protecting plants from blow over and insulating roots from heat and cold should be considered (Whitcomb and Whitcomb, 2003).

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×*Chitalpa*: The Next Generation©

Richard T. Olsen[1] **and Thomas G. Ranney**

North Carolina State University, Department of Horticultural Science, 455 Research Drive, Fletcher, North Carolina 28732-9244

Hybridizations between distantly related taxa are often sterile and are a barrier in breeding programs for the development of improved hybrids. One way to overcome this barrier is through the development of allopolyploid forms of sterile hybrids. In this study, we compared the pollen fertility and reproductive behavior of diploid ×*Chitalpa tashkentensis* Elias & Wis. 'Pink Dawn' and induced allopolyploid ×*C. tashkentensis* 'Pink Dawn'. Pollen fertility was analyzed using aceto-carmine staining techniques and pollen germination tests. Female fertility was assessed through a series of controlled crosses between diploid and allopolyploid ×*C. tashkentensis* 'Pink Dawn' and diploid *Catalpa* and *Chilopsis.* Diploid ×*C. tashkentensis* 'Pink Dawn' were both male and female sterile, whereas allopolyploid ×*C. tashkentensis* 'Pink Dawn' had pollen germination equal to that of *Catalpa* and *Chilopsis.* Allopolyploid ×*C. tashkentensis* 'Pink Dawn' also demonstrated restored female fertility as seen in successful pollinations and fruit set. The restoration of fertility in ×*C. tashkentensis* 'Pink Dawn' allows for the development of a breeding program for the introduction of new and improved cultivars of ×*Chitalpa.*

[1]1st Place Student Awardee for Graduate Student Research Competition

INTRODUCTION

The development of new nursery crops with novel characteristics, greater commercial merit, and improved resistances to abiotic and biotic stresses requires the hybridization of genetically diverse taxa. However, the usefulness of crosses between related taxa depends on the fertility of the hybrid progeny. In many cases, wide hybrids (e.g., interspecific, intergeneric, etc.) are sterile, due to chromosomal and genetic imbalances leading to meiotic irregularities between the two genomes (Hadley and Openshaw, 1980; Kalloo and Chowdhury, 1992). In order to facilitate the continued use of wide hybrids in a breeding program this sterility barrier must be overcome. The induction of polyploidy is a viable method for the improving meiosis and restoring fertility in wide hybrids (Hadley and Openshaw, 1980; Kalloo and Chowdury, 1992; Sybenga, 1992). The doubling of chromosomes in wide hybrids typically results in each chromosome pairing with its duplicate copy, regular segregation, and development of normal, albeit, doubled (2n) gametes, and restored fertility. This allows for the continued introgression of desired traits into advanced hybrids, and has been used for the overall genetic improvement of several agronomic crops, but has rarely been used in woody plant breeding, with the notable exception of the development of black-spot-resistant roses (Byrne et al., 1996; Ma et al., 1997).

The bi-generic hybrid ×*Chitalpa tashkentensis* Elias & Wis. (*C. bignonioides* Walt. × *Chilopsis linearis* (Cav.) Sweet) was first bred in Uzbekistan in the 1960s and introduced to the U.S.A. in 1977 (Elias and Wisura,1991). ×*Chitalpa* has performed well in the arid southwestern U.S.A., but is rarely encountered in eastern or southeastern gardens due to its limited cold hardiness and susceptibility to Catalpa worms (*Ceratomia catalpae* Bdv.) and powdery mildew (*Microsphaera elevata* Burr.). No further work has been done to improve ×*Chitalpa,* due principally to the sterility of the original hybrids. In order to initiate a breeding program for ×*Chitalpa*, we successfully developed allotetraploid forms of ×*C. tashkentensis* 'Pink Dawn' using the mitotic inhibiting herbicide oryzalin (Olsen et al., 2003). The objective of this research was to evaluate male and female fertility of the induced allotetraploid and explore new opportunities for the development of ×*Chitalpa*'s for the nursery industry.

MATERIALS AND METHODS

Diploid, L2 allotetraploid cytochimera (L2 histogenic layer was tetraploid, L1 and/or L3 tissue layers were diploid) ×*C. tashkentensis* 'Pink Dawn', and *C. linearis* 'Bubba' were grown in containers maintained in greenhouses at the Mountain Horticultural Crops Research Station, Fletcher, North Carolina. All pollen for viability tests was collected at anthesis and came from the stock plants growing in the greenhouses with the exception of *C. bignonioides*, which came from naturalized trees growing near the research station. Pollen viability was quantified using pollen staining and pollen germination assays (Sharma and Sharma, 1980). Pollen grains were treated using Müntzing's stain, 1 : 1 glycerol : 1% aceto-carmine, for 3 h. Stained pollen grains were scored as viable. Pollen germination tests were conducted utilizing spot tests with Brewbaker-Kwack media at 10% or 15% sucrose for 8 h. Pollen with pollen tubes greater than one-half the diameter of the pollen grain were scored as germinated. Pollen analysis was conducted using a compound light microscope (Micromaster, Fisher Scientific, Pittsburgh, Penn.) under 100×

Table 1. Pollen viability tests for diploid and allotetraploid ×*Chitalpa tashkentensis* 'Pink Dawn' and representative parental taxa *Catalpa bignonioides* and *Chilopsis linearis* 'Bubba'.

Taxa	Ploidy	Pollen tests[y] Staining (%)	Germination (%)
Catalpa bignonioides	2X	98.0a[z]	62.3b
Chilopsis linearis 'Bubba'	2X	94.9b	73.8ab
×*Chitalpa tashkentensis* 'Pink Dawn'	2X	0.8c	0.1c
×*Chitalpa tashkentensis* 'Pink Dawn'	2X + 4X	98.6a	65.9a

[y]Means are n=2 with 8 subsamples per replicate and a minimum of 100 pollen grains counted per subsample for each test.

[z]Any two means within columns not followed by the same letter are significantly different based on Tukey's HSD test at ∝=0.05.

and 400× magnification. Each pollen replicate contained eight subsamples, where ≥100 pollen grains were scored in a minimum of four fields of view (f.o.v.) of the microscope. For all crosses, flowers were emasculated prior to anthesis. The stamens were collected and dried overnight at 5 ºC (41 ºF) using indicator drierite (Drierite, Xenia, Ohio), and then stored at 5 ºC (41 ºF) for use in subsequent crosses. Stigmas were receptive to pollen when the stigma lobes separated, generally the afternoon after emasculation. Pollen was applied to stigmas using camelhair brushes. The number of pollinations ranged from 89 to 1,400 depending on the number of available flowers. Pollen viability and crossability tests were conducted in 2003 and repeated in 2004. The pollen viability test was a completely randomized design with 2 replicates (years) × 4 treatments (taxa) × 8 subsamples. Data were analyzed using SAS (SAS Institute, Cary, North Carolina) PROC ANOVA, and means compared using Tukey's Honestly Significantly Difference (HSD) test with the Type I error rate controlled at α=0.05.

RESULTS AND DISCUSSION

In *Catalpa* and *Chilopsis,* pollen grains are united into tetrads with coarsely reticulate areoles (Elias and Wisura, 1991; personal observation). Both parental taxa exhibited good pollen viability and germination, though pollen viability may be overestimated by the aceto-carmine stain (Table 1). In diploid ×*Chitalpa,* the pollen grains form highly variable polyads, but rarely, if ever tetrads (Elias and Wisura, 1991; personal observation), indicating a high degree of sterility. This was confirmed in the pollen viability tests, where pollen staining and pollen germination approached zero percent (Table 1). Solid allotetraploid ×*Chitalpa* 'Pink Dawn' have been reluctant to flower, however, we did identify several stable L2 cytochimeras where the L2 layer was tetraploid and the L1 and/or L3 layers were diploid (Olsen et al., 2003). These plants were used for the pollen viability tests, since, for breeding purposes they behave as allotetraploids. These L2 cytochimeras (designated 2X + 4X) produced well-formed tetrads and pollen grains, that stained and germinated in greater percentages than diploid ×*Chitalpa* 'Pink Dawn' and greater than or equal to the parent taxa, *Catalpa* and *Chilopsis* (Table 1).

Concomitant with restoration of male fertility in 2X + 4X ×*Chitalpa* is the restoration of female fertility (Table 2). Diploid ×*C.* 'Pink Dawn' failed to set fruit when it was self-pollinated, or pollinated with *Catalpa* and *Chilopsis* pollen (Table 2). Similarly, pollen of diploid ×*C.* 'Pink Dawn' failed to initiate fruit set when used to pollinate *C. linearis* 'Bubba'. However, the 2X + 4X ×*C.* 'Pink Dawn' were both male and female fertile, with fruit developing in both directions of the cross. Selfed 2X + 4X ×*C.* 'Pink Dawn' resulted in 30.6% fruit set compared to 20.7% and 6% when pollinated with diploid *Catalpa* and *Chilopsis*, respectively. Selfed flowers may have initiated more fruit because the cross is made at the same ploidy level (tetraploid), thus involving both 2n egg cells and 2n pollen. However, when 2X + 4X ×*C.* 'Pink Dawn' is pollinated with diploid *Catalpa* and *Chilopsis* pollen it is between ploidy levels (2n egg pollinated by 1n pollen sperm cells). Interploid crosses are often unsuccessful due to the abortion of embryos from genic imbalances in the developing endosperm (Johnston et al., 1980).

To date, we have germinated hundreds of seedlings from the selfed 2X + 4X ×*C.* 'Pink Dawn', and using flow cytometry, have verified that they are true allotetraploids (unpublished data). All interploid crosses, have thus far, resulted in mature fruit, with aborted embryos or nonviable seed, possible indicating the presence of a triploid block (unpublished data). We have initiated a series of ovule and embryo culture experiments to rescue developing embryos from interploid crosses. With the development of fertile allotetraploids, further progress can be made in breeding a new generation of ×*Chitalpa* with improved ornamental traits, cold hardiness, and pest resistances.

Table 2. Pollination and fruit set for diploid and allotetraploid ×*Chitalpa tashkentensis* 'Pink Dawn' and representative parental taxa *Catalpa bignonioides* and *Chilopsis linearis* 'Bubba'.

Maternal parent	Pollen source	Pollinations	Fruit (no.)	(%)
×*Chitalpa tashkentensis* 'Pink Dawn' 2X	Selfed	459	0	0.0
	Catalpa bignonioides	619	0	0.0
	Chilopsis linearis 'Bubba'	128	0	0.0
×*Chitalpa tashkentensis* 'Pink Dawn' 2X + 4X	Selfed	581	180	31.0
	Catalpa bignonioides	1400	292	20.9
	Chilopsis linearis	104	2	1.9
Chilopsis linearis 'Bubba'	×*Chitalpa tashkentensis* 'Pink Dawn'	103	0	0.0
	×*Chitalpa tashkentensis* 'Pink Dawn' 2X + 4X	89	19	21.3

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Starch Utilization During In Vitro Rooting of Easy- and Difficult-to-Acclimatize Sea Oats (*Uniola paniculata*) Genotypes©

Carmen Valero-Aracama[1], Michael E. Kane, and Nancy L. Philman
University of Florida, Environmental Horticulture Dept., Gainesville, Florida 32611

Sandra B. Wilson
University of Florida, Environmental Horticulture Dept., IRREC, Fort Pierce, Florida 34945

Starch content was evaluated during microcutting in vitro rooting of an easy- (EK 16-3) and difficult-to-acclimatize (EK 11-1) genotypes of *Uniola paniculata* L. (sea oats), a native dune species of the southeastern U.S.A. Excluding Week 0, EK 11-1 plantlets exhibited greater shoot starch reserves than EK 16-3. Starch content was lower in roots than in shoots at Weeks 6 and 9 and, during root elongation, root starch content decreased in both genotypes. The difficult-to-acclimatize genotype (EK 11-1) exhibited a lower shoot to root dry weight ratio and reduced leaf development compared to the easy-to-acclimatize genotype (EK 16-3). Sugar and starch reserves are reported to be critical for successful acclimatization. However, these results indicate that, while starch content is higher in EK 11-1 plantlets, it is insufficient for successful ex vitro acclimatization. This may be the result of a higher energy requirement of the extensive root system and the absence of photosynthetically competent leaves ex vitro.

[1] 2nd Place Student Awardee for Graduate Student Research Competition