Cytology and Morphogenesis of Embryo and Endosperm Tissues of Dendrophthoe and Taxillus

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Introduction

The cytology of cultured plant cells has been studied in a number of species. Occurrence of polyploidy, aneuploidy, and cells with giant nuclei have been observed, especially after repeated subcultures (Straus 1954, Norstog 1956, Mitra et al. 1960, Yamada et al. 1963, 1964, Cooper et al. 1964, Yamada and Sinotó 1967a, 1967b, Norstog et al. 1969, Heinz et al. 1969). However, these abnormalities in chromosomal behaviour in cultured tissues should not be generalized since instances do occur where the constancy in chromosome number is maintained (see Partanen 1963). Many investigators have recognised the constitution of the culture medium, or the abnormal environment of growth, as the causative agent for bringing about instabilities in chromosome numbers (Straus 1954, Cooper et al. 1964). Such a suggestion, however, fails to explain the situation where constancy in chromosome number is maintained. A pertinent question, therefore, is: Why is it that some of the plant species in cultures show frequent inconstancies whereas others normally do not show such a behaviour? A relevant observation is that of Partanen (1959). He demonstrated that Helianthus tuberosus, which exhibited a constancy of diploid chromosome number in the callus, also displayed an uniform diploid condition in the shoot, root and tuber tissues of naturally-growing plants. Reviewing the widespread occurrence of natural somatic endopolyploidy in angiosperms (see D’Amato 1952, 1965), and the earlier cytological investigations of the cultured tissues, Partanen (1965a) put forward a view that the behaviour of chromosomes in vitro is primarily the reflection of inherent tendencies of the plant implying a genotypic control. Experimental evidences to substantiate this hypothesis, however, are meagre.

A gradual loss in the capacity for root formation during repeated subcultures of pea (Pisum sativum) root callus was observed by Torrey (1967). This gradual loss was roughly accompanied by an increase in polyploidy and aneuploidy in the cells of callus. Torrey, therefore, suggested that “...the loss in organ-forming capacity is correlated with the increase in abnormality of chromosomal constitution”. Our investigations on the embryonal callus of Nuytsia floribunda (Loranthaceae), on the contrary, showed that even though the chromosome number remained at a constant diploid level, there occurred a gradual loss in the potentiality for root and shoot formation after repeated subcultures (Nag and Johri 1969).
In pea root cultures the original explant consisted of a mixture of diploid and tetra-
ploid cells, whereas in our studies it was the embryo of *Nuytsia* consisting of only
diploid cells.

The present paper deals with the cytological and morphogenetical findings
with reference to diploid (embryonal) and triploid (endosperm) tissues of three
mistletoes.

Materials and methods

Ripe fruits of *Dendrophthoe falcata* (L. f.) Ettings. were collected from Delhi
Ridge, of *Taxillus cuneatus* L. from Bombay, and *T. vestitus* Wall. from Shillong.
The endosperm and embryo were dissected out aseptically, and planted separately
on White’s modified medium (WM) containing indoleacetic acid (IAA), indolebuty-
ric acid (IBA), kinetin (KN), and casein hydrolysate (CH), either individually or in
various combinations (for details see Nag and Johri 1971). The cultures were
maintained under diffuse day-light at 25°±3°C and 50–60% relative humidity.

*Cytology of microspore mother cells, embryo, endosperm and fruit wall tissues
(in vivo)*: Young anthers of *D. falcata* were fixed in acetic alcohol (1:3). Squashes
were made in acetocarmine (2%) following the usual schedule. Embryo, endosperm
and inner fruit wall tissues were dissected out from young fruits (20–30 days after
anthesis) in the field, in the morning hours, and fixed in acetic alcohol for 24 hr.
In a few cases pre-fixation was done in a saturated solution of *p*-dichlorobenzene
for 1.45 hr. The tissues were hydrolysed in 1N-HCl for 12–15 min. at 60°C, and
rinsed with water. Storing the hydrolysed tissues in water for 6–8 hr facilitated
staining. The squashes were made in acetocarmine (2%), propionocarmine (2%),
or by Feulgen technique.

*Cytology of callus, shoot tip, leaf tip and haustorial tip (in vitro)*: In vitro-
grown callus, shoot and leaf tips from embryonal and endosperm tissues were
pre-treated with a saturated solution of *p*-dichlorobenzene for 2 hr, washed thorough-
ly in water, and fixed in acetic alcohol as usual; for control the material was fixed
without any pre-treatment. The callus and embryoids were hydrolysed for 7–10
min. in 1N-HCl at 60°C; the tips of leaf, shoot and haustorium for 12–15 min.

For anatomical studies of the leaves, customary methods of dehydration in
alcohol-xylol series were followed by infiltration and embedding in paraffin wax.
Sections were stained with safranin-fast green; the leaves were also cleared in KOH,
and chloral hydrate.

Observations

The endosperm, in *Dendrophthoe* and *Taxillus*, as in other members of Loranthaceae, is a ‘composite’ structure formed as a result of the fusion of several endo-
sperms developing from different embryo sacs within the same ovary (Narayana
1955, also see Johri and Bhatnagar 1971). At maturity, it becomes massive and
encloses the embryo except at the hypocotyl and radicular end. Its cells are filled
with starch, some of them also contain tannin. The mature embryo is deep-green
in both the genera; the endosperm is green in *T. cuneatus* and whitish in *D. falcata* and *T. vestitus*.

Shoot buds differentiated from the endosperm of both *T. cuneatus* and *T. vestitus*, on WM containing KN (5 ppm), in 6 to 10-week-old cultures (see Nag and Johri 1971). In *D. falcata* buds differentiated only when both KN (5 ppm) and IAA (2.5 ppm) were added to WM. In *T. vestitus* the buds organized directly on the surface of endosperm; whereas in *T. cuneatus* slight callusing always preceded organogenesis. Differentiation of haustoria, at the base of shoot buds, was ob-

Figs. 1 and 2. *Taxillus cuneatus*. 1, 12-week-old subcultures (after 3 passages, each of 10 weeks) of embryonal (a, b) and endosperm (c, d), callus on WM+IAA (2.5 ppm)+KN (5 ppm)+CH (2000 ppm); note the differentiation of leaves. ×1.8. 2, leaves from specimens shown in Fig. 1 (a and b from embryonal, and c and d from endosperm callus). ×3.

served in *T. cuneatus*, on WM+KN. In *D. falcata* and *T. vestitus* only haustoria differentiated when both IAA (or IBA) and CH were added to WM+KN. On WM+IAA (2.5 ppm)+KN (5 ppm)+CH (2000 ppm), in all the three species, shoot buds differentiated from the cultured endosperm and developed into normal shoots.

The response of the embryo was somewhat different (see Nag and Johri 1971, 1974). In *T. vestitus* only a few shoot buds differentiated on WM+KN (5, 8 and 10 ppm) but, when IAA and CH were added to this medium, the embryo callused
profusely and differentiated many shoots. The callus was maintained on WM+IAA (2.5 ppm)+KN (5 ppm)+CH (2000 ppm) for 30 months. WM+IBA (5 ppm)+KN (5 ppm)+CH (2000 ppm) promoted both shoot and haustorium formation, in contrast to only haustorium formation from endosperm.

The embryo of *T. cuneatus*, unlike that of *T. vestitus*, callused and differenti-

Figs. 3 and 4. *Taxillus cuneatus*. Anatomy of leaves differentiated from embryonal (diploid, Fig. 3) and endosperm (triploid, Fig. 4) callus. ×218.

ated shoot buds and haustoria on WM with KN (5, 8 and 10 ppm) alone, whereas in *D. falcata* only shoot buds developed on this medium. On WM+IAA (2.5 ppm)+KN (5 ppm)+CH (2000 ppm) the embryo of both *D. falcata* and *T. cuneatus* callused and differentiated shoot buds. This callus could be subcultured on the same medium (i.e. WM+IAA+KN+CH) for 30 months, without any loss in
the capacity for organogenesis.

Initially, the endosperm callus was whitish in *D. falcata* and *T. vestitus*, and slightly greenish in *T. cuneatus*, whereas the embryonal callus of all the species was greenish from the very beginning. During subcultures, however, the embryonal as well as the endosperm callus became compact and chlorophyllous. The growth of the callus was due to the activity of a peripheral meristematic zone.

In *T. cuneatus* the leaves (Figs. 1, 2) which differentiated from the endosperm callus subcultures were mostly linear (12.5 mm × 2.5 mm), in contrast to those from the embryonal callus which were ovate (14 mm × 4 mm). However, the leaves which differentiated after four subcultures, from endosperm or embryo callus, did not show any appreciable difference in morphology or anatomy (Figs. 3, 4).

*Dendrophthoe falcata—Meiosis in pollen mother cells (in vivo)*: At the onset of reduction division, the nucleolus became very prominent. A pair of chromosomes was always associated with the nucleolus. Nine bivalents were observed invariably (Fig. 5). The configuration of bivalents varied according to the nature and degree of terminalization of chiasmata. More than 1200 cells in metaphase I were screened; all of them invariably showed 9 bivalents (Fig. 6). Anaphase I and telophase I were also normal (Fig. 7). Meiosis II was regular and synchronous (Fig. 8). None of the chromosomes showed any lagging behaviour. The haploid nuclei moved normally to form tetrahedral spore tetrads (Fig. 9).

*D. falcata—Cytology of fruit wall, embryo and endosperm (in vivo)*: All the cells in the inner fruit-wall tissue, and of embryo and endosperm, were uninucleate. Mitosis was regular, and 18 chromosomes were invariably present at metaphase in the cells of fruit-wall (counted in 242 cells) and embryo (counted in 300 cells). Anaphase was normal; no lagging chromosome or abnormality of any type was seen.
There was no variation in the size and shape of nuclei in the cells of endosperm (Fig. 10). At prometaphase a prominent nucleolus in association with 3 nucleolar chromosomes was discernible (Fig. 11). Chromosome counts at metaphase in 754 cells from different endosperms revealed the presence of a triploid number (3n=27), without any exception (Fig. 12). Anaphase was normal but, in heavily squashed preparations of the pre-treated materials, the divided chromosomes were seen scattered throughout the cell (Fig. 13). Such preparations were observed very carefully so as to confirm whether they were hexaploid cells. 54 chromosomes could be counted in only 5 cells. Gradual tapping of an anaphase, size of chromosomes, and bending of arms at the centromere points, however, suggested that all figures were disrupted anaphases. Telophase was also normal.

**Cytology of callus and in vitro-differentiated organs:** In all the three mistletoes the cells of callus, and of the in vitro-formed organs, were invariably uninucleate. Chromosome counts in the cells of embryonal callus of all the three species, and leaf, shoot, haustorium, and embryoids differentiated from it showed 18 (2n) chromosomes (Figs. 14, 21–24); 27 (3n) chromosomes in the case of endosperm (Figs. 16, 17, 20). Irrespective of the number of passages (6–8 weeks each) through subcultures (8–16), the callus, as well as the differentiated organs, maintained 2n=18 (Figs. 15, 19, 23, 24) and 3n=27 (Figs. 17, 18, 20).

A difference in the size of chromosomes was noted in *T. vestitus*. In the initial culture the chromosomes in the cells of shoot and leaf (differentiated from the embryonal or endosperm tissue) measured 1.2–2.6 μ, whereas those in the cells of haustoria 1.8–4.0 μ. After 5 subcultures (each passage of 10 weeks) the size of chromosomes in the cells of haustoria and embryoids showed an approximately 3-fold increase, measuring 3.0–8.5 μ (Figs. 21, 22). This increase in size was noted
only up to the tenth passage; no significant increase occurred thereafter (Figs. 23, 24).

Such an increase in the size of chromosomes was not observed in *T. cuneatus*. Though the morphology and anatomy of the in vitro-differentiated diploid and triploid leaves were comparable, the nucleus in the tips of young leaves from em-

bryonal callus was roughly two-thirds (Figs. 25, 26) as compared to that from endo-
sperm callus.

**Discussion**

Unlike the inconstancies observed in the chromosome numbers in most of the
Figs. 14–18. *Dendrophyte falcata*. Cytology of tissues *in vitro*: 14, metaphase, from a leaf-tip cell of embryonal callus showing $2n=18$ chromosomes. $\times 2800$. 15, metaphase, from a shoot-tip cell developed in a callused leaf showing 18 chromosomes. $\times 3150$. 16, cells of endosperm callus; note the nuclei with 2 and 3 nucleoli (arrow-marked). $\times 290$. 17, metaphase, from a leaf-tip cell developed on callused endosperm showing $3n=27$ chromosomes; arrow-marked chromosomes broke at centromere points during tapping. $\times 2068$. 18, anaphase chromosomes in pretreated cells of endosperm callus showing 54 chromosomes. $\times 1034$. 

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cultured plant tissues (see Partanen 1963), the callus and organs differentiated in vitro in *Dendrophthoe* and *Taxillus* remain at a constant diploid, or triploid, level. In this feature these mistletoes resemble the tissues of *Helianthus tuberosus* (Partanen 1959) and *Nuytsia floribunda* (Nag and Johri 1969).

Are inconstancies due to the ingredients of the medium, and/or physical environment in which they are subjected to grow? Or, is it the inherent potentiality of the plant that is merely being reflected in the confinements of the cultural conditions? Different investigators have offered different explanations. Straus (1954), for example, concluded that it was possibly due to the nucleic acids introduced by the yeast extract in the medium that cultured endosperm tissues of *Zea mays* showed polyploidy and chromosomal aberrations. In the crown gall tissue of *Nicotiana tabacum* where even single-cell clones, maintained on almost the same medium for a long period, exhibited polyploidy in addition to the normal diploid chromosome level. Cooper *et al.* (1964) ascribed this change in the ploidy “... to wounding, to the constituent of culture medium, and/or to abnormal mitosis”. Likewise, Norstog *et al.* (1969) attributed the change in ploidy in 10-year-old endosperm tissue of *Lolium perenne* to the ingredients of medium. Yamada *et al.* (1963, 1964) and Yamada and Sinotō (1967a, 1967b), observed in the tissue cultures of *Tradescantia reflexa*, *T. paludosa* and *Paeonia japonica*, occurrence of polyploidy and aneuploidy on the same medium which supported the division of diploid cells. Considering the widespread occurrence of somatic polyploidy in plants, D'Amato (1965) considered that “... it can be regarded as the rule rather than the exception.”

![Figs. 19 and 20. *Taxillus cuneatus*. Cytology of organs differentiated in vitro. 19, metaphase, from a leaf-tip cell from embryonal callus showing 2n=18 chromosomes. ×2890. 2, same, from a leaf-tip cell from endosperm callus showing 3n=27 chromosomes. ×2800.](image-url)
Figs. 21–24. *Taxillus vestitus*. Cytology of organs differentiated from embryonal tissue. 21, metaphase, from a cell of shoot-tip developed from hypocotyl callus; note $2n=18$ chromosomes. $\times 1800$. 22, metaphase, from a cell of embryoid differentiated after 5 passages. $\times 2800$. 23, Same as 22, from a cell of embryoid differentiated after 2 years of subculture (through 9 passages), showing 18 chromosomes. $\times 2400$. 24, same as 23, from a cell of haustorium, differentiated after 2 years of subcultures (through 9 passages), showing 18 chromosomes. $\times 2200$.

Figs. 25 and 26. *Taxillus cuneatus*. Squash preparations from leaf-tip cells from embryonal (25) and endosperm (26) callus; note the difference in the size of nuclei. $\times 300$. 

The present investigation on *D. falcata* revealed a normal behaviour of chromosomes, both in vivo and in vitro. Even the cells of endosperm, which in most of the angiosperms contain polyploid and aneuploid nuclei (Kapoor 1962), displayed only triploid numbers. It was indeed remarkable that the stability in chromosome number was maintained—both in the diploid and triploid lineages of tissues—despite the repetitive dedifferentiation and redifferentiation. The demonstration that the nuclear divisions in vivo, in this plant, follow a regular sequence may, thus, be correlated with the regular diploid and triploid mitoses in calli and organs differentiated therefrom. The statement of Partanen (1965a) that “...what is observable in vitro is highly explicable as a reflection of potentialities in vivo” therefore seems justified.

Although the nutritional requirements for callusing and organogenesis in the diploid and triploid tissues of *D. falcata* and *T. vestitus* were markedly different in the initial cultures, no such difference was noted in *T. cuneatus*. For all the three taxa, in the repeated subcultures the requirements for callus formation and organ differentiation were, however, alike. The calli showed luxuriant growth and organogenesis on WM+IAA (2.5 ppm)+KN (5 ppm)+CH (2000 ppm). The morphology and anatomy of the diploid and triploid organs did not show much variation. The morphological appearance of *n*, 2n and 4n gametophytes of *Osmunda cinnamomia* has also been reported to be comparable (Partanen 1965b).

Torrey (1967) explained the loss in organ-forming capacity of pea root callus, after repeated subcultures, to the concomitant increase in the abnormality of chromosomal constitution. In the callus cultures of *Crepis capillaris*, Reinert and Küster (1966) observed a constancy at the diploid level, but found a gradual loss in the shoot-forming capacity at the end of one year. However, Yazawa’s (1967) studies on *C. capillaris* demonstrated that though till the 13th month the callus showed only diploid cells, squash preparations made at the end of the 17th month revealed 74% tetraploid and 16% octaploid cells. In the present investigation there was neither a change in the number of chromosomes, nor a loss in the potentiality for organ formation. Both the diploid and triploid tissues were capable of growth and organogenesis through repeated subcultures.

The differentiation of an organ from the callus is governed by many complex factors (see Gautheret 1966). In cultured tissues what role does the constancy of chromosomes, or aneuploidy and polyploidy, play in the differentiation of organs is not adequately understood. These problems deserve detailed experimentation which would no doubt be very rewarding.

**Abstract**

Cytological and morphogenetical studies were conducted on the tissues obtained in vitro, from the mature embryo and endosperm of three mistletoes: *Dendrophthoe falcata*, *Taxillus cuneatus* and *T. vestitus* (Loranthaceae). Differentiation of shoot buds and haustoria was observed during repeated subcultures of the embryo and endosperm tissues (on a similar medium). Cytological preparations of the embryonal and endosperm callus, and organs differentiated therefrom, showed the
diploid (2n=18) and triploid number (3n=27) of chromosomes, respectively. A cytological study of D. falcata (in vivo) indicated regular meiosis (n=9) during microsporogenesis. The developing embryo, endosperm, and fruit wall tissues exhibited constant chromosome numbers: 18, 27 and 18, respectively. The morphology as well as anatomy of diploid and triploid organs, differentiated in vitro, was more or less comparable.

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References


