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Biotechnological Approach to Increase the Metal Stress Tolerance Capacity of *Impatients balsamina* L.

Dhruv Pandya*, Archana Mankad and Himanshu Pandya

Department of Botany, Bio-informatics, Climate Change Impacts Management,
University School of Science, Gujarat University, Ahmedabad - 380 009, Gujarat, India

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A B S T R A C T

In this research heavy metal stress tolerance capacity of *Impatiens balsamina* L. was assessed. Here, two different approaches *in-vitro* and *in-vivo* were used for the production of plantlets. *In-vitro* approach involved tissue culture approach and *in-vivo* direct through media (soil, cocopeat, mosses). Seeds were used for the production of plantlets. After 30 days of seedlings development all the plantlets which are produced through *in-vitro* and *in-vivo* approaches and plants were transplanted in the pots and treated with two metals Lead and Cadmium in the form of Pb $(NO_3)_2$ and Cd $(NO_3)_2$. Different concentrations were selected for Lead 200mg, 400mg, 600mg, 800mg/Kg and for Cadmium 5mg, 10mg, 15mg, and 20mg/Kg. Each pot was filled with 5Kg of soil. The metals were given directly through root zone of plants in solution form. After incubation time of 75 days mature and treated plants were collected and root length, shoot length, number of branches was measured scientifically. On the basis of the results obtained of physiological parameters of the plants we concluded that for both the metals *in-vitro* produced plants has more capacity to tolerate the metal stress as compare to *in-vivo* produced plants.

Key words: *In-vitro* approach, *In-vivo* approach, Lead, Cadmium, *Impatiens balsamina* L., Physiological parameters

Impatiens balsamina L. belongs to Balsaminaceae family. It is also known as Garden Balsam or Rose Balsam. It is also cultivated in China and Myanmar as an ornamental herb. This is annual ornamental herb and cultivated as seasonal ornamental in India. The plant is generally growing up to 20-75cm length with succulent type of branched stem. The leaves are spirally arranged 2.5-9.0 cm long and 1-2.5cm broad and it has toothed margin. The flowers are pink, red, lilac or white and 2.5-5.0cm diameter (Waoo *et al*. 2015). Generally, it is bees or insect pollinated plant. It has short lifecycle, large number of flowers and various colors of flowers (Amin *et al*. 2013).

The plant is not directly used as fodder plant by animal or directly used by humans (Otaru *et al*. 2013). So many researchers worked on this plant and it also has different pharmacological activities like Anti-cancer, Anti-ulcer, Antioxidant, Anti-malarial, Antibacterial, Anti-fungal etc. it also has the weedicide potential. Even one researcher also assessed the naphthalene tolerance and remediate capacity of *Impatiens balsamina* L.

MATERIALS AND METHODS

The seeds were selected for the propagation of plants for both the *in-vitro* and *in-vivo* approaches. The experimental work was completed at Plant Biotechnology Laboratory and Botanical Garden, Department of Botany, Gujarat University, Ahmedabad - 380 009, Gujarat.

In-vitro production of plantlets:

Sources of Explant: Seeds were purchased of Fine Grow Company from Alpesh Nursery, Gandhinagar. So, seeds were used as an explant for the production of plantlets. All the seeds were sterilized with the help of 0.1% $HgCl_2$ solution and 70% methanol and rewashed with Grade-1 Distil water (Fig C).

***Corresponding author:** Dhruv Pandya (Ph. D. Scholar and Teaching Assistant), Department of Botany, University School of Science, Gujarat University, Ahmedabad - 380 009, Gujarat

e-mail: dhruvpandya1309@gmail.com

Aseptic conditions for production: Culture room and the laboratory or transfer room were sterilized through fumigation technique (Potassium iodide and formaldehyde were used for it with 2:4 ratio). All the glassware and miscellaneous agents were washed with soap solution and rapped with papers and then sterilized through Autoclave (121°C for 20 min). Laminar Air flow hood, weighing scale and all the other small equipment like micropipette were sterilized with 0.1% mercuric chloride solution and 70% methanol.

Preparation of M. S. Media for the production of plantlets

Here for the practical work most widely used media Murashige and Skoog's media (1962) was used. For the preparation first all the major, minor, iron and vitamin stalk solutions were prepared as per the (Table 1). PGRs were not used because in seeds generally we use to avoid PGRs in *in-vitro* condition and production of plantlets. Here all the chemicals used for the preparation of stalk solution were Hi Media and SRL company.

Different stalk solutions were prepared in the amount of 500ml (Major, Minor and Iorn) and 100ml (Vitamin) and then for the preparation of 1 liter M. S. Media 50ml from Major, 50ml from Minor, 50ml from Iron and 10ml from Vitamin stalk were taken and sequentially dissolved and other chemicals which were separately weighed like Myo Inositol, Agar-Agar, Glycine and Sucrose were added for the preparation of media. (Here Grade-1 Purified water was used for the preparation of media with the help of Genie Direct Pure (Rephile) Instrument was used for the preparation of Purified water). After the preparation of media, it was sterilized with the help of autoclave at 121°C temperature for 20 min. under the Laminar Air Flow Hood in all the sterilized culture flasks and Glass jars media was poured about 50ml in each vessel. And all the vessels with media were transferred in Culture room where 25±1°C temperature and sterilized conditions were maintained. After 24 hours media was ready for the Inoculation process.

Inoculation of explant

All the sterilized seeds were inoculated separately in the jars or culture flasks under the sterilized conditions of Laminar Air flow hood. Different small equipment's were used like forceps and scalpels for the inoculation process. After the inoculation of the seeds in the media all the jars and flasks were again transferred carefully at Culture room where 25±1°C temperature and 16hrs light and 8hrs darkness was maintained (In seed culture total darkness provided to all the cultures for first 3 days). Incubation time was of 30 days (Fig A-B).

Table 1 Showing the composition and components of M. S. Media (1962) preparation

Stock	Constituents	Quantity		Stock medium
		1 liter (gm)	10 liter (gm)	
A.	Major Stock (gm)	(gm)	(gm)	
	Ammonium Nitrate (NH_4NO_3)	1.65	16.5	
	Potassium Nitrate (KNO_3)	1.9	19	
	Calcium Chloride ($CaCl_2.2H_2O$)	0.44	4.4	500 ml
	Magnesium Sulphate ($MgSO_4.7H_2O$)	0.37	3.7	
	Monobasic Potassium (KH_2PO_4)	0.17	1.7	
B.	Minor Stock (mg)	(mg)	(mg)	
	Potassium Iodide (KI)	0.83	8.3	
	Boric Acid (H_3BO_3)	6.2	62	
	Manganese Sulphate ($MnSO_4.4H_2O$)	22.3	223	
	Cobalt Chloride ($CoCl_2.6H_2O$)	0.025	0.25	500 ml
	Zinc Sulphate ($ZnSO_4.7H_2O$)	8.6	86	
	Sodium Molybdate ($Na_2MoO_4.2H_2O$)	0.25	2.5	
	Copper Sulphate ($CuSO_4.5H_2O$)	0.025	0.25	
C.	Iron Stock	(mg)	(mg)	
	Sodium EDTA (Na_2 EDTA.$2H_2O$)	37.3	373	500 ml
	Ferric Sulphate ($FeSO_4.7H_2O$)	27.8	278	
D.	Vitamin Stock	(mg)	(mg)	
	Nicotinic Acid	0.5	5	
	Pyridoxine HCl	0.5	5	100 ml
	Thymine HCl	0.1	1	
E.	Myo Inositol	100mg	After the combination of all the required stocks for 1 litter all these weighed chemicals were added in that combination of solution for the preparation of media.	
F.	Glyine	2mg		
G.	Agar-Agar	8mg		
H.	Sucrose	30gm		

In-vivo production of plantlets

By same way sterilized seeds were directly sawed in the media (soil, cocopeat and mosses) in separate pots and regular irrigation process was maintained and up to 15 days the plantlets were produced. The production was carried out at Botanical Garden, Gujarat University.

Now same conditions were provided to all the *in-vitro* and *in-vivo* produced plantlets. 15 days all the plantlets were

transferred for the hardening process in the net house of Botanical Garden, Gujarat University where 60 percent moisture was maintained (Fig G). Here same media soil, cocopeat were applied for all the *in-vitro* and *in-vivo* produced platelets. After 15 days in the net house all the mature plants with 8-12 leaves they were transplanted in different pots separately with 5 kg of soil in each pot. *In-vitro* and *in-vivo* produced plants were segregated and potted individually in triplicate sets as seen in the (Fig H).

Fig A-B *In-vitro* production of plantlets from seeds

Fig C Sterilized seeds

Fig D-F *In-vivo* production of plantlets from seed

Fig G Hardening process of mature plantlets in net house

Fig H Transplantation of *in-vitro* and *in-vivo* produced plants in the pots seperately

Fig I-H Heavy metal treatment directly to the rootzones of the plants with different concentartions

Treatment of heavy metal to the plants

Lead and Cadmium metals were used for the treatment in the form of Lead nitrate and Cadmium nitrate. For the treatment lead the concentrations were selected 200 mg/kg, 400 mg/kg, 600 mg/kg, 800 mg/kg of soil. And for cadmium the concentrations were selected 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg of soil. One set was kept as control both the series and both the approaches. Lead nitrate and Cadmium nitrate solution series were prepared and the treatment was provided to individual directly through rootzone via digging the soil near by the roots (Fig I-J).

Incubation time of the plants

After the treatment to all the *in-vitro* and *in-vivo* plantlets all the plants are placed at Botanical Garden for 75 days incubation period. Regular irrigation was done to all the plantlets. After 75 days the plants were taken out (Fig K-L). Different parameters like root length, shoot length and total number of branches were measured and counted.

Fig K-L After 75 days of Incubation period taling the traeted plants outside for the physiological data analysis

Fig M Lead traeted plants at the time of measurement of root and shoot length

Fig NCadmium treated plants at the time of measurement of root and shoot length

RESULTS AND DISCUSSION

After 75 days incubation time period the root length, shoot length, number of branches was measured and counted. So, as the result graph shows that as the metal concentration of lead and cadmium increases the growth of the plants were decreased and cadmium effect was seen higher than the lead effect on the growth parameters. But here the *in-vitro* produced plants grow more healthily as compare to *in-vivo* produced plants and the treatment provided to all the plants were same with triplicate sets of both approaches produced plants (Waoo *et al*. 2014a). From root length to number of branches seen more in the *in-vitro* produced plants (As the data tables and graphs showing).

As the data depicted in (Table 1) shows that because of lead effect in *in-vitro* plants were not that much of effected as compare to *in-vivo* plants. So, may be the proteins which has capacity to accumulate the metal or the phytochemicals which are responsible for the stress tolerance they may be there in more amount in *in-vitro* produced plants. These observations are in accordance to the findings of (Cristiane *et al*. 2014, Yadav *et al*. 2016).

Table 1 Showing the effect of lead on root length, shoot length and number of branches of *in-vitro* produced plants of *Impatiens balsamina* L.

Treatment Series	Root length (cm)	Shoot length (cm)	Total No. of branches
Control	29	67	10
200mg/kg	24	61.3	5
400mg/kg	22.5	58.7	5
600mg/kg	20	48.2	3
800mg/kg	18	45.1	3

Table 2 Showing the effect of lead on the root length, shoot length and number of branches of *in-vivo* produced plants of *Impatiens balsamina* L.

Treatment Series	Root length (cm)	Shoot length (cm)	Total No. of branches
Control	26.5	64.3	10
200mg/kg	25.3	60.8	5
400mg/kg	22.4	56.9	3
600mg/kg	21.9	44.2	2
800mg/kg	18.2	40.5	1

Same way when we collected the data of cadmium treated plants in that also same thing that we observed that because of metal stress the plant growth was affected but in that also as compare to *in-vivo* plants *in-vitro* plants had much better growth in stressful condition (Geiana *et al*. 2011). So, on the basis of the research in future the proteins or the phytochemicals which are responsible for the stress tolerance that can be identified and even remediation study can be done (Jasrptia *et al*. 2015). As the data referred during the review that permissible limit for the lead in the soil was 200-250mg/kg and for cadmium it was 5 mg/kg but after providing the stressful conditions the *in-vitro* produced plants affected less by the heavy metal's high concentration as compare to *in-vivo* produced plants (Singh *et al*. 2012).

Table 3 Showing effect of cadmium on the root length, shoot length, number of branches of *in-vitro* produced plants of *Impatiens balsamina* L.

Treatment Series	Root length (cm)	Shoot length (cm)	Total No. of branches
Control	25.1	70.2	9
5mg/kg	22.4	65.6	5
10mg/kg	21.9	54.5	4
15mg/kg	20.8	57.8	3
20mg/kg	20	40.2	3

As the all graphical representation showing that for both the metals *in-vitro* produced plants has more capacity to tolerate the metal stress because the growth of all the plants were more as compare to *in-vivo* produced plants. Mohmmed Zaini Nawadi described in 2014 that Balsam has capacity to remediate Naphthalene and here heavy metal stress was provided to the plants to check the stress tolerance capacity of the *Impatiens balsamina* L (Waoo *et al*. 2014b).

Table 4 Showing effect of cadmium on the root length, shoot length, number of branches of *in-vivo* produced plants of *Impatiens balsamina* L.

Treatment Series	Root length (cm)	Shoot length (cm)	Total No. of branches
Control	24.8	52.5	7
5mg/kg	20.9	49.8	5
10mg/kg	20.2	44.4	3
15mg/kg	18.9	43.7	3
20mg/kg	17.7	42.2	2

Impatiens balsamina L. is heavy metal (Lead and Cadmium) stress tolerant plant. The plant can survive at high lead and cadmium metal stress. *In-vitro* produced plants has more capability to tolerate heavy metal stress as compare to *in-vivo* produced plants. Cadmium metal stress affected more as compare to lead metal stress to the plant growth. In future remediation capacity of the metal plant and proteins and phytochemicals can be identified which are responsible for stress tolerant capacity of the plant. This research also come up with application of plant tissue culture to increase stress tolerance capacity of the plant.

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