

The embryology of sterile F_1 hybrids between the sugarbeet and *Beta webbiana* Moq.

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Plant breeders often make use of wild species as a source of desirable resistance characters which could be incorporated into cultivated varieties by intercrossing. The *Beta* — species of the *Patellares* — section are known to be immune to beet nematodes (*Heterodera schachtii* Schmidt). Unfortunately, however, the obtaining of advanced generation hybrids between these species and the sugarbeet is extremely difficult as the F_1 hybrids are highly sterile. Cytological investigations, based on the analysis of PMC meiosis (Savitsky H. 1957, 1960; Kuzdowicz A. 1967) revealed an almost complete lack of homology between the genomes of both mating partners. Megasporogenesis and embryo sac formation in such F_1 hybrids have not been reported so far.

Our investigations were confined to the diploid hybrids of the F_1 generation. The object of the investigations was to find whether it is possible or not to obtain diploid hybrids of further backcross generations. So far the opinions in this respect are not unanimous. In most cases, including research carried out by our Institute, no such hybrids have been obtained (Rietberg 1951; Coe 1954; Gaskill 1954; Bandlow 1961; Szota 1964). Some authors, however, claim success in this field (Schneider 1937; Stewart 1950; Johnson and Wheatley 1960; Oldemeyer 1954; Oldemeyer and Brewbaker 1957; Curtis 1966). Also, the F_1 diploid hybrids obtained by H. Savitsky (1960) set some single seeds, but the author does not mention whether they germinated or not.

Unfortunately no cytological investigations on these advanced hybrids (except Curtis, 1966) or any detailed morphological descriptions are available. Therefore it cannot be ascertained whether they were true diploid backcross hybrids or polyploids resulting from asynaptic meiosis (as suggested by H. Savitsky, 1959), or else, types resulting from apomixis as is the case in some polyploid forms within the section *Corollinae* (Barocka 1966).

MATERIAL AND METHOD

For our investigations over two hundred buds and flowers of different ages were collected from F_1 hybrid sugarbeet \times *Beta webbiana* Moq. plants growing in the open and abundantly pollinated with sugarbeet pollen.

The specimens were fixed in chrom-acetoformol, embedded in paraffin and then microtome 7—12 μ slices were prepared and stained with iron-hematoxylin.

Specimens of both parental species were collected in the same way.

RESULTS

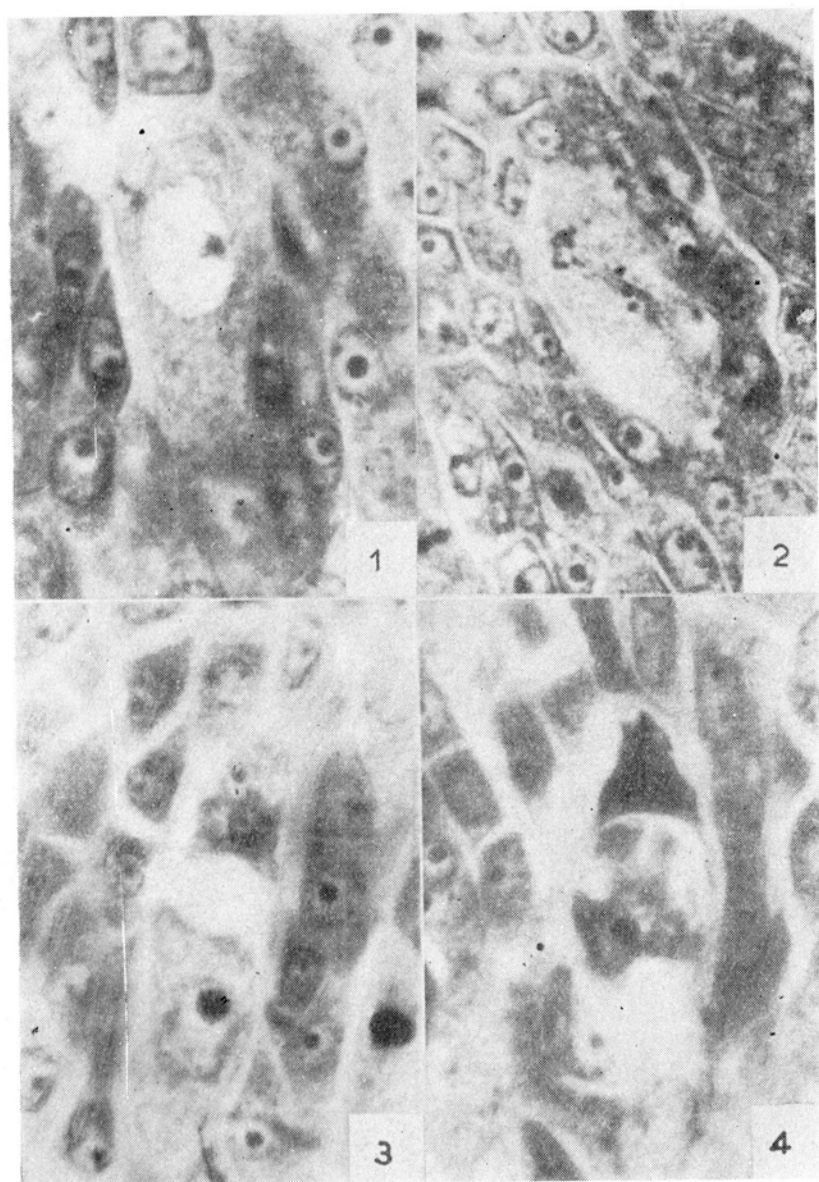
No essential differences between the parental species were observed as regards megasporogenesis and embryo sac development, which proceed in the way described minutely by Charetschko-Savitskaya (1942). In the *Beta* species studied by her as well as by ourselves the development of a triad of megaspores is a characteristic feature.

The diploid megaspore mother cell divides after meiosis into two haploid cells. In the subsequent somatic division only one of them participates, namely the cell located at the chalasal end of the dyad, whereas the other one does not divide. In this way a triad of megaspores is formed instead of a tetrad, as is the case in most plant species. Two cells located at the micropylar end of the triad abort and only the third one develops, owing to three successive divisions, into a normal bipolar eightnucleate embryo sac.

In the young buds of diploid hybrids regular development of the ovule and its differentiation into nucellus and integuments was observed. One of the sub-epidermal archesporial cells of the nucellus differentiates to a megaspore mother cell, much bigger than the surrounding cells. Many times different stages of prophase I in MMC were observed (photo 1). In spite of the abundant material investigated, we have not been able to trace the meiosis in detail. In one case only the MMC in anaphase I was observed (photo 2). On the ground of further disturbances in megasporogenesis, however, we can conclude that similar aberrations take place here as those described by H. Savitsky (1957, 1958, 1960) and by Kuźdowicz (1967) in PMC meiosis.

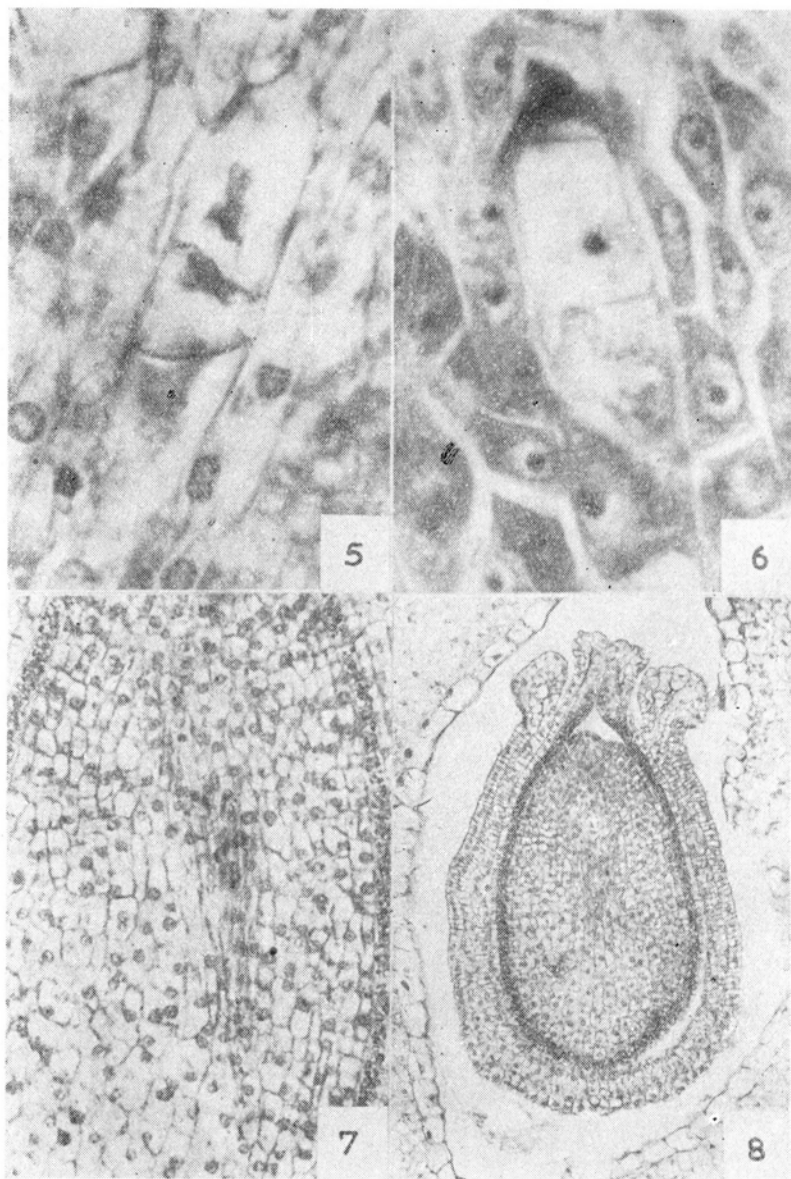
Owing to these disturbances the resulting dyad cells contain an unequal number of chromosomes. Dyads were observed in which sometimes one cell had a bigger nucleus than the other. Such dyads often collapse either simultaneously or one after the other (photos 3 and 4). or else the cell at the chalasal end divides and a triad is formed. As a rule all three megaspores declined, this being manifested by the irre-

Plate I



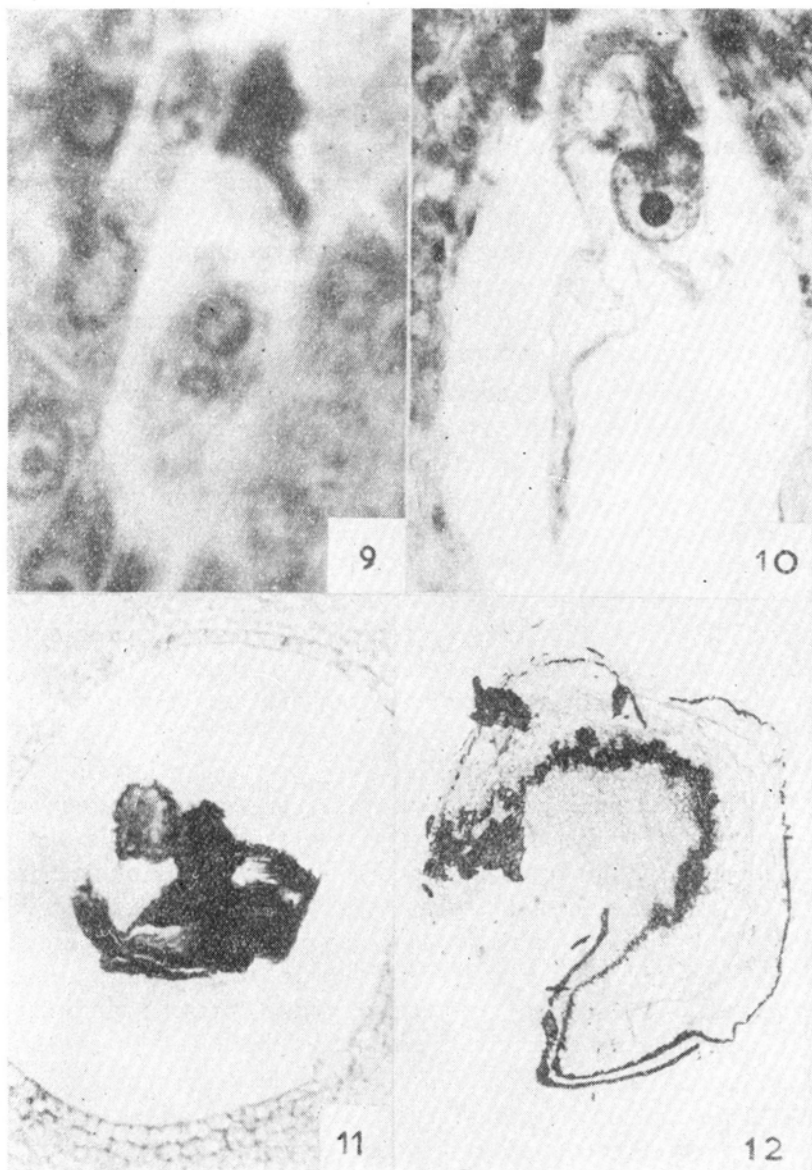
- 1 — Prophase I in megaspore mother cell
- 2 — Anaphase I in megaspore mother cell
- 3 — A declining dyad
- 4 — A declining dyad — one cell completely collapsed

Plate II



- 5 — A declining triad
6 — A declining triad — one cell completely collapsed
7 — Remnants of megaspores crushed in the nucellus tissue
8 — An old ovule with no embryo sac — only declining triad present (same as photo 5).

Plate III



- 9 — A declining binucleate embryo sac
- 10 — Deteriorating egg apparatus
- 11 — Collapsed ovule
- 12 — Blind seed with no embryo

gular shape of the nuclei, advanced plasmolysis or complete disintegration of the cell contents, in which no nuclei could any longer be traced (photo 5). Triads were also observed in which one megaspore was completely degenerated and formed a kind of a black cap, whereas in the remaining two cells the process of disintegration was not so far advanced (photo 6). Occasionally such triads were found in which two megaspores at the micropylar end collapsed. In a few cases the development of the gametophyte was still more advanced: binucleate, degenerating embryo sacs were observed (photo 9). It seems that further development of the embryo sac in the hybrids examined is an extremely rare feature. In two cases only we succeeded in finding structures resembling a mature embryo sac with degenerating egg apparatus. No antipodal cells or polar nuclei were found (photo 10). In no case was a normal eight-nucleate embryo sac or embryo observed.

The successive stages of megasporogenesis and embryo sac development are retarded as compared with the regular course of this process. This could be ascertained by comparing the localisation of the structures observed which (besides the megaspore mother cell itself) were often found in deeper layers of the nucellus than it was observed in parental forms. The relative age of the ovule can be approximately determined on the basis of the stage of integument development and also on the basis of the stage of microsporogenesis as observed in the anthers of the same flowers. At the time when in the anthers the presence of archesporial tissue was observed, in the ovaries young ovules were found in which no archesporium had yet formed. At the time when the meiotic division was observed in PMCs, archesporial cells were found in but a few ovules and a well developed megaspore mother cell was found in one case only. Only when in the anthers tetrads and pollen were formed, MMCs in prophase I were present in the ovules. In overblown flowers, only aborted megaspores (photo 8) or underdeveloped, collapsing embryo sacs were found. Also the ovules themselves were sometimes underdeveloped and distorted. In older ovules often only some remnants of crushed megaspores were present and the exact stage of abortion was difficult to establish (photo 7).

As mentioned before, our diploid F_1 hybrids were completely sterile, i.e. they never set any seeds (S z o t a 1964). Even the enlargement of the pericarp, often observed in tri- and tetraploid hybrids, was extremely rare in the diploid ones. Nevertheless, on shoots taken for analysis one such fruit increased in size was found. Inside the ovary an ovule over two millimeters in diameter was found, with dark and already lignified integuments, i.e. nearly ripe seed, only a little shrivelled. In microtome slides prepared from this ovule no embryo was found and its place was occupied by a sickle-shaped, elongated cavity with no

traces of any cellular structures or nuclei. The ovule was filled with degenerating perisperm tissue (photo 12).

It is evident that the increase in size of the ovary and even of the ovule itself is not necessarily stimulated by fertilization and by development of the embryo. Sometimes the ovary and/or the ovule can enlarge without previous fertilization and embryo development, resulting in the formation of empty fruits and blind seeds which fail to germinate.

DISCUSSION AND CONCLUSIONS

Our results obtained so far in embryological investigations on diploid sugarbeet \times *Beta webbiana* Moq. F_1 hybrids indicate that such hybrids are unable to produce normal embryo sacs and embryos. Hence, the obtaining of hybrids of further generations by embryo culture *in vitro*, as it is done with hybrids which fail to develop endosperm, is impossible. The so far described hybrids of the advanced generations were most probably a result of apomixis or were due to embryosac development from a restitutional asynaptic mother cell.

Despite our negative results, however, the possibility of obtaining in some exceptional cases viable F_1b_1 seeds cannot be ruled out provided different material and/or different conditions were present.

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Badania embriologiczne nad bezpłodnymi mieszańcami pokolenia F_1 buraka cukrowego \times *Beta webbiana* Moq.

STRESZCZENIE

Badania embriologiczne nad makrosporogenezą i rozwojem woreczka zalążkowego u diploidalnych mieszańców buraka cukrowego z *Beta webbiana* Moq. wykazały, że najczęściej następuje tutaj degeneracja w stadium diady lub triady, wynikająca z zaburzeń w mejozie (brak homologii genomów form rodzicielskich). Niekiedy dochodzi do wytworzenia się woreczka zalążkowego, który jednak także degeneruje na różnych etapach rozwoju. W ani jednym wypadku nie stwierdzono w zalążkach obecności prawidłowego, ośmiojądrowego woreczka zalążkowego ani zarodka.

Aczkolwiek wszystkie przebadane dotąd w naszym Zakładzie diploidalne mieszańce F_1 między burakiem cukrowym a gatunkami sekcji *Patellares* były całkowicie bezpłodne, to jednak nie można wykluczyć, że przy użyciu innego materiału wyjściowego lub w odmiennych warunkach mogą powstać w wyjątkowych wypadkach pojedyncze funkcjonalne makrogamety. Jest jednak bardziej prawdopodobne, że nasiona uzyskane z takich mieszańców (nieliczne doniesienia wskazują na możliwość otrzymania ich przy wstecznym krzyżowaniu) powstały w wyniku apomiksji lub innych anomalii w przebiegu procesu reprodukcyjnego.