

Inbreeding depression affects the growth of seedlings of an African timber species with a mixed mating reproductive system, *Pericopsis elata* (Harms) Meeuwen

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1 Inbreeding depression affects the growth of seedlings of an African timber species
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Abstract

Selfing or mating between related individuals can lead to inbreeding depression (ID), which can influence the survival, growth and evolution of populations of tree species. As selective logging involves a decrease in the density of congeneric partners, it could lead to increasing biparental inbreeding or self-fertilization, exposing the population to higher ID. We assessed the influence of inbreeding on the growth of a commercial timber species, *Pericopsis elata* (Fabaceae), which produced about 54% of self-fertilized seedlings in a natural population of the Congo basin. We followed the survival and growth of 540 plants raised in a plantation along a gradient of plant density (0.07 to 15.9 plants per m²). Parentage analysis allowed us distinguishing selfed and outcrossed seedlings. The annual growth was higher for outcrossed than selfed plants, on average by 10.8% for diameter and 12.9% for height growth. Based on the above ground biomass after 41 months, we estimated ID at $\delta = 0.32$, while a life-time estimate of ID based on the proportions of

selfed plants at seedling and adult stages led to $\delta = 0.7$. The level of ID on growth rate did not change significantly with age but tended to vanish under high competition. *P. elata* is a particularly interesting model because inbreeding depression is partial, with about 26% of reproducing adults resulting from selfing, contrary to most tropical tree species where selfed individuals usually die before reaching adulthood. Hence, the risks of ID must be considered in the management and conservation of the species.

Keywords: Inbreeding depression, Selfing, Nelder design, Growth performances, *Pericopsis elata*.

1. INTRODUCTION

Inbreeding depression (ID) is the reduction in fitness (W) of inbred offspring compared to noninbred ones due to increased homozygosity, and is often quantified by $\delta = 1 - W_{\text{inbred}} / W_{\text{noninbred}}$. Most ID results from the accumulation in the genome of deleterious recessive or partially recessive mutations that are maintained at low frequency in populations because their deleterious effect is not or minimally expressed in the heterozygous state (Crnokrak and Roff, 1999 ; Charlesworth and Willis, 2009). This phenomenon can occur following the mating between closely related individuals or, in self-compatible hermaphrodites, like many plant species, following self-fertilization (autogamy or geitonogamy). ID affects most sexual organisms, including hermaphroditic organisms (Lame and Charlesworth, 1993; Uyenoyama et al., 1993; Charlesworth, 1987; Crnokrak and Roff, 1999 ; Keller and Waller, 2002). Its severity may be sensitive to the way inbreeding occurs (Wang, 2000; Day et al., 2003; Glémin et al., 2003; Kristensen et al., 2003). Species or populations with an excess of consanguineous mating will express and purge more efficiently (partially) recessive deleterious mutations, eventually

reducing the intensity of ID, although they may still accumulate a genetic load due to the fixation of mild deleterious mutations. Therefore, ID is thought to be a key factor affecting the evolution of the mating system (Schemske and Lande, 1985; Charlesworth, 1987; Kristensen *et al.*, 2003 ; Husband and Schemske, 1996). Many theoretical works have questioned the origin and maintenance of a mixed reproductive system (i.e., coexistence of selfing and outcrossing) because the simplest models suggest that only complete outcrossing or complete self-fertilization are evolutionarily stable strategies (Holsinger, 2002). Nevertheless, nearly 40% of plant species maintain a mixed mating system (selfing rate between 20% and 80%; Goodwillie *et al.*, 2010) and these species seem to display as much ID as outcrossed species (Winn *et al.*, 2011).

ID is a major concern in conservation genetics because it is one of the main factors involved in the vortex of extinction following a reduction of population size, potentially bringing populations of threatened species at risk of extinction (Frankham, 1995, 2005; Frankham *et al.*, 2002; Spielman *et al.*, 2004). The level of ID (δ) varies among species but also among populations or lineages, and according to environmental conditions. Meta analyses show that ID is usually stronger under stressful conditions (Reed *et al.*, 2002; Fox and Reed, 2010; Sandner *et al.*, 2021), so that it could be minimized under experimental conditions even when it is strong in the wild, but exceptions are common, including cases where ID is stronger under optimal conditions for growth and reproduction (Armbruster and Reed, 2005 ; Rehling *et al.*, 2019; Sandner *et al.*, 2022; Sandner and Matthies, 2016). To assess how ID can affect the future of threatened species, it is therefore of interest to measure ID under a range of environmental conditions and for different populations, lineages or families.

In plants, ID has most often been studied on annual or perennial herbs, which are easier to manipulate than trees for example, for controlling mating or estimating life-time reproductive success. The deficit of experimental studies on ID in trees is particularly important for tropical tree species, except in the genus *Eucalyptus* (Byrne et al. 2008). In fact, most tropical tree species have developed strategies to avoid inbreeding and, in the case of hermaphroditic species, strategies to avoid self-fertilization (Dick *et al.*, 2008). In self-compatible tropical trees the selfing rate is generally low, typically <5% (e.g. Lourmas *et al.*, 2007), sometimes 10-30% (e.g. Duminil *et al.*, 2016b; Hardy *et al.*, 2019), and inbreeding detectable at the seed or seedling stage (heterozygote deficiency relative to Hardy-Weinberg equilibrium) usually disappears at the adult stage, indicating that self-fertilized individuals have not survived to the adult stage (e.g. Hardy *et al.*, 2019). However, there are exceptions, with some species having a truly mixed-mating system, like some *Eucalyptus* species (Byrne et al. 2008). In tropical Africa, a notable exception is *Pericopsis elata* (Harms) Meeuwen, a large legume tree from the dense forests, in which a selfing rate of 55% has been measured in seeds and young seedlings in a population from DRC (Angbonda *et al.*, 2021). In the studied population, the proportion of selfed adults dropped to about 26%, probably reflecting the impact of ID on survival rate. However, contrary to most self-compatible tropical tree species, ID would not be total (i.e. $\delta < 1$) in *P. elata* as a non-negligible proportion of selfed seedlings reach adulthood in the population.

Pericopsis elata (Fabaceae) is a large upper canopy tree of the African rainforest, highly traded in the Congo Basin due to its excellent wood quality (Bourland *et al.*, 2012). It is called by the trade names Afrormosia or Assamela, or sometimes "African teak" because it is often used as a substitute for teak. The species currently suffers from a regeneration deficit in the wild, so that it

is threatened by overexploitation linked to logging activities (Betti, 2008; Bourland *et al.*, 2012). The resulting reduction in the density of reproductive trees compromises the sustainability of its exploitation throughout its range. In addition to international export pressures, habitat degradation and insufficient regeneration issues are causing some of its populations to decline, and currently the species is rarely found in dense populations although locally we can find a fairly high density (Dickson *et al.*, 2005). Therefore, *P. elata* has been included in the IUCN Red List of Threatened Species (<http://www.iucnredlist.org>) and in Appendix II of the Convention on International Trade of Endangered Species (CITES). Managers of tropical forests are increasingly interested in making use of *P. elata* seedlings as a nature-based solution (Seddon *et al.*, 2020) to achieve conservation goals and possibly address climate change mitigation challenges (Griscom *et al.*, 2017). Such successful and cost-effective plantings require proper silvicultural methods (Preece *et al.*, 2023) and a good knowledge of the ecology of the species and its reproduction cycle, especially since we discovered that it is prone to high inbreeding and probably to ID. Seed quality is primordial for plantation operations, so that estimating ID, assessing how it varies according to environmental conditions and families, and assessing whether higher quality seeds could be obtained from seed trees less prone to selfing are important knowledge.

This study aims to analyse the effects of inbreeding on the growth and survival of *P. elata* seedlings raised in a nursery (for 9 months) and transplanted in an experimental setup with a gradient of plant density (Nelder design; 4 years of growth). We address the following questions: (1) What is the rate of selfing and does it vary among mother trees? (2) Does inbreeding affect seedling survival and/or sapling growth rate? (3) How important is the impact of inbreeding depression on growth compared to the impact of plantation density or of the maternal genotype?

(4) Does the impact of inbreeding depression on seedling growth vary according to (i) the age of plants, (ii) the level of intra-specific competition (density), and/or (iii) the maternal origin of plants? (5) What is the level of inbreeding depression using above-ground biomass accumulation as a proxy of fitness? Finally, we discuss the implications of our results for the management of *P. elata* and plantations.

2. MATERIALS AND METHODS

2.1 Study species

Pericopsis elata is an emblematic tree species of tropical rainforest from West and Central Africa. It can reach 1.70 m of diameter at breast height (dbh) with a total height varying between 30 to 50(60) m. It is indicative of disturbances (Boyemba, 2011; Bourland *et al.*, 2015), and is either ranked among the pioneer or non-pioneer light-demanding species, depending on the authors (Ampofo and Lawson, 1972; Hawthorne, 1995; Veenendaal *et al.*, 1996; Kyereh *et al.*, 1999). The minimum dbh of fertility is 32.3 cm and the mortality rate (recorded on 137 adult stems over a 30-year period) is 0.85% in eastern Cameroon (Bourland *et al.*, 2012b). Its flowers are bisexual and stand in short terminal panicles on a thin, hairy white rachis (15 mm long and 13-14 mm wide) (Boyemba, 2011). Its fruits are green to brown pods, containing 1 – 4(5) discoid seeds (Dickson *et al.*, 2005), dispersed by wind (Hawthorne, 1995), usually <100 m from the mother tree (Angbonda *et al.*, 2021). The seed is almost rectangular and about 15 mm wide (Toussaint *et al.*, 1953). Seed germination is rapid, sometimes even in the deep shade of the forest and in small forests, but seedling development remains dependent on gaps or other openings. In general, germination occurs between 6 to 12 days under controlled conditions, at a high rate (Ouedraogo *et al.*, 2014;

Umunay *et al.*, 2017) demonstrated that seedlings in large canopy gaps (from 50 m x 50 m) were more likely to survive than under closed canopy conditions, though more opening might be needed to reach optimal growth and survival dynamics (Fayolle *et al.*, 2015).

2.2. Sampling

2.2.1. Study Site and Sampling

To conduct our experiment, seeds of *P. elata* were collected in the Biaro natural forest (00 12 'N, 25 20' E), located approximately 38 km south of Kisangani at an average elevation of 435 m, Tshopo province, Democratic Republic of Congo (DRC). It is a minimally disturbed forest that has never been logged and has been granted full conservation status by the Ministry of the Environment (Lomba, 2011). All *P. elata* stems (N = 189) greater than 10 cm of dbh within a 400 ha plot were georeferenced using GPS and genotyped using microsatellite markers (Micheneau *et al.*, 2011) for a previous gene flow study (Angbonda *et al.*, 2021). From September 17 to December 12, 2016, we collected pods from under mature trees (N = 20), assuming these were mother trees for which we hoped to produce enough seedlings for this study. Pod collection was conducted within a maximum radius of 10 m from each focal tree to avoid contamination of our samples by the progeny of other nearby trees.

2.2.2. Germination in a nursery

Sampled pods were taken to a nursery located on the campus of the Science Faculty of the University of Kisangani where each pod was peeled to extract the seeds. Each pod contained between 1 and 4 seeds, with usually only one viable seed. The latter was placed in a well, in a polyethylene bag previously filled with potting soil to allow germination. The soil used came from superficial horizons found at the site.

In the nursery, the experimental setup consisted of six complete randomized blocks; each block contained between 300 and 400 randomized seedlings from different seed carriers, combined with four waves of planting (24-28 Sep, 31 Oct, 01-24 Nov, 06-19 Dec 2016). Seeds collected from twenty mother trees (named: S2, S4, S30, S40, S42, S64, S79, S82, S89, S90, S95, S109, S116, S124, S141, S146, S158, S160, S165, and S194) were planted individually with one seed per well. The planted seedlings were watered regularly to maintain sufficient moisture for germination. After 8 months, two leaflets were collected from the seedlings obtained (N = 1186 under 20 trees) and carefully dried in silica gel to preserve DNA.

2.2.3. Plantation following Nelder design and growth data

Seedlings raised in the nursery were transplanted in October 2017, when they had reached at least 40 cm in height, on three experimental plots in full sun on a land from the concession of a logging company called *Compagnie Forestière et de Transformation* (CFT), located approximately 9 km from Kisangani (25°15' 58" E, 0°30' 42" N, elevation 495 m). The soil, relatively homogeneous in each plot, consists of old ferrallitic layers rich in sand and clay (Mosango, 1983) with a pH between 3.5 and 5.5 (Colombaroli *et al.*, 2016), and low fertility (cation exchange capacity - CEC - varies between 2 and 8 meq per 100 g). On each plot, plants were spatially arranged according to one of Nelder's designs (Nelder, 1962), over a circular area of 908 m² as described by Ilunga-Ilunga-Mulala *et al.* (2021). This method consists of placing 18 plants on each of 12 concentric circles whose radius forms a geometric progression from 0.57 m to 15.57 m, so that the density of plants is very high in the center and decreases towards the edge. We installed three Nelder plots, hereafter referred to as replicates, each containing 180 measured plants labeled with a metal plate and whose growth was monitored for 4 years (up to March 2021). To avoid an edge

effect, plants located on the first and last circles were not considered in analysis. During transplantation we paid attention that plants from a same maternal origin were well distributed among concentric rings to avoid covariation between potential genetic and competition effects on growth. The height and diameter of seedlings were measured just after their transplantation to record their initial size. The selfed or outcrossed status of seedlings was unknown at the time of transplantation and is thus assumed randomly distributed.

Fifteen trees that died as a result of stress after transplanting were replaced, the last one in August 2018. Growth measurements were taken every three months using a caliper for diameter at 10 cm from the ground and a graduated ruler for total height. The measurements used for the present work are those from March 2018 (5 months after transplantation, to avoid the effect of transplant stress), 2019, 2020 and 2021 (i.e. one year apart). Plants had become saplings at the end of the experiment.

2.3. Genotyping and identification of selfed versus outcrossed seedlings

Total DNA of all seedlings included in the three Nelder devices was extracted using the NucleoSpin 96 Plant II kit (Macherey-Nagel, Dürren, Germany). We genotyped the seedlings using 11 nuclear microsatellite markers amplified in two multiplexes according to the protocol developed by Micheneau *et al.* (2011). Paternity analyses were conducted using the neighbourhood model implemented in the NM π software ver. 1.2 (Chybicki and Burczyk, 2010) as described by Angbonda *et al.* (2021), in order to assess for each seedling if it resulted from selfing or outcrossing. We completed the dataset available in Angbonda *et al.* (2021) by genotyping 250 additional seedlings.

2.4. Data analyses

Annual growth was calculated by the formula:

$$\Delta y = \frac{(y_{t_2} - y_{t_1})}{(t_2 - t_1)} 12 \quad (1)$$

with Δy the annual height (or diameter) increment, y the height (or diameter) measured at successive times t_1 and t_2 (in month), considering the following measurement times: 5, 17, 29 and 41 months after transplantation. Δy is thus computed at three age intervals, corresponding to plants aged approximately 2, 3 and 4 years since their germination. Hence, we will refer to growth of saplings aged 2, 3 or 4 years as the increase in their height or diameter observed, respectively, between 5 and 17 months, 17 and 29 months or 29 and 41 months after transplantation.

At each considered time t_2 , we estimated the level of competition that each plant had experienced from its neighbours (Hegyi, 1974; Uhl *et al.*, 2015) as described in Ilunga-Mulala *et al.* (2021) following the formula:

$$C_i = \sum_{\substack{j=1 \\ j \neq i}}^n \left(\frac{D_j}{D_i L_{ij}} \right) \quad (2)$$

where D_i et D_j are the respective diameters of subject i and competitor j and L_{ij} is the distance between i and j , and the sum is taken over neighbours within a radius around subject i equal to the mean height of plants within the respective Nelder plot.

Above-ground biomass (AGB) 41 months after transplantation was calculated for each plant according to the pantropical equation of Chave *et al.* (2014):

$$AGB = 0.0673 \times (\sigma D^2 H)^{0.976} \quad (3)$$

with AGB in kg, $\sigma = 0.64$ the wood density of *P. elata* provided by the Global Wood Density Database (Zanne *et al.*, 2009), D the diameter in cm and H the height in meter. Although this formula was adjusted for trees with $D > 5\text{cm}$ (Chave *et al.*, 2014) so that the parameters might be inaccurate for our saplings (mean $D = 5.1\text{cm}$), we still expect that AGB increases approximately proportionally with D^2 and H , so that it is a convenient formulae to integrate diameter and height growth in a single measure, which will be used as an early fitness component.

Modelling growth and assessing the impact of inbreeding

Height and diameter increments and AGB were modelled by linear mixed models (LMM). Two main classes of models were tested. The first class (Eq. 4) aimed to compare the percentage of variance explained by the following explanatory variables considered as random factors: replicate (3 categories), family (19 categories corresponding to mother trees), inbreeding (2 categories: outcrossed or selfed), age (3 categories) and plant density (10 categories, corresponding the Nelder circle number).

$$\Delta y_{ijklmn} = \mu + \sigma_i + \rho_j + \phi_k + \alpha_l + \iota_m + \chi_n + \varepsilon_{ijklmn} \quad (4)$$

where Δy_{ijklmn} is the response variable (annual height or diameter increment) for sapling i , in replicate j , of family k , at age l , with inbreeding m and in Nelder circle n . Parameter μ is the model intercept while σ_i , ρ_j , ϕ_k , α_l , ι_m , χ_n are random variables modelling the effects of sapling, replicate, family, age, inbreeding and density, respectively, and ε_{ijklmn} is the residual error. Except for μ , the other terms follow normal centred distributions whose variances were estimated by fitting the model using the 'lmer' function of package 'lmerTest' (Kuznetsova *et al.*, 2017).

In the second class of models (Eq. 6 and 7), while sapling, replicate and family were still treated as random factors, age, inbreeding and competition were treated as fixed effects. Age and inbreeding were still categorical variables, while competition was not treated as a category of plant density but as a quantitative variable depending on the relative sizes of the neighbours: the competitive index C (Eq. 2). Preliminary analyses showed that the relationship between growth and local competition was well adjusted by a 2nd order polynomial of $C^{1/2}$, so that our models involve $C^{1/2}$ and C among the explanatory variables. We also tested the effect of the initial size at transplantation, S , as a covariate. To quantify the impact of ID on growth and its potential interactions with age and competition, the model in Eq. 5 was used.

$$\begin{aligned} \Delta y_{ijkl} = & \mu + b_1 S_i + \mathbf{b}_2 A_l + b_3 I_i + b_4 C_{il}^{1/2} + b_5 C_{il} \\ & + \mathbf{b}_6 A_l I_i + \mathbf{b}_7 A_l C_{il}^{1/2} + \mathbf{b}_8 A_l C_{il} + b_9 I_i C_{il}^{1/2} + b_{10} I_i C_{il} \\ & + \sigma_i + \rho_j + \phi_k + \varepsilon_{ijkl} \quad (5) \end{aligned}$$

It includes as fixed effects (first line): initial size (height or diameter, S_i), age (A_l), inbreeding ($I_i = 0$ for selfed sapling, $I_i = 1$ for outcrossed sapling), competition (C_{il}), as well as (second line) the two-way interaction terms between age, inbreeding and competition, and finally (third line) the random effects of sapling, family and replicate. Parameters b_1 to b_{10} are the regression slopes of the fixed effects and interaction terms, with bold values being vectors when terms include a factor with more than two categories (age).

As interaction terms including age were often significant (see results), we also modelled annual height and diameter increment separately for each age (eq. 6), keeping the same terms as in eq. (5) except the terms including age and the random factor for sapling (as there was no more repeated measurements). We modelled the AGB at age = 4 years (eq. 7) as in eq. (6) but using the

cubic root of AGB to approach normality and stabilize the variance of the residuals, and without accounting for initial size as we had found nearly no impact on annual increments. Finally, we modelled the initial height and diameter of seedlings at the time of their transplantation (eq. 8, where S_{ik} is the initial height or diameter of individual i from family k) according to inbreeding (fixed effect) and family (random effect) to assess whether inbreeding depression already occurred in the nursery.

$$\mu + b_1 S_i + b_2 I_i + b_3 C_{il}^{1/2} + b_4 C_{il} + b_5 I_i C_{il}^{1/2} + b_6 I_i C_{il} + \rho_j + \phi_k + \varepsilon_{ijk} \quad (6)$$

$$AGB_{ijk}^{1/3} = \mu + b_1 I_i + b_2 C_{il}^{1/2} + b_3 C_{il} + b_4 I_i C_{il}^{1/2} + b_5 I_i C_{il} + \rho_j + \phi_k + \varepsilon_{ijk} \quad (7)$$

$$S_{ik} = \mu + b_1 I_i + \phi_k + \varepsilon_{ik} \quad (8)$$

The best model was selected based on a stepwise approach examining the Akaike Information Criterion (AIC). We estimated the coefficients of variation (CV) of the random factors by the ratio of the standard deviations of each factor provided by the best model to the mean of the variable being explained. The level of ID for growth was estimated from the mean AGB of selfed and outcrossed saplings: $\delta = 1 - AGB_{\text{selfed}} / AGB_{\text{outcrossed}}$.

To illustrate graphically the results, we plotted the raw annual height and diameter increments as a function of competition ($C^{1/2}$), separately for each year and distinguishing selfed and outcrossed saplings, adjusting a spline function for each case to account for the non-linear effects of competition. We also plotted the mean growth per family according to year, distinguishing again selfed and outcrossed plants. We performed all analyses in the statistical software R version

298 4.2.1 (R Core Team, 2022), using the 'ggplot2' (version 3.3.6) package (Wickham, 2016) for
299 plotting.

300 **3. RESULTS**

301 **3.1. Selfing rate**

302 In total, 494 *Pericopsis elata* seedlings were genotyped, among which 267 were selfed, 192 were
303 outcrossed, and 35 remained unassigned (i.e., their genotypes were too incomplete, or were
304 consistent both with a selfing or outcrossing with one of the potential parents of the population).
305 According to the neighbourhood model implemented in NM π , the self-fertilization rate was
306 estimated at 0.54 ± 0.02 (standard error), which is close to the proportion of selfed seedlings
307 (58%) if we excluded the unassigned cases.

308 When considering the 11 mother trees for which the selfed/outcrossed status of at least 12
309 seedlings was assessed, the selfing rate per seed tree ranged from 44% to 85%, a variation
310 statistically significant (Chi-square test p-value = 0.0011).

311 **3.2. Survival rate**

312 Among the 540 saplings transplanted in the Nelder plots, only 15 (2.8%) died naturally within the
313 first 41 months of planting and were replaced. Among those, 9 were selfed, 2 were outcrossed,
314 and 4 were undetermined (not genotyped or unassigned). Although the mortality rate seems
315 higher for selfed plants (3.4%) than outcrossed plants (1.0%), Fisher exact test is not significant
316 due to limited sample size (p-value = 0.13).

317 **3.3. Annual height and diameter increment**

318 Transplanted seedlings had a mean initial height of 73.5 cm (sd = 29.2, range = 24 - 160) and mean
 319 initial diameter of 7.68 mm (sd = 2.61, range = 3 - 16). Initial height and diameter did not differ
 320 significantly between selfed and outcrossed seedlings (model of eq. 8; height: $b = 0.72$, $t = 0.26$,
 321 p -value = 0.79; diameter: $b = 0.357$, $t = 1.49$, p -value = 0.137).

322 Variance partitioning using the purely random model (eq. 5, Table 1) shows that sapling age
 323 explained the highest part of variance in annual height (42.6%) and diameter (31.0%) growth,
 324 followed by plant density (Nelder circle number) for diameter growth (22.6%) but not height
 325 growth (3.5%). Substantial parts of variance (8.1% to 9.6%) are also due to differences between
 326 replicates (Nelder plot). Genetic effects were less important but higher for inbreeding (2.3% to
 327 3.6%) than for family (1.9% to 2.3%), with higher percentages for growth in diameter than in
 328 height. The residual variance reached 41.5% and 30.9% for height and diameter growth,
 329 respectively (Table 1).

330

331 Table 1. Percentage of the variability in annual height and diameter increments explained by the
 332 factors family (mother tree), density (Nelder circle number), replicate (Nelder plot), age (2, 3 or 4
 333 years old saplings) and inbreeding (outcrossed or selfed saplings), with n the number of categories
 334 for each factor.

	n	Height increment	Diameter increment
		Variance (%)	Variance (%)
Family	19	1.9	2.3
Density	10	3.5	22.6
Replicate	3	8.1	9.6
Age	3	42.6	31.0
Inbreeding	2	2.3	3.6
Residual		41.5	30.9

335

336

337 The mixed effects models (Eq. 6) show that both height and diameter annual growths were
 338 significantly affected by competition, age and inbreeding, with significant interaction between
 339 competition and inbreeding effects, and between competition and age effects, but little
 340 interaction between age and inbreeding effects (Table 2). The best models integrating these
 341 factors explained 64% of the variance in height growth and 79% of the variance in diameter
 342 growth. These effects are detailed hereafter.

343 Annual diameter increment was maximal when competition was lowest (up to c. 20 mm. yr⁻¹) and
 344 decreased as competition increased, with a nearly linear relationship with the square root of the
 345 competition index ($C^{1/2}$) for saplings 3 and 4 years old (Figure 1d, e, f). By contrast, annual height
 346 increment was maximal (up to c. 150 cm. yr⁻¹ for 2 years old samplings) at an intermediate level
 347 of competition ($C^{1/2}$ around 7; Figure 1a). Annual increments decreased with age (2, 3 and 4 years
 348 saplings) for both height (means of 153.7, 96.6 and 69.1 cm. yr⁻¹) and diameter (means of 20.0,
 349 12.4 and 9.6 mm. yr⁻¹) and the growth-competition relationships tended to be less linear with $C^{1/2}$
 350 at younger ages (Figure 1).

351 Outcrossed individuals showed higher growth rates than selfed individuals for both height (means
 352 $\pm$ standard deviation of 166.2 ± 59.3 cm. yr⁻¹ and 144.7 ± 58.9 cm. yr⁻¹; $t = 4.5$; p -value < 0.001) and
 353 diameter (21.3 ± 8.2 mm. yr⁻¹ and 19.0 ± 7.5 mm. yr⁻¹; $t = 3.4$; p -value < 0.001) for 2 years old
 354 saplings, a difference that tended to decay with age but remained statistically significant (Figure
 355 1). Interestingly, the effect of inbreeding was strongest under low competition, especially for

diameter growth, and vanished under the highest competition levels (when $C^{1/2} > 7$), a trend observed across ages (Figure 1).

Table 2. Parameter estimates of linear mixed models (μ and b coefficients of eq. 5) used to explain annual height and diameter increments of saplings aged 2, 3 or 4 years old. C is the Hegyi (1974) competition index; A_3 and A_4 age effects at 3 and 4 years, respectively, assuming no age effect at 2 years ($b(A_2) = 0$); $I_{outcrossed}$ the inbreeding effect for outcrossed saplings, assuming no effect for selfed ones ($b(I_{selfed}) = 0$); S the initial size (height or diameter) at transplantation; S.D., standard deviation; Std. Error, standard error and CV, the coefficient of variation. Significant relationships are shown (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

	Height increment			Diameter increment		
<i>Fixed effects:</i>	Estimate	Std. Error	<i>p</i> -value	Estimate	Std. Error	<i>p</i> -value
Intercept μ	149.90	13.52	**	16.68	1.91	**
Competition $C^{1/2}$	26.21	82.84	0.752	-176.45	10.08	***
C	-1338.00	87.85	***	-121.00	10.44	***
Age = 3 years A_3	-65.13	3.13	***	-6.85	0.30	***
Age = 4 years A_4	-83.11	3.13	***	-8.74	0.31	***
Inbreeding $I_{outcrossed}$	18.86	3.71	***	1.91	0.44	***
Initial size S	-0.06	0.04	0.193	0.16	0.07	*
$C^{1/2} * I_{outcrossed}$	-246.40	90.79	**	-45.83	12.67	***
$C * I_{outcrossed}$	-76.30	88.56	0.389	3.17	11.54	0.783
$C^{1/2} * A_3$	-873.40	94.53	***	7.52	9.40	0.424
$C * A_3$	1270.00	98.74	***	133.36	9.61	***
$C^{1/2} * A_4$	-938.60	95.47	***	-33.04	9.74	***
$C * A_4$	1397.00	93.39	***	167.74	9.19	***
$A_3 * I_{outcrossed}$	-3.98	4.82	0.409	0.77	0.47	0.102
$A_4 * I_{outcrossed}$	-10.97	4.82	*	-0.37	0.47	0.434
<i>Random effects:</i>	S.D.	CV		S.D.	CV	
Sapling	14.31	14.10		2.90	20.79	
Family	8.38	8.26		1.11	7.91	
Replicate	21.94	21.62		3.09	22.13	
Residual error	35.59	35.09		3.46	24.73	

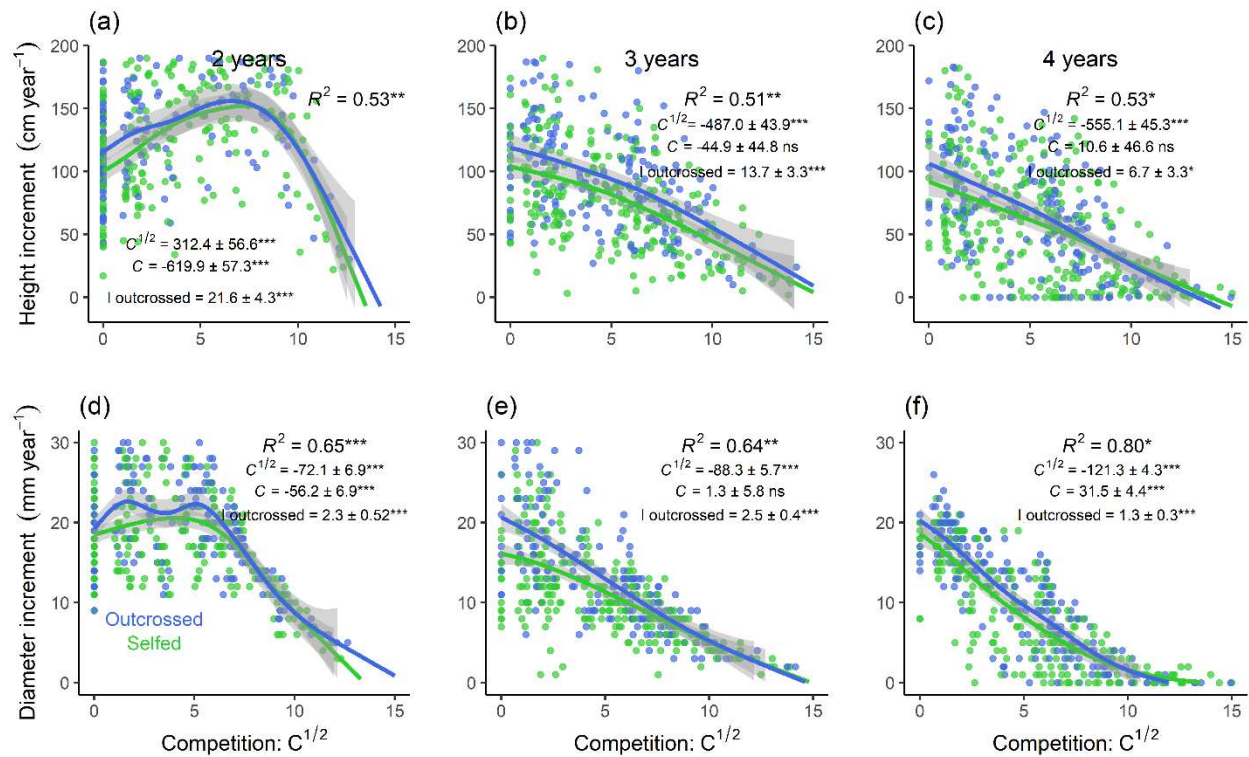


Figure 1. Relationship between annual height (a, b, c) or diameter (d, e, f) increment and local competition, $C^{1/2}$, for selfed and outcrossed *Pericopsis elata* saplings aged 2 (a, d), 3 (b, e) or 4 (c, f) years old (more specifically, growth from 5 to 17, 17 to 29, or 29 to 41 months after transplantation, respectively). The points are measured data. The shaded areas correspond to the 95% confidence interval of a spline function. Estimated models parameters following Eq. (6) and significant relationships (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant) are shown on each panel.

Among the ten families including at least six selfed and six outcrossed progeny (Figure 2), we observe that growth was consistently higher for outcrossed than selfed plants in five families (mother trees n° 4, 95, 116, 160, 194) and nearly so in two additional ones (n° 158, 165), while

there was no difference for one of them (n° 109) and even a slight trend of higher performances of selfed plants in two families (n° 89, 146). However, for the latter two families, selfed progeny appeared more often in the low density Nelder circles than outcrossed progeny, explaining the reverse pattern, while selfed and outcrossed progeny were well balanced across the density gradient of the Nelder plots in the other families.

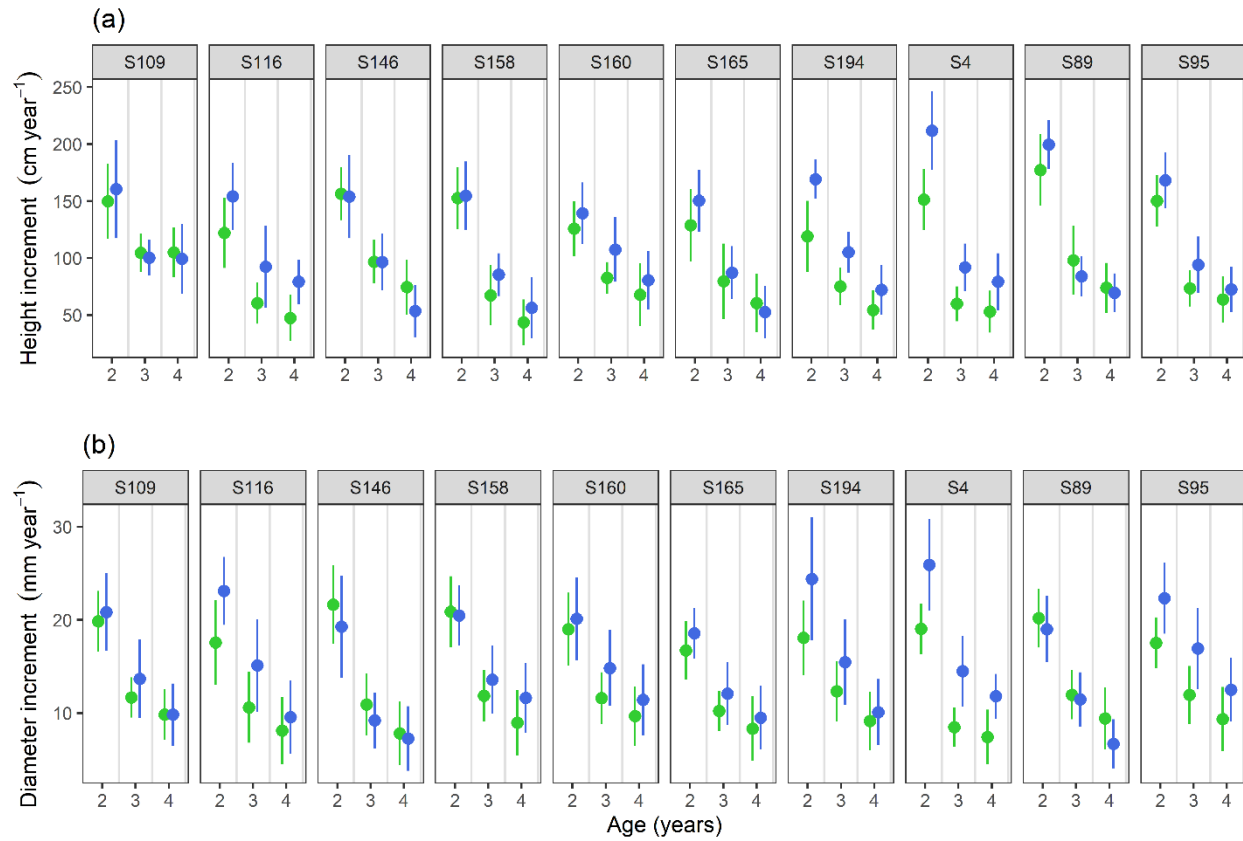


Figure 2. Variation of annual height (a) or diameter (b) increment of saplings from each of 10 families (same maternal origin, mother number indicated on top of each barplot), which have produced both selfed (green) and outcrossed (blue) saplings (at least 6 of each), according to age (2, 3 or 4 years old saplings). The dots are the means with standard deviations.

3.5. Inbreeding depression effect on above ground biomass (AGB) accumulation

Outcrossed saplings had higher AGB (6.3 ± 5.8 kg) than selfed saplings (4.2 ± 3.9 kg), leading to an ID of this fitness component of $\delta = 0.32$. However, the difference between selfed and outcrossed saplings was well marked when the level of competition was low ($\delta = 0.39$ when $C^{1/2} < 4$) but disappeared under high competition ($\delta \approx 0$ when $C^{1/2} > 10$; Figure 3). Therefore, both the outcrossing effect and the interaction between outcrossing and $C^{1/2}$ appeared significant in the model, which explains 74% of variance (Table 3). The relationship between AGB and $C^{1/2}$ is non-linear.

Table 1. Parameters and properties of a linear mixed model (Eq. 7) used to explain the above ground biomass (AGB) of *Pericopsis elata* saplings, 41 months after transplantation, according to local competition (C , considering a 2nd order polynomial of $C^{1/2}$), inbreeding (selfed or outcrossed) and the interaction between these explanatory variables. S.D., standard deviation, Std. Error, standard error and CV, the coefficient of variation. Significant relationships are shown (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

<i>Fixed effects:</i>	Estimate	Std. Error	<i>p</i> -value
Intercept μ	1.47	0.20	*
Competition $C^{1/2}$	-7.18	0.42	***
C	-1.81	0.43	***
Inbreeding $I_{outcrossed}$	0.20	0.03	***
$C^{1/2} * I_{outcrossed}$	-2.52	0.67	***
$C * I_{outcrossed}$	0.10	0.67	0.876
<i>Random effects:</i>	S.D.	CV	
Family	0.10	1.83	
Replicate	0.34	6.10	
Residual error	0.32	5.84	

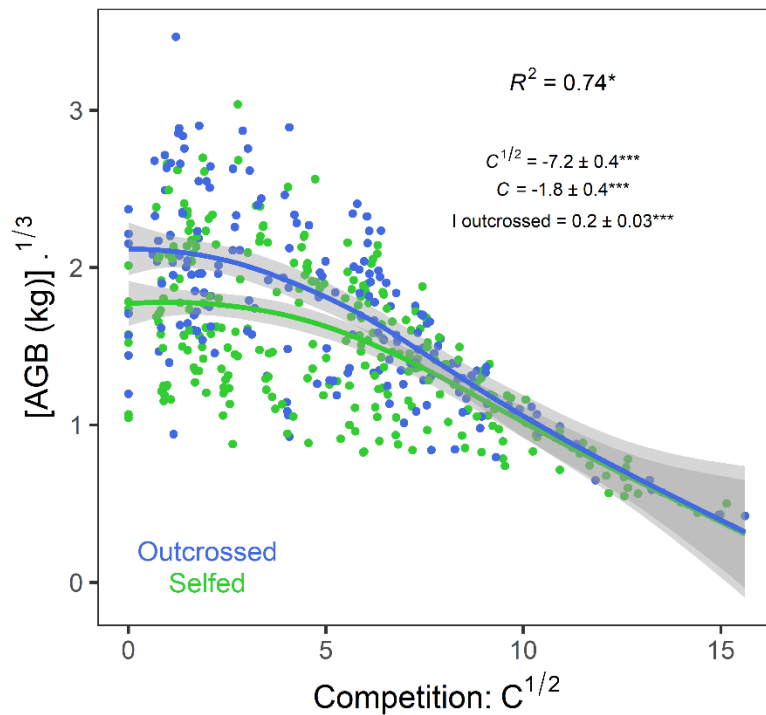


Figure 3. Above-ground biomass (AGB) of *Pericopsis elata* saplings 41 months after transplantation according to local competition and inbreeding (outcrossed in blue, selfed in green). The points are measured data, and the curves are smoothed using the "gam" method. The shaded areas correspond to the 95% confidence interval. Model parameters (Eq.7) and significant relationships ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$) are shown on the maps.

4. DISCUSSION

Understanding the effects of inbreeding on tropical rainforest tree species is of direct practical importance for genetic conservation, breeding, and environmental restoration to guide sustainable management of populations, particularly for threatened species. Relatively few studies have directly measured ID in tropical tree populations (Chaves *et al.*, 2011; Jolivet *et al.*, 2013; Bessega *et al.*, 2017; Pupin *et al.*, 2019; Aguiar *et al.*, 2020; Takeuchi and Diway, 2021), one

obvious reason being the difficulty to measure all fitness components in long-living organisms. For this study, we collected seeds of *P. elata* from known mothers in a natural forest and planted them following a Nelder design to compare the behavior of plants resulting from selfing with those resulting from outcrossing after monitoring growth during 41-month.

4.1. High self-fertilization rate reduces offspring growth

Pericopsis elata is an exceptional model due to its high proportion of selfed individuals (0.54 ± 0.02 among seedlings, 0.26 ± 0.10 among adults in the studied population; Angbonda *et al.*, 2021) in comparison with other tropical trees, which generally show a rate of self-fertilization $<10\%$ (Ward *et al.*, 2005; Dick *et al.*, 2008). Another tropical tree with similar selfing rate is the Asiatic *Rubroshorea acuminata* (Dyer) P.S.Ashton & J.Heck. (Naito *et al.*, 2008), and high selfing rates were also reported in *Eucalyptus* species (e.g. Byrne *et al.*, 2008; Griffin *et al.*, 2019), which include tropical species native to the Australian continent. Among African tropical trees, fairly high rates of selfing were also reported in *Erythrophleum suaveolens* (Guill. & Perr.) Brenan (c. 0.16; Duminil *et al.*, 2016) and *Baillonella toxisperma* Pierre (c. 0.3; Duminil *et al.*, 2016) based on seed or seedling genotypes but there was no evidence of selfed adults. Interestingly, we observed significant variation in selfing rates among *P. elata* families, although the minimal rate was 43%.

Inbreeding decays with age in *P. elata* (inbreeding coefficient $F = 0.3$ in seeds for $F = 0.15$ in adults; Angbonda *et al.*, 2021), as documented in most tropical trees producing a portion of selfed progeny (Cloutier *et al.*, 2007; Doligez and Joly, 1997; Gaiio *et al.*, 2010; Hufford and Hamrick, 2003). This is an expected manifestation of ID in species where a portion of juveniles are inbred, for example due to a mixed mating system (Hufford and Hamrick, 2003).

Our experimental results confirm that ID affects the growth of *P. elata* seedlings, particularly under low competition conditions (Table 1; Figure 1). ID often manifests in the traits of tree species (e.g., Tambarussi *et al.*, 2017). For example, the size, diameter at breast height (dbh) and volume of outcrossed progeny of *Eucalyptus regnans* F. Muell. at 45 months of age were on average 11, 18 and 37% greater, respectively, than those of the selfed progeny (Griffin and Cotterill, 1988). However, in *P. elata* we did not observe ID on growth in the nursery. In addition, we did not detect a significant effect of ID on survival rate but the mortality rate was probably too low in our experiment to be able to detect such an effect.

As often done with self-compatible organisms, we estimated ID from the relative loss of fitness of selfed individuals compared to outcrossed ones ($\delta = 0$ in the absence of ID while $\delta = 1$ if selfed progeny die before reaching adulthood or cannot reproduce). So, based on estimates of the above ground biomass (AGB) of selfed and outcrossed saplings 41 months after transplantation, ID was estimated as $\delta = 0.32$, and up to $\delta = 0.39$ under low intra-specific competition. AGB is only a proxy of a fitness component expressed here at an early stage. The actual level of ID for life-time reproductive success might be larger, for example if the initial growth disadvantage of selfed trees tends to worsen over time because faster growing neighbors increasingly compete for resources (Cheptou *et al.*, 2001), or if ID also affects seed set independently of tree size, or germination rate.

Estimating δ for all fitness components of trees is very laborious (but see Griffin *et al.*, 2019). However, an indirect estimate of δ for the lifetime fitness can be derived under specific assumptions from the comparison of the proportions of selfed seeds, s , and selfed adults, s' . Assuming that s and s' have reached a steady state, that inbreeding is only caused by selfing (no

biparental inbreeding) and that ID does not affect the reproduction or mortality of adults (i.e. assuming that ID affects only growth and mortality until adulthood), s' can be predicted from s and δ as following: $s' = s(1-\delta) / (s(1-\delta) + (1-s)) = s(1-\delta) / (1-s\delta)$, where the $s(1-\delta)$ term is the amount of remaining selfed adults after ID has acted and the $(1-s)$ term is the amount of outcrossed adults, which do not suffer from ID (Lande *et al.*, 1994). Hence, by reverting the equation, from estimates of s and s' , one can estimate $\delta = (s-s') / ((1-s')s)$. In the adult population used in this study and described in Angbonda *et al.* (2021), individual heterozygosity was not correlated with tree diameter, nor with their reproductive success assessed from the parentage analysis (unpublished results), so that all or most of the ID might be expressed during the seedling and sapling stages (lower growth and survival of selfed individuals) and the life time ID could be estimated from s and s' . Using $s = 0.54$ and $s' = 0.26$ for *P. elata* (Angbonda *et al.*, 2021), $\delta = 0.70$. In other words, the survival rate of selfed juveniles until adulthood is 70% lower than the one of outcrossed juveniles. This estimate is considerably higher than the one based on AGB. This should not be surprising as AGB at 41 months is only one of several fitness components but, in any case, the δ derived from s and s' reflects ID in natural conditions while the one derived from AGB concerns the experimental conditions of a plantation in full light, so that the two estimates are not directly comparable.

A literature survey of δ estimates in 58 plant species (Winn *et al.*, 2011) concluded that the lifetime ID averaged $\delta = 0.51$ for species showing a mixed mating system (selfing rate between 20% and 80%), and the only tree species with a mixed mating system included in this review, *E. regnans*, had $\delta = 0.80$, close to the value estimated for *P. elata*. Nevertheless, long-term follow up of ID in *Eucalyptus* species usually found that selfed individuals did not survive until the adult

stage (Griffin *et al.*, 2019; Nickolas *et al.*, 2019), contrary to *P. elata*, where we estimated that c. 26% of adults result from self-fertilization. The long-term maintenance of a mixed mating system in these conditions remains unexplained because deleterious recessive mutations should be purged efficiently. A model potentially explaining the maintenance of a mixed mating system, called selective interference (Lande *et al.*, 1994), occurs when many deleterious recessive alleles are generated by mutation so that few, if any, selfed individuals can eventually reproduce, but this is not the case in *P. elata*. Reproductive assurance, the capacity to reproduce under low pollinator or mate availability, might have been a strong enough selective force in favor of selfing, counter balancing the selection of mechanisms favoring outcrossing in response to ID (Ruan and da Silva, 2012).

Meta-analyses show that ID tends to be stronger under stressful conditions (Armbruster and Reed, 2005 ; Fox and Reed, 2010) and/or under higher intraspecific competition (e.g., Yun and Agrawal, 2014), especially when outcrossed and selfed individuals compete (Zanne *et al.*, 2009). ID in *P. elata* does not fit this pattern as it was maximal under low density and seemed to vanish under high competition. ID also did not appear in the nursery, where plant roots had access to a limited volume of earth (about 2 liter), limiting plant growth after several months. As *P. elata* is a light-demanding tree, which does not survive a long time in the shade of a closed canopy at the seedling or young sapling stage, only juveniles lucky enough to be in a large forest gap have a chance to grow fast enough to reach a reasonable size and eventually reproduce. In light of our experimental results, we hypothesize that it is in such favorable conditions that ID acts most against selfed juveniles.

4.2. Implications for the sustainable management of *P. elata* stands

This study has practical implications for genetic resource conservation and management of *P. elata* populations, including planting. Our variance partitioning of annual growth rate demonstrated a genetic determinism with higher inbreeding effect than family effect, highlighting the importance of ID (Table 1). More than half of open-pollinated progeny will grow slower due to ID, so that selecting early outcrossed progeny would be an advantage. However, ID was not detectable in the nursery, hence applying an early selection of faster growing seedlings at this stage does not seem efficient to select the outcrossed ones in the context of reforestation or planting of *P. elata*. Alternatively, we could select seed trees that deliver more outcrossed seeds for planting projects, as we observed variation in outcrossing rate among maternal families, although none of the family tested produced more than 56% outcrossed seeds. Given that we observed a significant family effect on growth rate, which has thus a non-null heritability, proper recording of the mother trees in plantations would be helpful to identify the best families (for growth rate but potentially also other traits related to disease or stress resistance or conformation) and later select the best performing seed trees for new plantations.

Thus, as recommended in good technical handbooks (e.g. Daïnou *et al.*, 2021), seed collection sources must be well selected to maintain the regeneration potential of this species. For plantations where a natural generation is projected in the future (e.g. plantation to restore degraded forests, enrichment of forest gaps), it is essential to keep a large genetic diversity of mother trees, avoiding sampling very close mother trees likely to be related, otherwise one would favor ID in the next generation due to consanguineous mating, even for outcrossed progeny (Bittencourt and Sebbenn, 2008; Angbonda *et al.*, 2021).

To reduce the risk of using and establishing inbred plants and to guarantee the genetic diversity of the population, a few measures can be considered when planting *P. elata*: (i) maintain the plants clearly identified by each mother tree in the nursery and keep the genetic identity. This has also the advantage of keeping an equal number of plants from each mother tree for planting, guaranteeing both genetic balance and diversity; and (ii) select a large number of mother trees, sufficiently spaced, to collect the seeds. This is of special importance since *P. elata* tends to be clustered, it is thus recommended to select mother trees at least from different clusters/stands/forest patches. This increases the chances of having sufficient genetic diversity while eliminating problematic material and allowing the selection of best performing mother trees for plantations primarily aiming wood production. As the density of mature trees is known to decline primarily due to logging, remnant populations of *P. elata* may be subject to increasing regeneration failure due to inbreeding depression, altered breeding systems mating and increased frequency of inbreeding (Cascante *et al.*, 2002; Fukue *et al.*, 2007; Naito *et al.*, 2008; Tani *et al.*, 2009). Leaving sufficient well-spaced mother trees after logging should be considered a responsible decision for any logger harvesting *P. elata* timber.

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