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Effect of Oryzalin on Growth of *Anthurium andraeanum* In Vitro

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ABSTRACT

Apical shoots and lateral buds of Anthurium andraeanum about 0.5 cm grew very well when cultured on MS medium supplemented with NAA, kinetin, sucrose and gelrite. When brought young plantlets (the same sized) of A. andraeanum soaked in various concentrations of oryzalin with different duration times. The A. andraeanum plantlets were subcultured into the same medium every 4 weeks for 3 times. It was found that 5.0 mg/l oryzalin with 24 and 72 hours gave the best average number of leaves per bunch, plant height and diameter of bunch. These parameters were reverse proportion, when increased concentration of oryzalin, the growth rate in each parameter was decreased with thick and pale green leaves.

1. Introduction

Anthurium is an ornamental plant that always use for decorating and landscape. Anthurium has beautiful bract and many colors, many types of leaf shapes, so this plant can use in many proposes. Propagation rate of this plant is cutting, and then we can get a few plants from one stock. Tissue culture is one technique used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium and growth regulator. This technique used to produce

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clones of a plant in a method known as micropropagation.

Oryzalin is a selective preemergence surface-applied herbicide of the dinitroaniline class. It acts through the disruption (depolymerization) of microtubules, thus blocking anisotropic growth of plant cells(Rye, et al. 2002)It can also be used to induce ploidity as an alternative to colchicine (Taiz, 2010). The present paper reports the effect of oryzalin on plant growth of Anthurium andraeanum) cultured in vitro.

2. Materials and Methods

Shoot tip and lateral bud of anthurium (*Anthurium andraeanum)* about 0.5 cm were used as explants, surface sterilized by 20% chlorox(v/v) for 20 min, followed by 10% chlorox for 20 min and rinsed three times with sterilized water for 3 min each. These explants were cultured on solid MS medium(1962) supplemented with 0.1 mg/l NAA and 0.1mg/l kinetin and subcultured for four times every 3 weeks until got enough plantlets for doing other experiments. Plantlets with the same sized were soaked with different concentration (0, 5, 10, 15, and 20 mg/l) of oryzalin and different duration times (0, 24, 48 and 72 hours). After soaking, plantlets were transferred to MS medium supplemented with 0.1 mg/l NAA, 0.1 mg/l kinetin 3% sucrose and 2.5 % gelrite. Plantlets were cultured for 12 weeks and subcultured for four times every 3weeks. The parameters: diameter of bunch, plant height and number of leaves per bunch and different characters were recorded.

3. Results and Discussion

Plantlets soaked with different concentration of oryzalin and different duration times were cultured on MS medium supplemented with 0.1 mg/l NAA, 0.1mg/l kinetin and 3% sucrose for 12 weeks. The diameter of bunch was recorded every four weeks. After cultured for four weeks, the result in diameter of bunch was significant difference ($p \leq 0.05$) (Table 1). The result showed that diameter of bunch increased when treated for 72 hours with 5mg/l oryzalin as showed in Figure 1g and 1h. It showed that different concentration and times for soaking in oryzalin would effect on growth of cells and tissue of anthurium. This result was similar as Rye et al., (2002) did with *Ilex parpguariensis*. It showed that after 2–3 weeks culture in various treatments with oryzalin profuse differentiation of somatic embryos was observed. Additionally, this is the first study to show that oryzalin can be used to promote somatic embryogenesis in zygotic embryo cultures of *Ilex paraguariensis*. Plantlets which treated with 20 mg/l oryzalin for 24, 48 hours dried after culturing

for two weeks. The reduction diameter of bunch obtained caused by highest antimicrotubule agent concentrations used in our study was expected and must be due to the strong toxic effect of the herbicide oryzalin in plant cells by destroying spindle fibers and modifying the differentiation process (Eigsti & Dustin, 1955); (Bartels et al., 1973). When treated plantlets with long period and high concentration of oryzalin, the result showed that some plantlets dried. This may be due to the strong toxic effect of oryzalin (Rose, 2006).

Table 1: Growth rate in bunch diameter of anthurium after soaking in various times with different concentrations of oryzalin and cultured for 4,8 and 12 weeks.

Oryzalin Conc. (mg/l)	Soaking (hrs.))	Diameter bunch (cm)		
		4 weeks.*	8 weeks.	12 weeks.
0	0	0.30b* ± 0.28	0.45 ± 0.85	0.50 ± 0.50
5	24	0.09ab ± 0.29	0.39 ± 0.25	0.35 ± 0.18
	48	0.07a± 0.05	0.22 ± 0.22	0.30 ± 0.20
	72	0.15ab ± 0.27	0.25 ± 0.41	0.37 ± 0.17
10	24	0.08a ± 0.05	0.39 ±0.34	0.41 ± 0.46
	48	0.08a ± 0.04	0.13 ± 0.13	0.25 ± 0.22
	72	0.08a ± 0.04	0.18 ± 0.15	0.34 ± 0.22
15	24	0.07a ± 0.05	036 ± 0.46	0.22 ± 0.28
	48	0.03a ± 0.06	0.50 ± 0.30	0.43 ± 0.12
	72	0.06a ± 0.05	0.08 ± 0.13	0.12 ± 0.18
20	24	0.10ab ± 0	–	–
	48	0.02a ± 0.04	0.00 ± 0	0.20 ± 0.21
	72	–	–	–

*ab - compared mean in the same row was not significant difference with Turkey test at $p \leq 0.05$.

3.1 Plant height

When examined height of *A. andraeanum* after treating with various times and different concentration of oryzalin. It was found that growth rate in plant height were significant difference ($p \leq 0.05$). The result showed that after four weeks, plantlets which soaked for 72 hours with 5 mg/l oryzalin gave the highest height. Eight weeks, plantlets which treated for 48 hours with 5 mg/l oryzalin were the best result and 12 weeks, plantlets which treated for 24 hours with 5 mg/l oryzalin gave the highest plantlets. When treated plantlets with higher concentration of oryzalin (20 mg/l) for 24 and 72 hours plantlet died quickly. This result was the same as Kimberly et al. (2006) treated on *Euphorbia pulchurrima* with 115.5 µm for 2 days failed to produce callus and died quickly after exposure to oryzalin. For Lilium (van Tuyl et al., 1992) and Nerine (Tosca et al., 1995), shoot regeneration was less inhibited by oryzalin and the same as Tandon et al.,(1965) did

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with *Torenia fournieri* . In the experiment found that at 15 mg/l oryzalin at different duration times (24,48 and 72 hours) showed that callus were formed at the base of explants as in Figure 3b ,3c 3d and 3e.

Table 2: Growth rate in plant height of anthurium after soaking in various times with different concentrations of oryzalin and cultured for 4, 8 and 12 weeks.

Oryzalin conc. (mg/l)	Soaking (hrs.)	Plant height (cm)		
		4 weeks*	8 weeks*	12 weeks*
0	0	0.41b ± 0.44	0.72bc ± 0.79	1.19d ± 0.82
5	24	0.09a ± 0.11	0.48abc ± 0.31	0.94bcd ± 0.49
	48	0.15ab ± 0.11	0.57abc ± 0.35	0.59abcd ± 0.34
	72	0.18ab ± 0.18	0.39abc ± 0.38	0.56abcd ± 0.40
10	24	0.07a ± 0.11	0.17ab ± 0.21	0.17a ± 0.25
	48	0.02a ± 0.06	0.20ab ± 0.24	0.20a ± 0.21
	72	0.09a ± 0.17	0.19ab ± 0.28	0.21ab ± 0.25
15	24	0.05a ± 0.1	0.36ab ± 0.46	0.43abc ± 0.58
	48	0.10a ± 0.17	0.93c ± 0.25	1.13cd ± 0.32
	72	0.02a ± 0.07	0.18ab ± 0.29	0.22ab ± 0.32
20	24	0.10a ± 0.17	–	–
	48	0.02a ± 0.58	0.01a ± 0.03	0.04a ± 0.05
	72	–	–	–

*ab - compared mean in the same row was not significant difference with Turkey test at $p \leq 0.05$

3.2 Number of leaves

It was found that number of leaves from bunch of anthurium in every four weeks was significant difference ($p \leq 0.05$). It was found that number of leaves was depending on concentration and time for soaking with oryzalin. In the first recorded (4 weeks) the number of leaves was the highest when treated with 5 mg/l oryzalin for 72 hours. Then, the result showed that 5.0 mg/l oryzalin at 8, 12 weeks gave the best number of leaves (Table 3). After cultured for 12 weeks, the result showed that low concentration of oryzalin and short duration time gave the highest number of leaves as showed in Table 3. Plantlet which treated with 20 mg/l oryzalin and soaked for 24 and 72 hours could not survival after culturing for 8 and 12 weeks. Oryzalin is known to be less toxic to plant tissues (Vainola.,2000).This result was the same as Lilium (van Tuyl et al., 1992), Nerine (Tosca et al.,(1995) and *Euphorbia pulcherrima* (Pickens et al.,2006). However, in winter rose, oryzalin was found to be unsuitable for inducing growth, the same as Eeckhaut *et al*., (2004) had been observed for Rhododendron species which soaked with oryzalin at higher concentrations regardless of duration of treatment.

Figure 1: *Anthurium andraeanum* cultured for 12 weeks after treating with 5 mg/l oryzalin and difference duration time : 1a,1b - 0 hours, 1c,1d - 24 hours, 1e,1f – 48 hours, 1g,1h – 72 hours.

Anthurium treated with different concentration of oryzalin and vary duration time were transferred to the same MS medium. After cultured for 12 weeks all parameters in diameter of bunch, plant height and number of leaves per bunch were collected and their picture were showed as in Figures 1, 2 and 3.

Plantlets treated with 15 mg/l for 48 and 72 hours gave abnormal characteristics such as slow

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growth rate, leaf with round shape, light green leaves , thick leaves and short leaf petriole (as showed in Figure 2c, 2d, 2e, 2f, and 2g, 2h. This worked was the same as Mandon *et al*. (2005) did with oil plam, and Jala *et al*. (2012) did with *Amethyst Curcuma*.

Figure 2: *Anthurium andraeanum* cultured for 12 weeks after treating with 10 mg/l oryzalin and difference duration time : 2a,2b - 0 hours, 2c 2d - 24 hours, 2e,2f – 48 hours, 2g,2h – 72 hours.

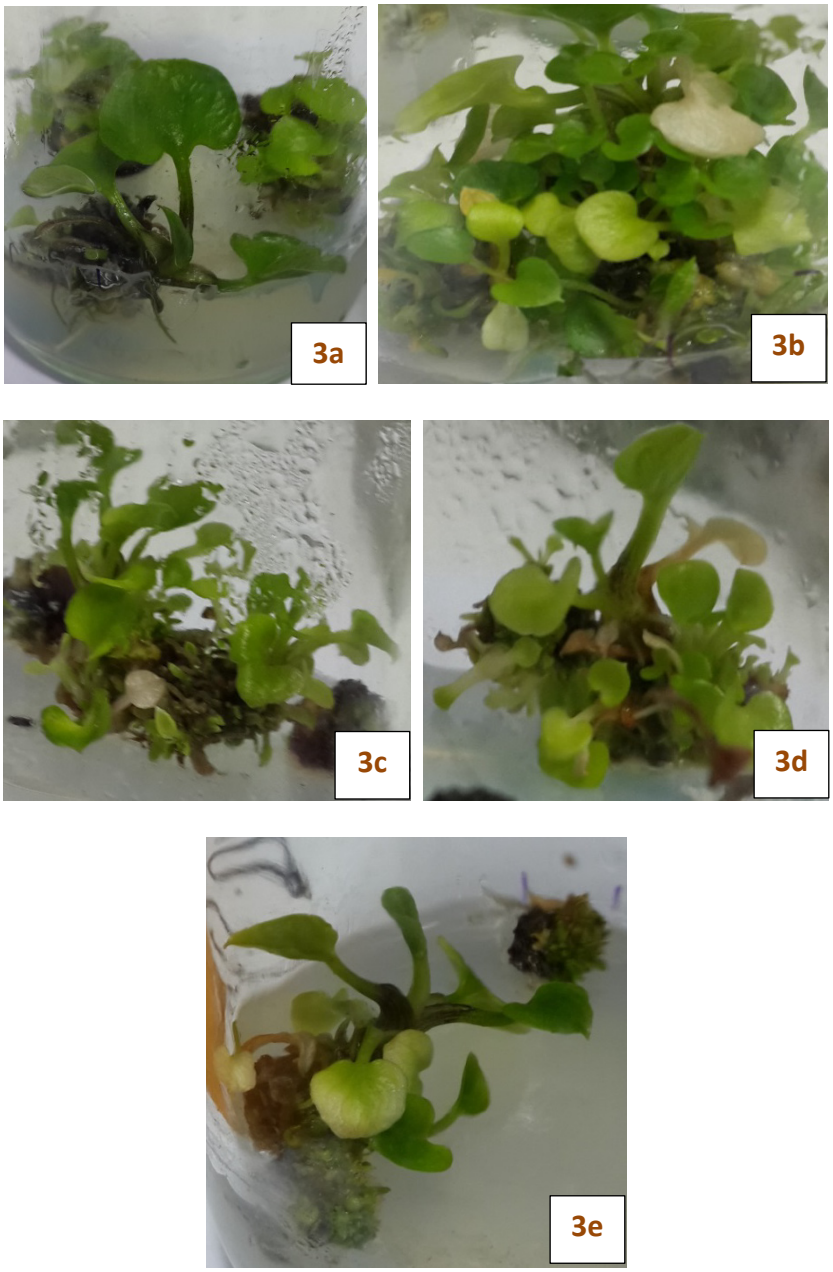

Figure 3: *Anthurium andraeanum* cultured for 12 weeks after treating with 15 mg/l oryzalin and difference duration time : 3a - 0 hours, 3b - 24 hours, 3c, 3d – 48 hours, 3e – 72 hours.

4. Conclusion

Shoot tip and adventitious bud of *Anthurium andraeanum* about 0.5 cm were used as explants. Explants cultured on MS medium supplemented with 0.1 NAA and 0.1 mg/l kinetin, 3% sucrose gave the best result in increasing number of plantlets. Plantlets treated with different concentration

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Table 3: Number of leaves in anthurium after soaking with oryzalin at different concentrations and duration times and cultured for 4, 8 and 12 weeks.

oryzalin Conc. (mg/l)	Soaking (hrs.)	Number of leaves (leaves)		
		4 weeks.*	8 weeks*	12 weeks*
0	0	8.25a ± 11.55	9.83ab ± 10.94	15.17ab ± 12.50
5	24	2.58ab ± 2.78	5.33ab ± 4.94	14.50ab ± 5.98
	48	3.00ab ± 3.44	7.22ab ± 5.61	11.56b ± 6.15
	72	4.08ab ± 4.66	8.25a ± 7.44	16.22a ± 8.97
10	24	1.33ab ± 2.50	3.67b ± 2.86	5.75b ± 8.98
	48	0.25a ± 0.87	2.00b ± 2.86	4.82b ± 4.83
	72	0.75a ±1.42	1.83b ± 2.79	4.92b ± 6.43
15	24	1.17ab ± 2.12	4.89b ± 6.25	6.22b ± 8.26
	48	2.67ab ± 4.62	6.33ab ± 3.79	9.00b ± 4.36
	72	0.22b ± 0.07	1.22b ± 1.99	3.67b ± 4.53
20	24	1.33b ± 2.31	–	–
	48	1.17b ± 0.58	0.11b ± 0.33	1.2.0b ± 2.17
	72	–	–	–

*ab - compared mean in the same row was not significant difference with Turkey test at $p \leq 0.05$.

of oryzalin with varied duration time. It was found that 5 mg/l oryzalin with 24 hours in 4 weeks gave the best average diameter of bunch but next times (8 weeks and 12 weeks) the result in diameter of bunch is not significant difference. Plantlets treated with 5 mg/l oryzalin for 24 hours gave the best result on plant height, treated with 5 mg/l oryzalin for 24 and 72 hours gave the best number of leaves per bunch. When treated with 20 mg/l oryzalin for 24 and 72 hours plantlet died within three weeks. Abnormal plantlets were found in plantlets which treated with 15mg/l and higher oryzalin and duration longer than 48 hours, slow growth rate, thick leaves and short leaf petriole.

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