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Seed Germination Studies in *Pterocarpus santalinus* L.f. – An Endangered and Endemic Medicinal Plant, and Relevance to Conservation

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ABSTRACT

Pterocarpus santalinus L.f. (Fabaceae), an endangered tree, is commercially and medicinally the most valuable endemic to the Deccan ecoregion of India. It is a highly recalcitrant leguminous species and the seeds show a dormancy period of approximately one year. Studies on seed germination from various locations were carried out with the aim of producing appropriate germination protocols for use in *ex situ* conservation. In field conditions 60% germination was observed from seeds collected from Balpally. *In vitro* studies revealed 100% germination with 3% sucrose solution i.e., in water and in different combinations of Murashige and Skoog medium, which is highly cost effective. Among all the locations of collection, seeds from Balpally and Papireddypally showed the highest percentage of seed germination.

Keywords: Deccan region, dormancy, *in vivo* and *in vitro* seed germination, leguminous tree, plant growth regulators

INTRODUCTION

Knowledge of seed germination and seedling establishment is a prerequisite for the successful restoration of forests seldom articulated in forest conservation and management plans especially for rare and an endangered tree species. The majority of dry tropical species possess orthodox seeds, which are characterized by dormancy due to a tough seed coat which is mostly overcome by mechanical, acid scarification or sometimes the transit through animal guts.

Pterocarpus santalinus L.f. (Fabaceae) is one among the above-mentioned species, commonly termed in the trade as "Red sanders", and locally considered as the "Pride of India" and the "State Tree of Andhra Pradesh". It is an endangered and endemic tree that occurs in Southern regions of Eastern Ghats (Ahmed and Nayar 1984; Jadhav *et al*. 2001). In Japan the heartwood is used for the manufacture of a famous musical instrument called "Shamisen". A natural dye santalin is extracted from the wood, and is used for colouring pharmaceutical preparations and food items. Extracts of wood and fruit have been extensively used as an antidiabetic, astringent, diaphoretic and for curing inflammations, dermal diseases, bilious infections, etc. (Anonymous 1969). Seed germination has been advocated as one of the most viable biotechnological tools for the *ex situ* conservation of threatened germplasm (Kameshwararao 2004). In view of its high timber price, overexploitation, restricted distribution and poor seed germination there is an urgent need for conservation of this plant species failing which the category of endangered may move to extinct.

Externally there has been unprecedented loss of forest genetic resources over the past two decades in the tropics due to habitat destruction, over-exploitation, pollution and conversion of forest area into non-forestry purpose (Padmalatha and Prasad 2006). According to the reports of Dayanand and Lohidas (1988) and Kesavareddy and Srivasuki (1990) various limitations in propagation of red sanders by natural means are prolonged dormancy, low germinability and poor viability of seeds. Clonal multiplication through rooted cuttings is also very difficult. Poor seed germination was reported by Kalimuthu and Lakshmanan (1995). *In vitro* multiplication also did not prove too successful as too few shoots were raised (Lakshmisita *et al*. 1992). Hence to build up proper conservation management practices which in turn may help in the improvement of elite germplasm which is biotechnologically relevant for mass propagation by using plant tissue culture, the present studies were primarily concentrated on standardization of seed germination technique of pods collected from different locations of Andhra Pradesh (AP). The viability of the seeds was checked periodically for every three months and the seedlings were raised in natural as well as in *in vitro* conditions.

MATERIALS AND METHODS

Mature dried pods from different locations i.e., Balpally, Papireddypally, Cuddapah (Cuddapah), Visakapatnam, Rapur, Veligonda Hills (Nellore), Talakona, Tirupathi and Papavinasanam (Chittoor) were collected during summer (March-June). All the locations in parenthesis mentioned are district names. The samples were collected at random from a particular location. The viability testing (germination percentage by visual observation) of the seeds was carried out by using mature pods (100 in number) by germinating them in the field and also in *in vitro* conditions from different locations. Since most of the chemicals are very harmful, when the seeds are treated with them, they may lead to loss of germination because the embryo may be affected. Thus pods were used directly. The mature stage of the pods was mostly characterized by seasonal variations as the fruit becomes mature during summer and the pod colour will also be brown in nature. For *in vivo* seed germination directly mature pods collected in the fruiting season were soaked initially in tap water for 48 hrs and germinated in field conditions. In another set of experiments pods were washed thoroughly with double distilled water (DDW) and were treated with different chemicals such as KNO_3, H_2O_2, KCl, HNO_3, H_2SO_4, GA_3 and KN (100, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500 and 5000 ppm) at different time intervals (5, 10, 15, 30 and

Received: 14 November, 2006. Accepted: 10 August, 2007.

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60 min) and also subjected to mechanical scarification apart from hot water and cold water treatments. Germination percentage was tabulated after 3 days.

For *in vitro* seed germination studies the viability of the seeds (100) was checked by using MS basal media for which mature dried pods were sterilized by subjecting to scarification by soaking them in tap water overnight or by using boiling water i.e., at 100°C (5 min) or sulphuric acid (5% for 10 min) and the seed coat was removed mechanically. Seeds were washed in tap water for 30 min and further treated with 2% Bavastin® (Carbendazime (BASF India Ltd, Maharashtra) for 30 min (seeds) followed by 70% (v/v) ethanol treatment for 2 min and with 0.1% (w/v) mercuric chloride for 12 min, under sterile conditions and finally rinsed 4-5 times in sterile DDW with duration of 5 min each.

Seeds were aseptically placed on different strengths of MS (Murashige and Skoog 1962) medium with different percentages of agar (Himedia Chemicals, India) {0.8% (PS 1), 0.7% (PS 2), 0.6% (PS 3) and 0.5% (PS 4)} and without agar (liquid medium) MS (PS 6), ¾ MS (PS 7), ½ MS with 0.8% agar (PS 5), full strength MS medium with different percentages of phytagel (Sigma Chemicals, USA) (0.1% (PS 10), 0.2% and 0.3%), different concentrations of GA_3 (gibberellic acid) (Sigma Chemicals, USA)(1.0 $mg.L^{-1}$, 2.0 $mg.L^{-1}$ and 3.0 $mg.L^{-1}$), in 3% sucrose (water) (PS 12) alone and on 1% agar (water) (PS 11). Seeds treated with cold (PS 8) and hot water (PS 9) were cultured on MS medium with 0.8% agar and percentage of germination was recorded after two weeks of culture. The data was analysed statistically by using ANOVA test (Analysis of Variance) with both Randomized and Critically randomized block designs for *in vivo* and *in vitro* germination studies.

RESULTS AND DISCUSSION

In *in vivo* conditions, maximum seed germination (50-60%) was observed from pods collected from Papireddypally and Balpally and the lowest percentage (5%) of germination was noticed from pods collected from Visakapatnam. The pods, when treated with different chemicals such as H_2SO_4, KNO_3, KCl and HCl for different time intervals, seed germination was observed only in pods treated with KNO_3 (100 ppm) for 30 min. The percentage of germination was much less (2%) (**Fig. 1A, 1B**).

In vitro seed germination studies revealed that overnight soaking in tap water followed by mechanical scarification was most effective. In *in vitro* conditions, 100% seed germination was observed from seeds collected from Balpally on MS (liquid), ½ MS (liquid), 3% sucrose, 1% agar and MS with 0.1% phytagel. Seed germination on MS medium after 15 days from seeds collected from various locations of Balpally is shown in **Fig. 1C-D**.

According to Kalimuthu and Lakshmanan (1995), *P. santalinus* shows poor seed germination. Factors which affect seed germination may be seed dormancy, temperature, water stress, predation, pod (seed) size, light intensity, soil moisture, seasonal variations and site of collection. The optimum temperature for seed germination in *P. santalinus* was between 20 and 25°C. Seeds from different locations exhibited differences in germination, seed size and seedling growth even in the natural forest areas as was noticed during a field survey in the forests of Cuddapah, Tirupathi, Nellore and Vishakapatnam. In Cuddapah, where *P. santalinus* is the most persistent and dominant species, the pods (seeds) are bigger than those collected from Vishakapatnam. Emergence, establishment and growth of seedlings face very heterogeneous situations in the natural dry deciduous forests. The difference in the size of the pods during our field observations in different locations may be purely due to the influence of the environment or it may be due to the difference at the genetic level which has to be studied further.

In vivo seed germination was found to be maximum in pods collected from Balpally compared to other locations (**Fig. 1B**). This may be due to the larger size of seeds compared to those collected from Vishakapatnam, which showed the lowest germination percentage (5%), which might be due to the local edaphic and environmental factors, which interfere with the seed production, germination, survival and seedling development (data not shown). A greater food reserve in larger seeds may enhance their ability to persist by providing metabolic requirements during the quiescence period, until suitable light or moisture conditions arise. Compared to smaller seeds, larger and heavy seeds contain more reserves to stimulate germination, seedling survival and growth (Teketay and Granstrom 1997). Young seedlings from large seeded species withdraw nutrients for their successful establishment, survival and early seedling growth more from the cotyledons than from the soil. Hence seed size represents a trade-off between seedling establishment and seed dispersal efficiency in wind-dispersed tree species i.e., *P. santalinus*.

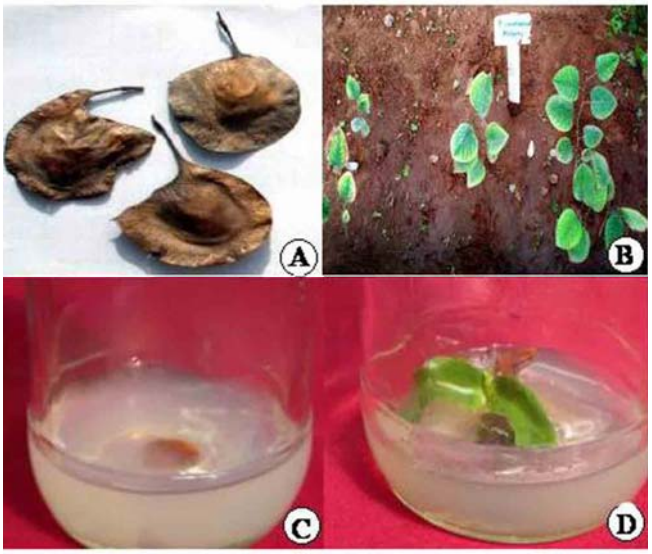

Fig. 1 (**A**) Pods of *Pterocarpus santalinus* collected from Balpally (Cuddapah District). (**B**) *In vivo* seed germination of *P. santalinus* in normal field conditions from pods collected from Balpally. (**C**) Seed of *P. santalinus* collected from Balpally on MS basal medium with micropylar end touching the medium. (**D**) *In vitro* seed germination of *P. santalinus* on MS basal medium.

During *in vivo* seed germination, acid, hot water and mechanical scarification have been found suitable, mechanical scarification being the most effective (data not shown) similar findings were also reported in *Olea auropaea* and *Podocarpus falacatus* by Milberg and Lamont (1997). Pods of *P. santalinus* collected from various locations responded only to potassium nitrate treatment (100 ppm) for 30 min but the percentage of germination was very low (2%). A hard seed coat prevents the entry of moisture during isolated showers in the middle of a long dry season while permitting it during a sustained rainy season. The extent of dormancy varies within a species, and as a result, individual seeds become permeable to water at different times, which results in staggered seedling recruitment providing an insurance against spells of unfavorable conditions. The soil seed bank thus considerably produces seedlings continuously for several years due to different periods of dormancy. Therefore in *P. santalinus*, seed germination in natural field conditions was more effective and among all of them pods collected from Balpally showed a higher germination percentage and hence could be considered as superior seeds (*in vivo*) compared to those from other locations (**Fig. 1A-D**).

In vivo seed germination of *P. santalinus* was much less during summer inspite of proper irrigation, which may be due to increased water stress. Germination and mortality were found to be highly seasonal. As the fruiting period is during summer, mature pods are obtained by the end of April or June and the germination of these pods starts immediately depending on the availability of the external resources like water in spite of the dormancy (1 year) as reported earlier. Competition from annual herbaceous flora

Table 1 Randomized Block Design Analysis representing the *in vivo* seed germination when seeds of various locations (Balpally, Papireddypally, Cuddapah, Visakapatnam, Rapur, Veligonda Hills, Talakona, Tirupathi, Papavinasanam) were used.

Source of variation	Degrees of freedom	Sum of Squares	Mean Squares	F Ratio
Replications	4	388.8542	97.2135	0.49
Treatments	11	16140.7246	1467.3386	7.35
Error	44	8780.7334	199.5621	-

Experimental Mean Value: 73.9776
Coefficient of Variation (CV): 0.19
Standard Error of Treatment Mean: 6.317
Standard Error difference of two Treatment Means: 8.93
Critical Difference at 5% level: 17.99

Table 2 Complete Randomized Design Analysis representing the *in vitro* seed germination when seeds are subjected to various treatments (MS +0.8% agar, MS + 0.7% agar, MS + 0.6% agar, MS + 0.5% agar, ½ MS + 0.8% agar, MS liquid, PS-7: 3/4th MS liquid, MS with cold water treatment, MS with hot water treatment, MS + 0.1% phytagel, PS-11: 1% Agar (water), 3% sucrose (water).

Source of variation	Degrees of freedom	Sum of Squares	Mean Squares	F Ratio
Treatments	8	319.8	39.9	6.95
Error	36	207.1	5.7	-

Experimental Mean Value: 5.75
Coefficient of Variation (CV): 0.41
Standard Error of Treatment Mean: 1.07
Standard Error difference of two Treatment Means: 1.51
Critical Difference at 5% level: 2.97

must be overcome for successful germination and survival during rainy season.

Low viability and decline in germination percentage may also be due to the loss of moisture, which is associated with a layered seed coat (3 layers), increased leachate conductivity and decreased fatty acid content due to aging in certain seeds (Thapliyal and Connor 1997).

To enhance the rate of germination, *in vitro* experiments carried out using Balpally seeds resulted in a 100% germination was observed on MS (liquid), ½ MS (liquid), 3% sucrose, 1% agar and MS with 0.1% phytagel. Among all of them 3% sucrose can be chosen for its cost effectiveness (**Fig. 1C-D**). Germination was more effective when seeds were cultured with the micropylar end touching the medium, which would facilitate direct nutrient uptake from the medium. Thus *in vitro* seed germination proved to be very useful for enhancing seed germination and building seedling stocks.

Statistical analysis using ANOVA showed that the differences in the mean values among the treatment groups in the case of seed germination are greater than would be expected by chance. This statistically significant difference observed may be due to environmental differences in case of *in vivo* seed germination or due to treatments of the seeds with different chemical agents in *in vitro* conditions (**Tables 1** and **2**, respectively). The critical difference at 5% level differs greatly in the case of both *in vivo* and *in vitro* seed germination studies, which is clearly in support of the above statement.

Usually seeds, being natural perennating structures of plants, represent a condition of suspended animation of embryos and are best suited for storage. By suitably altering their moisture content (5-8%), they can be maintained for relatively long periods at low temperatures (-18°C or lower).

Propagation from seed is a viable method for *ex situ* conservation of *P. santalinus*, although it has more stringent requirements for germination i.e., a sterile environment and supply of nutrients externally. Germination in non-sterile conditions and without plant growth regulators will allow widespread propagation by regional parks and botanical gardens without the use of specialist facilities. However seed storage and germination are only the first steps in the reinforcement of populations of this species. Studies to attain the baseline data on *ex situ* plant development and establishment in the field following transplantation are now required. As the species is already in the endangered status, extensive population reinforcement is necessary for subsequent multiplication. The results mostly suggest that germination behaviour depends on the site of collection also.

ACKNOWLEDGEMENTS

The authors thank the Department of Biotechnology, Govt. of India (Ref: BT/PR 2273/PBD/17/117/2000 dt. 7-9-01) for financial support from 2000-2004. Thanks are due to the principal Chief Conservator of Forests, Government of Andhra Pradesh, Hyderabad for permission to collect the germplasm from various forests division.

REFERENCES

Ahmed M, Nayar MP (1984) Red sanders tree (*Pterocarpus santalinus* L.f.) on the verge of depletion. *Bulletin of the Botanical Survey in India* **26**, 142-143

Anonymous (1985) *Pterocarpus*. In: Balaji S (Ed) *The Wealth of India. Raw Materials* (Vol 7) CSIR Publications, New Delhi, 422 pp

Dayanand T, Lohidas T (1988) Effect of different treatments on pod germination of Red sanders (*Pterocarpus santalinus* L.f.). *Indian Journal of Forestry* **11**, 87-88

Jadhav SN, Reddy KN, Reddy CS (2001) Conservation assessment and management planning for medicinal plants of Andhra Pradesh. Medicinal plants conservation center (MPCC) and FRLHT. Hyderabad, India, pp 1-39

Kameshwararao N (2004) Plant genetic resources: Advancing conservation and use through biotechnology. *African Journal of Biotechnology* **3**, 136-145

Kesavareddy K, Srivasuki KP (1990) Vegetative propagation of Red sanders (*Pterocarpus santalinus* L.f.). *Indian Forestry* **116**, 536-539

Kalimuthu K, Lakshmanan KK (1995) Effect of different treatments on pod germination of *Pterocarpus* species. *Indian Journal of Forestry* **18**, 104

Lakshmisita G, Sreenatha KS, Sujata S (1992) Plantlet production from shoot tip cultures of Red sandal wood *Pterocarpus santalinus* L.f. *Current Science* **62**, 532-534

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-497

Padmalatha K, Prasad MNV (2006) *Pterocarpus santalinus* L.f and *P. marsupium* Roxb. Potential source of phytomedicines. In: Sharma KK, Aurora R (Eds) *Herbal Drugs: A 21st Century Prospective*, Jaypee Brothers, New Delhi, pp 450-457

Teketay D, Granstrom A (1997) Germination ecology of forest species from the highlands of Ethiopia. *Journal of Tropical Ecology* **14**, 793-803

Milberg P, Lamont BB (1997) Seed/cotyledon size and nutrient content play a major role in early performance of species on nutrient-poor soil. *New Phytologist* **137**, 665-672

Thapliyal RC, Connor KF (1997) Effects of accelerated ageing on viability, leachate exudation and fatty acid content of *Dalbergia sissoo* Roxb. seeds. *Seed Science and Technology* **25**, 311-319