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Investigation note

Basic seed production of cassava clone Agrosavia Melúa-31

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Abstract

The cassava frogskin disease (CFSD) is one of the main phytosanitary constraints for the sustainable production of cassava because it restricts the tuberization and obtaining of healthy planting material from commercial plantations. The cassava clone Agrosavia Melúa-31 is of high socioeconomic relevance for the Colombian Orinoquía region; nevertheless, the high susceptibility to cassava frogskin disease restricts the commercial planting of this clone. Therefore, this research was conducted with the aim of developing a preliminary protocol for the production of basic seed of the cassava clone Agrosavia Melúa-31. This work was carried out at Agrosavia, Turipaná Research Center in three phases: *in vitro* micropropagation, *ex vitro* acclimatization and hardening, and adaptation to field conditions. The results revealed that, under *in vitro* growing conditions, the survival rate of the Agrosavia Melúa-31 clone ranged from 85 to 90%. In the acclimatization phase, the highest percentage of survival (87.5%), fresh (2.6 g) and dry (0.42 g) biomass production, and leaf area index (0.66) were obtained with a substrate based on sand, organic matter, and alluvium. In the phase of adaptation to field conditions, the survival rate was 98%, healthy roots and basic seed (Cangres) of Agrosavia Melúa-31 of high phytosanitary and genetic quality were harvested. The standardization of quality seed production protocols is an indispensable tool for the sanitization of vegetatively propagated materials.

Keywords:

micropropagation, plant health, sexual seed, tissue culture.

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Cassava (*Manihot esculenta* Crantz) is one of the most important tubers in family farming and self-consumption crops in tropical and subtropical regions of the world. It is grown in more than 100 countries and its annual global production exceeds 300 million tons (FAO, 2020). In Colombia, cassava is the fifth most produced agricultural product by volume, after sugarcane, banana, potato, and rice (Parra-Olarte, 2019).

In Colombia, cassava is grown in six regions, of which the Caribbean, Cauca, and Orinoquía are the main producing regions; this tuber is cultivated for industrial purposes, fodder production, and the self-sufficiency of families. Crop yields vary according to the region; in the Caribbean region, they are around 27 t ha^{-1} (sweet starch cassava), whereas in the Orinoquía region and the department of Cauca, main producers of bitter starch cassava, yields only reach 11.9 t ha^{-1} (Parra-Olarte, 2019).

The low yield of industrial cassava varieties in the Orinoquía and Cauca regions is associated with the high incidence of the cassava frogskin disease (CFSD), which affects the root of cassava plants, which do not tuberize and have a thick peel with a cork appearance and woody consistency.

To reduce the risks of CFSD infection and other diseases (brown streak disease and cassava mosaic disease), it is necessary to have clean seeds of resistant varieties that mitigate the persistent threat to cassava production from these diseases. *In vitro* micropropagation is a biotechnological strategy of great importance for the sanitary cleaning of cassava materials (Marín *et al*., 2009).

Nonetheless, it is necessary to standardize the conditions of *ex vitro* acclimatization and production under field conditions to ensure the quality of the plant material and survival rates. In this sense, this research was conducted with the aim of developing a preliminary protocol for the production of basic seed of the cassava clone Agrosavia Melúa-31.

The research was carried out from May 2021 to October 2022 at the Colombian Corporation for Agricultural Research (Agrosavia), Turipaná Research Center, located in the municipality of Cereté, department of Córdoba (Colombia) at 8° 50’ 79” north latitude and 75° 47’ 58” west longitude, at an altitude of 13 masl. Ecologically, this region corresponds to a humid savannah region according to the Köppen-Geiger climate classification (Kottek *et al*., 2006).

The area has an average annual temperature of 28 ±2 °C, annual rainfall of 1 200-1 300 mm and relative humidity of 80 to 90%. For the development of the protocol, we used the cassava clone Agrosavia Melúa-31 (SM 2792-31), obtained in the CIAT-Agrosavia breeding program from polycrossing sexual seeds (Rosero-Alpala *et al*., 2019).

The seed of Agrosavia Melúa-31 requires the cleaning of the plant material given its high susceptibility to CFSD under the conditions of the Colombian Orinoquía region and the high demand for healthy seed for the establishment of commercial lots.

To obtain the preliminary protocol, the research was developed in three phases: the first consisted of the multiplication of the plant material under *in vitro* conditions at the Plant Micropropagation Laboratory of Agrosavia, the second stage was developed in a greenhouse (*ex vitro*), and in phase three, the hardened seedlings were transplanted to field conditions and subjected to management and agronomic evaluations for the production of basic seed. The seedling production process (phases 1 and 2) was carried out under the specifications detailed in the standard operating plan for cassava micropropagation of Agrosavia (Espitia *et al*., 2019).

In phase two of acclimatization and hardening, three substrates were evaluated under a completely randomized design (CRD) with three replications. The substrate (S1) was made up of sand and alluvium (1:1). The substrate (S2) was made with 85% alluvium, 10% sand, and 5% organic matter (Lombriabono®) and the substrate (S3) was made up of peat (Pindstrup Substrate®).

Eight plants were used in each replication, which were planted in plastic germinators. For each treatment, 24 plants were used in total. The physicochemical characteristics of the substrates are detailed in Table 1.

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Table 1. Physicochemical characteristics of the substrates evaluated in the acclimatization phase.

Parameter	Unit	Substrate 1	Substrate 2	Substrate 3
Electrical conductivity (EC) (1:5)	(dS m^{-1})	0.1	0.22	0.9
Organic matter (OM)	(%)	0.23	0.49	0.2
Phosphorous (P)	(mg kg^{-1})	2.02	4.05	1.01
Cation exchange capacity (CEC)	(cmol kg^{-1})	3.05	6.48	2.06
Potassium (K)	(cmol kg^{-1})	<0.02	<0.09	<0.01
pH (1:2.5)	Not applicable	6	6.62	4.89
Texture	Not applicable	Loam-sandy	Sandy-loam	Not applicable

Before planting the vitroplants, the substrates were chemically disinfected with the commercial product Terrasafe West® (1,4-diformyl propane) in doses of 5 ml L^{-1}. The seedlings were kept for one week at 28 ±2 °C, relative humidity of 95%, shading of 70%, and frequent irrigation. At the end of this period, the Cerostress® foliar fertilizer was applied at doses of 5 ml L^{-1}. Next, the plants were moved inside the greenhouse to shading of 40% to facilitate light capture and maintain a relative humidity greater than 80%, irrigation was twice a day.

The seedlings were kept under this condition for three weeks. From the fifth to the sixth week, the plants were subjected to a luminosity of 60% and relative humidity of 65%. The Agrimins® foliar fertilizer (3 ml L^{-1}) and Amistar top® (2 ml L^{-1}) and Oxithane® (2 g L^{-1}) fungicides were applied weekly and irrigation was daily. After six weeks, the variables of survival (%) and leaf area index (LAI) were recorded with an Accupar LP-80® ceptometer; the production of fresh biomass (leaves + stems + roots) was determined by recording the weight with an Ohaus™ analytical balance.

To determine the dry biomass, the plant tissue was dried in an oven for 48 h at a temperature of 70 ±2 °C. An analysis of variance (Anova) was performed to evaluate the effect of substrates on plant survival and growth parameters. In case of significant differences between treatments, Tukey's test was used at the threshold of 5%. The analyses were carried out with the R v4.3.2 language in the Rstudio environment (R Development Core Team, 2011).

In phase three, the plants hardened in the best substrate were established under field conditions (October 2021) in a plot of 144 m^2, in a Vertic Endoaquept soil with a clayey loam texture. The lot was mechanically prepared and the Diuron herbicide was applied at 80% (1.5 kg ha^{-1}), and the establishment was carried out eight days later. The plants were spaced 1 m apart and the furrows 1 m apart (10 000 plants ha^{-1}). Daily manual irrigation was performed for the first 30 days after transplanting (DAT). There were two weed controls with glufosinate ammonium (2 L ha^{-1}) and a manual control. Fertilization was carried out in an edaphic way according to Cadavid (2002) recommendations.

In this phase, we evaluated the survival of the plants 30 DAT and the incidence of pests and diseases on a monthly basis. Manual harvesting was carried out after 362 DAT; 10 plants located in the central area of the plot were selected, and the health status of fresh cassava roots was evaluated using a visual scale for CFSD disease proposed by Álvarez *et al*. (2015).

Additionally, the weight of fresh roots per plant was recorded and the yield was estimated in kg ha^{-1}. The dry matter of the roots (%) and the number of cuttings or cangres per plant (basic category seed) were then determined. The results obtained allowed us to establish a preliminary protocol for the production of basic seed of the cassava clone Agrosavia Melúa-31, free of CFSD and of high genetic and sanitary quality.

The results obtained indicated that, during phase one of *in vitro* multiplication, the survival of the material ranged from 85 to 90%, with a multiplication rate of one in three. The variation in survival percentages is closely linked to the cassava variety and the concentrations of sucrose and growth

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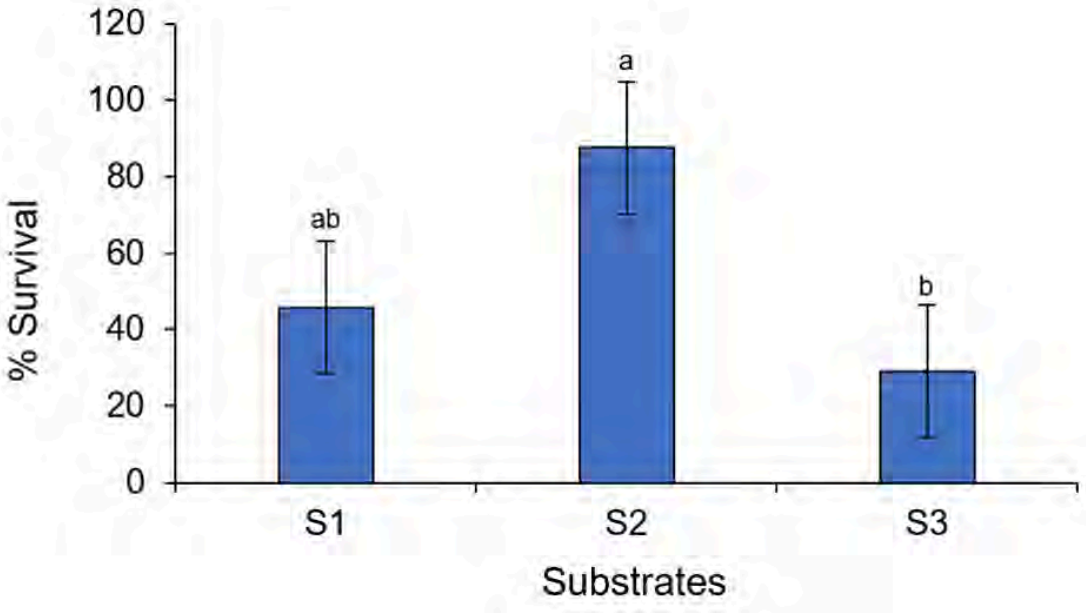
regulators (Marín *et al*., 2009). In phase two of *ex vitro* acclimatization and hardening, the S2 substrate allowed the survival of 87.5% of the vitroplants, compared to the S1 substrate with 45.83% and the S3 substrate with 29.17% (Figure 1).

Figure 1. Survival percentage of Agrosavia Melúa-31 cassava vitroplants in three types of substrates. Different letters by variable indicate significant differences between substrates ($p< 0.05$).



Since the survival percentage in S3 was statistically lower than in the rest of the substrates, biomass and LAI data were recorded only for S1 and S2 substrates. The analysis of variance detected highly significant differences for the variables of fresh biomass (p-value= 0.0052) and dry biomass (p-value= 0.0028). For LAI, significant differences were found (p-value= 0.02767).

The results detailed in sections 2a and 2b of Figure 2 indicate that the S2 substrate led to a greater accumulation of biomass and leaf area index, between 49 and 51% more compared to the S1 substrate. In this regard, some authors have mentioned that substrates with good organic matter content help to improve the growth parameters of cassava vitroplants, increase water retention, and stimulate the optimal development of the root system (Cacaï *et al*., 2021).

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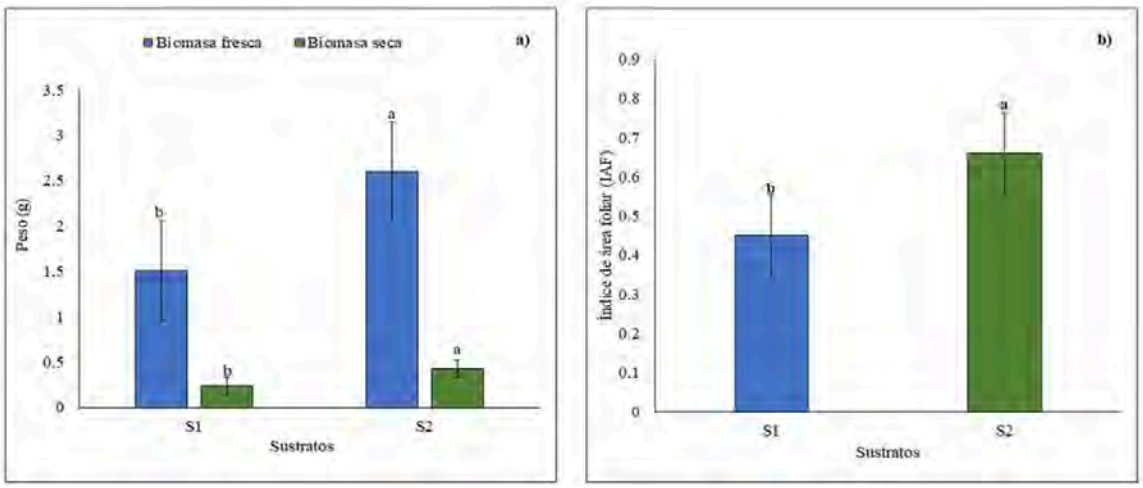
Figure 2. Growth variables of cassava vitroplants in two types of substrates. a) fresh and dry biomass and b) leaf area index (LAI). Different letters by variable indicate significant differences between substrates ($p< 0.05$).



Likewise, seedlings hardened in nutrient-enriched substrates may have greater leaf area, number of leaves and internodes, which translates into greater vigor of the material. Under open field conditions, the survival of the material from the S2 substrate was 98%. During the phenological cycle, the incidence of mites (*Mononychellus tanajoa* and *Oligonychus peruvianus*) and thrips (*Frankliniella* sp.) was less than 2%. No signs or symptoms of phytopathogenic infections were observed.

Likewise, visual evaluations of the health status of fresh cassava roots with Álvarez *et al*. (2015) scale allowed us to establish that the roots were free of CFSD. The plants had an average yield of 62.53 ±2.5 t ha^{-1}, dry matter production of 34.04%, and production of 22.2 basic seeds (cangres) per plant. The yields of Agrosavia Melúa-31 exceeded the technical reports for this variety, which correspond to 26.22 ±2.3 t ha^{-1}, and other varieties for industrial use under the usual planting conditions in the Colombian Orinoquía region (Rosero-Alpala *et al*., 2019). Likewise, the results of this research made it possible to obtain pathogen-free basic seed for the vegetative propagation of Agrosavia Melúa-31.

Conclusions

The development of protocols for the production of basic cassava seed is a useful tool to ensure the genetic, sanitary, and physiological quality of the seed; in addition, it reduces losses of plant material during the stages of *in vitro* micropropagation, *ex vitro* acclimatization, and adaptation to field conditions. Additionally, this process guarantees the refreshing of the seed (cangres) for the massive multiplication of genetic material. The development process of the preliminary protocol in this research for the production of seeds of Agrosavia Melúa-31 has allowed us to identify a highly effective substrate for phase two of the plant production protocol.

Bibliography

1 Álvarez, E.; Pardo, J. M.; Mejía, J. F.; Santos-Oliveira, S. A.; Zacher, M.; Cardozo, L. y Gómez, Y. 2015. Manejo del 'cuero de sapo' enfermedad limitante de la yuca. 1-20 pp. https://hdl.handle.net/10568/70086.

2 Cacaï, G. H. T.; Ahanhanzo, C.; Ahoya, D. K. D.; Houédjissin, S. S. and Houngue, J. A. 2021. Effects of non-conventional substrate and NPK fertilizer on cassava plantlets

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acclimatization for plant material production. Agricultural Sciences. 12(10):1058-1069. https://doi.org/1058-1069.10.4236/as.2021.1210068.

3 Cadavid, L. F. 2002. Suelo y fertilización para la yuca. La yuca en el tercer milenio. Sistemas modernos de producción, procesamiento, utilización y comercialización. CIAT, Cali, Colombia. 76-103 pp.

4 Espitia, M. A.; Regino, H. S. y Pérez, C. S. 2019. Procedimiento operativo estándar (POE). Micropropagación de yuca (*Manihot esculenta* Crantz). Agrosavia. 1-7 pp.

5 FAO. 2020. Organización de las Naciones Unidas para la Alimentación y la Agricultura. Bases de datos estadísticas sobre producción de yuca. https://www.fao.org/faostat/es/#data/QCL/visualize.

6 Kottek, M.; Grieser, J.; Beck, C.; Rudolf, B. and Rubel, F. 2006. World map of the Köppen-Geiger climate classification updated. Meteorologische Zeitschrift. 15(3):259-263. https://doi.org/10.1127/0941-2948/2006/0130.

7 Marín, A.; Albarrán, J. G.; Fuenmayor, F. y Perdomo, D. 2009. Evaluación del efecto de los reguladores de crecimiento en la regeneración *in vitro* de cinco cultivares élites de yuca (*Manihot esculenta* Crantz). Revista Científica UDO Agrícola. 9(3):556-562.

8 Parra-Olarte, J. L. 2019. Subsector productivo de la yuca: Dirección de cadenas agrícolas y forestales [Documento técnico]. Ministerio de Agricultura y Desarrollo Rural. Recuperado de https://acortar.link/FMbvob.

9 R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-070. http://www.R-project.org.

10 Rosero-Alpala, E. A.; Ceballos, H.; Calle, F.; Salazar, S.; Morante, N.; Beltrán, F.; Becerra-Campiño, C. J. y Sandoval, C. H. A. 2019. Agrosavia Melúa-31. Nueva variedad de yuca industrial para la Orinoquía colombiana. Mosquera, Colombia: Corporación Colombiana de Investigación Agropecuaria (Agrosavia). 7-17 pp. http://hdl.handle.net/20.500.12324/35643.

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