Cytological Studies in Diploid and Triploid *Hosta* *

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(With 3 Text Figures and One Plate)

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The chromosome constitution in *Hosta* is an interesting one from several points of view. STRASBURGER (1882) states in regard to the number of the chromosome pairs in the meiotic cells „In dem gedachten Falle, es handelt sich um die primäre Kernspindel der Pollenmutterzellen von *Funkia* [*Hosta*] *Sieboldiana*, war die Zahl der Elemente viel bedeutender, meist etwas grösser als 24.“ Afterwards, however, many workers described the haploid number in *Hosta* as 24 (STRASBURGER 1900, 1905, MIYAKE 1905, SYKES 1908, INARIYAMA 1928) until WHITAKER (1934) described the number in *Hosta coerulea* and determined its chromosome constitution as of the *Yucca-Agave* type (5 long and 25 short). IMAI and KANNA (1934) reported the polyploid series in this genus and determined its base number as 12. D. SATÔ, (1935) in his first paper described the constitution of the chromosome and put the number at 24, but later he gave the number as 30 (4 long and 26 short). AKEMINE (1935) also studied several species in this genus and reported the number as 30 in haploid denying the polyploidy in his materials.

The present writer, having been concerned with the cytogenetical study of this genus for several years, here reports some cytological results of the work.

Material and Method

The following nine species were used for the study.

Species	Japanese name	Species	Japanese name
1. <i>Hosta Foruni</i> var. <i>gigantea</i> BAILEY	Ohba-giboshi	6. <i>H. lancifolia</i> STERN (<i>H. japonica</i>) f. <i>albomarginata</i> MAKINO and its derivatives	Shiro-fukurin-midugiboshi
2. <i>H. Sieboldiana</i> HOSK.	Tô-giboshi	7. <i>H. undulata</i> BAILEY and its green bud-variation and albo marginata form	Suji-giboshi
3. <i>H. venusta</i> F. MAEKAWA	Otomé-giboshi	8. <i>H. sp.</i>	Tsubomi-giboshi
4. <i>H. lancifolia</i> STERN, var. <i>longifolia</i> NAKAI	Nagaba-midugiboshi	9. <i>H. clausa</i> NAKAI	
5. <i>H. plantaginea</i> Asch.	Tama-no-kanzashi		

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Materials were fixed with either BENDA's modification of FLEMMING's solution or with NAVASHIN's fluid, and stained either with HEIDENHAIN's iron-alum hämatoxylin or with NEWTON's gentian-violet. Also for some materials the aceto-carmin smear method was used. All text-figures are camera-lucida drawings.

Observations

The chromosome number in the root-tip cells in all species in the list except No. 9 was determined as 60 (Text-fig. 1, *a-h*), and in the meiosis in PMCs in Nos. 1-3 and 5-6, 30 gemini were counted (Text-fig. 2, *a-f*).

Text-Fig. 1. Chromosome garniture of the metaphase in the root-tip cells of *Hosta*. *a*, *H. Fortuni* var. *gigantea* BAILEY; *b*, *H. Sieboldiana* HOOK.; *c*, *H. venusta* F. MAEKAWA; *d*, *H. lancifolia* STERN. f. *albomarginata* MAKINO; *e*, *H. plantaginea* ASCH.; *f*, *H. lancifolia* STERN. var. *logifolia* NAKAI; *g*, *H. undulata* BAILEY; *h*, *H. sp.*; *i*, *H. clausa* NAKAI. $\times 1400$.

The division was regular generally resulting in the formation of good healthy pollen grains. The partial sterility of *H. plantaginea* (rarely fertile later, in the season), may perhaps be due to the slow growth of the pollen tubes in its own style; the meiosis in PMCs occurs regularly. No. 7 is the sterile cultivated species, but the behaviour of the chromosomes in PMC are generally regular; sometimes however there were found lagging chromosomes between two daughter chromosome groups or a chromosome bridge after the 1st division (Text-fig. 2, *e* and *f*).

Of the haploid chromosome set, four chromosomes (4L) are remark-

Text-Fig. 2. Meiotic phases in PMCs of *Hosta*. a, *H. lancifolia* f. *albomarginata*, diaphase; b, *H. venusta*, equatorial plate; c, *H. Sieboldiana*, equatorial plate; d, *H. plantaginea*, diaphase; e, *H. undulata*, early metaphase; f, the same, the later anaphase; two lagging, dividing chromosomes shown. ca. $\times 830$.

ably long, while, among the rest one (I1) is specially longer than others. The shorter 25 chromosomes vary in their length with small gradations, one of them representing quite a peculiar shape in the meiotic stage (Text-fig. 2, x).

H. clausa which was brought from Corea by Prof. T. NAKAI who has named it after its corolla which entirely fails to open; this is due to the stop of the growth before the opening of the flower and also to the fact that the petals do not separate from each other. The style and the filaments, however, can stretch quite normally, if the tip of the corolla is trimmed. This species is entirely sterile in nature, and hitherto artificial pollination has met with no success.

The chromosome number in the nucleus of the root-tip cells of this species is 90 (Text-fig. 1, i). The chromosome constitution of a set is the same as that of the diploid species above described, though here we have to do with a triploid. In the 1st pro- and metaphase of the meiosis in PMC there were observed trivalent chromosomes mostly, but a few

bivalents and univalents too were found among the small chromosomes (Text-fig. 3, *a-g*). In some of the trivalents all chromosomes were arranged side by side, though two of them generally conjugate closely, the third one joining them loosely with one or both ends (Text-fig. 3, *b, d, e*); in others the third joins the other two with one end only (Text-fig. 3, *c* and *f*). No ring formation was observed.

At the metaphase almost all chromosomes enter in the formation of the equatorial plate simultaneously (Plate 21, Fig. 1), but in some cases some small chromosome groups, probably bivalent, divide promptly and migrate into the poles much earlier than others. Most of small trivalents proceed to divide faster than the longer ones, and the univalents generally lag behind, so that they are often excluded from the daughter nuclei (Text-fig. 3, *g*). Some of these univalents show loosening spirals, and divide longitudinally, but the halves are joined together at the attachment point, which gives the appearance that the attachment points do not divide, consequently the halves do not separate from each other and migrate into the poles (Text-fig. 3, *h*). In some other PMC, a more irregular behaviour of chromosomes at anaphase were observed (Text-fig. 3, *i*). Some of the excluded chromosomes divide themselves simultaneously when the 2nd meiotic division occurs (Text-fig. 3, *j*).

The distributions of the chromosomes in the 1st meiotic division takes place at random, and consequently the two daughter nuclei are furnished with a different numbers of chromosomes, probably ranging from 30 to 60 or more, with the modal value about 45.

The second division of meiosis was generally regular, though in some cases irregular behaviour of chromosomes was observed as well as some lagging chromosomes. There were often found one or more extra nuclear chromosomes and the miniature pollen grain in PMC after the second division. Almost all pollen grains in the mature anther were sterile, though some showed a normal appearance (Plate 21, Fig. 6), the generative cells being mostly degenerated. Among those grains some were enormous (Plate 21, Fig. 8); dumb-bell shaped grains (Plate 21, Fig. 7) were also observed.

Beside the degeneration of the mature pollen grains, some PMCs degenerate in the early meiotic stage. When this occurs, it begins in the central part of the anther, the intercellular space among the PMCs becomes larger and the membrane of the PMC is the first to dissolve; later cytoplasm and finally the nucleus also dissolves. Therefore the only PMCs which complete the meiosis are some of those which are situated in contact with tapetum (Plate 21, Figs. 1-4). At the later stage, the cavity of the anther was filled with the mucilagenous fluid, which was well stained with gentian-violet, pollen grains being found floating in it (Plate 21, Fig. 5).

Text-Fig. 3. Meiotic phases and chromosomes in PMCs of *H. clausa*. *a*, diaphase; *b-f*, configurations of some trivalent chromosomes; *g*, 1st anaphase, small chromosomes at the poles followed by large chromosomes, some univalent chromosomes are seen; *h*, 1st telophase, daughter nuclei with hanging chromosomes and two extra nuclear univalents are seen; *i*, 1st anaphase, several chromosomes are lagging around at the equatorial region, are seen; *j*, 2nd later anaphase, two extra nuclear chromosomes also dividing are seen; *k*, 2nd telophase, four daughter nuclei having the hanging chromosomes, one small cell beside two main daughter cells in which extra nuclear chromosomes are scattering, are seen.

As sufficient material could not be secured the meiosis in the EMCs (embryo-sac mother cells) and the development of embryo-sac could not be observed thoroughly. A mature normal embryo-sac was observed in many ovules. Some embryo-sac had two egg cells beside two synergids.

These embryo-sac however, had nuclei which stained faintly; and in a later stage they, as well as the tissue in the ovule, degenerated, except one layer of cells which surrounded the embryo-sac before the flower drops (Plate 21, Fig. 9).

Discussion

1. Except IMAI and KANNA no investigators had recognized the polyploidy in this genus. As far as the present writer's investigation is concerned, all species, except *Hosta clausa*, are diploid plants which had a similar chromosome constitution, having 30 as its base number and not 12. *H. clausa* is the only triploid plant. The derivation of this plant is not known yet, but the shapes and sizes of the chromosomes and their configuration in the meiosis suggest that this species is an autotriploid.

WHITAKER's classification of the chromosome sizes in a nucleus of this genus (5 long and 25 short) and that of D. SATÔ's (4 long and 26 short) seem to be too simple. There are more gradations among them, and so the present writer proposes the chromosome formula $4L+1l+1M+3m+13S+8s$. Though the number and constitution of the chromosomes show much resemblance to the *Yucca-Agave* type, there is a difference between the two, in that none of the long chromosomes in the *Yucca-Agave* type has any sub-median attachment point as in this species (Text-fig. 3,b). Therefore the chromosome type of this genus can not be included in the *Yucca-Agave* type. The karyotype of *Hosta* however, is not very close to that of *Hemerocallis* with which it is included in Hemerocallineae. The base number of the chromosome in *Hemerocallis* is 11 (TAKENAKA, 1929), of which the variation in sizes are slighter than those in *Hosta*. Consequently, so far as the chromosome constitution is concerned this genus may be separated from Hemerocallineae.

2. On the causes of sterility of triploid plants in general several considerations have already been made; some authors emphasize the chromosome behaviour in the meiotic division while others lay stress upon physiological conditions. In the present case there are at least three different causes concerning the sterility. Firstly there is the degeneration of PMC (probably EMC too) in the early stages in meiosis; secondly the irregular chromosome behaviour in the meiosis which generally occurs in other auto- and allotriploid plants too. A supernumerally or infra-numerally chromosome constitution will have some effects on the viability of the gametes and of zygotes, even if they contain one or more complete genomes, as in well known case of *Datura*; thirdly the suppression of

pollination is due to the failing to open of the corolla. Some physiological condition may exist which influences the growth of corolla. This character may be due to the triploidy itself, because if the diploid ancestor had already such a character as a failure of opening of flowers, there would be some difficulties in forming the autotriploid.

3. There are many genera in which the chromosome number and constitution of species are constant, in spite of the varying habits and sizes of the plants. *Hosta* would have been classified among the latter, if the triploid species had not been found.

Summary

1. Eight diploid and one autotriploid species of *Hosta* were studied cytologically. The formula of the haploid chromosomes in this genus may be given as $4L+11+1M+3m+13S+8s$. This constitution shows great deviation from those of *Hemerocallis* not only in the chromosome number but also in their sizes, so the writer is of the opinion that the genus, as far as chromosome types are concerned, is to be separated from Hemerocallineae.

2. *H. clausa* is an autotriploid species. The irregular behaviour of the chromosomes in the meiosis in PMC is the cause of pollen sterility. Beside this the degeneration of PMC in the early meiotic phases, the degeneration of the embryo-sac, the non-opening of the corolla caused by the stultation of the growth of the corolla just before the opening stage, all tend to the entire sterility of this species. The cytological derivation of these characters as well as of the species itself is not yet known.

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Explanation of Plate 21

All figures are photomicrographs concerning *Hosta clausa* NAKAI.

- Fig. 1. Ist metaphase of a PMC. *t*, a part of tapetum cell. ×750.
- Fig. 2. Later anaphase of 2nd meiotic division of PMC., and the residue (*r*) of degenerated PMC. *t*, a part of tapetum cell. ×750.
- Fig. 3. A part of an anther, in whose central part degeneration of PMCs is shown, the living PMCs are situated at the periphery of the cavity. ×165.
- Fig. 4. The processes of degeneration of PMCs shown. *a*, PMC which is about to degenerate; *b*, later stage; *c*, still later stage, where the membrane and the cytoplasm have almost disappeared, the nucleus also being much affected; *d*, still later stage, nucleus only visible. ×750.
- Fig. 5. Cross section of an anther. The cavity filled with mucilaginous substance derived from the degenerated PMCs, in which sterile pollen grains are floating. ×333.
- Figs. 6-8. Several kinds of pollen grains in anthers, in which almost all pollen grains are sterile. *a*, healthy looking pollen grain; *b*, dumb-bell shaped two-nucleated pollen grain; *c*, an enormous pollen grain, probably reconstructed PMC; the small grains in these cells are starch grains. ×333.
- Fig. 9. Degenerated ovule *e*, degenerated embryo-sac. ×165.