

CALLUS CULTURING, ITS MAINTENANCE AND CYTOLOGICAL VARIATIONS IN *TRIGONELLA FOENUM-GRÆCUM* L.

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ABSTRACT

Studies on callus culture and cytological behaviour were performed in *Trigonella foenum-græcum* L. cotyledonary segment and it was found to be the best explant for callus initiation as well as callus growth and maintenance in MS basal nutrient medium supplemented with NAA in combination with coconut water. Cytological studies revealed the abundance of heterogeneous cells with different chromosome numbers varying from 16 to 48. The present results indicate variable frequencies of ploidy level and anomalies in different subcultures.

INTRODUCTION

TRIGONELLA FOENUM-GRÆCUM L. is traditionally cultivated for its spice-yielding seeds and leafy twigs. This plant is now gaining importance for its diosgenin content¹ and usefulness in the treatment of diabetes². Being self-pollinated, the plant offers very limited scope for improvement through conventional breeding methods. Genetic variability, however, can be induced by the exploitation of cell and tissue culture techniques. The possibility of utilization of these techniques needs proper assessment, but only a few relevant references are available^{1,3,4}. We therefore studied the behaviour of chromosomes in callus cells of *T. foenum-græcum*.

MATERIAL AND METHODS

Seeds of *T. foenum-græcum* (fenugreek) were surface-sterilized with 0.1% mercuric chloride for 5 min and then washed repeatedly with sterile distilled water. They were inoculated on Murashige and Skoog's (MS)⁵ medium without any hormone for germination. The cultures were maintained in the dark for rapid germination at $22 \pm 2^\circ\text{C}$. Cotyledonary and hypocotyledonary segments of the aseptically grown seedlings were used as explants for callus initiation. Small segments of the explants (4–6 mm thick) were cultured in MS medium (pH 5.8–6.0) supplemented with naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin and coconut water (CW) for initiation and growth of callus. The cultures were maintained at $22 \pm 2^\circ\text{C}$ and 55–60% RH under Philips fluorescent daylight tubes emitting $32 \times 10^8 \mu\text{mol sec}^{-1} \text{m}^{-2}$ in a 10/14 h

light/dark regime. Subcultures were carried out every 15 days.

For cytological observations cotyledon callus tissues were taken and fixed in acetic-ethanol (1:3). They were then slightly warmed in a 1% aceto-orcein–1 N HCl mixture (9:1), and kept for 1 h before washing in 45% acetic acid.

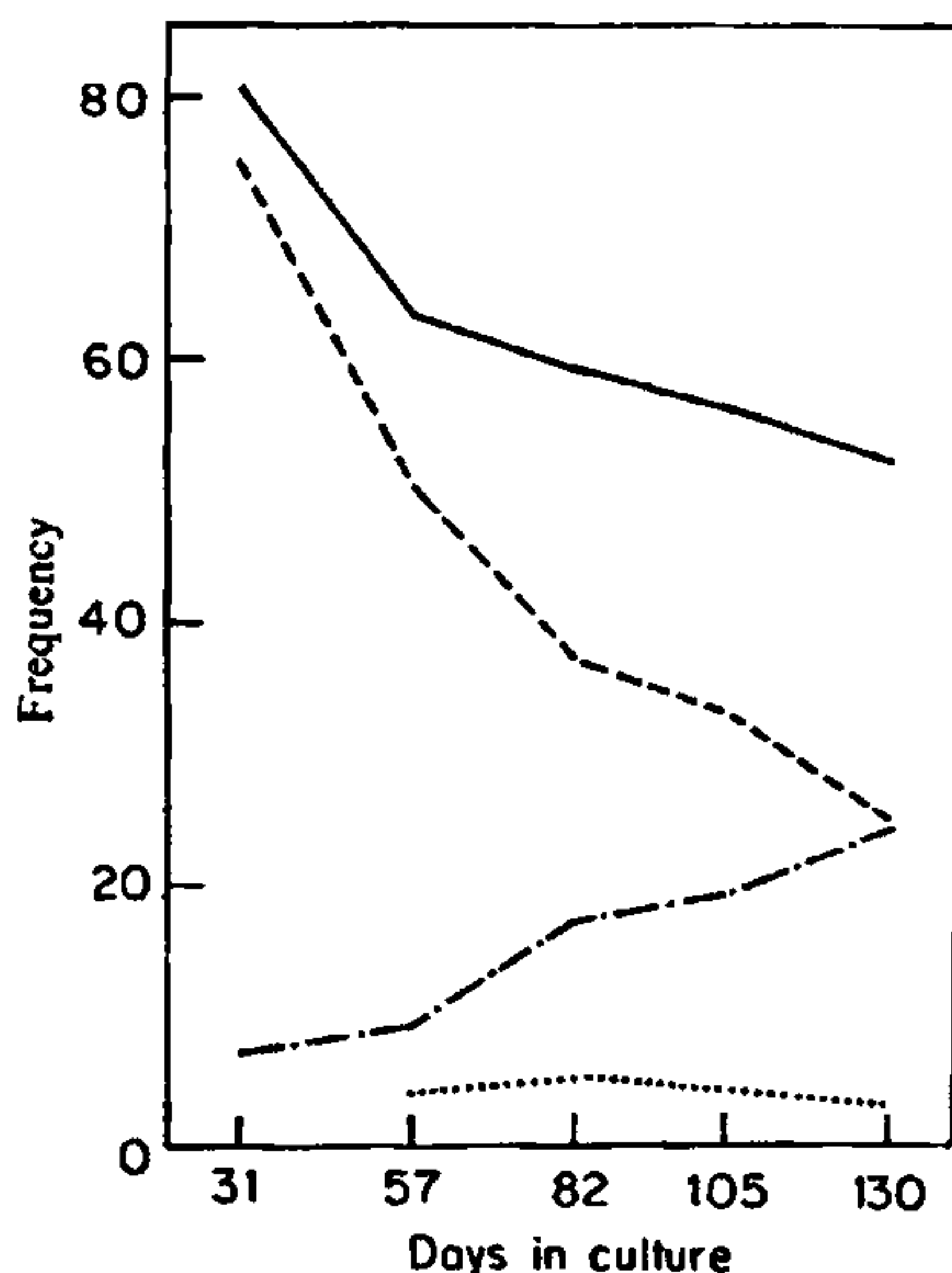
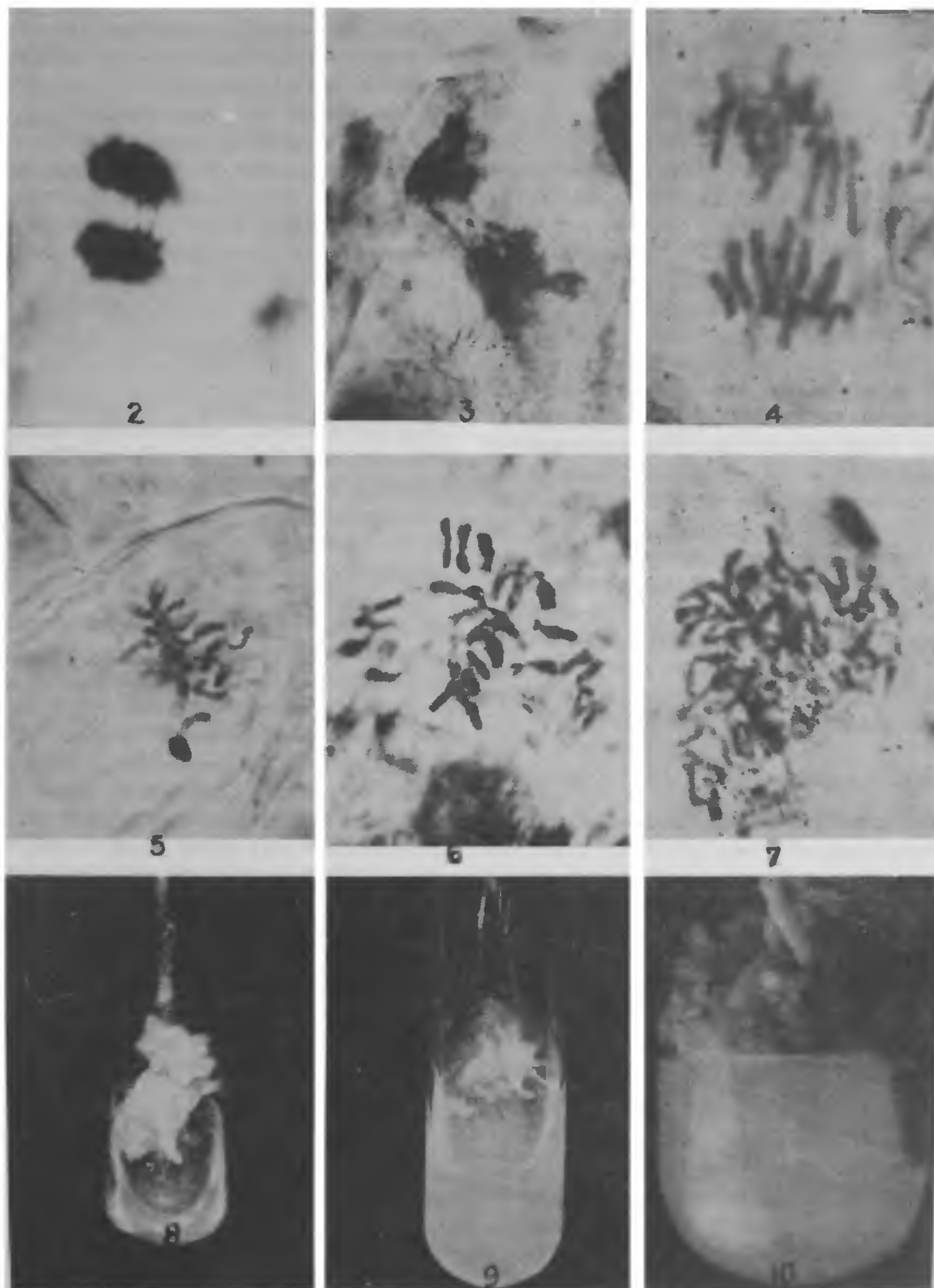


Figure 1. Frequency of diploid and polyploid cells in relation to age in culture (— dividing cells, — diploid cells, — tetraploid cells, higher-ployploid cells).



Figures 2-10. Cytology of cotyledon callus and callus growth and differentiation. **2,** Double bridge at anaphase. **3,** Bridge with laggard. **4,** Multipolar separation. **5,** Metaphase with $2n=16$. **6,** $4n=32$ at metaphase. **7,** Metaphase with hexaploid number. **8,** Callus from cotyledon. **9,** Root organogenesis. **10,** Callus with shoot buds.

RESULTS

Both cotyledonary and hypocotyledonary segments were swollen at the cut ends after two days. Callus tissue initiated on cotyledon segments within 5–6 days of inoculation but after 12–15 days in the case of hypocotyl. There was more callus proliferation from the cotyledon (figure 8). Among the growth hormones used NAA (2 mg/l) alone as well as in combination with 10% v/v CW resulted in better initiation and growth of the callus from the cotyledon. On the other hand neither 2,4-D nor kinetin alone could bring about any improvement in callus induction. Callus from cotyledon was soft, greenish white, friable and vigorously growing whereas callus from hypocotyl was milky to brownish white and compact. The appearance of whitish roots was noted on cotyledon callus at the third subculture, and at the end of this passage, both callus tissue and roots turned brown (figure 9). The callus in most cases turned brown to black and looked friable to granular, sticky and compact after two months (figure 10). There was no difference between cultures in callus initiation medium and in differentiating medium. The same growth hormone induced shoot bud formation and better callus development under identical conditions.

Cytological observations

Cytological studies on cotyledon callus revealed that mitotic index was very low in the initial cell population (table 1). The frequency of both diploid cells and total number of dividing cells decreased with age of the tissue but that of tetraploid and higher-polyploid cells increased (figure 1). The countable chromosome number in the somatic complement varied from a diploid number of 16 to 48 during growth and proliferation of cotyledon callus (figures 5–7). There was a high percentage

(45%) of normal diploid cells ($2n=16$) in cotyledon callus tissue at the first subculture. The percentages of triploid ($3n=24$), tetraploid ($4n=32$) and higher-polyploid ($6n=48$) cells were 30, 28 and 13% at the 4th, 5th and 3rd subculture respectively.

Among the observed chromosomal aberrations, in cotyledon callus cells, bridges, laggards and multipolarity were common and were maximum at the 6th subculture (figures 2–4). These were less common in hypocotyl callus cells. Chromosomal bridges were the most common aberration (table 1).

DISCUSSION

The cotyledon explant showed better response than the hypocotyl, which clearly indicates that different parts of the same plant may show different responses to callus-inducing medium. In the present investigation, chromosomal instability was indicated by the occurrence of cells with polyploid chromosome numbers and different types of mitotic anomalies. Calli constitute an unorganized system where chromosomal instability and karyotype changes are of predominant occurrence^{6–11}. Cytological and biochemical studies on callus cultures are useful in finding out the role of growth hormones in the production of chromosomal aberrations and in studying nucleic acid metabolism. A decrease in the frequency of dividing cells and increase in polyploid cells were seen with increasing age of culture. The polyploid cells may appear owing to either endomitosis or failure of cytokinesis and subsequent nuclear fusion, or a combination of both. Cell cultures with a mixed population of diploid and polyploid cells have been reported from time to time¹². Spindle irregularities have been said to account for the occurrence of polyploid and aneuploid cells in callus cultures¹³. In the present study, the polyploid cells were mainly tetraploid and cells with even higher

Table 1 Mitotic index and frequency of chromosomal abnormalities in *Trigonella foenum-graecum* cotyledon callus

	Days in culture				
	31	57	82	105	130
Mitotic index	2.69	2.17	2.07	2.02	1.77
Normal	90.1	89.2	87.1	78.5	68.3
Bridges	8.2	8.9	9.0	15.0	20.3
Laggards	1.7	1.9	3.1	5.3	8.6
Multipolarity	—	—	0.8	1.2	2.8
Mitotic cells scored	3005	2901	2850	2768	2931

ploidy occurred at a much lower frequency. A general tendency of change in ploidy under *in vitro* conditions has also been reported earlier¹⁴.

Different types of ana-telophase anomalies noted in *T. foenum-graecum* were found to increase with duration of culture but the frequency was low. Lagging chromosomes and multipolar separation have been cited as the main source of variation of chromosome number¹⁵. Such variations appeared partly as the manifestation of nuclear conditions in the primary explant and partly owing to nuclear changes occurring at the time of callus induction¹⁶.

It can be inferred that the cotyledon is the best material for morphogenetic studies in *T. foenum-graecum* under culture conditions. The rapid polyploidization in the callus of *T. foenum-graecum* may be utilized for regeneration of polyploid plants for successful exploitation of its medicinal properties.

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ANNOUNCEMENT

26TH ANNUAL CONVENTION OF THE INDIAN GEOPHYSICAL UNION AND SEMINAR ON GLOBAL CHANGE

The Indian Geophysical Union will hold its 26th Annual Convention during 14-16 December 1989 and organize a seminar on the theme 'Global Change'. Contributed original research papers are invited for presentation in the following general sessions of the Annual Convention: (i) solid earth geophysics/processes, (ii) oceanography, (ii) atmospheric sciences, (iv) space science and planetology, (v) solar-terrestrial relations, (vi) instrumentation.

The seminar on 'Global Change' will have invited papers. However, original research contributions are also welcome.

Abstracts must be sent by 30 October 1989 and full papers by 30 November 1989. For more details contact: Dr D. Atchuta Rao, Hon. Secretary, Indian Geophysical Union, NGRI Campus, Hyderabad 500 007, India.