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Research article

EFFECT OF SEED DIRECTION AND GROWTH MEDIA ON *IN VITRO* SEEDS GERMINATION AND SEEDLING ESTABLISHMENT OF *PTEROCARPUS MARSUPIUM* ROXB.

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ABSTRACT: In present study *in vitro* seed germination methods were developed for optimization of germination performance of medicinally important plant, *Pterocarpus marsupium* Roxb. *In vivo* seed germination through conventional method of this plant and seeds are facing so many problems due to its hard seed coat and poor viability. Application of PGRs is also not much affective in *in vivo* condition. Present study was conducted using in vitro culture technique the seeds were inoculated in different orientation in to different media type viz. M.S media, ½ M.S, Nitsch media. Among above results media and horizontal direction was found suitable for seed germination. So, with that selected condition different hormones were added in media for better germination. All together horizontal direction on MS media are the good for the population of the plant.
Key words: *Pterocarpus marsupium*, seed germination, seed direction, hormones.

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INTRODUCTION

Use of plants as a source of medicine has been an ancient practice and is an important component of the health care system in India. Among them *Pterocarpus marsupium* Roxb. is one of the most important medicinal tree commonly known as Indian kino or Malabar Kino found on hilly slope even in dry and fully exposed area above 750-3200m especially in Deccan Peninsula. It is also distributed in central India, certain part of Western and Northern India, especially in Gujarat, Rajasthan, Madhya Pradesh, Uttar Pradesh, Bihar, Orrissa and Tamilnadu, West Bengal and Goa [1]. In Gujarat it found in Dangs and Rajpipla hills [2]. Pterocarpus is a medium to large sized tree reaching height up to 15- 20 meter with dark brown to grey bark having swallow cracks tall deciduous tree. The older tree bark exudes a red gum resin substance called "Gum Kino" when injured. This tree also yields gum kino, which is powerful astrigent, used for treatments of diarrhea, dysentery, leucorrhoea, haemorrhages and toothache [3]. The water stored in vessels node of the wood is reputed to have antidiabetic properties due to transfer of glycosides into water [4, 5]. Two important phenolic constituents' marsupsin and pterostilbene, isolated from the heartwood of P. marsupium are reported to possess ant hyperglycemic activity [6, 7].

Pterocarpus marsupium Roxb. has been used as an anti-inflammatory, anti-elephantiasis, anti-leucoderma agent and often used to treat dysentery, cough and diarrhea. Traditionally the plant material has been used as a cooling external application for inflammations and headache, as antipyretic, anti-helminthic, aphrodisiac, alexetic and in biliousness, mental aberrations and ulcers [8, 9]. Parts of the Indian Kino (heart wood, leaves and flowers) have long been used as an astringent and in the treatment of inflammation. The mature tree harvested after 10-15 years has been estimated to produce approximately 500 kg of dry heart wood and the natural stands of these trees are rapidly disappearing due to illict felling for its significant multipurpose properties and current high market price of its dry heartwood ranging from Rs. 70-80/ kg [10].

The winged pod is the only propagating material, but its germination only 30% [11]. However, Propagation and cultivation of forest tree species including *Pterocarpus marsupium* Roxb. Through seeds are difficult due to pathogenic infection [12], which leads to low germination and causes its restricted natural regeneration. The exploitation of this medicinally as well as economically important tree from the natural habitat and scarce efforts for its cultivation resulted in marked decline in the population of the species and has, therefore, been included on the list of endangered category of IUCN red list [13, 14]. There are so many problems found in seeds germination for that in this study have been conducted with three different objective first to check whether the seed direction and different medium type affect the seed germination second one to check the hormone application will improve the seed germination and last to optimization effect of seed germination and their successful establishment in nursery stage.

MATERIALS AND METHODS

Pterocarpus marsupium roxb. seeds were collected from the Botanical garden, Vagai, South Gujarat in the month of March. Plant authentication was done by the Department of Life Sciences including botany at Hemchandracharya North Gujarat University, Patan. The fruit coat were removed manually with help of cutter and isolated seeds were washed properly then washed with 1% Teepol-B-300 liquid detergent again wash properly with tap water. The seeds were kept in water for 2 hour to remove any phenolic exudates. Whole vitro seeds germination method was used by Mishra *et al.,* [15].

In vitro seed germination

Seed surface sterilization treatment was conducted in laminar air flow chamber. Seed were dipped into 1% Dettol Solution for 1 minutes and again washed with distilled water then sterilized by 0.1% $HgCl_2$ for 2 minutes and rinsed four to five time with distilled water. For comparing the effect of seed orientation, sterilized seeds were implanted horizontally, vertically up and vertically down on three Nitch, MS and ½ MS medium with 0.7% agar. For speed up the seed germination sterilized seeds were inoculated in selected MS medium with different hormonal concentration of GA3, Kin, and BAP (0.5 to 3 mg/l) compare with plain MS medium as control. Seeds germination data was recorded up to 1 week and seedling data was recorded after four week. Seedling Vigour Index was calculated by following equation.

Seedling Vigour Index = % of Germination × [Total seedling length]

Hardening and Transplantation

Take an *in vitro* raised seedling from the test tube and remove the agar from the plants and dipped for 30 second in 0.2% (W/V) bavistin solution and washed with tap water and transferred to root trainers and filled with a mixture of soil: compost and maintained at 25±2°C under 16 h photoperiod for in vitro hardening. After acclimatization period of 2-3 weeks, the seedlings were transferred to polythene bags containing soil: manure (1:1) in the net house.

Culture Media and Condition

The pH of the medium was adjusted to 5.8 by 1N NaOH or 1N HCl before autoclaving at 121°C and 1.05 kg cm-2 pressures for 20 minutes. The medium was dispensed in20ml aliquots in 25x150 cm culture tubes. All the cultures were incubated in a culture room at an air temperature of 25º ±2ºC with 65% relative humidity under a 16h photoperiod with irradiation of 50µEm-2 s-1 photo synthetically active radiation (PAR) provide by cool white fluorescent tubes.

Statistical Analysis

Data were statistically analyzed by using SAS 9.3 computer software and SPSS ver. 17 to explore possible treatment variation. The analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were used for the analysis.

RESULTS AND DISCUSSION

The result of seed germination in different direction with different medium type was shown in table 1 and plate 1. The percentage of seed germination was significantly affected by the orientation in which seeds were inoculated. The highest percentage of seed germination (92.22) was recorded in horizontal position which was significantly higher than other direction. For seed germination various medium were used but a no more difference was observed during seed germination for that non- significant difference was found. Same thing was observed in comparison study of seed position with different medium strength, which showed not significant result.

In results of seedling performance seed germination, higher shoot length (11.68), higher root length (6.00) and vigor index also showed significantly (1638.22) result in horizontal direction but in medium strength and interaction study was showed a non- significant difference for the study. Regardless of the concentration of MS medium, the horizontally inoculated seeds enhanced germination rate, shoot length, root length and vigor index compare to the vertical up or down seeds under the *in vitro* condition. Similar study was done by Mishra *et al.*, [15] and found that inoculation of the seeds in particular direction affects the germination.

There were no seeds germination were observed in any concentration of Ki and BAP but only seed germination was found in all GA_3 concentration for that BAP and Ki data was not represented here. Results of GA_3 on seed germination and that data was recorded in table no.2 and plate 1. In percentage of germination, root length and in vigor index significance difference were observed but in shoot length no significant difference was observed. In set seeds were inoculated in plain MS medium as a control and in the other sets seeds were inoculated MS with different concentration of GA_3. In all the concentrations of GA_3, 1mg/l showed good result and that percentage of seed germination was 76.67. The shoot length was higher in 1.5 mg/l in GA3 concentration but root length was higher in 1 mg/l GA3 with MS medium. For the entire GA concentration, no major difference was observed in vigor index between 1mg/l and 1.5 mg/l GA3 concentration. If compare with control (MS plain medium) percentage of germination, shoot length, root length and vigor index were higher than the hormonal treatment on seed germination. Here, hormonal treatment compare with control was showed no significance effect was found. Here, if concentration increased, % of seed germination was decreased that means higher concentrations of GA_3 may be inhibit the seed germination. Similar type of result was also found in *Andrographis paniculata* but here compare with hormonal treatment with hot water with respect to the seeds germination and demonstrated that hormone treatment showed not significant germination percentage compare to hot water treated seeds [16]. Because in case of seeds germination of *Andrographis paniculata, Pterocarpus marsupim* Roxb. Dormancy can be due to its hard seed coat. Similar type of work was done *in Pterocarpus santalinus* Linn. f. [17], *Astragalus cyclophyllon* [18]. In some plants exogenous GA application has been substitute for stratification and has increased germination in many plant species, including *Leucospermum, Fagus sylvatica* and *Helianthus* and many times it is applied as substitute for scarification [19, 20,21]. That treatment with GA_3 can be added to the formation of rough endoplasmic reticulum and polymerase. More over it has been found that GA stimulates the synthesis of mRNA which is specific for α amylase release and which one of the reasons is for higher seed germination [21].

Table:1 Effect of seed position and media types on seed germination of *Pterocarpus marsupium* Roxb.

Position of Seeds inoculation	Seed germination % of different Medium types				Shoot length (cm) of different Media types				Root length (cm) of different Media types				Vigor index of different Media types			
	½ MS	MS	Nitch	Mean	½ MS	MS	Nitch	Mean	½ MS	MS	Nitch	Mean	½ MS	MS	Nitch	Mean
Horizontal	93.33	96.67	86.67	92.22	10.93	15.00	9.10	11.68	5.20	6.67	6.13	6.00	1565.00	1876..67	1473.00	1638..22
V D	70.00	73.33	66.67	70.00	6.67	11.67	12.33	10.22	5.13	5.63	5.00	5.26	856.67	1262.33	1133.33	1087.11
V U	56.67	56.67	43.33	52.22	2.67	4.50	2.33	3.17	3.10	2.83	4.67	3.53	315.00	400.00	276.67	330.56
Mean	73.33	75.56	65.56	71.48	6.76	10.39	7.92	8.36	4.48	5.04	5.26	4.93	915.22	1179.67	961.00	1018.63

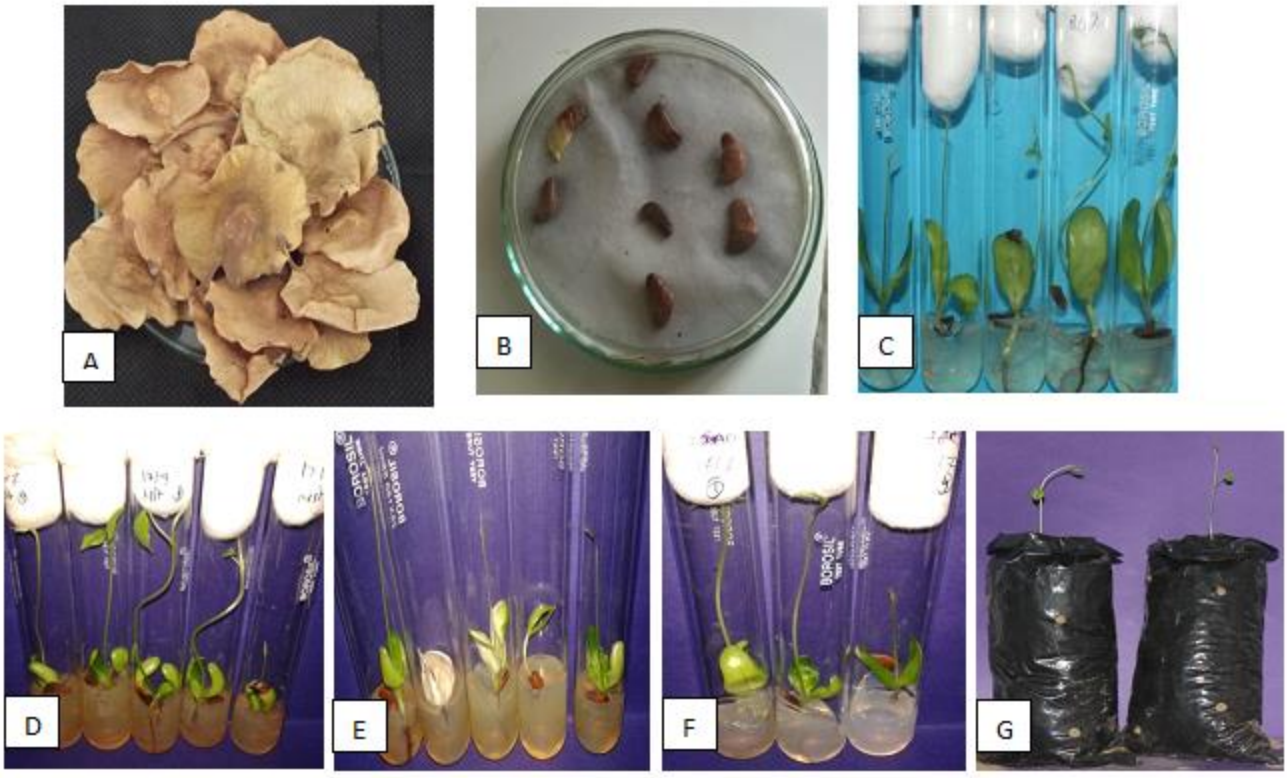
Note: VD= Vertical Direction, VU=Vertical up, NS= Not significant, MS= Murasig and Skoog. The data is represented in Mean of five replicates

Position of explants * Medium strength	LSD (0.05)		LSD (0.05)		LSD (0.05)		LSD (0.05)
Position of explants	9.14	Position of explants	0.299	Position of explants	1.15	Position of explants	222.516
Medium strength	NS	Medium strength	NS	Medium strength	NS	Medium strength	NS
Position * Medium strength	NS	Position * Medium strength	NS	Position * Medium strength	NS	Position * Medium strength	NS

Table: 2 Effect of GA_3 on seed germination of *Pterocarpus marsupium* Roxb

Name of Treatment	% of Seed germination	Shoot length In (cm)	Root length In (cm)	Vigor index'
M.S	93.33^{A}	14.67	6.33^{A}	1959.93^{A}
M.S+ 0.5 GA	56.67^{CD}	5.33	3.00^{B}	472.06^{E}
M.S+ 1.0GA	76.67^{B}	7.33	3.33^{B}	817.30^{BC}
M.S+ 1.5 GA	66.33^{BC}	10.50	2.00^{B}	829.12^{B}
M.S+ 2.0 GA	63.67^{BC}	9.33	1.67^{B}	700.3^{D}
M.S +3.0 GA	43.33^{D}	7.66	1.33^{B}	389.53^{F}
General Mean	66.67	9.14	2.94	861.39
p-Value			.	
CV (%)	12.25	42.59	42.36	1.11
SE(d)	6.666	3.178	1.018	7.932
LSD at 5%	14.525	NS	2.2188	17.282

Mean values in the same column followed by the same latter are not significantly different at the 0.05 level according to the Duncan test, the data is represented in Mean of five replicates



Note: S.G= Seed germination, **A.** Pterocarpus seeds with seed coat **B**. Isolated seed **C**. S. G in M.S medium **D.** S. G. in Nitsch medium **E**. S. Germination in1/2 M.S medium **F.** S. G in 1 mg/l with MS medium **F.** Hardening of seed.

Plate:1 Seed germination of *Pterocarpus marsupium* Roxb.

Hardening and Transplantation

In the results hardening of seedling transfer in to polythene bags containing soil: manure (1:1) and showed that 90% seedling successfully established and survived for field transfer.

CONCLUSIONS

For *in vitro* seed germination MS medium and horizontal direction are the best for the germination. Hormonal treatment does not improve seeds germination compare to Plain ms medium. So the MS medium and horizontal direction are good for the seed germination and seedling growth and that may be helpful for increasing the population of this plant.

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