

T.S. Fedorenko, USSR

CYTOEMBRYOLOGICAL PECULIARITIES
OF HELIANTHUS TOMENTOSUS MICH
AS A COMPONENT PART OF REMOTE
HYBRIDIZATION WITH SUNFLOWER

Wild species of the *Helianthus* genus are used as initial forms to breed cultivated sunflower for immunity. Of some practical interest is a crossing of sunflower (VNIIMK 8931) with the wild species *H. tomentosus*, the only one resistant to verticillium wilt.

However, in this case remote hybridization meets some difficulties. One of them is the low setting of hybrid fetuses in the crossing year.

As a result of four-year studies we have found that *H. tomentosus* plants are self-sterile and do not set seeds when stigmata are artificially pollinated by a pollen mixture of own variety. When they are pollinated by pollen of cultivated sunflower from 0-28 seeds set in one inflorescence. A reaction of incompatibility of remote forms comes in a different way. Some plants (65%) demonstrate partial incompatibility, and plant fertility is 7 seeds per head on average, others are completely incompatible and sterile.

We suggested that weak fertility of the *H. tomentosus* plants was due to the hybridogenic origin of this species and some deviations in the process of formation of generative elements, and was also explained by the incompatibility of the component parts crossed.

Experiments showed that anthers formed normally and that in the period of their maturation the fibre layer split and pollen got to the stigma.

The *H. tomentosus* pollen quantity is low. The impregnating ability of certain plants varies from 3.5 to 35% and does not exceed 21% on average. Viability of pollen seeds is 18.2%.

Pollen is uneven, and big, and small pollen grains and micronuclei form. The predominating fraction is 20-30 microns (Table 1).

One of the factors determining the quality and evenness of matured pollen is the correct meiosis process in maternal cells of microspores. In *H. tomentosus* plants meiosis in microspores' maternal cells is violated to a large extent (Table 1). Univalents, tri, tetra and polivalents are formed at the diakinesis stage along with the bivalents. From 4 to 10 univalents are located beyond the equatorial plate at the 1st metaphase stage. Together with diploid cells there may be cells with the chromosome number of $34+P$. Chromosome bridges and a diverse number of lame chromosomes (up to 17), as well as telophatic nuclei of varied sizes, microspores and micronuclei are formed as a result of uneven deviations of chromosomes in telophase I and II.

Pollen seeds begin to degenerate at the uninucleus stage and are fragmentized during the period of matured gametes. Pollen seeds have a different size.

Thus serious disfunctions take place in the chromosome apparatus during microsporogenesis and gametogenesis. The number of anomalies is 83% in the first reduction fission 77.8% in the second fission (Table 2).

The quantity, ploidy, fertility and viability of pollen seeds vary quite considerably as a result.

Besides male sterility the plants are characterized by a high female sterility. The analysis of unpollinated ovules has showed that 44.7% have morphologically normal embryo sacs with all components. The fertility of embryo sacs is 17.6% in the group of sterile plants. The female gametophyte sterility is different. Undeveloped ovules die at the stage of microspores maternal cell. There are cases when asynchronous fission of the embryo sac nuclei and the following violation of their dif-

Table 1
 Fraction Content of Fertile and Sterile Pollen Grains (%)

Pollen im- pregnating ability	Pollen grains size		
	big, 44 microns	average, 34-43 microns	small, 20- 30 microns 8-17 microns
Fertile	1.59	6.36	10.94
Sterile	1.00	7.29	72.53
			0.02
			0.37

Table 2

Anomalies in the First and Second
Meiosis Fissions (%)

Fission phases	Fission 1	Fission 2
Metaphase	76.2	69.4
Anaphase and telophase	83.0	76.0
Tetrads	-	77.8
Microspores	-	49.5

ferentiation result in the formation of components either unable to pollinate at all or in the pollination of 2 or 3 ovules at once. Meiosis disfunction takes place in the female generative sphere too. The testimony to this is the fact that a different number of micronuclei are isolated in ovules and central cells, and the female nuclei size varies considerably with no direct correlation between the size of the embryo sac and its components (Fig. 2).

Our experiments have proved that embryo sacs degenerate owing to (1) broken metabolism and (2) meiosis.

The female sterility rate changes depending on inflorescences location on the plant and the year conditions, but remains, constant for separate plants. It is possible therefore to select highly fertile plants for crossing with sunflower.

As is known, the effectivity of crossing remote forms depends on many cytoembryological traits and the more similar features the more effective hybridization is. Ploidness of crossed components, morphological peculiarities of chromosomes, pollen seeds and embryo sacs play an important part at remote hybridization.

Thus using wild varieties of diverse ploidness, cultivated sunflower and diploid inter-

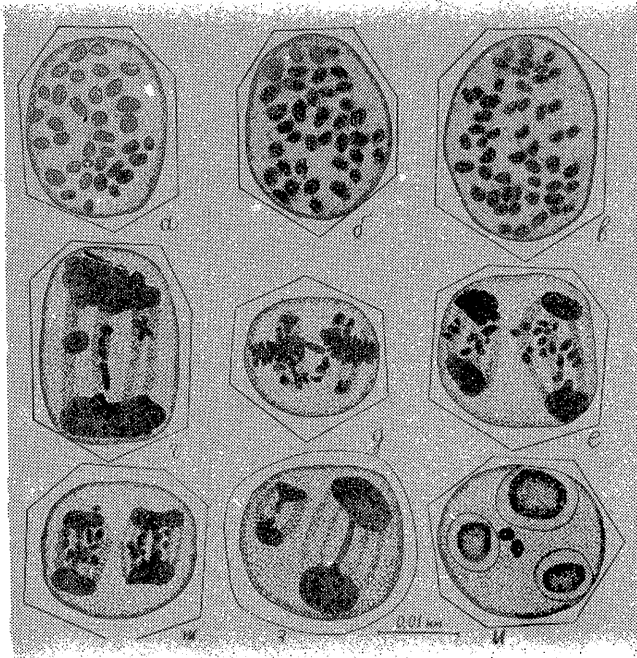


Fig. 1. Meiosis in maternal cells of microspores: **а-в** - metaphase I, **г** - telophase I, **д** - metaphase II, **е-з** - telophase II, **и** - three cells of microspores

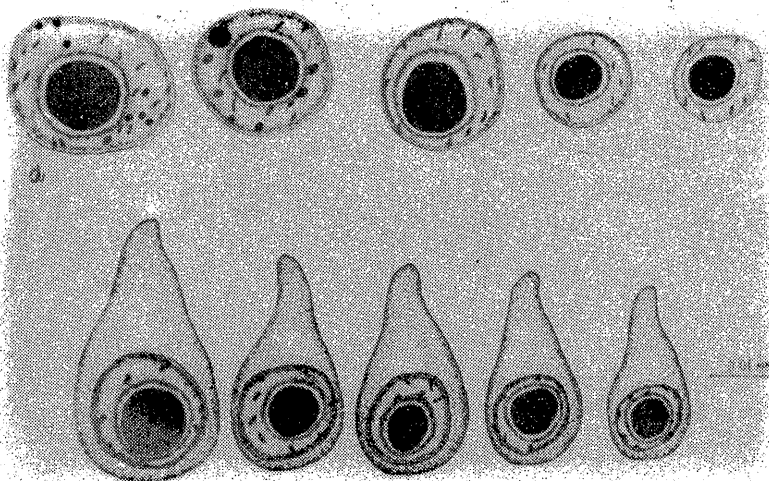


Fig. 2. Different size of female nuclei: **a** - secondary nuclei; **b** - ovicells

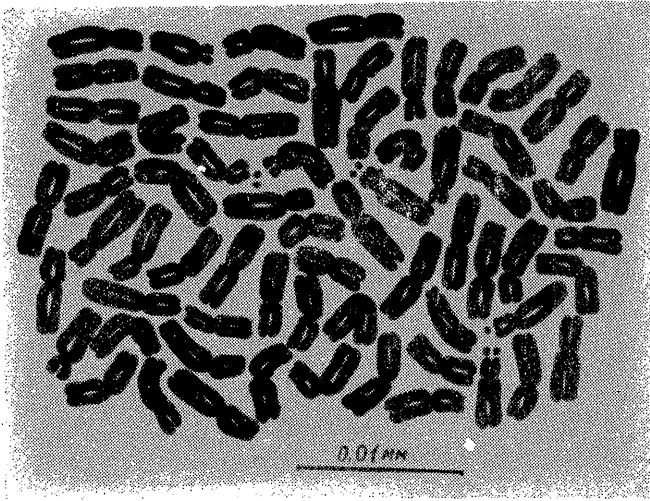


Fig. 3a. Somatic metaphase plate of *H. tementosus* Mich.

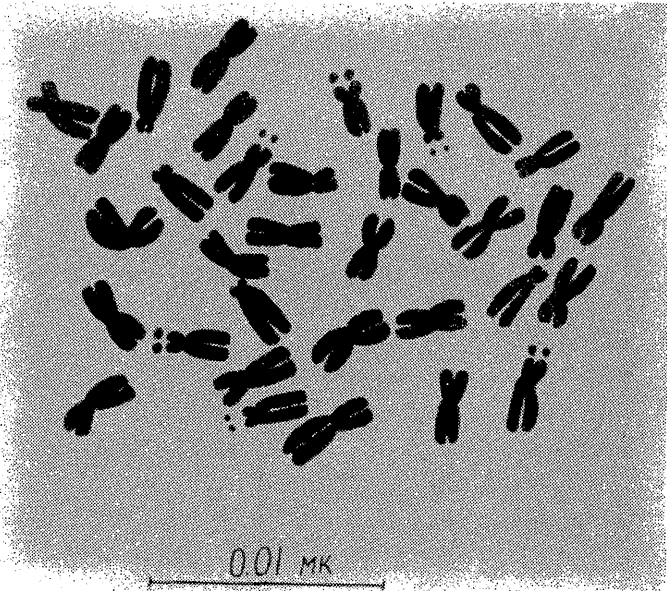


Fig. 3b. Somatic metaphase plate of *H. annuus* L.

varietal hybrids as pollinators, the most responsive *H. tomentosus* stigmata occur when they are pollinated by bigger pollen of diploid species. Two incompatibility types are manifest depending on the pollinator: (1) full when pollen seeds swell and then split without forming pollen tubes and (2) partial incompatibility characterized by the appearance of a reaction at diverse stages of pollination. The *H. tomentosus* ♀ x *H. annuus* ♂ crossing combination is an example of partial incompatibility.

The cytoembryology of these varieties is quite similar. The embryo sac develops according to a normal type (*Polygonum*), and polar nuclei fuse into one, secondary. According to Ye.N. Ustinova, the viability of sunflower ovules remains there during 8-10 days and that of *H. tomentosus* during 4-5 days. Genital nuclei fuse according to the premitotic type and the embryo develops according to the *Asteraceae* type.

The study of chromosome morphology of parental forms has showed that at the minimum contraction level chromosomes of cultivated sunflower are a little smaller than the *H. tomentosus* ones (Fig. 3). Chromosome distribution in a set is similar in both varieties. Two chromosome sets can be singled out when comparing morphological parameters of chromosomes in karyotype of a wild variety. One of them consists of acrocentric, submetacentric chromosomes and is similar to the cultivated sunflower karyotype (except a few pairs). The other 34 chromosomes differ slightly according to the location of the centromere and general length.

The similarity of cytoembryological features speaks for the efficacy of crossing these varieties.

Considerable delay in the of growth of pollen tubes is observed at remote hybridization in the beginning of the programme pha-

se of fertilization. Sunflower pollen germinates in *H. tomentosus* stigmata in 5 minutes after pollination in the quantity of 80-90 pollen seeds. This is slightly quicker than at intra-varietal pollination of sunflower (Fig. 4). In 15 minutes after pollination diverse anomalies appear in the majority of pollen tubes and their content pours into the stigma or stylus tissue. In 60 minutes after pollination many pollen tubes stop developing. However, some pollen tubes penetrate an embryo sac and in some cases an hour after pollinating double pollination takes place. A pollen tube often grows to normal embryo sac but meets a degenerating ovular apparatus (owing to the growth of endstiel cells). There is no fecundation in this case.

When foreign gametes fuse different deviations are also observed, viz. death of a pollinated ovule or an embryo in different periods of its development. Symptoms of death of hybrid embryos appear in 2 days after pollination and are expressed in the lag of their growth and differentiation.

It has been proved on the basis of experimental data that the weak fertility of *H. tomentosus* in the year of crossing with cultivated sunflower is explained by a high female sterility of plants and partial incompatibility of tissues of component parts crossed.