VEGETATIVE VIGOR CONILON COFFEE AND ITS POTENTIAL FOR IN VITRO CALLUS INDUCTION

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ABSTRACT: Considering the importance of the culture of *Coffea canephora* in the Brazilian Amazon and the need for genetic improvement of this species for cultivation in the state of Rondonia, he objective of this study was to evaluate the correlation between the initial vigor of clonal plants of *C. canephora* variety Conilon, under field conditions, and its potential for in vitro propagation. Promising clones belonging to the Genetic Improvement Program of Coffea sp were used Embrapa (Brazilian Agricultural Research Corporation) Rondonia. The plant height, number and length of orthotropic shoots in coffee at 10 months after planting were evaluated. In the laboratory, leaves of plants of each clone were segmented into fragments of 1 cm ² which were inoculated on MS medium containing half the concentration of salts, AIB (10 μM), 2,4-D (20 μM) and 2iP (10 μM), sugar (20 g cm^{-3}) and agar (6 g cm^{-3}). Cultures were maintained in a growth chamber under 16 h photoperiod at 2000 lux and 24 ± 2 º C. The callus induction and the percentage of leaf area covered with callus cells were observed within 90 days. Clone 9 showed greater vegetative growth in the field. Only clone 12 showed no callus induced, all other clones were highly responsive to callus induction, and clone 5 showed a higher percentage of leaf area covered with callus cells. There was no phenotypic correlation between early vigor in the field and calogênico potential in vitro, for clones of coffee used.

Index terms: *Coffea canephora*, vegetative growth, plant tissue culture, callus induction.

VIGOR VEGETATIVO DE CAFÉ CONILON E SEU POTENCIAL PARA INDUÇÃO DE CALOS *IN VITRO*

RESUMO**:** *Considerando a relevância da cultura de Coffea canephora para a Amazônia brasileira e a necessidade de melhoramento genético dessa espécie para cultivo no estado de Rondônia, objetivou-se, neste trabalho, avaliar a correlação existente entre o vigor vegetativo inicial de plantas clonais de C. canephora var. Conilon, em condições de campo, e seu potencial para propagação in vitro. Foram utilizados clones promissores pertencentes ao Programa de Melhoramento Genético de Coffea sp. da Embrapa Rondônia. Foram avaliadas as características altura da planta, número e comprimento de brotos ortotrópicos, em cafeeiros com 10 meses pós-plantio. Em laboratório, folhas de plantas de cada clone foram segmentadas em fragmentos de 1 cm² os quais foram inoculados em meio MS, contendo metade da concentração dos sais, AIB (10 µM), 2,4-D (20 µM) e 2iP (10 µM), açúcar (20 g cm^{-3}) e ágar (6 g cm^{-3}). As culturas foram mantidas em sala de crescimento, sob fotoperíodo de 16 horas a 2000 lux e 24±2ºC. A indução de calos e a porcentagem de área foliar coberta com células de calo foram observadas nos 90 dias subsequentes. O clone 9 demonstrou maior crescimento vegetativo em campo. Apenas o clone 12 não apresentou calos induzidos, todos os outros clones foram altamente responsivos à indução de calos, e o clone 5 apresentou maior porcentagem de área foliar coberta com células de calo. Não houve correlação fenotípica entre vigor vegetativo inicial em campo e potencial calogênico in vitro, para os clones de cafeeiro utilizados.*

Termos para indexação*: Coffea canephora, crescimento vegetativo, cultura de tecidos vegetais, calogênese.*

1 INTRODUCTION

The coffee agribusiness is among the most important in the world due to its economic and social importance (SOUZA et al., 2005). *Coffea canephora* Pierre ex A. Froehner takes part in approximately 35% of the financial operations regarding coffee in the world. In Brazil, *C. canephora* culture generates income of more than half a billion dollars, taking into account all of the business activities involved with this product (FAZUOLI et al., 2007).

The species *C. arabica* L. and *C. canephora* account for 75% and 25% of the Brazilian commercial production, respectively (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2013). These species are different in relation to the production system, agronomic and biochemical aspects, origins, cycle, size, number of chromosomes. *C. canephora* ('Conilon' and 'Robusta') has aroused great interest among plant breeders due to its strength, adaptation to a wide range of tropical climatic conditions, such as low altitudes and high

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temperatures, tolerance to rust (*Hemileia vastatrix* Berk. et Br.) and the major plagues that affect *C. arabica*. Its high yields and great amount of soluble solids in comparison to *C. arabica* makes the species highly attractive to the production of soluble coffee (CECON et al., 2008).

In the Brazilian Amazon region, specifically in the state of Rondonia, coffee is a dominant perennial crops (RESENDE et al., 2011). In this state, the coffee cultivations occur in small farms, under the family farm system and are a major source of family income (SOUZA et al., 2006). Rondonia has the combination of soil and climatic conditions for the cultivation of *C. canephora,* which has been increasing significantly in the last years (MARCOLAN et al., 2009). The cultivation of *C. canephora* is in the short term the most adequate alternative to increase productivity and quality of coffee produced in Rondonia, but there is need for breeding of this species. Due to these and other socioeconomic aspects, the tradition of cultivation and the hot, humid climate and low altitude of the state of Rondonia (SANTOS; FERREIRA; SARUBO, 2010). The botanical variety Conilon of *C. canephora* predominates in 95% of the coffee plantations of the state (RAMALHO; OLIVEIRA, VENEZIANO, 2009).

Among the conventional techniques for vegetative propagation of *Coffea*, the use of cuttings from orthotropic branches is the most adequate for *C. canephora* clones (BRAUN et al., 2007; FAZUOLI et al., 2007; PARTELLI et al., 2006). In general, this method is applied to 'Conilon' plants when they are two years old. The cuttings may have one or two lateral buds and are treated with auxinic compounds to promote rooting (FONSECA et al., 2007).

Currently, *in vitro* multiplication is an economically viable alternative when the goal is the large scale production of elite plants (AZEVEDO et al., 2008; CENTOFANTE et al., 2009; PASQUAL et al., 2008). Among the available techniques for micropropagation of coffee, the most common is callogenesis, a basic phase of massive propagation systems that are used in breeding programs of coffee. This technique is capable of rapid multiplication of certain genotype in short term, which allows accelerating the achievement of evidence of superior variety (SANTANNA-BUZZY et al., 2007).

Correlations have been established in several species between morphogenic events *in vitro*, as the rate of growth of tissues, and vigor in other agronomic traits. Some of the characteristics of in vitro cultures of closely related genotypes have been subjected to biometrical analyses and have been shown to be correlated in a complex fashion with apparently unrelated features of whole plants *in vivo* (GEORGE, 1993).

The objective in this this work was to evaluate clones of *C. canephora* var. Conilon in relation to their vegetative vigor under field conditions and their potential for callus induction under *in vitro* conditions, and the correlation between field and *in vitro* potentials. Although preliminary, this study provides data on the diversity of aspects among clones of the same variety of *C. canephora*, and will guide future proceedings related to their micropropagation taking into account the peculiarities of each clone.

2 MATERIAL AND METHODS

The experiment was established in December 2008 at the Experimental Station of Embrapa Rondonia, in Porto Velho city (08˚48'01"S, 63˚51'05"W, 288.7 feet height). The climatic Köppen classification is Aw, tropical rainy with average annual temperatures of 25.5°C and relative humidity of 81.5%, with no rainfall from June to August. The soil of the experimental area is a dystrophic oxisoil, medium texture (RONDÔNIA, 2009).

In the field phase ten months old plants of fifteen promising clones of *C. canephora* var. Conilon (Congolese Group SG_1) from the Coffee Breeding Program of Embrapa Rondonia were used. The evaluated characteristics were: plant height – determined from the soil surface to the tip of the dominant branch by using a ruler; number and length of orthotropic branches – with a ruler, from the insertion of the branch to its tip. The evaluation was carried out in October 2009. The experimental design was randomized blocks with three replications of six plants of each clone. The setting was planted in single row, one plant per hole (40×40×40 cm), spaced 2.0 m between plants and 3.0 m between rows. All the evaluated plants were surrounded by other plants from the same clone to avoid border effect. Plants were kept with only five orthotropic branches.

The *in vitro* phase was carried out at the Plant Biotechnology Laboratory of Embrapa Rondonia. One plant of each of the fifteen clones of *C. canephora* used in the field phase was randomly selected as a source of explants. Leaves were collected from the second pair of

orthotropic branches. At the laboratory they were washed with water and a detergent agent and immersed in 70% alcohol for 1 minute, NaOCl 1.25% for 30 minutes and rinsed three times in sterile distilled water. Under aseptic conditions the leaves were cut into fragments of about 1.0 cm^2, which were inoculated individually in assay tubes with the adaxial face in contact with the medium. This medium was supplemented with half the concentration of salts of the MS medium (MURASHIGE; SKOOG, 1962) and 10 g cm^{-3} thiamin, 1 g cm^{-3} pyridoxine, 1 g cm^{-3} nicotinic acid, 1 g cm^{-3} glicyn, 100 g cm^{-3} inositol, 100 g cm^{-3} casein, 400 g cm^{-3} malt extract, 20 g cm^{-3} sucrose, 8 g cm^{-3} agar, 10 µM indole-3-butyric acid (IBA), 20 µM 2,4-dichlorophenoxyacetic acid (2,4-D), and 10 µM 6-(γ,γ-dimethylallylamino) purine (2iP) (SANTOS; FERREIRA; SARUBO, 2010). The experimental design was entirely randomized with three replications of 10 tubes. The cultures were kept in a growth-chamber under 16 hour photoperiod (2000 lux) at 24±2°C. In periods of 10 days during 90 days the induction of callus and the percentage of leaf area covered by callus cells were evaluated by visual observation between 0 the 25%; 25 the 50%; 50 the 75% and 75 the 100%.

The field data and the percentage of callus induction were submitted to a variance analysis and the averages were compared by the Scott-Knott test at a 5% probability level. The laboratory data were submitted to descriptive statistics. Pearson correlation was used to compare field and laboratory data. All the analysis were carried out by using the Genes statistical program.

3 RESULTS AND DISCUSSION

Vegetative vigor

The number and length of orthotropic branches and the plants height were affected by the clones, with averages of 1.85 branches, 39.10 cm and 59.00 cm, respectively. The number of orthotropic branches ranged from 1.12 to 2.79. Clones 5 and 9 showed the highest number of branches, 2.72 and 2.79, respectively (Table 1). A high number of orthotropic branches can cause the "closing" of the coffee culture, because the weight of the fruits bends the branches in the middle of the street, which makes cultural treatment difficult (MARCOLAN et al., 2009). Lower values were observed in clones 3 (1.16), 4 (1.29), 13 (1.23) and 15 (1.12) (Table 1).

TABLE 1 - Number and length of branches, plant heights of Conilon coffee clones.

Clone	Vegetative vigor*		
	Number of branches	Lenght of branches (cm)	Plant heights (cm)
1	1.50 c	32.50 j	59.80 b
2	1.80 c	43.20 e	64.10 a
3	1.20 d	25.50 l	62.10 a
4	1.30 d	37.00 i	58.40 b
5	2.70 a	45.70 c	54.80 b
6	2.30 b	40.90 g	52.30 b
7	1.80 c	40.90 g	54.90 b
8	2.20 b	38.70 h	52.00 b
9	2.80 a	49.70 a	65.60 a
10	1.70 c	36.70 i	53.90 b
11	1.70 c	42.60 f	62.70 a
12	2.40 b	44.30 d	57.80 b
13	1.20 d	32.90 j	69.60 a
14	2.20 b	49.10 b	59.50 b
15	1.10 d	29.10 k	58.40 b

* Averages followed by the same letter within the column belong to the same group of similarity, by Scott-Knott criteria at a 5% level of probability.

Clones 9 and 14 showed the highest branch length, 49.73 and 49.06 cm, respectively. The lowest values were observed in clones 3 (25.51 cm) and 15 (29.05 cm), respectively (Table 1).

The highest plant height values were observed in clones 2, 3, 9, 11 and 13, with 64.1, 62.1, 65.6, 62.7 and 69.6 cm, respectively. Other clones did not differ in relation to plant height (Table 1). High plants can show high productivity; nevertheless this characteristic can make the harvesting process difficult. On the other hand, smaller plants make harvest and cultural treatments easier, and create an adverse environment for insects like the coffee berry borer (*Hypothenemus hampei* Ferrari) and diseases such as rust (*Hemileia vastatrix* Berk. et Br.), due to aeration and light penetration in the plant (MARCOLAN et al., 2009).

In general clone 9 showed the best values regarding the vegetative vigor for its highest values of plant height, number and length of orthotropic branches. This clone can be derived from natural crosses between 'Conilon' and 'Robusta' varieties, admittedly of high vegetative vigor, provided that these varieties were grown simultaneously in commercial fields over the years, since the introduction of coffee culture in the state of Rondonia.

Callogenesis

The callus induction in leaf explants of Conilon plants ranged from zero to 100% (Figure 1). Only clone 12 showed no callus induction 90 days after inoculation, all other clones were highly responsive to callus induction and formed callus, ranging from 8.33 to 100% of induction (Table 2). According to Santos, Ferreira and Sarubo (2010), the plant regeneration from callus in these conditions can be achieved from the 53rd day after inoculation by subculturing the callus onto another medium.

Clones 1, 2, 5, 6, 8, 11, 13, 14 and 15 were highly responsive to the induction medium and showed the best results in relation to the percentage of callus induction in leaf explants (Table 2). Clones 4 and 10 showed the lowest percentages of induction, 8.33 and 42.85, respectively.

Factors such as type of explant, composition of the medium and physical conditions of incubation, such as light and temperature, can affect the callus formation (MOREIRA-DIAS et al., 2001; RODRIGUES et al., 2009). In this study the type of explant, medium and physical conditions were the same, so the clones are responsible for the different callogenesis results for they have distinct genetic constitutions. The variation within each clone is generally low due to the high homogeneity enabled by the cloning process (WEIGEL; JURGENS, 2002).

Only clones 3, 4, 5, 6, 7, 8, 9, 10, 13, and 14 showed leaf area covered by callus cells between zero and 25%. Clones 1, 2, 11 and 15 had 100% of occurrence ranging from 25 and 50%. No clone had from 50 to 75% and only clone 5 had from 75 to 100% of the leaf area covered by callus cells (Table 2). George (1993) shows many reports that illustrate how the capacity of explanted tissues to form callus and the subsequent growth rate of callus cultures, can be genotype or variety-dependent.

These results suggest that the medium composition and the cultivation conditions cannot be adequate for all used genotypes. In addition to genetic factors, the time of leaf collection, plant age and other environmental conditions can affect the behavior within the same species (VENCOVSHY, 1987).

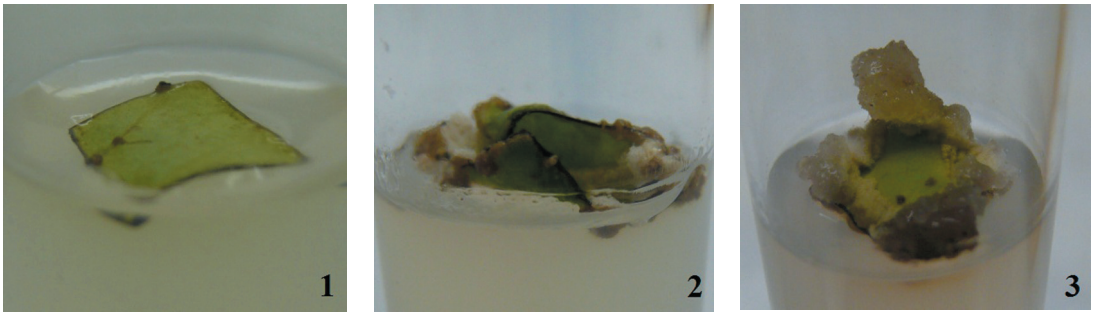

FIGURE 1 - *In vitro* cultivation of Conilon coffee leaves. (1) Leaf explant immediately after inoculation. (2) Leaf explant with swelling 30 days after inoculation. (3) Leaf callus, 60 days after inoculation. Photo: Carla Liegi L. G. de Oliveira.

TABLE 2 - Percentages of callus induction and leaf area covered by callus cells of Conilon coffee clones, 90 days after inoculation.

Clone	Callus induction (%)	% Leaf area covered by callus cells			
		0-25%	25-50%	50-75%	75-100%
1	100,00 a	0.00	100.00	0.00	0.00
2	100.00 a	0.00	100.00	0.00	0.00
3	60.00 c	20.00	40.00	0.00	0.00
4	8.33 e	8.33	0.00	0.00	0.00
5	100.00 a	16.67	16.67	0.00	66.66
6	100.00 a	33.33	66.67	0.00	0.00
7	91.66 a	83.32	8.34	0.00	0.00
8	100.00 a	45.45	54.55	0.00	0.00
9	88.88 b	88.88	0.00	0.00	0.00
10	42.85 d	42.85	0.00	0.00	0.00
11	100.00 a	0.00	100.00	0.00	0.00
12	0.00 f	0.00	0.00	0.00	0.00
13	100.00 a	100.00	0.00	0.00	0.00
14	100.00 a	94.12	5.88	0.00	0.00
15	100.00 a	0.00	100.00	0.00	0.00

*Averages followed by the same letter do not differ by Tukey test at a 5% level of probability.

Correlations

For the correlations between the traits under evaluation, the number of orthotropic shoots was the only variable that correlated positively with length of orthotropic shoots (r = 0.8664) (Table 3). Looking for genetic information on the breeding of Conilon coffee, Fonseca et al. (2006) evaluated the correlation between plant height and number of branches with several characteristics of productivity, but did not find any correlation. The author considered that these parameters are not appropriate to select promising Conilon clones.

The expected correlations between callus induction and number of shoots, shoot length, and plant height were not confirmed. In an overview, the results suggest that there is no correlation between vegetative vigor and cellular division in *in vitro* conditions.

According to George (1993), certain kinds of morphogenic responses in tissue cultures are seen to be directly related to the behavior exhibited by whole plants or parts of plants *in vivo*.

Keyes et al. (1981) working with tobacco cotyledons noted correlations between the rate of the tissues *in vitro* and vigor in other agronomic traits and suggested that characteristics of *in vitro* cultures might be of value for the rapid identification of hybrid combinations which could produce superior plants in the field. In *Petunia hybrid* E. Vilm. however, Hanson and Read (1981) found that although F_1 hybrids generally gave more vigorous plants and callus cultures than their inbred parents, there was no consistent correlation between tissue culture and field performance. Callus from the shoot meristems of the interspecific hybrid between *Trifolium hybridum* L. and *T. ambiguum M. Bieb* grew more slowly than those of either of the parental species (RUPERT et al., 1976).

TABLE 3 - Pearson correlation coefficient to the studied characteristics.

	Callus induction (%)	Plant height (cm)	Number of orthotropic branches
Branches length (cm)	0.0176	- 0.0656	0.8664*
Number of orthotropic branches	0.0762	- 0.0270	
Plant height (cm)	0.1309		

* Significance at a 1% level of probability.

4 CONCLUSIONS

In general clone 9 showed the best values regarding the vegetative vigor for its highest values of plant height, number and length of orthotropic branches.

All the clones were highly responsive to callus induction (except clone 12), but only clone 5 showed from 75 to 100% of the leaf area covered by callus cells, in 66.67% of the explants.

The results suggest that there is no correlation between callus induction and number of shoots, shoot length, and plant height.

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