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In Vitro Multiple Regeneration from Cotyledons and Hypocotyls of *Impatiens*

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ABSRACT

In vitro shoot regeneration from cotyledon and hypocotyl explants derived from germinating mature *Impatiens* seeds. The induction of organogenetic tissue was also influenced by the cotyledon and hypocotyl. Multiple shoot induction was higher in hypocotyl than in the cotyledon explant with Thidiazuron and a NAA medium.

Key words : *Impatiens*, shoot regeneration, Thidiazuron, hypocotyl, cotyledon

INTRODUCTION

The *Impatiens*, which grows in shady areas and is native to Tanzania and other equatorial countries, has been enjoyed in the western world since 1896(*Impatiens*, 1995). In 1972, the first hybrids (New Guinea *Impatiens*) burst onto the horticulture scene with their splashy foliage, festive color and blossoms of gargantuan size. New Guinea and Java *Impatiens* were introduced into the United States in 1970(USDA, 1972; Winters, 1973). Since then, cultivator' s interest of these species has steadily increased. While the seeds are ripening in the capsule, the cells at the tip of the capsule become swelled with sugar that the slightest touch triggers the dispersal process. The *Impatiens* is named so because of the nature of their seed pods, which burst open to scatter their seeds. A common name for the *impatiens* is the touch-me-not because of this seed dispersal method. Since the *Impatiens* is a large genus of widely distributed spurred, saccate flowers and dehiscent capsules, with its previously noted method of seed dispersal, gives an indication of the difficulties that this research encountered. The difficulty of collecting mature seeds made it necessary to use in vitro propagation methods to collect the needed specimens.

Today' s hybrids are available in more than a dozen brilliant, clear colors, and even a few with bold white stars for a striking contrasts(*Impatiens*, 1995).

There are three general height classifications that include tiny elfin plants, suitable for woodland planting, a medium-tall variety that reach about 12 inches in height, which are excellent for mass plantings and baskets, and a tall group which can grow up to 2 feet in length(1995). It has become the most popular bedding plant because of it' s ability to grow robustly in shaded or semi-shaded areas.

Another series is the New guinea *Impatiens*, which grow in areas that are exposed to strong sunlight.

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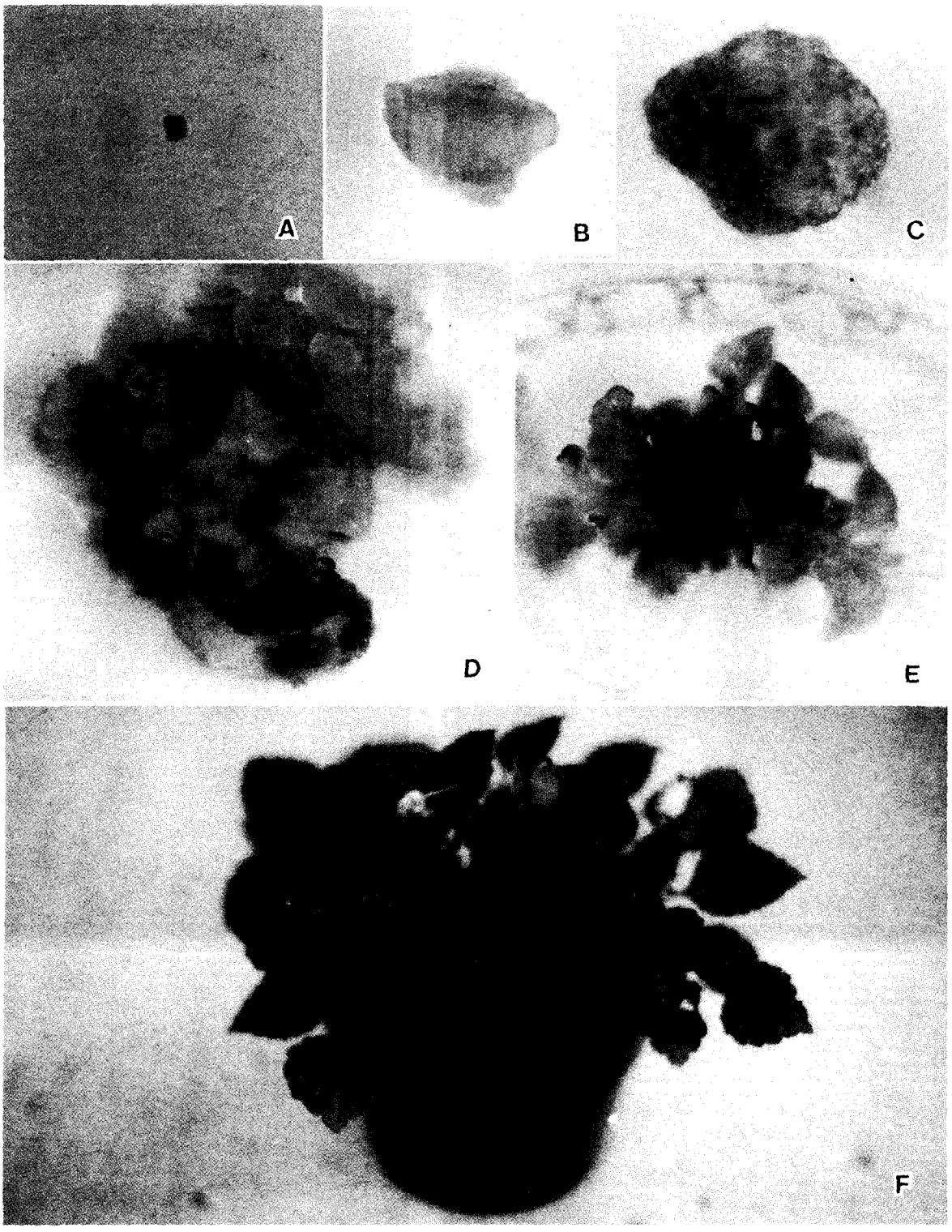

Fig. 1. A: Cotyledon explants of *Impatiens*(Impulse Bright Red) B,C: Callus of cotyledon(18 days). D: callus and shoot initiated (30 days) E: Explant of shoot organogenesis after 45 days of culture medium with 0.05mg/ *l* NAA and 0.5mg/ *l* BA. F: Developed *Impatiens*(Impulse Bright Red)(3 months).

This species has much more of a striking foliage than the *impatiens* that grow in the shade have. Because of this showy nature, the New Guinea *Impatiens* work well as specimen plants rather than incorporated with the annual bedding plants.

Several conventional breeding methods have been developed to improve *Impatiens* species(Arisumi, 1974, 1978, 1980, 1987 ; Pasutti and Weigle, 1980), but there is also growing interest among breeders in improving *Impatiens* by means other than conventional breeding techniques. More specifically, these breeders have become interested in somatic variation, in vitro cell selection, gene transformation, and other efficient regeneration systems(Han, 1987, 1994) requiring organogenesis and somatic embryogenesis.

Although the *Impatiens* have robust growth in shaded and partly shaded areas, their use is severely limited because of the *Impatiens* lack of resistance to direct sunlight when cultivated open areas. Because of this inability of the *Impatiens*, it is necessary to develop the *Impatiens* resistance to direct sunlight through gene transformation.

This research focused on the establishment of an efficient shoot and root regeneration system of the *impatiens* that both maximize the consistency and efficiency of shoot regeneration, as well as the number of plants obtained in cotyledon and hypocotyl explants.

MATERIAL AND METHOD

The species and genotype chosen for this research was the impulse bright red because it has more ovules per fruit and is very popular in ornamental flowers. The seeds were surface disinfected by immersing them into 70% ethanol for approximately 30 seconds. This was followed by immersing the seeds into 0.5% NaOCL(10%vol/vol commercial laundry bleach) for 15 minutes. After this was complete, the seeds were rinsed four times in sterile deionized water. All stock plants were grown in 1/2 MS medium.

The cotyledon and hypocotyl began to cut within 3 weeks of the germination process. Healthy green and cotyledons with thick petioles were selected and the cotyledon base attached to the petioles severed(Fig.1A). The base was removed to eliminate the possible contamination from meristematic and petioles tissues attached or adjacent to the cotyledons.

The basal medium was composed of inorganic MS

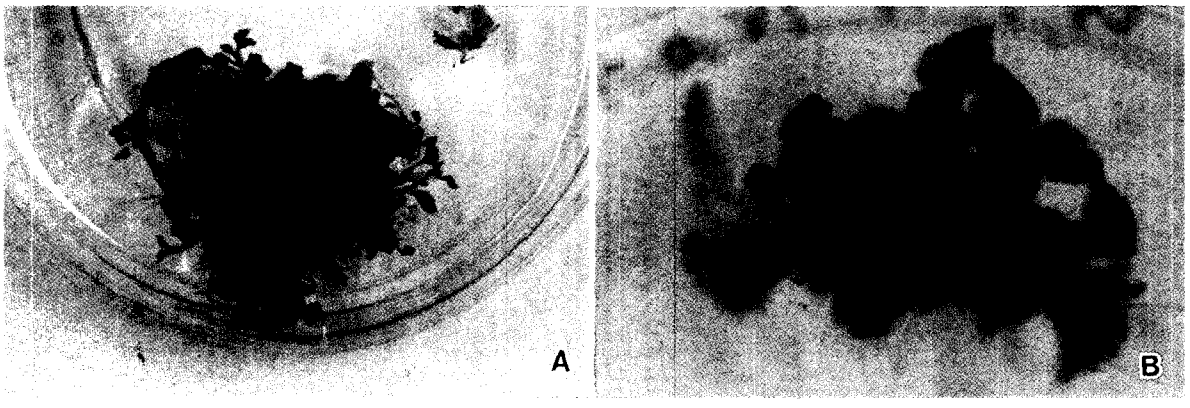

Fig. 2 A: Hypocotyl explants of *Impatiens*(Impulse Bright Red) after 40 days of culture medium with 0.05 mg/ *l* NAA and 0.5 mg/ *l* Thidiazuron(TDZ).
B: Cotyledon explants of *Impatiens*(Impulse Bright Red) after 40 days of culture medium with 0.05 mg/ *l* NAA and 0.5 mg/ *l* Thidiazuron(TDZ)..

medium adding 7.5g sugar content. Four BA concentrations (0, 1.5, 2.5, 3.5 mg/ *l*) combined with 4 NAA concentration (0, 0.125, 0.25, 0.5 mg/ *l*) were added to determine the best combination for the induction of organogenesis. Another experiment used 4 NAA concentrations(0.05, 0.25, 0.5 mg/ *l*) that were combined with Thidiazuron (0.25, 0.5, 1.0 mg/ *l*). The pH was adjusted to 5.8 before 8g L^{-1} Difco Bactor-agar was added. The medium was autoclaved for 10 minutes at 1.2kg cm^{-2} and 121° C to dissolve the agar. 25ml of medium was dipensed into disposable petri dishes. The plated explant was incubated in the growth room at 27° C under 24 h illumination with cool white fluorescent lamps.

All experiments were set up in completely random design. Each treatment was composed of 5 replication (petri dishes). Each petri dish contained 5 cotyledons and 5 hypocotyls explants. The data was statistically analyzed by using the Statistical Analysis System(SAS Institute, Inc., 1996).

RESULTS AND DISCUSSION

The explants of the cotyledon and hypocotyl were enhanced by BA concentrations from 1.5 mg/ *l* to 3.5 mg/ *l* BA combination with NAA concentrations from 0.125 mg/ *l* to 0.5 mg/ *l* NAA.(Table 1). The greatest number of roots per explant was obtained with 1.5 mg/ *l* BA concentrations. Shoot multiplication was enhanced for the explants of cotyledon with BA concentrations from 1.5 to 2.5 mg/ *l* , with NAA concentrtions from 0.125 to 0.5 mg/ *l* . Shoot production decreased steadily with an increase in NAA concentration. The greatest number of shoots per explant was obtained with 2.5 mg/ *l* BA and 0.125 mg/ *l* NAA. Compared

Table 1. The BA effect combined with NAA in MS medium containing 7.5g/ *l* liter^{-1} sucrose on the percentage of shoot and root induction of cotyledon and hypocotyl *Impatiens* (Impulse Bright Red).

Plant Growth Regulator		Explant (mean±sd)			
BA(mg/ *l*)	NAA(mg/ *l*)	Cotyledon		Hypocotyl	
		% of shoot	% of root	% of shoot	% of root
0	0	0	36.7^{ij*}±4.32	0	50.0^{g}±1.00
0	0.125	0	46.7^{hi}±2.70	0	100.0^{a}±0.00
0	0.25	0	50.0^{h}±1.00	3.3^{e}±0.29	100.0^{a}±0.00
0	0.5	0	76.7^{f}±3.42	0	93.3^{b}±1.78
1.5	0	0	3.3^{l}±0.49	10.0^{a}±0.76	26.7^{h}±3.42
1.5	0.125	10.0^{b}±1.16	100.0^{a}±0.00	10.0^{a}±0.76	86.7^{c}±2.43
1.5	0.25	3.3^{d}±0.57	100.0^{a}±0.00	0	96.7^{ab}±1.96
1.5	0.5	6.7^{c}±1.52	100.0^{a}±0.00	6.7^{b}±0.46	83.3^{cd}±4.32
2.5	0	0	6.7^{k}±1.83	0	3.3^{j}±0.52
2.5	0.125	14.3^{a}±2.24	90.0^{c}±4.47	3.3^{e}±0.29	96.7^{ab}±4.82
2.5	0.25	3.3^{d}±0.70	96.7^{ab}±3.20	0	96.7^{ab}±6.72
2.5	0.5	3.3^{d}±0.48	86.7^{cd}±0.97	3.3^{e}±1.20	96.7^{ab}±5.25
3.5	0	0	6.7^{k}±0.35	0	6.7^{i}±3.24
3.5	0.125	3.3^{d}±0.48	83.3^{de}±1.23	3.3^{e}±0.48	73.3^{ef}±2.78
3.5	0.25	3.3^{d}±1.25	73.3^{fg}±2.12	-	76.7^{e}±2.24
3.5	0.5	2.3^{de}±0.36	86.7^{cd}±3.23	-	83.3^{cd}±4.51

* : Duncan' s Multiple Range Test at 5% level.

** : Shoot and root were investigated 40 days after planting.

Table 2. The effect of various NAA and Thidiazuron combinations on multiple shoot formation in *Impatiens*.

NAA (mg/ *l*)	TDZ (mg/ *l*)	Percentage of multiple shoots (mean ± sd)	
		cotyledon	hypocotyl
control	control	0	0
0.05	0.25	$26.67^{b*} \pm 1.53$	$26.67^{b} \pm 1.00$
0.05	0.5	$35.53^{a} \pm 1.08$	$86.67^{a} \pm 3.00$
0.05	1.0	$13.33^{e} \pm 1.00$	$5.67^{d} \pm 1.53$
0.25	0.25	$22.20^{c} \pm 1.53$	$2.33^{efg} \pm 2.08$
0.25	0.5	$22.02^{cd} \pm 1.53$	$3.67^{def} \pm 1.53$
0.25	1.0	0	$12.67^{c} \pm 3.06$
0.5	0.25	$26.67^{b} \pm 2.65$	$4.33^{de} \pm 1.53$
0.5	0.5	$8.86^{f} \pm 0.58$	0
0.5	1.0	0	0

* : Duncan' s Multiple Range Test at 5% level.

** : Shoot numbers were counted after 40 days after planting.

with other treatments, the greatest shoot multiplication was obtained with a concentration of 0.125 mg/ *l* NAA. The combination of BA 1.5 mg/ *l* concentration and 0.125 mg/ *l* NAA stimulated the greatest number of shoots. More shoots were obtained from explants of hypocotyl at 0.125 mg/ *l* of NAA than higher concentration of BA.

Shoot multiplication was stimulated by a Thidiazuron concentration from 0.25 mg/ *l* to 0.5 mg/ *l* . Shoot elongation was enhanced with an NAA concentration from 0.05 mg/ *l* to 0.5 mg/ *l* concentration. Higher Thidiazuron concentration (1.0 mg/ *l*) inhibited shoot production. The greatest shoot multiplication of hypocotyl(35.5%) was obtained through a 0.5 mg/ *l* Thidiazuron concentration with an 0.05 mg/ *l* NAA concentration. Shoot production was 3 times of greater than were using a 1.5mg/ *l* BA and 0.125 mg/ *l* NAA treatment. The hypocotyl was to create more induced multiple shoots than the cotyledon at 0.05 mg/ *l* NAA with 0.5 mg/ *l* Thidiazuron. The greatest number of shoots from hypocotyl was obtained by using a combination of the 0.05 mg/ *l* NAA and 0.5 mg/ *l* Thidiazuron. Multiple shoot formation decreased with increasing NAA concentration. As the NAA concentration increased, the number of shoots per explant decreased. Higer concentrations of NAA inhibited multiple shooting from the explant of cotyledon and hypocotyl.

Thidiazuron was more effective in stimulating multiple shoot formation than BA. The finding that kinetin is ineffective in stimulating multiple shoot formation supports previous research(Stephens et al., 1985), showing that concentrations greater than 80 uM were required for the greatest shoot multiplication. One possible explanation for the lack of multiple shoot formation in kinetin-containing medium is that the uptake of kinetin by *Impatiens* explants is less efficient than the uptake of BA(Han, 1987). Another possibility is the cytokinin oxidase operating in a given species(Norton and Norton, 1985).

Since we wanted to compare with Thidiazuron and BA for multiple shoot formation, the optimum shoot-producing concentration is less than 10μM of BA(Han, 1987). This experiment indicates that the optimum shoot production is with 1.5 mg/ *l* of BA. It was same result for the BA effect on the shoot although that was not same unit. NAA was not a necessary medium component for shoot multiplication, which confirm

previous results(Stephens et al., 1985). This experiment also found that NAA stimulated shoot elongation at it lowest concentration. Thidiazuron should be used for woody plant multiple shooting. Thidiazuron induced more 2 or 3 times, more shoots than the BA medium. Hypocotyl induced more multiple shoots than cotyledons with Thidiazuron and an NAA medium

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