Cytology of Fenugreek Calli Cultivated *in vitro*

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Great strides have been made in the nutrition and physiology of plant tissue cultures, but cytology is still lagging behind (Partanen 1963, White 1967). It is only recently that the cytology of plant tissue cultures has begun to attract attention. A survey of the literature on the cytology of plant tissue culture revealed that attention has been directed to only a few of the species. It is imperative, therefore, that wider survey of the subject be made for better understanding.

A study of the histogenesis of calli developed from the hypocotyl explants of fenugreek, *Trigonella foenum-graecum* L. revealed that they originated mostly from the pericycle (Gupta 1969), which is always known to be diploid (Torrey 1959). Most of the investigators reported polyploidy and aberrations in callus cultures as common phenomena. Since fenugreek calli originated from cells having a diploid constitution, it was thought desirable to study the cytological changes that occurred during callus formation.

Materials and methods

The primary culture and 12th subculture were exclusively employed for the cytological investigations. The cultures raised from the hypocotyl explants of fenugreek were maintained on the agarified Murashige and Skoog's medium supplemented with 15% (v/v) coconut milk and 0.2% casein hydrolysate at 23°C (Gupta 1969), exposed to a light intensity of 1000 lux for 8 hours per day. The calli were fixed in acetic-alcohol (1:3) during morning hours and were processed according to Gupta's (1970) procedure for staining with Harris' haematoxylin.

The cells and nuclei were measured at a magnification of ca 400. Their area was calculated using the formula: $\text{area} = \pi a c$, where a and c are radii in directions at right angles to each other (Bucher 1954). Cells from ten random fields in a slide were measured. Cells and nuclei with irregular dimensions were omitted from measurements. The data from three slides were pooled for analysis. Since the cells were flattened during squashing, the cell and nuclear area were considered proportional to their volumes (Comings 1967). The area of the nuclei were plotted against the age of the culture utilizing the proportionality factor, 1.58 (Bucher 1954).

Some of the cells had a very large number of chromosomes. It was often difficult to count them exactly. Therefore, the ploidy is assigned arbitrarily.

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Observations

Shape and size of cells: The cells from the primary and the 12th subculture exhibited considerable variation in shape and size. The meristematic cells were generally small in size, densely protoplasmic and isodiametric. The mature cells, on the other hand, exhibited great diversity in their morphology ranging from long filamentous types to globular ones. Fig. 1 depicts a fusiform binucleate cell.

Graph 1. Cell sizes.

Cells often exhibited non-septate papillate or tubular projections. Figure 2 apparently is of an early stage of the development of a bud. The mother cell may be uni- or bi-nucleate. In the latter condition, one of the nuclei may enter the bud (Fig. 3) or both may remain in the mother cell (Fig. 4). Fig. 5 is that of a flash-shaped cell, in the neck of which the solitary nucleus of the cell has migrated.

The cell sizes, as judged by their area showed considerable variation and ranged from $500 \mu^2$ to $64,000 \mu^2$. Graph 1 emphasizes the relatively higher variability of cells sizes from primary than from 12th subculture: While the modes of cell sizes

Figs. 1-9. 1, a binucleate oblong cell ($\times 500$). 2, cell with a bud-like outgrowth. $\times 500$. 3, binucleate cell with a bud, both mother cell and the bud are having one nucleus in each. $\times 500$. 4, binucleate cell with a big bud-like growth, both nuclei are in the mother cell. $\times 400$. 5, flask-shaped cell with its nucleus in the neck $\times 500$. 6, highly vacuolated nucleus. $\times 1800$. 7 and 8, elongated and dumb bell shaped nuclei. $\times 500$. 9, binucleate cell with nuclei at either ends. $\times 700$.

of the primary culture shifted with the passage of time, those of 12th subculture remained apparently constant (at $3 \times 10^3 \mu^2$).

Number and morphology of nuclei: Generally the interphase nuclei had a spherical outline. About 5% of the nuclei, however, exhibited variation in their morphology. Some of the cells were bi- or multi-nucleate.

Primary culture: During the early stages of the growth of an explant all nuclei had a homogeneous interior, but differed widely in their size. After three weeks of growth some of the nuclei exhibited vacuolation. Fig. 6 is that of a highly vacuolated large sized nucleus. In four week old calli, the nuclei of some of the cells were spindle—or dumbbell-shaped (Figs. 7 and 8).

Binucleate and multinucleate cells were rather rare. The binucleate cell in Fig. 9 has a waist-like constriction with its nuclei in the dilated ends. Fusion- or fission- like structures were observed (Fig. 10). Figs. 11 and 12 of the nuclei connected by thread-like structures are difficult to interpret.

All the nuclei of one-week old explant had only a single nucleolus, while 20% of nuclei of two-week old explants had 2 to 4 nucleoli. The lobed nucleus in Fig. 13 has five nucleoli. From the third week onward only about 2% nuclei were multinucleolate. The cells exhibiting such a condition were usually smaller in size.

12th subculture: Unlike in the primary culture multinucleate cells were observed during all the six weeks of callus growth. The percentage of such cells was about 25.

During the first three weeks of growth about 30% nuclei were multinucleolate. The incidence of such nuclei is higher in this subculture than in the primary culture. During further growth, there was a reduction in the percentage of the multinucleolate nuclei. As in the primary culture the cells exhibiting the multinucleolate condition were smaller in size and had dense cytoplasm.

Nuclear size: Considerable diversity was observed in the size of the nuclei. While the distribution of the nuclear size from subculture was often unimodal, such a mode was usually absent from the primary culture (Graph 2).

Nuclear division: The cell division occurred only during the first week in the primary cultures. Division figures were observable within 24 hours of the inoculation. All the cells exhibited a diploid chromosome complement ($2n=16$) in 24 hour old primary cultures. The polyploid divisions observed in 48-hour old culture increased in percentage with passage of time.

Nuclear divisions were observed in the 12th subculture during the first two weeks after transfer. No division figures could be located after two weeks. Only polyploid and aneuploid division figures were observed but no diploid.

Whether it be a primary or the subculture, the cells in divisions were smaller in size, isodiametric and had dense cytoplasm. The nuclei of such cells were prominent and stained intensely. Rarely a large cell was seen dividing (Fig. 14). About 90% of the mitoses were normal. The aberrant mitoses had similar abnormalities in primary and in the subculture.

Figs. 15 and 16 illustrate the prophases of polyploid cells. Fig. 16 is probably of an octoploid cell ($2n=64$). A stained body of unknown identity

Graph 2. Nuclear area.

(arrow) is lying near the periphery of this nucleus.

Diploid metaphase from a primary culture is illustrated in Fig. 17. Fig. 18 and 19 are of metaphases exhibiting increased chromosome numbers. While Fig. 18 appears to be that of a tetraploid, Fig. 19 seems to be of an octoploid. It

Figs. 10-18. 10, fusion of nuclei or fission of a nucleus. $\times 500$. 11 and 12, nuclei with anomalous configurations. $\times 600$. 13, a pentanucleolate lobed nucleus. $\times 1550$. 14, large cell exhibiting metaphase. $\times 500$. 15 and 16, late prophase of polyploid cells. $\times 800$ and $\times 1400$ respectively. 17, diploid metaphase. $\times 2000$. 18, metaphase of a tetraploid (suspected) cell. $\times 2000$.

Figs. 19-26. 19, V-shaped metaphase plate of a highly polyploid cell. $\times 1200$. 20, metaphase of presumably an octoploid cell. $\times 1500$. 21, metaphase of an aneuploid cell. $\times 1500$. 22, highly polyploid anaphase, the anaphase movement of chromosomes appears asynchronous. $\times 800$. 23, multiple chromosome bridges at anaphase. $\times 1800$. 24, chromatin bridge between the telophasic groups. $\times 1400$. 25, anaphase groups still lying close to each other. $\times 1500$. 26, normal telophase of a highly polyploid cell. $\times 1800$.

is interesting that the metaphase plate in Fig. 19 has a 'V' shape. Polar views of the metaphases of polyploid and/or aneuploids are presented in Figs. 20-22. Fig. 20 is probably of an octoploid metaphase. The chromosomes in Fig. 21 are

Graph 3. Nuclear/cell ratio.

difficult to count exactly owing to its large chromosome numbers probably it represents a hexadecaploid or an aneuploid.

Fig. 22 presents an anaphase of a polyploid cell. It appears that the polar movement of chromosomes is not synchronous. Multiple chromosome bridges are exhibited in Fig. 23 while Fig. 24 exhibits a chromatin bridge between telophase groups.

Figs. 25 and 26 are of telophases. A pair of telophasic groups lying together (Fig. 25) is suggestive of a prelude to the formation of a binucleate cell.

Discussion

The tissues growing *in vitro* constitute an unorganized system. Their varying manifestations are sporadic and reflect perhaps the capacity of the genotype to respond to local changes in the environment.

Fenugreek cells cultivated *in vitro* exhibited great diversity in their shape and size. Similar observations were recorded in suspension cultures of several plant tissues (Nickell 1956, Street and Henshaw 1963). Steward (1958) considered the bud-like projections to be the consequences of scattered points of weakness in the cell wall. Street (1966) suggested that the appearance of giant cells is due to the absence of those factors which normally limits cell division. The greater uniformity of cell size in 12th subculture as compared to the primary culture, may be due either to the adaptation of certain cells to cultural conditions or a selection among them.

The nuclear configurations presented in Figs. 7, 8, 11 and 12 are rather interesting and need further elucidation.

Nuclei with many nucleoli were seen only in smaller, presumably meristematic cells. It would appear, therefore, that a multinucleolate condition of the nuclei may be a manifestation of greater activity of nuclei, since the occurrence of the multinucleolate condition corresponded with the occurrence of division in cultures.

A comparison of the morphology and size of the cells and nuclei in the primary culture than in the 12th subculture emphasizes the greater range of variation in the former. In spite of all variations the nuclear/cytoplasmic ratio is said, to remain constant (De Robertis, Nowinsky and Saez 1960). An attempt was made to check this postulate from the measurements of cell and nuclear areas from fenugreek cultures. The results are presented in Graph 3. An apparent constancy in the nucleus/cell ratio was observed in the primary cultures while no constancy was apparent in the subculture. These results suggest that much reliance should not be placed on such a constancy in cultured cells. Variations of the nucleus/cell ratio might be brought about by asynchronous nuclear and cytoplasmic growth during the interphase (De Robertis *et al.* 1960).

Generally a majority of the cells exhibited normal mitoses irrespective of their ploidy level. It is only a small percentage (10%) among them that mitotic abnormalities occurred. Partanen (1905) has presented a scheme as to the possible mode of origin of polyploidy. Aneuploidy is a corollary to multipolar spindles, lagging chromosome, and bridge formation during ana- and telophases. Partanen proposed that the behaviour of cells *in vitro* is largely explicable as a reflection of

their potentialities *in vivo*.

Chromosomal aberrations were observed in the cells of primary cultures and the subculture. Venkateswaran (1963) interpreted them to be the result of chromosomal breaks and the consequent structural rearrangements in resting or actively dividing cells. Such chromosomal rearrangements are often expressed as ana- and telophase bridges during the succeeding mitoses. The nucleic acids present in the media have been considered responsible for chromosomal breaks and consequent rearrangements by Straus (1954), Torrey (1959), and Mitra and Steward (1961). Venkateswaran and Spiess (1963), however, could obtain no evidence for the role of nucleic acids or their precursors as agents for chromosomal aberrations.

The morphological changes observed in the cells and nuclei from the cultures of fenugreek are in conformity with the observations recorded in many species. The differences between the species may only be quantitative.

Summary

The cytology of fenugreek calli from the primary culture and 12th subculture was studied. Cytometry and karyometry revealed a greater range of variation in the primary culture than in the subculture. In general mitoses were normal irrespective of their ploidy. Infrequently multinucleate cells, apparent nuclear fusion or fission, bridge formation at ana- and telophases were also observed in both the cultures.

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Literature cited

- Bucher, O. 1954. Caryometric studies of tissue cultures. *Internatl. Rev. Cytol.* 3: 69–110.
Combings, D. E. 1967. Sex chromatin, nuclear size and the cell cycle. *Cytogenetics* 6: 120–144.
De Robertis, E., Nowinsky, W. and Saez, F. 1960. *General Cytology*, Saunders Philadelphia.
Gupta, K. C. 1969. Culture of the organs and cells of *Trigonella foenum-graecum* L. and their cytology. Ph. D. Thesis, Indian Institute of Science, Bangalore, India.
— 1970. A simple squash technique for the tissue cultures of *Trigonella foenum-graecum* L. *Curr. Sci. (India)* 39: 40–42.
Mitra, J. and Steward, F. C. 1961. Growth induction in cultures of *Haplopappus gracilis* II. The behaviour of the nucleus. *Amer. J. Bot.* 48: 358–367.
Nickell, L. G. 1956. The continuous submerged cultivation of plant tissue as single cells. *Proc. Natl. Acad. Sci. (U.S.A.)* 42: 848–850.
Parthanen, C. R. 1963. Plant tissue culture in relation to developmental cytology. *Internatl. Rev. Cytol.* 15: 215–241.
Parthanen, C. R. 1965. Cytological behaviour of plant tissues *in vitro* as a reflection of potentialities *in vivo*. In 'Proc. Internatl. Conf. Plant Tissue Culture' 463–471. (Eds. P. R. White and A. R. Grove) McCulchan Publishing Co. Berkeley.

- Steward, F. C. 1958. Growth and organized development of cultured cells III. Interpretations of the growth from free cell to carrot plant. *Amer. J. Bot.* **45**: 709–713.
- Straus, J. 1954. Maize endosperm tissue grown *in vitro* II. Morphology and cytology. *Amer. J. Bot.* **41**: 833–839.
- Street, H. E. 1966. Growth, differentiation and organogenesis in plant tissue and organ cultures. In 'Cells and Tissues in Culture'. Vol. III 631–681. (E. N. Willmer) Acad. Press. Lond.
- and Henshaw, G. G. 1963. Cell division and differentiation in suspension cultures of higher plant cells. *Symp. Soc. Exptl. Biol.* **17**: 234–256.
- Torrey, J. G. 1959. Experimental modification of development in the root. In 'Cell, Organism and Milieu' 189–220. (Ed. D. Rudnick) Ronald Press. N.Y.
- Venketeswaran, S. 1963. Tissue culture studies on *Vicia faba* II. Cytology. *Caryologia* **16**: 91–99.
- and Spiess, E. B. 1963. Tissue culture studies on *Vicia faba* III. Effect of growth factors on chromosome morphology. *Cytologia* **28**: 201–211.
- White, P. R. 1967. The promises and challenges of tissue culture for biology and for mankind. In 'Seminar on cell tissue and organ culture', 12–19. (Ed. B. M. Johri) Delhi Univ. India.
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