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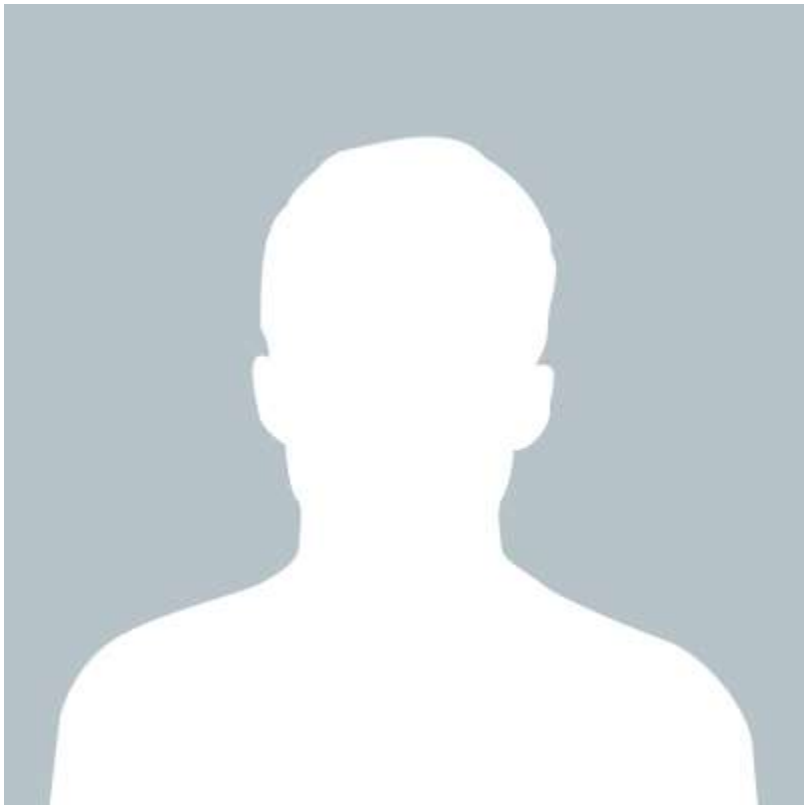

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Oryzalin Treatment ModifiedPlant Morphologyof *Impatiens balsamina* L

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ABSTRACT

Impatiens balsamina L. is well known as garden balsam that flowers are very usefull. The flowers can be arranged in coconut leaves for praying or as a decoration in pots. Garden balsam plants are tall. The flowers are easy to decay especially in rainy season. Vigorous plants with bigger flower for potted plant can be produced by using oryzalin throughseedlings treatment of garden balsam. Seedlings were treated in oryzalin at concentrations of 0, 0.01, 0.02 and 0.03% for 0, 12, 24, 36, and 48 hours, then grown in the field. As a result, for M1 generation, interaction between oryzalin concentration and incubation time was significant for plant height, number of branch and flower weight. Plants height decreased about 54% for treatmentof 0.01% oryzalin in 48 h incubation. Oryzalin application also increased the weight of flower. In the next generation (M3), mixoploid plants were obtained from treatment 0.02% oryzalin for 12 h incubation. Based on this study, oryzalin can be used for producing compact potted plants.

Key words: *Impatiens balsamina*, Oryzalin, Potted plant, Mixoploid.

INTRODUCTION

*Impatiens balsamina*L. (garden balsam) is a flowering plant that belonged to Balsaminaceae family.The plant has many flower colour like red, purple, pink and white. The flowers can be used for gardening and potted plants, nail polished and natural colouring agent[1], antibiotic activity against pathogenic bacteria and fungi[2], offerings in Balinese ceremony[3]. Seed extract of garden balsam contained antimicrobial activity against the growth of *E.coli* and *Bacillus anthrasis* and antifungal against *Aspergillus niger*and *Fusarium* sp.[4]Seeds also used for expectorant and treatment for cancer[5]. This plantis relatively tall with plant height[6] can reach up to 65 cm or higher that depend on type and fertility of soil. In the field, tall plants are susceptible to be lodged by strong wind and heavy rain[3].

Previously, colchicine is widely applied for plant modification such as *Rhododendron* to produce compact plants[7],*Impatiens balsamina* L. to obtain tetraploid plants[8], *Phlox subulata*[9], ornamental ginger (*Hedychium muluense*)[10], basil (*Ocimum basilicum* L.)[11] and yellow passion fruit[12], *Portulaca grandiflora*[13] to increase flower size. In fact, colchicine in a certain concentration had negative effect to the plant cell[14],environment[15] and people who are exposed to this chemical[16]. In contrast, oryzalin is easier to be degradabled by light and had similar effect to colchicineas antimitotic agent in order to inhibit mitosis[12].

Oryzalin is a herbicide that can be used for inhibit root growth[17]. As herbicide, oryzalin was applied preemergence for control seedlings of grasses and annual broadleaves plants[17]. As

mutagenic agent, oryzalin was used in some plants such as rose[18] and *Mecardonia tenella* a native plant from South America[19]to produce shorter plant with bigger flower, *Rhododendron* to obtain thicker leaf and vigorous plant[7], *Hibiscus acetosella*to have compact plant form[20], banana cultivar to increase microshoot production[21]. Oryzalin can replace colchicine application as antimitotic agent, that bind tubulin dimmer along division of cell and alter microtubules formation and spindle fibers[22]. Based on those previous study, oryzalin was applied to treat seedlings of *Impatiens balsamina* to modify plant form.

Preliminary study showed that the growth of radicles of garden balsam seedlings was altered when treated with oryzalin because roots elongation was very poor[3]. After 7 days of germination, control and treatment with 0.01% oryzalin showed the growth of roots and hypocotyl. In contrast, treatment with oryzalin higher than 0.01 % only showed the growth of hypocotyl, while the roots was very slow and stunted.

Effects of oryzalinin the field varied between plant species and oryzalin concentrations. In order to have shorter and compact plants, garden balsam seedlings were treated with oryzalin as a mutagenic agent with various time of soaking.Vigorous plant has advantage as border plant in a landscape or potted plants. In addition, lowered plants can enhance plant survival during heavy rain.

MATERIALS AND METHODS

Oryzalin treatment

Seeds of garden balsam with red flower were pretreated by soaking in distilled water for 12 hand germinated on filter paper in Petri Dishes. Germinated seeds (4 days old) were treated with oryzalin in different concentrations (0, 0.01, 0.02, 0.03)% for (0,12, 24, 36 and 48) h. Treated seedlings were rinsed in water and planted in polybags and grown in the field.The randomized block design was applied to allocate the treatment. There are 20 of combination treatments and each treatment was repeated three times. Thus, there are 60 experimental units and each of experimental unit consisting of 5 plants. The plants were maintained by watering every day and applying fertilizer (NPK 15:15:15) at 4 weeks and 8 weeks intervals after planted.

Plant Morphology

Morphology measurement includes plant height (from the stem base to the shoot tip), number of branch, weight of flower.

Flowcytometry analysis (FCM)

FCM analysis was sampled from M3 generation. Each treated plant that showed altered growth with bigger flower size was analysed to check its ploidy level. Sample of leaf tissue (0.5 cm^2) was put in 55 mm plastic Petri Dishes (Partec code 04-2005) and was added with 500 µL extraction buffer (Partec Kit). Material was chooped using a sharp razor blade for 30 to 60 seconds, then incubated for 90 seconds before filtered through a Partec 50 µm Cell Trics disposable filter (code 04-0042-2317). Staining solution (PI) and RNAse of 2 mL each were added to the test tube, then incubated and protected from light at least 30 min. Sample was then analysed with flow cytometer in the red channel (Partec GmbH Flow Cytometry, Germany).

Statistical analysis

All data were subjected to the analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at 5% level(CoStat Co.).

RESULTS AND DISCUSSION

Plant height

Twelve weeks after planted, plant height was measured to know the effect of oryzalin and time of incubation for plant performance. M1 generation showed significance of different for interaction of the treatment between oryzalin concentration and time of incubation on plant height (Table 1). Concentration of oryzalin 0.01% for 48 hand oryzalin 0.03% for 48 h reduced plant height significantly by 53.6 % and 53.9 %, respectively when compared to control.

Plant height of *Hibiscus acetosella* treated with colchicine and oryzalin were reduced and internode were shorter in octoploid plants when compared with diploid[20]. Reduction of plant size

had reported for some polyploid plant. In arrow root (*Maranta arundinacea*) plant, oryzalin treatment at high concentration (e"30 µM) inhibited plant growth. In contrast, at concentration of 10 µM enhanced growth of plant[23]. *Mecardonia tenella* treated with colchicine 0.01% for 48 h incubation produced tetraploid plant in the field and showed shorter and more compact plant than diploid[19].

Number of branch

Plant response to oryzalin treatment was varied in number of branch. The plants treated with 0.01% oryzalin for 48 h incubation did not produce any branch, eventhough higher concentration of oryzalin 0.02% for 48 h incubation produced 6 branches (Table 1), and significantly different to control.

Oryzalin treatment altered the growth of vegetative plants. Treated plant with 0.01 % for 48 h showed the shortest plant without any branch, the lowest weight and diameters of flower. In addition, in M1 generation, the treatment did not produce polyploid plant, but plant morphology showed a dwarf plant that suitable for potted plant or border plant in landscape garden. Inhibition of vegetative growth due to limited root growth after treated with oryzalin. Radicle was very slow to develop longer roots and even fail to produce root hairs. Seedlings stage is very critical. Limitation on root growth can alter further growth of shoots because the absorption of nutrients were very low.

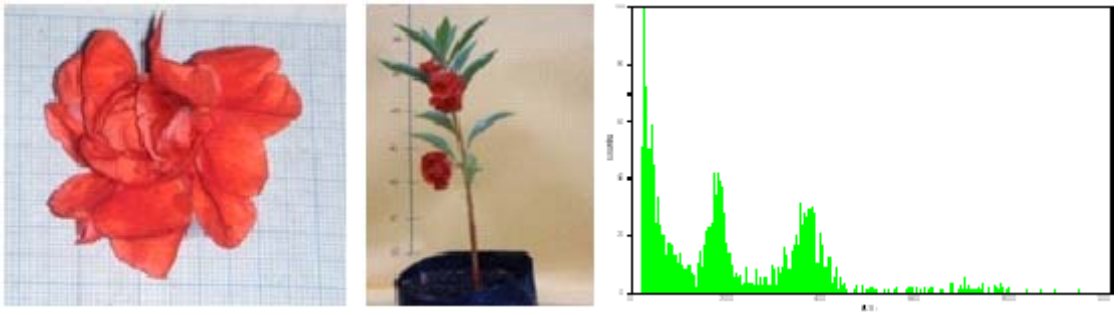
Flower weight

Weight of flower was not statistically different between all treatments including control in M1 generation (Table 1). Oryzalin 0.02% for 48 h incubation and 0.03% for 36 h incubation tended to produced more weight of flower (32.9 % and 48.8%, respectively). The flower weight did not

Table 1:Oryzalin concentration and duration of treatment modified plant morphology of garden balsam at 12 weeks after planted

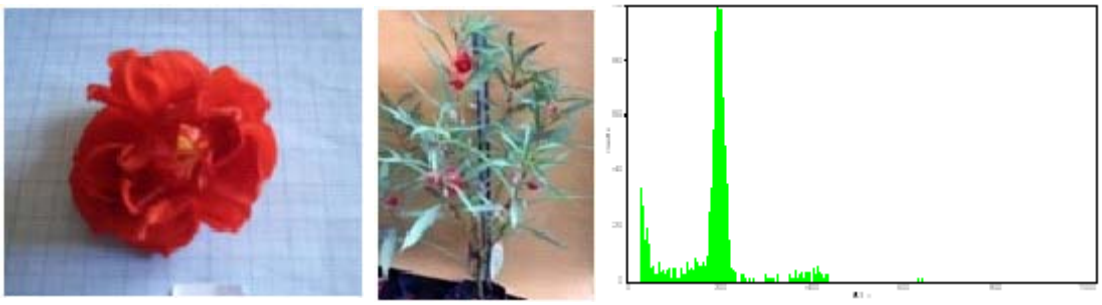
Oryzalin concentration (%)	Duration of soaking (hours)	Plant heigh t (cm)	Number of of branch	Flower weight (g)
0 (Control)	0	51.66^{bcdef}	3.17^{bc}	0.79 ^{bcd}
	12	67.89^{a}	2.22 ^{bcd}	0.99 ^{abc}
	24	47.22 ^{def}	2.5 ^{bcd}	0.63 ^{cd}
	36	48.22 ^{cdef}	2.67 ^{bcd}	0.83 ^{bcd}
	48	66.56 ^{ab}	2.44 ^{bcd}	0.82 ^{bcd}
0.01	0	48.44 ^{cdef}	2 ^{cd}	0.86 ^{bcd}
	12	57.33 ^{abcde}	2.33 ^{bcd}	0.73 ^{cd}
	24	38.67 ^{fg}	4.25 ^{ab}	0.83 ^{bcd}
	36	36.06 ^{fgh}	1.25 ^{cde}	0.74 ^{bcd}
	48	22.5 ^{h}	0 ^{e}	0.55 ^{d}
0.02	0	60.67 ^{abcd}	2.89 ^{bc}	0.82 ^{bcd}
	12	43.39 ^{efg}	2.55 ^{bcd}	0.86 ^{bcd}
	24	37.75 ^{fgh}	0.67 ^{de}	0.65 ^{cd}
	36	64 ^{abc}	4.25 ^{ab}	0.79 ^{bcd}
	48	36.44 ^{fgh}	6 ^{a}	1.09 ^{ab}
0.03	0	66.56 ^{ab}	2.55 ^{bcd}	0.86 ^{bcd}
	12	46.22 ^{defg}	2.39 ^{bcd}	0.79 ^{bcd}
	24	49.78 ^{cdef}	2.84 ^{bcd}	0.90 ^{bcd}
	36	37.84 ^{fgh}	2.33 ^{bcd}	1.28 ^{a}
	48	30.67 ^{gh}	2.67 ^{bcd}	0.66 ^{cd}

Note: Numbers in the same group followed by same letter in a column are not significantly different at the 5% level of DMRT



(a) Flower (b) Plant 12 weeks (c) FCM histogram

Fig. 1: Mixoploid (2x + 4x) plant



(a) Flower (b) Plant 12 weeks (c) FCM histogram

Fig. 2: Diploid (2x) plant

influenced by flower diameter. Increased in flower weight did not followed by enhanced of flower diameter (data not shown). Visually, flower with higher in weight obtained more petals even though in small size of petals. In *Rose*, tetraploid plants showed double the number of petals flower[18]. Tetraploid plants due to colchicine treatment of *Portulaca grandiflora* had a large number of petals than diploid plants[13] (Mishiba and Mii, 2000).*Mecardonia tenella* treated with colchicine 0.01% for 48 h then cultured in vitro,obtained bigger flowers compared to control plants[19]. In the field, compact shaped were shown by selected tetraploid plants of *M. Tenella*.

Ploidy analysis by FCM

Ploidy level of garden balsam was analysed by flowcytometry for further generation (M3). Concentration oryzalin 0.02% for 12 and 24 h incubation and oryzalin 0.03 % for 12 h, 36 h, 48 h showed mixoploid plants (2x+4x) (Fig. 1). Based on FCM analysis, oryzalin treatment to seedlings of garden balsam was unsuccesfull in inducing tetraploid plants on M3 generation, however mixoploid plants were obtained in the present study. Mixoploid plant (2x+4x) had shorter plant morphology and higher number of petal flower than diploid plants (Fig. 2).

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