

SCREENING OF SIX GRAPEVINE VARIETIES FROM FRANCE IN NURSERY FOR THEIR RESILIENCE TO THE PEDOCLIMATIC CONDITIONS OF COTE D'IVOIRE

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ABSTRACT

The obvious interest of Africans for the culture of the grapevine and in particular in Côte d'Ivoire, has only increased in recent years. In this context, grapevines from France have been investigated in nursery in the aim to find varieties resilient to the pedoclimatic conditions of Côte d'Ivoire, to develop its culture. To this purpose, cuttings of six grapevine varieties namely Aleatico, Béquignol, Muscat rouge, Italia blanc, Sulima blanc and Exalta blanc, were put in nursery in polyethylene bags containing a mixture of arable soil from the experimental plot of the University Nangui Abrogoua (Abidjan-Côte d'Ivoire) and of compost made from local debris. Two-weekly after sowing the cuttings, the hardiness of the developed grapevines was evaluated through some morpho-physiological parameters. The screening revealed that three varieties, which are Bequignol, Muscat rouge and Aleatico, presented good morpho-physiological profiles. These varieties seem to adapt to the soil and climate conditions of Côte d'Ivoire. On the other hand, the varieties Exalta white, Italia white and Sulima white did not perform well in the nursery. Indeed, a deleterious effect of the substrate was observed on the morpho-physiological parameters of these varieties, showing their non-adaptation to the pedological and climatic conditions of the region of Abidjan, representing the sampled zone in Côte d'Ivoire. Thus, it is possible to test the culture in planta of the varieties Béquignol, Muscat rouge and Aleatico, i.e. to try their development in Côte d'Ivoire.

Key words : Côte d'Ivoire, cutting, grapevine, hardiness, nursery, screening, variety.

1. Introduction

Grapevine is a perennial plant adapted to a variety of environments (Lacombe, 2012). It belongs to the Vitaceae family and the genus *Vitis*. The species *Vitis vinifera* is the most cultivated in the world for its fruit called grapes (Coutin, 2002). World grape production is estimated at over 69 million tons per year (Yara, 2022). In Africa, grape production is estimated at 3.4 million tons, or 4.4% of global production (FAO STAT and OIV, 2019). South Africa and Morocco are the main producers (Beatrice *et al.*, 2018). According to OIV (2019), viticulture is poorly developed in sub-Saharan Africa. Yet, very rich in phenolic compounds, vine products are highly prized by the population (Houel, 2011). In addition, the many jobs generated by viticulture are a way to reduce youth unemployment (Bastian, 2008). On the other hand, the cultivation of grapevines is facing enormous problems in Europe, linked to climate change and the reduction of cultivable areas (OIV, 2010). To manage these problems, grape growers are increasingly turning to new growing areas. Thus, the introduction of grapevine cultivation in Côte d'Ivoire could constitute for Ivorian farmers a lever for diversifying agricultural activities and a source of additional income. Unfortunately, sub-Saharan Africa, and particularly Côte d'Ivoire, is struggling to integrate grapevines into their agricultural systems due to a lack of grapevine varieties adapted to soil and climate conditions (Sophia, 2017).

Indeed, grapes, the fruit of the grapevine, are characterized by criteria and organoleptic properties that are influenced by environmental conditions (climate, soil type); but also by the age of the grapevines (Girard, 2017). Thus, the choice of a grape variety is based on the analysis of certain criteria such as hardiness, disease and insect resistance, grape sugar content, acidity content, aromas and wine yield per ton of grapes (Zerouala, 2006). Thus, winemakers are increasingly opting for hardy grape varieties with good physiological condition. Indeed, hardiness gives the vine the ability to withstand harsh living conditions, robustness and resistance to climatic variations and diseases in growing areas (Zerouala, 2006). Hence, the aim of this study was to investigate the search for grapevine varieties that are resilient to soil and climatic conditions, in order to develop the cultivation of this plant in Côte d'Ivoire and, consequently, diversify farmers' income.

2. Materials and methods

2.1. Plant material

Plant material consisted of cuttings of six grape varieties (*Vitis vinifera* L.), including three table grape varieties (Exalta Blanc, Sulima Blanc, Italia Blanc) and three wine grape varieties (Bequignol, Aleatico, and Muscat rouge), Fig. 1. These grapevines were provided by the Superior Institute of Vine and Wine of Bordeaux, France. The cuttings received were carefully wrapped in moisture-saturated blotting paper. They were unpacked and transferred to a polyethylene bag for planting.



Figure 1. Cuttings of the different varieties of vine studied

a: red Muscat; b: white Exalta; c: white Italia; d: white Sulima; e: Aleatico; f: Bequignol

2.2. Methods

2.2.1. Study zone

The study was carried out on the experimental site of Nangui Abrogoua University (Côte d'Ivoire). This site is a fallow land covered by vegetation composed of *Pueraria phaseolides* (Rosb.) and *Panicum maximum* (Jacq). The geographical coordinates of this site are: 5°17' and 5°31' North latitude between 3°45' and 4°22' West longitude (Koffi *et al.*, 2009). It reveals a high plateau of 37 to 80 m altitude and is endowed with loamy sand (Mobio *et al.*, 2017). The prevailing climate is of the humid tropical type with an average annual rainfall of 1545 mm (Avit *et al.*, 1999). The mean of annual temperature is between 24.2 and 30.6°C with a relative humidity of 83 %.

2.2.2. Compost production

The compost was made from vegetable and animal debris. It was made in a 3 m³ pit (2 m long, 1.5 m wide and 1 m deep) near the study site. The pit was built up by successive layers of 15 cm thick dry *Pueraria faciolodea* straw, 5 cm of sawdust, 5 cm of chicken droppings and at the end, a homogeneous spreading of 1 kg of urea. Regular watering with tap water helped to maintain optimal humidity. Then, the whole was covered with a black plastic film. A wooden rod was planted in the center of the pit as a means of monitoring the progress of mineralization, eight days after the composting process began. Indeed, the heating of the stick is the sign of a good decomposition of the organic matter. The compost was considered mature after eight months of fermentation and when the stick is no longer hot (Leclerc, 2012). Figure 2 shows the process of the composting pit installation.



Figure 2. Different stages of setting up a compost pit

- a: pit used for composting; b: application of a layer of *Pueraria faciolodea*; c: application of a layer of sawdust;
d: application of a layer of chicken manure; e: wooden stem planted in the center of the heap;
f: mature compost used to fertilize nursery substrates

2.2.3. Establishment of the nursery culture system

Nursery was established under a 10 m² (5 m x 2 m) and 4.30 m high shadehouse that was constructed from bamboo (*Bambusa vulgaris*). The shadehouse was covered with a transparent polyethylene film. Then, black polyethylene bags, perforated with a rectangular cross-section of 30 cm x 28 cm, previously filled with substrate were placed under the shadehouse (Figure 3). The substrate consisted of a mixture of compost and arable soil in the proportions 1:1 (v/v). The cuttings, which are 30 cm pieces of vine shoot with at least three axillary buds, were sown in polyethylene bags filled with substrate. Seeding was carried out at the rate of one cutting per bag of substrate. The basal part of the cuttings were inserted vertically to a depth of 10 cm into the substrate contained in the polyethylene bags (Chaireire, 2021). To avoid dehydration of the cuttings during the nursery phase, maintenance of the culture consisted of watering every three days.

The experimental setup used was a complete randomized block with three replications. Each block, sized 2 m x 1.60 m, was spaced 0.4 m from its immediate adjacent and contained 60 cuttings. Likewise, each block was composed of six rows in which the black polyethylene bags were also spaced 0.4 m.

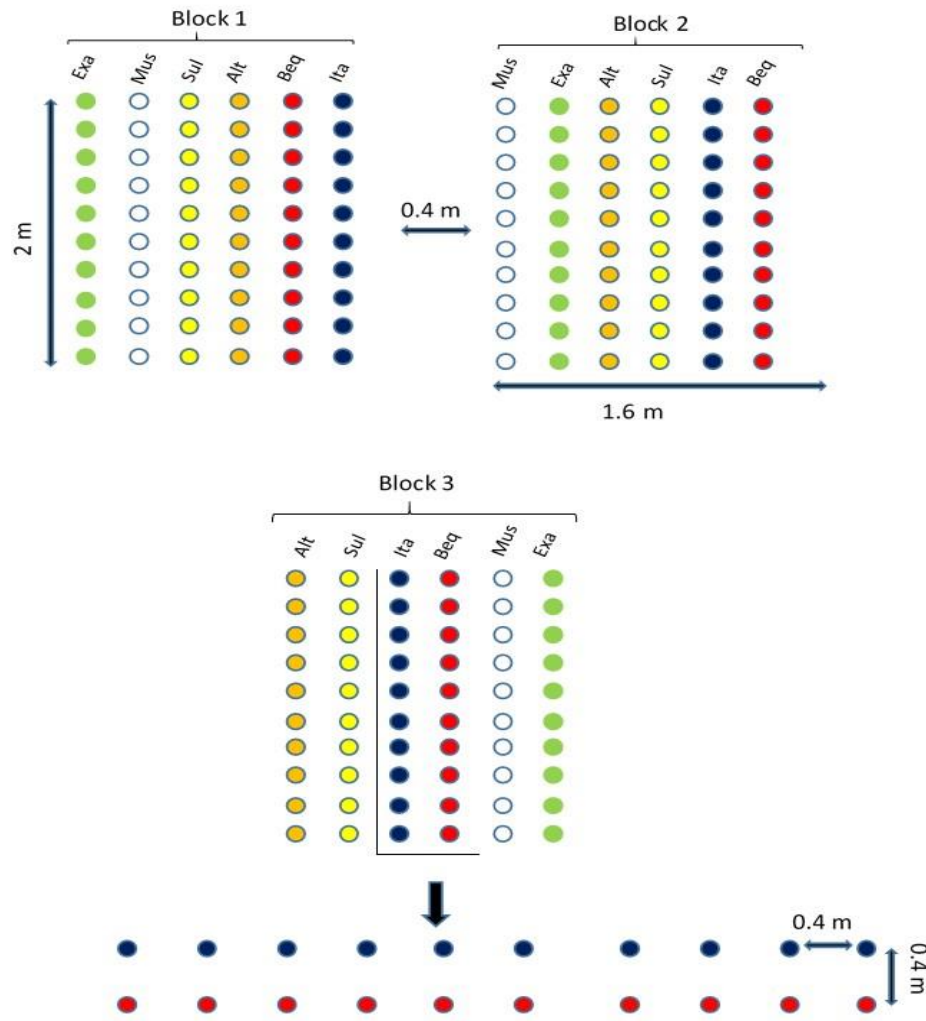


Figure 3. Experimental design of grapevine plants grown in nursery

Alt: Aleatico; Subl: white Sulima; Ita: Italia white; Beq: Bequignol; Mus: red Muscat; Exa: white Exalta

2.2.4. Evaluation of morpho-physiological parameters of grapevine plants in nursery

The hardiness of grapevine varieties in nursery was investigated through some morphological and physiological parameters.

2.2.4.1. Number of cuttings budded

The cuttings budded of grapevine, for each grapevine variety, were counted daily from the 6th to the 14th day of cuttings.

2.2.4.2. Rate of bud break

The budding rate (BR) corresponds to the number of cuttings budded per variety in relation to the total number of cuttings placed in nursery bags. It was evaluated each 5-day using the following formula:

$$BR (\%) = \text{number of cuttings budded} / \text{total number of cuttings bagged}.$$

2.2.4.3. Growth dynamics

The growth dynamics (GD) of grapevine plants in nursery was evaluated over a period of 30 days, from the 14th day of cutting. It concerned the first 30 cuttings of each variety. Using a tape measure, the distance between the budding point and the stem apex of the plant was measured every 5-day.

2.2.4.4. Number of leaves emitted

The number of leaves emitted by the grapevine plants was counted from the 14th day of cutting. The number of leaves emitted (NF) per plant and per variety was determined every 5-day for 30 days. A total of 30 leaves were involved per vine variety.

2.2.4.5. Width and length of leaves

The width (WiL) and length (LeL) of three previously marked leaves per plant on the 14th day of cuttings were measured after the leaves were spread. Measurements were taken every 5-day for 30 days. A total of 30 leaves were involved per grapevine variety.

2.2.4.6. Diameter at the collar of plants

The collar diameter (CD) of the plants of each vine variety was evaluated, using a digital caliper, from the 29th day of cutting. Measurements were made every 5-day for 15 days.

2.2.4.7. Robustness Quotient

The robustness quotient (RQ) was used to screen grapevine plants. Indeed, it allows to assess the plants having a height and a diameter likely to express a good resilience to the pedoclimatic conditions of the study area. In fact, it corresponds to the ratio of the height of the plants by the diameter at the collar and calculated according to the following formula: $QR = \text{length of plants} / \text{diameter at the collar of plants}$.

2.2.4.8. Number and length of roots

The number of roots (NR) was evaluated on 44-day-old seedlings. Three plants per variety were watered thoroughly before being gently uprooted from the bags. The roots were then counted and an average was taken per vine. In addition, the root length (RL), the three longest roots of each grapevine plant, was estimated using a tape measure.

2.2.4.9. Plant water content

The grapevine plants are gently uprooted from the nursery bags. The roots are washed with tap water to remove substrate debris. The plants are then spin-dried with blotting paper to remove the remaining water. Three plants were used per variety. The plants were weighed using a precision balance for fresh weight (FW) determination. These plants were then placed in an oven at 60°C until a constant weight was obtained, which represents the dry weight (DW). Plant water content, according to Henson et al. (1981), is determined by the following formula: $PWC (\%) = [(FWP - DWP) / DWP] \times 100$, where FWP is the fresh weight of plant, PDW is dry weight of plant and PWC is the plant water content.

2.2.4.10. Extraction and dosage of leaf pigments

For each vine variety, the leaves were harvested from the third leaf stage. They were then cut into small fragments and 0.2 g were ground in a porcelain mortar in the presence of 20% acetone and a pinch of calcium carbonate. The extraction residue was recovered by rinsing twice with 5 mL of 20% acetone. The filtrate was centrifuged at 5,000 rpm for 15 min at 4 °C. The resulting supernatant was the crude leaf pigment extract.

The determination of leaf pigments was performed according to the method of Lichtenthaler and Buschmann (2001).

The volume (V) of the crude pigment extract was determined for the mass (m) of leaves used. The absorbance of the pigment extracts was measured with a spectrophotometer at 470 nm for carotenoids and at 647 nm and 663 nm for chlorophylls, against a control without pigment extract and consisting of acetone. The calculation of the different concentrations was done according to the following formulas:

$$\text{Chl a (mg/g MF)} = [12.25 \times \text{OD}_{663} - 2.79 \times \text{OD}_{647}] \times V / 1000 \text{ m}$$

$$\text{Chl b (mg/g MF)} = [21.5 \times \text{OD}_{647} - 5.10 \times \text{OD}_{663}] \times V / 1000 \text{ m}$$

$$\text{Chl t (mg/g MF)} = [7.15 \times \text{OD}_{663} + 18.71 \times \text{OD}_{647}] \times V / 1000 \text{ m}$$

$$\text{Car (mg/g MF)} = [1000 \times \text{OD}_{470} - 1, 82 \times \text{chl a} - 85.02 \times \text{chl b}] / 189 \times V / 1000 \text{ m}$$

Where V denotes the volume of the crude leaf pigment extract in milliliters (mL) of the pigment extract and m is the mass in milligrams (mg) of the fresh leaves (MF), Car represents carotenoids, Chl a is chlorophyll a, Chl b is chlorophyll b and Chl t represents total chlorophyll.

2.2.4.11. Indicators of functional pigment equipment and leaf greenness

The indicator of functional pigment equipment and leaf greening status of the different grapevine varieties were evaluated by the ratios Chl a/Chl b and Chlt/Car respectively. As an indication, grapevine varieties with a Chl a/Chl b ratio higher than 1 and Chlt/Car higher than 3.5 develop a good photosynthetic activity. Conversely, those with a Chl a/Chl b ratio below 1 and Chlt/Car below 3.5 possess a low photosynthetic activity (Morot-Gaudry and Farineau, 2011).

2.2.5. Statistical analyzes

The data obtained from the evaluation of morpho-physiological parameters of the different grapevine varieties were analyzed using Statistica 7.1 software (StatSoft, 2005). Analyses of variance with one or two classification criteria were performed on the mean values of the measured parameters. Percentage data underwent an arcsin angular transformation (\sqrt{x}) before analysis of variances. When this analysis showed a significant difference ($p \leq 0.001$), the multiple comparison test of Newman-Keuls at 5% was conducted to classify the means into homogeneous groups.

3. Results

3.1. Effect of variety and cultivation time on the bud break rate of grapevine cuttings

Figure 4 shows the variation in the rate of cutting of the cuttings of six grapevine varieties as a function of time. The results indicate that the rate of cutting, which was not significant on the 6th day of culture, increased significantly to vary between 70 and 80% on the 10th day of culture. The red Muscat variety had the highest rate of cuttings followed by Bequignol, white Exalta, white Italia and then white Sulima. On the other hand, on the 14th day of cuttings cultivation, the rate of bud burst of the grapevine varieties drastically falls to reach the rates observed on the 6th day except with Aleatico where the rate of bud burst increases considerably to reach almost 80%.

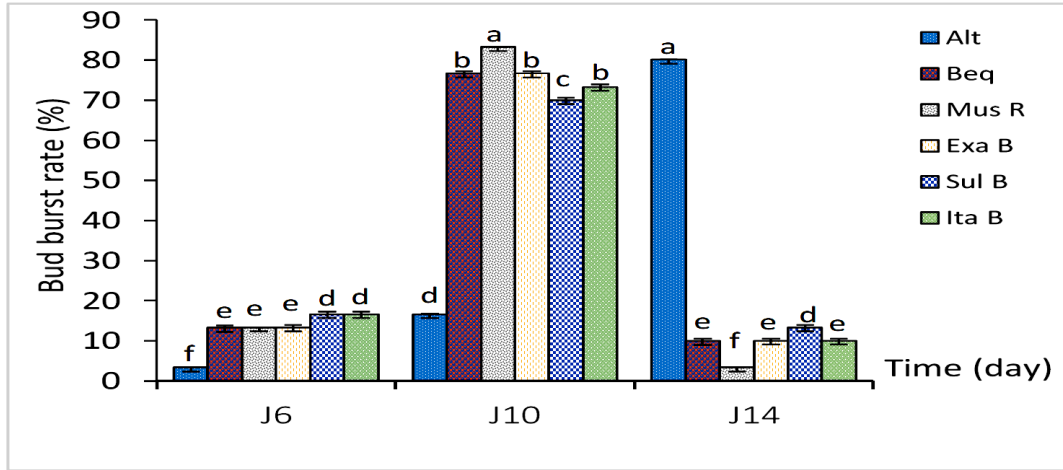


Figure 4. Variation in grapevine cuttings rate of bud burst with nursery cultivation time

Alt: Aleatico; Beq: Bequignol; Mus R: Red Muscat; Exa B: white Exalta; Sul B: white Sulima; Ita B: white Italia; J: day.

Histograms topped with the same letter are not significantly different (Newman-Keuls test at 5 %)

3.2. Effect of variety and cultivation time on growth dynamics of grapevine in nursery

The growth dynamics of grapevine plants in the nursery is shown in Figure 5. The results indicated that from Day 14 to Day 24, plant growth was identical and stable in the six varieties studied. However, from the 24th to the 44th day of cultivation, two groups of growth can be distinguished. Indeed, in the first group constituted by the grapevine varieties Aleatico, Bequignol and Muscat rouge, the growth of the plants is exponential. Thus, Aleatico had the most important growth of plants (35.51 cm) followed by Bequignol (29.57 cm) and Muscat rouge (23.37 cm). For group 2 composed of the vine varieties named white Exalta, white Sulima white and white Italia, the growth of the stems evolved very weakly compared to that of group 1. Therefore, the growth of the stems of Exalta white and Sulima white was around 6 cm, while that of Italia white, the weakest, oscillated around 3 cm.

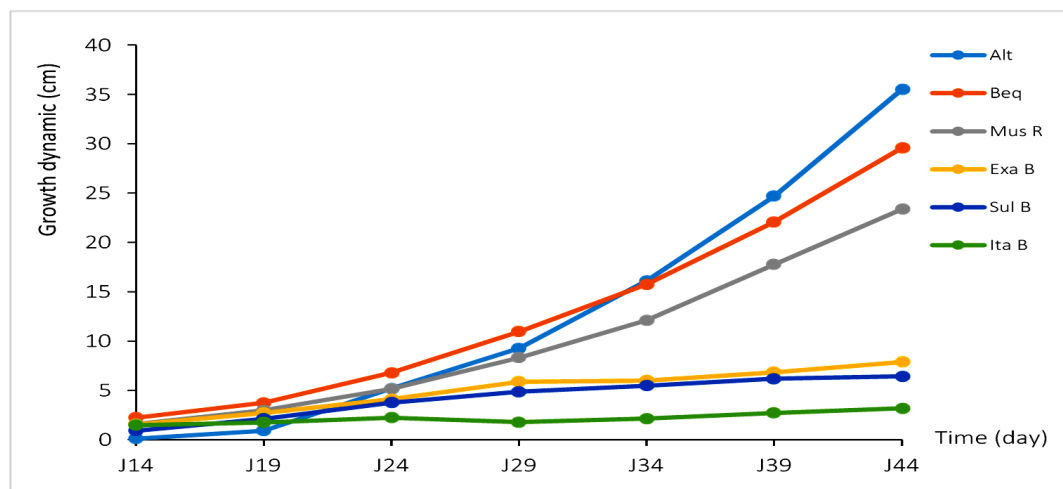


Figure 5. Changes in grapevine plants growth with nursery cultivation time

Alt: Aleatico; Beq: Bequignol; Mus R: Red Muscat; Exa B: white Exalta; Sul B: white Sulima; Ita B: white Italia; J: day.

Means followed by the same letter are not significantly different (Newman-Keuls test at 5 %)

3.3. Effect of grapevine variety and cultivation time on foliar emission

The interaction between vine variety and growing time on leaf emission is presented in Figure 6. The results show that leaf emission is influenced over time by vine variety. Indeed, leaf emission was regular in all vine varieties from Day 14 to Day 24. From Day 29 to Day 44, the grapevine varieties form two groups that are clearly distinguished by the number of leaves emitted. In fact, in the first group constituted by the grapevine varieties that are Aleatico, Bequignol and Muscat Rouge had a rather important phylogeny that evolved exponentially. Thus, Bequignol had the highest number of leaves emitted (21.53), followed by Aleatico and Red Muscat with, respectively, 17.46 and 16.03 leaves emitted on the 44th day of cuttings culture. Regarding group 2, composed of the grapevine varieties white Exalta white Sulima and white Italia, the number of leaves emitted was very low compared to group 1. As such, white Exalta and white Sulima emitted on average six leaves, while white Italia emitted only four leaves per plant.

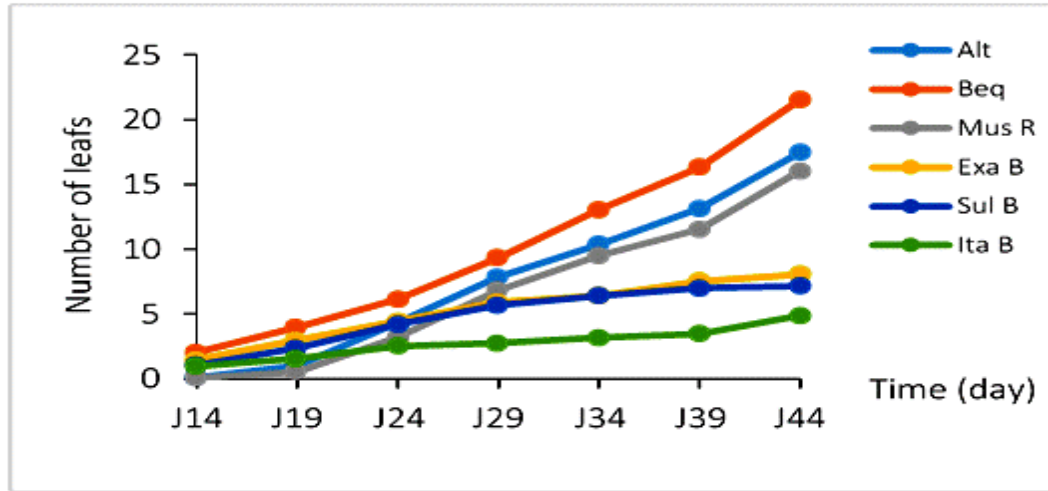


Figure 6. Changes in grapevine plants foliar emission with nursery cultivation time

Alt: Aleatico; Beq: Bequignol; Mus R: Red Muscat; Exa B: white Exalta; Sul B: white Sulima; Ita B: Italia white; J: day
Means followed by the same letter are not significantly different (Newman-Keuls test at 5 %)

3.4. Effect of grapevine variety and cultivation time on leaves width

Figure 7 shows the combined effect of grapevine variety and cultivation time on leaf width growth. The results show that the leaf width of the six grapevine varieties evolves in a similar way. However, two phases of leaf width growth of the plants appear. Therefore, from Day 14 to Day 34, the growth in width of the emitted leaves was significantly significant in all grapevine varieties. However, the leaves of the Aleatico variety plants were the widest (10.27 cm) on Day 34. On the other hand, in the second phase of growth, between Day 34 and Day 44, the study revealed that the growth in leaf width remained constant, regardless of the vine variety. However, the leaves of Aleatico had the greatest growth in width (10.50 cm), followed by red Muscat (8.34cm) and Bequignol (6.46 cm). On the other hand, white Silima and white Exalta then Italia white had the smallest leaf widths, with 5.39, 4.8 and 3.95 cm, respectively.

3.5. Effect of grapevine variety and cultivation time on leaves length

The interaction between grapevine variety and cultivation time of the cuttings was evaluated on the growth in length of grapevine leaves (Figure 8). The analysis revealed that the changes in grapevine plants leaves length with nursery cultivation time is similar to the width. Indeed, the length of the leaves increases rapidly until Day 34 and then remains constant until Day 44. Thus, from Day 14 to Day 34, the growth in length of emitted leaves was very significant in all grapevine plants varieties. However, the leaves of Aleatico and Bequignol were the longest (8.48 and 8.25 cm, respectively), followed by red Muscat (7.47 cm), white Exalta (6.95 cm) and white Sulima (6.29 cm) at Day 34 and this until Day 44. As for the variety Italia white, the growth in length of the leaves was the lowest (4.92.cm) during the plants culture in nursery.

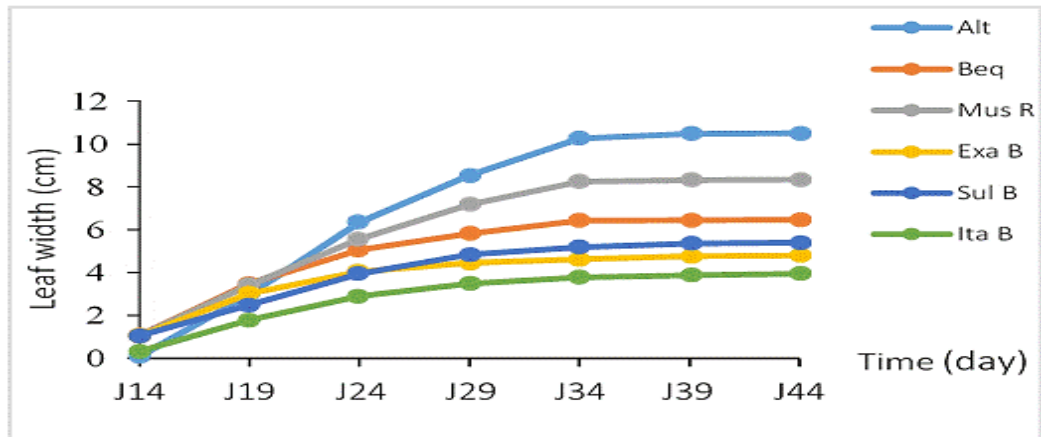


Figure 7. Changes in grapevine plants leaf width with nursery cultivation time

Alt: Aleatico; Beq: Bequignol; Mus R: Red Muscat; Exa B: white Exalta; Sul B: white Sulima; Ita B: Italia white; J: day
Means followed by the same letter are not significantly different (Newman-Keuls test at 5 %)

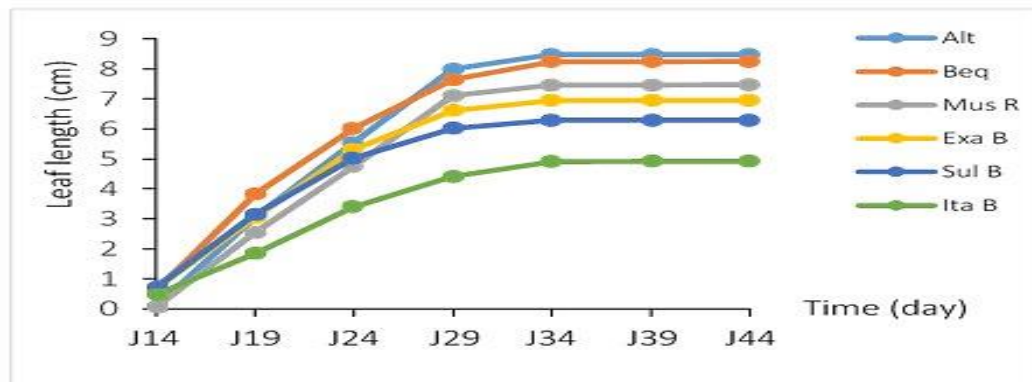


Figure 8. Changes in grapevine plants leaves length with nursery cultivation time

Alt: Aleatico; Beq: Bequignol; Mus R: Red Muscat; Exa B: white Exalta; Sul B: white Sulima; Ita B: Italia white; J: day
Means followed by the same letter are not significantly different (Newman-Keuls test at 5 %)

3.6. Effect of grapevine varieties and cultivation time on plant collar diameter

Figure 9 shows the variation in plants collar diameter of the six grapevine varieties as a function of the cuttings cultivation time. The measurements were carried out between the 29th and 44th day of culture. Overall, a regular change in the collar diameter of the grapevine plants, with the same trend was observed regardless of variety. However, Aleatico variety (6.58 cm), followed by those of Bequignol (6.07 cm) and red Muscat (5.29 cm) have developed the most collar diameter. On the other hand, plants of the grapevine varieties as white Sulima (4.27 cm) and white Italia (4.08 cm) have a collar diameter statistically similar but significantly lower than the two varieties above mentioned. Finally, white Exalta (3.46 cm) developed the weakest collar diameter compared to the other five studied grapevine varieties.

3.7. Influence of variety on root formation by grapevine plants

The results presented in Figure 10 indicate that the number of grapevine plants roots was significantly influenced by the grapevine varieties. Indeed, the number of roots formed decreased regularly from Bequignol to white Italia through Aleatico, red Muscat, white Exalta and white Sulima. Therefore, the plants of the Bequignol variety developed the best root system, with about 20 roots per plant. They were followed by plants of the varieties Aleatico (18 roots), red Muscat (14 roots) and white Exalta (11 roots). On the other hand, the varieties white Sulima and white Italia had the lowest number of roots, with 8 and 7 roots, respectively.

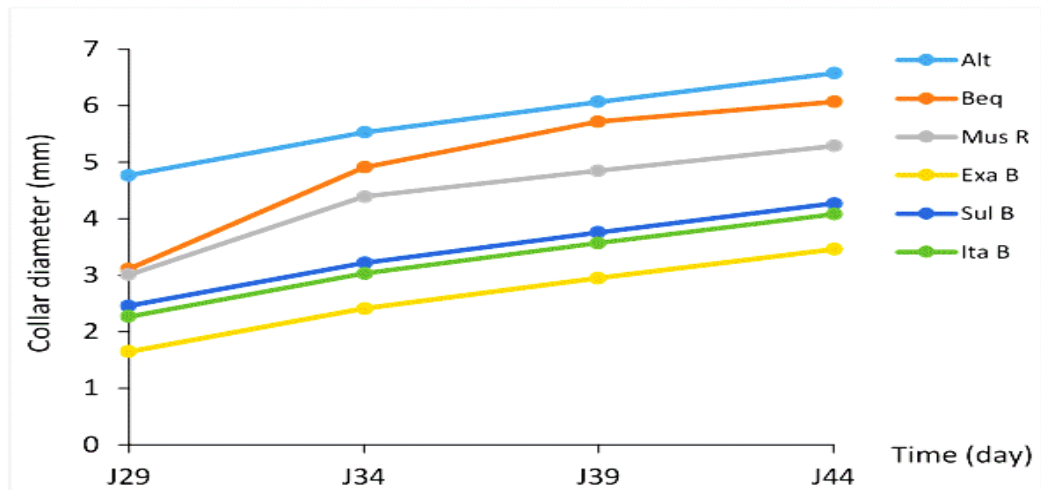


Figure 9. Variation in grapevine plants collar diameter with nursery cultivation time

Alt: Aleatico; Beq: Bequignol; Mus R: Red Muscat; Exa B: white Exalta; Sul B: white Sulima; Ita B: Italia white; J: day
Means followed by the same letter are not significantly different (Newman-Keuls test at 5 %)

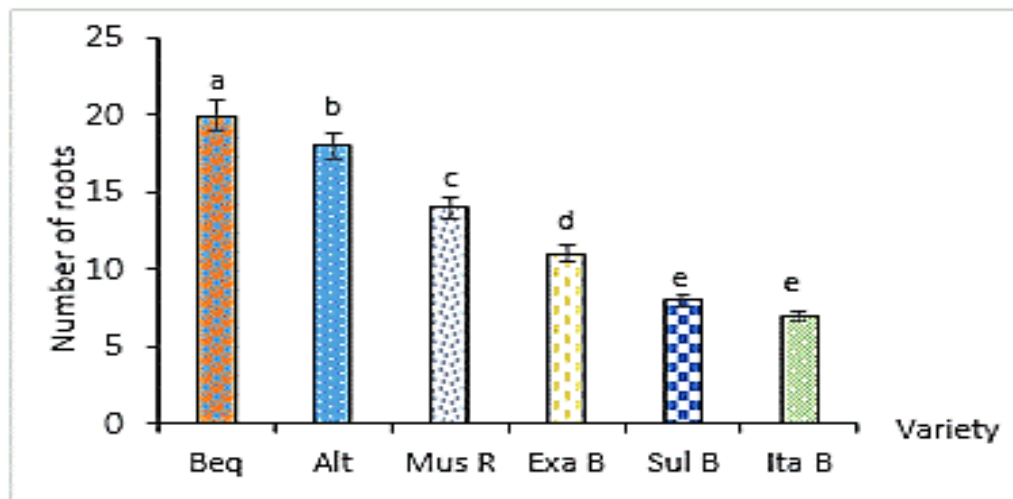


Figure 10. Variation of the plant roots number in six grapevine varieties

Alt: Aleatico; Beq: Bequignol; Mus R: red Muscat; Exa B: white Exalta; Sul B: white Sulima; Ita B: white Italia
Histograms topped with the same letter are not significantly different (Newman-Keuls test at 5 %)

3.8. Influence of variety on root length of grapevine plants

Figure 11 shows that the length of the vine roots varied greatly according to variety. Indeed, it decreases steadily from Aleatico to white Italia through to Bequignol, red Muscat, white Exalta and white Sulima. The analysis of variance revealed a highly significant varietal effect ($p > 0.005$) on root length. Hence, plants of the variety Aleatico (29 cm) developed the most significant growth in root length, followed by Bequignol (24.16 cm). Afterward, Muscat red (16.47 cm), Exalta white (14.08 cm), followed by white Sulima and white Italia induced the lowest root lengths, with 11.32 and 11.36 cm, respectively.

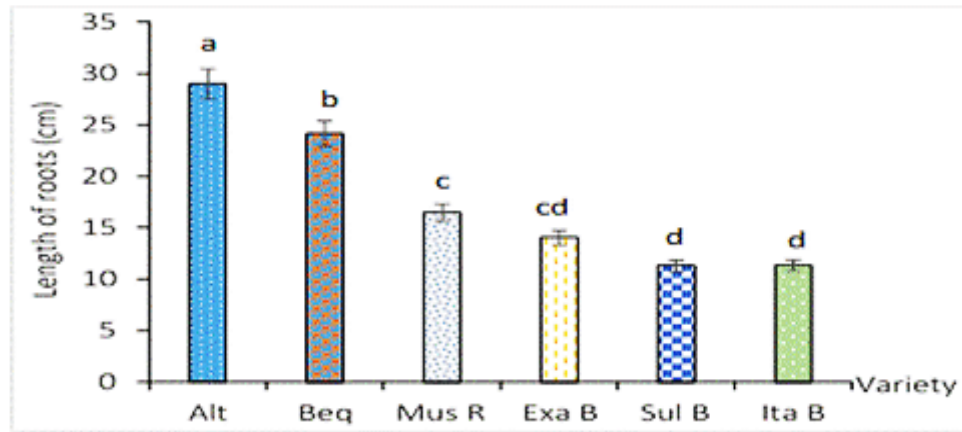


Figure 11. Variation of the plant roots length in six grapevine varieties

Alt: Aleatico; Beq: Bequignol; Mus R: red Muscat; Exa B: white Exalta; Sul B: white Sulima; Ita B: white Italia
Means followed by the same letter are not significantly different (Newman-Keuls test at 5 %)

3.9. Influence of variety on vigor of grapevine plants

The results of the influence of grapevine variety on the plants vigor in nursery were presented in Figure 12. Plant vigor decreases regularly from Aleatico to white Italia passing by Bequignol, red Muscat, white Exalta and white Sulima as previously. The results show a very highly significant ($p > 0.0001$) varietal effect of grapevine varieties on plant vigor. Thus, the highest plant vigor indices or robustness quotient were recorded in Aleatico (5.42 cm/mm), followed by Bequignol (4.45 cm/mm) and then Muscat rouge (3.87 cm/mm) and Exalta blanc (2.07 cm/mm). In addition, the plants of the grapevine varieties, white Sulima (1.61 cm/mm) and white Italia (0.79 cm/mm) were less vigorous compared to the plants of the other studied grapevine varieties.

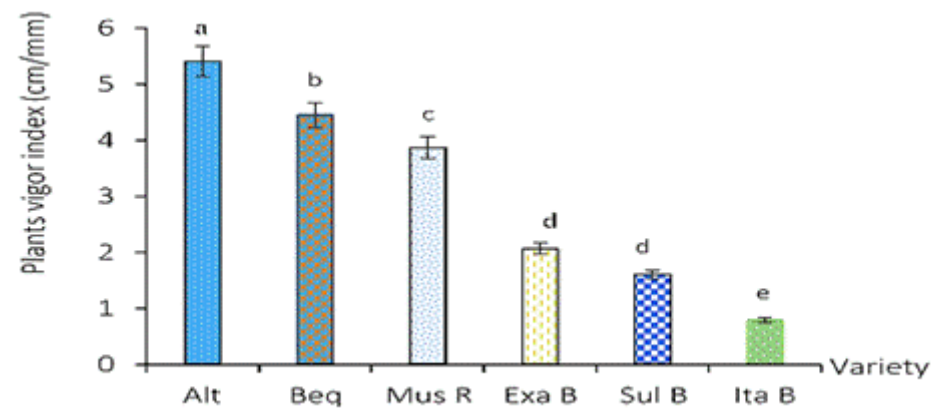


Figure 12. Variation of plants vigor in six grapevine varieties

Alt: Aleatico; Beq: Bequignol; Mus R: red Muscat; Exa B: white Exalta; Sul B: white Sulima; Ita B: white Italia
Histograms topped with the same letter are not significantly different (Newman-Keuls test at 5 %)

3.10. Influence of variety on water content of grapevine plants

The results in Figure 13 show that the grapevine variety has an influence on plant water content. In fact, water content decreased gradually in the plants of Aleatico to white Italia, via Bequignol, red Muscat, white Exalta and white Sulima. The analysis of the results revealed that the plant water content was significantly greater in Aleatico variety (95%), followed by Bequignol (69%) and red Muscat (45%) varieties. In contrast, the plant water content was significantly lower in grapevine varieties as white Exalta (22%), white Sulima (21%) and white Italia (20%) have registered statistically identical values.

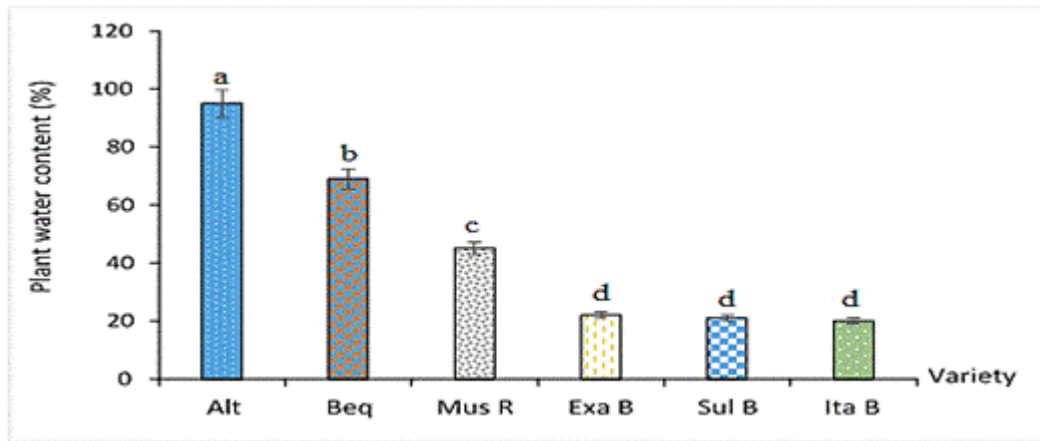


Figure 13. Variation of plants water content in six grapevine varieties

Alt: Aleatico; Beq: Bequignol; Mus R: red Muscat; Exa B: white Exalta; Sul B: white Sulima; Ita B: white Italia
Histograms topped with the same letter are not significantly different (Newman-Keuls test at 5 %)

3.11. Influence of variety on leaf pigment content of grapevine

The evaluation of the physiological status of the plants of six grapevine varieties was carried out by determining the chlorophyll a, b, total and carotenoid content of the leaves (Table 1). The analysis of the results revealed that the chlorophyll a content (0.78 mg/g MF) of the leaves of the Aleatico variety plants was the highest. It was followed by those of the varieties Bequignol (0.63 mg/g MF) and Muscat rouge (0.59 mg/g MF). On the other hand, this content was low in plants of the varieties Exalta white (0.25 mg/g FM), white Sulima (0.20 mg/g FM) and white Italia (0.15 mg/g FM). Regarding chlorophyll b, the highest content was observed with plants of Bequignol (0.44 mg/g FM), followed by Aleatico (0.38 mg/g FM) and red Muscat (0.39 mg/g FM). The varieties Exalta white (0.30 mg/g FM), together with Sulima white (0.25 mg/g FM) and Italia white (0.19 mg/g FM) recorded the lowest chlorophyll b content. As for the total chlorophyll content, it followed the evolution of chlorophyll a, with the highest content for Aleatico (1.16 mg/g FM), followed by Bequignol (1.07 mg/g FM) and red Muscat (0.98 mg/g FM). White Exalta (0.55 mg/g FM), white Sulima (0.45 mg/g FM) and white Italia (0.34 mg/g FM) had the weakest total chlorophyll contents.

In addition, for carotenoids, the highest levels were observed with Aleatico (0.30 mg/g FM), Bequignol (0.29 mg/g FM) and red Muscat (0.26 mg/g FM). As before, white Exalta (0.17 mg/g FM), white Sulima (0.14 mg/g FM) and white Italia (0.11 mg/g FM) were the grapevines that synthesized the lowest levels of carotenoids.

Table 1. Content of leaf pigment content in six grapevine varieties

Grapevine variety	Leaf pigment content (mg/g FM)			
	Chl a	Chl b	Chl t	Car
Alt	0.78 ± 0.03 ^c	0.38 ± 0.02 ^f	1.16 ± 0.05 ^a	0.30 ± 0.02 ^g
Beq	0.63 ± 0.02 ^d	0.44 ± 0.01 ^e	1.07 ± 0.03 ^a	0.29 ± 0.01 ^g
Mus R	0.59 ± 0.02 ^d	0.39 ± 0.02 ^f	0.98 ± 0.04 ^b	0.26 ± 0.03 ^g
Exa B	0.25 ± 0.01 ^g	0.30 ± 0.02 ^g	0.55 ± 0.01 ^d	0.17 ± 0.02 ⁱ
Sul B	0.20 ± 0.01 ^h	0.25 ± 0.02 ^g	0.45 ± 0.02 ^e	0.14 ± 0.01 ^j
Ita B	0.15 ± 0.01 ⁱ	0.19 ± 0.01 ^h	0.34 ± 0.02 ^f	0.11 ± 0.01 ^j
P	<0.001	<0.001	<0.001	<0.001

On a line and in a column, means with the same letter in the same column are not significantly different (Newman-Keuls test at 5%); ±S: Standard error; FM : Fresh matter; Chl a: Chlorophyll a; Chl b: Chlorophyll b; Chl t: Total chlorophyll; Car: Carotenoid; Alt: Aleatico; Beq: Bequignol; Mus R: red Muscat; Exa B: white Exalta; Sul B: white Sulima; Ita B: white Italia

3.12. Evaluation of indicators of functional pigment equipment and leaf greenness state

The results of the impact of grape variety on indicators of functional pigment equipment and leaf greenness of six grape varieties were reported (Table 2). The Chl a/Chl b ratio, which gives an indication of the functional pigment equipment of the leaves, was greater in Aleatico varieties (2.05), followed by Muscat rouge (1.51) and Bequignol (1.43). On the other hand, this ratio is less than 1 in the grapevine varieties as Exalta white (0.83 mg/g FM), white Sulima (0.80 mg/g FM) and white Italia (0.78 mg/g FM). In contrast, Chl t/Car ratio, which is the indicator of leaf greenness, was higher than 3.5 with Aleatico (3.86), followed by red Muscat (3.77) and Bequignol (3.68). On the other hand, with grapevine varieties such as white Exalta (3.23), white Sulima (3.21) and white Italia (3.09), the Chl t/Car ratio was lower than 3.5 which is the reference value.

Table 2. Indicators of functional pigment equipment and leaves greenness state in six grapevine varieties

Grapevine variety	Indicator	
	Functional pigments equipment	Leaf greenness status
Alt	2.05 ± 0.08 ^a	3.86 ± 0.07 ^a
Beq	1.43 ± 0.09 ^b	3.68 ± 0.09 ^b
Mus R	1.51 ± 0.06 ^b	3.77 ± 0.04 ^{ab}
Exa B	0.83 ± 0.03 ^c	3.23 ± 0.08 ^c
Sul B	0.80 ± 0.05 ^c	3.21 ± 0.06 ^c
Ita B	0.78 ± 0.04 ^c	3.09 ± 0.04 ^c
P	<0.001	<0.001

Means with the same letter in the same column are not significantly different (Newman-Keuls test at 5%); ±S: Standard error; FM : Fresh matter; Chl a: Chlorophyll a; Chl b: Chlorophyll b; Chl t: Total chlorophyll; Car: Carotenoid; Alt: Aleatico; Beq: Bequignol; Mus R: red Muscat; Exa B: white Exalta; Sul B: white Sulima; Ita B: white Italia

4. Discussion

The cuttings of the grapevine varieties initiated bud break on the 6th day to reach the maximum bud break on the 10th day of cultivation except for Aleatico where it is on the 14th day of cultivation. These results suggest that red Muscat, Bequignol, white Exalta, white Italia and white Sulima are precocious varieties while Aleatico is a tardy variety. Indeed, the date of bud break has an important impact on the grapes that will be harvested later in the season. If the bud burst is long and therefore late, it means that the different buds will have opened at different times, and thus that the bunches will be formed at different times. It is then that the levels of maturity of the grapes within the same grapevine may be disparate at the time of the harvest. This is a real problem, because the harvest can be lower, but also because it can cause acidity if some small bunches are harvested at the same time as those considered mature. On the other hand, if the budburst is short and therefore very early, the grapes will be synchronized and everything will be harvested at an optimal level of maturity to obtain beautiful bunches and hope for great wines. This stage of bud break is particularly striking in terms of the importance of understanding vintages (Covigneron, 2022). Late varieties bud much later than early ones. Thus, Merlot is often 15 days ahead of Cabernet Sauvignon (Covigneron, 2022). Moreover, during trials conducted on a collection of grape varieties located on the same plot, we noted differences in average bud break dates of 11 days between the most precocious, Chardonnay, and the most tardy, white Ugni. This shows an effect of the grapevine variety on bud break and is therefore dependent on the genotype.

In this study, the difference observed between early and late grapevine varieties is four (4) days. In view of the smaller gap, it would be wise to speak of very precocious varieties and precocious varieties rather than tardy varieties (Martinez, 2020). Therefore, red Muscat, Bequignol, white Exalta, white Italia and white Sulima would be in this study very precocious varieties and Aleatico, an precocious variety as reported by Uscidda (2014) among Corsican island varieties. But, the major risk of grapevine bud break is undoubtedly the frost, which with the negative night temperatures, can lead to the death of young buds and, consequently, the absence of cluster (Martinez, 2020). However, it is worth noting that in the case of this study, no constraint related to frost can be retained because the crop is grown in a tropical climate. Moreover, the interest of this study is to identify grape varieties that can adapt well to the soil and climate conditions of Côte d'Ivoire and therefore to the Ivorian terroir.

Morphological parameters of grapevine plants such as growth dynamics, leaf emission, leaf length and width were impacted by the variety and by the time the cuttings were grown in the nursery. The results of the analysis show that the evolution of these parameters occurred globally in two phases. Indeed, in the first phase, the growth in height of the plants and the leaf emission of the different varieties were very stationary in all the varieties from the 14 to 24th day of cutting. This could be explained by the fact that at the beginning of growth, the root system of the plants is not well established. The roots do not adequately meet the water and mineral needs of the plants (Doucet, 1992). Therefore, the low assimilation of organic nitrogen, necessary for the growth in height and the development of the plants leaves explains the low growth observed in all grapevines (Bacze, 1993). The second phase was characterized by a height significant growth of the plants and an important phylogenesis. In fact, at this stage (from the 24 to 44th day of cutting), the seedling has already a well-developed root system to ensure a good growth (Galzy, 1969). These perfectly developed roots will not only allow a good

fixation of the plant to the substrate to maintain its vigor, but also ensure a good absorption of the mineral elements released by the substrate (compost). This seems to contribute to the induction of twigs and leaves, the growth in height of the plants and the synthesis of photosynthetic products (Raphael, 1966).

Beyond the 24th day of cultivation, height growth and leaf emission of three grapevines, Aleatico, Bequignol, and red Muscat, were greatest. This suggests an intense mitotic activity of the cells in these grapevine varieties. Indeed, Benmahioul *et al.* (2009) reported that the increase in the growth of aerial plant parts and leaf draft is a function of the cell division intensity. On the other hand, Aleatico, Bequignol and red Muscat varieties developed the best root system. Plants of these varieties recorded the highest numbers and longest induced roots. This intense rhizogenesis allowed the plants of these varieties to get enough water and mineral elements to ensure good growth (Pradier, 2016). However, several studies have already reported that the varieties white Sulima, white Italia and white Exalta are vigorous (Groeninger, 2016; INRA, 2021). In this study, the low growth in height and leaf emission of these varieties seems to be related to the soil and climatic conditions of Côte d'Ivoire. These grapevine varieties would be more adapted to temperate climates, such as that of France where they originate, compared to the tropical and humid climate of Côte d'Ivoire. This resulted in a high mortality rate in these grapevine varieties during the experiment. The grapevine would thus be highly affected by the climate and the temperature. Moreover, Van Leeuwen (2010) reported that the adaptation of grape varieties is a function of climate or soil. Regarding the length and width of the leaves, the analysis also found two phases of growth. During the first phase (from day 14 to day 34), growth in leaf length and width was very significant in all grapevine varieties. This seems to suggest a non-significant effect of grape variety in the juvenile stages on growth in leaf length and width in grapevine. Thus, after bud break, plants of different grapevine varieties would allocate mobilized resources to the establishment of leaves as the first organs. This phenomenon would not be correlated with the grapevine variety. Concerning the second phase (from day 34 to day 44), the study showed a relatively low or even constant growth in all grapevine varieties. However, Aleatico, Bequignol and red Muscat varieties recorded the highest leaf lengths. Similar results were previously reported by Heller *et al.* (1995) who revealed that leaves have limited growth over time, despite being the first organs formed. On the other hand, a high diametric growth of stems was observed in all six grapevine varieties. This increase was progressive over time. Nevertheless, the largest diameters at the neck were recorded in the varieties Aleatico, Bequignol and red Muscat. The good diametrical growth of these three varieties could be explained by the fact that these varieties were able to develop a better root system. Moreover, Lamhamedi *et al.* (2007), showed that plants with a large collar diameter have generally a well developed root system. In addition, the Aleatico, Bequignol and red Muscat varieties recorded the highest vigor indices, reflecting a high robustness of these varieties. On the other hand, the low collar diameters observed in the varieties white Exalta, white Sulima and white Italia appear to be related to the less developed root system in these varieties. So, there would be a significant decrease in the hydric and mineral nutrition of these varieties plants, resulting in reduced cambial growth and plant vigor. Furthermore, according to El Boukhari *et al.* (2013), plant vigor is correlated with plant morphology and physiology. Moreover, physiological parameters of grapevine plants in the nursery were investigated in order to assess their influence on the grapevine varieties. The study concerned the water content of the plants and leaf pigments as well as indicators of the functional pigment equipment and the greenness status of the leaves of the different grapevine varieties. The greatest plant water content was obtained with the Aleatico, Bequignol and Red Muscat varieties. This high water content of these plants could be explained by the fact that these varieties have a large rhizosphere. This portion of the substrate subject to the influence of root activities allowed sufficient water supply to the plants (Van Leeuwen *et al.*, 2009; Pradier, 2016). These results corroborate those of Zufferey (2008) who showed that grapevine shows a very high resistance to drought which related to its great capacity of root exploration. The high water content of grapevine plants could also be explained by the fact that these varieties not only have a high number of leaves, but broad and long leaves. Indeed, according to Abbassenne (1997) and Jonckheere *et al.* (2004), the leaf area of a plant gradually determines both the quantities of water used by the plant in the form of transpiration and the quantities of carbon fixed photosynthetically. Thus, plants of the varieties Aleatico, Bequignol and red Muscat with high leaf surfaces would store more water than plants with low leaf surfaces. Furthermore, the photosynthetic efficiency of the plants of different grapevine varieties was assessed by the chlorophyll content. According to Garrity *et al.* (2011) and Barry and Newnham (2012), chlorophyll content is more reliable in assessing the photosynthetic capacity of the plants. Thus, the results obtained showed that there is a significant content of total chlorophyll and especially chlorophyll a in the leaves of Aleatico, Bequignol and red Muscat varieties in contrast to the leaves of the other three varieties (white Exalta, white Sulima and white Italia). Moreover, the Chla/Chlb ratio that is lower than 1 in the leaves of these last three grapevine varieties would mean that the plants of Exalta blanc, Sulima blanc and Italia blanc have not yet reached physiological maturity (Morot-Gaudry and Farineau, 2011). Indeed, the content of chlorophyll a, which is the main photosynthetic pigment, being low, photosynthesis would not be sufficient to make the plants truly autonomous and therefore vigorous (Berger *et al.*, 2007). Thus, these varieties would not be in a good physiological state to be transferred to the field. These plants would therefore be physiologically retarded under the study conditions compared to Aleatico, Bequignol and red Muscat varieties. On the other hand, the high content of chlorophyll a compared to chlorophyll b ($Chla/Chlb > 1$) in the leaves of Aleatico, Bequignol and red Muscat suggests good photosynthetic activity and good physiological status (Nguinambaye *et al.*, 2020). Moreover, Gamon *et al.* (1997) reported that photosynthetic activity of plants is significantly related to the ratio of total chlorophyll and carotenoid content ($Chlt/Car$). This ratio, which is an indicator of leaf greenness, should be greater than 3.5 (Morot-Gaudry and Farineau, 2011). So, in this study, the results obtained show that this ratio is higher than 3.5 for the varieties Aleatico, Bequignol and red Muscat. These three varieties seem to be better adapted to the pedoclimatic conditions of Côte d'Ivoire and could be the subject of in planta trials. On the other hand, white Exalta, white Sulima and white Italia, which have $Chlt/Car$ ratios below 3.5, would not have a good photosynthetic activity because of the weak green state of the leaves confirmed

by a low content of total chlorophyll as well as chlorophyll a contents lower than those of chlorophyll b. These varieties would not be in a good physiological state to be transferred to the field because of the low availability of nutrients. They do not seem to be well adapted to the pedoclimatic conditions of Côte d'Ivoire. Indeed, there is a need to adapt grapevines to the pedoclimatic conditions of new growing areas (Santillan *et al.*, 2019). Thus, as reported by Barreau *et al.* (2015), *in planta* trials will help to identify high-potential grapevines adapted to new growing areas.

Conclusion

The screening results obtained during these experiments show that the varieties Aleatico, Bequignol and red Muscat obtained better morpho-physiological profiles in nursery. In fact, unlike the varieties white Exalta, white Sulima and white Italia. Aleatico, Bequignol and red Muscat varieties had a good morphological growth and were quickly autonomous in nursery. Thus, taking into account considering the chlorophyll contents and the indicators of functional pigments equipment and greenness status of leaves, the screening in nursery made it possible to retain and to classify the varieties adapted to the pedoclimatic conditions of the Ivory Coast as follows Aleatico, Muscat then Bequignol. These three varieties can be transferred *in planta* in various agro-ecological zones of Côte d'Ivoire for behavioral trials.

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