International Journal of Pharmacy and Biological Sciences-IJPBS™ (2020) 10 (3): 331-336
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

Promotion of *in vitro* Seed Germination of *Semecarpus anacardium L.* an Important Medicinal Plant

Y. Sravanthi and MD. Mustafa*
Plant tissue Culture and Molecular Taxonomy Laboratory, Department of Botany, Kakatiya University, Warangal, Telangana, India.

Received: 12 Mar 2020 / Accepted: 8 Apr 2020 / Published online: 1 Jul 2020
***Corresponding Author Email:** mustafarz67@gmail.com

Abstract
A Protocol for *in vitro* seed germination of *Semecarpus anacardium* has been promoted in the present study. The collected seeds were surface sterilized and soaked in distilled water containing 0.1% GA_3 for about 7 days. Such treated seeds were transferred on paper bridges of 0.5 % GA_3 containing distilled water. These seeds germinated after 15 days of culture with 90% of response. The seedlings were transferred to field and some allowed in *in vitro* for further growth and then they were transferred to pots and field conditions after one month.

Keywords
Semecarpus anacardium, *in vitro,* Germination, Gibberelic acid.

*****

INTRODUCTION
Plants provide different kinds of drugs which are useful for curing our aliments. Therapeutic plants are the backbones of traditional remedy, which means more than two billion people in the less advanced countries, utilize medicinal plants on a regular basis. Therapeutic plants play a dynamic role to preserve our healthiness [1]. The genus *Semecarpus anacardium* is a deciduous forest tree belonging to the family Anacardiaceae. It is valued for its therapeutic properties. It is generally called as marking nut and the vernacular name is 'Bhallataka'. This plant has been demanded as Half-Physician (multi-purpose) in Ayurveda [2]. *Semcarpus anacardium* is a one of the therapeutically important plants which may be used as an additional remedy [3]. The nuts used everywhere in India as a substitute for marking ink for clothes by washer men. It is one of the finest, multipurpose, most commonly used herbs as household medicine [4]. The fruits, their oil and the seeds have great medicinal value, and are used to treat the wide range of diseases. Internally, bhallataka is extensively used in a vast range of illnesses because of its different possessions [5]. The fruit and nut extract shows various activities like antiatherogenic, anti-inflammatory, antioxidant, anti-microbial, CNS stimulant, hypoglycaemic, anticarcinogenic and anti-arthritic. Bhallataka has been described to be a potential plant for the remedy of ailments such as Diabetes, Asthma, rheumatoid arthritis, Piles, malignancy etc. [6]. Still the mechanism of the pharmacological action of *S. anacardium* nut can be greatly aided by the isolation of its active principle and purpose of structure and functional association [7]. Micropropagation of tree species offers a quick means to harvest clonal planting stock for afforestation, woody biomass invention and preservation of superlative germplasm [8].

BOTANICAL DESCRIPTION
S. anacardium is a moderate-sized deciduous tree with begin flexible leaves. Greeneries are 7-24 inches long, 2-12 inches wide, Obovate- oblong, rounded at the tip. Leaf base is rounded, heart-considered or pointed into the branch, leathery in texture. Flowers

are minor, borne in panicles shorter than the leaves. Fruit is a drupe 1 inch long, ovoid or oblong. smooth and shining, black when ripe, seated on a fleshy cup. The shoot yields, by tapping, an acid viscid juice from which a paint is prepared.

FLOWERING AND FRUITING

Blossoming period is May to October, whereas fruiting time is December to March.

DISTRIBUTION

S. anacardium grows in the forests of the sub-Himalayan tract, up to 3,500ft in Assam, Khasi hills, Madya Pradesh, Gujarat, Konkan and the deciduous forests of the southern states of India, including, Telangana *S. anacardium* is growing in Pakhal, Mulugu, and forests in Warangal district. The genus *Semecarpus* contains 40 species spread throughout the tropical regions of the biosphere. It is also widely spread in the forests of Similpal Biosphere Reserve, Odisha and central parts of India.

2. MATERIALS AND METHODS

Trees allow seeds in winter. Mature fruits of *Semecarpus anacardium* were collected from the trees growing (15 yrs. old) widely in dissimilar forest areas of Warangal and Adilabad districts in Telangana. The fruits were dried below sunlight for 15 days and cleaned by eliminating the false fruit and stored in a vessel. Mature nuts were used to raise *in vitro* seedlings. Seeds were washed under running tap water and then rinsed and immersed in sterile distilled water for 1-7 days followed by treated with Bavistin fungicide for 2hrs and M_{45} fungicide for 1hr and they were treated with soap and nip of detergent for 15 minutes then they were treated with 0.1% Dettol for 5 minutes and allowed tween - 20 for 7 minutes. Seeds were washed thrice with sterile distilled water. Then they were treated with 0.1%. Mercuric chloride for 6-7 min. The seeds were washed 3 to 5 times with sterile distilled water. Still $HgCl_2$ and NaOCl are the most extensively working surface sterilant additional excess of water were removed by chemicals [9]. Blotting on sterile tissue paper and after that they were inoculated into sterile wet cotton and GA_3 containing culture tubes and MS medium. Sterile seeds were germinated on MS medium and paper boats and sterile wet cotton screw cap bottles and culture tubes.

2.1 METHODS FOR SEED GERMINATION

2.2 GA_3 treatment: 70 decontaminated seeds were soaked for 3days, 5days and 7days with 0.1% GA_3 at room temperature.

2.3 Mechanical scarification

The mature seeds were nicked with a sharp small knife, sandpaper and seeds soaking in distilled water for the elimination of seed coat.

3. CULTURE MEDIUM AND CONDITIONS

Sterilized seeds were transferred on paper bridges containing MS medium fortified with o.5% GA_3.The pH of the medium was used to 5.8 before gelling with 0.8% Agar and autoclaved for 20 min at 121^0C with 15lbs psi pressure. Prepared solution was dispensed into the culture tubes containing paper bridges and plugged with non-absorbent cotton. The cultures were maintained in a culture room at (25 ± 2^0C) under cool-white, fluorescent light.

3.1 Paper boat method: The sterilized seeds were cultured on filter paper bridges having distilled water and GA_3.

3.2 Statistical analysis: All the experiments were repeated thrice 30 explants for each treatment and the seed germination percentage was noted occasionally.

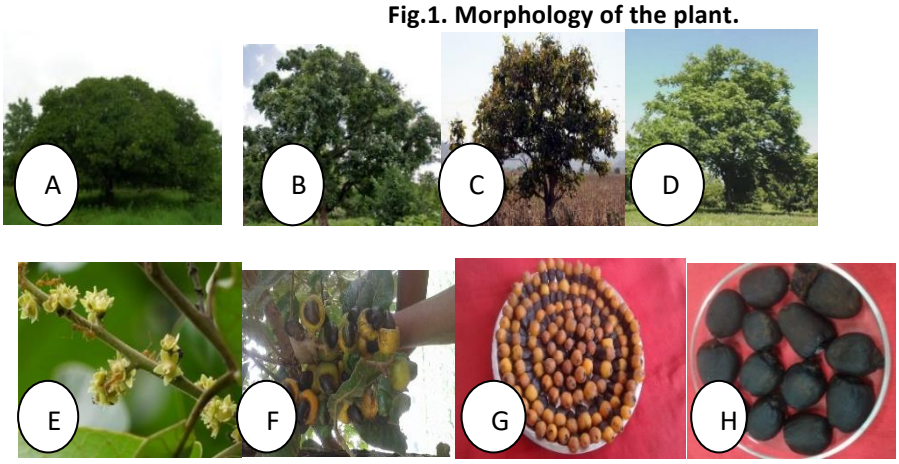
4. *IN VITRO* SEED GERMINATION

The seed germination was achieved both sterile cotton and paper boat method. The germination was increased when the hard seed coat was spilt after surface sterilization GA_3 treated seeds showed increase in germination in paper boat method and early seed germination with 90%.Normal seeds showed less ratio compared to GA_3 treated seeds. Different type of treatments was tested for germination (0.5 mg/l GA_3) treated seeds M.S medium fortified with 0.5% GA_3 was found more suitable for *in vitro* seed germination of seeds, when compared to the control seeds.

4.1 Transplanting

After germination seedlings were carefully removed and washed with water in test tubes and potted in pots containing sterilized soil, cocopeat, sand (1:1:1) and after 6 weeks transferred to green house. After the 25 days of germination, germinated seeds showed cotyledonary growth & long hypocotyl area having long roots.

Fig.1. Morphology of the plant.



A-D Trees of *Semecarpus anacardium* from Adilabad and Warangal districts.E.Flowers F-G. Ripening fruits.H- Seeds.

Fig.2.Germination of seeds and seedlings development of *S.anacardium* on paperboat

H

A-E Germination of Seeds on paper bridges treated with GA_3 F-H sprouting on MS medium. I, J. Seeds inoculated on sterile cotton. K- Fully developed plantlets. O. Potting.

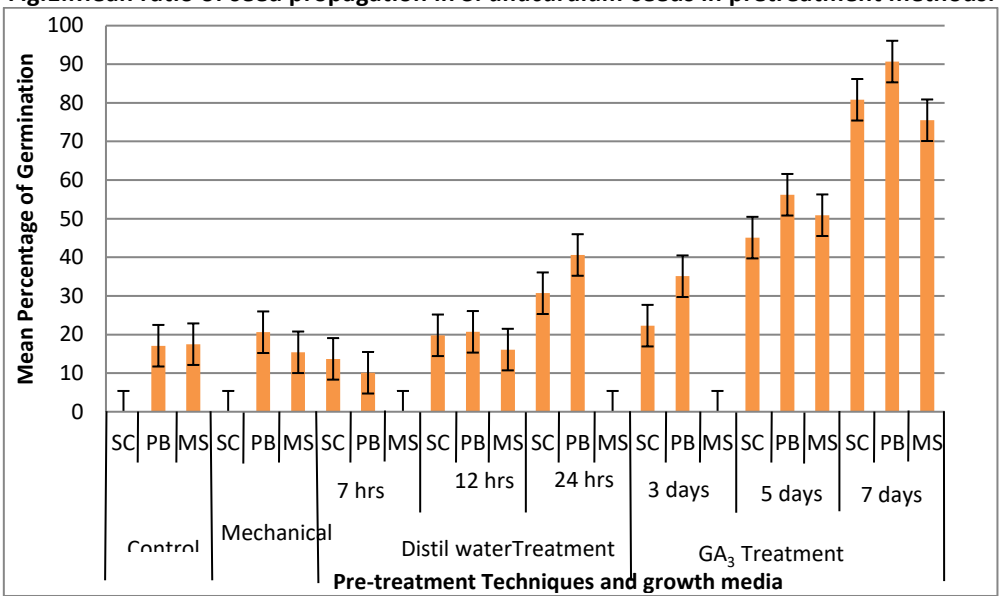
Table-1. Effect of different methodologies and techniques for *in vitro* seed germination

Methods for seed germination			Percentage of germination (SE±)	No. of days for germination (SE ±)	Average length (cms) of seedling
Control		**SC**	0.00±0.00	0.00±0.00	0.00±0.00
		PB	17.1 ± 0.33	45.5±0.36	7.1±0.24
		MS	17.5±0.38	41.1±0.36	7.4±0.24
Mechanical scarification		**SC**	0.00±0.00	0.00±0.00	0.00±0.00
		PB	20.6±0.32	36.2±0.30	5.5±0.42
		MS	15.4±0.35	33.3±0.38	4.6±0.32
Seeds soaking in sterile distilled water	**7hrs**	**SC**	13.7±0.31	30.4±0.35	9.3±0.41
		PB	10.1±0.33	34.2±0.37	8.5±0.42
		MS	0.00±0.00	0.00±0.00	0.00±0.00
	12hrs	**SC**	19.8±0.34	27.8±0.40	10.2±0.43
		PB	20.7±0.35	29.5±0.30	8.3±0.27
		MS	16.1±0.33	25.5±0.32	6.2±0.34
	24hrs	**SC**	30.7±0.31	42.4±0.28	5.1±0.33

		PB	40.6±0.32	47.3±0.38	3.8±0.34
		MS	0.00±0.00	0.00±0.00	0.00±0.00
GA3	**3days**	**SC**	22.3±0.38	30.9±0.42	6.5±0.47
treatment		**PB**	35.1±0.39	26.3±0.31	9.4±0.28
		MS	0.00±0.00	0.00±0.00	0.00±0.0
	5days	**SC**	45.1±0.33	27.1±0.33	10.1±0.33
		PB	56.2±0.30	14.3±0.31	9.7±0.31
		MS	50.9±0.36	21.5±0.32	6.3±0.38
	7days	**SC**	80.8±0.40	11.4±0.39	6.1±0.33
		PB	**90.7±0.35**	**10.2±0.34**	**8.8±0.34**
		MS	75.5±0.32	9.7±0.38	7.5±0.36

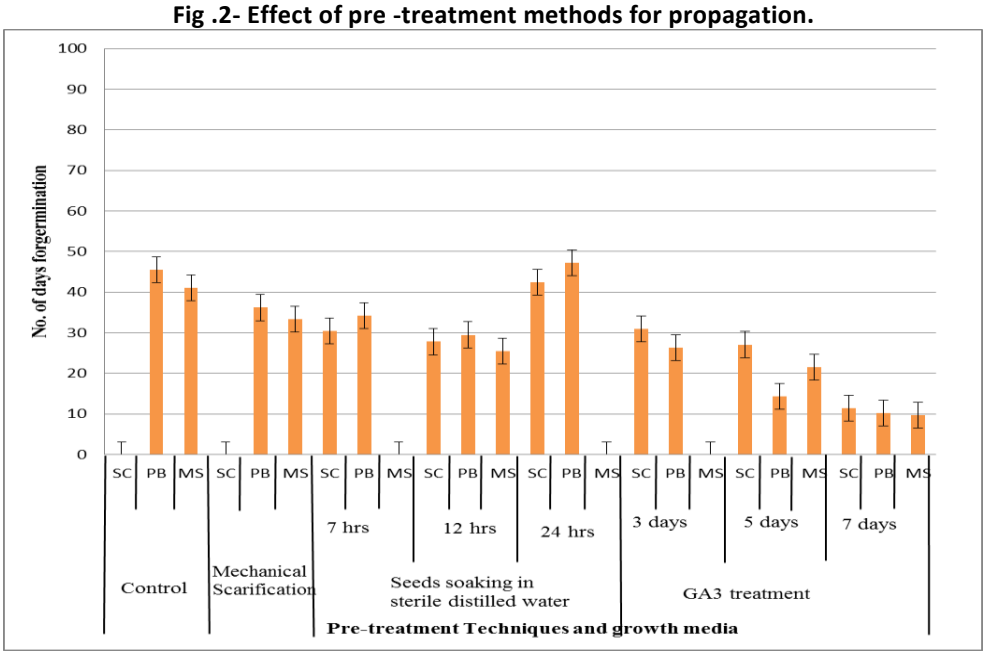
Mean±Standard Error; PB-Paper Boat; MS-Murashige and Skoog's medium; SC- Sterile cotton.

Fig.1.Mean ratio of seed propagation in *S. anacardium* seeds in pretreatment methods.



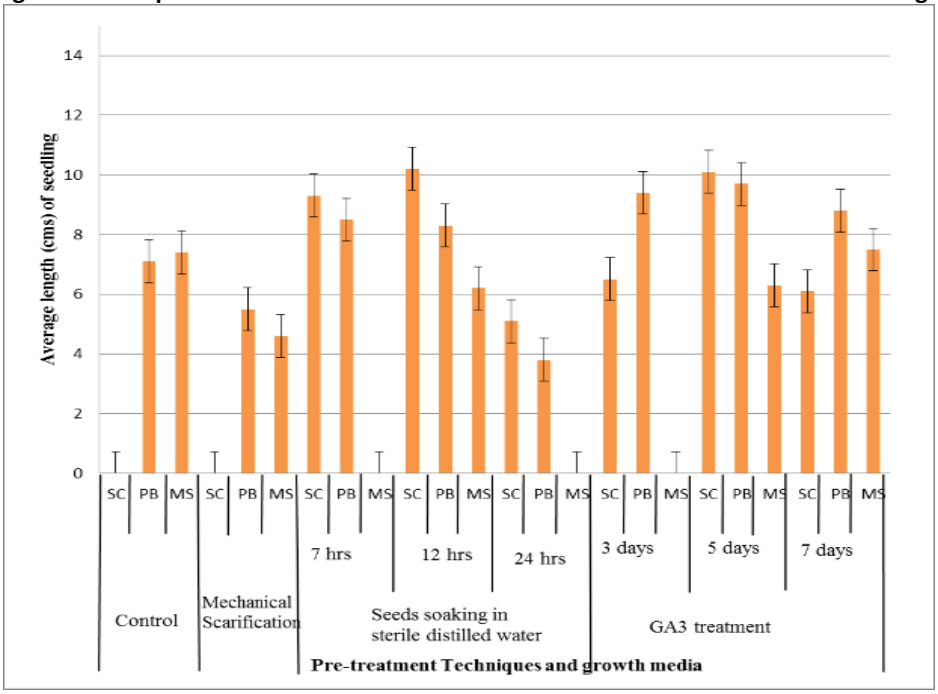
SC-Sterile cotton, PB- Paper-Boat, MS- MS (Murashige and skoog) medium

Fig .2- Effect of pre -treatment methods for propagation.



SC-Sterile cotton, PB- Paper-Boat, MS- MS (Murashige and skoog) medium

Fig.3.Effect of pre- treatment methods on measurement of *S. anacardium* seedlings.



SC-Sterile cotton, PB- Paper-Boat, MS- (Murashige and Skoog) medium

RESULT AND DISCUSSION

The Phenolic complexes markedly decayed in plants treated with the dissimilar levels of GA3 associated with the raw plants [10]. Presoaking of nuts in growth regulator similar gibberellic acid (GA3) and indole acetic acid (IAA) [11]. Are known to overcome in expression of seeds and can also enhance seed germination proportion. In tree species seed germination is hard task due to rigid seed coat and sleeping embryos [12]. *Elaeocarpus Prunifolius seed shows* joint inactivity (physical and physiological) as strong seed coat and application of GA3 successfully broke inactivity [13]. The seeds are bounded in tough coats and show very meager propagation rates in spite of several developments [14]. The explants resulting from mature trees produce a lot of phenolic compounds that result in- browning of the explants and the medium subsequent in tissue death (15).

Seeds were allowed for germination on 3 different approaches, sterile cotton, Paper boat and MS medium. Mechanically scarified decocted seeds germinate earlier than raw seeds. The month of October appears the most satisfactory time for highest ratio of germination and propagation. Mechanical scarification procedure was successfully removed seed coat with the support of small knife and uneven sandpaper. These seeds germinate early related to normal seeds. Seeds were soaking distilled water at dissimilar time stages 7hrs,12hrs,24hrs and allowed them to propagate three dissimilar procedures. Outcomes shows that low germination ratio was noted in seeds were soaked in 7hrs and 12hrs. Pre- soaking of seeds in distilled water for 24hrs demonstrations high germination ratio associated to control and mechanical scarified seeds. GA_3 acts as a straight on embryo releasing them from inactivity through promoting protein synthesis and GA_3 also stimulates seed germination by construction of a-amylase enzymes which change insoluble starch into soluble sugars and it also initiates the radical growth by eliminating some metabolic blocks [16]. Achievement of seeds with GA_3 resulted highest percentage (90%) of germination compared to other treatments. Among the different kinds of treatments Pre -soaking period of 7days improved *in vitro* seed germination on paper boat method compared to other 10 days in sterile cotton and 15 days in MS medium. Soaking in distilled water for 1-7days for the become softer of their seed coat. During imbibition, seeds prolonged and there were dissimilarities in size. The propagation process happening with speedy water absorption which happened during the primary 17 to 72 h imbibition, where, in assessment with the dehydrated seeds. [17]. Various methods have been used for breaking seed dormancy and seed propagation in *Semecarpus anacardium* [18]. *In vitro* regeneration of *S. anacardium* seeds appear to be problematic job due to seeds which have rigid seed coat, minor seed propagation rate and higher quantity of phenolics, long dormancy and microbial infection. Short seed capability has limited

conventional propagation. Added to this, since seeds are used in large scale medicine preparation and as nutrition, replanting becomes scarce. We developed simple and reproducible and enhanced *in vitro* seed germination and seedling development protocol for *S. anacardium* using mature seeds. Pre-soaking of seeds with 0.1% GA3 for 7 days and inoculating on PB with sterilized distilled water improve propagation percentage and successfully enhanced *in vitro* seed development compared to other methods. Different types of treatments were tested for germination 3-5mg/l GA_3 treated seeds was found more suitable for *in vitro* germination compared to other treatments.

ACKNOWLEDGEMENTS

The authors are grateful to the Head, Department of Botany, Kakatiya University for providing necessary facilities, employed atmosphere and author would like to thanks to University Grants Commission for providing the financial assistance under Rajiv Gandhi national fellowship.

REFERENCES

1. Patel L.S., Patel R.S. Rapid *In vitro* Micro Propagation of *Asparagus racemosus Wild* from Nodal explants. International Journal of Current Microbiology and Applied Sciences, 4 (5): 607-617, (2015).
2. Poornima M., Anita S, Vinod Kumar G. *Semecarpus anacardium*: A review. International Ayurvedic Medical Journal, vol 1(6):70-76, (2013).
3. Sanjeeb Kumar M., Prashant T . Pratap Kumar S. Pharmacology, Phytochemistry and Toxicology of *Semecarpus anacardium.*International journal of pharmaceutical Sciences Review and Research, 42(2): 25-31, (2017).
4. Amaley Kranti Kumar D., Jain Atul S. A Review article on Bhallataka (*Semecarpus anacardium)* Linn. International Ayurvedic Medical Journal, vol 3(1): 1-6, (2015).
5. Dr. Dattatray S., Kamini B, Jaiswal M. Bhallataka (Semecarpus anacardium Linn.f.): A comprehensive Ayurveda review. International Journal of advanced Research, 4(6): 445-457, (2016).
6. Sandip Patel., Dipa Raval. The role of bhallataka (*Semecarpus anacardium*) in the management of rheumatoid arthritis for clinical study. International Journal of Researh Trends in Science and Technology, 381-385, (2018).
7. Premalatha B., Semecarpus *anacardium* Linn nuts -A boon in alternative medicine. Indian journal of Experimental Biology. vol 38:1177-1182, (2000).
8. Rout G.R., Senapati SK, Aparajeta S. Micropropagation of *Acacia chundra* (Roxb) DC. Hort Sci, 35(1) :22-26, (2008).
9. Damiano C., Padro MDA, Frattarelli A. Propagation and establishment in vitro of myrtle (Myrtuscommunis L.) An international journal Propagation of ornamental plants, 8 (1):3-8, (2008).
10. Gehan , Mostafa G, Mona F, Abou Alhamd .Effect of Gibberellic acid and Indole -3-acetic acid on improving growth and accumulation of phytochemical composition Balanites aegyptiaca plants. American Journal of Plant Physiology, 6(1) :36-43(2011).
11. Chauhan J.S., Tomar Y. K, Indrakumar Singh N, Seema Ali, Debarati. Effect of Growth Hormones on Seed Germination and Seedling Growth of Black Gram and Horse Gram. Journal of American Science,5(5) :79-84, (2009)
12. Jaiswal P., Chaudhary S, Germination behaviour of some trees and grasses of arid lands Bull Nat Inst Ecol1, 5: 201-205, (2005).
13. Iralu V., Krishna Upadhaya, Seed dormancy, germination, and seedling characteristics of *Elaeocarpus prunifolius* Wall. ex Müll. Berol.: a threatened tree species of north-eastern India. New Zealand Journal of forestry Science, 48:16, (2018).
14. Joshi MD, Kelkar SP, Germination of seed in dry teak (Tectono grandis L) 1 Preliminary studies in fruit development and seed dormancy. Oldest international peer Reviewed Forestry journal ,97(4) : 210–212,(1971) .
15. Sweta Mishra., Yuvraj K Vala. Efficient plant regeneration protocol for *Simarouba glauca* DC: A medicinal tree and an alternative source of edible oil-using explants derived from physiologically mature trees. Journal of pharmacognosy and phytochemistry ,7(2): 91-96, (2018).
16. Patel M. S., Nurbhanej, K.H, Rathod M. J, Vyas S. V, N.H. Bhutiya. Effect of Media and GA_3 on Seed Germination of Custard Apple (*Annona squamosa* L.) cv. Sindhan. International Journal of Pure and Applied Bioscience, 5(4): 969-975, (2017).
17. Martinez-Maldonado F.E., Miranda- Lasprilla D, Magnitskiy S. Sugar apple (*Annona squamosa* L., Annonaceae) seed germination: morphological and anatomical changes. Agronomia Colombiana, 31(2) :176-183, (2013).
18. Panda B.M., Hazara S. Seedling culture of *Semecarpus anacardium* L. Seed Science and Biotecnology ,3(2) :54-59, (2009).