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Somatic embryogenesis-derived coffee plantlets can be efficiently propagated by horticultural rooted mini-cuttings: A boost for somatic embryogenesis

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ABSTRACT

In general, the current industrial somatic embryogenesis (SE) propagation processes for coffee are costly because they are not productive enough. We show that SE-derived plantlets from *C. arabica* hybrids were temporarily – between 10 and 25 weeks of development in nursery – able to root with a high success rate (up to 90%) whatever the genotype tested, before gradually losing that capacity. We took advantage of this transient rooting capacity, probably due to the rejuvenation process occurring during SE, to establish a new propagation system based on the continuous culture of rejuvenated SE plants and on the serial rooting of cuttings under nursery conditions, known as horticultural rooted mini-cutting (HRMC). The excessively low SE efficiency with an embryo-to-plantlet conversion rate of only 37% can be greatly offset by the much higher HRMC multiplication rate (14 in six months) and better overall quality. Fifteen week-old rooted mini-cuttings proved to be more uniform (2–4.5 vs.1–5.5 cm for plant height distribution) and vigorous (1.41 vs. 0.81 mm for stem diameter) than same-age somatic seedlings. This effect persisted for five years after field planting, mainly through a slightly greater collar diameter (43.3 vs 40.6 mm), whereas at root level no differences were found. The HRMC method is expected to dramatically reduce arabica hybrid production costs (by up to 50% at US$ 0.27/plant ready for field planting) and thus to promote the mass utilization of genetically superior hybrid clones of coffee.

1. Introduction

More than 85% of arabica coffee is produced in Latin America, from trees comprising a small number of so-called "American" varieties, derived from a narrow genetic base. These homozygous varieties, known as "lines", reproduce from seed. Some F1 hybrid varieties have been created by crossing traditional American varieties with some wild parents originating from Ethiopia (the center of origin of the *Coffea arabica* species) (Bertrand et al., 2011). The hybrid varieties produce 20–40% more than the best cultivated lines. Given their heterozygous structure, F1 hybrids must be vegetatively propagated. The best individual is selected from the best F1 families to be cloned, thereby creating an F1 hybrid clone (Van der Vossen et al., 2015).

It is difficult to propagate arabica coffee trees by conventional horticultural techniques such as cuttings, as they are rooting-recalcitrant, or by budgrafting, which requires too much manpower (Etienne et al., 2002). In *in vitro* conditions, micro-cutting techniques have been developed for coffee but cannot be considered for mass propagation, as it is very difficult to establish axenic cultures *in vitro* and it involves too much work for low multiplication rates (Bertrand-Desbrunais et al., 1991). Somatic embryogenesis (SE) has been effectively mastered on an industrial level for the *C. arabica* species (Bobadilla Landey et al., 2013). The CIRAD-ECOM group consortium has been producing between one and two million intraspecific F1 hybrid plants per annum in Nicaragua and Mexico since 2007 (Georget et al., 2010). In spite of these achievements, the arabica SE process is still impeded by two technical bottlenecks: an embryo-to-plantlet conversion rate that is too low, and excessive plant losses in the nursery at each step of the acclimatiza-

Abbreviations: RH, Relative Humidity; HRMC, Horticultural Rooted Mini-Cuttings; SES, omatic Embryogenesis.

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tion and hardening process, which renders this propagation system insufficiently cost-effective and productive (Etienne et al., 2013). At present, these excessive production costs are a major obstacle to the mass dissemination of coffee F1 hybrids in Central America. A similar situation is found with the *C. canephora* coffee species, but also in many other woody species, leading to somatic seedling production costs that are too high, thereby holding back the large-scale utilization of somatic seedlings as planting materials (Lelu-Walter et al., 2013).

Recently, we discovered that very young *C. arabica* plants derived from SE were able to root with a high success rate. In this paper, we describe the development of a mini-cuttings propagation system using somatic seedlings. Several issues are examined to assess the technique. First, we provide a reminder of the limitations of the SE method by measuring the biological efficiency of the final stages of this propagation system. We then go on to test the production of rooted mini-cuttings by evaluating the genotypic effect, the incidence of somatic seedling age on the capacity of rooting, and the feasibility of successive multiplication cycles (serial cuttings) from a single somatic seedling. We proceed to compare the vigor and the homogeneity of the material produced by rooted mini-cuttings compared to somatic seedlings at nursery and field level. Finally we perform a pilot production to estimate the gain in productivity achieved and the consequences for production costs.

2. Materials and methods

2.1. Producing somatic embryo-derived plantlets and assessing the efficiency of the last SE steps

Somatic seedlings derived mainly from six F1 hybrids obtained by crossing traditional dwarf American varieties (Caturra, T5296 and 17931 Sarchimor lines) and wild accessions originating from Ethiopia and Sudan (Bertrand et al., 2005, 2006) were used in this study. In this paper, these hybrids will be called H1, H3, H5, H10, H16 and H17.

The regeneration of cotyledonary somatic embryo of *C. arabica* species has already been described in detail (Etienne 2005; Bobadilla Landey et al., 2013). Plantlet conversion was obtained after direct sowing of cotyledonary somatic embryos in the nursery. Cotyledonary embryos were sown vertically on top of the substrate comprising a mix of Peatmoss (Pro-mix, Premier Tech Ltd, Canada) and sand. Somatic embryo culture density in the plastic boxes (l.w.h = 30/21/10 cm) was approximately 3600 per square meter. The cultures were placed under a transparent roof that provided 50% shade, and were watered for 2 mins twice daily. The conversion of the somatic embryos into plants was generally observed 12 weeks after sowing, and was characterized by the emergence of a stem bearing at least two pairs of true leaves. For the growth and hardening step in the nursery (21 weeks), plantlets grown from somatic embryos were transferred to 1 l plastic bags containing soil and coffee pulp (3/1, v/v) under conventional nursery conditions until they reached the required size for planting in the field (approx. 30 cm). During this stage, the shade (50% light interception) and relative humidity (RH, 90%) were gradually reduced over 4 weeks to 0% light interception, with natural RH ranging from 40 to 80%.

The biological efficiency of the last SE steps in the nursery, i.e. plantlet conversion average duration of the acclimatization step was measured from the results of the industrial production, mainly with H1 (Sarchimor T5296 x Rome Sudan) and H3 hybrids (Caturra x Ethiopian 531), of 5 million embryos regenerated in Nicaragua between 2010 and 2012. The embryo-to-plantlet conversion rate and the average duration of the weaning phase in the nursery were used as biological indicators.

2.2. Experimental design to establish a rooted mini-cutting vegetative propagation process

The technique known as 'horticultural rooted mini-cuttings (HRMC)' is illustrated in Fig. 1 and comprises the following stages:

2.2.1. Cutting origin and characteristics

The plants used to initiate the propagation cycle are 15-week-old nursery plantlets derived from somatic embryos (Etienne et al., 2012). The size of the plantlets is around 4–6 cm in height with 3 pairs of fully developed leaves (Fig. 1A). "Tip mini-cutting" – so called because it bears the terminal apex – is used. It is formed of two pairs of developed leaves (Fig. 1B). Fine secateurs are used to make the cut, just above the third pair of leaves, in order to keep a long enough internode. It is only 3–4 cm long, hence the term "mini-cutting" to signify the miniaturization of the plant material compared to the 20 cm-long conventional cuttings.

2.2.2. Rooting and hardening conditions

The rooting process consisted of inserting the mini-cuttings into trays (Fig. 1C) filled with substrate composed of a mixture of 30% sand and 70% commercial peat of the Peatmoss type (Pro-mix, Premier Tech Ltd, Canada). The trays were then placed in weaning greenhouses under a plastic tunnel with up to 95% RH and a temperature ranging from 18 to 20 °C at night to 25-30 °C during the day. The plastic was removed gradually 5–6 weeks later.

After a 6-week long weaning period (Fig. 1D), the rooted mini-cuttings were placed for 4–5 weeks under hardening conditions at a night/day temperature of 16–18/20–25 °C and a 60–80% RH. After the hardening, the rooted mini-cuttings reaching an average height of 8–10 cm (Figs. 1E, 1F) were transferred directly into 1.5 l polybags (Fig. 1G) in traditional coffee seedling nurseries for 4 months – the time needed for the plant to be developed enough i.e. 25–35 cm tall to be field planted (Fig. 1H).

2.3. Evaluation of the genotypic effect on the rooting capacity of mini-cuttings

The pilot production of 225,000 mini-cuttings derived from SE (15 weeks after somatic embryos were germinated and weaned in the greenhouse) was subjected to the protocol described above and rooting rates were then assessed. Rooting capacity was evaluated on 225,000 cuttings of H1 (T5296 × Rume Sudan), H3 (Caturra × Et 531), H5 (T5296 × Et06) and H10 (T5296 × Rume Sudan) clones (between 50,000 to 60, 000 cuttings for each clone) by observing the presence of the adventitious roots at the bottom of each mini-cutting, and calculating the rate between the number of cuttings initially set in trays and the number of rooted plants transferred to bags in nurseries after 10 weeks of the rooting process.

2.4. Rooting capacity of mini-cuttings depending on the SE-derived plantlet age

To establish the optimum age for somatic embryo-derived nursery plantlets to produce rooted mini-cuttings, mini-cuttings were produced from somatic seedlings at regular intervals over a 40-week nursery period and tested for their rooting capacity. To do this, three replications of 50 mini-cuttings were carried out with the H1 hybrid clone for each of the following periods of time: 10, 15, 20, 25, 30, 35 and 40 weeks after sowing the somatic embryos under *ex vitro* conditions. The rooting response was assessed 10 weeks after planting the generated mini-cuttings by noting the presence/absence of roots and measuring the length of the main roots. Plants with roots up to 3 cm in length were considered as rooted.

Fig. 1. Setting-up of an experimental rooted mini-cutting vegetative propagation process from rejuvenated somatic embryogenesis-derived plants. A: 15-week rejuvenated plants derived from the somatic embryogenesis process sown in a plastic box; B: Cuttings from somatic seedlings; C: Planting of the mini-cuttings in plug trays; D: Rooted mini-cuttings obtained after 6 weeks of acclimatization in the greenhouse; E: 8-week rooted mini-cuttings in plot substrate; F: 8-week bare rooted mini-cuttings; G: 3-month rooted mini-cuttings hardening in the nursery; H: 6-month rooted mini-cuttings in nursery bags ready to be planted in the field.

2.5. Propagation rates achieved over a 6-month period through successive multiplication cycles with rooted mini-cuttings

An initial pool of 26,000 somatic seedlings (H1 and H3 hybrids) underwent successive serial mini-cutting cycles within a period of 6 months. The number of cuttings obtained and the rooting rate of the cuttings were monitored throughout this period after each mini-cutting cycle. In order to maintain and to prolong the juvenile status, all the plants produced from the initial pool were kept in the greenhouse with strong mineral nutrition, and in total confinement for the duration of the experiment.

2.6. Qualitative comparison of plants derived from somatic seedlings vs from rooted mini-cuttings at nursery stage and 5 years later in the field

i) In the greenhouse: this study was carried out under commercial production conditions at the end of the acclimatization process, i.e. fifteen weeks after sowing plantlets (somatic embryos and rooted mini-cuttings respectively). The quality of the plantlets from batches of somatic seedlings was compared to that of rooted mini-cuttings under similar greenhouse conditions. Two hundred plants derived from somatic seedlings were compared to 200 plants derived from rooted mini-cuttings after 15 weeks' weaning in the greenhouse. Four replications of fifty plants each were carried out for the H1 hybrid and were randomly chosen in each type of container, i.e. plastic boxes for somatic seedlings or plug trays for rooted mini-cuttings. The plant batches were assessed by studying the variability in plant and root sizes, and stem and root diameters and for their uniform growth.

ii) In the field: H1 hybrid plants derived from somatic seedlings and rooted mini-cuttings were planted in 2010 in Nicaragua (Cumplida farm, Matagalpa) to carry out a first agronomic evaluation of the conformity of the material produced in both ways. Two hectares of each material were planted and phenotypically observed in the field (8000 plants), five years after planting. In the two parcels, 100 plants derived from somatic seedlings were compared to 100 plants derived from rooted mini-cuttings and parameters such as plant height, stem diameter, internode number, primary branch mortality, and primary branch length were measured. Moreover, among the studied plants, 10 plants of each material showing the same vigor (with the same stem diameter) were uprooted to compare the root systems. Root diameter, plant height, main root length, root fresh weight and stem fresh weight were assessed.

Table 1
Somatic embryo-to-plantlet conversion rate and average duration for plantlet conversion as obtained from production data in the framework of the commercial dissemination of *Coffea arabica* hybrids in Nicaragua (Exportadora Atlantic S.A., ECOM group, data from 2010 to 2012). The plantlet conversion duration values are means ± SD.

Hybrid clones	No. of embryos sowed	Embryo-to-plantlet conversion (%)	Duration for plantlet regeneration (weeks)
H1 (T5296 × RS)	3 478 141	35 ± 3.5	30 ± 7
H3 (Caturra × ET 531)	1 454 886	36 ± 2.8	34 ± 10
H10 (T5296 × RS)	78 543	56 ± 4.9	44 ± 12
H16 (T5296 × ET01A1)	142 964	41 ± 2.2	18 ± 1
H17 (Catuai × ET59A2)	264 344	31 ± 3.6	16 ± 2
Other hybrids	223 029	33 ± 2.8	19 ± 4
Total/Average	5 641 907	37 ± 3.5	30 ± 11

2.7. Production costs of rooted mini-cuttings compared to somatic seedlings and seedlings

The production costs for plants ready to be field planted (around 30–40 cm height) regenerated from different materials, i.e. seedlings, somatic seedlings and rooted mini-cuttings, were calculated in the production units and nurseries of Exportadora Atlantic S.A (ECOM group, Nicaragua) then compared with each other. The costs were calculated in 2014 from the pilot production of 300,000 rooted mini-cuttings derived from an initial pool of 20,000 somatic seedlings in greenhouses and transferred to nursery bags, 200,000 Caturra seedlings in nursery bags, 3 million somatic embryos from H1 and H3 hybrids produced in the Exportadora Atlantic S.A. laboratories and 650,000 somatic seedlings of both hybrids acclimatized in the greenhouse and then transferred to nursery bags.

2.8. Statistical analysis

Most of data were analyzed by ANOVA followed by the Fisher Least Significance Difference test (LSD). Various small letters in the tables indicate significant differences for each parameter between means ($P \leq 0.05$). The data are the means of measurement ± SD on independent samples. The bars represent the standard deviations (SD).

3. Results and discussion

3.1. The embryo-to-plantlet conversion step is a major bottleneck in the coffee SE propagation process

The embryo-to-plantlet conversion step in the greenhouse for cotyledonary embryos is the trickiest stage of the SE propagation procedure. At present, this stage is a major bottleneck in industrial terms as, for all the genotypes studied, on average only 37% of embryos regenerate plantlets suitable for subsequent transfer to nursery bags (Table 1). In addition, the average time taken to wean and acclimatize somatic embryos is also too long and confirms the inefficiency of this stage. On average, it takes 30 weeks to harvest all the plantlets having reached the expected size. In addition, plantlet conversion and growth is asynchronous within the same batch of plants and four to five successive harvesting rounds are often needed to select all the plantlets suitable for the following stages in the nursery (data not shown). This heterogeneous growth, which arises from the *in vitro* germination stage in a bioreactor, complicates management of the technical procedures and entails extra manpower, even though, in terms of development, most of the plants have caught up with seedlings by the end of the nursery stage (Barry-Etienne et al., 2002; Menéndez-Yuffá et al., 2010)

Industrial or pre-industrial application of SE currently involves only a few woody species, such as Douglas-fir (CellFor, Canada, http://www.cellfor.com/), white and Sitka spruces (Arborgen, USA, http://www.arborgen.com/), radiata, loblolly and slash pines (Forest Genetics, New Zealand, http://www.forest-genetics.com/) in the gymnosperms, along with the hybrid yellow poplar (International Paper, USA, http://www.internationalpaper.com/), oil palm (Felpa Inc., Malaysia, http://www.felpaglobal.com/), *C. canephora* (Agromod SA-de CV/Nestlé, Mexico, http://www.agromod.mx/), *C. arabica* (CIRAD/ECOM, Mexico, Costa Rica and Nicaragua, http://www.ecomtrading.com/) and Cocoa (Nestlé, Indonesia, http://www.nestle.co.id/ina/) in the angiosperms. Despite these few examples for which annual production now exceeds a few hundred thousand, or even a few million plants, most of the time the SE propagation procedures for woody plants are not cost-effective as there are many technical obstacles. The quality of regenerated somatic embryos, their low conversion rates for example in cocoa (Guillou et al., 2014) and in coffee (this paper), and the incidence of somaclonal variations on the conformity of the banana and oil palm plants produced (Côte et al., 2000; Jaligot et al., 2011) are major drawbacks.

3.2. Genotype effects

Fig. 2 shows that rooting capacity of mini-cuttings under nursery conditions was up to 90% with 15-week-old somatic seedlings. The genotypic effect is often high in vegetative reproduction processes (Lardet et al., 2009; Bonga and Durzan, 1987; Celestino et al., 2013). Here, with mini-cuttings harvested from 10- to 25-week-old young somatic seedlings, no genotypic effect was found in the various hybrids' responses for rooting capacity.

3.3. Evidence of rejuvenation in somatic seedlings through transiently recovered rooting capacity of Arabica mini-cuttings

The ability of *C. arabica* mini-cuttings to root was linked to the juvenile nature of the plant from which the cutting was taken. In a kinetic study, we took a closer look at this phenomenon. The rooting capacity of mini-cuttings taken from *C. arabica* somatic seedlings declined with the increasing age of the somatic seedlings from which they had been collected in the nursery (Fig. 3). This is particularly obvious between 25–35-week-old somatic seedlings with corresponding rooting rates of 80–20%, respectively. When mini-cuttings were harvested from 35 to 40-week-old nursery plants, rooting capacity was stabilized at around 15–20%. The transient rooting capacity of mini-cuttings derived from coffee somatic seedlings was probably linked to the rejuvenation process resulting from the successive cell dedifferentiation/re-differentiation mechanisms occurring during the onset of the SE programme.

For many woody species, mature trees are recalcitrant to vegetative propagation. Greenwood et al. (2001) showed that the decline in adventitious rooting capacity in forest tree cuttings is generally one of the most dramatic effects of the maturation process and is the most widely used morphological and physiological marker to assess maturation. Somatic embryogenesis *in vitro* is the most efficient method used for inducing rejuvenation (Pierik 1990; Carron et al., 1995; Perrin et al., 1997). For example, 43–55% rooting frequencies were obtained in *in vitro* shoots from two *H. brasiliensis* genotypes regenerated from somatic seedlings, whereas it was impossible to initiate rooting in shoots obtained from the mature plant material

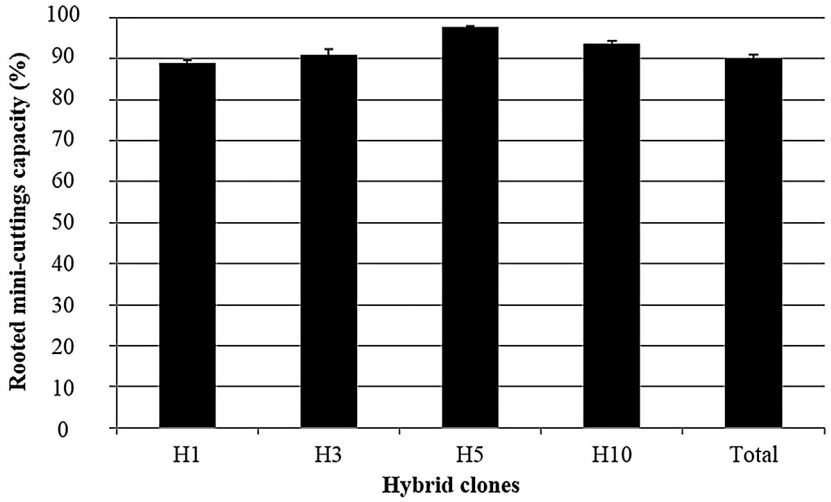

Fig. 2. Evaluation of mini-cutting rooting capacity of several *C. arabica* F1 hybrid clones. Rooting capacity was evaluated on 50,000 to 60,000 plantlets/clone by observing the presence of roots at the bottom of each plant ten weeks after rooting induction. Rooting capacity values are means of 50,000 measurements ± SD (standard deviation). The bars represent the SD.

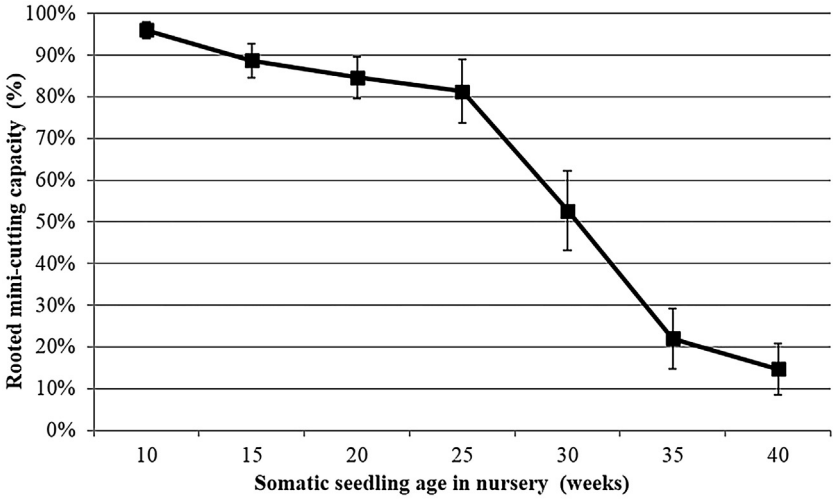

Fig. 3. Evolution of rooting capacity in mini-cuttings depending on the age of the somatic seedlings (i.e. growth duration in nursery) from which the mini-cuttings were taken. The kinetics were performed over a 10–40-week period of somatic seedling development in nursery bags. Each point represents an average of 150 values (3 replications of 50 mini-cuttings) ± SD.

(Lardet et al., 2009). Aging in plants was found to induce cyto-and histomorphological changes and modify certain metabolic pathways in *Sequoiadendron giganteum* (Mankessi et al., 2011). DNA methylation plays a key role in the various maturational changes observed in plants during their ontogenetic development – the prevailing hypothesis is that during higher organism development, genomic DNA becomes more methylated, resulting in the modification or the switching on and off of the gene expression responsible for the variation in maturational traits found (Finnegan and Kovac, 2000). For example, more percentage of DNA methylation is found from needle bases of mature *Pinus radiata* than juvenile ones, to the tune of respectively 60 vs 30% (Fraga et al., 2002). Recently, differences in gene expression were found between juvenile and adult material for the apple tree (Gao et al., 2014). In coffee, we used SE-induced rejuvenation to initiate a HRMC propagation process. We showed that the timing for implementing this propagation must be very accurate if rooting is to be successful, reliable and highly efficient. The "action window" is very ephemeral during early plant development – approx. between 10–25 weeks after planting somatic embryos in the nursery.

3.4. Possible serial multiplication cycles with rooted mini-cuttings process

Starting from a pool of 26,000 young somatic seedlings, 335,844 rooted mini-cuttings were produced in the greenhouse within a period of 6 months of cultivation, i.e. a multiplication rate of 14 (Fig. 4). Rooting capacity during the serial rooted cutting cycles over the 6-month period did not decrease and remained up to 90%. We observed that the juvenile status of the young mini-cuttings produced could be prolonged for more than 6 months without any loss of rooting capacity in the plants by influencing plant growth and forcing conditions in the nursery, through successive cycles of mini-cuttings, while keeping the plants confined with up to 90% RH and at a high temperature (ranging from 20 to 25 °C at night to 30–35 °C during the day). When extrapolated over a period of one year, the multiplication rate should be around thirty-seven mini-cuttings from one SE-rejuvenated plant. Similar combined two-step propagation processes have recently been established in conifers. Forest Genetics Ltd and Arbogen in New Zealand annually produce about 50,000 *P. radiata* somatic seedlings which are subsequently mass

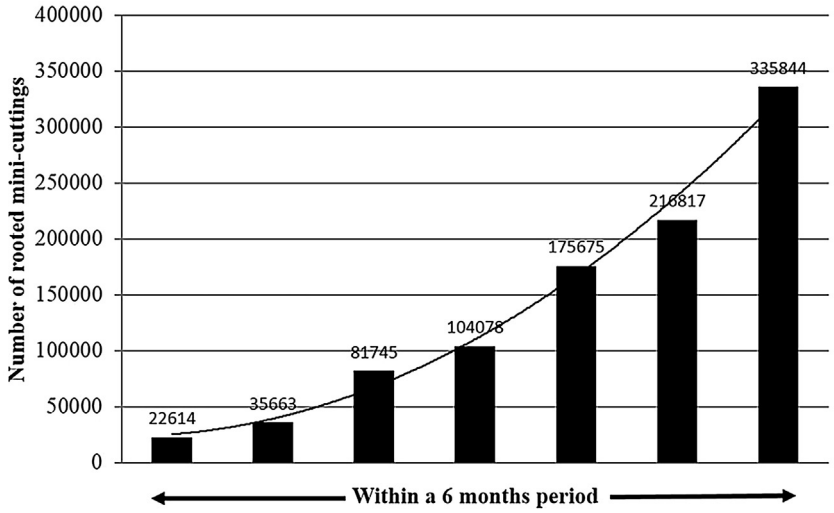

Fig. 4. Amplification rate of Arabica coffee hybrids through serial rooted mini-cutting process. We started from an initial batch of 22,614 rejuvenated somatic seedlings and serial cuttings were taken periodically from it within a 6 months period. During the experiment, plants were maintained under confined conditions in the greenhouse. The experiment was carried out in 2013 at the Exportadora Atlantic S.A Company, Sebaco, Nicaragua.

Table 2
Comparison of several morphological parameters between 15-week-old somatic seedlings and rooted mini-cuttings of H1 Hybrid (T5296 x Rume Sudan) in the greenhouse nursery. Morphological parameter values are means of 200 measurements ± SD on independent samples. The comparison was based on an analysis of variance (ANOVA) between somatic seedlings and rooted mini-cuttings. Values followed by different letters are significantly different at $P \leq 0.05$ (Fischer Least Significance Difference test).

Morphological parameters	Somatic seedling	Rooted mini-cutting	F	Prob
Plant height (cm)	3.14 ± 1.26^a	3.34 ± 0.92^a	1.08	0.31
Stem diameter (mm)	0.81 ± 0.17^a	1.41 ± 0.29^b	215.2	0.00
Root length (cm)	4.75 ± 2.04^a	4.15 ± 1.29^a	3.7	0.05
Root diameter (mm)	0.58 ± 0.24^a	0.94 ± 0.28^b	61.4	0.00

propagated by rooted cuttings to produce 2.5–3 million plants per year (Bonga 2015). In Sitka Spruce (Lelu-Walter et al., 2013), juvenile somatic seedling stock is intensively managed as outdoor stock plants, which are regularly hedged in order to stimulate the production of rooted axillary shoots. These somatic seedling-derived donor plants are renewed every 5 years, after each has produced 250 cuttings (Thompson 2014).

3.5. *Evidence of better vigor and homogeneity of rooted mini-cuttings compared to somatic seedlings*

Somatic seedlings and rooted mini-cuttings were compared for vigor and homogeneity 15 weeks after planting in the greenhouse nursery. Table 2 shows, for both, the different morphological parameter measurements on 200 plants sampled. As regards plant vigor, rooted mini-cuttings were significantly more vigorous than somatic seedlings with a larger stem (1.41 vs. 0.81 mm) and root diameter (0.94 vs. 0.58 mm). As regards the other parameters, no statistical differences were found. When compared to somatic seedlings, the rooted mini-cutting batches displayed greater homogeneity for plant height and root length distribution (Fig. 5). The majority of plants derived from mini-cuttings were grouped in a 2–4.5 plant size range, whereas most of the somatic seedlings were found in a larger 1–5.5 cm size range (Fig. 6A). Similar differences between rooted mini-cuttings and somatic seedlings were observed for root length (Fig. 6B). Somatic seedlings showed the widest distribution of frequencies for root length. Most of the plants derived from somatic seedlings had root lengths ranging from 3 to 7 cm long, whereas there was a clear peak in the distribution of rooted mini-cutting root lengths at around 4–6 cm. Current studies are aimed at an in-depth assessment of growth characteristics under field conditions in plants derived from rooted mini-cuttings. The first results have showed that, overall, there are no significant differences between somatic seedlings and rooted mini-cuttings, five years after field planting. Indeed, no significant difference was found for most of the aboveground parameters between the two types of material (100 plants for each material), except for the stem collar diameter, which was significantly bigger in plants derived from rooted mini-cuttings, which denotes slightly greater vigor (Table 3). As regards root system parameters, no significant difference was observed for any of the parameters studied for the two origins of plants (Table 4).

3.6. ***Markedly reduced propagation costs of rooted mini-cuttings compared to somatic seedlings***

In Central America, a six-month-old nursery plant derived from a seedling and ready for planting in the field has a production cost

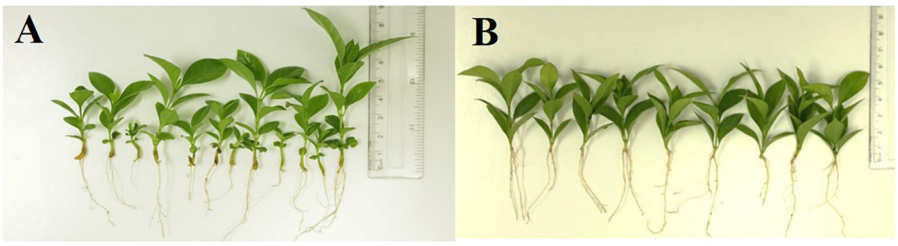

Fig. 5. Morphological appearance of somatic seedlings and rooted mini-cuttings. A: Heterogeneous appearance of somatic seedlings, 15 weeks after directly sowing embryos in plastic boxes; B Homogeneous appearance of rooted mini-cuttings, 15 weeks after sowing mini-cuttings in plug trays.

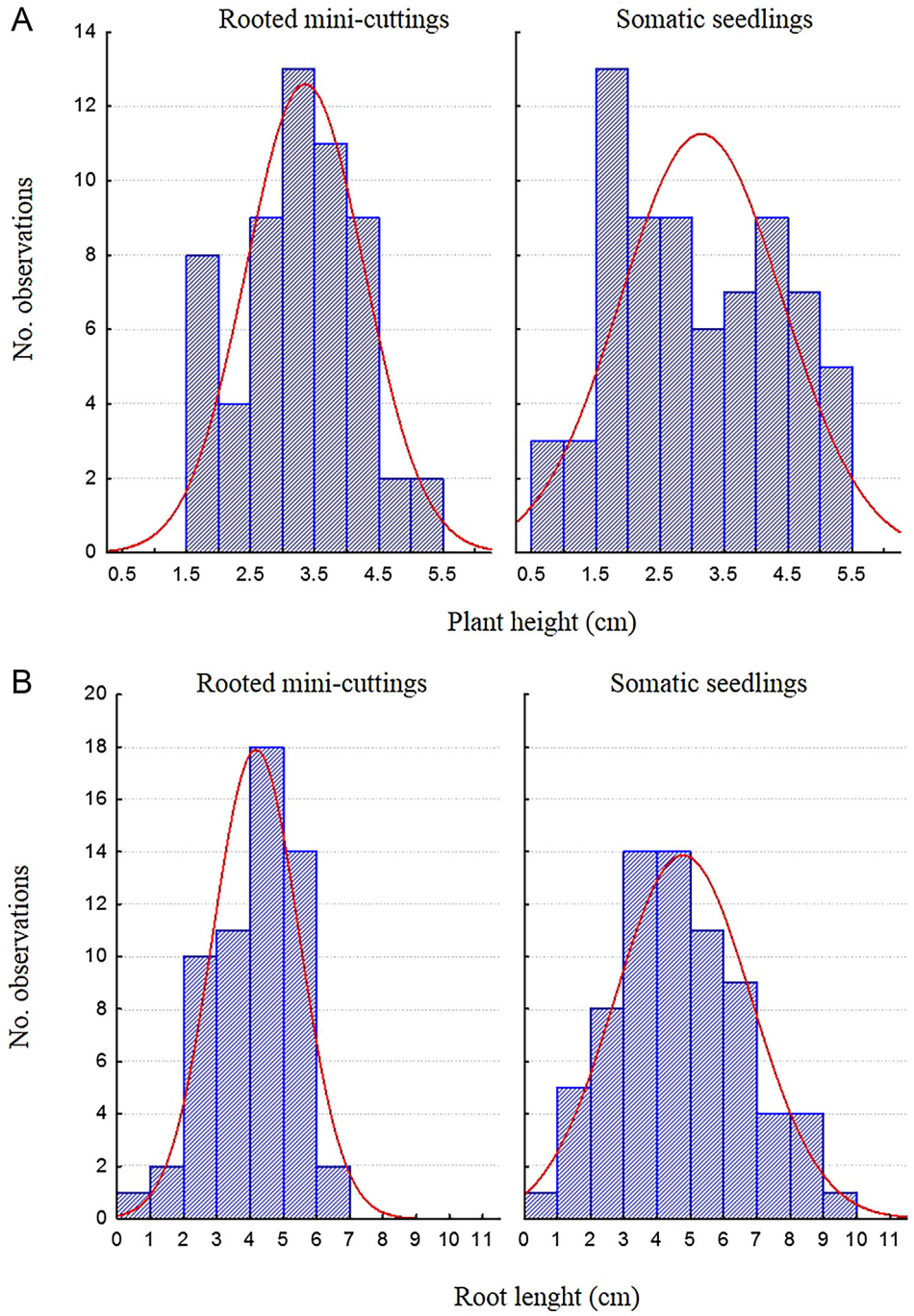

Fig. 6. Comparison of frequency distribution of plant height (cm) [A] and root length (cm) [B] parameters between 15-week-old nursery plants derived from somatic seedlings and rooted mini-cuttings from the H1 hybrid (T5296 × Rume Sudan). Two hundred plantlets derived from somatic seedlings were compared to 200 plants derived from rooted mini-cuttings through their distribution in size ranges for plant height (cm) and root length (cm).

of around US$ 0.20 and the selling price in the nurseries ranges from US$ 0.30–0.50 (depending on the country of Central America) which amounts to a total investment of US$ 1500–2500 per hectare for a renovation crop (5000 plants/ha). A six-month-old nursery hybrid plant propagated from a somatic seedling and ready for the field has a production cost of around US$ 0.60 and the selling price is around US$ 0.80-1, which represents three times the price of a seedling plant. A six-month-old hybrid plant derived from a rooted mini-cutting has a production cost of around US$ 0.27, which amounts to a cost reduction of around 50% compared to somatic seedlings and represents only 1.5 times the cost of a traditional seedling. Given the earliness of F1 hybrid production, the low plantation density (4000 plants/ha) and their higher productivity (up to 20–40%) (Bertrand et al., 2005, 2011), the additional investment compared to that of traditional seedlings (estimated at US$ 1500) is rapidly paid back three to four years after planting. A coffee plantation has a life cycle usually ranging from 15 to 20 years. But, the somatic seedling selling price of US$ 0.80–1 for hybrids, represents an important bottleneck for the large-scale commercialization of F1 arabica hybrids. By using the HRMC method, we estimated that *C. arabica* hybrid plants could be sold between US$ 0.65–0.70, which would be more affordable for farmers.

Very few studies have provided information about the production costs of plants derived from SE. In any case, based on the

Table 3
Comparison in the field between aerial parts of somatic seedlings and rooted mini-cuttings of H1 Hybrid (T5296 × Rume Sudan), five years after planting. Measurements were made on 200 plants (100 for each material) and plants were chosen randomly from the four hectare parcel. The comparison was based on an analysis of variance (ANOVA) between somatic seedlings and rooted mini-cuttings. Values followed by different letters are significantly different at $P \leq 0.05$ (Fischer Least Significance Difference test).

Morphological parameters	Somatic seedling	Rooted mini-cutting	F	Prob
Stem diameter at collar (mm)	40.6 ± 5.3^a	43.3 ± 3.2^b	6.5	0.01
Plant height (cm)	210 ± 2^a	214 ± 2.2^a	1.54	0.21
No. of plant internodes	38.5 ± 4.9^a	40.4 ± 4.9^a	2.75	0.10
Primary branch length (cm) *level 10 from the apex*	45.1 ± 8.7^a	45.5 ± 9.1^a	0.03	0.85
No. of primary branch internodes *level 10 from the apex*	11.8 ± 2.5^a	13.0 ± 2.8^a	3.64	0.06
Primary branch length (cm) *level 15 from the apex*	59.2 ± 6.7^a	57.2 ± 9.2^a	0.92	0.34
No. of primary branch internodes *level 15 from the apex*	15.0 ± 1.5^a	15.8 ± 2.2^a	0.00	0.92
Primary branch mortality percentage (%)	12 ± 4^a	11 ± 5^a	0.81	0.36

Table 4
Comparison in the field between root parts of somatic seedlings and rooted mini-cuttings of H1 Hybrid (T5296 × Rume Sudan), five years after planting. Measurements were made on 20 excavated plants (10 for each material). For the study, 10 plants of each material where chosen with the same vigor (diameter stem) and then extracted from the ground. The comparison was based on an analysis of variance (ANOVA) between somatic seedlings and rooted mini-cuttings. Morphological parameter values are means of 10 measurements ± SD on independent samples. Values followed by different letters are significantly different at $P \leq 0.05$ (Fischer Least Significance Difference test).

Morphological parameters	Somatic seedling	Rooted mini-cutting	F	Prob
Stem diameter at collar (mm)	40.0 ± 1.7^a	40.8 ± 1.4^a	0.71	0.42
Plant height (cm)	210 ± 0.2^a	197 ± 6.7^a	0.05	0.52
Root diameter (mm)	49.9 ± 5.3^a	51.4 ± 4.9^a	0.22	0.64
Main root length (cm)	31.2 ± 3.6^a	31.2 ± 4.4^a	0.00	1
Fresh biomass stump (kg)	0.5 ± 0.1^a	0.5 ± 0.1^a	1.11	0.32
Fresh biomass aerial part (kg)	2.6 ± 0.2^a	2.7 ± 0.3^a	0.46	0.51

limited data available, they are all currently too high to commercially be acceptable. In loblolly pine, the production costs of somatic seedlings (US$ 0.30–0.40) were between 7.5–10 times higher than those of plants conventionally produced by seedlings, i.e. US$ 0.05 (Bettinger et al., 2009). In Sitka spruce, somatic seedlings were estimated to cost between € 2–3/plant competing with the € 0.25 for a seedling transplant (Thompson, 2014). Hence, for these two species, the cost of a somatic seedling is about 10 times that of a seedling. Now, if Sitka spruce somatic seedlings are grown first in nursery beds to produce cuttings for rooting, the cost of Sitka spruce rooted cuttings decreases to twice the cost of a seedling. Seedling and planting a hectare of rooted cuttings thus becomes economically more realistic.

As regards coffee, Carvalho et al. (2013) studied the production costs of 400,000 plantlets of *C. arabica* produced by SE and reported a cost of US$ 0.37/plant, which is quite different from the cost obtained in our study (US$ 0.60). Recently, an interesting comparative cost study was done in *C. canephora* between plants produced by SE and traditional rooted cuttings mature trees from clonal gardens under field conditions (Alves dos Santos et al., 2015). The cost of a somatic seedling was evaluated at US$ 0.23 while a rooted cutting at US$ 0.12, which is approximately half of the cost we obtained for the *C. arabica* F1 hybrids.

4. Conclusion

This study shows that HRMC can efficiently complement SE for producing arabica hybrids in much larger quantities and at cheaper cost. We demonstrate that the low biological productivity observed for the SE procedures – particularly during the late stages of plantlet conversion – can be largely compensated by high-yielding horticultural propagation in the nursery. Plants derived from HRMC are more uniform and vigorous than somatic seedlings. We show that this effect persists for several years after planting out. Subsequently, in coffee the commercial vegetative propagation of hybrid plants should be envisaged as follows: laboratories still remain key to producing the rejuvenated material, the *ex vitro* rooted mini-cutting technique is outsourced to the best professionals in ornamental crop nurseries and the acclimatization is done by current nurseries or by farmers themselves, who are used to doing exactly that from seedlings. The methodology presented in this paper could be extended to the other crops where the SE propagation process is still not cost-effective and thus expand the general use of SE.

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