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# CHROMOSOME ANALYSIS OF CULTURED CELLS OF *VIGNA MUNGO* (L). HEPPER

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## ABSTRACT

A study on the variation in chromosome number of the cells from callus tissues of *Vigna mungo* L. Hepper for cotyledon, hypocotyl and embryonal axis explant was undertaken. The variation induced by treatment 2, 4-D and kinetin treatments at 10, 20 and 30 days was studied in all the three explants. The chromosome number showed a wide range of variation, however, the frequency of diploid cells (2n = 22) was higher in all the cultures obtained from each type of explant. Frequency of tetraploid cells was higher as compared to aneuploid and cells with higher ploidy level, the tetraploid cells increased with the increase in age of the callus in all the explants. The frequency of aneuploid and higher polyploid cells also showed increase with increase in age of culture. Plant regeneration was achieved after 30 days of inoculation. Hypocotyl explant cultured on MS medium supplemented with 0.5 mg/l kinetin identified as the best treatment for the induction of somaclonal variation in Urd been.

**Key Words :** Somaclonal variation, *Vigna mungo*, chromosomal aberrations, callus tissues

Callus tissues have a unique potential for generating genetic variation. Plant cells grown *in vitro* generally exhibit cytological variations, which may be because of mixoploid nature of the explant used or due to culture conditions. This type of variation is commonly described as 'Somaclonal variation' and that has been documented in several reviews[1-2]. Structural and numerical variations in chromosomes of cultured cells and subsequent recovery of whole plants from them may be useful in plant breeding programmes aimed at crop improvement. On the other hand structural and numerical change in chromosomes disturbs the physiological and genetical balance of the callus leading to loose the capacity to regenerate plants[3-4]. Thus, plant regeneration appears to be linked with the chromosomes behaviour of the source callus culture[5]. Therefore, there is a need to establish the nature and source of variation in culture cells and to regulate the degree of variation with a view to explore the possibility of regenerating plants with varying chromosome number[6-7]. In view of the meagre information available on callus culture and its

cytological behaviour in Urd bean (*Vigna mungo* (L.) Hepper), the present investigations were carried out to study the chromosomal variations induced by phytohormons 2,4-D and Kinetin in three different explants viz., hypocotyl, cotyledons and embryonal axis of Urd bean.

## MATERIALS AND METHODS

Seeds of blackgram (*Vigna mungo* (L.) Hepper), variety T-9 were washed under running tap water than surface sterilized in 70% ethanol for half minute and washed three times with sterilized distilled water. The second surface sterilization was carried out by 0.1% $HgCl_2$ solution for five minutes. Aseptic seedlings were raised on humified cotton in petriplates and explants such as cotyledons, hypocotyl and embryonal axis were prepared from five days old seedlings. These explants were cultured on MS basal medium[8] supplemented with phytohormons 2,4-D (2, 4-dichlorophenoxy-acetic acid) and kinetin and their combinations. The cultures were kept in growth chambers with 26°C ± 2°C and illuminated with Philips (India) fluorescent lamps (25 µmol $m=^2$ S-1) for eight hours daily. Callus tissues were harvested from each treatment after 10, 20 and 30 days for cytological studies. For cytological studies cells were collected from culture tubes and were separately treated with L-bromonaphthalene for 3 hours, washed with distilled water, fixed in acetoalcohol (1:3) for 8 hours and preserved in 70%, alcohol, stored in refrigerator at 4°C for subsequent use. Acetocarmine stain was used to prepare temporary slides by squash technique. Data were observed from 10-15 preparation per callus.

## RESULTS AND DISCUSSION

Detail cytological study of the plant material used to derive different explants (cotyledon, hypocotyl and embryonal axis) of *Vigna mungo* L. Hepper variety T-9 was done and it was confirmed that it has the diploid number of chromosomes 2n = 22. Callus culture derived from different explants were analysed for variation in chromosome number and structure after 10, 20 and 30 days of culturing. In general a wide variation in chromosome number (Fig. 1 A-D) and structure were observed in all types of the explants. However, a majority of cells in each culture type were observed to be diploid in nature (Table 2). It was observed that frequency of diploid cell decreased with increase in concentration of growth hormones and culture durations. Combination treatment of 0.5 mg/l kinetin + 2.0 mg/l 2,4-D exhibited minimum number of diploid cells with maximum number of tetraploid, aneuploid and higher ploidy cells in all the three explants viz., cotyledon, hypocotyl and embryonal axis at all culture duration.

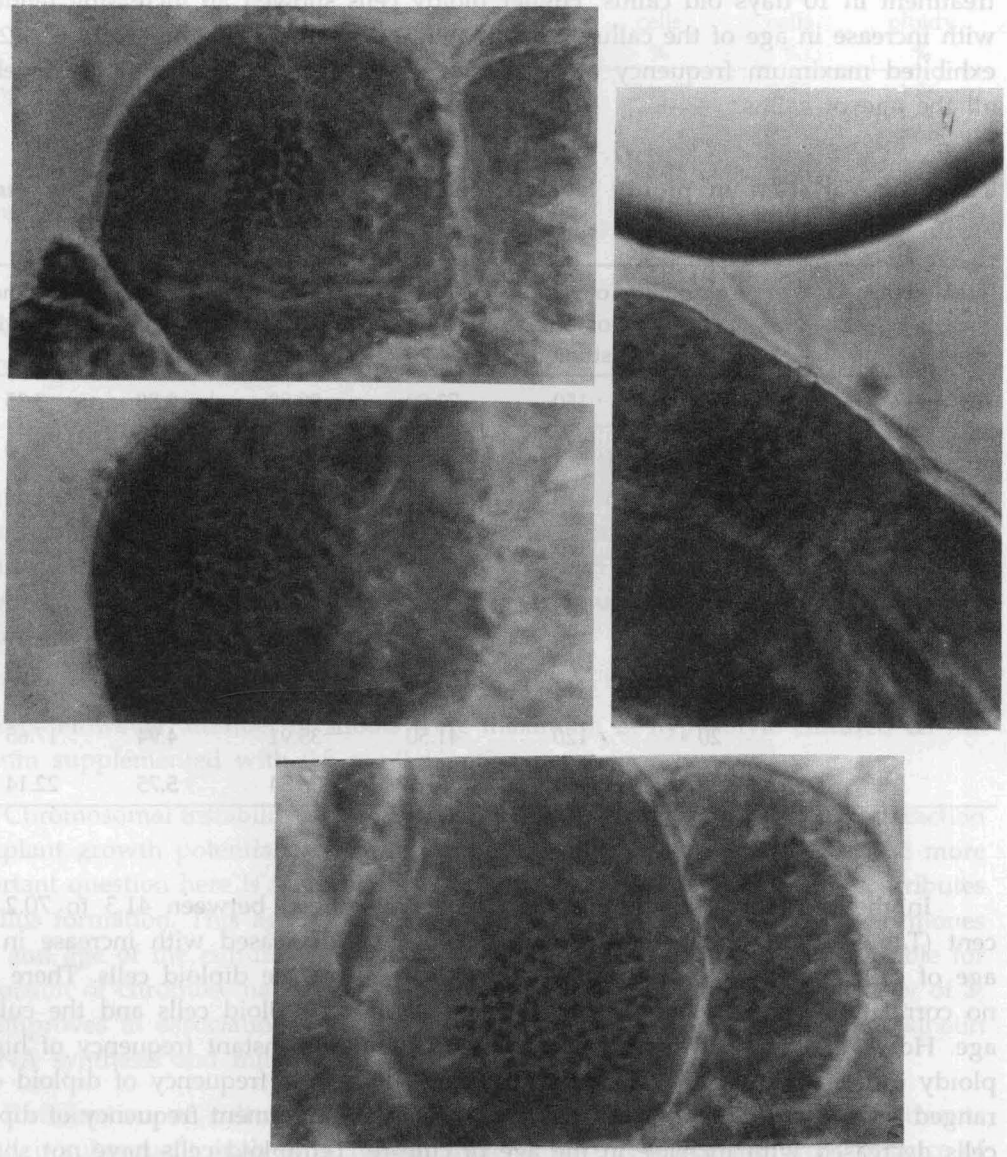

**Fig. 1A-D. Chromosome number variation in callus culture of *Vigna mungo* L. Hepper. A callus cells sharing normal diploid (2n = 22). B. callus cells sharing (2n = 55). C. callus cells sharing (2n = 66) D. callus sharing (2n = 13)**

In cotyledon culture the diploid cell frequency varied from 35.1 to 72.8 per cent. The frequency of diploid cell decreased with increase in the age of the culture at all the treatments (Table 1). It was maximum (72.8 per cent) at 0.5 mg/l kinetin treatment in 10 days old callus. Higher ploidy cells showed an increasing tendency with increase in age of the callus. Combination of 0.5 mg/l kinetin + 2.0 mg/l 2,4-D exhibited maximum frequency of tetraploid, aneuploid and higher ploidy levels at all the age of callus.

**Table 1. Variation in ploidy level in cotyledon Callus tissues of *Vigna mungo* (L.) Hepper**

| Treatments | Age of callus (days) | Total No. of cells studied | Diploid cells % | Tetraploid cells % | Aneuploid cells % | Higher ploidy % |
|---|---|---|---|---|---|---|
| 0.5 mg/l Kinetin | 10 | 150 | 72.89 | 20.88 | 2.88 | 3.35 |
| | 20 | 134 | 70.52 | 21.26 | 2.31 | 6.00 |
| | 30 | 145 | 66.95 | 19.78 | 1.73 | 11.54 |
| 2.0 mg/l 2,4-D | 10 | 128 | 58.02 | 27.89 | 4.88 | 8.21 |
| | 20 | 135 | 52.33 | 30.84 | 5.64 | 10.94 |
| | 30 | 140 | 46.42 | 31.02 | 5.35 | 17.21 |
| 0.5 mg/l Kinetin + 2.0 g/l 2,4-D | 10 | 144 | 52.25 | 35.11 | 4.18 | 8.46 |
| | 20 | 120 | 41.50 | 35.91 | 4.94 | 17.65 |
| | 30 | 108 | 35.17 | 36.94 | 5.75 | 22.14 |

In the hypocotyl the diploid cell frequency ranged between 41.3 to 70.2 per cent (Table 2). The frequency of diploid cells was decreased with increase in the age of callus. Tetraploid cells showed similar trend as the diploid cells. There was no correlation between increase or decrease of the aneuploid cells and the culture age. However, there always existed a low and almost constant frequency of higher ploidy cells in the culture. In embryonal axis culture the frequency of diploid cells ranged from 34.6 to 70.6 per cent (Table 3). In all the treatment frequency of diploid cells decreased with increase in the age of culture. Tetraploid cells have not shown any definite trend with culture age, similarity, aneuploid cells have not exhibited any relation with age of the culture cells. Higher ploidy cells showed an increasing tendency with increase in age of the callus.

**Table 2. Variation in ploidy level in hypocotyl callus tissues of *Vigna mungo* (L.) Hepper**

| Treatments | Age of callus (days) | Total No. of cell studied | Diploid cells % | Tetraploid cells % | Aneuploid cells % | Higher ploidy % |
|---|---|---|---|---|---|---|
| 0.5 mg/l Kinetin | 10 | 148 | 70.20 | 24.65 | 3.59 | 1.56 |
| | 20 | 144 | 66.82 | 26.44 | 2.88 | 3.86 |
| | 30 | 136 | 58.60 | 26.03 | 2.16 | 13.21 |
| 2.0 mg/l 2,4-D | 10 | 132 | 53.79 | 28.42 | 6.19 | 11.60 |
| | 20 | 140 | 50.40 | 32.89 | 5.51 | 10.20 |
| | 30 | 146 | 46.28 | 39.50 | 6.50 | 7.72 |
| 0.5 mg/l Kinetin + 2.0 mg/l 2, 4-D | 10 | 152 | 51.22 | 32.65 | 3.06 | 13.08 |
| | 20 | 147 | 44.60 | 36.51 | 5.58 | 13.31 |
| | 30 | 138 | 41.39 | 38.88 | 6.20 | 13.53 |

Besides, the variation in chromosome number, all the explants in all the treatments of phytohormones showed mieotic and interphase abnormalities, like anaphase bridges, chromosome fragments, clumping of chromosomes, laggards, binucleate to multinucleate cells, one or several micronuclei and cells with abnormal nuclear morphology. In general, it was observed that structural changes do not show any relationship with duration of culture. (Figs. 2 A-C).

After 30 days, shoots were observed in almost all treatments in all the three explants. However, number of shoots were maximum in hypocotyle cultured on MS medium supplemented with 0.5 mg/l kinetin.

Chromosomal instability in an *in vitro* system has been attributed to an interaction of explant growth potentiality with the environmental culture conditions. The more important question here is the type of tissue of a particular explant which contributes to callus formation. This again depends upon the concentration of growth hormones used and age of the culture, 2, 4-D besides an effective auxin is also responsible for production of chromosomal abnormalities noticed in the culture. The efficiency of 2, 4-D improves in association with kinetin has been attributed to the role of kinetin in DNA synthesis and mitosis[9].

Examination of cultures from different explant sources at various treatments and their subsequent analysis at regular interval have revealed the predominance of diploid cell populations in urd bean callus cultures. Occurrence of high frequency of diploid cell in callus cultures in barley has also been reported[10]. It has been observed that the frequency of diploid cells decreased with increase in the age of

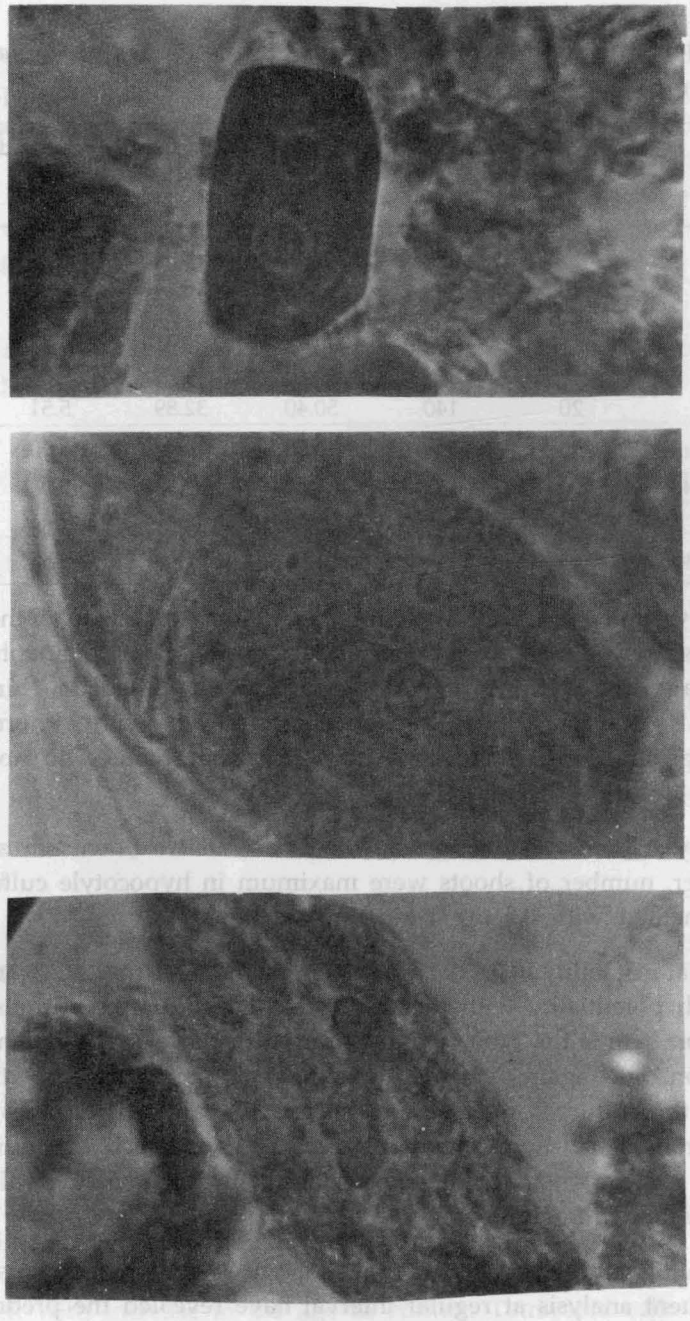

**Fig. 2A-C. Interphase abnormalities in callus cells of *Vigna mungo* L. Hopper A. Binucleate callus cell. B. callus cell sharing micro-nuclei formation. C. callus cell sharing abnormal nucleus shape**

the callus (Tawakley *et al.*,[11] in chickpea). It was observed that frequency of diploid cell was higher in individual treatments of phytohormone but it was reduced in their combination treatments. Similar results were reported by Kunakh *et al.*,[12] and Gostimskii *et al.*,[13] in pea.

**Table 3. Variation in ploidy level in Embryonal axis callus tissues of *Vigna mungo* (L.) Hepper**

| Treatments | Age of callus (days) | Total No. of cells studies | Diploid cells % | Tetraploid cells % | Aneuploid % | Higher ploid % |
|---|---|---|---|---|---|---|
| 0.5 mg/l Kinetin | 10 | 145 | 70.65 | 22.76 | 3.52 | 3.07 |
| | 20 | 136 | 66.19 | 22.98 | 1.72 | 9.11 |
| | 30 | 144 | 61.79 | 23.79 | 1.96 | 12.36 |
| 2.0 mg/l 2,4-D | 10 | 140 | 64.34 | 26.79 | 4.30 | 4.57 |
| | 20 | 148 | 59.85 | 27.14 | 4.64 | 8.37 |
| | 30 | 135 | 52.75 | 27.45 | 3.70 | 15.10 |
| 0.5 mg/l Kinetin + 2.0 mg/l 2,4-D | 10 | 136 | 41.32 | 38.81 | 4.79 | 15.08 |
| | 20 | 142 | 40.70 | 36.23 | 5.17 | 17.90 |
| | 30 | 147 | 34.69 | 34.21 | 6.26 | 24.84 |

Among the polyploidy induced, frequency of tetraploid cells was higher than aneuploids at higher ploidy levels. In majority of treatments, frequency of tetraploid cells increased with increase in the age of the callus. Similar results were reported by Tawakley *et al.*,[11] in chickpea and Gostimskii *et al.*,[13] in pea. Tetraploid cells in callus culture might be due to the endoreduplication or due to endomitosis[11]. Aneuploid cells were observed in all the treatments. The frequency of aneuploid cells increased with increase in the age of the callus. Gostimskii *et al.*,[13] reported 4% aneuploid cells in callus culture of pea.

Structural changes in chromosomes may be very important because they results in the loss of genetic material. The most frequent observed structural changes were bridges, fragments and stickiness. A low frequency of interphase abnormalities such as binucleated and multinucleated cells and cells with irregular shape of the nucleus were observed. Low frequency of structural changes may be due to low division of structurally changed cells which are not able to divide and propagate in a manner similar to the diploid cells. Abraham *et al.*,[14] in *Vicia faba* and Mohanty *et al.*,[15] in barley have also reported similar structural changes.

There was no apparent relationship between the ability of a callus to regenerate and the frequency of diploid, polyploid or aneuploid cells. Hypocotyl explant cultured

on 0.5 mg/l Kinetin after 30 days of inoculation exhibited moderate amount of chromosomal aberrations with high frequency of plant regeneration. Hence this treatment proved to be the best to explore the possibility of regenerating plants with varying chromosome number to evidence somaclonal variations in blackgram aimed to create novel genetic variation.

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