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

Kouakou Laurent Kouakou, Irié Arsène Zoro Bi, Yao Georges Abessika, Tanoh Hilaire Kouakou, Jean-Pierre Baudoin

*Université Abobo-Adjamé, UFR des Sciences de la Nature, Laboratoire de Biologie et Amélioration des Cultures Végétales, BP 801 Abidjan, Côte d'Ivoire*

*Gambleux Agricultural University, (FUSAGs), Tropical Crop Husbandry and Horticulture, 2 passage des Déportés, B-5030 Gembloux, Belgium*

ARTICLE INFO

Article history:
Received 15 May 2008
Received in revised form 22 September 2008
Accepted 24 October 2008

Keywords:
Rattan regeneration
Sucker
Seed
GA3
KNO3
Germination

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 unsheared seeds for 4 days in 1.01 g l−1 and 0.10 g l−1 KNO3, with 79% and 68% of germination, respectively and in 3.46 × 10−3 g l−1 GA3 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 sheared seeds in 3.46 × 10−3 g l−1 and 3.46 × 10−4 g l−1 GA3, 96% and 94% of germination, respectively. Dormancy, probably a combination of mechanical and chemical dormancy, is present in the two species.

© 2008 Elsevier B.V. All rights reserved.

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, 1959). Although less marketed compared to Asian species, the rattans from Africa can significantly contribute to both rural development and global market (Oteyn-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 (Padmanathan 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
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 (Suderland, 1999, 2001). According to Manokaran (1978) and Morj 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 (2004) 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. macroura, 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 clasée de N’Zooudji", Albé-Pôe 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. Organs collected are stored in hermetically sealed plastic bag and transported to the research station of the University of Abobo-Açamé where experiments were carried out. On the basis of the works of Yousoff and Manokaran (1985) on Calamus spp. and Zoro Bi and Kouakou (2004b) on L. secundiflorum, two organs were used: rhizome and sucker. As E. macroura 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 “îles Etillé” Adiaké, in the South-East of Côte d’Ivoire (5° 9’24” N latitude, 3° 16’42” W longitude). E. macroura 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° 36’13” 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 31°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 cm²), 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 vaporisation 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 (%) = (NVC/NVO) × 100, where NVO is the number of viable organ and NVO 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 (Chutham et al., 1989; Paranjathy, 1992), 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 (GA₂) 3.46 × 10⁻⁴ g l⁻¹ and 3.46 × 10⁻³ g l⁻¹, respectively. The three media were incubated in a growth chamber at 28 ± 2°C under darkness (Chutham et al., 1989; Shanith and Ramanayake, 1999). For each species and each medium, 72 excised embryos were arranged in three replicates. Each embryo was placed in a 35 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 (Pathmanathan 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 I. Treatments applied were based on previous results of germination experiments in Calamus spp. (Morj et al., 1990; 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. macroura seeds. Note that the E. macroura 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. macroura and 50
seeds of *L. secundiflorum*. 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 cloth, 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 \( \sum n t \) (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, non-parametric 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 4

Results of sucker regeneration in *Fremaspaha macrocarpa* under three nursery conditions.

<table>
<thead>
<tr>
<th>Nurseries</th>
<th>Organ size</th>
<th>Viability (%)</th>
<th>Viability (%)</th>
<th>Shoot emerg.</th>
<th>First leaf emerg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26 DAP</td>
<td>117 DAP</td>
<td>Time DAP</td>
<td>Time DAP</td>
<td></td>
</tr>
<tr>
<td>Suckers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greenhouse</td>
<td>Small (n = 45)</td>
<td>100</td>
<td>70</td>
<td>37 ± 13 a</td>
<td>43 ± 24 a</td>
</tr>
<tr>
<td></td>
<td>Medium (n = 45)</td>
<td>68</td>
<td>62</td>
<td>51 ± 22 b</td>
<td>76 ± 22 b</td>
</tr>
<tr>
<td></td>
<td>Large (n = 45)</td>
<td>50</td>
<td>30</td>
<td>80 ± 23 c</td>
<td>93 ± 29 b</td>
</tr>
<tr>
<td>Open site</td>
<td>Small (n = 90)</td>
<td>35</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Medium (n = 90)</td>
<td>32</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Large (n = 90)</td>
<td>27</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shaded site</td>
<td>Small (n = 90)</td>
<td>100</td>
<td>100</td>
<td>49 ± 23 a</td>
<td>62 ± 22 a</td>
</tr>
<tr>
<td></td>
<td>Medium (n = 90)</td>
<td>100</td>
<td>60</td>
<td>51 ± 22 a</td>
<td>60 ± 18 a</td>
</tr>
<tr>
<td></td>
<td>Large (n = 90)</td>
<td>47</td>
<td>19</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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 seedlings were immediately displaced in shaded site after first leaf 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 of 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 GA₃), 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 *Leucocepero secundiflorum* and *Fremaspaha macrocarpa* seeds sown in nursery.

<table>
<thead>
<tr>
<th>Applied treatment</th>
<th><em>L. secundiflorum</em></th>
<th><em>E. macrocarpa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>R</td>
</tr>
<tr>
<td>1. Control</td>
<td>26</td>
<td>0.0021</td>
</tr>
<tr>
<td>2. Uncscarified, 4 days H₂O</td>
<td>34</td>
<td>0.0012</td>
</tr>
<tr>
<td>3. Uncscarified, 4 days H₂O</td>
<td>62*</td>
<td>0.0016</td>
</tr>
<tr>
<td>4. Uncscarified, 4 days H₂O</td>
<td>44*</td>
<td>0.0020</td>
</tr>
<tr>
<td>5. Uncscarified, 4 days GA₃ (3.46 × 10⁻⁶ g⁻¹)</td>
<td>52*</td>
<td>0.0020</td>
</tr>
<tr>
<td>6. Uncscarified, 4 days GA₃ (3.46 × 10⁻⁸ g⁻¹)</td>
<td>68</td>
<td>0.0016</td>
</tr>
<tr>
<td>7. Uncscarified, 4 days GA₃ (3.46 × 10⁻⁸ g⁻¹)</td>
<td>54</td>
<td>0.0020</td>
</tr>
<tr>
<td>8. Uncscarified, 4 days GA₃ (3.46 × 10⁻⁶ g⁻¹)</td>
<td>-</td>
<td>0.0020</td>
</tr>
<tr>
<td>9. Uncscarified, 4 days KNO₃ (0.10 g⁻¹)</td>
<td>68</td>
<td>0.0017</td>
</tr>
<tr>
<td>10 Uncscarified, 4 days KNO₃ (0.10 g⁻¹)</td>
<td>79</td>
<td>0.0018</td>
</tr>
<tr>
<td>11 Uncscarified, H₂O (5 mm), 2 days H₂O</td>
<td>32</td>
<td>0.0012</td>
</tr>
<tr>
<td>12 Uncscarified, H₂O (10 mm), 2 days H₂O</td>
<td>38</td>
<td>0.0014</td>
</tr>
<tr>
<td>13 Uncscarified, Pre-chilling H₂O, 4 days</td>
<td>18</td>
<td>0.0022</td>
</tr>
</tbody>
</table>

* Indicate significant differences from the control at 95% probability.
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 Vissoff 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 nursery 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; Padmanathan 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 thwaitesi 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. thwaitesi 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. (Bradbury, 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,
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 higher germination percentages and first germinations were recorded within a 30 days period in the treatments containing GA3 might suggest chemical dormancy. Gibberellic acid treatment is typically used for leaching out inhibitors (Ellis et al., 1985; Rehman and Park, 2000; Delanyo et al., 2006). The most successful methods showed only 2% and 3% of fresh seeds, respectively for treatments 5 and 7. 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 GA3) were able to overcome dormancy. Gibberellinic acid is a costly phytohormone that is unaffordable for most farmers in developing countries. However, treatment 4 without GA3 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 and 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 GA3 and KNO3 improved germination percentage which could indicate the presence of chemical dormancy. Application of GA3 and KNO3 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 chemical and physical 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 21 °C and a low relative air humidity of 65%. Those of *E. macrocarpa* regenerated seedlings better under high relative air humidity (35%), coupled with relatively high temperature (38 °C).

The treatments using seed scarification combined with soaking in GA3 gave the highest germination percentages in *E. macrocarpa*. In *L. secundiflorum*, seed scarification led to death and KNO3 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.

Acknowledgements

Fellowship to Kouakou L. Kouakou was awarded by Agence Universitaire de la Francophonie (AUF), Abidjan (Côte d’Ivoire). Thanks also for the help of Didier Leurquin, a research technician of the Unit of Tropical Crop Husbandry and Horticulture of Gembloux Agricultural University.

References


