Journal of Soil Science and Plant Nutrition (2020) 20:933–940
https://doi.org/10.1007/s42729-020-00180-7

ORIGINAL PAPER

Propagation of Rust-Tolerant *Coffea arabica* L. Plants by Sprout Rooting in Microtunnels

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Received: 13 December 2019 / Accepted: 19 January 2020 / Published online: 27 January 2020

Abstract
Small Peruvian coffee producers face low yields per hectare, caused mainly by recent rust outbreaks and by natural crossing of coffee varieties, which are sown without specific order. Coffee rust has drastically reduced areas of susceptible but high-quality cup varieties as *caturra*, *pache*, and *nacional*, which have been replaced by others with high grain weight, but low-quality cup. This study aimed to determine the rooting capacity of sprouts subjected to different concentrations of indole-3-butyric acid (AIB) in three varieties of *Coffea arabica*, in the San Martín region, Peru. The most appropriate sprout rooting characteristics allowing to propagate rust-tolerant *C. arabica* plants were evaluated, under the effect of four doses of AIB (0, 1000, 2000, and 3000 ppm) in three coffee varieties (*caturra*, *pache*, and *nacional*), using microtunnels as rooting environments. A completely randomized design, consisting of three repetitions per treatment and six sprouts per repetition, was used. At the end of 50 days, the best rooting result (89%) was obtained with a dose of 2000 ppm of AIB in the *caturra* variety. The overall results are successful, since there is a conversion rate of sprouts into useful seedlings of more than 85%, if proper handling conditions are implemented. This method of propagation of rust-tolerant *C. arabica* sprouts by microtunnels enables a mass production of seedlings. The validation of these results could lead to the establishment of entire rust-tolerant coffee farms.

Keywords *Caturra* · Coffee · Coffee rust · Indole-3-butyric acid · Propagation · Sprouts

1 Introduction

Coffee is a crop of great commercial importance, being the second most commercialized product in the world (after oil) and being a livelihood source for more than 125 million people (ICO 2013). It belongs to the Rubiaceae family and its commercial production is limited only to two species belonging to the *Coffea* genus: *C. arabica* and *C. canephora* (the latter generally known as "robusta" coffee) (Charrier and Eskes 2004; Lashermes et al. 1999). Although the cup quality of *C. arabica* is considered to be superior to that of *C. canephora*, improving the cup quality and commercial production of *C. arabica* remains a priority for most breeding programs (Tran et al. 2016).

One of the most serious problems faced by small coffee producers in the region of San Martín, Peru, is a low yield per hectare, because almost all of the coffee farms have a great diversity of varieties planted in the same plot, without a specific order. This mixing possibly caused a natural crossing between varieties and possibly leads to the emergence of several unidentified hybrids. The genetic diversity of *C. arabica* in this region is likely to be low, which generates confusion or errors in the differentiation of varieties by farmers (Blas et al. 2011).

The low genetic diversity of *C. arabica* is mainly attributed to its particular reproductive biology and to the artificial selection processes that this species has undergone (Palomino et al. 2014). Thus, the introduction of very few accessions from coffee-producing countries as Costa Rica, Brazil, and Colombia, which in turn came from Africa (Palomino et al. 2014), can explain this low genetic diversity. These accessions formed the genetic basis of all breeding programs in Peru

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(Maluf et al. 2005). These genetic characteristics of coffee, coupled with the fact that there is little morphological differentiation between varieties, have allowed coffee rust (*Hemileia vastatrix* Berk. & Broome) to spread throughout the Peruvian territory, devastating not only crops but also coffee producers and their families.

The cultivars of *C. arabica* are predominantly self-pollinated and, consequently, homozygous. They are also quite uniform, which is why they are commonly propagated by seeds. However, these cultivars can reach up to 20% of allogamy, not being able to preserve heterozygous plants with loci conditioning for grain production' heterosis and pathogen resistance (Bergo and Mendes 2000). Jesús (2003) mentions that the preservation of heterozygous plants with the above-mentioned characteristics is possible through cloning by means of sprout rooting. Vegetative propagation allows to keep the genotype intact and to ensure the conservation of valuable germplasm; in addition, it allows to multiply higher-performance genotypes and to increase genetic gains in very short periods by using both the additive and non-additive components of the total genetic variance (Zobel and Talbert 1988).

The success of sprout rooting depends on several factors related to the minimization of water deficits in the cuttings, the optimization of photosynthesis during the propagation process, and the use of suitable substrates and growth regulators that favor the initiation and development of the roots (Leakey et al. 1990; Loach 1988; Mesen 1993). The increase in the rooting capacity of auxin-treated cuttings is attributed to the positive and well-recognized effects of auxins on cell division and on the transport of carbohydrates and foliar cofactors to the regions treated with them (Phillips 1975). Another effect of auxins on root formation is their ability to stimulate DNA synthesis, which also results in greater cell division (Gaspar and Hofinger 1988). Therefore, the objective of this study was to determine the rooting capacity of sprouts subjected to different concentrations of indole-3-butyric acid (AIB) in three varieties of *C. arabica*, in the region of San Martín, Peru, using microtunnels as rooting environments.

2 Materials and Methods

2.1 Study Site

This study was carried out in the experimental greenhouse of the Instituto de Investigaciones de la Amazonía Peruana, San Martin Region, Peru, (6° 35′ 28″ S, 76° 18′ 47″ W; altitude 330 m.a.s.l.).

2.2 Selection of Coffee Farms

Coffee farms with the *caturra*, *pache*, and *nacional* (or *typical*) varieties were identified in the provinces (of the San Martín Region, Peru) of Rioja, Moyobamba, Lamas, El Dorado, and Huallaga, all of which have a strong prevalence of coffee rust. For the selection of these five provinces and the study plots, semi-structured interviews were conducted with technicians, producers, and experts in the production of coffee in the region, in order to know the damage caused by the disease. Visits to the affected fields were made.

Coffee mother plants were selected during 3 years, in three production campaigns affected by the presence of coffee rust in the *caturra*, *pache*, and *nacional* varieties. In the first selection (2016), 104 plants were considered for the *caturra* variety, 102 plants for the *pache* variety, and 102 plants for the *nacional* variety with a total of 308 plants distributed in the five provinces of the San Martín Region, with degree of coffee rust impact-severity of 1 to 5 (Hiroshi et al. 2009). In the second selection (2017), 39 plants were considered for the *caturra* variety, 37 plants for the *pache* variety, and 36 plants for the *nacional* variety with a total of 112 plants selected, with a degree of coffee rust impact-severity of 1 and 2. For the third year (2018), 75 selected plants with 25 mother plants for each variety were considered, with a degree of coffee rust impact-severity of 1.

2.3 Characteristics Considered for the Selection of Mother Coffee Plants

- Degree of coffee rust attack according to the impact-severity scale (1 to 5) of Hiroshi (Hiroshi et al. 2009).
- Coffee plants of the *caturra*, *pache*, and *nacional* varieties, older than 3 years, and in each of the altitude ranges 800–1000 m.a.s.l., and 1000–1200 m.a.s.l.
- Good plant structure. For this, the number of productive branches per plant, number of productive nodes per branch, and number of floral internodes were evaluated.
- Plants with high productivity, with uniform maturation, and with good fruiting load.

A total of 75 mother plants of the three coffee varieties with degree of impact-severity 1 (Hiroshi et al. 2009) of coffee rust attack were selected. The determination of the percentage of incidence of coffee rust in the mother plants was made using the following formula (Barquero 2013):

$$\%\text{Incidence} = (\text{Number of rust–affected leaves} / \text{total number of sampled leaves}) \times 100$$

Once the coffee farms and mother plants of the three varieties were selected, each one of the mother plants was codified, georeferenced, and labeled. Also, it was explained to the coffee producer the importance of conserving these plants as genetic material with high productivity and resistance to coffee rust, thus counteracting the problems of this disease.

2.4 Sprouts Obtaining

To obtain sprouts, considered as the vegetative seed for rooting, the induction of coffee mother plants was carried out, starting with cleaning the aerial part and around the selected coffee plant in order to facilitate the entry of sunlight. In order to generate a greater number of buds, the "agobio" (plant bending) technique was used in the coffee mother plants. This technique consists of tilting the plant at an angle of 45° in relation to the ground, introducing a hook in the ground to keep the plant inclined, preventing it from returning to its normal position, and eliminating plagiotropic branches (Fig. 1a). This technique increases leaf area by inducing the central axis of the mother plant, and its purpose is to induce the development of orthotropic buds, stimulating lateral buds by the tilt effect. The "agobio" of the plant causes uneven distribution of the auxin content and stimulates the formation and activation of buds that give rise to the development of orthotropic buds. This procedure was performed in 112 initially selected plants with ages greater than 3 years (Ramírez 1996).

After 80 days of the induction, the buds were isolated and collected in early hours, thus avoiding water stress (Fig. 1b). This was carried out with hand scissors (disinfected with 96% alcohol), placing the sprouts in labeled paper bags in a cooler containing ice, allowing a homogeneous temperature, and avoiding water stress and deterioration of the sprouts due to transportation.

2.5 Rooting Procedure

To stimulate the growth of coffee sprouts, the auxin used was the indole-3-butyric acid (AIB) at 0, 1000, 2000, and 3000 ppm, dissolved in pure alcohol (96%)—used as a solvent. The application of AIB was directed to the base of the sprout with a micropipette of 10 μl to guarantee that all the sprouts received equal amount of this solution (Fig. 1c). Then, the alcohol was evaporated from the solution with a cold air stream at the base of each sprout for 30 s.

The sprouts were cut at an 8-cm size, making an oblique cut just above each knot, with a foliar area of 50 cm^2 (Fig. 1d), which allowed a better manipulation, as this high leaf area has a strong stimulating influence on root initiation, probably due to the translocated carbohydrates of the leaf and other substances (Hartman and Kester 1997). The sprouts were protected by depositing them in containers with water, separated by each variety. Fungicide was applied to avoid physiological stress.

For the sowing of the *Coffea arabica* sprouts, specialized substrates in light nursery trays were used. On these trays, small holes at a depth of 2 cm for the introduction of the sprouts were made. Finally, the sprouts were deposited in the microtunnels for rooting.

2.6 Rooting Environment

A 96-m^2 greenhouse with a metal frame and lined with plastic was used for placing the coffee sprouts in microtunnels. The greenhouse had 18 triple nebulizers to lower the temperature

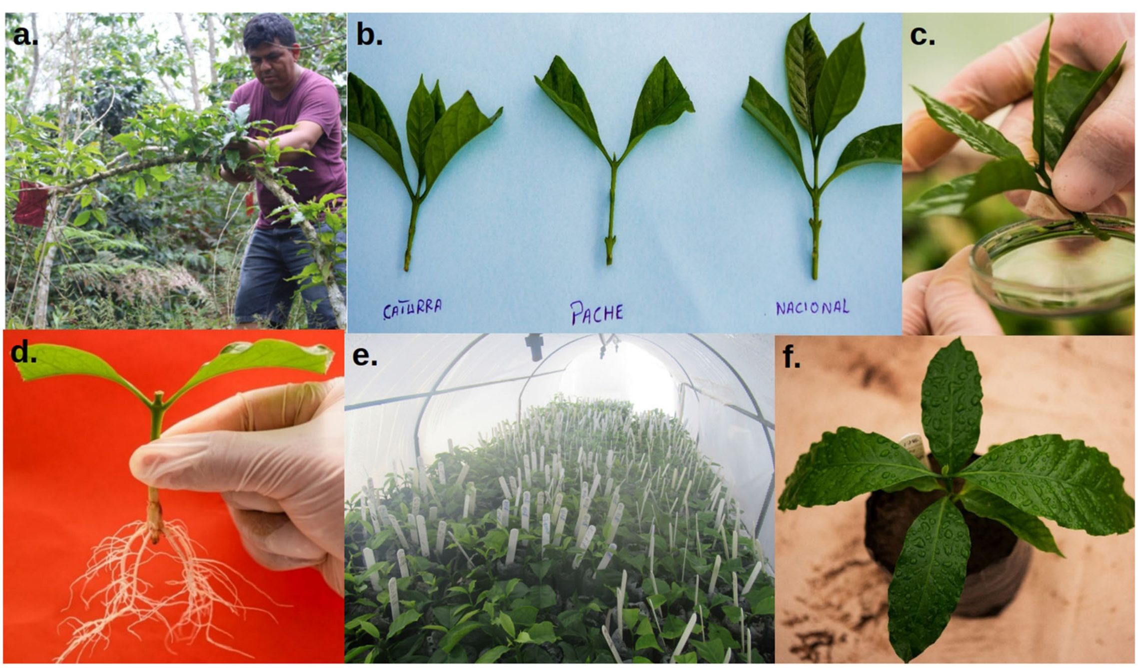

Fig. 1 Rooting of *Coffea arabica* L. **a** Rust-tolerant coffee mother plant. **b** Sprouts of the *caturra*, *pache*, and *nacional* coffee varieties. **c** Application of indole-3-butyric acid to the sprout base. **d** Rooted coffee sprout. **e** Establishment of the sprouts in substrates. **f** Cloned acclimated coffee plant

in hours of excessive heat, an automated irrigation system, and housed five rooting microtunnels for agroforestry species, having the capacity to produce 4000 plants every 2 months (Fig. 1e).

These microtunnels had the role of rooting *caturra*, *pache*, and *nacional* coffee varieties, presenting a painted or galvanized metal frame, with dimensions of 3 m long, 1 m wide, and 0.6 m high, lined with white transparent plastic to facilitate the passage of diffused sunlight. Internally, the microtunnels contained three nebulizers with an automated irrigation system that allowed to maintain the relative humidity above 75% and a temperature between 28 °C to 35 °C.

As a rooting substrate, a specialized substrate composed of 97% by Canadian *Sphagnum* spp. peat moss (Jiffy-7 Forestry Peat Pellet, Maruplast International Eirl, Lima, Peru) was used; this substrate is optimal for rooting percentages greater than 90%, thus converting collected sprouts into cloned coffee seedlings. Water was pumped by a 1-hp engine. The microtunnels environment was maintained clean and free of holes.

After 50 days of the sprout trays' establishment in the microtunnels, the trays were removed to extract the rooted sprouts; the sprouts had an average of 3.48 roots of 2.46 cm. Finally, sprouts rooted in the microtunnels were replicated in storage bags with soil substrate mixed with sand (Fig. 1f). At this stage, between 1500 and 2000 native arbuscular mycorrhizal fungal spores were applied on each bag. These spores consisted of a consortium of the species *Acaulospora mellea*, *Acaulospora spinosissima*, *Glomus trimurales*, and *Paraglomus occultum*, selected based on their efficiency of biofertilization and protection against pathogens (Aguilera et al. 2017). Each replicated sprout was taken to an acclimatization nursery with a favorable shade system, with Raschel meshes that allowed 50% of sunlight to enter and with frequent application of light water irrigation three to five times per day. Thus, turgor of the rooted sprouts was maintained, and hardening of the plants was achieved for a period of 2 months.

2.7 Experimental Design and Data Analysis

For this study, a completely randomized design with a factorial arrangement 3A (varieties *caturra*, *pache*, and *nacional*) × 4B (0, 1000, 2000, and 3000 ppm of AIB) with 3 repetitions was used. The data were analyzed by analysis of variance (ANOVA) and by the Tukey test with a level of $p < 0.05$ probability of error, to determine the nature of the differences between coffee varieties and AIB doses, as well as the differences that arised through the interactions between both factors. Prior to the analysis, the number (no.) of roots was transformed to $\sqrt{\text{no.} + 1}$ (Snedecor and Cochran 1980). Likewise, the percentage of rooting and mortality were transformed by the formula arcoseno$\sqrt{\%}$ of rooting.

3 Results

There were significant differences in the number of sprout roots in the *Coffea arabica* varieties, as two groups formed (*caturra* variety in the first group and the *pache* and *nacional* varieties in the second group). The best results were obtained with the *caturra* variety (4.5 roots by sprout) (Table 1). Similar results were obtained for the sprouting percentage, where the same two groups also formed, and where the *caturra* variety also showed a higher sprouting percentage (61.67%) (Table 1). Root length did not show significant differences within the three coffee varieties (Table 1). Regarding the rooting percentage, significant differences were obtained between the *caturra* and the *nacional* varieties, the first one showing the best results with 63.34% rooting (Table 1).

There were significant differences in the number of sprout roots with respect to the application of different doses of indole-3-butyric acid (AIB), where the 2000-ppm dose of AIB obtained the highest number of roots (6.09 roots per sprout) (Table 2). Similar results were obtained regarding root length, sprouting percentage, and rooting percentage, where the 2000-ppm dose of AIB resulted in the highest values (3.19 cm, 82.22%, and 86.67%, respectively) (Table 2).

Doses of 0, 1000, and 3000 ppm of AIB had rooting values of 20%, 66.67%, and 66.67%, respectively (Fig. 2). In contrast, the application of 2000 ppm of AIB in coffee sprouts of the *caturra* variety resulted in 100% rooting success and 0% mortality; this treatment also resulted in an average of 9.07 roots, 3.99 cm of root length, and 93.33% sprouting. Generally, the sprouts of the *caturra* variety with application of 2000 ppm of AIB behaved better, since all the sprouts with this treatment achieved successful results for the propagation of *C. arabica* plants by sprout rooting in microtunnels. In contrast, the less successful results were observed in the *nacional* variety that did not received applications of AIB, with a 0% rooting, 0.40 roots on average, 0.53 cm average root length, and 0% sprouting.

4 Discussion

Coffee (*Coffea arabica* L.) is one of the most consumed agricultural products in the world. In Peru, it is one of the most important agricultural products in the international trade (Ministerio de Agricultura y Riego 2014a), reaching in 2011 an historical trading figure in the agricultural sector, with sales of US$1.5 billion. Particularly, in the San Martín Region, coffee is a strong asset in the regional economy, as approximately 100,927 ha are grown, directly involving 44,857 producers, with approximately 224,285 people dependent on this crop (Ministerio de Agricultura y Riego 2014b). In this region, approximately 75% of the crop is cultivated above 1200 m.a.s.l., with an average yield of 910 kg/ha. However, in 2012, the

Table 1 Effect of varieties on the propagation of *Coffea arabica* L. plants by sprouts, evaluated 50 days after their establishment in microtunnels

Variety	Number of roots	Roots longitude (cm)	Rooting (%)	Sprouting (%)
Caturra	4.5[a]	2.68[a]	63.34[a]	61.67[a]
Pache	3.02[b]	2.41[a]	40[ab]	41.67[b]
Nacional	2.93[b]	2.29[a]	55[b]	48.33[ab]
Average	3.48	2.46	52.78	50.56
CV (%)	1.9	1.86	2.51	5.26

Tukey's test ($p < 0.05$)

Averages followed by different letters indicate statistically significant differences

yellow coffee rust spread, having great negative effects on the productivity of cultivated areas, with a devastating impact on the sector during 2013, when exports fell to US$700 million—from US$1.5 billion in 2011.

Coffee rust incidence, added to the low yields per hectare because multiple varieties of coffee are sown in the same plot at the same time without a specific order, has led to coffee plantations presenting problems such as pollution due to high demand for agrochemicals, reduction of associated biodiversity to the coffee plantation, and abandonment of coffee farms. Small farmers see their resilience reduced in periods of crisis when the international coffee price falls, and they can not cover the costs of fertilizers and pesticides. Likewise, sudden changes in temperature have caused coffee growers not to find alternative crops as a form of economic income or not to have many subsistence crops to reduce expenses (García Pineda 2013; Turbay et al. 2013). Also, it can be clearly seen that coffee plantations with a diversified canopy of shade trees have a greater potential for biodiversity conservation than plantations in full sun. Although agrobiodiversity is mostly managed on a plot/farm scale, many ecological and social factors influence its structure as part of a larger landscape (Brookfield and Padoch 2007; Phillips and Stolton 2008). An analysis of agrobiodiversity in small coffee farms is relevant, as these farmers are facing global challenges, such as climate change, instability of international coffee prices, and threats to their food security (FAO 2008; Petchers and Harris 2008).

The present study explored the vegetative propagation of *C. arabica* by sprouts rooting via microtunnels, as an alternative to propagate coffee from selected rust-tolerant plant material, using simple and medium-cost technologies available to coffee growers. With this, it is hoped to achieve, with time, clean genetic material that can help to address challenges of quality, intraspecific diversity, and accessibility of the material, by installing clonal propagation gardens as a source of continuous vegetative seed. This technology, in turn, seeks to combine the use of clean coffee genetic material with the use of arbuscular mycorrhizal fungi during its acclimatization stage, being an agroecological and environmentally friendly technology. This coffee cultivation technique would constitute a good agricultural practice that shows a trend of improving sustainability indicators and towards a continuity of organic management practices (De Muner 2011).

4.1 Rooting Process

An alternative way to propagate coffee plants is to use selected plant material, specifically material from rust-tolerant mother plants with high productivity. In the present study, it was possible to determine the effects of applications of different doses of indole-3-butyric acid (AIB) on the sprout rooting of the coffee varieties *caturra*, *pache*, and *nacional*. When 2000 ppm of AIB were applied to coffee sprouts of the *caturra* variety, 100% rooting was obtained, considering this result as very successful, since a conversion rate of cuttings into useful seedlings of more than 95% was also obtained with this treatment. The propagation method of *C. arabica* plants by sprouts rooting via microtunnels developed in this study could enable the mass production of seedlings. If validated, this method could lead to the establishment of entire rust-tolerant coffee farms.

Regarding the effects of the varieties on the propagation of *C. arabica* plants by sprout rooting, the highest rooting percentage was obtained with the *caturra* variety (63.34%), followed by the *pache* (40%), and *nacional* (55%) varieties, respectively (Table 1). The *caturra* variety has a vigorous and compact appearance, with short internodes and abundant lateral branches. This variety has a fast development and high productivity, so it requires proper management. These characteristics of the *caturra* variety could be explained by the amount of nutritional reserves present in the stem and by the amount of assimilates produced in the leaves (Ruíz-Solsol and Mesen 2010).

The presence of nutritional reserve substances and carbohydrates in sprouts of the *caturra* variety could lead to a greater length and number of roots, and to a greater percentage of rooting and sprouting. The number of roots emitted and the ability to restart the growth of the aerial part are influenced by the amount of nutritional reserves present in the stem and by the amount of assimilates produced in the leaves. Veierskov (1988) describes a positive relationship of carbohydrate content with rooting capacity and with the number of roots formed in sprouts of different woody and

Table 2 Effect of different doses of indole-3-butyric acid (AIB) on the propagation of *Coffea arabica* L. plants by sprouts, evaluated 50 days after their establishment in microtunnels

AIB dose (ppm)	Number of roots	Roots longitude (cm)	Rooting (%)	Sprouting (%)
0	0.6[c]	0.63[b]	6.67[c]	11.11[c]
1000	3.58[b]	2.87[a]	55.56[b]	51.11[b]
2000	6.09[a]	3.19[a]	86.67[a]	82.22[a]
3000	3.67*b*	3.14[a]	62.22[b]	57.78[b]
CV (%)	2.2	2.14	2.9	6.07

Tukey's test ($p < 0.05$)

Averages followed by different letters indicate statistically significant differences

herbaceous species. In turn, Hartman and Kester (1997) point out that thicker stems accumulate a higher content of reserve carbohydrates and, possibly, under the influence of substances that promote rooting, have a higher probability of inducing roots. This pattern fits and possibly explains the results obtained in the present study.

Regarding the effect of different doses of AIB on the propagation of *C. arabica* plants by rooting via microtunnels (Table 2), a greater root length, number of roots, and percentages of rooting and sprouting were obtained with the 2000-ppm dose of AIB, with significant differences with the other doses. The three doses applied (1000, 2000, and 3000 ppm) had statistically superior results to the control (0 ppm), which showed a rooting percentage of only 6.67%. This pattern has been found in a large number of other species (Blazich 1988; Hartman and Kester 1997; Mesen 1993), where there is usually an increase in the rooting capacity by increasing the dose of the auxin until reaching an optimum, after of which any increase results in a decrease in rooting due to the toxic effects of overdosing. The number of roots produced by the cuttings is highly influenced by the sprout's ability to supply carbohydrates either from reserves or via photosynthesis, directed to the area where the roots arise (Lovell and White 1986; Moe and Andersen 1988; Veierskov and Andersen 1982).

The treatment of 2000 ppm of AIB applied to the *caturra* variety resulted in the greatest number of roots, probably due

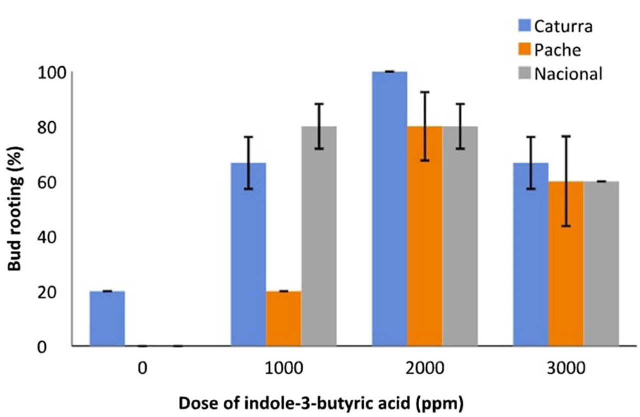

Fig. 2 Bud (sprout) rooting percentages formed as an effect of different doses of indole-3-butyric acid and varieties of *Coffea arabica* L.

to an adequate hormonal concentration and due to a balance between the photosynthetic and breathing processes of the sprout. This causes the formation and development of new roots; likewise, rooting is affected by radiation, affecting the leaves' turgor and the production of carbohydrates required for the initiation and growth of the roots (Grange and Loach 1985). Irradiation should not be so high as to inhibit rooting through its effects on sugar accumulation and loss of turgidity, but it should be sufficient to allow photosynthetic production of carbohydrates for root initiation and growth (Loach 1988). That is why, for this experiment, a greenhouse with white plastic was used, allowing the passage of diffused light protected with a shading mesh at 20% light transfer, during the 50-day rooting period.

5 Conclusions

In Peru, coffee is the main agricultural export product followed by asparagus and accounts for about half of agricultural exports. About 425,000 ha of the Peruvian territory are allocated to coffee production (Instituto Nacional de Estadística e Informática del Perú 2013), which are distributed in 338 districts of 16 administrative regions of the country (Junta Nacional del Café de Perú 2013). This crop is the direct economic support of more than 150,000 Peruvian families, generating approximately 2 million direct and indirect jobs in the productive chain. The development of the propagation of *Coffea arabica* L. plants by sprouts rooting via microtunnels can help to achieve coffee availability and stability with high-quantity and high-quality grains, free of contaminants, pests, and diseases such as the yellow coffee rust. This methodology allows the propagation and preservation of coffee clones from selected plant material, also allowing rescuing varieties that are likely to be lost over time. For example, this method would allow to assess the genetic material of *caturra*, *pache*, and *nacional* varieties, all of which have good cup quality; in turn, with this method, it is relatively easy to preserve genotypes with favorable genetic characteristics—as resistance to pests and diseases. All these factors lead to an improved food security, with coffee productivity being one of the sustainability indicators of small coffee farmers positioned in rural areas of

Peru. All measures aimed at reducing poverty and reducing the gap between urban and rural areas will have a positive effect on strengthening food security, with coffee being a direct source of biodiversity conservation and ecological sustainability.

Funding information To the "Programa Nacional de Inovación Agraria – PNIA" for funding this research work in the framework of the activities of the project "Biofertilización y bioprotección de plantas clonales de café (*Coffea arabica*) con micorrizas arbusculares en la Región San Martín," according to Contract No. 23-2015-INIA-PNIA/UPMSI/IE. To the project "Generación de tecnologías de adaptación a los efectos negativos del cambio climático como un modelo sostenible y exitoso de la caficultura en la región San Martín" (No. 20-2018-FONDECYT-BM-IADT-AV), for funding the doctoral thesis "Efectos de la inoculación de micorrizas arbusculares sobre la tolerancia a roya en clones de café (*Coffea arabic*a L.) var. *caturra* y *pache* en Moyobamba, Perú." C.M. thanks the Universidad de O'Higgins postdoctoral fund.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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