A Comparative Study in Umbellifers of Artificially Induced Polyploids and Structural Hybridity with Special Reference to Change in the Expression of Gene (Genes) Controlling the Pollen Shapes

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Abstract

The paper deals with a comparative data of cytological and palynological salient features of chemically induced autoploids and translocation heterozygotes of certain members of Umbelliferae (Anethum graveolens, Coriandrum sativum, Foeniculum vulgare, Pimpinella monoecia, Carum copticum, Cuminum cyminum and Daucus carota). In general the plants exhibit low multivalent frequency. At diakinesis Carum copticum has no univalents and trivalents while Anethum graveolens shows complete absence of trivalents, the remaining four genera have all the expected configurations. A comparative idea of the cytological anomalies exhibited by the plants understudy is also provided. A feature shared by all the plants investigated is the presence of nondividing pollen mother cells in their sporogenous tissue. The pollen of all the polyploids and some 2n treated plants show variability in shape while that of the controls is uniformly bipolar, radiosymmetric—characteristic of the family. The variable grains have been classified into definite types. Explanation has been put forward regarding this artificial induction of pollen variability and a scheme of the lines of development of the different shapes is also presented.

Introduction

The family Umbelliferae is distributed mostly in the northern hemisphere—widely dispersed throughout boreal, temperate and sub-tropical regions but rare in the tropics except in the mountains. The name is derived from the prevailing umbelate inflorescence; many species are cultivated for food, others for ornamentals and for medicinal products as well as condiments. Artificial induction of autoploids and translocation heterozygotes of certain members of this family has been undertaken. Since the plants investigated present a certain trend not common in other polyploids it was considered worthwhile to present a comparative account of the polyploids of different genera investigated.

The autotetraploids as well as certain diploid treated plants of different genera exhibited pollen variability while controls had only normal bipolar pollen grains. On the basis of shape these grains have been classified into different types. It was observed that some of the types were present in all the genera while others were peculiar to a particular genus. Besides com-
parative account of cytology of polyploids and structural heterozygotes of different genera, the paper presents the study of various pollen types, their frequency and mode of origin.

**Materials and methods**

Polyploids were produced by treating the seedlings with colchicine-gammexane solution making use of the technique developed in this laboratory (Raghuvanshi and Joshi 1965). Studies of the following genera are included in the present paper.

1. *Anethum graveolens* $2n=22$
2. *Coriandrum sativum* $2n=22$
3. *Foeniculum vulgare* $2n=22$
4. *Pimpinella monoecia* $2n=22$
5. *Carum copticum* $2n=18$
6. *Cuminum cyminum* $2n=14$
7. *Daucus carota* $2n=18$

The details of the technique followed for the study have already been given elsewhere (Raghuvanshi and Joshi 1966, Joshi and Raghuvanshi 1965a).

**Results**

A comparative account of different stages of nuclear division of the various genera is as follows.

**Diakinesis.** Tetraploids exhibited the classical configurations of quadri- valents, trivalents, univalents and the usual bivalents. The frequency of the multivalents in $4n$ plants of different genera is represented graphically in Fig. 9. In all the genera the frequency of quadri- valents and bivalents is inversely proportional while the values of trivalents and univalents over-lap each other. The highest frequency of quadri- valents was observed in *Carum copticum* which had the lowest percentage of bivalents. Inversely *Cuminum cyminum* had the lowest frequency of quadri- valents with the highest percentage of bivalents (Fig. 6) while the other four genera hold intermediate position between these 2 extremes.

In general in the autotetraploids of non umbelliferous plants the frequency of quadri- valents quite often exceeds that of the bivalents. For direct comparison of percentages observed in the tetraploids of *Torenia fournieri* (Joshi and Raghuvanshi 1966) and *Capsicum frutescense* (Raghuvanshi and Joshi 1964) are also given. It will be noted that the frequency of univalents and trivalents does not deviate much from that of the umbellifers but the quadri- valents and bivalents are in the reverse proportion. The absence of trivalents in *Anethum graveolens* (Fig. 4) and of trivalents as well as univalents in *Carum copticum* are quite outstanding in the graph. Quadri- valents may be chains or rings while in *Pimpinella* triple chiasma configurations have also been observed. The minimum frequency of IVs varies from 0 to 1
while in one PMC of *Carum copticum* all the 36 chromosomes were associated as quadrivalents.

**Metaphase I.** Most of the plants had one plate except in the case of *Pimpinella monoecia* and *Anethum graveolens* where multiple spindles were present. Bivalents off the metaphase plate were seen in *Foeniculum vulgare*, *Cuminum cyminum* and *Carum copticum*. Precocious separation of chromosomes from the metaphase plate was noted in *Foeniculum vulgare* and *Cuminum cyminum* while univalents in appreciable number were seen in *Pimpinella monoecia*. In mixoploids of *Coriandrum sativum* (E. 19) up to 88 chromosomes were found in a single plate (Fig. 1). A general trend to be noted in the umbellifers is the low frequency of univalents indicating high percentage of pairing in autotetraploids.

![Fig. 9. Comparison of the frequency of the configurations (I, II, III, and IV) in percentage in the tetraploids of the various genera.](image-url)
Anaphase I. Typical anaphase separation was found in Carum copticum while in other plants anomalies like laggards, strays, unequal separation, breakdown of the spindle mechanism, bridges, multipolar and multiple spindles were prevalent. Table 1 gives the comparative idea of the anomalies in the different plants investigated.

Telophase I. In cases where there was non-disjunction of chromosomes at A.I the resulting two nuclei are eventually of unequal size. Breakdown of the spindle has given rise to a mononucleate T.I instead of the two nucleate.

Figs. 1–8. 1, M. I in a 88 chromosome PMC of Coriandrum sativum mixoploid. 2, a non-dividing PMC along with normal tetrads at sporad stage in Coriandrum sativum. 3, nuclear migration from type a into type A grain in the form of a protuberance in Coriandrum sativum. 4, a metaphase plate showing multivalents in Anethum graveolens tetraploid. 5, a variable (type G) pollen grain showing chromosomes in Coriandrum sativum. 6, diakinesis showing all bivalents in Cuminum cyminum tetraploid. 7, and 8, diplotene, diakinesis in Cuminum cyminum showing a quadrivalent in the translocation heterozygote.
Metaphase II. *Foeniculum vulgare* and *Anethum* have strays. In *Coriandrum sativum* most of the PMC's have 2 plates while in a few microplates were also spotted. In certain cases chromosomes arising by precocious separation of the chromatids were noted at the poles in *Foeniculum vulgare* and *Pimpinella monoecia*. Instead of the normal 2 spindles 3, 4 and even up to 5 have been common in *Foeniculum vulgare* and *Pimpinella*.

Anaphase II. Multiple spindles in *Foeniculum*, *Pimpinella* and *Anethum* gave rise to confusing arrangement of chromosomes on the spindles, hence, the origin of each chromosome group from the spindle had to be traced with great caution. Strays and laggards were a common feature at this stage in *Pimpinella*, *Anethum*, *Coriandrum* and *Cuminum*. Breakdown of spindles was a common anomaly in *Cuminum cyminum* and rare in *Coriandrum*, *Pimpinella* etc.

Table 1. The comparative data of meiotic anomalies in percentage at anaphase I

<table>
<thead>
<tr>
<th>Plant</th>
<th>Normal</th>
<th>Lag-</th>
<th>Strays</th>
<th>Unequal separation</th>
<th>Breakdown of spindle</th>
<th>Bridges</th>
<th>Multiple spindle</th>
<th>Multiple spindles</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cuminum</em></td>
<td>39</td>
<td>28</td>
<td>9</td>
<td>16</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Coriandrum</em></td>
<td>59</td>
<td></td>
<td></td>
<td>13</td>
<td>11</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pimpinella</em></td>
<td>67</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td></td>
<td></td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td><em>Foeniculum</em></td>
<td>37</td>
<td>25</td>
<td>14</td>
<td></td>
<td></td>
<td>7</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td><em>Anethum</em></td>
<td>72.0</td>
<td>13</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Carum</em></td>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Daucus</em></td>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Telophase II. The meiotic anomalies of the preceeding stages are directly reflected at telophase II. Strays and laggards gave rise to micronuclei which after the sporad stage transformed into micro-pollen grains.

Sporad stage. Table 2 gives the frequency of microspores per sporad met in the different plants.

Non dividing pollen mother cells. A noteworthy feature in the genera under investigation is the occurrence of nondividing PMC's in their sporogenous tissue (Fig. 2). Within the sporogenous tissue certain cells do not undergo further division. The nucleus assumes a perfectly inter-phasic appearance and with the passage of time they get transformed into PMC's, sporads and finally pollen grains (Fig. 3), but, the nuclear condition remains the same. A detailed behaviour has been described in E.67 of *Coriandrum sativum* in which they appear in significant numbers (Joshi and Raghuvanshi 1965b). Such PMC's have also been observed in different species of *Allium*.

Structural heterozygosity. During the course of analysis of colchicine-gammexane treated plants translocation heterozygosity was observed in *Coriandrum sativum* and *Cuminum cyminum*. In the latter quadrivalents having
the shape of 8 were observed at diakinesis. In this 2n plant 34% cells had a quadrivalent (Figs. 7, 8) while in the tetraploids only 14% of the PMC’s had it.

The 2n plants exhibiting pollen variability. Certain 2n plants resulting from colchicine-gammexane treatment also exhibited pollen variability. Some of them had normal meiotic behaviour while others exhibited various anomalies.

Pollen grains. The unique feature of all the polyploids was the presence of variable shape of pollen grains while the grains of controls of all the genera are radio-symmetric, bipolar, 3 colporate- per prolate. The grains of the treated plants are asymmetric and nonfixiform. Among the variable grains the equatorial view exhibits variable shapes. The grains may be elliptical, oval, oblong, rounded, trilobed, 4-lobed, 5-lobed to 6-lobed. The arms of the lobed grains may be of equal size or they may show difference in length. The grains may have symmetric arrangement of the lobes or may be placed in an asymmetric fashion.

The poles may be round, pointed or rather tapering while they may also be flattened. At the equatorial region there may be a bulge or a constriction. In the former case the grain may appear rhomboidal while in the latter case it may be knuckle bone shaped. The grains are colporate, colpi usually long. The apertures are non-operculate. Sculpture on sexine almost psilate. Local thickenings may occur anywhere on the surface of the grain. Ectosexine tenuitegillate but it may be crassitegillate while it may also be an admixture of the two on one and the same grain. The grains may be conspicuously baculate, simple or hetero-brochate. The apertures may be at the equatorial region, Umb angular. The ontogenetical studies of the grains showed that these different grains arose from a single microspore. The grains developing from one sporad were not of one shape but an admixture of the various types encountered in that particular plant. The normal type as well as the variable types developed together within the same sporad.

In order to give a comparative idea of the various variable grains, they have been classified into different types, and their frequency in different genera

Table 2. Frequency of microspores per sporad (in percentage) in the tetraploids of the various genera

<table>
<thead>
<tr>
<th>Plant</th>
<th>No. of microspores per sporad</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>Anethum</em></td>
<td>0.18</td>
<td>12.84</td>
</tr>
<tr>
<td><em>Pimpinella</em></td>
<td>1.57</td>
<td>12.89</td>
</tr>
<tr>
<td><em>Cuminum</em></td>
<td>2.74</td>
<td>23.36</td>
</tr>
<tr>
<td><em>Coriandrum</em></td>
<td>2.92</td>
<td>3.5</td>
</tr>
<tr>
<td><em>Foeniculum</em></td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td><em>Carum</em></td>
<td>Majority have four, rarely a few with 1-3 micro also noted</td>
<td></td>
</tr>
</tbody>
</table>
is presented in Table 3.

The various types of grains have already been described in Coriandrum sativum (Joshi and Raghuvanshi 1965a) but a brief idea may be given below. Type A is the normal control type. Type B is a bicelled grain. Type C is trilobed with a side protrusion having a prominent beak at the equator. Type D a small rectangular grain. Type E a trilobed grain; Type F a triangular grain. Type G is a 4 lobed grain in which the lobes are not in one plane, Fig. 5 shows such a grain with chromosomes. Type H also a 4-lobed grain but with all the lobes in one plane. Type I is a circular grain. Type J a thick walled, four nucleate giant grain which is the transformation of the entire PMC into a single grain. Type K is like Type J but with thin walls. Type L is also a giant grain in which the ornamentation develops in a crust like fashion, the ornamentation is so
dense that the nuclear behaviour could not be studied. *Type M* includes all the polyads. *Type N* is a J or L shaped grain. *Type O* is a 3 lobed grain in which 2 of the lobes develop at one of the poles in opposite directions. *Type P* is a dumbell shaped grain constricted at the equator with flat poles. *Type Q* is a small grain like *Type D* with lobed poles rather than flat. *Type R* is a 5-lobed grain. *Type S* is a 6-lobed grain. *Micropollen* are grains which may be round, *Type A* like or may have the shapes of the variable grains. Another interesting feature was the presence of joined grains in *Anethum* which were like *Type A*; 4 nucleate joined giant grains were present in *Foeniculum vulgare*.

From the transitional stages come across during the course of study Fig. 10 brings out the possible mode of development of the various types of grains.

![Fig. 10. A possible mode of development of the variable grains as indicated by the ontogenetical study.](image)

**Discussion**

The cytological behaviour of the polyploids brings out the following salient features.

*Quadrivalents.* Autotetraploidy is generally accompanied by high quadrivalent frequency but the autotetraploids of umbellifers have a low frequency of quadrivalents. The highest frequency has been observed in *Carum copticum* while the lowest is in *Cuminum cyminum*. Out of all the plants studied only in one PMC of *Carum copticum* all the 36 chromosomes gave rise to 9
quadrivalents. The instance of *Cuminum cyminum* clearly indicates that in general there is a strong tendency among autotetraploids of umbellifers towards bivalent formation. The predominance of bivalent formation may be similar to hexaploids of wheat (Riley and Chapman 1958). The small size of the chromosomes may also be responsible for this.

*Trivalents* occur in very low numbers, the highest frequency being 2 while in *Carum copticum* and *Anethum graveolens* they are altogether absent.

*Univalents*. They are altogether absent in *Carum copticum* while in *Cuminum cyminum* they are significant. In the rest of the genera the percentage ranges between 0.09–0.6.

**Multiple and multipolar spindles.** These have been observed at the first metaphase in *Pimpinella monoecia*, and *Anethum graveolens*, while at the 2nd division in *Foeniculum vulgare*, *Pimpinella monoecia* and *Anethum graveolens*. As suggested by Swanson and Nelson (1942) and Walters (1958), they may be due to asynchronous division of chromosomes and pole determinants or spindle organisors. In *Mentha* Swanson and Nelson (1942) attributed multipolar spindles to extra pole determinants probably arising *de novo*. The complex spindles are produced by subdivisions or misdivisions of the spindle organisors. The newly formed spindle organisors attract proportional number of chromosomes and the spindles are organised. Depending upon the kind and the number of divisions the spindle organisor undergoes the spindles are multipolar or multiple as the case may be.

**Breakdown of the spindle.** A feature exhibited by *Cuminum cyminum*, *Coriandrum sativum*, *Pimpinella monoecia*, and *Anethum graveolens* at the first division, while at the second division one or two spindles show prominent breakdown in *Cuminum cyminum*. This obviously results in the production of pollen grains with higher number of chromosomes than the normal.

**Nondisjunction of chromosomes.** This results in unbalanced gametes; at A the tetraploids of *Cuminum cyminum* as well as *Coriandrum sativum* display this anomaly.

**Most stabilised behaviour of the tetraploids.** On the whole it may be concluded that *Carum copticum* has most stabilized tetraploids. It is unique in having no trivalents or univalents and high quadrivalent frequency—a character not common to tetraploids of other umbellifers.

**Non dividing PMC’s.** The presence of nondividing PMC’s is a spectacular feature of E.67 of *Coriandrum sativum* (Joshi and Raghuvanshi 1965b) as well as other members investigated. Their frequency may vary in the different genera. As postulated by the French scientists Jacob and Monod (1961) it might be possible that the switch genes controlling the nuclear division do not function in certain cells, hence the nucleus in the nondividing PMC’s has the same appearance throughout the sequence of events taking place in the other PMC’s. Their transformation into pollen indicated that these developments are not associated with nuclear division and both are
Pollen. Pollen grains in the controls of all the genera are uniformly of the same shape, typical for the family. From the studies of Cerceau (1959) and Ting (1961) on umbellifers it is clear that the great diversity of forms encountered in the plants subjected to chemical treatments is a unique feature. The grains show diversity not only in shape but also in size and this coupled with intensification and minimization of the development of bacula further magnified the spectrum of variability.

A survey of Table 3 brings out clearly the frequency of variability of the grains in the different plants. Amongst the polyploids type A grains are maximum in Anethum graveolens and minimum in Cuminum cyminum. In the 2n plants cited in the Table E.139 of Foeniculum vulgare has the lowest number of Type A grains and maximum number of Type G. Although in certain plants the frequency of Type A grains is high, yet, they exhibit a wide spectrum of diverse types and they had certain new types, not found in other plants. Examples may be sought in Daucus carota which has Type Q not present in other plants. E.120 of Anethum graveolens had joined grains. Type B occupies a prominent position in E.178, a mixoploid of Foeniculum vulgare; while in other plants it is insignificant. Type C is also well represented in E.128 while in the tetraploids of Carum coptium also it is outstanding. Type D has a wide distribution in polyploids as well as 2n plants, the highest number being in Daucus carota. The trilobed grain Type E is in maximum frequency in Cuminum cyminum and is present in all the plants studied. Type F is also represented in all except 2n plants of Anethum and Carum. Type G, the 4 lobed grain is without any exception present in all the plants, the highest frequency being in the tetraploid of Foeniculum vulgare. Type H was observed in a few cases only. Type J and K, the thick and thin wall giants were most frequent in Foeniculum, Cuminum and rare in Anethum. They represent the transformation of the entire PMC into a pollen in which cytokinesis has been arrested. The four nuclei within the grain are always noted to remain separate. Polyads (Type M) were observed in few cases. Type N is best represented in Coriandrum sativum while it is altogether absent in Foeniculum vulgare and Pimpinella monoecia. Type O is prominently represented in Pimpinella while it is absent in Foeniculum vulgare. Type P, present only in Anethum and Carum. Type Q is observed in 2n plants of Daucus only. Type R, the five lobed grains and Type S are present in Carum coptium only, Type T is represented in Cuminum cyminum only.

In Anethum graveolens in the 2n plant (E.210) joined grains were observed in acetolysed as well as carmine preparation. Normal type of grains from 2 to 5 were joined in one unit.

A unique feature of Coriandrum sativum was the transformation of the sporogenous cell into PMC, sporad and finally into pollen without under
going any nuclear division. The nucleus remained throughout deeply stained without revealing any chromatin network. These grains Type α were smaller than Type A with scanty cytoplasm. Even at pollen stage, when the rest of the grains were at 2-nucleate condition, this type has only one deeply stained nucleus. However, in the progeny of a tetraploid of Coriandrum sativum it was noticed that one of such grains with deeply stained nucleus showed transfer of nuclear material into another normal control type grain through the pores (Fig. 3). The 2 grains were closely appressed and a small protrusion from the smaller grain was observed. Such a movement of nuclei between fully developed grains is not a normal feature (Joshi and Raghuvanshi 1965a).

In Foeniculum vulgare the enlargement of certain giant grains took place to such a size that they resembled embryosac like structures.

In order to show that colchicine alone as well as in combination with gammexane are not the only chemicals which could induce variability of pollen, treatment of shoots of Pimpinella monoecia with α-bromonaphthalene, saponin, aesculine, and 8-hydroxyquinolene also resulted into pollen variability (Joshi and Raghuvanshi 1967a). These experiments further substantiate the instability of pollen shape which could be altered by the application of diverse chemicals and still certain types of grains obtained being common. Further our studies have shown that change in pollen shape can also be induced by irradiation (Joshi and Raghuvanshi 1967b). The treatment with the chemicals disturbs the polarity of the grains, ultimately resulting into different pollen shapes. It is possible that the expression of the genes (gene) responsible for pollen development may be changed due to treatment with different chemicals.

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