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SOMACLONAL VARIATION OF LILY AND THEIR CHROMOSOMES STABILITY

VARIASI SOMAKLONAL LILI DAN KESTABILAN KROMOSOMNYA

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ABSTRACT

Somaclonal variation of lily after long term in vitro culture was studied by means of cytological approach. *Lilium* × *formolongi* cv. Azusa was subcultured regularly every two weeks for over than two years. Cell suspension growth rate was determined by measuring packed cell volume. Artificial mutation was induced by irradiating the cells with X-ray at their growing peak.

The results showed that lag phase of cell suspension growth rate occurred during the first two days after subculture. Number of metaphase cells decreased due to irradiation treatment. Furthermore, the range of chromosome number variation has not changed but the amplification of variation due to irradiation occured.

Keywords: somaclonal variation, irradiation, cell suspension, lily.

INTISARI

Penelitian ini bertujuan untuk mengetahui variasi somaklonal yang terjadi pada sel-sel lili yang telah dipelihara secara in vitro dalam waktu yang lama. Bahan yang digunakan adalah kultur suspensi sel Lilium × formolongi *cv. Azusa dan disubkultur setiap dua minggu sekali selama lebih dari dua tahun. Laju pertumbuhan sel diukur dengan mengukur volume suspensi sel. Mutasi buatan dilakukan dengan penyinaran sinar- X.*

Hasil penelitian menunjukkan bahwa pertumbuhan suspensi sel mengikuti kurva sigmoid. Perlakuan radiasi menurunkan jumlah sel-sel yang mengalami pembelahan. Kisaran variasi jumlah kromosom tidak menunjukkan perbedaan antara perlakuan radiasi dengan tanpa radiasi tetapi memperlihatkan amplifikasi yang berbeda di antara keduanya.

Kata kunci: variasi somaklonal, iradiasi, suspensi sel, lili.

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INTRODUCTION

Plant tissue culture has enormous potential as a tool in plant breeding programs. It was often considered a sophisticated method of asexual propagation enabling a more rapid rate of propagation. Therefore, by tissue culture application, it become an accepted dictum that all plants regenerated from tissue culture should be identical to the parental form. In fact, there are possibilities to find some plants subsequently expressing phenotypic or genetic differences from their parent plant. The differences could have occurred during the culture and their subsequent maintenance in the culture over a long period of time.

Moreover, tissue culture provides a new and exciting option for obtaining increased genetic variability in shorter time and without sophisticated technology. Tissue culture per se appears to be an unexpectedly rich and novel source of genetic variability. All of those are very valuable and necessary for breeding programs.

Various types of genetic changes have been associated with somaclonal variation, such as numerical and structural chromosomal changes, deamplification and amplification of genes, single gene mutations, transposable element activation, biochemical changes, quantitative trait variation and DNA hypo- and hyper-methylation (Philip *el al.*, 1990; Scowcroft, 1985). Nevertheless, chromosomal rearrangements and/or numerical variation in chromosomes are still considered as playing major role in describing somaclonal variation (Raja *et al.*, 1992).

Larkin and Scowcroft (1981) mentioned that one method that can be used in order to know the origin of somaclonal variation is karyotype changes. Karyological analysis of plants can very often reveal significant chromosomal changes such as alteration in ploidy levels, as well as structural rearrangements (Brown *et al.*, 1993). However, chromosomal changes cannot reveal alterations in individual genes. In reference to these limitation, RAPD (Random Amplified Polymorphic DNA) is an alternative method that can be easily applied to determine the level of variation in plant material at all culture and the growth stage.

This study tried to elucidate the nature of and artificial induction of chromosomes variation of lily after long term culture and their stability, through cytological approach.

MATERIAL AND METHOD

Cell culture of *Lilium* × *formolongi* cv. Azusa was used in these experiments. The materials were subcultured regularly every 2 weeks for more than 2 years.

Artificial mutation was induced by X-ray irradiation (25 rad for 10 minutes) at the peak of cells growth. Karyotype analysis was conducted two months after irradiation.

Cell suspension growth rate was determined by measuring packed cell volume. One gram of cell suspension was centrifuged at 1000 rpm for 5 minutes. Measurement was conducted 2 days after subculture.

For cytological observations, suspension cultures were pretreated with cold water for 24 hours. Cells were fixed in 3:1 ethanol (99%) : acetic acid (v/v) for at least one day and then rinsed three times with distilled water before maceration and then randomly stained with aceto-carmine for 30 minutes. Cells from samples were squashed with 45% acetic acid on the slide glass and observed under microscope.

RESULTS AND DISCUSSION

Cell suspension growth rate is showed in figure 1. The curve shows a sigmoid curve with a lag phase during first 2 days after subculture followed by a rapid growth phase from second up to the fourth day. This periodic growth pattern seems to repeat itself over several times. During these growth phases, cells seem like have greatest activities for cell division.

Metaphase analysis was measured both in cell suspension culture, with and without irradiation (Fig.2). Cell suspension without irradiation showed a tendency to increase the number of metaphase cells continuously after subculture. However, the cell suspension with

irradiation showed tendency to decrease the number of metaphase cells. This difference is assumed to be due to mutation induced in the cell suspension culture by irradiation.

The relationship between cell growth rate and number of cell in metaphase stage of the cell suspension culture without irradiation is shown in Figure 3. The value of regression coefficient is 60.1 (R^2=0.93). It shows that with the increase in packed volume of cells, there is also increase in the number of cells in metaphase stage.

Chromosome number variation of cell suspension culture without irradiation treatment ranges from 20 to 48 (Fig. 4). Most of them are diploid with $2n$=24 (70.25%), and the rest are $2n$=20 (13.98%); $2n$=22 (1.43%); $2n$=23 (2.51%); $2n$=25 (0.36%); $2n$=26.28 (0.72%) and $2n$=48 (10.03%). The irradiated cell suspension culture has the same range of variation in chromosome number. However, the amplification of variation is larger (Fig. 4). Percentage of diploid cells decrease (54.05%) and cells with other ploidy levels are more frequent. Since the ploidy levels caused by X-ray irradiation are more varied, there is great possibility to get a mutant plants from this treatment.

Metaphase analysis as a method of analyzing aneuploid variation has often been criticized as a tool for obtaining information about chromosome number distribution due to over- or underestimation. Therefore, it is necessary to minimize errors during observation, such as by making multiple count per cells and observations should be taken only from intact cells.

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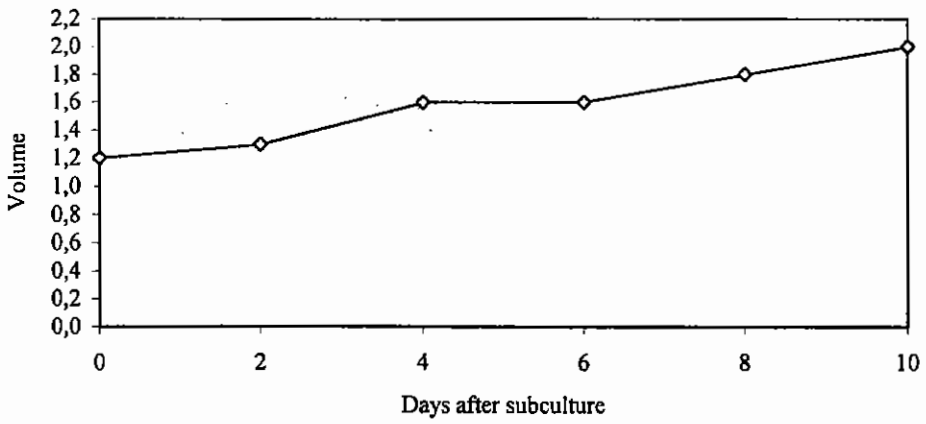

Figure 1. Cell growth rate of cell suspension culture *of Lilium* × *formolongi* cv. Azusa. No irradiation was applied.

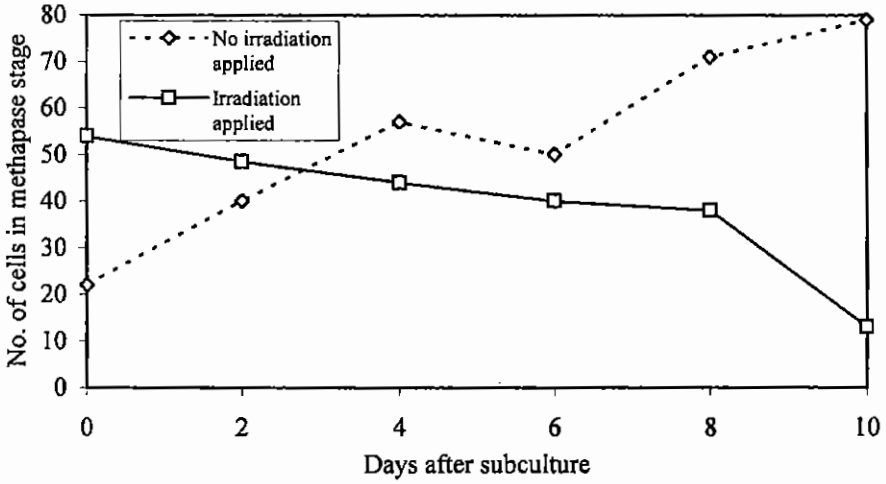

Figure 2. Number of cells in metaphase stage.

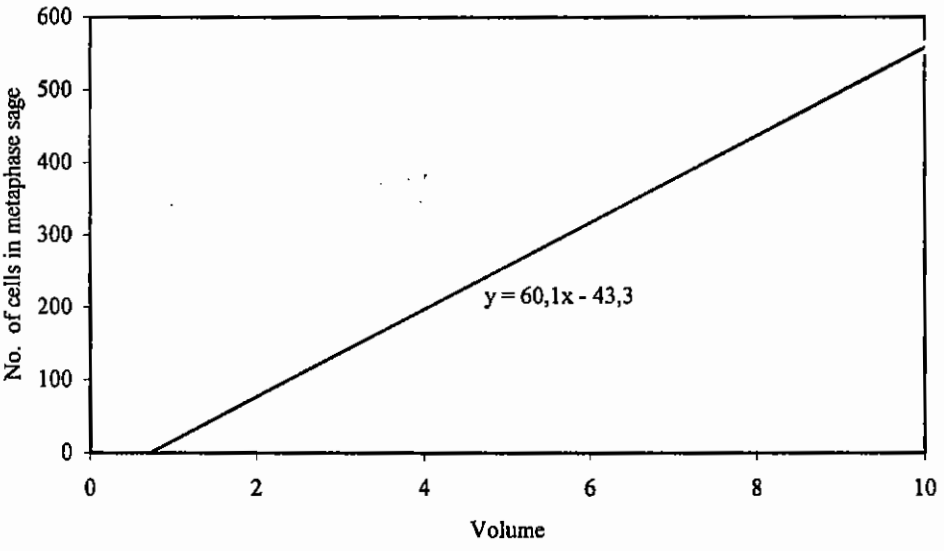

Figure 3. Regression curve between cell growth rate and number of cells in metaphase stage (R^2=0,93).

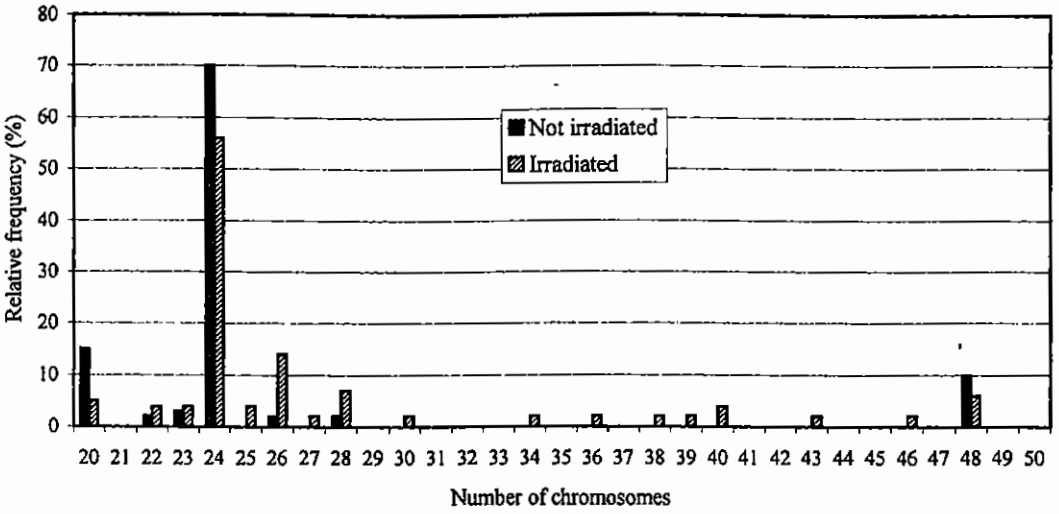

Figure 4. Distribution of chromosome number variation in cell suspension culture.