SPROUTING INDUCTION FOR MICRO-CUTTING ON *IN VITRO* CLONED ARABICA COFFEE PLANTS

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ABSTRACT: Vegetative propagation of arabica coffee plants selected by their agronomic value has been accomplished routinely in Brazil for scientific purposes, through somatic embryogenesis and rooting of stem cuttings. Somatic embryogenesis is the election method when a very high number of cloned plants is demanded. Nevertheless, the costs of *in vitro* multiplication make difficult to explore it commercially. The experiments described herein aimed to amplify the number of *in vitro* cloned plants, post acclimatization, to reduce costs. Different concentrations of 2,3,5-triiodobenzoic acid (TIBA) and its association with benzylaminopurine (BAP) were applied, as successive pulses, in the 3rd, 8th and 13th months after transference to the greenhouse, on the same set of Catucaí and Siriema *in vitro* cloned plants, to induce sprouting. At the 8th month, the experiments with *in vitro* cloned Catucaí plants were reproduced in the nursery, for comparison. Best results were observed for the association TIBA 1000 mg.mL^{-1} + BAP 60 mg.mL^{-1} applied in the greenhouse, at the 13th month, when, on average, 8.5 and 7.0 micro-cuttings above 1 cm in length were produced using sprouts taken from each Catucaí and Siriema acclimatized plant, respectively. Applying this treatment twice a year, and harvesting induced sprouts each six months after the induction treatments, approximately 15 plants per each acclimatized one can be produced. The most important effect of TIBA was the induction of sub-apical sprouting. Greenhouse would be the best environment to apply successive pulses of sprouting inducers to coffee *in vitro* cloned plants.

Index terms: Cloning, micropropagation, cutting, growth regulators, *Coffea arabica* .

INDUÇÃO DE BROTAÇÕES PARA MICROESTAQUIA EM CAFEEIROS ARABICA CLONADOS *IN VITRO*

RESUMO: A propagação vegetativa de cafeeiros arábica selecionados em função de seu valor agronômico tem sido realizada rotineiramente no Brasil para fins científicos, através de embriogênese somática e enraizamento de estacas. A embriogênese somática é o método eleito quando há demanda por um número muito grande de plantas clonadas. No entanto, os custos da multiplicação *in vitro* a tornam difícil de explorar comercialmente. Os experimentos descritos neste trabalho visaram amplificar o número de plantas clonadas *in vitro*, após a aclimatização, com o objetivo de reduzir o custo de clonagem. Diferentes concentrações de ácido 2,3,5-triiodobenzóico (TIBA) e sua associação com benzilaminopurina (BAP) foram aplicadas, na forma de pulsos sucessivos, no 3º, 8º e 13º meses depois da aclimatização, em um mesmo conjunto de plantas de Catucaí e Siriema clonadas *in vitro*, para induzir brotações, em casa de vegetação. No 8º mês, os experimentos com plantas de Catucaí foram reproduzidos em viveiro telado, para comparação. Os melhores resultados foram observados para a combinação de 1000 mg.mL^{-1} de TIBA + 60 mg.mL^{-1} de BAP, aplicados na casa de vegetação, no 13º mês, quando foram obtidas, em média, 8,5 e 7,0 microestacas para cada planta de Catucaí e de Siriema aclimatizada, respectivamente. Aplicando este tratamento duas vezes por ano, aproximadamente 15 plantas por cada planta aclimatizada podem ser produzidas. O efeito mais importante do TIBA foi estimular brotações subapicais. A casa de vegetação pareceu ser o melhor ambiente para aplicar pulsos sucessivos de indutores de brotações em cafeeiros clonados *in vitro*.

Termos para indexação: Clonagem, micropropagação, estaquia, regulador de crescimento, *Coffea arabica.*

1 INTRODUCTION

The agronomic value of coffee plants highly productive and resistant to diseases, which are selected during long lasting breeding programs, justifies the use of vegetative propagation methods in order to capture all of the plants best characteristics at the end of a reduced period of time (VOS; SNIJDER, 2000).

Vegetative propagation through the rooting of cuttings has been routinely used to clone *Coffea canephora* plants (SANTOS et al., 2013; AQUINO et al., 2017). More recently, *C. arabica* cuttings have been rooted as well (JESUS; CARVALHO; SOARES, 2006; CARVALHO et al., 2008; OLIVEIRA et al., 2010; REZENDE et al., 2010; BALIZA et al., 2013). Micropropagation *in vitro* through somatic embryogenesis also has been used

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for *C. arabica* (REZENDE et al., 2012; ETIENNE et al., 2012; CARVALHO et al., 2013). The use of nodal segments taken from the main orthotropic stem to prepare micro-cuttings was demonstrated by Vos and Snijder (2000). A process similar to this last, the use of successive cycles of production and rooting of mini-cuttings obtained in the first cycle from *in vitro* cloned coffee plants, with no use of plant growth regulators. was recently taken to the commercial scale, and proved to be very effective in reducing the costs of the *in vitro* cloning process (GEORGET et al., 2017).

Nevertheless, branching can be useful as a preparation to collect vegetative organs (SANSBERRO; MROGINSKI; BOTTINI, 2006). In trees, the shoot apex exerts apical dominance by inhibiting the outgrowth of the axillary sub-apical buds (MÜLLER; LEYSER, 2011) and, for arabica coffee plants, only orthotropic stems, which are normally under strong apical dominance, can be used to prepare cuttings. However, coffee seedlings grown from the seeds, decapitated and treated with auxin antagonists were able to produce additional orthotropic branches (CARVALHO et al., 2007).

The aims of this work were to test different concentrations of an inhibitor of auxin translocation (triiodobenzoic acid - TIBA) and its association with a cytokinin (benzylaminopurine - BAP) to produce sprouts, which are sources of micro-cuttings for *C. arabica* plants cloned *in vitro*. Two different experimental environments - the greenhouse and the nursery - were tested as well. As far as we know, this is the first report on the post-acclimatization application of plant growth regulators as an aid to the vegetative propagation of arabica coffee varieties cloned *in vitro*. These procedures may contribute to bring down the costs for producing *C. arabica* plantlets *in vitro*, promoting their introduction in the coffee market processes.

2 MATERIAL AND METHODS

Plant material

C. arabica L. varieties Catucaí (567) and Siriema (3) were cloned *in vitro* through somatic embryogenesis (REZENDE et al., 2012). These varieties are very productive and Siriema is resistant to the leaf rust caused by *Hemileia vastatrix* Berk. & Broome (MATIELLO et al., 2016). Clones were rooted and hardened *in vitro*, before the transference to a compost for horticulture made from processed coconut husks. The compost was added with slow release fertilizer granules (NPK 14-14-14) and potted in 300 cm³ plastic conical containers placed in trays. Acclimatization took place in the greenhouse, under controlled fogging, temperature and humidity maintained around 27 ºC and 80%, respectively.

Experimentation

From the end of the third month of acclimatization and ahead, the *in vitro* cloned plants were subjected to a series of experiments to stimulate sprouting.

Experiment I

256 *in vitro* cloned plants, organized in four replicas of eight plants per variety and treatment were maintained in the greenhouse and subjected to successive pulses of sprouting induction. PULSE 1: at the 3rd month of acclimatization, decapitation only (control) and decapitation and application of 2,3,5-triiodobenzoic acid (TIBA) as ethanolic solutions in the concentrations of 200, 400 and 600 mg.mL^{-1}, sprayed on the leaves of the *in vitro* cloned plants. Apical sprouts (those coming out of the buds in the most apical node resting in each cloned plant) induced by these treatments were counted, measured and harvested two months after the stimulation pulse and again (spontaneous apical sprouts) five months after the pulse. Sub-apical sprouts (those coming out of buds in any node except the most apical one) were harvested five months after the pulse. PULSE 2: the same as pulse 1, applied to the same plants at the 8th month of acclimatization, with a single sprouts harvest made three months later. PULSE 3: at the 13th month of acclimatization, decapitation only and decapitation and application of 200, 600 and 1000 mg.mL^{-1} TIBA or 1000 mg.mL^{-1} TIBA plus 60 mg.mL^{-1} benzylaminopurine (BAP) as ethanolic solutions sprayed on the leaves of the *in vitro* cloned plants. Sprouts harvest took place six months later.

Experiment II

128 *in vitro* cloned plants of Catucaí, organized in four replications of eight plants per treatment were transferred from the greenhouse to the nursery (50% shadowing and automated irrigation), on the 5th month of acclimatization. In the 8th month, these plants were subjected to the same procedures for sprouting stimulation and sprouts harvests described for Experiment I PULSE 2.

After counting and measuring, apical and sub-apical orthotropic sprouts were collected, divided in nodal segments of length equal or above 1 cm, and the leaves in each node were cut by their halves, to prepare the micro-cuttings. Micro-cuttings were planted in multi-cellular plastic trays (50 cells with capacity to hold 90 cm³ each) filled

with compost for horticulture added of fertilizers, as aforementioned for the acclimatization of the primary clones, and maintained in the greenhouse. Three months after planting, micro-cuttings survival, rooting, stem elongation and production of leaves were evaluated.

Statistics

The effect of a same concentration of TIBA on the number and length of apical and subapical sprouts produced following the different PULSES of induction were evaluated by linear regression analyses.

PULSES 1 to 3 in Experiment I were compared using multiple comparison procedures and Holm-Sidak's methods to identify the sources of variation influencing the number of micro-cuttings produced by the same set of *in vitro* cloned plants while subjected to the three different pulses of sprouting induction. The mean values of the different treatments and the control, regardless the PULSE, were compared by analysis of variance and the Tukey's test. Tests between treatments were performed using Student's tests (paired t tests) when necessary.

In vitro cloned plants of Catucaí subjected to PULSE 2 in Experiment I were compared to *in vitro* cloned plants of the same variety and cloning batch transferred to the nursery for Experiment II through linear regressions and rank sum paired tests of variance.

Overall, the variables analyzed were the number of apical and sub-apical sprouts, the length and the number of nodes in apical and sub-apical induced sprouts, micro-cuttings produced per *in vitro* cloned plant using nodal segments of apical and sub-apical sprouts, and the percentage of surviving micro-cuttings that developed roots and retrieved aerial part expansion 90 days after planted. The factors analyzed were the three different PULSES of growth regulators used to stimulate sprouting, the environment (greenhouse x nursery) where sprouting stimulation took place, the dosage of plant growth regulators and the genotype of the *in vitro* cloned plants (Catucaí x Siriema). Statistical analyses were performed using SigmaPlot (version 11.2).

3 RESULTS AND DISCUSSION

A trend to the inhibition of apical sprouting and the stimulation of sub-apical sprouting correlated to the increase in TIBA concentration was found for all the PULSES of plant growth regulators throughout Experiment I. Invariably, increases in the concentration of TIBA were significantly correlated to the inhibition in number and length of apical sprouts (Figures 1A and 1B). Nevertheless, sprouts were, at least one of them and frequently two or even more than two in control plants and plants treated with the lower concentrations of TIBA, invariably observed coming out of the buds in the most distal nodes of the stems, which had been promoted to apical nodes by decapitation. On the other hand, the occurrence of orthotropic sprouts in nodes below the most apical one was extremely rare in the absence of TIBA. The number and the length of these sub-apical sprouts was improved by the increase in TIBA concentration (Figures 1C and 1D).

TIBA is an inhibitor of auxin translocation and when applied to the distal half of the shoots it reduced the ability of the distal buds to establish dominance in apple (COOK; VERHAEGEN; KEULEMANS, 2000). TIBA can enhance *IPT* expression by inhibiting auxin transport along the stem (TANAKA et al., 2006). *IPT* genes code for adenosine phosphate–isopentenyltransferases, which are key enzymes in the cytokinin biosynthesis pathway. By reducing auxin transport and consequently apical dominance, while contributing to enhance the synthesis of cytokinins, TIBA induced axillary buds to develop. And this explains the trends to inhibit apical buds reorganization that was observed. For a while, the sprouting of the axillary buds became favored. Finally, the re-establishment of apical dominance, exerted by new formed apical orthotropic branches, took place. In control plants, the reorganization of newly formed apical meristems was not impaired, apical dominance was more promptly re-established and sub-apical sprouting was inhibited.

To identify the factors influencing the production of micro-cuttings throughout Experiment I, the results obtained for each pulse were compared. The treatments were the only source of variation identified (F_{TREATS} = 8.041; p_{TREATS} = 0.006) and the subjects (the *in vitro* cloned plants) were not identified as a source of variation that could influence the results by the analysis of repeated measures. Indeed, PULSE 3 was effective (F = 8.445; p = 0.002) in reason of the treatments applied to the plants (Figure 2A). The highest concentrations of TIBA and mainly the association of TIBA and BAP applied to the *in vitro* cloned plants were essential to attain the best results of Experiment I (Figure 2B).
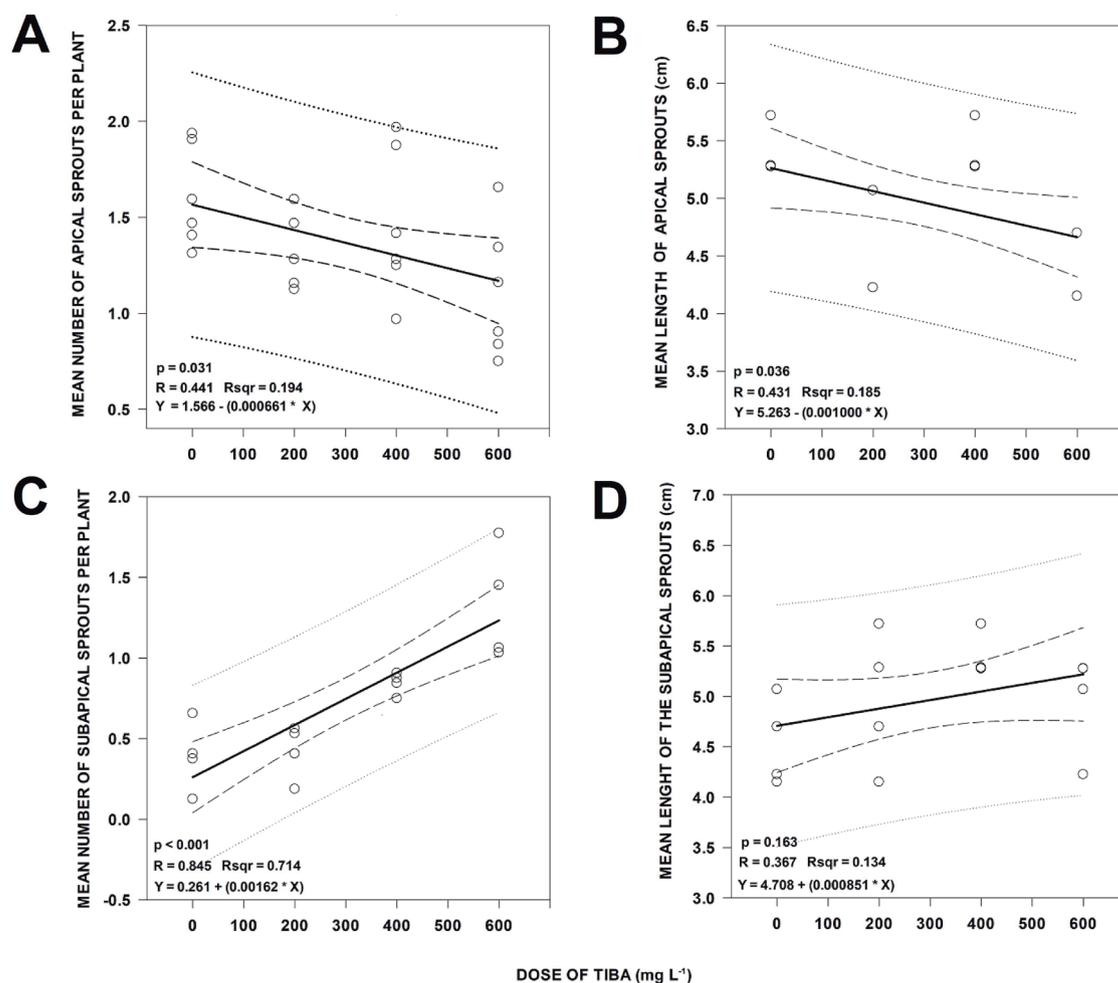
FIGURE 1 - Number and length of sprouts induced on *in vitro* cloned coffee plants by the use of the auxin translocation inhibitor TIBA, in the greenhouse. Means represent plants of Catucaí and Siriema induced to sprout at the third and at the fifth months, all together (PULSES 1 and 2 of sprouting induction).

The analyses of the results obtained for all the *in vitro* cloned plants regardless of genotypes, supports what is stated above. Micro-cuttings yield observed for TIBA + BAP was significantly (t = 2.904; p = 0.043) higher than the yields observed for control plants of all the PULSES in Experiment I. The association TIBA + BAP did not differ from the highest concentration of TIBA but it promoted higher (t = 2.635; p = 0.039) micro-cutting yields than 600 mg.mL^{-1} TIBA applied in the PULSES in Experiment 1.

The statistical difference between genotypes regardless the PULSE could not be detected, certainly in reason of the variability represented by the opposite results of plants of different genotypes to a same concentration of growth regulator (Figure 2B). Nevertheless, analyzing the results for each genotype independently, it was possible to determine that the micro-cuttings yields of Catucaí *in vitro* cloned plants treated with 1000 TIBA or 1000 TIBA + 60 BAP were not statistically different. On the contrary, for Siriema *in vitro* cloned plants, 1000 TIBA or 1000 TIBA + 60 BAP were statistically different (t = 3.517; p ≤ 0.001; df = 89) and the higher micro-cutting yield was observed under the association TIBA + BAP (Figure 2B). Regardless genotypes, the association of TIBA + BAP induced a higher number of sub-apical sprouts than 1000 mg.mL^{-1} TIBA (t = 5.536; p ≤ 0.001; df = 87) or 600 mg.mL^{-1} TIBA (t = 4.709; p ≤ 0.001; df = 114).
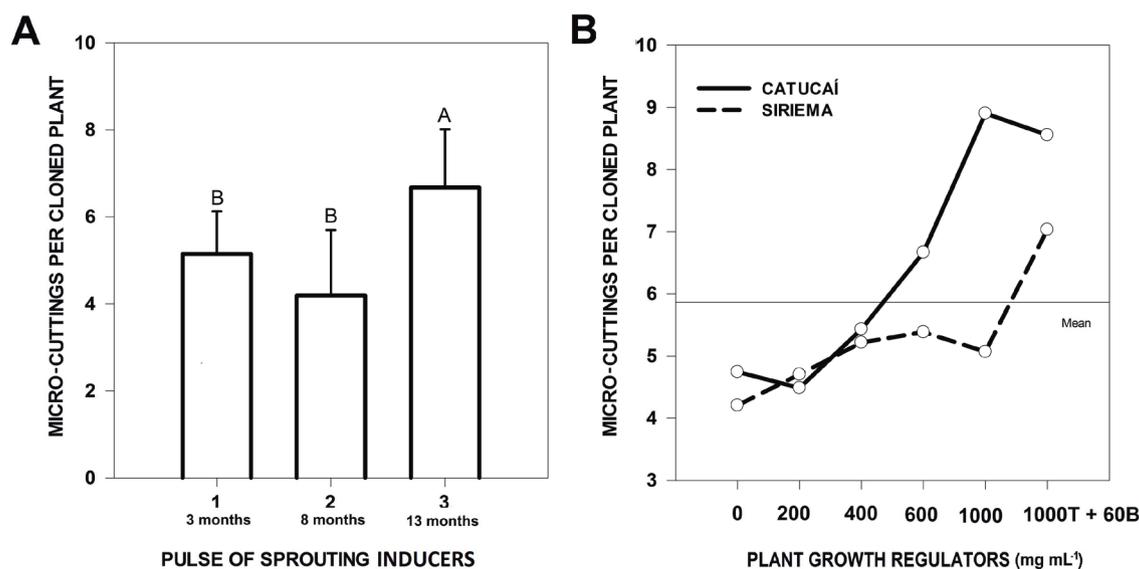
FIGURE 2 - Number of micro-cuttings produced with the sprouts induced on *in vitro* cloned coffee plants following decapitation and the use of plant growth regulators, in the greenhouse. **A** - means represented are for micro-cuttings produced by cloned plants of Catucaí and Siriema all together induced to sprout by decapitation and application of three consecutive PULSES (1, 2 and 3) of plant growth regulators. **B** - means represented are for micro-cuttings produced by cloned plants of Catucaí and Siriema decapitated and subjected to the application of different concentration of TIBA and the combination of TIBA (**1000T**) and BAP (**60B**) in order to induce sprouting.

BAP, an exogenous cytokinin, contributed to the increase in the concentration of this class of growth regulators in the stem, eventually promoting their absorption by the sub-apical axillary buds. Inside the buds, cytokinins activate the cell cycle and promote cell multiplication (MÜLLER; LEYSER, 2011) for buds to growth. The higher number of sub-apical sprouts contributed for the higher yield of micro-cuttings in PULSE 3 when compared to PULSES 1 and 2. Induction using the highest concentration of TIBA and its association with BAP granted the production of 8.5 and 7.0 micro-cuttings per *in vitro* cloned plant of Catucaí and Siriema, respectively, in six month. These results can be related to differences between the genotypes: Siriema received contributions from *Coffea racemosa* Lour., a species described as "profusely branching" (SURESHKUMAR; PRAKASH; MOHANAN, 2010). Lower concentrations of regulators could work better for this last variety.

PULSES 1 and 2 together produced around 10 micro-cuttings per *in vitro* cloned plant treated with 600 mg.mL^{-1} of TIBA in a year. Harvesting apical sprouts each three months would produce the same 10 to 12 micro-cuttings per decapitated plant in a year with no need to induce sprouting by the application of plant growth regulators. Nevertheless, two pulses of 1000 TIBA + 60 BAP applied to the same *in vitro* cloned plants and two sprouts harvests would result, in average, in 15 micro-cuttings produced per *in vitro* cloned plant each year. If each one of these micro-cuttings, once rooted and already growing, had been subjected to the procedures to induce sprouting or even to the dissection of their main orthotropic stems in nodal segments to produce mini-cuttings with no use of plant growth regulators (GEORGET et al., 2017) clonal amplification in the greenhouse would be exponential.

Experiment II was planned to evaluate the influence of the environment where sprouting induction was accomplished on the production of micro-cuttings. *In vitro* cloned plants of Catucaí at the 8th month of acclimatization were tested for the micro-cuttings production in the greenhouse (PULSE 2) and the nursery. Besides being grown in those two different environments, part of the plants under controlled temperature and humidity and maintained under approximately 70% shadowing

in the greenhouse and the other part growing in an environmen deprived of those controls, under approximately 50% shadowing in the nursery, another difference between the two sets of clones was that the plants in the greenhouse had already been subjected to PULSE 1 of sprouting induction and two sprouts harvests during the previous eight months.

As mentioned above, in the greenhouse, the higher the concentration of TIBA the lower the number of apical sprouts (Figures 1A) and, consequently, micro-cuttings prepared with nodal segments taken from apical sprouts produced in the greenhouse following PULSES 1 and 2. However, the correlation between these two factors was not maintained in the nursery (Figures 3A). On the contrary, the trend observed, in the greenhouse, for results coming from three months old plus eight months old plants (PULSES 1 and 2, Figure 1A) to the inhibition of apical sprouting following TIBA application was confirmed for eight months old plants solely (PULSE 2), when cultivated in the greenhouse, despite the reduction in strength and lack of statistical significance (Figure 3B). Regarding sub-apical sprouts, on the other hand, the positive correlation between the concentration of TIBA and the number of sub-apical sprouts for the eight months old plants solely was observed to be significant and considerable in both environments (Figures 3C and 3D).
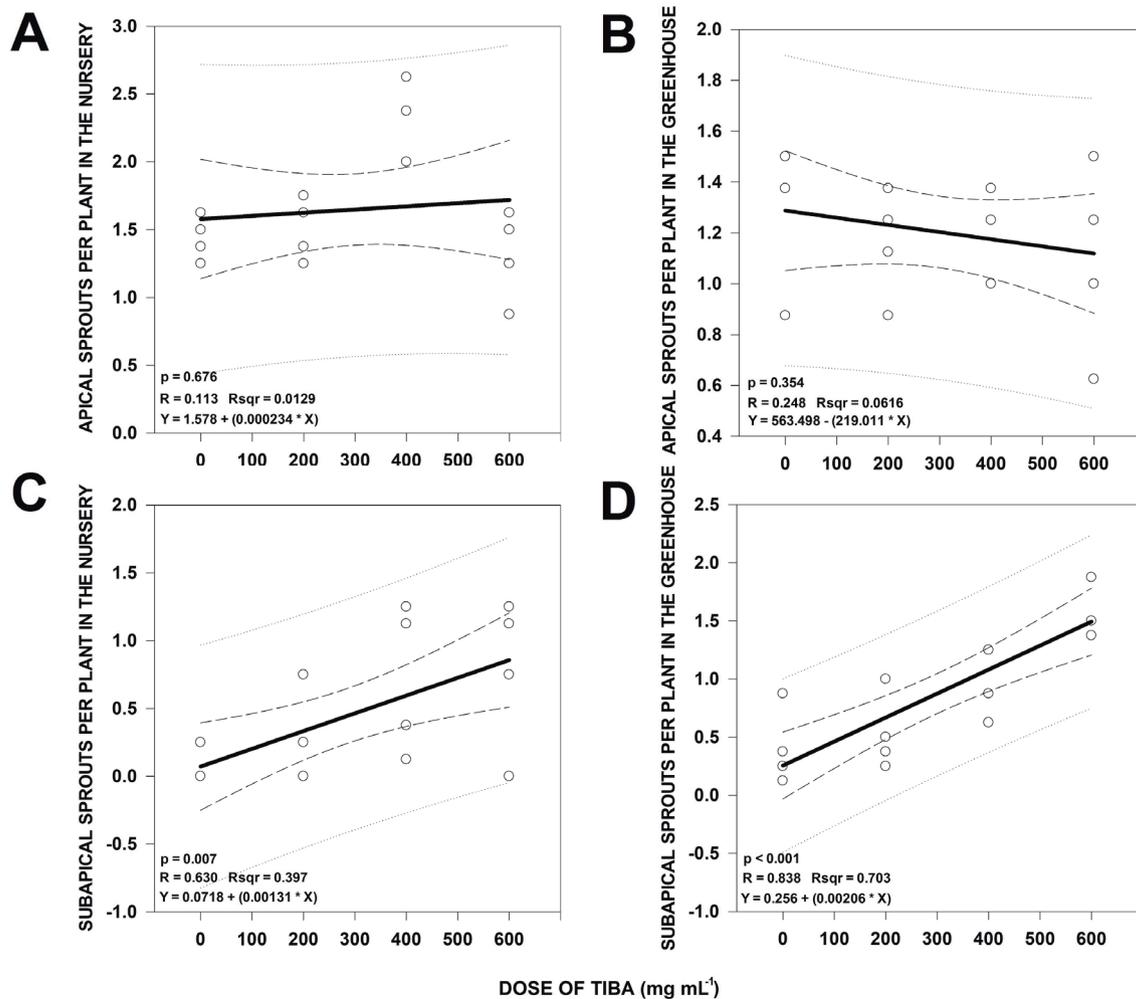

FIGURE 3 - Mean number of sprouts induced on *in vitro* cloned Catucaí coffee plants by the use of the auxin translocation inhibitor TIBA at the eighth month of acclimatization. **A** and **C** – induction of sprouts in the nursery. **B** and **D** – induction of sprouts in the greenhouse (part of PULSE 2, Experiment I).

In addition, the length of induced apical (p ≤ 0.001) and sub-apical (p ≤ 0.001) sprouts was expressively higher in the nursery (Figures 4A and 4B), and at a first sight nursery could have been inappropriately declared as the ideal place to induce sprouting. Nevertheless, the number of nodes in apical sprouts was just slightly higher (p ≤ 0.001) in the nursery (Figure 4C), and the numbers of micro-cuttings produced by *in vitro* cloned Catucaí plants did not differ significantly (p = 0.079) in the nursery and the greenhouse, for the eight months old plants, because numbers of micro-cuttings are related principally to the numbers of nodes in the sprouts, which reached 7.41 and 6.55, respectively, regardless of the concentration of TIBA applied. In the nursery, longer internodes were produced and apical sprouting was not inhibted as harder as it was in the greenhouse by the application of TIBA, but these reactions were not sufficient to lead to the production of a higher number of micro-cuttings

when compared to the greenhouse, where a higher number of sub-apical sprouts (Figure 3C x 3D) displaying shorter internodes (Figure 4B) were produced under the influence of TIBA. Regardless of environments or genotypes, the increase in TIBA concentration was significantly correlated to the increase in the number of micro-cuttings produced by linear regression analysis (p = 0.003, R = 0.418 and R^2 = 0.174) throughout Experiment II, in agreement to Experiment I. This positive correlation was undoubtedly a consequence of the induction of sub-apical sprouting. In the nursery, the plants probably produced more auxins, and TIBA provided in the same concentration was not sufficient to inhibit translocation as well as in the greenhouse.

It still needs experimentation, but considering that the stems lignification in the greenhouse was clearly slower, and the diameter of the main orthotropic stem of *in vitro* cloned plants growing in the greenhouse had never reached that
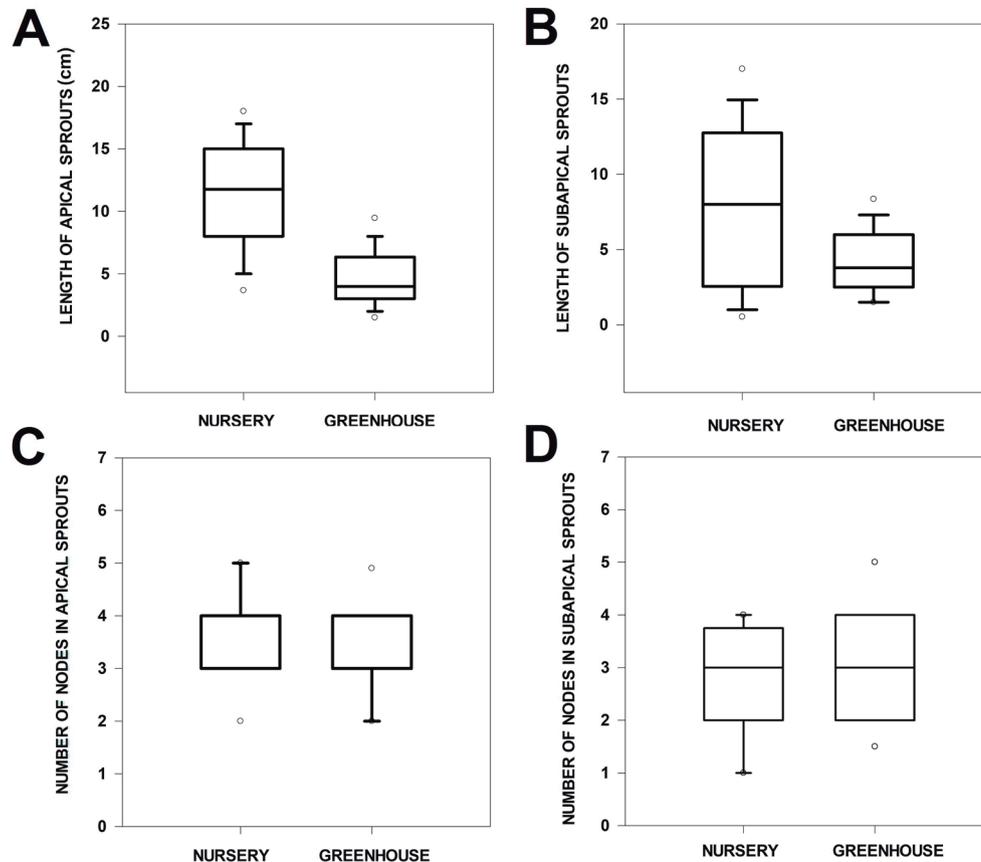

FIGURE 4 - Mean length and number of sprouts induced on *in vitro* cloned Catucaí coffee plants by the use of the auxin translocation inhibitor TIBA on the eighth month of acclimatization, in the nursery and the greenhouse. **A** and **B** - length of apical and subapical sprouts. **C** and **D** - number of nodes in apical and subapical sprouts.

of *in vitro* cloned plants, at the same age, while growing in the nursery, the induction of sprouts and rooting of micro-cuttings in short time lapses, induced by successive pulses of plant growth regulators, applied each three or six months, would probably be better in the greenhouse. An additional experiment (results not shown) aiming to induce the five or six basal nodes to sprout, following the harvest of the upper ones, did not succeed. The basal nodes of the *in vitro* cloned plants lost leaves as the time went by under the harder conditions of the nursery, exposed to variations in the temperature and to diseases. In these reasons sprouting for the basal nodes was not comparable with that of their counterparts in the greenhouse that produced more micro-cuttings after PULSE 3 than after PULSE 1 of inducers (Figure 2B). These last results were in part due to the maintenance of sprouts growing for six months on the *in vitro* cloned plants following PULSE 3 of plant growth regulators instead of harvesting sprouts in the third month after the stimulation, as done following PULSE 2. The association of TIBA and BAP and the use of a higher concentration of TIBA was advantageous to reach PULSE 3 results, as well. Nevertheless, the good physiological status of the plants, preserved from diseases and stresses in the greenhouse, which made possible their fast reactions to the sprouting inducers, even 13 months after potted, was also important.

Regarding rooting, retrieving of stem elongation and production of leaves, there was no statistical difference among micro-cuttings just above 1 and up to 3 cm in length and those lengthy, prepared with stem segments holding two nodes and four leaves. On the contrary, micro-cuttings below 1 cm in length rooted 11% less frequently and retrieved the growth of aerial parts 30% less frequently than those above 1 cm (p < 0.001). Micro-cuttings of Catucaí rooted 80% and the surviving ones developed new nodes and leaves in 90 days. Micro-cuttings of Siriema that produced new nodes and leaves in 90 days were 90% and all of them rooted.

4 CONCLUSIONS

In conclusion, the most remarkable effect of the treatments with TIBA was the induction of sub-apical orthotropic sprouts, which contributed importantly to the production of micro-cuttings. Regarding the ideal environment to induce sprouting, the number of nodes and nodal segments of the sprouts were not higher in the nursery. In addition, the possibilities to control the environment in the greenhouse would favor the micro-cuttings production through the application of successive pulses of sprouting inducers. There was no important difference between genotypes, and micro-cuttings above 1 cm rooted and formed new aerial organs 90 days after planting.

6 REFERENCES

AQUINO, L. P. et al. Cortes basais e substratos na formação de mudas de cafeeiro canéfora. **Coffee Science**, Lavras, v. 12, n. 1, p. 9-16, Jan./Mar. 2017.

BALIZA, D. P. et al. Antecipação da produção e desenvolvimento da lavoura cafeeira implantada com diferentes tipos de mudas. **Coffee Science**, Lavras, v. 8, n. 1, p. 61-68, Jan./Mar. 2013.

CARVALHO, C. H. S. et al. **Efeito de reguladores de crescimento sobre a produção de brotos ortotrópicos axilares em mudas de café.** In: SIMPÓSIO DE PESQUISA DOS CAFÉS DO BRASIL, 5., 2007, Águas de Lindóia. **Anais**... Brasília, DF: Embrapa Café, 2007.

CARVALHO, C. H. S. et al. **Custo de produção de mudas clonais de café arábica produzidas por embriogênese somática**. Brasília - DF: Embrapa, 2013. (Embrapa Café. Circular Técnica, 3).

CARVALHO, M. et al. Comportamento em condições de campo de cafeeiros (*Coffea arabica* L.) propagados vegetativamente e por semeadura. **Coffee Science**, Lavras, v. 3, n. 2, p. 108-114, July./Dec. 2008.

COOK, N. C.; VERHAEGEN, K.; KEULEMANS, J. Manipulation of acrotony in one-year-old apple shoots. **South African Journal of Plant and Soil**, Cape Town, v. 17, p. 108-112, 2000.

ETIENNE, H. et al. Un exemple de transfert de technologie réussi dans le domaine de la micropropagation: la multiplication de *Coffea arabica* par embryogenése somatique. **Cahiers Agricultures**, Paris, v. 21, n. 2/3, p. 115-124, 2012.

GEORGET, F. et al. Somatic embryogenesis-derived coffee plantlets can be efficiently propagated by horticultural rooted mini-cuttings: a boost for somatic embryogenesis. **Scientia Horticulturae**, Amsterdan, v. 216, p. 177-185. 2017.

JESUS, A. M. S.; CARVALHO, S. P.; SOARES, A. M. Comparação entre sistemas radiculares de mudas de *Coffea arabica* L. obtidas por estaquia e por sementes. **Coffee Science**, Lavras, v. 1, n. 1, p. 14-20, Jan./June. 2006.

MATIELLO, J. B.; SANTINATO, R.; ALMEIDA, S. R.; GARCIA, A. W. R. Variedades de Café. In*:_____.* **Cultura de Café no Brasil: manual de recomendações.** São Paulo: Futurama, 2016. cap. 3, p. 61-100.

MÜLLER, D.; LEYSER, O. Auxin, cytokinin and the control of shoot branching. **Annals of Botany**, Exeter, v. 107, n. 7, p. 1203-1212, May. 2011.

OLIVEIRA, D. H. et al. Influência do comprimento de estacas e ambientes no crescimento de mudas cafeeiras obtidas por enraizamento. **Coffee Science**, Lavras, v. 5, n. 2, p. 183-189, 2010.

REZENDE, T. T. et al. Types of stem cuttings and environments on the growth of coffee stem shoots. **Scientia Agraria**, Curitiba, v. 11, n. 5, p. 387-391, 2010.

REZENDE, J. C. D. et al. Multiplication of embryogenic *calli* in *Coffea arabica* L. **Acta Scientiarum**, Maringá, v. 34, n. 1, p. 93-98, Jan./Mar. 2012.

SANSBERRO, P.; MROGINSKI, L.; BOTTINI, R. Stimulation of lateral branch formation on *Ilex paraguariensis* (Aquifoliaceae) seedlings. **Australian Journal of Experimental Agriculture**, Clayton, v. 46, n. 5, p. 707-710, May. 2006.

SANTOS, M. R. A. D. Vegetative vigor of conilon coffee and its potential for *in vitro callus* induction. **Coffee Science**, Lavras, v. 8, n. 4, p. 432-438, Oct./ Dec. 2013.

SURESHKUMAR, V.; PRAKASH, N.; MOHANAN, K. A study of *Coffea racemosa* x *Coffea canephora* var. *robusta* hybrids in relation to certain critically important characters. **International Journal of Plant Breeding and Genetics**, New York, v. 4, n. 1, p. 30-35, Jan./Mar. 2010.

TANAKA, M. et al. Auxin controls local cytokinin biosynthesis in the nodal stem in apical dominance. **The Plant Journal**, London, v. 45, n. 6, p. 1028-1036, Mar. 2006.

VOS, J. E.; SNIJDER, B. *In vitro* rejuvenation techniques for the rapid commercial release of clonal coffee plants. **Acta Horticulturae**, Belgica, v. 520, p. 119-126, 2000.