Effect of Strain Age and Substrate on the Production of Pineapple (*Ananas comosus* L.) Extra Sweet (Md2) Vivo Plants in Greenhouse

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**Authors’ contributions**

This work was carried out in collaboration among all authors. Authors KNM, CM, KT, SS and KAN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TS, AAM, KKJFM and BEL managed the analyses of the study. Authors CB, SF, DAE and KD managed the literature searches. All authors read and approved the final manuscript.

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**ABSTRACT**

Pineapple has several types of organs that can be used for its multiplication. Its natural multiplication is particularly slow, as it is necessarily vegetative because the species is self-sterile (on average 2 suckers per strain in six months). The supply of pineapple rejects is not always easy for those who want to grow pineapple on large areas. This study was initiated to improve the
production of MD2 pineapple seed (suckers or vivo plant) per pineapple strain fragment. Thus, the production of two types of pineapple strains, young and old strains (The young strains bear green leaves and make up all the strains that have produced fruit. Their ages range from 0 to 12 months after the fruit has been harvested. The old strains without green leaves are older than 12 months of age), was evaluated on three types of substrates, namely: S1, 100% coarse coconut fibre; S2, 2/3 coarse coconut fibre + 1/3 sawdust and S3, 3/4 coarse coconut fibre + 1/4 chicken droppings. The seeding of the fragments on the substrates it’s made in a completely randomized device in a greenhouse. The study carried out was repeated twice during the same period. Results showed that vivo plant production varied according to the age of the pineapple strain. With fragments of young strains, a mean of 3.96 ±1.74 (average ± Ecart-type) vivo plants per fragment was obtained compared to 3.07±1.63 (average ± Ecart-type) vivo plants at fragments of old strains. This difference was significant with P = 0.000. The effect of substrate was significant on the number of plants produced per strain fragment. More plants per fragment were obtained on S2 with 3.53±1.11 (average ± Ecart-type) vivo plants than on the other substrate types S1 and S3 with 3.22±1.3 and 3.09±1.02 (average ± Ecart-type) vivo plants per strain fragment, respectively. This difference was significant with P= 0.002. This study showed that a strain fragment after fragmentation gives only 3.96 ± 1.74 plants in six months. This production of pineapple vivo plants is influenced by the type of substrate and the age of the strain. Thus, young strains grown on a substrate that maintains moisture should be recommended for sustainable production of pineapple vivo plants.

Keywords: Greenhouse; MD2 pineapple; substrate; vivo plants; young and old strains.

1. INTRODUCTION

Ananas comosus (L.) [1] is a Bromeliaceae, mainly grown in the humid tropics. Its fruit is eaten fresh or canned (slices, pieces, juice). This tropical plant, native to South America, is cultivated for its edible fruit [2-3]. Its yellow, fragrant and generally sweet flesh is covered with a scaly bark with greenish-brown or reddish hues. The leaves can be used for their fibre or in livestock feed. It is one of the major tropical fruits whose demand on the international market is increasing nowadays. Its cultivation is an important source of income for rural populations [4]. Pineapple is the third most important tropical fruit produced in the world [5] and plays an important role in the economies of many countries. In 2018, 27.92 million tons of pineapples were produced worldwide [6]. The leading producer is Costa Rica with a production of 3.42 million tons. In Africa, Nigeria (1.66 million tons in 2018) remains the leading producer [7]. However, Ghana, Benin, Cameroon and Kenya with 677,112, 360,257, 351,574 and 204,850 tonnes respectively in 2017 remain major producers on the African continent. In 2018, African production was 5.50 million tons from an area of 408,648 ha [8]. Côte d'Ivoire's pineapple production in 2018 is estimated at 49,000 tons [9]. In the national economy, it contributes 1.6% to agricultural GDP and 0.6% to national GDP [10]. However, its intensive production requires a large number of rejects (suckers) which unfortunately are poorly produced by the plant. The average sucker production per plant is 1.5 [11]. Thus, the uniformity of planting material to ensure grouped and homogeneous production is a real problem. Thus, the scarcity of shoots leads to the use of shoots of different types, sizes and origins, thus affecting the quality of the harvest. The supply of good quality and sufficient rejects is even more acute in the event that a new cultivar is sought for release[12], as it is necessary between 40,000 to 70,000 plants per hectare [13]. In vitro pineapple propagation techniques are not adapted to farmers' conditions because they require a lot of financial means and are practiced by highly equipped specialists, which has led to the development of in vivo techniques accessible to farmers that have increased the number of plants (rejects) per strain [14]. However, since the introduction of the pineapple variety MD2, which is sweeter and less acidic [15-16] on the international fruit market, export from Côte d'Ivoire is based essentially on the smooth Cayenne have continued to decline. Indeed, Ivorian pineapple exports to Europe, which amounted to 150,000 tons in 1990, have been reduced to 49,000 tons in 2018 [9]. In order to regain its market share, the country needs to adopt MD2 cultivation with a well-developed technical production itinerary [13]. The introduction of this new variety in Côte d'Ivoire is faced with an increased lack of supply of rejects (the reject is a vivo plant).
The general objective of this study is to improve the production of MD2 pineapple rejects by strain (strain is the pineapple stem) fragmentation and more specifically is to:

- evaluate the effect of different substrates on strain fragments to produce vivo plants;
- know the effect of time on the ability of strain fragments to produce vivo plants;
- evaluate the effect of the age of the strain to produce vivo plants.

1.1 Study Site

The study was carried out in the compound at the University Nangui Abrogoua (UNA) in Abidjan, located in the south of Côte d'Ivoire at 4° W; 5°23 W and 100 m altitude. The soils of UNA (Abidjan) are deeper with a sandy to sandy-clay texture [17]. The vegetation in UNA is that of the ombrophilous sector. It is the continuity of the Banco National Park [18]. The rainforest sector shelters hydrophilic or ombrophilic forests that are rich in lianas and epiphytes [19]. The climate is subdivided into four seasons: a large and a small rainy season from March to July and October to November respectively, and a large and a small dry season from December to April and August to September respectively. The average annual temperatures in Abidjan city are between 25 and 29°C. The study was conducted from July 2018 to December 2018.

2. MATERIALS

2.1 Plant Material

The plant variety consisted of young and old pineapple strains of the Extra Sweet or MD2. The young strains bear green leaves and make up all the strains that have produced fruit. Their ages range from 0 to 12 months after the fruit has been harvested. The old strains without green leaves are older than 12 months of age (Fig. 1).

2.2 Technical and Chemical Equipment

The experiments required the construction of a greenhouse (Fig. 2) with dimensions equal to 4 m long, 1.5 m wide and 1.15 m high. The greenhouse was built in the open air in the field under a shady roof. For the disinfection of the strains the fungicide Mancozan (Mancozan 80 wp, Mancozeb 800g/kg;) was used. The concentration of fungicide used to disinfect the strains was 80 to 100 g per 15 litres of water. A digital scale was used for weighing these different chemicals and the vivo plants produced.

Fig. 1. MD2 pineapple strain harvested from the field; A. Old pineapple strain MD2; B. Young pineapple strain MD2
3. METHODS

3.1 Production of Germinating Vivo Plants

The young and old MD2 pineapple strains harvested from the field were split longitudinally from the insertion of the peduncle on the stem to the base of the stump (Fig. 3). Thus, each strain was split into two approximately equal parts. After this step, the fragments were soaked in a contact fungicide (Mancozan) for 30 min. Then they were put in greenhouses on different substrates. Each greenhouse contained only one type of substrate and both ages of strains.

Fig. 3. MD2 pineapple strains after fragmentation

The substrates tested consisted of three components: coarse coconut fibre, chicken manure composted for two months, and sawdust (Fig. 4), the combination of which resulted in three types of substrate:

- S1, 100% coarse coconut fibre (pH = 6.9);
- S2, 2/3 coarse coconut fibre + 1/3 sawdust (pH = 6.7);
- S3, 3/4 coarse coconut fibre + 1/4 chicken droppings (pH = 7.2).

3.2 Experimental Device

The experimental design used is a completely randomized design. The study carried out was repeated twice during the same period. The number of fragments contained in each greenhouse were 150 at each repetition. The different fragments were seeded onto different substrates. The spacing between rows was 3 cm. The stump fragments were arranged in a furrow, the injured side after the fraction was placed against the substrate and the other uninjured side was oriented towards the roof of the greenhouse. The average temperature was 42 °c, the Humidity was 75 %. A 15-litre watering can was used for watering. 30 litres of water was brought in once a week per greenhouse for the six months of the study.

3.3 Weaning and Evaluation of the Physiological and Health Parameters of the Plants

From the sixth week after embedding the strain fragments in the substrate all plants that appeared on the surface of the substrate (Fig. 5) and had at least 5 leaves were weaned (Fig. 6). The period required for the first leaves of the releases from both types of strains was 4 weeks.
Fig. 4. Components of the different substrates used during the production of pineapple vivo plants; A. Chicken manure composted for two months; B. Sawdust; C. Coarse coconut fibre

on average. This weaning operation was possible three times in six months. During weaning, parameters such as the average number of plants produced per fragment (Fig. 7) and the mass of vivo plants at weaning were evaluated.

3.4 Average Number of Plants Produced Per Fragment

The average number of plants produced per fragment (NMP) is the ratio of the total number of weaned plants (NPS) to the number of fragments that produced plants (NFP).

$$NMP = \frac{NPS}{NFP}$$

3.5 Determination of the Mass of Vivo Plants

A Roberval scale was used to evaluate the mass of vivo plants. After tare, the reject or fruit was placed on the scale and the mass displayed was noted in grams. The average mass (M) is the ratio of the sum of individual mass (MI) to the total number of plants harvested (NP).

$$M = \frac{\sum MI}{NP}$$

3.6 Statistical Analysis of the Data

The data collected during this study have been subjected to an analysis of variance (ANOVA) using XLSTAT software, version 7.1. In case of significant differences, the means were compared according to the Newman-Keuls test at the 5% threshold.
4. RESULTS

4.1 Number of Vivo Plants Produced

4.1.1 Influence of substrate on the production of vivo plants after six months of cultivation

The results reported in Table 1 show the influence of substrate on vivo plant production after six months of cultivation as a function of strain age. Analysis of these results indicates that plant production is influenced by culture substrates. Thus, with young strains the best results were obtained on substrates S2 and S3 compared to S1. This difference was significant ($P= .001$) depending on the substrates. The number of plants produced was 3.35, 3.96 and 3.69 plants per fragment on S1, S2 and S3 respectively. No significant difference was found in old strains. The number of plants produced was 3.07; 3.07 and 2.56 plants per strain fragment on S1, S2 and S3 respectively. On S1, the results showed that between young and old strains the production of plants was statistically identical ($P = .054$). For both young and old strains, 3.35 and 3.07 were noted. On S2 and S3, the results showed that young strains produced more plants per fragment than old strains. Statistical analyses showed that this difference was significant. There were 3.96 and 3.07 plants for young and old strains on S2 ($P= .000$). On S3, we noted 3.69 and 2.56 plants for young and old strains with $P= .000$.

4.1.2 Influence of substrate on the production (per quarter) of vivo plants from fragments of young strains

Table 2 shows the production (per quarter) of vivo plants by young strain as a function of substrate. The results obtained show that vivo plant production is higher in the first quarter compared to the second quarter regardless of substrate type. This difference according to the statistical analyses is significant. On S1, 2.26 and 1.21 were noted in the first and second quarters respectively ($P= .000$). On S2, 2.57 and 1.58 plants were obtained in the first and second quarter ($P= .000$). With S3, the number of plants obtained per strain fragment was 2.35 and 1.50 in the first and second quarter ($P= .000$). However, in the first quarter, more plants were obtained with substrate 2 (S2). There were 2.26; 2.57 and 2.35 plants respectively on S1, S2 and S3 ($P= .007$). In the second quarter, there were 1.21; 1.58 and 1.50 plants respectively on S1, S2 and S3 ($P= .000$).

Table 1. Influence of substrate on the production of vivo plants after 6 months of cultivation according to the age of the strains

| Treatments | S1            | S2            | S3            | P    |
|------------|---------------|---------------|---------------|------|
| Young strains | 3.35 ± 1.34 b(a) | 3.96 ± 1.74 a (a) | 3.69 ± 1.12 a (a) | .001 |
| Old strains  | 3.07 ± 1.04 a(a) | 3.07 ±1.63 a (b) | 2.56 ± 1.42 a (b) | .134 |
| P           | .054          | .000          | .000          |      |

In the same line, the numbers followed by the same letter (out of parenthesis) are statistically identical to the threshold $\alpha = 5\%$ and also in the same column, the numbers followed by the same letter (in parenthesis) are statistically identical to the threshold $\alpha = 5\%$ (Newman-Keuls test); average ± Ecart-type.

S1, 100 % coarse coconut fibre; S2, 2/3 coarse coconut fibre + 1/3 sawdust; S3, 3/4 coarse coconut fibre + 1/4 chicken droppings. P: probability.
4.1.3 Influence of substrate on the production (per quarter) of vivo plants from fragments of old strains

The data in Table 3 shows that the production of vivo plants was very high in the first quarter compared to the second quarter regardless of the type of substrate. This difference according to the statistical analyses was significant. Thus on S1, 2.16 and 1.13 plants per strain fragment were obtained in the first and second quarter with $P = .000$. On S2, 2.26 and 1.52 plants were obtained in quarters 1 and 2 ($P = .000$). In S3, there were 1.95 and 1.38 plants per strain fragment in quarters 1 and 2 ($P = 0.000$). However in the first quarter substrates 1 and 2 (2.16 and 2.26 plants on S1 and S2) allowed to have more plants per fragment than substrate 3 (1.95 plants) with $P = .000$. In quarter 2, the production of plants was 1.13; 1.52 and 1.38 plants respectively on S1, S2 and S3 ($P = .000$).

4.1.4 Influence of substrate type on the production of vivo plants after six months of cultivation

Fig. 8 shows that substrate type significantly influences production ($P = .002$). The highest number of plants was recorded on S2 while S1 and S3 were statistically identical. We noted 3.22, 3.53 and 3.09 plants respectively on S1, S2 and S3.

4.1.5 Influence of strain age on the production of vivo plants per quarter

The data in Table 4 shows that, regardless of strain age, it is in the first quarter that the major production of vivo plant per strain fragment takes place. With the young strains, 2.39 and 1.84 plants were obtained in quarters 1 and 2 ($P = .000$). With the old strains, 1.84 and 1.23 plants per strain fragment were obtained in the first and second semester ($P = .000$). In quarters 1 and 2, it was found that the young strains produced more plants than the old strains. Thus, 2.39 and 1.84 plants were noted in the first quarter for young and old strains respectively ($P = .000$). In the second half of the year, 1.42 plants per fragment were obtained with the young strains compared to 1.23 with the old strains ($P = .000$).

4.2 Mass of Vivo Plants at Weaning

4.2.1 Influence of strain age and substrate on the mass of vivo plants after each weaning

Fig. 9 showing the mass of vivo plants at weaning as a function of the age of the strains shows that plants from young strains had a greater mass than those from old strains. According to statistical analyses this difference was significant ($P = .000$). Thus, 10 g of seedlings were obtained from the young strains and 7.33 g from the old strains. Fig. 10 shows that the substrates significantly influenced the mass of the vivo plants produced ($P = .000$). It was obtained 8.24, 6.17 and 11.67 g respectively on substrates S1, S2 and S3.

5. EFFECT OF TECHNICAL ROUTES ON THE GROWTH OF REJECTS PRODUCED BY MD2 PINEAPPLE STRAIN

The study in Table 5 shows that the highest number of plants per strain fragment was obtained with the FQ/JS/S2 route, with 2.6 plants in the quarter. The lowest value was 1.1 plants with the SQ /VS/S1 route in the second quarter. The FQ /JS/S3 and SQ /JS/S3 routes resulted in the highest mass values of vivo plants with 12.3 and 13.2 g respectively. Plants from the SQ /JS/S1 route had a smaller mass than the others at 5 g.
Table 3. Production (per quarter) of vivo plants by fragments of old strains in substrate function

| Treatments | Number of vivo plants produced from 0 to 3 months | Number of vivo plants produced from 3 to 6 months | P    |
|------------|--------------------------------------------------|---------------------------------------------------|------|
| S1         | 2.16 ± 0.88 a (a)                                | 1.13 ± 0.34 b (c)                                 | .000 |
| S2         | 2.26 ± 1.09 a (a)                                | 1.52 ± 0.50 b (a)                                 | .000 |
| S3         | 1.95 ± 0.82 a (b)                                | 1.38 ± 0.50 b (b)                                 | .000 |
| P          | .000                                             | .000                                              |      |

In the same line, the numbers followed by the same letter (out of parenthesis) are statistically identical to the threshold $\alpha = 5\%$ and also in the same column, the numbers followed by the same letter (in parenthesis) are statistically identical to the threshold $\alpha = 5\%$ (Newman-Keuls test); average ± Ecarr-type.

S1, 100% coarse coconut fibre; S2, 2/3 coarse coconut fibre + 1/3 sawdust; S3, 3/4 coarse coconut fibre + 1/4 chicken droppings; P: probability.

Fig. 8. Number of vivo plants produced per fragment in 6 months as a function of the substrate

Means assigned different letters on the histograms are significantly different at the 5% threshold (Newman-Keuls Test) S1, 100% coarse coconut fibre; S2, 2/3 coarse coconut fibre + 1/3 sawdust; S3, 3/4 coarse coconut fibre + 1/4 chicken droppings.

Table 4. Production of vivo plants per quarter according to the age of the strains

| Treatments     | Number of vivo plants produced from 0 to 3 months | Number of vivo plants produced from 3 to 6 months | P    |
|----------------|--------------------------------------------------|---------------------------------------------------|------|
| Young strains  | 2.39 ± 0.86 a (a)                                | 1.42 ± 0.50 b (a)                                 | .000 |
| Old strains    | 1.84 ± 0.95 a (b)                                | 1.23 ± 0.42 b (b)                                 | .000 |
| P              | .000                                             | .000                                              |      |

In the same line, the numbers followed by the same letter (out of parenthesis) are statistically identical to the threshold $\alpha = 5\%$ and also in the same column, the numbers followed by the same letter (in parenthesis) are statistically identical to the threshold $\alpha = 5\%$ (Newman-Keuls test); average ± Ecarr-type.

P: probability.
Fig. 9. Mass of vivo plants at weaning as a function of strain age
Means with different letters on the histograms are significantly different at the 5% threshold (Newman-Keuls test). S1, 100 % coarse coconut fibre; S2, 2/3 coarse coconut fibre + 1/3 sawdust; S3, 3/4 coarse coconut fibre + 1/4 chicken droppings

Fig. 10. Mass of vivo plants at weaning as a function of substrate
Means with different letters on the histograms are significantly different at the 5% threshold (Newman-Keuls test). S1, 100 % coarse coconut fibre; S2, 2/3 coarse coconut fibre + 1/3 sawdust; S3, 3/4 coarse coconut fibre + 1/4 chicken droppings
Table 5. Number of discharges produced and average mass of discharges or vivo plants according to technical routes

| Technical routes | Number of vivo plants produced | Technical routes | Weaning vivo plants mass |
|------------------|--------------------------------|------------------|--------------------------|
| FQ / OS /S3      | 1.6 ± 0.8 d                    | FQ / OS /S3      | 11.0 ± 3.2 b             |
| FQ / OS /S2      | 1.9 ± 0.9 c                    | FQ / OS /S2      | 4.8 ± 3.6 c              |
| FQ / OS /S1      | 2.1 ± 0.9 c                    | FQ / OS /S1      | 5.8 ± 1.8 bc             |
| FQ / YS /S1      | 2.2 ± 0.8 b                    | FQ / YS /S1      | 10.2 ± 4.5 b             |
| FQ / YS /S3      | 2.3 ± 0.5 b                    | FQ / YS /S3      | 12.3 ± 3.5 a             |
| FQ / YS /S2      | 2.6 ± 1.0 a                    | FQ / YS /S2      | 7.5 ± 2.7 b              |
| SQ / OS /S1      | 1.1 ± 0.2 f                    | SQ / OS /S1      | 10.0 ± 3.5 b             |
| SQ / YS /S1      | 1.2 ± 0.4 fe                   | SQ / YS /S1      | 5 ± 3 d                  |
| SQ / OS /S3      | 1.3 ± 0.4 e                    | SQ / OS /S3      | 7 ± 2.1 c                |
| SQ / OS /S2      | 1.4 ± 0.4 d                    | SQ / OS /S2      | 11 ± 3.5 b               |
| SQ / YS /S3      | 1.5 ± 0.5 d                    | SQ / YS /S3      | 13.2 ± 2 a               |
| SQ / YS /S2      | 1.6 ± 0.4 d                    | SQ / YS /S2      | 8 ± 3.2 c                |
| P                | .00                            | P                | .00                      |

In the same line, the numbers followed by the same letter (out of parenthesis) are statistically identical to the threshold α = 5% (Newman-keuls test); average ± Ecart-type

S1, 100% coarse coconut fibre; S2, 2/3 coarse coconut fibre + 1/3 sawdust; S3, 3/4 coarse coconut fibre + 1/4 chicken droppings; P: probability FQ : First quarter ; SQ : Second quarter ; YS : Young strains ; OS : Old strains

6. DISCUSSION

The significant difference observed between the young and old strains on the number of plants produced per fragment would be justified by the fact that the old strains have their meristematic tissues in a senescent state. The young strains produced more plants than the old strains because their meristematic tissues would contain more nutrient reserves that would favour bud development. These results are similar to those of Fletcher [20]. Indeed, this author observed an identical phenomenon with Arabidopsis thaliana (lady's thumb). According to him, the meristematic tissues of old strains would have difficulties to differentiate to produce plants. However, Tchoa [21] had opposite results as he observed that older strains of banana produced more plants [22], as they were more mature than younger ones and contained more nutrient and hormonal reserves favourable to bud development. Bonté et al. [23] and Bakelana and Mpanda [24] also obtained results identical to those of Tchoa [21].

The substrate (S2) composed of 2/3 coarse coconut fibre + 1/3 sawdust produced more plants than the other substrates composed of 100% coarse coconut fibre (S1) and 3/4 coarse coconut fibre + 1/4 chicken droppings (S3). The sawdust mixed with the coconut fibre provided a warm and moist containment environment that favoured the conditions necessary to allow expression of axillary meristems [25]. It should be noted that the coarse coconut fibre provided water permeability that may have reduced plant production in substrates 1 and 3 because the cells were slowly imbibed, which delayed budding. These results were similar to those of Tchoa [21]. Tchoa's observations showed that the production of banana vivo plants was influenced by the production substrate. Thus, substrates that maintained more moisture allowed the proliferation of buds than those that maintained little water.

However, Substrate 3 resulted in higher plant mass than Substrates 1 and 2. This is explained by the presence of chicken droppings in the substrate. Chicken manure is an excellent fertilizer rich in nitrogen, phosphorus, potassium and calcium. This richness in mineral and organic elements is easily assimilated by the plants as soon as the first roots appear. Thus, in addition to drawing on the nutrient reserves present in the explant, these plants had another source of nutrient supply, unlike plants growing on substrates 1 and 2, whose composition is low in nutrients.

Production time significantly influenced plant production. Results showed that plant production was higher in the first quarter compared to the second quarter. These observations can be explained by the depletion or decrease in carbohydrate assimilation within strain fragments over time, which would allow them to produce plants quickly [26]. Indeed, these authors showed
that the production of shoots in pineapples would require carbohydrate assimilation at least until these plants develop sufficient leaf area to become autotrophic. This could explain this decrease in production in the second quarter.

7. CONCLUSION

It should be noted that the production of pineapple vivo plants is influenced by the cultivation substrates. Thus, a substrate that maintains a relatively constant moisture content should be recommended for sustainable production of pineapple vivo plants. Our results showed that the young strains produced more plants than the old strains. However, it should be noted that old strains are more available than young strains as suitable material. Based on the results obtained, it was found that a fragmented strain gives 3.96 ± 1.74 plants in six months. These results are better than those obtained with the direct strain maintenance technique, which produces only 2 plants per strain in six months on average. It should also be remembered that the first three months have a high production of vivo plants, so the grower should pay particular attention to this.

With the aim of further research on the production of pineapple vivo plants, in another trial, it would therefore seem appropriate for further study:

- The effect of several fertilizers and substrates on the growth of vivo plants in the nursery.
- The agronomic performance of vivo plants (vegetative growth, yield and organoleptic quality of the fruit).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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