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Original article

The effect of various seed pretreatments to improve germination in eight indigenous tree species in the forests of Cameroon

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Abstract – Techniques were tested for improving germination in eight tree species indigenous to Cameroon forests. Manual scarification was the most efficient treatment for all species, although a significant interaction between treatment and species was found. Only one species was sensitive to all treatments. The use of sulphuric acid was not an effective alternative to manual scarification. © 1999 Inra/Éditions scientifiques et médicales Elsevier SAS.

scarification / boiling water / sulphuric acid / sowing depth

Resumé – Effet de divers prétraitements sur l'amélioration de la germination de huit essences locales des forêts camerounaises. Des études ont été menées sur la germination de huit essences agroforestières indigènes des forêts camerounaises. La scarification manuelle était le meilleur traitement pour toutes les espèces, bien qu'il existait une interaction significative entre traitement et espèce. Une seule espèce a été sensible à tous les traitements. L'utilisation de l'acide sulfurique n'a pas été une alternative effective à la scarification manuelle. © 1999 Inra/Éditions scientifiques et médicales Elsevier SAS.

scarification / eau bouillante / acide sulfurique / profondeur de semis

1. Introduction

Rapid population growth and demands for increases in land production, especially from agriculture, have led to a rapid disappearance of tropical rain forests and to land scarcity for peasant farmers [11]. As a result, the traditional fallowing system is disintegrating. Fallow periods are being shortened and cropping periods lengthened. This leads to declining soil fertility, falling yields and a serious problem of weed invasion into cropland. These substantial constraints reveal the need to introduce a land use system which can allow the farm-

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ers to save the forest while sustaining agricultural production. Some agroforestry technologies (alley farming, fodder banks, simple improved fallow, home gardens, live fences) have been developed with the objective to improve soil fertility and reduce the fallow period through the use of nitrogen fixing trees and prunings [7]. Most agroforesry trees used in the development of the novel technologies are exotic (*Cajanus cajan, Sesbania sesban, Crotalaria anagyroides, Calliandra callothyrsus*) and are not well known by the farmers. However, there are several local varieties of agroforestry trees including *Alstonia boonei.*De Wild., *Ceiba pentandra* (L.) Gearth., *Cordia platythyrsa* Bark., *Milicia excelsa* (Welw.) c.c Berg., *Pycnanthus angolense* (Welw.) Warb., *Ricinodendron heudelotii* (Baill.) Pierre ex Pax., *Terminalia superba* Engl. & Diels. and *Triplochiton scleroxylon* K. Schum, all of which are well known locally as sources of food, fodder timber and medicine, but their potential as substitutes for the exotics has not been investigated [17, 18]. Even less is known about propagating them from seeds, creating a serious constraint for use in the new agroforestry technologies. Preliminary studies have shown that they have generally poor germination [15]. There are two factors which influence the dormancy degree of the seed: 1) the relative maturity of the seed and 2) the humidity level during maturation [12,13]. The author suggests also that the dormancy level is higher in drier environments. All the influences which occur before the dispersion are 'maternal environment' [22].

The present study aimed at identifying easily applied pretreatments that can be used to treat large quantities of seeds to assure fast, homogeneous and synchronised germination. One specific objective was to overcome the dormancy of *Ricinodendron heudelotii.* The underling hypothesis is that there could be a polymorphism of the germination behaviour of the seeds depending on the presowing techniques applied.

2. Materials and methods

2.1. Description of location

The study site is located in humid lowlands of Cameroon (situated: latitude 2°56'N–3°52'N; longitude 11°32'E–11°57'E; altitude 813 m). According to data collected between 1980 and 1992, mean annual rainfall is 1 269 mm usually distributed in two rainy seasons with a distinct dry period, although rainfall pattern varies from year to year. The average temperature is 23.4 °C; while relative humidity averages 75.1 %. Ferralitic soils predominate. Fallowing is the most commonly used technique for ameliorating soil fertility on farming land, the main crops being cassava, groundnut and maize. Poultry, pigs and small ruminants are the major domestic source of animal protein in the region [8].

2.2. Selection of species

An earlier ethnobotanical survey carried out in the area [8] showed that local farmers had good knowledge of indigenous agroforestry species, the ten most promising being *Ceiba pentandra* (70 % = interviews in which the species was mentioned), *Terminalia superba* (57 %), *Triplochiton scleroxylon* (56 %), *Cordia platythyrsa* (24 %), *Milicia excelsa* (24 %), *Pycnanthus angolense* (24 %), *Alstonia boonei* (18 %), *Ricinodendron heudelotii* (18 %), *Ficus exasperata* (12 %) and *Ficus mucuso* (10.5 %). In the present paper the two *Ficus* species were excluded.

2.3. Seeds collection, processing and handling

Seeds for all species were collected in 1992. Mature and healthy seeds for each species used in different trials were from four seed bearers. After harvesting, they were mixed in view of minimising inter genetic variations. They were screened and handled differently according to their morphological structures. In *T. superba, T. scleroxylon*, the wings of the seeds were removed. Concerning *A. boonei* and *C. pentandra*, the hair and cotton were removed, respectively, before drying. For *M. excelsa*, fruits were crushed by hand in a container with water, fertile seeds falling to the bottom. They were extracted and washed several times with water from the cold supply. As far as *R. heudelotii* is concerned, the seeds were left for about 2 weeks to enable disintegration of the mesocarp after which they were crushed in a container as above. The seeds were washed many times and dried for about 4 weeks. The seeds of *C. platythyrsa* and *P. angolense* were collected, extracted and dirt removed, then pretreated and sown immediately because they are refractory. The seeds of the species which did not fall into this category were dried in the open for 2–4 weeks, after which they were weighed and put in sealed sample bags from International Board of Plant Genetic Resources (IBPGR), put in large, dark polythene bags and conserved in the freezer (20–25 °C) pending the sowing period.

2.4. Methodology

Seeds were germinated in petri dishes lined with filter paper sprayed with 25 mL of water. Ambient temperature in the laboratory varied from 25 to 30 °C.

The treatments used were: 1) seeds soaked for 12 h in water from the cold supply; 2) seeds soaked for 3 min in boiling water; 3) seeds soaked in approximately 0.5 L of 98 % sulphuric acid (just enough to cover the seeds) for 20 min, and stirred every 3 min; 4) seeds hand scarified by puncturing the seedcoats at both the micropyle and the opposite end; and 5) untreated seeds (control). A split-plot experimental design with four replications was employed. The main components were species, with sub-components represented by the various treatments.

In a second germination trial carried out on *R. heudelotii*, seeds were soaked in sulphuric acid (98 %) at different times: 20, 35, 45, 60, 75, 90 and 180 min. The experimental design was a randomised complete block with four replications.

To determine the effect of alternating hot and cold water immersions on the germination of *R. heudelotii*, seeds were soaked as follows: 1) cold (0 °C) water for 12 h; 2) boiling (100 °C) water for 3 min; 3) cold (0 °C) water for 12 h followed by boiling water for 3 min (0, 100 °C); 4) boiling water for 3 min followed by cold water for 12 h (100, 0 °C); 5) cold water for 12 min followed by boiling water for 3 min followed by cold water for 12 h (0, 100, 0 °C); 6) boiling water for 3 min followed by cold water for 12 h followed by boiling water for 3 min (100, 0, 100 °C); and 7) no treatment (control). Four replications were tested in a completely randomised block design.

Concerning the test of the effect of sowing depth on germination of *R. heudelotii*, seeds were sown in a polyethylene bag containing a soil/sand (70/30) mixture at 0, 2.5, 5 and 10 cm. This experiment was set up as a randomised complete block design with four replications.

For each of the above-mentioned experiments, the experimental unit was made up of 60 seeds. The first two experiments were carried out in the open laboratory under ambient conditions, whereas the third was undertaken in the nursery ambient conditions. The seed was considered germinated if the radicle goes through the seedcoat in the first two experiments and when the seedling appears above the substrate for the third one. Data were collected at 3-day intervals. The germination duration ranged from 2 to 9 weeks.

The number of seeds germinated were counted and the percentage germination computed. Variance and correlations were calculated using the statistical package Bstat. Means were compared using Duncan's multiple range test [10].

3. Results

Highly significant ($P < 0.001$) differences were found among species and among pretreatments, resulting in a highly significant species × treatment interaction. Germination ranged from 0 % in *A. boonei* seeds treated with boiling water to 100 % in those of *C. platythyrsa*, *M. excelsa*, *P. angolense* and *T. superba* that had been hand scarified (*table I*). The overall mean germination for hand scarification was over 85 %, indicating that this was the most efficient treatment. Whereas seeds of most species responded well to only one or two treatments, seeds of *C. pentandra* were sensitive to all treatments. Boiling water

Table I. Effect of five pretreatments on percentage germination of *Alstonia boonei* (AB), *Ceiba pentandra* (CE), *Cordia platythyrsa* (CO), *Milicia excelsa* (ME), *Pycnanthus angolense* (PA), *Ricinodendron heudelotii* (RH), *Terminalia superba* (TE) and *Triplochiton scleroxylon* (TR).

		Germination (%)				
Species	Control	Hot water	Ordinary water	Hand scarification	H_2SO_4 98 %	Means
AB	56.7	0	75	82.6	0	42.86
CE	49	59.5	56.6	68.5	77.5	62.22
CO	61	0.5	1.5	100	55.50	43.70
ME	64.6	2.5	91.00	100	0.00	51.62
PA	79.2	1.70	43.3	100	0	44.84
RH	0	1.3	0	61.6	3.30	13.24
TE	61.3	0	71.70	100	63.70	59.34
TR	6	0	5.0	69.0	2.0	16.40
Means	47.23	8.19	25.25	85.21	42.98	41.78

LSD.05 between species: 10.01; between treatments: 5.15.
CV % between species: 23.94; between treatments: 12.33.

killed most if not all the seeds of *A. boonei, C. platythyrsa, T. superba* and *T. scleroxylon,* but not those of *C. pentandra, M. excelsa, P. angolense* and *R. heudelotii,* while sulphuric acid killed the seeds of *A. boonei, M. excelsa* and *P. angolense* (*table I*). Similar results with sulphuric acid have been reported for *Casuarina equisetifolia* even after a 5-min immersion [9]. However, 77.5 % of *C. platythyrsa* seeds germinated and almost 64 % of *T. superba* when soaked in acid for 20 min (*table I*). Cold water alone had very little effect on seeds of *C. platythyrsa* and *R. heudelotii* but had varying effects on the other species. This wide variation in responses to the treatments indicates considerable differences among species in the structure of the seedcoat as protective barrier. Hand scarification produced 100 % germination in seeds of four species, demonstrating that once the seedcoat had been scarified, that is, punctured at both ends, water and oxygen were able to move into the seed tissues and stimule blastogeny. The entry of water modifies the physiological status of the seed [2]. Puncturing at the micropyle is probably significant because the radicle then encounters least resistance to its elongation.

The effectiveness of hand scarification in enhancing germination in the species studied here is supported by observations on *Terminalia ivorensis* [1], *Leucaena leucocephala* [6], *Myrica faya* [14], *Tetrapleura tetraptera* [19, 20], *Ricinodendron heudelotii* [16], *Vitellaria paradoxa* and *Lophira lanceolata* [18] and *Canarium schweinfurthii* [21]. In this study hand scarification significantly increased germination rate in all species. Although sulphuric acid had been recommended as one of the best treatments to overcome seed dormancy in some species [6], the results from the first experiment did not confirm this for *R. heudelotii* seeds. This may have been due to the soaking time being too short. In a second experiment the soaking time in sulphuric acid was increased up to 180 min, resulting in germination from 0 % for soaking duration longer than 60 min, to 15 % (maximum) after soaking for 60 min (*table II*). Despite the differences being statistically significant ($P < 0.05$), germination after soaking in sulphuric acid was inferior to that obtained by hand scarification. Soaking time in acid was not significantly related to germination (dl = 6, r = –0.24, $P > 0.05$), which contrasts with the effects on *L. leucocephala* in which germination increased with longer treatment duration up to 60 min; the seedcoats of the Euphorbiaceae (*R. heudelotii*) is tougher than that of the Leguminoseae (*L. leucocephala*).

When seeds of *R. heudelotii* were subjected to various pretreatment combinations of hot and cold water, no clear trend in germination was found (*figure 1*). No significant differences between treatments occurred during the first 3 weeks after planting, but differences became significant after the 4th week. Exposing the seeds to a 12-h soak in cold water (0 °C) followed by a 3-min soak in boiling (100 °C) water gave the best germination, approximately 65 %. Other combinations of hot and cold water treatments gave less than 50 % germination 2 months after planting. Changing the water temperature creates a mechanical shock which causes a change in the seedcoat, thereby facilitating the incursion of water and oxygen indispensable for germination [3].

It was observed that many seeds died indicating that cold or hot water had made contact with the embryos. This occurred because the seedcoats, which normally regulate the uptake of water, had been damaged and the rapid increase in water caused irreversible damage.

Germination of *R. heudelotii* seeds increased as sowing depth increased (*figure 2*). Best germination, 60 %, occurred at 10 cm, which is contrary to the results for *Metrosideros polymorpha* in which germination decreased with increasing depth [5]. Our study did not, however, investigate sowing depths beyond 10 cm.

4. Discussion

Our results demonstrate that each species has its own characteristic set of germination requirements with a particular threshold of response according to its peculiar degree of heterochrony: the most heterochronic species, the seeds of which are subjected the most to environmental variations during their development, will present the highest plasticity response. It appears that hand scarification significantly improved germination in all species. *C. pentandra* was sensitive to all treatments while *R. heudelotii* responded to only a few treatments. Hand scarification could be regarded as a feasible alternative to sulphuric acid treatment. However, the quantity and the size

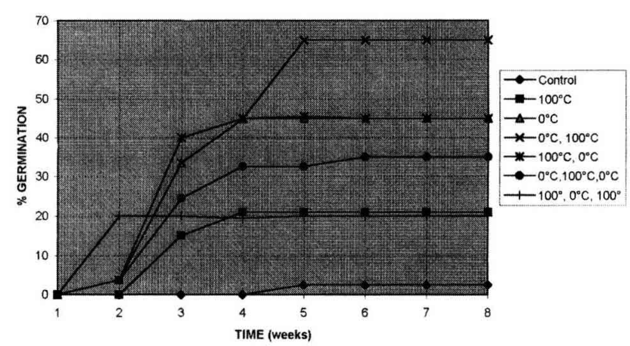

Figure 1. Effect of hot and cold water soaks on the germination of *Ricinodendron heudelotii.*

Table II. Effect of soaking time in sulphuric acid on germination percentage of *Ricinodendron heudelotii*.

Time (min)	Germination (%)
20	1.67
35	1.67
45	1.67
60	15.00
75	0
90	0
180	0
Mean	3.57
LSD.05	4.95
CV %	47.00

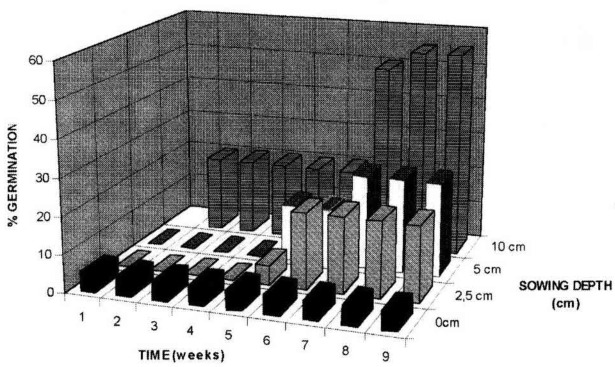

Figure 2. Effect of sowing depth on germination of *Ricinodendron heudelotii* seeds.

of the seed can be a constraint. More research is required to improve the efficiency of this approach. It should be based on the effects on germination of seed maturity, seed position on the branch and position of the branch.

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