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POLYPLOIDY IN *DIOSCOREA*

by

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INTRODUCTION

With the advancement of the knowledge of chromosome behaviour in plants, the role of polyploidy in speciation is being more and more appreciated (BHADURI, 1933; MÜNTZING, 1936; MANTON, 1937; EMSWELLER and BRIERLY, 1940; BLAKESLEE, 1941). Since the classic works of BLAKESLEE, GATES, DARLINGTON, MANTON, MÜNTZING etc. bringing forth the evidences of innumerable polyploid forms in nature, investigations on this aspect have been carried out in different centres. These researches have established the significance of this particular phenomenon in evolution, and practically speaking, very few families are on record, whose diversifications have not been influenced by polyploidy.

Not only the families show a series of different polyploid genera starting from the basic set, a number of species are on record of the

same genus where also such a series is found (SAKAMURA, 1918; HAGERUP, 1932; BLAKESLEE, 1941; CLAUSEN, 1941). The evidences of its occurrence in practically all the plant sections, – strongly justify the practice of considering this as a criterion in forming a natural and phylogenetic system of classification (GUNDERSON, 1950).

In addition to the presence of polyploid representatives in different genera and species, a number of varieties of a single species have been claimed to possess polyploid constitution. Such a phenomenon is well exemplified in the plants by diploid, tetraploid and hexaploid representatives of *Solanum nigrum*, recorded by BHADURI (1933). This series is often cited as an example warranting the invalidity of the theory of "weediness- an after-effect of polyploidy".

A number of polyploid species is reported in the genus *Dioscorea* (DARLINGTON and JANAKI AMMAL, 1945). It is well represented in Bengal by a variety of species all occurring mostly as climbers. As the role of polyploidy in the speciation in this genus is well established, it was thought desirable to have a further investigation on this aspect amongst the representatives of Bengal. Members of the same species were collected from diverse habitats and were grown in the College compound. It was presumed that an investigation of individuals of the same species might reveal evidences of the occurrence of the polyploid types as obtained in *Solanum nigrum*. It is interesting to note that the presence of different polyploid individuals within the same species has been demonstrated in the present investigation.

MATERIALS INVESTIGATED

The materials investigated in the present case were collected from the suburban areas of Calcutta, and they were grown in the College garden. All the members are typical stem-climbers on trees, belonging to various genera. Bulbils were collected and sown in soil and profuse roots could be obtained within four to five days of sowing. Root-tips were collected both directly from germinating bulbils and from plants growing in pots.

The various types of these plants differend mainly in the structure of leaf and stem. At first they were collected and numbered as type A, type B etc., and later were identified from the Indian Botanic Garden, Sibpur, as different forms of *Dioscorea alata* L. and of *Dioscorea sativa* L.

It is to be stated here that for the last four or five years, some individuals are growing in the Ballygunge Compound without producing any flowers whatsoever. As such, the observations had to be restricted mainly to the somatic chromosomes. It is desirable to study the meiotic stages, if flowering is noted at any time of the year.

METHODS

Dioscorea has been found to be a very difficult material for cytological studies. Trials for fixation in different chemicals proved to be of no use as the sections are very difficult to stain in crystal violet, and practically speaking impossible in orcein.

Various fixatives involving both metallic and non-metallic constituents in varying proportions were used.

The difficulties in fixation and staining were later found to be due to different cytoplasmic constituents causing hindrance to the adsorption of stain particles. Rinsing in normal Hydrochloric acid for two minutes of the root-tips before fixation proved essential for the removal of cytoplasmic granules.

After such treatment, healthy root-tips were fixed in acetic-alcohol mixture (1 : 1) for about forty-five minutes at room temperature. Subsequent to fixation, the root-tips were hydrolysed in N.HCl for fifteen minutes at 58° C. Feulgen squashing was performed in the usual manner. The best result was obtained on mounting them in aceto-carmine solution, instead of in 45% acetic acid.

For the fixation of flower buds, Belling's mixture of Navashin A and B in equal proportions was found to be most advantageous. For flower buds and root-tips, paraffin sections were cut at a thickness of 16 μ and 14 μ respectively. The slides were stained following the usual schedule of Newton's crystal violet-iodine technique. Thirty seconds iodine-mordanting instead of the usual forty-five seconds was found to be highly effective in case of flower buds.

The figures were drawn using Leitz compensating eye-piece of X 18 and X 20 and a 1.3 apochromatic – objective with an aplanatic condenser of 1.4 N.A. at table magnification of approximately X 3600.

OBSERVATIONS

The extremely minute size of the chromosomes is characteristic of the genus, all of which are more or less of the same size and shape in different species. Most of the chromosomes possess median primary constrictions and a few are satellited. The size difference of the chromosomes varies between 0.45 μ and 1.55 μ.

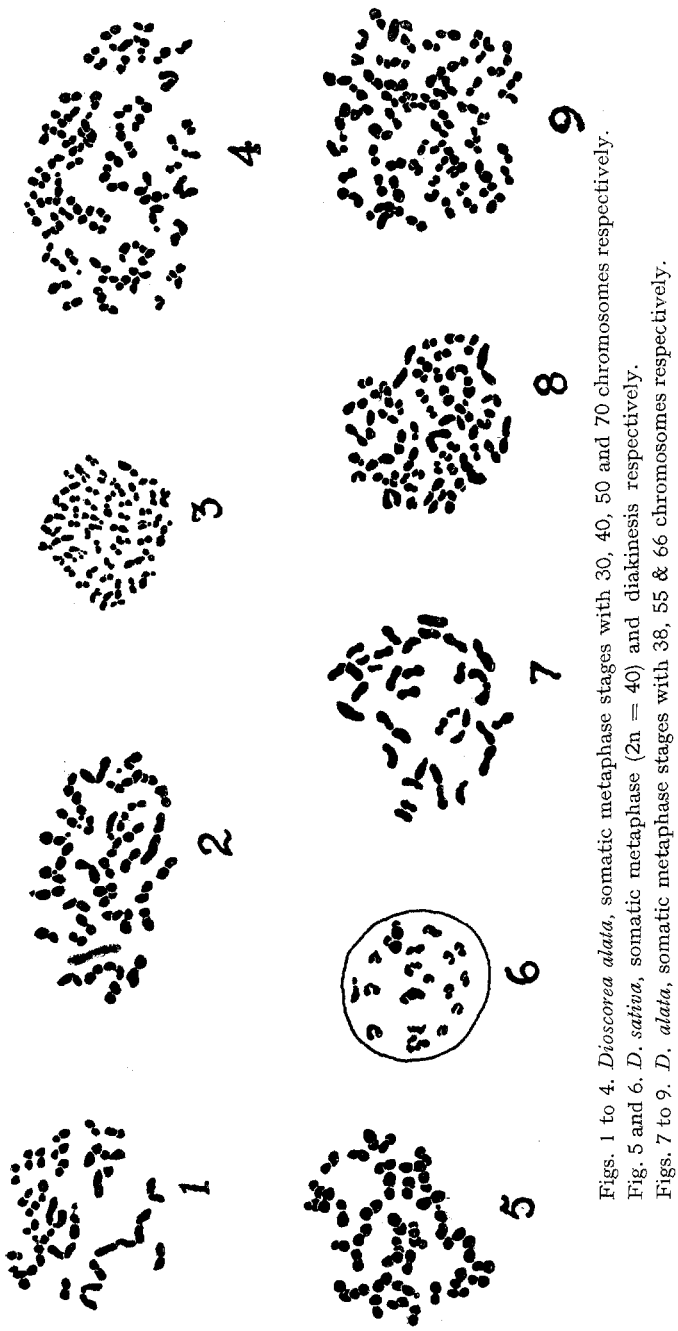
The chromosome counts in *Dioscorea sativa* show the number to be $2n = 40$ (Fig. 5), but the case with *Dioscorea alata* is different, different types having different chromosome numbers as thirty, forty, fifty, seventy together with many variations in details and chromosome number (Figs. 1–4 and 7–9).

Chromosome counts in different types of *Dioscorea alata* are given below:

Type	Chromosome No.	Satellites noted
A	70	6
B	50	4
C	40	6
D	30	2

It is evident from the table that the four individuals so far studied differ widely in their chromosome number, all being multiples of ten. Satellite numbers too have been found to vary, but not in direct proportion to the chromosome numbers. It is quite likely that the minute size of the chromosomes and their satellites render their visibility difficult. In addition to different chromosome numbers in different individuals, variations in number were noted even in the same root-tip in a very negligible percent of the cases. This might be due to non-disjunction of certain chromosomes during the mitotic division. No marked variation in chromosome morphology could be found in the different types of *Dioscorea alata*. The satellite number noted in *Dioscorea sativa* is two only.

Meiotic studies were only possible in *Dioscorea sativa*, as luckily it was once found to flower in a suburban area of Calcutta. It was at once fixed in Belling's mixture of Navashin A and B, and the only divisional stage that could be noted was diakinesis, in which clear twenty bivalents were exhibited, with no abnormality (Fig. 6).



Figs. 1 to 4. *Dioscorea alata*, somatic metaphase stages with 30, 40, 50 and 70 chromosomes respectively.
Fig. 5 and 6. *D. sativa*, somatic metaphase (2n = 40) and diakinesis respectively.
Figs. 7 to 9. *D. alata*, somatic metaphase stages with 38, 55 & 66 chromosomes respectively.

DISCUSSION

The members of the genus *Dioscorea*, most of which are inhabitants of the tropics, serve as very good examples for demonstrating the role of polyploidy in speciation within the genus. All the species are characterized by having 2n numbers as derivatives of ten, and thus indicate the role of a homogeneous and single line of evolution within the genus. In this polyploid series, at one extreme lie species like *D. caucasica* (SMITH, 1937), *D. tokoro* (NAKAJIMA, 1933) etc. having twenty as the somatic number. At the other extreme are species like *D. oppositifolia* (SMITH, 1937), *D. cyannensis* (SMITH, 1937) etc. having as many as one hundred and forty chromosomes in the body cells. A series of polyploid forms is traced between the two extremes.

How far simple polyploidy alone can be responsible for the origin of species is still a much debated problem. No doubt, with the attainment of the polyploid condition, the interaction of the genes and their expression at the same time are markedly affected. This may result in the expression of characters hitherto suppressed. But even in that case, in order to attain a specific status, that is to maintain their individuality, certain amount of sterility barrier is needed. This is in most cases, assumed to be brought about by gene mutation.

But whether simple polyploidy associated with gene mutation for sterility can bring about the origin of different species of *Dioscorea* is questionable in view of the findings of the present investigation. It has been clearly demonstrated here that individuals of a single species may behave as members of a polyploid series. In case of *D. alata*, for example, numbers of thirty, forty, fifty and seventy have been reported in different individuals. It is interesting to note that their phenotypic characters show no evidences of marked difference from one another. It may be claimed, therefore, that polyploidy alone in *Dioscorea* does not result in the manifestation of characters which can be detected morphologically.

If such polyploid forms, during evolution become associated with gene mutation inhibiting cross fertiiization, one cannot expect the origin of types with phenotypic differences as marked as to raise them in the status of different species. In the light of these findings, it may be concluded, therefore, that speciation within the genus *Dioscorea* has been caused not solely through polyploidy, but by the latter

being associated with certain other changes responsible for phenotypic differences, at the same time being supplemented with gene mutation for inducing a sterility barrier. These changes, which are responsible for phenotypic difference, might involve only gene mutation or might have evolved from gross structural changes of chromosomes. The species having very short chromosomes provide immense difficulties in the study of their structure in detail. A critical investigation into the karyotypes of different species of *Dioscorea* with the aid of the refined techniques at present available may provide facts of fundamental importance. An investigation in this direction is highly desirable.

The formation of twenty bivalents in *D. sativa* does not necessarily indicate that the species is not an autopolyploid. It is an established fact (DARLINGTON, 1937), that chiasma frequency is significantly controlled by the length of the chromosome. The chromosomes of *Dioscorea* being extremely short may normally possess a very low chiasma frequency, a fact which would necessarily stand against the formation of multivalents in the polyploids.

Varying chromosome complements in somatic cells and their significance

In addition to the occurrence of polyploid series in different individuals of the same species, variations in chromosome number have been recorded even within the same individual. Cases of polysomaty no doubt are known in certain species, but as far as this record goes, such variations do not include multiplications of the whole set, but rather it is at random involving no particular chromosome. This results in the origin of cells with numbers, such as thirty-eight, fifty-two, fifty-five, sixty-six etc. in the same individual.

The survival of these cells and their subsequent entrance into the formation of the germ tissues seems remote. This is in view of their less chance of survival in competition with the normal ones, which are found in much larger numbers than in these cells.

The means of propagation of the species is quite remarkable. It is mainly vegetative, facilitated by the formation of the bulbils at the nodes. During the favourable season, innumerable bulbils are formed, which being detached off from the plant in the matured state, give rise to a number of individuals. Reproduction through sexual means,

though a natural process, is very infrequent. If the cells with abnormal number of chromosomes in the somatic tissue survive and enter into the growing point of the bulbils, new individuals, with chromosome numbers different from those in the polyploid series, can easily come out. Moreover, such individuals have not so far been recorded. In view of these present cytological findings, a thorough investigation into a large number of individuals of the same species is highly desirable. The evidences of origin of new types through somatic cells in the vegetatively propagated plants have been gathered in a number of species of Aroids and Amaryllids (SHARMA and DAS, 1953; SHARMA and GHOSH, 1953; SHARMA and Bal, 1954; SHARMA and BHATTACHARYYA, 1954; SHARMA and MOOKERJEA, 1955). The presence of such varying numbers in the same tissue was also reported by CHAKRAVORTI (1951) in *Musa*.

The present investigation may, therefore, be considered as a preliminary report on the cytology of the genus *Dioscorea*, which has further opened up possibilities of investigation in the genus. The necessity of a thorough research of karyotypes of different species with the aid of refined technique has been revealed. Further, their importance has been emphasized in the studies of the different individuals of the same species. It is expected that if these two aspects of research be successfully carried out, some of the fundamental problems regarding the evolution of the genus *Dioscorea* will be solved.

SUMMARY

1. Bulbils of *Dioscorea* of different species and individuals were collected and grown in the garden. Somatic number was found out following Feulgen squash technique.

2. *Dioscorea sativa* has a chromosome number of $2n = 40$ and *D. alata* has a polyploid series of thirty, forty, fifty and seventy chromosomes in different individuals. Satellite counts were also taken.

3. Certain individuals of *D. alata* revealed the presence of different chromosome numbers, viz., thirty-eight, fifty-two, fifty-five, sixty-six in the somatic cells of the same individual.

4. Meiotic divisional stages were only obtained in *D. sativa* showing clear twenty bivalents.

5. Significance of a polyploid series in the same species and different

chromosome numbers in the same individual, especially in plants propagating mainly through vegetative means, from the point of view of speciation has been discussed.

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