Determination of genetic stability of *Agapanthus praecox* ssp. *minimus* grown *in vitro* via cytological analysis

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Abstract Summary: Ploidy analysis revealed percentage of polyploid cells *in vitro* increased concomitantly with culture time, indicative of genetic instability under in vitro conditions. Chromosome number was stable (2n=2x=30) throughout culture period, indicating no occurrence of early somaclonal variation.

Introduction: Meristematic cells of roots have long been employed as the standard tissue or cells for cytological studies as well as in cycling-cell studies [1]. Somaclonal variation can easily be detected through distinct morphological abnormalities that distinguish a plant from the rest of its siblings. Plant species are commonly characterized based on its morphology and chromosomal stability [2]. Repeated DNA replications and doubling of chromosomes (from 2n to 4n, 8n, 16n and et cetera) can yield polyploid cells through 'endomitosis' where sister chromatids would separate without the help of spindle fibers within the nuclear membrane [3,4].

Methodology: Seeds of *A. praecox* ssp. *minimus* collected from Cameron Highlands, Malaysia were surface sterilized in decreasing concentrations of commercial bleach (Chlorox) and alcohol, then cultured on MS [5] media without hormone to produce aseptic seedlings. Bulb explants excised from aseptic seedlings were cultured on full strength or half strength MS media supplemented with 1.0 – 2.0 mg/L auxins, indolebutyric acid (IBA) and α-naphthalene acetic acid (NAA), with the presence of 1.0 – 2.0 mg/L 6-furfurylaminopurine (Kinetin). All culture media was added with 30 g/L sucrose, pH 5.6 $\pm$ 0.1, solidified with 2 g/L Gelrite Gellan gum and autoclaved at 120°C for 20 minutes. The cultures were maintained in the culture room at 25 $\pm$ 1°C with 16 hours light and 8 hours dark, with illumination at 1000lux and relative humidity of 90 – 100% [6] for 2 months. Root segments of resulting plantlets were treated with 5M Hydrochloric acid (HCl) and stained with Feulgen for 2 hours. Stained root tips without the root caps were transferred onto glass slides, wetted with 1-2 drops of 45% (v/v) acetic acid and then made permanent based on the quick-freeze method [7]. DPX (Di-N-Butyle Phthalate in Xylene) was used to mount the cover slides. The chromosomes of *A. praecox* ssp. *minimus* cells were visualized using Axioskop Zeiss (Germany) microscope attached to AxioCam MRc video camera and were then analyzed using AxioVision 4.7 software.

Results: Regeneration of *Agapanthus praecox* was successfully obtained *in vitro* and induction of roots *in vitro* was significantly improved when 1 mg/L IBA and 1 mg/L Kinetin were added to the growth media. The transfer from *in vivo* to *in vitro* conditions was found to have an immediate effect on mitotic index of *A. praecox*, where the MI value was found to increase slightly when cells are transferred into *in vitro* conditions, and when hormones were added to the media (Table 1). However, the increase in MI values was not significant. Chromosome number (2n=2x=30) was maintained when *A. praecox* entered tissue culture system and remained stable throughout the culture period (Table 1). Ploidy analysis also revealed that no polyploid cells was observed in *in vivo* grown *A. praecox*, but present when cells are transferred *in vitro* (Table 2). The presence of polyploid cells became more prominent and the percentage increased with culture time (Table 2).

Table 1: Mitotic index and chromosome number of *in vitro* and *in vivo* grown *A. praecox* ssp. *minimus* root meristems.

Growth media	Week	Mitotic Index, MI (Percentage, %)	Number of Chromosomes
MS + 1 mg/L IBA + 1 mg/L Kinetin	2	$16.23 \pm 0.66_a$	$32.07 \pm 2.3_a$
	3	$13.00 \pm 1.46_a$	$29.93 \pm 1.4_a$
	4	$15.71 \pm 4.53_a$	$29.50 \pm 2.4_a$
	5	$15.69 \pm 3.35_a$	$30.53 \pm 0.9_a$
MS basal	5	$11.81 \pm 1.84_a$	$30.00 \pm 1.3_a$
In Vivo	-	$10.84 \pm 1.84_a$	$30.20 \pm 0.7_a$

Mean values with different letters within a column are significantly different at $p < 0.05$.

Table 2: Percentage of nuclei in root tip meristem of *in vitro* and *in vivo* grown *Agapanthus praecox* ssp. *minimus* in various cell cycle phases.

Growth media	Week	Cell Cycle Phase (%)			Polyploidy (%)
		G_1	S	G_2	
MS + 1 mg/L IBA + 1 mg/L Kinetin	2	52.67_d	34.67_c	8.67_c	4.00_b
	3	49.33_c	46.00_e	3.33_a	1.33_a
	4	20.00_b	40.67_d	19.33_e	20.00_d
	5	7.33_a	20.00_a	10.67_d	62.00_e
MS basal	5	59.33_e	26.67_b	6.67_b	7.33_c
In Vivo	-	100.00_f	-	-	-

Mean values with different letters within a column are significantly different at $p < 0.05$.

Acknowledgements

This work has been supported by the University of Malaya, under PPP grant no: PS294-2009B.

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