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In vitro Polyploidy Induction of *Anthurium andreanum* Lind. Plants by Colchicine Treatment for Enhanced Floriculture Applications

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ABSTRACT

The *A. andreanum* Lind. (commercially known as Flamingo flower) plays a significant role in the global floricultural trade. It is traditionally propagated by seeds, however, it is not desired because of cross-pollination and the progenies are heterozygous. This study aims to develop faster method to obtain new variety(s) of *A. andreanum* Lind. through *in vitro* polyploidization using antimitotic agent, colchicine. Three different explants (petiole, shoot tip and root) of *A. andreanum* Lind. clones (namely A1 and A5) were treated with colchicine (0.3 mg/L and 0.4 mg/L) at different exposure times (2, 3 and 5-hours) and transferred into shoot regeneration medium. The explants were kept for 3 months under control conditions *in vitro*. Survival rates and regeneration ability were recorded. Leaves developed from each colchicine-treated explant were excised and evaluated for ploidy level using flow cytometry (FCM). Results showed ploidy level changes detected at less than 30 % in all samples. Aneuploidy and haploidy were detected in leaf samples regenerated from A1 shoot tip after treated with 0.4 mg/L colchicine for 3 and 5-hour exposure time. Changes in ploidy levels were detected in shoot tip after treated with 4 mg/L colchicine at 2 and 3-hour exposure time however, no ploidy changes from leaf developed from petiole explants in both clones. This study showed that types of explants, antimitotic agent concentration and genotypic differences play a role in the susceptibility in the success of polyploidy induction in *A. andreanum* Lind.

1. Introduction

Anthurium andreanum belongs to Araceae family and a very popular flowering plant among the gardeners and florists thus, contributing substantially to the floricultural trade [1-3]. The species is native to Colombia and Ecuador and can be found in abundance in South East Asian countries such as Thailand, Taiwan, Malaysia and Indonesia [3-5]. It is a perennial

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herbaceous plant cultivated for its long-lasting and attractive heart-shaped inflorescence. *Anthurium* has a modified leaf (spathe), bearing numerous pencil-like protrusion (spadix) with a vase life of 14-28 days [3,4]. *Anthurium* is traditionally propagated by seeds, however propagation through seeds is not desired because of cross-pollination and the progenies are heterozygous [3,4]. Moreover, it is hampered by the poor germination rate and low viability of the seed. Seed cannot be conserved and must be collected immediately after fruit maturation.

Due to difficulties of propagation with seeds, macropropagation such as cuttings and bud cultures were applied, however these conventional methods of vegetative propagation are slow and cannot keep pace with the increasing demand of these ornamental plant species. Therefore, micropropagation through tissue culture is a suitable option to facilitate in producing of high-quality planting material in large quantities [4-6]. Today, the competitive world on producing high quality and unique ornamental plant varieties, including *Anthurium* has initiated an alternative way to produce special hybrids to the floriculture world.

In the past few decades, polyploidy induction has been one of the ornamental breeding methods used to induce novel variation, overcome crossing barriers or create homogenous lines [7-9]. The purpose of breeders is primarily focused on aesthetically relevant parameters including plant shape, flower colour, form and architecture of flowers, flowering time, flower shelf life or fragrance and resistance to biotic and abiotic stresses [8,9]. Polyploid plants also have lower growth rates, and tend to flower later or over a longer period of time than related diploids, which is a desirable feature for ornamental breeding [8,9]. Concentration and exposure time are important parameters, but there is an evident interaction between them.

There are many methods for antimitotic agent application such as by dropping the solution on apical meristem, seed soaking, *in vitro* media or short application in liquid medium followed by culture of new and/or putative polyploidy explants on a regeneration medium [9-11]. The success of polyploidization, however is unpredictable as many factors involved including plant species, cultivar, explant used and laboratory conditions [9-11]. Several explant types have been successfully used are shoot, bud or shoot tip, callus, somatic or zygotic embryos, seed, seedling, nodal segment and tuber segment [9-14]. The effects of explants and genotype-dependent efficiency is also observed. They are several antimitotic agents used such as colchicine, trifluralin and oryzalin. In general, colchicine is the most commonly used anti-mitotic agent, but binds only poorly to plant tubulins, thus it must be used in relatively high concentrations whereas the lower cost of oryzalin over trifluralin made this antimitotic agent more beneficial than colchicine [8,13,15].

Several approaches are available for determining the ploidy level of regenerated plants such as chromosome number and enhancement in stomatal sizes. Direct chromosome number determination through counting is a reliable method. However it can be labor-intensive and time-consuming particularly when dealing with large number of samples. Stomata density and size as micromorphological traits are simple and low-cost techniques to measure the ploidy level. The morphological variation caused by the use of antimitotic agents can be due not only to increasing the ploidy level, but also to chromosomal and DNA mutations [14-17] because the extent and character of morphological changes can be related to parental genotype and/or mitotic inhibitors. Therefore, flow cytometry (FCM) is an indirect method which are fast and accurate and has become the predominant technique for establishing plant genome size particularly when dealing with large number of samples [14-

17]. This study aimed to determine the most suitable explants to establish polyploidization using different concentrations of colchicine and exposure time.

2. Methodology

2.1 Plant Materials and In Vitro Polyploidization Induction

In the experiments, two clones of *A. andreanum* Lind. (clone A1 and A5) grown *in vitro* at the Center for Biotechnology Bioenterpreneur (CBB), FRIM were selected. Three different explants (shoot, tip and root) from the two clones were excised and cultured *in vitro* in modified MS medium supplemented with 1, 2 and 3 mg/L 6-benzylaminopurine, (BAP) for two weeks to initiated small amount of callus production at the cutting edges. Then, the explants were treated accordingly with different concentrations of colchicine (0.2, 0.3 and 0.4 mg/L) at various exposure times (2, 3 and 5-hours). After the treatment, the explants were rinsed 3 times with sterile distilled water and blot dry before culture into fresh [18], MS hormone-free medium. The explants were incubated for 3 months under control conditions of 16/8 h light regime (2,000 lux light intensity) at 22°C ± 2 with ≤ 40 % humidity. The control cultures (without exposure to colchicine) were kept under the same conditions for comparison. By the end of the 3 months, clumps of young shoots emerged from explants. These young shoots were transferred into fresh MS-hormone-free medium for further development until mature leaves developed and ready to be excised for ploidy level determination.

2.2 Ploidy Level Determination

The ploidy level was measured using FACS Calibur flow cytometer (Becton Dickinson, USA) FCM analyser at the Advance Biotechnology and Breeding Center, Malaysia Palm Oil Board (MPOB). The FCM measurements were made following the procedure set up by MPOB. The leaf samples were excised from *in vitro* culture and kept in Petri dishes containing wet kitchen towels to maintain the humidity and prevent samples from drying. Nuclei suspensions were prepared by excision of about 1 cm^2 piece of leaf sample and placed onto Petri dish containing 1 ml LB01 solution and rapidly chopped the leaf sample to release their nuclei into the solution. The solution was collected using a syringe and transferred into FCM valve with 10 ml of fluorescent. The relative fluorescence intensity (FL2) was measured from at least 5,000 particles per sample. Control samples were used as control for the measurement of ploidy level.

2.3 Optimizing Nuclei Preparation

The nuclei suspensions from samples were isolated using LBOI buffers to obtain highly resolved fluorescent histogram peaks with a CV of 1 to 3 %. The samples were placed in a Petri dish and 1 ml of each lysis buffer was added. Next, the leaves were finely chopped using a scalpel and the suspended released nuclei were removed using a 1 ml pipette and filtered into a 5 mL Falcon tube. All lysis buffers were supplemented with 50 mg propidium iodide (PI) and RNaseA, followed by incubation for 15 mins. The nuclei suspension was analyzed using FCM. PI was measured at 585 nm to read a 2C nuclei histogram of 5,000 nuclei per sample. Histograms were collected over 1024 channels. The peak means from the samples and soybean histograms were obtained using 3 the CellQuest software (11-10865-01 Rev B, 1998). Soybean Glycine max cv. Polanka was used as an external reference standard (2C=2.5 pg) as

reported by Madon *et al.*, [19]. Using these values, the ploidy level was calculated by the sample ratio to diploid control fluorescent intensity. FCM analysis was carried out using LBOI and Otto isolation buffers to determine the ploidy level in each sample using the Eq. (1):

$$\text{Ploidy level of samples} = \frac{\text{Samples G1 mean FL}}{\text{Control diploid G1 mean FL}} \quad (1)$$

3. Results and Discussion

3.1 Regenerants Ability and Ploidy Level Changes with Colchicine

Regenerants between three different samples (petiole, shoot tip and root) of *A. andreanum* Lind. (clone A1 and A5) was compared after 1 month in culture. These samples were transferred into BAP (1, 2 and 3 mg/L) to initiate shoot development for 1 month. Results showed petiole sample of clone A1 gave the highest percentage of regenerants was from treatment with 0.4 mg/L colchicine for 5-hours exposure time, cultured on 3 mg/L BAP (40.00 ± 41.95 % regeneration rates), followed by 0.3 mg/L colchicine for 3-hours exposure time on 3 mg/L BAP (30.00 ± 37.42 % regeneration rates) and 0.4 mg/L colchicine for 2-hours on 1 mg/l BAP (30.00 ± 24.49 % regeneration rates) and 0.3 mg/L colchicine and 3-hours exposure time on 2 mg/L BAP(30.00 ± 24.50 % regeneration rates), respectively (Table 1). In root sample, high percentage of regenerants from root samples came from treatment with 0.4 mg/L colchicine for 3 hours on 1 mg/L BAP (83.33 ± 23.38 % regeneration rates), followed by 0.4 mg/L colchicine for 2-hours, on 1 mg/L BAP (76.67 ± 29.44 % regeneration rates) and 0.4 mg/L colchicine for 2-hours and 3 mg/L BAP (63.33 ± 26.58 % regeneration rates), respectively. Treatment with 0.4 mg/L colchicine for 5-hours on 2 and 3 mg/L BAP gave regenerants rated at 53.33 ± 45.02 % and 53.33 ± 43.21 %, respectively. Further observation showed lower regenerants percentages from shoot tip samples with 0.4 mg/L colchicine for 3-hours and 5-hours resulting in less than 30 % regenerants (Table 1). In clone A5, the highest regenerants from petiole came from treatment with 0.3 mg/L colchicine for 3-hours exposure time on 3 mg/L BAP (36.67 ± 42.74 % regeneration rates), while root and shoot tips sample gave high regenerants less than 30 % (Table 2).

The results showed the concentration of colchicine, plant growth regulator (PGR) and exposure time correlated with the regenerants ability of the samples tested. In this study, the highest regenerants percentages came from the most suitable combination treatments on the samples. Samples treated with high concentration of colchicine and supplementation of PGR, BAP play a higher role compared to exposure time to facilitate regenerants ability. The type of samples also plays a significant role for the success of regenerants which showed here shoot tip gave the highest number of regenerants compared to petiole and root, together with PGR are more suitable for initiation of callus and organogenesis from shoot tips rather in petiole and root samples. The regenerants were left for further development until mature leaf available for further analysis in ploidy level changes.

Table 1
Percentage of regenerants ability (%) and ploidy level changes (%) in petiole, shoot tip and root of *A. andreanum* Lind. (clone A1) after treatment with colchicine

Concentration of colchicine, mg/L	Treatment duration, hour	BAP concentration, mg/L	% Regenerants and % Ploidy level changes from different explants					
			Petiole		Shoot tip		Root	
			% Regenerants	% Ploidy changes	% Regenerants	% Ploidy changes	% Regenerants	% Ploidy changes
		1.0	0.00 ± 0.00	n.a	6.67 ± 16.33	0.00	0.00 ± 0.00	n.a
0.3	2	2.0	6.67 ± 16.33	0.00	30.00 ± 24.49	0.00	0.00 ± 0.00	n.a
		3.0	0.00 ± 0.00	n.a	6.67 ± 16.33	0.00	26.67 ± 27.33	0.00
		1.0	0.00 ± 0.00	n.a	26.67 ± 27.33	0.00	6.67 ± 16.33	0.00
0.3	3	2.0	30.00 ± 24.50	0.00	0.00 ± 0.00	n.a	26.67 ± 27.33	0.00
		3.0	26.67 ± 27.33	0.00	0.00 ± 0.00	n.a	0.00 ± 0.00	n.a
		1.0	3.33 ± 8.17	0.00	33.33 ± 27.33	0.00	6.67 ± 16.33	0.00
0.3	5	2.0	6.67 ± 16.33	0.00	50.00 ± 27.57	0.00	6.67 ± 16.33	0.00
		3.0	0.00 ± 0.00	n.a	33.33 ± 27.33	0.00	20.00 ± 21.91	0.00
		1.0	30.00 ± 24.49	0.00	96.67 ± 8.17	0.00	76.67 ± 29.44	0.00
0.4	2	2.0	26.67 ± 27.33	0.00	100.00 ± 0.00	0.00	23.33 ± 26.58	0.00
		3.0	16.67 ± 15.06	0.00	33.33 ± 37.24	0.00	63.33 ± 26.58	0.00
		1.0	0.00 ± 0.00	n.a	100.00 ± 0.00	0.00	83.33 ± 23.38	0.00
0.4	3	2.0	11.67 ± 20.41	0.00	100.00 ± 0.00	20.00 ± 0.49 (A)	36.67 ± 36.69	0.00
		3.0	30.00 ± 37.42	0.00	100.00 ± 0.00	20.00 ± 0.16 (A)	36.67 ± 36.69	0.00
		1.0	16.67 ± 19.66	0.00	66.67 ± 51.64	26.67 ± 40.00 (A)	60.00 ± 47.33	0.00
0.4	5	2.0	13.33 ± 32.66	0.00	63.33 ± 49.67	25.33 ± 40.00 (A)	53.33 ± 45.02	0.00
		3.0	40.00 ± 41.95	0.00	53.33 ± 46.76	0.00	53.33 ± 43.21	0.00

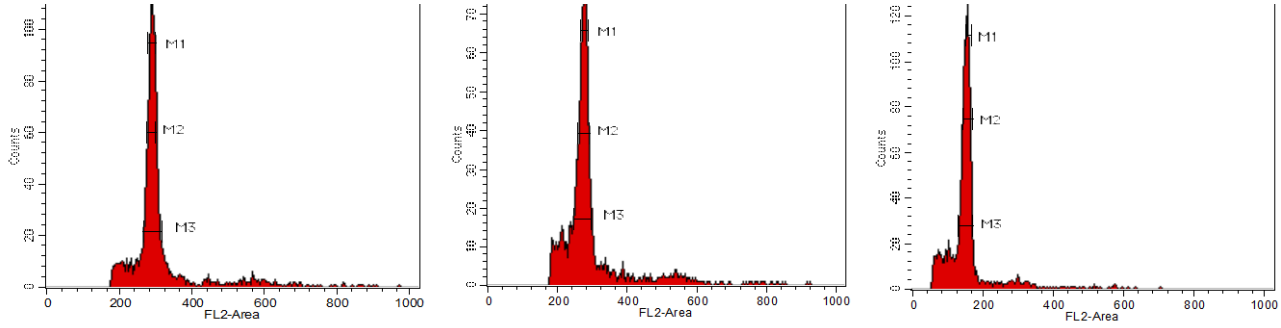
Note: *n.a=100 % mortality; 0.00=no ploidy level changes; A=aneuploidy; H=haploidy*

Table 2
Percentage of regenerants ability (%) and ploidy level changes (%) in petiole, shoot tip and root of *A. andreanum* Lind. (clone A5) after treatment with colchicine

Concentration of colchicine, mg/L	Treatment duration, hour	BAP concentration, mg/L	% Regenerants and % Ploidy level changes from different explants					
			Petiole		Shoot tip		Root	
			% Regenerants	% Ploidy changes	% Regenerants	% Ploidy changes	% Regenerants	% Ploidy changes
		1.0	13.33 ± 20.66	0.00	3.33 ± 8.17	0.00	0.00 ± 0.00	n.a
0.3	2	2.0	0.00 ± 0.00	n.a	3.33 ± 8.17	0.00	0.00 ± 0.00	n.a
		3.0	0.00 ± 0.00	n.a	0.00 ± 0.00	n.a	3.33 ± 8.17	0.00
		1.0	3.33 ± 8.17	0.00	36.67 ± 42.74	0.00	3.33 ± 8.17	0.00
0.3	3	2.0	3.33 ± 8.17	0.00	0.00 ± 0.00	n.a	0.00 ± 0.00	n.a
		3.0	36.67 ± 42.74	0.00	0.00 ± 0.00	n.a	0.00 ± 0.00	n.a
		1.0	0.00 ± 0.00	n.a	13.33 ± 20.66	0.00	6.67 ± 16.33	0.00
0.3	5	2.0	3.33 ± 8.17	0.00	23.33 ± 19.66	0.00	3.33 ± 8.17	0.00
		3.0	0.00 ± 0.00	n.a	6.67 ± 16.33	0.00	0.00 ± 0.00	n.a
		1.0	0.00 ± 0.00	n.a	80.00 ± 17.89	16.00 ± 8.16 (H)	63.33 ± 23.38	0.00
0.4	2	2.0	13.33 ± 20.66	0.00	80.00 ± 17.89	16.00 ± 0.09 (H)	63.33 ± 19.66	0.00
		3.0	0.00 ± 0.00	n.a	96.67 ± 8.17	19.33 ± 8.17 (A)	50.00 ± 41.47	10.00 ± 10.95 (A)
		1.0	0.00 ± 0.00	n.a	36.67 ± 42.74	0.00	53.33 ± 37.24	0.00
0.4	3	2.0	20.00 ± 21.91	0.00	100.00 ± 0.0	20.00 ± 19.66 (A)	43.33 ± 40.83	0.00
		3.0	10.00 ± 10.95	0.00	90.00 ± 10.95	18.00 ± 20.66 (A)	63.33 ± 19.66	0.00
		1.0	3.33 ± 8.17	0.00	33.33 ± 51.64	0.00	30.00 ± 46.90	0.00
0.4	5	2.0	13.33 ± 23.10	0.00	33.33 ± 51.64	0.00	30.00 ± 46.90	0.00
		3.0	0.00 ± 0.00	n.a	30.00 ± 46.90	6.00 ± 42.74 (H)	33.33 ± 51.64	6.67 ± 0.09 (H)

Note: *n.a=100 % mortality; 0.00=no ploidy level changes; A=aneuploidy; H=haploidy*

Flow cytometry is a quick and convenient technique for determining ploidy level of plants already grown *in vitro*. FCM was used to estimate the ploidy level of *A. andreanum* Lind. samples clone A1 and A5. A minimum of 5,000 nuclei were analyzed and the ratio mean was calculated. The results revealed high regenerants does not correlate with changes in ploidy level whereby most samples were diploids. Figure 1 shows the FCM histograms of nuclei suspensions of *A. andreanum* Lind. in LBOI and FCM histograms. Based on FCM analysis results, we observed 4 aneuploids were detected in clone A1 whereas 4 haploids and 4 aneuploids were detected in clone A5. The frequency of haploids obtained was from leaf derived from stem samples (> 5 %) and mixoploids occurred from leaf samples derived from shoot tip and root samples (3 %), both obtained from treatment combinations of 0.4 mg/L of colchicine at 5-hours exposure time. On the other hand, haploid was determined from leaf samples derived from root samples exposed to 0.4 mg/L at 2-hours exposure time. Therefore, several regenerants and the percentage of leaves with changed ploidy levels depended on the colchicine concentration. Higher concentrations of colchicine therefore are necessary to induce polyploidy. Shoot development generally decreased at longer exposure time but the effect of the colchicine was stronger.



(a) (b) (c)

Fig. 1. Histogram of different ploidy levels detected in *A. andreanum* after treatment with colchicine. (a) Diploid, 2n ploidy level from untreated sample; (b) Aneuploid, 2n* level; (c) Haploid, n ploidy level

4. Conclusions

Polyploidy was obtained in *A. andreanum* Lind. with colchicine but at a low rate. Higher concentrations of colchicine are recommended to obtain higher number of polyploidy. Chromosome count and length of stomata can be included to confirm the ploidy level further. In future studies, the regenerants could be monitored and once the plant mature and develop flowers, it could be examined for morphological changes such as in petal size, petal shape, color intensity and ploidy level, therefore recognizing that polyploidization is a promising technique for enhancement in agronomic traits of *A. andreanum* Lind.

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References

[1] Chen, F.C., Kuehnle, A.R. and Sugii, N. "*Anthurium* roots for micropropagation and *Agrobacterium tumefaciens*-mediated gene transfer." *Plant Cell, Tissue and Organ Culture*, no. 49 (1997):71-74. https://doi.org/10.1023/A:1005812802655

[2] Dufour, L. and Guerin, V. "Growth, Development Features and Flowering Production of *Anthurium andreanum* Lind." *Scientia Horticultureae* 98, no. 1 (2003):25-35. https//doi.org/10.1016/S0304-4238(02)00196-6

[3] Li, J., Tan, Q., Yi, M., Yu, Z., Xia, Q., Zheng, L., Chen, J., Zhou, X., Zheng, X.Q. Guo, H.R. "Identification of key genes responsible for green and white colored spathes in *Anthurium andreanum* (Hort.)" *Frontier in Plant Science* 14, (2023). https://doi.org/10.3389/fpls.2023.1208226

[4] Gantait, S., Mandal, N., Bhattacharyya, S. and Das, P.K. "*In vitro* mass multiplication with pure genetic identity in *Anthurium*." *Tissue Culture and Biotechnology* 18, no. 2 (2008):113-122. https://doi.org/10.3329/ptcb.v18i2.3361

[5] Gantait, S. and Mandal, N. "Tissue Culture of *Anthurium andreanum*: A Significant Review and Future prospective." *Botany* 6, no. 3 (2010):207-219. https://doi.org/10.3923/ijb.2010.207.219

[6] Oropeza M., Mejías, A. and Vargas, T.E. "Micropropagation and organogenesis of *Anthurium andreanum* Lind. cv Rubrun." *Methods in Molecular Biology* 589, (2010):3-14. https:// doi.org/10.1007/978-1-60327-114-1_1

[7] Eeckhaut, T., Samijn, G. and Van Bockstacle, E. "*In vitro* polyploidy induction in *Rhododendron simsii* hybrids." *Acta Horticulturae* 6572, (2002):43-49. https://doi.org/10.17660/ActaHortic.2002.572.4

[8] Stanys, V., Weckman, A., Staniene, G. and Duchovskis, P. "*In vitro* induction of polyploidy in japanese quince *(Chaenomeles japonica).*" *Plant Cell, Tissue and Organ Culture* 84, (2006): 263–268. https://doi.org/10.1007/s11240-005-9029-3

[9] Miri, S.M. "Artificial polyploidy in the improvement of horticultural crops." *Plant Physiology and Breeding*, (2008) https://doi.org/10.22034/JPPB.2020.12490

[10] Roughani, A., Miri, S.M. "Polyploidy induction in ornamental plants." *Conference paper* (2018). https://www.researchgate.net/publication/328601169

[11] Dhooghe, E., Van Laere, K., Eeckhaut, T., Leus, L. and Van Huylenbroeck, J. "Mitotic chromosome doubling of plant tissues *in vitro*." *Plant Cell, Tissue and Organ Culture* 104, no. 3 (2011):359-373. https://doi.org/10.1007/s11240-010-9786-5

[12] Sattler, M.C., Carvalho, C.R. & Clarindo, W.R. "The polyploidy and its key role in plant breeding." *Planta* 243, no. 2 (2016):281-296. https:// doi: 10.1007/s00425-015-2450-x

[13] Manzoor, A., Ahmad, T., Bashir, M.A. and Ahmad Hafiz, I. "Studies on Colchicine Induced Chromosome Doubling for Enhancement of Quality Traits in Ornamental Plants." *Plants* 8, no. 7 (2019):194. https://doi.org/10.3390/plants8070194

[14] Mo, L., Chen, J.H., Chen, F., Xu, Q.W., Tong, Z.K., Huang, H.H., Dong, R.H., Lou, X.Z., Lin, E.P. "Induction and characterization of polyploids from seeds of *Rhododendron fortunei* Lindl." *Journal of Integrative Agriculture* 19, no. 8 (2020): 2016–2026. https://doi.org/10.1016/S2095-3119(20)63210-5

[15] Mohammadi, M., Kaviani, B. and Sedaghathoor, S. "*In vivo* polyploidy induction of *Phalaenopsis amabilis* in a bubble bioreactor system using colchicine." *Ornamental Horticulture* 27, no. 2 (2021):204-212. https://doi.org/10.1590/2447-536X.v27i2.2275

[16] Xie, L., Ke, L.Z., Lu, X.Q., Chen, J.J. and Zhang Z.S. "Exploiting unreduced gametes for improving ornamental plants." *Frontier in Plant Science* 13, (2022). https://doi.org/10.3389/fpls.2022.883470

[17] Banjade, G., Chaudhari, G., Mahato, A. and Uprety, S. "Polyploidy Breeding In Agriculture: An Overview." *Reviews In Food And Agriculture (RFNA)* 4, no. 1 (2023):25-27. https://doi.org/10.26480/rfna.01.2023.25.27

[18] Murashige, Toshio, and Folke Skoog. "A revised medium for rapid growth and bio assays with tobacco tissue cultures." *Physiologia plantarum* 15, no. 3 (1962).

[19] Madon, M. A. R. I. A., L. Q. Phoon, M. M. Clyde, and A. Mohd Din. "Application of flow cytometry for estimation of nuclear DNA content in Elaeis." *Journal of Oil Palm Research* 20, no. June (2008): 447-452.