EMBRYOLOGICAL INVESTIGATIONS ON THE FORMATION OF HAPLOIDS IN THE POTATO (*SOLANUM TUBEROSUM*)

By

K.-H. v. WANGENHEIM, S. J. PELOQUIN* and R. W. HOUGAS*

With 14 Figures in the Text

(Received November 3, 1960)

From crosses between tetraploid (*2n = 48*) *S. tuberosum* (*♂*) and diploid (*2n = 24*) *S. phureja* (*♀*), containing marker genes, HOUGAS and PELOQUIN (1960) have obtained 500 *S. tuberosum*-haploids (*2n = 24*) from 31 different potato varieties and breeding stocks. Because of the practical and theoretical importance of this method (HOUGAS and PELOQUIN 1958b), it is desirable to know more about the embryological events which lead to the formation of these haploids. Furthermore this knowledge should give some information about the cause for seed failure in intraspecific crosses between diploids and polyploids (v. WANGENHEIM 1954, 1957), which is a common phenomenon in many plant families.

Material and Methods

The cross between the potato variety "Merrimack" and a selected clone of P. I. 225 682 of *S. phureja* yielded the highest frequency of haploids (PELOQUIN, HOUGAS and GABERT 1960). Seed balls of this cross were opened 14 to 21 days after pollination (cut stem method, McLEAN and STEVENSON 1952) and generally only those ovules were fixed, which were obviously bigger than the majority of the ovules, which could be expected to have died already by this time after pollination.

For squash-preparations the ovules were fixed in alcohol-acetic acid 3:1 and stained with Feulgen and acetic orcein. For paraffin sectioning MIANTING's modification of NAVASHIN-KARPECHENKO was used as a fixative after a pre-treatment with alcohol-acetic acid. The sections were cut at 20 μ and stained with crystal violet and light green. Usually there were many cell divisions in the endosperm, but few in the embryos. Thus the chromosome number of only little more than a third of the embryos could be counted. Where no good metaphases could be found, reliable counts of prophases were made with the aid of pencils of different colours for different optical levels. Since single ovules had been embedded, which were usually well-oriented, measurements of the approximate length could be made.

Results

In squash preparations of 33 relatively big ovules from two seed balls, one ovule contained twin embryos without cell divisions, 13 ovules contained tetraploid embryos, 2 ovules contained single embryos with no cell divisions, and 17 ovules (51.5 %) contained no embryo at all. The chromosome number of the endosperms could not be counted, possibly because of the advanced stage of development of the ovules (the embryos were already in the torpedo stage). Since the structure and type of endosperm cells could not be judged in squash preparations, more emphasis was placed on paraffine sectioned material.

* Crops Research Division, ARS, USDA and the University of Wisconsin.
Some of the ovules, which had been fixed for paraffine embedding and were thought to have developed vigorously, contained a dead endosperm as is common in crosses between *S. tuberosum* (or artificial tetraploids) and diploids of *Solanum* (v. Wangenheim 1957), or in crosses between hexaploid *S. demissum* and diploid species (Beamish 1955, Walker 1955). Sometimes parts of the embryos were still visible, surrounded by dead endosperm and meristematic endothelium (Fig. 1). As is shown in the Table, the average length of these ovules was smaller than that of those with a „living” endosperm, even though a few of the older ovules with a dead endosperm were longer than the average length of ovules with “living” endosperm.

From 26 different seed balls, a total of 118 ovules with “living” endosperm were fixed for paraffine embedding. Nine of these seed balls contained no ovules with “living” endosperm at all, while the other 17 contained from 1 to 13. Considering that the seed ball usually contains up to 600 ovules and many of these ovules could have been fertilized, in the 26 seed balls examined by us the majority of the endosperms must have died. Thus only the exceptional cases had developed as far as this stage observed in preparations made 14 to 21 days after pollination.

The chromosome numbers of 97 endosperms have been counted. 96 of them were hexaploid and only one was pentaploid, though the latter constitution is expected in this type of cross. This suggests that the hexaploid constitution of the endosperm facilitates normal endosperm differentiation in this cross, while the pentaploid endosperm usually dies, as it did in the majority of the ovules.

A feature of younger stages in normal endosperm development was the presence of less vacuolized cells around the embryo and in the neighbourhood of the micropyle, and bigger and more vacuolized cells at the chalazal pole of the embryosac (Fig. 2). In the latter region the cell formation was often somewhat disturbed (Figs. 3, 4). One hexaploid endosperm was found, which was very similar to a normal nuclear endosperm with only a slight suggestion of cell walls and 12 pairs of nuclei simultaneously being in telophase (Figs. 10, 11), though the normal endosperm of *Solanum* is cellular from the beginning and the nuclei divide independent of each other. In some other endosperms huge nuclei were found and in one case two metaphase plates with about 12 chromosome sets (137 chromosomes were counted in one) were detected, while other nuclei of the same endosperm were hexaploid and the embryo tetraploid.