International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2023; 11 (1): 89-91
ISSN:2320-7817(p) | 2320-964X(o)

Research Article Open Access

Cytological studies on tissue culture raised plants

Deora Narpat Singh

Associate Professor, SMPBJ Govt. college Sheoganj (Sirohi) 307027, RJ.

Manuscript details:

Received: 12.03.2023
Accepted: 28.03.2023
Published: 24.04.2023

Cite this article as:
Deora Narpat Singh (2023) Cytological studies on tissue culture raised plants., *Int. J. of Life Sciences*, 11 (1): 89-91.

Available online on http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

The actively growing root tips of various in vitro raised plantlets i.e. *Anogeissus spp*., *Capparis decidua* and *M. emarginat*a were taken and pretreated with colchicine and cold treatment (-4%) for overnight. These pretreated root tips were fixed in 1:3(glacial acetic acid and absolute ethanol). Then these root tips were stained with basic fuchsine (0.5%) prior to make squash. The stained root tips were squashed in 1% of acetocarmine mordanted with ferric chloride. The squashed root tips were observed under microscope for study of chromosome Most of the in vitro raised plantlets shows chromosome stability but some plantlets shows aneuploidy.

Keyword: stain, colchicine, regenerants, plantlets, aneuoploidy,

INTRODUCTION

Documentation of basic information regarding the genome structure and verification of the same for variation if any, is essential for in ascertaining the trueness of the in vitro regenerants. Cytological studies will offer an authentic rather fundamental information regarding genome integrity of the plants raised through tissue culture. A critical perusal of earlier published literature reveals that tissue culture scientists have made extensive use of such studies not only to analyse the regenerants but also for testing the genetic stability in them (Evans et al., 1983 ; George and Bingham, 1984; Nagarajan, 1987 ; Rao et al., 1993; Shekhawat et al., 1993). In addition to this, karyology of cultured plant cells and tissue can provide a realistic bridge between the need for nutritional requirement of cultured plant tissue and the factors regulating morphological competence and genetic improvement of plant species (Papes et al., 1983). Catlin et al., (1988) have estimated that only 20% of the regenerants in Celery are of true-to-type, while the rest showing significant variation in karyotypes owing to various reasons.

In views of this, an attempt has been made to analyse the regenerants, produced through different modes of regeneration, using cytological techniques.

MATERIAL AND METHODS

Actively growing root tips, either from seedlings or regenerated plantlets of 8-10 days old, were excised and pretreated with saturated solution of paradichlorobenzene (aqueous) for 2 ½ to 3 hrs at room temperature, or subjected to cold treatment (-4˚C) in distilled water for 24 hrs. Besides, 0.025% Colchicine aqueous solution was also tried as pretreating reagent, treating the root tips for 3 hrs at room temperature. After Pretreatment, root tips were fixed overnight in 1:3 glacial acetic acid and absolute ethanol. Thereafter root tips were stored in 70% ethanol at 10˚C till squash preparation were made from them. For making squashes, the stored root tips were slowly hydrated by adding distilled water to the fixative, and subsequently acid hydrolysed using IN HCL at 60˚C for 8-10 minutes. This was followed by thorough washing of the material with distilled water. Fuelgen's stain (0.5% leucobasic feuchsin, aqueous solution) has been used for staining the root tips for about 1 to 11 hrs in dark. The stained root-tips were squashed in drop of 1% acetocarmine mordanted with ferric chloride solution to get well stained chromosome preparation. The staining of cytoplasmic background posed a great deal of problem which was resolved by taking microphotographs of the cells immediately and recording observation from temporary preparations.

For counting of chromosomes on average 25 cells from 5 randomly selected root tips of different regenerants were selected and cells only with intact cell wall and clear of chromosomes were considered. Cells with broken cell wall or fractured chromosomes were eliminated from the counting.

RESULTS AND DISCUSSION

From the preliminary experiments, it was observed that of all the three reagents used for pretreatments, 0.025% of aqueous colchicine gave satisfactory results and this alone was used in subsequent experiments.

In *Anogeissus acuminata* the root tips cells, obtained from the germinating seed, had shown the occurrence of 24 chromosomes. Such number has been reported in other genus of A. seresia by Gill et al, [1978]. The regenerants obtained through direct regneration using seedling explants (nodal portion and shoot segment with 1 - 2 nodes) have shown 2n=24 in majority (96%) of the root tip cells studied. The remaining 4% cells showed numerical variation. These regenerants in their three successive passage have maintained the normal chromosomes number in most of the cells and significantly the third passage regenerants had no cell with deviant chromosome number. Such stability of chromosome numbers in regenerants was reported in this genus, earlier by Singh (1992) in *A., rotundifolia*.

In *Capparis decidua* the normal chromosome number has been recorded as 2n=38, from the analysis of root tips obtained from germinating seeds as opposed to 2n=44 reported by Panikkar (1962). The regenrants in this case, obtained from seedling explant have shown 2n=38 chromosome in most of the cells analyzed cytologically. However, few cells with aneuploid numbers (2n=44, 41,42) were also encountered, but never exceeded 5% of the total cells analysed. It is generally observed that the type of the explants (hypocotyl, epicotyl and cotyledon) did not affect the stability of chromosome in regenerants. The occurrence of some deviant aneulpoid numbers in this case is considered as exception rather than rule and such observations were made earlier by Rao et al. (1993).

In Matyenus emarginata the normal diploid chromosome number was recorded as 2n=54 as reported earlier by Adiata and Gavde (1962). The regenerants obtained by direct regeneration from woody nodal shoot segment as explant source had thin roots and very few meristematic cells and their tips. Therefore, detailed cytological studies could not be carried out in these regenerants. However, attempts were made to analyse the cells from well-developed roots of field transferred plantlets of Maytenus emarginata and very few cells that could be analyzed here had shown more or less the same chromosome number as observed in control material.

Conflict of Interest: None of the authors have any conflicts of interest to disclose. All the authors approved the final version of the manuscript.

REFERENCES

Adatia RD and Gavde G (1962) Embryology of the celastraceae. In:Plant Embryology Symposium,CSIR, New Dehli pp. 1-11.

Catlin D, Ochoa A Mac Cormick S and Quiros C (1988) Celery transformation by A tumefaciens. Cytological and genetic analysis of transgenic plants. Plant Cell Report 7:100-103

Evans DA, Sharp WR and Amiratto PV (1983) Hand book of plant cell culture vol. 4 Technique and Application Mcmillon New York, London.

George R and Bingham E (1984). An unstable karyology has also been reported among alfa alfa plants regenerated from callus culture crop. Sci.24:655-658

Gill BS, Bir SS and singhal VK (1979) IOPB chromosome number reports. Taxon 28:391-408.

Nagarajan P and Walton P (1987) A Comparison of samatic chromosomes instability in tissue culture regenerants from medicga media pers Plant cell report 6:10 9-113.

Panikkar AON (1962) chromosome number in capparis decidua, Curr,Sci. 31-32.

Papes D ,Garaj-Vrhovac V, Jelaska S and kovlevska B (1983) chromosome behavior in cultured cell population of higher plants kew chromosome conference 11 George Allen and Unwin London.

Rao SR, Pandey R and Chandel KPS (1993). Genetic stability studies in regenerated plants of Allium tuberosum Rottle ex. Spreng-A cytological approach cytologia 58:31-38.

Shekhawat NS Rathore TS Singh RP Deora NS and Rao SR 1993 Factor affecting in vitro clonal propagation of P. cineraria. Plant growth regu. 12:273-280.