CHROMOSOME STUDIES IN DIPLOID AND TRIPLOID FORMS OF *DISPORUM SESSILE*

By

Nobumi HASEGAWA

(Received Nov. 27, 1932)

In his preceding paper, the writer (1932) described that a special form of *Disporum sessile* D. Don. ($2n=16$) which has 24 somatic chromosomes, bore fruit with no seeds by artificial as well as by natural pollinations.

The chromosome behaviour in PMC-s of this form is reported in the present paper together with some chromosome observations of the diploid form of the same species for the comparison.

The anthers were fixed in CARNOY's fluid, and BELLING's iron aceto-carmin was employed yielding very satisfactory results.

All Text-figures are drawn from PMC-s except Text-fig. 4 which shows a pollen-grain. Text-figs. are all made on a drawing stand by the aid of ABBE's large camera using ZEISS fluorite objective $100\times$ and compensation ocular $10\times$, giving a magnification of $\times 1430$. They are reduced to $\times 950$ in reproduction.

DIPLOID FORM

The diploid form of *D. sessile* has 16 somatic chromosomes in root-tip cells (HASEGAWA, 1932). In the 1st metaphase in PMC-s, 8 bivalents of varying size were observed (Text-figs. 1-2).

At the 1st anaphase, the disjunction of bivalents is probably begun with the shorter chromosomes and then that of the longer ones follows. Text-fig. 3 shows a side view of the 1st anaphase in which the bivalents are already separated or going to be so. The halves of bivalents show the homotypic split. Two long chromosomes are being separated in the equatorial region from their partners at one end, while they are attached to each other at the other end. Shorter chromosomes are already disjoined and most of them are found near the poles.

At the 1st anaphase the constrictions are clearly seen on the split halves of univalents. The types of chromosomes classified by the writer (1932) according to the length and relative position of constrictions in somatic

Text-figs. 1-4. Diploid form, $\times 950$. 1 and 4 polar views; 2 and 3 side views.

1. 1st metaphase, showing 8 bivalents.
2. 1st metaphasic chromosomes from one PMC.
3. 1st anaphase.
4. Haploid group of metaphasic chromosomes in the division of pollen-grain nucleus.

chromosomes of this form are clearly seen. These chromosome types were also observed in the chromosomes in metaphase of division of the pollen-grain nucleus (Text-fig. 4).

The positions of constrictions or the proximal ends of the short arms made by the constrictions were pointed in the ordinary way to the poles.

Throughout the whole course of the meiotic division in PMC-s, the diploid plants showed the regular behaviour of chromosomes, and they, consequently, produced functional pollen-grains.

The fruits contain some perfect seeds surrounded by white pulp.

TRIPLOID FORM

The plants which were reported by the writer (1932) to have 24 somatic chromosomes in root-tip cells were found to be autotriploids by the present study.

In the polar view of 1st metaphase of PMC-s, in most cases 8 trivalents each consisting of three homologous chromosomes can be seen (Text-fig. 5). In side view of 1st metaphase, they are arranged more or less irregularly, and there is difficulty to some extent in counting them (Text-fig. 6).

At metaphase, three elements of trivalents are conjugated end to end to form Υ , $\P$ and $\dagger$ as was reported in triploid *Datura* (BELLING and BLAKESLEE, 1923) and in other triploid plants. Trivalents in other forms more or

Text-figs. 5-9. Triploid form, $\times 950$. 5 and 8 polar views; 6, 7 and 9 side views.

5-6. 1st metaphase, showing 8 trivalents.

7. Several types of trivalents in 1st metaphase from different PMC-s.

8. 1st late metaphase or early anaphase, showing 3 trivalents, 5 bivalents and 5 univalents.

9. 1st early anaphase, showing 24 univalents.

less resembling them were also observed. Different forms of trivalents are shown in Text-fig. 7.

In some trivalents in late metaphase or early anaphase, one of the three elements is disjoined from the other two, and they appear as a univalent and a bivalent. In Text-fig. 8, 5 bivalents and 5 univalents derived from 5 trivalents are shown together with 3 trivalents.

At 1st anaphase, all elements of trivalents and bivalents are detached from their partners, and we observe there 24 univalents with homotypic split (Text-fig. 9). The constrictions are seen on the split halves. The positions of the constrictions or the proximal ends of the short arms made by the constrictions were pointed to the poles as was the case in the diploid form.

The distribution of the elements of trivalents in 1st telophase is usually irregular as one of the three elements in each trivalent goes to a pole and the other two toward the other, thus the number of chromosomes at the poles is determined at random. The most usual numbers found at the two poles are 12-12, 11-13, 10-14 (Text-figs. 10-11), but 9-15 and 8-16 can be seen in a few cases.

Text-figs. 10-15. Triploid form, $\times 950$. 10 and 11 oblique views; 12 and 13 side views.

10-11. 1st late anaphase, showing irregular distribution of chromosomes.

12. 1st anaphase, showing lagging chromosomes.

13. 2nd anaphase, showing chromosome bridge and lagging chromosomes.

14-15. Pollen tetrads.

A few split halves of univalents in 1st anaphase were observed to become lagging chromosomes (Text-fig. 12) and some of them made bridges between two nuclei. The similar chromosome behaviours were observed seldom at 2nd anaphase (Text-fig. 13), and some of the lagging chromosomes would give rise to the extra micro-nuclei in the pollen tetrads (Text-fig. 15).

Though the pollen tetrads somewhat differ in their size, the writer could not observe the monad and dyad cells or the polyspory, as were reported hitherto to have been observed in other triploid plants.

As stated above, the chromosome distribution is irregular in 1st anaphase or rarely in 2nd anaphase also, so that the chromosomes contained in pollen-grains of the triploids should present a great range of number. In some cases the number must be markedly small as is recognized in Text-fig.

14 showing tetrad cells, in one of which only a few chromosomes are observable. The mature pollen-grains differ in their size. Many of the pollen-grains appear as if they are normal but, a few are shrunken.

Triploid plants usually bear seedless fruit. When it is matured the empty space is observable at the position within the white pulp where the seeds are to be formed, and only the ovules not developed normally or their rudiments are found there.

No seeds were formed in the fruits except in only a case in which a small seed was obtained from fruits on over a hundred plants during three years cultivation.

DISCUSSION

The triploids must be classified into two categories, i. e. auto- and allotriploids.

In diploid form of *D. sessile*, 8 haploid and 16 somatic chromosomes were counted, the same number in other species in this genus (HASEGAWA, 1932).

A form in *D. sessile* which was reported previously to have 24 somatic chromosomes, showed in the present studies 8 trivalents in 1st metaphase and 24 univalents in 1st anaphase of PMC-s. So that this form can be recognized to be an autotriploid having 8 as the basic number.

Examples of triploid plants are hitherto known in *Morus*, *Canna*, *Datura*, *Hyacinthus*, *Heimerocallis*, and other genera. The chief characteristic of them is the high sterility, and for this reason, the triploids are most frequently found in perennial plants which are able to be propagated vegetatively. Most of these triploids are reported or seem to be autotriploids. The triploid *Disporum* is also highly sterile, and it is propagated by rhizomes.

As already discussed by BELLING (1925 a, b) and others, the origin of the autotriploid is attributed to the union of a diploid gamete with a haploid one produced in a diploid form, and the diploid gamete may often be formed by unsuitable temperature in the course of meiosis.

The possibility of the formation of diploid gamete in *Disporum* was well proved by our colleague WASHIASHI (unpublished), who found giant pollen-grains in young flower-buds of diploid plants of *D. sessile* which were kept, in the course of the meiosis, at 7°C in a thermostat during 6–9 days in May (room temp. 16°–18°C in day time). The possibility of producing 2x pollen-grains by a similar way was studied in various plants by BELLING (1925 a), SHIMOTOMAI (1927) and others.

Diploid *D. sessile* begins its meiosis in PMC-s in about April. Though it is rather warm in this season, the temperature is changeable, and occasionally exceptional cold with snow or frost comes.

So, the origin of the triploid form of *D. sessile* might be suggested in that a haploid gamete united with a diploid one formed by the abnormal meiosis caused by chilling the young flower-buds of the diploid form.

Diploid plants of *D. sessile* produced the normal pollen-grains through the regular division of their PMC-s, and they accordingly bore fruit with some perfect seeds. Triploid plants, however, produced pollen-grains of diverse size some of which are shrunken. But not few of the pollen-grains appeared to be morphologically normal.

The difference of size of pollen-grains in triploid owes probably to the varied chromosome number contained in them, and this may be caused by the irregular distribution of chromosomes, as was stated by BELLING (1924) in triploid *Hyacinthus*.

In view of the irregular meiosis in PMC-s, it might be conceivable that the megasporogenesis of EMC-s are also incapable of the production of functional female gametes.

But, as a very rare instance, the writer found by chance a fruit with only a small seed. So, it can be said that there are formed occasionally at least female gametes which are functional.

In the triploid form the ovary, however, developed easily to the seedless fruit, showing a large tendency of parthenocarpy. This phenomenon was observed also in *D. smilacinum* var. *ramosum* pollinated, without forming any seed, with the pollen of diploid *D. sessile* as well as in the castrated but unpollinated flowers of diploid *D. sessile*.

The writer is indebted to Prof. Dr. F. KAGAWA for his helpful suggestion and guidance of this work.

Utsunomiya Agricultural College,
Utsunomiya, Japan.

LITERATURE CITED

- BELLING, J. (1924) The distribution of chromosomes in the pollen-grains of a triploid *Hyacinth*. Amer. Nat., **58**: 440-446.
- (1925a) The origin of chromosomal mutations in *Uvularia*. Jour. of Genet., **15**: 245-266.
- (1925b) Production of triploid and tetraploid plants. Jour. of Heredity, **16**: 463-464.
- BELLING, J. and BLAKESLEE, A. F. (1923) The reduction division in haploid, diploid, triploid and tetraploid *Daturas*. Proc. of Natio. Acad. of Sci., **9**: 106-111.
- HASEGAWA, N. (1932) Comparison of chromosome types in *Disporum*. Cytologia, **3**: 350-368.
- SHIMOTOMAI, N. (1927) Über Störungen der meiotischen Teilungen durch niedrige Temperatur. Bot. Mag., Tokyo, **41**: 149-160.