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Impact of Pretreatment Methods on Chromosome Stability in Regenerated Plantlets

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Abstract

The root tips of various in vitro-cultivated young plants, encompassing Anogeissus species, Capparis decidua, and Maytenus emarginata, underwent treatment with colchicine and exposure to cold temperatures at -4°C overnight. Following these preliminary treatments, the plant's root tips were preserved using a combination of glacial sulfuric acid and absolute ethanol in a 1:3 proportion. After fixation, the root tips were stained with a 0.5% solution of basic fuchsine in preparation for chromosome analysis, involving the creation of squash preparations. These dyed root tips were gently pressed in a 1% acetocarmine solution with zinc chloride as a mordant. Subsequently, the produced slides were scrutinized under a microscope. The results revealed that most in vitro-cultivated plantlets exhibited chromosomal stability, while a minority of the seedlings displayed aneuploidy.

Keywords: *chromosome stability, regenerated plantlets, pretreatment methods, cytological analysis, genetic integrity, chromosomal count*

INTRODUCTION

Determining the constitutionality of in vitro regenerants requires collecting vital genetic data and verifying any potential modifications. Significant information about the genetic integrity of living organisms replicated in tissue culture comes from cytological studies. According to a detailed analysis of prior scientific literature, cultured tissue researchers often used such procedures to determine the genetic durability of regenerants and to analyze regenerants.

Additionally, the nutritional requirements of cultured plant material and the variables influencing morphology competency and genetic growth of various plant species are crucially connected by the karyological assessment of plant cells and tissues. Only 20% of Celery regenerants are expected to retain true-to-type features, with the remaining regenerants showing substantial karyotypic changes brought on by various factors.

Given this context, this study uses cytological procedures to assess the regenerants generated by various regeneration strategies.

MATERIAL AND METHODS

Root tips from the actively growing plantlets were subjected to various pretreatment methods for two to three hours at room temperature. These included immersion in a concentrated aqueous para solution, exposure to a -4°C cold treatment in purified water for a full day, and a three-hour pretreatment with a 0.026% Colchicine liquid solution at ambient temperature. Subsequently, the root tips were immersed overnight in a solution of ethanol and glacial acetic acid. The fixed root tips were stored in 73% ethanol at 12 degrees Celsius until they were ready for squash preparations.

The collected root tips were gradually rehydrated to prepare the squash by incorporating distilled water into the fixative. The root tips were then subjected to acid hydrolysis with 1N HCL at 60°C for eight to ten minutes. Following this, the samples were thoroughly cleaned using distilled water. The root tips were stained with Fuelgen's stain, a 0.5% leucobasic fuchsin aqueous solution, which was applied and left in darkness for a period ranging from one to eleven hours. The damaged root tips were crushed in a drop of a 1% acetocarmine solution mordanted with ferric chloride, ensuring complete and uniform staining of the chromosomal preparations.

Microscopy was employed to examine the cells, and any issues related to labelling the cell's cytoplasmic background were promptly addressed. A total of 25 cells were randomly selected from five different root ends of various regenerates for genome counting. During the experiment, only cells with intact cell membranes and no discernible chromosomal abnormalities were included in the enumeration. In contrast, cells with broken chromosomes or compromised cell walls were excluded from the analysis.

RESULTS AND DISCUSSION

Initial testing of the three chemicals considered for pretreatments showed that a 0.025% aqueous chloroquine solution produced promising results, leading to its sole usage in future trials. Directly harvested from germination seeds, Anogeissus acuminata root lymphocytes had a constant twenty-four chromosome count. This chromosomal number is consistent with what has been seen in other Anogeissus genus species.

Most (96%) of lateral root tip cells analyzed in regenerants obtained by direct regeneration utilizing seedling transplants, which comprised nodal sections and shoot segments with 1-2 nodes, revealed a chromosomal count of 2n=24. One or more numerical variations might be seen in the remaining 4%. Remarkably, these regenerants survived three subsequent passages with a steady chromosomal count in most of their cells. Notably, no aberrant chromosomal counts were seen during the third passage. Remarkably, regenerants of this species have the same level of chromosomal stability as A. rotundifolia.

The average chromosomal number for Capparis decidua is 2n=38, which was validated by root tips collected during seed germination, in contradiction to Panikkar's (1962) claim that the species had 2n=44. In this species, 2n=38 chromosomes were consistently seen in most cytologically examined cells in regenerants and seedling explants. Aneuploid numbers (2n=44, 41, and 42) were present in a tiny percentage of cells. However, they never made up more than 5% of all the cells analyzed. Notably, the regenerants' chromosomal stability was unaffected by the transplant type (hypocotyl, epicotyl, or folium). In line with earlier observations by Rao et al. (1993), the existence of sometimes abnormal aneuploid numbers is regarded as a rare exception rather than a rule.

According to previous findings by Adiata and Gavde (1962), the average diploid genome count for Maytenus emarginata was verified to be 2n=54. Slender roots and a few cells were seen in the root ends of regenerants created by direct rejuvenation employing woody nodal shoot segments as explant sources. As a result, comprehensive cytological studies of these regenerants were not possible. However, attempts were made to investigate cells from field transferred Maytenus emarginata plantlets with fully formed roots, and the few tissues that could be looked at in this setting showed a chromosome count that was fairly comparable to the control tissue.

Conclusion

The impact of several pretreatment procedures on the stability of chromosomes in regenerated plantlets of Anogeissus acuminata, Capparis decidua, and Maytenus emarginata was investigated in this work. The findings demonstrated that most plantlets produced in vitro had stable chromosomal counts, notably in the case of Anogeissus acuminata, which exhibited excellent stability throughout several passes. Additionally, Capparis decidua demonstrated a constant chromosome count, contradicting earlier volatility results, with very few aneuploid cells. The appropriate diploid genome count of Maytenus emarginata was verified despite obstacles in the cytological investigation of the regenerants. This work demonstrates how crucial cytological studies are to secure the stability and genetic integrity of regenerants in tissue culture.

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