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Polyploidy and Pollen Variability in *Pimpinella monoecia*

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Abstract. Treatment of seedlings of *Pimpinella monoecia* with colchicine-gammexane lead to the production of tetraploids. The tetraploids besides showing the meiotic anomalies like univalents, split spindles, multiple spindles, micro-nuclei, nondisjunction, strays, etc., exhibited pollen grains which were triradiate, triangular and quadriradiate while the controls had only one type of bipolar radiosymmetric grains. They have been classified into different types and their frequency has been determined. A scheme has been presented representing the development of these variable grains from the control type A, which is based on the observation of grains showing intermediate stages. Explanation of this pollen variability has been presented.

The artificial induction of chromosome doubling has wrenched the initiative from nature in the evolution of new types. That this has been nature's main tool of evolution is clear from the abundance of polyploids in the plant kingdom. Nature appears to have solved many of its tricklish problems of hybrid sterility and adaptability by increasing the level of ploidy. Though no longer considered to be the sole solution of improving varieties, yet, still polyploidy continues to have its sway among ornamentals where large attractive polyploid flowers offer nobility of a most valued type. Ornamental lovers are constantly hunting for nobility and polyploidy most often than not meets their requirements. *Pimpinella monoecia*, with its imposing umbells of white flowers, is much sought after ornamental. Artificial induction of polyploidy in this species was undertaken to improve the ornamental valve.

Materials and Methods

The seedlings of *Pimpinella monoecia* were treated with colchicine-gammexane following the technique developed in this laboratory (RAGHUVANSHI and JOSHI, 1965). Material for cytological study was fixed in 1 : 3 acetic-alcohol. Slides were made permanent by ethanol-butanol schedule. To calculate the frequency of variable grains and for the study of structure of grains, slides were prepared by the classical method of acetolysis (ERDTMAN, 1952).

Results

Morphology.

Phenotypically the diploids and tetraploid plants were quite similar in height. There was increase in size of various floral parts (Fig. 1). The flowering period of tetraploids was extended.

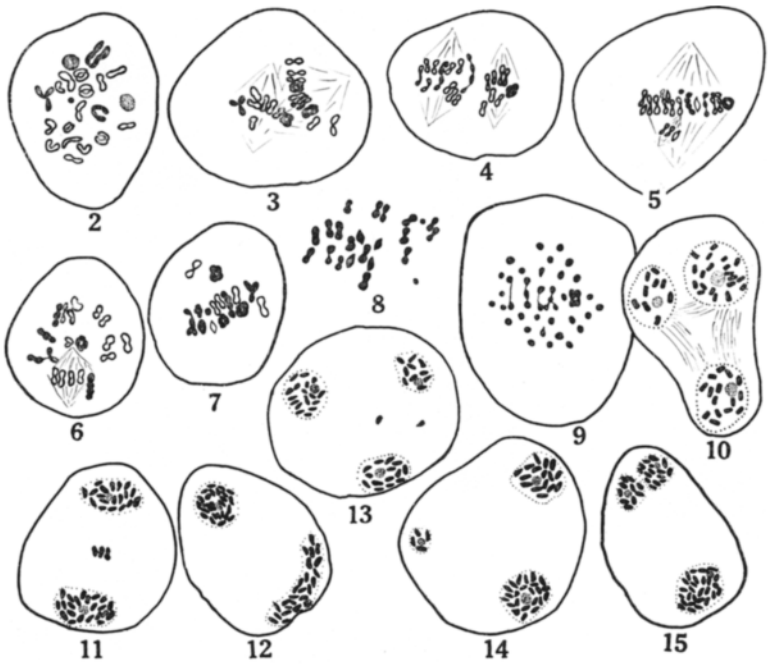
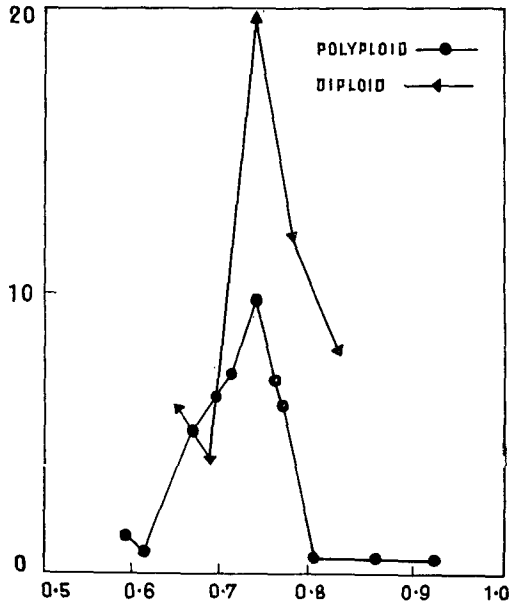


Fig. 2. Diakinesis showing multivalents.
Figs. 3 and 4. Two M. I spindles of almost equal and unequal size respectively.
Fig. 5. A microspindle having only three bivalents along with the normal one.
Fig. 6. Multiple spindles at M. I.
Fig. 7. M. I showing multivalents.
Fig. 8. M. I having no multivalents.
Fig. 9. Late separating multivalents at A. I.
Fig. 10. T. I resulting from a tripolar separation.
Fig. 11. T. I showing three laggards.
Fig. 12. Lax nucleus (sickle-shaped) along with the normal one at T. I.
Fig. 13. T. I resulting from a tripolar separation and stray chromosomes.
Fig. 14. T. I. with two micro-nuclei having 2 and 4 chromosomes respectively.
Fig. 15. T. I with 3 nuclei resulting by a split at one of the poles along with stray chromosomes.

Cytology.

Meiotic study of the controls revealed 22 chromosomes which formed bivalents only. The average number of chiasmata per PMC was 16·24, while the range was from 14 to 18. Meiosis was regular. However, tetraploids exhibited considerable meiotic instability.

In 4n plants at M. I one or two univalents occurred in 70% of the cells, out of which in 52% cases they accompanied a trivalent (Fig. 2). In certain cases the chromosomes were orientated on two equal or unequal equatorial plates Figs. 3, 4, 5). The two groups may or may not be synchronised. The number of quadrivalents ranged from 0 to 7 (Figs. 8, 7). The comparison of frequency of half chiasmata per chromosome of diploids and tetraploids is graphically represented in Graph 1. Although the lowest and highest value of half chiasmata shows a wider range in the tetraploids, yet the highest frequency of both the groups falls on a common figure, i.e. 0·73 half chiasmata per chromosome. Statistical analysis of chiasma frequency was made in the tetraploids and controls following the half-chiasmata-per-chromosome method. Some of the statistical constants are represented below.



Graph 1. Frequency of half chiasmata in diploids and tetraploids. Relationship between frequency of half chiasmata per chromosome (ordinate) and number of half chiasmata per chromosome in different PMCs (abscissa).

Since the difference between the two means is less than S.E.D X 2 in the tetraploids, the observed difference between the frequency of half chiasmata is statistically insignificant.

Statistical constants	Controls	Tetraploids
Assumed mean	0·74	0·73
Standard deviation (S.D.)	0·117	0·01974
Standard error (S.E.)	0·06155	0·02791
S.E.D		0·6754
S.E.D X 2		1·3514
Difference between the two means		0·01
Results significant or not		not significant

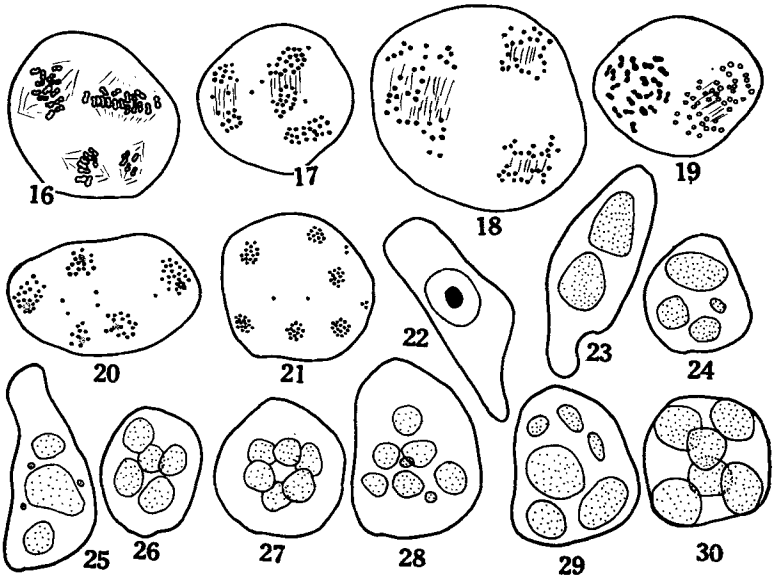


Fig. 16. Four spindles at M. II.
Fig. 17. Three spindles at A. II.
Fig. 18. A. II in a PMC which may have undergone tripolar separation at A. I.
Fig. 19. Spindle breakdown at A. II.
Fig. 20. Five nuclei at T. II along with strays.
Fig. 21. Six nuclei at T. II with strays.
Fig. 22. Mononucleate sporad resulting from a non-dividing PMC.
Figs. 23—30. PMCs having a varying number of microspores at sporad stage.

Out of 109 cells analysed at M. I 82 were normal, while anomalies observed were 2 spindles (8 PMCs), 3 spindles (1 PMC), split nature of spindle (2 PMCs) and 16 showed stray chromosomes. At A. I besides other anomalies multivalents separated later than bivalents (fig. 9). Out of 160 PMC's, 105 were normal, 18 showed tripolar spindles (Fig. 10), 11 had stray chromosomes, 16 had 1 to 2 laggards, while 6 showed 4 nuclei instead of the normal 2 — direct result of 2 spindles instead of 1, and lastly 4 PMCs indicated breakdown of the spindle mechanism. Similarly, at T. I there were laggards (Fig. 11), sickle shaped nuclei (Fig. 12) and 3 nuclei (Figs. 13, 14). It may be added that in a few buds more than 60% of the PMCs showed sickle-shaped nuclei instead of the normal round one (Fig. 12). The split nature of one of the poles is indicated in Fig. 15. At M. II the number of spindles corresponds to the number of nuclei at T. I. Out of 120 PMCs 85 were normal, 6 showed 3 spindles, 4 had 4 spindles (Fig. 16), while 25 had 1 to 2 stray chromosomes. Fig. 17 shows anaphasic separation in 3 spindles along with stray chromosomes. There are three spindles indicating the origin from a tripartite separation at A. I in Fig. 18, while breakdown of spindles is shown in Fig. 19. These meiotic anomalies give rise to a T. II having a variable number of nuclei (Figs. 20, 21). The sporads exhibited a wide range of variation both in size and number of spores (Figs. 22—30).

Pollen grains.

Although the grains of the controls were all radiosymmetric, 3-colporate, perprolate, that of tetraploids were an admixture of normal type as well as asymmetric non-fixiform grains which exhibited different shapes in equatorial view. Ontogeny of the variable grains revealed similar lines of development as observed in *Foeniculum vulgare*, *Anethum graveolens* and *Coriandrum sativum* (RAGHUVANSHI and JOSHI, 1964, 1966a-c: JOSHI and RAGHUVANSH, 1965b). Such variable grains have been observed in the first generation of the tetraploids of other umbellifers and to determine their frequency they have been classified on the basis of their shape into different types as found in *Foeniculum vulgare* which had types A to M (RAGHUVANSHI and JOSH, 1965d). In *Coriandrum sativum*, while some of the variant types observed in *Foeniculum* were missing, there were three new additions of types N, O and α, the last being the result of direct transformation of a PMC into a pollen grain without the intervention of meiosis (JOSHI and RUGHUVANSH, 1965d). In the present case tetraploids of *Pimpinella* show the following frequency in a count of about a thousand grains. Types A (120), O and E (348), F (12), G (498) and micropollen (18). Besides these there were certain very rare shapes. The different types are described below and their development has been presented in Fig. 45. It may be mentioned that this scheme is based on the observation of various transitional stages of different types of rains observed during the course of study. Type A (Fig. 31). The grains are radiosymmetric, 3 colporate, prolate-perprolate. Sexine tegillate. The size of this type in controls and tetraploids does not show any difference. Types E and O (Figs. 34, 35, 36, 37, 41, 42, 43, 44). Both these types are triradiate but type E arises due to development of a lobe at the equatorial region of type A, while in the type O two arms arise at one of the poles in opposite directions. Type F. These grains are triangular. Type G (Figs 38, 39, 40). This is tetralobed grain in which four arms are not in the same plane. The size of the four arms may be same or they may differ. Depending upon the plane in which the grain is lying it may show three arms in one plane with the fourth more or less at right angles (Fig. 38), or two arms in one plane and two in another placed in a cruciate pattern (Figs. 39, 40). The poles of the arms may be rounded or pointed. Micro-pollen. These small grains may be circular, oval, rectangular or have the shape of variants. Their origin has been from micro-spores which have

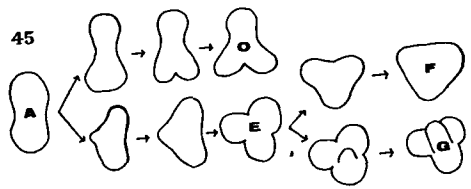


Fig. 45. Scheme of development of variable grains.

less than the normal chromosome complement. The ornamentation on the walls is the same as in the variable grains. Fig. 33 represents type N, a rare occurrence in the present study, although in *Coriandrum sativum* they were present in appreciable frequency (JOSHI and RAGHUVANSHI 1965b). In this

type a side protrusion arises at one of the poles making the grain look like the letter L. or J. Differential development of bacula also give rise to variants (Fig. 32).

Discussion

The morphological characters of the tetraploids as regards the flower size are superior to the diploids, thus improving its ornamental value. The frequency of univalents in 4n is very low, never exceeding 2 and invariably accompanying trivalents. Our experience with umbelliffers indicates that they have a low frequency of univalents in general (JOSHI and RAGHUVANSHI 1965b; RAGHUVANSHI and JOSHI 1966a). The highest configuration was quadrivalent, the number of which never exceeded 7 in any PMC. Multiple chiasmata was a special feature of the present study.

The chromosome complement was subdivided into two or more groups that functioned independently inside a PMC. This phenomenon designated as "complement fractionation" by THOMPSON (1962) has also been described in the colchiploids of *Capsicum frutescens* variety "Oshkosh" (RAGHUVANSHI and JOSHI 1964a). The presence of more than two spindles at M. II may also be the result of multiple spindles at M. I. At T. I the chromosome complement may be separated into two, three or four nuclei and regardless of the number of chromosomes in the nuclei they regularly divide and pass on to the further division stages.

Another phenomenon encountered in the present study is non-disjunction, which results in the formation of diads or monads with double or four times the gametic number. It may be mentioned that certain PMCs having single deeply stained nucleus do not represent the outcome of non-disjunction but are the PMCs in which division has been arrested completely, perhaps at the premeiotic stage. Such cells were encountered in considerable frequency in *Coriandrum sativum* (JOSHI and RAGHUVANSHI 1965a, 1965b). The behaviour of such PMCs has been explained on the basis of Operon theory of JACOB and MONOD (1961).

Unequal distribution of chromosomes at A. II further gives rise to gametes with a varying number of chromosome.

Although pollen variability is well-exhibited by the tetraploids, pollen of the controls was uniformly of the same shape. CERCEAU (1959) and TING (1961) have studied the pollen of some umbellifers. Our investigations with umbellifers indicate that there exists some sort of tendency in them towards pollen variability, which is expressed by the application of certain chemicals. Disturbance from the normal conditions of the factors governing the development of pollen grains results in their variation. The various transitional stages observed clearly justify the assumption that the different shapes result due to a disbalance in the polarity in pollen grains caused by treatment with various chemicals.

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Aplikace kolchicinu — gammexanu na semenáčky *Pimpinella monoecia* vede ke vzniku tetraploidů. U tetraploidů se vyskytují meiotické anomálie jako univalenty, rozštěpy vřetena, mnohonásobná vřetena, mikronuklea, neoddalování aj. Pylová zrna jsou tří- až čtyřpaprsčitě souměrná, zatím co u kontrol se vyskytují jen dvoupaprsčitě symetrická. Jsou v práci roztříděny v typy a určena jejich frekvence. Uvedeno schéma vývoje těchto tvarů pylových zrn vycházející z kontrolního typu a založené na pozorování intermediárních stádií. Podává se vysvětlení variability pylu.

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Применение колхицина-гаммексана на сеянцы *Pimpinella monoecia* приводит к образованию тетраплоидов. У тетраплоидов появляются мейотические аномалии как унивалеты, расщепления веретен, многократные веретена, микронуклеи, неудаления и др. Симметрия пыльцевых зерен три- и четыре-лучевая, в то время как у контрольных лишь двулучевая. Приводится классификация типов зерен и их частота, схема развития форм пыльцевых зерен построенное от контрольного типа и основанное на наблюдении интермедиарных форм. В работе объясняется вариабильность пыльцы.