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Original article

Morphological changes in *in vitro* propagated pineapple varieties ('MD-2' and 'Champaka F-153') during the growth phase under field conditions

Cambios morfológicos en variedades de piña propagadas *in vitro* (´MD-2´y ´Champaka F-153´) durante la fase crecimiento en condiciones de campo

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ABSTRACT: *In vitro* multiplication is a feasible technique for the accelerated propagation of pineapple (*Ananas comosus* var. *comosus*), particularly when introducing new varieties at a commercial scale. This study was conducted at the "Pineapple Production" Base Business Unit, part of the Ceballos Agroindustrial Company, to compare the field performance of two micropropagated pineapple varieties, hybrid 'MD-2' and 'Champaka F-153', under the edaphoclimatic conditions of Ciego de Ávila, Cuba. Plants were established at a density of 68,000 ha^{-1} in double rows on raised beds covered with plastic mulch. A randomized complete block design with three replications per treatment was used. Every 90 days, 20 homogeneous plants per plot were randomly selected and evaluated for: survival rate, plant fresh mass, plant length, number of roots, number of leaves, and fresh mass, length, and width of the "D" leaf. Results indicated that 'Champaka F-153' reached 99 % survival after 90 days, while 'MD-2' reached 96 %. During this period, fresh mass increased by 226 g in 'Champaka F-153' and 156 g in 'MD-2', with significant differences between varieties. However, at 270 days, no significant differences were observed in the number of leaves, the number of roots, or the fresh mass of the "D" leaf. These findings suggest initial differences in adaptation and growth that tend to level off during later vegetative stages.

Key words: Adaptation, micropropagation, morphology, pineapple, vitroplants.

RESUMEN: La micropropagación constituye una alternativa viable para la multiplicación acelerada piña (*Ananas comosus* var. *comosus*), especialmente cuando se requiere introducir nuevas variedades a escala productiva. El presente estudio se desarrolló en la Empresa Agroindustrial Ceballos, con el objetivo de comparar el crecimiento en campo de dos variedades de piña micropropagadas (el híbrido ´MD-2´ y ´Champaka F-153´) bajo condiciones edafoclimáticas de Ciego de Ávila, Cuba. Las plantas se establecieron a una densidad de 68 000 plantas ha^{-1} en canteros cubiertos con manta plástica y dispuestos en doble hilera. Se empleó un diseño experimental de bloques al aza con tres réplicas por tratamiento. Cada 90 días se evaluaron 20 plantas homogéneas seleccionadas aleatoriamente por parcelas: Las variables medidas incluyeron: porcentaje de supervivencia (%), masa fresca de la planta (kg), longitud de la planta (cm), número de raíces, número de hojas emitidas, masa fresca de la hoja "D" (g), longitud de la hoja "D" (cm), ancho de la hoja "D" (cm). Los resultados mostraron que ´Champaka F-153´ alcanzó un 99 % de supervivencia a los 90 días, mientras que ´MD-2´ alcanzó un 96%. Durante este periodo, la masa fresca ´Champaka F-153´ se incrementó en 226 g, frente a 156 g en ´MD-2´ con diferencias significativas entre ambas. No obstante, a los 270 días no se observaron diferencias significativas en el número de hojas, número de raíces y masa fresca de la hoja ¨D¨. Estos resultados evidencian diferencias iniciales en la capacidad de adaptación y crecimiento, las cuales tiende a atenuarse durante etapas avanzadas del ciclo vegetativo.

Palabras clave: Adaptación, micropropagación, morfología, piña, vitroplantas.

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INTRODUCTION

Pineapple (*Ananas comosus* var. comosus) is the most economically important species within the Bromeliaceae family. It contributes more than 20 % of the global volume of tropical fruits, ranking only behind banana and mango. Currently, the 'MD-2' hybrid is the most widely marketed and demanded variety for fresh consumption internationally (1). Its introduction transformed the global pineapple market, increasing per capita consumption in the United States and Europe, and required the development of logistic and technological strategies to meet the growing demand (2).

'MD-2' is characterized by its high yield potential (up to 100 t ha^{-1}) and superior organoleptic attributes. However, it has greater susceptibility to fungal diseases and requires more intensive agronomic management than varieties such as 'Cayena lisa' or 'Champaka F-153', which increases its production costs, although these are offset by its better market price (2). In the Cuban context, the need to diversify the cultivated varieties is recognized as part of a sustainable production strategy (3,4).

'Champaka F-153', a selection of 'Cayena lisa', is distinguished by its vigor, spineless leaves except at the apex, intense dark green color with reddish hues, and low offshoot emission, which favors the development of higher quality fruits. Under optimal conditions, it can achieve yields close to 100 t ha^{-1} with densities of 60 000 plants ha^{-1} (5,6).

In Cuba, pineapple production was historically based on 'Española roja' and, to a lesser extent, on 'Cayena lisa Serrana', genotypes well adapted to the country's agroecological conditions (7). However, during the 1990s, the reduction of cultivated areas and the consequent shortage of offshoots severely limited production (8,9).

From 2008 onwards, the Ceballos Agroindustrial Enterprise led a program for the recovery of the crop in Ciego de Ávila, as part of a national strategy. In 2009, the 'MD-2' hybrid (Gold Extra Sweet) was introduced from Costa Rica, and its adaptation to local conditions proved successful. Nevertheless, the availability of quality planting material remains the main limitation for the expansion of 'MD-2' and 'Champaka F-153' (10).

In 2019, the Center for Plant Biotechnology of the University of Ciego de Ávila initiated a technological innovation project aimed at standardizing the micropropagation of both varieties, in order to establish basic seed banks at the UEB "Producción de Piña" (10). The objective of this work was to characterize the morphological changes of two *in vitro* propagated varieties ('MD-2' and 'Champaka F-153') during their vegetative growth cycle under field conditions.

MATERIALS AND METHODS

Here is the English translation of the provided text, maintaining a formal, academic tone. Personal pronouns have been avoided where possible (e.g., using passive constructions or impersonal phrasing).

The study was conducted at the Basic Business Unit (UEB, according its acronyms in Spanish) "Producción de piña" (21°47'N, 78°48'W), belonging to the Ceballos Agroindustrial Enterprise. Micropropagated plants of pineapple (*Ananas comosus* var. comosus) hybrid 'MD-2' and 'Champaka F-153' were used, obtained from the Scale-up and Technology Transfer Unit of the Center for Plant Biotechnology, after 6.5 months of acclimatization and hardening.

The soil of the experimental area was classified as Typical Red Ferrallitic. Before planting, a chemical analysis was performed (Table 1) according to the standards of the National Institute of Soils and Fertilizers (NR. 279, 1987). Crop management followed the protocol established by the UEB for micropropagated plants, with modifications to the fertilization regime. During the vegetative cycle, total doses of 615, 310, 550, 38.25, and 0.55 kg ha^{-1} of N, P, K, Mg, and Ca, respectively, were applied, as well as micronutrients: Fe (1.50), Mn (6.28), Zn (1.80), B (0.57), Cu (0.17), and Mo (0.08 kg ha^{-1}). Foliar fertilization was carried out with a CLAXON sprayer (76 nozzles, 3200 L capacity). Sprinkler irrigation was performed using a center-pivot system (Western, TUSA SA).

Experimental design and treatments

A randomized block design with a 2 × 4 factorial arrangement (two varieties and four evaluation times) was used. Each treatment was replicated in three plots of 13 × 6.70 m (six double-row furrows covered with 90 µm plastic nylon), planted in April 2019 at a density of 58,600 plants ha^{-1}.

Variables evaluated

Twenty homogeneous plants randomly selected per plot were evaluated at 0, 90, 180, and 270 days after planting (dap). The recorded variables were: survival (%), plant fresh mass (g), plant length (cm), number of leaves, number of roots, fresh mass of leaf "D" (g), length of leaf "D" (cm), and width of leaf "D" (cm). Additionally, the absolute growth rate (AGR) was calculated: $AGR = (FM_2 - FM_1) / (t_2 - t_1)$, where FM is fresh mass and t is time.

Statistical analysis

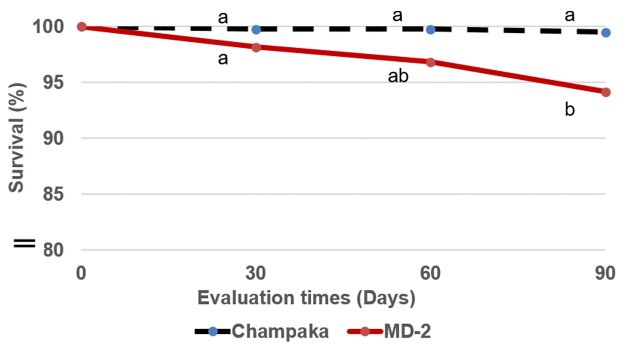
Data were analyzed using STATISTIC 8.0 software. Normality (Kolmogorov-Smirnov, $P \leq 0.05$) and homogeneity of variances (Levene, $P \leq 0.05$) assumptions were verified. A factorial ANOVA and Tukey's test ($P \leq 0.05$) were applied for mean comparison. Survival percentages were transformed using $y' = 2 \arcsin (y/100)^{0.5}$.

Table 1. Chemical characteristics of the soil in the experimental area

K_2O (mg 100^{-1} g S.)	P_2O_5 (mg 100^{-1} g S.)	M.O (%)	Mg (Cmol kg^{-1})	Ca (Cmol kg^{-1})	pH (KCl)
29.40	15.0	2.46	2.27	2.86	4.5

RESULTS AND DISCUSSION

La Figura 1 muestra el comportamiento de la supervivencia de las dos variedades en los primeros 90 días luego de su plantación en condiciones de campo.



Means with different letters indicate significance (factorial ANOVA, Tukey P≤0.05). Data were transformed according to y' = 2 arcsin (y/100)^0.5. Each data point represents the mean for n=20

Figure 1. Survival behavior (%) of 'MD-2' and 'Champaka F-153' pineapple plants during the first 90 days after planting under field conditions

At 90 days after planting (dap), 'Champaka F-153' showed significantly higher survival (99.5 %) than 'MD-2' (94.1 %) (Figure 1). However, no significant differences were detected at previous evaluation times (30 and 60 dap). These results indicate a rapid adaptation capacity of both varieties to conditions of high radiation and water stress, although with an advantage for 'Champaka F-153' in the final phase of the establishment period. The survival values exceed those reported for 'MD-2' (90.19 %) (7), which could be attributed to the larger size of the plants at the time of transplanting (11).

The yellowish coloration observed during the first months, more pronounced in 'Champaka F-153', is indicative of photoinhibition, probably associated with high solar radiation and temperature. These symptoms gradually decreased, suggesting an effective recovery of the photosynthetic systems and the activation of constitutive CAM metabolism (12,13). Damage to photosystems caused by high radiation triggers the production of reactive oxygen species. These species damage protein structures, cell membranes, and cellular components such as DNA and RNA (14-16).

The first 90 days are crucial for the adaptation of plants to the new and stressful environmental conditions to which micropropagated plants are transferred to field conditions. Table 2 shows the development achieved by both varieties during the first 90 days.

During the first 90 days (dap), 'Champaka F-153' showed significantly higher increases than 'MD-2' in fresh mass (226 vs. 156 g), plant length, number of leaves, and number of roots (Table 2). No differences were observed in root length. The AGR was 2.5 g day^{-1} for 'Champaka F-153' and 1.7 g day^{-1} for 'MD-2'. These results confirm a greater initial vigor of 'Champaka F-153' under field conditions (17).

The results demonstrate that the variety 'Champaka F-153' has a higher growth rate than 'MD-2' during the first 90 days after transfer of the plants to field conditions. This seems to indicate that it accumulates greater reserves, based on the emission of new organs, which favors increasing survival levels (Figure 1).

In Figure 2, the development of 'MD-2' and 'Champaka F-153' plants under field conditions can be observed.

Figure 2. 'MD-2' (top) and 'Champaka F-153' (bottom) plants during the growth process in the field at 90 days (left) and 180 days (right)

In the Figure 2, the typical green coloration can be observed on the leaves of 'MD-2' (top), while a green-reddish color is seen on the leaves of 'Champaka F-153' (bottom). From the moment the plantlets were exposed to the drastic conditions of the natural environment (higher light intensity and temperature), these typical appearances of both varieties were present (2,18).

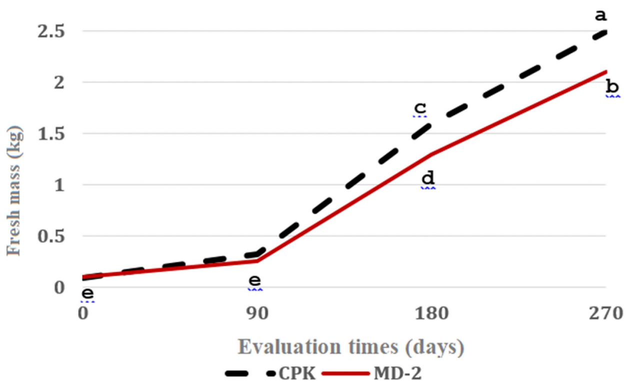
Table 2. Behavior of some morphological variables of 'MD-2' and 'Champaka F-153' (CPK) plants during the first 90 days after planting in the field

Evaluation times	Fresh mass (g)		Plant length (cm)		Number of leaves		Number of roots		Root length (cm)	
	CPK	MD-2	CPK	MD-2	CPK	MD-2	CPK	MD-2	CPK	MD-2
Sowing	96.0 c	104.5 c	33.0 b	34.0 b	26.0 b	23.5 c	12.8 c	14.2 c	28.2 b	38.5 a
90 days	322.0 a	261.0 b	39.9 a	34.6 b	29.6 a	26.9 b	51.3 a	35.3 b	34.4 a	34.6 a
SE.	35.8		1.3		0.7		4.8		3.2	

For each variable, different letters indicate significant differences according to Student's t-test. Each data point represents the mean for n = 20. SE = Standard error

A slight yellowish discoloration of the leaves was also observed, typical of chlorophyll denaturation, as symptoms of photoinhibition. However, a rapid recovery from these symptoms could be seen in subsequent evaluations, indicating a quick adaptation and fast recovery to the environmental changes characteristic of CAM plants (19). On the other hand, for ‘Champaka F-153’, the leaves were shown to be thicker and more drooping than those of ‘MD-2’, which tend to be narrower and longer and with a more vertical position relative to the central axis of the plant (2,18).

Figure 3 shows the behavior of fresh mass of the plants during their development under field conditions.



Means with different letters indicate significance (factorial ANOVA, Tukey P ≤ 0.05). Each data point represents the mean for n = 20

Figure 3. Fresh mass behavior of ‘MD-2’ and ‘Champaka F-153’ (CPK) plants during the 270 days after planting under field conditions

The results show that during the first 90 days, although a slight increase in fresh mass occurred, no significant differences were observed between the two varieties. However, at the evaluation carried out at 180 days, significant differences were seen between both varieties and with respect to the previous evaluation, with values of 1.27 kg for ‘Champaka F-153’ and 1.03 kg for ‘MD-2’ in just 90 days. At the end of the vegetative phase (270 days), significant differences were observed between both varieties, as well as with all previous evaluation times; the mass increases at this stage were 900 g and 800 g for ‘Champaka F-153’ and ‘MD-2’, respectively (20).

From planting time to just before artificial flowering induction, the variety ‘Champaka F-153’ increased by 2.49 kg, while ‘MD-2’ achieved 2.09 kg, with a difference of 400 g during the same period under the same environmental and agronomic conditions. These results confirm that during the first three months, pineapple plants are adapting to the new conditions where they were planted and must develop new organs (leaves and roots), so the plant depends on previously accumulated reserves; after this new leaf and root emission, they achieve exponential growth of fresh mass (7,17).

However, when the Absolute Growth Rate (AGR) was analyzed during the last 90 days in the field (180 to 270 dap), it was shown that ‘Champaka F-153’ plants reach 10 grams per day, while ‘MD-2’ reaches 8.8 grams (a daily difference of 1.2 g). These results demonstrate that in vitro propagated ‘Champaka F-153’ plants have a higher growth rate than ‘MD-2’. These results differ from those reported in the literature, where it is stated that the ‘MD-2’ plant reaches the weight for induction at least one month ahead of other commercial varieties (2,21).

Table 3 shows the behavior over time of some morphological variables evaluated in the plants from planting until just before artificial flowering induction (270 days).

At 270 days after planting (dap), ‘Champaka F-153’ significantly surpassed ‘MD-2’ in plant length (97.7 vs. 77.3 cm), although no differences were observed in number of leaves, number of roots, or fresh mass of leaf “D” (Table 3). This latter variable did not reach the recommended values for floral induction (70-80 g), suggesting that environmental or management conditions may have limited reserve accumulation (12).

It appears that the increases in fresh mass of ‘Champaka F-153’ plants are related to the higher values achieved in plant length (20.4 cm) and number of leaves produced. The greater fresh mass found in leaf “D” may be associated with a greater development of reserve tissues in its leaves under the same growing conditions, since ‘MD-2’ is less rustic than ‘Champaka F-153’.

It is well known that pineapple plantlet growth is slow, due, among other causes, to their Crassulacean Acid Metabolism (CAM) (22). Their metabolism can operate as “inducible CAM” or “facultative CAM” under in vitro conditions and during acclimatization stages (19,23), but under field conditions, pineapple plants behave as “constitutive CAM” or

Table 3. Behavior of some morphological variables of ‘MD-2’ and ‘Champaka F-153’ (CPK) plants from planting time up to 270 days in the field

Evaluation moment	Plant length (cm)		Number of leaves		Fresh mass of leaf "D" (g)		Number of roots	
	CPK	MD-2	CPK	MD-2	CPK	MD-2	CPK	MD-2
Planting	33.0 d	34.0 d	26.0 bc	23.5 c	-	-	12.8 d	14.2 d
90 days	39.9 d	34.6 d	29.6 b	26.9 bc	21.8 d	20.3 d	51.3 b	35.3 c
180 days	61.6 c	63.1 c	31.6 b	28.1 b	33.3 c	31.2 c	58.2 a	61.3 a
270 days	97.7 a	77.3 b	50.4 a	48.9 a	55.0 a	58.0 a	58.5 a	57.6 a
SE	10.5		2.6		3.1		3.6	

For each variable, different letters indicate significant differences (factorial ANOVA, Tukey P ≤ 0.05). Each data point represents the mean for n = 20. SE = Standard error "obligate CAM". These variations mean that their growth and development behavior is closely related to the agronomic and cultural practices applied to the plants under field conditions (24,25). However, if the conditions where the crop develops are favorable (irrigation, fertilization, health, temperature, etc.), they can behave as facultative and inducible plants (26). This metabolism will stimulate greater growth in pineapple plants and thereby reduce production time, which may be occurring with these varieties under the conditions where the experiment was conducted.

CONCLUSIONS

- Micropropagated plants of 'MD-2' and 'Champaka F-153' showed high survival percentages under field conditions, although 'Champaka F-153' presented significantly higher values at 90 days.
- During this phase, 'Champaka F-153' showed faster growth, reflected in greater increases in fresh mass, number of leaves, and number of roots. However, at 270 days, the morphological differences between the two varieties diminished, with no significant differences detected in leaf number, root number, or fresh mass of leaf "D".
- These results suggest that, despite a slower initial establishment, 'MD-2' has the ability to compensate during advanced stages of the vegetative cycle. Continuation of the evaluation until harvest is recommended to determine the impact of these differences on fruit yield and quality.

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