Maternal Haploids of *Nicotiana tabacum* L. from Seed

Abstract. *Abundant seeds of high germinability are obtained when* Nicotiana tabacum *is pollinated by* Nicotiana africana. *Most of the seedlings die at the cotyledonary stage. The remaining seedlings are viable F_1 hybrids or maternal haploids that can be easily distinguished. This simple method of producing* Nicotiana tabacum *haploids offers an alternative to anther culture.*

Haploid plants of *Nicotiana tabacum* ($n = 24$) have been derived from interspecific hybridizations (*1*). They also occur among self-pollinated progeny, but are very difficult to distinguish from diploid seedlings. Haploids have been observed in *N. tabacum* (*1*) and *Capsicum frutescens* L. (*2*) after treatments with x-rays, and as members of twin-seedling pairs (*3*) they have also been observed in reciprocal intraspecific crosses of *N. tabacum* marked with a recessive aureate seedling trait (*4*) or a dominant gene for resistance to a virus disease (*5*). During the last 10 years methods have been developed for producing haploids of *N. tabacum* through anther culture (*6*). The low frequency of naturally occurring haploids, the difficulty of detecting them, and the reliability of the anther culture method have all but eliminated consideration of seed-derived haploids of *N. tabacum* for biological investigations.

We have found that, when *N. africana* Merxm. (*7*) is used to pollinate *N. tabacum*, most of the seeds germinate; but the majority of the seedlings die before they develop true leaves. From 0.25 to 1.42 percent of the seedlings grow well and are readily identified as either F_1 hybrids or maternal haploids. Although these percentages appear small, the vast yield of tiny seeds and the early death of most seedlings provide significant numbers of viable hybrids and haploids in relatively small soil containers. This method of interspecific pollination with its built-in screening mechanism offers a practical way of producing seed-derived haploids of *N. tabacum*, either as an alternative or a complementary method to anther culture.

Interspecific pollinations included monosomics of *N. tabacum* (*8*), the disomic line (Red Russian) used to maintain the monosomics, a burley hybrid designated VT-9, and an inbred strain of the variety NC 95. The monosomic haplo "H" crossed to *N. africana* produced many viable hybrid plants, but the other monosomics behaved in the manner of the three disomics (Table 1) by producing many lethals and rarely viable hybrids or haploids. The lethals are an important attribute of the pollen parent.

Plants of disomic Red Russian, VT-9, and NC 95, when pollinated by *N. africana*, produced about 2800 seeds per capsule. Germination varied from 94 to 100 percent. Seeds were germinated in clear plastic preparation dishes (about 8 by 8 cm) filled with about 4 cm of sterilized soil. Sterilized soil and sterile water prevented the introduction of algae that would have inhibited seedling development. Initially, 1000 seeds were sown on the soil surface of each dish and were moistened with sterile water. The dishes were then covered and placed in a growth chamber at 30°C, with a light intensity of about 800 lux. Germination was evident in 5 days. Most of the seedlings had small cotyledons, failed to develop true leaves, and eventually died. In about 8 days, it was possible to distinguish viable seedlings from lethals by their larger cotyledons, continued growth, and development of true leaves. After 14 days, these seedlings were transferred to other dishes of soil. About 30 days later they were transplanted to pots of soil in greenhouses. Sowing 2000 or more seeds to a dish increased the number of viable plants per dish but did not alter their frequency. Space and time were also saved by sowing seeds heavily on flats of soil in a greenhouse and removing the hybrids or haploids when they were large enough to transplant.

Hybrid plants were distinguished from haploids by the greater length and density of trichomes on their stems and leaves. The leaves of haploids have a characteristic graininess or puckered surface and a non-undulate margin. The glands on their trichomes are distinctly larger than those of the hybrid. These morphological differences permit early separation of hybrids from haploids.

The haploids flowered earlier than the hybrids, had an abundance of flowers of reduced size, aborted pollen, and had anthers subordinate to the stigma. Somatic chromosome preparations of a sample of these haploid plants indicated a chromosome number of 24 in all instances but one. The exception ($n = 23$) was derived from one of the monosomic maternal parents.

Table 1. Percentages of interspecific F_1 hybrids and maternal haploids among surviving seedlings from crosses of *N. tabacum* with *N. africana*.

Cross	Hybrids (%)	Haploids (%)
VT-9 × *N. africana*	95.77	4.23
Red Russian × *N. africana*	84.75	15.25
NC 95 × *N. africana*	84.06	15.94

Monosomics of *N. tabacum* yielded fewer seeds than disomics when pollinated by *N. africana*. An exception was the cross haplo "H" × *N. africana*, which yielded an abundance of seeds that germinated well and resulted in a large number of viable hybrid plants. The disomic Red Russian, VT-9, and NC 95 sources yielded maternal haploids in the percentages shown in Table 1. These percentages are comparable to those obtained from reciprocal crosses of *N. tabacum*, in which one of the parents possessed a recessive seedling marker (*4*). This suggests that the frequencies obtained in our experiments probably reflect a normal rate of spontaneous gynogenic haploidy.

We recommend the seed-derived method of obtaining haploids of *N. tabacum* to those who may not have the laboratory facilities for producing anther-derived haploids or who may wish to compare haploids of different derivations. This method is successful because of the high yield of seeds per pollination, the indeterminate flowering of *N. africana* and *N. tabacum*, and the self-elimination of most hybrid seedlings. Seed of *N. africana* are available in limited quantities (*9*).

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9. Seed of *N. africana* were obtained through the courtesy of Professor H. Merxmüller. The seed has been increased for the *Nicotiana* germ plasm collection, U.S. Department of Agriculture, Tobacco Research Laboratory, Agricultural Research Science and Education Administration, Oxford, N.C. 27565.

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