OPEN ACCESS

African Journal of **Plant Science**

January 2021
ISSN 1996-0824
DOI: 10.5897/AJPS
www.academicjournals.org

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Vol. 15(1), pp. 1-7, January 2021
DOI: 10.5897/AJPS2020.2009
Article Number: 5E060BD65835
ISSN 1996-0824

http://www.academicjournals.org/AJPS

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African Journal of Plant Science

Full Length Research Paper

Association between agro-morphological traits in common bean under organic fertilization management in Brazil

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Received 2 May, 2020; Accepted 27 November, 2020

The objective of this work was to analyze the association between agro-morphological traits of the common bean cultivar "BRS Esplendor" under organic fertilization management. The experiment was implemented in the field, in a randomized block design, with three replications, in a split plot scheme, with two types of organic compounds (grass enriched with cattle manure and bean straw enriched with cattle manure) applied in six doses (0.0, 33.32, 66.65, 100.00, 133.32 and 166.65%). The control treatment comprised the recommended mineral fertilization. The characteristics include total number of pods, plant height and pod lengths are determinant to directly increase grain yield. The indirect determinant includes total weight of pods, total number of grains, plant height, root length and length of pods that had a positive effect with high magnitude on the characteristic total number of pods.

Key words: *Phaseolus vulgaris*, correlations, track analysis.

INTRODUCTION

Brazil is the world's leading producer of common beans (*Phaseolus vulgaris* L.), with production of 2.7 million tons and average productivity around 1,964 kg ha^{-1} in the 2019/2020 crop, and its cultivation was carried out in almost all regions of the country (CONAB, 2020). One of the options to leverage the stability of its commercialization is the aggregation of value to the grain, which can be desired with the use of the organic production system. Thus, the demand for this organically produced food has increased, even with values that are 30-40% higher than conventionally grown beans (Pereira et al., 2015).

The predominant bean cultivation system is conventional planting associated with the abusive use of nitrogen fertilizers in addition to pesticides; these factors influence the loss of soil quality, in addition to degradation by erosive processes (Ferreira et al., 2010). Darolt (2000) cited by Pereira et al. (2015) found that the barriers to organic cultivation is directly related to the lack of credit programs to finance such activity, in addition to the difficulties in marketing the products and the lack of technical information. Consequently, there is a need for examination that shows increased productivity and the expression of phenotypic characteristics. The variability in the expression of the results is essential for the success of the selection of phytotechnical characteristics and the breeder, with a focus on the main characteristic of economic importance (Cabral et al., 2010; Silva et al., 2008; Vieira et al., 2008 cited by Cabral et al., 2011).

With the use of appropriate statistical analysis, important information can be extracted for productivity gain; using trail analysis for yield and related components directly and indirectly. Hoogerheide et al. (2007) report that knowledge of the degree of this association, through correlation studies and trail analysis and possibilities identify characters that can be used as selection criteria for productivity. In addition, these analyses can be used for the selection of characters using different organic fertilizers via soil as treatments, favouring phytotechnists when performing similar work, avoiding waste of time and manpower.

In view of this context, Kurek et al. (2001) commented that path analysis is a tool that phytotechnist and improver has to understand the causes and effects involved in the combinations of characters and dissociate the correlation into direct and indirect effects, through a main variable. The trail analysis is used by several researchers in several crops of economic importance such as cotton (Hoogerheide et al., 2007), wheat (Vieira et al., 2007), beans (Kurek et al., 2001), and exotic forest species (Lorentz et al., 2006). This can be obtained from phenotypic, genotypic, environmental correlations, among others (Cruz and Carneiro, 2003); and phenotypic correlations are the most promising by phytotechnists and improver. Thus, the objective of this work was to analyze the associations between agro-morphological traits of the common bean cultivar "BRS Esplendor" under organic fertilization management.

MATERIALS AND METHODS

Study area

The experiment was conducted in the municipality of Campos dos Goytacazes, Rio de Janeiro State, Brazil (21°44'47" S and 41°18'24" W, and an average altitude of 10 m in relation to sea level). According to the Köppen climate classification, the climate of the Norte Fluminense region is classified as Aw, humid tropical climate, with rainy summer, dry winter and colder month temperature above 18°C.

Compost types

Two types of composts were formulated: the first based on elephant grass (*Pennisetum purpureum* Schum.) plus bovine manure and the second was based on bean straw with the addition of bovine manure. The materials used were dried for about 30 days in shade before being used in the composters. The windrow was installed in PESAGRO-RIO from June to September 2018, in a flat area protected from rain, sun and strong winds, with dimensions of 1.5 m^2. Each windrow was made by alternating layers of 20 (cm) in height of the bovine section (about 10 l) with grass or bean straw. During the production process of the compounds, the windrows were turned over and the temperature and humidity monitored, determining factors for the production of quality compost (Nunes, 2009). At the end of the composting process, a sample was taken for chemical analysis. The samples were ground and submitted to nitric-perchloric digestion in a digestor block. For the resulting extract, chemical characterization was performed to determine the nutrients content (Table 1), according to the methodologies described by Malavolta et al. (1997).

Soil

The soil of the experimental area is an Argissol, according to the Brazilian soil classification system (Santos et al., 2014). Ten simple soil samples were collected at PESAGRO - RIO, using a stainless probe and a depth of 0-20 cm. Composite samples, originated from the homogenization of simple samples were sent to the laboratory of the Federal Rural University of Rio de Janeiro (UFRRJ), in the municipality of Campos dos Goytacazes - RJ. The chemical characteristics of the soil were determined according to the methodology described by Teixeira et al. (2017). The results of the soil analysis of the experimental plot are shown in Table 2.

Experimental design

The experiment was implemented in the field, in a randomized

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Table 1. Chemical characterization of organic composts

Parameters	Elephant Grass and Dung	Bean Straw and Manure
pH (water)	6.9	7.5
N g / kg^{-1}	11.67	12.32
P_2O_5 g / kg^{-1}	8.87	9.57
K_2O g / kg^{-1}	7.01	9.53
Ca g / kg^{-1}	9.31	15.77
Mg g / kg^{-1}	4.37	4.7
C g / kg^{-1}	127.2	148.8
S g / kg^{-1}	2.02	1.19
Fe mg / kg^{-1}	14436	14496
Cu mg / kg^{-1}	26	40
Zn mg / kg^{-1}	276	276
Mn mg / kg^{-1}	480	456
B mg / kg^{-1}	37.95	80.42

pH = acidity; N = nitrogen; P_2O_5=phosphorus oxide; K_2O= potassium oxide; Ca = calcium; Mg = magnesium; C =organic carbon; S = sulfur; Fe = iron; Cu = copper; Zn = zinc; Mn = manganese; B = boron.

Table 2. Chemical attributes of the soil used in the study

Parameters	Soil
pH (water)	5.6
P mg dm^{-3}	7
K mg dm^{-3}	29
Ca $cmol_c$ dm^{-3}	2.2
Mg $cmol_c$ dm^{-3}	1.4
Al $cmol_c$ dm^{-3}	0.00
H+Al $cmol_c$ dm^{-3}	2.71
Na $cmol_c$ dm^{-3}	0.06
C %	1.24
N %	0.17
MO g dm^{-3}	2.1
SB $cmol_c$ dm^{-3}	3.7
T $cmol_c$ dm^{-3}	6.4
t $cmol_c$ dm^{-3}	3.7
m %	0.0
V %	57.9
Fe mg dm^{-3}	78
Cu mg dm^{-3}	1.0
Zn mg dm^{-3}	4.9
Mn mg dm^{-3}	12.6
S mg dm^{-3}	9.83
B mg dm^{-3}	0.80

pH= measurement of acidity and alkalinity (water); P= phosphorus (Extractor Mehlich 1); K= potassium; Ca= calcium; Mg= magnesium; Al= aluminum; H+Al=Hydrogen aluminum; Na = sodium; C = carbon; N = nitrogen; OM = organic matter; SB = sum of bases; T = CEC = cation exchange capacity; t = effective CEC; m = aluminum saturation; V = base saturation; Fe = iron; Cu = copper; Zn = zinc; Mn = manganese; S = sulfur; B = boron.

Table 3. Phenotypic (r_f) correlations related to agro - morphological characteristics of common beans in response to organic fertilization.

Variable	TNP	TWP	TNG	W100	PH	RL	SD	PW	PL	LAI
TNP	-	0.96	0.99	0.56	0.92	0.85	0.72	0.64	0.79	0.02
TWP			0.97	0.69	0.95	0.87	0.79	0.69	0.79	0.02
TNG				0.58	0.91	0.80	0.67	0.59	0.76	0.02
W100					0.64	0.61	0.55	0.67	0.43	0.07
PH						0.93	0.84	0.85	0.90	0.29
RL							0.96	0.88	0.90	0.18
SD								0.84	0.86	0.11
PW									0.85	0.54
PL										0.43
LAI										-

TNP - total number of pods; TWP - total weight of pods; TNG - total number of grains; W100 - weight of one hundred grains; PH - plant height; RL - root length; SD - stem diameter; PW - pod width; PL - pod length; LAI - leaf area index.

block design, with three replications, in a split plot scheme, with two types of organic compounds (grass enriched with cattle manure and bean straw enriched with cattle manure) applied in six doses (0.0, 33.32, 66.65, 100.00, 133.32 and 166.65%). The control treatment consisted of the mineral fertilizer recommended for the bean-producing region (Freire, 2013). The experimental plots contained 3 planting lines with 2 m linear each, being considered for evaluation; 1 m linear axis contains 10 plants.

The evaluated variables

The variables evaluated were: total number of pods (TNP), total number of grains (TNG), and total weight of pods (TWP) expressed in kg, obtained by means of precision electronic scale, performed in the useful area of the plot (10 plants). The other variables such as plant height (PH), root length (RL) and pod length (PL) were obtained using a ruler graduated in cm. For stem diameter (SD) and pod width (PW) (in mm) a digital pachymeter was been used. Leaf area index (LAI) was determined using AccuPAR (model 80) meter equipment configured in m^2 m^{-2} (Tewolde et al., 2005).

Pods were removed manually during harvest, then, their weight was determined on the same day, when they were collected as samples. Due to the difficulty in opening the pods on the same day of harvest, without damaging the seeds, it was preferred to wait four days at room temperature (25 to 30°C) and, after natural loss of moisture as pods began to open, the removal of seeds from the pods became easy.

Statistical analyses

Phenotypic correlation analyses were performed (r_f) through the following expressions: $r_F = \frac{PMG_{XY}}{\sqrt{QMG_X\, QMG_Y}}$. Where, PMG_{xy} = average product among genotypes for the characters of X and Y; QMG_x = square between genotypes for character X; QMG_y = square between genotypes for character Y. The trail analysis consisted of studying the direct and indirect effects of the explanatory variables mentioned above (X) on grain yield, the main dependent variable (Y). As Y is considered a complex feature, resulting from the combined action of other characteristics, the following model can be established: $Y = \beta_1 X_1 + \beta_2 X_2 + \cdots \beta_n X_n + \varepsilon$, in that: $X_1, X_2, \ldots, X_n$ are the explanatory variables, and Y is the dependent variable. The direct and indirect effects of explanatory variables are estimated on the dependent variable. Like this, $r_{iy} = p_i + \sum_{j\neq 1}^{n} p_{ij}\, r_{ij}$ where: correlation between the dependent variable (Y) and the i-th explanatory variable; p_i: direct effect of the variable *i* on the dependent variable; and $p_j r_{ij}$: indirect effect of the variable *i* variable route *j*, about the dependent variable (Almeida et al., 2014; Cabral et al., 2011; Coimbra et al., 2000; Cruz and Carneiro, 2003; Dalla Corte et al., 2010).

RESULTS

Phenotypic correlation (r_f)

The estimates of phenotypic correlation coefficients (r_f) evaluated for agro-morphological traits are presented in Table 3. The magnitudes of phenotypic correlation (r_f) between the characters ranged from 0.02 for LAI characteristics correlated with TNP, TWP and TNG and 0.99 for the TNG characteristic correlated with TNP. With the exception for W100, all correlations of TWP, TNG, PH, RL, SD and PL characteristics had positive values with high magnitude. The correlation of these characteristics on TNP varied with magnitudes of 0.96, 0.99, 0.92, 0.85, 0.72 and 0.79, respectively. The LAI characteristic had correlation of mean magnitude only with the PW characteristic with 0.54. The PW characteristic showed a correlation of medium magnitude with the characteristics TNP, TWP, TNG and W100 with 0.64, 0.69, 0.59 and 0.67, respectively, and of high magnitude with the characteristics PH, RL and SD with 0.85, 0.88 and 0.84 in this order.

Phenotypic (r_f) track coefficients

In the trail analysis, phenotypic (r_f) correlation coefficients ranged from negative high values to high magnitude positive levels, including direct and indirect effects for all evaluated characteristics, respectively (Table 4). Investigating the positive direct effects of the primary components on productivity, the main variable, the

Table 4. Direct and indirect effects of agro - morphological variables of common bean in response to organic fertilization.

Variable	Effect	Via	Coefficients(r_f)	Variable	Effect	Via	Coefficients(r_f)
	Direct	GY	0.6687		Direct	GY	-0.0590
		TWP	0.1061			TNP	0.5702
		TNG	-0.8412			TWP	0.0964
		W100	0.2228			TNG	-0.6810
		ALT	1.0000			W100	0.2424
TNP	Indirect	RL	-0.0503	RL	Indirect	PH	1.0000
		SD	-0.4329			SD	-0.5743
		PW	-0.2178			PW	-0.3003
		PL	0.4071			PL	0.4615
		LAI	-0.0087			LAI	-0.0536
	Total		0.9666		**Total**		0.8258
	Direct	GY	0.1097		Direct	GY	-0.5944
		TNP	0.6466			TNP	0.4870
		TNG	-0.8245			TWP	0.0868
		W100	0.2725			TNG	-0.5728
		ALP	1.0000			W100	0.2180
TWP	Indirect	RL	-0.0518	SD	Indirect	PH	1.0000
		SD	-0.4703			RL	-0.0570
		PW	-0.2357			PW	-0.2855
		PL	0.4042			PL	0.4406
		LAI	-0.0060			LAI	-0.0329
	Total		0.9843		**Total**		0.7066
	Direct	GY	-0.8486		Direct	GY	-0.3379
		TNP	0.6629			TNP	0.4311
		TWP	0.1066			TWP	0.0766
		W100	0.2290			TNG	-0.5089
		PH	1.0000			W100	0.2664
TNG	Indirect	RL	-0.0473	PW	Indirect	PH	1.0000
		SD	-0.4012			RL	-0.0524
		PW	-0.2026			SD	-0.5022
		PL	0.3908			PL	0.4365
		LAI	-0.0073			LAI	-0.1620
	Total		0.9782		**Total**		0.6729
	Direct	GY	0.3943		Direct	GY	0.5114
		TNP	0.3778			TNP	0.5323
		TWP	0.0758			TWP	0.0867
		TNG	-0.4927			TNG	-0.6485
		PH	0.7749			W100	0.1704
W100	Indirect	RL	-0.0363	PL	Indirect	PH	1.0000
		SD	-0.3286			RL	-0.0532
		PW	-0.2283			SD	-0.5122
		PL	0.2209			PW	-0.2884
		LAI	-0.0211			LAI	-0.1278
	Total		0.7368		**Total**		0.75
	Direct	GY	1.0000		Direct	GY	-0.2971
		TNP	0.6213			TNP	0.0195
		TWP	0.1044			TWP	0.0022
PH	Indirect	TNG	-0.7767	LAI	Indirect	TNG	-0.0210
		W100	0.2551			W100	0.0281
		RL	-0.0553			PH	0.3498
		SD	-0.5046			RL	-0.0106

Table 4. Cont'd

	PW	-0.2894		SD	-0.0658
	PL	0.4608		PW	-0.1843
	LAI	-0.0867		PL	0.2200
Total		0.9267	**Total**		0.0408
Coefficient of determination				1.0000	
Effect of residual variable				0.00	

TNP - total number of pods; TWP - total weight of pods; TNG - total number of grains; GY - grain yield; W100 - weight of one hundred grains; PH - plant height; RL - root length; SD - stem diameter; PW - pod width; PL - pod length; LAI - leaf area index.

primary variables (TNP, PH and PL) presented the greatest effects, especially PH, which obtained maximum direct effect with (0.6687, 1.0000 and 0.5114 (r_f)) respectively. Indirect effects were relatively high, for some characteristics such as TWP, TNG, PH, RL and PL on the TNP variable of 0.6466, 0.6629, 0.6213, 0.5702 and 0.5323 (rf), respectively. This result is indicative of the feasibility of indirect selection to obtain gains in the character of greater primary importance. In general, all primary variables presented high values of indirect effect on the PH variable, ranging from 0.7749 to 1.00 (rf), except for the characteristic LAI.

DISCUSSION

Correlations between agro-morphological characters

According to Dalla Corte et al. (2010), higher grain yield was obtained with smaller seeds, through high and negative correlations between seed width and thickness. The maximum correlation between PL and TNG shows the strong relationship between them, and their importance for productivity, since larger pods tend to provide a greater number of grains (Table 3). According to Carvalho et al. (2003), there is a positive correlation between chlorophyll concentration with N content in leaves and grain yield in beans. In this sense, the strong correlation between SD and PW with RL suggests that larger roots tend to increase the crude sap content in xylem in transport to shoots, showing that the plant nutrition factor is determinant for the performance of these characteristics that have a strong association with grain yield.

Path analysis between agro-morphological characters

The selection for any secondary character has no value if its performance does not correlate with the primary character (Coimbra et al., 2000). Also, according to Coimbra et al. (2000), the characters number of vegetables per plant and mass of one thousand grains showed a high degree of association with grain yield. The greater direct effect (rf) of PH on productivity is a complement that the increase in production has cause and effect relationship with the variable pod weight (Santos et al., 2014). The TNP and the PL are determinants for the increase in grain yield, since they presented positive direct effect values and high magnitude with GY (Table 4). According to Coelho et al. (2002), the number of pods per plant showed a high correlation with grain yield in the summer-autumn season. According to Ribeiro et al. (2016), the direct effects of phenotypic correlations indicate the true association between architecture and precocity of grain production.

The coefficient of determination was similar to that found by Almeida et al. (2014), when concluding that the number of pods per plant, and the number of grains per pod had a greater direct effect on yield. Higher PL indicates that the number of grains will be higher, possibly with lower thickness within the pods, which contributes to the increase in productivity. On the other hand, with lower PL, the number of grains for the plant will invest photoassimilates, making it possible to obtain larger grains (Table 4). According to Moura et al. (2012) the number of grains per pod correlates positively with grain yield, but the negative correlation between number of grains per pod, protein content and iron content suggests that the increase in the number of grains per pod decreases the protein and iron content in the grains. It is observed that the TNG characteristic negatively influences GY directly. According to Correa et al. (2015), the mass of five pods and the number of grains per pod are the components that contribute most to the production of grains in cowpea, surpassing the mass of one hundred grains. Both the number of grains and pod per plant and the number of grains per pod should be prioritized in indirect selection, since they have a higher genetic correlation with grain yield (Ribeiro et al., 2001). The negative direct effects of SD, PW, RL with GY characteristics suggest that the increase in productivity can be obtained by indirect selection with the characteristics TNP and PH, respectively.

Although the total coefficient (r_f) showed high magnitude, it was observed that there was no direct effect of the W100 variable with GY. The indirect effect via

positive PH and high magnitude should be considered for the increase in productivity. A direct negative effect with yield was expected with this characteristic as an important aspect for the increase in grain yield, since smaller grains would be obtained. The selection of plants with larger grains causes a decrease in yield, considering that the average weight of the grains presents a negative correlation with the production (Coelho et al., 2002). The increase in productivity can also be obtained by indirect correlation of TWP and TNG characteristics both with TNP and PH, respectively. The selection based on grains of higher weight consequently leads to reduction in grain yield; and the number of pod/plants is the greatest contribution to higher yield (Kurek et al., 2001).

Conclusion

The associations between agro-morphological characters show that TNP, PH and PL are determinant to directly increase grain yield. Indirectly, there was gain in the characteristics, TWP, TNG, RL and PL, that had positive effect and with high magnitude on the characteristics, TNP and PH.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 15(1), pp. 8-19, January 2021
DOI: 10.5897/AJPS2020.2058
Article Number: 735F30465894
ISSN 1996-0824

http://www.academicjournals.org/AJPS

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African Journal of Plant Science

Review

Natural rubber (*Hevea brasiliensis* Müell.-Arg.) production, processing, and rubber wastes utilization: Challenges and prospects for economic diversification and sustainable development of Nigeria

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Received 15 August, 2020; Accepted 30 December, 2020

Over 80% of Nigeria's foreign exchange earnings come from the sales of crude petroleum. Nigeria has a landmass of 910,768 km^2 with 38.97% arable, while 3.46% is suitable for the cultivation of permanent cash crops such as rubber, cocoa and palm trees. High-latex yields (3,000-3,500 kg dry natural rubber (NR)/ha/year) of Nigeria's hybrid rubber seedlings is a preferred choice to most foreign species (900-1,600 kg dry NR/ha/year) for cultivation in sub-Saharan Africa. In 2018, Nigeria's rubber export of US$41.8 million for global sales of US$13.1 billion was considered low compared to other African leading producers (Côte d'Ivoire-US$752.6 million, and Liberia-US$126.2 million). The present government's efforts to increase rubber cultivation at an annual growth rate of 5.7%, could be instrumental to diversifying its revenue base as demand for rubber-derived products is on a global increase. The challenges faced by small-scale rubber farmers are being addressed by government through the provision of affordable credit facilities and improved genetic seedlings for planting. Potential markets exist for micronized rubber powders sourced from waste tyres for the production of value-added fine chemicals, road construction, athletic and recreational facilities etc. Also, slurries and biogas obtained from natural rubber processing plants can also act as catalysts for sustainable development of the economy.

Key words: Nigeria, Agriculture sector, cash crops, rubber farming, rubber export, gross domestic product.

INTRODUCTION

This review paper discusses the impact of agriculture sector on the Nigerian economy in the 1960s, and the neglect of the sector as a result of the oil boom of 1970s. The natural rubber (NR) industry was a major contributor to the growth of Nigeria's gross domestic product (GDP) with robust foreign reserves in the pre-oil boom era.

The challenges and prospects of rubber farmers in Nigeria, assessment of natural rubber production and processing in pre- and post-civil war era in Nigeria, and conversion of rubber wastes to wealth for increased revenue generation are reviewed and discussed.

Nigeria has total landmass coverage of 910,768 km^2 with about 38.97% arable, while 3.46% is available for the cultivation of permanent cash crops (Chevron Nigeria Limited, 2015; Odetola and Etumnu, 2013) such as rubber, cocoa and palm trees. From 1960 until the oil boom of the 1970s, the main source of revenue in Nigeria was agriculture with surplus food produced for her entire population, such that over 70% of the total export came from natural rubber, cocoa, palm oil, and groundnuts (Oluwaseyi, 2017; Abolagha et al., 2016). About 60% of Nigerians were directly or indirectly employed in the agriculture sector. The sector also produces millet, tomatoes, gum arabic, sesame seeds, cotton, cashew nuts, citrus fruits, maize, cassava, yam, and sugarcane on a smaller scale. Infrastructural developments then, were quite visible with the prospects of a bigger bio-based economy in future. In the 1970s, the agriculture sector was neglected because of the oil boom and efforts by previous governments to revive this sector that produces natural rubber, cocoa, palm oil, and groundnuts, seen as the biggest foreign exchange earners for Nigeria failed as oil money was handy (Oluwaseyi, 2017). The agriculture sector at that time contributed about 64% to the total GDP, but this gradually declined to 8% in the 1970s, and 19% in 1985 (Izuchukwu, 2011). In addition, a report by the World Bank indicated a declined percentage of employment (public and private) in the agriculture sector as against the total employment in Nigeria between 1991 and 2019 at an average of 44.01% with a minimum of 36.38% in 2019 and maximum of 50.17% in 1992 (World Bank, 2019) compared to 60% prior to oil boom era. Revenue generation from the sales of about 2.1 million barrels of crude oil/day from the estimated oil reserves of 37.2 billion barrels, and sales of gas from the reserves of 187 trillion standard cubic feet of natural gas deposits did not adequately impact positively on the GDP's growth in the later years (Ishola et al., 2013; Ohimain, 2013; Omo-Ikerodah et al., 2009). Recently, the government introduced the Economic Recovery and Growth Plan (ERGP: 2017-2020) as a road map to diversify the economy, which depended on the sales of crude oil with emphasis on agriculture to create jobs, reduce food imports and support the growth of her GDP (Ejeh and Orokpo, 2019). *Hevea brasiliensis* Müell.-Arg tree (Figure 1) (Willd. ex Adr. de Juss.) is purposely cultivated for the production of latex as a source of NR for the production of over 50,000 products (Takase et al., 2015; Ng et al., 2013; Atabani et al., 2013). NR latex [$(C_5H_8)_n$] is a polymeric material of *cis*-1,4 polyisoprene (2-methyl-1, 3-butadiene) with a molecular weight in the range ≥300,000. It contains 30-35% rubber, 2-3% proteins and lipids, 0.3% resins, 1.5-4% glycosides, and the remainder is water (>50%) (Kumar and Nijasure, 1997). Due to the global shortage of NR for rubber-related products, the current rubber market is dominated by synthetic rubbers (SRs) sourced from fossil petroleum and vulcanized with sulphur atoms to produce chain of artificial polymers (e.g., styrene/1, 3-butadiene, and isobutylene/isoprene). For instance, styrene-butadiene rubber (SBR), isobutylene-isoprene rubber (that is, butyl rubber, abbr. BR), and *cis*-polybutadiene rubber (CBR) are the three most common SRs used in tyre production (Kan et al., 2017). A tyre may contain synthetic rubber, natural rubber, carbon black, steel wires, fabric, plasticizers, lubricants, antioxidants, and inorganic materials (e.g., calcium carbonate and silica). Synthetic rubbers from petrochemicals could be in short supply when the uses of fossil materials are reduced in the nearest possible future arising from the global Paris Agreement on climate change (UNFCCC, 2015). It is therefore envisaged that NR may be seen as the 'oil' of the future especially with the current expansion of the automobile industry and the global drive to using rubber-modified products, industrially.

The Nigerian government introduced a 12-year (2006-2018) Rubber Initiative Plan for increased rubber production for local use and export through aggressive cultivation of new plantations and rehabilitation of old plantations to achieve a target of 360,000 ha (Umar et al., 2010), which outstandingly yielded 371,775 ha of harvested rubber plantations (FAO, 2017). Thus, with the government's current expansion of rubber plantations, export earnings are projected to increase significantly by end of the current decade considering the renewed drive by the government to diversify the economy through the agriculture sector. NR production by small-scale rubber farmers was generally as low as 500 kg dry NR/ha/year in recent years, which was attributed to over-aged rubber trees (≥ 30 years), poor maintenance culture, lack of credit facilities, and non-availability of certified rubber seedlings for planting as reported by a cross section of small-scale rubber farmers (Field surveys). The primary objective of the initiative is to increase the income of the rubber farmers through increased local production and

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utilization of NR, creation of employment, and export the excess to boost the economy. This is perceived to ultimately diversify the economy and enhance the income of small-scale rubber famers who account for about 85% of hectares of rubber plantations (Umar et al., 2011). Recent survey shows that Nigeria produced 159,264 tons NR in 2017, second in Africa after **C**ôte d'Ivoire (580,000 tons), and ranked 13th in global production (FAO, 2017). Nigeria's annual export of 127,000 metric tons NR brought in export earnings of US$37 million in 2017 (NEPC, 2018). Global sales from NR exports by producing countries in 2018 totalled US$13.1 billion, and this reflected an average decline of 22.1% since 2014, when the world's shipment of NR was US$16.8 billion (Workman, 2019). In 2018, Nigeria's rubber export was US$41.8 million (0.3% global sales), while **C**ôte d'Ivoire (5.7% global sales-US$752.6 million) and Liberia (1% global sales-US$126.2 million) were the biggest exporters from Africa (Workman, 2019). Industrial applications of rubber tree products, apart from the products from NR and biofuels production, are numerous. With encouragement from government at all levels, through the provision of credit facilities for plantation owners, Nigeria may closely approach the production levels of Southeast Asian countries that account for over 90% of global NR production (Umar et al., 2011). Globally, the production of NR was reported at a 5.2% growth rate, Africa at 2.2%, while Nigeria's growth rate estimated at 5.7% (Umar et al., 2011). Onoji et al. (2016) reported that 18 million ha of land is available in South-South geopolitical region of Nigeria with good climatic factors (20-35°C, and 1800-4000 mm annual rainfall) required for the growth of rubber trees and good yields of NR. Unsuccessful trial cultivations were carried out in some Northern States of Nigeria where annual rainfall rarely exceeds 650 mm in the months of June to August considered as peak periods of rainy season (Umar et al., 2010). Rubber tree has a growth period of 6 to 9 years before commercial production of latex, but the seeds spring out between 4 and 6 years of growth (Eka et al., 2010). At present, non-edible rubber seed oil has the potential to generate about 8,000 tons/annum biodiesel in Nigeria using waste rubber seed shell as bio-catalyst (Onoji et al., 2017). The collection of rubber seeds for biodiesel production can be an additional source of income to rubber farmers who also practice intercropping with arable crops such as cassava, pineapple, yam, pepper, bitter leaf, maize, groundnut, and rice for sustenance, soil improvement and weed control mechanisms before NR production commences (Esekhade et al., 2019). The Rubber Research Institute of Nigeria (RRIN) genetically developed Nigerian rubber seedlings code named NIG800 and NIG900 series with yields 3,000 to 3,500 kg dry NR/ha/year when compared with imported seedlings from Southeast Asia countries, whose yields range from 900 to 1,600 kg dry NR/ha/year (Umar et al., 2010). The Nigerian seedlings are presently made available to small-scale rubber farmers and estate rubber planters for cultivation to address the challenges of low NR production. It is expected that in the next two decades, Nigeria will play a leading role in global NR production in a diversified sustainable agricultural-based economy. Due to expansion of the transportation sector in Nigeria, expired and used tyres are commonly dumped in unregulated dumpsites. This constituted an environmental hazard and exacerbated health problems to humans when these wastes are not properly disposed of. Pyrolysis is a thermochemical process that can be scaled-up to process the waste tyres in Nigeria into useable products, and recover the energy for domestic and industrial applications.

METHODOLOGY

Google scholar, a fast internet-based search engine was used to obtain information from online databases which include: Directory of Open Access Journals, Web of Science, African Journals Online, ScienceDirect, Research4Life, ResearchGate, etc.

Furthermore, publications from peer reviewed journals, conference papers, proceedings of national and international workshops, RRIN's manuals, government and biennial reports, FAO and corporate organizations' publications, book chapters and postgraduate theses published from year 1997 to 2020; and field interviews with rubber farmers were considered for this review. The materials were screened and relevant data extracted, analysed/or studied, and recorded for the review write up.

FINDINGS AND DISCUSSION

Challenges and prospects of rubber farms in Nigeria

Nigeria presently stands as the second largest producer of natural rubber in Africa with only 50% of the rubber plantations exploited (Ogbebor, 2013). Small-scale rubber farmers opted for food crops production and felling of aged-rubber trees for firewood as a source of energy for sustenance. The long gestation period of rubber tree, without income before maturity for latex production was perceived as non-beneficial to the plantation owners (Esekhade et al., 2019). Major stakeholders in the rubber industry believed that the Presidential Initiative on rubber cultivation needed to be reinvigorated for greater impact. Government at all levels, is expected to assist in the development of seed gardens/nurseries to produce quality budded materials for distribution to local rubber farmers at subsidized rates. This will prevent the use of uncertified seedlings for planting that result in low yield of NR and rubber seeds.

The rubber industry in Nigeria provided employment opportunities for the locals, and served as a major source of revenue in the 1960s (Otene et al., 2011). The mandate of RRIN is to carry out research into genetic

Figure 1. Typical latex collection from tapped rubber tree.

improvement and development of high yield rubber hybrids to assist the small-scale, and large-scale farmers such as RRIN Estates, Michelin Rubber Estate, Pamol Rubber Estate Calabar, Rubber Estates Nigeria Limited Udo, and Rubber Plantation Estate Urhonigbe (Umar et al., 2010; Otene et al., 2011). RRIN is also required to provide technical supports and information through improved technologies and diversified farming systems to enhance and improve the activities of the rubber farmers. Genetically developed Nigerian rubber hybrids (NIG800 and NIG900 series) used for inter-location trials at Akwete (Abia State), Calabar (Cross-River State), and Okho in Edo State in Nigeria have outstanding results in terms of natural rubber yields (Umar et al., 2010). It is expected that these high yield Nigerian rubber hybrids, which are resistant to wind and disease (Onoji et al., 2020), should dominate rubber plantations in Nigeria for bigger export in future. Available reports showed that in the past years, budded rubber stumps for these hybrid rubber seedlings are in limited supply at RRIN; and thus they are not available in required quantity for the small-scale rubber farmers that owned a majority of cultivated and tapped rubber plantations in Nigeria (Umar et al., 2010). The foregoing necessitated their engagement in the practice of small-size farming of other cash crops to complement income from rubber farms (Esekhade et al., 2019; Umar et al., 2011). RRIN's operations also include amongst others, collaboration with the Nigerian Export Promotion Council (NEPC), and their foreign partners such as the International Rubber Study Group (IRSG), Common Fund for Commodities (CFC) and World Agroforestry Centre (also known as ICRAF-International Council for Research in Agroforestry) to promote development of economically viable small-scale rubber businesses in Nigeria. NEPC is already intervening by providing farmers with 5,000 improved rubber budded seedlings and coordinating synergetic cooperation among rubber producers, processors, and exporters in Nigeria.

Other challenges facing the small-scale rubber farmers in Nigeria as reported by Abolagha and Giroh (2006) include:

(1) Low levels of mechanization for yield improvement;
(2) Aging rubber trees;
(3) Lack of commodity Boards to support investors;
(4) Poor investments in rubber farming;
(5) Inadequate supply of raw materials (latex and cup lumps) to the processing plants;
(6) Withdrawal of subsidies from pesticides, chemicals and farming implements;
(7) Inadequate provision of credit facilities to the smallholders of rubber farms;
(8) High production cost of NR;
(9) Diversion of loans by farmers to other areas of needs;
(10) Inadequate database for policy formulation and program planning;
(11) Rural-urban migration culminating in scarcity of

Table 1. Technical specifications of NSR**.**

Constituent	NSR10	NSR20
Dirts wt.%	≤0.1	≤0.2
Ash wt.%	≤0.75	≤1
Nitrogen wt.%	≤0.6	≤0.6
Volatile matter wt.%	≤0.8	≤0.8
Initial plasticity (P_o), min	≥30	≥30
Plasticity retention index (PRI, min) ISO 2930:2017	≥50	≥40

NSR: Nigerian standard rubber; min: minutes.
Source: Ogbebor (2013).

labour;
(12) Weak agricultural extension delivery services with poor feedback mechanism;
(13) Inconsistency and instability in macro-economic policies which do not engender confidence in the economy and tend to discourage medium- and long-term investments;
(14) Lack of involvement of stakeholders in program design;
(15) Poor monitoring, evaluation and implementation of farming programs.

Climatic factors are critical to NR production in Southern Nigeria, with the highest production attained in the months of December-February when a hazy harmattan wind persists. The rainy season peaks between the months of June and July, with drought in the latter part of August within the rubber producing states in Southern Nigeria. Rains hinder tapping during these periods, and this result in low outputs (field interviews with farmers). Labour is oftentimes mobilized to other sections such as field maintenance, cleaning of rubber cups, preparation of tapping utensils, and intercropping activities within the perimeter of the rubber plantation. Multiple intercropping with revenue from pineapple, cassava, pepper, okra, cocoyam, and maize crops in the vast interior of young rubber plantations holds the key to attracting small-scale rubber farming (Otene et al., 2011). In Nigeria, a majority of the rubber tappers are younger, matured workers within the age bracket of 30 and 40 years, and over 70% of them are male with little or no formal education. An experienced rubber tapper in Nigeria can tap about 450 to 600 trees/day on the average of 30s/tree for a standard half-spiral system.

Assessment of NR production in Africa-Nigeria in focus

Natural rubber played a dominant role in the economic development of Nigeria in the 1960s placing third to cocoa and palm oil as major foreign exchange earners (Umar et al., 2011).

Nigeria was the biggest producer of NR from 1961 (58,000 tons) to 1966 (73,500 tons) with Liberia being the runner-up (FAO, 2017). NR in different forms is processed into products that meet Nigerian standards (Table 1). These products such as concentrated latex, baled crumb rubber, ribbed smoked sheet (Figures 2 and 3), and crepe rubber are consumed locally and the excess are exported to boost foreign reserves. With the outbreak of the Nigerian civil war in 1967, production from Nigeria declined to 52,000 tons compared to Liberia's 62,290 tons in 1967 (FAO, 2017). Liberia continued to be the leading producer of NR in Africa, even after the post-war era in Nigeria. Liberia's production declined to 40 tons in 1990 as a result of the outbreak of the Liberian civil war in 1989. While the civil war in Liberia lasted, Nigeria regained the leading role as an Africa producer of NR till 1998. With the decline of NR production in Nigeria from late 1990s to 2002 due to aged trees (≥ 30 years), lack of trained labour and incentives from government, Côte d'Ivoire became Africa's biggest producer with a production of 580,000 tons compared with Nigeria's 159,264 tons in 2017 (FAO, 2017). During the peak production periods, the rubber industry in Nigeria had 54 factories with an overall installed processing capacity of 600,000 tons NR/year, but operated at 20% capacity. This gradually reduced to about 20 factories due to government neglect of the sector which resulted in a loss of about 40% in rubber exports (Gaille, 2018). A cross-section of small-scale rubber farmers interviewed at RRIN recommended that the government should subsidize 80% of the cost associated with plant budded seedlings for the farmers. This will cover long-term expenses that are necessary to bring rubber trees to maturity in order to earn more income from sales. The international price of NR steadily increased to about US$1,930/ton between the months of January and June 2017 (Thomas et al., 2019). There are expectations from stakeholders and industrialists that the changes will become a huge source of revenue to producers in the near future. On a global scale, and to meet increased demand for rubber, increase in hectares

Figure 2. Rubber sheets hung on reapers in a smokehouse.
Source: Aigbodion (2017).

Figure 3. Ribbed smoked sheet awaiting bailing process for export.
Source: Aigbodion (2017).

Table 2. Compositional analysis of biogas from NR processing plant.

Component	%
Methane	65
Carbon dioxide	30
Hydrogen sulphide	2.5
Ammonia and water vapour	2.5

Source: Aigbodion (2017).

under new cultivation by small-scale and large-scale rubber farmers should be intensified in Nigeria and other rubber producing nations. To achieve this, for instance, cooperative societies in Delta State, Nigeria, acquired over 75,000 ha of land for cultivation of new rubber plantations. This will engage large number of unemployed youths; which may accelerate the transformation of Nigeria into a leading producer of NR in the global market. In the same vein, the International Tripartite Rubber Council (ITRC) with memberships drawn from the major NR producers in Southeast Asia (that is, Thailand, Indonesia, and Malaysia) accounted for about 63% of World's NR production in 2017. Members of the Council implemented their collective "Agreed Export Tonnage Scheme (AETS)" for an export reduction of about 700,000 tons in 2019 as a response to a request from other producers for an increased market price of NR (Thomas et al., 2019). This intervention will further stabilise the international price of NR and enables it to compete favourably with other cash crops in revenue generation for rubber producing countries. However, the ITRC member nations are constrained with land challenges for NR cultivation due to severe competition for land by other cash crops such as palm trees. With the vast arable land in West and Central Africa sub-regions, Nigeria is a targeted choice destination for foreign investments in rubber plantations. The natural rubber industry in Nigeria has a potential for sustainable growth and development considering the government's policy on diversification of the economy through the sustainability of the private sector-driven agricultural sector.

Renewable energy from natural rubber factories

The NR processing factories in most rubber producing West Africa countries are perceived as filthy places of work because health, safety and environment laws are not often fully complied with. The effluents (serum wastewater, etc.) from such factories are not adequately disposed of; hence they constitute a menace to the environment. However, if properly digested, the effluents could be sources of renewable energy like biogas (methane) for heat and power generation purposes. A compositional analysis of effluents from a typical NR processing plant in Nigeria (Table 2), has been reported by Aigbodion (2017). The heating value of the biogas falls within the range of 18.6 to 26.1 MJ/m^3, and therefore suitable for domestic applications such as heat generation, while the slurry obtained after the digestion process has potential application to be converted to bio-fertilizer similar to rubber seed oil cake. A typical rubber factory processing 2.1 metric ton NR/day could generate 40 m^3/day of biogas, thus providing a minimum 744 MJ/day of energy (Aigbodion, 2017). Production of biogas from NR processing plants would most certainly be an additional source of revenue to Nigeria as reported in countries such as Sri Lanka, Malaysia, and India that are currently using biogas obtained from rubber factories (Aigbodion, 2017).

Industrial uses of rubber wood

The economic life span of rubber trees ranges from 25 to 30 years; thereafter, the trees are felled and replanted to maintain high latex production (Coulen et al., 2017). However, there is a growing interest in technical applications of rubber wood for furniture factories, energy generation (as firewood and charcoal), and in pulp and paper industry. In the Asian continent, studies have shown that rubber wood is significantly being utilized for various purposes in Thailand, Malaysia, Cambodia, and India. Rubber wood is considered a source of cellulose nanofibers which possess a vast range of potential applications in areas such as biomedical, electronics, packaging, nanocomposite, gas barrier films, and optically transparent functional materials (Aigbodion, 2017). There are limited technical applications of rubber wood in African countries especially the major rubber producers such as **C**ôte d'Ivoire, Nigeria, Liberia and Cameroon. In these countries, the wood is often burnt off or used as firewood and charcoal for heat generation by the locals, but this could generate substantial revenue if properly harnessed. Table 3 depicts the typical properties of rubber wood in comparison with other common tree woods in Nigeria.

Table 3. Comparison of properties of rubber wood with other tropical rainforest woods in Nigeria (at 12% moisture content).

Species	Density (kg/m^3)	Static bending		Volume Shrinkage (%)
		MOR (N/mm^2)	MOE (N/mm^2)	
Rubber wood (*Hevea brasiliensis*)	570	66	9,240	11.5
Apa (*Afzelia africana*)	823	136	6,313	7.6
Ita (*Celtis mildbraedii*)	732	149	7,088	12.2
Iroko (*Meliceae excelsa*)	650	90	5,765	9.1
Mahogany (*Khaya ivorensis*)	525	94	8,192	12.9
Obeche (*Triplochiton scleroxylon*)	372	30	3,937	6.9

MOR: Modulus of rupture; MOE: Modulus of elasticity
Source: Aigbodion (2017).

Rubber wastes to wealth?

Global population explosion, industrialization, urban development, and change in consumption patterns are responsible for the present huge global waste generation. A differential part of this waste comes from the auto industry as waste tyres (Li et al., 2016). Disposal of waste, used tyres in particular, is a major concern to waste management authorities in Nigeria because it is seen as a service to be rendered by the government. However, from the perspective of sustainable development and need for a cleaner environment, it is a collective responsibility of every individual in the society (Oh and Hettiarachchi, 2020).

In Nigeria, waste tyres are stockpiled at homes, auto-service centres, roadsides, and often disposed of in unregulated landfills and dumpsites that pave way for the proliferation of disease vectors such as the *Aedes aegypti* mosquito, Zika (Zanchet and de Sousa, 2020), and breeding sites for rodents (Umeki et al., 2016). In most cases, waste tyres are burnt during protests (e.g., #EndSARS; that is, Police brutality against youths in Nigeria), riots, and other civil disturbances, creating hazardous gases and dirt which result in serious health problems and environmental challenges. In extreme cases, they are used as roasting materials in abattoirs, which could cause serious health problems from consumption of the meat (Harrison-Obi, 2019).

The shortcomings of the conventional approach to managing disposal of waste tyres can be addressed through the use of more friendly green technologies to recover material and generate energy for domestic and industrial applications. With the fast depletion of natural resources, and to meet stringent emissions standards, reduction in waste tyres by reincorporating them into production processes will add value to what was hitherto considered valueless (Ramirez-Canon et al., 2018).

Waste valorization via thermochemical process is a promising method used to create wealth from waste tyres because of its simplicity, cost-effectiveness, and high purity of products. Pyrolysis is a valorization process that can be used to recover energy and value-added products from waste tyres due to challenges involved in tyre recycling and reuse (Kan et al., 2017; Smelik et al., 2015). The pyrolysis process conditions can be optimized to favour products of interest such as bio-char, oil (C_5-C_{24}), and gases (C_1-C_4) (Osayi et al., 2018). Studies have shown that pyrolysis at different temperatures can be used to monitor the compositions of the value-added products and by-products. Due to the rate of evaporation of chemical compounds used in tyre formulation, the yield of pyrolytic fractions from waste tyres can be varied with temperature (Ramirez-Canon et al., 2018; Osayi et al., 2018). High yield of heavy oil fractions, such as tar, have been reported at low temperatures ranges (300-400°C); thus, necessitating use of an energy intensive high temperature (Ramirez-Canon et al., 2018). Several studies reported in the literature supported high yield of lighter oil fractions and value-added gas fractions (such as CO, H_2, CH_4, etc.) between the temperature range of 400 and 600°C (Kan et al., 2017). However, other studies reported that the reactor configuration, feed type, and particle size also determine the level of dependence of liquid yield on temperature (Osayi et al., 2014). Some researchers reported that simple volatile compounds such as oil, plasticizer and additives, and moisture were released during thermal degradation of waste tyres at lower temperatures (220-250°C) (Ramirez-Canon et al., 2018). High volume of liquid fractions were reportedly obtained from the decomposition of styrene-butadiene-rubber at higher temperatures (400-500°C), but lower volume recovered from the butyl rubber (Ramirez-Canon et al., 2018). From the economic point of view, pyrolysis has not been commercialized in most advanced economies because of low volumetric yields (RMA, 2009). Therefore, its application in Nigeria may not be of potential interest to investors in energy and materials recovery from waste tyres, except on a laboratory scale for research purposes.

Another technology of interest to manage solid municipal waste and waste tyres disposal, is waste incineration. Incineration technology, using incinerators,

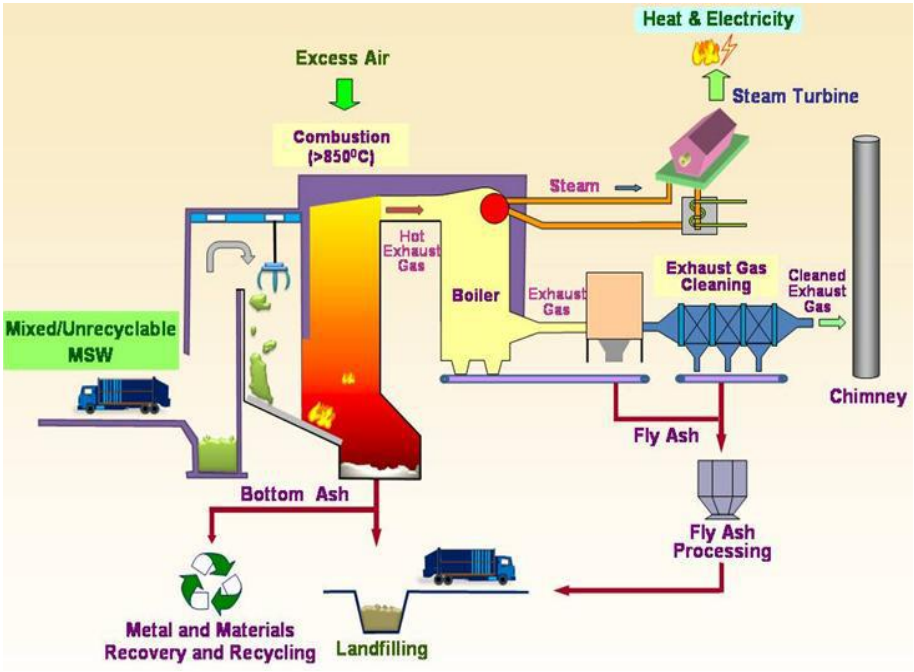

Figure 4. Schematic diagram of municipal solid waste (MSW) incinerator. Source: Lam et al. (2010).

Figure 5. Waste incinerator at Petroleum Training Institute (PTI), Effurun, Nigeria.

has been deployed to manage waste successfully in countries such as Japan and Sweden to recover energy for heat and electricity generation (Figure 4 shows a typical example of an incinerator) (Lam et al., 2010). For instance, a pilot-plant waste incinerator (Figure 5) manufactured by INCINCO UK Limited, and installed at

the Petroleum Training Institute, Effurun, Nigeria, has been used at a demonstration-scale level to showcase the benefit of incineration as a solid-waste management technology in Nigeria. Major products recovered from the process such as bottom ash and fly ash (used after pre-treatment to remove heavy metals, salts, chloride, organic pollutants, etc.) are sources of chemicals and valuable compounds such as CaO, SiO_2, Fe_2O_3, and Al_2O_3. These compounds could find applications in Nigeria, especially in the cement and concrete factories, ceramic industry, stabilizing agent, adsorbents and zeolite production. Most cement factories produce CaO from the thermal decomposition of $CaCO_3$ generating large emission of CO_2, a greenhouse gas (GHG) contributing substantially to global warming. Therefore, the production of CaO through combined waste (tyre, solids, etc.) incineration is considered environmentally friendly, as the process produces nitrogen, phosphorus, and potassium from the ash, for fertilizer production (Lam et al., 2010).

With the expansion of economic activities in Nigeria, the networks of roads across the six geopolitical zones are in deplorable conditions. Waste tyres can be shredded into crumb rubber and processed through ambient grinding or cryogenically turbo-milled (Lehigh technologies) to produce very fine micro-particles of free flowing rubber materials known as micronized rubber powder (MRP). Studies have shown that MRP can be efficiently and economically used as rubberized-asphalt concrete when mixed with conventional aggregate materials for road construction (RMA, 2009). MRP utilized in road pavements results in the followings:

(1) longer lasting and enhanced road surfaces;
(2) reduced road maintenance;
(3) lower road noise;
(4) cost effectiveness over long term;
(5) shorter breaking distances;
(6) skid resistance and better tyre traction.

Kim and Burford (1998) observed that when MRPs of diameter between 100 and 200 µm were incorporated into uncured NR as a filler, a better modulus match exists between the crumb and matrix compounding; making MRP a better filler for tyre manufacturing. MRP can also be utilized in the production of roof coatings, moulded and extruded products, adhesives, asphalt, plastic resins, sealants, brake pads and brake shoes with enhanced properties. Other promising areas where MRPs could be applied include athletic and recreational facilities such as ground cover for sports (e.g. football fields), playing fields, running tracks, and children playground (RMA, 2009). Therefore, proper understanding and utilization of MRPs can result in significant and environmental benefits when this green technology is deployed in Nigeria to create a sustainable economy.

Conclusion

Natural rubber was one of the major export products that earned Nigeria much revenue in the 1960s. However, rubber production declined in the 1970s because of heavy inflow of revenue from crude oil sales. Recently, a Presidential Initiative on rubber cultivation, production and export approved by Nigeria government for a 12-year period (2006-2018) targeting expanding rubber cultivation to 360,000 ha intended to boost the revenue generation from the agriculture sector, yielded positive results as hectares of rubber cultivated increased to 371,775. This initiative was borne out of the need to diversify the economy and enhance the income of the small-scale rubber farmers that account for about 85% of natural rubber production. The initiative improved Nigeria's export of natural rubber from US$37 million in 2017 to US$41.8 million in 2018. The provision of high yield Nigerian hybrid rubber seedlings (NIG800 and NIG900 series) to the small-scale farmers across the entire rubber producing areas will ultimately boost the production of natural rubber in the future. In addition, the value-added synthesis gas from the rubber processing plants will meet the energy requirements of local communities where most of the plants are located and possibly in excess, if any, that can be transmitted to the national grid. Additionally, waste tyres that constitute environmental hazards and health problems to humans can be properly harnessed through pyrolysis to recover energy and useful hydrocarbon oil and gas as sources of chemicals for a variety of industries and research institutes. Solid wastes of natural rubber origin, which significantly include waste tyres, can also be co-incinerated efficiently to recover energy for heat and electricity generation. In addition, production of chemicals and valuable metallic oxides from pre-treated bottom ash and fly ash from the incinerators have significant technical applications in cement and concrete production processes, ceramic industry, adsorbents and zeolite production. Potential markets such as construction of road pavements, athletic and recreational centres, agric and horticultural applications, horse arena flooring, etc., exist in Nigeria for the use of micronized rubber powders (MRPs) sourced from waste tyre (crumbs), and may remain a single viable commercial route for large-scale reuse of abundant waste tyres. The overarching benefits of rubber production and exploitation in Nigeria on improved GDP, sustainability of locally-acquired material resources, the revitalization of the agro-industry, and improving the country's ranking amongst NR producers cannot be overemphasized owing to the numerous unquantified outcomes to the government and its citizens.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors appreciate the support provided by the Petroleum Training Institute, Effurun, Nigeria, and the University of the Witwatersrand (Wits), Johannesburg, South Africa.

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Vol. 15(1), pp. 20-27, January 2021
DOI: 10.5897/AJPS2020.2018
Article Number: 77FE89765899
ISSN 1996-0824

http://www.academicjournals.org/AJPS

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Variation of *Parkia biglobosa* morphological traits according to land use and agro-climatic zones in Southern Mali

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Received 12 May, 2020; Accepted 9 December, 2020

A study was conducted in southern Mali to contribute to the domestication of *Parkia biglobosa*. Three agro climatic zones (North Sudanian "NS", South Sudanian "SS" and North Guinean "NG") and two stands (field and fallow) were concerned. Three plots of 0.25 ha each, were installed in each stand. Diameter at Breast Height (DBH), Total Height (TH) and Crown Diameter (CD) of adult trees were measured. The effect of agro-climatic zone on growth parameters was significant. The South and North Sudanian zones showed significantly higher means of DBH, TH and CD compared to the North Guinean zone. The mean DBH varied from 45.46 cm (NG) to 65.96 cm (NS). The mean TH varied from 10.68 m (NG) to 12.59 m (NS). The mean CD in the field stand varied from 10.50 m (NG) to 16.12 m (SS) and in fallow stand it varied from 11.21 m (SS) to 13.64 m (NS). Stand effect was not significant but the interaction zone*stand was significant. The effect of agro-climatic did not display an influence of the climatic gradient, suggesting that management practices played an important role in the growth of this species.

Key words: Domestication, fallow, field, growth parameters, management practices, Parkland species.

INTRODUCTION

Forest tree species are characterized by high genetic diversity. This diversity is important for the adaption of species to various climatic and environmental conditions. The high diversity is linked to geographical origin and to the difference between individuals within the same population (Goba et al., 2019). The diversity within a species can be assessed through morphological and molecular traits (Ikabanga et al., 2017; Avana-Tientcheu et al., 2019).

However, within climatic zones, environmental conditions can cause significant variations in the morphological characteristics of species populations (Dicko et al., 2019). Variability studies are needed to increase plant productivity and also for future breeding work (Freigoun et al., 2017). Phenotypic variability of a species could be assessed by identifying morphological descriptors and morphological data from geographical origins have been used in first studies of genetic diversity of tree species

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(Kouonon et al., 2020). According to Samim et al. (2018), morphological descriptors are the basis for the characterization of plant genotypes on the basis of their phenotype.

Parkia biglobosa is a forest tree species of the family of Leguminosae/Fabaceae (Sacande et al., 2016), common in agroforestry parklands in the Sudanian Zone. It is an agroforestry species of major socio-economic importance in Benin, but also in the whole West African region (Ayihouenou et al., 2016). These authors reported that its conservation and domestication for the diversification of agricultural production depend on its ability to adapt to climate change. But, populations of this species are highly threatened in large parts of its range due to overexploitation and environmental degradation (Lompo et al., 2017).

According to Lompo et al. (2017), a sound conservation strategy for *P. biglobosa* and the promotion of its sustainable management should be based on scientific information about threats as well as ecological and genetic processes affecting this species. Assessment of the variation of the morphological traits of this species in relation to agro-climatic zones could contribute to this scientific information needed for a successful conservation strategy. Also, it is essential to know the phenotypical variability of this species for domestication purposes to preserve goods and services provided by *P. biglobosa* (Kouonon et al., 2020). Very recent studies have focused on the variability of morphological traits and to the identification of morphological descriptors of several trees species like *Adansonia digitata* (Bamba et al., 2019), *Lophira lanceolata* (Dicko et al., 2019; Lankoande et al., 2020), *P. biglobosa* (Avana-Tientcheu et al., 2019; Kouonon et al., 2020), and *Pterocarpus erinaceus* (Johnson et al., 2020).

In Mali, *P. biglobosa* is one of the most important parkland tree species, present in the north and the south of Sudanian Zones in the regions of Kayes, Koulikoro, Ségou, Sikasso; and in the north Guinean zones in the regions of Kayes and Sikasso (FAGUI, 2015). It is a forest tree species which regenerates naturally. The cultivation of *P. bigolobsa* began only recently and it is still very limited. The limitation of its cultivation in Mali was linked to some traditional considerations in rules that were established during the recent two to three decades. First, nursery experiments for seedling production and on-field plantation experiments started in Mali in the 1990's, and a relative success was only observed in the NG zone.

P. biglobosa is a useful, multi-purpose tree species having almost the same uses in the three study sites. The species provides food for human beings (such as, pulp and grains used to produce the spice called "soumbala" or "dawadawa"). This spice is rich in proteins and contains lipids, essential amino acids, essential fatty acids, vitamins and mineral compounds (Ouoba et al., 2003). It is particularly appreciated and widely used in Africa. *P. biglobosa,* which also provides food for animals (pulp) and contributes to income generation for rural populations; and therefore it contributes to fighting poverty. It provides medicine and sometimes craft wood (mainly in the NS and SS zones).

In all study zones, the main constraint in conducting this study was the low density of *P. biglobosa* populations in farmed fields as well as in fallows. The low density could be explained by several causes like natural mortality, density reduction by farmers in the field to reduce competition with associated crops (mainly cash crops like cotton which was in expansion in the whole southern Mali) and the weak natural regeneration in the fallow. Despite its importance, very few studies, if any, were published on the state of this resource despite climatic changes and the several constraints; while according to Lompo et al. (2017), in the light of climatic changes, safeguarding the genetic diversity of the species is crucial to foster adaptation and to support its long-term survival. Hence, to contribute to fill this knowledge gap in Mali, this study was funded by the Malian Government within the frame of the Competitive Funds for Research and Technological Innovation (CFRTI).

The objective of the study was to contribute to the domestication of the species in Mali. More specifically, it aims to (i) understand the state of the resource *P. biglobosa* in different agro-climatic zones, (ii) identify morphological descriptors important for the resilience of the species and (iii) develop strategies of renewal of *P. biglobosa* Parklands.

MATERIALS AND METHODS

Study sites

The study was conducted in three agro-climatic zones (the North Sudanian NS, the South Sudanian SS, and the North Guinean NG). These zones were selected based on climatic and environmental conditions as well as management practices (land use system and tree management). In the NS zone, the mean annual rainfall varies from 500 to 800 mm. It is a zone of slightly undulated plains, lowlands and depressions with heavy soils quite wet, and actively cultivated. It also contains extensive, fine-textured plains. The natural vegetation is constantly being degraded, and the existing woody species are those spared by man. In the SS zone, the mean annual rainfall varies from 800 to 1100 mm. Soils are deep alluvial, often the most fertile in the country, used for continuous cultivation and short fallow systems. The soils on rocky foundations are shallow or moderately deep. There are open or moderately dense woody stands on shallow soils. In the NG zone, the rainfall is over 1100 mm per year. The valleys in this area are cultivated in a continuous regime. Fallow system is longer and the density of woody species is higher. It is an excellent zone of timber exploitation. It is important to notice that the average altitude of parcels of studied populations of *P. biglobosa* increased from north to south but the difference in altitude was not very substantial. These altitudes in average were 276 m in NS zone, 312 m in the SS zone and 332 m in the NG zone.

Land and parkland trees are managed differently in the study sites. In the NG zone, land is less scarce and shifting cultivation still

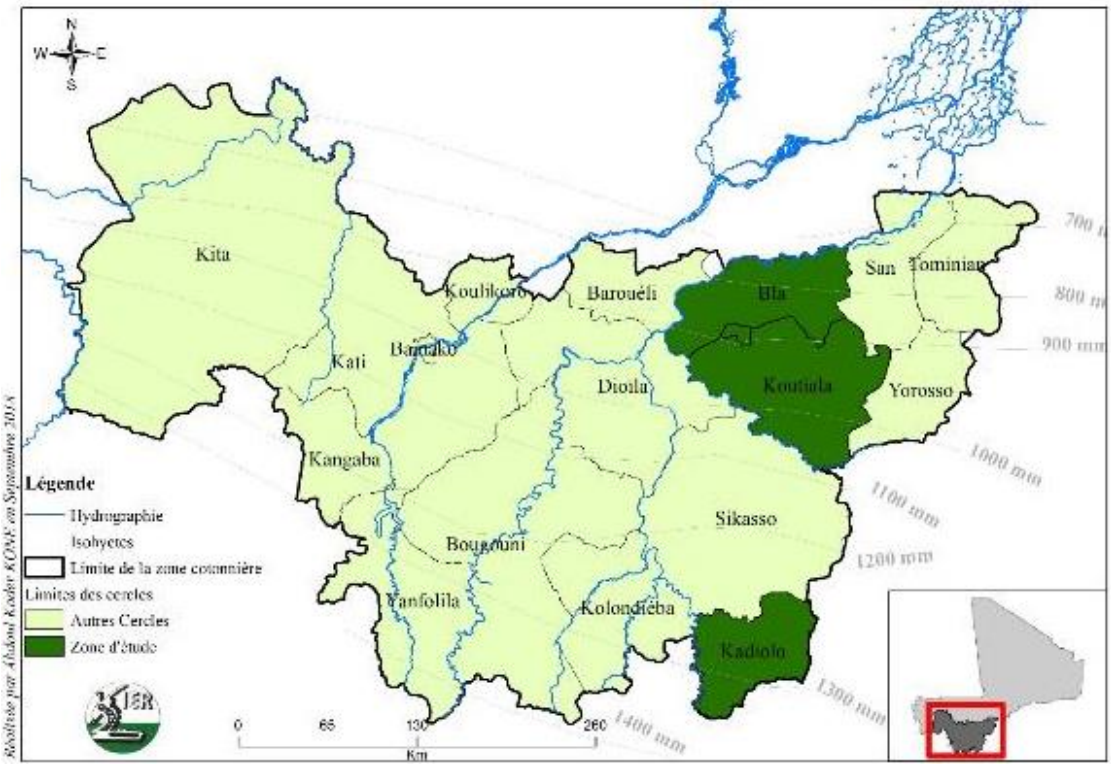

Figure 1. Map1 showing the study zones (green areas).

exists whereas in the other two zones, because of land scarcity, the same parcels are used continuously or with short fallow period. Also, like Burkina Faso (Lompo et al. 2017) and other West African countries, *P. biglobosa* is officially protected by national legislation in Mali. However, despite this protection, the species is cut for various purposes according to zones. In the NG zone, due to the presence of relative abundant vegetation, *P. biglobosa* trees are less exploited for purposes like fuel wood, charcoal or craft wood in contrary to the Sudanian zones where cases of their exploitation for various purposes were observed. Hence, *P. biglobosa* tree densities in the Sudanian zones are lower compare to the NG zone and because of the use of the same parcels continuously, *P. biglobosa* trees are older and bigger and therefore pruned to favour associated crops.

In each zone, one site was selected based on the availability of *P. biglobosa* populations in fields and fallows, the accessibility in all seasons, and the willingness of farmers to collaborate in research activities. The selected sites were Somasso (district of Bla) in the NS zone, Zanzoni (district of Koutiala) in the SS zone and Diou (district of Kadiolo) in the NG zone. Map 1 (Figure 1) shows the three study zones in green; and Map 2 (Figure 2) shows site localizations within the respective districts.

The site of Somasso (51°31'N, 36°27'W) in the NS zone has a little uneven relief composed of cultivable plains. The climate is North Sudanian, characterized by two seasons (the long dry season from October to May and the short rainy season from June to September). Agriculture is the main activity and the cultivated areas are large, dominated by cereal crops. Cotton and groundnuts are the cash crops. Vegetation is shrubby savannah with some big trees spared in the fields such as *Parkia biglobosa*, *Vitellaria paradoxa*, *Faidherbia albida* (PDESC-Somasso, 2019). The site of Zanzoni (36°52'N, 32°05'W) in the SS zone has little hilly relief composed of plains favourable for off-season crops. The climate is South Sudanian, with also two seasons with lengths similar to those of Somasso. The agriculture comprises food and cash crops such as cotton and peanuts. Vegetal resources are similar to those of Somasso but some protected forests and sacred woods are present (PDESC-Zanzoni, 2019). The site of Diou (35°46'N, 58°33'W) in the NG zone has a slightly uneven relief. The climate is North Guinean, with a dry season from November to May and a rainy season from May to October. Agriculture is the main activity and cereal production is mainly composed of Maize, while Cotton is grown as a cash crop. There are important forest resources consisting of natural formations, artificial plantations and sacred woods (PDESC-Diou, 2017).

Study design

The study design consisted of square plots of 50 x 50 m = 2500 m^2 (0.25 ha). Two factors were studied: the agro-climatic zones (ACZ) factor with three levels (NS, SS and NG) and the land use factor "called stand in the paper" with two levels (fields and fallows). Three plots were installed in each stand within each zone, giving six plots per agro-climatic zone. The total number of *P. biglobosa* populations was 18 (6 plots x 3 agro-climatic zones). All adult *P. biglobosa* trees (DBH ≥ 10 cm) in the plots were marked and measured. The geographical position of each tree was recorded using a GARMIN eTrex 10 GPS (accuracy ± 3 m).

Data collection and analysis

The variables measured were the diameter at 1.30 m above the ground (DBH) measured with a forest compass, the total height (TH) measured with a 12 m ruler, the crown diameter (CD) in the

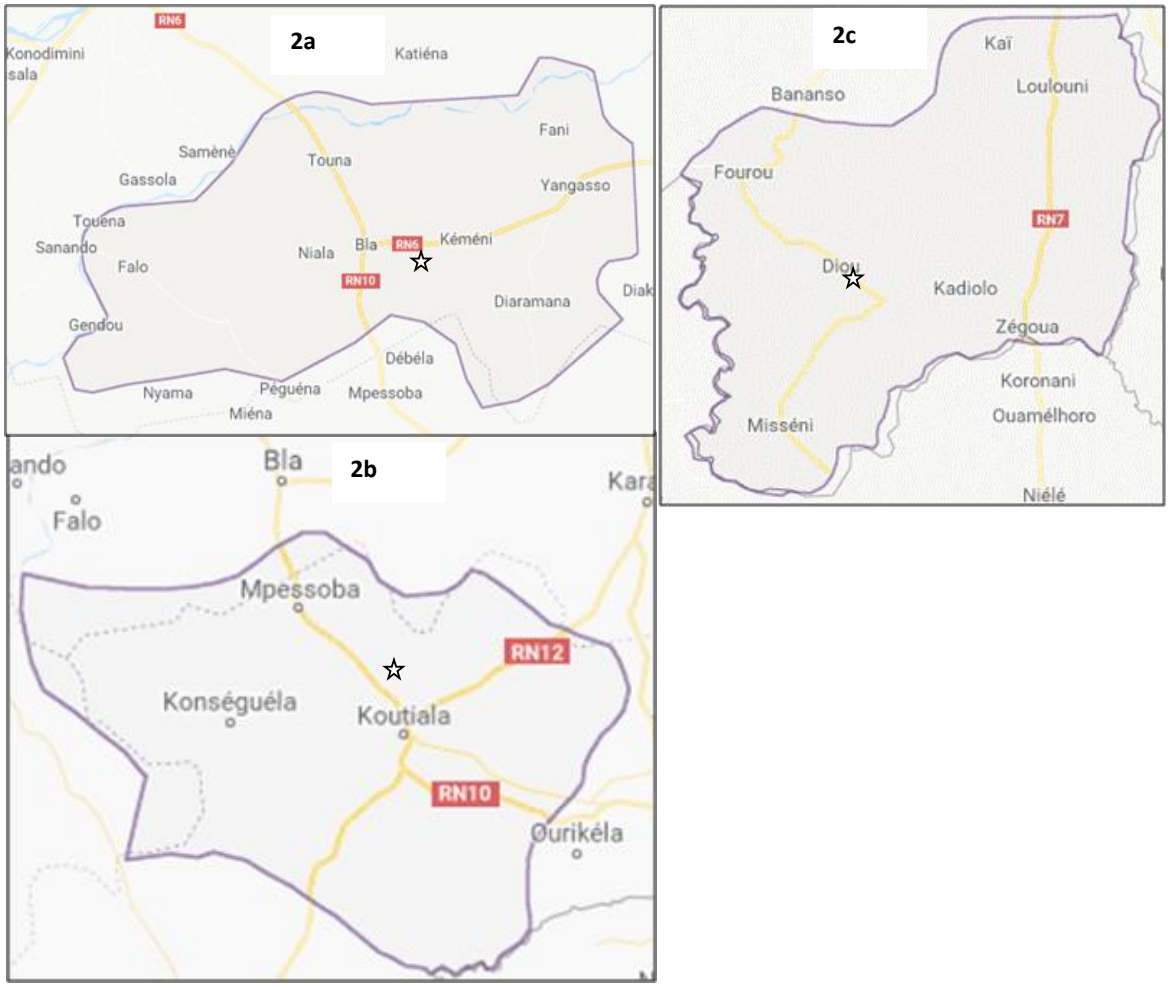

Figure 2. Map 2 showing sites localized in the respective districts each indicated by a star (2a Somasso in the district of Bla, 2b Zanzoni in the district of Koutiala and 2c Diou in the district of Kadiolo).

east-west and north-south directions measured with a 30 m measuring tape. Collected data were analysed using SYSTAT9 FOR WINDOWS software. Descriptive statistics and analysis of variance (ANOVA) were used as analysis methods. For the factors whose effects were significant, multiple comparisons of the means were made to distinguish the levels of the factor that were significantly different according to Bonferonni's method. The density of *P. biglobosa* in field and fallow stands in each zone was estimated based on the number of trees measured in the plots. Correlation coefficients between measured variables were also computed.

RESULTS AND DISCUSSION

Density and growth of *P. biglobosa* according to agro-climatic zones (NS, SS, NG) and stands (field, fallow) are presented and discussed as follows.

Density

The density of *P. biglobosa* by stand in each agro-climatic zone is shown in Table 1. The mean density of *P. biglobosa* increased from north to south (Table 1). The density was almost the same for the two stands (14 trees ha^{-1} and 13 trees ha^{-1} for fields and fallows, respectively). The same density was observed for the fields of the NS and SS zones (13 trees ha^{-1}). Higher density was observed for those of the NG zone (17 trees ha^{-1}). For fallows, the density increased from north to south (Table 1). The density of *P. biglobosa* was higher in the NG zone and a decreasing trend was observed from south to north for all stands together as well for fallow stands. For field stand, the highest density was observed in the NG (17 trees ha^{-1}), while the SS and NS zones had the same density (13 trees ha^{-1}). In the NG zone, the more abundant vegetal resources implying less pressure on *P. biglobosa trees* and the shifting cultivation implying new cleared parcels with more possibility to spare young *P. biglobosa* trees could explain the higher density observed in this zone. Mechanization of the agriculture could also explain the differences observed between zones. The mechanisation is more developed in the Sudanian zones

Table 1. Density of *P. biglobosa* by Agro-climatic zone and Stand.

Parameter	Density (ha^{-1})		
Agro-climatic zones (ACZ)	**Fields**	**Fallows**	**Mean ACZ**
North Sudanian (NS)	13	9	11
South Sudanian (SS)	13	13	13
North Guinean (NG)	17	18	18
Mean stands	14	13	

Table 2a. Mean diameter at body height (DBH) by stand and agro-climatic zone.

Agro-climatic zones (ACZ)	Mean DBH (cm)		
	Fields	**Fallows**	**Mean ACZ**
North Sudanian (NS)	70.53±21.43	61.40±8.57	65.96^{a}
South Sudanian (SS)	78.40±17.94	54.13±13.59	66.26^{a}
North Guinean (NG)	37.46±11.40	53.46±18.01	45.46^{b}
	Df	**F-ratio**	**P**
ACZ	2	17.121	0.000
STAND	1	3.039	0.085
SITE*STAND	2	12.456	0.000

Means with the same letter were not significantly different.

because of the intense production of cotton. Engines used require more space, which implies significant reduction of densities in production parcels. Also, because of this intense cash crop production to reduce competition between crops and trees, farmers reduce tree densities and prune mainly during cropping season.

For *P. biglobosa*, Dotchamou et al. (2016) reported a significant difference in density in both agro-ecological zones in Benin and the density of *P. biglobosa* is highest in the North (Sudanian zone 13 tree ha^{-1}) compared to the South (Sudano-guinean zone about 10 trees ha^{-1}). Their results contrast with ours as we observed the inverse but the density they observed for the Sudanian zone in Benin is the same as observed for the Sudanian zones in Mali (13 trees ha^{-1}) and the density they observed for Sudano-guinean is much lower than what we observed for NG zone (18 trees ha^{-1}). Avana-Tientcheu et al. (2019) reported a mean density of *P. biglobosa* in Tchad varying from 18 ± 12.73 to 28 ± 5.7 trees ha^{-1} for open forest and agricultural field systems, respectively. From our results, in the driest zone (NS) the density was higher in field stand compare to fallow (13 trees ha^{-1} vs 9 trees ha^{-1}). The scarcity of wood and land tenure system (resources in fallows are a common good of the community) could explain this result. Due to this system and to the need of craft wood, *P. biglobosa* trees in fallow stands are more exposed to exploitation. Studies carried out in the NS, SS and NG zones in southern Mali have reported densities of *P. biglobosa* varying from 4 to 16 trees ha^{-1} along the north south gradient (Diarra, 2017).

Growth parameters

The global analysis of variance on growth parameters of *P. biglobosa* showed a significant effect of ACZ on the diameter at body height ($p < 0.001$), the total height ($p = 0.001$) and the crown diameter ($p = 0.022$). The effect of stand was not significant for all variables, but the interaction between the two factors was significant. The means of measured variables as well as ANOVA output are shown in Table 2. Marginal means were shown for significant factors with F-ratios higher than that for the interaction. The mean of diameter at body height by stand and agro-climatic zone were shown in Table 2a.

The highest (78.40 cm) and lowest (37.46 cm) mean DBH values were observed in the Fields, in the SS and NG zones, respectively. The NG zone showed mean DBH significantly lower than those of Sudanian zones, which are not significantly different (Table 2a). Despite the non-significant effect of stand, in the Sudanian zones, fallows showed lower means compared to fields, while the inverse was observed in the NG zone. The Mean of total height by stand and agro-climatic zone were shown in Table 2b.

The highest (13.57 m) and lowest (10.22 m) means TH also were observed in the Fields in the SS and NG

Table 2b. Mean total height (TH) by stand and agro-climatic zone.

Agro-climatic zones (ACZ)	Mean TH (m)		
	Fields	Fallows	Mean ACZ
North Sudanian (NS)	12.53±1.83	12.65±189	12.59[a]
South Sudanian (SS)	13.57±1.26	10.89±1.82	12.23[a]
North Guinean (NG)	10.22±1.59	11.15±2.98	10.68[b]
	Df	F-ratio	P
ACZ	2	8.020	0.001
STAND	1	1.710	0.195
SITE*STAND	2	7.059	0.001

Means with the same letter were not significantly different.

Table 2c. Mean crown diameter (CD) by stand and agro-climatic zone.

Agro-climatic zones (ACZ)	Mean CD (m)		
	Fields	Fallows	
North Sudanian (NS)	14.62±3.22	13.64±4.00	
South Sudanian (SS)	16.12±2.04	11.21±2.65	
North Guinean (NG)	10.50±2.89	13.31±3.98	
	df	F-ratio	P
SITE	2	4.015	0.022
STAND	1	2.300	0.133
SITE*STAND	2	10.827	0.000

Table 3. Pearson correlation matrix.

	DBH	HT	CD
DBH	1		
HT	0.702	1	
CD	0.7	0.713	1

zones, respectively. The NG zone showed mean TH significantly lower than those of Sudanian zones, which are not significantly different (Table 2b). Stand effect was not significant; but in the SS zone, Fields showed higher means compared to Fallows and the inverse was observed in the NG zone. The Mean of crown diameter by stand and agro-climatic zone are shown in Table 2c.

The same trend as for the DBH was observed for the variation of the mean CD according to Stands and Agro-climatic zones. A relative high correlation was observed between the three measured variables (Table 3). Correlation coefficients between the three variables are of same magnitude (70 to 71%).

The analysis of variance showed non-significant effects of Agro-climatic zone on growth variables for Fallow stands; whereas, the effect was significant on the three variables for Field stands. Means of measured variables by Agro-climatic zone in Field stands are shown in Table 4.

For the three Agro-climatic zone variables, the NG zone showed significantly lower means than the Sudanian zones, which are not significantly different (Table 4).

The mean DBH varied from 45.46 to 66.26 cm, the mean TH varied from 10.68 m to 12.59 m and the mean CD varied from 10.50 m to 16.12 m, according to Agro-climatic zones. Differences between zones were significant for all these variables, and lowest means were observed in the NG zone which was the wettest zone. This result could be explained by changing cultivation patterns, as the new cleared parcels have relatively young *P. biglobosa* trees with small sizes compared to Sudanian zones where the same parcels are being used for very long cultivation times. Also, due to the presence

Table 4. Means of measured variables by agro-climatic zone in field stand.

Agro-climatic zones (ACZ)	Mean DBH (cm)	Mean TH (m)	Mean CD (m)
North Sudanian (NS)	70.53±21.43[a]	12.53±1.89[a]	14.62±3.22[a]
South Sudanian (SS)	78.40±17.94[a]	13.57±1.82[a]	16.12±2.04[a]
North Guinean (NG)	37.46±11.40[b]	10.22±2.98[b]	10.50±2.89[b]
F-ratio	23.28	18.38	16.61
Probability	0.000	0.000	0.000

Means with the same letter are not significantly different.
DBH = diameter at body height, TH = total height, CD = crown diameter.

of relative abundant vegetation and the production system in the NG zone, *P. biglobosa* trees are subject to higher competition and lower benefit from cultivation activities.

Several authors have reported morphological variation of *P. biglobosa* according to study zones and sites. In southern Mali, Diarra (2017) observed the highest mean DBH (25.8 cm) and mean CD (9.44 m) of *P. biglobosa* in the NS zone compared to the SS and NG zones. Other characteristics of the species were also found to vary according to study zones. For instance, Dembélé (2019) observed that the onset of the flowering and the fruiting progressed from the south (NG zone) to the north (NS zone); but the length of flowering (4-5 months) and fruiting (5-6 months) is almost the same for the three zones. Traoré (2019a) observed that some leaf morphological traits were discriminant between zones and the NS and SS zones had lower number of pairs of pinnae and leaflets compared to the NG zone. Regarding fruit production of *P. biglobosa*, Traoré (2019b) observed that there was not a climatic gradient effect; but the production was rather explained by farmers' land use and tree management practices according to zones.

In the south-west of Nigeria, Oyerinde et al. (2018) observed a difference between three sites with respect to CD of *P. biglobosa*; the least rainy site (Ekiti) have the highest average CD (13.71 m), but they did not observe a significant difference between sites with respect to TH and DBH. The TH mean values in the three sites (6.92 m; 7.11 m and 7.43 m) observed by these authors are lower than that of this study, whereas the means DBH (147 cm, 150 cm and 161 cm) they observed are higher. Variation according to climatic zones was also reported for other forest tree species regarding various aspects. For instance, Kelly et al. (2018) observed the highest mean DBH and mean CD of *V. paradoxa* in the NS zone compared to the SS and NG zones in southern Mali. Houètchégnon et al. (2015) reported that for *Prosopis africana*, variables discriminating climatic zones were: DBH, trunk height, diameter and number of leaflets, number of seeds and pod diameter. Thangjam et al. (2019) observed highly significant differences ($p<0.001$) between the agro climatic zones of *Parkia timoriana* in the North-Eastern states of India for the seed and pod traits.

For all growth variables, the difference between stands was not significant in all agro-climatic zones. A similar result was observed by Avana-Tientcheu et al. (2019) in Chad. These authors reported that growth parameters of *P. biglobosa* such as tree diameter, total height, and basal area are not significantly influenced by the type of production system (open forest, agricultural field, fallow and home garden) despite the observed differences in values. The means they observed (62.97±16.53 cm in fallow to 73.92±14.10 cm in field for the diameter, and 12.54 m in open forest to 14.68 m in fallow for total height) are close to the results of this study.

The differences observed between agro-climatic zones did not display an effect of climatic gradient. This result suggests that climatic conditions are not the only important factor influencing *P. biglobosa* growth and in determining phenotypic traits of adult trees. The way farmers are managing parklands and the production systems would be important factors. Another factor influencing phenotypic traits of *P. biglobosa* trees would be the genetic factor. For instance, according to Lompo et al. (2017), *P. biglobosa* is structured according to a South-North gradient and there would be a phenotypic variation, depending on the origin, due to the environment and the genetic difference according to latitude. Oyerinde et al. (2018) also, in explaining their results, referred to the genetic factor as a source of differences displayed in relation to the growth parameters of *P. biglobosa* in addition to environmental (rainfall, humidity, temperature), agro-ecological zone and soil factors. The same phenomenon was observed for other forest tree species as well. Goba et al. (2019) observed geographical structure of phenotypic variation in *Pterocarpus erinaceus* Poir in Côte d'Ivoire.

Conclusions

The results of this study revealed significant effect of climatic zone on growth parameters of *P. biglobosa,* which did not display a climatic gradient trend. This result suggests that other factors like production systems and management practices have influence on the growth

parameters of *P. biglobosa* as has been observed for other Parkland tree species, such as *V. paradoxa*. The effect of stand was not significant; but for most cases, variable means were higher in field stand compared to fallow. This result could also be explained by management practices and by the care brought to the crops that can benefit the trees. Hence, growth parameters of *P. biglobosa* highlighted the importance of management practices and any domestication strategy must take this into account.

CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to the Malian government for funding the *Parkia* project through the Competitive Fund for Research and Technological Innovation (CFRTI). They also appreciate Somasso, Zanzoni and Diou villages' authorities and populations for the permission given to conduct this study on their field and fallow parcels and also for cooperating in field activities. They are also grateful to the field technicians (Isaïe Dembélé, Younoussa Diarra and Mamadou Diabaté), who carried out the work of monitoring and data recording.

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Vol. 15(1), pp. 28-38, January 2021
DOI: 10.5897/AJPS2020.2077
Article Number: 91E94B565901
ISSN 1996-0824

http://www.academicjournals.org/AJPS

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African Journal of Plant Science

Full Length Research Paper

Genetic characterization of Cape gooseberry (*Physalis peruviana* L.) accessions in selected counties in Kenya using SSR markers

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Received 12 October, 2020; Accepted 4 December, 2020

Cape gooseberry (*Physalis peruviana* L.) is a neglected high potential crop, knowledge of the genetic diversity of the genotypes domesticated in Kenya is limited. To understand the genetic diversity and structure within and between Cape gooseberry germplasm, 70 accessions from six selected counties were analyzed using 15 pairs of highly polymorphic SSR primers. In this study, a total of 61 polymorphic SSR alleles were identified with mean polymorphic information content (PIC) of 0.43. Analysis of Molecular Variance (AMOVA) revealed that 92.8% of the total genetic variation was within accessions whereas variation among accessions accounted for 7.2% of the total genetic variation. Genetic diversity parameters among the 70 accessions revealed that Cape gooseberry was more diverse than previously recorded. Based on the SSR data, the 70 accessions were classified into five main phylogenetic groups, which corresponded to the county of origin through factorial analysis, principal component analysis (PCA), and phylogenetic analysis. Seven core SSR primer pairs, namely SSR1, SSR2, SSR10, SSR11, SSR123, SSR138, and SSR146 were found to have a wide applicability in genotype identification of cape gooseberry, and thus they are recommended for use in genetic characterization of germplasm collected from other counties not covered by the present study. This study demonstrated the existence of considerable genetic diversity in Cape gooseberry accessions growing in selected counties in Kenya and can therefore be used as a basis for future breeding programs in the development of hybrids with desirable traits. This wider genetic diversity is vital for posterity as it will help cope with unpredictable climatic changes and human needs.

Key words: Simple sequence repeats (SSRs), genetic diversity, germplasm, *Physalis peruviana* L., polymorphic information content (PIC).

INTRODUCTION

Physalis peruviana L. is a species from the family *Solanaceae* and genus *Physalis*, commonly known as Cape gooseberry, ground cherry, Cape gooseberry, or winter cherry. It contains high amounts of vitamins (A, B,

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and C), micronutrients (iron, phosphorous, and calcium), has anti-inflammatory, antioxidant, and anti-hepatotoxic activities (Wu et al*.,* 2009). The vitamin C content in this fruit is reported to be the highest among all other fruits and plants, thus its reference as a “super fruit”, Cape gooseberry contain up to twenty times vitamin C as that found in oranges (Villacorta and Shaw, 2013). Owing to such concentrated levels of nutrients in this fruit, Villacorta and Shaw (2013) posit that it is useful for medicinal purposes in restoring vitality and boosting immunity by fortifying the liver, supporting cardiovascular activity, strengthening lungs, and enhancing fertility and food absorption.

Investigation of genetic diversity in both wild and domesticated species is essential. Assessment of available genetic diversity is a preliminary stage in genetic improvement in crop plants (Bhandari et al., 2017). Wild populations of different crop species are known to be a potential source of useful genes and traits which could be introduced into the domesticated gene pool (Campisano et al*.,* 2015). There are various ways in which diversity analysis can be carried out including cytological, morphological, biochemical, and molecular approaches. With the advent of genomic tools, assessment of genetic diversity at the molecular level has proven to be more useful as compared to that at the phenotypic level because the latter entails analysis of data on morphological traits, which are generally influenced by environments (Myers et al*.,* 2000). Different molecular marker systems have been proved to be valuable tools in assessing the genetic diversity of plants between and within species regardless of environmental interferences on the phenotype (Demir et al*.,* 2010). Research has shown that different markers reveal different classes of variation (Virk et al*.,* 2000). Simple Sequence Repeat (SSR) markers have become the marker of choice for many researchers as they offer many advantages including technical simplicity, feasibility of automation, even distribution throughout the genome, higher frequency of polymorphism, rapidity, requirement of little and not necessarily high-quality DNA, and no requirement of prior information of any DNA sequence (Mason, 2015). Molecular marker analysis work would be of great help for analyzing genetic diversity, and exploiting genetic resources for identification, isolation, conservation, and utilization (Ravi et al*.,* 2010).

The availability of enough food to meet 95% of the world’s requirements is dependent on only a few crop species which are widely and intensively cultivated crops. These have been developed by extensive selection from available large agro-biodiversity pool (Ochatt and Jain, 2007). There is a great need to expand the exploitation of the plant genetic diversity that would broaden the crop diversity for food supply to feed the ever-growing human population and avoid dependence on few food crops. Wild relatives and neglected crops could become an excellent source of useful gene pool.

The average yields of Cape gooseberry are still below the maximum potential mainly due to fruit cracking, small fruits, and premature fruit drop (Ali and Singh, 2016). Also, the poor quality of fruits in some cultivars in terms of the levels of total soluble solids (TSS) and total titratable acidity (TTA) make them unattractive for large scale agriculture (Herrera et al*.,* 2011). No improved cultivars have been developed yet although Leiva-Brondo et al*.,* (2001) reported to have used a simple breeding strategy employed in other Solanaceae crops to develop hybrids with superior yield characteristics by exploiting heterosis. Genetic diversity is important in this context as it serves as the reservoir of many novel traits related to yield, quality as well as tolerance to abiotic and biotic stresses. A thorough understanding of the diversity in the Cape gooseberry genome is necessary before implementation of any breeding program in breaking these yield and quality barriers. The objective of this study was to determine the genetic diversity of Cape gooseberry genotypes in Kenya using SSR markers for use in present and future breeding schemes and conservation programs. This information will contribute to understanding the genetic relationship between and within different genotypes and provide basic information on parental selection for Cape gooseberry breeding material.

MATERIALS AND METHODS

Experimental materials

Seventy dry leaf samples were collected from accessions of Cape gooseberry collected from six selected counties of Kenya (Figure 1). The counties selected were Kiambu (1.0314°S, 36.8681°E), Muranga (0.7839°S, 37.0400°E), Kericho (0.1828ºS, 35.4782°E), Nakuru (1.3665°S, 35.3905°E), Nyandarua (0.1804°S, 36.5230°E), and Laikipia (0.3606°N, 37.7820°E). The six counties were selected because they have Cape gooseberry germplasm. The experiments were carried out during the rainy season in May 2019. The young leaves were sampled because they yield DNA better and have low concentrations of phytates which contaminate DNA. The samples were stored on Silica gel (Loba Chemie). The samples were coded based on the county. For instance, a collection was done at Nyandarua County, Olkalou district, Kipipiri division, Miharati location, Mara village and was the seventh Cape gooseberry crop to be sampled; the code assigned was NYD/OLK/KIP/MHT/MAR/07.

DNA extraction and quantification

Total nucleic acid was extracted from the dry young leaf tissue that had been stored in silica gel for one month using a modified CTAB protocol (Porebski et al., 1997). Modifications involved the introduction of the initial wash stage using 2-Mercaptoethanol to remove the aromatic compounds and phytates. Centrifugation time for initial stages was also increased to 10 min to ensure that cell debris and the proteins were well decanted to minimize contamination. Final centrifugation time was reduced to 3 min while centrifugation speed was increased to 14000rpm to avoid pelleting of carry over impurities. Precipitation time was also increased from the recommended time of 2 to 18 h to increase DNA precipitation

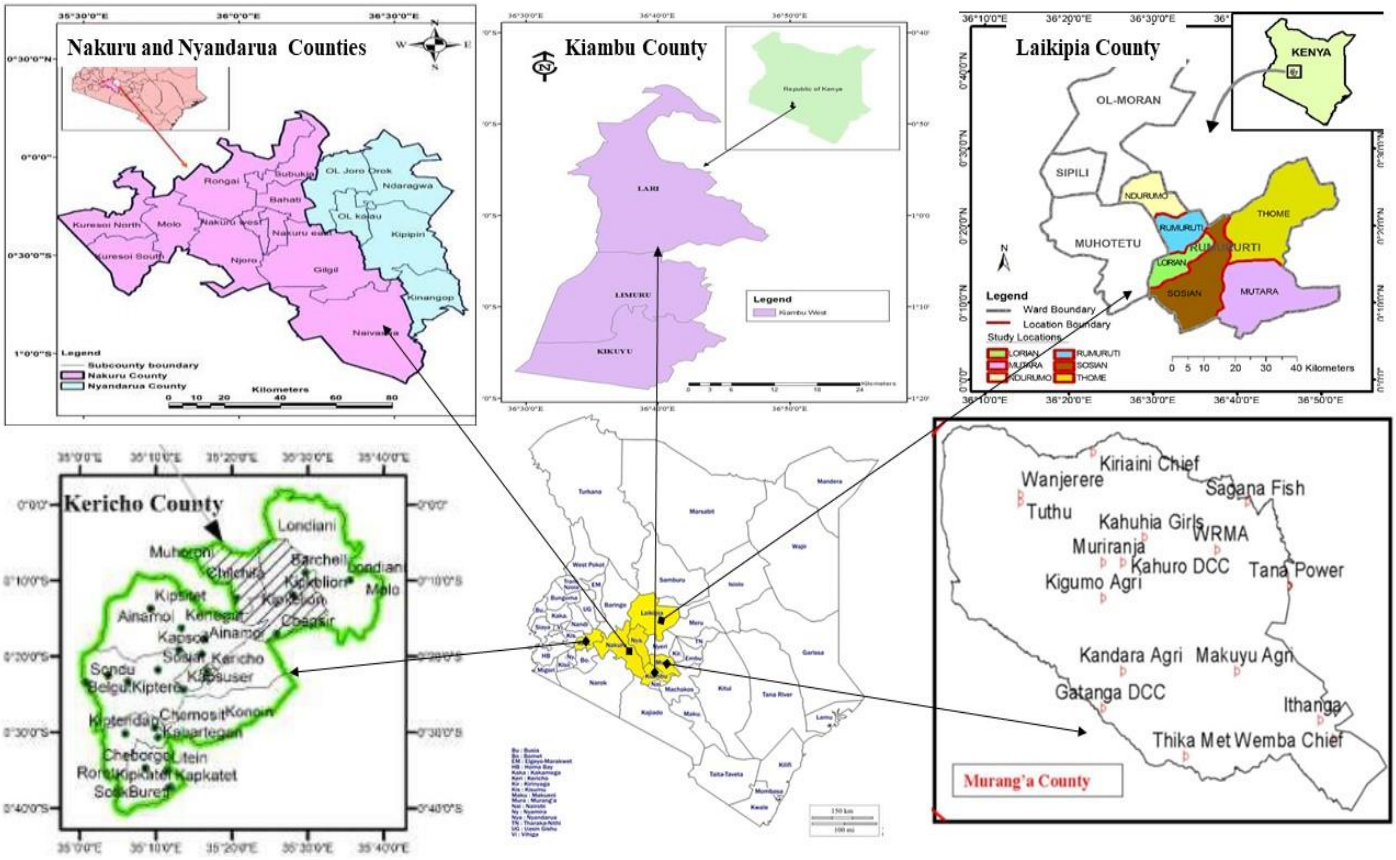

Figure 1. Map showing Cape gooseberry germplasm occurrence in six selected counties in Kenya.

and recovery.

Nanodrop spectrophotometer (Applied Biosystems) and agarose gel electrophoresis assays were used to determine the purity and concentration of DNA in the samples. Nanodrop spectrophotometry involved reading the concentration of DNA from the absorbance of the sample at 260 nm (1OD (A260) = 50 µg for doubled stranded DNA/µl). The purity of the DNA sample was determined by A260/A280 ratio (1.6±1.8 for pure DNA). Agarose gel electrophoresis quantification involved resolving the samples in agarose gel 0.8% (0.8g agarose and 100ml Sodium Borate) containing 3ul ethidium bromide staining dye at voltage of 100 volts and 400 mA current for 30 min. The DNA was visualized on a UV transilluminator (Applied Biosystems).

Selection and genotyping of SSR markers

A set of 15 SSR markers selected from earlier published reports (Table 1) were used to determine the diversity of Cape gooseberry collections. The SSR markers were selected based on high polymorphic information content (PIC) values (>0.4) in earlier published studies; the maximum number of alleles detected, genome coverage, and distribution on linkage groups. The primers for the SSR markers were synthesized on contract from Inqaba Biotech in South Africa and genotyping of the SSR markers was carried out at Marker Assisted Breeding Laboratory at Kenya Agricultural and Livestock Research Organization (KALRO) Njoro Center.

Polymerase chain reaction (PCR) was done in a 2720, 96 universal gradient thermocycler (Applied Biosystems) in 20 µl final volume containing 10±20 ng DNA template, 5.0 pmol forward and reverse primers, 1x PCR buffer, 2.5mM of each dNTPs, 1.5 mM MgCl and 0.5 U of Taq DNA polymerase (Biolabs). The amplification conditions for PCR profile were: 95°C for 5 min, 35 cycles of 95°C for 30 s, specific annealing temperature for each SSR for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 10 min. The PCR amplicons were run in a 1.8% agarose gel containing ethidium bromide staining dye at a voltage of 80 volts and a current of 400 mA for 1 h and visualized in a UV transilluminator (Applied Biosystems).

Data analysis

SSR marker alleles were scored manually from the gel images using a simple numerical scoring method. When the expected band was present it was scored as 1 while 0 was used to code for absence. Number of alleles, observed heterozygosity (HO) and expected heterozygosity (HE), Shannon's diversity index (I), gene flow (Nm), and gene differentiation coefficient (FST) executed in POPGENE Version 1.32 (Yeh et al., 2000) were used to determine genetic diversity. Chi-squared was used to determine Hardy - Weinberg equilibrium (HWE) while genetic diversity and polymorphic information content (PIC) were computed in Power Marker 3.25 (Liu and Muse, 2005).

Unweighted Pair Group with Arithmetic Mean (UPGMA) algorithm was used to construct an unrooted phylogram from a distance

Table 1. Selected SSR markers used in the study.

Marker	Forward primer	TM	Range of observed bands		Amplicon Size (Bp)	Repeat type	Location
SSR1F SSR1R	AGAGGACTCCATTTGTTTGCT TGAGGGTGTTGGATGTTTTCT	50	170	210	206	AT	39 UTR
SSR2F SSR2 R	CATTGGGTTTCGCATCCAT AGACAAGCCTAGGGGAAAGG	50	230	250	237	AG	39 UTR
SSR10F SSR10R	GCTTCCTATTGTGTTGCCTGA ACTTTGGGTTTCGGGAATTG	50	220	240	185	AG	59 UTR
SSR 11F SSR11R	CAGCTGAAATAAGAGAGTGATTGG CCCTCTTTTTCTCCTCCGAGT	50	170	190	180	AT	39 UTR
SSR15F SSR15R	GCTTGTTGATCAGCTTTCTTTG TGGATCATAACCTTGCTAATGC	50	180	210	172	AG	39 UTR
SSR54F SSR54R	CGGCTGGTATGCTTACAAAGAT GCACTTCCACTGTTTTTAACTTCC	50	160	210	197	AC	59 UTR
SSR72F SSR72R	GTGCTCGCAGTTTCTTCAAA CCGCCGTTACTTCCTAATCA	50	200	220	158	AT	39 UTR
SSR77F SSR77R	CATACCATAACTCCCCATCTCTC TGCCGATTCTGATTTCTTCC	50	160	180	216	AT	39 UTR
SSR112F SSR112R	CTACGCCTACCACTTGCACA CAGTGGAAGCCTCAAGATCC	50	180	230	203	AC	39 UTR
SSR118F SSR118R	AATCAAGGGTCAGAAGAAATGG GCAAGAATGGATGTGGGTGT	50	170	220	180	AT	39 UTR
SSR121F SSR121R	AGCAACCTCCCAATCAGCTA TGGTGAGTAAATGGGGGAAA	52	170	240	170	AG	39 UTR
SSR123F SSR123R	TCAGTGGAGCGCGTATATCT GCGATCTCACCAAACCTCTC	50	260	330	216	AG	39 UTR
SSR126F SSR126R	TCCAAAAAGAAAACAAAAACACT TTGAATGCATGTTTGATGGA	50	190	210	202	AC	39 UTR
SSR138F SSR138R	TCCGATCACTACTTCAGCACG CAATTCGGGTTGTGAATCGGGT	50	200	210	138	AG	59 UTR
SSR146F SSR146R	AGGCTAATGAGGACGAAGCA GGTTGCATTACAAAGCACTGA	50	200	210	187	AT	39 UTR

matrix based on Nei's (1973 and 1978) genetic distances, using Darwin 6.0 software (Perrier and Jacquemoud-Collet, 2006) and Power Marker 3.25 (Liu and Muse, 2005).

RESULTS AND DISCUSSION

DNA extraction and PCR

All the samples yielded good quality and quantity of DNA to enable genotyping (Plate 1). Genotyping of markers resulted in the amplification of expected regions resulting in single or multiple bands (Plate 2a). Minimal contamination of samples by proteins and phenolic compounds was observed in this study.

This study evaluated the genetic diversity and population structure of 70 accessions of Cape gooseberry that originated from six selected counties of Kenya (Nakuru, Laikipia, Nyandarua, Kericho, Murang'a, and Kiambu) by 15 SSR primer pairs. The 15 primer pairs amplified a total of 71 polymorphic SSR alleles (Plate 2b). Every primer pair was able to amplify varying the number of SSR alleles ranging from 100 to 300 bp from all accessions tested, regardless of the county of origin.

Marker polymorphism and genetic diversity of Cape gooseberry accessions

The number of major alleles per primer ranged between 2 (SSR54, SSR15, SSR72, SSR77, SSR112, and SSR 126) and 11(SSR 2). A total of 61 alleles were detected by the 15 primers with a mean of 4.07(Table 2). Allele frequency ranged between 0.26 and 0.96 with a mean of 0.64. The number of observed alleles was 2 for all markers while the number of effective alleles ranged

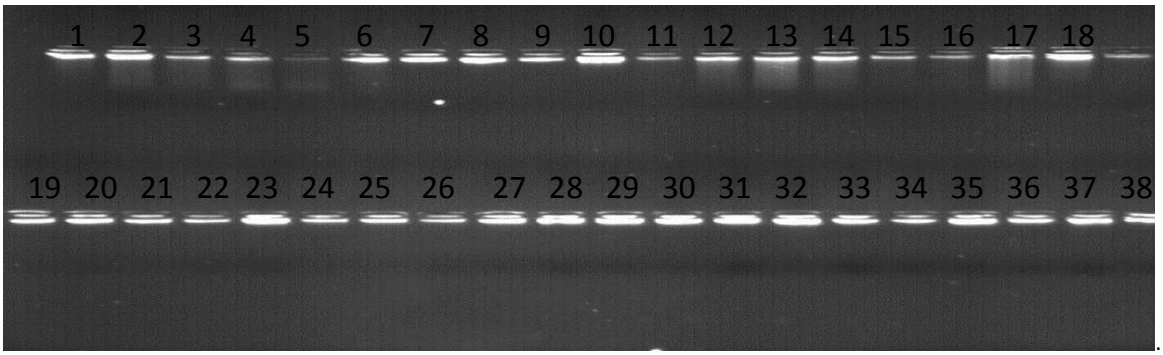

Plate 1. Representative quantification gel image of cape gooseberry accessions.

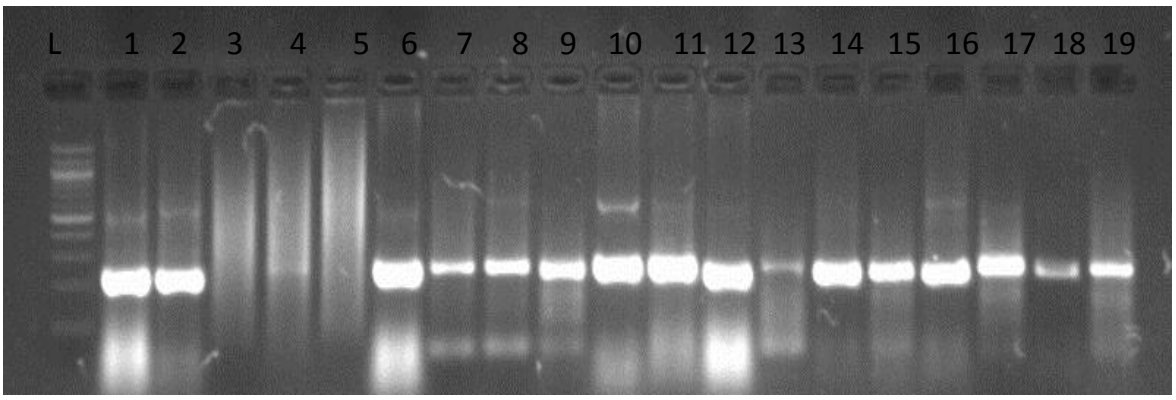

Plate 2a. Representative PCR image of single bands of the cape gooseberry accessions.

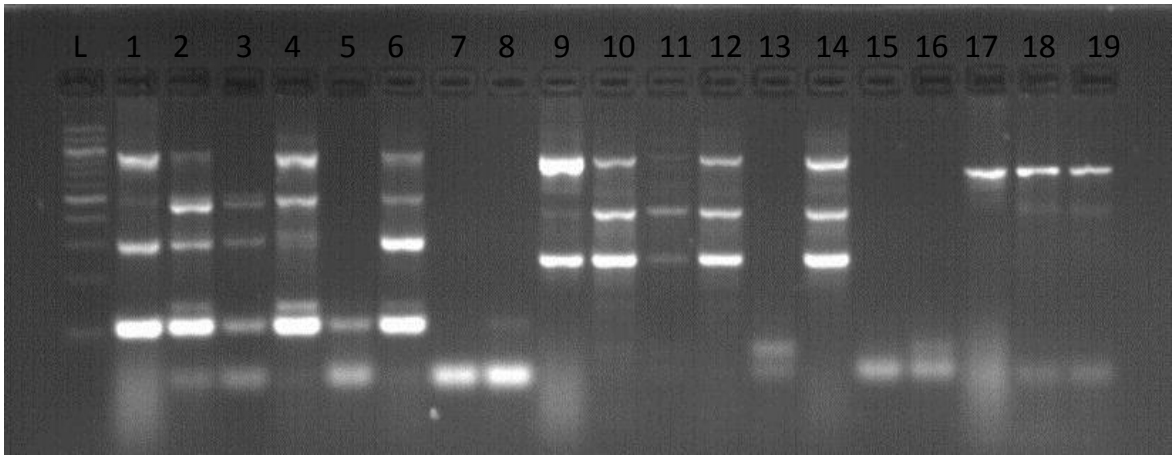

Plate 2b. Representative PCR images of multiple bands of varied sizes of Cape gooseberry accessions.

between 1.10 (SSR112) and 2.00 (SSR1) with a mean of 1.28 (Table 2). The lowest gene diversity was observed for SSR112 (0.08) while the highest was 0.82 for SSR1. All the markers used in the study were polymorphic, SSR1 was the most polymorphic marker (PIC = 0.77) while SSR112 was the least polymorphic (PIC = 0.079) while the mean polymorphic content was 0.432 (Table 2). The average PIC for the entire population (0.432) classifies the markers used in this study within the range of loci with intermediate polymorphism (0.25 to 0.5) according to Ge et al., (2013). The calculated Shannon's Information indices averaged at 0.25 and ranged between

Table 2. Summary statistics of Marker polymorphism and genetic diversity indices of cape gooseberry accessions.

Locus	Sample size	Allele No.	MAF	*Na	*Ne	H*	I*	PIC	Ht	Hs	Fst	Nm
SSR1	70	7	0.257	2.00	1.9984	0.4996	0.6927	0.793	0.4994	0.0300	0.9399	0.0320
SSR2	70	11	0.386	2.00	1.9935	0.4984	0.6915	0.758	0.4996	0.1214	0.7571	0.1604
SSR10	70	7	0.271	2.00	1.9600	0.4898	0.6829	0.78	0.4983	0.0537	0.8923	0.0604
SSR11	70	6	0.471	2.00	1.9935	0.4984	0.6915	0.662	0.3533	0.0781	0.7789	0.1420
SSR15	70	2	0.814	2.00	1.4336	0.3024	0.4799	0.257	0.2778	0.0000	1.0000	0.0000
SSR54	70	2	0.557	2.00	1.9742	0.4935	0.6866	0.371	0.4941	0.1769	0.6419	0.2789
SSR72	70	2	0.771	2.00	1.5448	0.3527	0.5375	0.291	0.3394	0.0700	0.7938	0.1299
SSR77	70	2	0.771	2.00	1.5448	0.3527	0.5375	0.291	0.3394	0.0700	0.7938	0.1299
SSR112	70	2	0.957	2.00	1.0894	0.0820	0.1769	0.079	0.0740	0.0592	0.2000	2.0000
SSR118	70	3	0.857	2.00	1.3243	0.2449	0.4101	0.239	0.3193	0.0586	0.8166	0.1123
SSR121	70	3	0.657	2.00	1.6897	0.4082	0.5983	0.409	0.4362	0.0221	0.9493	0.0267
SSR123	70	4	0.771	2.00	1.4706	0.3200	0.5004	0.356	0.3333	0.0773	0.7682	0.1509
SSR126	70	2	0.871	2.00	1.2888	0.2241	0.3837	0.199	0.3084	0.0408	0.8676	0.0763
SSR138	70	4	0.714	2.00	1.5817	0.3678	0.5544	0.426	0.4220	0.1322	0.6867	0.2281
SSR146	70	4	0.529	2.00	1.5817	0.3678	0.5544	0.583	0.3288	0.1449	0.5592	0.3941
Mean	70	61	0.257	1.51	1.2673	0.3668	0.2464	0.432	0.4083	0.1089	0.7333	0.1818
St. Dev				0.50	0.3479	0.1877	0.2709		0.1721	0.0321		

Na = Observed number of alleles, Ne = Effective number of alleles (Kimura and Crow, 1964), H* = Nei's (1973) gene diversity, I* = Shannon's Information index (Lewontin, 1972) MAF = Major allele frequency, PIC = polymorphic information content, Ht = genetic distance for the among populations, Hs = genetic distance within the population and Gst = degree of differentiation among populations and Nm = estimate of gene flow from Gstor Gcs. E.g., Nm = 0.5(1 - Gst)/Gst; (McDermott and McDonald, 1993).

0.1769 (SSR1) and 0.6927 (SSR112) (Table 2). Seven SSR primer pairs (SSR1, SSR2, SSR10, SSR11, SSR123, SSR138, and SSR146) produced more than five alleles among the 70 Cape gooseberry accessions. The seven SSR primer pairs would have a priority of choice in evaluating cape gooseberry because they were more informative in their ability to segregate between the accessions and had PIC values above 0.4. The highest number of SSR loci detected in this study contained dinucleotide (two nucleotide units) and hendecanucleotide (eleven nucleotide units) repeats characteristic of markers located along untranslated regions (UTR). This is in line with the hypothesis by Morgante et al. (2002) which posit that in most plants the SSR loci are found along the UTR's and may have accounted for the markers with low PIC because SSRs found in untranslated regions have been reported to be less polymorphic than genomic markers (Ellis and Burke, 2007). The 61 polymorphic SSR alleles detected in this study were higher than the 6 alleles reported by Chacón et al. (2016) and the 30 polymorphic alleles reported by Simbaqueba et al. (2011). These differences may be due to different accessions used in previous studies or the stringency of scoring. The differences could also be due to the relatively narrow genetic base of commercial Cape gooseberry varieties used in the previous studies.

Population structure of Cape gooseberry accessions

Genetic distances between gooseberry accessions within counties

Plant breeding applications such as germplasm collections, selection of parental materials, identification of quantitative trait loci, linkage, and association mapping are dependent on previous genetic diversity information (Rao and Hodgkin, 2002; Zhu et al., 2008; Rauf et al., 2010). In this study, the application of SSR markers on the

Table 3. Average genetic distances of cape gooseberry accessions within counties.

County	Ho	He	Mean Fst	HWE
Nakuru	0.500	0.1021	0.0031	
Nyandarua	0.456	0.0832	0.1778	
Kericho	0.488	0.1018	0.0076	
Kiambu	0.394	0.0973	0.0668	
Murang'a	0.384	0.0968	0.0662	
Laikipia	0.277	0.0375	0.5874	
HWE	-	-	-	70.00

Ho = Observed heterozygosity, He = Expected heterozygosity, Fst = gene differentiation coefficient, HWE = Hardy Weinberg equilibrium as calculated from Chi square.

Table 4. Genetic diversity of cape gooseberry accessions between counties.

County	Nakuru	Nyandarua	Kericho	Kiambu	Murang'a	Laikipia
Nakuru	0.000					
Nyandarua	0.0007					
Kericho	0.0000	0.0007				
Kiambu	0.0001	0.0008	0.0001			
Murang'a	0.0001	0.0003	0.0001	0.0003		
Laikipia	0.0067	0.0048	0.0066	0.0063	0.0061	

whole collection of P. peruviana from six selected counties of Kenya revealed a moderate to high genetic diversity. Average distances between individuals in the same cluster (expected heterozygosity) ranged from 0.03 in Laikipia county to 0.102 in Nakuru county indicating that Cape gooseberry accessions in Nakuru county have higher genetic diversity than those in Laikipia county. Genetic distance value recorded in this study (He=0.3668) is higher than those reported by Bonilla et al*.* (2008) (He = 0.2559) and lower than those reported by Garzón-Martínez et al*.* (2015) (He = 0.40). However, it is noteworthy that Bonilla et al*.* (2008) used RAM markers which are dominant in nature and therefore have the inherent limitation of underestimating allelic diversity as compared to the co-dominant SSR markers used in this study; while Garzón-Martínez et al*.* (2015) used genomic SNP markers which have a higher resolution than SSR markers.

Mean fixation index (F_{ST}) ranged between 0.003 and 0.58 for Nakuru and Laikipia respectively. Cape gooseberry accessions in Nakuru and Kericho counties exhibited insignificant genetic differentiation (Fst < 0.05) indicating that the accessions in the two counties are interbreeding freely. Cape gooseberry accessions in Nyandarua, Kiambu, Murang'a, and Laikipia showed significant genetic differentiation (Fst < 0.05). This great genetic differentiation is a sign of geographic isolation and a high inbreeding rate (Table 3). Overall, genetic differentiation reported in the present study is very high (Fst = 0.7333). This may be attributed to the fact that Kenyan Cape gooseberry cultivars have no history of domestication. Lagos et al. (2008) reported that *P. peruviana* is more than 53% outcrossing and its domestication from the wild did not involve a long process as compared to fruit-bearing relatives such as tomato (Labate et al*.*, 2009; Sim et al*.,* 2012). It is therefore probable that natural selection is still an important factor in retaining heterogeneous populations with broad genetic adaptability and variability (Rauf et al*.,* 2010).

Genetic distances of Cape gooseberry accessions among the six selected counties

For this study, the allele frequency of Cape gooseberry between counties was significantly lower than diversity within the counties (Table 4). Allele-frequency divergence among counties was the highest between Laikipia and Nakuru (0.0067) and the lowest between Kericho and Nakuru (0.00). Overall there was a very small allele divergence observed in Cape gooseberry across counties. This shows that the gooseberry accessions from the selected counties share most of the alleles evaluated, and this is an indicator of low geographic differentiation among these accessions. The findings of this study show that the population showed a slight deviation from the Hardy Weinberg equilibrium (HWE = 0.7). This may be due to the significant effects of natural selection resulting from the limited domestication of Cape gooseberry

Table 5. Analysis of molecular variance (AMOVA) for the 70 cape gooseberry accessions from 6 select counties of Kenya.

Source	SS	Df	MS	F	Prob > F
Between accessions	10.36	5	2.07	92.80	0.0000
Within acce	1.429	64	0.02	7.20	
Total	**11.79**	**69**	**0.17**		

SS = sum of squares, MS= expected mean squares, F = F-statistics, >F = Significance level.

Table 6. Factorial analysis of the Gooseberry accessions from the five select counties.

County	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5
Nakuru	31.906	40.492	1.271	2.584	0.641
Nyandarua	0.116	0.010	0.062	0.113	-0.048
Kericho	0.038	0.098	0.101	-0.100	-0.014
Kiambu	-0.056	0.099	0.034	0.034	0.041
Murang'a	-0.063	0.047	-0.128	0.008	-0.010
Laikipia	-0.227	-0.212	0.083	-0.007	-0.016
Proportion of Variance	31.714	40.533	1.422	2.632	0.592
Cumulative variance	31.714	72.247	73.67	76.303	76.89
Eigen values	**0.013**	**0.012**	**0.008**	**0.005**	**0.002**
% Inertia	27.74	**26.27**	**17.62**	**10.35**	**4.37**

collections in Kenya. This value is slightly higher than those reported by Tian et al. (2008) (HWE = 0.5481) though Tian's study used cultivated varieties which may have higher domestication influence. Partitioning of the genetic variation of the Cape gooseberry accessions was done using Analysis of Molecular Variance (AMOVA) to determine whether variation observed between the accessions was due to genetic makeup or microclimatic factors. A total of 92.80% of the variation was found among the accessions while a total of 7.20% of the variation was revealed within the accessions (Table 5). This is a further proof that the Cape gooseberry accessions in Kenya have a broad genetic diversity.

Factorial analysis

Factorial analysis was performed to analyze the genetic relationship and population structure of the accessions within and between the counties. A dissimilarity matrix calculated using raw data from the SSR "1" and "0" matrix was used for factorial analysis using Darwin 6.0.21 software. The first five axes accounted for 76.81% of all the variance observed in the test samples, with 41.2, 52.71, and 1.85% explained by PC axes 1, 2, and 3, respectively. The highest variance was observed in Nakuru county (31.90) indicating that cape gooseberry accessions in this county have high genetic diversity and the lowest variance was recorded in Laikipia (-0.21) showing comparatively genetic diversity in the accessions in this county (Table 6).

The high percentage of variation explained by the first three components in the factorial analysis shows that the differentiation of most of the individuals was well captured. However, it is noteworthy to consider the use of a larger number of high-resolution markers and platforms such as SNPs and genotyping by sequencing (GBS) due to the outcrossing nature of the species (Zhu et al., 2008).

Factorial analysis grouped the accessions into five distinct clusters depending on the county of origin. Accessions from Kiambu and Murang'a counties, however, clustered together showing that they were genetically more identical (Figure 2). Individuals were distinct within clusters with little overlap between individuals indicating that the collections are genetically diverse. The collections show a higher level of diversity within and across the clusters (Figure 2).

Phylogenetic analysis

A distance tree was constructed in Darwin 6 using the UPGMA method. The robustness of the node of the phylogenetic tree was assessed from 1000 bootstrap replicates. In this study, the minimum dissimilarity value for the phylogenetic tree was 0.027 while the maximum value was 1. This high dissimilarity value is further proof of high genetic diversity found in the Kenyan gooseberry accessions. Tree length varied between 0 for duplicates

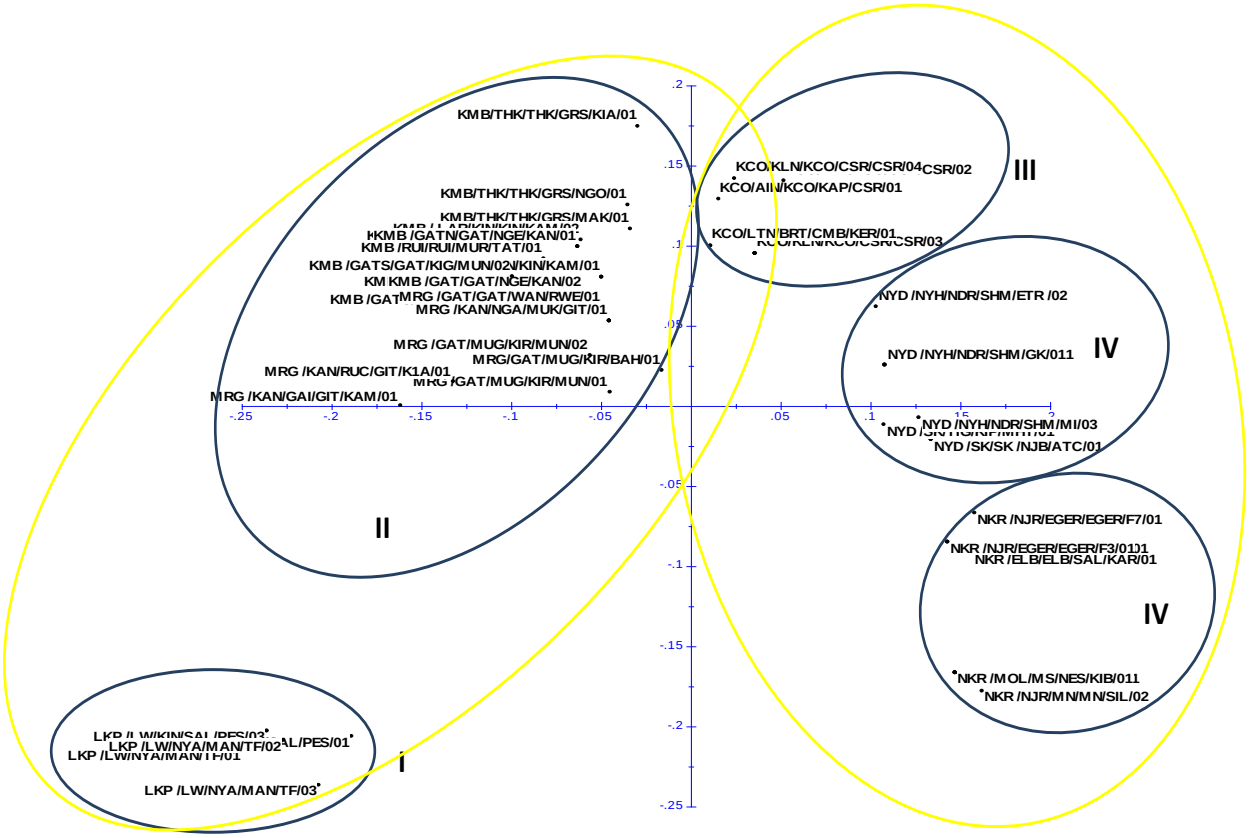

Figure 2. Factorial analysis of cape gooseberry accessions from the six selected counties in Kenya; Names in the circles correspond to individual accession names.

and 0.161 for distinct individuals, the edge length sum was 2.04 indicating that most of the cultivars are distinct. The collections were grouped into two major clusters I and II. Cluster I contained accessions sampled from Nyandarua (1.A), Nakuru (I.B), Kericho (I.C), and Laikipia (I.D) counties while cluster II contained accessions from Kiambu (II.A) and Murang'a (II.B). The phylogenetic tree further clustered the accessions into six sub-clusters (I.A, I.B, I.C, I.D, II.A, and II.B) based on the county of origin (Figure 3).

The clustering analysis applied was able to detect a geographical distribution pattern. This observation may be due to lack of frequent gene flow through the exchange of seeds among the counties by humans as is often the case in heavily domesticated species. This finding is in disagreement with the findings of Garzon-Martínez et al. (2015) who failed to deduce any geographical clustering of Columbian *P. peruviana* varieties using SSR and InDels markers. This may be because the study by Garzón-Martínez et al. (2015) used well defined domestic commercial gooseberry accessions. Both factorial and phylogenetic population analyses show that the whole Cape gooseberry population has two different genetic populations. Using the PCA approach, two different populations within the *P. peruviana* were identified.

CONCLUSION AND RECOMMENDATION

This is the first study in Kenya that used SSR markers to genetically characterize Cape gooseberry. The study established that Cape gooseberry in the six target counties of Kenya have a broad genetic diversity. Based on the SSR data, the 70 accessions were classified into two main phylogenetic groups and six sub-clusters which corresponded to the county of origin through factorial analysis, principal component analysis (PCA), and phylogenetic analyses. The study also established that seven SSR primer pairs with higher polymorphism namely, SSR1, SSR2, SSR10, SSR11, SSR123, SSR138, and SSR146 have a wide applicability in genotype identification and characterization of the population structure of Cape gooseberry. The information generated by this study contributes to understanding diversity and population structure and enhances the management of Cape gooseberry genetic resources in Kenya.

This study enhances understanding of levels of genetic variations among Kenyan Cape gooseberry germplasm

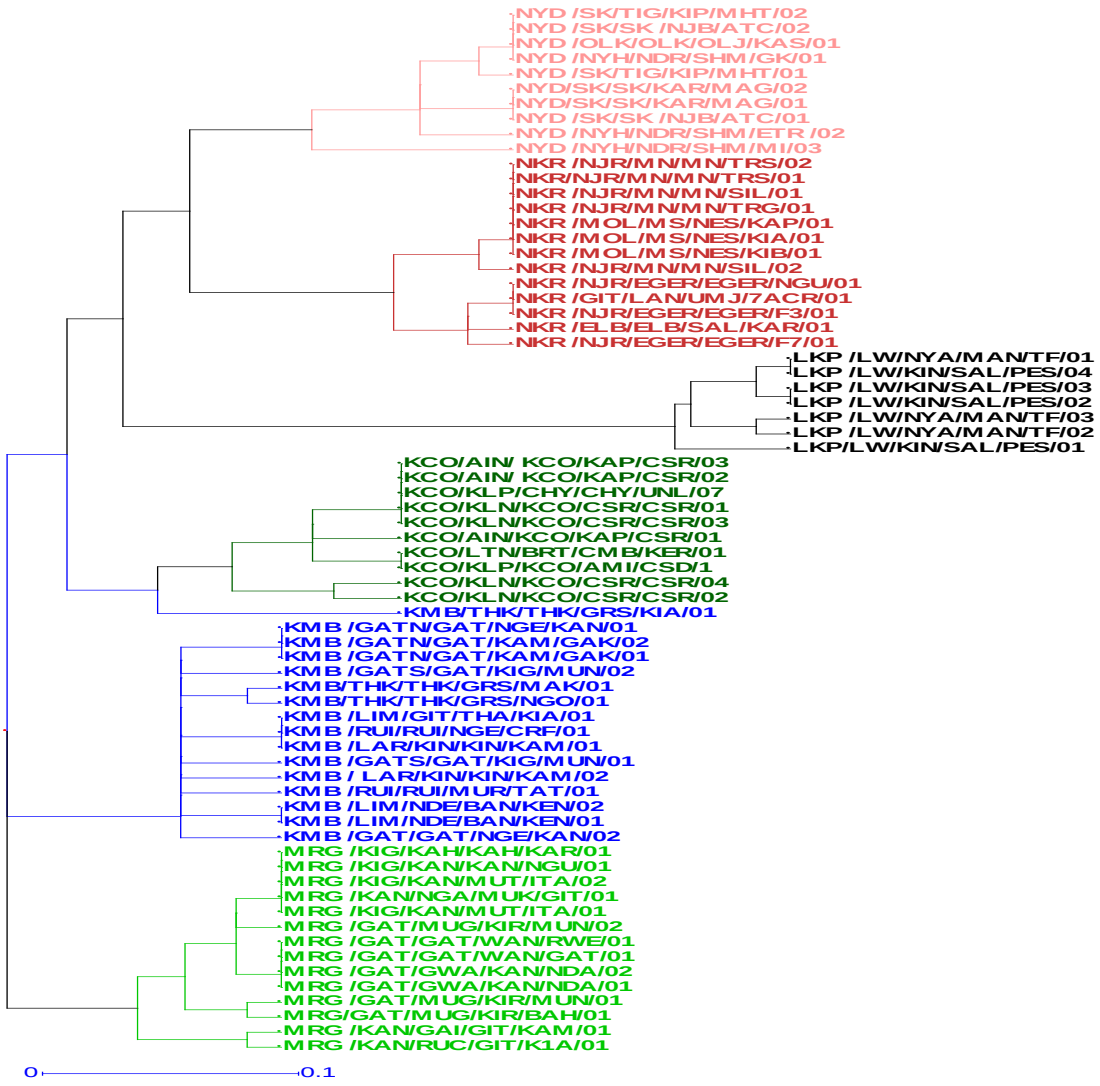

Figure 3. Phylogenetic trees of cape gooseberry accessions based on SSR data.

and informs the need to introduce commercial Cape gooseberry varieties as sources of genetic variation for breeding and hybridization purposes. The findings of the study also inform on the need to use more advanced molecular platforms such as genome-wide sequencing to establish more diversity in wild and cultivated Cape gooseberry in Kenya.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

This work was funded by RUFORUM in collaboration with Egerton University TAGDEv program under the nurturing Grant award, Kenya. I would like to thank the staff at KALRO-Njoro (Food Crops Institute) for the assistance and insight accorded to me during my lab work, colleagues, and staff from the Department of Crops, Horticulture and Soils, Egerton University for their support during the research.

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Vol. 15(1), pp. 39-48, January 2021
DOI: 10.5897/AJPS2020.2074
Article Number: 382755465909
ISSN 1996-0824

http://www.academicjournals.org/AJPS

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African Journal of Plant Science

Full Length Research Paper

Genetic relationship and the occurrence of multiple gene resistance to coffee berry disease (*Colletotrichum kahawae,* Waller & Bridge) within selected *Coffea arabica* varieties in Kenya

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Received 24 September, 2020; Accepted 23 November, 2020

The Coffee Berry Disease (CBD) epidemics destroy up to 100% of the crop on a susceptible variety. Resistance to CBD is conferred by the T-gene in Hibrido De Timor (HDT), R-gene in Rume Sudan (RS), and k-gene in K7, which were assembled in resistance varieties Ruiru 11(R11) and Batian. This study aimed to evaluate the genetic relationship between R11 and Batian with their parents' HDT, RS, SL8 and confirm the occurrence of T and R genes using DNA markers. Genome-wide single nucleotide polymorphism (SNP) markers were obtained through Diversity Arrays Technology sequencing (DArTseq). The genetic relationship was analyzed by Principal Component Analysis and hierarchical clustering. The Tgene was confirmed using Microsatellite primer, Sat 235 while the R gene was by marker sequence search within the DArTseq result files. The PC1 accounted for 42% of the total variation. Hierarchical clustering revealed less than 10% dissimilarity index apart from HDT that recorded above 20%. All the R11 and Batian genotypes carry the T gene. Eleven genotypes carry both T and R genes, therefore, with broad-based resistance to CBD. The study confirmed the narrow genetic relations within the *Coffea arabica* coffee varieties and further confirmed the occurrence of multiple gene resistance in R11 and Batian that will not break easily to new pathogen races.

Key words: Coffee berry disease, diversity arrays technology sequencing, T gene, R gene, Ruiru 11, Batian, SL 28, codominant.

INTRODUCTION

Coffee belongs to the family Rubiaceae and the genus *Coffea* with over 124 species that have been characterized (Davis et al., 2011). Despite the diversity of this genus, only two species, *Coffea arabica* L. and *Coffea canephora*

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P. are of economic importance contributing about 70 and 30% of the total world market share, respectively (Setotaw et al., 2020). The *C. arabica*. is an allotetraploid species (2n = 2x = 44) that exhibits a diploid-like meiotic behavior (Lashermes and Combes, 2018). *C. arabica* is the only tetraploid species of the *Coffea* genus while the rest of the species are diploid (Spiniso-Castillo et al., 2020). The *C. arabica* is believed to have been formed as a result of spontaneous hybridization between two diploid species, *C*. *canephora* and *Coffea eugenioides* (Lashermes et al., 1999, 2011). The species is autogamous with about 10% out-crossing (Bikila et al., 2017). On the other hand, *C. canephora* is diploid (2n = 2x = 22), highly diverse (Bertrand et al., 2003), with resistance to common disease and thus a good source of genes for disease resistance (Ky et al., 2001).

Next-generation sequencing (NGS) technologies, such as genotyping-by-sequencing (GBS) and Diversity Arrays Technology sequencing (DArTseq), provide markers that are widely used in genome-wide analysis (Spiniso-Castillo et al., 2020). The GBS approach is more informative than predesigned single nucleotide polymorphism (SNP) arrays especially on wild germplasm as it is unbiased and provide information on rare alleles while the DArTseq method is based on the complexity reduction approach, using restriction enzymes that target the genome coding regions (Pailles et al., 2017). The restriction enzymes separate low copy sequences from the repetitive regions of the genome that are more informative for marker discovery for breeding purposes (Courtois et al., 2013; Pailles et al., 2017).

The genetic variation is controlled by the segregation of multiple genes such that the variances of individual loci are so small that they cannot be investigated individually and thus the need to analyze sets of these loci (Bikila et al., 2017). The DArTseq based SNP markers were successfully utilized in the determination of genetic relations in the *Coffea* genus by Garavito et al. (2016) and Spiniso-Castillo et al. (2020).

The coffee berry disease (CBD) caused by a specialized hemibiotrophic fungal pathogen, *Colletotrichum kahawae* (Waller & Bridge) (Waller et al., 1993), is a key constraint of Arabica coffee production in Africa (Hindorf and Omondi, 2011; Van Der Vossen et al., 2015; Diniz et al., 2017; Vieira et al., 2019). The CBD epidemics can destroy 50 to 80% (Van Der Vossen and Walyaro, 2009; Diniz et al., 2017) and at times up to 100% (Giddisa, 2016) of the developing berries, 4 to 16 weeks following anthesis on a susceptible variety when no control measure is applied (Gichuru et al., 2012). The control of CBD using intensive fungicide spray programs (8-12 rounds per year) increases the cost of production by up to 40% (Van der Vosen and Walyaro, 2009). The use of these chemicals also contributes to environmental pollution (Gichuru et al., 2008), thus the use of resistant varieties is the most cost-effective and environmentally friendly approach for CBD management. Resistance to CBD is governed by three genes in the varieties Rume Sudan (R genes), HDT (Tgene) and K7 (k gene) where R and T are dominant while k is recessive (Van Der Vossen and Walyaro, 1980).

The breeding program for resistance to CBD in Kenya started in 1971 with the main breeding goal of developing cultivars that combine resistance to CBD, high production, good beverage quality, and compact growth that will be amenable to high-density planting (Van Der Vossen and Walyaro, 1980). Using conventional approaches, genes for resistance to CBD were introduced to *C. arabica* coffee varieties that are susceptible by crossing with donor varieties and backcrossing to standard varieties to restore desirable attributes (Walyaro, 1983). However, this approach takes a long time to develop a coffee variety due to the long juvenile nature of the *Coffea* genus (Moncada et al., 2016). The low genetic diversity of *C. arabica* also hinders the identification and selection of superior genotypes using traditional breeding methods (Sousa et al., 2017). To overcome this constraint, molecular markers have been used as a supporting tool to accurately discriminate genotypes and accelerate coffee breeding programs (Sousa et al., 2017). The DNA marker for the T gene was identified by Gichuru et al. (2008) and linked to Simple Sequence Repeats (SSR) primer locus Sat 235, popularly designated as *Ck*-1 (Gichuru et al., 2008). This marker was validated by Alkimim et al. (2017) who confirmed that Sat 235 marker co-segregate with the T-gene. A recent study by Gimase et al. (2020a) identified the putative DNA marker for the R gene using Single Nucleotide Polymorphism (SNP) markers.

The CBD resistant cultivar R11 is an F1 hybrid derived from a cross between a specific female and male population (Omondi et al., 2001), while Batian is a pure line that was selected from the R11 pollen parents (Omondi et al., 2001), after several generations of selfing to fix the CBD resistant genes (Gichimu et al., 2014). SL 28, is a Bourbon type single-tree selection that combines high yield, high quality, and drought tolerance but highly susceptible to CBD (Walyaro, 1983). The main objective of this study was to evaluate the genetic relationship within selected *C. arabica* cultivars R11, Batian and their resistance donor parent HDT, RS and the recurrent parent SL28 using DArTseq based SNP markers and identify genotypes within R11 and Batian with multiple gene resistance to CBD conferred by the T and R genes.

MATERIALS AND METHODS

This study was carried out at the Kenya Agricultural and Livestock Research Organization – Coffee Research Institute (KALRO-CRI) in Ruiru, Kenya. Ruiru is located within the upper midland (UM2) at 1° 06'S and 36° 45'E and an altitude of 1620 m above sea level. The rainfall pattern is bimodal with 1063 mm per annum and the annual average temperature is 19°C with a range of 12.8 to 25.2°C (Jaetzold et al., 2006).

The study materials were 91 genotypes comprising of 61 crosses

of the variety of R11 and 27 families of the variety Batian. Also included were SL 28, HDT and Rume Sudan.

Sample collection, genomic DNA extraction and genotyping of SNP markers

Fresh leaves were randomly picked from each of the 91 coffee genotypes, kept in cool boxes and taken to the laboratory for DNA sample extraction. The LGC genomics plant sample collection kit (www.lgcgenomics.com) was used in sample collection, where 6 disks were cut and placed in each strip of the 96 deep well sample plate, and sent to the Integrated Genotyping Service and Support (IGSS) platform (https://ordering.igssafrica.org/cgibin/order/login.pl) for DNA extraction and genotyping.

Genomic DNA samples were extracted from genotypes using a standard cetyltrimethylammonium Bromide (CTAB) protocol of Doyle and Doyle (1987). The quality and quantity of the DNA samples were evaluated by running it through 0.8% agarose gel electrophoresis. The DNA concentration was adjusted to 50 ηg/µl. The genomic DNA samples were sent to Diversity Arrays Technology (DArT) Pty Ltd., in Canberra-Australia (http://www.diversityarrays.com) for sequencing and identification of SNP markers. The GBS-SNP was performed following the standard protocol as described by Elshire et al. (2011). Next-generation sequencing was carried out using the Hiseq2500 Illumina platform.

The SNP calling was carried out by the DArT-soft14 algorithm within the KDCompute pipeline developed by Diversity Arrays Technology (https://kdcompute.seqart.net/kdcompute/plugins). In the primary pipeline, the FASTQ files were first processed to filter poor quality sequences to ensure that the assignments of the sequences to specific samples carried in the barcode split region were consistent and reliable (Nemli et al., 2017; Barilli et al., 2018). The identical sequences were collapsed into FASTQ call files that were used in the secondary pipeline for DArT P/L's proprietary SNPs calling algorithms (DArT-soft14) pipeline in the processing of the sequence data (Barilli et al., 2018). Since the allotetraploid *C. arabica* open-access genome assembly, with a reliable sorting of homoeologous sequences, is not yet available (Scalabrin et al., 2020), the filtered sequence reads were aligned against the finer and publicly available diploid *C. canephora* genome (http://coffee-genome.org/coffeacanephora) as a reference to find the SNP markers in *C. arabica* genome (Sant'Anna et al., 2018) and to determine their corresponding genomic positions.

The SNP marker quality analysis and evaluation of the genome-wide relations

The SNP loci with >30% missing data and rare SNPs with less than 5% minor allele frequencies (MAF) and heterozygosity (Ho) above 90% were removed (Garot et al., 2018). The genetic relationships within the study genotypes were determined using the Principal Component Analysis (PCA) and hierarchical clustering, both implemented within the clustering analysis component of the KDCompute plugins system (https://kdcompute.seqart.net/kdcompute/plugins).

Genomic DNA extraction, amplification, and electrophoresis using SSR primer locus Sat 235

A total of 59 genotypes comprising 27 R11 crosses, 27 Batian families, five control genotypes, HDT, Robusta, Rume Sudan, and susceptible cultivars SL28 and Caturra were analyzed. Healthy leaves were picked and genomic DNA extracted following the method of Diniz et al. (2005) with minor modifications in the extraction buffers. About 500 mg of fresh leaves were ground and transferred to 2 mL Eppendorf tubes. After grinding, 1 mL extraction solution was added and the tubes shaken vigorously for 5 min and immediately put in a 65°C water bath for 40 min. After which the samples were centrifuged for 5 min at 13000 rpm and the supernatant transferred to a new tube, to which 1 mL CIA (chloroform: isoamyl 24:1) was added and the tubes were shaken for 10 min and centrifuged for 5 min at 12000 rpm. The supernatant was transferred to another tube and the same volume of frozen Isopropanol was added and maintained at -20°C for 1 h.

The content was centrifuged at 1300 rpm for 5 min, the supernatant discarded and the pellet washed with 70% ethanol. This step was repeated twice and after drying, the pellets were treated with 190 µL TE (Tris-EDTA buffer plus RNAse 10 mg µL-1) for 30 min at 37 and 65°C for 5 min. The DNA was then purified with the addition of 100 µL TE, 100 µL water, 100 µL NaCl 5 M and 100 µL EDTA 0.5 M. The samples were homogenized and incubated on ice for 30 min and centrifuged for 5 min at maximum speed and isopropanol added. After drying, the pellet for each genotype was diluted in an appropriate amount of TE buffer as per the amount of DNA quantified using a spectrophotometer and stored at 4°C. The extracted DNA quality was determined by running the samples in 1% agarose gel alongside a Lambda standard with a known concentration of DNA fragments for comparison and quantification of the samples.

The Polymerase Chain Reaction (PCR) was carried out in a total volume of 25 µL, containing 10 ng/µL template of genomic DNA, 0.4 µM of Sat 235 SSR primer, 75 µM dNTPs (each), 2.5 µM $MgCl_2$, PCR buffer 1x TBE [75 mM Tris-HCl; 0.5 Na_2 EDTA (pH 8.0)], 20 Mm Boric acid and 1 unit Taq DNA polymerase (from Gene - on company, Germany). Amplification was carried out in a Eurogene thermocycler (TECHNE, UK). The amplification program was one cycle of initial denaturation at 94°C for 5 min followed by 35 cycles of 30 s at 94°C (denaturation), 30 s at 55°C for primer annealing, and 1 min and 30 seconds at 72°C for elongations with a final extension at 72°C for 10 min.

The amplification products with SSR primer Sat 235 were electrophoresed in 2.3% (w/v) agarose gel with a 1x TBE buffer system and then visualized in a UV light trans-illuminator after staining in 60% ethidium bromide solution.

Confirmation of the occurrence of multiple gene resistance to CBD conferred by the T and R genes

The presence/absence of the Tgene was confirmed by observation of the amplified fragment, based on the standard HDT, Robusta, and SL28 while the genotypes carrying DNA marker for R genes were identified by searching the SNP marker sequences within the GBS-based DArTseq marker result files of the study genotypes.

RESULTS

The analysis of the SNP marker and evaluation of the genome-wide relations

The DArTseq generated 2280 good quality SNP markers (MAF>5% and Ho<90%), out of which 1575 were aligned on the 11 chromosomes based on the *C. canephora* reference genome, markers that were well distributed with the genome (Figure 1) and therefore used in further analysis. The PCA results revealed that all the Batian genotypes from the three crosses clustered together apart from two genotypes, CR8-155 and CR30-809 that

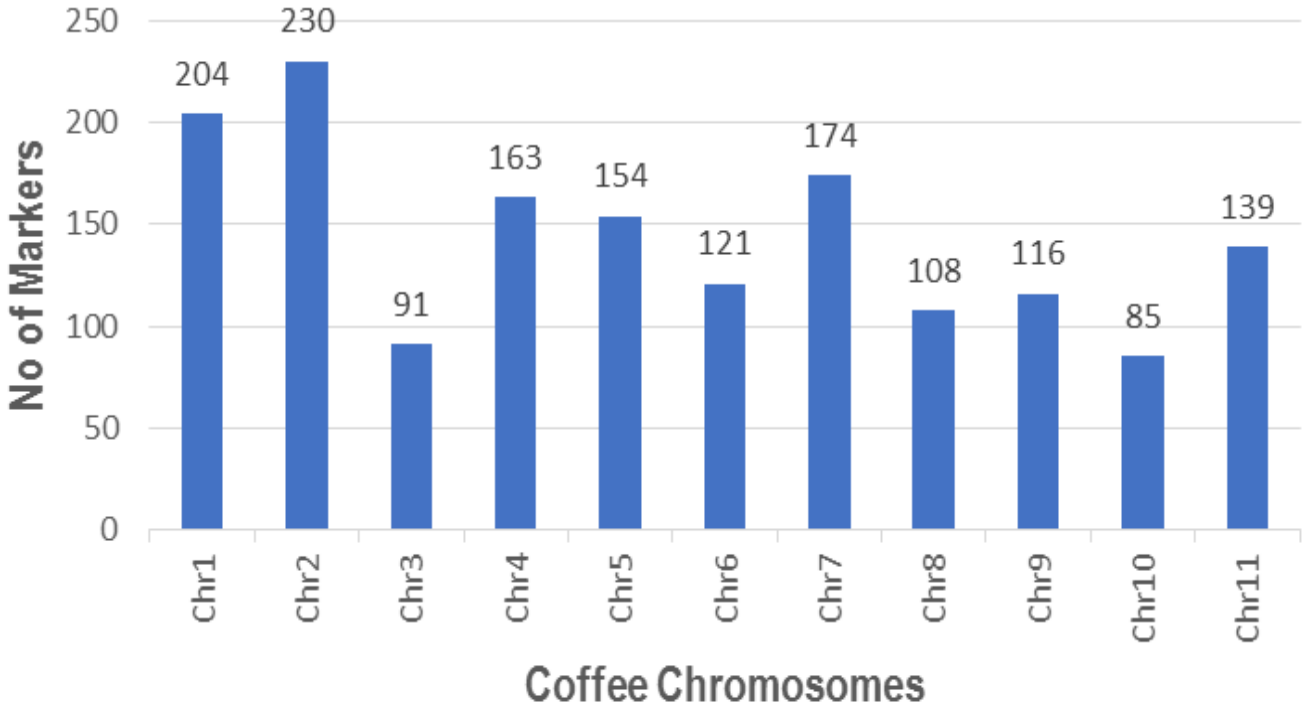

Figure 1. The genome-wide distribution of DArTseq-based SNP markers.

deviated from this cluster (Figure 2). The two genotypes had a close relation with Rume Sudan. The Batian genotypes were closely related to the cultivar SL 28. About 60% of the R11 crosses (38) formed one main cluster while the rest (23) were evenly distributed within the PCA plot. HDT did not portray any close relationship with any of the genotypes in the study. PC1 was the most important and accounted for 42% of the total phenotypic variation.

Similar to the PCA, the hierarchical clustering analysis (dendrogram) revealed that all the Batian formed one cluster together with SL 28 apart from two genotypes, CR8-155 and CR30-809 that showed a close relationship with Rume Sudan (Figure 3). 38 R11 crosses formed one major cluster while the rest (23) formed four different clusters. HDT did not cluster with any of the study genotypes while Rume Sudan showed a closer relationship with Batian and three R11 genotypes (R11-6, 22 and 195). The cluster dendrogram also revealed that all the R11 crosses, Batian families from the three crosses, RS and SL 28 had low genetic diversity of about 10% or less while HDT had a relatively high diversity of about 20%.

The occurrence of the T (*Ck-1*) gene in variety R11 and Batian

The amplification products revealed that all the 27 R11 crosses evaluated carry the DNA fragment for the T-gene (*Ck*-1) that is introgressed from the *C. canephora* genome (Plate 1). The *Ck*-1 DNA fragment was heterozygous for all the R11 genotypes. The *Ck*-1 DNA fragment was also present in HDT and Robusta in a homozygous state but absent in Rume Sudan, SL 28, and Caturra. Similarly, all the 27 Batian families analyzed carry the *Ck*-1 fragment (Plate 2), which was also present in HDT and Robusta but absent in SL28 and Rume Sudan. Out of the 27 Batian genotypes, 15 genotypes namely CR8-423, CR8-760, CR8-149, CR8-155, CR8-419, CR22-109, CR22-108, CR22-350, CR22-635, CR22-357, CR22-639, CR30-807, CR30-813, CR30-244 and CR30-809 were homozygous for the Ck-1 gene while 12 genotypes CR8-136, CR8-154, CR8-761, CR8-420, CR22-759, CR22-114, CR22-353, CR22-111, CR30-812, CR30-242, CR30-233 and CR30-236 were heterozygous for the Ck-1 gene (Pate 2).

The occurrence of the R-gene within the variety R11 and Batian

A total of 91 genotypes comprising 61 R11, 27 Batian and three control coffee varieties (Rume Sudan, SL 28 and HDT) were sequenced. The sequences search using the two SNP marker sequences (100025973|F|0-59:T>C-59:T>C and 100034991|F|0-44:C>T-44:C>T), that were significantly associated with CBD resistance through GWAS and QTL mapping (Gimase et al., 2020a, b), were carried out within the sample GBS result files. The

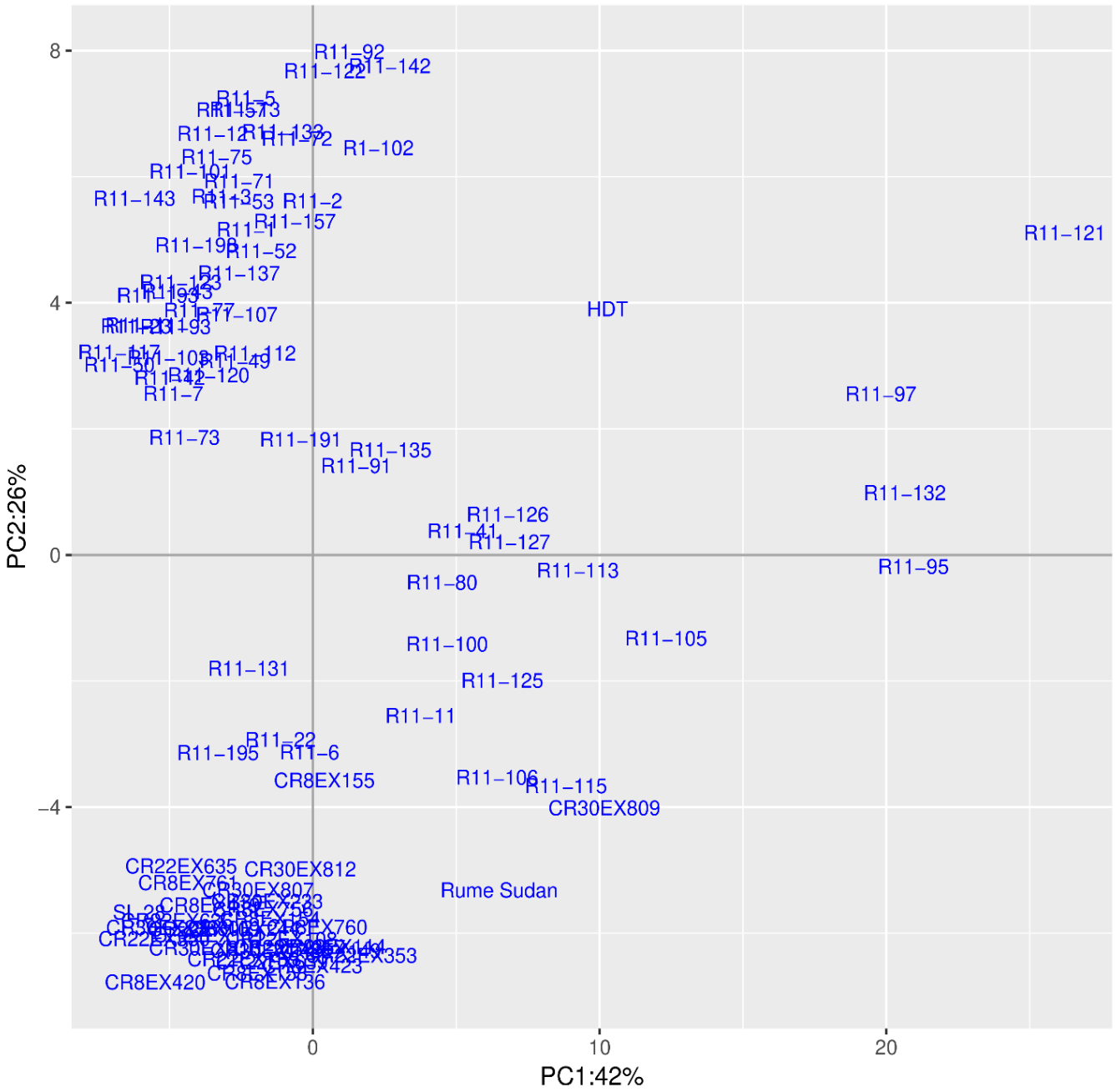

Figure 2. The Principal Components Analysis (PCA) plot of the individual genotypes within the selected Arabic coffee varieties.

search revealed the occurrence of the SNP marker 100034991|F|0-44:C>T-44:C>T in a total of 11 genotypes comprising eight R11 and three Batian (Table 1). The R11 genotypes were R11-157, R11-22, R11-121, R11-195, R11-6, R11-135, R11-123 and R11-11 while the Batian genotypes were CR30-809, CR8-155 and CR8-136 (Table 1). Out of the eight R11 genotypes, four genotypes were homozygous for the R-gene as revealed by the SNP marker 100034991|F|0-44:C>T-44:C>T. These were R11-22, R11-195, R11-6, and R11-123 while R11-157, R11-121, R11-135, and R11-11 were heterozygous. On the same note, two Batian individuals were homozygous, CR30-809 and CR8-155 while CR8-136 was heterozygous for the R-gene marker. This marker was also present in Rume Sudan but absent in SL28 and HDT, therefore polymorphic between the two parents of the mapping genotypes. The SNP marker 100025973|F|0-59:T>C-59:T>C sequence was absent in all the study genotypes. The 11 genotypes confirmed for the occurrence of the R-gene marker had also been

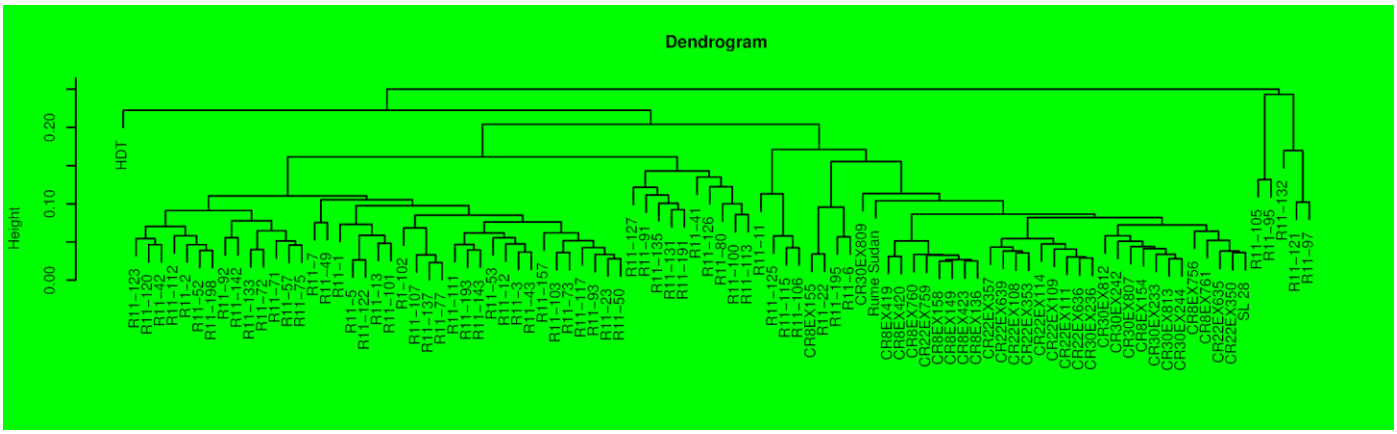

Figure 3. The clustering dendrogram of the genotypes within the selected coffee varieties and their relationships.

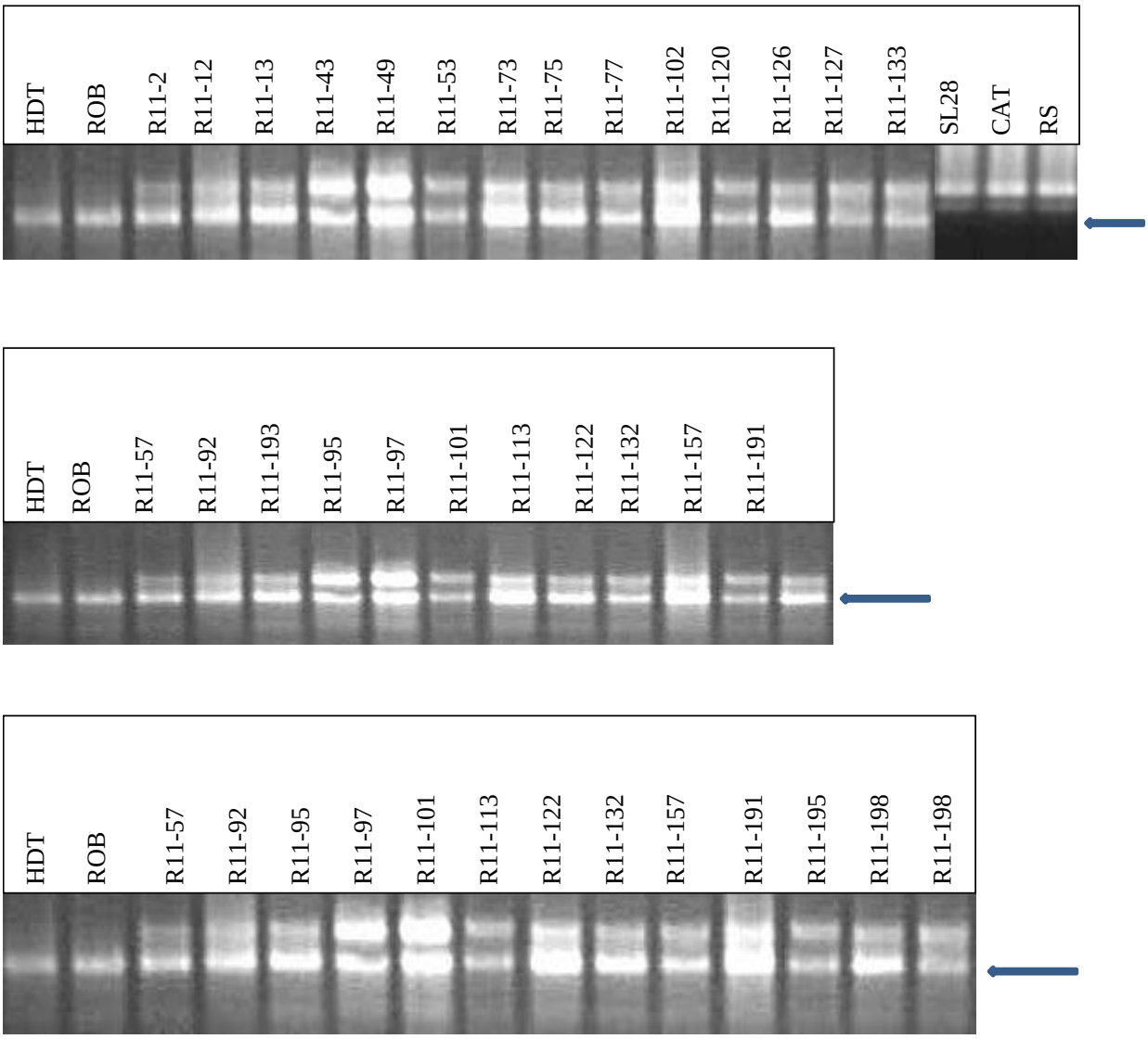

Plate 1. The occurrence of *Ck*-1-gene fragment within cultivar R11 crosses. The fragment is indicated by the arrow. Rob – Robusta, CAT – Caturra, HDT – Hibrido de Timor.

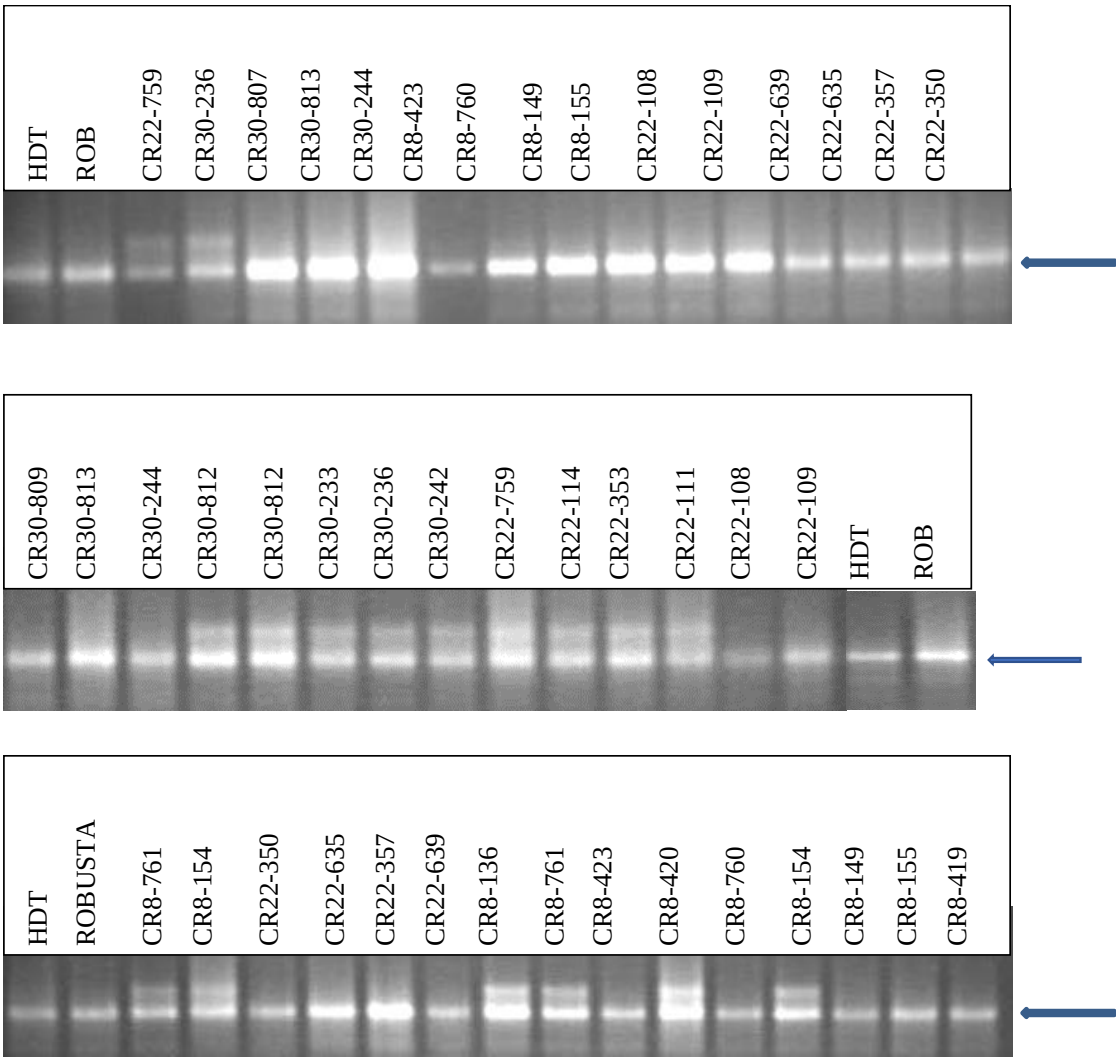

Plate 2. Occurrence of *Ck*-1 gene fragment within the families of Batian crosses, indicated by the arrow.

Table 1. Occurrence of the R-gene marker within the variety R11 and Batian.

Genotype	CR30-809	R11-157	CR8-155	R11-22	R11-121	R11-195	R11-6
Marker (100034991|F|0-44:C>T-44:C>T)	1	2	1	1	2	1	1

Genotype	R11-135	R11-123	CR8-136	R11-11	Rume Sudan	SL 28	HDT
Marker (100034991|F|0-44:C>T-44:C>T)	2	1	2	2	1	0	0

1 - Homozygous, 2 - Heterozygous, 0 - Absent.

confirmed to carry the T-gene (*Ck*-1).

DISCUSSION

The genome-wide relationship within the study varieties

The genome-wide analysis revealed a narrow genetic base within *C. arabica* coffee varieties R11, Batian, SL 28 and Rume Sudan, with a diversity index of less than 10%. This is attributed to the fact that these varieties are derived from a few individual collections and whose subsequent dispersal has progressively narrowed further their genetic base (Baruah et al., 2003; Setotaw et al., 2013). The variety of HDT had a higher diversity index of about 20%. The variety HDT is a natural interspecific cross between *C. arabica* and *C. canephora* and usually shows

a divergence from commercial cultivars for most of the agronomic traits (Agwanda et al., 1997). The expressed diversity in HDT is due to the introgressed genes from the *C. canephora* genome (Lashermes et al., 1999). Although HDT was utilized as a donor parent for resistance to CBD in the varieties' R11 and Batian, the high diversity was not reflected in these genotypes as in HDT. This is most likely due to filial advancement from the original crosses, such that the existing progenies contain less of the initial *C. canephora* genome (Gichuru, 2007).

The study revealed uniformity within individuals of the three crosses that make up the variety Batian and revealed further that they are closely related to SL 28. The variety Batian was obtained from complex crosses between CBD donor parents (HDT, Rume Sudan and K7) and susceptible varieties (SL 28, SL 34, Bourbon and Tanganyika drought-resistant selections) and then backcrossed to SL28 and selfed to restore and fix genes for desirable attributes of superior beverage quality (Gichimu et al., 2014). From this result, it is most likely that this attribute was successfully restored as SL 28 was closely related to the individual genotypes of Batian.

The cultivar R11 is also relatively uniform as 60% of the crosses clustered together while the rest were in four different small clusters. R11 is an F1 hybrid between complex crosses (that were selected as Batian) as pollen parents and Catimor (a cross between HDT and Caturra) isolines as seed parents (Omondi et al., 2001). Variation within R11 crosses on various traits have been reported in previous studies; resistance to CBD (Omondi et al., 2001; Gichimu et al., 2014); beverage quality (Gichimu et al., 2012); and yield (Gichimu et al., 2013).

The occurrence of the T-gene (*Ck*-1) within *C. arabica* variety R11 and Batian

All the 27 R11 crosses were confirmed for the occurrence of the T-gene. R11 is a composite F1 hybrid made up of 66 different crosses (Omondi et al., 2001). The previous study by Gichimu et al. (2014), confirmed the occurrence of T-gene in 34 R11 crosses and therefore this study brings the total number of R11 crosses confirmed to carry the T-gene through the marker-assisted selection to 61. The cultivar R11 inherited the T-gene from two different sources, the seed parent Catimor and Pollen parents. The seed parent Catimor comprises several lines derived from a cross between HDT (a spontaneous cross between *C. canephora* and Caturra, a *C. arabica* cultivar, highly susceptible to CBD) (Gichuru et al., 2008). The pollen parents are complex crosses between CBD resistance var HDT, Rume Sudan and susceptible SL28, SL34, SL4, N39 and Bourbon (Van Der Vossen et al., 1981; Agwanda et al., 1997; Omondi et al., 2001).

Similarly, all the 27 Batian genotypes analyzed were confirmed to carry the *Ck*-1 gene where 15 genotypes were homozygous and hence stable for the gene. Batian is a selection from the pollen parents of R11 hence inherited the T-gene from HDT, one of the resistance sources in the complex crosses (Gichimu et al., 2014).

A study by Alkimim et al. (2017) using three CBD resistance genotypes in Brazil revealed the occurrence of *Ck*-1, within the genotypes, where two genotypes were homozygous while one was heterozygous and confirmed that Sat 235 marker co-segregates with the gene. Similarly, a study by Mtenga (2016) reported the occurrence of *Ck*-1 in CBD resistant genotypes from Tanzania and Ethiopia accessions. In the study, Sat 235 could not amplify the T-gene fragment in Rume Sudan since the gene for resistance to CBD in this variety is in a different locus. This therefore confirmed further, the findings by Mtenga (2016) as the *Ck*-1 gene was not amplified in Rume Sudan.

The occurrence of the R-gene within *C. arabica* variety R11 and Batian

Eleven genotypes were confirmed for the occurrence of the SNP 100034991|F|0-44:C>T-44:C>T while none of the genotypes carry the SNP marker 100025973|F|0-59:T>C-59:T>C. The SNP marker 100025973|F|0-59:T>C-59:T>C was comparatively a rare variant where minor allele frequency (MAF) was lower (0.28) as opposed to 100034991|F|0-44:C>T-44:C>T (0.42) out of the possible maximum of 0.5 (Zhang et al., 2015; Gimase et al., 2020a). The SNP marker 100025973|F|0-59:T>C-59:T>C could have been lost in both R11 and Batian during backcrossing of the complex crosses to SL28 to restore good cup quality and high yield (Agwanda et al., 1997) since the selection process was not guided by the use of DNA markers (Omondi, 1998). This, therefore, confirmed that the SNP marker 100034991|F|0-44:C>T-44:C>T as a reproducible marker within *C. arabica* genotypes carrying resistance gene inherited from Rume Sudan. The polymorphic occurrence of this locus in Rume Sudan and SL 28 signifies its ability to discriminate variants in terms of resistance to CBD and its suitability for MAS (Rouet et al., 2019). The polymorphic genomic loci are used as genetic markers in the determination of the co-segregation of genetic alleles with qualitative traits emanating from populations of crosses or naturally occurring populations (Motazedi et al., 2019). The SNP marker 100034991|F|0-44:C>T-44:C>T was found to be linked to two genes by Gimase et al. (2020b), a finding that is further supported by the inheritance study by Van Der Vossen et al. (1980) that reported that the occurrence of two alleles for R gene in Rume Sudan as R1R1.

Conclusion

The study confirmed the close relationship within the *C. arabica* coffee varieties as exhibited by a narrow genetic base, with Batian as a very uniform cultivar and genetically very close to SL 28 while R11 was fairly

uniform. As per the previous studies, this work affirmed further the uniformity within the *C. arabica* genome. The study also revealed that all the genotypes within the CBD resistant varieties R11 and Batian carry the T gene, while 11 carries the R gene and therefore with multiple gene resistance to CBD. The study further confirmed the codominant nature of the DNA marker for T gene (*Ck*-1) and R gene due to their ability to discriminate between homozygous and heterozygous variants within the resistant genotypes. From this study, it is evident that the selection of arabica coffee varieties with multiple gene resistance to CBD is a reality. The genotypes that were confirmed to carry the two genes for resistance to CBD are recommended for further distribution to growers since resistance will not break easily to new disease races.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was supported by KALRO-CRI, International Livestock Research Institute (ILRI), and the National Research Fund (NRF). The authors thank the Director-General, KALRO (through the Institute Director, CRI), the Director-General ILRI, and the Chief Executive officer, NRF. This work is published with the permission of the Institute Director, CRI.

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