HISTOLOGICAL EVALUATION OF SEED FAILURE IN THREE *NICOTIANA* INTERSPECIFIC HYBRIDS

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A histological analysis of seed development in *N. repanda* x *N. tabacum*, *N. stocktonii* x *N. tabacum* and *N. nesophila* x *N. tabacum* indicated that the cause of seed failure in these hybrids was a cessation in endosperm development. The endosperm had stopped growing by 9 days after pollination in the *N. stocktonii* x *N. tabacum* hybrid and by 7 days after pollination in the other two hybrids. In all three hybrids, the embryo began to assume a highly irregular shape as the endosperm ceased its development. *N. repanda* x *N. tabacum*, *N. stocktonii* x *N. tabacum* and *N. nesophila* x *N. tabacum* hybrids were successfully obtained by fertilized ovule culture apparently because the artificial nutrient medium substituted for the endosperm as a source of nutrition for the developing embryos.

INTRODUCTION

The more than 64 species in the genus *Nicotiana* provide a potentially useful gene pool for the improvement of cultivated tobacco (*Nicotiana tabacum* L.). This potential has not been fully realized because crosses between *N. tabacum* and some of the wild *Nicotiana* species have not resulted in viable seeds. In particular, a transfer of genetic information controlling resistance to race 1 black shank (*Phytophthora parasitica* (Dast.) var. *nicotianae* (B. de Haan) Tucker) to *N. tabacum* has been hindered by the difficulty of hybridizing *N. tabacum* with the three species in sub-generic section Repandae that are resistant to race 1 black shank. In most cases, attempts to directly hybridize *N. tabacum* with *N. repanda*, *N. stocktonii* and *N. nesophila* have resulted in the production of inviable seeds (3, 6, 8, 9, 10). Recently, we reported the production of *N. repanda* x *N. tabacum*, *N. stocktonii* x *N. tabacum* and *N. nesophila* x *N. tabacum* through *in vitro* culture of fertilized ovules (8).

In the present study, a histological study of seed development in the three hybrids was performed. The purpose of this analysis was to determine the cause(s) of seed failure in the hybrids and to relate these causes to the successful production of the hybrids via fertilized ovule culture.

MATERIALS AND METHODS

Plants used in this study were grown in the greenhouse from seed stocks maintained either at the University of Kentucky or by L. G. Burk, Science and Education Administration, U.S.D.A., Tobacco Research Laboratory, RR 2, Box 16G, Oxford, N.C. 27565. The species and their identification numbers were *N. tabacum* 'Burley 21,' *N. repanda* S-47-B Ky , *N. stocktonii* S-57-A Ky and *N. nesophila* 34a-G-61 Beltsville. Stigmas of the three wild species were pollinated with either self-pollen or pollen of *N. tabacum*. Hand pollinations were made by emasculating unopened flowers and pollinating with donor pollen which had been collected on the previous day. The stigma and style of each pollinated flower were covered with a paper straw to insure against contaminant pollination.

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Crosses from interspecific hybridizations and self-pollinations were collected 2, 3, 5, 7, 9 and 12 days after pollination. A small section of the basal end of each capsule was removed to allow better penetration of the fixative and embedding solutions. Fixation was in 17:2:1 (v:v:v) 70% ethanol, formaldehyde and glacial acetic acid at room temperature for at least 24 hours. Dehydration was in a tertiary-butyl alcohol series (5). A rotary microtome was used to obtain 12 μ serial sections from paraffin embedded seed capsules. Staining was in a safranin and fast green series (5).

Serial sections of developing seeds from self-pollinations were compared with those from interspecific hybridizations. Observations were recorded as a range of number of embryo and endosperm cells present in the ovules. At least 25 ovules were observed for each of the collection periods.

RESULTS

At 2 days after pollination, fertilization had not yet occurred in *N. stocktonii* or in the *N. stocktonii* x *N. tabacum* hybrid. In both crosses, fertilization had occurred by 3 days after pollination and a 2-celled endosperm was present. At 5 days after pollination, the majority of the *N. stocktonii* ovules observed contained a 2-celled proembryo **(Figure 1A)**. In the remaining ovules, a 4-celled embryo was present. The endosperm in most of the ovules observed was composed of 8 to 12 cells. In the *N. stocktonii* x *N. tabacum* hybrid only about half of the ovules contained a 2-celled proembryo. In the rest of the ovules, the zygote had not yet divided **(Figure 1B)**. From four to eight cells were seen in the endosperm of most of the hybrid ovules. The appearance of the ovules of *N. stocktonii* and *N. stocktonni* x *N. tabacum* was similar at 7 days after pollination. In both cases, most of the embryos were composed of four cells **(Figures 1C-D)**. An 8- to 24-celled endosperm was present in the majority of the ovules. Two days later large differences between *N. stocktonii* and *N. stocktonii* x *N. tabacum* ovules became apparent. The *N. stocktonii* embryo was growing rapidly and the endosperm was multicellular. The embryos of hybrid ovules were larger than in previous collections, but they had begun to assume highly irregular shapes. The greatest abnormality, however, was observed in the endosperm of hybrid ovules. The hybrid endosperm contained no more cells at 9 days after pollination than it did at 7 days after pollination. A few endosperm cells appeared to be disintegrating. By 12 days after pollination, the difference between selfed and hybrid ovules was even greater. While a large heart-shaped embryo was present in *N. stocktonii* ovules **(Figure 1E)**, the *N. stocktonii* x *N. tabacum* embryos had stopped growing and the endosperm had almost completely disintegrated **(Figure 1F)**.

The pattern of seed development for the hybrids involving *N. nesophila* and *N. repanda* was similar to that observed in *N. stocktonii* x *N. tabacum*. However, seed failure appeared to occur slightly earlier in the *N. nesophila* x *N. tabacum* and *N. repanda* x *N. tabacum* hybrids than in the *N. stocktonii* x *N. tabacum* hybrid. Fertilization had occurred in *N. nesophila, N. repanda, N. nesophila* x *N. tabacum* and *N. repanda* x *N. tabacum* by 2 days after pollination. In *N. nesophila* and *N. repanda* most of the ovules collected 3 days after pollination contained a two-celled proembryo. The endosperm was composed of 8 to 14 cells. The zygote of the *N. nesophila* x *N. tabacum* and *N. repanda* x *N. tabacum* hybrids had not yet divided in most of the ovules collected 3 days after pollination and the primary endosperm nucleus had undergone only one division. By 5 days after pollination, the ovules of *N. nesophila* and *N. repanda* contained a four-celled embryo and a 10 to 24-celled endosperm. Most of the *N. nesophila* x *N. tabacum* and *N. repanda* x *N. tabacum* ovules observed had a two-celled embryo and a four- to eight-celled endosperm. The endosperm of the hybrids had ceased growing by 7 days after pollination and the embryo had started to assume an irregular shape. In contrast, a globular embryo and a multicellular endosperm were present in *N. nesophila* and *N. repanda* ovules. By 9 days after pollination the embryo and endosperm in the hybrid crosses were in an advanced state of degeneration. At 12 days after pollination, when *N. nesophila* and *N. repanda* ovules contained a heart-shaped embryo, most of the embryos of *N. nesophila* x *N. tabacum* and *N. repanda* x *N. tabacum* had completely disintegrated. Those few embryos that remained were highly disoriented structures. No endosperm was visible in any of the hybrid ovules.

DISCUSSION

In the three hybrids examined in this study, a cessation of development of the endosperm appears to be directly related to the death of the embryo. Unlike the *N. rustica* x *N. glutinosa* and *N. rustica* x *N. tabacum* hybrids studied by Brink and Cooper (1, 4), hypertrophy of the integuments was not observed in the *N. stocktonii* x *N. tabacum, N. nesophila* x *N. tabacum* and *N. repanda* x *N. tabacum* hybrids. However, the pattern of seed abortion seen in these three hybrids has frequently been observed in other inviable interspecific hybrids (2, 7).

In those hybrids where embryo abortion has been found to directly or indirectly result from endosperm malfunction, embryo culture has frequently been successful in rearing otherwise inviable hybrids (7). The embryo culture medium apparently substitutes for the endosperm in providing nutrition to the developing hybrid embryo, thereby allowing it to continue its development. From the histological analysis presented in the present study, it appears that the cause of seed failure in the three *Nicotiana* hybrids which have been produced only through fertilized ovule culture is similar to that found in hybrids that require the use of embryo culture. Thus, it appears that fertilized ovule culture provides an alternative to embryo culture for producing hybrids that fail because of endosperm malfunction. In particular, fertilized ovule culture should be useful in small-seeded species such as *Nicotiana* where seed size makes embryo excision highly impractical.

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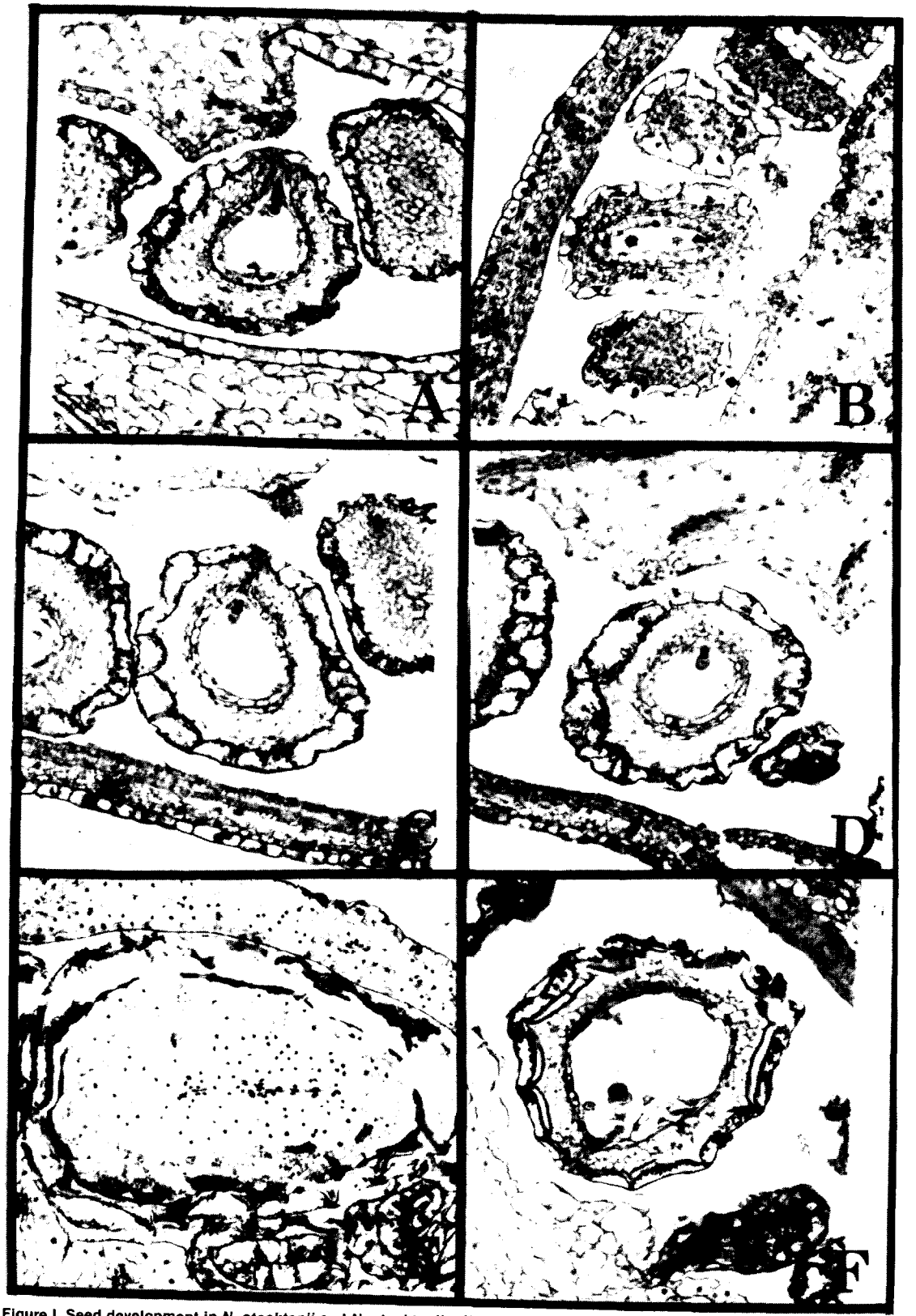

Figure I. Seed development in *N. stocktonii* and *N. stocktonii* x *N. tabacum*; A. *N. stocktonii* ovule 5 days after pollination; B. *N. stocktonii* x *N. tabacum* ovule 5 days after pollination; C. *N. stocktonii* ovule 7 days after pollination; D. *N. stocktonii* x *N. tabacum* ovule 7 days after pollination; E.*N. stocktonii* ovule 12 days after pollination; F.*N. stocktonii* x *N. tabacum* ovule 12 days after pollination.