Clonal evaluation and recurrent flowering of ornamental pineapple hybrid for use as miniature potted plant

Avaliação clonal e florescimento recorrente de híbrido ornamental de abacaxi para uso como planta miniaturizada em vaso

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ABSTRACT - The objective of this study was to evaluate the cloning and recurrent flowering of the miniature pineapple hybrid ORN-MUT based on quantitative and qualitative morphological descriptors for possible use as a potted plant. Besides its reduced size, the ORN-MUT hybrid is notable for its pink fruit, absence of thorns and recurrent flowering at the crown, formed after the plant’s complete cycle (562 days). The hybrid was assessed in the experimental field of the Embrapa Cassava and Fruits research unit in two flowering stages (first flowering and recurrent flowering) and was characterized by quantitative and qualitative morphological descriptors. A joint analysis of the data (multivariate analysis) was performed to verify the distinguishability and uniformity of the clones and parents. ORN-MUT presented uniformity in the first flowering, completing the phenological cycle at 75.70 days (first cycle) and 72.35 days (second cycle) after floral induction, respectively. The recurrent flowering occurred 170.60 days after induction. The peduncle is straight, with average length of 17.80 cm. The syncarp is small and pink, with conical-cylindrical shape. The crown has a moderately sharp tip with greenish central color and pink edges. In the recurrent flowering stage, the plants are smaller, with average size of 24.45 cm. The syncarp is miniaturized, with average of 1.20 layers of fruitlets and length of 1.61 cm. The quantitative and qualitative morphological traits allow classifying the hybrid as a potential potted plant.

Key words: Ananas comosus (L.) Merrill. Morphological characterization. Phenology. Floriculture.
INTRODUCTION

The pineapple plant [Ananas comosus (L.) Merrill], belonging to the Bromeliaceae family, is native to tropical and subtropical regions of the Americas (PALMA-SILVA et al., 2016). Its fruit has a peculiar shape, including an exuberant crown that has led to the moniker “king of fruits”. It is popular throughout the world, both fresh and in processed foods (CRESTANI et al., 2010).

In recent years, some pineapple varieties (the standouts being Ananas comosus var. erectifolius, A. comosus var. bracteatus and A. comosus var. microstachys) are being used in a genetic improvement program for development of ornamental cultivars (COSTA JUNIOR et al., 2016; SOUZA et al., 2012, 2014). The new hybrids stand out for their durabilty, beauty, originality and exuberance, and can be used in floral arrangements, for landscaping of parks and gardens, to produce cut flowers and mini-fruits, and as potted plants (SOUZA et al., 2014).

The varieties for growth in pots must be compact, meaning small fruits, leaves and stems and architecture based on open growth habit. Nevertheless, considering the characteristics of the botanical varieties, attaining these traits, whether in pineapple germplasm or improved progenies, is not easy, since pineapple plants can often reach one meter in height. Therefore, the adjustment of a pineapple variety for use as a potted plant has been carried out in the production system, which is complex, laborious and relatively slow (SOUZA et al., 2012).

Pineapple hybrids suitable for landscaping and producing cut flowers and mini-fruits have been developed and commercially launched, but plants for growth in pots are still under development (SOUZA et al., 2012, 2014). According to Souza et al. (2014), two hybrids resulting from crossing A. comosus var. erectifolius and A. comosus var. microstachys have reduced height and leaf length and width and can be selected as potted plants. However, these hybrids, despite having traits suitable for cultivation in pots, still require many adjustments in their production system, principally the need for further size reduction (TANIGUCHI et al., 2015).

On the other hand, among the progenies evaluated, an ornamental pineapple called ORN-MUT stood out for being moderately compact, with an attractive pink fruit and no thorns. However, besides these characteristics, this hybrid presents the trait called recurrent flowering at the top of the fruit crown (syncarp), which occurs after the plant’s complete cycle. The result of this phenomenon is a “new plant” that is very small and compact, formed by a new floral stem and infructescence emerging from the mother plant. The clonal evaluation of this genotype is a fundamental step to confirm the morphological traits selected and obtain a differentiated product suitable for the flower segment, i.e., a miniature pineapple plant suitable for growth in pots. The tests of ornamental pineapple plant in Brazil are typically based on official morphological descriptors (DOU 02/2013). The characteristics evaluated during the vegetative and reproductive cycle of the species encompass the growth habit; height, length and width of the main plant, fruits and crown; and color of the different structures of the cultivar, these evaluated based on the catalog of colors of the Royal Horticultural Society (BRASIL, 2013).

Therefore, the objective of this study was to evaluate the cloning and phenology as well as the recurrent flowering of the miniature pineapple hybrid ORN-MUT based on quantitative and qualitative morphological descriptors, for its use as a potted plant.

MATERIALS AND METHODS

The study was conducted in the experimental field of the Embrapa Cassava and Fruits research unit (Embrapa Mandioca e Fruticultura), located in the municipality of Cruz das Almas, Bahia, Brazil. The climate in the region is transition between Am and Aw zones according to the Köppen classification (KÖPPEN, 1936), with average annual rainfall of 1,143 mm, average temperature of 24.28 °C and relative humidity of 60.47%. The soil of the experimental area is a typical dystrophic Yellow Latosol, A moderate, sandy clay loam texture, kaolinite, hypoferic, transition zone between subperennial and semideciduous rainforest, with slope of 0–3%. Weather data were obtained from an agrometeorological station at Embrapa Cassava and Fruits. Climate data (rainfall and average, minimum and maximum air temperatures) referring between Jan 2015 and Feb 2017 are shown in Figure 1.

The miniature hybrid denominated ORN-MUT is the result of crossing A. comosus var. bracteatus X A. comosus var. erectifolius. The evaluation of the clones was carried out simultaneously with the parents in side-by-side competition, in two phases (first flowering and recurrent flowering), with 20 repetition (1 repetition = 1 plant).

After cultivation of the seedlings, irrigation and phytotechnical and phytosanitary treatments were performed as necessary, during all phases of the clonal evaluation. Twelve months after initial planting, flowering was induced with Ethrel 2400® (ethephon) at 500 ppm of the active ingredient plus 3% urea by spraying on the rosette (SOUZA et al., 2009). No inducement was performed for evaluation of recurrent flowering, since this was expected to occur naturally. The recurrent flowering is the emergence of the new inflorescence on the crown that is unusual in pineapple. The
following phenological variables were evaluated: emergence of the floral bud (number of days after induction); opening of the first flower (number of days after emergence of the floral bud); and closure of the last flower (number of days after emergence of the floral bud), separately for the first flowering phase and the recurrent flowering phase. At the end, these variables were recorded considering the complete cycle, from planting to closure of the last flower, according to the criterion established by Souza et al. (2012).

For clonal evaluation, nine quantitative descriptors were used: plant height (cm); length of leaf D (cm); width of leaf D (cm); length of the peduncle (cm); diameter of the peduncle (cm); length of the syncarp (cm); diameter of the syncarp (cm); length of the crown (cm); and diameter of the crown (cm). Besides these, we recorded the number of layers of fruitlets, evaluated horizontally along the syncarp, and the number of leaves of the mother plant's crown and secondary crown in recurrent flowering. We also applied 21 qualitative descriptors (growth habit; leaf variegation; distribution of leaf variegation; main color on the upper leaf face; anthocyanin pigmentation; presence of thorns on the leaves; color of thorns; undulation of the leaf edges; peduncle shape; external color of the fruit skin; syncarp shape; shape of fruitlet bract tips; fruitlet bract color; presence of bracts at the base of the crown; overlap of bracts in relation to fruitlets; color of bracts in relation to crown; color of crown bracts; ratio of crown length and fruit length; ratio of crown diameter and fruit diameter; number of crown colors; and shape of the crown tip). Quantitative and qualitative morphological descriptors developed by the International Board for Plant Genetic Resources (INTERNATIONAL BOARD FOR PLANT GENETIC RESOURCES, 1991) and National Service to Protect Cultivars applicable to ornamental pineapple varieties (BRASIL, 2013) were applied and used for clonal evaluation. The colors were evaluated according to the Royal Horticulture Society (RHS) color chart, adopted for protection of plants by the International Union for the Protection of New Varieties of Plants (UPOV). For the quantitative data, the following descriptive statistics were calculated: mean, standard deviation and coefficient of variation, using the SAS program (SAS INSTITUTE, 2010).

Joint analysis of the qualitative and quantitative data was performed to determine the genetic distance between the hybrid’s clones and the parents, based on Gower’s algorithm (GOWER, 1971). The hierarchical groups of the plants were ascertained by the Unweighted Pair-Group Method Using an Arithmetic Average (UPGMA), based on the mean Euclidian distance. The dendrogram cutoff point was estimated based on the mean of the matrix. The validation of the groupings was

**Figure 1** - Data of temperature and rainfall of Cruz das Almas, Bahia obtained during the period of clonal evaluation in the field with the ornamental pineapple hybrid (ORN-MUT). PL) Planting; IN) Floral induction; BU) Flower bud emergency; F FL) Period of opening of the first flower; L FL) Period of closing of the last flower. Cruz das Almas, Bahia, Brazil, 2015-2017
determined by the cophenetic correlation coefficient (r) (SOKAL; ROHLF, 1962). The data obtained for genetic distance, hierarchical groupings and cophenetic correlation were analyzed with the R program (R DEVELOPMENT CORE TEAM, 2011). The correlation between the matrices (first flowering and recurrent flowering) was calculated by the t-test and Mantel test (10,000 permutations). The dendrogram was generated based on the matrix of distances by the MEGA 6 program (TAMURA et al., 2013).

RESULTS AND DISCUSSION

The ornamental pineapple hybrid ORN-MUT presented uniformity among plants in both cycles. In the first cycle, the emergence of floral buds occurred 48.80 days after floral induction, the first flower opened 17.40 days after floral bud emergence, and closure of the last flower occurred 9.50 days after opening of the first flower. The fruiting concluded 75.70 days after induction and the complete cycle lasted 445.70 days (Table 1). The complete fruit formation, for genotypes for ornamental use, is considered to occur at the time of closure of the last flower (SOUZA et al., 2014).

In the second cycle, the floral bud emergence was recorded 47.20 days after floral induction, opening of the first flower 15.70 days after floral bud emergence, and last flower closure 9.45 days after first flower opening. The complete fruiting happened 72.35 days after floral induction and the complete cycle lasted 442.35 days (Table 1). There was no influence of temperature and rainfall in the months of emergence of the flower bud, opening and closing of the last flower, according to Figure 1, with a few variation in the climatic data during evaluation stages. The recurrent flowering and fruiting cycle on the crown of the mother plant of the ORN-MUT hybrid started 170.60 days after induction. The opening of the first flower in this cycle occurred 18.20 days after floral bud emergence, and the last flower opened 3.80 days after opening of the first flower. Therefore, the fruiting of ORN-MUT, considering the first flowering and what occurred on the fruit crown, took 562.60 days (Table 1). The recurrent flowering was not homogeneous for this morphological trait, being divided into two distinct groups (Figure 2C). Therefore, this lack of homogeneity observed in the second flowering cycle clearly implies that unknown mechanisms are involved in regulating the phenology of this second plant. The plants of A. comosus var. bracteatus (feminine parent) were large, with average height of 104.85 cm and semi-erect growth habit (Figure 3A, B). Their green leaves (FAN3 146A) were long (92.20 cm) and wide (4.10 cm) with presence of anthocyanin and thorns with different color than the leaves. The peduncles were straight and had average length of 37.22 and diameter of 1.96 cm. The syncarps had cylindrical cone format and red color (FAN1 46B), with average length of 17.99 cm and diameter of 9.24 cm. The bracts were red (FAN1 54B), with average length of 17.99 cm and diameter of 6.49 cm. The shape of the leaf tip on the crown was pointed, with thorns on the edges (Tables 2 and 3).

A. comosus var. erectifolius (masculine parent) diverged in the morphological traits (Figure 3A, C) in relation to the feminine parent. The genotype presented erect growth habit and average height of 80.63 cm. The leaves were grayish purple (FAN4 187A), without thorns, and had length of 70.83 cm and width of 3.18 cm. The peduncle was longer, with length of 41.66 cm, and had smaller diameter, of 0.82 cm. The syncarps were smaller, with length of 5.91 cm and diameter of 5.73 cm. The color of the outer layer was reddish purple (FAN4 183B) with conical shape and obtuse bracts that partially covered the fruitlets. The crown was red (FAN1 184A) with broad pointed tip smaller than the syncarp, having average length of 4.55 cm and diameter of 4.10 cm (Tables 2 and 3).
Table 1 - Phenology of the miniature ornamental pineapple hybrid ORN-MUT in the first production cycle and recurrent cycle

| Phenological phases (days) | Mean ± Standard Deviation | CV (%) |
|---------------------------|---------------------------|--------|
| Flowering of the matrix plant | First cycle |        |
| Induction/bud emergence | 48.80 ± 5.11 | 10.46 |
| Induction/first flower | 66.20 ± 4.06 | 6.13 |
| Induction/last flower | 75.70 ± 8.01 | 10.59 |
| Bud/first flower | 17.40 ± 3.36 | 19.32 |
| Bud/last flower | 26.90 ± 5.97 | 22.20 |
| First flower/last flower | 9.50 ± 4.89 | 51.51 |
| Planting/bud emergence | 418.80 ± 5.11 | 1.22 |
| Planting/first flower | 436.20 ± 4.06 | 0.93 |
| Planting/last flower | 445.70 ± 8.01 | 1.80 |
| | Second cycle |        |
| Induction/bud emergence | 47.20 ± 3.09 | 6.54 |
| Induction/first flower | 62.90 ± 3.26 | 5.18 |
| Induction/last flower | 72.35 ± 6.17 | 8.52 |
| Bud/first flower | 15.70 ± 3.60 | 22.93 |
| Bud/last flower | 25.15 ± 6.75 | 26.82 |
| First flower/last flower | 9.45 ± 3.41 | 36.09 |
| Planting/bud emergence | 417.20 ± 3.09 | 0.74 |
| Planting/first flower | 432.90 ± 3.26 | 0.75 |
| Planting/last flower | 442.35 ± 6.17 | 1.39 |
| | Recurrent flowering |        |
| Induction²/secondary bud emergence | 170.60 ± 1.85 | 1.08 |
| Induction/first flower | 188.80 ± 0.41 | 0.22 |
| Induction/last secondary flower | 192.60 ± 0.82 | 0.43 |
| Bud/first flower | 18.20 ± 1.82 | 10.02 |
| Bud/last flower | 22.00 ± 1.89 | 8.60 |
| First flower/last flower | 3.80 ± 0.41 | 10.80 |
| Planting/secondary bud emergence | 540.60 ± 1.85 | 0.34 |
| Planting/first flower | 558.80 ± 0.41 | 0.07 |
| Planting/last secondary flower | 562.60 ± 0.82 | 0.15 |

¹Referring to the new inflorescence emitted at the crown of the matrix plant; ²Induction of the matrix plant

The hybrid ORN-MUT (Figure 3D) had semi-erect growth habit with height of 35.45 cm. Its leaves were green (FAN3 138A), short (52.10 cm) and broad (4.00 cm), without thorns, without marginal variegation and had little anthocyanin pigmentation. The peduncle was straight and short, with length of 17.90 cm and diameter of 1.55 cm. The syncarps were small (length of 4.00 cm and diameter of 3.50 cm) and had pinkish color (FAN1 51B), with cylindrical cone format. The bracts were acute and totally covered by fruitlets, like the feminine parent (A. comosus var. bracteatus). The crown had a moderately acute tip with green central color (FAN3 138 A) and pink edges (FAN1 51B).

The plant resulting from the recurrent flowering (Figure 3E) was even smaller, with average height of 24.45 cm and a more compact and consolidated appearance. The peduncle was pink, with length of 7.95 cm and diameter of 1.05 cm, while the syncarps were very small, presenting 1.20 layers of fruitlets,
length of 1.61 cm and diameter of 2.14, characterizing a miniature pineapple plant. Not all the clones from recurrent flowering presented fruitlets. The crowns were smaller than the syncarp, with length of 1.58 cm and diameter of 1.74 cm (Table 2 and 3).

To summarize, the clonal evaluation of the recurrent flowering stage indicated lack of uniformity, mainly in relation to the formation and size of the syncarp. Of the 20 plants evaluated, 11 presented syncarp with 1 to 2 layers of fruitlets (Figure 2F) and 9 formed only a new crown, without fruitlets (Figure 3G). The larger crowns generated syncarps with a larger number of fruitlets, while on the small crowns the fruitlets were absent or very small. It should be stressed that the recurrent flowering occurred naturally, without any floral induction, making it even more complex to understand what determines the presence and size of the syncarps.

The hybrid ORN-MUT was initially selected as a progeny for use as a potted plant, due to its much smaller size than other pineapple varieties, but the presence of a new plant formed on the crown of the ripe fruit made the possibility of obtaining a miniature pineapple plant evident, something that would be virtually impossible by adjustments to production systems (Figure 4). Therefore, the evaluation of the clones, in particular the stability of their traits, should take into consideration the plant’s two flowering cycles.

The results demonstrated the uniformity of flowering and fruiting in the first cycle, before the emergence of the new inflorescence on the crown, a
process we call recurrent flowering. Renewed flowering on the crown of the fruit is not common in ornamental pineapple varieties or even commercial ones for food production. To the best of our knowledge, there are no reports in the literature of this type of mutation.

The uniformity of flowering observed in the first cycle did not repeat in the recurrent flowering of the crown, for which there were variations in the timing of the new flowering and in the layers of fruitlets forming the small syncarps, with some plants only producing a crown.

This hybrid has been maintained in a basic mother plants since 2010, and this trait (recurrent flowering) has remained stable for several cycles, suggesting it is the result of a mutation involving alterations in the pattern of morphogenesis and cell differentiation of the apical meristem (RODRIGUES; KERBAUY, 2009).

Flowering starts with an increase in the division rate of the central cells of the apical meristem, which is

Table 2 - Quantitative morphological traits of the feminine (Ananas comosus var. bracteatus) and masculine (A. comosus var. erectifolius) parents and the ornamental pineapple hybrid ORN-MUT 12 months after planting

| Variables (cm)            | A. comosus var. bracteatus | A. comosus var. erectifolius |
|---------------------------|-----------------------------|-------------------------------|
|                           | 1st cycle                   | 2nd cycle                     | 1st cycle                   | 2nd cycle                     |
| Plant height              | 98.20 ± 12.33               | 111.51 ± 10.22                | 79.14 ± 6.15                | 82.13 ± 7.10                  |
| Leaf length               | 90.00 ± 5.18                | 94.40 ± 4.65                  | 71.45 ± 4.25                | 70.22 ± 5.13                  |
| Leaf width                | 4.22 ± 0.15                 | 3.98 ± 0.45                   | 3.21 ± 0.44                 | 3.16 ± 0.48                   |
| Peduncle length           | 36.23 ± 6.38                | 38.22 ± 4.45                  | 40.80 ± 1.32                | 42.53 ± 1.59                  |
| Peduncle diameter         | 1.97 ± 0.22                 | 1.95 ± 0.22                   | 0.81 ± 0.08                 | 0.83 ± 0.12                   |
| Syncarp length            | 18.23 ± 4.32                | 17.75 ± 3.25                  | 5.53 ± 0.68                 | 6.30 ± 0.55                   |
| Syncarp diameter          | 9.44 ± 2.08                 | 9.04 ± 2.00                   | 5.49 ± 0.33                 | 5.98 ± 0.48                   |
| Crown length              | 7.12 ± 0.55                 | 6.95 ± 0.77                   | 4.12 ± 0.87                 | 4.98 ± 0.98                   |
| Crown diameter            | 6.44 ± 0.83                 | 6.55 ± 0.44                   | 4.05 ± 0.44                 | 4.15 ± 0.23                   |

Ornamental pineapple hybrid ORN-MUT

| Variables                | 1st cycle | 2nd cycle |
|--------------------------|-----------|-----------|
| Plant height              | 39.30 ± 7.05 | 31.60 ± 3.35 |
| Leaf length               | 56.30 ± 10.08 | 47.90 ± 5.24 |
| Leaf width                | 4.30 ± 0.70   | 3.70 ± 0.60  |
| Peduncle length           | 19.30 ± 3.83  | 16.50 ± 2.50 |
| Peduncle diameter         | 1.60 ± 0.30   | 1.50 ± 0.24  |
| Syncarp length            | 3.90 ± 0.83   | 4.10 ± 0.55  |
| Syncarp diameter          | 3.50 ± 0.40   | 3.50 ± 0.33  |
| Number of fruitlet layers | 3.74 ± 0.73   | 3.37 ± 0.60  |
| Crown length              | 4.10 ± 0.90   | 4.20 ± 0.78  |
| Crown diameter            | 2.50 ± 0.50   | 2.60 ± 0.24  |
Table 3 - Qualitative morphological traits of the ornamental pineapple hybrid ORN-MUT and its parents

| Descriptor¹ | ORN-MUT | ORN-MUT/ recurrent² | A. comosus var. bracteatus | A. comosus var. erectifolius |
|-------------|---------|---------------------|---------------------------|-----------------------------|
| Growth habit | Semi-erect | Semi-erect | Semi-erect | Erect |
| Leaf variegation | Absent | Absent | Absent | Absent |
| Distribution of variegation | - | - | - | - |
| Main color of the upper leaf face | Green FAN3 138 A | Green FAN3 138 A | Green FAN3 146A | Grayish purple FAN4 187A |
| Anthocyanins in leaves | Present | Present | Present | Present |
| Thorns on leaves | Absent | Absent | Present | Absent |
| Color of thorns | - | - | Different | - |
| Undulation of leaf edges | Absent | Absent | Absent | Absent |
| Peduncle shape | Straight | Straight | Straight | Straight |
| External color of the skin of the syncarp | Pink FAN1 51B | Pink FAN1 51B | Red FAN1 46B | Reddish purple FAN4 183B |
| Syncarp shape | Cylindrical cone | Cylindrical cone | Cylindrical cone | Cone |
| Shape of the tip of the fruitlet bracts | Acute | Acute | Acute | Obtuse |
| Overlap of bracts in relation to fruitlets | Total | Total | Total | Partial |
| Color of fruitlet bracts | Pink FAN1 51B | Pink FAN1 51B | Red FAN1 54B | Red FAN1 51B |
| Bracts at base of the crown | Present | - | Absent | Absent |
| Color of bracts at base in relation to crown | Different | - | - | - |
| Color of bracts of the crown | Pink FAN1 51B | - | Red FAN1 52B | Red FAN1 184A |
| Crown length-syncarp length ratio³ | High | High | High | Low |
| Crown diameter-syncarp diameter ratio | Low | Low | Low | Low |
| Number of colors of the crown | Two | Two | Two | Two |
| Format of crown leaf tip | Moderately acute | Moderately acute | Pointed | Enlarged pointed |

¹Descriptors developed by the Brazilian Ministry of Agriculture for testing of distinguishability, homogeneity and stability of cultivars, published in the Official Federal Gazette (DOU), second edition, on January 3, 2013, section 1, pages 4 and 5; ²Referring to the new inflorescence emitted from the crown of the mother plant fruit; ³Corresponds to the “plant height” variable measured in the main plant.
Comparison of the characteristics of ORN-MUT with those of its parents reveals why it was one of the hybrids selected as having potential as a potted plant. Its significantly smaller size, semi-erect growth habit and compact canopy are all traits necessary to meet the standards of the potted plant market.

The plant formed on the crown after flowering is a product with strong commercial interest, since it is a miniature pineapple plant with extremely compact architecture, impossible to obtain by adjustment of existing production systems. The development of the syncarp in pineapple is closely related to the plant’s physiological maturation at the moment of floral induction. Very young plants produce smaller syncarps when flowering is induced, due to the impairment of the layer of fruitlets, forming fruits outside the standard for the variety. The adjustment of this physiological maturation stage of this “second plant” will be a determinant to transform this miniature pineapple plant into a marketable product.

**CONCLUSIONS**

The ornamental pineapple hybrid ORN-MUT presented uniform plants only regarding flowering of the matrix plant. The plants formed after recurrent flowering presented lack of uniformity in relation to the syncarp development, with absence of fruitlets in some clones, besides variation of the timing of flowering. However to use the hybrid as potted plant for the floral market more studies with focus on the establishment of an agricultural system in order to adjust and standardize the fruit size and the control of flowering are needed.

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