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FRUITS PRODUCTION OF PINEAPPLE (*Ananas comosus* (L.) Merr.) MD-2 FROM VITROPLANTS

Producción de frutos de piña (*Ananas comosus* (L.) Merr.) MD-2 a partir de vitroplantas

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ABSTRACT. Pineapple (*Anana comosus* (L.) Merr.) is a species of high commercial demand, MD-2 is among the most promising varieties, which has captured the consumer appeal in recent years. For this reason micro-propagation techniques are used to achieve plants with better agronomic traits and seed production of excellent quality. In Cuba, although there have been some attempts to introduce the plantlets under production conditions of State Entities and Agricultural Enterprise, for various reasons the goal has not successfully been achieved. For this reason, we began working introducing vitroplants of pineapple MD-2 in collaboration with selected farmers and their families with a view to establishing agro-technics that allow to have an alternative planting material, procedures, necessary for productive reanimation pineapple cultivation and develop the technological basis to ensure continuous generation of planting material needed to maintain varietal biodiversity strategies and according to market demand. The results achieved to date demonstrate higher survival rates *in vitro* plants (90 %) during the first three months of field evaluation. The agrotechnic management established, allowed the increase of all variables evaluated in vitro plants (No. of leaves, No. of roots, length of major root and ¨D¨ leaf width, fresh mass and length of plant) tested in field conditions. It was also shown by bromatology analyzes, done to the fruits that they comply with such attributes internationally for pineapple MD-2 and that the agrotechnics applied to this crop influenced the results achieved.

Key words: farmers, micro-propagation, pineapple, vitroplants

RESUMEN. La piña (*Anana comosus* (L.) Merr.) es una especie de gran demanda comercial, entre las variedades más promisorias se encuentra la MD-2 la cual ha acaparado la atracción del consumidor en los últimos años. Por esta razón se emplean las técnicas de micropropagación para lograr plantas con mejores caracteres agronómicos y producir semillas de excelente calidad. En Cuba, aunque se han realizado algunos intentos de introducir las vitroplantas bajo las condiciones de producción de Entidades Estatales y Empresas Agrícolas, por diversas causas no se ha logrado satisfactoriamente este objetivo. Por esta razón, se comenzó a trabajar la introducción de vitroplantas de piña MD-2 con la colaboración de campesinos seleccionados y sus familiares, con vista a establecer procedimientos agro-técnicos que permitan contar con un material de siembra alternativo, necesario para la reanimación productiva del cultivo de la piña y así desarrollar las bases tecnológicas para garantizar la generación continua del material de siembra necesario para mantener las estrategias varietales, acorde a la biodiversidad y exigencia del mercado. Los resultados alcanzados hasta la fecha demuestran que se logran altos porcentajes de supervivencia en las vitroplantas (90 %) durante los primeros tres meses de evaluación en campo. El manejo agro-técnico que se estableció, permitió el incremento de todas las variables evaluadas en las vitroplantas (número de hojas, número de raíces, longitud de la raíz mayor, longitud y ancho de la hoja ¨D¨, masa fresca y longitud de la planta) en las condiciones de campo ensayadas. También se pudo demostrar, por análisis bromatológicos realizados a los frutos, que éstos cumplen con las características descritas internacionalmente para la piña MD-2 y que las atenciones agrotécnicas realizadas al cultivo influyeron en los resultados.

Palabras clave: campesinos, micropropagación, piña, vitroplantas

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INTRODUCTION

Pineapple (*Ananas comosus* (L.) Merr.) has been for years as one of the economic resources of export in many countries, especially the cultivar Gold "Extra Sweet" MD-2, that by its content of soluble solids, smell and color has been preferred and has remained the number one in the world markets. The variety MD-2, also called Yellow or Golden, it is a cultivar product of the crossing of two hybrids (PRI 581184 x PRI 59443) and it is known that one of its progenitors comes from smooth Cayenne. The company Del Monte Fresh Produce in Hawaii Inc. markets it as extra sweet Gold (Gold extra sweet, Golden Ripe or Gold) since 1996. The plant is fast growing and shorter production cycle. In addition, fruit yields and fruit size are higher and it is a very sweet and juicy fruit, although it is recognized that it is more susceptible to mechanical damage and to *Phytohpthora* than Champaka (1, 2, 3). The production of pineapple in Cuba, presented a gradual decrease until 2009[A] and as a strategy for the economic development of the country, since that year a program is developed for the recovery of its production. In the province of Ciego de Ávila, strategies were drawn up led by the "Ceballos" agro-industrial enterprise, which aimed at the development of plantations, involving not only state entities and cooperatives, but also independent peasants from all municipalities in the province.

Replacing lower-yielding cultivars with better ones is a difficult task, considering that pineapple is one of the high-density fruit trees, about 62,000 propagules per hectare for 'MD-2' and, at the same time , it is the one that produces the least propagules naturally (4). The need to introduce this new pineapple variety into Cuban plantations requires high seed production in the shortest time possible to cover the area, which can be achieved with the efficient use of micropropagation techniques. This new variety was introduced in Cuba from Costa Rica by the Bioplant Center since 2005 and approximately 1 million vitroplants have been commercialized to Ghana (Africa), from that same year to 2008. Under the climatic conditions of Cuba this hybrid has presented a good adaptation, reason why the national extension of planting has been increased since 2009 by the "Ceballos" Agroindustrial Company, of Ciego de Ávila, which has allowed him to export fresh fruit to the European market. However, in vitroplants has not been able to implement a methodology that allows the establishment of these in the field and evaluate the complete cycle of the crop (until the fruits), an objective that will be treated in this work.

[A] FAOSTAT. *Helping to Build a World without Hunger* [en línea]. Food and Agriculture Organization of the United Stated Nations, 2013, [Consultado: 1 de febrero de 2016], Disponible en: <http://www.ljemail.org/reference/ReferencesPapers.aspx?ReferenceID=1627280>.

MATERIALS AND METHODS

Researchers at the Bioplants Center have developed a novel micropropagation protocol, based on the use of the liquid medium and the technology of temporary immersion, coupled with the implementation of a semi-automated system, which makes it possible to reduce the time needed to generate sufficient amounts of vitroplants for the creation of basic seed banks that allow the promotion of pineapple plantations with quality seed (Figure 1) (5, 6).

Figure 1. General process of micropropagation of pineapple MD-2

Six-month-old MD-2 pineapple seedlings, which had already completed the cycle of acclimatization, nursery and sun-hardening phases were homogeneously selected to be planted under field conditions. The morphological characteristics of the seedlings are shown in Table I. In May 2012 the seedlings were transferred to the farm “Los Rabelos”, where they continued the hardening process (natural environment conditions) for another 15 days and at the end of this month and beginning of June, 5,000 vitroplants of pineapple MD-2. The conditions for transplantation of vitroplants were adjusted to 25,0 kg of decomposed cattle manure + 12,5 kg of crystalline NPK complex fertilizer (Haifa Chemicals Ltd., Haifa Bay 26120, Israel) at Bottom of the stonemason of 100 m long. Subsequent to these applications light irrigation was performed before planting. The planting distance used was 0,40 cm between rows and 0,30 cm between plants, at the rate of 55 000 plants per hectare, as established for this cultivar. Before the plantation an analysis of the chemical properties of the soil was carried out and the result showed that it meets the characteristics of the typical Red Ferralitic soils of the area and that are acts to produce the pineapple crop (Table II).

The results of analyzes show that the soil where the MD-2 pineapple vitroplants were planted complies with the chemical characteristics demanded by this crop. The pH is between 5,5 and 6,5,good availability of potassium (K_2O); Relatively low phosphorus (P_2O_5) content since the optimum range is 10 to 50; However, this element is corrected with the background application of 5 g per plant. On the second day of planting, a foliar application of a fungicide (Mancozeb 2 kg ha^{-1}) was carried out for the protection of vitroplants against possible fungal diseases (*Phytohpthora* sp.) as there was high soil moisture in the soil due to consecutive rains.

Determination of morphological indicators of plant quality

The evaluations of the morphological indicators were performed every 30 days and were the percentage of survival (%), the number of leaves, the plant length (cm), the ¨D¨ leaf length (cm), ¨D¨ Leaf width (cm), number of roots and the plant fresh mass (g).

Table II. Chemical properties of soil

pH	P_2O_5	K_20
5,94	7,50	60,36

The analyzes were carried out in the Provincial Laboratory of Soils of the Agriculture Ministry (MINAG) in Ciego de Avila province of, according to NR. 279, 1987. MINAG)

Floral induction

At 17 months flowering was induced at night (9:00 a.m. to 10:00 p.m.). Each plant received 50 mL of the final solution applied to the center of the plant rosette. The induction solution was prepared at 1 ha: Ethrel® 480 (4.0 L^{-1}) + urea (30 kg) and calcium carbonate ($CaCO_3$) 2 kg. The sprays were performed with a MATABY backpack with a capacity of 16 L, previously calibrated according to the floral induction treatments. The efficiency of the flowering inducer was evaluated by visual counting of inflorescences in the center of the plant leaf rosette, when white coloration was observed at 40, 45 and 50 days after the floral induction treatment (TIF). Statistical analysis of the results was carried out using the “STATGRAPHICS Plus” utility (7). The standard distribution (Kolmogorov-Smirnov, $P < 0.05$) and the homogeneity of the variances (Levene, $P < 0.01$) 05).

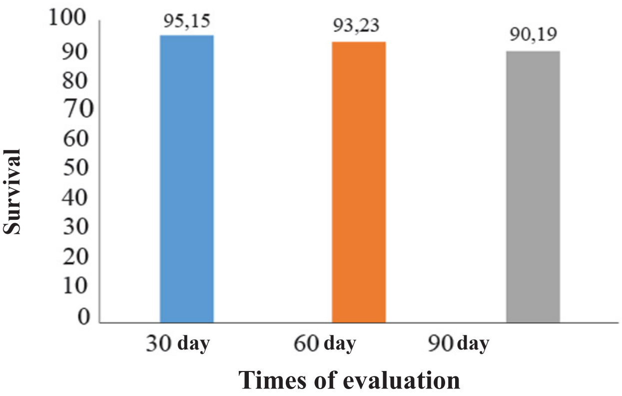
RESULTS AND DISCUSSION

In the evaluation of the mortality performed at 30 days after planting date, only 242 dead plants (4,85 %) had been quantified for various causes (*Phytophthora* ssp., soil at the heart of the plant and), suggesting that the phytosanitary treatments used at these early stages were efficient, as demonstrated by the 95,15 % survival achieved by the seedlings at these stressful moments (Figure 2). When this variable was evaluated at 60 and 90 days after the plants were in production conditions and in the natural environment, it was observed that the survival rates (93,23 and 90,19 %, respectively) were still high and without significant differences between the moments of evaluation. These results indicate the rapid and favorable adaptation of pineapple seedlings when subjected to new and stressful environmental conditions.

Table I. Morphological variables evaluated to the seedlings in the transfer from nursery to field conditions

Age	Number of leaves	Number of roots	Root length (cm)	Leaf length ¨D¨ (cm)	Width leaf ¨D¨ (cm)	Plant length (cm)	Dry mass (g)
6 months	15,05	18,85	17,89	21,65	2,11	23,91	27,78

Each data represents the mean of de n=40



The data were transformed according to y = 2arcosen (y / 100) 0,5 each data represents the mean for n = 20

Figure 2. Behavior of seedling survival during the first 90 days under field conditions

From the moment they exposed the seedlings to the drastic natural environment conditions (greater intensity of light and temperature), they presented a whitish appearance, similar to the typical burns of this species (8), although in this case they did not cause death. Yellowing was also observed in the leaves of the seedlings proper to the denaturation of chlorophylls, symptoms of the photo inhibition process (9). Although a rapid recovery of these symptoms could be observed in the subsequent evaluation, indicating the early adaptation and rapid recovery to the characteristic environmental changes in CAM plants (10, 11).

Although there have been some deaths, a very good population and adequate vegetative development of the crop can be observed. Up to now, two applications of bottom fertilizer (NPK) have been applied in the leaves and three fungal products have been applied to prevent the onset of fungal diseases (Mancozeb, Aliette and Ridomil). The irrigation was indicated with a frequency of 30 minutes every two or three days, if the crop demands it and in coordination with the farmer, it was agreed to apply foliar fertilizer weekly to half of the recommended dose and when the seedlings fulfilled 45 days of planted.

It is widely recognized that soils with pH higher than 6,5 plants do not fix iron, there is a greater presence of Phytophthora spp., which reproduce rapidly in the same and do more damage to the crop, when climatic conditions increase soil moisture and the temperature, it is therefore the need to make applications of fungicides to prevent the presence of the same and to make applications of foliar fertilizers (12). These results are encouraging if one takes into account that this is a very stressful stage for seedlings due to sudden environmental changes, soil and the risk to which they are exposed, among others. After reaching high percentages of survival in the seedlings, it is necessary to evaluate the behavior of growth variables, in order to know if the agricultural technique used is adequate and to be able to correct them in time to reach dynamic growth rates (Figure 3).

In the figure it can be seen that the seedlings at 45 days remain green, there is no great damage from burns and the emission of a new and autotrophic foliage is observed, allowing a greater growth. Table III shows the increases of the morphological variables evaluated in the seedlings under field conditions.

Figure 3. Development of the crop after 45 days of planting the vitroplants of pineapple MD-2

Table III. Development of the morphological variables evaluated during the stay of the pineapple seedlings under field conditions

Age	Number of leaves	Number of roots	Root length (cm)	¨D¨ leaf length (cm)	¨D¨ leaf width (cm)	Plant dry mass (g)	Plant length (cm)
60 days	16,15 f	23,41 f	18,03 g	24,72 g	2,82 ef	35,16 f	26,11 f
90	20,40 ef	28,70 e	22,84 g	29,38 g	3,54 e	62,31 f	31,22 ef
120	26,52 e	33,21 def	31,53 f	37,29 f	3,89 de	163,61 f	43,21 de
150	32,81 d	38,16 cde	43,11 e	49,82 e	4,12 d	225,31 e	49,12 d
180	40,20 c	43,67 cd	59,35 d	62,16 cd	4,53 cd	376,81 de	59,60 cd
210	45,12 bc	48,35 c	63,54 cd	68,81 cd	4,78 c	563,81 d	63,58 c
240	49,37 b	54,31 bc	68,32 bc	74,63 bc	5,12 b	1325,75 c	79,92 b
270	52,82 ab	62,87 ab	72,92 ab	83,49 b	5,63 ab	2359,28 b	89,71 b
300	55,41 a	71,41 a	75,41 a	97,22 a	5,86 a	3259,32 a	102,3 a
ES X	3,25	5,12	3,16	4,74	0,73	155,16	5,81
CV	7,21	6,54	7,75	9,32	0,75	52,21	8,42

Means with different letters in columns indicate significance (simple Anova and Tukey test (p <0.05) Each data represents the mean for n = 20

In the evaluation carried out at 90 days of planting the seedlings under field conditions, no statistical differences were observed in most of the variables, with respect to the 60 days, only in the number of roots were quantified increases that mark statistical differences. It is noteworthy that already since the evaluation carried out at 120 days a constant and significant increase of the fresh mass is observed, after the seedlings are able to adapt to the new environmental conditions; All variables related to length (roots, leaf ¨D¨ and plant) contributed significantly to the increase of this variable. Again the results show how the pineapple seedlings recovered quickly from the stress imposed by the change of environment and the conditions of edaphoclimatic soil transplants. It is well known that these changes cause stress in plants, expressed in terms of accumulation of ABA, proline and reactive oxygen species (13), so it is necessary to attenuate the negative effects of stressful conditions so that the plants maintain a constant growth and this is achieved with an adequate agrotechnical management.

The results obtained in this work have served as a basis for other peasants bordering the farm where the experiment is performed, they are motivated to introduce this new and promising hybrid in their productive farms. Already at this moment ,several farmers have been training and familiarizing with this crop, coming from vitroplants in the own farm “Los Rabelos”.

After 14 months of planting the crop and after a characterization of the plants in large, medium and small, the flowering of the plants was induced (Table IV).

As can be seen the plants considered as large differ statistically from the other categories, the values reached in these plants are in the ranges that have been achieved for this hybrid propagated from agile seed. Experimental results have correlated that plants with a fresh mass greater than 3 000 g achieve fruit with a weight of 2 000 g. No significant differences were observed between the medium and small plants in the fresh mass, leaf length and ¨D¨ leaf width. Table V shows the application effect of the flowering inducer on the classifications previously established in pineapple plants.

The results achieved reaffirm the role of Ethrel® 480 as an inducer of flowering in pineapple plants. However, the different behavior among the classifications studied could be related to the fact that in small plants the levels of ethylene needed to reach the threshold of induction are lower than in the larger plants. This is related to a spacial and temporal regulation of the ethylene in the plant tissue, the small ones perhaps had greater content of ethylene in the tissues and for that reason they flourished more easily in the evaluation realized at 45 days. Regarding planting material, the observed variations in relation to flowering are consequences of differences in the tenor of nutritional reserves and the physiological state of propagules. It has been observed that the mass of the propagule influenced decisively in the cycle of the plant. However, small plants have been observed that have responded satisfactorily to natural and artificial induction. It is recognized that floral induction in pineapple MD-2 is also achieved by different types of stress and small plants respond very easily to these (4).

Table IV. Development of morphological variables evaluated before floral induction of pineapple seedlings under field conditions

Plant material	Number leaves	Number roots	¨D¨ leaf fresh mass (g)	¨D¨ leaf length (cm)	¨D¨ leaf width (cm)	Plant length (cm)	Fresh mass (g)
large	57 a	72 a	113,39 a	93,2 a	5,9 a	105,3 a	3538 a
medium	42 b	47 b	56,70 b	69,9 b	4,4 b	77,2 b	1134 b
small	31 c	34 c	36,85 bc	59,8 bc	4,0 bc	68,7 bc	512 c
ES	0,23	0,81	12,97	5,66	0,18	5,12	62,8

means with different letters in columns indicate significance (simple Anova and Tukey test p <0.05)
Each data represents the mean for n = 10

Table V. Effect of the application of Ethrel® 480 on floral induction of pineapple at 40, 45 and 50 days after induction

Classifications	40 days	45 days	50 dayss
large	21,5 c	68,2 a	92,6 a
Medium	32,2 b	73,1 a	90,4 a
Small	42,7 a	79,4 a	81,9 b
ES	3,2	6,5	3,3

Means with different letters in columns indicate significance (simple Anova and Tukey test p <0.05)
Each data represents the mean for n = 40

In the experimental conditions tested, good flowering percentages were obtained at all evaluated moments, after the floral induction treatment with Ethrel® 480, with 92,6 % as the maximum value in plants classified as large at 50 days. This ensures a high homogeneity in the development of the inflorescence and the fruit, as well as a concentrated harvest. It has also been considered that an efficient floral induction should exceed 90 % of flowering plants (14).

The growth of the pineapple in its vegetative development goes through stages of slow growth until its physiological maturity is reached. Its cycle can be divided in three phases: the vegetative one that implies from the plantation to the floral differentiation; the reproductive (flowering fructification), which includes the floral differentiation until the ripening of the fruit and, finally, the propagation, which begins in the productive phase but continues after the fruit is harvested. Among these phases the less flexible is the reproductive, regardless of whether the flowering is natural or artificial.

Figure 4 shows the process of natural maturation of pineapple fruits produced by vitroplants according to the established categories.

The quality of the fruits includes the external and internal aspects of the fruits, so it is important to carry out a detailed analysis of the chemical and physical variables of these (15).

The results of the chemical analyzes performed on the MD-2 pineapple fruits at harvest time, according to the classifications established above, can be observed in Table VI.

Although differences in soluble solids concentrations according to the established categories are noteworthy in the values, they are low compared to the results obtained in MD-2 produced in Ghana (16). However, the fruits of the vitroplants exceeded the values in pH, ascorbic acid (vitamin C) and soluble solids content. The ascorbic acid content in the fruits is very important as it is recognized its influence on health, especially as an antioxidant agent in cellular metabolism. In MD-2 pineapple plants in Ghana, ascorbic acid values of 51,33 mg 100 mL of fruit juices have been found (16), which are lower than those found in this study (70,1 on average). These differences could be influenced by the climatic conditions and the agro-technology used in this experiment, which differs from those used in the production plantations established for this crop by other countries (Ghana, Costa Rica and Panama) (17) and the agribusiness company of Ceballos in Cuba.

In the fruits obtained in this experiment the maturation occurred naturally, the technique of fruit degreening was never used, although it is recognized that the fruit's translucency prevails over the soluble solids content to make the decision to degrease them, to homogenize the color of the fruits for the harvest. It has also been shown that the different colors in the pineapple fruits are closely related to the degree of maturity and the physicochemical properties of these in storage, showing large changes in sugar contents, pH and soluble solids (18, 19).

Figure 4. Natural ripening of the fruits of pineapple vitroplants MD-2

Tabla VI.Chemical variables evaluated in fruits obtained from vitroplants of pineapple MD-2

Evaluated variables	Large	Medium	Small
Content of soluble solid (ºBrix)	13,57	13,27	13,20
Ascorbic (mg 100 mL jugo)	74	68,6	67,7
Acidity (% m:m)	1,57	1,83	2,09
Solid soluble/acidity	8,53	7,25	6,6
Units of pH	3,7	3,48	3,54
Temperature (ºC)	22,6	22,0	22,1

The pH is the result of the biochemical changes that the fruit of pineapple undergoes during the period of maturation outside the plant, thinking conceptually, that as the pineapple matures, the pH increases, it tends to basic, therefore in the first days is within the range of acidity, due to the fact that ripening has been caused in the first instance by the stress of harvesting, and from the successive days tends to accelerate the process of maturation and future senescence of the fruit as a process (Table VII).

The results of experiments with vitroplants show that the fruits reach average values between 1,68, 1,52 and 1,03 kg, in correspondence with the established categories of large, medium and small. The size of the fruit is very important for the commercialization of pineapple. The final size of the fruit is controlled by internal development signals and modulated by environmental signals. The intrinsic mechanisms responsible for variation in organ size have not been well studied, but changes in cell number and size are the most important characteristics during fruit development, as they directly influence the final size of them (20). These ones average 77,5 tons per hectare if a planting density of 55,000 ha^{-1} plants is used.

The weight of the fruits decreases as the density increases, but the yield per hectare increases until reaching the maximum (21), since the yield is a function of the photosynthetic efficiency per unit area of leaf (22, 23). These same authors mention that the photosynthetic efficiency at the time of floral induction influences significantly the yield. The shape of the fruit is an important quality feature for the three types of existing market. In the national fresh market, the very cylindrical fruit is less appreciated and distances itself from the characteristics of the variety MD-2, which makes for the international market is the so-called rejection fruit and it is not exported. However, in the case of the industrial market this cylindrical pineapple has a higher yield in slices, which means that this important production is not lost (8).

In Table VIII it is possible to observe the results obtained with the physical analyzes realized to the crowns of the fruits.

The results show that there were no deformations in the crowns and that they met the parameters established in the fruits of MD-2. It has been found that when the established subcultures are not met, multicore or mismatch can occur in field conditions. Aspects that were not observed in vitroplants during the stay in the field.

Table VII. Results of the physical analyzes performed on fruits of pineapple MD-2 at the time of harvest

Evaluated variables	Large	Medium	Small
Form	Barrel	Barrel	Cylindrical
Length (cm)	15,15	14,15	11,53
Thickness bark	0,15	0,4	0,5
Upper diameter (cm)	11	10,6	10
Lower diameter (cm)	11,1	11,95	10,53
Cylindricity Index	0,99	1,78	0,95
Depth of eyes	0,35	0,4	0,33
Mass without crown (kg)	1,52	1,34	0,89
Mass with crown (kg)	1,68	1,52	1,03
External color	Orange	Orange	Orange
Internal color	Yellow	Light yellow	Yellow
Number of eyes	93,5	93	77,5
Number of spirals	11,5	10	10
Number of eyes greater spiral	12	11,5	10,3
Spiral orientation	2,5	2,4	2,6

Table VIII. Results of the physical analyzes performed on the crowns of fruits of pineapple MD-2 at the time of harvest

Evaluated variables	Large	Medium	Small
Mass (g)	0,17	0,16	0,15
Lenght (cm)	18,5	17,45	15,45
Ratio fruit lenght/crown	0,83	0,84	0,75
Multi-derforming	NO	NO	NO
Multi-crown	NO	NO	NO
Single crown	SI	SI	SI

The results indicate that fruits obtained from vitroplants have the same chemical and physical characteristics as pineapple fruits MD-2 achieved by conventional propagation

CONCLUSIONS

- Agrotechnical management has allowed to reach high survival rates (95 %) in pineapple MD-2plants.
- The morphological characteristics of the vitroplants delivered, together with the agrotechnical management established with the peasants, allowed a good adaptation to the field conditions, which motivated the increase in the time of all variables evaluated and a suitable flowering and fruiting.
- The fruits maintain the organoleptic characteristics established for this hybrid, demonstrated by the bromatological results made to them, under the conditions tested.

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Note:

During the editing process it was not possible to access the work of retouching and improvement of images, so they have been inserted with the same quality as the ones sent by their authors.

The editorial

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