A HAPLOID PLANT IN TORIA (BRASSICA CAMPESTRIS L.)

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1. Introduction

HAPLOIDY in flowering plants was first discovered by Blakeslee et al. in 1922. By subjecting pollinated flowers of Datura stramonium to low temperature at approximately the time of fertilization, they obtained two haploid plants each of which had 12 somatic chromosomes. Since that time numerous haploids have been discovered and described from a considerable number of different species belonging to widely different families. A detailed discussion of haploidy in flowering plants is contained in a recent paper by Ivanov (1938).

Haploids are usually reduced replicas of their parent plants although in some cases they have been found to show variations in this respect. Thus in Triticum vulgare (Namikawa and Kawakami, 1934) and T. compactum (Gaines and Aase, 1926) the haploid plants were absolutely identical with the mother plants except in their ill-developed generative organs and high sterility. In T. dicoccum and Tr. persicum (quoted by Ivanov, 1938) the haploids showed a marked change in their morphological characters. Another feature generally characteristic of the haploids is their high sterility following an absence of conjugation of the chromosomes and random assortment of univalents at meiosis. Consequently haploids produce few progenies. But the occasional
progenies produced by them consist mostly of diploids and rarely of poly-
ploids and trisomics. By far the most interesting progeny of the haploid, from the point of view of the breeder, is the diploid which by reason of its origin is fertile and homozygous. The importance of the production of homozygous diploids from haploids for breeding purposes is emphasised by R. C. C. (1936) and Blakeslee (1939). The latter author commenting on this subject remarks, "Now that we have a means of readily doubling the chromosome number (by the use of colchicine) the great need in our genetic programme is a method whereby we can reduce the number by half". If we can discover a method by which haploids could be artificially produced at will, then starting with a highly heterozygous plant, such as a fertile species hybrid, we can obtain homozygous lines in two jumps.

As regards the origin of the haploids, they are known to result from generative parthenogenesis or androgenesis either spontaneously with no known cause or under the action of certain agencies which are supposed to stimulate the development of unfertilized egg cells. Among the agents that are known to produce haploids in plants are distant hybridization, and pollination with X-rayed pollen; in both these cases the male gamete penetrates the embryo-sac but does not fuse with the nucleus of the egg cell. Subjection of pollinated flowers to low temperature at the time of fertilization is also known to induce haploidy. In this case also it is presumed that the male nucleus in the embryo-sac without fusing with the egg nucleus stimulates its development. These methods, however, are neither universally successful in producing haploids nor do they produce haploids in large numbers wherever they are successful.

So the problem of inducing haploidy artificially in plants is still unsolved and is receiving increased attention since the discovery by Blakeslee et al. (1937) that the chromosome number in plants can be doubled by the use of colchicine. Connected with this problem is the study of the origin and behaviour of new haploid plants. In this paper, the study of a haploid plant occurring for the first time in B. campestris is reported.

2. Previous Work in Brassica

In the genus Brassica haploids have been reported only in the species B. napella (Morinaga and Fukushima, 1933). They were highly sterile indivi, duals occurring rather frequently in rape fields. According to these authors, "Half a day's excursion through the rape field, when the flowers are passing away, will suffice to find dozens of such haploid individuals." The cause of their occurrence in such large numbers is, however, not known. The leaves and flowers of the haploid were obviously smaller than those of the diploid
but the height, though generally lower than that of the diploid, ultimately surpassed the latter on account of continuous development of inflorescences. In the root tip cells, 19 chromosomes were found in the haploid compared to 38 in the diploid. The meiosis in the haploid was irregular following the production of 0–6 or 7 bivalents at division I. The formation of so many bivalents in the haploid is explained on the basis that *B. napella* is an allopolyploid species (Morinaga, 1929) with two duplicated genomes. Most of the pollen grains formed in the haploids were small and shrivelled while some were large and perfect.

At the Imperial Agricultural Research Institute, where cytogenetic investigations on the oleiferous *Brassicae* are in progress, a spontaneously occurring haploid plant was discovered in one of the cultures of *rai*, *B. juncea* (Ramanujam, unpublished). This species with \(2n = 36\) chromosomes, like *B. napella*, is an allopolyploid species (U, 1935) and as a consequence, the haploid showed varying numbers of bivalents at meiosis.

The occurrence of haploid plants in elementary species of *Brassica* with \(n = 8\), 9 and 10 chromosomes is so far unknown and this paper reports the first occurrence of a haploid plant in such an elementary species, *viz.*, *B. campestris* with \(n = 10\) chromosomes.

3. *Origin and Description of the Haploid*

During the year 1940–41, in one of the cultures of *toria* grown from an open-pollinated plant, a dwarfish looking plant with smaller leaves and flowers compared to other plants (Pl. II, Figs. 1–4) in the line was noticed. It flowered almost at the same time as the other plants and set a fair amount of seed under conditions of open-pollination. As the plant was in every respect a reduced replica of the other plants, it was suspected to be a haploid. An examination of its pollen was undertaken and it was found that they were fairly fertile with only about 40 per cent. empty and shrivelled grains. This was rather unusual, as in most of the haploids previously described the sterility of pollen was very high as much as 90 per cent. or more in some cases. The epidermal cells of the leaf in the haploid were examined and the size of stomata guard cells was compared with that of the normal plants. Although many of the stomata of this abnormal plant were considerably smaller than those of the normal plants, a fair number of them was similar in size to those of the latter. An examination of the chromosomes definitely showed that this abnormal plant was a haploid. The plant was selfed and crossed reciprocally with diploid plants in the row, while a few branches were left for open-pollination. No seeds were obtained from selfed flowers, presumably owing to the self-sterile nature of the species, but a good amount of seed was
obtained from open- and cross-pollination. Unlike the haploid plants of B. napella (loc. cit.), this haploid did not exhibit a continuous development of inflorescences but died with the rest of its sister plants in spite of careful attention to perpetuate it by cultural means. It is probable that the fairly high degree of seed setting in the present haploid did not promote its continuous vegetative development.

4. Cytological Technique

The somatic chromosomes were studied in the meristematic cells of young leaves smeared and stained with aceto-carmine adopting Baldwin's technique (1939). Meiosis in pollen mother cells was studied in permanent aceto-carmine smears (McClintock, 1929). Drawings were made with the aid of a camera lucida at bench level using a 2 mm. apochromatic objective and 15X and 20X compensating oculars.

5. Cytology of the Haploid

The somatic chromosome numbers of the haploid and the diploid were counted in the meristematic cells of young leaves and found to be 10 (Text-Fig. 1, and Pl. III, Fig. 4) in the case of the former and 20 (Text-Fig. 2 and Pl. III, Fig. 3) in the case of the latter. In the haploid smears examined, a few big cells with double the number of chromosomes, i.e., 20, were found intermingled with normal cells with 10 chromosomes. The occurrence of bigger stoma guard cells in the epidermis of the haploid, which was mentioned earlier, is perhaps due to the presence of such diploid cells. Diploid cells in haploid tissue have been recorded in several plants (Ivanov, 1938). In the diploid material examined in the present case, however, no duplicated cells were observed.

The meiosis in the haploid was followed from diakinesis to pollen formation and compared with that in the diploid. In the pollen mother cells of the diploid 10 bivalents were observed at diakinesis and metaphase I (Text-Fig. 3). Subsequent stages of the meiosis were carried out regularly and normal tetrads were formed, which gave rise to fertile pollen. In the haploid, at the stage of diakinesis there could be clearly seen in each pollen mother cell 10 univalents scattered about the periphery of the nucleus. At a later stage, in which the distinction between metaphase I and anaphase I was obscured, the ten univalents were found distributed on a faintly differentiated spindle. Text-Figs. 4, 6 and 9 show the disposition of univalents at this stage. Bivalents were rarely met with except in a few cells in each of which one rod-shaped bivalent was noticed (Text-Figs. 5, 7 and 8). In such cells the bivalents soon separated out into their components. The later behaviour of the univalents
was found to take one of two courses. In a few cells the scattered univalents were seen to segregate into two groups (Text-Fig. 6) following a random distribution, while in a large majority of cases, they seemed to collect together and form an interphase nucleus containing all the ten chromosomes. In the former case, where there was random segregation of chromosomes, the division II proceeded fairly normally and gave rise to tetrads with unequal
pairs of spores. In the latter case, where the segregation was suppressed, the division II yielded dyads with two equal spores. This kind of suppression of the first division and the formation of dyads were noticed to a large degree in haploid *Matthiola* (Lesley and Frost, 1928) and to a lesser degree in haploids of other species. In the case of *Matthiola* (*loc. cit.*), however, the univalents at metaphase were long and bent, simulating the somatic chromosomes. Another interesting feature of meiosis in the haploid was the occurrence of diploid pollen mother cells. These were bigger than the haploid cells and were found usually scattered in the anther locules. They showed normal pairing of chromosomes into ten bivalents at metaphase I (Text-Fig. 10) and produced normal tetrads following a regular meiosis. Pl. III, Figs. 1 and 2 are photomicrographs showing pollen mother cells of the haploid plant in various stages of meiosis. In Pl. III, Fig. 2, two diploid cells, which are bigger than the surrounding haploid cells, may be seen at side view of metaphase I with ten bivalents in each. The occurrence of pollen mother cells with double the number of chromosomes has been recorded in several plants. In *Oryza officinalis* (Ramanujam, 1938) the occurrence of big pollen mother cells intermingled with normal ones was recorded. Tetraploid pollen mother cells were also noticed in the anther locules of diploid *Aegothera* (Cleland, 1929) and *Brassica* (Fukushima, 1931), etc. These polyploid cells may arise by the duplication of chromosomes in the last premeiotic division or earlier during the formation of the sporogenous tissue; in the former case isolated big cells and in the latter, groups of them will be formed. In the present case, the diploid cells were found mostly scattered and, therefore, may have resulted from sporadic autopolyploidy in cells at the last premeiotic division. The occurrence of diploid pollen mother cells considered in relation to the presence of scattered diploid cells in the epidermis and meristematic cells of leaves would appear to indicate that the haploid has a tendency to produce doubled cells throughout its ontogeny. How exactly this doubling is brought about is not known. One other fact worth mentioning in this connection concerns the pairing behaviour of the chromosomes in the diploid pollen mother cells of the haploid. While in the case of *Brassica* (*loc. cit.*) the groups of tetraploid cells in the diploid, which according to Fukushima arose from initial archesporial cells, did not show any appreciable tetravalent formation which would be expected in such autopolyploid cells, in the case of the diploid cells in the present case, the chromosomes were perfectly paired into bivalents according to expectation. It may be mentioned in this connection that in autotetraploid Kale (Howard, 1939) and autotetraploid *toria, B. campestris* (Ramanujam, unpublished) varying numbers of quadrivalents were regularly found at meiosis. This difference in the pairing properties
of homologous chromosomes only emphasises the complicated nature of the phenomenon of chromosome conjugation (Ramanujam, 1937).

An estimation of sterility of pollen in the haploid was made by counting the perfect and well filled (fertile) and the shrivelled and empty (sterile) grains in several anthers. A count in five different samples gave 617 fertile and 440 sterile grains which works out 41·6 per cent. sterility in the haploid compared to practically no sterility in the diploid. A similar count of tetrads and dyads was made from several anthers and the figures obtained were 472 dyads and 174 tetrads. Assuming that the tetrads would give rise to sterile pollen on the expectation that they would contain unbalanced chromosome numbers and the dyads to fertile pollen as they would have the haploid complement of chromosomes, 944 fertile and 696 sterile pollen grains would be obtained from the sporads examined. These figures work out to 42·4 per cent. sterility in the haploid which is more or less in agreement with the result obtained by directly counting the pollen grains. It may be pointed out that the slight increase in sterility obtained from sporad counting may be due to the fact that some of the tetrads which would be obtained from diploid pollen mother cells and which would yield fertile pollen are included in the sterile class. Pollen grains of the diploid and the haploid are shown in Pl. III, Figs. 5 and 6. The fertile pollen grains of the haploid were similar in size to those of the diploid. The fairly good setting of seed obtained in reciprocal crosses between the haploid and the diploid shows that many of the pollen grains and egg cells are functional in the haploid. Selfing the haploid did not produce any seed presumably due to self-sterility, which is characteristic of the species.

6. Discussion

The importance of the production of haploid plants for breeding purposes has already been referred to. Although it is obvious that the egg cells of many of the flowering plants are capable of developing into haploid plants, the question as to what precisely stimulates their parthenogenetic development is far from solved. Several investigators have endeavoured to obtain haploid plants by artificial means with very little success. Ivanov (1938) tried several methods for stimulating egg cells of Nicotiana to develop into haploids and succeeded in getting only a few by pollination of plants with X-rayed pollen. He also tried, among other things, pollinating Nicotiana plants with pollen from plants belonging to the other genera of the Solanaceae without any success. In the genus Brassica, a number of interspecific and a few intergeneric crosses were made and a good amount of crossed seeds was obtained by the author of the present paper; these seeds in many cases, however, gave rise to plants resembling the mother parents. Noguchi (1928),
Ali Mohammad and Sikka (1940) also reported the occurrence of maternal offspring in the progenies of interspecific crosses in *Brassica*. The maternal offspring in all these cases may have developed from the vegetative tissue of the mother plant or have arisen due to induced parthenogenesis with ensuing diploidy. East (1930) working with *Fragaria* showed that, in some cases at least, the latter process was involved and pointed out its significance to practical breeding. If a reasonably large percentage of parthenogenetic embryos can be forced to develop into diploids by interspecific hybridization or other means, the results would be of great value for breeding purposes. Starting with a highly heterozygous plant, such as a species hybrid, we could directly obtain from it homozygous lines with different combinations of the characters of the two parents. This method of obtaining homozygotes eliminates the necessity of obtaining haploids and then doubling the chromosomes, which was indicated earlier in the paper; the doubling in this case takes place earlier in the developing haploid embryo without the influence of any external agency. Experiments are in progress in the Institute to determine the mode of origin of diploid offspring in interspecific and intergeneric crosses of *Brassica* with a view to the practical application of the knowledge for breeding superior varieties.

The haploid *Brassica* described in this paper resembles most of the other haploids in being a reduced replica of the parent plant. Its tendency to double the chromosomes in the somatic and sporogenous cells, however, is a noteworthy feature which accounts for its high fertility. The study of the progeny of this plant will be taken up next year. Any diploids that may be obtained in the progeny will not be homozygous as selfed seeds were not obtained.

7. **Summary**

A spontaneously occurring haploid plant in one of the open-pollinated cultures of *toria* is described for the first time.

The haploid was a reduced replica of the diploid plant with smaller leaves and flowers. It flowered at the same time as the diploid and after setting a good amount of seed under conditions of open-pollination died with the rest of its sister plants.

The somatic chromosome number of the haploid was determined in meristematic cells of young leaves as 10 and that of the diploid as 20. A few cells with the diploid number of chromosomes were found scattered in the leaf tissue of the haploid.

At meiosis, the haploid showed 10 univalents from diakinesis to anaphase I in each pollen mother cell. Occasionally, however, a loosely paired
bivalent was noticed in some cells. At division I the univalents either segregated at random into two groups or collected to form an interphase nucleus. Division II in the former case produced tetrads of unequal spores, and in the latter dyads of equal spores.

A few pollen mother cells with the diploid number of chromosomes were also noticed in the haploid; these were seen to undergo normal meiosis producing tetrads with equal spores.

As a result of dyad formation in a large number of haploid pollen mother cells and tetrad formation in the few diploid pollen mother cells, the pollen fertility of the haploid was found to be as high as about 60 per cent. Seed-setting in the haploid was also fairly high under conditions of open and cross-pollination.

The possible utilisation of haploidy in practical breeding is discussed.

REFERENCES


15. ——— and Fukushima, E. .. “Karyological studies on a spontaneous haploid mutant of Brassica napella, Cytologia, 1933, 4, 457-60.


EXPLANATION OF PLATES AND TEXT-FIGURES

PLATE II

Figs. 1-2.—Photographs of diploid and haploid toria, respectively.

Figs. 3-4.—Photographs of flowers of the diploid and haploid toria, respectively.

PLATE III

Figs. 1-2.—Photomicrographs showing pollen mother cells of the haploid plant in various stages of meiosis.

(Note in Fig. 2 two diploid cells, bigger than the surrounding haploid cells, in side view of metaphase I with ten bivalents.)

Figs. 3-4.—Photomicrographs of somatic chromosomes in meristematic cells of young leaf of the diploid and the haploid, respectively.

Figs. 5-6.—Photomicrographs of pollen grains of the diploid and the haploid, respectively.

TEXT-FIGS. 1-10

Figs. 1-2. Somatic chromosomes of the haploid and the diploid, respectively, in the meristematic cells of young leaves. Fig. 3. Pollen meiosis in the diploid, showing side view of metaphase I. Figs. 4, 6 and 9. Pollen meiosis in the haploid, showing the disposition of univalents at metaphase I and anaphase I. Figs. 5, 7 and 8. Pollen meiosis in the haploid, showing one rod shaped bivalent in each cell. Fig. 10. A diploid cell in the haploid at metaphase I (side view with ten bivalents.)