ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 17, Issue, 05, pp.32841-32848, May, 2025
DOI: https://doi.org/10.24941/ijcr.48597.05.2025

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ANALYSIS OF DROUGHT TOLERANCE IN COCOA (*THEOBROMA CACAO* L.) GENOTYPES AND THEIR HYBRIDS BY PHYSIOLOGICAL PARAMETERS AND MOLECULAR MARKERS

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ARTICLE INFO

Article History:
Received 09th February, 2025
Received in revised form
21st March, 2025
Accepted 19th April, 2025
Published online 28th May, 2025

Key words:

Cocoa, Drought Tolerance, Molecular Markers.

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ABSTRACT

Cocoa (*Theobroma cacao* L.), in its present growing condition, has to undergo a minimum of four to six months of dry period both in the traditional belts of Kerala and Karnataka and non-traditional areas of Tamil Nadu and Andhra Pradesh. The crop is sensitive and susceptible to altering temperatures and varying environmental conditions in South India. In this study, systematic screening for drought tolerance of Nigerian and Malaysian cocoa collections and their hybrids was carried out using physiological parameters and microsatellite markers. Initially, screening of the 10 parents using nine microsatellite markers revealed a total of 43 alleles with an average of 4.77 alleles per locus. The observed heterozygosity was higher is Malaysian collections (0.43) compared to Nigerian collections (0.36). The fixation index, in contrast, was lower in Malaysian collections (0.29) compared to Nigerian collections (0.37). The primer mtcCIR8 primers mtcCIR11, mtcCIR7 and mtcCIR18 generated an allelic richness and a high level of heterozygosity. There was a clustering of collections showing drought tolerance. A total of 108 progenies of cocoa, comprising of 8 parental plants and 10 hybrids, were analyzed for photosynthesis, leaf water potential, chlorophyll fluorescence and stomatal conductance. The parents, NC23/43, NC29/66 I-14, I-29 and II-67 had drought tolerant nature and the hybrids, I-21 x NC 29/66, I-29 x NC 23/43, II-67 x NC 29/66 and II-67 x NC 42/94 exhibited positive physiological traits for drought tolerance along with high yield.

Citation: M'bo Kacou Antoine Alban, Elain Apshara, S., Shafeeq Rahman, M., Rajesh, K. and Balasimha, D. 2025. *"*Analysis of drought tolerance in cocoa (*Theobroma cacao* L.) genotypes and their hybrids by physiological parameters and molecular markers*". International Journal of Current Research, 17, (05), 32841-32848.*

INTRODUCTION

Cacao tree (*Theobroma cacao* L.) belonging to Malvaceae family (Alverson *et al*., 1999) is crops which have a great economic value. Cocoa dry bean supports nearly 6 million of persons in the tropic area. Native to the lower floors of tropical forests of Amazon basin and other tropical regions of South and Central America, it is now grown under shade in all producing countries to limit harmful effects of environmental factors upon its growth and development (Wood et Lass, 2001). The sensitivity of cocoa tree to environmental constraints particularly high temperatures and drought was revealed by several authors (Balasimha *et al*, 1991; Baligar *et al*, 2008; Daymond *et a*l, 2011, Joly et Hahn, 1989; Raja Harun et Hardwick, 1988a, 1988b). However, drought like other environmental stress is the most common adverse constraint affecting replanting, rehabilitation and productivity of cocoa tree (Boyer, 1967; Alvim, 1977). Due to frequent changes in environmental factors, it is difficult to carry out selection trials genotypes tolerant to drought stress quantitatively and reproductively. These difficulties significantly impede the drought tolerance genotypes identification, in particular on cocoa. The biological basis of drought tolerance is still largely unknown, but some morpho-physiological traits of tolerance were identified by Balasimha *et al*, (1985), Balasimha et Rajagopal, (1988), Ludlow et Muchow, (1990); Bohnert *et al*., (1995); Araus *et al.,* (2002); Bruce *et al*., (2002). The delay and difficulties in revealing mechanisms of drought tolerance has hampered traditional selections efforts. Thus, use the new approach of modern genetics is one of the popular ways to improve the drought tolerance of plants. In cocoa, improvement involves very complex character, with mostly a continuous distribution (D. Crouzillat, *et al.,* 2000). One of the methodologies for studying such as traits is the use of methods of association phenotypes/genotypes (E. Lerceteau *et al*, 1997), through molecular genotyping techniques using molecular markers. The objective is to perform a statistical correlation between the observed phenotypic variation for each character and the

observed allelic variation at a genetic level. The most commonly used are genomic polymorphism markers such as microsatellite. Microsatellites are highly polymorphic by varying the number of repetitions and therefore very informative. In addition, they are easily detectable by the polymerase chain reaction technique (PCR) and reproducible. However, these markers are frequently used because more than 2000 highly polymorphic microsatellites were ordained accurately (on average one every 1.6 cM) (Dib *et al.,* 1996). Also, the high degree of polymorphism of microsatellite is highly appreciated for genetic diversity studies or for mapping of genes involved in tolerance to abiotic and biotic stresses .

The selection of drought-tolerant varieties using SSR markers lead to a better understanding of the genetic and physiological determinism of response to the lack of water and thus improve tolerance to drought in cocoa. The objective of this work is to study through phenotypic and genotypic characterization based on the analysis of cocoa yield and physiological traits and molecular using microsatellite markers, the drought tolerance of 10 parental cocoa clones and their 10 hybrids progenies and to derive correlations between morpho-physiological and molecular markers for the relevant selection for drought tolerance.

MATERIAL AND METHODS

Plant material: The plant material is constituted of samples of cocoa leaves collected from ten clones and their hybrid progenies

Table 1. Cocoa parental clones and their hybrids used in this study

N°	Clones	Origins	N°	Hybrids
1	I-14	Malaysian	**1**	I-14 X NC 29/66
2	I-21	Malaysian	**2**	I-14 X NC 23/43
3	I-29	Malaysian	**3**	I-21 X NC 42/94
4	I-56	Malaysian	**4**	I-21 X NC29/66
5	II-67	Malaysian	**5**	I-21 X NC 42/94
6	NC-29/66	Nigerian	**6**	I-56 X NC 23/43
7	NC-23/43	Nigerian n	**7**	I-29 X NC 23/43
8	NC-42/94	Nigerian	**8**	I-29 X NC 42/94
9	III-105	Malaysian	**9**	II-67 X NC 42/94
10	I-35	Malaysian	**10**	II-67 X NC 29/66

Molecular primers: SSR molecular markers used are shown in Table 2

Tableau 2. List of primer used

Primer N°	Primer	Séquence (5'-3')	Tm
1	*MTcC1R7F*	*ATGCGAATGACAACTGGT*	*58*
	MTcC1R7R	*GCTTTCAGTCCTTTGCTT*	
2	*MTcC1R8F*	*CTAGTTTCCCATTTACCA*	*52*
	MTcC1R8R	*TCCTCAGCATTTTCTTTC*	
3	*MTcC1R17R*	*AAGGATGAAGGATGTAAGAGAG*	*58*
	MTcC1R17F	*CCCATACGAGCTGTGAGT*	
4	*MTcC1R18R*	*GATAGCTAAGGGGATTGAGGA*	*58*
	MTcC1R18F	*GGTAATTCAATCATTTGAGGATA*	
5	*MTcC1R22F*	*ATTCTCGCAAAAACTTAG*	*52*
	MTcC1R22R	*GATGGAAGGAGTGTAAATAG*	
6	*MTcC1R24F*	*TTTGGGGTGATTTCTTCTGA*	*58*
	MTcC1R24R	*TCTGTCTCGTCTTTTGGTGA*	
7	*MTcC1R25R*	*CTTCGTAGTGAATGTAGGAG*	*52*
	MTcC1R25F	*TTAGGTAGGTAGGGTTATCT*	
8	*MTcC1R26F*	*GCATTCATCAATACATTC*	*52*
	MTcC1R26R	*GCACTCAAAGTTCATACTAC*	
9	*MTcC1R11R*	*TTTGGTGATTATTAGCAG*	*52*
	MTcC1R11F	*GATTCGATTTGATGTGAG*	

Methods

Phenotypic assessment: Field measurements were done using a portable photosynthetic system LCA-4 (ADC Bioscientific Ltd, UK) for photosynthesis and related parameters (Transpiration, Conductance, and Water used efficiency). Plant efficiency analyzer (Hansatech Instruments Ltd., UK) was used to determine cocoa chlorophyll fluorescence and the Pressure chamber (Soil Moisture Corp., USA) was utilized for water potential (WP) (Balasimha, 1992; Balasimha, *et al.,* 1999; Scholander *et al*., 1965). Three replicated plants from each genotype were sampled and six leaves were measured per plant with at least 4-6 values in each leaf. Fully expanded healthy third to fourth leaf from distal portion was used for measurements. Cocoa yield data of dry beans was collected for three years (1995-1998) and tabulated as means.

Molecular assessment

Sample collecting: Early in the morning, cocoa leaves specimens were collected from ten cocoa parental genotypes and their ten hybrids from progeny trial IV, at CPCRI research Center of KIDU (Table1). Leaves specimens were wrapped into foil, labeled and immediately kept in ice. Then they were transported into lab and were stored in freezer under temperature -20 ^{0}C.

Genomic DNA extraction and quality checking: DNA was extracted by CTAB method following the protocol of the manufacturer, NucleoSpin^{R}, Plant II. The frozen leaves tissues of cocoa hybrids were grounded to fine powder in liquid nitrogen. 400 µl of preheat warmed extraction buffer (65°C) containing 10 mM Tris (pH 8), 1.4M NaCl, 2% CTAB, 2-mercarptoethanol (100%) and 20 mM EDTA, was added to the lysed tissue in the proportion 1:4 (Tissue: buffer). After added 10 µl of RNase, The suspension was mixed thoroughly by inverting tubes several times and then incubated at 65°C for 10 min in water bath.

The resulting powder was transferred into a new collection tube (2ml) and the lysate was loaded onto the column. The clear flow-through was collected into a new micro-centrifuge tube after centrifugation for two minute at 11 000 trs /min at 4°C. The DNA binding volume was adjusted by adding 450 µl of PC buffer. The filtrate containing the genomic extracted was eluted in a new column by centrifugation at 11000 trs / min. for 2 min at 4 °C, and the flow-through was discarded. Before eluting cocoa leaves samples DNA, the column of silica membrane was washing at three replicates, successively with 400 µl buffer PW1, 700 µl PW2 and 200 µl PW2 by centrifugation at 11000 trs / min throughout 2 min at 4°c, at each washing step. The NucleoSpin column was transferred into a new micro-centrifuge tube (1.5 ml). Then, the dried pellet was eluted at two replicate with 50 µl of preheat buffer PE (65°C). DNA quality was check by electrophoresis on agarose gel (0.8%).

PCR SSR process: The annealing temperatures was determined for each primer pair using gradient PCR. Once optimized, the PCR reaction was conducted with 12 high polymorphic SSR markers (Table 2). PCR completed in 0.2 µl volumes of genomic DNA, 3 µl each of forward and reverse primers, 0.1 µl of each dNTPs, 1X buffer (10 mM Tris-Hcl (pH8.3), 50 mM KCl, 1.5 mM MgCl2) and 0.5 µl of Taq DNA polymerase and 13.4 µl of milliQ water. PCR amplifications

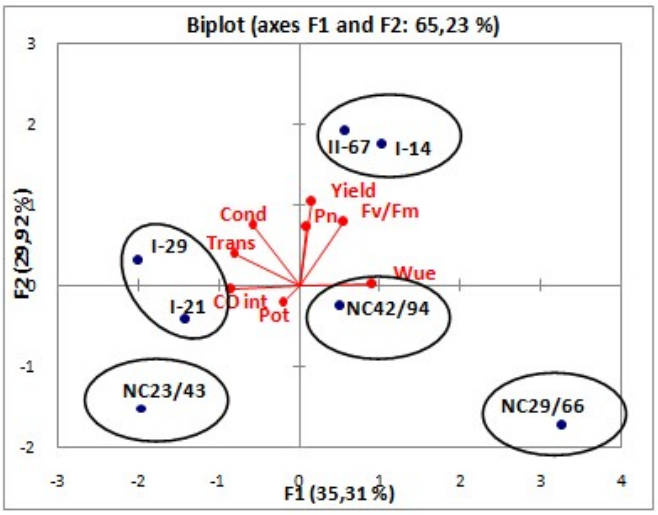
were performed on a BIORAD gradient thermal cycler with a PCR profile of 94 °C for 5 min followed by 30 cycles of 1 min at 94°C, 2 min at the different annealing temperatures standardized for the individual SSR locus, and 2 min at 72 °C with a final extension for 5 min at 72 ºC. DNA amplification products were analyzed by Multina capillarity electrophoresis with Shimadzu MCE-202 MultiNA device (Shimadzu Corporation, Kyoto Japan).

Phenotypical and molecular data analysis: Multivariate analysis was performed using phenotypic data collected to distinguish cocoa genotypes on the joint distribution of the variables using the software Xlstat 2013. These analyzes were used to determine the one hand, the links between the variables and, secondly, the proximities between cocoa genotypes depending on different variables. The method used multivariate analyzes is the principal component analysis (PCA). The PCA's policy condensation of more or less correlated variables together into a smaller number of independent variables called synthetic axes of principal components (Dagnelie, 1986). This approach allows to assess the degree of similarity between analyzed units and also to identify the variables that contribute most to the differentiation of units (Palm, 1998).Molecular data was analyzed with GDA (Genetic Data Analysis) Software version 1.1. The amplification data from the SSR markers was scored (marker presence = 1, absence = 0, lost information = 3) and converted into a numerical matrix from which the coefficients of genetic similarity were calculated and the grouping analysis performed for the cocoa hybrids. Coefficients of genetic similarity were calculated by the arithmetical complement of Dice's similarity coefficient (Nei and Li 1979, Corrêa *et al*. 1999). The Unweighted Pair Group with Arithmetic Mean (UPGMA) method was be used as a grouping criterion for the calculate matrix of genetic similarity among cocoa clones. Genetic diversity was calculated using five parameters that are allelic richness or the average number of alleles per locus (A), polymorphism rate (P), the average rate of observed heterozygosity (Ho), the average rate of 'expected heterozygosity (He) and the fixation index (F) of Wright.

RESULTS

Principal component analysis assessment: Principal component analysis (PCA) with a data table from genotypes X physiological and agronomic variables for phenotypic characterization of clones and their progenies, revealed the strongest correlations between variables, their place in the main component was used to assess the discriminant values. Thus, the descriptive variables were grouped into synthetic factors corresponding to the underlying dimensions of the mass of initial data. The results of PCA on clones studied are presented in Figure 1 and those of their hybrids in Figure 2. The number of selected factors was set at three (03) in the two cases of study based on the criterion of eigenvalue greater than or equal to 1.

Cocoa clones phenotypic characterization: The results of the PCA performed on the parent clones are shown in Figure 1 (Biplot : variable- clones), and the correlation matrix table (Table 3). The share of information returned by the three components retained is 83.89 pc of the total variability. Axis 1 with an eigenvalue of 2.825 expressing 35.31 pc of the total variability, is the most important for the agro-physiological characterization of clones. The variables that contribute to this axis are in order the water use efficient (Wue) at chloroplast level in photosynthetic respiratory chain and the concentration of intracellular CO2 (CO int), parameters that are associated with the photosynthetic activity. Axis 2, with an eigenvalue of 2.394 corresponding to 29.92 pc of the total variability is represented by the yield (yield), the photochemical reaction (Fv / Fm) and conductance (Cond) stomatal cacao. These parameters are related to each other with significant correlation coefficients at the 0.05 pc (Table 3). Axis 3 with 1,494 as an eigenvalue explains 18.66 pc of total variability and is characterized by net photosynthesis (Pn) and leaf water potential (Pot). Principal component analysis allows to show the main agro-physiological characteristics of Nigerian and Malaysian clones. Clones II-67 and I-14 are characterized by a yield, a high photochemical and photosynthetic activity, the clones I-29 and I-21 have a high water potential, conductance and an internal CO_2 concentration while the clone NC42/94 is identified by its water used efficiency in water stress condition and to a lesser extent by its performance and cellular photochemical activity. Two distinct groups are formed with genotypes NC23/43 and NC29/66.

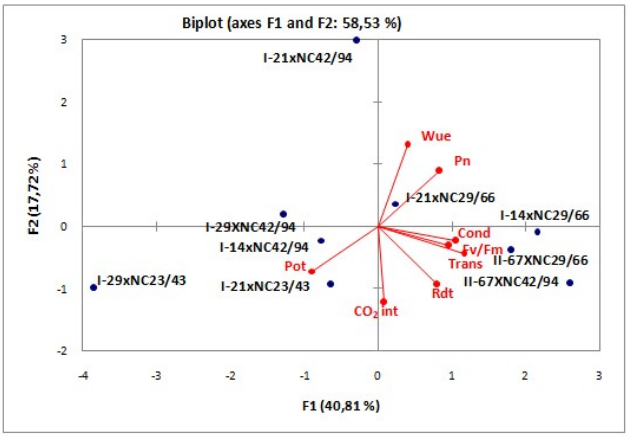


Yield = Yield, Cond = Conductance, Trans = Transpiration, Pn = Net Photosynthetic activity, Pot = water potential of leaves, Fv / Fm = photochemical reaction, Wue = Water use efficiency

Figure 1. Biplot variables-clones showing the relationship between physiological and agronomic parameters observed and clones cocoa characterized

Cocoa progenies phenotypic characterization: Phenotypic characterization from the multivariate analysis (PCA) is presented in Figure 2 (Biplot: variables-hybrids), and the correlation matrix table (Table 4). The share of information returned by the three components retained is 83.01 pc of the total variability. Axis 1 expressing 49.2 pc of the total variability is the most important for the agro-physiological characterization of cocoa hybrids. The variables that are related to this axis are in the order the parameters associated to photosynthesis that are transpiration (Trans) and conductance (Cond), leaf water potential (Pot) and photochemical activity (Fv/Fm). Axis 2 with an eigenvalue of 3.977 restores 19.00 pc of the total variability, and is characterized by the yield (yield), photosynthesis (Pn) and water use efficient (Wue). Axis 3 explaining 14.8 pc of total variability with an eigenvalue of 1.52, is characterized by the intracellular concentration of CO2. These parameters are related to each other with significant correlation coefficients at the 0.05 pc (Table 4).

PCA allowed highlighting important agro-physiological characteristics of progenies from cross of Nigerian and Malaysian parental clones. Hybrids II-67xNC29/66, II-67xNC42/94 I-14xNC29/66 and I-21xNC29/66 are characterized by a high water use efficiency, yield, photochemical and photosynthetic activity, the hybrids I-29xNC42/94, I-14xNC42/94, I-21xNC23/43 and I-29xNC23/43 had a high water potential and a more lesser high CO_2 intracellular concentration. One group is formed with progeny I-21xNC42/94 characterized by its water used efficiency and photosynthetic activity.



Yield = Yield, Cond = conductance, Trans= Transpiration, Pn = Net Photosynthetic activity, Pot = water potential of leaves, Fv/Fm = photochemical reaction, Wue = Water use efficiency.

Figure 2. Biplot Hybrids-variables indicating the relationship between the physiological and agronomic parameters observed and characterized cocoa hybrids

Molecular assessment: Molecular characterization of clones and their hybrid progenies was performed using nine genomic polymorphic microsatellite markers (Table 2).

Genetic diversity of microsatellite markers (SSR): Initially cocoa clones screening with nine polymorphic SSR markers result is presented in a table 5. The analyses revealed a allelic diversity of 71 alleles with an average of 7,8 alleles per locus. The allelic richness per locus ranged from 2 to 15, the high allelic number was obtained by locus mtcCIR18 (15), against the least number was got by mtcCIR22 (2). The observed heterozygosity (Ho) and expected heterozygosity (He) or gene diversity vary greatly across the nine loci, The average of observed heterozygosity (Ho) and gene diversity were 0,38 and 0,69 respectively. The locus mtcCIR7 (Ho = 0,89) following by loci mtcCIR25 (Ho = 0,8), mtcCIR11 (Ho = 0,58) and mtcCIR8 (Ho = 0,47) had high level of observed heterozygosity. However, loci mtcCIR26 and mtcCIR22 had a lowest value of Ho (0,00). Excepted mtcCIR7 and mtcCIR25, a heterozygosity deficit (He > Ho) is registered for all the SSR markers used. The locus with a smaller number of allele such as mtcCIR22, tend to have a lower heterozygosity. Without locus mtcCIR7 and mtcCIR25, the inbreeding coefficient or index of fixation which is the deviation from the Hardy-Weinberg structure was positive for all locus, ranging from -0,04 to 1. Cocoa progenies screening with nine polymorphic SSR markers result is shown in a table 6. A total allelic number discovered in cocoa progenies was 81 allele, with a range of 3 to 21 allelic richness per locus. The mean of expected heterozygosity ranged from 0,32 to 0,89 with an average of 0,68. Among a nine locus, locus mtcCIR11 (Ho=1), follow by locus mtcCIR25 (Ho=0,83), mtcCIR18 (Ho=0,8) and mtcCIR7 (Ho=0,78) had a high level of observed heterozygosity. The inbreeding coefficient move from -0,19 to 1 with an average of 0,36, a lowest value of fixation index was obtained from mtcCIR25 and mtcCIR11. In this study microsatellites markers were able to discriminate difference between cocoa clones and their progenies. Among nine genomics microsatellites markers, four primers such as mtcCIR8, mtcCIR11, mtcCIR7, and mtcCIR18 were sufficient to distinguish both cocoa clones and their progenies. Since, they generated a high allelic richness and high heterozygosity level. The primers mtcCIR24, mtcCIR17, and mtcCIR22 were less polymorphic, with lowest allelic frequency and lowest heterozygosity in cocoa genotypes.

Genetic variability among cocoa clones and their progenies: Gene diversity statistic were calculated based on nine polymorphic microsatellites for cocoa clones (Table 7, Figure 3). Allelic richness was high Malaysian collection (4,22) than Nigeria collection (3,66). However, the proportion of polymorphic loci obtained is identical for both Nigeria and Malaysian collection (P= 88). While observed heterozygosity was high Malaysian collection (0,43) than Nigeria collection (0,36). The fixation index was lowest in Malaysian (0,28) than Nigeria (0,37). The Nigeria clones NC29/66, NC23/43, and NC42/94 had identical percentage of polymorphic (P=78%), while it was least in some Malaysian clone such as III-35, III-105, I-56, and I-21 (P=44). The allelic richness ranged from 1,4 to 2,56 with an average of 1,93. For all the clones, the heterozygosity deficit was observed, excluded three Malaysian clones III-105, III-35 and I-56 which had same value of observed and expected heterozygosity. This clone had also a lowest fixation index (F=0), indicated a non-differentiation on this group. A remaining clones shown a high level of differentiation (F>0). Characterization of cocoa clones on the basis of data from genomic SSR markers is shown in Figure 4.

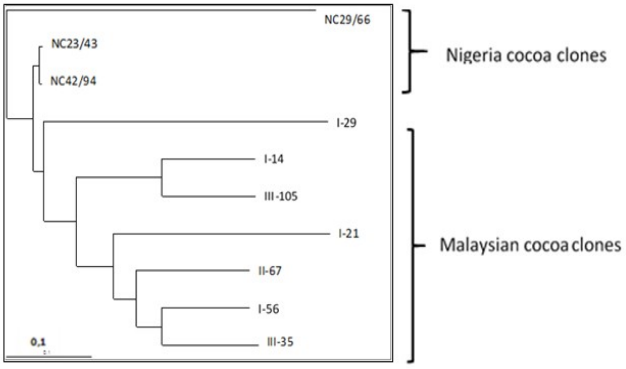


Figure 3. UPGMAdendrogram of ten cocoa clones based on Nei Distances (1978)

The two axes considered on the basis on their eigen value (≥ 1), axis 1 (61,27 pc) and the axis 2 (26,74 pc) express 88,01 pc of the total variability. These two axes distinguish cocoa clones into three groups. Molecular analysis derived from cocoa progenies with nine genomic polymorphic markers was presented in table 8 and Figure 9. A total of 33 alleles were obtained with an allelic richness average of 2.28. The proportion of polymorphic loci for all primers indicated a capacity of SSR markers to detect allelic variation among cocoa progenies. Heterozygosity expected was varied for all hybrids, I-21xNC23/43 (He = 0,75) recorded the highest value, it following by I-29xNC42/94 (0,67), II-67xNC29/66 (0,65),

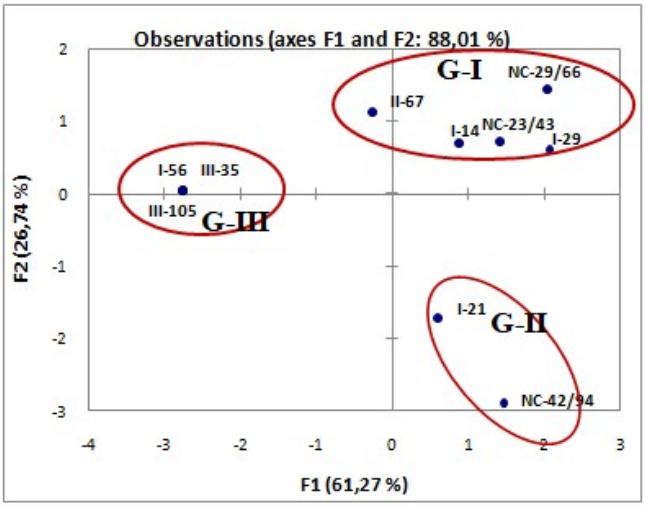


Figure 4. Grouping of cocoa clones on the basis of the ACP performed using genomic SSR primers (G: Group)

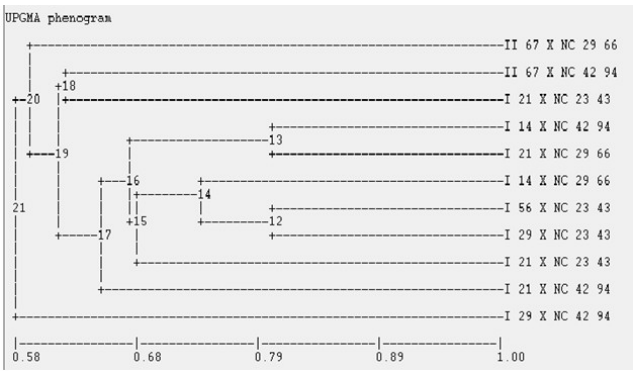


Figure 5. UPGMA dendrogram of ten cocoa progenies based on Nei Distances (1978)

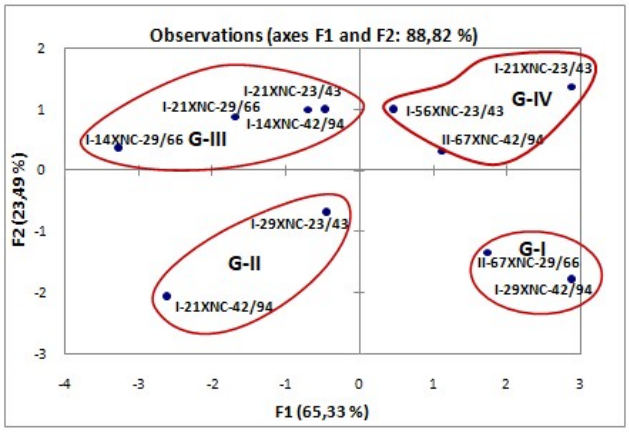


Figure 6. Grouping of cocoa hybrids from the ACP performed with genomic SSR primers

and II-67xNC42/94 (61). For the all progenies heterozygosity deficit was observed excepted I-14xNC29/66 and I-21xNC42/94. While, the high level of observed heterozygosity was obtained which a value of 0,5. The fixation index value obtained indicate the differentiation level for each hybrids, excepted I-14xNC29/66 and I-21xNC42/94 (F=0,00). The characterization of the hybrid progeny of cocoa based on SSR genomic markers is shown in Figure 6. The two axes considered on the basis of their eigenvalue (≥ 1), axis 1 (65.33 pc) and axis 2 (23.49 pc) express 88.82 pc of the total variability. These two axes distinguish hybrids progenies into four groups.

DISCUSSION

In the current context of climate variability, sustainable cocoa production must be based on a drought tolerant cocoa genotypes distribution program. Drought is a complex phenomenon that leads to a change of morphological, phenological and agronomic important and heritable traits in plants (Barakat and Handoufe F. A., 1997; H. Mellouli, 2005). Such circumstances limit conventional breeding and requires further molecular genetic methods. Concomitant use of morpho-physiological and agronomic descriptors based on Balashima *et al.* (2008) data were used to assess the potential of discriminating characters studied. Morpho-physiological and agronomic parameters showed phenotypic traits associated with genotypes. The results of the multivariate analysis indicated a good performance of the Clones II-67 and I-14 with an average weight of dry bean greater than 1 gram. The clones I-29 and I-21 were characterized by their ability to fix intracellular CO2 coupled with a rational use of water and to a lesser extent their yield at the field. The clones NC42/66 and NC29/94 are distinguished for their water use efficient, low transpiration rate and low conductance. Physiologically, the soil water deficit leads to regulation of stomatal (Clavet, 2000) where the decrease in conductance and transpiration which limit the water loss by transpiration. These criteria have long been used to distinguish plants in different tolerance levels (Levitt, 1980). Characterization of the hybrid progeny revealed four groups according to their agro-physiological characteristics.

The first group is distinguished from others by its high photochemical activity, efficient water use and technological characteristics of the beans. This group consists of hybrid II-67xNC29/ 66, II-67xNC42/94, I-21xNC29/66 and I-14xNC29/66. The second group constituted of hybrids I-29xNC42/94, I-14xNC42/94, I-21xNC23/43 and I-29xNC23/43 are characterized by a high water potential, low conductance and a low rate of transpiration. The third group of hybrids is formed by the hybrids I-21xNC42/94 which also featured significant drought tolerance traits. These physiological traits contribute to tolerance mechanisms to drought implemented by plants (Balashima *et al,,* 1991, 1999; Daymond and Harley 2003). All SSR primers used in this study were developed by CIRAD Lanaud *et al.* (1999). They presented a high degree of polymorphism with allelic richness 152 alleles detected in cocoa clones and their progenies studied. Polymorphism rate observed was 100% in the clones and an average value of 66% at the cocoa progenies. According Vaiman *et al.,* (1994), the loci are considered polymorphic if they reveal more than 50% of polymorphic information content. Among the primers used, our results showed that the four genomic primers mtcCIR7, mtcCIR8, mtcCIR11 and mtcCIR18 generated greater allelic richness and a high level of heterozygosity, able to distinguish the cocoa genotypes. Heterozygosity and polymorphism SSR primers rates revealed useful information in the analysis of genetic diversity and for the screening of cacao tolerant to water stress (Dapeng Zhang *et al.,* 2009; Fabiana *et al*, 2009. Elaina S. *et al*., 2013). The results are in agreement with those of Efombagn *et al*., (2006), who studied the genetic diversity of cacao grown in southern Cameroon and showed high polymorphism of SSR markers. SSR primers are a powerful tool for genomic analysis of the cocoa tree, multivariate analysis performed using genomic primers differentiated the

Table 3. Matrix of correlation between studied variables according to test of Spearman

Variables	Pot	Pn	Trans	Cond	Co int	Wue	Fv/Fm	Yield
Pot	1							
Pn	0,321	1						
Trans	-0,036	0,357	1					
Cond	0,144	0,360	0,577	1				
Co int	0,071	-0,464	0,429	0,523	1			
Wue	-0,071	0,143	-0,714	-0,342	-0,750	1		
Fv/Fm	-0,321	0,250	-0,357	0,144	-0,286	0,357	1	
Yield	-0,357	0,429	0,143	0,487	-0,036	0,107	0,821	1

Values in bold are different from 0 with a significance level alpha=0,05

Table 4. Matrix of correlation between studied variables according to test of Spearman

Variables	Pot	Pn	Trans	Cond	Co int	Wue	Fv/Fm	Yield
Pot	1							
Pn	-0,533	1						
Trans	-0,700	0,450	1					
Cond	-0,644	0,557	0,836	1				
Co int	0,360	-0,033	0,109	0,284	1			
Wue	-0,368	0,552	0,142	0,118	0,008	1		
Fv/fm	-0,483	0,450	0,783	0,583	-0,008	0,067	1	
Yield	-0,467	0,083	0,817	0,479	0,084	-0,075	0,533	1

Values in bold are different from 0 with a significance level alpha=0,05

Table 5. Allelic richness and heterozygosity parameters observed in cocoa parental clone studied by microsatellite

Locus	N	P	A	AP	H_e	H_o	F
mtcCIR8	19,00	1,00	7,00	7,00	0,81	0,47	0,42
mtcCIR11	19,00	1,00	14,00	14,00	0,90	0,58	0,36
mtcCIR22	19,00	1,00	2,00	2,00	0,10	0,00	1,00
mtcCIR25	15,00	1,00	8,00	8,00	0,79	0,80	-0,02
mtcCIR26	19,00	1,00	5,00	5,00	0,79	0,00	1,00
mtcCIR7	19,00	1,00	8,00	8,00	0,86	0,89	-0,04
mtcCIR17	18,00	1,00	7,00	7,00	0,70	0,11	0,85
mtcCIR18	19,00	1,00	15,00	15,00	0,92	0,32	0,66
mtcCIR24	19,00	1,00	5,00	5,00	0,33	0,26	0,21
All	18,40	1,00	7,80	7,80	0,69	0,38	0,45

Sample size (N), Proportion of polymorphic loci (P), Allele per locus (A), Allele per polymorphic loci (AP), Heterozygosity expected (He), Heterozygosity observed (Ho) and Fixation index (F)

Table 6. Allelic richness and heterozygosity parameters observed in cocoa progenies studied by microsatellite primers

Locus	**N**	**P**	**A**	**Ap**	**H_e**	**H_o**	**F**
mtcCIR8	21,00	1,00	7,00	7,00	0,81	0,24	0,71
mtcCIR11	23,00	1,00	16,00	16,00	0,89	1,00	-0,13
mtcCIR22	20,00	1,00	4,00	4,00	0,35	0,00	1,00
mtcCIR25	23,00	1,00	6,00	6,00	0,70	0,83	-0,19
mtcCIR26	23,00	1,00	8,00	8,00	0,66	0,26	0,61
mtcCIR7	23,00	1,00	12,00	12,00	0,88	0,78	0,11
mtcCIR17	22,00	1,00	3,00	3,00	0,32	0,00	1,00
mtcCIR18	20,00	1,00	21,00	21,00	0,96	0,80	0,17
mtcCIR24	21,00	1,00	4,00	4,00	0,54	0,05	0,91
All	**21,78**	**1,00**	**9,00**	**9,00**	**0,68**	**0,44**	**0,36**

Sample size (N), Proportion of polymorphic loci (P), Allele per locus (A), Allele per polymorphic loci (AP), Heterozygosity expected (He), Heterozygosity observed (Ho) and Fixation index (F)

Table 7. Gene diversity parameters obtained among cocoa clones with nine microsatellites primers

Populations	**% P**	**A**	**AP**	**He**	**Ho**	**F**
NC-29/66	78	2,56	3,00	0,53	0,44	0,20
NC-23/43	78	2,33	2,71	0,52	0,41	0,25
NC-42/94	78	1,89	2,14	0,54	0,17	0,77
I-21	63	2,00	2,60	0,46	0,25	0,56
I-14	78	2,11	2,43	0,54	0,44	0,24
II-67	78	1,89	2,14	0,52	0,50	0,07
I-56	44	1,44	2,00	0,44	0,44	0,00
I-29	78	2,22	2,57	0,61	0,44	0,43
III-105	44	1,44	2,00	0,44	0,44	0,00
III-35	44	1,44	2,00	0,44	0,44	0,00
Means	**66**	**1,93**	**2,36**	**0,50**	**0,40**	**0,37**
Collection						
Nigerian	88	3,66	4,00	0,56	0,36	0,37
Malaysian	88	4,22	4,62	0,59	0,43	0,28
Means	**88**	**3,94**	**4,31**	**0,58**	**0,39**	**0,32**

Rate of polymorphic loci (P), Allele per locus (A), Allele per polymorphic loci (AP), Heterozygosity expected (He), Heterozygosity observed (Ho) and Fixation index (F)

Table 8. Gene diversity parameters obtained among cocoa progenies with nine microsatellites primers

Populations	% P	A	Ap	He	Ho	F
II-67 X NC-29/66	78	2,56	3,00	0,65	0,39	0,50
I-29 X NC-42/94	89	3,00	3,25	0,67	0,39	0,48
II-67 X NC-42/94	78	2,56	3,00	0,61	0,50	0,25
I-14 X NC-42/94	67	2,11	2,67	0,54	0,50	0,13
I-14 X NC-29/66	56	1,67	2,20	0,39	0,39	0,00
I-21 X NC-23/43	100	2,63	2,63	0,75	0,50	0,43
I-56 X NC-23/43	78	2,33	2,71	0,59	0,50	0,22
I-21 X NC-42/94	44	2,00	3,25	0,39	0,39	0,00
I-21 X NC-23/43	67	2,11	2,67	0,56	0,50	0,18
I-21 X NC-29/66	67	1,89	2,33	0,48	0,44	0,11
I-29 X NC-23/43	63	2,25	3,00	0,52	0,44	0,22
Means	**71**	**2,28**	**2,79**	**0,56**	**0,45**	**0,28**

Rate of polymorphic loci (P), Allele per locus (A), Allele per polymorphic loci (AP), heterozygosity expected (He), Heterozygosity observed (Ho) and Fixation index (F)

cacao clones into three groups (Figure 4) and the hybrid progeny into four groups (Figure 6). The first group of clones (II-67, NC29/66, I-14, NC23/43 and I-29) and the second group (I-21 and NC29/66) were phenotyped at field as having agronomic and physiological traits of drought tolerance (Balashima *et al.,* 1999). Hybrid groups I, II and III were distinguished in the field based on physiological and agronomic traits conferring tolerance to drought (Balashima *et al.,* 2008).

CONCLUSION

This study provides details of genetic and phenotypic analyzes of cacao genotypes. The data reveal the ability of genomic SSR markers to distinguish cocoa clones and their progeny for their agronomic performance and tolerance to drought. Some of microsatellite primers revealed good polymorphism rate, high allelic richness and high heterozygosity rates.

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