APPLICATION OF ANTIMITOTICS IN ANGIOSPERM EMBRYOLOGY

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INTRODUCTION

ANY substance which prevents a cell from producing two cells, or which decreases the number of mitosis in a cell population (whatever be the mechanism underlying this decrease) is an antimitotic. Antimitotics therefore delay, decrease, or inhibit cell division, and in the process may induce varied cytologic aberrations. Todate over 120 substances, both naturally occurring and synthetic, have been found to have antimitotic effects. All natural antimitotics of plant origin are secondary metabolites. Synthetic antimitotics include among them certain insecticides, fungicides, weedicides and medicinal compounds. Majority of the antimitotics are preprophase inhibitors, some are spindle inhibitors and a few are cytokinesis inhibitors. Preprophase inhibitors prevent the nucleus from entering G_2 phase of mitosis. Antibiotics are among the best-known preprophase inhibitors. Alkylating agents and antimetabolites which affect DNA synthesis are also efficient mitodepressors. Mitodepressive effect leads to a decrease in mitotic index; mitostasis decreases in mitotic index to zero.

Spindle inhibitors (also termed mitoclasic agents) inhibit the formation of spindle apparatus and thus arrest mitosis at metaphase. The net result is the doubling of chromosome number in the resulting cell. Colchicine is a classical mitoclassic agent.

Cytokinesis inhibitors inhibit the formation of cell plate; the result is the formation of a binucleate cell. The purine derivatives caffeine, theobromine, and theophylline (all three are also methylxanthines) belong to this class of antimitotics.

Sequence of events in wall ontogeny constitutes a central theme in plant cell differentiation. In developmental embryology of angiosperms cell plate formation leading to partition of the progenitor cell serves as a cardinal marker for classifying the types of sporogenesis, gametophytogenesis, endosperm development and embryogenesis. On the doctrine that reproductive traits are conservative, these embryologic features have been ascribed taxonomic significance and have been routinely used to 'settle' phylogenetic disputes. Regrettably, however, embryologic variations encountered are dubbed as abnormalities and often conveniently ignored while considering taxonomic relations. It must be emphasized that variations not only help decide as to what is normality (for a given taxon) but also afford cues for experimentation.

From the latest general review on antimitotics[1] it can be inferred that antimitotics have been seldom employed in studies of angiosperm plant embryology. However, from 1970s onward there has been an upsurge of plant embryologic studies involving antimitotics; our survey covers this literature and refers to early works only where necessary to make the account more comprehensive. Additionally the review points out the prospects in studies of antimitotics in plant reproductive biology and calls for an intensive analysis of long-range effects of antimitotics used as agricultural chemicals on crop yield.

Because an antimitotic may belong to more than one category, generally a phenomenon-oriented account is given. By far pollen development has been the most extensively studied embryologic phenomenon and colchicine the most employed antimitotic. Among the other embryologic phenomena studied are pollen germination, formation of male gametes and development of endosperm and embryo and among the other antimitotics used in these studies are caffeine, chloral hydrate, cycloheximide and myrmicacin.

POLLEN DEVELOPMENT

The earliest reports on the effect of an antimitotic on microsporogenesis are those of Walker[2] and Derman[3] respectively on *Tradescantia paludosa* and *Rhoeo discolor*. Both species are characterized by successive type of development of microspores, *i.e.* wall formation occurs after each nuclear division. Colchicine treatment (0.05%—1.0%) of flowering materials suppressed spindle mechanism at either division or both divisions of meiosis in the microsporocytes leading to the formation of diploid or tetraploid microspores. Working on microsporogenesis in *Allium cernuum* Levan[4] was the first to report that colchicine suppressed not only the spindle apparatus but also the formation of chiasmata which led to a noticeable increase in the number of univalents (see also Barber[5]). If colchicine (0.01–1.0%) treatment was given to inflorescences when the microsporocytes were still differentiating from the archesporium, tetraploid or octoploid microsporocytes were formed. Colchicine treatment of microsporocytes lasting during both meiosis I and meiosis II resulted in the formation of tetraploid pollen. Colchicine treatment given during first pollen mitosis led to the formation of a diploid nucleus instead of the two haploid nuclei[4].

In the hexaploid *Triticum aestivum* some dodecaploid microsporocytes were obtained 7 days from treatment of an immature spike with 1 or 2.5% colchicine; the dodecaploid microsporocytes showed formation of fairly regular bival ents, synapsis and chiasmata[6]. Colchicine treatment given after completion of the premeiotic mitosis resulted in asynapsis and formation of multivalents. Thus colchicine seems to inhibit premeiotic pairing of homologous chromosomes. To test whether or not the premeiotic determination of chromosome pairing is dependent on centromeric spindle microtubules Dover and Riley[7] treated developing intact spikes as well as excised anther cultures of six genomes of *T. aestivum* including some four of its hybrids with *Aegilops mutica* and with *Secale cereale* (characterized by different degrees of chromosome pairing) with two antimitotics, colchicine and chloral hydrate, an organo-aliphatic compound. In spike treatment of the euploid genome with 0.01% colchicine some microsporocytes having 42 chromosomes resulted. Such microsporocytes showed some asynapsis and occasional trivalents and quadrivalents. When 2 ml of 0.5% colchicine was injected into tillers at premeiotic mitosis asynapsis was observed in all the genotypes. Tetraploid and octoploid microsporocytes resulting from disruption of premeiotic spindles by colchicine treatment of tillers showed only bivalent pairing of chromosomes even in those genotypes characterized by a high degree of multivalent pairing. But a 2 ml injection of 0.5% chloral hydrate into premeiotic tillers caused no disturbance in meiotic pairing. In colchicine-treated cultures of anthers excised immediately prior to or at leptotene both synapsis and chiasma formation occurred. Based on these experimental results Dover and Riley[7] concluded that the meiotic chromosome pairing presumably involves centromeric spindle microtubules. Using pentaploid *Triticum durum* × *Triticosecale* hybrids Thomas and Kaltsikes[8] showed that the sensitivity to colchicine leading to inhibition of chiasma began in premeiotic interphase and ended in mid-zygotene.

In *Secale cereale* colchicine-sensitive stage extended from early premeiotic interphase to leptotene only[9]. In anthers of this species excised at late leptotene stage of microsporocytes and cultured for 12–24 hr on a medium supplemented with 0.1% colchicine meiosis progressed to pachytene; however, in anthers cultured at mid-zygotene synapsis was inhibited[10] and in those cultured at zygotene crossing over was inhibited[11]. A single injection of 0.1% aqueous solution of caffeine to tillers of *S. cereale* until ooze-out-point 7-28 days before meiosis caused binucleate and tetraploid conditions, decrease in chiasma frequency and chromosomal abnormalities in microsporocytes[12]. The maximal frequency of binucleate or tetraploid uninucleate microsporocytes and the maximal decrease in chiasma frequency were obtained from tillers treated respectively 14 days and 10 days before

meiosis. Treatment given 7 days before meiosis caused formation of chromosomal bridges and fragments, laggards, absence of anaphase migration and formation of micronuclei.

In *Lilium* colchicine could render the microsporocytes achiasmatic although they were treated at leptotene-zygotene; given at preleptotene, however, colchicine arrested prophase in the microsporocytes[13]. Microsporocytes of *L. speciosum* contain a nuclear membrane protein (NMP) only during prophase I and NMP has high affinity for single-stranded DNA[14]. Studies on ^{3}H-colchicine-treated 2-day-old cultures mostly of microsporocytes of *L. speciosum* and occasionally of *L. henryi* and *L. tigrinum* excised at prophase I showed that by binding to nuclear membrane colchicine decreased this affinity and that the amount of membrane-bound colchicine increased as the meiotic nuclei progressed to pachytene. Thus a tubulin-like protein is present in the nuclear membrane also, and "the process of synapsis occurs at least partly in association with the nuclear membrane"[15]. Colchicine therefore seems to have different target sites at different stages of meiosis. Colchicine treatment during mid-zygotene to late pachytene hardly affected the lateral elements of the synaptonemal complex[13,16]. Through pulse treatment studies Stern's school further demonstrated that microsporocyte sensitivity to colchicine starts at premeiotic interphase and extends up to late zygotene, but once synapsis is initiated colchicine does not interfere with it[17]. Colchicine treatment of microsporocytes of *Triticum aestivum* and *L. speciosum* after the onset of meiosis and up to the initiation of synapsis decreased chiasma frequency in them[15,18]. Driscoll and Darvey[18] attributed this decrease to destruction of microtubules by colchicine. In microsporocytes of *L. lancifolium*, *L. longiflorum* and *L. speciosum* colchicine has been reported to disorganize the chromosomal spindle fibres leading to the formation of incomplete beaded fibrils[19]. On transfer of colchicine-treated material to water, only a partial recovery occurred.

Like colchicine, cycloheximide (3.5×10^{-6}M) also, if applied during late zygotene to early pachytene blocked chiasma formation *in vitro* in microsporocytes of *Lilium*[20]. Reports on the effects of cycloheximide on synaptonemal complex are contrary. In microsporocytes of lily var. Enchantment Orange treatment at leptonema and zygonema with 0.25 μg/ml ($=0.89\times10^{-6}$ M) of cycloheximide did not prevent the formation of synaptonemal complex[21]. But in var. cinnabar microsporocytes up to pachynema treated with 1.8×10^{-6} M cycloheximide for 4 days showed lack of major portion of the lateral component of the synaptonemal complex. However, by 4–5 days from transfer of the microsporocytes to the control medium the synaptonemal complex reappeared in those microsporocytes that were not beyond early zygonema at the time of culture[22]. In var. Enchantment Orange, mitomycin C unlike cycloheximide, prevented the formation of synaptonemal complex in microsporocytes cultured at leptonema and ceased further formation of synaptonemal complex in microsporocytes cultured at zygonema[21].

Mercaptoethanol, a thioglycol, acts as a mitoclassic agent. A 30 min treatment of microsporocytes of *Lilium* with 10^{-1} M mercaptoethanol led to disintegration of chromosomal fibres near the spindle poles and their subsequent disorientation. However, the inhibitory effect of mercaptoethanol on spindle activity unlike that of colchicine is reversible[19]. Sato[19] further reported that the alkylating agents, nitrogen mustard A and B (0.05 and 0.25%) also induced breakage and stickiness of chromosomes and formation of micronuclei in lily microsporocytes treated at prophase for 4 to 48 hr; of the two, nitrogen mustard B was more effective. Satō[19] suggests that the nitrogen mustards react with ionized –SH, –COOH and $-PO_4$ groups and induce cross-linking with proteins and nucleoproteins.

Increasing periods of treatment (24–120 hr) of anthers of *Pierotheca falconeri* with increasing concentrations (2.5–105 mM) of three mutagenic alkylating compounds, namely ethylene imine, ethylene oxide and ethyl methanesulphonate (EMS) increased the incidence of meiotic abnor-

malities in the microsporocytes[23]. Ethylene imine was the most potent of the three substances.

In M_1, M_2 and M_3 generations of pea plants raised from seeds treated with the mutagen EMS (0.25%) or MMS (methyl methanesulphonate, 0.025%) for 10 hr both the mutagens caused asynapsis and formation of multipolar spindles and unoriented chromosomes in up to 6% microsporocytes[24].

As already mentioned some insecticides, fungicides, and weedicides also act as antimitotics. Spray treatment of plants of *Vicia faba* with any of the three insecticides Dimecron-100[25], Rogor-40[25,26], or Phosvel (Leptophos)[27], or with either of the weedicides[26] isopropyl N-phenyl carbamate or Duphar induced chromosomal aberrations during microsporogenesis. Both Dimecron and Rogor caused a total loss of the spindle mechanism in some microsporocytes; Rogor-40 was more effective, and it induced tetraploidy also in a few microsporocytes[25]. At heading stage, plants of *Triticum aestivum*, *T. durum* and *Aegilops ligustica* raised from grains treated with the fungicide Vitavax-200 or Dithane S-60 showed an increased number of microsporocytes characterized by laggards and single and multiple chromosome bridges[28]. Spraying of 15-day-old seedlings and 35-day-old flowering plants of *V. faba* with 0.005% of the weedicide monochloroacetic acid (MCA) or trichloroacetic acid (TCA) decreased the production of viable pollen grains due to lagging, stickiness and fragmentation of chromosomes[29]. TCA treatment produced multipolar spindles also.

Besides affecting spindle fibres colchicine has been reported to affect germpore position and shape of generative cell. In pollen grains germpore position is a marker of polarity. In *Triticum aestivum* polarity in the developing pollen grain is established at microsporocyte stage itself. During meiosis I the long axis of the single spindle is parallel to that of the anther; during meiosis II the long axes of the two spindles are perpendicular to that of the anther. In the resulting microspore tetrad the single germpore in each microspore differentiates opposite a spindle pole. But a treatment of flowering spikes with colchicine upset the spindle polarity; based on the concentration of colchicine (0.01 or 0.5%) and the stage at which the treatment was given Dover[30] reported the formation of tetraploid pollen monads having four randomly placed germpores, pollen monads having more than four or no germpores, and multipore polyads. Dover[30] interpreted that the number of germpores in a pollen tetrad is related to that of spindle pole determinants at meiosis.

In *Haemanthus katherinae* initially the generative cell is spheroidal and its microtubules are scattered in the cytoplasm. Before travelling down the pollen tube the generative cell supported by the axial (parallel to the long axis of cell) orientation of its microtubules elongates to an ellipsoidal shape. A 24 hr treatment of excised anthers at the ellipsoidal stage of the generative cell with 0.2% colchicine or with 0.01% of the weedicide IPC caused the microtubules in the generative cell disappear and consequently the generative cell regressed to spheroidal shape. Washing the treated anthers for 24 hr with 0.05 M phosphate buffer (pH 7) induced the reappearance of microtubules in many generative cells. Although the reappeared microtubules aligned in bundles parallel to the cell axis they were not evenly distributed around the cell wall as in the control cells[31].

GAMETOCIDAL EFFECTS OF ANTIMITOTICS

In the angiosperms which shed pollen at 3-celled stage the twin male gametes (sperms) are formed as a result of mitosis of the generative cell (often referred to as second mitosis in pollen) prior to pollen germination. In the majority of angiosperms, however, second pollen mitosis occurs during pollen tube growth. Effects of antimitotics on sequential events namely pollen germination, pollen tube growth and gamete formation are therefore for convenience reviewed under a common title, "Gametocidal effects of antimitotics".

Eigsti[32] studied the effects of colchicine on pollen cultures of five species belonging to *Poly-*

gonatum, *Lilium* and *Tradescantia*. Colchicine decreased the bursting of pollen tubes, but inhibited normal gametogenesis; instead of two haploid gametes only one nucleus was formed. In its source plant *Colchicum autumnale* colchicine (0.1–1.0%) depressed pollen germination and decreased pollen tube length, but increased pollen tube width *in vitro*. Pollen tubes wider than the grain diameter have been called pollen tube tumors[33]. In pollen cultures of *Clivia miniata* and *Lilium longiflorum* colchicine (10^{-8} to 2×10^{-4}M) did not affect pollen tube growth up to a length of 5 mm, although microtubules were lacking in the treated materials; contrastingly vinblastine sulphate (5 μg/ml) inhibited both pollen germination and pollen tube growth in both species[34].

Of the 25 purine and pyrimidine derivatives tested 2-thiouracil most effectively stimulated tube growth in *Petunia hybrida* and *Nicotiana alata*[35]. Actinomycin D, a RNA synthesis inhibitor, did not affect pollen germination, initial pollen tube growth and migration of nuclei into pollen tube in *Tradescantia*. However, it inhibited subsequent pollen tube growth and generative cell division. That treatment with actinomycin D 2 hr after pollen germination did not affect generative cell division has demonstrated that RNA essential for generative cell division synthesized during the first 2hr of pollen tube growth[36]. In many other taxa also actinomycin D did not affect pollen germination and initial pollen tube growth although the extent of pollen tube growth varied from species to species[37–40].

Mature pollen of *Ornithogalum virens* is characterized by spontaneous mitotic arrest at prometaphase in generative cell. Upon hydration in normal culture medium the generative cell mitosis proceeds gradually and is completed in 140–150 min. Treatment of pollen cultures with 1 ppm cycloheximide, an inhibitor of protein synthesis, at prometaphase or metaphase delayed the generative cell mitosis[41,42]. This effect was more marked when pollen was treated at prometaphase because of the suppression of kinetochore fibre development[41].

Myrmicacin found in secretions of *Atta sexdens*, a South American leaf-cutting ant, also delayed second pollen mitosis in pollen cultures of *O. virens*. But unlike cycloheximide and colchicine, myrmicacin (0.01%) was effective even at anaphase[42]. With increase in concentration of myrmicacin from 0.0005 to 0.005% in culture medium both per cent germination and pollen tube growth of *Antirrhinum majus*, *Camellia japonica*, *Hyacinthus orientalis*, *Impatiens balsamina* and *Lilium speciosum* decreased progressively; no pollen germination occurred at 0.005% in any of the species tested[43]. In *I. balsamina* myrmicacin inhibited gamete formation also, but its inhibitory effect could be reversed on control culture medium[43]. Recently Iwanami *et al.*[44] have shown that at 0.005 or 0.01% myrmicacin decreased the speed of protoplasmic streaming but at 0.02% totally stopped the streaming in growing pollen tubes of *C. japonica*. The streaming recovered totally on transfer of treated pollen tubes to myrmicacin-free sucrose solution. In myrmicacin-treated pollen tubes the golgi-derived vesicles disappeared from the tube tips; it is assumed that myrmicacin interrupts the supply of wall material-containing golgi vesicles resulting in inhibition of pollen tube growth.

In their studies on effects of cycloheximide on pollen germination and pollen tube growth in *Luffa* and *Lilium* Lin *et al.* (cited in Shivanna *et al.*[45]) concluded that protein synthesis was required for pollen germination in *Lilium* but not in *Luffa*. Based on their studies on nine other taxa Lin *et al* found that cycloheximide inhibition of pollen germination was moderate in taxa in which flowers withered the day of anthesis and was drastic in taxa in which flowers withered more than 2 days from anthesis. In *Clivia miniata* and *Lilium longiflorum* cycloheximide (10^{-4}M) prevented pollen germination and caused 80% inhibition of pollen tube growth[34]. At all the concentrations tested (1–200 μg/ml) cycloheximide considerably decreased pollen germination and pollen tube growth in *Trigonella foenum-graecum* but not in *Impatiens balsamina*. All concentrations of cycloheximide

tested inhibited gamete formation in both species presumably by preventing the synthesis of kinetochore fibre proteins and in turn the separation of sister chromatids. These effects of cycloheximide treatment for 3 hr in hanging drop cultures and for 6 hr in test tube cultures of pollen were reversible[46,47]. In decreasing pollen tube growth in *Catharanthus roseus* and *Plumeria alba* cycloheximide was equally effective at 1 and 100 μg/ml, although it did not affect pollen germination in either taxon[48]. Like cycloheximide, chloral hydrate also inhibited gamete formation in pollen cultures of *I. balsamina*[49].

Unpublished observations of Janaki Subramanyan on effects of caffeine and theophylline on pollen germination and pollen tube growth in *Coccinia grandis* have shown that increasing concentrations (10^{-7} — 10^{-2} M) of both antimitotics increased the per cent inhibition of pollen germination and at the three highest concentrations tested the inhibition was total. Statistical data on pollen tube growth (every hr for 3 hr, have shown that with caffeine the inhibitory effect of concentrations was greater than that of incubation periods, while with theophylline the concentrations as well as the incubation periods were equally effective. In many determinations the maximal length of pollen tubes was less than the minimal tube length attained in control cultures.

From time to time some aliphatic acids and their derivatives, auxins, antiauxins and gibberellins have been tested for their phytogametocidal action. The gametocidal effect of the two rather extensively used substances namely Mendok (FW-450) and Dalapon and their efficacy as consequent inducers of male sterility have not been unequivocally established[50].

ENDOSPERM DEVELOPMENT

Studies on effects of antimitotics on endosperm development are limited. Rau[51] studied endosperm development in pollinated flowers of *Phlox drummondii* cultured on Nitsch's medium supplemented with 0.15% colchicine. Mitotic abnormalities such as scattering of chromosomes at metaphase and formation of micronuclei in endosperm were observed 3 days from culture. In *Argemone mexicana* treatment of developing fruits with 0.1% colchicine usually inhibited wall formation during endosperm development; together with the cytoplasm the endosperm nuclei aggregated into a single mass in which random nuclear fusions occurred. Most seeds that developed for 10 days from colchicine treatment lacked endosperm or showed a degenerating cellular endosperm. Treatments with 0.01 and 0.001% colchicine favoured normal endosperm development[52]. In cultures of pollinated ovaries of *Tropaeolum majus* on a colchicine-supplemented medium the developing seeds showed endosperm nuclei of varied sizes attributed to nuclear enlargement or fusion; some seeds, however, showed degenerated endosperm[53].

Through cine-micrographic studies Mole-Bajer[54,55] demonstrated that in endosperm cells of *Haemanthus katherinae* colchicine arrests spindle formation and consequently leads to the formation of restitution nuclei, while chloral hydrate (0.02% for 2 hr) causes one or the other of the following mitotic disturbances: (i) one or a few chromosomes not lying on metaphase plate, (ii) formation of tri- or multi-polar anaphase, (iii) diffuse anaphase in which the spindle lacked distinct poles and (iv) c-mitosis. Ultrastructural studies showed that colchicine (0.005%) largely destroyed spindle microtubules[56], and that in chloral hydrate-treated endosperm cells the "clear zone" containing numerous microtubules, kinetochore fibres and continuous spindle fibres were lacking[57]. However, by anaphase the kinetochore regions resumed normal structure[57].

Three other substances, namely ipc and trifuralin (both weedicides), and *Nerium oleander* glycosides have also elicited antimitotic effects on endosperm cells of *H. katherinae*. ipc (0.001%) treatment caused multinucleate condition; the spindle microtubules lost their parallel alignment and became orientated in radial arrays. This resulted in a multipolar condition and the chromosomes aggregated into

micronuclei[56] Pooled results of the treatments of endosperm cells with six concentrations (0.00001-0.01%) of trifuralin show that trifuralin inhibited the progress of mitosis from prophase to pro-metaphase; only 50% of the treated cells could form a cell plate. Trifuralin decreased the number of microtubules and favoured accumulation of large vesicles in the presumptive cell plate region. The net result was the formation of polyploid restitution nucleus[58]. *Nerium oleander* glycosides caused multipolar anaphases followed by formation of restitution nuclei; ultrastructurally the continuous fibres were lacking, while the kinetochore fibres were distinct[59].

EFFECTS OF ANTIMITOTICS ON EMBRYO AND SEEDLING

The earliest work on effects of antimitotics on embryo development is that of Rau[51] on *Phlox drummondii*. Rau compared embryo development in pollinated flowers of *P. drummondii* cultured on Nitsch's medium and on Nitsch's medium supplemented with 0.15% colchicine. Mitotic abnormalities such as dispersed chromosomes at metaphase and formation of micronuclei were observed 7 days from culture in embryo development. In *Argemone mexicana* treatment of developing fruits with 0.1% colchicine allowed embryo development usually up to globular stage and rarely up to pre-heart-shaped stage, whereas treatments with 0.01 and 0.001% colchicine not only permitted embryo development to maturity but also advanced embryo maturation by 8–12 days[52]. A somewhat similar effect of colchicine on embryo maturation *in vitro* has been described for *Tropaeolum majus*[53]. Treatment of *Lactuca sativa* achenes with 0.25% colchicine for 3 days resulted in inhibition of cytokinesis in all organs and tissues of the resultant seedlings[60]. Eight-day-old seedlings showed bi- and multi-nucleate cells and micronuclei, but not polyploid restitution nuclei common in colchicine-treated cells.

Gichner *et al.*[61] reported that a 3 hr treatment of excised barley embryos with the antimitotic methyl methanesulphonate (5 mM) followed by a 3 hr washing with tap water postponed the onset of mitosis and induced chromosomal aberrations in cells of the root tip. Mitosis was initiated only 54 hr from transfer of the treated material to water or control nutrient medium. Up to 30 hr from culture control embryos showed an increase in mitotic index. Two pyrimidine derivatives, 5-aminouracil and 5-fluorooxyuridine (FUdR) have also been demonstrated to be antimitotic with barley embryos. During a 24 hr treatment with 0.02 or 0.075% 5-aminouracil the mitotic index in root meristem of treated embryos decreased from 5–12% to 2–5%[62]. In a medium supplemented with 10^{-3}–10^{-8} M FUdR the root meristems of cultured embryos showed a gradual decrease in mitotic index up to 24 hr from culture, and thereafter the mitotic index declined to below 2%. FUdR induced chromosomal aberrations also, their frequency increasing with the period of treatment[63]. Increased production of bi-, tri- and poly-nucleated cells in meristems of barley embryos cultured on caffeine (0.05 or 0.1%) medium has been reported[64] but the increase in frequency of polynucleated cells was not directly proportional to that in duration of treatment. Longer durations of treatment resulted in fusion of nuclei leading to formation of polyploid mononucleate cells. Mitodepression and several mitotic aberrations in root tip cells of seedlings raised from seeds/grains treated with the insecticide Dimecron-100[25], Rogor-40[25,65], or Phosvel[66], or with the fungicide Vitavax-200 or Dithane S-60[67], or with the weedicide IPC or Duphar[68] have been reported for *Vicia*[25,65,66,68], *Gossypium*[65,68] and *Aegilops* and *Triticum*[67].

CONCLUSIONS

Irrespective of the chemical class to which the antimitotics belong, they affect both mitosis and meiosis at one or more phases. Their effect is dependent on their concentration, the stage at which the material is treated, and the duration of treatment. An easily visible expression of antimitotic effects is the formation of a polyploid nucleus or a bi-nucleate cell.

In angiosperms antimitotics impair microsporogenesis and lead to the formation of diploid and polyploid pollen grains; also, antimitotics may affect the number of germ pores and the shape of generative cell. Timed treatment with mitoclassic agents such as colchicine can prevent the formation of vegetative and generative cells. Certain antimitotics, some of which are also inhibitors of protein synthesis (*eg* cycloheximide) block the formation of male gametes. Nevertheless, many substances reported to have gametocidal action in angiosperms bring about undesirable effects, especially low crop yield. Likewise, some systemic insecticides, fungicides, and weedicides evoke long-range antimitotic effects which may well be deleterious traits related to yield. Therefore, caution has to be exercised in using such antimitotics as agricultural chemicals. Further, gossypol has been claimed to be spermatocidal in human species. Whether gossypol or any antimitotic acts as a selective male gametocide in plants is yet to be investigated.

While studies on effects of antimitotics on microsporocytes have been extensive, that on megasporocytes in angiosperms is restricted to a solitary report. Bennett *et al.*[17] studied the effects of 10^{-3} M colchicine on both micro- and megasporocytes of *Lilium speciosum*, and found the microsporocytes to be more sensitive to colchicine than the megasporocytes. That even in hermaphrodite angiosperms the events of meiosis in the two types of sporocytes differ in duration, synapsis, number and position of chiasmata, and survival of meiotic products[69] should prove future studies on effects of antimitotics on sporogenesis in angiosperms more illuminating.

Regrettably, todate no work is available on effects of antimitotics on developmental patterns of embryo sac, endosperm, and embryo. Studies on interactions of antimitotics with cytokinins and carcinogens (for sole purpose of terminology the latter two may be considered 'mitogens' or 'mitotics') during microsporogenesis, megasporogenesis, embryo sac development, endosperm formation, and embryogeny either to inhibit or to induce cell plate formation may eventually lead to a reconsideration of the rather rigid taxonomic value attributed to cell plate formation in these embryologic phenomena.

Whereas at least a few ultrastructural studies on spindle inhibitors are known in angiosperm embryology, none is available on molecular basis of action of either spindle inhibitors or cytokinesis inhibitors. In fact the molecular studies which explain the mechanism of action of spindle inhibitors have been conducted almost entirely on animal systems[70,71]. Regarding cytokinesis inhibitors, the studies, although fewer, concern both animal and plant systems; those on animal systems explain the molecular basis of cytokinesis; those on plants, both molecular and ultrastructural, are confined to vegetative parts of angiosperms[72]. To critically examine whether there is any basic mechanism of action of antimitotics common to both higher plants and animal cells parallel studies on plant systems are urgently needed. In this context, use of antimitotics in studies on liquid endosperms and synchronous endosperms such as those of coconut palm and *Acalypha*, and on coenocytic embryos of *Paeonia* and gymnosperms may prove challenging.

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ANNOUNCEMENT

SECOND AFRICAN NUTRITION CONGRESS AND THE WORKSHOP ON 'FOETAL AND NEONATAL DEVELOPMENT'

The Second African Nutrition Congress was organised in Ibadan, Nigeria from February 27 to March 3, 1983 by the Nutrition Society of Nigeria and the Department of Human Nutrition, University of Ibadan, Nigeria with support from various international organizations like, WHO, LAMBO Foundation, COSTED, etc.

The theme of the Congress was 'Foods and Nutrition in the 80s in Africa'. There were 161 delegates from Nigeria, Cameroun, Kenya, Tansania, Ghana, Zambabwe, Angola, Egypt, Sierra-Leone, Congo, Gambia, Lesotho, Senegal, Ivory Coast, Uganda, India, Australia, France, and Denmark. The Congress was inaugurated by the Vice President of Nigeria.

There were plenary sessions in the morning on 'Foods and Nutrition in Africa in the 80s', 'Nutrition and Primary Health Care', 'Food and Nutrition Policy', 'Food, Nutrition and Population', 'Nutrition Training and Education'. There were free communications in the afternoons on 'Nutritional requirement, Deficiencies and Diet therapy', 'Food Consumption and Nutrition Education', 'Maternal and Infant Nutrition', and 'Composition and Nutritive value of foods'. The young African scientists took very active part in the discussions.

From the deliberations it is found that the countries of Africa do not produce enough good quality foods and they are poorly and inequitably distributed. The Congress, therefore, felt that African Governments should give priority to Food and Nutrition in their development plans; promote rural development and control production and marketing of proprietary infant foods, especially breast milk replacers.

After the Congress, a training workshop on 'Foetal and Neonatal development in relation to maternal nutrition and ecological factors was organized from March 3–6, 1983 at Ibadan University by Prof. R. Rajalakshmi of Baroda University, India, Prof. A. Omololu of Ibadan University, Nigeria and Prof. C. V. Ramakrishnan of Baroda University, India, supported by COSTED, WHO, UNICEF and LAMBO Foundation.

The Workshop was attended by 36 African scientists.

Data on prepregnant nutrition, foetal growth, lactation and milk composition, breast feeding, infant-weaning practices and public health aspects were presented and discussed.

In the light of the discussions, Prof. Rajalakshmi and Prof. Omololu prepared a questionnaire to elicit information from different African Countries on various points discussed in the workshop and requested the participants to fill up the questionnaire and indicate the lines of research which could be carried out in their respective countries. A detailed technical report will appear in 1983 issue of Baroda Journal of Nutrition which has also published a book on Nutrition and Development of the Child in 1982.