Soumis le: 23/03/2018
Forme révisée acceptée le: 20/02/2019
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Nature et Technologie
Nature & Technology
http://www.univ-chlef.dz/revuenatec
ISSN: 1112-9778 – EISSN: 2437-0312

Contribution to the domestication of indigenous *Fabaceae* species of Burundi: *Entada abyssinica* seedling production

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Abstract

In Burundi, species used in reforestation are quasi-exclusively exotic. Indigenous species and their ecosystems are continuously being degraded due to demographic pressure. It is crucial to domesticate indigenous species particularly those with socio-economic role such as *Fabaceae* woody species. This study aims at (i) optimizing seed germination of *E. abyssinica*, (ii) evaluating the mechanical scarification effect on seedling growth and (iii) seedling growth parameters. We used mechanical and boiling water to scarify *E. abyssinica* seeds and imbibed at two duration times. Seeds were germinated at three different temperatures. The results reveal the effectiveness of the mechanical scarification up to 100 % germination compared to boiling water, 75 % (or non-scarified seeds, 4 %) and a quick germination (mean germination time and time to 50 % germination). Deep-scraping on seed hilum differs from a superficial one and produces excised seed radicles and seedlings with abundant and fasciculate root system. Growth parameters reveal fastness of *E. abyssinica* growth approaching one meter per year. The present study concludes that *E. abyssinica* is a fast-growing species that should be adopted in afforestation, reforestation and ecosystem restoration programs. Further research should investigate field growth parameters of *E. abyssinica* and pursue domestication trials of other indigenous plant species with high socio-economic and ecological importance.

Keywords: Burundi; seedling production*; Fabaceae*; indigenous species*; reforestation; d*omestication.

1. Introduction

Species used in reforestation in Burundi are almost exclusively exotic, while around the world, indigenous species represent 3/4 of species used in artificial reforestation [1]. Leakey and Newton [2] observe that 'Cinderella' species remain ignored by researchers and farmers who master only a handful of exotic species domesticated and bred from immemorial time.

Until recently, the relative low level of tropical species domestication might be associated with high tropical biodiversity that satisfied the needs of the market without any production investment [3]. Nonetheless, this is no longer the case. Since colonial times, forestry research on Burundi indigenous tree species has been found unsuccessful for artificial reforestation due to its slow-growing compared to exotic species[4; 5]. Thus, many exotic species were introduced. For instance, *Eucalyptus* seduces foresters and population due to its capability of a quick growth [5;6]. A status quo was then set up: the exclusive use of exotic species whose seedlings are easy to produce in nurseries with a high productivity in plantations. Yet, in recent decades, these species have been criticized for their negative impact on ecosystems such as soil acidification and drying, on the one hand, and decrease of exchangeable phosphorus stock, on the other hand [6-7].

We assume that the exclusive use of exotic species would be explained by the lack of diligent researches to master agronomic techniques of tropical indigenous species [8]. We think that further researches would suggest efficient indigenous species to replace exotic species commonly used in reforestation programs. This may contribute to

A. Reduce the adverse effects of exotic species on soil and biodiversity,
B. Conserve indigenous species,
C. Preserve their natural ecosystems and
D. Serve as a tool for afforestation, reforestation and restoration of ecosystems. In addition, this would efficiently contribute to mitigate species loss, restore degraded ecosystems and sustainably preserve biological resources [9-10].

Our long-term vision is (A) to master the farming techniques (germination, seedling production, etc) of indigenous plant species, and (B) to establish a list of plant species to be adopted in reforestation and agro-forestry programs instead of exotic species.

As there exist a large number of indigenous species, those with obvious socio-economic and ecological interests would be prioritized. This study focuses on *Entada abyssinica (Fabaceae*), indigenous to tropical Africa and adapted to degraded ecosystems.

Thus, the purpose of this study is to contribute to the domestication of *Entada abyssinica* species. We carried out (A) the optimization of seed germination, (B) the evaluation of mechanical scarification effect on seedling growth and (C) the seedling growth parameters.

2. Material and Methods

2.1. Plant material

Entada abyssinica seeds were collected from the savannah of Ruvubu National Park. Seed collection was carried out from July to August 2014. Ruvubu National Park is located in North-East of Burundi between 1350 m and 1900 m of altitude [11; 12]. *Entada abyssinica* generally grows on fertile and deep soil. However, the physionomy of Ruvubu savannah is particularly prone to quasi-annual wild fires. Collected seeds were stored at room temperature in airtight jars.

2.2 Experimental germination protocol

Seeds were sorted out and infected ones or those showing abnormalities were discarded. In order to optimize and homogenize the germination of *E. abyssinica* seeds with coat dormancy, we combined scarification treatment, seed imbibition and germination temperature.

- Scarification treatment: two treatments (in addition to the control) were applied: (i) mechanical scarification and (ii) boiling water scarification. Mechanical scarification was performed by scraping with Emery paper on seed hilum while scarification with boiling water was carried out by soaking seeds in freshly boiled water until complete cool down.
- Seed imbibition: seeds from each scarification treatment were imbibed with water for 0 and 24 hours (0 h means no imbibition).
- Germination temperature: seeds from both scarification and imbibition treatments were set to germinate under three different temperatures (20 ± 0.8; 28 ± 1.4 and 35 ± 0.6 °C).

A set of 8 seeds with 3 replicates was used in each treatment (scarification, imbibition and germination temperature), thereby making a total of 144 seeds used. We used virgin peat containing 67.39 ± 0.03 % of water and supplemented with around 150 ml of water per litre. The germination process was recorded on a daily basis for a period of four weeks. Seeds were considered to have germinated when the radicle was about 2 mm long, and seeds were systematically discarded once they germinated.

2.3. Mechanical scarification effect on growth parameters of *E. abyssinica*

Mechanical scarification of *E. abyssinica* by scraping on the hilum may be superficial or deep. The latter may affect the root embryo. We evaluated the effect of deep scraping on seedling growth parameters at two- and three-month growth period.

2.4. Seedling production

Seedlings were grown on a mixture of peat and river sand in equal volume in plastic bags of approximatively 2 litres (10 cm diameter and 25 cm height). Pre-germinated seeds were transferred to the bags and twenty-five seedlings were grown in a completely randomized design in greenhouse of experimental station of Faculty of Science-Oujda, Morocco in December 2014. Watering was done daily using tap water.

2.5 Studied parameters

2.5.1 Seed germination parameters

Seed germination parameters and the corresponding formulas were expressed and detailed in Nkengurutse *et al.* [13] :

A. Final germination percentage (FGP): corresponds to the ratio of the number of germinated seeds on the total number of seeds at the end of the experiment
B. Time to 50 % germination (T50): is the time required for 50 % of seeds to germinate
C. Mean germination time (MGT): is the average time (number of days) for seed germination taking into account the time taken by delayed seeds to germinate.

2.5.2 Seedling growth parameters

Following parameters were assessed:

- Height (cm): measured from the collar to the terminal bud of the main stem;
- Diameter (mm): assessed by caliper directly from upper collar,
- Biomass: dry weight of aerial part (stem from collar and leaves) and underground part (roots from the collar). Aerial and underground parts were dried separately at 80 °C for 6 days [14].
- Leave number: evaluated taking into account leaves present at the time of the data collection and those that may have fallen.

2.6 Statistical analyses

Values of different parameters are expressed as mean ± standard deviation (x ± SD). Statistical analysis using T-student test and one- and two-ways analysis of variance (ANOVA) followed by Tukey's test were performed using IBM SPSS statistics software version 21; $p < 0.05$.

3. Results and discussions

3.1. Optimization of *E. abyssinica* seed germination

3.1.1. Final germination percentage (FGP)

Results of final germination percentage (FGP) of *E. abyssinica* seeds shown in figure 1 reveal the effectiveness of the mechanical scarification by scraping with up to 100 % of germination while boiling water pre-treatment yield a maximum of 75 % of germination. However, non-scarified seeds do not reach 5 %. These results confirm previous studies [15], and are better than those of Teketay [16]. Gashaw & Michelsen [17] reported that dormancy breaking can be achieved by shock temperature up to 150 °C for a minute, simulating wild fires. Seeds of *E. abyssinica*, unlike other *Fabaceae* species studied by Nkengurutse *et al.*[13], present coat dormancy as most of other *Fabaceae* of savannahs, semi-arid and arid regions [18–21]. It is worth mentioning that *E. abyssinica* is a savannah species [11]. The optimization germination of *E. abyssinica* seeds can be then achieved using mechanical scarification by scraping on seed hilum. This method is less expensive and can be adopted at the local level for the benefit of rural nursery holders. Statistical analyses show no significant (NS) effect of germination temperature of seeds scarified by scraping on FGP (F=1.44, ddl=2; NS). Similar results are found for seeds scarified by scraping. Nevertheless, previous studies reported the influence of germination temperature. Teketay [16] showed that at 15 °C, *E. abyssinica* seed had very low germination rate, under 5 %. Germination rate increased with raising temperatures, up to 30 °C. Our results show that *E. abyssinica* seeds germinate at higher temperatures up to 35 °C. Similarly, to the germination temperature, there is no effect of imbibition of seeds scarified by scraping or by boiling water on FGP ($F = 1.23$; $ddl = 5$; $p > 0.05$ and $F = 0.8364$; $ddl = 5$; $p > 0.05$, respectively). In addition to the importance of FGP assessment; the relative time of germination is also crucial.

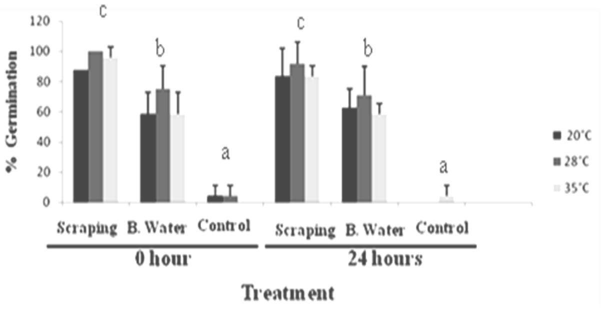

Figure 1. Final germination percentage of *Entada abyssinica* seeds scarified by scraping and by boiling water treatment; control seeds were not scarified. Significant differences (by ANOVA followed by Tukey's test) are shown by different letters (a, b, c) for scarification types but no difference between imbibition times and germination temperatures; B. Water = boiling water; $p < 0.05$

3.1.2. Mean germination time (MGT) and Time to 50 % of germination (T50):

MGT and T_{50} of *E. abyssinica* seeds are presented in table 1. All treatments (scarification, imbibition and germination temperature) significantly influence the germination time. These results show a remarkable role of the scarification type on germination time up to five times in favour of the scarification by scraping (Table 1). Seeds scarified by scraping need 4.19 ± 0.16 days to germinate while those handled by boiling water take 13.16 ± 1.01days in similar germination conditions. Imbibition and germination temperature also shorten significantly the germination time ($F = 20.38$; ddl =2; $p < 0.05$ for effect of germination temperature on MGT of seeds scarified by boiling water). At 20 °C, germination takes an average of 2.56 ± 0.66 and 4.19 ± 0.16 days respectively for imbibed and non-imbibed seeds. Concerning the temperature, non-imbibed seeds germinate respectively after 4.19 ± 0.16; 3.04 ± 0.07 and 2.82 ± 0.06 days in 20; 28 and 35 °C.

Table 1

Mean germination time and time to 50 % of germination expressed in number of days for *Entada abyssinica* seeds scarified by scrapinging or by boiling water and imbibed during 0 and 24 h (0h = non-imbibed seeds) germinating at 20, 28 and 35 **°C**. Control = no-scarified seeds

		Scraping		**Boiling water**		**Control**	
T °C	Imbibition	TMG	T_{50}	TMG	T_{50}	TMG	T_{50}
20 °C	0 h	$4.19 \pm 0.16^{b,y}$	$3.68 \pm 0.17^{b,y}$	13.16 ± 1.01^{b}	13.00 ± 0.86^{b}	5	4.5
	24 h	$2.56 \pm 0.66^{b,x}$	$2.44 \pm 0.27^{b,x}$	13.36 ± 1.24^{b}	13.36 ± 1.12^{b}	-	-
28 °C	0 h	$3.04 \pm 0.07^{a,y}$	$2.52 \pm 0.04^{a,y}$	10.65 ± 0.89^{a}	9.89 ± 1.04^{a}	7	6.5
	24 h	$1.80 \pm 0.40^{a,x}$	$1.21 \pm 0.36^{a,x}$	10.71 ± 0.04^{a}	10.38 ± 0.58^{a}	-	-
35 °C	0 h	$2.82 \pm 0.06^{a,y}$	$2.93 \pm 0.05^{a,y}$	9.27 ± 1.75^{a}	9.11 ± 1.61^{a}	-	-
	24 h	$1.86 \pm 0.29^{a,x}$	$1.26 \pm 0.39^{a,x}$	9.53 ± 1.41^{a}	10.33 ± 2.02^{a}	8	7.5

Significant differences in the same column are shown by different letters for germination temperature (a, b) and imbibition (x, y); the statistical analyses by ANOVA shows a very significant difference between the types of scarification $p < 0.05$; - : data unavailable (because of no-germination and data on control seeds were not compared with those of scarified seeds).

3.2 Effect of deep or superficial seed scraping on growth parameters of *E. abyssinica* seeds

The mechanical scarification by scraping on hilum was adopted according to Teketay [16]. The latter recommends a careful scraping. So, we proceeded to evaluate the difference between a superficial scraping and a deep scraping on seedling growth behaviour. Germinated seeds present morphological differences: deep-scraped seeds are characterized by excised radicles (Figure 2.A), unlike superficial-scraped seeds, characterized by normal radicles (Figure 2.B). Deep-scraped seeds produce seedlings with abundant and fasciculated root system (Figure 2.C) while the latter have a well-individualized and tuber-like taproot with fewer second-order roots (Figure 2.D). Seedlings produced from deep-scraped seeds have two to six small tuber-like roots with less biomass accumulation (Figure 2.C) compared to the other category of seedlings (Figure 2.D).

Figure 2. Effects of mechanical scarification on germinated seeds and seedling morphology.
A: excised radicle of deep-scraped seeds, B: intact radicle of superficial-scraped seeds producing respectively
C: seedlings with abundant and fasciculated root system and D: seedlings with tuber-like taproot

During the first two months of growth, decapitation of the embryonic radicle did not show any impact on growth parameters (height, leave number, stem diameter and biomass) (Table 2). At three months, except root biomass of seedlings from superficial-scraped seeds which was significantly important, all other growth parameters evaluated here remained comparable (Table 2). Whatever deep-scraping affects the root biomass, other parameters were not affected. This would suggest that deep-scraping has no impact on the general growth of the seedlings. We believe that, although fasciculated root system accumulates less nutritive reserves (compared to tuber-like taproot), it remains effective for the physiological role of soil nutrient absorption. Moreover, fasciculated root system allows the seedling to grow enough in plastic bag in nursery. In any case, it seems that the scarification of *E. abyssinica* seeds by scraping is well indicated for seed dormancy breaking and would not require any special care.

Table 2
Growth parameters of seedlings from deep and superficial scraped seeds of *E. abyssinica*

	2 months		**3 months**	
Parameters	Deep-scraping	Superficial-scraping	Deep-scraping	Superficial scraping
Height (cm)	25.53 ± 4.02	23.05 ± 3.42	45.89 ± 7.62	45.41 ± 5.04
Diameter (mm)	2.60 ± 0.30	2.75 ± 2.38	4.72 ± 0.43	4.68 ± 0.75
Leave number	7.50 ± 0.97	7.10 ± 0.99	9.60 ± 1.74	10.00 ± 0.81
Root biomass (g)	0.09 ± 0.01	0.11 ± 0.03	0.42 ± 0.11*	0.84 ± 0.25*
Aerial biomass (g)	0.48 ± 0.12	0.52 ± 0.12	1.57 ± 0.58	1.49 ± 0.54

*shows significant differences $p< 0.05$ by T-student test

3.3 Growth parameters of *E. abyssinica* seedlings of three-months

Mean values of *E. abyssinica* seedling growth parameters recorded at 3 months in nursery are presented in table 3. To evaluate the growth performance of *E. abyssinica* seedlings, we presented in the same table, the growth parameters of two other species, *B. microphylla* and *P. angolensis*, raised under the same conditions (data not published). The height growth of *E. abyssinica* seedlings is 23.68 ± 2.95 cm, corresponding to 3÷3.75 times higher than the two other species. Similar results are noticed for other parameters such as diameter, leave number and biomass. Their values (*E. abyssinica* seedlings) are at least double of those of *B. microphylla* and *P. angolensis* (Table 3). Munyanziza [21] reported that miombo woodland species like *B. microphylla* and *P. angolensis* would favour development of deep taproot during the early stage of growth to the detriment of aerial biomass to adapt the harsh environment. While keeping an important aerial growth, *E. abyssinica* seedlings from savanna showed also an important accumulation of reserves in the taproot. We believe that these reserves allow *E. abyssinica* seedlings to relaunch easily the growth after adverse periods such as fire.

Table 3
Growth parameters of *E. abyssinica* seedlings of three-months

Parameters	***E. abyssinica***	***B. microphylla*#**	***P. angolensis*#**
Height (cm)	23.68 ± 2.95	8.08 ± 1.75	6.31 ± 0.91
Leave number	8.00 ± 1.04	4.18 ± 0.93	4.15 ± 0.70
Diameter (mm)	3.47 ± 0.52	1.51 ± 0.99	2.19 ± 0.19
Root biomass (g)	0.67 ± 0.17	0.23 ± 0.04	0.20 ± 0.05
Aerial biomass (g)	0.83 ± 0.38	0.36 ± 0.07	0.31 ± 0.09

#: Data not published

Some three-month seedlings of the three species were transplanted in 5 liter plastic pot in same condition. *E. abyssinica* seedlings almost reached one-meter height (86.55 cm) after one year while *B. microphylla* and *P. angolensis* seedlings did not attain 10 cm height. Thus, *E. abyssinica* can be qualified as a fast growing species as well as those used in reforestation programs such as *Eucalyptus* [22], *Acacia* [23] and *Pinus halepensis* [24]. Duponnois *et al.* [23] recommend fast-growing leguminous species for reforestation, soil nitrogen improvement and ecosystem restoration.

4. Conclusion

Strategies to master seedling production constitute an important step towards indigenous species and ecosystem conservation. The present study contributes to the domestication of *E. abyssinica* species through:

- The optimization of seed germination;
- The evaluation of mechanical scarification effect on seedling growth and;
- The seedling production of *E. abyssinica* species. The mechanical scarification by scraping constitutes a better method of coat dormancy breaking of the seeds. This method shows a quick seed germination up to five times compared to those using boiling water. The mechanical scarification is less expensive and can be adopted at the local level for the benefit of rural nursery holders. Superficial scraped seeds produce seedlings with well-individualized and tuber-like taproot allowing them to accumulate considerable biomass. Seedlings from deep-scraped seeds have abundant and fasciculated root system that could be better in soil nutrient absorption. Evaluated growth parameters reveal *E. abyssinica* to be a fast-growing species as those generally used in reforestation programs such as *Eucalyptus*, *Acacia* and *Pinus*. Further investigations should focus on field growth parameters of *E. abyssinica* and domestication trials of other indigenous plant species with high socio-economic and ecological importance.

Acknowledgments

This study was supported by Moroccan–Burundian cooperation through “Agence Marocaine de la Coopération Internationale”[1], Burundian government and “Réseau des Institutions de Formation Forestière et Environnementale d’Afrique Centrale (RIFFEAC)”[2]. We would like to express our gratitude to the “Office Burundais pour la Protection de l’Environnement (OBPE)”[3], particularly Mr. Anselme Rufyikiri and Léonidas Baryanintimba for their help during seed collection period. Sincere thanks to Jean Bosco HARERIMANA for his contribution in improvement of the revised manuscript.

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