

Cutting propagation of 'Cambona 4' yerba mate clones

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Abstract

The 'Cambona 4' yerba mate (*Ilex paraguariensis* A. St. Hil. - Aquifoliaceae) stands out for the high productivity and soft taste of the raw material obtained. The seedlings come from the controlled crosses of a female and a male plant. In order to guarantee the planting of even more uniform and productive herbs, the cutting is an alternative of propagation. The objective of this research was to evaluate the rooting capacity of apical and subapical cuttings of six 'Cambona 4' clones (C7, C8, C9, C10, C11 and C13) in two seasons, in January (summer/autumn) and April (autumn/winter) treated with 2.000 mg/kg of IBA in the form of talc. The cuttings were standardized with 10 cm, keeping a pair of leaves cut in half. The cutting was done in an agricultural greenhouse, with irrigation by intermittent nebulization, the cuttings were placed to rooting in plastic tubes containing carbonized rice husk. After 120 days, it was verified that the cutting season influenced the response of each clone, but for most clones rooting was higher when performed in summer/autumn, with better rates varying between 78.2% to 90.6% (C8, C9, C11 and C13), while in autumn/winter it was from 59.4% to 75.0% (C7, C10, C11 and C13). In the summer/autumn cutting period, subapical cuttings showed greater survival and rooting than the apical ones, but they didn't differ when performed in autumn/winter. Leaf retention positively influenced the survival.

Keywords: *Ilex paraguariensis*, Indolbutiric acid, cutting seasons, types of cuttings, rooting

Introduction

The use of yerba mate is associated with tea preparation and, more often, with typical beverages, popularly known as "chimarrão" (hot) and "tererê" (cold), by the infusion of grinded aerial part of the plant (leaves and thin branches). Besides its bitter and strong taste, it has phytochemical compounds of medicinal properties, such as polyphenols and antioxidants, having also anti-inflammatory, antimicrobial and diuretic action (Filip et al., 2001; Gonçalves et al., 2005; Meinhart et al., 2010; Carelli et al., 2011).

The 'Cambona 4' yerba mate was obtained in the city of Machadinho, Rio Grande do Sul, Brazil, which is considered the first bi-parental progeny of yerba mate (Corrêa et al., 2009). The exclusivity of pollination between only two selected plants, one pistillate and one staminate, reduced the variability between the seedlings, but a certain degree of heterogeneity is observed

(Hettwer, 2013), resulting from genetic recombination and segregation.

Vegetative propagation by cuttings is a procedure that allows for quality seedlings in a short time, with the possibility of selecting good quality genotypes in the field for the production of plants with suitable characteristics (Fachinello et al., 2005; Loss et al., 2009). Among the challenges of cutting, it can be cited initially the selection of agronomically superior matrix trees that should be tested for rooting capacity, which may vary between: genotypes, cutting season, mother plant physiological condition, mother plant and cuttings juvenility, cut type and treatments with phytohormones, besides other factors (Wendling & Xavier, 2001; Wendling, 2004; Wendling et al., 2014; Wendling & Santin, 2015).

Several studies have shown that the rooting capacity of yerba mate cuttings varies according to the age of the matrix trees (Wendling & Xavier, 2001;

Wendling, 2004; Wendling et al., 2014; Wendling & Santin, 2015). However, higher rooting rates can be achieved using mother plants cuttings previously cloned, established in so-called clonal gardens that are usually made in hanging flower beds or pots (Wendling et al., 2013). The most common and effective methods of rejuvenation are based on micropropagation, cutting or serial grafting (Wendling et al., 2014).

For this research, the differentials that can be pointed out regarding other works are: the use of 'Cambona 4' cultivar as the study subject; the use of cuttings collected from this cultivar clones of genotypes, rescued by cuttings and planted in the field, and not from plants of seminal origin; and the use of AIB in talc, in a lower concentration (2.000 mg/kg), while in general treatments are carried out with higher doses of hydro-alcoholic solutions.

This work sought to evaluate whether the rooting potential differs between clones, cutting types (apical and subapical) and cutting periods. The proposed hypotheses were: that there are genotypes standing out for their greater capacity to form adventitious roots; that apical and subapical cuttings differ in terms of rooting potential; and that the cutting period influences the results.

The goals of this study were, therefore, to evaluate in two periods (summer/autumn and autumn/winter) the rooting capacity of apical and subapical cuttings of 'Cambona 4' yerba mate clones of genotypes.

Material and Methods

The yerba mate plants (*Ilex paraguariensis* A. St. Hil. - Aquifoliaceae) studied were 'Cambona 4' clones, about three years old and established in the orchard of the Extension Center and Agricultural Research (Cepagro) of the Faculty of Agronomy and Veterinary Medicine (FAMV) from the University of Passo Fundo (UPF), in the city of Passo Fundo, RS/Brazil. The clones correspond to the seminal genotypes G7, G8, G9, G10, G11 and G13, studied and rescued by cuttings in the work of Hettwer (2013), selected in a seven-year-old commercial herb, in Machadinho, RS. Subsequently, the material was identified by the following exsiccate numbers: Clone 7 - RSPF 14443, Clone 8 - RSPF 14444, Clone 9 - RSPF 14445, Clone 10 - RSPF 14446, Clone 11 - RSPF 14447, Clone 13 - RSPF 14449 and they are stored at Muzar, in the Institute of Biological Sciences of UPF.

The study was conducted in a plastic agricultural greenhouse, internally coated (on the top and sides) with a shade screen (70% shading) to reduce temperature and insolation. The irrigation system was intermittent

misting, with watering for 10 seconds every 8 minutes.

The research was carried out in two periods, between January 26 to May 25 (summer/autumn), and March 28 to July 25, 2017 (autumn/winter), considering each period as one experiment. Six clones (C7, C8, C9, C10, C11 and C13) were studied comparing apical and subapical cuttings. The experimental design was in completely randomized blocks, with the treatments arranged in a 2 x 6 factorial scheme, with four repetitions and eight cuttings per plot.

Six months in advance of the first cutting period, the plants were submitted to drastic pruning, lowering the branches at an average height of 0,50 m above ground level to stimulate new shoots and to promote a rejuvenation effect.

Cuttings of semi-woody consistency were standardized with 10cm in length, apex elimination and a bevel base cut, keeping two leaves reduced in half. In carrying out the cutting, the basal region of the cuttings (3-4 cm) was treated with IBA in talc, at a dose of 2,000 mg/kg and then planted to an average depth of 4 cm in tubes of 140 cm³, using carbonized rice husk as substrate.

After 120 days of cutting, the percentage of leaf retention, survival, rooting and callus rooted cuttings were determined; as well as the average length of the three largest roots and the fresh and dry root mass. Data were subjected to analysis of variance and the differences between means were compared by Scott-Knott test at 5% probability of error. Among the percentage variables of leaf retention, survival, rooting and callus rooted cuttings, the Pearson correlation coefficient was determined.

Results and Discussion

In the summer/autumn cutting period there was significant interaction for leaf retention and survival rates between clones and cut types (Table 1). The subapical cuttings of four clones stood out for the higher leaf retention (C8, C9, C11 and C13), between 71.9% and 79.7%, considered satisfactory results for yerba mate. Only C7 and C10 didn't differ in leaf retention of apical and subapical cuttings. The average retention in apical cuttings was 36.7%, not differing between clones.

The survival of subapical cuttings was higher in the same clones that stood out for leaf retention, with 84.4% to 93.8% of cuttings remaining alive. In apical cuttings the survival rate did not differ among the clones (mean of 46.9%), also consistent with the results obtained for leaf retention. The greater survival of subapical cuttings (except in clones C7 and C10) can be justified, probably for presenting more mature tissue (slightly more lignified) in relation to the apical, younger ones. This characteristic

gives them a condition of greater tolerance to the elevated summer temperatures and less dehydration, even though the greenhouse has intermittent mist irrigation. Fachinello et al. (2005) corroborate, stating that cuttings collected during spring/summer are more herbaceous and more susceptible to water loss at higher temperatures.

Table 1. Percentage of leaf retention and survival of apical and subapical cuttings of six 'Cambona 4' yerba mate clones after 120 days of cutting in summer/autumn

Clones	Leaf retention (%)		Survival (%)	
	Apical	Subapical	Apical	Subapical
C7	32,8 Aa	34,4 Ab	40,6 Aa	50,0 Ab
C8	26,6 Ba	75,0 Aa	37,5 Ba	84,4 Aa
C9	40,6 Ba	71,9 Aa	50,0 Ba	93,8 Aa
C10	40,6 Aa	40,6 Ab	50,0 Aa	56,3 Ab
C11	34,4 Ba	79,7 Aa	43,8 Ba	90,6 Aa
C13	45,3 Ba	78,1 Aa	59,4 Ba	87,5 Aa
Mean	36,7	63,3	46,9	77,1
C.V. (%)	25,03		23,88	

Means followed by the same capital letter in the line and small letter in the column do not differ by the Scott-Knott test at 5% probability of error.

Hettwer (2013) studied the rooting potential of seminal genotypes (G7, G8, G9, G10, G11 and G13) that gave rise to the clones studied in this research, using apical cuttings in summer, with and without the treatment of 6,000 mg / L. from AIB. With IBA, the leaf retention results were similar to those obtained for the apical cuttings of this study, except for G13, which presented high leaf retention, close to what was verified for the subapical ones. The survival of seminal genotypes and clones cuttings, on the other hand, presented less similarity from the obtained results. The comparison of results of both studies shows the possibility of getting different responses when collecting cuttings from seminal or cloned plants of different ages or degree of juvenility.

In the autumn/winter period there wasn't interaction between cut types and clones (Table 2). Clone C9 stood out for having the highest leaf retention (94.6%), with 100% survival, not differing from clone C11, with 89.1% of live cuttings. Regarding the other clones, it was found that leaf retention remained between 46.1% and 60.9%, except C9, and the survival between 70.3% and 76.6%, except for C9 and C11.

Differently from the summer/autumn cuttings, the apical and subapical cuttings didn't differ regarding leaf retention and survival percentage, with means for the respective variables of 62.5% and 81.0% (Table 1). The rates of leaf retention were superior to those for apical cuttings in summer and close to those observed for subapical cuttings (Table 2), especially C9, which exceeded all results. Likewise, the survival of cuttings in autumn/winter was higher than those obtained for apicals in summer/

autumn. Comparing with the subapicals in summer/fall, it is possible to state that C9 and C11 presented similar rates, being therefore clones that stood out in the two studied periods. In its turn, there was improvement in survival for C7 and C10, between 20.3% and 23.4%, but small reduction for C8 and C13, between 11% and 14%.

Table 2. Percentage of leaf retention and survival of apical and subapical cuttings of six 'Cambona 4' yerba mate clones after 120 days of cutting in autumn/winter

Clones	Leaf retention (%)	Survival (%)
C7	60,2 b	73,4 b
C8	46,1 b	70,3 b
C9	94,5 a	100,0 a
C10	52,3 b	76,6 b
C11	60,9 b	89,1 a
C13	60,9 b	76,6 b
Cuttings		
Apical	60,9 ^{ns}	81,3 ^{ns}
Subapical	64,1	80,7
Mean	62,5	81,0
C.V. (%)	23,98	17,28

Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability of error. ^{ns} - not significant by the F test.

Differences between genotypes in leaf retention and survival were found in other studies, proving to be one of the biggest challenges of yerba mate cuttings propagation. Santos (2011) concluded that leaf retention and survival varied among genotypes, cutting season (spring/summer or autumn/winter) and the use or not of IBA (6,000 mg/L), showing a possible phytotoxic effect of IBA on these variables at the tested concentration. Tres (2016), performing the cutting of thirty genotypes in January, obtained satisfactory leaf retention rates in eleven genotypes (76.0% to 88.5%), and only five genotypes had the survival below 68.8%, ranging up to 95.8%. However, Teixeira et al. (2017), combining substrates with and without the application of 3,000 mg/L of IBA, found a drastic reduction in survival for the autumn/winter period, ranging from 30% to 55% after 60 days and at 180 days not exceeding 30%.

Higher leaf retention and survival, generally achieved with cuttings in the autumn/winter period, may be explained by combined factors such as: higher lignification of cuttings, less intense solar radiation and milder temperatures than in summer. Oliveira et al. (2012) also attribute it to the parent plant and branches physiological condition at the time of collection, because it's during this period that plants begin to store reserves in the stem, being readily available to the cuttings during periods of higher growth, reducing mortality.

In the cuttings conducted during summer/autumn, subapical cuttings of clones C8, C9, C11 and C13 had higher rooting, between 78.2% and 90.6%

(Table 3), with a highlight also observed in relation to leaf retention and survival (Table 1). The clones didn't differ regarding rooting of the apical cuttings, with an average of 45.8%. In both clones C7 and C10, rooting rates didn't differ between apical and subapical cuttings. Almost all rooted cuttings presented callus formation (Table 3).

Table 3. Percentage of apical and subapical cuttings rooted and rooted with callus of six 'Cambona 4' yerba mate clones after 120 days of the summer/autumn cutting

Clones	Rooting (%)		Rooted with callus (%)	
	Apical	Subapical	Apical	Subapical
C7	40,6 Aa	50,0 Ab	31,3 Aa	46,9 Ab
C8	37,5 Ba	84,4 Aa	37,5 Ba	81,3 Aa
C9	43,8 Ba	78,2 Aa	43,8 Aa	65,6 Aa
C10	50,0 Aa	56,3 Ab	50,0 Aa	43,8 Ab
C11	43,8 Ba	90,6 Aa	40,6 Ba	87,5 Aa
C13	59,4 Ba	84,4 Aa	59,4 Ba	84,4 Aa
Mean	45,8	74,0	43,8	68,2
C.V. (%)	25,50		29,70	

Means followed by the same capital letter in the line and small letter in the column do not differ by the Scott-Knott test at 5% probability of error.

The obtained data show that, when performing the cutting in January (summer), it's possible to get more promising answers by using subapical semi-wood cuttings, slightly more lignified. The lowest rooting rates of apical cuttings were similar to those achieved by Hettwer (2013), with the seminal genotypes that gave rise to the studied 'Cambona 4' clones, also using apical cuttings. He concluded that the use of IBA increased rooting for several genotypes and also that in any case there was damage, obtaining between 25.0% to 52.6% of rooted cuttings.

It is verified that the genetics of each plant decisively interferes in the yerba mate's rooting capacity, as observed by Brondani et al. (2009), studying substrates and rooting environment of yerba mate clones, treated cuttings with 8.000 mg/L of IBA. In the cutting of thirty genotypes in summer, Tres (2016) achieved rooting rates between 2.1% and 85.4%, also with 8.000 mg / L of IBA.

In the autumn/winter period, the rooting percentage didn't differ between the two types of cuttings and was higher in clones C7, C10, C11 and C13 (Table 4), between 59.4% and 75.0%. Almost all rooted cuttings presented callus formation, such as also observed in summer/autumn cutting period.

The unobserved difference between rooting of apical and subapical cuttings is possibly justified by the more similar degree of physiological and morphological characteristics of the two branch segments, such as: degree of lignification (juvenility), reserve accumulation, concentration of auxins and cofactors.

Making a comparison between cutting periods, it turns out that the clones responded in part differently.

In the summer/autumn cuttings, the rooting rates were superior in clones C8, C9, C11 and C13 (Table 3), but in the autumn/winter period the best ones were C7 and C10, while C8 and C9 showed lower rates and C11 and C13 remaining prominent (Table 4).

Table 4. Percentage of cuttings rooted, rooted with callus and non-rooted with callus of six 'Cambona 4' yerba mate clones after 120 days of the autumn/winter cutting

Clones	Rooting (%)	Rooted with callus (%)	Non-rooted with callus (%)
C7	59,4 a	59,4 ^{ns}	14,1 b
C8	51,6 b	48,4	18,8 b
C9	42,2 b	42,2	56,3 a
C10	60,9 a	59,4	15,6 b
C11	68,8 a	65,6	20,3 b
C13	75,0 a	73,4	1,6 b
Cuttings			
Apical	58,9 ^{ns}	57,3 ^{ns}	21,9 ^{ns}
Subapical	60,4	58,9	20,3
Mean	59,6	58,1	21,1
C.V. (%)	32,37	34,48	76,98

Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability of error. ^{ns} - not significant by the F test.

Lower rooting rates in autumn/winter, when compared to subapical cuttings in summer/autumn cuttings may be attributed to slower metabolism, which gives them less favorable physiological conditions for growth and development, as also corroborated by Nery et al. (2014). Besides, low temperatures of the rooting bed (lower air and irrigation water temperature) reduce the exchange activity and the rhythm of induction and root development. The average temperatures outside the greenhouse, respectively in the summer/autumn and autumn/winter seasons were: maximum of 24.8 °C and 20.8 °C; means of 19.2 °C and 15.1 °C; and minimum of 15.1 °C and 11.0 °C.

The lower rooting potential of yerba mate in winter was found by Stuepp et al. (2015), studying the bud cuttings from coppicing tree over 80 years old, and by Stuepp et al. (2017a), in 12 years old and over 80 year old tree canopy shoots. The 12-year-old mother cuttings showed greater rooting, with no influence of IBA, and cutting in the autumn was more favorable, followed by spring.

In another study, Stuepp et al. (2017b), testing rescue methods by coppicing and stem girdling of 17 and over 80 years mother plants, in winter and summer, found greater rooting in cuttings of epicormic shoots arising from the stem girdling of younger mother plants in the winter season (88.7%). The results found for the older mother plants were also satisfactory (47.5% to 57.1% of rooting), considering yerba mate as a hard-to-root species.

Considering that in the last month of cuttings

(July) there was already a stability in the survival rate (average of 81.0%) (Table 2), perhaps the permanence of the cuttings beyond 120 days would give better results, taking advantage of the increasing temperature period in August and September. This hypothesis is reaffirmed by analyzing the percentages of unrooted living cuttings with callus. While in the cutting of summer/autumn period no cuts were observed in this condition except C9, but at a reduced rate (10.9%), during autumn/winter the callus formation, except C13 (1.6%), ranged from 14.1% to 20.3%, with greater emphasis on C9 (56.3%) (Table 4).

Stuepp et al. (2015) and Stuepp et al. (2017a) found that there were higher rates of callus induction in winter compared to summer, and cuttings from plants older than 80 years formed more callus (Stuepp et al., 2017a). High percentage of callus cuttings (50%) was verified by Stuepp et al. (2017b) in sprouts after coppicing and stem girdling of plants older than 80 years, emphasizing that the permanence in a greenhouse for a period longer than 90 days could have increased the rooting percentage. Wendling et al. (2014), in turn, mention that high percentages of callus formation may be indicative of a higher degree of maturation of the branches, which would in part justify the higher callus formation in the autumn/winter period.

Although adventitious root and callus formation are independent processes, stemming from cell division, and dependent on environmental and internal cuttings conditions, the induction of both may or may not occur at the same time. In some species, callus formation is a precursor to adventitious root formation, while in others; excess callus can prevent rooting (Hartmann et al., 2011). According to Quadros (2009), yerba mate rooting is positively or negatively affected by the presence of callus, leaves and bud emission, depending on the material source and the rejuvenation degree of the cuttings. Callus formation in cuttings is directly linked to the endogenous auxin content available in the cut (Nery et al., 2014).

Therefore, performing cuttings in autumn/winter, although it may seem at first to be the least appropriate time to induce rooting due to less exchange activity, can be considered a good strategy for reducing cuts mortality in the first weeks of cutting, reaching the end of winter and early spring better acclimatized, in order to start or accelerate the rooting process.

Having determined the Pearson correlation coefficient between the variables of leaf retention, survival, rooting and callus rooted percentages, it was possible to confirm, in the two cutting periods that the

clones which maintained more leaves ensured longer survival ($R^2 = 0,99$ in summer/autumn and $R^2 = 0,78$ in autumn/winter) and that the callus and root formation have a high correlation ($R^2 = 0,98$ in the two periods).

Tarragó et al. (2005) verified the existence of correlation between leaf retention and survival ($R^2 = 0,72$), a fact attributed to the supply of auxin and nutrients provided by the leaves. Also Tres (2016) confirmed the positive correlation ($R^2 = 0,93$), as well as these variables with the rooting, contrary to what was found in the present study.

The positive relationship between leaf retention capacity and cuttings survival should be highlighted, since the leaves guarantee a good supply of photoassimilates, such as carbohydrates, and are sources of cofactors and auxins, as also stated by several authors (Pacheco & Franco, 2008, Hartmann et al., 2011). Quadros (2009) states that the presence of leaves was fundamental for the survival and rooting of the yerba mate. For Lima et al. (2011), leaf fall can lead to a carbohydrate deficit, as a result of insufficient stored reserves, or lack of timely to transportation and rooting effects.

The average length of the three largest roots, as well as the fresh and dry root mass, did not differ in the summer/autumn season between clones and cut types, with the largest roots presenting an average of 8,7 cm, and fresh and dried mass per cut of 0,80 g and 0,11 g, respectively (Table 5). The results were superior to those verified by Hettwer (2013) in the 'Cambona 4' seminal cuttings, concluding that the use of 6.000mg/L of IBA increased by up to 60% the length of the largest root.

Table 5. Average length of the three largest roots, fresh and dry root mass of apical and subapical cuttings of six 'Cambona 4' yerba mate clones, after 120 days of the summer/autumn cutting

Clones	Three largest roots (cm)	Fresh root mass (g)	Dry root mass (g)
C7	7,6 ^{ns}	0,81 ^{ns}	0,11 ^{ns}
C8	8,9	0,88	0,12
C9	9,7	0,75	0,10
C10	8,0	0,80	0,11
C11	9,0	0,91	0,13
C13	9,1	0,68	0,09
Cuttings			
Apical	8,9 ^{ns}	0,81 ^{ns}	0,11 ^{ns}
Subapical	8,6	0,80	0,11
Mena	8,7	0,80	0,11
C.V. (%)	11,07	27,85	30,42

^{ns} - not significant by the F test.

When cutting in autumn / winter, no significant differences were found between clones and cut types (Table 6), with the three largest roots presenting average length of 3,0 cm, fresh mass of 0,87 g and dry mass of

0,017 g per cut, values much lower than those obtained in the summer/autumn cutting.

Table 6. Average length of the three largest roots, fresh and dry root mass of apical and subapical cuttings of six 'Cambona 4' yerba mate clones, after 120 days of the autumn/winter cutting

Clones	Three largest roots (cm)	Fresh root mass (g)	Dry root mass (g)
C7	3,5 ^{ns}	0,73 ^{ns}	0,016 ^{ns}
C8	3,4	0,93	0,023
C9	1,8	0,67	0,011
C10	2,8	0,58	0,011
C11	3,3	1,03	0,016
C13	3,5	1,26	0,025
Cuttings			
Apical	2,9 ^{ns}	0,80 ^{ns}	0,016 ^{ns}
Subapical	3,2	0,93	0,018
Mean	3,1	0,87	0,017
C.V. (%)	42,27	71,41	69,57

^{ns} - not significant by the F test.

Brondani et al. (2008) state that for the production of yerba mate seedlings, the quality and vigor of the root system is essential for growing in the field. Thus, for the autumn/winter cutting, considering the satisfactory leaf retention and cuttings survival, it would be possible betting that the technique may be viable, as long as the cutting time for the months of temperature rise in southern Brazil be extended, for effective adventitious root formation to occur. Another advantage of the autumn/winter would be the possibility to use, with higher survival rate, both apical and subapical cuttings, increasing cuttings yield per mother plant.

The results show that the combination of procedures, such as the cutting period, application of IBA in lower concentration and in the form of talc, and also the use of clonal mother plants, may favor better results. It is necessary to emphasize that the rescue of mother plants, being the first step, will usually start from seed genotypes, native or not, often not being possible to obtain higher rooting rates.

Conclusions

The conclusions of this work were that: the cutting period influences the response of each clone, but for most of them the rooting is higher when performed in summer/autumn, with emphasis on C8, C9, C11 and C13; in autumn/winter the rooting rate is lower, keeping C11 and C13 with a better response, in addition to C7 and C10; in the summer/autumn cutting period, subapical cuttings have greater survival and rooting than the apical ones, but they don't differ when performed in autumn/winter, which makes it possible to collect a greater number of cuttings per plant; and that leaf retention positively influences survival.

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