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In-vitro Plant Production Approach to Increase Heavy Metal Stress Tolerance Capacity of *Polyscias fruticosa*

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ABSTRACT

Pollutants are increasing day by day in the environment. Mitigation of pollutants from the environment is really very difficult task and specially when we are focusing on the soil pollutants, heavy metals are major soil pollutants. Phytoremediation is only the approach by which we can remove the heavy metal from the soil. For that first identification of metal tolerant species is pioneer phase. In this research Heavy Metal stress tolerance capacity of *Polyscias fruticosa* (L.) Harm. 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). Shoot apexes 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 were 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: MICROPROPAGATION, PLANT PRODUCTION, STRESS TOLERANCE CAPACITY, POLYSCIAS FRUTICOSA (L.) HARM, PHYSIOLOGICAL PARAMETERS.

INTRODUCTION

Environmental factors can be of abiotic and biotic nature. Biotic environmental factors, resulting from interactions with other organisms, are, for example, infection or mechanical damage by herbivory or trampling, as well as effects of symbiosis or parasitism. Abiotic environmental factors include temperature, humidity, light intensity, the supply of water and minerals, and heavy metals these are the parameters and resources that determine the growth of a plant. Heavy metals are the major soil pollutants that are emitted from different industries like battery, chemical or steel (Ashwini, Khare and Ganguly 2014). Some plants can survive at high stressful conditions which can be identified and should be grown at high stressful conditions. Some of the stress tolerant plants also has the remediation capacity so the phyto accumulation of the pollutant from the environment can be identified. There are two ways to produce the plants. *In-vitro* production and *In-vivo* production. In *In-vitro* production plants has to be produced under controlled environmental

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conditions like in Green house or in culture room etc. Stress tolerant capacity of all the plants are different. *In-vitro* produced plantlets are healthier and stress tolerant after hardening process (Kozminska et al. 2018).

Polyscias fruticosa (L.) Harm is plant which belonging to Araliaceae family, also known as Ming Aralia. It is dicot shrub native to India. This is shade loving and planted for its foliage purposes. It has compound leaves with seven or more than seven leaflets. Generally, the leaves are deeply lobbed and opposite arrangement is observed. The growth of the plant is seen highest from 19-29°C temperature. Its sensitive plant for any type of stress specially it cannot survive at high temperature. It bears rare flowers and mostly used as an ornamental foliage plant. It is not directly edible by any animals or humans. The leaves have so many important phytochemical constituents and that can be utilized for drug designing. Yang et al. (2009) discovered remediation capacity of Octane through *Polyscias fruticosa* (L.) Harm. Stanley in 2011 described and reviewed indoor phytoremediant plant species and Polyscias was one of the plants that he reviewed. So, here in this research heavy metal stress was provided to the plant *Polyscias fruticosa* (L.) Harm (Yang et al. 2009).

MATERIAL AND METHODS

The shoot apexes (tips) of *Polyscias fruticosa* (L.) Harm were selected for the propagation of plants through In-vitro and *In-vivo* approaches. The Experimental work was completed at Plant Biotechnology Laboratory and Botanical Garden of Gujarat University.

Sources of Explant: Shoot tips were collected with sterilized scalpel from Botanical Garden of Gujarat University. So, shoot tips were used as an explant for the production of plantlets. All the shoot tips were sterilized with the help of 0.1%$HgCl_2$ solution and 70% methanol and rewashed with Grade-1 Distil water (Kanwar, Yu and Zhou 2018).

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 (Ijaz et al. 2016). Different stalk solutions were prepared in the amount of 500ml (Major, Minor and Iorn) and 100ml (Vitamin) and then for the preparation of 1 litre 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 minutes. After Autoclave sterilization the kinetin 0.5mg was added in the media and then under the Laminar Air Flow Hood in all the sterilized culture flasks and Glass jars media was poured about 50ml in each vessel. All the vessels with media were transferred in Culture room where 25±1°C temperature and sterilized conditions were maintained. After 24 hrs media was ready for the Inoculation process (Yang et al. 2009).

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 was 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. Incubation time was of 40 days.

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 40 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. 40 day's all the plantlets were transferred for the hardening process in the net house of Botanical Garden, Gujarat University where 60% moisture was maintained. Here same media soil, cocopeat were applied for all the In-vitro and *In-vivo* produced platelets. After 40 days in the Net house all the mature plants with 8-12 compound leaves, they were transplanted in different pots separately with 5kg of soil in each pot. In-vitro and In-vivo produced plants were segregated and potted individually in triplicate sets.

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 200mg/kg, 400mg/kg, 600mg/kg, 800mg/kg of soil. And for cadmium the concentrations were selected 5mg/kg, 10mg/kg, 15mg/kg, 20mg/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.

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.

Table 1. Showing the Composition and Components of M. S. Media (1962) preparation

Stock	Constituents	Quantity		Stock medium
		1 litter (gm)	10 litter(gm)	
A.	Major Stock (gm)			
	Ammonium Nitrate (NH_4NO_3)	1.65	16.5	500 ml
	Potassium Nitrate (KNO_3)	1.9	19	
	Calcium Chloride ($CaCl_2.2H_2O$)	0.44	4.4	
	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 (MnSO4.4H2O)	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 (Na2 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		

Figure 1: Showing *In-vitro* production of aralia

Figure 2: Showing *In-vivo* production of Aralia

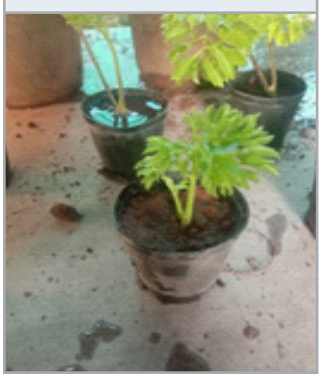


Figure 3: Showing Hardening of the plantlets

Figure 4: Showing ready plants for transplantation

Figure 5: Showing Treatment to the plants in pots

Figure 6: Showing Plants after metal treatment

RESULTS AND DISCUSSION

After 75 days the plants were taken out. Different parameters like root length, shoot length and total no. of branches were measured and counted. As the table data and graphical representation shows that as the metal concentration increases the root length, shoot length and no. of branches were decreased. For *In-vitro* plants lead 200mg concentration in the soil plants growth parameters showed 20.4cm root length, 36.8cm shoot length and 4 number of branches but as the concentration increases 800mg concentration in the soil showed that decreased plant growth included 13.9cm root length, 29.8cm shoot length and 2 number of branches. For In-vivo plants lead 200mg concentration in the soil showed 15.9cm root length, 33.9cm shoot length, 3 number of branches and 800mg concentration of lead showed decreased physiological parameters of the plant included 9.8 root length, 20.8 shoot length and 1 branch.

Figure 7: Showing lead treated *In-vitro* plants

Figure 8: Showing Lead treated *In-vivo* plants

Figure 9: Showing Cadmium treated *In-vitro*

For Cadmium *In-vitro* produced plants at 5mg concentration in the soil showed 15.3cm root length, 40.2cm shoot length, 3 number of branches and highest concentration 20mg in the soil showed that decreased physiological data included 12.3cm root length, 32.0 cm shoot length and 2 number of branches. For *In-vivo* plants cadmium 5mg concentration in the soil showed at 15.9cm root length, 32.9cm shoot length, 3 number of branches and highest concentration 20mg in the soil showed 9.1cm root length, 28.4cm shoot length and 2 number of branches. Even the cadmium effect on the plant growth was more than the lead because at lower concentration also it showed the effect on the growth parameters of the *Polyscias fruticosa* (L.) Harm (Kays 2011; Waoo, Khare and Ganguly 2014).

Figure 10: Showing Cadmium treated In-vivo plants

Table 1. Showing Effect of Lead on the *In-vitro* produced *Polyscias fruticosa* (L.) Harm

Treatment Series	Root length (cm)	Shoot length (cm)	Total No. of Branches
Control	22.2	40.6	5
200mg/kg	20.4	36.8	4
400mg/kg	16.8	35.4	4
600mg/kg	15.1	31.9	4
800mg/kg	13.9	29.8	2

Table 2. Showing Effect of Lead on *In-vivo* produced *Polyscias fruticosa* (L.) Harm

Treatment Series	Root length (cm)	Shoot length (cm)	Total No. of Branches
Control	17.4	38.7	4
200mg/kg	15.9	33.9	3
400mg/kg	13.2	30.1	2
600mg/kg	11.6	24.3	1
800mg/kg	9.8	20.8	1

In the comparison of *In-vitro* and *In-vivo* produced plants In-vitro produced plants has more capacity to tolerate metal stress because the growth of these plants was observed higher than the In-vivo produced plants. As the table shows that as compare to control the metal stressed plant growth rate of the plants (root length, shoot length, no. of branches) were lower for both the metals lead and cadmium (Mojiri et al. 2013). Thach et al. (2016) worked on the propagation of the plant as a medical plant because of the volatile compounds found in the leaves of *Polyscias fruticosa* (L) Harm. Boye et al (2018) discovered the effect of the extract of the plant in male rat.

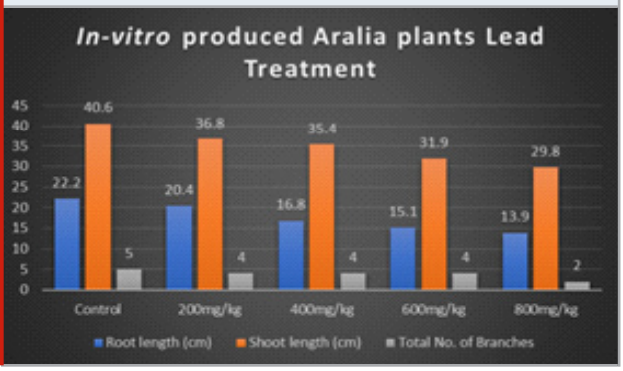
Table 3. Showing Effect of Cadmium on *In-vitro* produced *Polyscias fruticosa* (L.) Harm

Treatment Series	Root length (cm)	Shoot length (cm)	Total No. of Branches
Control	18.9	42.6	6
5mg/kg	15.3	40.2	3
10mg/kg	14.9	33.5	3
15mg/kg	13	32	2
20mg/kg	12.3	29	2

Table 4. Showing Effect of Cadmium on *In-vivo* produced *Polyscias fruticosa* (L.) Harm

Treatment Series	Root length (cm)	Shoot length (cm)	Total No. of Branches
Control	18.8	36.9	4
5mg/kg	15.9	32.9	3
10mg/kg	13.7	30.8	3
15mg/kg	11.8	29	2
20mg/kg	9.1	28.4	2

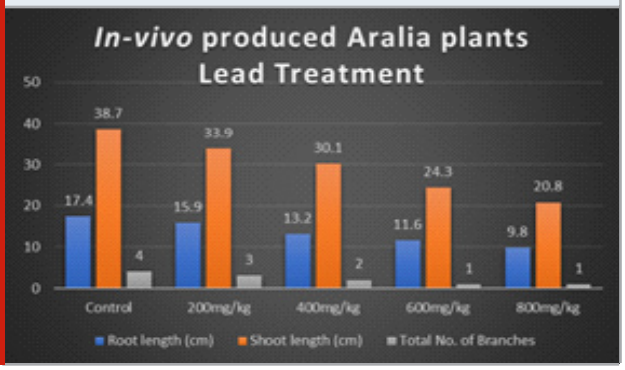
Graph 1: Showing Effect of Lead on the *In-vitro* produced *Polyscias fruticosa* (L.) Harm



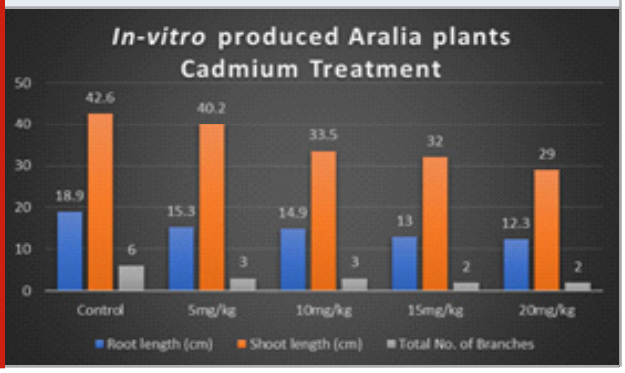
Koffur et al. (2014) discovered the anti-inflammatory effect of the plant. Salva S. Sakar et al. (2014) worked on *In-vitro* production of *Polyscias fruticosa* (L) Harm. In this research work heavy metal stress was provided to the

different method (*In-vitro* and *In-vivo*) produced plants. So many researchers worked on the phytochemicals and different activities of Polyscias fruticosa (L.) Harm there was no any record or the review of articles which showed heavy metal or stress tolerance activity or its effects on the growth parameters of the plant. In future the proteins or the phytochemicals can be identified which are responsible to increase the metal stress activity of the plants. For this study in future phytoremediation study can be assessed of the plants and with the help of In-silico analysis the binding capacity of metal and plant proteins can be analysed and protein molecules can be identified where the metal binds strongly (Hussain et al. 2018).

Graph 2: Showing Effect of Lead on the *In-vivo* produced *Polyscias fruticosa* (L.) Harm



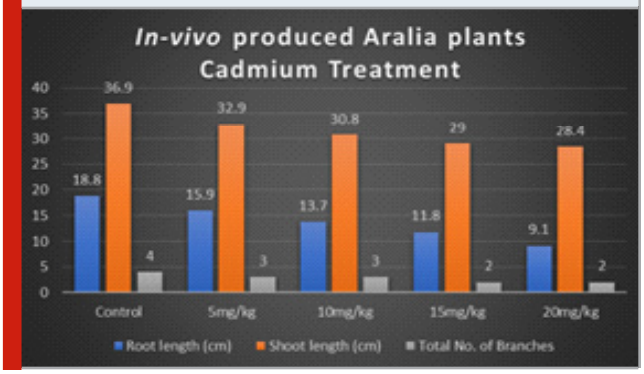
Graph 3: Effect of Cadmium on the *In-vitro* produced *Polyscias fruticosa* (L.) Harm



CONCLUSION

Polyscias fruticosa (L.) Harm is highly metal stress tolerant plant. The plant can survive at high lead and cadmium stress. *In-vitro* produced plants has more capacity to tolerate metal stress compare to In-vivo produced plants. Cadmium effect on the plants was higher as compare to lead metal stress. The research is also coming up with new application of plant tissue culture too increase metal stress tolerance capacity. After the treatment the plant material can be used for the production of Biochar which can be used in different industries like tier industries, varnish industries. After the identification of proteins which provided stress tolerant activity to the plants, the genes which are responsible for the production of that proteins can be identified and with the help of genetically modified technology it can be inserted in another plants and stress tolerant plants can be produced. So, it can be applicable to molecular genetics level and to improve the crop stress tolerant capacity.

Graph 4: Effect of Cadmium on the *In-vivo* produced *Polyscias fruticosa* (L.) Harm



REFERENCES

Ashwini A., Khare Swati, and Ganguly Sujata (2014). Comparative tissue culture on Lantana camera and Datura innoxa at heavy metal contaminated site and phytoremediation approach at industrially contaminated sites. International Journal of Advances in Biology, 1 (1); 55-62.

Bui Dinh Thach, Le Nguyen Tu Linh, Diep Trung Cang, Trinh Thi Ben, Tran Thi Linh Giang, Nguyen Pham Ai Uyen and Ngo Ke Suong (2016). Protoplast establishment for the multiplication and regeneration of *Polyscias fruticosa* (L) Harm. As medicinal plant. European Journal of Biotechnology and Genetic Engineering, 3(1); 31-37.

Hussain, Z., Arslan, M., Malik, M. H., Mohsin, M., Iqbal, S., and Afzal, M. (2018). Integrated perspectives on the use of bacterial endophytes in horizontal flow constructed wetlands for the treatment of liquid textile effluent: phytoremediation advances in the field. Journal of environmental management, 224, 387-395.

Ijaz, A., Imran, A., ul Haq, M. A., Khan, Q. M., and Afzal, M. (2016). Phytoremediation: recent advances in plant-endophytic synergistic interactions. Plant and Soil, 405(1-2), 179-195.

Kanwar, M. K., Yu, J., and Zhou, J. (2018). Phytomelatonin: recent advances and future prospects. Journal of pineal research, 65(4), e12526.

Kays, S. J. (2011). Phytoremediation of indoor air—Current state of the art. The value creation of plants for future urban agriculture. Nat. Inst. Hort. Herbal Science, RDA, Suwon, Korea, 3-21.

Kozminska, A., Wiszniewska, A., Hanus-Fajerska, E., and Muszynska, E. (2018). Recent strategies of increasing

metal tolerance and phytoremediation potential using genetic transformation of plants. Plant biotechnology reports, 12(1), 1-14.

Mojiri Amin, Aziz Abdul Hamidi, Aziz Quarani Shuokr, Razip M., and Selmat B. (2013). Phytoremediation of Heavy metals from Urban Waste Leachate by Typha domengenis.

Waoo, A. A., Khare, S., and Ganguly, S. (2014). Comparative In-vitro studies on native plant species at heavy metal polluted soil having phytoremediation potential. International Journal of Scientific Research in Environmental Sciences, 2(2), 49.

Yang, D. S., Pennisi, S. V., Son, K. C., and Kays, S. J. (2009). Screening indoor plants for volatile organic pollutant removal efficiency. HortScience, 44(5), 1377-1381.