CYTOMIXIS AND MEIOTIC ABERRATIONS IN TWO SPECIES OF *ABUTILON* MILL FROM PAKISTAN

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Cytomixis and meiotic aberrations have been reported in *Abutillon grandifolium* (Wild.) Sweet and *A. pannosum* (Forst. f.) Schelecht. from Pakistan. Cytomixis was observed in the two species includes migration of chromatin matter to entire chromosomes or whole nucleus. The meiotic aberrations such as laggards, uni – or multivalents, multinucleate PMCs; curved, open, triangular, multipolar, or multiple spindles; incomplete tetrad or polyspory, observed in the two species are attributed to their cytomictic behaviour.

Key words: Cytomixis, Meiotic aberrations, Spindle anomalies, *Abutilon*.

INTRODUCTION

Fifteen species of *Abutilon* Mill. are native to Pakistan [1]. Two species (*A. grandifolium* (Wild.) Sweet and *A. theophrasti* Medic.) are confined to the northern hilly region, two (*A. ramsoum* (cav.) Guill. and Perr. and *A. ghafoorianum* S. Abedin) to Punjab, seven (*A. alii* S.A. Hus. and S.R. Baq., *A. figraianum* Webb, *A. hirtum* (Lamk.) Sweet, *A. karachianum* S.A. Hus. and S.R. Baq., *A. pakistanicum* Jafri and Ali, *A. pannosum* (Forst. f.) Schlecht and *A. sepalum* S.A. Hus. and S.R. Baq.) to Karachi and adjoining areas. The remaining four (*A. bidentatum* A. Rich, *A. fruticosum* Guill. and Perr., *A. indicum* (Linn.) Sweet, and *A. muticum* (Del. ex DC.) Sweet) commonly occur throughout Pakistan. Thus out of 15 species 11 are found in a small area comprising Karachi and lower parts of Baluchistan and Sind, adjoining to Karachi. It is important to note that five species are endemic to Pakistan, four of them are confined to the above mentioned region. The presence of 4 endemic species, besides seven other in such a geographically small area is quite significant; primarily because the natural phenomena of polyploidy, chromosome aberrations or gene mutations seem to have played more active role in this region to create and/or establish the above mentioned taxa; secondly because these 11 species, sharing more or less similar habitats, have had ample opportunities of inter-hybridization. The cytological studies were, therefore, initiated to identify chromosomal races and natural hybrids if any, and to determine the role and distribution of polyploidy with in the genus. These studies have recently been published separately [2]. This paper deals with two species viz; (1) *A. grandifolium* (Wild.) Sweet = *A. molle* (Orgega) Sweet and [2] *A. pannosum* (Forst f.) Schelecht. = *A. glaucum* (Cav.) Sweet in which cytomixis has been found. It may be noted that former species occurs at 34°N 72°E while the later at 25°N 67°E in Pakistan.

MATERIALS AND METHODS

Meiotic studies were made from pollen mother cell (PMC) squashes. Suitable yound buds were fixed in Carnoy solution (alcohol-chloroform-acetic acid in a ratio of 6:3:1) for 24 hours and stored in 70% alcohol. Anthers were squashed in aceto-carmine as well as in aceto-iron-hematoxylin which gave good results for spindle observations. The buds of *A. pannosum* were collected from 250 plants, growing wild in different localities of Memon goth, Jam goth, Malir, Landhi, PCSIR Laboratories and Karachi University Campuses, Nazimabad and Manghopir and meiotic abnormalities were recorded in 55 of the plants. The buds of *A. grandifolium* were collected from 52 plants which were raised in an experimental field at PCSIR Laboratories Karachi, from the seeds obtained from Peshawar University by one of the authors himself. Eighteen of these plants exhibited abnormalities presented in this paper. The voucher specimens are deposited in the herbarium of PCSIR Laboratories, Karachi. Camera lucida drawings were made at a magnification of approximately 2000x. The taxonomic work of Abedin [1] and Afaq-Husain and Baquar [3] was consulted for identification and nomenclature.

Observations

A. grandifolium (Wild) sweet. Approximately 1300 PMCs were studied in the plants showing cytomixis twenty one percent exhibited cytoplasmic connection and 9% cytomixis. The total meiotic abnormalities observed in this species were not more than 32%. The haploid complement of this species comprises 21 chromosomes.

(a) *Cytoplasmic connections between meiocytes.* Cytoplasmic connections have been observed between 14 to 60% of meiocytes in different anthers. The meiocytes are found connected in the form of ring of three to four cells or in open chain consisting of up to 20 or more cells; 1-4 connections are present between two PMCs and they usually

occur at prophase but rarely at Metaphase-I or Telophase-I (Fig. 2). Cytoplasmic connections are occasionally seen between PMCs at later stages of meiotic division. Long chains are also diminished as meiosis advances; there were only three or four cells seen connected in later stages of meiosis. Portions of chromatin and sometimes the entire nucleus has been observed either attenuated in the cytoplasmic connections or partly moved from one PMC in to another through the cytoplasmic channel (Fig. 2). 13 cells are

also observed containing an extra amount of chromatin; in figure one PMC contains a small amount of chromatin in the form of a micronucleus, besides a large nucleus in prophase and the cell is stretched into a beak like projection which is most probably a *remnant* of the cytoplasmic channel.

(b) *Abnormal behaviour of chromosomes*. The Metaphase-I plate is mostly compact and normal but at Metaphase-II, 1-4 chromosomes or a group of chromatin bodies do not arrange on the equator and remain scattered either on the spindle or outside the spindle (Fig. 4). 1-2 precocious chromosomes are also not uncommon at Anaphase-I or II.
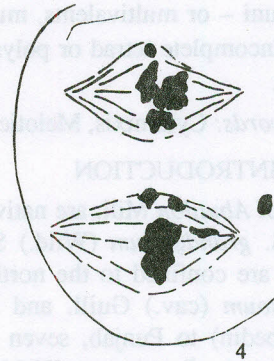

Fig. 1. PMC stretched into a beak like projection containing one micronucleus besides one large, normal looking nucleus.
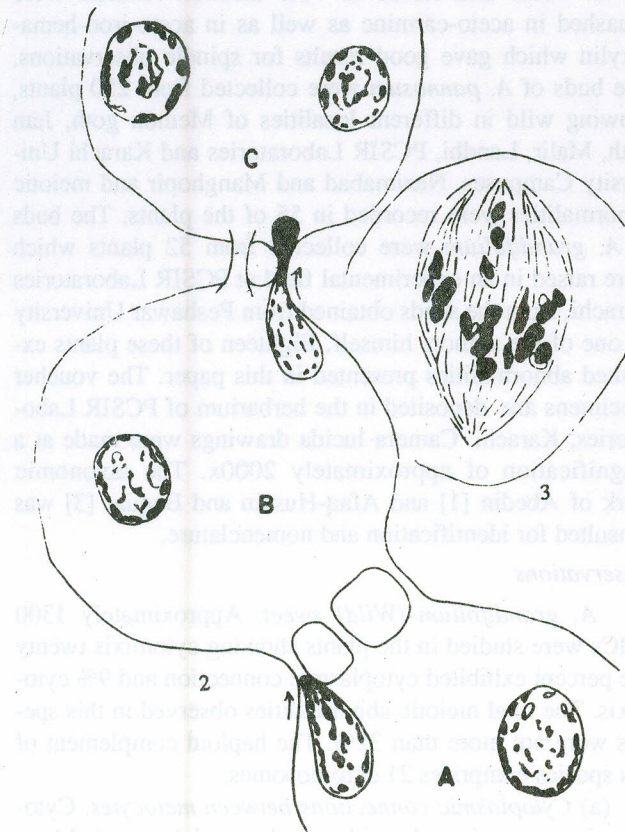
Fig.2. Chain of 3 PMCs, all at Telophase-I; attenuation of one nucleus in the cytoplasmic channel in cell A; one nucleus stretched between cells B and C through the channel.

Fig. 3. Triangular spindle with 3 separate fiber bundles and 3 apices at one pole.

Fig. 4. Metaphase-II, One chromosome lying away from the spindle and 3 chromosomes lying scattered on one spindle.

(c) *spindle aberrations*. The spindles are mostly normal but in some anthers 3 to 5% of PMCs are found exhibiting separate fiber bundles at Metaphase-I or II. Twenty one such cells were observed at Metaphase-I and 8 at Metaphase-II. In figure 5 the PMC contains more or less a triangular spindle with three separate fiber bundles at Metaphase-I; one pole is normal conical and the other is broad consisting of three apices which appear to correspond to the three fiber bundles. In the central bundle the chromosomes are distributed throughout the fibers between the two poles. At Metaphase-II (Fig. 5) each spindle is divided into two
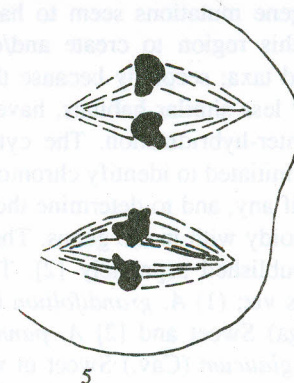

Fig. 5. Metaphase-II, each spindle divided into two fiber bundles, each bearing one compact group of chromosomes, in equatorial region.

bundles each of which bears a compact group of chromosomes in the equatorial region. Sometimes the spindles are broad as described by Walters [16] with the chromosomes found distributed either towards one pole or both the poles however the fibers are not divided into bundles. At Metaphase-II the two spindles are mostly parallel but several cases have been observed where the spindles are at right angles to each other. The frequency of total spindle abnormalities was in the vicinity of 3%.

A. pannosum (Forst. f.) Schlecht. The haploid complement of this species is also 21 but it shows more structural variations in spindle form as compared to *A. grandifolium*. Approximately 2400 PMCs were studied into the plants which showed meiotic aberrations. Fifty three percent of these showed different types of meiotic aberrations.

(a) *Cytoplasmic connections between meiocytes.* In *A. pannosum* cytomixis is observed in 19% of PMCs and cytoplasmic connections or channels in about 35% of PMCs; their breadth measured up to 9 um. Mostly 2 to 3 cells are found connected either as chains or rings and 1 to 3 connections were observed between two meiocytes (Fig. 6-8). Cytoplasmic connections and cytomixis have been observed in all stages from Prophase-I to Metaphase-II, however higher frequency of abnormalities were observed in early stages of meiosis. Cytomixis included migration of small units of chromatin, large chromatin amounts or whole nuclei from one PMC to other through the intercommunicating cytoplasmic channels, (Fig. 6-8). In figure 6 one of the two connected PMCs, bears 2 nuclei in diakinesis while
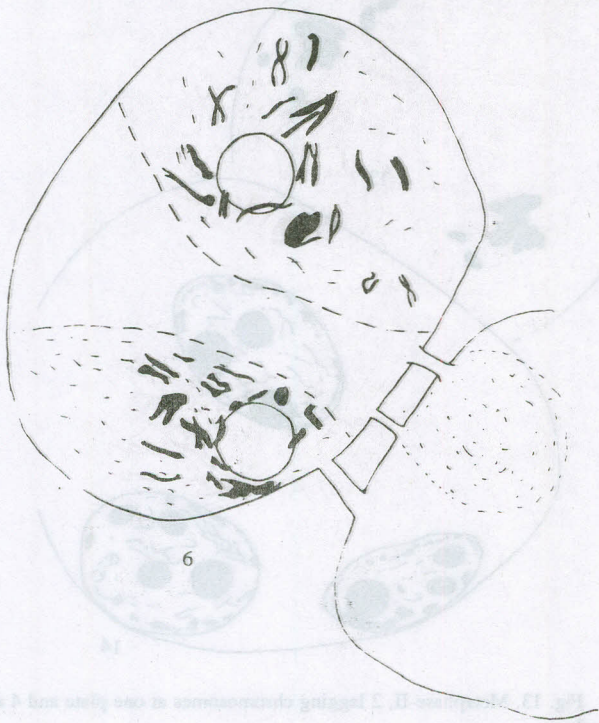
the other cell shows some signess of the previous nucleus near the channels. The two nuclei in the recipient cell remain separated from each other. In figure 7 one PMC bearing 3 nuclei is connected to two PMCs which do not contain any nucleus, however, a little part of nuclear matter is found stretched in each isthmus showing that the two extra nuclei have migrated from the other two cells. Moreover few fragments of chromatin remained unmoved in one of the cells.
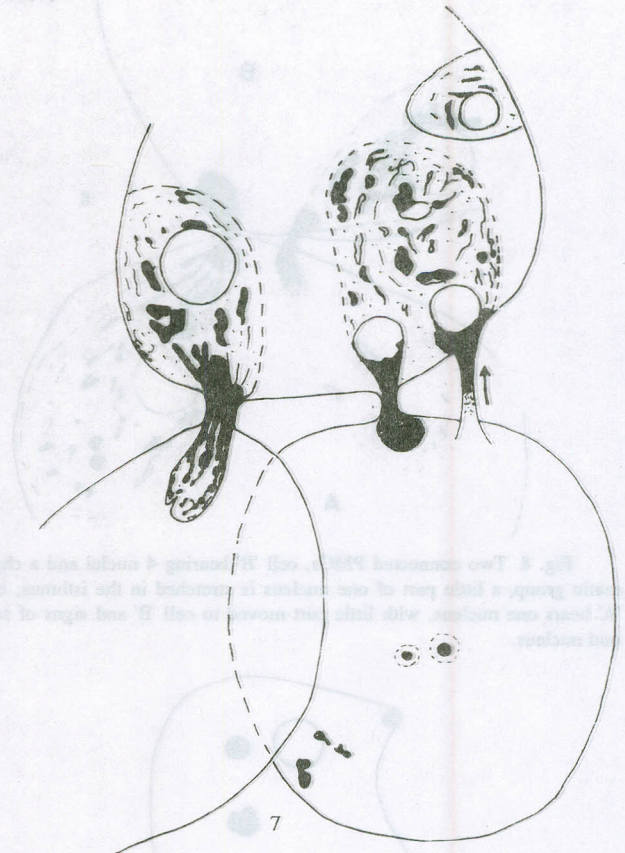

Fig. 7. Three connected PMCs, one bearing 3 nuclei others none except a little part stretched in the isthmus besides few chromatin fragments in one cell.

Figure 8 also presents an interesting anomalous situation. Out of two connected PMCs, one bears 4 nuclei and a chromatin group; a little part of one of the nuclei still present in the isthmus as if this nucleus is migrating from cell 'A' which also bears another nucleus which is migrating to cell 'B'; both of these nuclei are in prophase. The remaining three nuclei in cell B bear less chromatin than the migrating nucleus. Bi-or trinucleate PMCs have also been observed (Fig. 14). The PMCs with beak like projections and with less chromatin matter than normal have also been observed which probably indicate the presence of earlier cytoplasmic connections as well as migration of some of the chromatin matter to other cells (Fig. 9).

(b) *Abnormal behaviour of chromosomes.* Lagging chromosomes, 1-4 univalents, bivalents or a group of ill de-

Fig. 6. Two connected PMCs, one with no nucleus and the other with 2 nuclei in Prophase-I.

Fig. 8. Two connected PMCs, cell 'B' bearing 4 nuclei and a chromatin group, a little part of one nucleus is stretched in the isthmus; cell 'A' bears one nucleus, with little part moved to cell 'B' and signs of second nucleus.
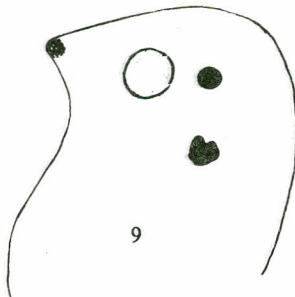

Fig. 9. PMC with beak like projection a little chromatin alongwith a nucleolus.

and tripolar (Fig. 17). The aberrations are mostly found at Metaphase-I and the chromosomes usually occur scattered along the spindle fibers. Univalents and multivalents besides bivalents are usually seen on these spindles. Occa-

Fig. 10. Constricted spindle with two lagging chromosomes.

Fig. 11. Doubly curved and broad spindle, chromosomes scattered along the fibers.

Fig. 12. Triangular spindle with 3 apices at broader pole corresponding to 3 fiber bundles.
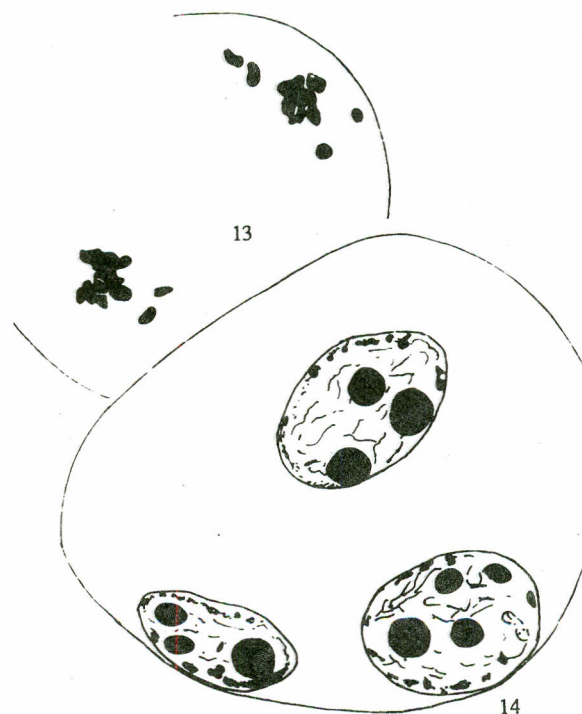

Fig. 13. Metaphase-II, 2 lagging chromosomes at one plate and 4 at the other.

Fig. 14. PMC with 3 nuclei.

fined chromosomes, are of common occurrance at Metaphase-I, and Metaphase-II (Fig. 10, 13). At telephase-I the chromosomes generally form a compact group but the lagging chromosomes do not associate with them. Precocity and non-disjunction are also exhibited by few chromosomes at Anaphase-I.

(c) *Spindle aberrations.* In *A. pannosum* the normal spindles are elongate, narrow with pointed conical poles with nearly compact metaphase plates. Many spindles are found oriented on one side instead of in the center of the cell. The aberrant spindles observed are curved (Fig. 15), constricted (Fig. 10), open (Fig. 16), triangular (Fig. 12)

sionally a spindle is doubly curved and broad (Fig. 11). In three PMCs the spindle apparatus was found constricted at metaphase plate giving it more or leas dumble shaped appearance (Fig. 10). Some cells were also observed with a supernumerary spindle which was usually narrow and open type carrying very few chromosomes (Fig. 16). In triangu-
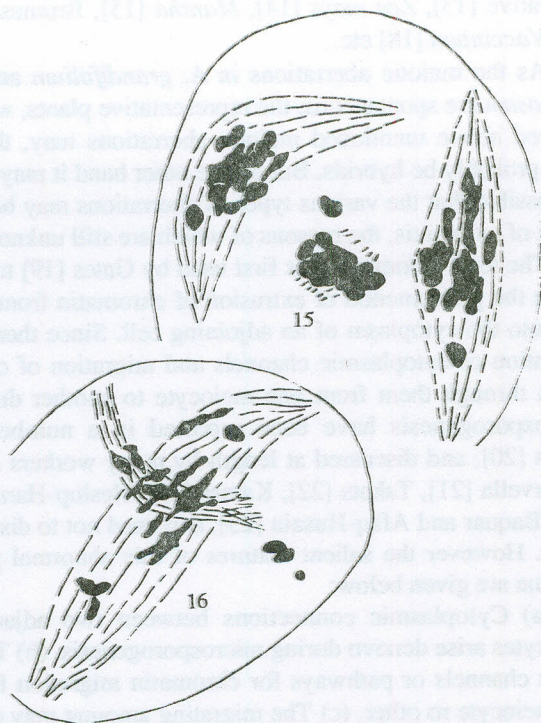

Fig. 15. One PMC with 3 separate spindles each at Metaphase-I and chromatin amount apparently equal to normal; one is curved; one chromosome lying away from metaphase plate in the central spindle.

Fig. 16. *Supernumerary* spindle-open type, crossing the large spindle; chromosomes scattered on the spindles; 3 chromatin bodies lying away from the spindles.

lar spindles which are very broad at one pole, the fibers and chromosomes look divided into 2 to 3 separate groups and 2 to 3 apices are also seen at broader pole (Fig. 12). In tripolar spindles the distribution of chromosomes on 3 inter connected spindles is such that one bears a nearly entire complement while others, two chromosomes to none (Fig. 17). One case was observed where 3 separate non aligned spindles were present in a single cell, each with a compact Metaphase-I plate and chromatin amount apparently equal to normal (Fig. 15). Two interesting aberrations are seen in two cells (Figs. 18, 19). In one cell (Fig. 19) one pole is broad and not defined, entire chromatin matter concentrating about it and the fibers are diverging broadly, other pole could not be seen, or most probably not formed. In another cell (Fig. 18) the spindle is very broad and round consisting of separate fiber bundles bearing nearly half of the normal chromosome complement; 2 spindles (open type) over lapping each other passing across the first large spindle. The

total spindle anomalies were observed in 8% of the PMCs.

(d) *Pollen irregularities*. Dyads, triads and polyads containing up to 12 sporads have been observed besides
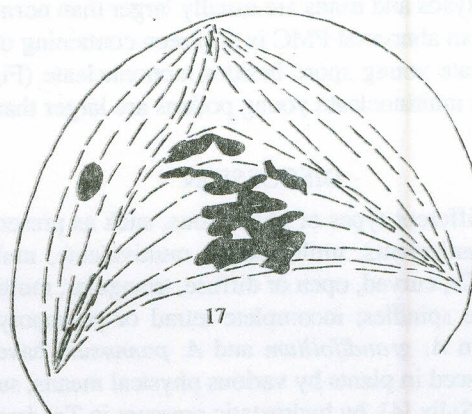

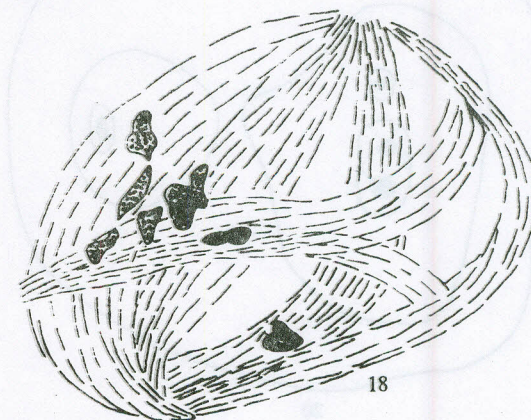Fig. 17. Tripolar spindle; one bearing one chromosome and the other none.

Fig. 18. Broad and round spindle with several fiber bundles running in different directions; chromatin matter appear to be half of the normal.

Fig. 19. Spindle with broadly diverging fibers, one pole ill defined and chromatin matter concentrating about it, other pole not formed or not visible.

normal tetrads (Fig. 20, 21). PMCs with abnormal number of pollens are not more than 2%. The extra pollen in a polyad may be smaller or equal in size than the other; the spores of dyads and triads are usually larger than normal. In rare cases an abnormal PMC is also seen containing one bi- or trinucleate young spore besides mononucleate (Fig. 20, 21). These multinucleate young pollens are larger than others.

DISCUSSION

The different types of aberrations, such as presence of laggards, univalents, multivalents, multivalents, multinucleate PMCs; curved, open or diffuse, triangular, multipolar or multiple spindles; incomplete tetrad or polyspory etc., observed in *A. grandifollium* and *A. pannosum* have also been produced in plants by various physical means, such as by cold in Salix [4], by hydrostatic pressure in *Tradessantia*
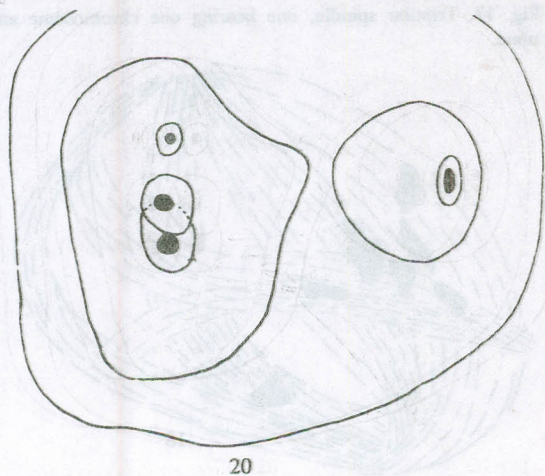
Fig. 20. Dyad; one spore with 3 nuclei and the other with one.
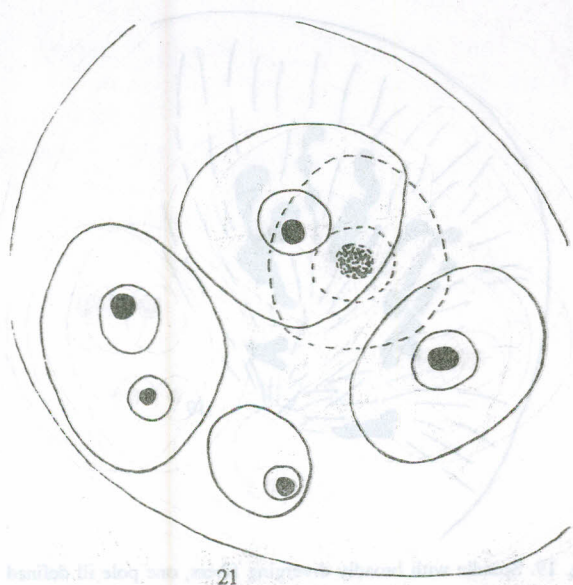
Fig. 21. Pentad with one micro and one binucleate spore.

[5]; or by chemical treatments, such as by chloralhydrate in *Vicia* [6], by colchicine in *Agropyron - cristatum* [7] by isopropyl N-phenylcarbamate (IPC) in *Haemanthus* [8], Oedogonium [9] and common wheat [10] etc. These aberrations have been reported to occur spontaneously in hybrids, like *Lilium* [11], *Malandrium* [12], *Festuca-lelium* derivative [13], *Zea mays* [14], *Mantha* [15], Bromus [16, 17], *Vaccinium* [18] etc.

As the meiotic aberrations in *A. grandifolium* and *A. pannosum* are spontaneous, the representative plants, which showed above mentioned meiotic aberrations may, therefore, probably be hybrids. But on the other hand it may also be possible that the various types of aberrations may be the result of cytomixis, the reasons of which are still unknown.

The term cytomixis was first used by Gates [19] to describe the phenomenon of extrusion of chromatin from one cell into the cytoplasm of an adjoining cell. Since then the formation of cytoplasmic channels and migration of chromatin through them from one meiocyte to another during microsporogenesis have been reported in a number of plants [20], and discussed at length by many workers such as Sarvella [21], Takats [22], Kamra [23], Heslop-Harrison [24], Baquar and Afaq-Husain [25], and need not to discuss again. However the salient features of this abnormal phenomena are given below:

(a) Cytoplasmic connections between two adjacent meiocytes arise denovo during microsporogenesis. (b) They act as channels or pathways for chromatin migration from one meiocyte ro other. (c) The migrating amoung may consist of a little part to large chromatin amounts or whole nuclei, chromosome/s or part thereof. (d) This migration is a natural phenomenon.

The present studies further strengthen the above mentioned views. In the present species the phenomena of chromatin migration and cytomixis may be clearly understood from: (1) attenuation of chromatin matter or nucleus towards the cytoplasmic connections (Fig. 2) showing that chromatin matter started moving towards the channels; (2) the presence of chromatin matter in the isthmus or stretched between two meiocytes (Fig. 2, 7) showing that the chromatin matter has partly moved to another cell; (3) among connected meiocytes one cell bearing more than one nucleus and the other none (Fig. 6, 7) showing that the nucleus of one cell has migrated to the other.

Furthermore, the cells with beak like projections and increased or decreased amount of chromatin (Figs. 1,9) might be indicative of the fact that these cells were previously connected to other cells and the chromatin matter was donated to or received from the connected meiocytes.

Recently the gain of chromatin by some PMCs and loss by others in *Agropyron cristatum* is also attributed to cytomixis [20]. The cells with abnormal chromosome complement usually get disintegrated during the meiotic cycle. However, few cells reach the end of meiosis and produce

abnormal number of spores. The presence of only 1 to 4 laggard chromosomes at various stages (Figs. 4,10,17) show that the meiocytes which receive or donate 1 to 4 chromosomes may take part in further meiotic division. The additions or loss of more than 4 chromosomes (Figs. 8, 9) causes disintegration of the nuclei or cells - such cells have not been observed. The occurrance of dyads and triads may be due to the disintegration of the nuclei with abnormal chromosome complement at Metaphase-I or II. The lagging chromosomes may be included in a nucleus when they happen to be present either on the spindle or near a pole but when they are away they may get dissolved in the cytoplasm or form one or more micro nuclei which give rise to extra abortive sporads; when a micro-nucleus is present near a normal nucleus, it is included in the same cell forming a binucleate pollen (Fig. 21). Trinucleate pollen (Fig. 20) may be formed in the same way. In these species it also appears that when entire nucleus migrates from one PMC to other (Figs. 6, 8), it keeps its identity in the recepient cell and may be have independently in further meiotic division. Bi- or trinucleate PMCs (Fig. 14), cells with 3 plates at Metaphase-I (Fig. 15) and 8 or 12 pollens in a PMC might be the result of such a behaviour.

As far as spindle aberrations are concerned, Walters [16, 17] has suggested that multipolar and multiple spindles arise from splitting or extra division of spindle organisers which may occur at various times throughout the meiotic cycles. There will be no difference in understanding the origin of multipolar and multiple spindles if the term spindle organisers in Walter's work is replaced by "Microtubule Organising Centers" (MTOCs) of the modern science. It has been proved that the spindle structuraly consists of microtubules whose initiation, orientation and directionality are governed by MTOCs which are structure-less but may be characterized by the presence of an amorphous, floculent material [26-31]. Multipolar and multiple spindles have been produced in Haemanthus [8], Oedogonium [9], and common wheat [10] by the treatment of IPC and griseofulvin. The latter produced 5 to 9 poles in more than 90% of cells in wheat plants [10]. Both the drugs act on MTOCs (and not on spindle microtubules or fibers) due to which microtubule orientation and directionality are markedly disturbed [8, 9, 11, 32, 33]. Thus any disturbance in MTOCs causes anomalies in spindle orientation.

In the present two species the spindle anomalies may also be due to disturbance in MTOCs which in turn may be the result of cytomixis. MTOCs may also migrate along with chromatin, either as aggregate units or part thereof (sub-unit) or in the form of diffuse state. The migrating matter of MTOCs may interect with the MTOC of the recepient cell, thereby disturbing the orientation of the spindle in that cell. When MTOCs migrate as aggregate units multipolar (Fig. 17) or multiple spindles may be produced; Fig. 18 may indicate the accumulation of several MTOCs in one meiocyte producing several spindles/fiber bundles, running in different directions. If the migrating matter of MTOC remains in diffuse state open or divergent spindles may be produced (Figs. 16, 19). Spindles with separate fiber bundles (Figs. 3, 5, 12) may be formed by two or more sub-units of MTOCs which come closer, (but do not merge to form an aggregate unit, probably because they belong to different cells) forming a pole. In triangular spindles separate apices have been observed at broader pole, corresponding to fibre bundles in the present work (Fig. 12 as well as by Walters [17]. In figure 4 and 6 the MTOCs sub-units are close enough to form a conical pole, keeping individuality to form separate fiber bundles but in Fig. 12 the MTOCs sub-units may be bit apart to form separate polar apices and separate fiber bundles. Using modern methods more work is to be done to correlate different types of aberrations in spindle orientation with different states of MTOCs described above.

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