Sains Malaysiana 46(12)(2017): 2417–2423
http://dx.doi.org/10.17576/jsm-2017-4612-17

Kinetin Induces Chromosomal Abnormalities in African Blue Lily (*Agapanthus praecox* ssp. *minimus*) Grown in *In Vitro*

(Kinetin Mencetus Keabnormalan Kromosom dalam Lili Biru (*Agapanthus praecox* ssp. *minimus*) yang Dihasilkan secara *In Vitro*

JAMILAH SYAFAWATI YAACOB* & ROSNA MAT TAHA

ABSTRACT

Kinetin has been reported to exert inhibitory effect when used in tissue culture and in some cases reverse the action of auxin and cause growth inhibition and retardation of root formations. Kinetin also acts as 'mitotic poison', mimicking the effect of pesticides and toxic chemicals and interferes in mitosis mechanism of plants. The effect of kinetin on size of cell and nucleus as well as chromosome behaviour in root tip meristems of Agapanthus praecox *ssp.* minimus *was studied. The results showed that prolong exposure to kinetin caused chromosome abnormalities to occur more frequently. Chromosome breakage yielded fragmented chromosomes, while abnormal spindle fibers caused delay in chromosome movement, termed as laggard chromosomes. Abnormal nucleus was also observed with kinetin treatments, such as micronucleus, binucleated and tripolar cells.*

Keywords: Binucleated; chromosome behavior; cytomixis; fragmented chromosome; kinetin; laggard

ABSTRAK

Kinetin telah dilaporkan memberi kesan merencat apabila digunakan dalam kultur tisu dan dalam beberapa kes membalikkan tindakan auksin dan menyebabkan pertumbuhan dan pembentukan akar terencat. Kinetin juga bertindak sebagai 'racun mitosis', meniru kesan racun perosak dan bahan kimia toksik dan mengganggu mekanisma mitosis dalam tumbuhan. Kesan kinetin pada saiz sel dan nukleus dan juga tingkah laku kromosom dalam hujung meristem akar Agapanthus praecox *ssp.* minimus *telah dikaji. Keputusan kajian menunjukkan bahawa pendedahan berpanjangan kepada kinetin telah menyebabkan keabnormalan kromosom berlaku lebih kerap. Pecahan kromosom menghasilkan kromosom terserpih, manakala gentian gelendong yang tidak normal menyebabkan kelewatan dalam pergerakan kromosom, yang digelar sebagai kromosom yang terkemudian. Keabnormalan nukleus juga diperhatikan dengan rawatan kinetin, seperti nukleus mikro, binukleus dan sel tripolar.*

Kata kunci: Binukleat; kelakuan kromosom; kinetin; kromosom terserpih; sitomiksis; terkemudian

INTRODUCTION

Agapanthus praecox or 'Lily of the Nile' or the 'African Lily' originated from South Africa and is gaining popularity around the world as an ornamental and for landscaping. *Agapanthus* plants are popular among native tribes of Africa as a traditional remedy to ease prolonged labour (Varga & Veale 1997). The plant was reported to contain active pharmaceutical agent such as phytoecdysteroids (Savchenko et al. 1997), sapogenins and saponins and showed anti-inflammatory activities. *Agapanthus* extracts also possess fungitoxic properties against various bacterial and fungal pathogens (Pretorius et al. 2002; Singh et al. 2008; Tegegne et al. 2008). Coloured extract from *Agapanthus* flowers was reported to contain anthocyanin pigments (Bloor & Falshaw 2000; Yaacob et al. 2011), with potential for production of highly commercialized salt and heat-stable organic dye (Yaacob et al. 2011). The vast benefit and potential of *Agapanthus* plants had rendered this species to be an interesting candidate for mass propagation through tissue culture.

Tissue culture technique however can result in some limitations or implications, which arise from strong selection pressure imposed by prolonged culture conditions. Various genetic and epigenetic irregularities can occur in cultured cells, giving rise to non-preferred somaclonal variants (Bayliss 1980; Kaeppler et al. 2000; Kovarik et al. 2012). Somaclonal variation resulted from genetic changes at DNA sequence or chromosomal level and epigenetic regulation during translation (such as DNA methylation and histone deacetylation) can hamper mass propagation of clonal plants. Somaclonal variation is also triggered by genomic shock or plasticity (Cullis 2005), especially when plants have exhausted its normal physiological response mechanism to withstand environmental stress (Cullis 1999). Other external factors have also been reported to contribute to development of somaclonal variants, such as type and concentration of plant hormones and explant source (Karp 1991). The exact role of each and relationship between contributing factors underlying the occurrence of somaclonal variation are

yet to be fully understood, however any attempts towards better understanding of this phenomenon is welcomed to exert more control on stable tissue culture system.

Previously, we had reported successful propagation of *A. praecox* ssp. *minimus* via somatic embryogenesis (Yaacob et al. 2012) as well as through direct organogenesis from bulb explants (Yaacob et al. 2014). We found that addition of kinetin to the root induction media promoted efficient rhizogenesis from bulb explants of *A. praecox* ssp. *minimus*, contrary to most reports in other species (Yaacob et al. 2014). In tissue culture of *D. cumminsii*, kinetin was shown to inhibit growth and callus formation (Oselebe & Ene-Obong 2007). Kinetin also caused cell death in root cortex of *Vicia faba* ssp. *minor* seedlings (Kunikowska et al. 2013). In the present study, we aim to elucidate the effect of kinetin at chromosome level; to observe the consequence of exposure to Kinetin on cell division and chromosomal behaviour.

Materials and Methods

Sterilization of seeds and determination of standard growth of primary roots

Seeds of *A. praecox* ssp. *minimus* were obtained from several locations at Cameron Highlands, Malaysia. Standard growth of primary roots was determined using 100 pre-sterilized seeds. Sterilization was achieved by washing the seeds using sterile distilled water and treatments with decreasing concentrations (100 %, 70% and 30% v/v) of commercial bleach (clorox). Two drops of Tween-20 were also added to the 100% clorox to reduce surface tensions and aid sterilization process. The adhering clorox was removed by submerging the seeds in sterile distilled water. Sterilization was completed in the Lamina flow, by immersing the seeds in 70% (v/v) ethanol followed by 3 times rinse with sterile distilled water.

Sterilized seeds were germinated on pre-autoclaved moist cotton wools to induce root formations. The seeds were kept in the culture room, with incubation temperature of 25 ± 1°C with 16 h light and 8 h dark and light illumination of 1000 lux. The growth (length) of primary roots were measured every 24 h and used in determination of standard growth of the primary roots. Standard curve showing linear regression of primary root length against time was plotted and optimum (standard) root length determined from the graph was used in all experiments.

Rooting induction and treatment with kinetin

Seeds of *A. praecox* ssp. *minimus* were surface sterilized as previously described and cultured on MS (Murashige & Skoog 1962) basal media (without hormone) to produce aseptic seedlings of this species. Cultures were kept in the culture room at 25 ± 1°C with 16 h light and 8 h dark, illumination at 1000 lux and relative humidity of 90-100% (Yaacob et al. 2012). Bulbs were excised from 4-week-old aseptic seedlings and used as explants to produce *in vitro* plantlets of this species. The MS media was supplemented with 30.0 gL^{-1} sucrose, 2.0 gL^{-1} Gelrite Gellan gum and 1.0 mgL^{-1} auxin IBA (indolebutyric acid) to encourage root formations. Cultures were treated with kinetin (6-furfurylaminopurine) by adding 1.0 mgL^{-1} kinetin to the root induction media. The concentration of kinetin was standardized at 1.0 mgL^{-1} because low kinetin concentrations can lead to mitotic abnormality (Soh & Yang 1993) and was previously determined as the most optimum concentration for efficient rhizogenesis of *A. praecox* ssp. *minimus* (Yaacob et al. 2012). The cultures were maintained in the culture room at 25 ± 1°C with 16 h light and 8 h dark, with illumination at 1000 lux and relative humidity of 90-100% for 2 months.

Feulgen squash technique and preparation of permanent slides

Roots of *in vitro* plantlets with standard length of 12.12 ± 1.07 mm (based on standard curve) were fixed in Farmer's fluid, which consisted of 3:1 (v/v) of absolute ethanol and glacial acetic acid for 30 min, then washed in 3 changes of water and hydrolyzed in 5N HCl for 50 min at room temperature with occasional agitation. Following that, the roots were washed in water for 1 min to remove any adhering acid and then stained using Feulgen (Schiff's) reagent for 2 h. The root segments were then rinsed in several changes of tap water for 5 min and were washed in three changes of SO_2 water for 5 min each. Subsequently, the root segments were rinsed in several changes of distilled water and any excess water was blotted using a filter paper. The apical meristem of the root segments were subdivided and squashed followed by addition of a small drop of 45% acetic acid on clean slides. The slides were then heated gently by passing over a spirit lamp a few times. The slides were made permanent by following the method described by Conger and Fairchild (1953). The permanent slides were then viewed using Axioskop Zeiss (Germany) microscope attached to AxioCam MRc video camera and were then analyzed using AxioVision 4.7 software.

Chromosomal behaviour and cell and nuclear areas

Non-squash permanent slides of *A. praecox* ssp. *minimus* roots were also prepared as previously described. The slides were counterstained in 0.2% (w/v) light green in absolute ethanol for 1 min, followed by immersion in absolute ethanol for another 10 min. The slides were then cleared in xylene for 10 min and mounted using DPX. The mean nuclear and cell areas were measured from 150 prophase cells from 3 slides. The ratio of nuclear to cell area was also determined from each sample. The chromosomes in each cell were carefully observed to detect any chromosomal irregularities that might have occurred due to treatments with kinetin.

STATISTICAL ANALYSIS

Randomized complete block design (RCBD) with 30 replicates was employed in tissue culture experiments, to decrease error and enhance accuracy. Statistical analysis was conducted using statistical variance test (ANOVA) and compared using Duncan's multiple range test (DMRT) with least significant differences at 5% level.

RESULTS AND DISCUSSION

MEAN CELL AND NUCLEAR AREAS

Mean nuclear areas, cell areas and ratio of nuclear areas to cell areas were also calculated from three replicates consisting of 150 prophase cells. The ratio of nuclear area to cellular area in *A. praecox* ssp. *minimus* grown *in vivo* and on MS basal media were quite low, indicating that the size of the nucleus was quite small as compared to the cellular area (Table 1). Similar observation were recorded in *in vitro* plantlet supplemented with hormones (1.0 mgL^{-1} IBA and 1.0 mgL^{-1} kinetin), but after the third week, the ratio of nuclear area to cellular area had significantly increased.

MITOSIS STAGES

The cells of primary roots obtained from *A. praecox* ssp. *minimus* grown *in vivo* were quite large in size (Table 1), therefore cell division and cells undergoing interphase could be easily identified. Most cells from both *in vivo* and *in vitro* samples exhibit normal mitosis. The 4 phases of mitosis had been successfully observed from root tips of *A. praecox* ssp. *minimus* grown *in vivo* and *in vitro*. During prophase, the chromatin becomes condensed via a process called chromatin condensation which is mediated by the condensing complex. The chromatin becomes more visible as it gets fatter and shorter when it condenses into double rod-shaped structures called chromosomes (Figures 1 & 2). During prophase, the chromosomes are homologous, with two identical copies of each chromosome called sister chromatids and are attached to each other at the centromere. Metaphase occurs after prophase, in which the condensed chromosomes become aligned in the

TABLE 1. The mean nuclear and cell areas and their ratios in root meristem cells of *in vitro* and *in vivo* grown *Agapanthus praecox* ssp. *minimus* plants

Growth media	Age (weeks)	Mean area (μm^2)		
		Nucleus (N)	Cell (C)	Ratio (N/C)
MS + 1 mgL^{-1} IBA + 1 mgL^{-1} Kinetin	2	$248.04 \pm 24.79_{ab}$	$813.51 \pm 85.12_{ab}$	$0.31 \pm 0.03_{ab}$
	3	$382.55 \pm 31.13_{b}$	$981.05 \pm 99.89_{ab}$	$0.40 \pm 0.02_{c}$
	4	$265.97 \pm 32.98_{ab}$	$847.15 \pm 174.09_{ab}$	$0.35 \pm 0.03_{bc}$
	5	$205.64 \pm 11.75_{a}$	$586.54 \pm 48.18_{a}$	$0.36 \pm 0.02_{bc}$
MS basal	5	$153.28 \pm 4.62_{a}$	$604.42 \pm 33.87_{a}$	$0.26 \pm 0.01_{a}$
In Vivo	2	$742.20 \pm 68.27_{c}$	$2738.96 \pm 220.54_{c}$	$0.28 \pm 0.02_{a}$

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

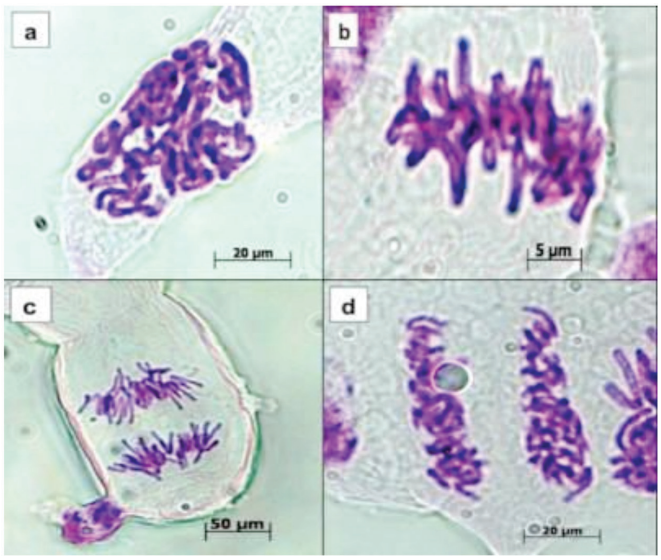

FIGURE 1. A cell undergoing mitosis; (a) Prophase, (b) Metaphase, (c) Anaphase and (d) Telophase, observed from squashed preparation of *in vivo A. praecox* ssp. *minimus* root tip meristem cell

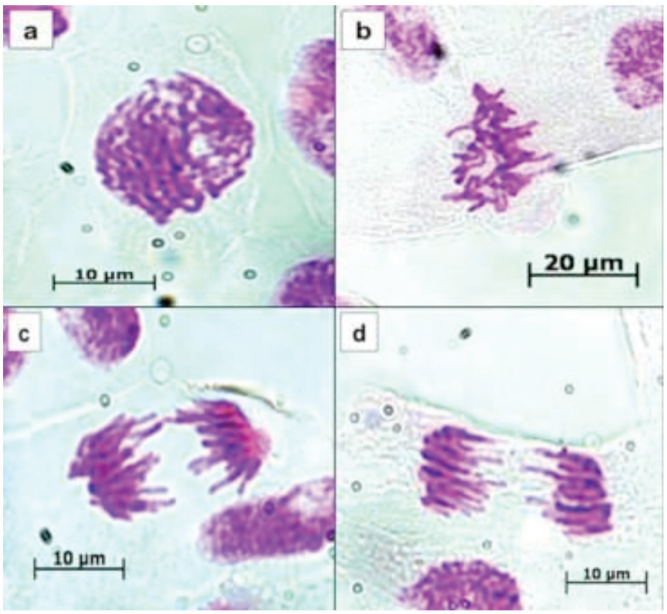

FIGURE 2. A cell undergoing mitosis; (a) Prophase, (b) Metaphase, (c) Anaphase and (d) Telophase, observed from squashed preparation of *in vitro A. praecox* ssp. *minimus* root tip meristem cell

middle of the cell (Figures 1 & 2). During metaphase, the chromosomes are at their most condensed state and are attached to the spindle fibers by the centromeres. The centromeres arranged themselves on the metaphase plate, a term describing an imaginary line in the middle of two centrosome poles. Microtubules that were formed during prophase have also become attached to the kinetochores. Following metaphase is anaphase. During anaphase, the chromosomes split and migrate to opposite poles of the cell (Figures 1 & 2). The chromosomes are drawn to separate sides of the cell, whereby the non-kinetichore spindle fibers will push each other while the kinetichore spindle fibers pull the separated chromosomes to the different poles, stretching the cell into an oval. Telophase is the last phase in mitosis, in which, the spindle fibers continue to lengthen and nuclei would start to from at the opposite poles (Figures 1 & 2). The chromatin fibers of the chromosomes would uncoil and the genetic information (DNA) of each cell would then be equally divided into two, after completion of telophase.

CHROMOSOMAL BEHAVIOR AND IRREGULARITIES

Meanwhile, prolong exposure to kinetin was observed to yield some degree of chromosomal irregularities in root meristematic cells of *in vitro* plantlets, compared to plantlets grown on MS basal media and *in vivo* plants. Significantly high percentage of occurrence of cytomixis, bridging and binucleated or tripolar cells were observed in 5-week-old meristematic roots of *in vitro* plantlets compared to meristematic roots of *in vivo* plants (Table 2, Figure 3). Frequencies of chromosomal irregularities also increased with prolong duration of culture conditions and exposure to kinetin (Table 2, Figure 3). The results indicated that *in vitro* conditions yielded a small degree of chromosomal abnormalities, compared to none observed in root meristematic cells of *in vivo* plants, possibly due to

TABLE 2. Percentage of chromosomal irregularities observed in root meristem cells of *in vitro* and *in vivo* grown *Agapanthus praecox* ssp. *minimus* plants

Growth media	Age (weeks)	Chromosomal irregularities (%)					
		Cytomixis	Laggard	Bridge	Micronucleus	Binucleated cell	Tripolar cell
MS + 1.0 mgL^{-1} IBA + 1.0 mgL^{-1} Kinetin	2	$1.72 \pm 0.02_{c}$	$2.94 \pm 0.01_{b}$	$0.43 \pm 0.01_{a}$	$3.96 \pm 0.25_{b}$	$1.42 \pm 0.33_{b}$	$1.24 \pm 0.02_{a}$
	3	$1.46 \pm 0.02_{b}$	$8.33 \pm 1.31_{d}$	$1.76 \pm 0.17_{b}$	$5.87 \pm 0.45_{c}$	$3.37 \pm 0.14_{c}$	$4.37 \pm 0.13_{d}$
	4	$2.07 \pm 0.15_{d}$	$5.56 \pm 0.79_{c}$	$4.33 \pm 0.35_{c}$	$11.91 \pm 1.3_{d}$	$4.64 \pm 0.86_{e}$	$3.87 \pm 0.35_{c}$
	5	$2.56 \pm 0.11_{e}$	$11.11 \pm 2.31_{e}$	$7.53 \pm 0.62_{d}$	$13.15 \pm 0.55_{e}$	$4.11 \pm 0.45_{d}$	$5.42 \pm 0.91_{e}$
MS basal	5	$1.22 \pm 0.01_{a}$	$2.56 \pm 0.14_{a}$	$0.32 \pm 0.02_{a}$	$1.42 \pm 0.02_{a}$	$0.85 \pm 0.01_{a}$	$1.62 \pm 0.22_{b}$
In Vivo	2	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

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

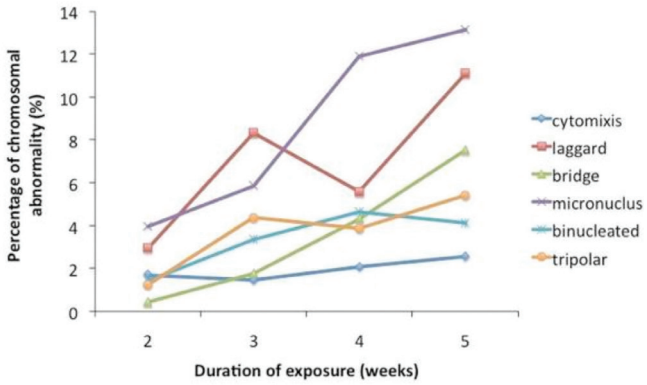

FIGURE 3. A dose-response curve depicting effect of duration of exposure to kinetin (weeks) on percentage of chromosomal abnormality

adaptations to restricted and/or controlled growth supplied by tissue culture conditions. However, chromosomal irregularity frequencies were significantly augmented with increasing exposure to Kinetin (based on plantlet age). Cells showing abnormal chromosome behaviours are depicted in Figure 4.

The mean nuclear areas, mean cell areas and ratio of nuclear areas to cell areas of meristematic cells of *in vivo* and *in vitro* grown *A. praecox* ssp. *minimus* roots were investigated in the present study. Addition of hormones to the culture media was observed to affect the size of nucleus, causing the nucleus size to be augmented. However, prolong exposure to hormones, possibly due to kinetin had caused the nuclear size to initially increase, but is subsequently reduced over time. However, analysis of results showed that nucleus of cells grown *in vivo* was significantly larger than cells grown *in vitro*, implying the effect of tissue culture stress. Similar observations were obtained in measurement of cellular areas. Interestingly, ratio of nuclear to cell area was not significantly different between *in vivo* plants and *in vitro* plantlets grown without hormones, suggesting that relative cell and nuclear size was maintained when cells are transferred to *in vitro* conditions. However, the ratio was significantly elevated with prolong exposure to hormones, possibly due to kinetin. Various published literatures showed that kinetin could cause inhibitory effects when used in tissue culture. Kinetin was reported to induce cell death in *Vicia faba* ssp. *minor* root cortex cells (Kunikowska et al. 2013). Kinetin also functions similar to Ethionine and Chloramphenicol, causing complete inhibition of root formation from pea stem sections (Kaminek 1967). Kinetin can also cause the action of auxin to reverse, for example in *in vitro* grown *Secale cereale* var. Petkus supplemented with IAA and

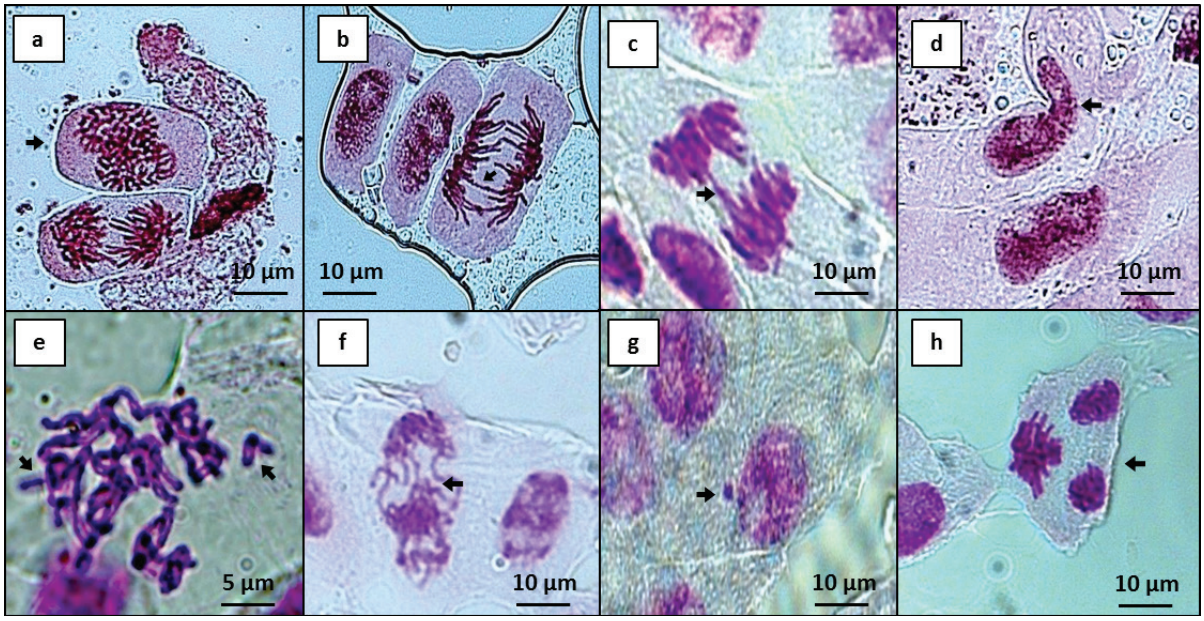

FIGURE 4. Abnormal chromosome behaviours in root meristem cells of *in vitro A. praecox* ssp. *minimus* supplemented with kinetin; (a) Binucleated cell, (b) - (c) Bridge during anaphase (d) Cytomixis, (e) Fragmented chromosomes, (f) Laggard chromosomes during anaphase, (g) Micronucleus and (h) Tripolar cell

Kinetin, lateral roots formation was retarded (Yang & Dodson 1970).

According to Partanen (1963), meristematic regions in shoot and root tips normally undergo diploid chromosomal complement, unlike non-meristematic regions, which may contain varied chromosome numbers caused by somatic chromosomal inconsistency. Many factors could be involved in causing this incidence, including abnormal mitotic spindle that disturbs normal cell multiplication, chromosome breakage and continuous DNA replications without mitosis (Partanen 1963). As a result, chromosome content in the cells might be augmented or significantly reduced. In the present study, meristematic region of *A. praecox* ssp. *minimus* roots was used in the analysis. The results suggested that exposure to kinetin yielded abnormal chromosomal behaviour, such as fragmented and laggard chromosomes. Fragmented chromosomes are caused by chromosome breakage, which can subsequently alter the chromosome count within a nucleus and affect the structural arrangements between the chromosomes (Sunderland 1973). On the other hand, abnormal spindle fibers can affect the separation of chromosomes (Sunderland 1973), yielding laggard chromosomes as observed in the present investigation. This can concomitantly increase DNA content of the cell.

Cytokinins played a major role in various aspects of plant growth and development, especially in regulation of cell proliferation. Cytokinins involve in induction of CycD3 expression encoding a D-type cyclin, which play a role in transition from G1 to M phase during cell division. Nevertheless, specific involvement of cytokinins in other aspects of cell cycle is yet to be uncovered (D'Agostino & Kiebe 1999). Kinetin especially was proposed to exert stimulatory effect on seed germination and affect mitotic activity (Das et al. 1956; Haber & Luippold 1960; Reynolds & Thompson 1973), parallel to our findings in the current investigation. Soh and Yang (1993) showed that low concentrations of cytokinins, specifically kinetin and BAP increased mitosis activity and caused mitotic aberrations in *Allium cepa*. For example, 50 and 100 ppm of kinetin and BAP yielded abnormal chromosomal behaviour such as laggards, anaphase or telophase bridges, C-mitosis, multipolar spindle formation, endoreduplication and micronuclei. Our results also showed similar observations, whereby increasing timely exposure to low concentration of kinetin at 1 mgL^{-1} resulted in more pronounced chromosomal aberrations.

The action of kinetin in mitotic interference is to some extent similar to effects of toxic chemical such as pesticides, often termed as 'mitotic poison' due to their acute interference and disturbance of the spindle apparatus; in serious cases, causing the C-mitosis effect or complete absence of spindle apparatus (Ateeq et al. 2002; Yuzbasioglu et al. 2009). On the other hand, a weak and less pronounced C-mitosis effect yielded laggard chromosomes and cell multipolarity (Badr et al. 1985; Haliem 1990). Cytomixis was also observed to occur more frequently with increasing exposure to kinetin. Cytomixis describes the migration of nuclei from a cell to another, which can subsequently alter DNA content and cause variance in chromosome number. Repeated DNA replication accompanied by the lack of mitosis can also result in augmented DNA content and chromosome girth, but without affecting the chromosome number (Sunderland 1973). Furthermore, repeated DNA replications and chromosome doubling (from 2n to 4n, 8n & 16n) can yield polyploid cells through 'endomitosis', allowing sister chromatids to separate without the help of spindle fibers within the nucleus (Sunderland 1973; Nagl 1974).

Conclusion

Prolong exposure to kinetin was observed to cause abnormal chromosome behaviour. For example, micronuclues, binucleated and tripolar cells occurred more frequently with increasing exposure to kinetin. Restricted growth conditions *in vitro* caused the size of cell and nucleus to reduce significantly, but improved with further addition of hormones. However, prolong exposure to kinetin caused the cell and nuclear size to eventually decrease.

Acknowledgements

The authors would like to thank University of Malaya for the facilities and financial support provided to successfully carry out this research. This project was supported by postgraduate research grant (PPP) no. PS294/2009B. Both authors contribute in conceptualization of the experiment; J.S. Yaacob conducted the experiments, analysis of data and wrote the manuscript. The authors declare no conflict of interest.

References

Ateeq, B., Abdul, F.M. & Niamat, A.M. 2002. Clastogenicity of Pentachlorophenol, 2, 4-D and Butachlor evaluated by *Allium* root tip test. *Mutation Research* 514: 105-113.

Badr, A., Hamoud, M.A. & Haroun, S.A. 1985. Effect of the herbicide Gespax on mitosis, mitotic chromosomes and nucleic acids in *Vicia faba* L. root meristems. *Proceedings of the Saudi Biological Society* 8: 359-370.

Bayliss, M.W. 1980. Chromosomal variation in plant tissues culture. *International Review of Cytology* 11A: 113-144.

Bloor, S.J. & Falshaw, R. 2000. Covalently linked anthocyanin-flavonol pigments from blue *Agapanthus* flowers. *Phytochemistry* 53: 575-579.

Conger, A.D. & Fairchild, L.M. 1953. A quick freeze method for making smear slide permanent. *Stain Technology* 28: 281-283.

Cullis, C.A. 2005. Mechanisms and control of rapid genomic changes in flax. *Annals of Botany* 95: 201-206.

Cullis, C.A. 1999. Environmental stress - a generator of adaptive variation?. In *Plant Adaptations to Stress Environments*, edited by Lerner, H.R. New York: Marcel Dekker. pp. 149-160.

Das, N.K., Patau, K. & Skoog, F. 1956. Initiation of mitosis and cell division by kinetin and indolacetic acid in excised tobacco pith tissue. *Physiologia Plantarum* 9: 640-651.

D'Agostino, I.B. & Kieber, J.J. 1999. Molecular mechanisms of cytokinin action. *Current Opinion in Plant Biology* 2: 359-364.

Haber, A.H. & Luippold, H.J. 1960. Effects of gibberellin, kinetin, thiourea and photomorphogenic radiation on mitotic activity in dormant lettuce seed. *Plant Physiology* 35: 486-494.

Haliem, A.S. 1990. Cytological effects of the herbicide sensor on mitosis of *Allium cepa*. *Egyptian Journal of Botany* 33: 93-104.

Kaeppler, S.M., Kaeppler, H.F. & Rhee, Y. 2000. Epigenetic aspects of somaclonal variation in plants. *Plant Molecular Biology* 43: 179-188.

Kaminek, M. 1967. Root formation in pea stem sections and its inhibition by kinetin, ethionine and chloramphenicol. *Biologia Plantarum* 9: 86-91.

Karp, A. 1991. On the current understanding of somaclonal variation. In *Oxford Surveys of Plant Molecular and Cell Biology,* edited by Miflin, B.J. Oxford: Oxford University Press. pp. 1-58.

Kovarik, A., Lim, K.Y., Souckova-Skalicka, K., Matyasek, R. & Leitch, A.R. 2012. A plant culture (BY-2) widely used in molecular and cell studies is genetically unstable and highly heterogenous. *Botanical Journal of the Linnean Society* 170: 459-471.

Kunikowska, A., Byczkowska, A. & Kazmierczak, A. 2013. Kinetin induces cell death in root cortex cells of *Vicia faba* ssp. *minor* seedlings. *Protoplasma* 250: 851-861.

Murashige, T. & Skoog, F. 1962. A revised medium for rapid plant growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473-497.

Nagl, W. 1974. The *Phaseolus* suspensor and its polytene chromosomes. *Zeitschrift für Pflanzenphysiologie* 73: 1-44.

Oselebe, H.O. & Ene-Obong, E.E. 2007. Organogenesis in *Dioscoreophyllum cumminsii* (Stapf) Diels. *Tropicultura* 25: 37-43.

Partanen, C.R. 1963. Plant tissue culture in relation to developmental cytology. *International Review of Cytology* 15: 215-243.

Pretorius, J.C., Zietsman, P.C. & Eksteen, D. 2002. Fungitoxic properties of selected South African plant species against plant pathogens of economic importance in agriculture. *Annals of Applied Biology* 141: 117-124.

Reynolds, T. & Thompson, P.A. 1973. Efffects of kinetin, gibberellins and abscisic acid on the seed germination of lettuce (*Lactuca sativa*). *Physiologia Plantarum* 28: 516-522.

Savchenko, T., Whiting, P., Sarker, S.D. & Dinan, L. 1997. Phytoecdysteroids in the genus *Agapanthus* (*Alliaceae*). *Biochemical Systematics and Ecology* 25: 623-629.

Singh, D.N., Verma, N., Rahuwanshi, S., Shukla, P.K. & Kulshreshta, D.K. 2008. Antifungal activity of *Agapanthus africanus* extracts. *Fitoterapia* 79: 298-300.

Soh, Y.W. & Yang, W.Y. 1993. Effect of plant growth regulators on mitotic chromosomes in *Allium cepa* L. *Nucleus* 36: 109-113.

Sunderland, N. 1973. Nuclear cytology. In *Plant Tissue and Cell Culture: Bot Monographs,* edited by Street, H.E. Oxford: Blackwell Scientific Publication. pp. 161-190.

Tegegne, G., Pretorius, J.C. & Swart, W.J. 2008. Antifungal properties of *Agapanthus africanus* L. extracts against plant pathogens. *Crop Protection* 27: 1052-1060.

Varga, C.A. & Veale, D.J.H. 1997. Isihlambezo - Utilization patterns and potential health effects of pregnancy-related traditional herbal medicine. *Social Science & Medicine* 44: 911-924.

Yaacob, J.S., Taha, R.M. & Mohajer, S. 2014. Organogenesis induction and acclimatization of African blue lily (*Agapanthus praecox* ssp. *minimus*). *International Journal of Agriculture and Biology* 16: 57-64.

Yaacob, J.S., Mad Yussof, A.I., Taha, R.M. & Mohajer, S. 2012. Somatic embryogenesis and plant regeneration from bulb, leaf and root explants of African blue lily (*Agapanthus praecox* ssp. *minimus*). *Australian Journal of Crop Science* 6: 1462-1470.

Yaacob, J.S., Mad Yussof, A.I., Abdullah, S., Ramesh, K. & Taha, R.M. 2011. Investigation of pH varied anthocyanin pigment profiles of *Agapanthus praecox* and its potential as natural colourant. *Materials Research Innovations* 15(S2): 106-109.

Yang, D. & Dodson, E.O. 1970. The effect of kinetin on the growth of diploid and autotetraploid root tips of rye *in vitro*. *Canadian Journal of Botany* 48: 19-25.

Yuzbasioglu, D., Unal, F. & Sancak, C. 2009. Genotoxic effects of herbicide Illoxan (Diclofop Methyl) on *Allium cepa* L. *Turkish Journal of Biology* 33: 283-290.

Jamilah Syafawati Yaacob*
Centre for Research in Biotechnology for Agriculture (CEBAR)
Faculty of Science
University of Malaya
50603 Kuala Lumpur, Federal Territory
Malaysia

Jamilah Syafawati Yaacob* & Rosna Mat Taha
Institute of Biological Sciences
Faculty of Science
University of Malaya
50603 Kuala Lumpur, Federal Territory
Malaysia

*Corresponding author; email: jamilahsyafawati@um.edu.my

Received: 28 January 2015
Accepted: 14 April 2017