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RESEARCH ARTICLE

ENTADA PHASEOLOIDS SEED DORMANCY AND GERMINATION: IMPLICATIONS FOR CONSERVATION AND RESTORATION.

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Abstract

Entada phaseoloids is a woody liana growing in the dense forest on huge trees. Its seed posses many medicinal properties and its main activity is anti inflammatory. Anti inflammatory activity is due to the presence of high saponin content. Hence the seeds are collected in huge quantity by pharmaceutical and cosmetic companies. Due to the change in climatic scenario and human intervention in all over the world the forest cover is shrinking and so the natural habitat of the plant is reducing. In addition, due to the thick seed coat and high saponin content, *Entada* seeds remain dormant for more than 5 years. With indeterminate overexploitation of seeds from natural source to meet growing demand by industries, the numbers of wild plants are decreasing day by day. Moreover as the germination is poor, natural multiplication of this valuable medicinal plant is seriously handicapped. Hence it has been listed as endangered**. (**Report,ayush, 2004**)** Realizing the threat of extinction, we have taken up the study of seed dormancy breaking methods and seed germination. The study was carried out to investigate the effect of mechanical scarificatin followed by soaking in cold water for 36 hours on the germination of seeds of *Entada*. The treated seeds were sown directly in the garden soil in small plastic bags. One seed with three replicates were used and 100% germination was obtained after 14 days.

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Introduction:-

Entada phaseoloids belonging to the family Mimosae, sub family Mimosidae which consists of about 30 species in the tropics and subtropics (Luckow, 2005;). *E. Phaseoloids* is an endemic woody liana distributed in the subtropical ever green forests of western ghats of Karnataka, Kerala & Tamilnadu, eastern ghats of Andhra Pradesh.(Sai Vishnupriya,2008). In the Indian system of medicine the plant is called as Bidhanta (Sanskrit), and locally Garambi(Marathi). The seed kernel is potential source of drug for various ailments such as cancer, diabetes, liver disorders, cuts, wounds, snake bite, inflammation etc.

The importance of medicinal plant is well known worldwide. The recent trend of herbal remedies has caused a revival and restoration in traditional systems of medicine. The medicinal plants as such are facing another dimension of loss because of indiscriminate harvesting to meet the increased demand of the herbal drugs. This trend has resulted into over exploitation of the natural resources.

Globally about 10% of world flora may be threatened. According to the 1997 IUCN Red list; about 12.5 % of World's flora is facing the threat of extinction. The same or probably more serious threat is there for the medicinal plants. Using the current global rate of species extinction, about 10-12 % of the medicinal plants of India (i.e. about 800-1000 species) are likely to be threatened. About 95% of medicinal plants in trade in India are obtained from the wild.

The objective of conservation of medicinal plant diversity depends on maintaining stability of their ecosystems and utilization of the inherent phytochemicals for developing high-value products (Ratnam and Teik, 1999).

Investigations on assessment loss of medicinal plant diversity will help the understanding of forces of interference, needs for its re-instatement, management and execution of different creative measures for its sustainable utilization and conservation. Nevertheless, work on biodiversity of these received much less attention as compared to the food plant species. Commercial utilization including cultivation is a new phenomenon in medicinal plants

Entada phaseoloids is under severe threat due to destruction of tropical evergreen forest and unscientific exploitation of the plant parts like bark &seeds for pharmaceutical and cosmetic purpose, which has resulted in the dwindling of population in the wild.

Conventionally the plant is propagated only through seeds which are largest (6-8cm diameter) among the angiosperms. The pod is 1.5 to 2m long and 10-12cm broad. The seed coat is very thick and hard. Dormancy period of seeds prolong up to 5 yrs. (Vidya S.M, 2004)

Based on numerous studies, it is obvious that many seeds are dormant at maturity and, further, that there are various innate mechanisms for delaying germination. *Entada* species show great dormancy. A freshly matured dormant seed is said to have primary dormancy, which develops during seed maturation on the mother plant (Hilhorst, 1995; Bewely, 1997). A classification system for seed dormancy has been published which shows different classes of dormancy like physiological, morphological, morphophysiological, physical dormancy and combinational dormancy. (Baskin &Baskin, 2004). Dormancy of seeds leads to leaving the species for ageing and death without replacements and make the species endangered. Knowledge on seed germination requirements is a critical factor in conservation of plants which produce seeds with high dormancy periods.

Studies have been made by us to evaluate the germination capacity of *Entada* seeds and conserve this rare and important plant genetic resource through dormancy breaking methods.

Materials and methods:-

The *Entada* seeds for the experiments were collected from Kankeshwar near Alibaugh, Maharashtra. Damaged, diseased and undersized seeds were discarded. The healthy seeds were washed and stored under room temperature in dry place.

Viability of seeds were tested by 0.5% aqueous solution of 2, 3, 5-triphenyl tetrazolium chloride as per procedure described in ISTA (Anonymous, 1985).

Seeds for experiment were kept in triplicate. Seeds were surface sterilized with alcohol and sown in plastic bags containing garden soil (50%sand and 50% clay). Plastic bags were kept under sterilized condition in germination chamber.

At all times, during the test due care was taken to maintain sterilized condition by fumigating the germination chamber and wiping it with spirit swabs. The double distilled water was sprinkled if required to keep substrate moist to provide suitable condition for germination. Watering was done daily. The seeds were observed regularly on alternate days for germination. It was continued till the seeds showed no further signs of germination. Criterion for germination was visible protrusion of radicle.

Seeds were kept for normal germination. With their failure to germinate under favourable condition it was confirmed that seeds show dormancy. In order to investigate the mechanism associated with the failure of germination, the seeds were subjected to various treatments of dormancy breaking.

Physical Methods:-

1. Mechanical scarification: The healthy seeds were rubbed with polish paper/sand paper so as to reduce seed thickness. The treated seeds were sown in soil and kept in germination chamber.
2. Piercing of seed coat: The healthy seeds were pierced with hand drill at hylum. These were sown in soil in the plastic bags and kept in germination chamber.

3. Slitting/incising the seed coat: The seeds were slitted/incised at the sides and sown in soil and kept for germination.
4. Heat shock treatment: Healthy seeds were taken and they were first kept in high temperature at about 45^0C and then immediately kept at cold temperature at around 5^0C. These seeds were also kept for germination in germination chamber.
5. Chemical methods
6. Water soaking treatment: The seeds were soaked in the hot water and cold water for 12hrs, 24hrs and 36 hrs. Soaked seeds were sown in garden soil and kept in germination chamber.
7. Combination treatment: Pierced and slitted seeds were immersed in the cold water for 36 hrs and sown in the garden soil and kept in germination chamber for germination.

Observation:-

It was observed that there was no germination noted in mechanically scarified seeds, pierced seeds, slitted seeds, heat treated seeds and water soaked seeds. 70% of germination was shown after 14 days in the combined treatment i.e., pierced seeds soaked in water for 36 hrs. . (Photoplate1 fig.2) 100% germination was noted in slitted and water immersed seeds after 14 days. .(Photo plate 2 fig.1)

Before seeds were kept for germination their viability was tested. Seeds showed 100% viability. .(Photo plate 1fig.1) The seeds subjected to piercing, slitting followed by soaking in cold water for 36hrs kept under favourable conditions showed 70% and 100% germination respectively.

Seedlings were growing luxuriantly after 15 days. (Photo plate 2 fig.2). 30 days after germinated plantlets were noticed to be growing nicely. It is observed that the seedling planted in college garden is growing rapidly.

Discussion:-

The primary reason for the dormancy is because of the thickness of seed coat. According to the classification scheme of seed dormancy it is called as physical dormancy (Baskin&Baskin, 2004)

The strong inhibitory effect of the seed coat on seed germination may be caused by several possible mechanisms, including mechanical constraint, prevention of water and oxygen uptake, and retention or production of chemical inhibitors (Taiz and Zeiger, 2002). The integument breaking or softening, for instance, is needed to remove dormancy imposed by seed coat hardness or impermeability. However, it is very difficult to use mechanical scarification to break the hard seed coat of *E. Phaseoloids.* . Therefore, piercing, slitting of the seed coat, heat treatments, water soaking treatment were used to remove exogenous dormancy. In the present study, it was found that response to the combination treatment was positive (mechanical scarification combined with chemical treatment i.e.,soaking in coldwater for 36hrs after piercing& slitting of the seed coat)

If only physical dormancy was present, the seeds would have germinated by mechanical or chemical scarification. But in case of *E.phaseoloids*, the seeds were found to be unable to germinate after piercing or slitting of the seed coats they germinated only when pierced or slitted seeds were further soaked in cold water for 36 hrs indicating that permeability of the thick seed coats as well as removal of inhibitory substances is required to break the dormancy.

Entada phaseoloids. have saponin content between 8-9%. The effect of high content of saponins is inhibitory for germination. (Siddhuraju, 2002). The combination treatment of piercing and/or slitting seed coat and soaking same seeds in water which was used promoted germination because piercing or slitting of seed coat and soaking removed accumulated saponions which promoted germination and germling emergence was allowed through hole or slit in the seed coat. (Table 1)

This proves that piercing &slitting must have removed seed coat induced dormancy and water soaking must have leached out inhibitory saponins. Thus we conclude that best dormancy breaking treatment for *Entada phaseoloids* seeds is combination treatment.

As germinated seedlings are growing rapidly in our college garden, we suggest that the seeds should be pierced or slitted and then should be thrown in the forests just before beginning of rainy season where they will germinate soon

and will grow rapidly. Thus the natural regeneration of this endangered species will be assured. And this important medicinal, endangered plant can be conserved in its natural habitat.

Table.1:- Percentage of germination after various treatments.

Treatment	No. Of seeds used for treatment	No. Of seeds germinated	% of germination
Mechanical Scarification	3	0	0%
Piercing	3	0	0%
Incised	3	0	0%
Heat treatment	3	0	0%
Water treatment	3	0	0%
Combination treatment(pierced&water treatment)	3	2	66%
Combination treatment(incised &water treatment)	3	3	100%

Photoplate 1:-

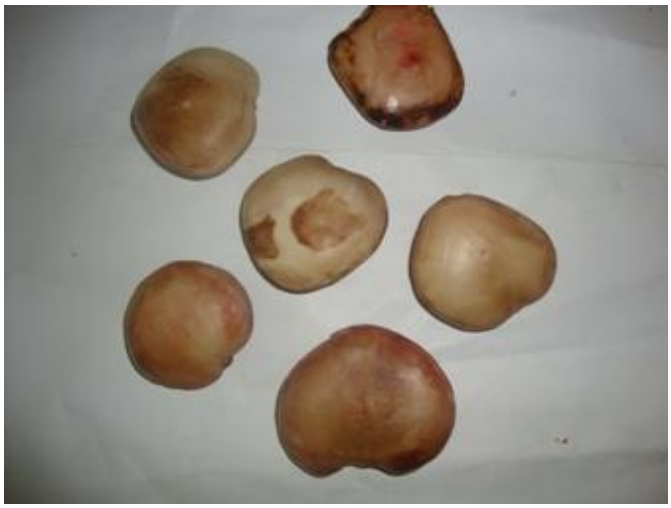

Fig.1:- Seeds showing 100% viability.

Fig.2: Pierced and soaked seeds showing 66% of germination.

Photo plate 2:-

Fig.1: Slitted/incised and soaked seeds showing 100% germination.

Fig.2: Germinated seeds after 15 days.

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