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Differences in germination capacity and seedling growth between different seed morphotypes of *Afzelia africana* Sm. in Benin (West Africa)

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SUMMARY
Afzelia africana is a multi-purpose woody species threatened by a lack of natural regeneration in the wild. In the present study, differences in seed germination capacity and seedling growth between morphotypes of the seeds of *A. africana* Sm. were evaluated. A total of 600 seeds were collected in the Sudanian and Guinean climatic zones of Africa and their lengths, widths, thicknesses, and weights were recorded. A hierarchical classification and canonical discriminant analysis were applied to the above traits of seeds from the different climatic zones. An analysis of variance with repeated measures was applied to seed morphotypes identified by hierarchical classification to test for the effect of these morphotypes on seed germination and seedling growth. Hierarchical classification helped to identify three seed morphotypes. Canonical discriminant analysis performed on these morphotypes revealed highly significant differences. Morphotype 1 consisted of seeds from the Guinean zone, while seeds from the Sudanian zone were clustered in morphotypes 2 and 3. Morphotype 1 had the longest and heaviest seeds, while the shortest and lightest seeds were from morphotype 3. Morphotype 1 and morphotype 3 seeds showed rapid germination, while only morphotype 1 seedlings displayed rapid growth. Morphotype 1, consisting of large seeds, was superior in terms of its germination ability and seedling growth, and represents the best choice for species restoration purposes.

Afzelia africana Sm. is one of the most threatened multi-purpose woody forest tree species in Africa. The species is used by local people for animal feed (Sinsin, 1993; Onana, 1998), traditional medicine (Sinsin *et al*., 2002), and wood fuel (Ahouangonou and Bris, 1997; Bayer and Waters-Bayer, 1999). The species is found in several types of natural forest ranging from the dense forests of the Guineo-Congolian zone, to the woodland forests of the Sudanian zone (White, 1983). In the Guinean zone of Benin, *A. africana* trees exist at high density, with large diameters (100 – 110 cm) and great heights (31 – 33 m) in typically dense forests, while the opposite is found in fallow trunks (Bonou *et al*., 2009). It is common to observe adult *A. africana* trees in savannah, as well as in woodlands and dense forests, but natural regeneration is rarely observed in these habitats. This situation has been reported across the range of distribution of the species (Sinsin, 1993; Bationo *et al*., 2000). The species is considered to be vulnerable at an international level (www.UICNredlist.org) and in danger of extinction in Benin (Adomou, 2005). It is therefore important to identify those factors that influence the regeneration and development of the species.

A. africana fruit are hard and woody, almost black, and burst violently to discharge the seed (Ren *et al*., 2005). Historically, the seed have been used as a famine food, but required roasting and soaking for several days to render them non-poisonous (Keay *et al*., 1964). In Africa, *A. africana* seed are not available in commercial quantities, but are sold as a food condiment in local markets. Flour made from the seeds is traditionally used for thickening soups and stews (Ren *et al*., 2005). The multiple uses of *A. africana* in West Africa, particularly use of the seeds, has led to a permanent pressure on natural populations. Moreover, the rate of seed germination in the wild has been found to be low.

Since seed germination and seedling growth are influenced by seed size (Rai and Tripathi, 1982; Harms *et al*., 2000; Leishman *et al*., 2000; Mamo *et al*., 2006), this study aimed to assess differences in seed germination capacity and seedling growth between different morphotypes of *A. africana* Sm. based on seed size. The first objective was to assess the level of natural variation in seed size in morphotypes of the species. If morphological variability in seed size could be correlated with their provenance, and linked to their germination parameters, it may be possible to determine which seed provenances would be most suitable for propagation. In addition, the study aimed to compare the rates of germination and seedling growth of different morphotypes, the main hypothesis being that there was a strong link between variation in seed size, germination ability, and seedling growth. This study therefore sought to contribute to the ecological restoration of the species in the wild.

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MATERIALS AND METHODS

Seeds were collected in the Sudanian and Guineo-Congolian climatic zones of Benin. The Sudanian zone is located between 9º45' and 12º25' N, while the Guineo-Congolian zone is located between 6º25' and 7º30' N. The mean annual rainfall in the Sudanian zone is often less than 100 cm, and the relative humidity varies from 18 – 99% (highest in August). The temperature varies from 24º – 31ºC. The Sudanian zone has hydromorphic, well-drained soils, and lithosols. The vegetation in this zone is composed mainly of savannas with trees of smaller size.

In the Guineo-Congolian zone, the rainfall is bimodal, with a mean annual rainfall of 120 cm. The mean annual temperature varies between 25º – 29ºC and the relative humidity ranges from 69 – 97%. The soils are either deep ferralitic, or rich in clay, humus and minerals. The vegetation in this zone is mainly dense semi-deciduous forest.

Thirty mature trees of *A. africana*, located at least 100 m from each other, in order to avoid narrowing-down the genetic base due to relatedness or inbreeding, were sampled in each climatic zone, as recommended by Turnbull (1975). A total of 300 seed were collected at random in each climatic zone between November - December 2009. The collected seed were kept under ambient temperature conditions for 10 d prior to sowing. The length, width, and thickness of each seed were measured using electronic calipers during these 10 d. The weight of each seed was measured using an electronic balance with a sensitivity of 0.0001 g. Seeds were subjected to a viability test using the flotation method. Those seeds that floated on water after 24 h of soaking were considered to be non-viable and were discarded.

The seed length, width, thickness, and weight data were subjected to Ascending Hierarchical Classification (AHC) using SAS 9.1 statistical software (SAS, 2003). This enabled the classification of seeds by size. Canonical discriminant analysis was performed on the morphotypes identified from the AHC in order to validate and test the differences between morphotypes. The morphotypes of *A. africana* seed were also described according to their differences, using canonical discriminant axes defined by seed size. The same analysis was also performed to test and describe the differences between the climatic zones according to seed size.

For germination tests on *A. africana* seed, and measurements on seedling growth, a nursery experiment was carried out in January 2010 at the University of Abomey-Calavi, Benin (between 6º21' and 6º42'N; 2º13' and 2º25' E in the Guineo-Congolian climatic zone). Three morphotypes of seed were identified from the hierarchical classification described above. For each morphotype, one seed was sown in a pot (5.5 cm × 18 cm) made from a polythene bag and filled with forest soil. The seeds were sown at an equal depth and the pots were watered twice daily (morning and evening) throughout the duration of the experiment, and equally between morphotypes. Thirty seeds of each morphotype were used. The experimental units were arranged in a randomised complete block design with three replicates of 30 pots per morphotype (n = 90). The experimental units were kept in a weaning shed to reduce the rate of evaporation. The number of seeds of each morphotype that germinated was recorded daily over a 60 d period. At the end of this period, the collar diameter, stem height, and number of leaves were measured each week for 30 d on five seedlings selected at random for each morphotype per replicate block.

The germination percentage of each morphotype of *A. africana* seed was calculated each day over 60 d, and the data were used to test the effects of time and morphotype on the germination of *A. africana* seed. The statistical test used for this purpose was analysis of variance with repeated measures (Crowder and Hand, 1990) available in SAS software (SAS, 2003), using the mixed model. In this model, the factor “block” was considered to be random, whereas the factor “morphotype” was considered to be fixed. No data transformation was applied to germination percentages because normality and homoscedasticity were checked without transformation using the Ryan-Joiner test of Normality, and the Levene test for homogeneity of variances (Glèlè Kakaï *et al*., 2006). The effects of morphotype on seed germination rate and on seedling growth were assessed using these statistical methods.

RESULTS

Identification of A. africana *morphotypes*

Three morphotypes were identified from the hierarchical classification based on 54.3% of the information on all seeds saved. The results of canonical discriminant analysis performed on the morphotypes of *A. africana* seed showed that the Mahalanobis distances between pairs of the three morphotypes identified were all highly significant ($P \leq 0.001$). The morphotypes identified from the hierarchical classification were thus highly different according to the size of the *A. africana* seed.

Other results from the canonical discriminant analysis performed on individuals of the three seed morphotypes revealed that the first two axes were highly significant ($P \leq 0.001$) and explained 100% of the variation between morphotypes. The coefficient of correlation between the two canonical axes (Can) and the morphological traits of *A. africana* seed indicated that the first axis (Can 1) discriminated between morphotypes according to seed length and weight. On this axis, heavy seeds were also often long. The second axis (Can 2) discriminated between morphotypes according to the thickness of the seed. All seeds from morphotype 1 were heavy and long, while seeds from morphotype 3 were lightweight and short. Seeds from Morphotype 2 were intermediate in weight and length between Morphotypes 1 and 3. However, Morphotype 2 included the thickest seed (Figure 1). A more detailed description of each morphotype is provided in Table I, from which we noted that seeds of Morphotype 1 originated mostly from the Guinean zone (93.86%), while morphotypes 2 and 3 seeds were predominantly from the Sudanian zone (94.85% and 87.82% respectively). Morphotype 1 had the longest seed (mean length 26.44 mm), while the shortest seed were from Morphotype 3 (mean length 18.67 mm). The heaviest seed were found in Morphotype 1 (mean weight 3.05 g), whereas the lightest seed were from Morphotype 3 (mean weight 1.97 g). With regard to seed thickness, seed of Morphotype 2 were the thickest (mean thickness 13.15 mm), while Morphotype 3 seed were the thinnest (mean thickness 10.01 mm).

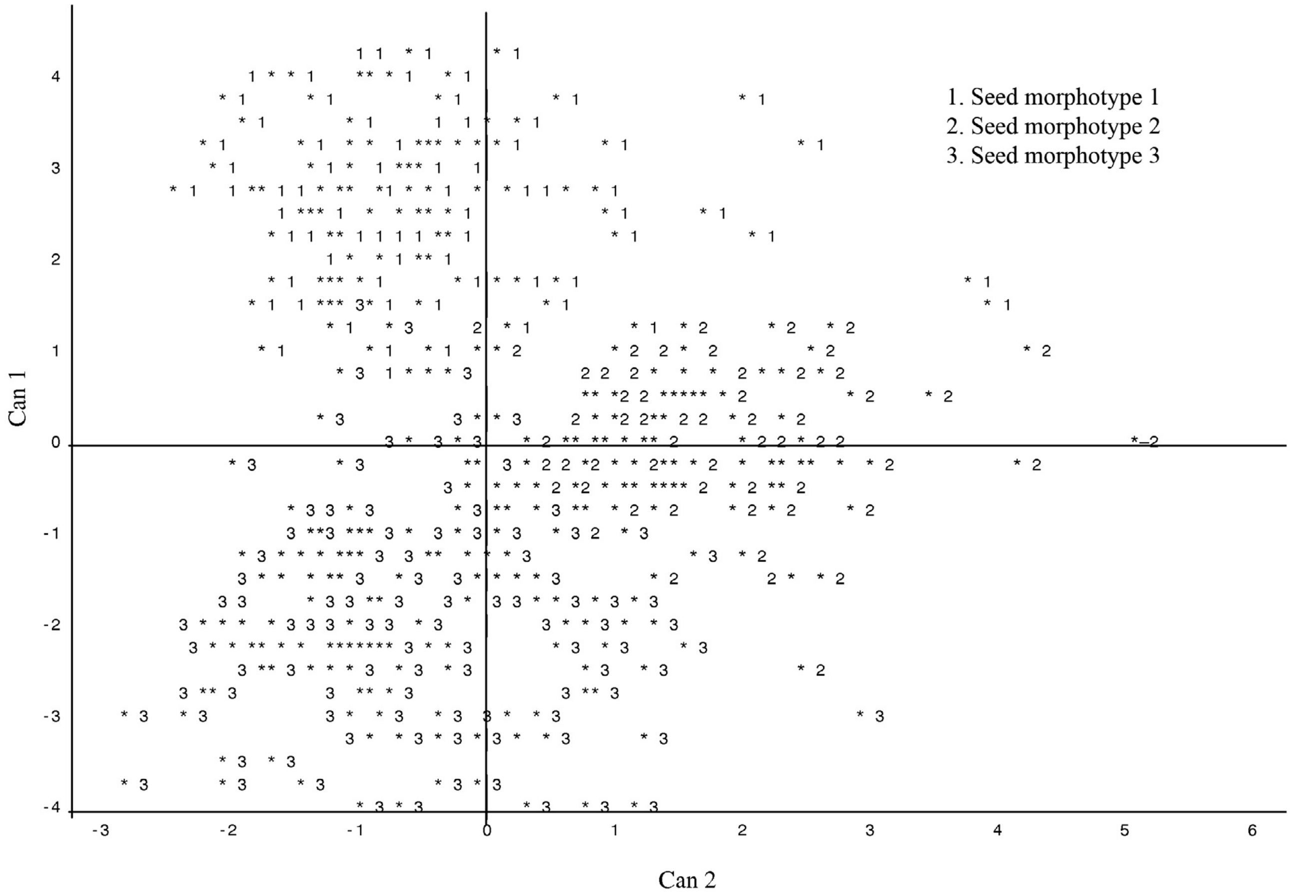

FIG. 1
Projection of the three morphotypes of *A. africana* seed on the canonical axes defined by seed size. Canonical axis Can 1 discriminated between morphotypes based on seed length and weight. Canonical axis Can 2 discriminated according to the thickness of the seed.

The longest seeds were from the Guinean zone (mean length 25.58 mm), while the shortest seeds were from the Sudanian zone (mean length 19.45 mm). The Guinean zone was also characterised by having heavier seeds (mean weight 2.81 g), while the lightest seeds originated mostly from the Sudanian zone (mean weight 1.93 g). The widths and thicknesses of seeds were similar in the Guinean and Sudanian zones.

Germination ability of A. africana *seeds according to morphotype*

The germination ability of each morphotype varied over time between sowing and 60 d after sowing (Table II). The blocking factor and all interactions were non-significant, indicating homogeneity of the environmental characteristics within and between blocks. Trends in the germination percentages of the three morphotypes of seed were assessed by least-square means computed from an analysis of variance on repeated measures (Figure 2). Seeds of Morphotypes 1 and 3 showed rapid growth from 5 – 25 d after sowing, compared to Morphotype 2 which were characterised by having a lower rate of germination over time.

Growth of A. africana *seedlings according to morphotype*

Growth of the three different morphotypes of seedlings, in terms of collar diameter, stem height, and number of leaves produced (Table III), varied between the end of germination (60 d after sowing) and 30 d later (90 d after sowing). The blocking factor and all its interactions were non-significant, indicating homogeneity of the environmental characteristics within and between blocks. Morphotype 1 seedlings showed the highest rates of growth in terms of collar diameters, stem heights, and numbers of leaves during this period, while seedlings of Morphotypes 2 and 3 showed the slowest growth rates (Figure 3). Seedling growth was therefore

TABLE I
Mean values and standard deviations of the morphometric traits of three morphotypes of A. africana *seed*

Distribution/Trait	Morphotype 1	Morphotype 2	Morphotype 3	Guinean zone	Sudanian zone
Sudanian zone (%)	6.14	94.85	87.82	–	–
Guinean zone (%)	93.86	5.15	12.18	–	–
Length (mm)	26.44 ± 1.70[†]	20.59 ± 1.63	18.67 ± 2.38	25.58 ± 11.82	19.45 ± 11.39
Width (mm)	13.91 ± 0.92	12.76 ± 1.20	13.01 ± 1.54	3.00 ± 1.33	2.32 ± 1.96
Thickness (mm)	12.28 ± 1.26	13.15 ± 0.93	10.01 ± 1.19	13.56 ± 2.81	13.04 ± 1.93
Weight (g)	3.05 ± 0.36	2.19 ± 0.28	1.68 ± 0.39	1.22 ± 0.63	1.41 ± 0.48

[†]Values are means ± SD (n = 600).

TABLE II
ANOVA with repeated measures related to the germination ability of three morphotypes of A. africana *seed*

Source	DF‡	Type III SS‡	Mean square	F-value¶
Time (T)	36	3,289.23	91.37	222.78 ***
Block (B)	2	10.66	5.33	0.21 ns
T × B	72	27.04	0.37	0.92 ns
Morphotype (M)	2	1,433.75	716.87	28.45 ***
T × M	72	769.06	10.68	26.04 ***
B × M	4	208.82	52.2	2.07 ns
T × B × M	144	146.59	1.02	2.48 ***

‡DF, degrees of freedom; Type III SS, Type III Sum of Squares; F-value, Fisher value.
¶ns, non significant at $P \geq 0.05$; ***, significant at $P \leq 0.001$.

influenced by seed size, and morphotype 1 seed were considered most suitable for restoration of the species.

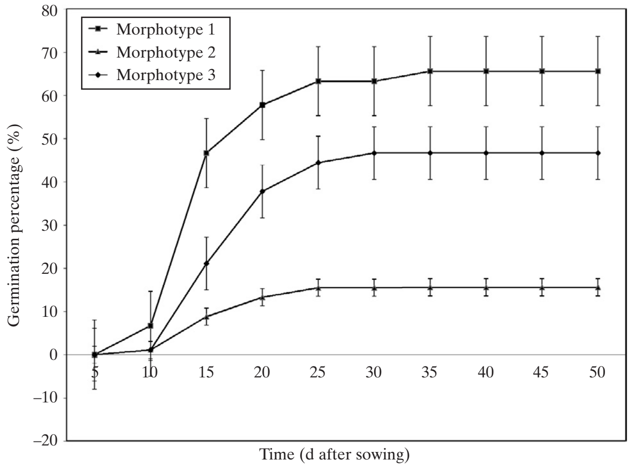

FIG. 2
Cumulative germination rates of *A. africana* seed according to morphotype.

DISCUSSION

Morphological variations in A. africana *seed*

The occurrence of *A. africana* over a wide range of environmental conditions, encompassing diverse edaphic and climatic conditions, was expected to be reflected in the morphometric traits of seed from diverse populations. In the present study, considerable variations in seed characteristics were observed among different *A. africana* populations. The longest seeds were in morphotype 1, while the shortest seeds were from morphotype 3. The heaviest seeds were in morphotype 1 whereas the lightest seeds were from morphotype 3. The thickest seeds were in morphotype 2, while the thinnest seeds were grouped in morphotype 3.

Most of the phenotypic variations observed in these traits were attributed to variations in populations within the different climatic zones. Indeed, the longest seeds were from the Guinean zone, while the shortest seeds were from the Sudanian zone. The Guinean zone was also characterised by having heavier seeds, while seeds from the Sudanian zone were lighter. This observation suggested that environmental factors play an important role in determining these seed characteristics and confirmed the influence of environmental factors on seed morphology, as established previously by Salazar and Quesada (1987), Maranz and Wiesman (2003), Soloviev *et al*. (2004), Assogbadjo *et al*. (2005; 2006), and by Mamo *et al*. (2006).

Seed germination and seedling growth in A. africana

Seed size is regarded as an important aspect of the reproductive strategy of plants, as it plays a key role in the establishment of the juvenile phase of the life cycle (Rai and Tripathi, 1982; Mamo *et al*., 2006). A number of selective advantages of having a large seed size have been documented such as prolonged dormancy during unfavourable light conditions, the development of large amounts of photosynthetic tissue, rapid seedling growth, and modes of seed dispersal (Harms *et al*., 2000; Leishman *et al*., 2000). Therefore, seed size is considered to be an indicator of seed quality. In this respect, we expected some variation in the rates of seed germination among the three morphotypes of *A. africana* grown under the same environmental conditions.

In the present study, rapid growth (as indicated by germination percentage) and seedling growth were observed in morphotype 1, which had the longest and heaviest seeds of the three morphotypes. This result confirmed the hypothesis that seed size permitted rapid seed germination and seedling growth. Morphotype 3, which had the smallest seeds (in length, weight, and thickness), also showed rapid growth (in terms of seed germination), but slow seedling growth. Rapid seed germination in this morphotype suggests the existence of other factors that influence seed germination. It has been reported that seed dormancy status and/or external environmental conditions such as temperature, humidity and light have a strong influence on seed germination (Amusa, 2011; Albrecht and McCarthy, 2006). As environmental variation at the experimental site was negligible, and the experimental design reduced any residual variation that could persist on site, the variation in seed germination between morphotypes may be due

TABLE III
ANOVA with repeated measures related to seedling growth on collar diameters, stem heights, and numbers of leaves in three morphotypes of A. africana *seed*

		Collar diameter		Stem height		Number of leaves	
Source	DF‡	Mean square	F-value‡	Mean square	F-value	Mean Square	F-value
Time (T)	4	1.69	61.94***¶	11.96	39.13	1.03	1.57ns
Block (B)	2	0.44	3.68ns	43.43	1.05	3.24	1.26ns
T × B	8	0.05	2.19ns	2.17	0.76ns	0.52	0.79ns
Morphotype (M)	2	4.92	40.94***	265.21	6.41*	5.67	2.20ns
T × M	8	0.08	2.99*	6.55	2.29*	1.49	2.26*
B × M	4	0.01	0.16ns	42.52	1.03ns	5.56	2.16ns
T × B × M	16	0.06	2.35*	6.94	2.43*	1.06	1.62ns

‡DF, degree of freedom; F-value, Fisher value.
¶ns, non-significant; *,significant at $P \geq 0.05$; ***, significant at $P \leq 0.001$.

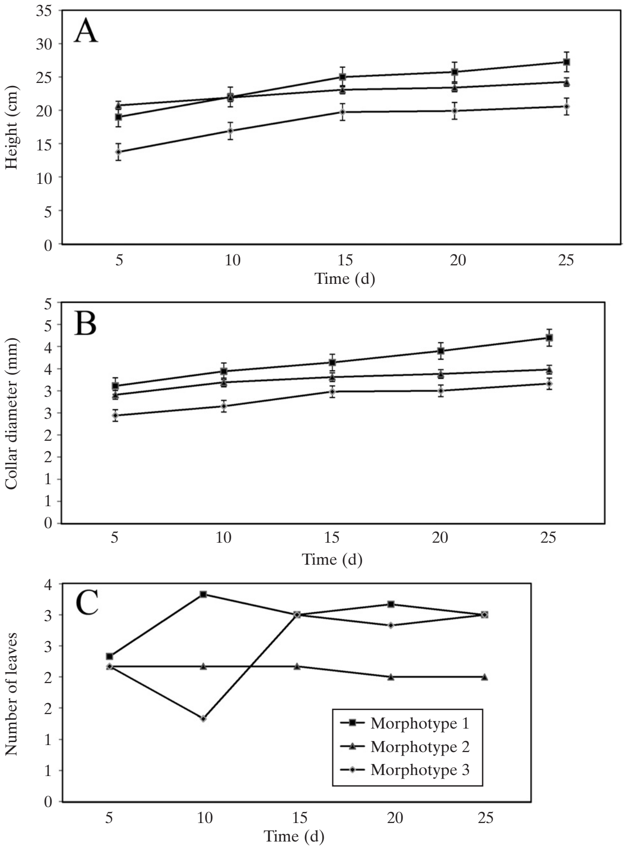

FIG. 3
Trends in seedling traits over time (in d) following germination in *A. africana* based on collar diameters (Panel A), stem heights (Panel B), and numbers of leaves (Panel C) according to seed morphotype.

to seed dormancy status. Since morphotypes consist of seeds from different sources, samples of *A. africana* seed may have various dormancy statuses which may lead to discontinuous variation in germination characteristics.

Despite the rapid germination of morphotype 3 seed, their seedling growth was slow. This may have been due to the small size of these seeds, and again confirmed the importance of seed size for seedling growth since large seeds have a positive influence on photosynthate reserves and the establishment and early growth of seedlings (Rai and Tripathi 1982; Mamo *et al*., 2006). Even if seed size was not the only factor to influence seed germination, the rate of growth of seedlings was strongly determined by seed size. Since it is possible to produce seedlings of *A. africana* easily, *ex situ*, the poor regeneration of the species *in situ* may be attributed to perturbations in the ecological characteristics of its natural habitat. Forests in West Africa have been subjected to degradation (FAO, 1999) which affects biodiversity and plant habitats. Some vascular plants require ectomycorrhizae in order to grow (Agerer, 2001; Bending *et al*., 2002; Hobbie, 2006; Artursson *et al*., 2006). *A. africana* may depend on such fungi for seedling growth *in situ*, and these may be lost following habitat degradation. Further exploration of this issue may help contribute to the restoration of this species *in situ*.

CONCLUSIONS

This study revealed considerable variation between two populations of *A. africana* with respect to seed size, germination rate, and seedling growth. On the basis of these results, we conclude that morphotype 1, consisting of large seeds from the Guinean zone, was superior in terms of germination ability and seedling growth among the three morphotypes and would be the best choice for species restoration purposes.

More research is required on the possible role of ectomycorrhizae in stimulating the growth of seedlings of this species *in situ*, and on their restoration in natural habitats before starting to restore the tree species *in situ*.

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