Reproductive Behavior of Diploid and Allopolyploid ×*Chitalpa tashkentensis*

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Nature of Work: Plant breeders often utilize wide hybridization to combine desirable traits among distantly related taxa. Wide crosses, either at the interspecific, intersubgeneric, or intergeneric level, are often sterile as a result of meiotic irregularities (e.g., pairing failure) during gametogenesis (2). In order to further improve these hybrids this sterility barrier must be overcome.

The induction of polyploidy has been used to reestablish meiotic pairing and restore fertility in wide hybrids (2). Chemicals that induce polyploidy, such as the alkaloid colchicine, inhibit the formation of the spindle apparatus during mitosis, allowing for replication of the DNA, but preventing cell division. The resulting cells have additional sets of chromosomes and are referred to as polyploids. When polyploidy is induced in wide hybrids (allopolyploidy), the doubling creates an exact duplicate of the chromosomes from both parents, which facilitates proper pairing during meiosis, gametogenesis, and restored fertility.

Although colchicine has traditionally been used as a doubling agent, safer, more effective chemicals include mitotic inhibiting dinitroaniline herbicides, such as oryzalin [4(dipropylamino)-3,5-dinitrobenzenesufonamide] (Surflan®) (5). Although these herbicides have been shown to be effective in tissue culture systems (3), the cost of micropropagation, and the lack of culture protocols for many ornamentals, precludes this method for most nurseries and researchers involved in plant improvement. The development of protocols for the rapid and efficient induction of polyploidy in *ex-vitro* vegetative meristems could allow for restoration of fertility in sterile hybrids and provide for newfound plant breeding opportunities. Therefore, our objectives were to evaluate 1) the efficacy of oryzalin as a chromosome-doubling agent, and 2) evaluate the fertility of induced allopolyploids of ×*Chitalpa tashkentensis* Elias & Wisura.

The bi-generic hybrid ×*Chitalpa tashkentensis* (*Catalpa bignonioides* Walt. × *Chilopsis linearis* (Cav.) Sweet) was chosen as a model for this study due to its known sterility (1), ease of propagation and growth, and the potential for further breeding improvements. Thirty-two 1-quart (1-liter) plants were selected from stock material at the Mountain Horticultural Crops Research Station, Fletcher, N.C. Each plant was pruned to three, actively growing, indeterminate shoots. Leaves were removed from the terminal end of the shoot to expose the apical meristem, and the apical buds were treated with a saturated solution, 0.004% (150 μM), of oryzalin for one of four durations: 0, 6, 12, or 24-hours. After growth resumed, ploidy analysis was conducted using a flow cytometer (Partec PA Ploidy Analyzer, Münster, Germany). The experimental design was a completely randomized design with eight single plant replicates, with three subsamples (treated shoots) per plant. Data were analyzed using regression analysis.

Pollen viability was quantified using pollen staining and pollen germination assays (4). Pollen was collected from diploid and allopolyploid ×*Chitalpa* and both parent taxa, *Catalpa bignonioides* and *Chilopsis linearis*. Pollen was collected at the time of anthesis and stained using Müntzings stain, 1:1 glycerol: 1% aceto-carmine, for three hours. Stained pollen grains were scored as viable. Pollen germination tests were conducted using spot tests using Brewbaker-Kwack media with 10% sucrose for eight hours. 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, PA) under 100x and 400x magnification. Eight replicates were observed per viability and germination test, except for the mixoploid, where n = 4. Due to uneven and non-overlapping flowering periods, data for both staining and germination tests are presented as means and standard error of the means only.

Results and Discussion: The duration of the oryzalin treatment had a significant influence on the percentage of diploids, mixoploids, and mortality of shoot meristems (Fig. 1.). As treatment duration increased the percentage of diploids decreased and the percentage of mixoploids (up to the 12-hour duration) and mortality increased. As duration increased beyond 12 hours, the percentage of diploids continued to decrease, and the percentage of mixoploids decreased as mortality increased. A few completely tetraploid shoots were found at the 24-hour treatment.

In *Catalpa* and *Chilopsis*, pollen grains are united into tetrads with coarsely reticulate areoles (2; personal observation). Both parent taxa exhibited good pollen viability and germination, although pollen viability may have been overestimated by the aceto-carmine stain (Table 1). In diploid ×*Chitalpa*, the pollen grains form highly variable polyads, but rarely, if ever tetrads (2; personal observation), indicating a high degree of sterility, which we confirmed using pollen viability and germination tests (Table 1). Although most mixoploids observed thus far have similar pollen characteristics as diploid ×*Chitalpa* and are sterile (results not shown), one mixoploid produced viable pollen that germinated in high percentages (Table 1). This mixoploid most likely represents a stable cytochimera of the L2 histogenic layer. As of this time, our completely tetraploid ×*Chitalpa* have not flowered.

Significance to Industry: Oryzalin was effective at inducing a large percentage of mixoploids across all treatments, which may, through selective pruning and selection, be stabilized as tetraploids. Several completely tetraploid ×*Chitalpa* were also induced. One induced mixoploid (2x + 4x) formed viable pollen grains which germinated *in vitro*. These results demonstrate that oryzalin can be used for inducing polyploidy in vegetative shoots and for restoring fertility in wide hybrids, e.g. ×*Chitalpa tashkentensis*. The development of fertile allopolyploids will allow for further development of new ×*Chitalpa* cultivars with greater pest resistance, tolerance to environmental stresses, and improved ornamental traits. Furthermore, this technique may be applied to other sterile hybrids, creating exciting new breeding opportunities.

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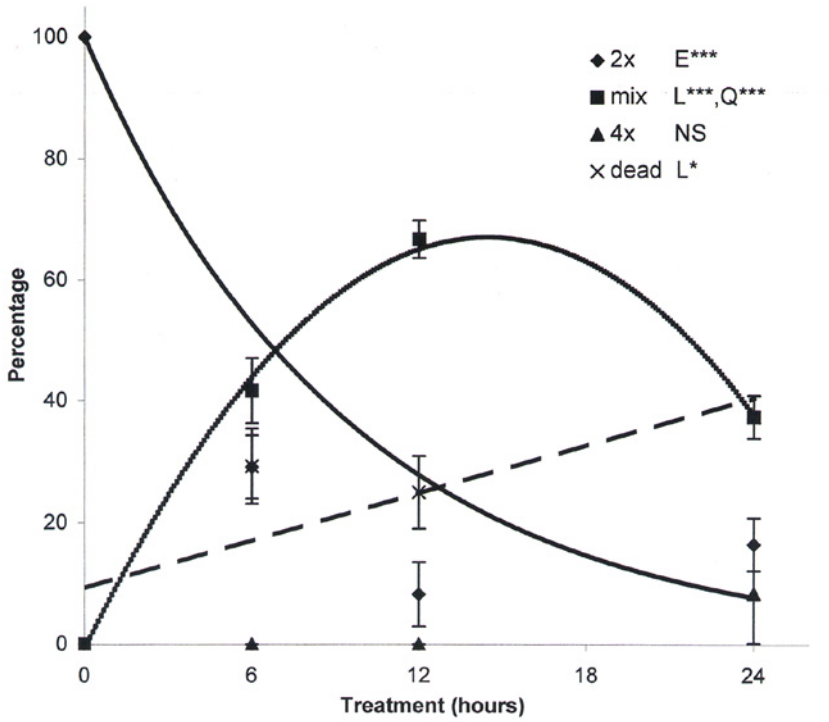

Figure 1. Efficacy of oryzalin as a chromosome-doubling agent in ×*Chitalpa tashkentensis*. Regression analysis: linear (L), quadratic (Q), and exponential (E) at $P < 0.05$ (*), 0.01 (**), 0.001 (***) and nonsignificant (NS) levels. Symbols are means ($n = 8$) ± 1 standard error of the mean.

Table 1. Results of pollen staining and germination tests.

		Pollen Texts[y]	
Taxa	Ploidy	% Viability	% Germination
Catalpa bignonioides	2x	97.8 ± 0.2[z]	56.8 ± 3.1
Chilopsis linearis	2x	95.1 ± 0.3	64.3 ± 1.0
×*Chitalpa tashkentensis*	2x	0.2 ± 0.8	0.1 ± 0.7
	2x + 4x	84.1 ± 0.2	81.1 ± 0.8

[y]Minimum pollen counted n = 100 per replicate for each test.

[z]Means (n = 8, except mixoploid, n = 4) followed by ± 1 standard error of the mean.