

Intergeneric Hybrids Between *Weigela* and *Diervilla* (Caprifoliaceae)

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Significance to Industry: *Weigela* Thunb. consists of 12 species endemic to northeast Asia (1). Several species are grown in North America as ornamental shrubs and have significant commercial value to the ornamental horticultural industry. The most common ornamental species is *W. florida* (Bunge.) A. DC. (old fashioned weigela). This species typically grows to 3-8 feet tall and produces abundant showy flowers. It is pest resistant, tolerant to a wide range of environmental conditions, and cold hardy to USDA zone 5. *Weigela* is closely related to the endemic North American genus *Diervilla* Mill. (bush honeysuckle) (1). *Diervilla* are typically more compact, growing to 2-4 feet tall, adaptable to a wide range of environmental conditions and possess a higher level of cold tolerance (USDA zone 3-5), depending on the species. Intergeneric hybridization between these genera could potentially combine the superior cold hardiness and compact stature of *Diervilla* with the showy flowers and foliage of *Weigela*.

Nature of Work: Intergeneric hybridization is often difficult or impossible due to genetic and reproductive incompatibilities. However, in some cases, biotechnology tools can be used to facilitate wide hybridization and to confirm hybridity of the resulting progeny. Our prior attempts to hybridize *Weigela* and *Diervilla* resulted in seed capsules with underdeveloped and aborted seeds that failed to germinate. In the present study we report on the development of intergeneric hybrids of *Weigela* and *Diervilla*, using a combination of tissue culture and molecular screening techniques. Specifically, we used ovule culture and micropropagation techniques to obtain plants from immature seed prior to embryo abortion (2). We then used Cleaved Amplified Polymorphic Sequence (CAPS) techniques to assist in verifying hybridity (3).

Four intergeneric crosses were attempted (Table 1). Ovules were collected at 30, 41, 49, 56 or 63 days after pollination (DAP) depending upon intergeneric cross (Table 1.). Germination of ovules was conducted in vitro on media containing Woody Plant Medium (WPM) basal salts and vitamins or Schenk and Hildebrandt (SH) basal salts and vitamins supplemented with either 1, 2, 3 or 4 μM gibberellic acid and 30 g/l sucrose. Germinated ovules were transferred to a maintenance medium consisting of Murashige and Skoog basal medium supplemented with 2 μM Benzylaminopurine.

CAPS analysis was used to assess hybridity (1). DNA from parents and hybrids was extracted using a modified CTAB method (3). The Internal Transcribed Spacer (ITS) region was amplified using PCR primers and conditions described

by Kim and Kim (1), producing a 670 bp fragment. Sequence analysis of the ITS region of the parent plants revealed that *W. florida* contained a restriction site (cccggg) at 480 bp, recognized *Sma*I, that was absent in all other taxa. *W. middendoriana* contained a restriction site (tataa) at 538 bp, recognized by *Psi*I, that was absent in other taxa. To confirm hybridity, the amplified ITS region of plants derived from ovule culture was subjected to digestion by *Sma*I and *Psi*I and the digestion profiles compared to parents.

Results and Discussion: A total of 1873 ovules were obtained from four intergeneric crosses. Ovules began germinating 2 months after culture, with new germinants being recorded up to 1 year after culture. Ovule maturity was key factor in influencing germination with highest germination generally occurring in ovules collected from 41 to 63 DAP (Table 1.). For *D. lonicera* 'Copper' X *W. florida* higher germination was observed at 56 DAP compared to 63 DAP which may indicate increase in embryo abortion in the more mature ovules. For all intergeneric crosses medium composition did not have an influence on ovule germination (data not shown).

D. lonicera 'Copper' x *W. middendoriana* grew well in tissue culture, but were later confirmed not to be hybrids and were discarded. From the remaining three crosses, over 85% of plantlets were albino or had low vigor, leaving only 80 plantlets maintained in tissue culture. Only 10 hybrids have been successfully transferred *ex vitro*. Further, only 2 hybrids of *D. sessifolia* 'Butterfly' x *W. florida* are maintaining good growth under greenhouse and field conditions.

Using CAPS analysis hybrids could be confirmed if they shared banding patterns from both parents (Figure 1.). Using this procedure, all plant derived from *D. lonicera* 'Copper' x *W. middendoriana* were confirmed not to be hybrids. Random analysis of plants from *D. sessifolia* 'Butterfly'x *W. florida* indicated that all plants were hybrids, while 60% of plants from *D. lonicera* 'Copper' X *W. florida* were hybrids. To further describe these hybrids, morphological assessment of leaves from the *D. sessifolia* 'Butterfly' x *W. florida* hybrid growing in the field was conducted. Leaves from the hybrid shared characteristics with both the parents (Table 2.).

In conclusion, this study demonstrates how biotechnology can be used to assist traditional plant breeding strategies. We successfully obtained and verified hybrids from intergeneric crosses between *Weigela* and *Diervilla*. However, the intergeneric hybrids resulting from our study have low vigor and poor growth under greenhouse and field conditions. Further research into mechanisms to improve growth and vigor is currently being conducted. One approach being explored is improving vigor through ploidy manipulation.

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Table 1. Percentage germination of ovules harvested from different crosses between *Diervilla* and *Weigela* at different number of days after pollination.

Intergeneric cross	Percentage Ovule Germination	
	30 DAP	41 DAP
<i>D. sessifolia</i> 'Butterfly' X <i>W. florida</i> (Clone 1)	28.5 ± 8.2	39.0 ± 10.5
<i>D. sessifolia</i> 'Butterfly' X <i>W. florida</i> (Clone 2)	15.5 ± 6.9	44.0 ± 3.3
	56 DAP	63 DAP
<i>D. lonicera</i> 'Copper' X <i>W. florida</i>	57.4 ± 6.7	43.3 ± 1.2
	49 DAP	63 DAP
<i>D. lonicera</i> 'Copper' X <i>W. middendoriana</i>	28.4 ± 4.3	44.0 ± 3.3

Table 2. Comparison of leaf characteristics of a hybrid from *D. sessifolia* 'Butterfly' X *W. florida*.

	<i>D. sessifolia</i> 'Butterfly'		<i>W. florida</i>
Characteristic		Hybrids	
Leaf Shape	Lanceolate	Ablanceolate	Ablanceolate
Leaf Apex	Acuminate	Acuminate	Acuminate-slightly Cirrose
Leaf Base	Rounded	Cuneate	Cuneate
Leaf Margin	Crenulate	Crenulate	Serrulate
Lamina length	5-7.5 cm	4-6 cm	3-5 cm
Lamina width	2-3 cm	1.5-2.5 cm	1-2.5 cm
Lamina lateral vein number	7-8	6-7	5-7

Figure 1. Cleaved Amplified Polymorphic Sequence analysis verifying hybridity based on shared banding patterns of hybrids with both parents.

