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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.