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A study of morphology, cytology and sterility in interspecific hybrids and amphidiploids of *Nicotiana knightiana* × *N. umbratica*

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Summary. Interspecific hybrids and amphidiploids of *Nicotiana knightiana* Goodspeed (n = 12) × *N. umbratica* Burbidge (n = 23) resembled either parent in some characters and were intermediate in other characters. The F_1 hybrids (2n = 35) showed mostly univalents during meiosis, while the amphidiploids (2n = 70) formed bivalents almost regularly. The former were completely sterile and the latter fully male fertile but predominantly female sterile. This female sterility was due to disintegration of the embryo sacs leading to collapsed ovules. The few fertile ovules, however, showed normal development of embryo sac and embryo. The occurrence of fertile and sterile ovules was believed to be due to segregation of the genes governing sterility.

Key words: *Nicotiana knightiana* – *N. umbratica* – Interspecific hybrids – Cytology – Female sterility

Introduction

As part of a project on the assessment of interspecific relationships of *Nicotiana umbratica* within the genus, cytogenetic studies were made in interspecific hybrids and their amphidiploids involving this species, with *N. knightiana*.

Materials and methods

N. umbratica Burbidge (n = 23) belongs to section Suaveolentes and *N. knightiana* Goodspeed (n = 12) to section Paniculatae. Flower buds were fixed in Carnoy's fluid and anthers were squashed in Carbol-Fuchsin (modified from Miller et al. 1971). Microtome preparations of ovaries were made following Johansen (1940). In vivo pollen germination and pollen tube growth were studied following Murthy (1971).

Results and discussion

F_1 hybrids

The cross was successful when *N. knightiana* was used as the female parent. The F_1 plants resembled *N. knightiana* in their habit and in having all five stamens subequal with half-free filaments. They had the cordate leaf and lobed corolla of *N. umbratica.* In plant height, and flower size and colour they were intermediate between the parents (Figs. 1–3). They had 2n = 35 chromosomes which remained mostly as univalents (Fig. 4) during meiosis. One or two bivalents were found in 9.4% of the pollen mother cells (PMC) (Fig. 5). This very low pairing indicates a distant relationship of the parental species. Meiosis was irregular and the range of pollen fertility was 0.3%. There was no seed set.

Amphidiploids

Two F_1 plants were treated with 0.5% colchicine. One doubled in chromosome number (C_1 generation amphidiploid) from which the C_2 and C_3 generations were raised. Except for a slight increase in plant height and flower size they were morphologically similar to the F_1 plants. They showed 2n = 70 chromosomes with regular meiosis (Fig. 6). The plants were fully male fertile (range 96–100%). Pollen germination on the stigma and pollen tube growth were normal. However, the seed-set was very low (4–6%) although seed

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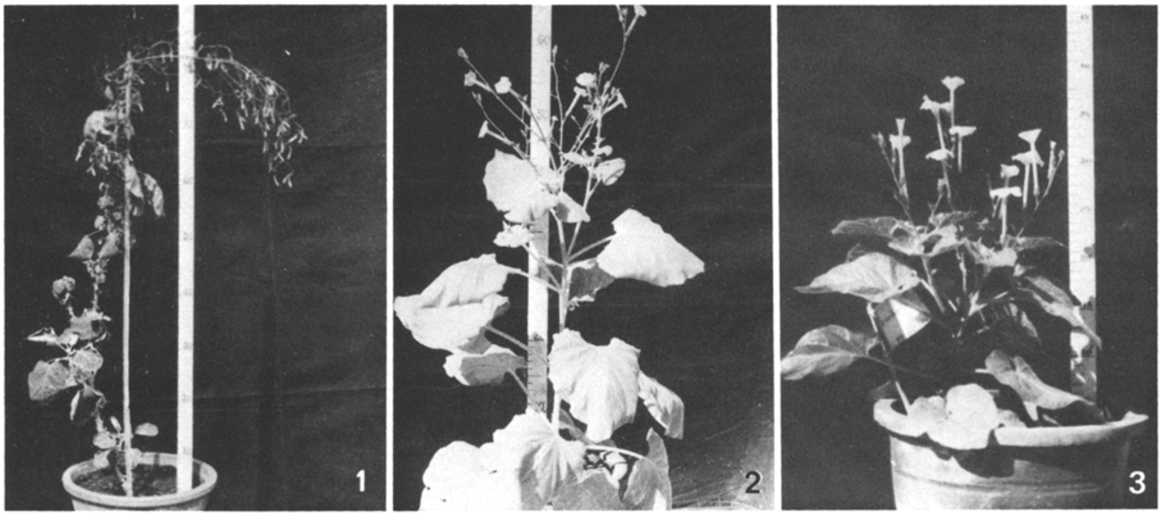
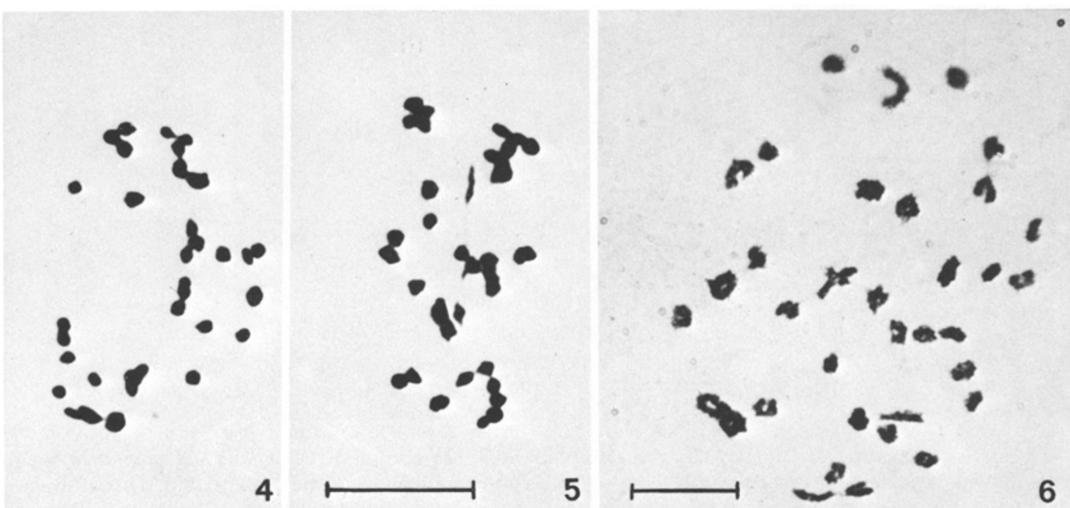


Figs. 1–3. Plants of parental species and F_1 interspecific hybrids of *Nicotiana:* **1** *N. knightiana;* **2** *F_1* hybrid; **3** *N. umbratica*



Figs. 4–6. Meiotic chromosome associations in interspecific hybrids (2n = 35) and amphidiploid (2n = 70) of *N. knightiana* × *N. umbratica:* **4** meta-anaphase I in interspecific hybrid with 35 I; **5** meta-anaphase I in interspecific hybrid with 1 II and 33 I; **6** diakinesis in amphidiploid with 35 II

germination was very good. Microtome preparations of ovaries revealed that the embryo sac development did not proceed beyond the 4-nucleate stage in 94.3% of the ovules. The four nuclei were found near the chalazal end (Fig. 7) which subsequently degenerated. The endothelium became hypertrophied (Fig. 8) and ultimately the ovules collapsed (Figs. 9 and 11). A few ovules which were fertile, showed normal development of embryo sac and embryo (Figs. 10 and 11).

This high female sterility despite normal meiosis and full male fertility is presumed to be governed exclusively by genic factors. It is thus believed that due to segregation of genes, such of those ovules with megaspores possessing sterility genes collapsed, whereas ovules having megaspores without such genes functioned normally, producing seeds. Similar female sterility in certain other amphidiploids of *Nicotiana* was attributed to complementary gene action (Greenleaf 1941).

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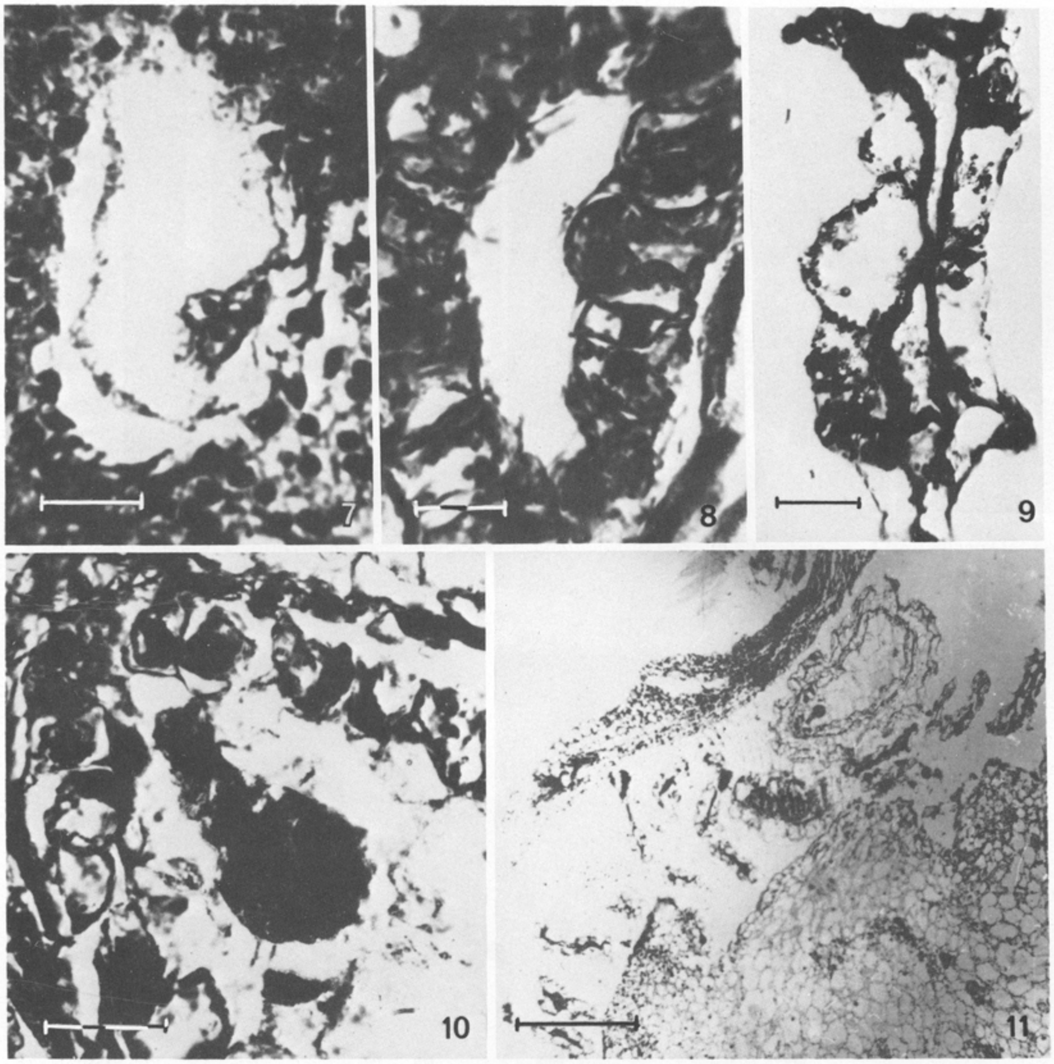

Figs. 7–11. Embryo sac formation in sterile and fertile ovules in the amphidiploids (2n = 70) of *N. knightiana* × *N. umbratica:* **7** embryo sac in a sterile ovule showing 4 nuclei (one nucleus out of focus), after second karyokinesis, at the chalazal end; **8** hypertrophied endothelium sorrounding the degenerated embryo sac; **9** collapsed ovule; **10** normal developing embryo in a fertile ovule; **11** vertical section of an ovary showing several sterile and collapsed ovules and one fertile ovule with cellular endosperm and developing embryo

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