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NUCLEAR CYTOLOGY OF CALLUS DERIVED FROM SEEDS AS EXPLANT IN *CARTHAMUS TINCTORIUS* L.

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ABSTRACT

Fresh seeds were used as sources of explant from *Carthamus tinctorius* plants and those of their derived calli were analyzed for chromosomal variation. Although the callus was obtained from different combinations and concentrations of phytohormones but the best callus was induced in different concentrations of 2, 4 – D. It was maintained for five subcultures and cytology was done after every subculture. The majority of the calli (75%) cells showed normal mitosis, however, some cells with mitotic abnormalities were also found.

INTRODUCTION

Callus tissues are good source of chromosomal variation. Callus tissue is obtained from root, hypocotyls, leaves and other parts of regenerated plants. These organs are made of numerous cells which remain in different states of differentiation. Normally, *in vivo* meristematic diploid cells undergo selective division for the growth of an organ. The degree of endoreduplication depends upon the degree of cellulalr differentiation. Therefore, the genomic constituent is heterogeneous in original explant. Callus tissue may get such genomic heterogeneity possibly due to non-selective induction of asynchronous division of both diploid and endoreduplicated cells. So, the pre-existing genomic heterogeneity of explant may be a source of chromosomal variation in the callus tissue.

Carthamus tinctorius L. is a highly branched, herbaceous, thistle like annual usually with many long sharp spines on the leaves. Plants are 30 to 150 cm tall with globular flower heads (capitula) and commonly brilliant, yellow, orange or red flowers which bloom in July. It is cultivated more or less over India in account of the florets, which are used as dye stuff and the seeds as a source of oil. The plant is also important economically and medically. There are many wild and weedy relatives of Safflower. This species has chromosome no. 2n = 24. Owing to the economic importance of the plant, different subcultured callus cytology was undertaken. Some attempts have been made on the normal cytology of this plant (Ashri and Knowles, 1960; Harvey and Knowles, 1965; Khidir and Knowles, 1970a and b; Peirce, 1992; Garnatje *et al.*, 2006; Malik and Srivastava, 2008; Gregory, 2009). Ashri and Knowles, 1960 regarded *C. lanatus* as an interspecific hybrid between one x = 10 ancester of another x = 12 ancester. However, it is also possible that *C. lanatus* is an autopolyploid (Vilatersana, unpublished,) originating from x = 11 ancester, such as *C. divaricatus* Beg, and Vaceari (Estilai and Knowles, 1976).Mutagenic mitotic aberration in plant tissue cultures is well known for the prolonged subcultures as it is was suggested by Torry that 2,4 – D induces chromosomal variation in the callus tissue (Karp and Bright, 1985; Lee and Philips, 1988). Mitotic aberrations in the callus tissue of coffee plant was observed by Larkin and Scowcraft, (1981). It was maintained upto five subcultures and the cytology was done after every subculture.In present investigation the chromosomal variation in the callus subculture has been studied.

MATERIALS AND METHODS

Tissue culture: For callus induction, seeds of Safflower (*Carthamus tinctorius* L.) were cultured in Murashige and Skoog media with different combinations and concentrations phytohermones, like 2, 4 - D + IAA, 2, 4 - D + IBA, IAA + KN, 2, 4 - D + KN. Highest callus was seen in 2, 4 - D alone from different parts of explants with different concentrations of phytohormones starting with 0.5 ppm to 5 ppm. The present findings are in accordance with Bahrany (2000) and Hayati (2004). Apart from this, there was also good response of callus in different combinations of phytohormones.

The resulting calli from 2, 4 - D phytohormone were transferred to a medium of similar composition for further subculture. The subculturing of calli was maintained upto five subcultures.

Chromosome squashes: Chromosome squashes were prepared from subcultured embryogenic calli after every subculture. The plant callus after every subculture were fixed for at least 12 - 24 hr in fixative (I part of acetic acid: 3 parts of

ethanol) followed by transfer into 70% ethanol. The callus was fixed at regular interval of one hour from 6.00 am to 6.00 pm for knowing the actual time of division and it was observed that in the morning at 8.00 am, there was high rate of cell division and slide preparation was done of the Callus fixed at that time. It was preceded by hydrolysis in 1N HCl for 30 - 60 sec. and then placed on slides and stained with acetocarmine during 30 - 60 minutes. The callus tissue was squashed under a coverslip in a drop of 45% acetic acid.

RESULTS AND DISCUSSION

The embryogeic calli showed several types of cells including non-dividing cells with small rounded nuclei, meristematic cells, which are small with densely stained cytoplasm and a prominent nucleus with condensed chromatin, and parenchyma cells, which are large and elongated. All these types of cells are characteristic of calli and have been previously described by Yeoman and Street (1973).

Fluminhan and Kamaya, (1996) reported mitotic aberrations in tissues incubated *in vitro* during long periods of time.The chromosome squashes of calli showed that majority of cells (75%) undergo normal mitosis and 25% of the cells displayed abnormal mitosis, including changes in chromosome number

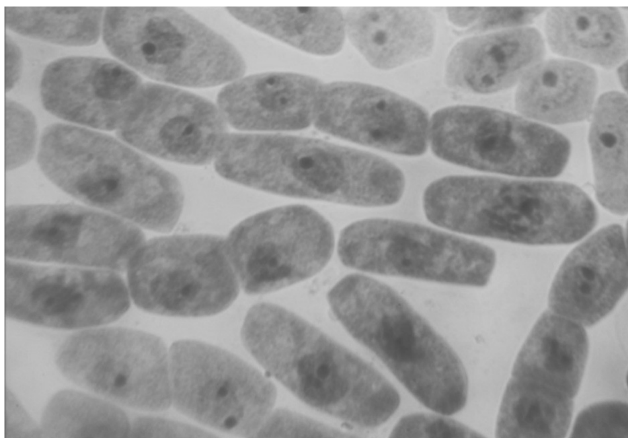

Figure 1: 10X Showing cytology of callus with mitotic aberration like binuclei and vacuolation

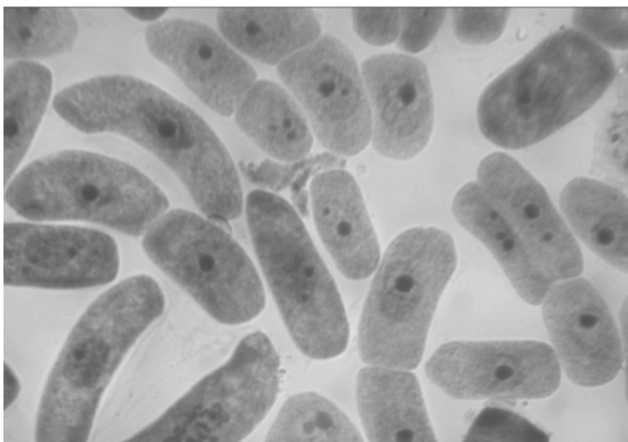

Figure 2: 10X Showing cytology of callus with mitotic aberration like micronuclei

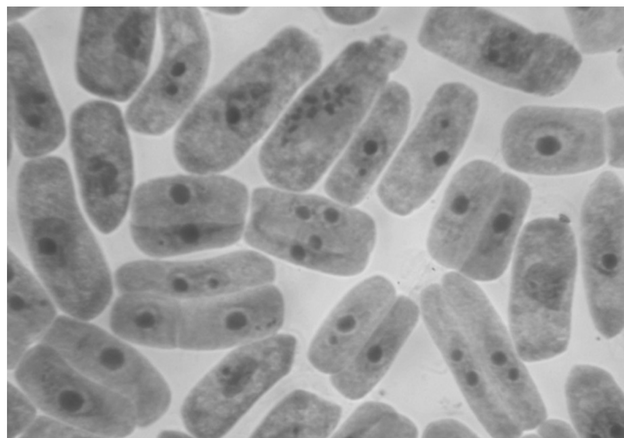

Figure 3: 10X Showing cytology of callus with mitotic aberration like binuclei and aneuoploidy

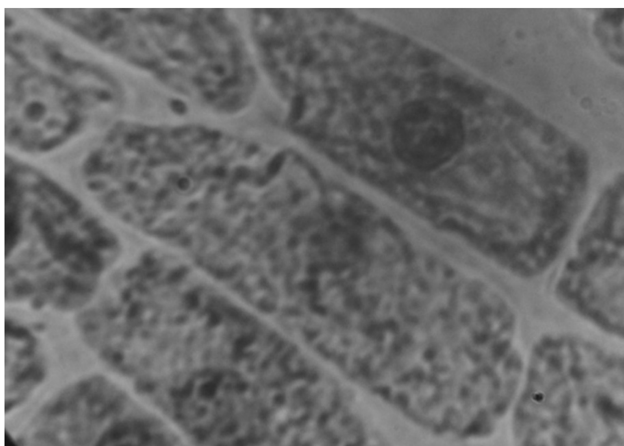

Figure 4: 1500X Showing cytology of callus with mitotic aberration like vacuolation and nondividing cell

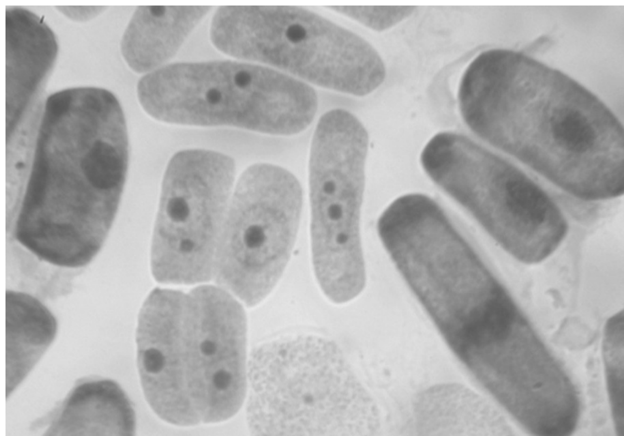

Figure 5: 10X Showing cytology of callus with mitotic aberration like binuclei

such as aneuploids with chromosome number lower that 2n = 24 and polyploids, cells with chromosomal aberrations such as bridges, stickiness, binuclei, micronuclei, laggards etc. cells with double prophase and multipolar metaphases were also observed (Figs. 1 to 5). Some of these mitotic aberrations have also been reported in embryogeic calli of

Maize (Fluminhan and Kamaya, 1996), and potato (Ramulu *et al.,* 1985), in cell suspensions of Daucus (Bayliss, 1975) and in cultured cells of Rauwolfia (Kunakh, 1996).

In conclusion, a number of evident mitotic aberrations are preset in calli derived from seed of the plant. This finding suggests that most of the abnormal cells are incapable of regeneration and that there is auto-selection of normal cells, which are capable of differentiating into somatic embryos.

REFERENCES

Ashri, A. and Knowles, P. F. 1960. Cytogenetics of Safflower (*Carthamus* L.) species and their hybrids. *Agronomy J.* **52:** 11 – 17.

Bahrany, A. L. and A. M. 2000. Effect of phytohormones on in vitro shoot multiplication and rooting of lime (*citrus aurantifolia* (christm) Swing. *Scientia Hortic.* **95:** 285 - 29.

Bayliss, M. W. 1975. The effect of growth *In vitro* on the chromosome complemet of *Daucus carota* (L.) suspesio cultures. *Chromosome.* **51:** 401 - 411.

Estilai, A. and Knowles, P. F. 1976. Cytogenetic studies of *Carthamus divaricatus* with eleven pairs of chromosomes and its relationship to other *Carthamus species* (Compositae). *American J. Botany.* **63:** 771 – 782.

Evans, D. A., Sharp, W. R. and Medina – Filho, H. P. 1984. *Somaclonal and gemetoclonal variation. American J. Botany.* **71:** 759 – 774.

Fluminhan, A. and Kamaya, T. 1996. Behaviour of chromosomes in anaphase cells in embryogenic callus cultures of maize (*zea mays* L.). *Theoretical and Applied Genetics.* **92:** 982 - 990.

Garnatje, T. G. S., Vilatersane, R. and Valles, J. 2006. Gename size variation in the genus *Carthamus* (Asteraceae, Cardueae): Systematic Implications and additive changes during alloplyploidization. *Annals of Botany*. **97(3):** 461 – 467.

Gregory, P. J. 2009. Cytological studies in Safflower (*Carthamus tinctorius* Linn.). *Springer Link.* **1(11):** 763 – 777.

Harvey, B. L. and Knowles, P. F. 1965. Natural and artificial alloploids with 22 pairs of chromosomes in the genus *Carthamus* (compositae). *Canadian J. Genetic and Cytology.* **7:** 126 – 139.

Hayati, A. 2004. In vitro regeneration and callus formation of different hybrid of sunflower (*Helianthus annus* L.) yielding in Turkish Trakya region. *Asian J. Plant Scis.* **3(6):** 747 - 751.

Karp, A. and Bright, S. W. J. 1985. On the causes and origins of somaclonal variation. *Oxford Surveys of Plant Molecular and Cell Biology*. **2:** 199 – 234.

Khidir, M. O. and Knowles, P. F. 1970a. Cytogenetic studies of *Carthamus* species (compositae) with 32 pairs of chromosomes. I. Intrasectional hybridization *American J. of Botany.* **57:** 123 – 129.

Khidir, M. O. and Knowles, P. F. 1970b. Cytogenetic studies of *Carthamus* species (compositae) with 32 pairs of chromosomes. II. Intersectional hybridization. *Canadian J. Genetics and Cytology*. **12:** 90 – 99.

Kunakh, V. A. 1996. Somaclonal variation in Rauwolfia. In: Biotechology in agriculture and Forestry. **36:** Somaclonal variation in crop Improvement II. Y.P.S. Bajaj.Berlin Heidelberg Springer - Verleg. pp. 315 - 332.

Larkin, P. J. and Scowcroft, W. R. 1981. Somaclonal variation – A novel source of variability from cell cultures for plant improvement. *Theoretical and applied Genetics.* **60:** 197 – 214.

Lee, M. and Philips, R. L. 1988. The chromosomal basis of somaclonal variation. *Annual Review of Plant Physiology and Plant Molecular Biology.* **99:** 413 – 437.

Malik, A. and Srivastava, A. K. 2008. 3 D somatic keryotype Analyses in *Carthamus tinctorius*. *Cytologia.* **73(4):** 365 – 370.

Peirce, J. R. 1992. The biology of Australian weeds 23 *Carthamus lanatus* L. *Plants protection quarterly.* **7:** 86 – 95.

Ramulu, S. K. Dijkhuis, P. Hanish Ten Cate, Ch. H. and Groot, B. 1985. Patterns of DNA and Chromosome variation during in vitro growth in various genotypes in potato. *Plant science.* **41:** 69 – 78.

Yeoman, M. M. and Street H. E. 1973. General cytology of cultured cells. In: plant tissue and cell culture. *Street H .E. Oxford, Blackwell Scientific Publicatios.* pp. 121 - 160.