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ASSESSMENT OF CITRUS DIVERSITY IN GHANA

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ABSTRACT

Citrus is among the plant germplasm conserved at the CSIR - Plant Genetic Resources Research Institute (CSIR - PGRRI), Bunso in Ghana; however, due to lack of information on the specific traits of this germplasm at the Institute, the potential diversity within the collection is not yet fully exploited. The objective of this study was to evaluate the morphological diversity among the citrus accessions under conservation at the field genebank of the CSIR - PGRRI, Bunso, Eastern region of Ghana. The various accessions were raised on rough lemon rootstocks, planted using the augmented design. The citrus accessions evaluated include different citrus types suitable for different purposes, such as Flying Dragon for rootstock, Sweet Lime for medicine and Mediterranean Sweet for fruit drink. Late Valencia and Mediterranean Sweet were used as checks. Planting was done at 8 m by 8 m. Apart from watering immediately after planting, the plants grew under natural condition without irrigation. Variability was revealed by all the traits studied, including scion trunk surface, tree shape, tree growth habit and branch angle, based on IPGRI descriptors for citrus. The resulting phylogenic tree clustered the citrus accessions mainly based on known genetic relationships, using the clustering away of citrons from mandarins as an example. Although the morphological traits used in the characterisation were not sophisticated, they were useful in discriminating among the accessions. Based on this study, the citrus genebank at CSIR - PGRRI, Bunso, Eastern region of Ghana contains 44 morphologically distinct accessions. The information generated in this study is important for guiding conservation and utilisation decisions geared towards the improvement of the citrus sub-sector in the country.

Key Words: Genebank, germplasm, mandarins, phylogenic, scion

RÉSUMÉ

Les agrumes font partie du germoplasme végétal conservé au CSIR - Institut de recherche sur les ressources génétiques végétales (CSIR - PGRRI), Bunso au Ghana ; cependant, en raison du manque d'informations sur les traits spécifiques de ce matériel génétique à l'Institut, la diversité potentielle au sein de la collection n'est pas encore pleinement exploitée. L'objectif de cette étude était d'évaluer la diversité morphologique parmi les accessions d'agrumes en conservation à la banque de gènes de terrain du CSIR - PGRRI, Bunso, région orientale du Ghana. Les différentes accessions ont été élevées

sur des porte-greffes bruts de citronniers, plantés selon le dispositif augmenté. Les accessions d'agrumes évaluées comprennent différents types d'agrumes adaptés à différents usages, tels que Flying Dragon pour le porte-greffe, Sweet Lime pour la médecine et Mediterranean Sweet pour la boisson aux fruits. Late Valencia et Mediterranean Sweet ont été utilisées comme témoins. La plantation a été faite à 8 m sur 8 m. Outre l'arrosage immédiatement après la plantation, les plantes ont poussé dans des conditions naturelles sans irrigation. La variabilité a été révélée par tous les traits étudiés, y compris la surface du tronc du greffon, la forme de l'arbre, le port de croissance de l'arbre et l'angle des branches, sur la base des descripteurs IPGRI pour les agrumes. L'arbre phylogénique résultant a regroupé les accessions d'agrumes principalement sur la base de relations génétiques connues, en utilisant le regroupement des cédrats des mandarines comme exemple. Bien que les traits morphologiques utilisés dans la caractérisation n'aient pas été sophistiqués, ils ont été utiles pour discriminer les accessions. Sur la base de cette étude, la banque de gènes d'agrumes du CSIR - PGRRI, Bunso, région orientale du Ghana contient 44 accessions morphologiquement distinctes. Les informations générées dans cette étude sont importantes pour guider les décisions de conservation et d'utilisation axées sur l'amélioration du sous-secteur des agrumes dans le pays.

Mots Clés : banque de gènes, matériel génétique, mandarines, phylogénétique, greffon

INTRODUCTION

Citrus is a major sub-sector in Ghana, employing more than 20,000 producers (Akosah *et al.*, 2021). The citrus sub-sector contributes over US$ 350,000.00 annually to Ghana's foreign exchange earnings (Trend Economy, 2021). As of 2016, Ghana was ranked as the 18th highest producer of citrus in the world (CDC consult, 2016). Citrus is mostly cultivated in the semi-deciduous forest zone, with the major production areas located in the Eastern, Central and Ashanti regions of Ghana (CDC Consult, 2016). This notwithstanding, some cultivation is done in other vegetation zones like the coastal savannah, humid forest and the guinea savannah zones. The types of citrus grown in Ghana are diverse, including sweet oranges, tangerine, lime, lemon, grape and pumelo (Velasco and Licciardello, 2014).

Citrus is among the germplasm conserved at the CSIR - Plant Genetic Resources Research Institute (CSIR - PGRRI), Bunso in Ghana; however, due to lack of information on the specific traits of the accessions, the potential diversity within the collection has not been fully exploited. The purpose for the conservation of germplasm at the CSIR - PGRRI includes protection to forestall irreversible loss and usage for research and utilisation for food. Due to lack of information on the specific traits of the citrus germplasm at the CSIR-PGRRI, the potential diversity within the collection has not been fully understood. In order to optimise the utilisation of the citrus genetic resources conserved, there is the need for characterisation to identify important traits that can be utilised for different purposes.

Some citrus accessions have differences that are obvious to the untrained eye; however, expert knowledge is needed to objectively differentiate between some accessions. Comprehensive characterisation is, thus essential for effective utilisation of these materials. For example, in seedling production, it is particularly important to accurately distinguish between different varieties because the effect of choosing a wrong seedling may not be realised until the citrus starts fruiting, many years after planting. Therefore, the objective of this study was to evaluate the morphological diversity among different citrus accessions being conserved at the field genebank of the CSIR – PGRRI, Bunso, Eastern region, Ghana.

MATERIALS AND METHODS

This study used citrus accessions available in the citrus field genebank of the CSIR-PGRRI, Bunso in the Eastern region of Ghana. The citrus field genebank was established in 2011 at N06°17'09.4" W000°27'58.8", which is a location in the moist semi-deciduous forest agro-ecological zone. Scions (bud woods) used for the establishment were collected throughout Ghana. The passport information of the bud woods is kept at the institute (CSIR - PGRRI), which is the official genebank for Ghana.

The land used for the study was bush cleared with machetes. Lining and pegging were done to ensure accurate and uniform planting distance of 8 m by 8 m. Four seedlings per accession were used to establish the field genebank. The four seedlings of an accession followed each other before the subsequent accession, in a randomised manner using the augmented design, with Late Valencia and Mediterranean Sweet as the checks. All seedlings were produced using rough lemon rootstocks. Regular weed control, pruning and other recommended cultural practices were performed as and when necessary.

Characterisation was done five years after plantation establishment, using thirteen qualitative and three quantitative morphological traits, based on the International Plant Genetic Resources Institute (IPGRI) crop descriptor for citrus (IPGRI, 1999). The qualitative traits included scion trunk surface, tree shape, tree growth habit, density of branches, branch angle, leaf division, leaf blade intensity and leaf colour variegation. The other qualitative traits included leaf lamina attachment, lamina shape and margin, petiole wings width and petiole wing shape. The quantitative traits included leaf lamina width and length; and fruit diameter.

Statistical analysis of quantitative traits was done using analysis of variance of GenStat statistical package, edition 12 (Payne *et al.*, 2009). Qualitative traits were assessed using cluster analysis, followed by drawing of the phylogenic tree.

RESULTS

Quantitative traits. Leaf lamina length and width; and fruit diameter were highly significant among citrus accessions ($P < 0.001$) (Table 1). Leaf lamina length ranged from 21.6 mm for "Water Grape" to 198.6 mm for "Bengia". Leaf lamina width ranged from 11.0 to 78.0 mm for leaf lamina width for "Water Grape" and "Late Valentia". On the other hand, fruit diameter ranged from 8.0 mm for Kumquat to 65.0 mm for Late Valencia (Table 1; Fig. 1).

Qualitative traits. Forty-nine of the citrus accessions had simple leaves; except, Flying Dragon which was trifoliate (Fig. 2). The rest of the 13 qualitative traits scored are presented in Table 2.

Five types of leaf lamina shapes were observed in the citrus collection; these included elliptic, ovate, obovate, lanceolate and orbicular (Table 2). Leaf lamina shapes were most informative among the traits used for the characterisation. This information and the rest of the traits scored are presented in Table 2.

TABLE 1. Size of leaf lamina blade and fruit diameter of the citrus accessions available at the field genebank of the CSIR – PGRRI, Bunso, Eastern region, Ghana

Trait	Minimum (mm)	Mean (mm)	Maximum (mm)	F-probability
Leaf lamina length	21.6	91.95	198.6	0.001
Leaf lamina width	11.0	53.75	78.0	0.001
Fruit diameter	8.0	22.77	65.0	0.001

Figure 1. Disparity in mature fruit sizes of two citrus accessions from the field genebank of the CSIR - PGRRI, Bunso, Eastern region, Ghana.

Figure 2. Trifoliate leaf (left) of flying dragon and simple leaf (right).

The data collected were used to generate a dendrogram as shown in Figure 3.

DISCUSSION

Quantitative traits. Differences between accessions in terms of some quantitative traits were easily distinguishable (Table 1). For example, fruit sizes (diameter) of Late Valencia and Kumquat, were 65 and 10 mm, respectively. Disparities between trees of different accessions for other quantitative traits (leaf sizes and canopy spread) were also easy to detect. Leaf lamina length and width can be used to distinguish among most of the citrus accessions in the study germplasm.

In the case of canopy spread, Flying dragon was less than 45 cm; while that of Mediterranean sweet was more than 4 m. Citrus canopy spread is highly determined by the rootstock and the scion (Stuchi *et al.*, 2003). Since the same rootstock (rough lemon) was used for all the accessions, the differences in canopy spread can be attributed to the particular accession.

Qualitative traits. The differences between citrus accessions in terms of some qualitative traits were more difficult to detect with reasonable precision compared to quantitative traits (Table 1). For example, it was difficult to classify the density of branches as medium

TABLE 2. Morphological traits observed on fifty citrus accessions at the field genebank of the CSIR – PGRRI, Bunso, Eastern region, Ghana

Accessions	Traits																			
	Scion trunk surface		Tree shape			Tree growth habit		Density of branches			Branch angle			Leaf division		Leaf blade intensity		Leaf colour variegation	
	Smooth	Grooved	Obloid	Ellipsoid	Spheroid	Spreading	Erect	Medium	Dense	Sparse	Medium	Narrow	Wide	Simple	Trifoliate	Dark	Medium	Present	Absent
Abo	x			x			x			x		x		x		x			x
Ade	x			x			x		x			x		x			x		x
Ano	x				x		x		x		x			x		x			x
Asu	x			x			x	x			x			x		x			x
Ban	x				x	x			x				x	x		x			x
Ben	x			x			x			x		x		x		x			x
Blo II	x			x			x			x		x		x		x			x
BOPF	x			x			x	x				x		x		x			x
Cle	x			x			x		x			x		x			x		x
Dun	x			x			x			x	x			x		x			x
Fav	x			x			x		x			x		x			x		x
Fly	x			x			x			x		x			x		x	x	
Ham	x			x			x	x			x			x			x		x
Imp	x			x			x	x				x		x		x			x
Kara	x			x			x	x				x		x		x			x
King	x			x			x	x				x		x		x			x
Kum	x			x			x			x		x		x			x		x
Kwe	x				x		x		x			x		x		x			x
Lake	x			x			x	x			x			x			x		x
Late	x					x	x				x			x	x		x		
LateV	x					x		x			x		x		x			x	
LateV	x				x			x	x				x		x		x		
May		x				x	x			x				x	x		x		
May	x					x		x	x				x		x			x	

TABLE 2. Contd.

Accessions	Traits																			
	Scion trunk surface		Tree shape			Tree growth habit		Density of branches			Branch angle			Leaf division		Leaf blade intensity		Leaf colour variegation	
	Smooth	Grooved	Obloid	Ellipsoid	Spheroid	Spreading	Erect	Medium	Dense	Sparse	Medium	Narrow	Wide	Simple	Trifoliate	Dark	Medium	Present	Absent
Min	x			x			x		x					x	x			x	
Abo	x			x				x				x				x			
Ade		x	x								x		x						
Ano	x		x					x				x			x				
Asu	x			x				x				x		x					
Ban	x		x								x	x			x				
Ben	x		x					x					x						
Blo II	x		x								x	x			x				
BOPF	x		x					x			x					x			
Cle		x	x					x				x							
Dun	x						x	x					x						
Fav	x			x				x					x						
Fly	x				x				x				x						
Ham	x		x								x	x			x				
Imp	x		x					x					x						
Kara	x		x					x					x						
King	x		x					x					x						
Kum		x		x				x			x								
Kwe		x	x					x					x						
Lake	x			x				x				x			x				
Late	x	x		x				x				x		x					
LateV	x	x		x					x				x						
LateV	x	x			x				x				x						
May	x		x			x						x		x					

TABLE 2. Contd.

Accessions	Traits																			
	Scion trunk surface		Tree shape			Tree growth habit		Density of branches			Branch angle			Leaf division		Leaf blade intensity		Leaf colour variegation	
	Smooth	Grooved	Obloid	Ellipsoid	Spheroid	Spreading	Erect	Medium	Dense	Sparse	Medium	Narrow	Wide	Simple	Trifoliate	Dark	Medium	Present	Absent
May	x	x			x				x				x						
Min	x	x		x								x	x						
Mrs	x					x		x	x			x			x		x		
Nat	x				x			x			x		x		x		x		
Nig	x					x		x	x			x			x			x	
Obu	x				x			x	x				x		x		x		x
Oku	x				x			x		x			x		x			x	
Orl	x					x		x		x		x			x			x	
Ort	x			x			x		x					x	x			x	
Ova	x				x			x	x				x		x		x		
PinS	x				x			x	x				x		x		x		
Pom	x				x			x			x	x			x		x		x
Pon	x				x			x		x			x		x		x		
Port	x				x		x				x	x			x	x			x
Rho	x					x		x	x			x			x			x	
Rob	x					x		x		x				x	x			x	
Rub	x			x			x		x					x	x			x	
Sat	x				x			x	x			x			x		x		
Sek	x			x				x		x		x			x		x		
SGT She	x					x		x	x			x			x		x		
Subi	x				x			x	x				x		x		x		
Swt	x					x		x	x					x	x			x	
Tho	x					x		x	x			x			x		x		

TABLE 2. Contd.

Accessions	Traits																			
	Scion trunk surface		Tree shape			Tree growth habit		Density of branches			Branch angle			Leaf division		Leaf blade intensity		Leaf colour variegation	
	Smooth	Grooved	Obloid	Ellipsoid	Spheroid	Spreading	Erect	Medium	Dense	Sparse	Medium	Narrow	Wide	Simple	Trifoliate	Dark	Medium	Present	Absent
Unk	x					x		x	x					x	x		x		
Was	x				x			x			x	x			x			x	
Wat	x			x			x		x			x			x		x		x
Mrs	x	x		x					x				x						
Nat	x	x		x					x				x						
Nig	x	x		x					x				x						
Obu		x			x					x			x						
Oku	x	x			x				x				x						
Orl	x	x			x				x				x						
Ort	x	x			x							x	x						
Ova	x	x		x								x			x				
PinS	x	x		x					x				x						
Pom		x						x		x			x						
Pon	x	x		x					x				x						
Port			x				x		x			x		x					
Rho	x	x			x							x			x				
Rob	x	x		x								x			x				
Rub	x	x			x					x			x						
Sat	x	x		x								x				x			
Sek	x		x	x					x					x					
SGT She	x	x						x	x				x						
Subi	x	x		x					x				x						
Swt	x		x		x							x		x					

TABLE 2. Contd.

Accessions	Traits																			
	Scion trunk surface		Tree shape			Tree growth habit		Density of branches			Branch angle			Leaf division		Leaf blade intensity		Leaf colour variegation	
	Smooth	Grooved	Obloid	Ellipsoid	Spheroid	Spreading	Erect	Medium	Dense	Sparse	Medium	Narrow	Wide	Simple	Trifoliate	Dark	Medium	Present	Absent
Tho	x	x				x			x				x						
Unk	x	x			x				x				x						
Was	x	x				x			x				x						
Wat		x		x					x				x						

or dense. However, in general, the qualitative traits were quite informative as they were able to differentiate between most of the accessions (Fig. 3). The least distinguishing qualitative traits were scion trunk surface, leaf division, petiole wings, leaf blade colour intensity and leaf lamina attachment. Apart from the accession "Mayer Citron", which had grooved and ridged scion trunk surface, the rest of the accessions had smooth scion trunk surface.

In terms of leaf division, all the citrus accessions had simple leaves, except Flying Dragon which had trifoliate leaves. Similarly, only Kumquat had lanceolate type of leave lamina shape; while the others had elliptic, ovate, obovate or orbicular shapes. Leaf blade colour intensity was either dark or medium; while leaf lamina attachments were brevipetiolate or sessile.

The traits studied were unable to discriminate among some of the citrus accessions. Bengia and Natal Valencia were not separated and also, Mayer Sweet and Sekan. Similarly, three accessions, namely BOPF, Pineapple sweet and Subi were not discriminated by the qualitative traits (Fig. 3). The failure to discriminate among accessions could imply that the materials were the same or the markers used were not sufficient to do the discrimination. In cases where the reason was that the accessions were genetically the same, differences in the accession names could be the reason why they were considered to be different and, therefore, conserved as different accessions.

In some cases, those accessions which were not discriminated may be both morphologically and genetically similar as revealed in the case of the accessions "Kera de Semis" and "King de Semis" (Fig. 3). In other cases, clustering together may not mean genetical similarity, in spite of the existence of morphological similarity, for instance in Bengia and Natal Valencia (Knoema, 2020).

Accessions, Kara de semis, Imperial mandarin and King de semis were morphologically similar, hence they were not

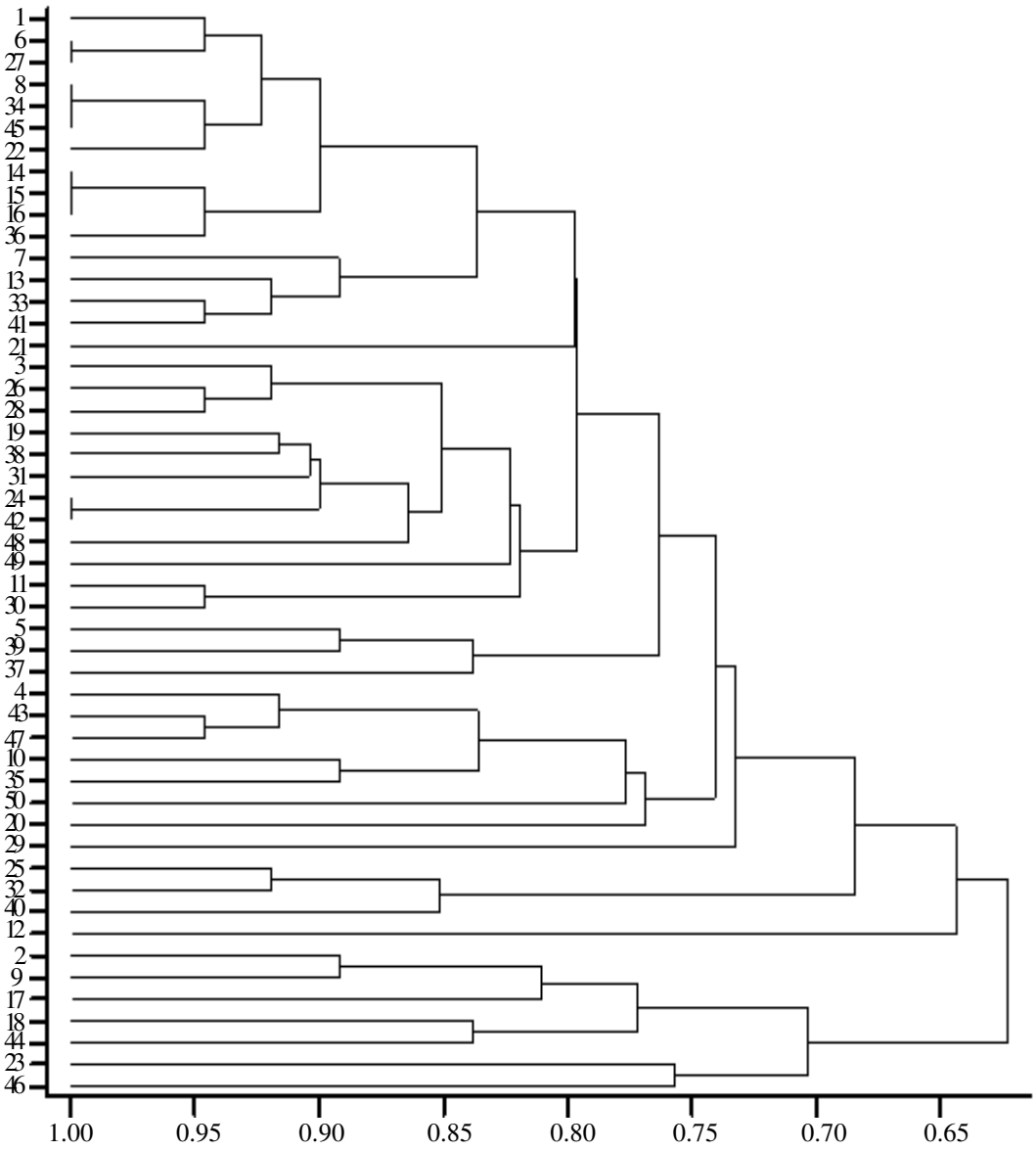

Figure 3. Phylogenic tree of 50 citrus accessions based on 13 qualitative morphological traits using the group average method. Similarity index is on the horizontal axis and the accession numbers on the vertical axis. The numbers represent the corresponding accession names at CSIR - PGRRI. Abofour (1); Adeiso (2); Anomabo (3); Asuansi (4); Baninhia (5); Bengia (6); Blood orange II (7); BOPF (8); Cleopatra (9); Duncan grape (10); Favi child (11); Flying dragon (12); Hamilin (13); Imperial mandarine (14); Kara de semis (15); King de semis (16); Kumquat (17); Kwesi Nyarko (18); Lake tangelo (19); Late (20); Late Valencia (21); Larttey (22); Mayer citron (23); Mayer sweet (24); Minneola tangelo (25); Mrs. Wright (26); Natal Valencia (27); Nigerian green (28); Obuasi (29); Okumaning (30); Orlando (31); Ortanique (32); Ovaleto (33); Pineapple sweet (34); Pomelo (35); Ponkan (36); Portuguese (37); Rhode Red Valencia (38); Robinson (39); Ruby (40); Satsuma (41); Sekan (42); SGT Delta (43); Shekwansha (44); Subi (45); Sweet lime (46); Thompson pink (47); Unnamed (48); Washington Navels 49); Water grape (50).

discriminated (Fig. 3). These three accessions are all descendants of *Citrus reticulate,* as explained by Velasco and Licciardello (2014). However, they should not be presumed to be the same, since fruit quality traits including aroma-volatile profiles and other chemical constituents can be used to differentiate between these mandarin type citruses (Goldenberg *et al*., 2015; Aboagye *et al*., 2017).

Since citrus is grown widely in Ghana, the assessment done at one location, Bunso, which is in the semi-deciduous forest ago-ecological zone, needs to be extended to other locations charaterised by different agroecologies and with different management regimes. According to Verreynne (2010), several factors such as weeds, insect pests and disease management; and fertiliser use affect growth, development and reproduction of citrus.

Phylogenic relationship of the citrus accessions. The first clustering was formed at a 65% level of similarity, with the smaller cluster having only seven members and an outlier, Flying Dragon (Fig. 3). The Flying Dragon was the only trifoliate citrus in the collection, with other unique features like long curved thorns and twisted branches (Cheng and Roose, 1995). The Flying Dragon is used more for medicinal purposes and as a root stock, than as food (Suozhan and Roose, 1995; Stuchi *et al*., 2003). The utility of the accession as rootstock stems from the fact that it is more resistant to a number of important citrus diseases; including phytophthora, nematode and tristeza (Stuchi *et al*., 2003).

The smaller cluster had only Kwesi Nyarko from the sweet orange group; while the rest were from the lime and lemon groups (Knoema, 2020). The members of the small cluster such as Sweet Lime, Mayer Citron and Kumquat, are morphologically distinct from most of the other accessions, implying that they are not from the sweet orange group.

Citrus collection at the citrus genebank of CSIR - PGRRI, Bunso in Ghana is dominated by sweet orange varieties; which were distributed throughout the clusters in the dendrogram (Fig. 3). Accessions SGT delta and Thompson pink, which were all grape types, and were the nearest neighbours at 95% similarity. This shows how reliable the traits were in discriminating among the accessions. Other grapes like Ruby were however, quite a distance from these grapes (Fig. 3), implying possession of different morphological traits. Clustering with genetically distant, rather than similar members may, have been caused by morphological similarity, which might not have a genetic base. This phenomenon, which may be misleading can result in customers or farmers purchasing undesirable planting materials. In cases where the information obtained is deemed unreliable, other traits such as number of seeds per fruit, leaf size and spine length, which were not included in this study, should be used in discriminating among citrus accessions.

CONCLUSION

This study is the first report on citrus diversity in Ghana from the national genebank located at CSIR - PGRRI, Bunso. Accessions with unique features, including Mayer Citron and Flying Dragon, were easy to distinguish morphologically. Because of its disease resistance ability accession Flying Dragon will be promoted for rootstock production and for other horticultural purposes in Ghana. On the other hand, accessions Ovalento and Satsuma need a critical expert observation before they can be separated and this will be best done using the descriptors. The morphological diversity observed among citrus accessions in the Ghanaian citrus germplasm was immense and useful for crop improvement programmes in Ghana.

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