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Rapid seedlings regeneration from seeds and vegetative propagation with sucker and rhizome of *Eremospatha macrocarpa* (Mann & Wendl.) Wendl and *Laccosperma secundiflorum* (P. Beauv.) Kuntze

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ABSTRACT

To develop efficient seedling production methods for *Laccosperma secundiflorum* and *Eremospatha macrocarpa*, a study was conducted to examine regeneration using offsets combined with several physical and chemical treatments of seeds. Offsets categorized into small, medium and large diameters, were planted in three conditions: shaded and open nursery, and greenhouse. We tested sucker from *E. macrocarpa*, and sucker and rhizome from *L. secundiflorum*. For both species, high viability percentage (ranging from 55% to 100%) were observed for small and medium suckers planted in shaded nursery and greenhouse, against less than 49% for sucker planted in open nursery. The mean seedling emergence times were estimated to 84, 77 and 75 days after planting (DAP) for small, medium and large sucker of *L. secundiflorum*, respectively under open nursery condition, and 76, 75, 95 DAP for small, medium and large suckers of the same species, respectively in shaded condition. Greenhouse has a significant positive effect on *E. macrocarpa* seedlings emergence time. For this species, the mean seedling emergence times were estimated to 43 DAP for small sucker and 76, 93 DAP for medium and large suckers. No seedling was obtained from rhizome planted in all the growing conditions tested. Concerning seed dormancy breaking, germination percentages and rates were determined for 13 treatments. The best treatments were pre-soaking unscarified seeds for 4 days in 1.01 $g\,l^{-1}$ and 0.10 $g\,l^{-1}$ KNO_3, with 79% and 68% of germination, respectively and in $3.46 \times 10^{-3}\,g\,l^{-1}$ GA_3 for 68% of germination. These methods are suggested to improve germination of *L. secundiflorum* seeds. Successful and recommended methods for *E. macrocarpa* are pre-soaking scarified seeds in $3.46 \times 10^{-3}\,g\,l^{-1}$ and $3.46 \times 10^{-4}\,g\,l^{-1}$ GA_3, 96% and 94% of germination, respectively. Dormancy, probably a combination of mechanical and chemical dormancy, is present in the two species.

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1. Introduction

Palms have been recognised as one of the most interesting and diversified groups of monocotyledons. Many palm species are economically important as sources of oil, timber, fibre, fermented liquors, sugars and starch. Among the palms, the rattans are unique on the basis of their distribution, morphology and uses. Like their Asian relatives, the rattans of Africa form an integral part of subsistence strategies for many rural populations and provide the basis of a thriving cottage industry (Howthorne, 1990). Although less marketed compared to Asian species, the rattans from Africa can significantly contribute to both rural development and global market (Oteng-Amoako and Obiri-darko, 2002; Zoro Bi and Kouakou, 2004a). Presently, most African rattan canes are obtained from natural forests. Consequently, there is a rapid depletion of natural population as a result of overexploitation, industrial and urban development activities (Padmanadhan and Ilangovan, 1989). The most seriously affected species are *Laccosperma secundiflorum* (P. Beauv.) Kuntze and *Eremospatha macrocarpa* Mann & Wendl, due to their high economic value (Zoro Bi and Kouassi, 2004). The harvest of mature plants leads to seed scarcity and regeneration problems, and causes a decrease in rattan natural populations. In addition, rattan seeds are characterised by poor germination percentage and rate. Animals including primates, birds and rodents are known to consume mature fruits falling to the ground as well as vast quantities of rattan seeds when they are mature (Sunderland, 2001). Seeds

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hidden by rodents for later consumption can occasionally provide the opportunity for some of the seeds to germinate if they are buried for a long time (9–12 months) in moist humus (Sunderland, 1999, 2001). According to Manokaran (1978) and Mori et al. (1980), rattan seeds germinate between 8 weeks and 41 weeks and the germination percentage vary from 0.2% to 79%, depending on the species and time of seed maturity.

African Rattan Research Programme (ARRP) has initiated various research activities on rattan throughout Africa, namely to promote and improve rattan cultivation. Other research activities on rattan have been investigated by Zoro Bi and Kouakou (2004a,b) and Zoro Bi and Kouassi (2004). These investigations were aimed at the implementation of strategies for the conservation and sustainable use of some taxa. Supplying households and forest managers with vigorous and genetically uniform seedlings is a prerequisite for success in rattan implementation. This work presents the results of the investigations carried out on the regeneration through offsets of two rattan species as well as the effect of different treatments to overcome exogenous and endogenous dormancy of their seeds. Particularly, the objectives of the present work were to: (1) identify the type and size of organ suitable for vegetative propagation; (2) identify nursery conditions for better vegetative multiplication and (3) develop methods speeding up seed germination in order to develop an efficient rattan seedlings production protocol.

2. Materials and methods

2.1. Plant material

Two rattan species, *L. secundiflorum* and *E. macrocarpa*, widely used in crafts and industry throughout tropical Africa, were used for this study. Offsets were collected on several tufts of rattan growing in a sandy clay soil in a primary rain forest: "*Forêt classée de N'zodji*", Alépé-Côte d'Ivoire (5°22′40″N latitude, 4°40′00″W longitude), during the rainy season (August 2004). Mean annual precipitation and temperature of this site were 1727 mm and 26.2 °C, respectively. Organ collected are stored in hermetically sealed plastic bag and transported to the research station of the University of Abobo-Adjamé where experiments were carried out. On the basis of the works of Yusoff and Manokaran (1985) on *Calamus* spp. and Zoro Bi and Kouakou (2004b) on *L. secundiflorum*, two organs were used: rhizome and sucker. As *E. macrocarpa* does not develop rhizome, only sucker was assayed with this species. Rhizome and sucker fragments have been classified into three categories with respect of the organ diameter at the basis: small diameter ≤2.5 cm; medium diameter between 2.5 cm and 4.0 cm; large diameter ≥4.0 cm. For the seed germination test, *L. secundiflorum* seeds were collected in dry season (December 2004) from mature fruits of three individuals growing in evergreen swampy forest of 2 ha of "*Iles Eotilé*" Adiaké, in the South-East of Côte d'Ivoire (5°9′24″N latitude, 3°16′42″W longitude). *E. macrocarpa* seeds were collected in January 2005 from more than 10 individual trees from "*Forêt classée de la haute Dodo*" Tabou, in the South-West of Côte d'Ivoire (4°41′21″N latitude, 7°06′13″W longitude). Ripe fruits collected were transported to the laboratory and stored in plastic bags. The latter were kept under laboratory climatic conditions (23–25 °C, 35–50% RH) during 4 days before seed germination test. The time between the fruit harvest and the germination test was 7 days.

2.2. Methods

2.2.1. Vegetative multiplication

To test the effect of air moisture, temperature and light on the regeneration aptitude of vegetative organs, trials were established in three nurseries. The first nursery was covered with a shade built using bamboo and palm fronds, with an average air temperature of 31 °C and a mean relative humidity of 66%. The second nursery was an open site where the average air temperature and the relative humidity were 33 °C and 62%, respectively. The third nursery was a greenhouse built with polyethylene plastic, with an average air temperature and a relative humidity respectively, of 38 °C and 85%. Suckers and rhizomes were planted in plastic bags (750 cm^3), filled with a sandy topsoil with high amount of organic matter (pH 5.31) collected in a 5-year-old fallow plot. This substrate was disinfected with a fungicide. Pots were watered daily for the two first nurseries but only twice a week for the third nursery. The watering regime in the greenhouse is justified by the lowest water evaporation of the substrate due to polyethylene plastic.

Three traits were observed: (1) the viability percentage estimated 24 and 117 DAP; (2) the mean shoot emergence time expressed as days from planting to shoot appearance; and (3) the mean first leaf emergence time.

The durations 24 and 117 days corresponded to the time after which the first shoot and the last single-leaf seedling were observed in the shaded nursery. Non-viable organs show mould and decay. The viability was calculated according to the following formula: viability (%) = (NVO/NVOI) × 100, where NVO is the number of viable organ and NVOI is the initial number of organ.

The experiment was laid out in randomized block design with two factors, organ diameter (3) and nursery (3), resulting in nine treatment combinations. In shaded and open sites, 90 pots were arranged in three replicates while in the greenhouse 45 pots were arranged in three replicates.

2.2.2. Seed germination test

2.2.2.1. In vitro germination. Cleaned seeds were shaken for 30 min in 3.6% NaOCl solution for disinfection and they were rinsed in distilled water (Rehman and Park, 2000; Goh et al., 2001; Delanoy et al., 2006). Embryos were then carefully excised (Chuthamas et al., 1989; Paranjothy, 1993), washed in sterile distilled water and planted on medium (M1) made of Murashige and Skoog (MS) salt (Murashige and Skoog, 1962) supplemented with *myo*-inositol (0.1 $g\,l^{-1}$) and casein hydrolysate (0.5 $g\,l^{-1}$); media M2 and M3 consisting of M1 supplemented with gibberellic acid (GA_3) $3.46 \times 10^{-4}\,g\,l^{-1}$ and $3.46 \times 10^{-3}\,g\,l^{-1}$, respectively. The three media were incubated in a growth chamber at 28 ± 2 °C under darkness (Chuthamas et al., 1989; Shantha and Ramanayake, 1999). For each species and each medium, 72 excised embryos were arranged in three replicates. Each embryo was placed in a 55 ml culture tube containing 15 ml of medium. Seeds were considered as germinated when the embryos swelled and the emerging radicle was approximately 2.5 mm long (Padmanadhan and Ilangovan, 1989). Germination was evaluated every 2 days.

2.2.2.2. Seed germination in nursery. The cleaned seeds were subjected to treatments indicated in Table 1. Treatments applied were based on previous results of germination experiments in *Calamus* spp. (Mori et al., 1980; Bradbeer, 1988) and in several ornamental palms (Nagao et al., 1980; Bronschat and Donselman, 1988). The scarification consisted to remove carefully with a sharp blade the part of pericarp at the hilum side of seeds. For the chemical treatments seeds were soaked under total darkness in beakers containing 100 ml and 300 ml of the testing solutions, respectively for *L. secundiflorum* and *E. macrocarpa* seeds. Note that the *E. macrocarpa* seeds are on average four times wider than those of *L. secundiflorum*. After soaking, seeds were rinsed in distilled water prior to germination test. Each treatment consisted of three replicates, each one including 25 seeds of *E. macrocarpa* and 50

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Table 1
Treatments for seed germination in nursery.

1. Control (no treatment)
2. Unscarified seeds soaked in distillate water for 4 days
3. Unscarified seeds soaked in distillate water for 8 days
4. Scarified seeds soaked in distillate water for 4 days
5. Scarified seeds soaked in 3.46×10^{-3} g l^{-1} GA_3 for 4 days
6. Unscarified seeds soaked in 3.46×10^{-3} g l^{-1} GA_3 for 4 days
7. Scarified seeds soaked in 3.46×10^{-4} g l^{-1} GA_3 for 4 days
8. Unscarified seeds soaked in 3.46×10^{-4} g l^{-1} GA_3 for 4 days
9. Unscarified seeds soaked in 0.10 g l^{-1} KNO_3 for 4 days
10. Unscarified seeds soaked in 1.01 g l^{-1} KNO_3 for 4 days
11. Unscarified seeds soaked in H_2O_2 25% for 5 min and in distillate water for 2 days
12. Unscarified seeds soaked in H_2O_2 25% for 10 min and in distillate water for 2 days
13. Prechilling unscarified seeds at 4 °C for 4 days

seeds of *L. seccundiflorum*. The nursery consisted of eight seedbeds of 1 m × 2 m each. Seedbeds were filled with a sandy topsoil disinfected with a fungicide and covered with a shade built with bamboo and palm fronds. Temperature varied between 26 °C and 31 °C with an average of 29 °C. Substrate was watered every 2 days. Germination was evaluated every 2 days. Seeds were classified as germinated when the emerging shoot was approximately 3 mm above the substrate.

Two parameters of germination were determined: germination percentage (evaluated up to 270 days after sowing for *E. macrocarpa* and 300 days for *L. secundiflorum*) and mean germination rate (R), equal to $\Sigma n/\Sigma(tn)$, where t is the time in days and n is the number of seeds germinated the day t (Edwards et al., 1986; Bewley and Black, 1994). At the end of the test, non-germinated seeds were cut open using a pruner and classified in two categories following Ellis et al. (1985): (1) fresh seeds (firm and healthy); (2) dead seeds (showing mould, empty and decay).

2.3. Statistical analysis

Data were processed using SAS version 6 (SAS, 1999). Before carrying out all the following statistical analyses, normal distribution tests were evaluated using Kolmogorov–Smirnov. As the normal distributions were not significant ($P > 0.001$) no transformation was necessary.

A Kruskal–Wallis test was applied in order to find significant differences (at $p < 0.05$) between treatments. Correlation between germination percentage and germination rate was calculated using Pearson correlation. For the experiments on vegetative multiplication, analysis of variance (ANOVA) and Student t-test for two means comparison were applied.

Table 3
Viability percentages of rhizomes in *Laccosperma secundiflorum* under three different nursery conditions.

Nurseries	Organ diameters	Viability (%) 26 DAP	Viability (%) 117 DAP
Rhizomes			
Greenhouse	Small (n = 45)	86	60
	Medium (n = 45)	85	55
	Large (n = 45)	84	50
Open site	Small (n = 90)	51	29
	Medium (n = 90)	49	16
	Large (n = 90)	46	14
Shaded site	Small (n = 90)	95	67
	Medium (n = 90)	65	55
	Large (n = 90)	58	47

DAP = days after planting.

3. Results

3.1. Vegetative multiplication

Mean values of the characters, with respect to species, organ size and growing condition are indicated in Tables 2–4.

The highest viability percentages for rhizome and sucker were obtained in the greenhouse and shaded site. However, suckers and rhizomes with large diameter had the lowest viability percentages whatever the nursery and the species.

Raised from sucker of *L. secundiflorum*, the first shoots from shaded nursery were observed 24, 26 and 29 DAP for small, medium and large suckers, respectively and 30 DAP for large sucker in open site. No shoot emergence for the three sucker diameters was observed under greenhouse for *L. secundiflorum*. No significant effect on shoot emergence in open site was observed between the three sucker diameters of *L. secundiflorum*, but a significant improvement of shoot emergence in shaded nursery was obtained with small sucker and decrease of sucker diameters (Table 2). Results also showed no significant difference between shaded and open site on shoot emergence by sucker whatever their diameter.

For *E. macrocarpa*, no shoot emergence has been observed in open site and no large sucker emitted shoots under shaded site. We observed the first shoots 24 DAP for both small and medium sucker in shaded nursery, 21 DAP for small and 35 DAP for both medium and large suckers under greenhouse. Small and medium sucker diameters showed no significant effect on shoot emergence in shaded site (Table 4). The three sucker diameters affected significantly shoot emergence in greenhouse ($P < 0.05$).

Table 2
Results of sucker regeneration in *Laccosperma secundiflorum* under three nursery conditions.

Nurseries	Organ diameters	Viability (%) 26 DAP	Viability (%) 117 DAP	Shoot emerg. time DAP	First leaf emerg. time DAP
Suckers					
Greenhouse	Small (n = 45)	98	82	–	–
	Medium (n = 45)	91	71	–	–
	Large (n = 45)	78	62	–	–
Open site	Small (n = 90)	60	45	49 ± 19 a	84 ± 13 a
	Medium (n = 90)	59	49	40 ± 09 a	77 ± 23 a
	Large (n = 90)	54	44	53 ± 23 a	75 ± 17 a
Shaded site	Small (n = 90)	100	67	35 ± 11 a	76 ± 17 a
	Medium (n = 90)	80	63	44 ± 16 ab	75 ± 25 a
	Large (n = 90)	75	58	56 ± 25 b	95 ± 14 b

DAP = days after planting. Means followed by same letters are not significantly different at P = 0.05.

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Table 4
Results of sucker regeneration in *Eremospatha macrocarpa* under three nursery conditions.

Nurseries	Organ size	Viability (%) 26 DAP	Viability (%) 117 DAP	Shoot emerg. Time DAP	First leaf emerg. Time DAP
Suckers					
Greenhouse	Small (n = 45)	100	70	37 ± 13 a	43 ± 24 a
	Medium (n = 45)	98	62	52 ± 22 b	76 ± 22 b
	Large (n = 45)	50	30	80 ± 23 c	93 ± 26 b
Open site	Small (n = 90)	35	15	–	–
	Medium (n = 90)	32	10	–	–
	Large (n = 90)	27	7	–	–
Shaded site	Small (n = 90)	100	100	49 ± 23 a	62 ± 22 a
	Medium (n = 90)	100	60	59 ± 22 a	60 ± 18 a
	Large (n = 90)	47	19	–	–

DAP = days after planting. Means followed by same letters are not significantly different at $P = 0.05$.

Open site did not affect significantly leaf emergence time of *L. secundiflorum* whatever the diameter. Nevertheless, after the emergence of the first leaf, about 95–98% of *L. secundiflorum* seedlings did not survive. Shaded site has significantly accelerated leaf emergence of small and medium sucker ($P < 0.05$).

For *E. macrocarpa*, a significant difference was obtained between small sucker and the two others diameters in the greenhouse for leaf emergence time ($P < 0.05$). The shaded site did not affect significantly leaf emergence time for small and medium suckers.

Unlike open site, the viabilities percentages recorded on seedlings after first leaves emergence under shaded site were 98% and 95%, respectively for *L. secundiflorum* and *E. macrocarpa*, regardless of sucker diameter. With *E. macrocarpa* seedlings from greenhouse, we obtained 70% viability percentage if seedling were immediately displaced in shaded site after first leave emergence. This percentage was lower if seedlings remained for a longer time in the greenhouse after first leaf emergence.

3.2. Germination test

3.2.1. In vitro germination

According to the three *in vitro* treatments, embryos swelled rapidly after 6 and 10 days with *E. macrocarpa* and *L. secundiflorum*, respectively. All embryos have germinated after 22 days of incubation with *E. macrocarpa* against 26 days for *L. secundiflorum* (data not shown). In the treatment M1 without regulator growth, embryos swelled slowly and failed to form seedling

3.2.2. Seed germination in nursery

After 9 months (270 days) in nursery, only treatments 5 and 7 reached 90% of germination for *E. macrocarpa*. Seeds were still germinating at a slow rate and germination test was stopped 270 days after sowing (DAS) for *E. macrocarpa*. Average germination percentages and rates per treatment are given in Table 5. The first germination in the control treatment was observed 150 DAS for *E. macrocarpa*. Treatments 11, 12 and 13 were not significantly different from the control. There was a significant difference between control and treatments 2 up to 8. The highest germination percentages were obtained with treatments 5 (96%) and 7 (94%). Germination started in the first month and 50% of final germination percentage was achieved 110 and 180 DAS for the treatments 5 and 7 (containing GA_3), respectively (data not shown). The germination rates of all treatments were not significantly different from the control. Germination percentage and rate were not significantly correlated. Contrarily to treatments 3, 5, 6, 7 and 8, treatments 4, 11 and 12 presented significantly higher percentages of dead seeds compared to control (Fig. 1B).

For *L. secundiflorum*, the germination tests were extended to 300 days because at 270 days, the rates of germination in tests that promote seed germination of this species are still high. In the control treatment, the first germination was observed 240 DAS. Treatments 2, 11, 12, and 13 were not significantly different from the control. Germination percentage of the control was significantly different from the treatments 3 up to 10. The highest germination percentages were obtained with treatments 10 (79%),

Table 5
Germination percentages (%) and germination rates (R) of *Laccosperma secundiflorum* and *Eremospatha macrocarpa* seeds sown in nursery.

Applied treatment	*L. secundiflorum*		*E. macrocarpa*	
	%	R	%	R
1. Control	26	0.0021	36	0.0017
2. Unscarified, 4 days H_2O	34	0.0012	53*	0.0015
3. Unscarified, 8 days H_2O	62*	0.0016	58*	0.0015
4. Scarified, 4 days H_2O	44*	0.0020	70*	0.0017
5. Scarified, 4 days GA_3 (3.46×10^{-3} g l^{-1})	52*	0.0020	96*	0.0010
6. Unscarified, 4 days GA_3 (3.46×10^{-3} g l^{-1})	68*	0.0016	74*	0.0011
7. Scarified, 4 days GA_3 (3.46×10^{-4} g l^{-1})	54*	0.0020	94*	0.0011
8. Unscarified, 4 days GA_3 (3.46×10^{-4} g l^{-1})	–	–	72*	0.0011
9. Unscarified, 4 days KNO_3 (0.10 g l^{-1})	68*	0.0017	–	–
10. Unscarified, 4 days KNO_3 (1.01 g l^{-1})	79*	0.0018	–	–
11. Unscarified, H_2O_2 (5 mn), 2 days H_2O	32	0.0012	40	0.0010
12. Unscarified, H_2O_2 (10 mn), 2 days H_2O	38	0.0014	40	0.0013
13. Unscarified, Pre-chilling H_2O, 4 days	18	0.0022	26	0.0013

* Indicate significant differences from the control at 95% probability.

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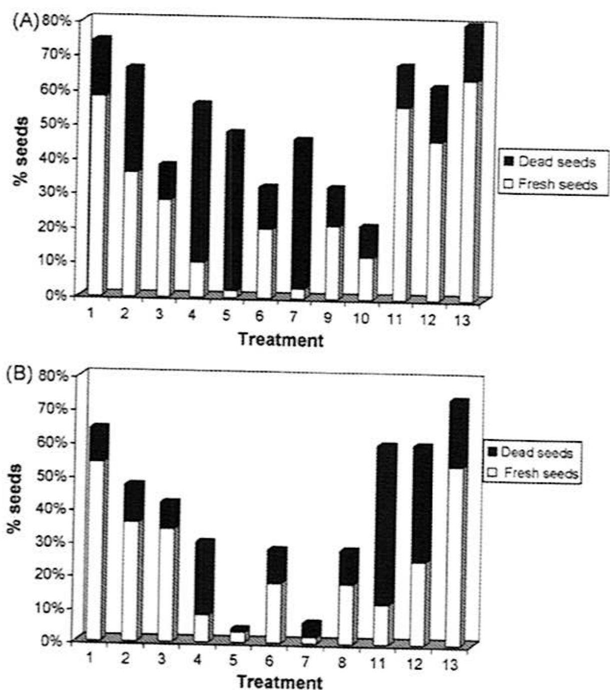

Fig. 1. Evaluation of non-germinated seeds of (A) *Laccosperma secundiflorum* and (B) *Eremospatha macrocarpa* according to treatment.

9 and 6 (68%). All treatments did not differ significantly for germination rates, which were also not significantly different from the control. There was no significant correlation between germination percentage and germination rate. The majority of non-germinated scarified seeds were decayed (Fig. 1A). Treatments 2, 3, 11, 12, 13 and the control had significantly high number of fresh seeds compared to the others.

4. Discussion

4.1. Vegetative multiplication

Seedlings mass-production by means of vegetative propagation using sucker is possible for both *E. macrocarpa* and *L. secundiflorum*. Seedling production by rhizome has been reported by Yusoff and Manokaran (1985) on *Calamus caesius* Blume, a rattan in clump. In this study, although rhizome presented viability percentage higher than 50% under shaded and greenhouse conditions, it did not develop shoot for *L. secundiflorum*. Different explanations could be mentioned for this discrepancy. The rhizome of *L. secundiflorum* includes one apical meristem and less than three axillary buds. This offset, contrarily to sucker, once separated to mother plant, may lost its capacity of regeneration. *L. secundiflorum*, naturally abundant in swampy areas (Sunderland, 2005), could have some difficulties to regenerate on a substrate consisting of a sandy topsoil with a low capacity for water retention. The high death percentage noted for *L. secundiflorum* seedlings under open site could be explained by the high solar radiation combined with the low air humidity that characterize the growing environment. No significant difference was observed between small and medium suckers for seedling emergence. Both shaded nursery and open site did not affect significantly seedlings emergence. The two sucker diameters, open site and shaded nurseries could be used in seedlings mass-production of *L. secundiflorum*. Zoro Bi and Kouakou (2004b) obtained the first shoot emergence 74 DAP for *L. secundiflorum* suckers planted under shaded site. In the present study, the mean emergence time of seedlings raising from small suckers under shaded site was 43 days. Note that Zoro Bi and Kouakou (2004b) did not categorise sucker in different sizes. The utilization of inadequate suckers (such as large diameters) could explain their results.

Concerning *E. macrocarpa*, the viability percentage of sucker under open site was very low and ranged from 35% to 27% after 26 DAP and 15% to 7% after 117 DAP. Although, this species is extremely light demanding, it occurs naturally in gap vegetation and forest margins where high air humidity is prevailing (Dransfield, 1996; Sunderland, 2001). This could explain the best emergence of seedling recorded in greenhouse where the mean relative air humidity was 85%. Results showed a significant difference between the three sucker diameters planted in greenhouse and shaded nursery. The mean emergence time of seedlings of small and medium sucker diameters ranged from 43 to 76 DAP against 93 DAP for large suckers under greenhouse. Small and medium suckers were more adapted in seedling regeneration under greenhouse and shaded nurseries. The shaded site and greenhouses appeared to be more adapted to *E. macrocarpa* seedling regeneration.

Therefore, our results showed that for both species, large suckers were less appropriate for regeneration and rattan seedlings required more shading and humidity at the early stage than at the old stage.

4.2. Germination test

4.2.1. In vitro germination

Excised embryos germinated 6–10 DAS. Embryos germination was facilitated by exogenously supplied hormone and water. The presence of growth regulators in the media (M2 and M3) enhanced the development of normal healthy plantlets, while in absence of growth regulators (M1), plantlets did not grow normally and were stunted (Patena et al., 1984; Padmanadhan and Ilangovan, 1989; Hugh et al., 2000; Goh et al., 2001). The positive results obtained in our study are almost similar to those reported by Shantha and Ramanayake (1999) on *Calamus thwaitesii* Becc. excised embryos cultured on MS medium supplemented with 0.05 mg l^{-1} of benzylaminopurine and 20 g l^{-1} of sucrose. The presence of gibberellins in our germination media has accelerated excised embryos germination of *L. secundiflorum* and *E. macrocarpa* (100% germination after 26 days) contrarily to *C. thwaitesii* for which maximum germination was obtained after 58 days of incubation.

4.2.2. Seed germination in nursery

Various treatments improved germination percentage of *L. secundiflorum* and *E. macrocarpa*. Pre-chilling seeds for 4 days in water did not improve germination percentage or rate for the two species. This result confirms findings reported by Mori et al. (1980): the latter noted that pre-chilling seeds of *C. manan* at 10–14 °C for 7 days did not improve the germination percentage and germination rate. Pre-chilling is a treatment which appears to improve seed germination of most temperate plants such as *Corylus avellana* L. (Bradbeer, 1988; Rehman and Park, 2000). In our case, the inhibitor effect observed could be explained by the fact that *L. secundiflorum* and *E. macrocarpa* have recalcitrant seeds which were damaged by the chilling (Hong and Ellis, 1997). Pre-soaking seeds of these two species for 8 days in distillate water improved significantly germination percentage compared to control. Overall, there was no significant difference in germination rates between the control and the treatments. In both species,

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sometimes once embryos swelled, it could take 30–90 days to observe seedling emergence above the substrate. This may suggest the presence of mechanical restriction of embryos growth. The radicle was probably unable to develop sufficient thrust to cause testa rupture.

For *E. macrocarpa*, treatments 5 and 7 resulted in significantly highest germination percentages. High number of non-germinated seeds in the control treatment at the end of germination test (54% of fresh seeds) indicated the presence of dormancy. As soaking scarified seeds in water for 4 days (treatment 4) improved germination percentage and excised embryos germinated well, dormancy was probably exogenous (Bewley and Black, 1994; Schmidt, 2000). The fact that highest germination percentages and first germinations were recorded within a 30 days period in the treatments containing GA_3 might suggest chemical dormancy. Gibberellic acid treatment is typically used for leaching out inhibitors (Ellis et al., 1985; Rehman and Park, 2000; Delanoy et al., 2006). The most successful methods showed only 2% and 3% of fresh seeds, respectively for treatments 7 and 5. These two treatments showed a low level of dormant seeds at the end of the test. This could indicate that both treatments (scarified seeds and co-applying GA_3) were able to overcome dormancy. Gibberellic acid is a costly phytohormone that is unaffordable for most farmers in developing countries. However, treatment 4 without GA_3 application showed 70% germination percentage. Depending on the cost and effectiveness of treatments, treatments 4, 5, and 7 should be recommended.

At the end of the test, control treatment resulted in 58% of fresh seeds, indicating a high level of dormancy in *L. secundiflorum* seeds. Treatments 4, 5 and 7, although improving significantly the germination percentage compared to the control, resulted in significantly higher percentage of dead seeds compared to other treatments using unscarified seeds. *L. secundiflorum* possesses small seeds, and damage could have occurred during scarification, given that the micropylar region is very sensitive. The use of scraper paper that performs low nicks in the seed could preserve the embryos survival. As excised embryos germinated very well and scarified seeds soaked in water for 4 days (treatment 4) improved germination percentage, the presence of exogenous dormancy can be suggested (Bewley and Black, 1994; Schmidt, 2000). Treatments using GA_3 and KNO_3 improved germination percentage which could indicate the presence of chemical dormancy. Application of GA_3 and KNO_3 showed positive effect in overcoming dormancy caused by inhibitors (Mori et al., 1980; Bradbeer, 1988; Bewley and Black, 1994; Rehman and Park, 2000). Therefore, we suggest that both mechanical and chemical dormancy might be present in seeds of *L. secundiflorum*.

5. Conclusion

Better seedling regeneration was obtained from small and medium sucker. *L. secundiflorum* suckers regenerated better under 31 °C and a low relative air humidity of 66%. Those of *E. macrocarpa* regenerated seedlings better under high relative air humidity (85%), coupled with relatively high temperature (38 °C).

The treatments using seeds scarification combined with soaking in GA_3 gave the highest germination percentages in *E. macrocarpa*. In *L. secundiflorum*, seed scarification led to death and KNO_3 application on unscarified seeds improved significantly percentage of germination. The two species showed dormancy of both chemical and mechanical nature. These two types of dormancy have been reported in several species of Arecaceae, in particular in *Areca triandra* Roxb. (Yang et al., 2007).

Further investigations are needed to improve the seedlings mass-production: this concerns substrate type and fertilization, soil moisture content, identification of dormancy causes and treatment to overcome them.

Vegetative propagation and seedlings production by seeds could be helpful to improve the well being for many rural populations and provide the basis of a thriving cottage industry, given that presently, the application of biotechnology (namely the tissue culture) seems inappropriate, due to the current economic status of people of those communities.

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References

Bewley, J.D., Black, M., 1994. Seeds Physiology of Development and Germination. Plenum Press, New York.
Bradbeer, J.W., 1988. Seed Dormancy and Germination. Blackie, New York.
Bronschat, T.K., Donselman, H., 1988. Palm seed storage and germination studies. Principe 32, 3–12.
Chuthamas, P., Prutpongse, P., Vongkaluang, I., Tantiwiwat, S., 1989. In vitro culture of immature embryos of *Calamus manan* Miq. in: Rao, A.N., Vongkaluang, I. (Eds.), Recent Research on Rattan. Thailand, pp. 144–147.
Delanoy, M., Damme, P.V., Scheldeman, X., Beltran, J., 2006. Germination of *Passiflora mollissima* (kunth) L.H. Bailey, *Passiflora tricuspis* Mast. and *Passiflora nov* sp. seeds. Sci. Hortic. 110, 198–203.
Dransfield, J., 1996. The Rattan Taxonomy and Ecology. INBAR, Sarawak, Sabah.
Edwards, M.D., Lower, R.L., Staub, J.E., 1986. Influence of seed harvesting and handling procedures on germination of Cucumber seeds. J. Am. Soc., Hortic. Sci. 111, 507–512.
Ellis, R.H., Hong, T.D., Robrechts, E.H., 1985. Handbook for Seed Technology for Genebanks. IBPGR, Rome, Italy.
Goh, D.K.S., Bon, M.-C., Alliotti, F., Escoute, J., Ferriere, N.M., Monteuuis, O., 2001. In vitro somatic embryogenesis in two major rattan species: *Calamus merrillii* and *Calamus subinermis*. In vitro cell develop. Biol. Plant. 37, 375–381.
Hong, T.D., Ellis, R., 1997. A Protocol to Determine Seed Storage Behaviour. IPGRI, UK.
Howthorne, W.D., 1990. Field Guide to the Forest Trees of Ghana. Natural Resources Institute (NRI), London (UK).
Hugh, W., Louisa, A., Davies, R., 2000. Role of embryo culture in the seed conservation of palms and other species. In: Razdan, M., Cocking, E. (Eds.), Conservation of Plant Genetic Resources in vitro. Science Publishers, USA, pp. 88–166.
Manokaran, N., 1978. Germination of fresh seeds of Malaysian rattans. Malay. For. 41, 319–345.
Mori, T., Bin, Z., Rahman, A.B.D., Tan, H., 1980. Germination and storage of rotang manau (*Calamus manan*) seeds. Malay. For. 43, 44–55.
Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473–497.
Nagao, M.A., Kanegawa, K., Sakai, W.S., 1980. Accelerating palm seed germination with gibberellic acid, scarification, and bottom heat. Hortic. Sci. 15, 200–201.
Oteng-Amoako, A., Obiri-darko, B., 2002. Rattan as sustainable industry in Africa: the need for technological interventions. In: Dransfield, J., Tesoro, F., Manokaran, N. (Eds.), Rattan Current Research Issues and Prospects for Conservation and Sustainable Development. FAO-INBAR, Rome, pp. 89–100.
Padmanadhan, D., Ilangovan, R., 1989. Studies on embryo culture in *Calamus rotang* Linn. RIC Bull. 8, 5–9.
Paranjothy, K., 1993. Tissue culture of Palms. In: Andover, U.K., Hampshire, (Eds.), Plant Biotechnology: Commercial Prospects and Problems. Palm Oil Research Institute of Malaysian, Royaume-Uni, pp. 73–83.
Patena, F.L., Mercado, M.S., Barba, R.C., 1984. Rapid propagation of rattan (*Calamus manillensis* H. Wendl.) by tissue culture. Philipp. J. Crop Sci. 9, 217–218.
Rehman, S., Park, I.-H., 2000. Effect of scarification, GA and chilling on the germination of goldenrain-tree (*Koelreuteria paniculata* Laxm.) seeds. Sci. Hortic. 85, 319–324.
SAS, 1999. SAS/ETS User's guide. SAS Inst., Cary.
Shantha, M., Ramanayake, S.D., 1999. Viability of excised embryos, shoot proliferation and *in vitro* flowering in a species of rattan *Calamus thwaitesii* Becc. J. Hortic. Sci. Biotechnol. 74, 594–601.
Schmidt, L., 2000. Guide to Handing of Tropical and Subtropical Forest Seed. Danida Forest Seed Centre, Denmark.
Sunderland, T.C.H., 1999. Recent research into *African rattans* (Palmae): a valuable non-wood forest product from the forests of Central Africa. http://www.fao.org/docrep/x2161e/x2161e09.htm.
Sunderland, T.C.H., 2001. The Taxonomy, Ecology and Utilisation of African Rattans (Palmae: Calamoieae). University College, London.

Author's personal copy

Sunderland, T.C.H., 2005. A Field Guide to the Rattan Palm of Africa. Royal Botanic Gardens, INBAR, CARPE, Kew.

Yang, Q.-H., Ye, W.-H., Yin, X.-J., 2007. Dormancy and germination of *Areca triandra* seeds. Sci. Hortic. 113, 107–111.

Yusoff, A.M., Manokaran, N., 1985. Seed and vegetative propagation of rattans. In: Wong, K.M., Manokaran, N. (Eds.), Proceedings of Rattan Seminar. The Rattan Information and Forest Research Institute, Kuala Lumpur, pp. 13–21.

Zoro Bi, I.A., Kouakou, L.K., 2004a. Etude de la filière rotin dans le district d'Abidjan (Sud Côte d'Ivoire). Biotechnol. Agronom. Soc., Environ. 8, 199–209.

Zoro Bi, I.A., Kouakou, L.K., 2004b. Vegetative propagation methods adapted to two rattan species (*Laccosperma secundiflorum* and *L. laeve*). Tropicultura 22, 163–167.

Zoro Bi, I.A., Kouassi, I.K., 2004. Rattan abundance and collection in N'zodji forest (South Eastern Côte d'Ivoire). Rev. Ivoir. Sci. Technol. 5, 185–197.