Seed Set and Germination for Interspecific and Intergeneric Hybrids in Two Genera of *Fabaceae*©

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Interspecific and intergeneric crosses were performed between species in the genera *Baptisia* and *Thermopsis* in an attempt to create hybrids with the best qualities of both parents. Interspecific crosses produced a higher percentage of fertile crosses and number of seeds per fertile cross than intergeneric crosses. Germination rate was not different between interspecific and intergeneric crosses. When comparing species to determine the best female parents, we found no difference between female parents for percentage of fertile crosses or germination rate. However, *Thermopsis* female parents produced a higher number of seed per cross than *Baptisia* female parents. When comparing species to determine the best male parents, crosses with *Thermopsis* male parents produced more seed per cross than those with *Baptisia* male parents, but were not different for percentage of fertile crosses or germination rate. Since seedlings could be obtained from both interspecific and intergeneric crosses, production of a *Baptisia-Thermopsis* hybrid is feasible. Steps to increase the percentage of fertile crosses and number of seedlings include new species as parents, use of bridge parents, embryo rescue, and selection of the male and female parents that produced the most fertile crosses for further breeding efforts.

INTRODUCTION

Interspecific and intergeneric crosses are often used to create new hybrids in ornamental plants. As well as combining good ornamental traits, wide crosses are also used to introgress such traits as disease or pest resistance, heat or cold tolerance, drought tolerance, or greater ease of propagation into a hybrid. *Artemesia*'s resistance to chrysanthemum aphids was conferred on a chrysanthemum (*Dendranthema morifolium*) by *Artemesia vulgaris* hybrid (Deng et al., 2010). A cross between chrysanthemum (*Chrysanthemum grandiflorum* (Ramat.) Kitamura) 'Zhongshanjingui' and *Artemisia vulgaris* produced a hybrid that rooted more easily than its chrysanthemum parent (Deng et al., 2012). Interspecific crosses between *Hibiscus paramutabilis*, *H. sinosyriacus*, and *H. syriacus* were made to incorporate the vigor of the first two species into *H. syriacus* (Van Laere et al., 2007).

In the genus *Baptisia*, interspecific crosses have been used to create many novel cultivars (Avent, 2002; Cullina, 2011). Another genus in the *Fabaceae*, *Thermopsis*, has not been used to create hybrids to our knowledge and is also not much known or used in the ornamental plant industry. Yet several species of *Thermopsis* have good horticultural qualities. Many species of *Thermopsis*, particularly *T. villosa*, are very tolerant of both drought and heat, making *Thermopsis* a good substitute for lupines in the Southeastern United States (Armitage, 1989). *Thermopsis* roots more easily than *Baptisia* (Hawkins et al., 2013) and will bloom 2 years after germination, whereas *Baptisia* requires 3 years to bloom from seed (pers. commun., Heather Alley, State Botanical Garden of Georgia, 2013).

To attempt to introgress these desirable traits into a hybrid between *Thermopsis* and *Baptisia*, intergeneric crosses were made between several species of *Baptisia* and *Thermopsis*. Interspecific crosses were also made, since such a cross might produce a hybrid of good ornamental quality, or one that might be used as a bridge parent for further intergeneric crosses.

MATERIALS AND METHODS

Three species of *Thermopsis* were used in the crosses: *Thermopsis chinensis*, *T. lupinoides*, and *T. villosa* (Table 1). *Baptisia australis* was the only species in its genus to be used as the female parent. To increase the diversity of male *Baptisia* parents, pollen was collected from *B. alba*, *B. bracteata*, and *B. lanceolata* and used to make crosses (Table 1). *Baptisia australis*, *T. chinensis*, and *T. villosa* plants were obtained from Northcreek Nurseries, Inc., Landenberg, Pennsylvania, as seed-grown liners. *Thermopsis lupinoides* plants were vegetatively produced from a stock plant obtained from Plant Delights Nursery, Raleigh, North Carolina. Reciprocal crosses were made where possible. Since not all species had blooming periods that overlapped or that overlapped for only a brief time, this could not always be accomplished. The crosses were carried out in an enclosed shade house (50% shade) at the University of Georgia Horticulture Farm in Watkinsville, Georgia, from March to May 2013.

Flowers of the female parents were emasculated. Pollen was collected from the male parent onto a small paintbrush and used to pollinate the female parent. Crosses were tagged and seed pods were collected once ripe in May and June 2013. Seeds were extracted from the pods and counted. Seeds were scarified in 0.1 M sulfuric acid for 20 min, rinsed, and soaked for several hours in plain water to imbibe before being sown in a potting substrate containing bark, peat moss, and perlite. Germination data was taken at 4 weeks after sowing.

Statistical analysis on the fertility of the crosses, number of seed per cross, and germination rate was performed using SAS 9.3 (PROC GLM) (SAS Institute, Cary, North Carolina). Percentages were transformed before analysis to normalize data, and retransformed for reporting. Differences were considered to be significant at the level of P=0.05.

RESULTS

Interspecific crosses had a higher percentage of fertile crosses than intergeneric crosses (P=0.005). Number of seed per fertile cross was also higher in interspecific crosses (P=0.008). However, the germination rate was not different between interspecific and intergeneric crosses (P=0.238).

No differences in fertility were found among female parents in the percent of fertile crosses (P=0.760) or in germination rate (P=0.182). The number of seeds per fertile cross was different (P=0.019), with crosses having *Baptisia* as the female parent producing much fewer seed on average than any of the crosses having *Thermopsis* as the female parent. Intergeneric crosses were also compared to determine whether female parents differed in fertility. Average number of seeds per fertile cross was not different among female parents (P=0.084). Neither was germination rate (P=0.155) or the percent of fertile crosses (P=0.231).

Interspecific crosses were also compared to determine which male parents had highest percentage of cross fertility, germination rate, and seed per cross. We found no difference among male parents for percentage of fertile crosses (P=0.370) or for germination rate (P= 0.087). However, we found a difference in the average number of seeds per cross (P=0.008), with all crosses with a male *Thermopsis* parent having higher numbers of seed than crosses with a male *Baptisia* parent.

In intergeneric crosses, the male parent made no difference in percentage of fertile crosses (P=0.661), number of seeds per fertile cross (P=0.573), or germination rate (P=0.656).

DISCUSSION

Pre- or post-zygotic barriers between parents in interspecific and intergeneric crosses will often preclude fertilization of the ovule after pollination, resulting in low seed set. Since percentage of fertile crosses and number of seed per fertile cross was higher in interspecific crosses than in intergeneric crosses, barriers to fertilization seem to be much lower in the interspecific crosses. Though such a result is typical in many species, it is not always the case. Intergeneric crosses in brooms (genera *Genista* and *Cystisus*, family *Fabaceae*) showed greater fertility than interspecific crosses (Bellenot-Kapusta et al., 2006).

The number of seeds obtained per cross when *B. australis* was used as a female parent was less than when *Thermopsis* was used (Table 1). Apparently, this lower seed set is due to factors other than ovule number, as *B. australis* is reported to have numerous seeds per pod, as is *T. villosa* (Cronquist, 1980). Counts of 30 seeds per pod have been reported for *B. australis* (Evans et al., 1989). Southeastern *Thermopsis*, such as *T. villosa*, have been reported to have 12-16 ovules and Asian *Thermopsis*, such as *T. chinesis* and *T. lupinoides*, have been reported to contain 10-14 ovules (Chen et al., 1994).

Since the germination rates for interspecific and intergeneric crosses were not different, mature seeds from intergeneric crosses have a good chance of producing a viable intergeneric hybrid. The challenge for this breeding program is to increase the number of fertile crosses, especially intergeneric crosses. Several techniques could be used to reach this goal. Performing more crosses, especially more intergeneric crosses, would likely result in additional hybrids. Using more diverse genotypes within each species, storing pollen of an early-blooming species to use on a late-blooming species, or using different species in each genus as parents, might also be helpful. Reciprocal crosses should be evaluated for cross fertility, seed set, and germination rate to determine the best cross. For example, the *T. chinensis* × *T. lupinoides* cross had a lower percentage of fertile crosses compared to the reciprocal cross (21.1 vs. 44.0%), but a higher number of seeds per cross (12.1 vs. 8.4) and a higher germination rate (89.4 vs. 81.1%). If these averages held true for the next round of crossing, *T. lupinoides* would prove to be the superior female parent, as 100 crosses of *T. chinensis* × *T. lupinoides* would produce 228 seedlings while the same number of reciprocal crosses would produce 301 seedlings.

Techniques to overcome pre- and post-fertilization barriers could also be used to increase the percentage of fertile crosses. Treatment of the stigma of the female parent with calcium chloride and boron might increase fertilization (Jayavalli et al., 2011). Embryo rescue could also be attempted for intergeneric crosses that had low seed set, since fertilization was proven to be possible for these crosses. In some intergeneric crosses, such as that between *Ascocenda* and *Phalaenopsis* or between *Alstroemeria* and *Bomarea*, embryo rescue has been needed to produce viable progeny (Kashihara et al., 2010; Tsai et al., 2009).

Since putative interspecific *Thermopsis* hybrids were obtained, use of these hybrids as bridge parents should also be investigated. It is possible that cross fertility will be higher with the bridge parents than with the species used in the initial crosses. The blooming period for *T. lupinoides* and *T. chinensis* did not overlap substantially with the blooming period for *B. australis*, while that of *T. villosa* did. A hybrid between *T. villosa* and either *T. lupinoides* or *T. chinensis* might have an intermediate blooming period, making it a more suitable parent in an intergeneric cross with *B. australis*.

Much work remains to be done to create a desirable hybrid between *Baptisia* and *Thermopsis*. However, the initial results indicate that creating such a hybrid is feasible.

Literature Cited

Armitage, A.M. 1989. Herbaceous Perennial Plants: A Treatise on Their Identification, Culture, and Garden Attributes. Varsity Press.

Avent, T. 2002. Revenge of the 'Redneck Lupines'. Horticulture 99:70.

Bellenot-Kapusta, V., Pesteil, C. and Cadic, A. 2006. Diversity study and breeding of brooms (tribe of Genisteae). Acta Hort. 714:29-36.

Chen, C.J., Mendenhall, M.G. and Turner, B.L. 1994. Taxonomy of *Thermopsis* (*Fabaceae*) in North America. Ann. Missouri Bot. Gard. 81:714-742.

Cronquist, A. 1980. Vascular Flora of the Southeastern United States. Chapel Hill: University of North Carolina Press, 116 South Boundary Street, Chapel Hill, North Carolina 27514-3808.

Cullina, W. 2011. Gardener's challenge. Horticulture 108:14-19.

Deng, Y., Chen, S., Lu, A., Chen, F., Tang, F., Guan, Z. and Teng, N. 2010. Production and characterisation of the intergeneric hybrids between *Dendranthema morifolium* and *Artemisia vulgaris* exhibiting enhanced resistance to chrysanthemum aphid

(*Macrosiphoniella sanbourni*). Planta 231:693-703.
Deng, Y.M., Chen, S.M., Chang, Q.S., Wang, H.B. and Chen, F.D. 2012. The chrysanthemum × *Artemisia vulgaris* intergeneric hybrid has better rooting ability and higher resistance to alternaria leaf spot than its chrysanthemum parent. Scient. Hort. 134:185-190.
Evans, E.W., Smith, C.C. and Gendron, R.P. 1989. Timing of reproduction in a prairie legume: seasonal impacts of insects consuming flowers and seeds. Oecologia 78:220-230.
Jayavalli, R., Balamohan, T.N., Manivannan, N. and Govindaraj, M. 2011. Breaking the intergeneric hybridization barrier in Carica papaya and Vasconcellea cauliflora. Scient. Hort. 130:787-794.
Kashihara, Y., Hirano, T., Murata, N., Shinoda, K., Araki, H. and Hoshino, Y. 2010. Evaluation of pre-fertilization barriers by observation of pollen tube growth and attempts for overcoming post-fertilization barriers in intergeneric hybridization between Alstroemeria and Bomarea by ovule culture. Acta Hort. 855:159-164.
Tsai, C.C., Chiang, Y.C., Huang, S.C., Liu, W.L. and Chou, C.H. 2009. Intergeneric hybridization, embryo rescue and molecular detection for intergeneric hybrids between Ascocenda and Phalaenopsis. Acta Hort. 829:413-416.
Van Laere, K., Huylenbroeck, J.M. and Van Bockstaele, E. 2007. Interspecific hybridisation between *Hibiscus syriacus*, *Hibiscus sinosyriacus* and *Hibiscus paramutabilis*. Euphyt. 155:271-283.

Table 1. Interspecific and intergeneric crosses of *Baptisia* and *Thermopsis*.

Female parent	Male parent	No. crosses	Crosses with seed (no.)	Crosses with seed (%)	Seeds per cross	Seeds sown (no.)	Seed germinated (no.)	Germination (%)
T. chinensis	*T. lupinoides*	635	134	21.1	12.1	1,617	1,445	89.4
T. chinensis	*T. villosa*	130	25	19.2	9.9	248	158	63.7
T. chinensis	*B. alba*	25	3	12.0	7.3	22	19	86.4
T. lupinoides	*T. chinensis*	600	264	44.0	8.4	2,228	1,808	81.1
T. lupinoides	*T. villosa*	160	21	13.1	10.5	221	171	77.4
T. lupinoides	*B. australis*	10	0	0.0	0.0	0	0	0.0
T. lupinoides	*B. alba*	25	0	0.0	0.0	0	0	0.0
T. villosa	*T. lupinoides*	10	4	40.0	11.5	46	43	93.5
T. villosa	*B. australis*	459	46	10.0	1.4	63	51	80.9
T. villosa	*B. lanceolata*	15	3	20.0	5.7	17	13	76.5
T. villosa	*B. alba*	15	4	26.7	6.5	26	23	88.5
B. australis	*T. chinensis*	5	0	0.0	0.0	0	0	0.0
B. australis	*T. villosa*	340	41	12.1	1.0	42	36	85.7
B. australis	*B. lanceolata*	10	4	40.0	3.00	12	7	58.3
B. australis	*B. bracteata*	5	1	20.0	3.00	3	1	33.3