ORIGINAL ARTICLE

A Study on Biocharification of *In vitro* Seedlings of *Buchanania Lanzan* Using Different Soil Compositions

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ABSTRACT Healthy and uninfected planting material is an essential factor for sustainable production of Chironji (*Buchanania Lanzan*). Ancient propagation methods need to be added with modern propagation techniques to fulfill the constantly increasing requirement of elite planting material. This ever increasing demand of quality planting material of *B. Lanzan* can only be met when conventional and non-conventional methods of *B. Lanzan* propagation are exploited at commercial scale with need based alterations involving modern propagation technologies. But exertion is still under progress to produce bio-hardened and better field performing Chironji plants using tissue culture technique in an economic way. In-vitro culture methods facilitate obtaining large number of high-quality genetically uniform and disease free plants in less time. A study was performed at science department RKDF University Bhopal to study the biocharification of *In vitro* seedlings of *Buchanania lanzan* using different soil compositions. The results suggest that highest existence rate is shown by sand: soil: wormy compost + mixture of *Glomus* species followed by sand: soil: cow dung + mixture of *Glomus* species. This shows that *Glomus* when used alone does not show much increase but when used in mixture having different species of *Glomus* shows percent existence.

Keywords: *Buchanania lanzan*, Micro propagation, Bio-hardening, *Glomus macrocarpum*

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INTRODUCTION

Micro propagation is the technique of *in-vitro* multiplication of large number of plants from its parts, whether it is leaves, seeds, shoot tips, nodes and root, etc. It is the fast and dependable method for production of a large number of plantlets in a short time. Moreover, the plant multiplication can continue throughout the year irrespective of season and the stock of germplasm can be maintained for many years. Micro propagation can be used to produce disease free plants. It is only possible through *in-vitro* technique. *In-vitro* comes from the Latin term "in glass". The term refers to studies of biological properties that are done in a test tube (i.e., in a glass vessel) rather than in a human or animal. Tissue culture is the growth of tissues or cells in an artificial medium separate from the organism. The micro-propagated plants are prone to various pressures after transfer from *in-vitro* to ex-vitro greenhouse environments because of the unexpected environmental changes and certain incompleteness of physiological improvement of the plant system. These *in-vitro* grown up plantlets have poorly formed a weak root system (Hazarika, 2003) unfavorable nutritional and environmental conditions (Schubert *et al.*, 1990) poorly established cuticle and non-functional stomata (Hazarika, 2003). The ex-vitro performance of tissue cultured raised up plantlets can efficiently be improved by utilizing plant beneficial bio-agents or microbes like arbuscular mycorrhizal fungi (AMF) and Aspergillus niger strain AN-27 in the hardening procedure (Rupnawar and Navale, 2000; and Mondal *et al.*, 2000). Bio-hardening of *in-vitro* grown up plantlets utilizing plant beneficial microbes and placing their preparations in rhizosphere and phyllosphere of *in-vitro* grown plants, determine the improved performance by virtue of upgraded morphological, physiological and biochemical functioning

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bio-hardened plants. Arbuscular Mycorrhizal Fungi develop a symbiotic relationship with the plants that improves the development of the root. *Aspergillus niger* strain AN-27 can be used as a bio-fertilizer as well as a bio-control agent hence, using it for bio-hardening of *B. Lanzan* plantlets prior to planting in field-as well as field application of this bio-formulation-will safeguard energetic plants with good yield (Sen, 2000). Therefore, a study was carried out with the objective of evaluation of performance of tissue cultured Chironji plantlets by ex-vitro bio-hardening.

Buchanania lanzan

Kingdom: Plantae

Phylum: Trichophyta

Class: Magnoliopsida

Order: Sapindales

Family: Anacardiaceae

Genus: Buchanania

Species: Buchanania lanzan

Buchanania lanzan is a very hard plant is grown well on rocky and red soils. Though it is hard tree but it doesn't survive under waterlogged conditions. Drain deep loam soil is ideal. Seeds of *B. lanzan* are a major source for regeneration of Chironji in India but the major problem is that it has a low percentage of germination due to its hard seed coat. The seeds of Chironji lose its viability soon even after three months of its harvesting. Methods like vegetative propagation as chip budding (Tiwari and Bajpai, 2000) and softwood grafting (Singh and Singh, 2014) are also reported in case of Chironji but these methods are less effective due to less availability of rootstocks and seasonal conditions. Also, propagation by root cutting is very slow (Singh *et al.*, 2002). The oil extracted from kernels is applied on skin diseases and also to expel spots and imperfections from the face. The root is utilized as expectorant, in biliousness and also for curing blood diseases. The juice of the leaves is stomach related, expectorant, sexual enhancer and laxative. The gum subsequent to mixing with goat milk is utilized as a pain relieving. Products of Chironji are diuretic and used to ease thirst, burning of the body and fever. Kernels of organic products are utilized as ointment in skin diseases (Das and Agrawal, 1991). The tree is leafless or about in this way, for a short time during the late spring season. Blossoms show up from January to March and their color is greenish-white. Natural products mature in the long periods of May-June (Troup, 1986). The organic products become red in the wake of ripening. The organic product assortment begins from mid-April and finishes by mid-June; however, its harvesting is commonly finished in 15-20 days as it were. The harvesting time frame may fluctuate with the end goal of organic product assortment in various agro-climatic zones. In many pieces of Chotanagpur locale, natural products are gathered before ripening. Subsequently, it gets a lot of lower cost in the stamped in view of little seed size and low seed quality even. This tree is trimmed regularly for the motivation behind gigantic and fast assortment. In forests, its regular regeneration is extremely insufficient because of informal and pre-full grown harvesting of its seeds and site debasement by virtue of growing biotic weight. Chironji is an income generating produce of forest ward networks. On a normal, 40-50 kg new organic products are delivered per tree, which yields 8-10 kg on drying, resulting in 1-1.5 kg of finished produce per tree (Tiwari, 1995). Normal yearly seed assortment is 300 to 1200 quintals in Madhya Pradesh (Prasad, 1989). In the ongoing past, because of unnecessary felling of trees and overgrazing, impressive decrease in the number of inhabitants in Chironji in the forest and non-forest regions has been recorded (Singh *et al.*, 2002). It is included in the Red Data Book distributed by International Union for Conservation of Nature and Natural Resources (IUCN) as it is a powerless medicinal plant. Chironji crop assume an important job in rustic and ancestral network of province of Jharkhand. It isn't developed industrially. Be that as it may, it contributes 10% of all out forest item in assortment based employment of forest inhabitants of this locale (Govt. of Jharkhand, 2013). The ancestral individuals frequently gather the products of this tree to acquire their vocation, through its deal, the tree is thusly overexploited. A wide assorted variety of Chironji exists in Chotanagpur level area. In any case, a high pace of hereditary disintegration saw in forest region because of deforestation. The seeds are the major wellspring of regeneration of Chironji in India. The major issue in the reforestation is low seed germination. The fundamental issue in germination is the nearness of a hard seed coat which hinders germination.

MATERIAL AND METHOD

Hardening and Planting of Tissue Cultured Raised Plants

The process of acclimatization involves transfer of tissue cultured regenerated plantlets from aseptic condition to green house and ultimately to the final location natural condition (environment). Plantlets developed within in vitro culture vessel under aseptic condition, on a rich nutrient medium containing carbohydrate and required nutrients to allow heterotrophic growth and in an atmosphere with high relative humidity and low levels of light, these all contribute to a phenotype that cannot survive the environmental conditions when directly placed in green house or field. Thus, it is necessary to acclimatize plantlets gradually to ensure survival until they develop new leaves that are more adapted to ambient

conditions under which plants are normally grown. High relative humidity and normal low temperature has to be maintained during hardening process to protect the plants from desiccation and enable them to initiate new roots and shoots. Healthy plants with well-developed roots (5-7 cm) from the shoot multiples were taken out of the culture room and kept for pre- acclimatization at room temperature under diffused sunlight for a period of 2 days.

Healthy plants with well-developed roots (5-7 cm) from the shoot multiples were taken out of the culture room and kept for pre- acclimatization at room temperature under diffused sunlight for a period of 2 days. The plants were then removed from the culture vessels, roots were washed thoroughly in running tap water to ensure removal of traces of agar, and plants were planted in to glass jam jars filled-up to quarter level with vermiculite and soaked with half strength MS salt solution. After 4-5 weeks when plantlets showed new growth the plastic cap of the glass jar was unscrewed gradually over a period of 2-3 days to reduce relative humidity in the jar, then finally the caps were removed completely from the jars on the third day. The pots were transferred to greenhouse inside an artificially created mist chamber covered with plastic sheets. The plantlets were also transferred to thermocol (Styrofoam) cups containing sand: vermiculite in the ratio of 1:5 soaked with one fourth strength MS salt solution at one-week interval. These plantlets were placed in mist chamber. After two weeks these were then transferred to vermicompost: soil in the ratio of 1:2 mixtures in plastic plantation bags (polythene-bags). In mist chamber, 90 second misting at ten minutes' interval was given to maintain RH between 85% to 95%. The temperature of mist chamber was maintained between 28-30 °C. After one month of transfer to poly-bags plantlets were transferred under green-75% agro-net shade.

RESULTS

Hardening of *in-vitro* Plantlets of *B. Lanzan*

Hardening of the *in-vitro* grown rooted plants is a very critical process. *In-vitro* grown plants several times do not survive when they are taken out from the conditions of optimized culture rooms. When culture is brought up in controlled environment it's not able to bear external environment as *in-vitro* grown plantlets are very delicate to the temperature, humidity and photoperiod. The *in-vitro* grown Chironji plantlets were tried to harden. Well rooted plants were taken out from containers. After taken out they were washed thoroughly by sterile double distilled water to remove the unwanted material. After washing the plants were potted in sterilized sand: soil 2:1 mixture. 25 saplings were transferred in one replicates. Equally, 25 saplings were transferred in sterilized soil; sand and cow dung 2:2:2 mixtures in each replicates. Sand, soil, and wormy compost were also studied in 2:2:2 proportions. Four replicates for each treatment were studied. Firstly, these *in-vitro* saplings were shifted in plastic pots for one week and maintained at high humidity of 80-90% in growth chamber. After one week these saplings were shifted in the same mixture in earthen pots/polythene bags maintained in high humidity controlled green house. After 30, 60, 90 and 120 days of transfer of pots in green house observations were noted for their existence rate. The results of existence percentage of *in-vitro* grown saplings are presented in the below Table 1.

Table 1 indicates survival rate of *in-vitro* hardened plants using different soil combinations in different ratios. In sand and soil mixture which was taken in the ratio of 2:2 indicates 6.31 mean number of plants lasted after 30 days while after 60 days the survival rate falls to 5.55, after 90 days it falls to 4.00

Figure 1: Pictorial Representation (A) *In-Vitro* Raised Plantlets Which were Maintained in Growth Chamber for about 10 Days Transferred to Plastic Tray for Hardening and (B) Plants Maintained in Green House are Transferred to Polybags

Table 1: Existence Rate of *in-vitro* Harden Plants in Different Soil Mixtures After 30, 60, 90 and 120 Days of Transfer in Green House

Parameters	Treatments	Plants Transferred in Greenhouse	Days			
			30	60	90	120
Percentage existence of plants after days of transfer in green house	SM1	25	27.60%	22.80%	16.00%	12.30%
	SM2	25	41.40%	39.60%	35.00%	29.03%
	SM3	25	51.50%	49.00%	41.50%	33.07%
Number of plants survived after days of transferred in green house (AVERAGR±SE)	SM1	25	6.31±0.50	5.55±0.30	4.00±0.67	3.37±0.95
	SM2	25	9.08±0.43	8.8±0.49	7.9±0.63	5.7±0.75
	SM3	25	11.02±0.65	10.7±0.59	9.08±0.67	7.07±0.73

Note: SM: Soil Mixtures; SM1 = Soil: sand (2:2); SM2 = Sand: soil: cow dung (2:2:2); SM3 = Sand: soil: vermicompost (2:2:2).

and it again goes down to 3.37 in 120 days. In case of sand, soil and cow dung mixture which was taken in the ratio of 2:2:2 indicates 9.08 mean number of plants lasted after 30 days while after 60 days the survival rate falls to 8.8, after 90 days it falls to 7.9 and it again goes down to 5.7 in 120 days. In case of sand, soil and wormy compost mixture which was taken in the ratio of 2:2:2 indicates 11.02 mean number of plants lasted after 30 days while after 60 days the survival rate falls to 10.7, after 90 days it falls to 9.08 and it again goes down to 7.07 in 120 days. This shows that sand: soil: wormy compost at the proportion of 2:2:2 is the best mixture for hardening of *in-vitro* plants. The survival percentage of *in-vitro* hardened plants was seen increasing in different mixtures like it was 12.3% in case of sand and soil after 120 days then it increased to 29.03% in case of sand: soil: cow dung and finally it went upto 33.07% which was the maximum survival rate in 120 days.

Bio-Hardening of *in-vitro* Grown Plantlets of *B. Lanzan*

Hardening through biological means is taking new heights in this field, for this VAM (Vesicular Arbuscular Mycorrhiza) is a famous biological agent. It helps in the development of strong root system and increases the potential of the roots to establish in vast areas. The plants that are grown on soil which is poor in phosphorous is provided with VAM as it increases the phosphorous uptake of many plants. Plants provided with VAM will use all the available nitrogen. This fungal association stops the attack of soil born pathogens as the plants grown in aseptic conditions are very prone to pathogens. External stress to plants such as toxic metals, saline soil, drought and pH all are neutralized by this association. Even the symbiotic relation between the VAM fungi and roots of plants make plants enough strong to

Table 2: Bio-Hardening of *in-vitro* Grown Plants of *B. lanzan* by Using Glomus Macrocarpum and Mixture of Glomus Species in Different Sand, Soil and Compost Mixture

Parameters	Treatments	Plants Transferred in Green House	Days			
			30	60	90	120
Number of plants survived after days of transferred in green house (AVERAGR±SE)	T1	25	17.9±0.29	13.03±0.89	12.04±0.21	10.05±0.17
	T2	25	13.9±0.99	11.02±0.13	11.6±0.13	10.5±0.9
	T3	25	20.79±0.90	19.7±0.81	18.0±0.05	16.03±0.04
	T4	25	14.03±0.45	12.06±0.18	11.17±0.41	10.15±0.40
Percentage existence of plants after days of transfer in green house	T1	25	85%	61%	56.30%	50.20%
	T2	25	65%	51.60%	53.60%	50.50%
	T3	25	99.90%	94%	86%	83%
	T4	25	66%	56.40%	56.90%	55.80%

Note: T = Treatments; T1 = *Glomus species* + SM2; T2 = *Glomus macrocarpum* = SM2; T3 = *Glomus Species* = SM3; T4 = *Glomus macrocarpum* + SM3.

withstand with any kind of external environmental stress (Table 2).

DISCUSSION

Bio-hardening of *in-vitro* raised plantlets of *B. Lanzan* using *Glomus macrocarpum* and mixture of *Glomus* species containing *Glomus Geosporum, Glomus Faciculatum* and *Glomus Mosseae.* In Table 3.6 It was witnessed that *in-vitro* raised plants survive more in case of sand: soil: cow dung at the ratio of 2:2:2 and more in case of sand: soil: wormy compost at the rate of 2:2:2. So the above two combinations mixed with cultures of Glomus species will be valuable. In case of sand: soil: cow dung 2:2:2 with combination of Glomus macrocarpum shows 65% existence rate after 30 days while the percentage decreased slightly to 53.6% and after 120 days it again goes down to 50.5%. In case of mixture of Glomus species, the percentage after 30 days' rises upto 85% and then decreases to 61% after 60 days and again slightly decreases to 56.3% after 90 days and again drops down to 50.02% in 120 days. In case of sand: soil: wormy compost in combination with Glomus macrocarpum shows 66% existence rate after 30 days and after 60 days the percentage slightly decreases to 56.4%, it decreased to 56.2 in 90 days and again dropped down to 55.8% in 120 days which is inadequate compared to percent survival in other combination with mixture of Glomus species which showed 99.9% survival after 30 days however the percentage decreased slightly after 60 days i.e., 94%, after 90 days to 86% and after 120 days it again showed decrease to 83%.

CONCLUSION

In-vitro grown plantlets have delicate roots as they are raised-up through high nutrition medium. Thus, the roots are not more potent to struggle for the survival of plant. At the moment they are taken out from the controlled conditions of nutrient rich medium, they have to struggle for their survival. When they are transferred in different soil combinations the entire medium gets changed. So from all these noted observations it gets clear that highest existence rate is shown by sand: soil: wormy compost + mixture of *Glomus* species followed by sand: soil: cow dung + mixture of *Glomus* species. This shows that Glomus when used alone does not show much increase but when used in mixture having different species of Glomus shows percent existence.

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REFERENCES

1. Das D. and Agrawal V. S. (1991.) Fruits drug plants of India", Kalyani Publishers, New Delhi, p. 250.
2. Prasad R. (1989). Research Needs in Minor Forest Produce in Madhya Pradesh. In: Minor Forest Produce and Tribal Development. Proceeding National Seminar October, Institute of Deciduous Forests, Jabalpur, India, pp. 32-34.
3. Singh J., Banerjee S. K. and Francis A. (2002). Vegetative propagation of Buchanania lanzan Spreng. Root cuttings. Indian Forester, 128(6): pp. 700-704.
4. Tewari (1995). Bulletin on Buchanania lanzan (Chironji), Indian Council of Forest Research and Education, pp. 1-6
5. Troup R. S. (1986). The Sylviculture of Indian Trees. IBP Publishers, Dehra Dun (Reprinted). 1: pp. 240-243.