Theoretical and Applied Genetics 44, 138–140 (1974)

Brief Notice / Kurze Mitteilung

Experimental Androgenesis *in vitro* in *Nicotiana clevelandii* Gray and *N. sanderae* hort.

B. VYSKOT and F. J. NOVÁK

Department of Genetics, University of J. E. Purkyně, Brno, and Vegetable Research Institute of Czech Academy of Agriculture, Olomouc (ČSSR)

Summary. Androgenic haploids were found in two species of the genus *Nicotiana* in which they have not been reported previously. Anthers at the stage of uninucleate microspores were cultivated on synthetic medium with the addition of indole-3-acetic acid.

Haploid plants are of great importance in plant genetics and breeding (Melchers and Bergmann 1958, Melchers 1960, Kimber and Riley 1963, Katayama and Nei 1964, Chase 1969, Melchers and Labib 1970, Melchers 1972, and other authors). The latest experimental method, by which haploid sporophytes can be obtained in large numbers, has been termed the induction of androgenic plants in anther and pollen cultures in vitro. This induced in vitro androgenesis has so far been successful with approximately 35 plant species, but direct development of the pollen into haploid embryoids has been observed in only four genera – *Datura* (Guha and Maheshwari 1964, 1966), *Nicotiana* (Bourgin and Nitsch 1967), *Atropa* (Zenkteler 1971) and *Lycium* (Zenkteler 1972). In the remaining species, only growth of the haploid callus could be induced, and in this organogenesis of the tops was induced, or a callus of pollen origin could be obtained which showed reduced morphogenetic potency. The present state and significance of experimental androgenesis have been summarised by Sunderland (1971), Nitsch (1972), and Melchers (1972). It would appear highly desirable that the method be applicable to most higher plants, particularly crops of economic importance.

In the experiments reported here the anthers of four species of the *Nicotiana* genus were cultivated; for two of the species – *N. sanderae* and *N. clevelendii*

Table 1. *In vitro induction of haploid plants, Nicotiana genus*

	n	Basal medium	Author
S. 1. Petunioides			
N. alata Otto et Link	9	Nitsch (1969)	Nitsch (1969) Sunderland (1971)
N. sanderae hort. (*N. forgetiana* × *N. alata*)	9	Nitsch (1969)	Vyskot and Novák (unpublished)
N. sylvestris Speg. et Comes	12	Bourgin and Nitsch (1967)	Bourgin and Nitsch (1967)
N. clevelandii Gray	24	Nitsch (1969)	Vyskot and Novák (unpublished)
S. 2. Rustica			
N. rustica L.	24	Nitsch (1969)	Nitsch (1969)
S. 3. Tabacum			
N. glutinosa L. 2n	12	Nitsch (1969)	Nitsch (1972)
4n	24		Sunderland (1971)
N. otophora Griseb.	12	Murashige and Skoog (1962)	Collins, Legg and Kasperbauer (1972)
N. tabacum L.	24	Bourgin and Nitsch (1967), Hildebrandt (1962), Linsmaier and Skoog (1965), Nitsch (1969)	Bourgin and Nitsch (1967), Nakata and Tanaka (1968), Sunderland and Wicks (1971), Nilsson-Tillgren and Wettstein-Knowles (1970), Melchers and Labib (1971)
S. 4. Suaveolentes			

— no induction of haploid sporophytes had been reported before. A summary of the in vitro androgenic haploids hitherto obtained in the *Nicotiana* genus, the present experiments included, is shown in Tab. 1.

The anthers to be used for the experiments were collected from blooming plants of *N. tabacum* cv. 'Samsun' (2 n = 48), *N. tabacum* cv. 'Xanthi' (2 n = 48), *N. sanderae* (2 n = 18), *N. clevelandii* (2 n = 48), and *N. glutinosa* (2 n = 24), at three stages of microsporogenesis — tetrads, uninucleate microspores, binucleate pollen. A uniform growth medium was used for all variants, as suggested by Nitsch (1969), with the addition of 0.1 mg · l^{-1} IAA. The cultures were maintained in continuous illumination of approx. 1,500 lux, at temperatures ranging from 25 to 32 °C. Regenerated plants were transferred to test tubes filled with liquid medium, containing only minerals as suggested by Nitsch (1969), and reduced amounts of sucrose, 10 g · l^{-1}. The plants grown in this medium developed rich root systems.

Squash preparations from anthers and root tips, after pretreatment in 0.002 M solution of 8-hydroxyquinoline for 3—4 hours, were stained with lactopropionic orceine (Dyer 1963).

The optimum stage of anther development for the growth of androgenic embryoids was determined on the basis of these experiments. With the exception of *N. glutinosa*, all the studied species developed seedlings exclusively in anthers with uninucleate microspores, approximately at the time of the first pollen mitosis. The results of anther cultivation at this stage of development are shown in Tab. 2. In *N. glutinosa*, no development of embryoids was observed. All the seedlings obtained from the remaining three species showed haploid numbers of chromosomes when cytologically analysed.

The production of haploid androgenic seedlings in *N. sanderae* and *N. clevelandii* has not been reported by any other author concerned with the matter. Sunderland (1971) observed, in his *N. sanderae* cultures, proliferation of the callus, presumably of pollen origin, from inside the pollen sac, but cytological analysis revealed the diploid number of chromosomes. He concluded that endomitotic doubling of chromosomes might have occurred in the callus cells. Considering that only one anther out of a total of 225 included in our experiments produced seedlings, i.e. only 0.44 per cent anthers gave rise to haploids (Fig. 1), it will be necessary to test other culture media with various concentrations of growth regulators.

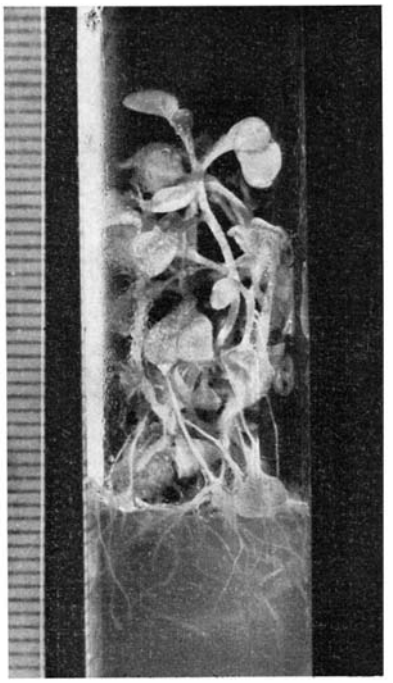

Fig. 1. Haploid plants of *Nicotiana sanderae* in anther culture after 12 weeks cultivation

However, the results of our experiments suggest that induced androgenesis is possible in this species although at a very low frequency under the given conditions. In no other experimental way has a haploid plant of *N. sanderae* been obtained to date, and its spontaneous occurrence has not been described either. It might be that the low frequency of haploid sporophytes in this interspecific hybrid is genetic in nature.

Table 2. *Experiments with anther cultivation, Nicotiana genus*

Species	Total number of anthers under cultivation	Number of anthers producing *n* seedlings	Total number of *n* seedlings produced	% of anthers producing *n* seedlings	Number of anthers developing callus	% of anthers developing callus
N. tabacum cv. 'Samsun'	40	24	117	60	0	0
N. tabacum cv. 'Xanthi'	40	14	42	35	0	0
N. sanderae	225	1	2	0,44	34	15,11
N. clevelandii	50	1	5	2	0	0
N. glutinosa	100	0	0	0	4	4

The other species under study, *N. clevelandii*, also showed a low frequency of successfully induced androgenesis, amounting to 2 per cent. Sunderland (1971), too, attempted to cultivate anthers of this species but failed to obtain growth. He explained this failure as the result of insufficient understanding of the growth and hormonal requirements in this species. As in *N. sanderae*, neither a spontaneous haploid sporophyte nor a haploid seedling induced experimentally has yet been described for *N. clevelandii*.

Literature

1. Bourgin, J. P., Nitsch, J. P.: Obtention de Nicotiana haploides à partir d'étamines cultivées in vitro. Ann. Physiol. Vég. **9**, 377–382 (1967). — 2. Chase, S. S.: Monoploids and monoploid — derivatives of maize (Zea mays L.). Bot. Rev. **35**, 117–167 (1969). — 3. Collins, G. B., Legg, P. D., Kasperbauer, M. J.: Chromosome numbers in anther-derived haploids of two Nicotiana species. J. Hered. **63**, 113–118 (1972). — 4. Dyer, A. F.: The use of lactopropionic orcein in rapid squash methods for chromosome preparation. Stain Techn. **38**, 85–90 (1963). — 5. Guha, S., Maheshwari, S. C.: In vitro production of embryos from anthers of Datura. Nature **204**, 497 (1964). — 6. Guha, S., Maheshwari, S. C.: Cell division and differentiation of embryos in the pollen grains of Datura in vitro. Nature **212**, 97–98 (1966). — 7. Hildebrandt, A. C.: Tissue and single cell cultures of higher plants as a basic experimental method. In: Linskens, H. F., Tracey, M. V. (ed.): Modern Methods of Plant Analysis, pp. 383–421. Berlin: Springer-Verlag 1962. — 8. Katayama, Y., Nei, M.: Studies on the haploidy in higher plants. Univ. Miyzaki, Japan, 1964. — 9. Kimber, G., Riley, R.: Haploid angiosperms. Bot. Rev. **29**, 480–531 (1963). — 10. Linsmaier, E. M., Skoog, F.: Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. **18**, 100–127 (1965). — 11. Melchers, G.: Haploide Blütenpflanzen als Material der Mutationszüchtung. Beispiele: Blattfarbmutanten und mutatio wettsteinii von *Antirrhinum majus*. Züchter **30**, 129–134 (1960). — 12. Melchers, G.: Haploid higher plants for plant breeding. Z. Pflanzenzücht. **67**, 19–32 (1972). — 13. Melchers, G., Bergmann, L.: Untersuchungen an Kulturen von haploiden Geweben von *Antirrhinum majus*. Ber. Dt. Bot. Ges. **71**, 459–473 (1958). — 14. Melchers, G., Labib, G.: Die Bedeutung haploider höherer Pflanzen für Pflanzenphysiologie und Pflanzenzüchtung. Ber. Dtsch. Bot. Ges. **83**, 129–150 (1970). — 15. Murashige, T., Skoog, F.: A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. **15**, 473–498 (1962). — 16. Nakata, K., Tanaka, M.: Differentiation of embryoids from developing germ cells in anther culture of tobacco. Japan J. Genet. **43**, 65–71 (1968). — 17. Nilsson-Tillgren, T., von Wettstein-Knowles, P.: When is the male plastom eliminated? Nature **227**, 1265–1266 (1970). — 18. Nitsch, J. P.: Experimental androgenesis in Nicotiana. Phytomorphol. **19**, 389–404 (1969). — 19. Nitsch, J. P.: Haploid plants from pollen. Z. Pflanzenzüchtg. **67**, 3–18 (1972). — 20. Sunderland, N.: Anther culture: a progress report. Sci. Progr., Oxf. **59**, 527–549 (1971). — 21. Sunderland, N., Wicks, F. M.: Embryoid formation in pollen grains of *Nicotiana tabacum*. J. Exp. Bot. **22**, 213–226 (1971). — 22. Zenkteler, M.: In vitro production of haploid plants from pollen grains of *Atropa belladonna* L. Exp. **27**, 1087 (1971). — 23. Zenkteler, M.: Development of embryos and seedlings from pollen grains in *Lycium halimifolium* Mill. in the in vitro culture. Biol. Plant. **14**, 420–422 (1972).

Received December 18, 1972

Communicated by G. Melchers

B. Vyskot
Department of Genetics,
University of J. E. Purkyně
Kotlářská 2
60000 Brno (ČSSR)

F. J. Novák
Vegetable Research Institute
of Czech Academy of Agriculture
P.O. Box 36
77236 Olomouc (ČSSR)