http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 16 (13): 630-635, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.630.635

Determination by Flow Cytometry Polyploidy Inducing-capacity of Colchicine in *Cajanus cajan* (L.) Mill sp.

[1]O.U. Udensi and [1,2]V. Ontui
[1]Department of Genetics and Biotechnology, University of Calabar, PMB 1115, Calabar, Nigeria
[2]Laboratory of Plant Cell Technology, Graduate School of Horticulture, Chiba University, Japan

Abstract: The need to optimize flow cytometric analysis for the determination of ploidy level is a worthwhile venture to precisely know at what concentration of a mutagen and at what time of exposure polyploidy could be induced. Flow cytometry was used to determine the polyploidy inducing-capacity of colchicine in pigeon pea (*Cajanus cajan* (L.) Mill sp). Seeds of pigeon pea were soaked in three different concentrations of colchicine-5 mg, 10 and 15 mg L^{-1} for 24, 48 and 72 h, respectively, while the control group was soaked in water. Treated seeds and those from the control were planted in a greenhouse using a Completely Randomized Design (CRD). Results show that colchicine induced tetraploids (4n) and mixoploids (2n+ 4n) as the concentration of colchicine increased and soaking duration. Days to seedling emergence increased as concentration of colchicine and duration of soaking increased while germination rate decreased proportionately with the increase in colchicine concentration and soaking duration but did not significantly affect percentage seedling survival. Explicitly, colchicine has the capacity of inducing polyploidy; especially tetraploids on the seeds of pigeon pea, which obviously could be harnessed for further breeding and improvement of the pigeon pea.

Key words: Colchicines, polyploidization, pigeon pea, yield improvement

INTRODUCTION

The need to explore and exploit extinct threatened crop plants should not be over-emphasized, especially to the sub-Saharan African counties that are bedeviled with food insecurity amidst ever-increasing population (Udensi *et al*., 2011, 2012a). Landraces of pigeon pea, which have been reported to be highly adaptive to environmental stresses, heritable and superb nutritive values (Udensi *et al*., 2011) is one of such crops that needs exploitation.

Chemicals such as oryzalin, colchicines, amiprophos methyl, etc., widely used as antimitotic; agents have the intrinsic capacity to inhibit spindle formation during cell division. This results to the induction of cells with extra chromosomal copies, which are known as polyploids (Goyal and Khan, 2009). The ability of each of these chemicals to induce polyploidy in crops species depends on the chemical concentration, duration of exposure and species of plant being investigated (Udensi *et al*., 2012a-c).

Cytogenetic studies are pivotal for obtaining information pertaining the role and effects of various mutagens and also elucidating the responses of different crop genotypes to a particular mutagen (Khan and Tyagi, 2009). Before now, standard cytological techniques have been employed to decipher the effects of mutagens on the polyploidization of crop plants. As good as these techniques were, flow cytometry provides a faster and efficient means of determining relative genome size and associated ploidy levels in cell samples, which is obviously cardinal to the plant breeder. It does therefore suggest that instead of carrying out a trial and error experiment and speculating a possible polyploidy induction, flow cytometric analysis gives this answer at a particular concentration of mutagens, and possibly duration of soaking.

According to Wendel (2000), ploypoids population often demonstrate extensive genomic rearrangement posses a certain amount of genetic redundancy implying that extra copies of genes can mutate and diverge resulting in new traits without necessarily compromising essential functions (Ranney, 2006); they display heterosis relative to their parental species and may also display novel variation or morphologies that may contribute to the processes of speciation and ecoloniche exploitation (Comai, 2005). Additionally, Ranney (2006) noted that since polyploids exhibit more heterozygousity than their diploid counterparts, the degree of this condition could possibility be the underlying factor in their growth performances and adaptability to environmental stressors.

This work is therefore hinged on the precise determination of the polyploidy-inducing capacity of colchicine on pigeon pea, which will serve as a spring board to its improvement through mutagenesis.

Corresponding Author: O.U. Udensi, Department of Genetics and Biotechnology, University of Calabar, PMB 1115, Calabar, Nigeria

MATERIALS AND METHODS

Collection of materials and seed treatment: Pigeon pea seeds were collected from the germplasm collection of Dr. Ugorji O. Udensi of the Department of Genetics and Biotechnology, University of Calabar, Nigeria. The seeds were soaked in water (control) and three different concentrations (5, 10 and 15 mg L^{-1}) of colchicine for 24, 48, and 72 h, respectively at room temperature. Seeds were later washed several times in running tap water and then planted in a green house for germination.

Flow cytometry analysis: Approximately, 50 mm^2 of young fresh intact leaves of *Cajanus cajan* (grown in the greenhouse) was chopped in 1 mL of ice-cold nucleic isolation buffer from DNA kit (Cysteine UV precise P. Partec GMbH Germany) using a razor blade. The crude suspension was then filtered through a 30 μm nylon and stained with 4`,6`-diaminodino-2-phenylindole (DAPI). Fluorescence intensity of isolated nuclei was measured using a partec PA II flow cytometry equipped with an argon ion laser (488 nm). The flow speed was 30 fluorescent events per sec. in all cases and the fluorescence of at least 5,000 particles (counts) was recorded. This was done in the faculty of Horticulture, Chiba University, Chiba, Japan.

Data collection and analysis: Data collected on days to seedling emergence and germination rate was subjected to analysis of variance (ANOVA) using Predictive Analytics SoftWare (PASW), version 18.0.

RESULTS

The flow cytometry profiles of pigeon pea seeds treated with colchicine are as presented on Fig. 1-4.

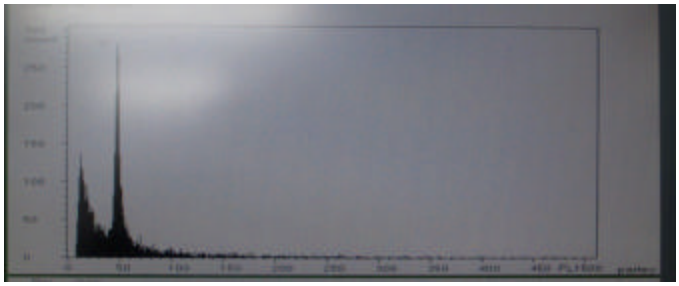

Fig. 1: DNA content of pigeon pea seeds of treated with colchicine (Diploid = 2n)

Fig. 2: DNA content of pigeon pea seeds of treated with colchicine (Triploid = 3n)

Fig. 3: DNA content of pigeon pea seeds of treated with colchicine (Tetraploid = 4n)

Fig. 4: DNA content of pigeon pea seeds of treated with colchicine (Mixoploid = 2n+4n)

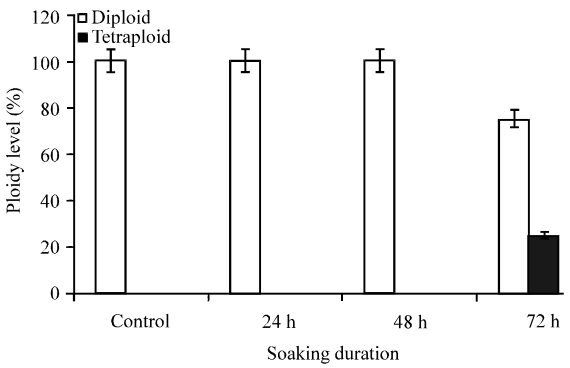

Fig. 5: Ploidy level at 5 mg L^{-1} colchicine induction

The peaks of the horizontal axis correspond to the relative nuclear DNA content, which is represented as the fluorescence intensity while the vertical axis represents the number of nuclei. It was observed that as the fluorescence intensity increased, ploidy level also increased. It revealed that colchicine induced tetraploids (4n) and mixoploids (2n+4n) which is the combination of a diploid and a tetraploid.

These result showed that APM concentration and soaking duration affected ploidy levels as expressed in percentage. As APM concentration increased, ploidy levels also increased. The ploidy level for the diploid cell across the treatments were 100% (control), 96% (5 mg L^{-1}), 70% (10 mg L^{-1}) and 50% (15 mg L^{-1}) while for tetraploid it was 8% (5 mg L^{-1}), 20% (10 mg L^{-1}) and 30% (15 mg L^{-1}), respectively. For the mixoploid, ~18% was observed at the highest concentration of 15 mg L^{-1} APM. Additionally, increase in seed soaking duration, increase in ploidy levels. For instance, diploid cell ranged from 100-30% at 72 h of soaking while tetraploid increased from 20% (24 h) to 50% (72 h). However, mixoploid occurred more at 24 h of soaking (25%). Generally, polyploidization was concentration of antimitotic agent and duration of soaking dependent (Fig. 5-8).

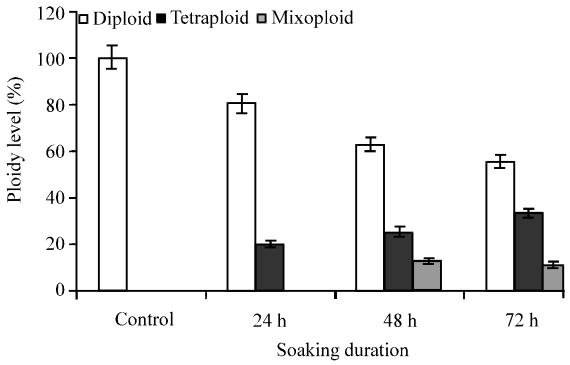

Fig. 6: Ploidy level at 10 mg L^{-1} colchicine induction

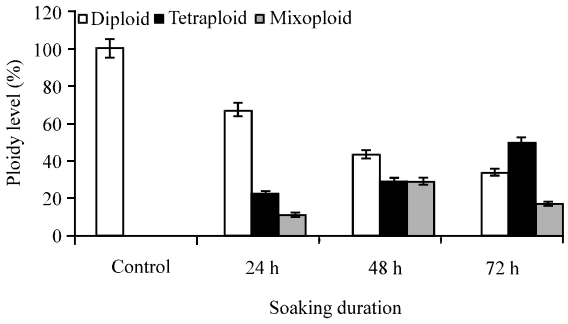

Fig. 7: Ploidy level at 15 mg L^{-1} colchicine induction

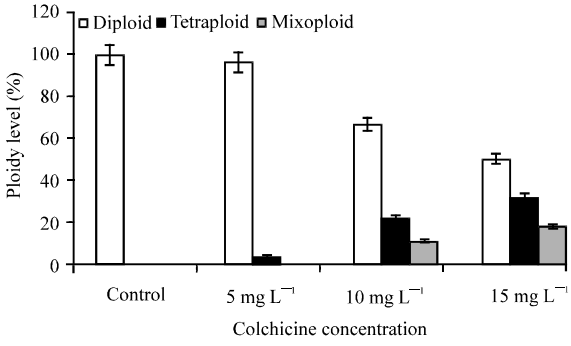

Fig. 8: Ploidy induction at different colchicine treatments

Table 1: Phenological response of pigeon pea to colchicine treatment

Colchicine concentration (mg L^{-1})	Soaking duration	Days to seedling emergence	Germination rate	Plant survival (%)
Control	24	10.00±0.71^{a}	100.00±0.41^{h}	100
	48	9.50±0.29^{a}	99.75±0.48^{h}	100
	72	10.25±0.25^{a}	100.00±0.48^{h}	100
5	24	15.25±0.63^{b}	92.49±0.30^{g}	100
	48	15.50±1.04^{b}	56.56±0.30^{c}	100
	72	15.25±0.46^{b}	56.43±0.25^{c}	100
10	24	21.25±0.48^{e}	71.22±0.12^{f}	100
	48	21.00±0.82^{e}	57.19±0.28^{d}	100
	72	20.75±0.63^{e}	63.93±0.16^{e}	100
15	24	14.75±0.85^{b}	63.93±0.17^{e}	100
	48	22.50±0.85^{cd}	49.95±0.05^{b}	100
	72	26.25±0.48^{e}	42.20±0.48^{a}	100

Superscript with the same case letters indicates no significant difference ($p>0.05$)

Phenological traits were affected significantly by the concentration and soaking duration, especially on days to seedling emergence and percentage germination. Days to seedling emergence increased with increase in APM concentration and soaking duration. For instance, at the different concentrations and 72 h of soaking, it took approximately 10, 15, 21 and 26 days for seeds to emerge while germination rate reduced from 100 to 42.20%. Additionally, treatment did not affect the survival rate as all the seed sown survived (Table 1).

DISCUSSION

Ployploidization is undoubtedly very crucial for crop development and improvement. This is because ployploidized crops display heterosis that leads to enhanced growth, yield and adaptive potentials. There are reports on the efficacy of antimitotic agents in inducing polyploidy in crops, including pigeon pea (Mahandjiev *et al*., 2001; Udensi *et al*., 2011; Brisibe *et al*., 2011).

These results revealed that colchicine treatment of pigeon pea seeds induced ployploids, which was concentration and soaking duration specific (Jaskani *et al*., 2004; Udensi *et al*., 2011; Brisibe *et al*., 2011). There were basically two types of ployploids observed - mixoploids (2n+4n) and the tetraploids (4n). It should be understood that mixoploids contain either diploid-triploid (2n+3n) or diploid-tetraploid (2n+4n) (Comai, 2005). Interestingly, the mixoploid observed in our present report was diploid-tetraploid. From our result when the seeds were soaked in 15 mg L^{-1} colchicine for 48 h, the mixoploid percentage was approximately 30% when compared with the 72 h soaked seeds that were 18%. The possible implication of this condition could be that the induction of ploidy levels though concentration-specific, is critically tied to the duration of exposure of the mutagen. It thus revealed that mixoploidy induction was more favoured at lower soaking duration. Explicably, colchicine affects microtubule depolymerization through the formation of colchicine-tubulin complex. The intensity of the complex should be proportional to the polyploidy inducing capacity of this mutagen. This intensity is however, a function of the mutagen concentration and duration of exposure of the seeds to the mutagen. This implies that the higher the concentration/soaking duration, the higher the complex intensity, which could lead to increase in ploidy levels. This interaction could also result to increase chromosomal aberrations.

During meiosis, ployploids could give rise to univalent, divalent, trivalents, tetravalents or qaudravalents. However, the pairing behaviours of these pairs determine the success of fertility or sterility. It does therefore suggest that diploid-tetraploids mixoploids and the tetaploids will be more beneficial in pigeon pea improvement due to equal separation to the poles during anaphase. Many studies have confirmed that the major cause of sterility in plants is the frequency of univalents and trivalents that might be more common in mixoploid than in tetraploids. If the former is the case, it does suggest that it will lead to the production of unbalanced gametes, which could cause sterility and impaired seed production (Pandit *et al*., 2011; Acquaah, 2007; Chen *et al*., 2007; Dhawan and Lavania, 1996). Tetraploids on the other hand, will give rise to equal gametes and the separation of chromosomes during anaphase will be uniform. This will lead to increase in the proportion of balanced and functional gametes (Comai, 2005). It does suggest that the higher the frequency of tetraploid induction, the higher the gametes fertility and seed sets.

Days to seedling emergence was increased significantly as the concentration of the mutagen increased. This was confirmed by Mensah *et al*. (2005) in cowpea, Udensi *et al*. (2012a-c) in cowpea and pigeon pea following gamma seed irradiation and amiprophos methyl treatments, respectively. The delay in seedling emergence could be attributed chiefly to physiological disturbances, which might have probably affected some

biochemical pathways in the treated seeds. This might have delayed seedling emergence and finally reduced the germination percentage (Udensi *et al*., 2011; Brisibe *et al*., 2011; Mahandjiev *et al*., 2001). It is probable that the seeds soaked in higher concentrations of colchicines have to readjust in response to the treatment thus delaying the time it took the seeds to emerge. The decline in percentage germination as reported in the current result corroborates the earlier reports of (Udensi *et al*., 2012a-c). This might not be unconnected to some adverse effects of the mutagens. Obviously, though colchicines and indeed any other mutagen might present beneficial effects, there could be adverse effects such as induction of chromosomal aberration that might be responsible to the distortions in chromosomal behavior during metaphase.

The percentage survival was not significantly affected. This negates the report of Udensi *et al*. (2012d) using gamma irradiation and amiprophos methyl in cowpea and pigeon pea and Brisibe *et al*. (2011) in Egusi melon. According to Tosca *et al*. (1995), Mensah and Akomeah (1992), Mensah *et al*. (2005), Mensah *et al*. (2007) the result on percentage seedling survival could mean either that the treatment did not significantly perturb the physiological mechanisms underlying the seedling survivability. A striking possibility about the non effect of the treatment to the percentage seedling survival is the fact that these colchicine-soaked seeds might have developed tolerance after the initial inhibitory effect of the treatments, readjusted to the condition and improved their physiological conditions resulting to delayed seed germination and elongated the days to seedling emergence (Udensi *et al*., 2012b).

CONCLUSION

It is concluded that colchicine can induce polyploidy in *Cajanus cajan* at higher concentrations and longer durations which is very pivotal in crop development and improvement as we seek to enhance food sustainability and security in sub-Sahara African nations like Nigeria.

REFERENCES

Acquaah, G., 2007. Principles of Plant Genetics and Breeding. Blackwell Publishing Ltd., Malden, Pages: 569.

Brisibe, E.A., O. Udensi, V.O. Ntui, P.A. Otu and P.N. Chukwurah, 2011. Sensitivity of some quantitative and yield characters of Egusi melon (*Colocynthis citrullus* L.) to treatment with microtubule inhibitors. Afr. J. Plant Sci., 5: 759-766.

Chen, L., Q. Lou, Y. Zhuang, J. Chen, X. Zhang and J.N. Wolukau, 2007. Cytological diploidization and r apid genome changes of the newly synthesized allotetraploids *Cucumis* x *hytivus*. Planta, 225: 603-614.

Comai, L., 2005. The advantages and disadvantages of being polyploid. Nature Rev. Genet., 6: 836-846.

Dhawan, O.P. and U.C. Lavania, 1996. Enhancing the productivity of secondary metabolites via induced polyploidy. Euphytica, 87: 81-89.

Goyal, S. and S. Khan, 2009. A comparative study of chromosomal aberrations in *Vigna mungo* induced by ethylmethane sulphonate and hydrazine hydrate. Thai J. Agric. Sci., 42: 117-182.

Jaskani, M.J., H. Raza, M.M. Khan and S.W. Kwon, 2004. Effect of antimitotic agent colchicine on *in vitro* regeneration of watermelon. J. Plant Biotechnol., 6: 247-252.

Khan, M.H. and S.D. Tyagi, 2009. Cytological effects of different mutagens in soybean (*Glycine max* (L.) Merrill). Front. Agric. China, 3: 397-401.

Mahandjiev, A., G. Kosturkova and M. Mihov, 2001. Enrichment of *Pisum sativum* gene resources through combined use of physical and chemical mutagens. Israel J. Plant Sci., 49: 279-284.

Mensah, J.K. and P.A. Akomeah, 1992. Mutagenic effects of hydroxylamine and streptomycin on the growth and seed yield of cowpea, *Vigna unguiculata* (L.) Walp. Legume Res., 15: 39-44.

Mensah, J.K., B.O. Obadoni, P.A. Akomeah, B. Ikhajiagbe and J. Ajibolu, 2007. The effects of sodium azide and colchicine treatments on morphological and yield traits of sesame seed (*Sesame indicum* L.). Afr. J. Biotechnol., 6: 534-538.

Mensah, J.K., P.A. Akomeah and E.O. Ekpekurede, 2005. Gamma irradiation induced variation of yield parameters in cowpea (*Vigna unguiculata* (L.) Walp.). Global J. Pure Applied Sci., 113: 327-330.

Pandit, M.K., M.J.O. Pocock and W.E. Kunin, 2011. Ploidy influences rarity and invasiveness in plants. J. Ecol., 99: 1108-1115.

Ranney, T.G., 2006. Polyploidy: From evolution to new plant development. Combined Proc. Int. Plant Propagators Soc., 56: 137-142.

Tosca, A., R. Pandolfi, S. Citterio, A. Fansoli and S. Sgorbati, 1995. Determination by flow cytometry of the chromosome doubling capacity of colchicine and oryzalin in gynogenetic haploids of gerbera. Plant Cell Rep., 14: 455-458.

Udensi, O., E.A. Edu, V.O. Ntui, P.A. Otu, I.S. Urua and G.A. Akpan, 2011. Cyto-sensitivity of some locally grown pigeon pea [*Cajanus cajan* (L.) Millsp] to amiprophos methyl treatment as a guide for improvement. Int. J. Curr. Res., 3: 176-180.

Udensi, O., E.A. Edu, E.V. Ikpeme, J.K. Ebiwgai and D.E. Ekpe, 2012a. Biometrical evaluation and yield performance assessment of cowpea [*Vigna unguiculata* (L.) Walp] landraces grown under lowland tropical conditions. Int. J. Plant Breed. Genet., 6: 47-53.

Udensi, O., E.V. Ikpeme, E.A. Edu and D.E. Ekpe, 2012b. Relationship studies in cowpea (*Vigna unguiculata* L. walp) landraces grown under humid lowland condition. Int. J. Agric. Res., 7: 33-45.

Udensi, O., G.A. Arong, J.A. Obu, E.V. Ikpeme and T.O. Ojobe, 2012c. Radio-sensitivity of some selected landraces of pulses to gamma irradiation: Indices for use as improvement and preservation techniques. Am. J. Exp. Agric., 2: 320-335.

Udensi, O.U., E.V. Ikpeme, J.A. Obu and D.E. Ekpe, 2012d. Assessing the mutagenic effects of gamma irradiation on *Cajanus cajan* (L.) Huth and *Vigna unguiculata* (L.) Walp landraces using morphological markers. Comunicata Scientiae, 3: 271-281.

Wendel, J.F., 2000. Genome evolution in polyploids. Plant Mol. Biol., 42: 225-249.