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Effect of oven - heat and boiling on the germination and seedling development of *Dichrostachys cinerea* (L) Wight and Arn (Fabaceae)

Macdonald Idu^{a*}, Asotie Conrad Omonhinmin^b

Department of Botany, University of Benin, Benin City, Nigeria
University of Benin Department of Botany, P.MB, 1154, Benin City, Nigeria

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Abstract – *Dichrostachys cinerea* (L.) Wight and Arn. seeds were heated to various temperature ranges in the oven and immersed in boiling water to improve germination and determine the effect of the different pre-treatments on germination and vigour growth of the resultant seedlings. Immersing seeds in boiling water (100 °C) for 10 s recorded the highest germination percentage and energy (94 %; 85 %). Treatment for 40 s also gave good results. Incubating seeds in oven at 80 °C for 360 min recorded high germination percentage (84 %) and germination energy (70 %), seeds heated at 80 °C for 60 min and at 100 °C for 360 min gave encouraging results. Majority of the seeds germinated within the first nine days, and subsequent seedlings from this pre-treatment were mostly of the high vigour category. © 1999 Inra/Éditions scientifiques et médicales Elsevier SAS

Dichrostachys cinerea / germination / seedling development / oven-heat / boiling

Résumé – Effet de la température et du trempage dans l'eau bouillante sur la germination et le développement des plantules de *Dichrostachys cinerea* (L.) Wight et Arn. Les graines de *Dichrostachys cinerea* (L.) Wight et Arn. ont été chauffées à l'étuve à différentes températures ou trempées dans l'eau bouillante afin d'améliorer la germination et de déterminer l'action de ces différents traitements sur la germination et la croissance de la plantule. Les graines trempées dans l'eau bouillante (100 °C) pendant 10 secondes ont montré le plus fort pourcentage de germination et la meilleure énergie de germination (94 % ; 85 %). Le trempage dans l'eau bouillante pendant 40 secondes donna aussi un bon résultat. L'incubation des graines à l'étuve à 80 °C pendant 360 min montra un pourcentage élevé de germination (84 %) et d'énergie de germination (70 %). Des graines chauffées à 80 °C pendant 60 min et à 100 °C pendant 360 min donnèrent des résultats encourageants. La plupart des graines ont germé pendant les neuf premiers jours et les plantules issues de ces germinations ont montré une forte énergie de germination. © 1999 Inra/Éditions scientifiques et médicales Elsevier SAS

Dichrostachys cinerea / germination / développement des plantations / température / eau bouillante

Communicated by Gérard Guyot (Avignon, France)

* Correspondence and reprints
mcdonald@uniben.nigol.net.ng

1. Introduction

Dichrostachys cinerea (L.) Wight and Arn. subsp. *africana* Brenan and Brummitt (*Fabaceae*) belongs to a small genus of the sub-family Mimosoideae. It is wide spread in the tropical savannah region of Africa. It is, however, the only known member of the genus in Nigeria [10]. This multi-purpose and indigenous, but under-exploited, tree species is well known for its fodder, tanning and fuel uses, as well as its soil stabilisation ability in the area where it grows. Its prospective use as an ornamental plant for urban beautification schemes is also high.

Dormancy breakage and germination improvements by heating and boiling treatments have been reported for a large number of seeds with hard coat [3, 4, 5, 6, 7, 9]. Boiling probably stands out as one of the most extensively and successfully employed methods of terminating hard-coat seededness, particularly for seeds of members of the family Fabaceae [9, 13]. To overcome poor germination and achieve maximum nursery recovery in seeds of a lesser-known species, such as *D. cinerea*, a specialised nursery technique, rather than a generalised method is more suitable [12]. However, such pre-treatments can be fully categorised only after its effect on germination, vigour and development of raised seedlings has been assessed. This study is part of an ongoing research into the effect of various pre-treatments on germination, germination vigour and seedling development (performance) of *Dichrostachys cinerea*.

2. Materials and methods

2.1. Source of seed and collection method

Seeds for the study were collected from Gieri, Adamawa State in the Sudan savanna vegetation belt (12 °, 20E; 90 °, 14N), Nigeria. Rip pods were collected, seeds were removed and stored at ambient temperature (28 ± 3 °C) during the experimental period.

2.2. Pre-treatments

Seeds were surfaced sterilised by stirring in 0.1 % mercuric chloride for 1 min and rinsed thoroughly thereafter in several changes of distilled water. Heat and boiling pre-treatment techniques are as outlined by [6, 9].

2.2.1. Oven heat

The effect of heating was monitored by incubating seeds in the oven at 40, 60, 80 and 100 °C for 30, 60, 180, 360 and 720 min. Heated seeds were allowed to cool to ambient temperature before being planted for germination.

2.2.2. Boiling

Seeds were immersed in boiling water (100 °C) for 10, 40, 60, 300 and 900 s. Treated seeds were immediately transferred to cold water.

2.3. Germination

A set of untreated seeds served as control for both the oven-heat and boiling pre-treatments. The treated and untreated seeds (control) were allowed to imbibe distilled water in glass Petri dishes lined with filter paper, following a randomised design of five replicates of 20 seeds each for the different pre-treatment under the prescribed exposure time. Germination was recorded daily for 30 days. A radicle length of 3 mm served as criterion for germination. The whole experimental set-up was allowed to stand under continuous fluorescent light at bench level. After germination, seedlings were transplanted at a planting depth of 2 cm into segmented wooden trays measuring 240 × 120 × 30 cm each and filled with sterile soil (pH = 6.90). Each segment represented the different pre-treatment. Watering was done daily with Harris culture medium. Transplanting was carried out at a three-day interval following a completely randomised design. The height of the seedlings was measured before transplanting.

2.4. Seedling development

The height of transplanted seedlings was recorded at 3-days interval. Seedlings were grouped into vigour categories based on germination and seedling height. The vigour index of Germination Vigour was estimated by calculating the germination energy-maximum daily germination [14]. The experiment lasted for 14 weeks after germination (30 days).

3. Results

3.1 Germination

3.1.1 Oven heat pre-treatment

Table I shows the germination percentages after 30 days, germination energies at eight days and vigour categories of oven-heat treated seeds. Seeds incubated at 80 °C for 360 min recorded the highest germination percentage (84 %) obtained for the oven heat pre-treatment. Similarly heating seeds at 80 °C for 60 min and at 100 °C for 360 min gave good results (75, 55 %). Poor germination was recorded for temperatures below and above 80 °C.

High germination energies were obtained for seeds heated at 80 °C for 30, 60 and 360 min and at 100 °C for 360 and 720 min. The majority of the seeds germinated within the first nine days. Other heat pre-treatments showed lower energies.

3.1.2. Boiling (100 °C) pre-treatment

Immersion of seeds in boiling water (100 °C) for 10 s recorded the highest germination percentage of 94 % (table III). The 40-s treatment also recorded high germination percentage (88 %). Other boiling pre-treatments recorded lower germination. Higher germination energies of 85 and 65 % were recorded for 10 and 40-s boiling treated seeds than for other boiling treatments (table III). Most of the seeds in the high-energy category germinated before the first eight days.

3.2. Height of seedling

Analysis of variance for a complete randomised design was carried out on the height data for seedlings grown from the oven heat and boiling pre-treated seeds to test for the effect of the two pre-treatments. The oven-heat pre-treatment was

Table I. Percentage of germination after 30 days, germination energies after eight days period and vigour categories based on germination height after 30 days for oven - heat pre-treatment.

Pre-treatment	% Germ.	Germ En.	HV	LV
40 °C 30 min	08	03	02	06
40 °C 60 min	10	04	07	03
40 °C 180 min	15	05	10	05
40 °C 360 min	18	05	10	08
40 °C 720 min	10	06	02	08
60 °C 30 min	10	04	04	06
60 °C 60 min	13	08	08	05
60 °C 180 min	16	08	09	07
60 °C 360 min	30	21	18	12
60 °C 720 min	20	14	12	08
80 °C 30 min	40	20	20	20
80 °C 60 min	75	48	58	17
80 °C 180 min	36	20	20	16
80 °C 360 min	84	70	56	18
80 °C 720 min	34	15	18	16
100 °C 30 min	11	04	06	05
100 °C 60 min	10	03	03	07
100 °C 180 min	13	06	34	09
100 °C 360 min	55	18	30	25
100 °C 720 min	48	25	18	30
Control	14	08	05	09

* Values are means of five replicates. % Germ, percentage germination; Germ En., germination energy; HV, high vigour (above mean); LV, low vigour (below mean). Experimental mean height, 6.45cm after 30 days.

associated with an F-Ratio 32.14 (2.04 *table* value), suggesting treatments difference at 5 % level of significance. Comparison of mean height was performed using the Least Significance Difference (LSD) multiple range test (*table II* and *IV*). The comparison of pre-treatments mean height show that heating seeds at 80 °C for 360 and 720 min and at 100 °C for 30 and 360 min, produced seedlings of a higher vigour than the other heat pre-treatments. Similarly, boiling (100 °C) gave greater vigour growth for seedlings raised from the 10 and 40 s pre-treated seeds. Significant differences in seedling height were observed for the pre-treatments under the various temperature ranges and exposure time (*table II* and *IV*).

Table II. Comparison of treatment mean height for *D. cinerea* seedlings raised from oven-heat pre-treated seeds.

Pre-treatment	Ranked mean	LSD(H) + mean
Control	5.80 a	6.89
40 °C 720 min	6.50 ab	7.59
40 °C 30 min	6.70 ab	7.79
100 °C 60 min	7.00 bc	8.09
100 °C 180 min	7.10 bc	8.19
100 °C 720 min	7.10 bc	8.19
60 °C 30 min	7.20 bc	8.19
40 °C 360 min	7.50 bc	8.29
40 °C 180 min	7.80 cd	8.59
60 °C 180 min	8.37 de	9.46
40 °C 60 min	8.40 de	9.49
60 °C 60 min	8.40 de	9.49
60 °C 720 min	8.50 de	9.69
80 °C 180 min	8.60 de	9.89
60 °C 360 min	8.80 de	9.99
80 °C 30 min	8.90 e	9.99
80 °C 720 min	8.90 e	9.99
100 °C 30 min	9.00 f	–
100 °C 360 min	9.10 f	–
80 °C 60 min	9.20 f	–
80 °C 360 min	9.40 f	–

** Mean followed by the same letter are not significantly different at 5 % (LSD = 1.09). Multiple range test (F-ratio = 33.14).

4. Discussion

Dormancy in *Dichrostachys cinerea*, may be due primarily to the impermeability of the waxy seed coat to water and gases, as well as the presence of inhibiting substances which work concurrently with the former to suppress germination to the low level observed under natural conditions, even though the inhibitory substances might not be hormonal in nature [11]. This implies that to overcome dormancy and improve germination in *D. cinerea* seeds, pre-treatments that are severe enough to breakdown the seed coat barrier as well as destroy or dislodge the inhibitory substances will be required.

Seeds differ in their response to heat treatment. The precise sensitivity differs with species resistance to high or low temperature, and principally linked to the duration of exposure. During the present study, oven-heat pre-treatment at 80 °C recorded the highest results for the dry-heat pre-treatment. At 80 °C, 360 min of treatment gave 84 % germination and germination energy of 75 % low germination percentages were observed for lower temperature (40 and 60 °C) and for higher temperature (100 °C). While poor germination in the former (40 and 60 °C) may be due to the less severity of the temperature ranges to crack the seed coat, protein denature at high temperature may account for the low germination recorded for the latter (100 °C). High germination percentages observed for the 80 °C range may be due to the effective disintegration of the seed coat, thereby allowing entry of water and gases and is an indication that the temperature range of 80 °C is probably closer to the seed optimal germination temperature. This view is also shared by others [11]. Similarly, high percentages were obtained for *Parkia clappertoniana* and *Bixa orellana* seeds, respectively [3, 8].

Seed coat barrier breakdown by rupture of the lens tissue [2], or by raising and splitting of the hard coat, creating a passage for entry of water into the seed interior, may have been responsible for the high germination percentages recorded for boiling water pre-treatment for 10 and 40 s. However, pro-

long immersion, which leads to protein denature and seed death due to oxygen scarcity, a condition commonly associated with water at high temperature, probably accounts for the progressively lower germination percentages observed with increased exposure time for boiling pre-treatments (table III). Data from other reports [1, 7, 9] support this view. High germination energies were also recorded for 10 s (85 %) and 40 s (65 %) pre-treatments. The pre-treatments with high energies for the oven-heat and boiling (tables I and III) can be applied in nursery settings to produce uniform planting stock, with a good nursery recovery of high quality seedlings. Poor nursery recovery of seedlings of low planting value will result from the pre-treatments of low germination energies, as they produced seedlings of vary sizes [12]. Of interest is the observation that the control treatments in both cases (oven-heat and boiling) recorded one of the lowest percentage germination and germination energy, and produced seedlings of the lowest mean height. This suggests an initial poor germination energy of the untreated seeds, indicating that the seeds require scarification or other pre-treatments before better seedling performance can be achieved. In conclusion, it is evident that boiling (100 °C) and oven-heat treatment had variable effect on germination and vigour of seedling growth on *D. cinerea*. Boiling water (100 °C) treat-

Table III. Germination % after 30 days, germination energies after eight days period and vigour categories based on germination height after 30 days for Boiling (100 °C) Pre-treatment.

Pre-treatment	%Germ.	Germ. En.	HV	LV
Boiling 10 s	94	85	70	24
Boiling 40 s	88	65	58	30
Boiling 60 s	50	35	28	22
Boiling 300 s	03	20	18	12
Boiling 900 s	05	02	01	04
Control	11	07	89	07

* Values are means of five replicates. % Germ, germination energy; HV, high vigour (above mean); experimental mean height-6.45 after 30 days. LV - low vigour (below mean).

Table IV. Comparison of treatment mean height for *D. cinerea* Seedlings raised from boiling (100 °C) Pre-treated.

Pre-treatment	Ranked mean*	LSD(H)+mean
Control	5.70 a	7.43
Boiling 900 s	6.50 ab	8.23
Boiling 300 s	7.80 bc	9.53
Boiling 60 s	8.05 bc	9.78
Boiling 40 s	8.80 d	-
Boiling 10 s	9.40 d	-

* Mean followed by the same letter are not significantly different at 5 % (LSD 1.73). Multiple range test (F-Ratio = 22.36).

ment for 10–40 s was the most effective and hence present the most ideal pre-treatment for germinating *D. cinerea* seeds and raising high quality seedlings with maximum nursery recovery.

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