of the bigleaf hydrangea cultivars are cold sensitive, requiring protection from bud damage with low winter temperatures, early fall, and late spring freezes often remaining their flowering potential (Dirr, 2004). Cold hardy bigleaf hydrangea is, therefore, one of the major breeding goals of horticulture. Although most cultivated bigleaf hydrangea are sterile, some wild species in genus *Hydrangea* macrophylla ssp. *serrata* hydrangea species (wild bigleaf hydrangea) are the maternal parent and producing outdoor hydrangea cultivars have sterilized mutant (Cai et al., 2011; Kudo and Nakai, 1999; Hood et al., 2001). Cipriani (1962) and Hood et al. (2001) found that the cultivar 'Lanarth' (*H. serrata* 'Beni Kanaboshi') was the parent of commercial cultivar 'Veitchiella' (2n=2x=36) had 34-38 chromosomes with nearly half of Fertile/Sterile hybrid (1:1) (Fig. 4). The fertile color of the hybrids was red or blue, when blue of the maternal parent and paternal parent were pigment available, cyanidin-3-glucoside, cyanidin-3-sambubioside, and cyanidin-3-rutinoside, and hermaphrodite or fertile hybrids were dominant over sterile mutations.

Figure 4. Chromosomes of (F1) of the Fertile/Sterile hybrid of 'Veitchiella' (2n=2x=36) and 'Lanarth' (*H. serrata* 'Beni Kanaboshi') (2n=2x=36). Bar = 5 µm. Adapted from Kudo and Nakai (1999). Cultivar 'Veitchiella' (2n=2x=36) had nearly half of the fertile/sterile hybrids (F1) of the cultivar 'Lanarth' (*H. serrata* 'Beni Kanaboshi') (2n=2x=36) (Kudo and Nakai, 1999).

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Plant Materials

Hardwood stem cuttings of bigleaf hydrangea 'Endless' (plants) in greenhouse were collected in the early morning in UGA Horticulture Farm (Athens/Athens, GA). They were then rinsed with tap water and placed in 1:100 (per 95%) and rinsed with 1:100 (per 95%) and then 1:100 (per 95%) and then 1:100 (per 95%) and then