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Analysis of the Genetic Diversity within Ten Citrus Species in Burkina Faso

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Abstract

Citrus fruits are plant species that grow in tropical and subtropical zones, particularly in Burkina Faso. The aim of this study is to characterize citrus species in Burkina Faso. To this end, 10 citrus species were evaluated in a Fisher block with two replications. The IPGRI citrus descriptor was used to select the variables to be measured. The morphological evaluation concerned the leaves and fruits of the 10 citrus species. The results show very remarkable variations, especially in incisions on the edge of the leaf blade, the shape of the top of the leaf blade, the shape of the leaf blade and the presence of wings on the petiole. As for the fruits of citrus species, variations in shape and flesh are observed. Qualitatively, the fruits are recognizable by their shape and by the presence or absence of a neck, a depression at the distal end and an outgrowth. The results of the data analysis of the quantitative fruit variables showed that there was a very highly significant difference at the 5% threshold. In view of the results obtained, it is necessary to study the variability within each citrus species.

Keywords

Citrus, Species, Genetic Diversity, Burkina Faso

1. Introduction

Agriculture in Burkina Faso is facing a multitude of challenges, including climatic constraints and increasing food production requirements. In this context, citrus stands out as a crop with high economic potential, not only for its profitability, but also for its role in improving agricultural biodiversity. Citrus production is growing rapidly in Burkina Faso, with the Hauts-Bassin, Central West and Central South being the main production areas [1]. It is estimated that 42,875 tons of citrus will be produced in the 2019/2020 season [2] and around 50,000 tons in 2022 [1]. Several species belonging to the Citrus, Fortunella and Poncirus genera contribute to the high diversity of citrus fruits [3]. Of these species, citrus is the most widely produced in the world, with 161.8 million tons produced in 2021 [4]. Citrus fruits, which are the result of complex hybridizations between several species, such as mandarins, lemons and grapefruit, have significant genetic diversity that can be exploited to improve their resistance to disease and their yield under a variety of climatic conditions [5].

However, the genetic performance of citrus species, although crucial to their adaptation and productivity, remains an under-exploited area in Burkina Faso. The study of citrus species in West Africa, and more specifically in Burkina Faso, is all the more relevant as these plants can play a major role in sustainable agriculture, offering solutions to the challenges of food security and income diversification for farmers [6]. Using advanced population genetics techniques, it is possible to gain a better understanding of the adaptation mechanisms of citrus species and select the best performers for particular local conditions. This work explores prospects for improvement by analyzing the diversity of citrus species in Burkina Faso, in order to better guide the choice of species adapted to local conditions.

2. Material and Methods

2.1. Geographical Location of the Site

The study was conducted from June to October 2024 at the Kouentou Agricultural Farm. Kouentou is located in arrondissement 3 of the Bobo-Dioulasso commune in the Houet province at coordinates 11˚19'33'' North and 4˚07'24'' West. It is a village located on the Bobo-Dedougou road, at 26 km from Bobo-Dioulasso center (**Figure 1**).

2.2. Material

The plant material for the study was composed of 10 citrus species. These species are listed in **Table 1**.

2.3. Methods

2.3.1. Plant Preparation and Maintenance

The different species of citrus fruits were purchased from nurseries recommended by INERA. For the planting of different citrus species, the plants were arranged in a simple block device with two repetitions for their agro-morphological characterization. The 2-year-old plants were planted in August 2024. Thus, five plants per species are used for study. Five leaf samples were taken from each plant per species. A total of 50 leaves were studied. Maintenance of the trial consisted of manual weeding as needed to limit competition with weeds in the trial.

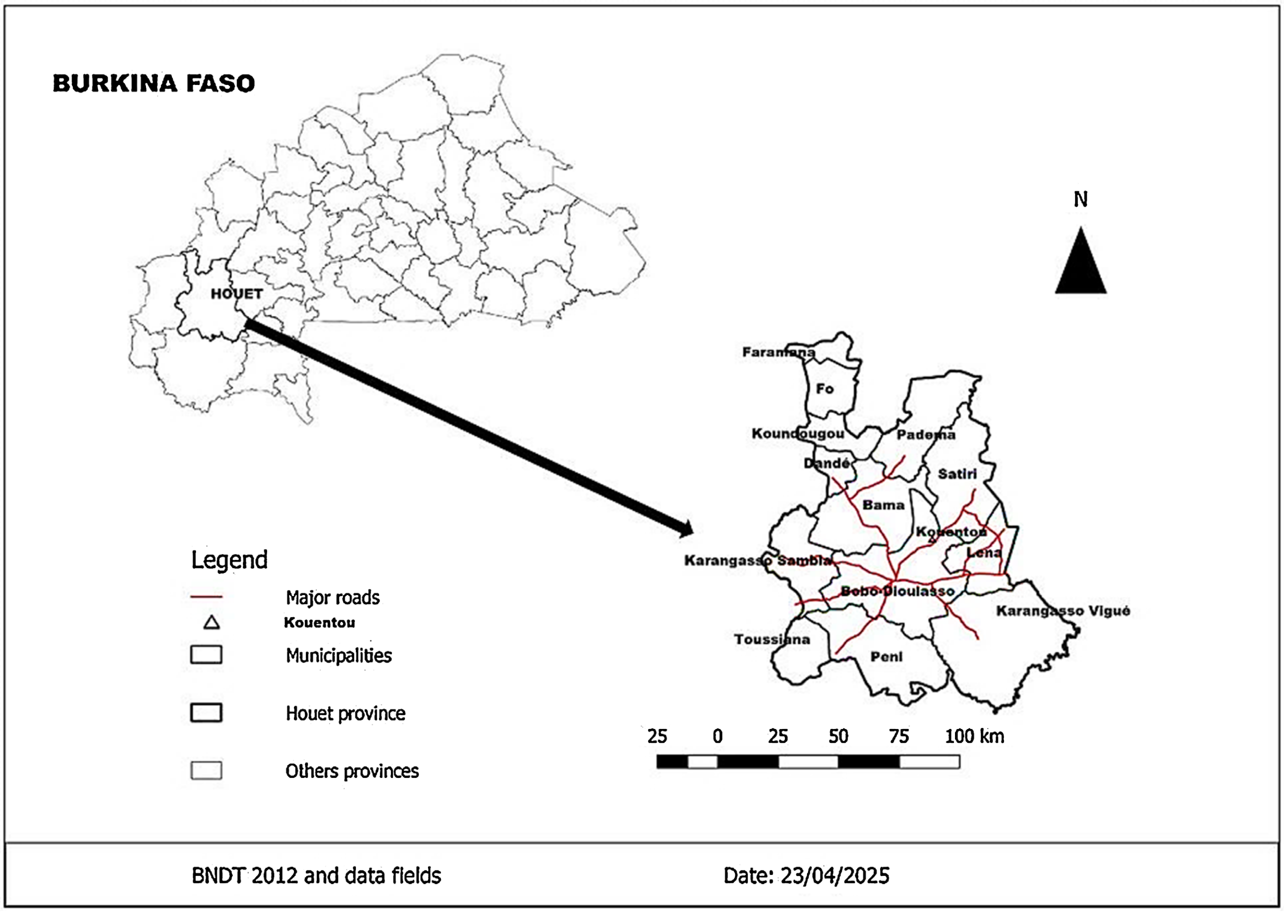

Figure 1. Geographical location of the Kouentou Agricultural Farm.

Table 1. List of the 10 citrus species characterized.

N°	Species	Scientific name	Characteristics
1	Sweet orange	*Citrus sinensis*	Hybrid
2	Yuzu	*Citrus junos*	Hybrid
3	Pomelo	*Citrus maxima*	Ordinary
4	Grapefruit	*Citrus paradisi*	Hybrid
5	Frimo	*Cirus frimo*	Hybrid
6	Tangelo	*Citrus tangelo*	Hybrid
7	Lime	*Citrus aurantiifolia*	Ordinary
8	Volka	*Citrus volkameriana*	Ordinary
9	Mandarin	*Citrus reticulata*	Ordinary
10	Clementine	*Citrus clementina*	Hybrid

2.3.2. Leaf Quality Variables

The variables measured on the leaves during the experiment were only of a qualitative type. The variables measured are: blade twist (BT), leaf blade blister (LBB), leaf blade green color (LBGC), blade edge ripple (BER), blade edge incisions (BEI), blade tip shape (BTS), blade vertex shape (BVS), blade shape (BS), the indentation at the end of the blade (IEB) and presence of wings at the petiole level (PWPL).

2.3.3. Qualitative and Quantitative Fruit Variables

The variables measured on the fruit during the experiment were qualitative [cross-sectional shape of the fruit (CSSF), General shape of the proximal part (GSPP), presence of a fruit neck (PFN), presence of a depression at the distal end (PDDE), Roughness (R), general shape of the distal part (GSDP), flesh colour (FC), presence of a growth (PG) and predominant colour of the fruit surface (PCFS)] and quantitative type [Fruit length (FL), Fruit diameter (FDiam), Ratio (R), Fruit neck length (FNL), Heart diameter of fruit (HDiamF), Fruit mass (FM) and Pericarp thickness (PT)].

2.3.4. Data Collection

Qualitative data for leaves and fruits were obtained by observing the variables described by [7] and taking into account the frequency of observation of these variables. For the quantitative fruit data, the variables fruit length, fruit diameter, fruit neck length, fruit core diameter and pericarp thickness were measured using a caliper. As for the mass of the fruits, it was measured with a precision 0.1 g electronic scale of the WH-B05 brand.

3. Data Analysis

The data collected was captured, organized and processed using the Microsoft Excel spreadsheet version 2016. This spreadsheet was also used to calculate the frequencies of the qualitative variables. The XLSTAT 2016 software was used for the analysis of variances (ANOVA), and the means were compared with the Newman and Keul test at the 5% level.

4. Results and Discussion

4.1. Results

4.1.1. Foliar Qualitative Variables Discriminating between the 10 Citrus Species

The leaves of citrus species show variations in leaf shape (**Figure 2**). Thus, the twisting of the blade (TB) is observed in the leaves of the sweet orange, Mandarin tree, Clementine tree, Tangelo plants, Frimo and Volka plants. However, it is absent on the leaves of the grapefruit tree, the plants of the grapefruit, the Yuzu tree and the lime tree. The variable leaf blade blister (LBB) is present on the leaves of Tangelo plants but absent in the leaves of the sweet orange Tree, the Grapefruit Tree, the Mandarin Tree, the Clementine Tree, the Yuzu Tree, the Lime Tree, Frimo Plants, Pomelo and Volka Plants. Concerning the leaf blade green color (LBGC), the leaf blade is dark green on the leaves of the Grapefruit tree, the Lime tree, the Frimo plants, the Pomelo and the Volka plants. However, it is moderately light on the leaves of the sweet orange, mandarin, clementine, Yuzu and tangelo

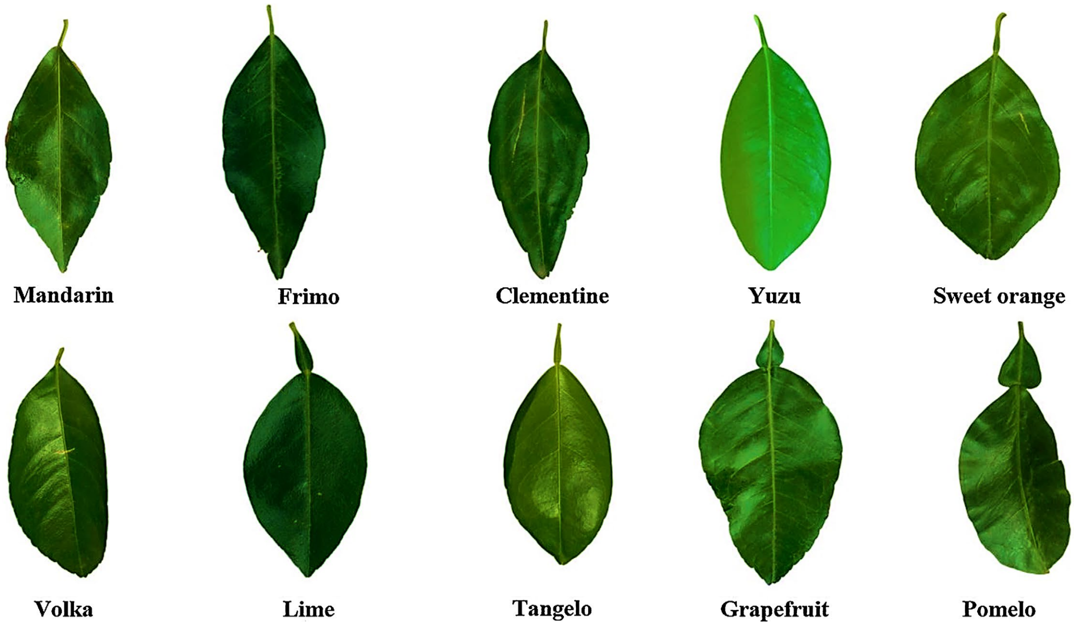

Figure 2. Variations in the leaf shapes of 10 citrus species.

plants. As for the blade edge ripple (BER), the edges of the blade of the sweet orange, the Grapefruit, the Mandarin, the Clementine, the Tangelo plants, the Frimo plants, the Pomelo and Volka plants are undulated. On the other hand, the edges of the leaf blades are not wavy at the level of the leaves of the Lime and Yuzu. For the blade edge incisions (BEI), they are serrated at the level leaves of the sweet orange, the lime tree, the Tangelo and Volka plants. However, they are crenulated at the level of the leaves of Grapefruit, Mandarin, Clementine, Yuzu, Pomelo and Frimo plants. As for blade tip shape (BTS), it is acute on the leaves of the sweet orange, grapefruit, lime, tangelo, Yuzu, Pomelo and Volka plants. On the other hand, there is no shape of the tip of the blade of Mandarin, Clementine and Frimo plants. In the blade vertex shape (BVS) of the blade, the apex of the leaves of the lime trees and Tangelo plants is acuminate. On the other hand, mandarin trees, Frimo and Clementine plants have leaves with pointed tops. As for the leaves of the Yuzu trees, the Sweet orange trees, the Volka plants, the Grapefruit trees and the Pomelo plants, have an acute top. For the blade shape (BS), the leaves of Mandarin trees, Frimo plants, Clementine trees, Lime trees and Sweet orange trees are elliptical. As for the leaves of Yuzu trees, Volka and Tangel plants, their blades have an oblong shape. Grapefruit and Pomelo plants have leaves with oval blades. The variable indentation at the end of the blade (IEB) is present in the leaf blades of Sweet orange trees, Grapefruit trees, Mandarin trees, Clementine trees and Frimo plants. However, it is absent in the leaf blades of the Lime Tree, Tangelo Plants, Yuzu Plant, Pomelo and Volka Plants. For the variable presence of wings at the petiole level (PWPL), the wings are observable at the level of the petioles of the leaves of Grapefruit trees, Volka plants, Sweet orange trees, Lime trees, Tangelo and Pomelo plants. However, they are absent in the petioles of the leaves of Mandarin trees, Clementine trees, Frimo plants and Yuzu trees (**Table 2**).

Table 2. Leaf quality variables of 10 citrus species.

N°	Species	BT	LBB	LBGC	BER	BEI	BTS	BVS	BS	IEB	PWPL
1	Mandarin	+	−	Medium	+	crenulated	None	Pointed	Elliptical	+	−
2	Frimo	+	−	Dark	+	crenulated	None	Pointed	Elliptical	+	−
3	Clementine	+	−	Medium	+	crenulated	None	Pointed	Elliptical	+	−
4	Yuzu	−	−	Clear	−	crenulated	Acute	Acute	Oblong	−	−
5	Sweet orange	+	−	Medium	+	Serrated	Acute	Acute	Elliptical	+	+
6	Volka	+	−	Dark	+	Serrated	Acute	Acute	Oblong	−	+
7	Lime	−	−	Dark	−	Serrated	Acute	Acuminate	Elliptical	−	+
8	Tangelo	+	+	Medium	+	Serrated	Acute	Acuminate	Oblong	−	+
9	Grapefruit	−	−	Dark	+	crenulated	Acute	Acute	Oval	+	+
10	Pomelo	−	−	Dark	+	crenulated	Acute	Acute	Oval	−	+

Legend: Present (+); Absent (−); Blade twist (BT); Leaf blade blister (LBB); Leaf blade green color (LBGC); Blade edge ripple (BER); Blade edge incisions (BEI); Blade tip shape (BTS); Blade vertex shape (BVS) of the limb; Blade shape (BS); Indentation at the end of the blade (IEB); Presence of wings at the petiole level (PWPL).

4.1.2. Fruit Quality Variables Discriminating between the 10 Citrus Species

The fruits of citrus species show variations in shape and flesh (Figure 3). Thus, for the cross-sectional shape of the fruit (CSSF), it is circular in the species Pomelo, Grapefruit, Mandarin, Clementine, Lime, Volka, Yuzu, Sweet orange and Frimo. On the other hand, the cross-section of the fruit has a somewhat angular shape in the Tangelo. For the variable presence of fruit neck (PFN), the neck is present in Pomelo, Grapefruit and Clementine but it's absent in Mandarin, Lime, Volka, Tangelo, Yuzu, Sweet orange and Frimo. The predominant colour of the fruit surface (PCFS) was green to yellow in the species Pomelo, Grapefruit, Mandarin, Clementine, Lime, Volka, Tangelo and Sweet orange. On the other hand, it is green to orange in Frimo and Yuzu.

The flesh colour (FC), it is white in the Pomelo, Grapefruit, Lime, Volka. On the other hand, it is orange in the Frimo, the Clementine, the Mandarin and Yellow in the Tangelo, Sweet orange, and the Yuzu. As for the presence of a growth (PG), the growth is observed in Grapefruit, Mandarin and Frimo. On the other hand, it is absent in the Grapefruit, Clementine, Lime, Volka, Tangelo, Yuzu and Sweet orange. For the variable presence of a depression at the distal end (PDDE), depression is observed in the species Grapefruit, Mandarin and Clementine). However, it is absent in the species Pomelo, Lime, Volka, Tangelo, Yuzu, Sweet orange and Frimo. The general shape of the distal part (GSDP) has been slightly rounded in Pomelo, Mandarin, Clementine and Frimo. It is flattened in Grapefruit, Tangelo, Yuzu and Sweet orange. However, it is strongly rounded in Lime and Volka. The general shape of the proximal part (GSPP) was slightly rounded in Mandarin, Clementine and Frimo. It is flattened in Grapefruit, Tangelo, Yuzu and Sweet orange. However, it is strongly rounded in Pomelo, Lime and Volka. For the variable roughness (R), no species characterized showed fruits with high roughness.

All fruits showed a smooth appearance in all characterized citrus species (Table 3).

Figure 3. Variations in fruit shapes of 10 citrus species.

Table 3. Fruit quality variables of 10 citrus species.

Species	CSSF	PFN	PCFS	FC	PG	PDDE	GSDP	GSPP	R
Pomelo	Circular	+	Green to yellow	White	+	−	Slightly rounded	Strongly Rounded	Smooth
Grapefruit	Circular	+	Green to yellow	White	−	+	Flattened	Flattened	Smooth
Mandarin	Circular	−	Green to yellow	Orange	+	+	Slightly rounded	Slightly rounded	Smooth
Clementine	Circular	+	Green to yellow	Orange	−	+	Slightly rounded	Slightly rounded	Smooth
Lime	Circular	−	Green to yellow	White	−	−	Strongly Rounded	Strongly Rounded	Smooth
Volka	Circular	−	Green to yellow	White	−	−	Strongly Rounded	Strongly Rounded	Smooth
Tangelo	A little angular	−	Green to yellow	yellow	−	−	Flattened	Flattened	Smooth
Sweet orange	Circular	−	Green to yellow	yellow	−	−	Flattened	Flattened	Smooth
Yuzu	Circular	−	Green to yellow	yellow	−	−	Flattened	Flattened	Smooth
Frimo	Circular	−	Orange	Orange	+	−	Slightly rounded	Slightly rounded	Smooth

Legend: Present (+); Absent (−); Cross-sectional shape of the fruit (CSSF); General shape of the proximal part (GSPP); Presence of a fruit neck (PFN); Presence of a depression at the distal end (PDDE); Roughness (R); General shape of the distal part (GSDP); Flesh colour (FC); Presence of a growth (PG); Predominant colour of the fruit surface (PCFS).

4.1.3. Quantitative Fruit Variables Discriminating between the 10 Citrus Species

The results of the data analysis of the quantitative fruit variables showed that there is a very highly significant difference at the 5% threshold (Table 4). For the variable fruit length (FL), Pomelo had the longest fruits with an average of 17.75 ± 0.35 cm. As for the Yuzu (2.20 ± 0.03 cm), it has the shortest fruits. Regarding the fruit diameter (FDiam), Pomelo presented the widest fruits with an average of 13.50 ± 0.70 cm. As for the Yuzu (2.10 ± 0.03 cm), it had the narrowest fruits. For the variable ratio (R), Pomelo also had the highest ratio with an average of 1.31 ± 0.04 cm. The lowest ratios were presented by the Tangelo and Mandarin species with the respective averages of 0.89 ± 0.03 cm and 0.89 ± 0.03 cm. At the variable fruit neck length (FNL), Grapefruit has the highest length of the fruit neck with an average of 4.90 ± 0.14 cm. As for the species Volka, Sweet orange, Frimo, Mandarin, Clementine, Lime and Yuzu, their fruits do not have a collar. For the variable heart diameter of fruit (HDiamF), clementines had the highest diameters of the heart of the fruit with an average of 2.50 ± 0.71 cm. It is statistically equivalent to Pomelo and Grapefruit species because they are assigned the same letter "a". The Volka had the smallest fruit heart diameter (0.60 ± 0.14 cm). Regarding the fruit mass variable (FM), Pomelo had the highest average fruit mass (1410 ± 70.71 cm) compared to Yuzu, which had the lowest average fruit mass (6 ± 0.35 cm). As for the variable pericarp thickness (PT), Pomelo had the highest thickness of the pericarp with an average of 1.55 ± 0.07 cm compared to the Yuzu, which had the lowest thickness of the pericarp, 0.10 ± 0.03 cm.

Table 4. Quantitative fruit variables of 10 citrus species.

Species	*FL*	*FDiam*	R	*FNL*	*HDiamF*	*FM*	*PT*
	Means (cm) ± standard deviations						
Pomelo	17.75^{a} ± 0.35	13.50^{a} ± 0.70	1.31^{a} ± 0.04	3.50^{b} ± 0.70	2.50^{a} ± 0.70	1410^{a} ± 70.71	1.55^{a} ± 0.07
Grapefruit	8.90^{b} ± 0.14	9.75^{b} ± 0.35	0.91^{e} ± 0.01	4.90^{a} ± 0.14	1.75^{a} ± 0.35	375^{b} ± 1.41	1.28^{b} ± 0.03
Volka	6.68^{c} ± 0.45	5.82^{cd} ± 0.26	1.14^{b} ± 0.03	0.00^{d}	0.60^{b} ± 0.14	118.5^{de} ± 0.71	0.48^{c} ± 0.03
Sweet orange	6.67^{c} ± 0.46	6.63^{c} ± 0.53	1.01^{d} ± 0.01	0.00^{d}	0.90^{b} ± 0.14	154.5^{cd} ± 34.65	0.40^{d} ± 0.01
Tangelo	5.98^{d} ± 0.02	6.75^{c} ± 0.35	0.89^{e} ± 0.03	2.75^{c} ± 0.35	0.75^{b} ± 0.07	197^{c} ± 21.21	0.40^{d} ± 0.01
Frimo	5.25^{ef} ± 0.35	4.75^{ef} ± 0.35	1.11^{bc} ± 0.01	0.00^{d}	0.90^{b} ± 0.14	104^{def} ± 5.66	0.43^{cd} ± 0.03
Mandarin	5.83^{de} ± 0.23	5.88^{cd} ± 0.17	0.99^{d} ± 0.01	0.00^{d}	0.80^{b} ± 0.14	113.5^{def} ± 17.67	0.40^{d} ± 0.02
Clementine	4.98^{f} ± 0.03	5.63^{de} ± 0.52	0.89^{e} ± 0.07	0.00^{d}	2.50^{a} ± 0.71	85.5^{ef} ± 20.51	0.40^{d} ± 0.03
Lime	4.80^{f} ± 0.28	4.65^{f} ± 0.49	1.03^{cd} ± 0.04	0.00^{d}	0.80^{b} ± 0.14	54^{fg} ± 12.73	0.40^{d} ± 0.02
Yuzu	2.20^{g} ± 0.03	2.10^{g} ± 0.03	1.05^{cd} ± 0.01	0.00^{d}	0.90^{b} ± 0.14	6^{g} ± 0.35	0.10^{e} ± 0.03
F	423.761	109.039	28.268	108.149	8.567	455.589	470.175
Pr > F	0.000	0.000	0.000	0.000	0.001	0.000	0.000
Signification	THS	THS	THS	THS	HS	THS	THS

Legend: Fruit length (FL); Fruit diameter (FDiam); Ratio (R); Fruit neck length (FNL); Heart diameter of fruit (HDiamF); Fruit mass (FM); Pericarp thickness (PT); THS: Very highly significant; HS: Highly significant. NB: The values of numbers bearing the same letters in the same column are statistically equivalent at the 5% threshold according to the Newman Keuls test.

5. Discussion

The results obtained on the morphological variables of the leaves and fruits of the ten citrus species studied illustrate a great phenotypic diversity and an important capacity to adapt these species to the local conditions of Burkina Faso. The leaf variables observed, such as the twisting and undulation of the leaf blade, the colour of the leaves and the presence or absence of wings at the petiole, provide discriminating elements for the identification and distinction of species. According to [8], differences in blade width contribute to variation between accessions and even between species. In addition, the twisting of the blade and the dark green colour of some leaves (especially those of Volka) are traits often associated with better efficiency in managing dehydration and increased tolerance to water stress. Finally, the presence of wings at the petiole discriminates between the citrus species in two groups. It is linked to climatic conditions and could change from one area to another. Whereas for these same authors, the appearance of the leaves would be negatively correlated with temperature and positively correlated with precipitation. These morphological variations, including the presence of wings and the twisting of the blade, can provide better flexibility and resistance of the leaves to winds and optimization of photosynthesis in tropical and subtropical areas. According to [9], two structures are observed in the citrus genus and the family Rutaceae: the three-leaved multifoliolate form and the presence of petiolar leaves (wings) in tropical regions such as the case in Burkina Faso, of Grapefruit, Volka, Sweet orange, Lime, Tangelo and Pomelo. All of these foliar characteristics of citrus trees were confirmed by [10] for a few species and by [7] for all species studied. At the end, SSR markers can permit to compare and to identify specific structures of citrus [11]. As for the qualitative variables of the fruits studied, they reveal important morphological differences, highlighting the phenotypic diversity among these species, a crucial aspect for varietal identification and selection. The characteristics analyzed, such as the shape of the fruit, the colour of the flesh, the presence of particular structures, such as the neck or the outgrowth, are essential to distinguish the species. Indeed, they influence not only the visual appearance of the fruit but also their adaptation to specific growing conditions. Organo-leptid and sensory characteristics are also used to identify the fruits of different citrus species [12]. The circular cross-sectional shape for most species can be associated with the genetic characteristics of each species, and it can be used for the identification and classification of citrus fruits. In addition, the presence or absence of a neck in other species, such as the Pomelo and the Grapefruit, reinforces the interest of this characteristic to distinguish these species from the Lime or the Volka, in which the neck is absent. The color of the surface of the fruit is an indicator of ripeness and visual quality of citrus fruits. In this study, the green color that turns yellow in some species, such as the Grapefruit and the Mandarin, and the green-orange in the Frimo and the Yuzu, highlights a significant phenotypic variation. The colour of the flesh is also discriminating, with white flesh (Lime, Volka, Pomelo, Grapefruit), yellow flesh (Sweet orange, Tangelo, Yuzu) orange (Frimo, Clementine, Mandarin), these variations can be related to the content of pigments such as carotenoids and flavonoids, often influenced by environmental and genetic factors. The outgrowth in some species, such as the Pomelo and the Mandarin, can be interpreted as an evolutionary trait contributing to the identification of species. According to [13], the presence or absence of a growth can also be influenced by mutations or variations in growing conditions. Distal depression, present in species such as the Grapefruit and the Clementine, is a trait that could result from adaptations to specific ecological niches or from local varietal selection for aesthetic or gustatory qualities. The rounded or flattened shape of the distal and proximal parts of the fruit is another distinguishing feature among citrus species. Species such as Lime and Volka, which have a rounded distal shape, may have evolved to optimize their resistance to mechanical stresses, such as wind and shock during harvest, while species with flattened ends, such as Tangelo and Yuzu, may have been selected for aesthetic preferences or storage qualities [14]. This type of morphology is often appreciated in the citrus market because it facilitates packaging and transport. The smooth structure of the fruits studied is a desired trait, as it facilitates cleaning and improves the visual appearance of the fruit, both of which are important for marketing. In addition, a smooth surface is less likely to retain dust and contaminants. This local diversity of citrus fruits has also been studied in Argentina by [15]. Indeed, the work of [15] revealed about thirty varieties of cultivated citrus.

6. Conclusion

The objective of this study was to characterize citrus species in Burkina Faso. At the end of this study, the morphological characterization of the leaves of the 10 citrus species revealed a large variation. These different variations observed on the leaves are mainly linked to the climatic conditions of the country. They may change slightly from one area to another. As for the fruits, morphological traits favor the distinction of species. From the measurements made on the leaves and fruits of the different citrus species, an identification key can be established.

Authors' Contributions

This work was carried out in collaboration with all the authors. CD and PS wrote the manuscript. ROFO carried out the activities. JS initiated and directed the work. MS supervised the work of this study. All authors have read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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