The Interspecific Hybrid *Petunia parodii* × *P. inflata* and its Relevance to Somatic Hybridization in the Genus *Petunia*

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Summary. Attempts at the reciprocal cross between *Petunia parodii* and *P. inflata* using standard emasculation and pollination techniques failed. Limited pollen tube growth down the style in reciprocal crosses led to reproductive isolation between the self-compatible *P. parodii* and self-incompatible *P. inflata*. The interspecific hybrid was successfully produced by bud-pollination of *P. parodii* with *P. inflata* as the male parent in 22 percent of attempts, but not in the opposite direction. In vitro pollination of *P. parodii* ovaries with *P. inflata* pollen also produced hybrids. The small size of the ovary made it technically impossible to use *P. inflata* as the female parent for in vitro pollination. The interspecific hybrids were intermediate, as compared to the two parents, for six of the seven plant and flower characters measured. Furthermore, the hybrids had high pollen fertility, set abundant seed upon self-pollination, and readily inter-crossed with the parental species. The results are consistent with a high degree of chromosomal homology in the parental species and with minor genetic divergence leading to reproductive isolation that is pre-zygotic in nature. Overcoming the barriers to cross-incompatibility by practical techniques resulted in fertile interspecific hybrids that segregated for parental characters. The potential value of employing the parental species in somatic hybridization experiments is discussed.

Key words: *Petunia* — Unilateral cross-incompatibility — Bud-pollination — In vitro pollination — Somatic hybridization

Introduction

Our studies on the genus *Petunia* have been primarily concerned with the inheritance of monogenic traits (Sink 1973), their action in morphological development (Natarella and Sink 1971) and biochemical expression (Knowlton and Sink 1977) and the regeneration to whole plants of cultured protoplasts (Hayward and Power 1975; Power et. al., 1976b). The crossing behaviour of *P. hybrida* genetic lines with *P. axillaris*, *P. inflata*, *P. parodii* and *P. violacea* has also been examined (Sink 1975). During these studies, the intercrosses among these selected *Petunia* species were made not only to determine the origin of the cultivated types, but to identify possible new sources of variation, and to define taxonomic relationships. We observed that all the *Petunia* species employed readily intercrossed among themselves and with selected *P. hybrida* cultivars. An exception proved to be the attempted cross between *P. parodii* and *P. inflata*. We report here our subsequent studies in which we have examined by histological procedures, the stage where failure occurs in the reproductive cycle between these two species and the successful production of the interspecific hybrid by the in vitro pollination method and by bud-pollination. The information gained is important in view of the current aim of plant breeders and geneticists to extend the degree of genetic variation that can be incorporated into economically important crop and horticultural species. The results are discussed in relation to two in vitro approaches that are presently being intensively studied and further developed for their potential in overcoming sexual incongruity at various taxonomic levels. These methods include in vitro pollination (Rangaswamy 1977) and somatic hybridization (Smith 1974).

Materials and Methods

The *Petunia* species *P. inflata* Fries (Fries 1911) 2n = 14, and *P. parodii* W.C.S. (Steere 1930) 2n = 14 were obtained (Natarella and Sink 1975) and verified by Dr. L.B. Smith (Personal Communication 1972). Voucher specimens of these two species and their interspecific hybrid have been deposited in the U.S. National Herbarium. The plants used in this study were grown from seed ob-
Table 1. Plant and flower character means and standard deviations for the interspecific hybrid *Petunia parodii* by *P. inflata* and the parental species. Measurements are in centimeters.

<table>
<thead>
<tr>
<th>Species</th>
<th>Veg lv length</th>
<th>Veg lv width</th>
<th>Pedicel length</th>
<th>Total length</th>
<th>Tube length</th>
<th>Lobe diameter</th>
<th>Length filament attach</th>
<th>Percent filament attach</th>
<th>Flower color</th>
<th>Percent viability</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>inflata</em></td>
<td>6.0 ± 0.86</td>
<td>2.9 ± 0.48</td>
<td>3.5 ± 0.47</td>
<td>2.7 ± 0.19</td>
<td>1.5 ± 0.15</td>
<td>2.8 ± 0.31</td>
<td>0.4 ± 0.40</td>
<td>26.6</td>
<td>magenta</td>
<td>95.6</td>
</tr>
<tr>
<td><em>parodii</em></td>
<td>8.8 ± 1.54</td>
<td>3.1 ± 0.51</td>
<td>4.0 ± 0.85</td>
<td>9.1 ± 0.50</td>
<td>6.9 ± 0.38</td>
<td>4.2 ± 0.61</td>
<td>3.8 ± 0.25</td>
<td>55.0</td>
<td>white</td>
<td>99.0</td>
</tr>
<tr>
<td><em>parodii inflata</em></td>
<td>8.6 ± 1.58</td>
<td>3.5 ± 0.68</td>
<td>3.8 ± 0.92</td>
<td>4.7 ± 0.94</td>
<td>3.0 ± 0.67</td>
<td>3.5 ± 0.73</td>
<td>1.1 ± 0.37</td>
<td>36.7</td>
<td>lt. magenta</td>
<td>93.4</td>
</tr>
</tbody>
</table>

Results and Discussion

Cross-incompatibility between *P. parodii* and *P. inflata* was confirmed using the standard pollination technique for hybridizing *Petunia*. Approximately 500 pollinations were conducted in each direction. In addition, pollination of mature stigmas throughout different growing seasons failed to produce hybrid seed. The corolla of pollinated flowers of both species wilted and abscissed, the ovary turned brown and shrank within 10-14 days, but the calyx persisted. Histological observations of pollen grain germination and pollen tube growth in the styles of the reciprocal crosses indicated the cross-incompatibility was pre-zygotic in nature. Twenty-four hours after pollination, the pollen grains of *P. parodii* had germinated readily on the stigmatic surface but the pollen tubes failed to penetrate further than the neck region of the style of *P. inflata*. In the reciprocal cross, *P. inflata* pollen grains germinated but were arrested at the neck region of *P. parodii* styles.

Fig. 1. Flowers of, top to bottom, *Petunia inflata*, interspecific hybrid *P. parodii X P. inflata*, *P. parodii*.