Genetic stability of \textit{in vitro} propagated African blue lily (\textit{Agapanthus praecox} ssp. \textit{minimus})

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The present study presents cytological investigations of meristematic root cells of African lily (\textit{Agapanthus praecox} ssp. \textit{minimus}) grown \textit{in vivo} and \textit{in vitro}. Cellular parameters including mitotic index (MI), chromosome count and ploidy level (nuclear DNA content) were determined from 12 mm root tip segments of this species. The MI value was found to increase when cells were transferred from \textit{in vivo} to \textit{in vitro} conditions, perhaps due to early adaptations of cells to \textit{in vitro} environment. However, addition of plant hormones in the culture medium was found to increase the MI values recorded for root cells grown \textit{in vitro}. The mean chromosome number was generally stable (2n = 2x = 30) throughout the five-week culture period, indicating no occurrence of early somaclonal variation. Ploidy level analysis revealed that \textit{A. praecox} root cells contained a high percentage of polyploid cells when grown \textit{in vitro} after prolonged culture period. This showed that \textit{Agapanthus praecox} ssp. \textit{minimus} was not genetically stable when cultivated \textit{in vitro}, especially when subjected to prolonged culture period.

\textbf{Keywords:} \textit{Agapanthus praecox} ssp. \textit{minimus}; cellular behaviour; mitotic index; DNA C-value; somaclonal variation; genetic stability

1. Introduction

Growth of an organism is supported by a process called mitosis, which involves replications of DNA and cell divisions to generate more cells in order for an organism to grow or for tissue repair. Mitosis is essential in keeping the number of chromosomes within each daughter cell constant by ensuring that every mother cell is uniformly divided into two daughter cells with similar nuclear gene complement (DNA content). However, DNA replication does not occur during mitosis but during interphase, a process that occurs prior to mitosis (Swift 1950). Interphase involves three important phases: G1, S and G2 (Howard and Pielc 1953). Interphase coupled with mitosis is termed the “cell cycle” (Howard and Pielc 1953).

G1 phase (DNA pre-synthetic phase) is a when protein and RNA (ribonucleic acid) are synthesized; in this phase the nuclei have a nuclear DNA C-value of 2C. During the S phase (DNA phase), DNA is synthesized and histones are formed; at the beginning of this phase the nuclei have a nuclear DNA C-value of 2C, but this doubles to 4C by the end of the phase (Van’t Hof 1973). This is followed by G2 phase (DNA post-synthetic phase) in which the nuclei still have a nuclear DNA C-value of 4C. Following interphase, cell division or mitosis divide the mother cell into two daughter cells consisting of equal amounts of DNA (with nuclear DNA C-values of 2C in each cell).

Somaclonal variation can easily be detected through distinct morphological abnormalities that distinguish a plant from the rest of its siblings. According to Partanen (1963), plant species are commonly characterized based on its morphology and chromosomal stability. In general, the meristematic regions such as the root or shoot tips will undergo diploid chromosomal complement, although non-meristematic regions (within the same individual plant) may have varied chromosomal numbers triggered by somatic chromosomal instability (Partanen 1963). This is associated with many factors, such as disturbance in the normal cell multiplication due to abnormal mitotic spindles, or continuous DNA replications accompanied by lack of mitosis and chromosome breakage, which may lead to an increase of chromosomal material (Sunderland 1973). Chromosome breakage can affect the chromosome count within a nucleus and alter the structural arrangements between the chromosomes, which in turn would affect the DNA content of the cell (Sunderland 1973). Furthermore, abnormalities in spindle fibres can affect the separation of chromosomes and result in changes of nuclear DNA amount (Sunderland 1973). On the other hand, repeated DNA replication without mitosis would result in an increase of DNA content and chromosome girth, without affecting the chromosome number (Sunderland 1973).

Repeated DNA replications and doubling of chromosomes (from \textit{2n} to \textit{4n}, \textit{8n}, \textit{16n}, etc.) contributes to the occurrence of polyploid cells through “endomitosis” where sister chromatids separate without the help of spindle fibres within the nuclear membrane (Sunderland 1973; Nagl 1974). As a result, the diploid cell consists of four sets of chromosomes (\textit{4n}) instead of \textit{2n}, therefore