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Effect of physical and chemical treatments on breaking the seed dormancy of *Caesalpinia bonduc* (L.) Roxb.

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ABSTRACT

Caesalpinia bonduc (L) Roxb. is a medicinal plant belonging to the family Caesalpiniaceae was used for the present study. It is a prickly shrub widely distributed all over the world. Keeping the economic and social medicinal uses of C. bonduc seeds are being used widely in Folk, Ayurvedha, Siddha, Unani medicines to treat skin disease, eyesores, cancer, asthma, tuberculorosis, fever, toothaches etc. The aim of this study is to determine the requirements for breaking seed dormancy and germination of C. bonduc. The germination is prevented due to hard seed coat. C. bonduc seeds were experimented with various physical and chemical treatments to break the dormancy. The seeds were subjected to various treatments like mechanical scarification, dry heat method, light, hot water, acid scarification, inorganic compounds, plant growth regulators etc. The seeds treatment with mechanical scarification at 50, 40 and 30 seconds showed 100%, 80% and 10% of germination, whereas no changes was observed in dry heat. White light treatment showed 100% germination at 48 hrs, whereas darkness and red light showed least germination of about 10%. The hot-water treatment showed 100% germination. In chemical treatments, concentrated sulphuric acid scarification showed highest germination percentage, whereas lowest germination was found in nitric acid. Among the plant growth regulators, Gibberellic acid showed 100% germination whereas 2-isopentyl adenine showed least germination of 10% at 50 ppm. Results of this study prove that mechanical scarification was the most effective treatment to overcome dormancy of seeds in C. bonduc.

Introduction

Forest cover on earth support countless species as well as human livelihoods. Forest covers 30 % of the land. Forests are the most biologically diverse and ecologically complex of terrestrial ecosystems that are disappearing at alarming rates. The trees frequently have viable seeds of low life expectancy or seeds of dormant nature. The average dormancy period of *Caesalpinia bonduc* seeds has a longer period, the reason may be due to soil, climate and other factors. The seed dormancy is a block to the completion of germination of an intact viable seed under favourable conditions (1-5).

The term dormancy means seeds will not germinate under unfavourable conditions of temperature, moisture and light. The seed dormancy is under five classes such as physiological, morphological, morpho-physiological, physical and combinational dormancy was understood (6). A seed is a small embryonic plant enclosed in a covering called the seed coat. Seeds of different tree species germinate at different time duration of the year. Keeping in mind the economic, social, medicinal and ecological importance, the seed dormancy have to be broken to induce germination. *Caesalpinia bonduc* (L.) Roxb. is a prickly shrub widely distributed all over the world especially in India including Andaman and Nicobar islands and Sri Lanka. The leaves are bipinnate. Flowers produced in dense terminal racemes and the fruits are brown and downy, bracts squarrose, linear, acute, reaching 1 cm long. Pods shortly stalked, oblong, 4.5 cm, densely armed with wiry prickles. The hard and shiny seeds are 1-2, oblong, up to 1.3 cm long green turning grey.

It is widely distributed in tropical and subtropical regions of Asia and is abundant in Western Ghats. The different parts of the plant is used in popular indigenous system of medicine like folk medicines, Ayurveda, Siddha, Unani and Homoeopathy due to its high medicinal value for the treatment of various diseases. It has been reported that the seeds possess anti diarrhoeal, anti filarial, antiviral, antibacterial, anti microbial, anti fungal, anti diabetic, anti tumar,

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analgesic, anti inflammatory, immunomodulatory, adoptogenic, anticonvulsant, antipasmodie, nootropic, antimocbic, diuretic properties (7-12). However, C. bonduc shows poor germination and low seedlings establishment. The information on seed biology and germination behavior and dormancy pattern of this valuable species is lacking. Therefore, the purpose of the study is to investigate the aspects germination ecology of such as fruit-seed characteristics and germination employing various physical and chemical treatments to standardise the best treatment to break dormancy (13).

Materials and Methods

Study area

Madras Christian College campus, Chennai, Tamil Nadu, India is located at 12°55' N latitude and 80°7' E longitude. The average elevation of the campus is about 30 m above sea level (14).

Source of Seeds

The healthy, viable, uniform sized seeds were collected from the campus. Herbarium specimen has been prepared and deposited in the Laboratory of Ecology (Ref. No. JR 1012), Madras Christian College, Chennai.

Surface Sterilization

The seeds were sterilized with two chemical disinfectants namely 1% $HgCl_2$ for 5 min, followed by 3 min exposure to 70 % C_2H_5OH . 1% $HgCl_2$ is prepared by dissolving 1 gm of $HgCl_2$ in 100 ml of distilled water. The petridishes used for the experimental purpose was first sterilized and lined with Whattman filter paper. The treatment seeds were watered with 5 ml distilled water once in two days throughout the germination period.

Moisture Content of Fruits and Seeds

A batch of 10 leguminous pods and seeds were taken and fresh weight of the individual pods and seeds were analyzed using an electronic balance. The fruits were then dried in a hot air oven at 80 °C for about 12 hours and the dry weight of the fruits were also calculated using the formulae: (15).

Percentage (%) of moisture content= $\frac{FW - DW \times 100}{FW}$

Where, FW – Fresh weight; DW – Dry weight

Germination Percentage

The germination percentage of the seeds of *C. bonduc* in each treatment in our present study was calculated by the formula (16).

Germination percentage (%) =

No. of Seeds Germinated × 100

Total number of seeds

Seed Viability

Test for seed viability was carried out on a weekly basis. This test was done to ensure that the seeds to be used for the experiment were viable and of high quality. Seeds was soaked overnight in beaker containing water. Imbibed seeds were then cut into equal halves using a sharp blade and immersed in 0.1% (TTC) solution. Observation was made at hourly intervals and gradual changes in the color of seed halves were noted. The seed halves which turned pink were viable/Tetrazolium positive and those that did not show any color change were non-viable/Tetrazolium negative and it does not germinate in less scarification action of microbes insects. The results were tabulated and the percentage of viability was calculated (16). The seed viability test was done in the initial stage of the work and the average number of seeds used for each experiment was 10.

Physical Treatments

Mechanical Scarification

The seed batches were subjected to mechanical scarification by rubbing against the floor for 10, 20, 30, 40, 50 and 60 sec duration (17).

Dry Heat Scarification

The seeds were exposed to dry heat in a hot air oven maintained at 10 °C, 20 °C, 30 °C, 40 °C, 50 °C and 60 °C for 5 min along with a control maintained at room temperature (18).

Leaching

The seeds were mechanically scarified by rubbing on the ground. Batches of 10 seeds of were pre-soaked in distilled water for 12, 24, 36 and 48 hrs duration under lab condition (19).

Light Treatment

The seeds were subjected to different radiation like white light, red light, far red and complete darkness in the seed germination chamber. The respective lamps are used in the germination chambers for about 12, 24, 36 and 48 hrs duration (20).

Hot water Scarification

Ten seeds were subjected to hot water scarification by heating the seeds over electric hot water heater. The different temperature is reached by turning the temperature knob and the required temperature is maintained by adding cold water and hot water displaced using a small breaker. The seeds are treated at varying temperatures of 60 °C, 70 °C, 80 °C, 90 °C and 100 °C for about 30 min (21).

Chemical Treatments

Acid Scarification

Sulphuric acid Treatment (H₂SO₄)

Three batches of 15 seeds were pretreated with concentrated (H_2SO_4) for varying durations of time of 10, 20, 30, 40, 50 and 60 min respectively. After the acid treatment, the treated seeds were subjected to leaching in running water. The seeds were then kept separately in petriplates for observation (22).

Nitric acid (HNO₃)

Three batches of 15 seeds were pretreated with concentrated HNO_3 for varying duration of 10, 20, 30, 40, 50 and 60 min to test their germination inability.

After the acid treatment, the treated seeds were subjected to leaching in running water respectively.

Hydrochloric acid (HCl)

Three batches of 15 seeds were pretreated with concentrated HCl for varying duration of 10, 20, 30, 40, 50 and 60 min. After the acid treatment, the seeds were subjected to leaching in running water and kept for germination in petriplates respectively.

Inorganic Compounds

Potassium nitrate (KNO₃)

The different concentrations of KNO_3 such as 0.25M, 0.5M, 1.0M, 1.5M and 2.0M were prepared with distilled water. The seeds were rubbed against the concrete floor, then soaked in the respective concentrations of potassium nitrate for a day and control (23).

Ammonium nitrate (NH₄NO₃)

The different concentrations of NH_4NO_3 such as 0.1%, 0.2%, 0.3%, 0.4% and 0.5% were prepared by adding 0.1 gm, 0.2 gm, 0.3 gm, 0.4 gm and 0.5 gm in 100 ml of distilled water. The seeds were rubbed against the concrete floor, then soaked in the respective concentrations of NH_4NO_3 for a day and control (24).

Plant Growth Regulators

Gibberellic acid (GA₃)

The different concentrations of GA such as 10 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm were prepared with distilled water. The seeds were rubbed against the concrete floor, then soaked in the respective concentrations of GA for a day and control.

2-Isopentyl adenine

The different concentrations of 2- Isopentyl adenine such as 10 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm respectively were prepared with distilled water. The seeds were rubbed against the concrete floor, then soaked in the respective concentrations of 2-Isopentyl adenine for a day and control. The growth hormone has no specific reaction in breaking up of seed dormancy in a very low ratio.

Statistical Analysis

All analyses were carried out in triplicate and the results reported as mean \pm Standard Deviation (SD). The Student's *t* test and ANOVA was used to compare the means between three groups.

Results and Discussion

Seed Viability, Fresh Weight, Dry Weight and Moisture Content Percentage

The percentage of seed viability in *C. bonduc* was found to be 75%. The average fresh weight of the pod was about 4.97 gm while the average dry weight was about 4.81 gm. The average moisture content percentage of the pod was found to be 3.8 % (Table 1). The average fresh weight and dry weight of the seed were analyzed (0.48 gm and 0.44 gm). The average moisture content percentage of the seed is noted to be 8.02 (Table 2).

Physical treatment

Mechanical scarification

The mechanically scarified seeds had significant impact on breaking the dormancy. The seeds that were mechanically scarified for 50, 40 and 30 sec showed 100%, 80% and 10% germination percentage respectively; whereas the germination percentage of seeds that were mechanically scarified for about 10 and 20 sec doesn't show any germination (Table 3). In the present study, it was observed that mechanically scarified seeds showed very high germination percentage. The study (24) revealed that the seeds mechanical scarification improved seed germination and seedling growth. Seeds mechanically scarified with sandpaper had germination of about 83.3% respectively in the seeds of Parkia biglobosa. This shows that mechanically scarified may be effective for breaking dormancy and improving the seedling vigour (25).

Dry Heat Treatment

The seeds treated at different temperatures in hot air oven were found not very effective on breaking the dormancy. The seeds treated at 30 °C, 40 °C, 50 °C, 60 °C, 70 °C and 80 °C didn't show any significant change in the breaking up of seed dormancy. However, incubating seeds at 30/15 °C, 40/25 °C and 80 °C-100 °C were ineffective in breaking dormancy in *Senna marilandica* (6).

Leaching Treatment

The seeds treated in leaching treatment with water at room temperature showed highest of 100%, 80% and 80% germination at 72, 48 and 36 hrs respectively. No germination was seen at 12 and 24 hrs interval (Table 4).

Light Treatment

At different light treatments (White light, Red light, Darkness) the seeds showed a highest of 100% germination at 48 hrs in white light and least germination of 10% were observed in darkness and red light at the same time interval. Germination percentage was observed to be very low in all light treatments. White light shows better result in all time interval when compared to other light treatments. Among the lights that were used red light favours the germination of seeds next to darkness was observed in the present study (Fig. 1).

Hot Water Treatment

The seeds treated at different temperatures in hot water bath treatment showed highest of 100%, 80%, 40% and 30% germination at 100 °C, 90 °C, 80 °C and 70 °C respectively. No germination was seen at 60 °C

Table 1. Moisture content of pods

Fresh weight of pods (gm)	Dry weight of pods (gm)	Average moisture content of pods (gm)
4.97	4.81	3.8
Fable 2. Moisture c	ontent of seeds	
Table 2. Moisture c Fresh weight of seeds	ontent of seeds Dry weight of seeds	Average moisture content of seeds

	•		
Sl. No	Time (in seconds)	No. of seeds germinated	
1	10	-	
2	20	-	
3	30	1.2 ± 0.71(10)	
4	40	8.33 ± 1.24(80)	
5	50	10.33 ± 0.46 (100)	

 Table 3.
 Seed germination in response to mechanical scarification in *Caesalpinia bonduc* (L.) Roxb.

(Fig. 2). Similar results were observed in an earlier report (26).

Chemical Treatment

Acid Scarification

The experimental seeds soaked in concentrated H₂SO₄ at different time intervals showed significant difference in seed germination. The treatment promoted seed dormancy breaking in all time intervals. The highest germination percentage of the seeds in different time intervals such as 10, 20, 30, 40, 50 and 60 minutes are 10%, 10%, 20%, 30%, 50% and 100% respectively. The germination percentage is highest in seeds soaked for 50 and 60 min (50% and 100%) and the lowest germination percentage for seeds treated for 10 min, 20 min, 30 min and 40 min (10%, 10%, 20% and 30%) (Fig. 3). Among these acids used for scarification treatment, concentrated H₂SO₄ promoted seed dormancy breaking more significantly than concentrated HNO₃, and concentrated HCl. The seeds treated with concentrated HCl showed very poor or no germination.

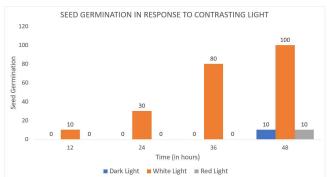
Germination percentage analysis of seed germination effect with HNO_3 showed similar results in lower time intervals when compared to concentrated H_2SO_4 . Higher percentage of 100% result was significantly seen in seed soaked for 50 min in concentrated H_2SO_4 (25).

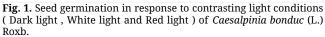
Table 4. Seed germination in response to leaching in Caesalpiniabonduc (L.) Roxb.

Sl. No	Time (in hrs)	No. of seeds germinated
1	12	-
2	24	-
3	36	8.33 ± 1.24 (80)
4	48	8.33 ± 1.24 (80)
5	72	10.33 ± 0.46 (100)

Inorganic compounds

The treatment with KNO_3 didn't have much significance on the germination of *C. bonduc* seeds in all concentrations. The highest germination percentage was observed in 0.5M concentration (40%). Whereas, other concentration with KNO_3 were noted to be 0% respectively. Like KNO_3 , HNO_3 also responded only in low concentration (1M) of 10%, the rest of the concentrations (0.25M, 0.5M, 1.5M and 2M) didn't show any sign of germination (Fig. 4). When comparing both the chemical treatments, no significant effect on breaking the seed dormancy of *Caesalpinia* was observed.





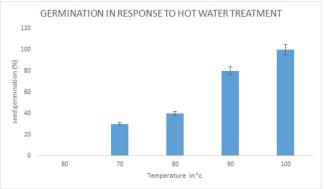


Fig. 2. Germination in response to hot water treatment in *Caesalpinia bonduc* (L.) Roxb. seeds.

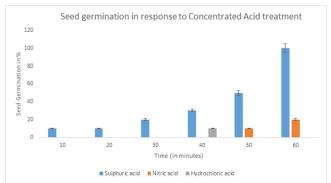


Fig. 3. Response to concentrated acids in seed germination of *Caesalpinia bonduc* (L.) Roxb.

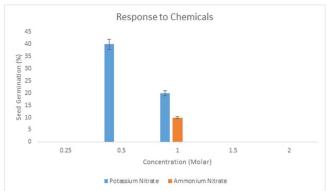


Fig. 4. Effect of chemicals (KNO $_3$ and NH $_4$ NO $_3$) on seed germination of Caesalpinia bonduc (L.) Roxb.

Plant growth regulators

Observations were made in breaking the seed dormancy of *C. bonduc* in plant growth hormones by soaking the seeds for about 24 hrs in both GA₃. The

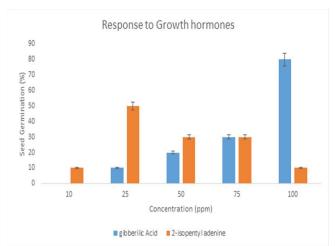


Fig. 5. Effect of growth hormones on seed germination of *Caesalpinia bonduc* (L.) Roxb.

highest germination percentage was percentage at 50 ppm of GA₃ (60%). The germination percentage at 10 ppm, 25 ppm, 75 ppm and 100 ppm were 10%, 20%, 30% and 10% respectively (Fig. 5). When the rate of seed germination of both the growth regulators were compared and analysed 2ip showed about 10% less germination at 50 ppm concentration than GA₃. results were obtained in Echinacea Similar angustifolia (25). General uses of both the growth hormones were found in this growth of plants but no specific use is mentioned in breaking up of seed dormancy.

Conclusion

The seeds of the wild spiny shrub *Caesalpinia bonduc* (L.) Roxb. are dispersed by wind when the mature pods dry up. The seeds were experimented with various physical and chemical treatments to break the seed dormancy. The seeds have physical dormancy due to the hard impermeable seed coat. As it prefers to both shade and direct sunlight, it thereby reduces germination percentage. This study provides data about the germination of seeds which will be useful in regeneration of this species. The study develops an efficient method to break the dormancy. The treatment was given to break the seed coat dormancy. Among the different treatment methods, the hot water scarification of seeds showed 100% germination and rapid growth was significantly observed. This wild spiny plant is not only ecologically important but also serves as a source of medicine and livelihood. So it is important to such wild species. More conserve intense investigation on the reproductive biology of C. bonduc is the need of the hour for conservation and regeneration.

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Authors' contributions

All authors have read and approved the final manuscript. As the corresponding author, I certify that the submission is an original work and is not under review at any other publication, nor has it previously been submitted to any other journal.

Conflict of interests

Authors do not have any conflicts of interests to declare.

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