Seed cassava cuttings production: alternative use of growing substrates

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ABSTRACT

This experiment was realized to improve the rapid propagation technique in different substrates applied to cassava crops in order to multiply traditional genotypes based on selected characteristics. The first stage the study was carried out in September 2016 in open air beds covered with transparent plastic. Four traditional cassava genotypes and one cassava cultivar (Fepagro RS13) were tested. Measurements of air and soil temperatures were carried out throughout the experiment until more than half of the planted manures were sprouted. Measurements stopped when most plantlets had sprouted. The second stage of the experiment was carried out in November 2016 (cutting in different substrates) when plants presented four fully expanded leaves. Shoots were cut and transplanted to individual containers with four different substrates (water, sand, soil and commercial substrate). After transplanting, seedlings were stored in greenhouse, under controlled temperature and irrigation. Leaves, stem and root dry and fresh masses,

and the size of the three longest roots per plant, were evaluated 40 days after the start of the experiment. Cultivars Fepagro RS13 and SJ13 stood out among the assessed varieties, because they presented good potential for rapid multiplication. Commercial substrates can be an alternative for rapid propagation.
***Keywords**: Manihot esculenta* Crantz; stem cutting; multiplication; substrate.

1. INTRODUCTION

Manihot esculenta Crantz is relevant for the world economy (Schons *et al*., 2009), since its tuberous roots have high carbohydrate and protein contents, which turn it into an option for animal feed (Machado *et al*., 2016). The species is widely cultivated in African and Latin American tropics as the main source of energy for millions of people (Gárcia-Segovia *et al*., 2016). According to Ribeiro *et al*. (2012). This plant adapts to different environments; however, its exposure to several biotic and abiotic stresses ~~that~~ can negatively influence its growth, development and yield.

The rapid propagation method developed by the International Center for Tropical Agriculture (CIAT) in Colombia is inexpensive and simple. The method can increase cassava multiplication rates up to 100 times (Silva *et al*., 2002). Seedlings presenting better physiological and sanitary quality show multiplication increase in the short-term, since stem cutting have two to three buds, whereas the traditional cultivation have approximately seven buds. Different from traditional multiplication in the field, which generates at most four stems, sprouting buds subjected to rapid multiplication re-sprout, depending on the environmental propagation conditions.

The rooting substrate is a determining factor for seedling development, since it is the first nutrient source; therefore, some change in its composition can influence plant formation. Substrate works as structural support for plants, besides providing water and nutrients (Fermino *et al*., 2010). Substrate type, irrigation, proper fertilization and production management provide the conditions to generate high quality plants in the field (Costa *et al*., 2012). Seedlings presenting appropriate quality standards show favorable growth and competition conditions for environmental factors such as water, light and nutrients (Caron *et al*., 2010).

The aim of this study was to analyze the growth and development processes of four cassava accessions and Fepagro cultivars rescued in rural properties and through fast propagation technique adaptation using different substrates.

2. MATERIALS AND METHODS

The research started by rescuing cassava accessions in rural properties in Alto Jacuí Region, after the implementation of a Germoplasm bank. Two experiments (stage 1 and stage 2) were installed after the branches were collected in the Unicruz University germoplasm bank.

Stage 1 – The stage one was conducted in open masonry beds covered with a transparent plastic structure (protection tunnel against weather changes and insect visitations). Experimentation in masonry beds (tunnel) started on September 21, 2016. Eighty (80) stem cutting from 4 cassava accession tables (FV13, SJ 08, SJ10, XV04) and one cultivar (Fepagro RS13) were planted. The five treatments were composed of the four accessions and ~~one~~ cassava cultivar, planted in two beds, considered as two

replications and each replication consisted of 40 stems cutting. Segments containing only two buds were cut with manual saws, weighed and planted approximately 3 cm down the ground, spaced 20 cm from each other. The number of shoots and their relations with the weight of the stem cutting were evaluated at the end of the experiment.

Minimum and maximum daily air temperatures inside the tunnel were read with digital thermometer. Soil temperature was measured at 09:00 am, 12:00 pm and 03:00 pm, with digital thermometer installed 5cm down the ground, when 51% of planted cassavas had sprouted. The second stage of the research started after shoots reached height higher than 15 cm in order to perform the rooting phase, which corresponded to stage 2.

Stage 2 –The stage two was carried out on November 10, 2016 - 50 days after stage 1 was implemented. The experiment was conducted in greenhouse and followed a completely randomized design, based on 4 x 5 factorial arrangement. Factor "A" referred to four different treatments: 1) Substrate commercial Plantmax ®, 2) soil, 3) sand 4) water (control), and Factor "B" accession: 1) FV13, 2) SJ 08, 3) Fepagro RS13, 4) SJ10 and 5) XV04.

The sample counted on four cassava accessions and one cultivar, thus totaling 20 treatments, with 10 replications, each one composed of 1 (sprout) cutting. Shoots were cut with blade sterilized in alcohol. Forty (40) shoots from each accession were used in the experiments. These shoots were immediately placed in water to avoid losing sprouting turgescence. Subsequently, they were placed in seedling packages (dimension 15 x 20 x 0.10 cm) with 1 kg of substrate for rooting. Different substrates were used in the experiment. Samples were stored in greenhouse at 25ºC under controlled irrigation.

Weekly evaluations were performed throughout this period by analyzing cuttings' survival rates and the total number of leaves. It was done in order to seek knowledge about the most appropriate substrates for shoot development. The final evaluation was carried out 40 days after the second stage was installed. It was done by assessing leaves, stem and root fresh and dry masses, as well as the size of the three longest roots per plant.

Data normality in all experiments was assessed through Komogorov-Smirnov test and variance homogeneity was calculated through Bartlett test. Data were subjected to Analysis of Variance, and the mean values of the characteristics evaluated in the cassava accessions were grouped by means of Scott Knott test, at 5% error probability, when "F" was significant.

3. RESULTS AND DISCUSSION

Stage 1

The accessions FV 13 and SJ 08 recorded the largest number of shoots, (1.1 and 1.0 shoots per plant, respectively). They were followed by Fepagro RS13 (0.71 shoots). The smallest number of shoots obtained for accessions SJ10 and XV04 (Table 1). With regard to stem cutting mass, FV13, XV04, Fepagro RS13 and SJ10 recorded the highest values (Table 1). Data in the present research evidence that stem cutting mass influences the number of shoots; therefore, accessions recording the highest masses were the ones presenting the best shoot emissions, which can be related to the greatest stem cutting reserve. Besides

the inherent characteristics of each genotype, the difference in the number of roots may be related to the vigor of the planted seed cassava cuttings associated with its origin and the capacity of root formation.

Table 1: Number of shoots and stem cutting mass (g) of different cassava accessions for seedling formation.

Accession/Cultivar	Features	
	Number of shoots**	Stem cutting mass (g)
FV13	1.12 a	17.00*a
SJ 08	1.00 a	17.00 a
Fepagro RS13	0.71 b	18.37 a
SJ10	0.42 c	16.75 a
XV04	0.29 c	9.87 b
CV (%)	16.00	17.82

* means not followed by the same lowercase letter in the columns differed from each other in the Scott Knott test at 5% probability level.

Differences were verified between the genetic materials used in relation to the weight of the stem cutting (Table 1), which may influence the development of the seedlings and it could compromise the production. It is noteworthy that there are no studies in the literature related to the production of cassava seedlings from stem cutting. Different results recorded for different cassava cultivars were also observed in other studies with other cultivars. Rós Golla *et al*. (2010) found differences to the number of leaves and root diameter of different cassava varieties. Silva *et al*. (2011) concluded that, overall, cultivars with larger stem diameters (25 to 30 mm) present the best performance.

The Figure 1 depicts the mean air and soil temperature during the experimental period. One-hundred forty-four (144) soil temperature and air temperature records were registered throughout the assessed period (from September 21, 2016 to October 10, 2016). Average soil temperature and average air temperature were 26.18 ºC and 23.60 ºC, respectively. According to Alves (2006), environmental factors such as temperature and photoperiod affect cassava growth and development. Air temperature affects sprout budding, formation, size and the useful life of plant leaves. Plant growth benefits from mean annual temperature variation from 25 ºC to 29 ºC and the plants have the ability to tolerate temperature variation from 16 ºC to 38 ºC.

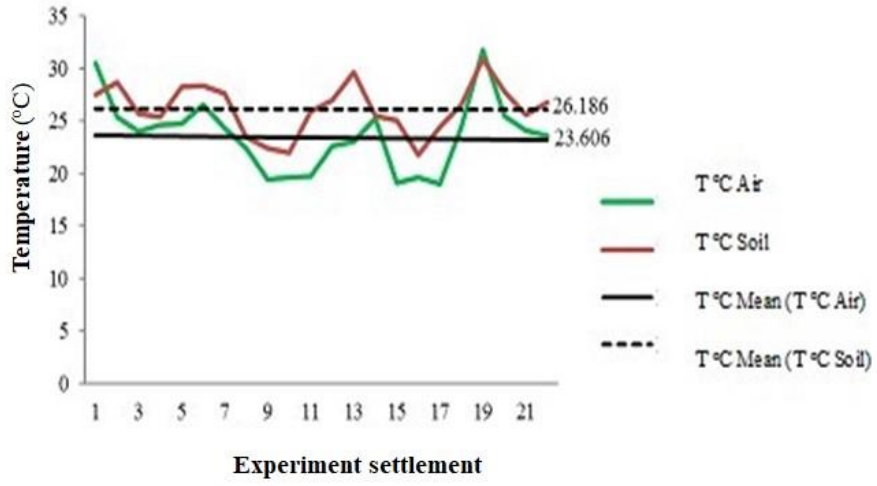

Figure 1: Air and soil temperature from 09/21/2016 to 10/12/2016 in the study site.

Results as shown in Figure 1 the average soil temperature was 26.1 °C during the experiment, and it is worth to note that our results comply with results recorded by El-Shakawy, (2004). According to these author', sprouting buds in stem cutting benefit from soil temperature from 28 to 30 °C. However, sprouting is interrupted at temperatures above 37 °C and below 17 °C.

Stage 2

For the plant height variable, no interaction between accession/cultivar and substrates was obtained. When the individual factors were analyzed, for plant height, accessions SJ08 and cultivar Fepagro RS13 showed the greatest values for plant heights. Regarding cultivations, water was superior to the other treatments, showing a height of 11.32 cm (Table 2). Water can be a good alternative for rooting of cassava seedlings.

Table 2. Plant height (cm) in different system of cultivate with cassava accessions.

Plant height (cm)			
Accession/Cultivar		Cultivation	
SJ08	10.40 a*	Water	11.32 a
FepagroRS13	9.60 a	Sand	8.16 b
FV13	8.35 b	Substrate	8.00 b
XV04	7.95 b	Soil	7.84 b
SJ10	7.85 b		
CV (%)		16.21	

* means not followed by the same lowercase letter in the columns differed from each other in the Scott Knott test at 5% probability level.

In relation to cultivar x substrate, accessions FV13, SJ08 and cultivar Fepagro RS13 had the highest survival rate in water. In all the other substrates, all accessions had excellent performance, with no significant differences among them (Table 3). The variable number of leaves showed interaction between accessions/cultivar and substrates. For cultivar Fepagro RS13, the largest number of leaves was observed in the commercial substrate, sand and water crops, which were superior to the soil. For the FV13 accession, the best result was obtained in the sand, followed by commercial substrate and soil, all of them superior to water. For accession SJ10, the soil and commercial substrate were superior to water and sand. For SJ08 accession, the best results were obtained for cultivation in water and commercial substrate, which were superior to sand and soil, and for accession XV04, the water was superior followed by substrate. The lowest results were found in sand and soil (Table 3).

Table 3: Survival (%), number of leaves, plant height, length of the three largest ~~h~~accession roots / cassava cultivar in different culture media.

Survival (%)				
	Cultivation			
Accession/Cultivar	Water	Sand	Soil	Substrate
Fepagro RS13	100 aA*	100 aA	100 aA	100 aA
FV13	100 aA	100 aA	100 aA	100 aA
SJ10	60 cB	100 aA	100 aA	100 aA
SJ08	100 aA	100 aA	90 aA	100 aA
XV04	80 bB	100 aA	90 aB	100 aA
CV (%)		17.33		
Number of leaves				
	Cultivation			
Accession/Cultivar	Water	Sand	Soil	Substrate
Fepagro RS13	7.00 cA	7.20 bA	6.00 bB	7.20 bA
FV13	6.00 dC	7.80 aA	6.80 aB	7.00 bB
SJ10	6.00 dB	6.00 cB	7.00 aA	7.20 bA
SJ08	8.00 aA	7.00 bB	6.80 aB	7.80 aA
XV04	7.60 bA	6.00 cC	6.00 bC	7.00 bB
CV (%)		4.24		
Plant height (cm)				
	Cultivation			
Accession/Cultivar	Water	Sand	Soil	Substrate
Fepagro RS13	11.30 aA	13.20 aA	13.00 aA	12.00 aA
FV13	9.00 aB	9.90 bB	11.80 aA	13.50 aA
SJ10	5.00 cC	9.00 bB	11.60 aB	14.70 aA
SJ08	12.50 aA	12.80 aA	13.60 aA	14.90 aA

XV04	7.10 bB	8.90 bB	9.40 aB	12.90 aA
CV (%)		17.05		

Mean length of the three longest roots (cm)

	Cultivation			
Accession/Cultivar	Water	Sand	Soil	Substrate
Fepagro RS13	3.70 bB	14.30 aA	12.50 aA	17.10 Aa
FV13	2.80 bC	9.30 bB	10.40 aB	14.90 aA
SJ10	2.60 bB	10.30 bA	13.10 aA	14.50 aA
SJ08	5.90 aC	10.90 bB	10.00 aB	16.00 aA
XV04	3.40 bC	9.40 bA	7.20 bB	12.40 aA
CV (%)		19.04		

* Means followed by the same lowercase letter in the columns and uppercase in the row do not differ from each other by the Scott-Knott test at 5%.

The comparison between number of leaves in each substrate evidenced that accession SJ08 recorded the best results in water, which was followed by XV04, Fepagro RS13 - the worst results were recorded for accessions FV13 and SJ10. The largest number of leaves was recorded for accession FV13 in sand, which was followed by cultivar Fepagro RS13 and accession SJ08 - the lowest values obtained for SJ10 and XV04. FV13, SJ10 and SJ08 had better results than Fepagro RS13 and XV04 in soil. Accession SJ08 showed larger number of leaves than other varieties in commercial substrate (Table 3).

The highest plant-height values obtained in commercial substrate. All culture media behaved similarly for cultivar Fepagro RS13 and accession SJ08, did not significantly differ from each other. Commercial substrate and soil were the best substrates for accession FV13, which were followed by sand and water. The commercial substrate was better for accession XV04 than the other ones (Table 3).

For the variable mean length of the three longest roots, the commercial substrate was the best treatment. The data are similar to those obtained for plant height. Comercial substrate, soil and sand were superior to water in cultivar Fepagro RS13 and in the SJ10 accession. For FV13 and SJ08, commercial substrate and soil were superior to sand and the lowest values were obtained for water. For accession XV04, commercial substrate and sand were the best treatments followed by soil. However, the lowest values were obtained in water.

Viana *et al*. (2001) conducted a study under field conditions and they found that the stem cutting size effect on the number of shoots per plant is linear. High stem cutting mass can be associated with longer stem cutting length; therefore, heavier stem cutting stem cutting tend to produce a larger number of shoots corroborating with Rós-Golla *et al*. (2010) that observed different plant height in the same cassava cultivar; overall, greater diameters generate larger plants.

For fresh mass of cassava roots, it was observed that the commercial substrate showed the highest values (Table 4). Regarding root dry mass, the highest values for all accessions were also obtained in

commercial substrate cultivation, and the lowest values were found in water. Based on the results of this study, it can be inferred that in the methodology proposed by Silva *et al.* (2002), who developed the method of rapid propagation, the authors describe that this method allows an increase of up to 100 times in multiplication rate of cassava using water for the rooting process and that after rooting, the seedling should be placed in the commercial substrate. The results obtained in this work allows to observed that it is evident that the process of passage through water is unnecessary since the seedling can be passed directly to the commercial substrate, already that it shows a high cost in in time and labor.

Table 4: Fresh and dry mass (g) of cassava accessions / cultivars in different culture media.

Accession/Cultivar	Root fresh mass (g) Cultivation			
	Water	Sand	Soil	Substrate
Fepagro RS13	1.00 aC	3.50 aB	3.10 aB	5.10 aA
FV13	1.50 aD	4.10 aB	3.20 aC	6.70 aA
SJ10	0.90 aC	3.40 aB	3.50 aB	6.30 aA
SJ08	2.00 aC	4.30 aB	3.30 aB	7.90 aA
XV04	1.60 aA	1.60 bA	1.30 bA	2.40 bA
CV (%)	23.69			
Accesso/Cultivar	Root dry mass (g) Cultivation			
	Water	Sand	Soil	Substrate
Fepagro RS13	0.00 aB	0.40 aA	0.50 aA	0.60 bA
FV13	0.00 aB	0.60 aA	0.60 aA	0.90 aA
SJ10	0.00 aC	0.40 aB	0.60 aA	0.90 aA
SJ08	0.00 aC	0.50 aB	0.40 aB	1.30 aA
XV04	0.00 aA	0.00 bA	0.20 aA	0.10 cA
CV (%)	22.69			

* means not followed by the same lowercase letters in the columns and by the same uppercase letters on the lines differed from each other in the Scott Knott test at 5% probability level.

The Table 5 shows no accession/cultivar and substrate and, accession/cultivar substrate interactions in leaf fresh and dry masses - accession XV04 presented the lowest leaf fresh and dry mass. Growth was higher in commercial substrate, sand and soil than in water. The total fresh mass of all accessions and cultivar Fepagro RS13 were higher than that recorded for accession XV04; its cultivation in commercial substrate stood out, since it recorded better results than all other (Table 6). Fepagro RS13, FV13, SJ08 recorded higher total dry mass than SJ10 and XV04; the commercial substrate was better than sand and soil in these cultures. SJ10 and XV04did not significantly differ from each other.

Table 5: Fresh and dry mass (g) of cassava accessions / cultivars in different culture media.

Leaf fresh mass (g)			
Cultivar		Cultivation	
Fepagro RS13	2.57* a	Substrate	2.86 a
FV13	2.40 a	Sand	2.50 a
SJ10	2.40 a	Soil	2.64 a
SJ08	2.85 a	Water	1.60 b
XV04	1.77 b		
CV (%)		24.22	
Leaf dry mass (g)			
Cultivar		Cultivation	
Fepagro RS13	0.77 a	Substrate	0.80 a
FV13	0.75 a	Sand	0.70 a
SJ10	0.57 b	Soil	0.72 a
SJ08	0.82 a	Water	0.44 b
XV04	0.40 b		
CV (%)		24.75	

* means not followed by the same letter differed from each other in the Scott Knott test at 5% probability level.

Table 6: Fresh and total dry mass (g) of cassava accessions / cultivars in different culture media.

Total fresh mass (g)			
Cultivar		Cultivation	
Fepagro RS13	7.30 a	Substrate	10.26 a
FV13	7.40 a	Sand	7.04 b
SJ10	7.10 a	Soil	6.86 b
SJ08	8.60 a	Water	3.62 c
XV04	4.32 b		
CV (%)		25.61	
Total dry mass (g)			
Cultivar		Cultivation	
Fepagro RS13	1.62 a	Substrate	1.98 a
FV13	1.55 a	Sand	1.56 b
SJ10	1.40 b	Soil	1.58 b
SJ08	1.82 a	Water	0.80 c
XV04	1.00 b		
CV (%)		23.04	

* means not followed by the same letter differed from each other in the Scott Knott test at 5% probability level.

Stem fresh mass of accessions FV13, SJ10 and SJ08, and cultivar Fejagro RS13 was higher than the values obtained for accession XV04 grown in commercial substrate, which were followed by results observed in sand and (Table 7). There was no stem dry mass difference between stratum accessions, all results were better than values observed in water.

Table 7: Fresh and dry mass (g) of cassava accession / cultivar stem in different culture media.

Stem fresh mass (g)			
Cultivar		Cultivation	
Fepagro RS13	1.50 a	Substrate	1.72 a
FV13	1.22 a	Sand	1.22 b
SJ10	1.22 a	Soil	1.40 b
SJ08	1.47 a	Water	0.70 c
XV04	0.87 b		
CV (%)		24.60	
		Stem dry mass (g)	
Cultivar		Cultivation	
Fepagro RS13	0.22^{ns}	Substrate	0.26 a
FV13	0.05	Sand	0.18 a
SJ10	0.22	Soil	0.22 a
SJ08	0.27	Water	0.06 b
XV04	0.12		
CV (%)		24.11	

* means not followed by the same letter differed from each other in the Scott Knott test at 5% probability level.

The highest root/shoot ratio values obtained for SJ08 and XV04, which were followed by SJ10, FV13 and Fepagro RS13. Water and soil were the best culture media, but they were followed by sand and substrate (Table 8). There was no statistical difference in leaf area between accessions, since substrate, sand and soil were better than water in the crops.

Table 7: Root / shoot ratio and leaf area of cassava accessions / cultivars in different culture media.

Root / shoot ratio/(g)			
Cultivar		Cultivation	
FepagroEPAGRO RS13	2.60 b	Substrate	1.75 b
FV13	2.35 b	Sand	2.18 b
SJ10	2.00 b	Soil	3.02 a
SJ08	2.87 a	Water	3.62 a
XV04	3.35 a		
CV (%)		25.57	
		Leaf area cm^2	
Cultivar		Cultivation	

Fepagro RS13	139.42 a	Substrate	142.93 a
FV13	133.08 a	Sand	124.47 a
SJ10	139.75 a	Soil	140.47 a
SJ08	125.58 a	Water	96.00 b
XV04	92.00 a		
CV (%)		28.70	

* means not followed by the same letter differed from each other in the Scott Knott test at 5% probability level.

Seed cassava cuttings remained in greenhouse under intermittent misting, which had positive influence on their development. This outcome corroborated the results recorded by Fachinello *et al*. (1994), who state that intermittent nebulization provides lower moisture reduction due to water-film formation on the leaves and to temperature decrease. Seed cassava cuttings were stored under the aforementioned conditions, which proved to favor seedling development.

S and density (Kämpf, 2000) favors cuttings' support, which is an important characteristic of substrates used for rooting, the same author recommends that extremely porous substrates for the propagation of cuttings presenting low water retention ability. Oliveira *et al*. (2001) recommend avoiding excessive moisture in substrates favoring the emergence of fungal diseases that affect cuttings' rooting. Accordingly, it can be inferred that cuttings laid in sand are more stable; the high drainage ability of this substrate provides better conditions for mini-ixora rooting (Ixora coccinea 'Compacta') under the herein assessed experimental conditions. Similar results were recorded for cassava, because sand recorded good results for some of the assessed variables.

According to Garay *et al*. (2014), there is no ideal substrate, the choice for a good material depends on crop features and on purchasing costs; therefore, it is necessary testing different substrates or substrate mixtures for each species.

4. CONCLUSIONS

The present results validate the potential of rapid cassava multiplication strategy in comparison to the conventional multiplication technique. Cultivar Fepagro RS13 and accession SJ13 stood out among other varieties, because they have good potential for rapid multiplication; therefore, they can be used by farmers who aim at producing stem cutting

The commercial substrate can be an alternative for rapid cassava propagation.

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