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MICROPROPAGATION OF FOUR VARIETIES OF CASSAVA (MANIHOT SCULENTA CRANTZ) WITH EXPORT AND INDUSTRIAL POTENTIAL IN THE DOMINICAN REPUBLIC

Héctor Rafael Peralta Corona, Julio Bolívar Mejía Brea Rosario, Carmen María de Jesús, Manuel Díaz Román, Daniel Martínez Tejada, Roosevelt Humberto Escobar Pérez

Institute for Innovation in Biotechnology and Industry (IIBI). P.O. Box 329-2. Santo Domingo. Dominican Republic
Ministry of Higher Education, Science and Technology (MESCYT) / National Fund for Innovation in Scientific and Technological Development (FONDOCYT).

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ABSTRACT

Cassava (Manihot sculenta Crantz) has been a source of carbohydrates for low-income sectors of the population worldwide. Cassava is implicit in food security and sovereignty. The limitations of this crop to produce it are the lack of healthy material for planting and low yield levels. Cuttings from conventional cassava plantings are infested with pathogens and these are difficult to eliminate with existing procedures. This affects growth and yield. The massive in vitro multiplication of cassava contributes to the food security of Dominicans and the recovery of export markets. In vitro cassava plants were obtained to supply producers with high quality and yield varieties. In addition to being used in agro-industry, strengthening exports and satisfying the demand for material, it is important that the extraction of healthy material exceeds the traditional supply. For this reason, in vitro culture techniques are playing an important role in the maintenance of collections, sanitation and rejuvenation of the material whose yields are tripled when using in vitro cassava plants.

Keywords: Organogenesis, Temporary Immersion System, In vitro Conservation, Micropropagation.

1. INTRODUCTION

Agrobiodiversity is exposed daily to the following factors: (1) climate change, (2) the increase in demand for natural resources, (3) mining, (4) exploitation of hydrocarbons, (5) expansion of the border agriculture, (6) industrial pollution, (7) deforestation, loss of genetic diversity and (8) soil erosion as well as agro-ecosystems (Sans, 2007; González and Martín, 2011; Mohan, 2011; Reed et al: 2011) cited by Bonilla et al: (2015). Agrobiodiversity is important for its present and future development, growth and permanence; and for this reason, the world strategy for plant conservation, signed by more than 180 countries in 2002. It is proposed to stop this loss through the understanding and conservation of plant diversity, and the promotion of sustainable use through conservation techniques in field and laboratory (Mohan, 2011; Reed et al: 2011) cited by Bonilla et al: (2015).

Cassava is native to South America where its domestication dates back 5,000 years. This is extensively cultivated in tropical and subtropical areas, specifically at latitudes below 30º and altitudes ranging from sea level to 1800 meters above sea level. (Ceballos 2002). The importance

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of cassava cultivation lies in the diversity of its uses, since its roots and leaves can be used for human or animal consumption. Likewise, starch and alcohol can be obtained from cassava that can be used in industry (Ceballos 2002).

In the Dominican Republic, cassava is grown on marginal soils by low-income farmers. The limitations of the crop are the lack of material or healthy seed and, therefore, low levels of yield. Because sexual seed confers great genetic variability to plants, farmers have perpetuated the crop through vegetative propagation using asexual seed (cuttings) in repeated plantings. The asexual seed is obtained by cutting 20 cm long stem sections with several buds, from selected crop plants. The selection of cuttings and their storage prior to a new planting cycle influence crop yields, so not all planted cuttings sprout, causing losses and the use of cuttings infected with pathogens, difficult to eliminate with the procedures. existing. This affects growth and consequently the yield obtained by producers.

Vegetative propagation ensures the conservation of varietal characteristics during successive generations, which represents an advantage for this crop. However, the multiplication rates are low and the cultivation of cassava by cuttings constitutes a means of dissemination and transmission of pests and diseases, mainly viruses and mycoplasmas (Roca and Jayasinghe, 1982) cited by Bonilla et al et al; (2015). The massive *in vitro* multiplication of cassava would increase the national yield of the crop, would contribute to the food security of Dominicans and the recovery of export markets for the crop.

Tissue culture is a method of propagation that begins with the isolation of a part of the plant (cell, tissue or organ) to be grown in an aseptic nutrient medium. The culture was carried out under controlled conditions of light, temperature and humidity, until obtaining or regenerating a new individual. Rapid clonal multiplication of plants brings disease-free plants in a sterile and controlled environment, independent of the time of year and with a limited space requirement. In cassava, the *in vitro* culture of meristems has allowed the propagation of virus-free plants and any other systemic pathogen, which facilitates the international exchange of germplasm. On the other hand, the cultivation of nodal segments of the stem allows the rapid multiplication of easily acclimatized plants. At the same time, it contributes to the *in vitro* conservation of different varieties for an indefinite time, in a limited space with less exposure to natural disasters and easy multiplication when required (Ashmore, 1997) cited by Bonilla et al; (2015). The general objective of this research was to micropropagate four varieties of Cassava (*Manihot sculenta* Crantz) with export and industrial potential in the Dominican Republic.

2. MATERIALS AND METHODS

2.1 Location of the Investigation

This research was carried out over a period of three years in the tissue culture laboratory of the Center for Plant Biotechnology (CEBIVE) of the Institute for Innovation in Biotechnology and Industry (IIBI), Santo Domingo, Dominican Republic. It is a project for the *in vitro* production of roots and tubers, the product of an agreement between the IIBI and the Dominican Agrarian Institute (IAD). Within the IIBI and IAD agreement, the latter was considered committing to acquire the production of said project. The goal in the present work establishes that after three (3) years of work, there will be a sufficient number of *in vitro* cassava plants. These will be

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acquired by the IAD to be planted in experimental plots of 50 tasks (3.13 ha) each for the production of healthy and rejuvenated cassava material (cuttings). In addition, the IAD is committed to supply cassava producers for fresh consumption, export and the cassava industry in Cibao and the Northeast Line of the country. *In vitro* laboratory conservation of the four cassava varieties is also considered.

2.2 Plant Material

Four cassava varieties (Valencia, Lima 40, Perla 83 and Señorita) were obtained from the North Research Center of the Dominican Institute of Agricultural and Forestry Research (IDIAF) in La Vega from the International Center for Tropical Agriculture (CIAT) of Colombia for the experiments and mass propagation.

2.3 Organogenesis Process

In the laboratory, for the organogenesis process, the material was disinfected using the following process. The apices were washed with deionized water and liquid soap, disinfected with 70% v/v isopropyl alcohol for 30 seconds, and introduced into a 20% Sodium Hypochlorite solution (5.25% v/v of active ingredient). In the laminar flow chamber, the stipules were removed until a 1 mm apex was available, which was cut and quickly transferred to the flask with culture medium. Seeding was done in vials with 10 ml of MS medium (Murashige and Skoog, 1962) supplemented with: sucrose (20 g/L), Thiamine. HCl (0.4 mg/L), m-Inositol (100 mg/L), Benzylaminopurine (BAP) (0.05 mg/L), Gibberellic Acid (GA) (0.05 mg/L), Naphthaleneacetic Acid (ANA) (0.02 mg/L), agar (7 g/L), at pH 5.8. From this medium, the concentrations of 6-BAP and ANA were varied for Medium 1 and Medium 2. M1= (BAP 0.04 mg/L + ANA 0.02 mg/L). M2= (1.0mg/L + ANA 0.05mg/L). The explants were kept in a growth chamber with temperatures that fluctuated between 27 and 30 ºC and 16 hours of artificial light (Laynez and Sánchez, 2006).

2.4 Temporary Immersion System.

The protocol used by Medero et al will be used; (2015) in the Production of Planting Material of high Genetic and Phytosanitary Quality in Cassava. In this, the culture medium formed by the "MS" salts supplemented with 0.13 mg/L of Thiamine and 0.01 mg/L of Naphthalene Acetic Acid (ANA) was used for the multiplication of the explants in a Temporary Immersion System. A frequency of immersion every six hours (four immersions per day) and an immersion time of 2 minutes was used, taking into account the results described for other cultures in these systems at a frequency of every six hours. The volume of medium to be used was 250 / bottle. 50 explants per flask were used. The evaluations were carried out at 30 days of culture and the explant length, number of internodes per explant, number of active leaves and multiplication coefficient were evaluated.

2.5 Conservation

Nodal segments of cassava seedlings established in vitro were used for conservation according to the protocol used by Rayas et al; (2002). The MS culture medium (Murashige and Skoog, 1962) was used, supplemented with 0.02 g/L of 6-Benzylaminopurine (BAP), 0.1 g/L of Gibberellic Acid (GA3) and 0.01 g/L of Naphthalene Acetic Acid (ANA) and 40 g/L) of sucrose and 10 g/L of mannitol. One explant per test tube and three repetitions were used. The test tubes were kept

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at 25 °C. The evaluations were carried out nine months after *in vitro* implantation, taking into account: height (cm), number of internodes per plant, number of active leaves and survival percentage. The explants that survived, after conservation, were incubated for their recovery in the culture medium described for the *in vitro* growth of cassava.

2.6 Acclimatization and Planting in Nursery Conditions

This process, which is essential to achieve the highest percentage of plant survival and this depends on multiple factors: (1) the quality of the plant, (2) the skill in handling the material and (3) the cultivation conditions, among other. For optimal acclimatization, the next steps to follow were the following: trays were filled with sterile sand-earth substrate (3:2). Then, 5 ml of N-P-K fertilizer solution were added to the substrate, and the roots of the plants were washed to eliminate the culture medium, with enough distilled water. The vitroplants 5 cm tall were placed on the substrate. 5 ml of fertilizer solution was added. The trays with the plant were placed in a humid microchamber, formed with a transparent plastic bag. They were closed well to prevent the escape of water vapor. They were placed under shade in the greenhouse (Aguilar et al; 2016).

2.7 Planting in the Field

The regional banks were the cassava seed multiplication and management centers (experimental plots), where the genetic material (vitro plants) was sown with the aim of producing cuttings to be transferred to the farmers of each region, who began the process of cassava production. The plants were transferred in the trays to the experimental field. Planting was carried out in furrows with a separation of 1 m and a distance between plants of 0.80 m. The plants were removed from the trays, placed in the hole and the roots were covered with sufficient soil, verifying that the plant remained upright. Irrigation was carried out at the end of sowing and later once a week depending on the sowing season.

2.8 Experimental Design

For the organogenesis experiment, a complete randomized design was used with a 4 x 2 x 10 factorial arrangement (4 varieties x 2 Means x 10 replicates) with a total of 80 experimental units. In the Automated Temporary Immersion System, immersion frequencies of 3 min every 3 hours were applied for a period of six to eight months. In it, there was growth at a temperature of 25–30 0C, photoperiod of 11 or 12 hours per day under fluorescent white light. For the Conservation of the four cassava varieties, nodal segments of seedlings established in vitro were used according to the protocol used by Rayas et al; (2002). The MS culture medium (Murashige and Skoog, 1962) was used, supplemented with 0.02 g/L of 6-Benzylaminopurine (BAP), 0.1 g/L of Gibberellic Acid (GA3) and 0.01 g/L of Naphthalene Acetic Acid (ANA) and 40 g/L) of sucrose and 10 g/L of mannitol.

2.9 Statistical Analysis

The data from the experiments were processed statistically represented by the mean ± SD. The statistical analysis is analyzed by variance and its multiple comparison according to Duncan with a significance level of $p<0.05$. InFoStat Version 12 statistical software was used for all trials.

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3. RESULTS AND DISCUSSION

Tissue culture is a technique used for plant micropropagation. In cassava (*Manihot sculenta* Crantz) cultivation, plant micropropagation has been successfully applied to obtain cassava vitro plants. This technology allows the mass production of seedlings free of pests and pathogens, thus increasing their productivity and, in certain cases, their longevity. In relation to cassava, in vitro micropropagation is used to produce pathogen-free seedlings, such as toad skin, cassava mosaic and bacteriosis, among others. This micropropagation can be carried out by the traditional means, with low multiplication rates, or improved through more efficient multiplication systems. An example thereof is: the Automated Temporary Immersion Vessel (RITA) and the Automated Temporary Immersion System (SITA) (Segovia et al; 2015). In this study, micropropagation of four varieties of cassava (Manihot sculenta Crantz) was used using two culture media.

3.1 Explant Length

The length of the plants is an important variable in micropropagation, since taller plants have a greater number of nodes, cutting is easier and more explants are obtained for subsequent propagation. The analysis of variance was carried out and highly significant differences ($p<0.05$) were found between the culture media, but not between the cassava varieties in the different variables evaluated. According to Duncan's test Medium 2 (1.0 mg/L + ANA 0.05mg/L). it reached the greatest length of the explants with 6.24 cm, while the lowest value of the explant length was obtained with medium 1 of 2.38 cm (BAP 0.04 mg/L + ANA 0.02) (Graph 3.1).

Yandia et al; (2018) reported an average height of 5.28 cm in the Yalipe cassava cultivar grown in MS + ANA (0.02 mg /L) + BAP (0.05 mg /L) + GA3 (0.02 mg /L). Mehmood et al. (2016) observed that the greatest lengths in plants of two potato varieties (10.3 cm and 10 cm) were obtained when applying 0.25 and 0.5 mg /L of GA3 to the culture medium, but higher concentrations produced seedlings. weak and tender.

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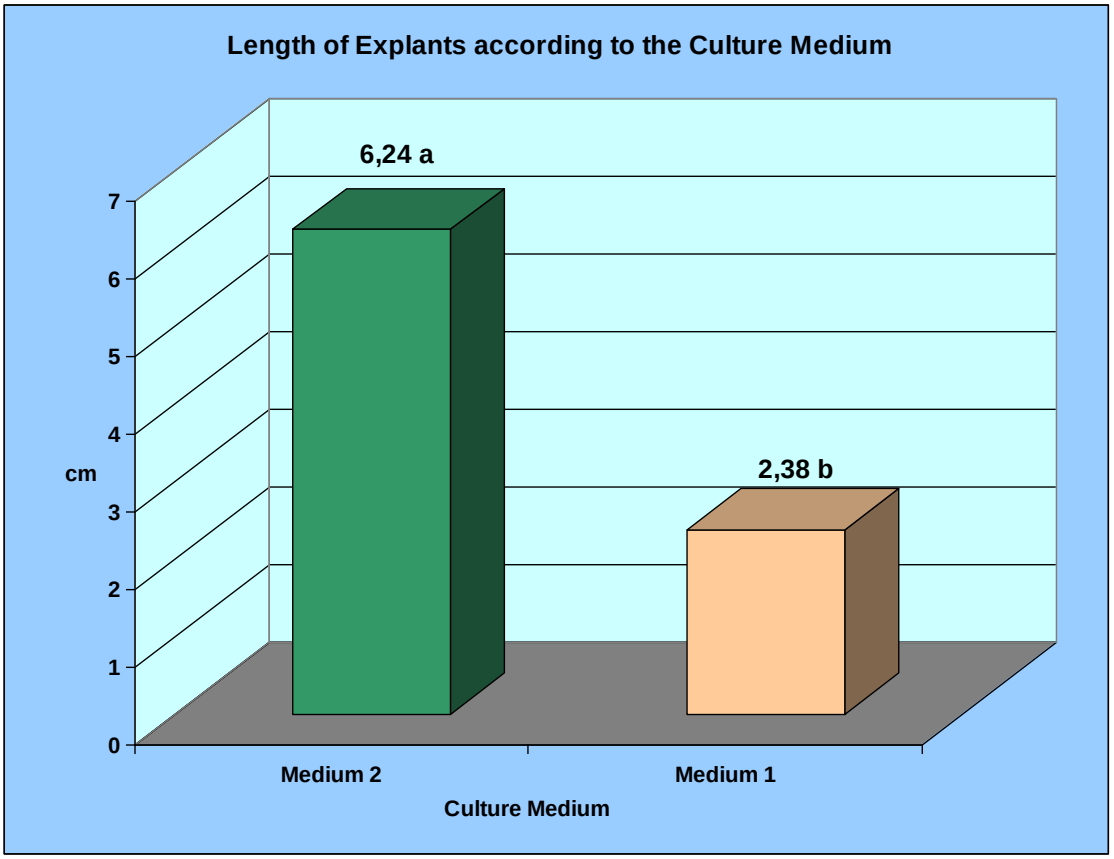

Graph 3.1: Length of the Explants according to the Organogenesis Culture Medium in the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

The length of the explants regarding the varieties was statistically the same ($p<0.05$) with means of 4.61, 4.56, 4.05 and 4.02 cm for the varieties Valencia, Lima 40, Señorita and Perla 83 (Graph 3.2).

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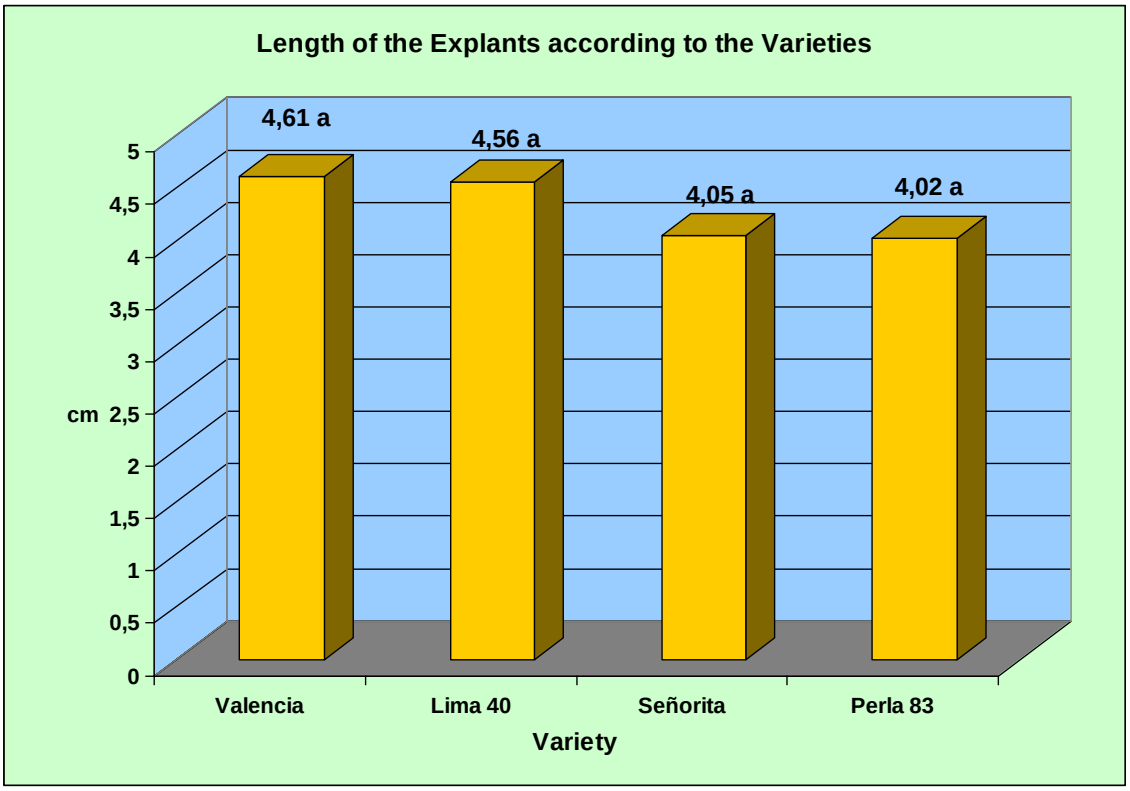

Graph 3.2: Length of the Explants according to the Varieties in the Organogenesis in the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

3.2 Number of Active Leaves of the Explants

The leaves, like those of any other plant, are the organs in which photosynthesis occurs, which transforms the sun's radiant energy into chemical energy. In cassava, the leaves are formed from the axillary meristems located at the nodes of the stem, and are arranged in a spiral shape. The leaves are simple and are composed of the leaf blade and the petiole. The size of the leaf is a typical characteristic of each cultivar, although it depends a lot on the environmental conditions. The leaves that the plant produces between the first and fourth month after planting are larger than the leaves that the plant develops between the fourth month and harvest. The upper side of the leaf is covered by a shiny waxy cuticle, while the underside is opaque and is where most of the stomata are located, although some varieties have abundant stomata on the upper side (Calderón and González, 2009).

The analysis of variance showed highly significant differences ($p<0.05$) between the culture media for the number of active leaves of the explants, while in the varieties there were no significant differences between the means of the same. According to Duncan's test, medium 2 reached the highest average number of active leaves of the explants with 4.52, while medium 1 obtained the lowest average with 2.70 active leaves (Graph 3.3).

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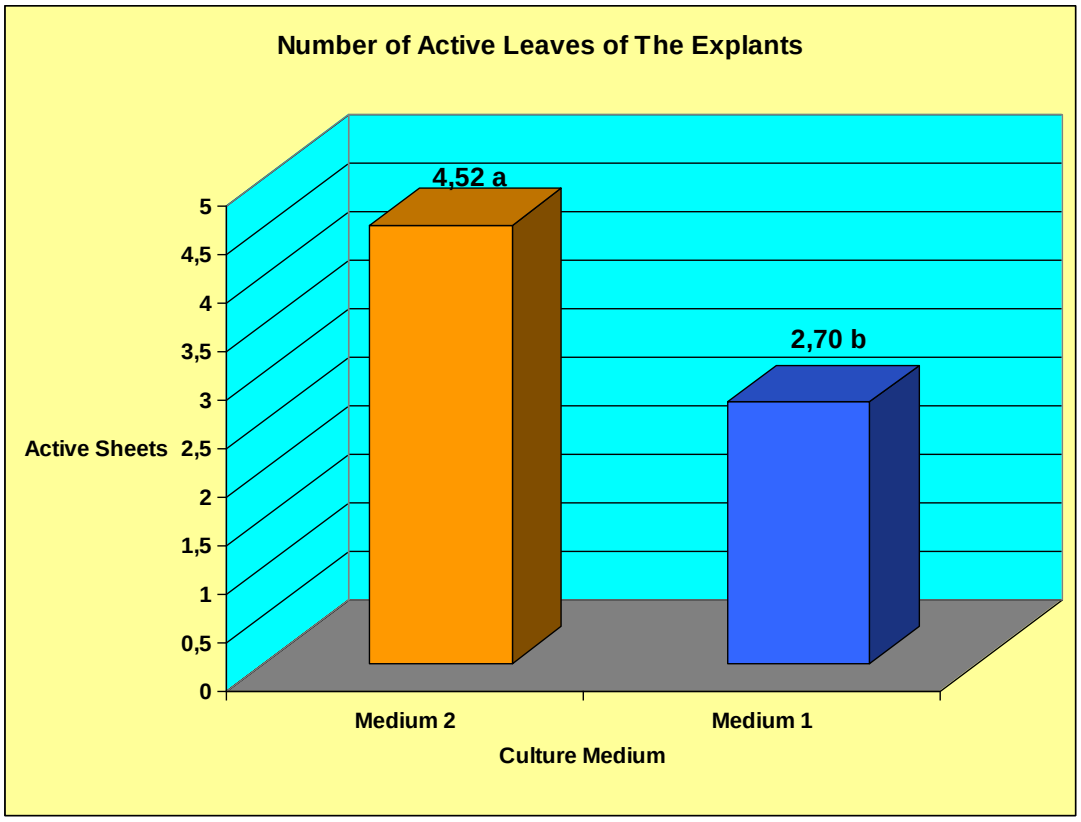

Graph 3.3: Number of Active Leaves of the Explants according to the Culture Media of Organogenesis in the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

Regarding the varieties and according to the analysis of variance, the means of the number of active leaves were statistically similar in the four varieties with 3.82, 3.60, 3.54 and 3.48 active leaves, respectively (Graph 3.4).

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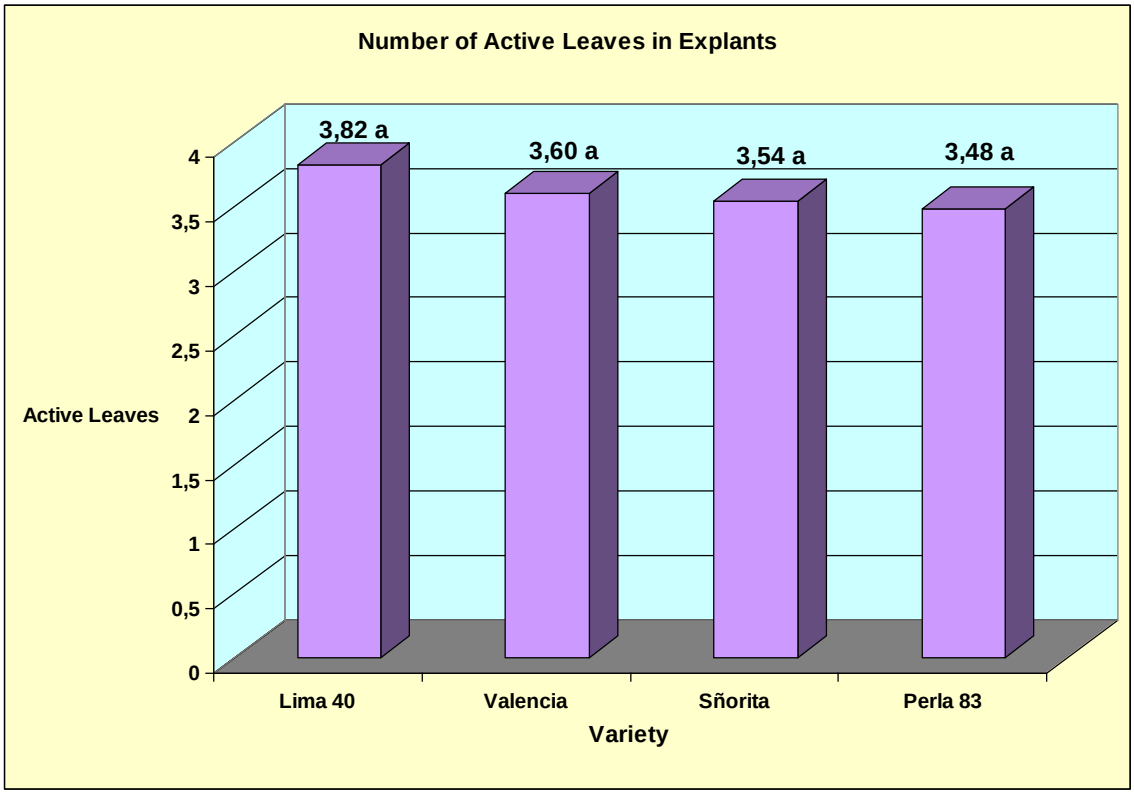

Graph 3.4: Number of Active Leaves of the Explants according to the Organogenesis Varieties in the Micropropagation of Four Cassava Varieties (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

The leaves are the main site of production of processed substances for the plant, and a well-developed leaf system will be important for the survival of the plant during acclimatization (Ogero et al; 2012). Lolaei et al; (2013) cited by Corozo et al; (2020). Gibberellic acid intervenes to a great extent in the growth and development stages of plants and increases the number of leaves when applied in low concentrations. Xu et al; (2016) pointed out that the treatment with GA3 is determinant in the elongation of the leaves and their productivity.

3.3 Number of Internodes of the Explants

The analysis of variance showed highly significant differences ($p<0.05$) for the crop media in this variable, in addition they also presented significant differences between the media of the varieties. In the Duncan test, medium 2 obtained the highest mean number of internodes for the explants with 6.10, while the mean of medium 1 was 4.27 internodes (Graph 3.5).

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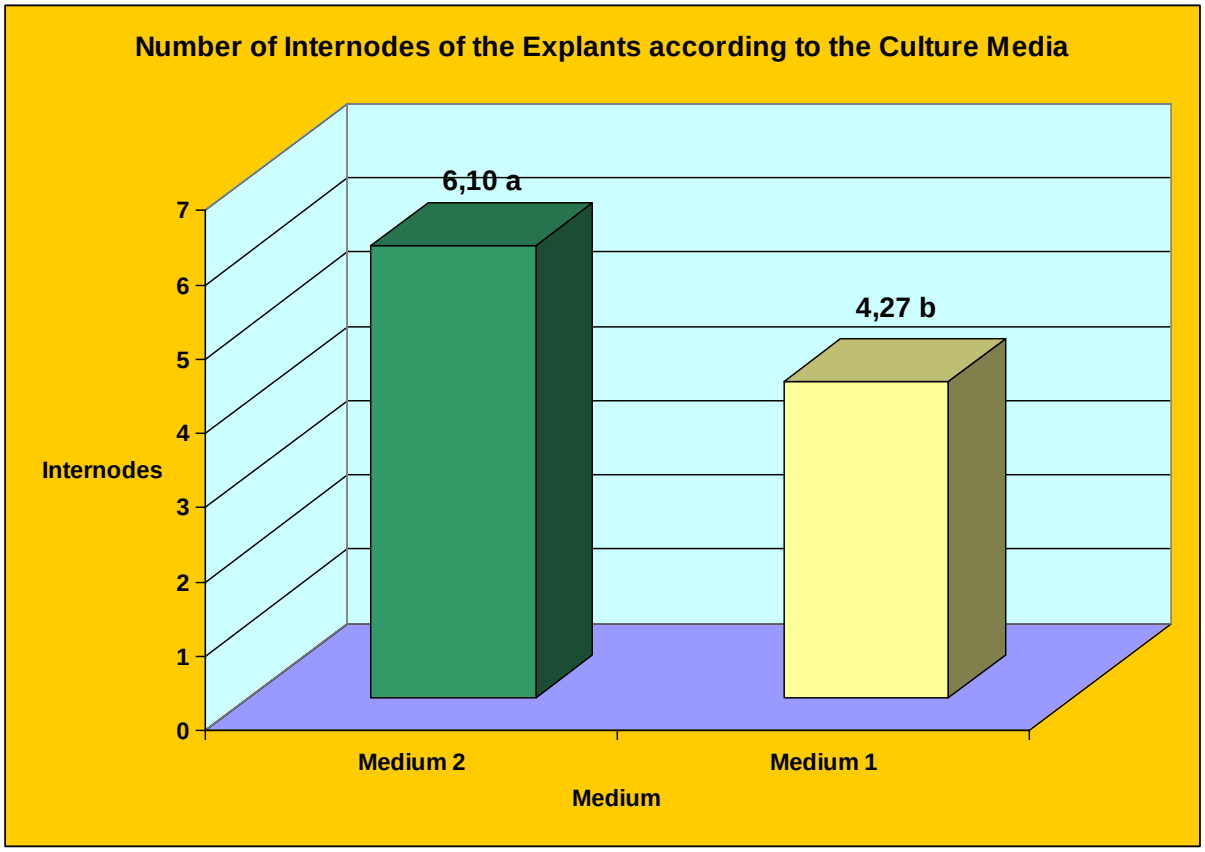

Graph 3.5: Number of Internodes of the Explants according to the Culture Media of Organogenesis in the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

In the varieties, the greatest number of internodes was obtained by the Valencia variety with 6.10 internodes followed by the Lima 40 and Señorita varieties, which were statistically equal with means of 5.38 and 4.37 internodes, while the Perla 83 variety had the lowest mean number of internodes with 4.39. (Graph 3.6). These values are similar to those obtained by Smith et al; (1986) cultivating axillary buds in DM added with 0.23 mg/L of BAP and 0.05 mg/L of ANA.

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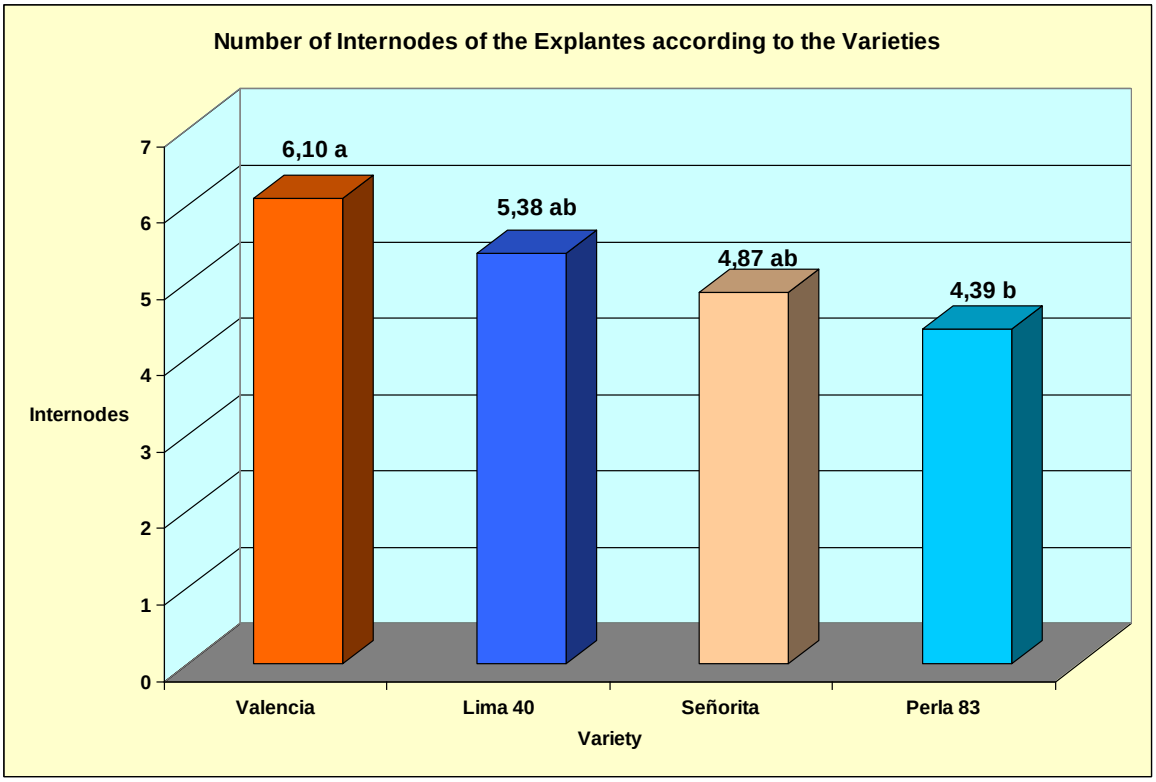

Graph 3.6: Number of Internodes of the Explants according to the Varieties of the Organogenesis in the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

3.4 Number of Explant Shoots

The shoots have a marked apical dominance and sequentially produce new leaves, however, when the apex becomes reproductive, one to six axillary buds develop (usually only 2 or 3) and produce the characteristic division or branching of cassava. Lateral shoots develop from axillary buds in the basal part of the stem. This type of branching is common, indicating that apical dominance is dependent on the erect position of the main stem. Each nodal unit is composed of a node from which a leaf and an internode arise. The knot production rate in each branch changes from approximately one knot per day in the initial growth period to one knot per week in one-year-old plants, showing little varietal variation. The total number of nodal units per plant depends not only on the production of nodes per branch, but also on the number of branches or apices per plant (Calderón and González, 2009).

The causes of branching in cassava are not fully known; some branch early and continue to branch while others do not branch. Under constant environmental conditions, the interval between ramifications tends to be constant, according to Tan and Cock, 1979, CIAT, 1978 (Domínguez et al; 1971) cited by Calderón and González, 2009. The number of ramifications is reduced with low fertility levels. from the soil, or increases due to lack of water during the

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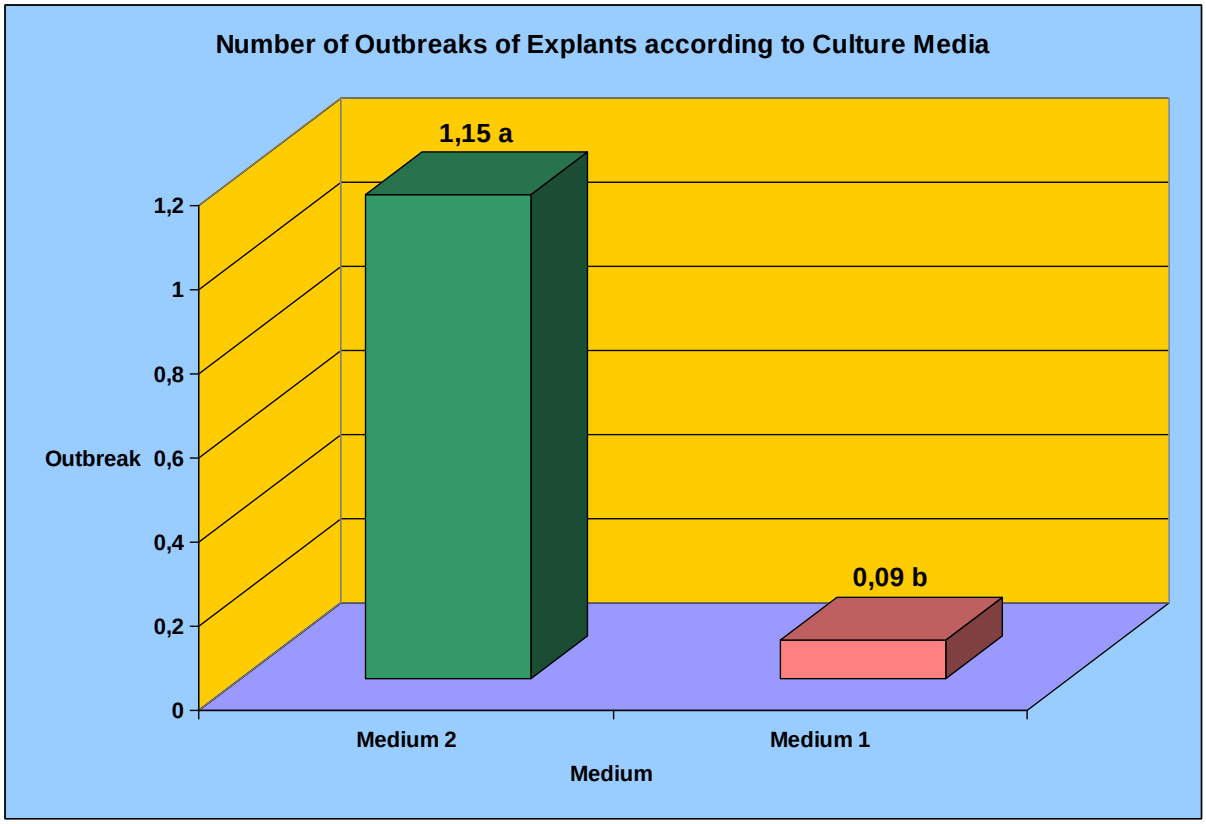
growth cycle; the influence of temperature reduces or increases the ramifications (Domínguez et al. 1971).

The analysis of variance showed highly significant differences ($p<0.05$) between the culture media, but there were no significant differences between the varieties in the number of shoots of the explants. According to Duncan's test, the medium that reached the highest number of shoots of the explants was obtained by medium 2 with 1.15 shoots, while medium 1 obtained the lowest mean with 0.09 shoots (Graph 3.7).



Graph 3.7: Number of Sprouts of the Explants according to the Culture Media of Organogenesis in the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

The means of the shoots of the explants in the varieties according to Duncan's test were statistically equal with 0.76, 0.67, 0.57 and 0.49 for the varieties, respectively (Graph 3.8).

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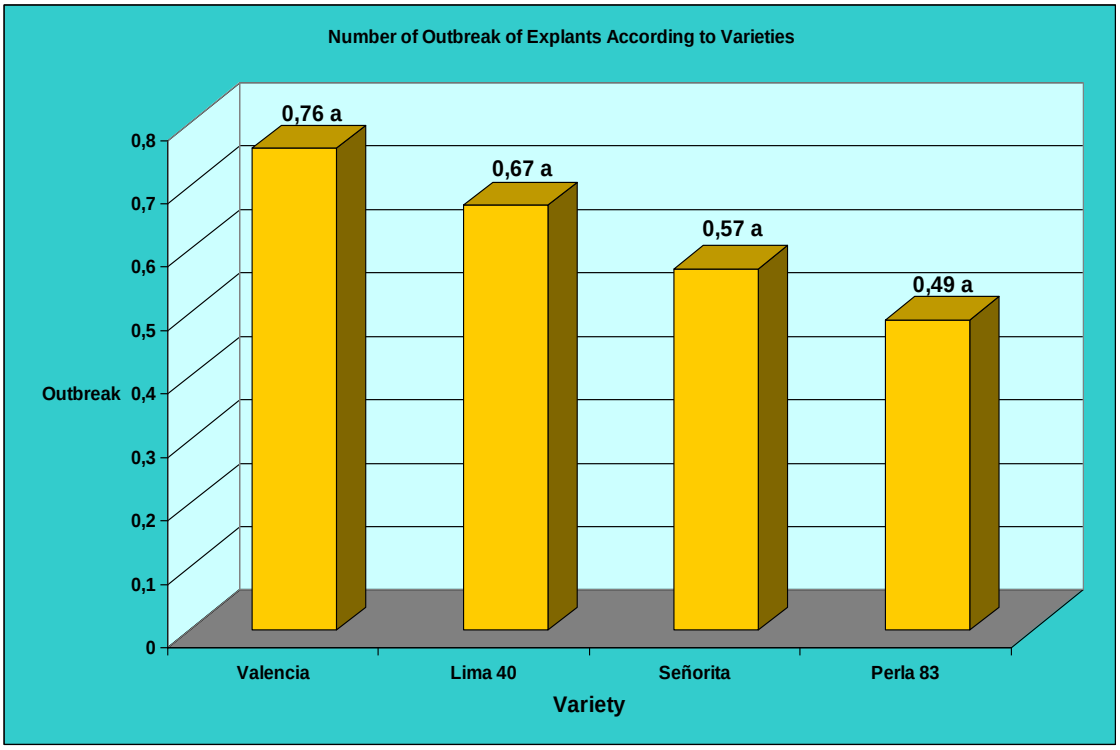

Graph 3.8: Number of Sprouts of the Explants according to the Varieties of the Organogenesis in the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

Suárez and Hernández, 2008, cited by Calderón and González, 2009 report that, when applying ANA with other hormones, it causes callus formation, without obtaining a difference in the increase in shoots, this contradicts the authors Albarrán et al; 2003. In it, they indicate that the concentration of 0.02 mg/l of ANA forms complete cassava plants without callus formation, which the authors Medina et al; 2000, cited by Calderón and González, 2009. When applying ANA plus BAP, it generates callus formation and growth of complete plants, this corroborates the results obtained in this work.

Ulloa, (2017) carried out a study on the effect of 6-BAP and ANA on the in vitro production of cassava nodal segments, in which cassava microcuttings were transferred in the multiplication stage, subculture one, to Murashige culture media and Skoog modified and supplemented with phytohormones. Four treatments were evaluated: the control without phytohormones and three treatments supplemented with BAP at 0.5 mg/L and ANA at 0.01 mg/L in different combinations. The number of buds was higher with BAP 0.5 mg/L and BAP 0.5 mg/L + ANA 0.01 mg/L since they produced a greater number of buds per microcutting (9.64 and 9.12, respectively). The vitro-cuttings obtained in these treatments had growth in rosette forms (dwarf and succulent). The number of roots and height of the plant was higher in the control medium (without phytohormone supplementation). It is recommended to evaluate the effect of gibberellic

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acid in combination with the treatments supplemented with BAP, which presented the best response for the variable number of buds (Ulloa, 2017).

Marin et al; (2009) carried out a work with the objective of evaluating the effect of growth regulators on the in vitro regeneration of five elite cassava cultivars from CIAT. The selected clones were BRA 383, PER 183, CM 523-7, CM 3306-4 and SM 1565-15. Two semisolid culture media made up of mineral salts from Murashige and Skoog (1962) were used, differentiated by the following combination of growth regulators: M1 (ANA 0.02 mg L-1 + AG3 0.05 mg L-1) and M2 (ANA 0.02 mg L-1 + AG3 0.05 mg L-1 + BA 0.5 mg L-1). The M1 medium was the best inducer for the regeneration of most of the evaluated cultivars, since there was a good development of shoots and roots. On the contrary, the M2 culture medium showed little development of shoots and roots, in most of the clones evaluated. A differential response of the genotype was observed in the *in vitro* development of the seedlings. The cultivars CM 523-7, PER 183 and BRA 383 showed the highest values for most of the variables evaluated, such as: number of nodes, length of shoots and roots.

3.5 Organogenesis protocol for cassava reproduction

The protocol was formulated from Culture Medium 2 (BAP 1.0 mg/L + ANA 0.05 mg/L) with which the best results were obtained with respect to the 4 variables, greater length of the explants, greater number of active leaves, greater formation of internodes and a greater number of shoots.

3.6 Length of the Explants in the Temporary Immersion System

The analysis of variance reflected significant differences ($p<0.05$) between the varieties in the Temporary Immersion System for the length of the explants. According to Duncan's test, the highest average length of the explants was reached by the Valencia variety with 6.80 cm, followed by the Señorita variety with 6.15 cm. The Perla 83 and Lima 40 varieties, which were statistically equal, obtained the lowest means with 5.56 and 5.45 cm, respectively (Graph 3.9).

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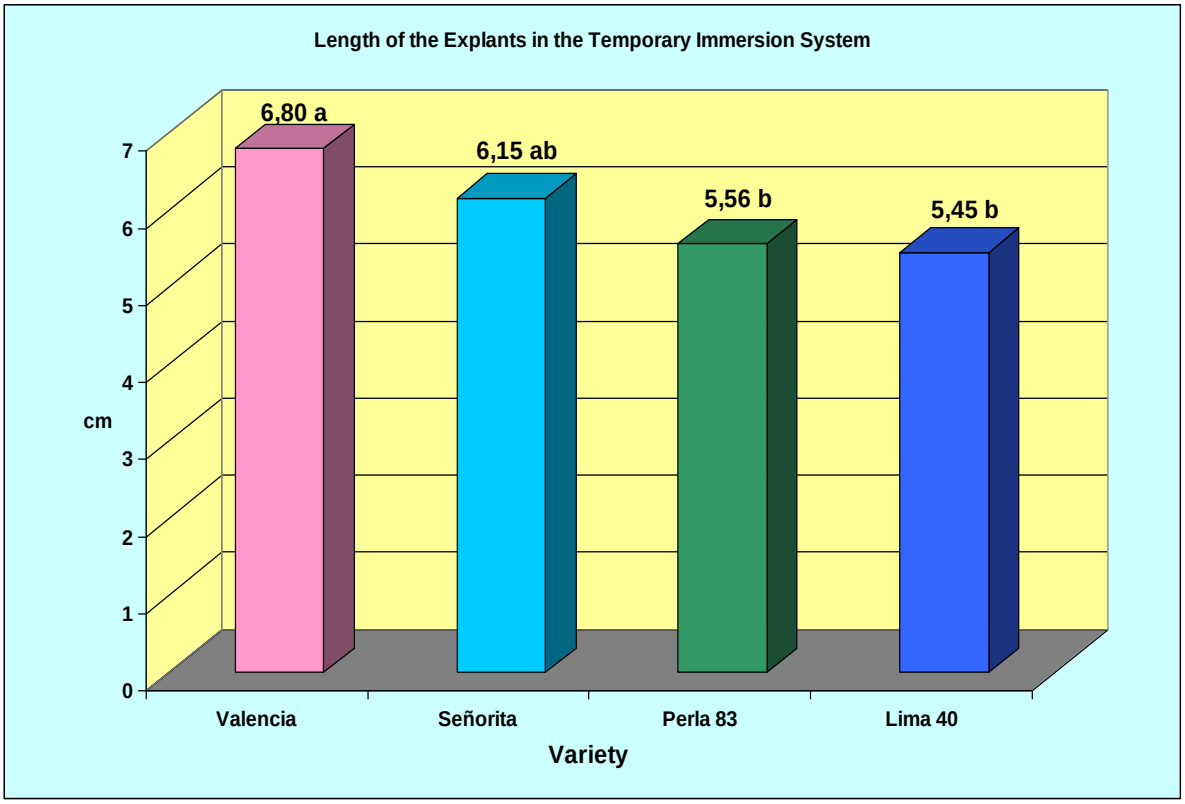

Graph 3.9: Length of the Explants according to the Varieties of the Temporary Immersion System in the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

3.7 Number of Active Leaves of the Explants in the Temporary Immersion System

The analysis of variance showed highly significant differences ($p<0.05$) between the varieties in the Temporary Immersion System for the number of active leaves of the explants. In the Duncan test, it is evident that the Perla 83 variety obtained the highest average number of active leaves with 4.62 followed by the Señorita and Valencia varieties with 2.90 and 2.27 active leaves, while the Lima 40 variety obtained the lowest average number of active leaves in the Temporary Immersion System with 1.67 (Graph 3.10).

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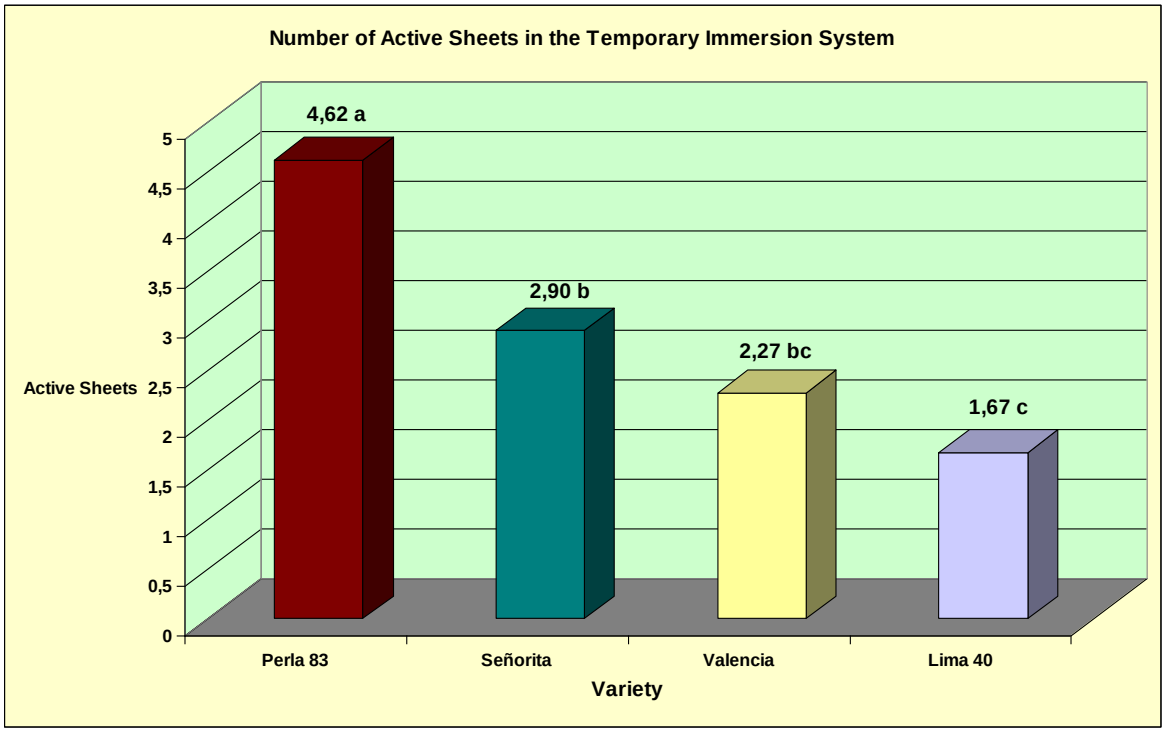

Graph 3.10: Number of Active Leaves of the Explants according to the Varieties in the Temporary Immersion System in the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

3.8 Number of Internodes of the Explants in the Temporary Immersion System.

The statistical analysis showed significant differences ($p<0.05$) for this variable in the different varieties used in the Temporary Immersion System. In the Duncan test, the varieties Perla 83, Valencia and Señorita were statistically equal with means of 7.29, 6.67 and 6.45 internodes, respectively, while the variety Lima 40 obtained the lowest mean number of internodes with 4.83 (Graph 3.11).

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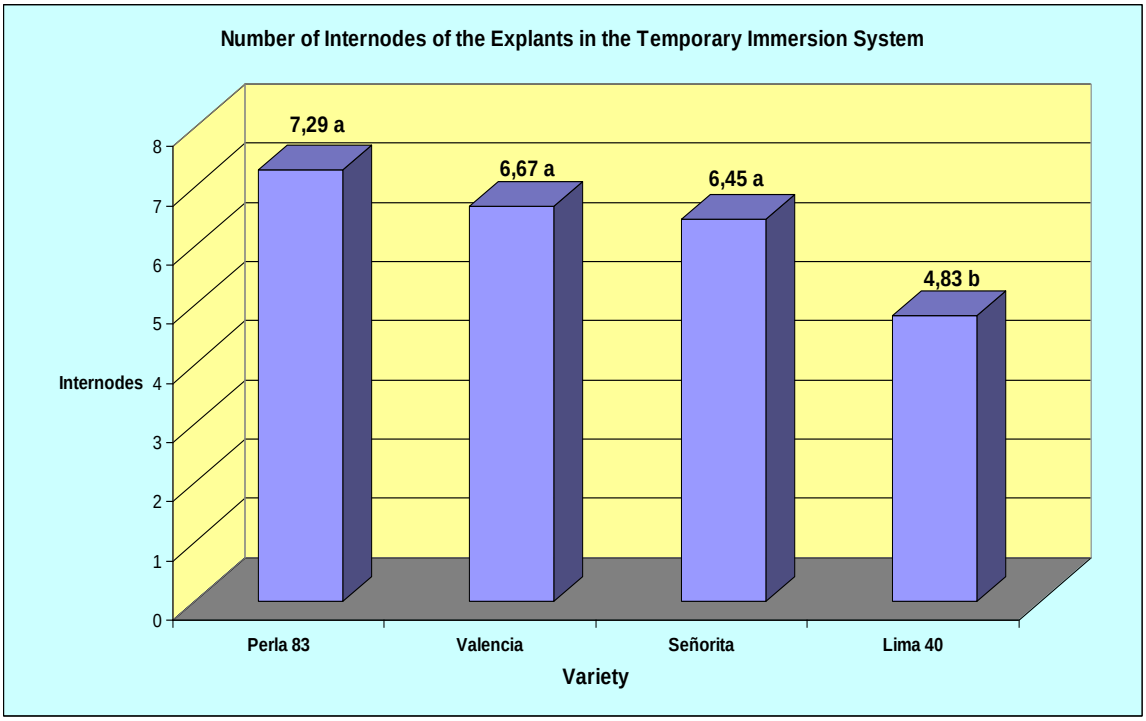

Graph 3.11: Number of Internodes of the Explants according to the Varieties in the Temporary Immersion System in the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

3.9 Number of Shoots of the Explants in the Temporary Immersion System

The analysis of variance did not show significant differences ($p<0.05$) for the number of shoots of the explants in the Temporary Immersion System. In the Duncan test, the Perla 83, Valencia, Lima 40 and Señorita varieties obtained similar means of the number of shoots: 1.05, 0.79, 0.77, and 0.73, respectively (Graph 3.12).

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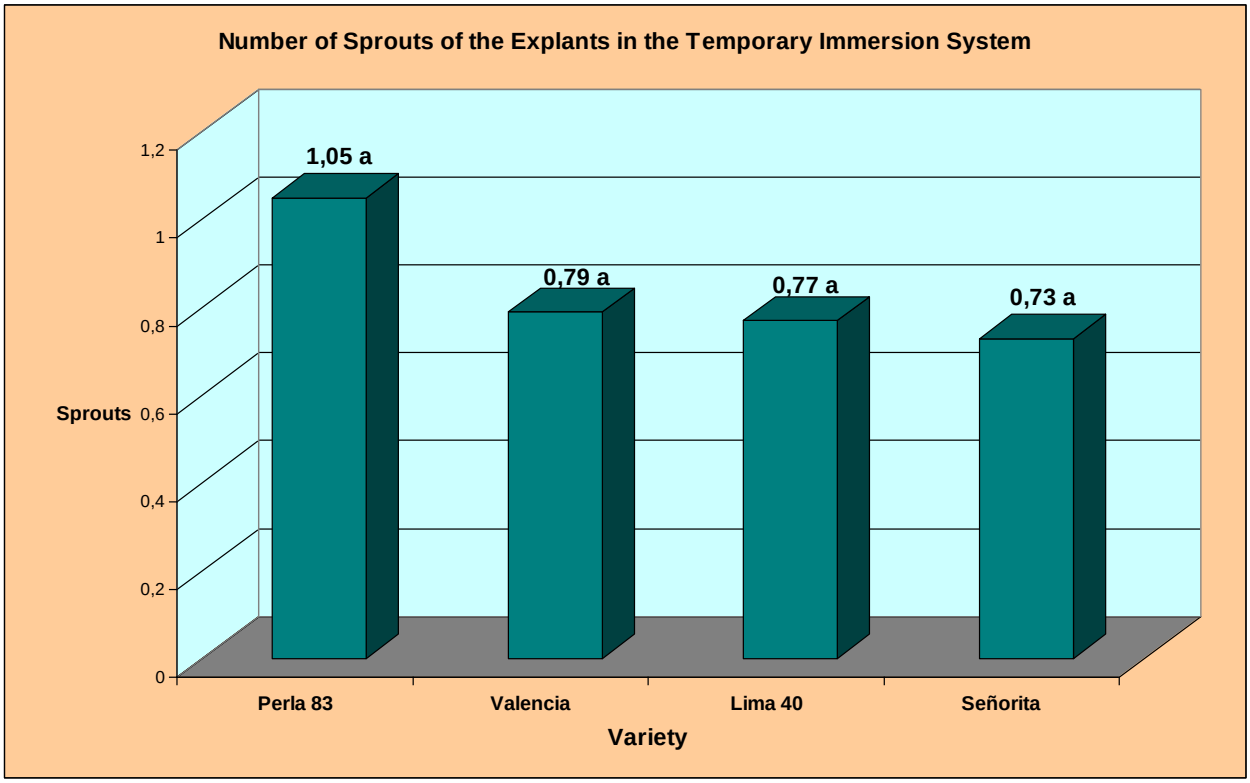

Graph 3.12: Number of Outbreaks of the Explants according to the Varieties in the Temporary Immersion System in the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

3.10 Length of Explants in *In vitro* Conservation

The analysis of variance did not show significant differences ($p<0.05$) for the length of the explants of the conserved varieties. In the Duncan test, the varieties Señorita, Valencia, Lima 40 and Perla 83 obtained similar means of the length of the explants with 4.40, 3.70, 3.41 and 3.33, respectively (Graph 3.13).

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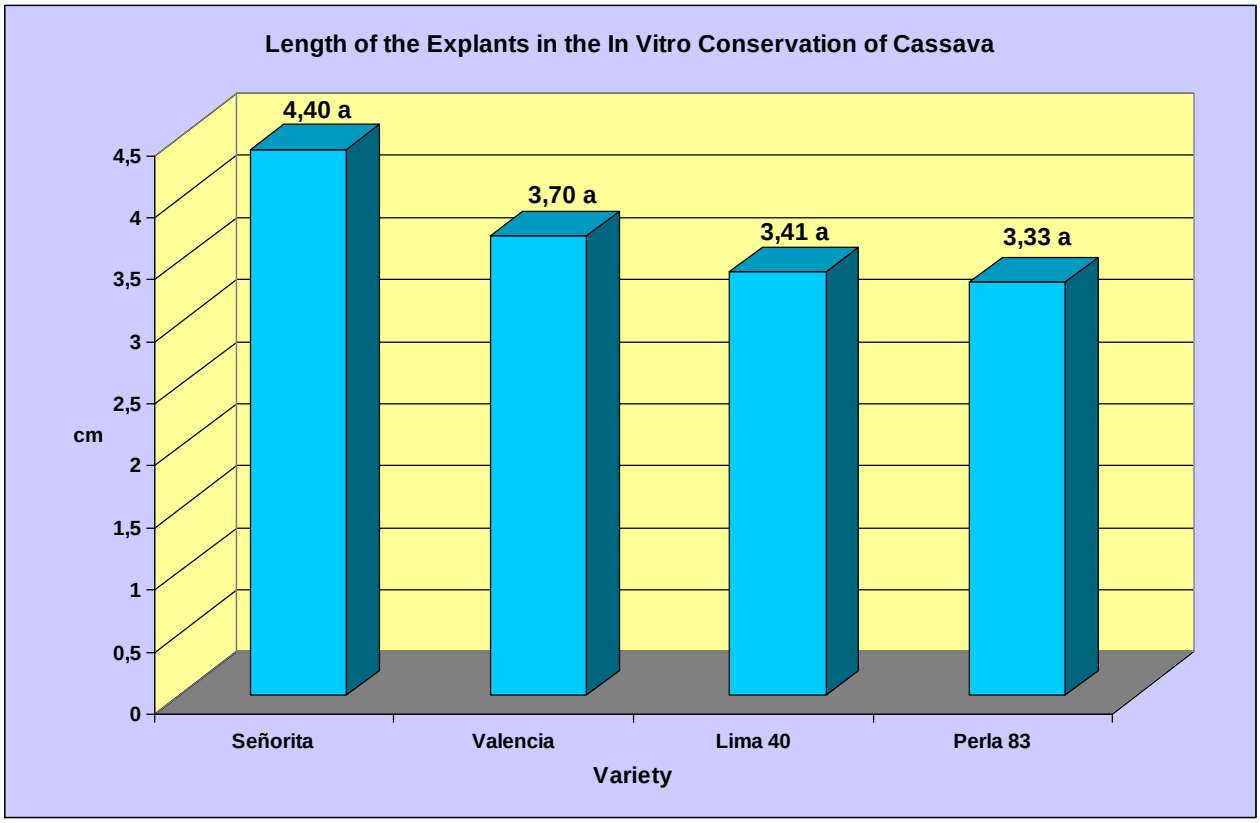

Graph 3.13: Length of the Explants of the Explants according to the Varieties of the *In vitro* Conservation in the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

3.11 Active Leaves of the Explants in the *In vitro* Conservation of Cassava

The analysis of variance did not reflect significant differences ($p<0.05$) for the Active Leaves of the explants of the conserved cassava varieties. In the Duncan test, the varieties Señorita, Lima 40, Valencia, and Perla 83 obtained similar means of the active leaves of the explants with 2.17, 2.10, 1.97, and 1.93, respectively (Graph 3.14).

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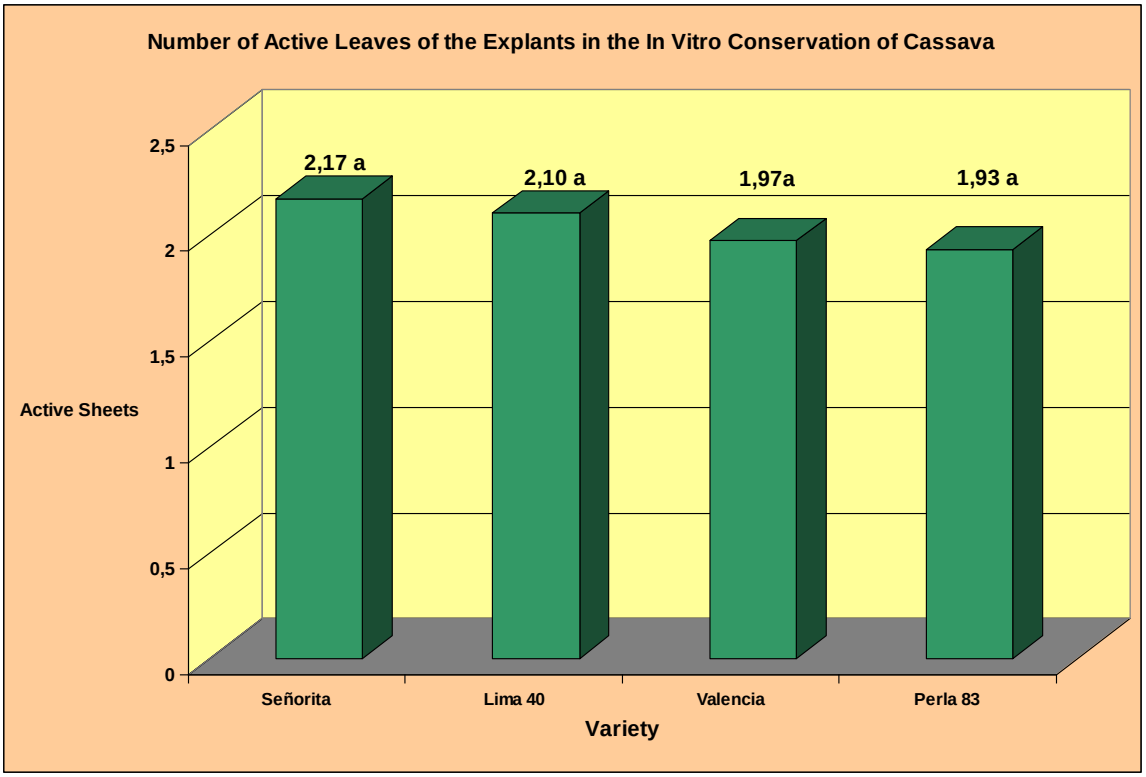

Graph 3.14 Number of Active Leaves of the Explants of the Explants according to the Varieties in the *In vitro* Conservation of the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

3.12 Number of Internodes of Explants in the *In vitro* Conservation of Cassava

The analysis of variance showed significant differences ($p<0.05$) for the number of internodes of the explants of the cassava varieties preserved *in vitro*. In Duncan's test, the Señorita variety surpassed the other varieties with the highest mean number of internodes with 3.97 nodes, while the Perla 83 and Valencia varieties obtained statistically similar means of 3.37 and 3.27 internodes. The Lima 40 variety was the one that obtained the lowest mean number of internodes with 2.50 (Graph 3.15).

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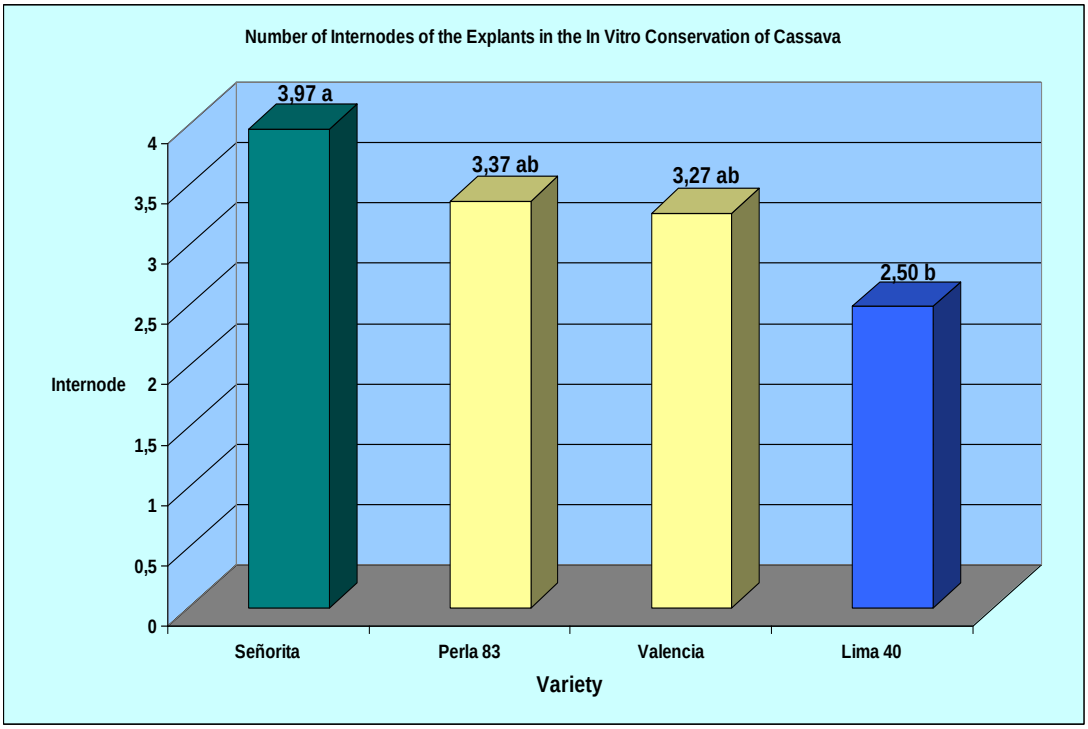

Graph 3.15: Number of Internodes of the Explants of the Explants according to the Varieties of the *In vitro* Conservation in the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

3.13 Number of Shoots of Explants in In vitro Conservation of Cassava

The analysis of variance showed significant differences ($p<0.05$) for the number of shoots of the explants of the cassava varieties preserved *in vitro*. In Duncan's test, the Señorita variety surpassed the other varieties with the highest mean number of shoots with 0.30, while the Valencia and Perla 83 varieties, being statistically similar, obtained means of 0.10 and 0.10 shoots. The Lima 40 variety did not obtain any sprouts (Graph 3.16).

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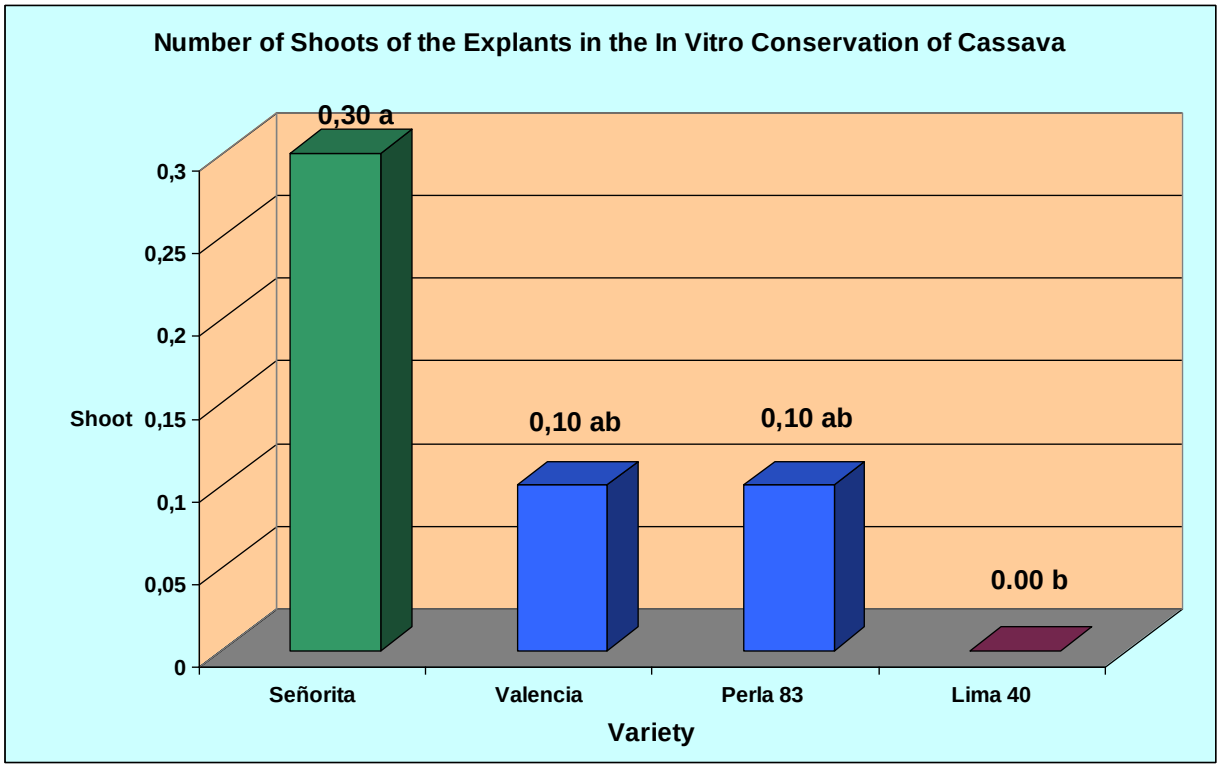

Graph 3.16: Number of Outbreaks of the Explants of the Explants according to the Varieties of the *In vitro* Conservation in the Micropropagation of Four Varieties of Cassava (*Manihot sculenta* Crantz) with Export and Industrial Potential in the Dominican Republic, Santo Domingo, 2022.

3.14 Validate a cassava acclimatization technology in vitro in a nursery whose plants were transferred to the producers.

Number of *In vitro* cassava plants that were acclimatized and delivered to the producers

Señorita variety: 656 vials (4,592 explants)

Variety Lima 40: 1,737 vials (12,159 explants)

Valencia variety: 113 vials 791 explants)

Variety Perla 83: 1,879 vials (13,153 explants)

Taverita variety: 1,011 vials (7,077 explants)

Total 5,396 vials (37,772 explants).

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This process, which is essential to achieve the highest percentage of plant survival, depends on multiple factors, such as: the quality of the plant, the skill in handling the material and the growing conditions, among others. The steps to follow are the following: Fill trays with sterile sand-earth substrate (3:2). Add 5 ml of N-P-K fertilizer solution to the substrate. Wash the roots of the plants to remove the culture medium, with enough distilled water. Place vitroplants 5 cm high on the substrate and add more substrate. Add 5 ml of fertilizer solution. Place the trays with the plants in a humid microchamber, formed with a transparent plastic bag. Close well to prevent the escape of water vapor. Place under shade in the greenhouse according to the following figure (Aguilar et al; 2016).

Survival at this stage was 87% for the Señorita variety, 83% for the Valencia variety, 85% for the Lima 40 variety, and 86% for the Perla 83 variety. This is a very favorable result, since the losses in the acclimatization stage are critical in the micropropagation process (Segovia et al; 2002).

Cassava is one of the species that suffer significant losses in this phase (Laynez and Sánchez, 2006; Marín et al; 2009) cited by Corozo et al; (2020), since plants must adjust their stomatal physiology to the prevailing evapotranspiration conditions and activate the uptake of water and nutrients through the root system (Bonilla et al; 2015) cited by Corozo et al; (2020). Oliveira et al; (2000) cited by Corozo et al; (2020), reported the successful acclimatization of cassava plants obtained by tissue culture; other authors, on the other hand, reported a high mortality in this stage (Zimmerman et al; 2007; Marín et al; 2008) cited by Corozo et al; (2020), which can reach up to 95% if it is not carried out with the appropriate technology (Segovia et al; 2002; Albarrán et al; 2011) cited by Corozo et al; (2020).

During this stage, the plants go from experiencing conditions of high relative humidity and low levels of light intensity to the comparatively lower levels of relative humidity and higher light intensity present in the greenhouse or in the field (Jorge et al; 2000) cited by Knight, (2011). To this is added the mechanical damage caused to the roots (Segovia et al; 2002) cited by Cavallero, (2011); the modification in the nutritional conditions, since the plants must go from a

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heterotrophic state to an autotrophic state, with different levels of nutrients and the modification in the aseptic conditions, since in this stage the plants can be exposed to the attack of microorganisms saprophytes and eventually phytopathogens (Grattapaglia and Machado, 1990; Vilchez et al; 2007) cited by Cavallero, (2011).

There are works that report a high mortality for cassava in the acclimatization stage, which can reach 95% if it is not carried out with the appropriate technology (Segovia et al; 2002) cited by Cavallero, (2011). In this sense, the substrates used exert a great influence on the architecture of the root system and on the biological associations that are established, affecting the nutritional status and the translocation of water in the soil-plant-atmosphere system (De Rezende et al; 2000) cited by Cavallero, (2011).

In this research, the high survival rates achieved may be due to pre-acclimatization, as recommended by Bonilla et al; (2015) cited by Corozo et al; (2020), before exposing the plants to natural conditions. The present investigation allowed to determine the essential conditions for the micropropagation of the varieties Lima 40, Perla 83, Valencia and Señorita. The results obtained from the propagation of these genotypes, of interest for production, can be useful in their accelerated multiplication with a view to providing propagules to farmers.

4. CONCLUSIONS

It was possible to validate an Organogenesis protocol for the micropropagation of four varieties of cassava (Manihot sculenta Crantz).

Surviving shoots in all organogenesis treatments, Temporary Immersion System and in vitro conservation of cassava. In vitro cassava plants produced roots and showed 85 % survival during the acclimatization stage. After they were acclimatized, they looked vigorous and developed normally when transferred to the soil.

The reduction in height and presence of axillary shoots observed in mannitol treatments would be of interest for in vitro conservation. It is concluded that the use of mannitol can be a new alternative for the in vitro conservation of the germplasm of these species.

Cassava plants were preserved in vitro under conditions of slow growth caused by mannitol, which retarded growth without affecting survival. Therefore, this system makes it possible to maintain collections of viable germplasm with a minimum investment of time and resources.

In vitro preserved cassava plants can be transferred to multiplication culture medium to obtain an unlimited source of plant material required for plant bank establishment, germplasm exchange, or breeding programs.

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