map and our preliminary phenotypic data for leaf unfolding rate, we identified three quantitative trait loci (QTL) for leaf unfolding rate, two on linkage group 1, and one on linkage group 5. Together, these three QTL explained 49% of the variability for leaf unfolding rate observed in this mapping population. We are currently developing recombinant inbred lines for these interspecific hybrid populations to evaluate the robustness of the identified QTL.

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(393) Discovery of a New Diploid Cytotype of *Fothergilla*

Thomas G. Ranney*
North Carolina State University, Mills River, NC; tom_ranney@ncsu.edu

Ron Miller
Pensacola, FL; rhodokiller@cox.net

Rick Lewandowski
Greenville, DE; rick1517@gmail.com

Jenny Xiang
North Carolina State University, Raleigh, NC; jenny_xiang@ncsu.edu

Fothergilla (Hamamelidacea) is a small genus of uncommon, deciduous shrubs found exclusively in the southeastern United States. Two species of *Fothergilla* are currently recognized: *F. gardenii* and *F. major*. However, variation in the genus is considerable and as many as four taxa have been recognized in the past. *Fothergilla gardenii* is found in the coastal plains of North Carolina, South Carolina, Georgia, Florida, and Alabama and is tetraploid with $2n = 4x = 48$. In contrast, *F. major* is found on upland sites in the piedmont and mountains of North Carolina, South Carolina, Georgia, Alabama, Tennessee, and Arkansas and is hexaploid with $2n = 6x = 72$. No diploid cytotypes of *Fothergilla* have previously been known. The objective of this study was to survey DNA contents and ploidy levels of *Fothergilla* spp. from throughout its range. Samples from thirty populations were collected and tested. Flow cytometry was used to determine DNA contents and associated ploidy levels. As expected, tetraploid and hexaploid plants were identified, consistent with *F. gardenii* and *F. major*, respectively. However, populations of diploid plants were also discovered in a few locations. Considering that diploid plants differ morphologically and are isolated both geographically and cytogenetically from other *Fothergilla* spp., this cytotype may represent a new, distinct, and rare taxon. Additional work is continuing to reassess the systematics and phytogeography of *Fothergilla* in order to elucidate the diversity and evolutionary relationships among species, properly classify this new cytotype, and to help guide future conservation efforts.

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(394) Effects of Oryzalin and Trifluralin in Polyploidy Induction in In Vitro *Cattleya walkeriana* Gardner (Orchidaceae) Seedlings

Renato Galdiano*
Universidade Estadual Paulista (UNESP), Jaboticabal; renatofgaldianojr@yahoo.com.br

Wagner Vendrame
University of Florida, Homestead, FL; vendrame@ufl.edu

Eliana G.M. Lemos
Universidade Estadual Paulista (UNESP), Jaboticabal; egerle@fcav.unesp.br

Orchidaceae is one of the largest and most evolved families of flowering plants found especially in tropical and sub-tropical regions. Orchids are undoubtedly the ornamental elite because of their perplexingly complex flowers of exquisite beauty. Polyploidy induction is a common technique utilized to obtain plants with enhanced characteristics. Polyploid orchids might present larger flowers, larger number of flowers per inflorescence, enhanced vigor, and intensified coloration. The traditional polyploidy inductor used worldwide is colchicine, but this alkaloid is extremely toxic to human manipulation due to its high affinity to microtubules of animal cells. Oryzalin and trifluralin are herbicides recognized for their antimitotic activity and suitable plant polyploidy induction ability. They can be effective in very low quantities, present more affinity for plant tubulin dimers and consequently have reduced human toxicity. The objective of this study was to evaluate the effects of oryzalin and trifluralin in the induction of polyploidy in in vitro *Cattleya walkeriana* orchids. Seeds from *C. walkeriana* were germinated in vitro and 90-day-old protocorms were treated with different concentrations (0, 15, 30, 50, and 100 μM) of both antimitotic agents for 3 and 6 days. After that they were transferred to 180-mL flasks containing 50 mL of semi-solid ½ strength MS culture medium. The experimental design consisted of 20 treatments, 5 flasks per treatment and 10 protocorms per flask, with a total of 1,000 explants. The evaluation included biometrical responses (protocorm survival and number of shoots recorded at 8 weeks), and cytogenetic and flow cytometry analyses 22 weeks after the experiment establishment. Data were subjected to analysis of variance (ANOVA), and means compared using Tukey test ($\alpha = 0.05$). Treatments for both antimitotic agents decreased rooting and dramatically increased the number of shoots compared with controls. Trifluralin was significantly more toxic than oryzalin, particularly when protocorms were exposed to it for 6 days, and showed lower survival rates and high callus formation. The cytogenetic and flow cytometry analyses of regenerated plantlets confirmed the level of polyploidy induction. This study demonstrated that oryzalin showed more promising results and it was more effective in polyploidy induction as compared to trifluralin. Renegerated plants of *C. walkeriana* will be further evaluated for growth ex vitro and subsequently for floral characteristics.

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