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A computational statistical approach to assess cowpea [*Vigna unguiculata* (L.) Walp.] cultivars diversity and select elite genotypes from Agro-morphological and biochemical traits

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ABSTRACT

Computational statistic approaches play an essential role in the evaluation and processing agronomic, biological, and bio-medical big data. The complexity and large size of those data make computational statistic a crucial tool in bio-statistical analysis. Based on this evidence, we characterized phenotype performances of cowpea cultivar in the Northern of Côte d'Ivoire, developing own computational statistical approach, exclusively in the R programming language. Several packages of R have been executed to assess cowpea cultivar agro-morphological and biochemical performances. Z-score clustering analysis revealed four groups of cowpea based on agro-morphological parameters. K-mean clustering survey revealed four and two groups of cowpea cultivar respectively for agro-morphological and biochemical parameters. The Horn parallel analysis highlighted two, four, two and two agro-explanatory components and/or agro-morphological parameters as influencing data variability respectively in the first, second, third, and fourth groups of cowpea cultivars previously revealed by the k-mean analysis. The same analysis exhibited two components in terms of biochemical parameters, inducing the variability in the two cluster groups of cowpea cultivar revealed by the k-mean survey. Integrative analysis of the ANOVA test and Tukey's multi-comparative analysis displayed yield (agro-morphological parameter) and cowpea energetic content (biochemical parameter) as main sources of cowpea cultivar phenotypical variability ($P < 0.05$). Of note, receiver operating characteristic predictive analysis showed the excellent performance of both cowpea yield and energetic content in selecting cowpea genetic germplasm area under the curve (AUC = 0.9). Considering as a whole, the present computational statistical approach shows excellent performance in the evaluation, characterization, and management of agro-morphological (yield parameter) and biochemical (energetic content) features of cowpea in genetic selection procedures.

1. INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.], very resistant to semi-arid and arid climates, is one of the main food and fodder legumes grown and consumed in tropical and subtropical areas of Africa, Asia, Europe, and America [1]. Cowpea offers many agronomic and nutritional benefits to smallholder farmers. Of note, cowpea improves soil fertility by fixing atmospheric nitrogen and provides cover by protecting it against erosion and the invasion of weeds [2,3]. In Africa, cowpea is grown primarily for its dry seeds, cooked in the most diverse forms. In several areas, its young leaves, fresh or dried, and its immature pods are also part of the diet [4]. Cowpea represents an important protein and energy source for humans and animals in developing countries where animal protein accessibility is limited [5]. Of note, several studies revealed agro-morphological and biochemical features as useful factors in characterizing cowpea cultivar's agronomic and nutritional performances [5-7]. Given the socio-demographic constraints, the use of cultivars combining good nutritional and agronomic values could fill the many nutritional deficits recorded in developing countries where food deficiencies are still a major current scourge. In Africa and Côte d'Ivoire in particular, cowpea remains a marginal crop although it is widely consumed [8]. However, it is noteworthy to underline that agricultural extension services reported cowpea crop activities in northern Côte d'Ivoire [9]. In addition, a recent study suggested morphological variability in cowpea seeds in the northern of Côte d'Ivoire [10]. Because of that, we believe that integrating agro-morphological and biochemical parameters could contribute to highlighting cowpea characterization and performance in terms of morphological and nutritional properties. For this purpose, integration of several biological fields, i.e., genetic, agronomy as well as rigorous computational statistical demarche is needed. Therefore, adequate integration of these methodologies can

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strongly contribute to identifying accurate and estimated unbiased cowpea cultivars genetic diversity [11]. A correct interpretation of biological and agronomic experimental data needs rigorous data harvesting, and accurate data pre-treatment followed by an appropriate statistical analysis procedure depending on data feature as well as experimental context. In view to make straightforward the integration of these complex procedures, adequate experimental schemes followed by a rigorous statistical demarches are required, playing attention to analyzed data typology [12,13]. Numerous researchers continue to generate and analyses quantitative and qualitative phenotypical data in their agronomical experimentations. Considering the impressive heterogeneity as well as size and complexity of that data, we believe that computational statistical approaches could play an essential role in processing and analyzing these data helping in improving bio-statistical inference. Indeed, the present study aims to highlight the performances of cowpea cultivars yield and grown process in Northern Côte d'Ivoire by integrating agro-morphological and biochemical features by performing a multivariate statistical survey by developing a computational statistical approach and/or script in R programing environment.

2. MATERIALS AND METHODS

2.1. Cowpea Agro-morphological Features

The plant material consists of 32 cowpea accessions from the seed bank of Peleforo Gon Coulibaly University [10]. The assessment of cowpea accessions agro-morphological features was carried out on experimental plots of the vegetable plot of Peleforo Gon Coulibaly University botanical garden characterized by the Sudo-Sahelian dry tropical climate [14]. The soils of this region are of the Ferralsols, Cambisols, Fluvisols and Luvisols types and the vegetation consists of gallery forests, woods, and savannah shrubs [15,16]. The seeds of the cowpea accessions were sown according to an experimental device in Fisher blocks with three repetitions. Each of the three blocks constituting the sowing device was made up of 32 lines representing 20 individuals from each accession. The area of a block is 220.75 m² (23.75 m by 9.5 m). The three blocks are equidistant from each other by 2 m and the plots inside a block are spaced 0.75 m apart as proposed by Dansi *et al*. [6]. We randomly assigned the cultivars to the lines according to the permutation table of Dagnelie [17]. Sowing was done flat in a line and by hand at a rate of 2–3 seeds per pocket at a depth of < 5 cm, with a spacing of 0.5 m between the pockets [7]. Each line or experimental unit has 20 pockets. 15 days after sowing, thinning of one plant was carried out to leave the most vigorous plant, which gives a density of 26667.67 plants/ha [7]. We considered 16 quantitative variables for the agro-morphological characterization [Table 1]. We organized data for a computational statistical analysis, according to the recommendations listed in the cowpea descriptors [18].

2.2. Cowpea Biochemical and Nutritional Features

We measured cowpea biochemical and nutritional parameters at the biotechnology laboratory of the said University. Eight biochemical parameters were evaluated i.e.

i. Dry matter according to the AOAC 925.40 method [19]
ii. The water content was also deduced from the same method [19]
iii. Lipids or fat produced by the classic Soxhlet method according to the modified ISO/659 standard, known as giant Soxhlet
iv. Reducing sugars were measured by Bernfeld method by quantifying 3,5-dinitro salicylic acid (DNS) [20]
v. Protein assay was carried out by KJELDAHL method
vi. Ash content was measured according to AOAC (2000) [21]
vii. The total carbohydrate and starch levels were determined by Bernard and Thomas formulas [22]
viii. The energy value was determined applying Coleman formula [23] by using Atwater and Rosa coefficient [24]

2.3. Computational Statistical Analysis

Computational statistical analysis is mainly based on our previous pipeline for agronomic metric data analysis [12]. Because of cowpea agro-morphometric and biochemistry parameters heterogeneity, we performed logarithmic data transformation as suggested by Dago *et al*. [13] to assess the linear regression relationship between the above-mentioned parameters through the ANOVA test. We integrated the aforementioned agronomic metric data analysis pipeline with z score, Horn parallel test, k-mean analysis, and receiver operating

Table 1: Quantitative characters, codes and measurement methods used for the agro-morphological characterization of the 32 cowpea accessions.

S. No.	Characters	Observation stage and measurement method
1	Plant height	Plant height (cm) measured 6 weeks after sowing
2	Plant width	Plant width (cm) measured 6 weeks after sowing
3	Number of nodes	Count of nodes on the main stem from the 6th week after sowing
4	Leaflet length	Average measurement taken on three terminal leaflets of the plant at the 6th week after sowing
5	Leaflet width	Average measurement taken on three terminal leaflets of the plant at the 6th week after sowing
6	Flowering time	Number of days from sowing to start of flowering
7	Maturity time	Number of days from sowing to onset of maturity
8	Number of pods per plant	Pod count per plant after harvest
9	Pod width	Average measurement made using a digital caliper on 5 pods per plant after harvest
10	Pod length	Average measurement made using a millimeter tape on 5 pods per plant after harvest
11	Pod weight	Average measurement made using a digital scale on 5 pods per plant after harvest
12	Number of pod chambers	Cell count in the pod after harvest on 5 pods per plant
13	Number of seeds in the pod	Seed count in the pod after harvest on 5 pods per plant
14	Seed length	Average measurement made using a digital caliper on 5 seeds per plant after harvest
15	Weight of 100 seeds	Counting and weighing 100 dried seeds after harvest
16	Seed yield per hectare	Estimate in Kg/ha from the average production of a plant with a seeding density of 26667.67 plants/ha

characteristic (ROC) predictive model. Of note, a z score describes the position of a raw score in terms of its distance from the mean when measured in standard deviation units. The z score is positive if the value lies above the mean and negative if it lies below the mean. We based cowpea cultivar clustering by submitting cowpea agro-morphometric and biochemistry data to z score normalization procedure. Indeed, the formula for calculating a z score is $z = (x-\mu)/\sigma$, where x is the raw score, μ is the population mean, and σ is the population standard deviation [25,26]. Z score data interpretation is as follows: the value of the z score tells you how many standard deviations you are away from the mean. If a z-score is equal to 0, it is on the mean. A positive z score indicates the raw score is higher than the mean average. Clustering is one of the most common exploratory data analysis techniques used to get an intuition about the structure of the data. K-means algorithm is an iterative algorithm that tries to partition the dataset into K pre-defined distinct non-overlapping subgroups (clusters) where each data point belongs to only one group [27]. It tries to make the intra-cluster data points as similar as possible while also keeping the clusters as different as possible [28]. Horn's parallel analysis (PA) is the method of consensus in the literature on empirical methods for deciding how many components and/or factors to retain [29] showed that Horn's PA may be reliably performed with the computationally simplest distributional assumptions about the simulated data. ROC curves provide a graphical representation of the range of possible cut points with their associated sensitivity versus 1-specificity, (i.e. false positives rate). This illustrates the merit of the particular predictor and/or predictive model, making it possible to identify different cut-points for specific applications depending on the cost of misclassification. Estimates of the area under the curve provide an indication of the utility of the predictor and a means of comparing (testing) two or more predictive model. Of note, cut-off values for the present ROC predicting model referred to the average values of each parameters variable.

3. RESULTS

3.1. Euclidian Distance of Pearson Correlation Assessing Cowpea Cultivar Plants Interactions by Agro-morphological and Biochemical Parameters

Pearson correlation analysis by characterizing cowpea cultivar plant phenotypes suggested two and four cluster groups of those plants by processing respectively nutritional [Figure 1a] and agro-morphological [Figure 1b] parameters. The same analysis exhibited a high data variability with regard cowpea cultivar clustering groups by processing biochemical and/or nutritional parameters as opposite to those plants agro-morphological features. Indeed, Pearson correlation coefficients (R^2) regarding cowpea agro-morphological parameters ranks between −0.26 and 1 ($-0.26 \leq R^2 \leq 1$) [Figure 1b], while those of biochemical and/or nutritional parameters rank between −0.41 and 1 ($-0.41 \leq R^2 \leq 1$) [Figure 1a], suggesting data variability difference between cowpea agro-morphological and biochemical parameters. Interestingly, the present survey showed a dissymmetric distribution of Pearson correlation values by processing agro-morphological and biochemical parameters of the cowpea cultivar [Figure 1].

3.2 Z-score Analysis Assessing Agro-morphological and Biochemical Features Interaction Characterizing Cowpea Cultivars

We assessed the interactions between agro-morphological and biochemical parameters in influencing cowpea crop clustering. This analysis highlighted respectively four and five groups with regard to agro-morphological and biochemical parameters evaluating cowpea cultivar plants data variability aptitude [Figure 2]. Indeed, z-score survey based on cowpea nutritional and/or biochemical features exhibited four clustering groups as follows: (i) Ash and sweet residues, (ii) protein, lipid, and water content, (iii) energetic value and (iv) glucose and dry mass [Figure 2a]. The same analysis

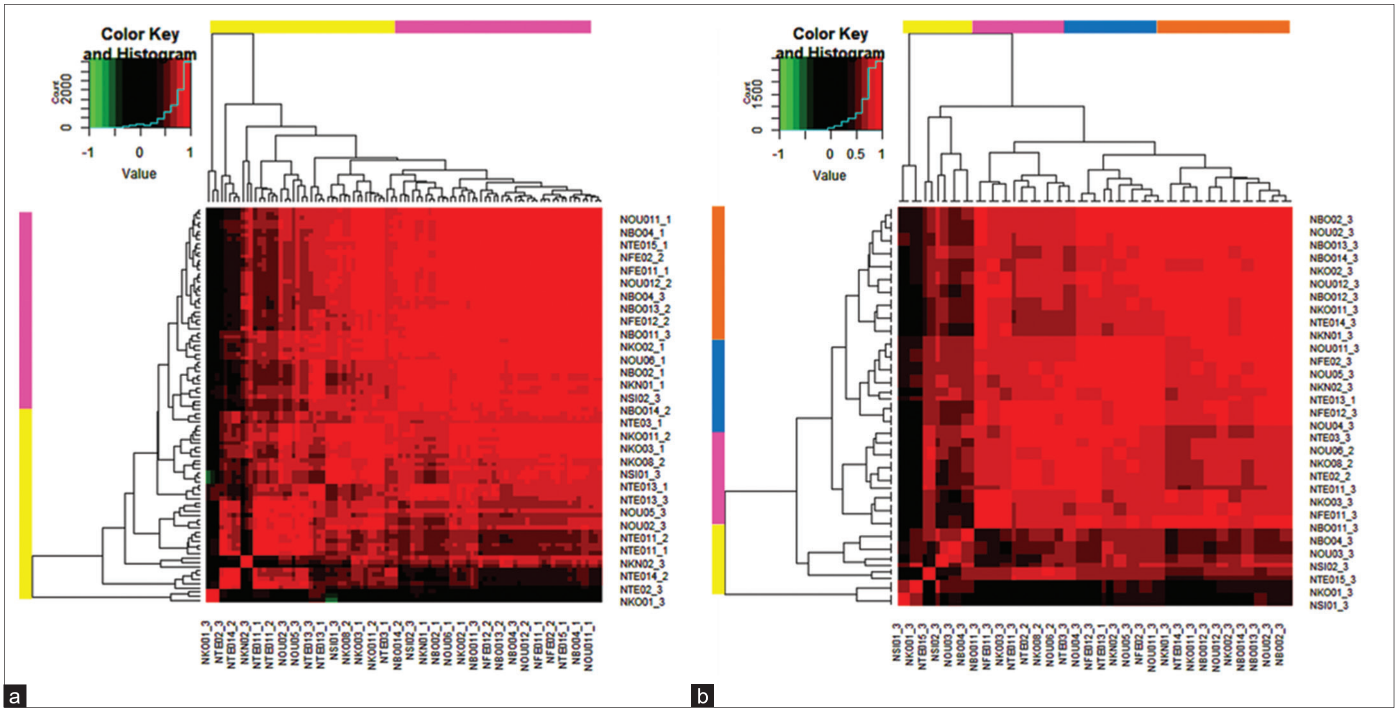


Figure 1: Biochemical (a) and agro-morphological (b) parameters Pearson correlation heatmap in evaluating cowpea cultivar angiosperm clustering group.

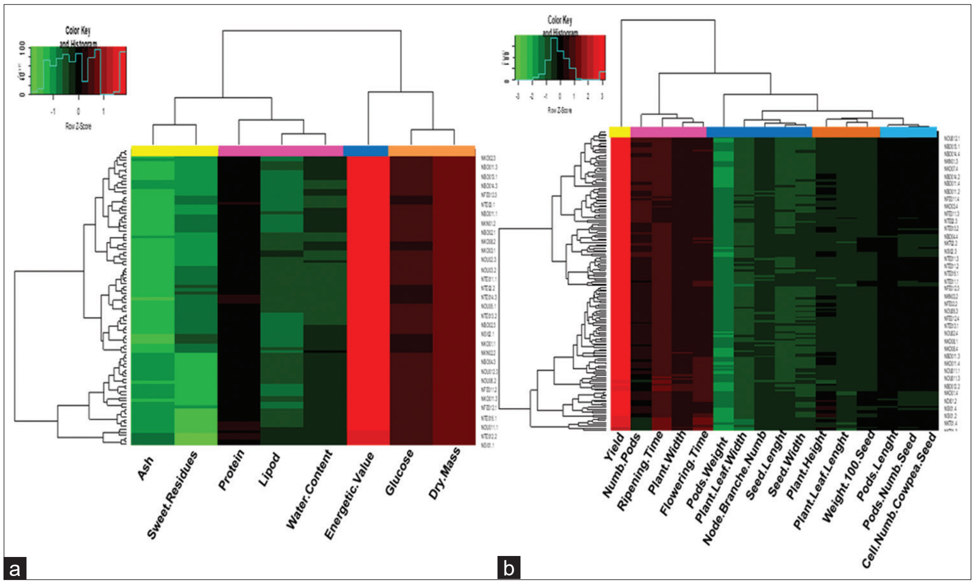


Figure 2: Z-score analysis evaluating the impact of the interaction between biochemical and nutritional (a), agro-morphological (b) parameters on cowpea cultivar plants variability.

referred five clustering groups for agro-morphological parameters as follows: (i) Yield, (ii) pods number, ripening time, plant width and flowering time, (iii) pods weight, plant leaf width, plant nodes and branches number, cowpea seed length and width, (iv) plant height, plant leaf length and 100-cowpea seeds weight and (v) pods length, pods seed number and seed cell number [Figure 2b]. Z-score survey highlighted data variability by processing cowpea cultivar biochemical and agro-morphological parameters [Figure 2] confirming previous results [Figure 1]. Of note, the present analysis suspected energetic value and yield components respectively of cowpea nutritional and agro-morphological parameters as main sources of data variability [Figure 2]. In the same tendency, protein (cowpea nutritional parameter, Figure 2a) and pods width (cowpea agro-morphological features, Figure 2b) have been revealed as alternative and additional sources of data variability in the cowpea plant population.

3.3. K-mean PA Discriminating Clustering Groups by Processing Cowpea Cultivar Agro-morphological and Biochemical Features

K-mean PA has been executed for a number of clusters k = 10 for cowpea cultivar agro-morphological and biochemical and/or nutritional parameters. We checked optimal number of clusters assessing cowpea phenotype performances by the agro-morphological and biochemical features, revealing k = four and two as clustering number for each considered cowpea cultivar features. Indeed, K-mean parallel survey suggested four and two clustering groups with regards cowpea cultivar based respectively on agro-morphological [Figure 3a] and biochemical [Figure 3b] parameters. Of note, K-mean analysis suggested average silhouette width coefficient values raking between 0.5–0.6 and 0.3–0.6 respectively for agro-morphological and biochemical cowpea parameters. Following the k-mean analysis, we clustered cowpea cultivar in two and four groups based respectively on biochemical and agro-morphological parameters [Supplementary Figure 1a and b]. As previously suspected each discriminated group for each parameters typologies seem to exhibit data variability among them [Figure 1 and Supplementary Figure 1]. In addition principal component analysis clustering analysis grouped cowpea cultivar by the first component that explains data variability at 87.45% and 99.87%, respectively, for biochemical and agro-morphological parameters [Supplementary Figure 1].

3.4. Assessment of Data Variability by Processing Cowpea Cultivar Biochemical and Agro-morphological Parameters

We carried out a variance variability test with regard to the four clusters and/or group of cowpea plants obtained via the agro-morphological parameters by the aforementioned k-mean survey [Figure 3]. ANOVA test evaluating variance variability supported a strong significant difference of the variance parameters ($P = 0.001$) between above mentioned generated cowpea cluster groups by the k-mean survey [Figure 4a and Table 2]. We performed the same variance variability test assessing variance differences between the two cluster groups generated by k-mean survey by processing cowpea cultivar biochemical and/or nutritional parameters. Analysis of variance exhibited a strong significant difference between the two cluster groups generated by the k-mean analysis [Figure 4b and Table 2], thought cowpea biochemical parameters ($P = 0.00$). Multivariate boxplot survey showed that cluster groups 2 from the cowpea cultivar for agro-morphological and biochemical features, displayed a median value higher than the others generated cluster groups [Figure 4]. In addition, it is noteworthy to underline the asymmetry distribution of agro-morphological and biochemical parameters data in the above-mentioned generated cowpea cluster groups [Figure 4].

3.5. Horn's PA Discriminating Data Variability Sources in Generated Cowpea Cluster Groups Based on Agro-morphological and Biochemical Features

Horn PA predicting factor and/or component explaining data variability for cowpea agro-morphological parameters, exhibited two, four, two,and two agronomic parameters and/or components as impacting data variability respectively in generated cluster group 1, 2,

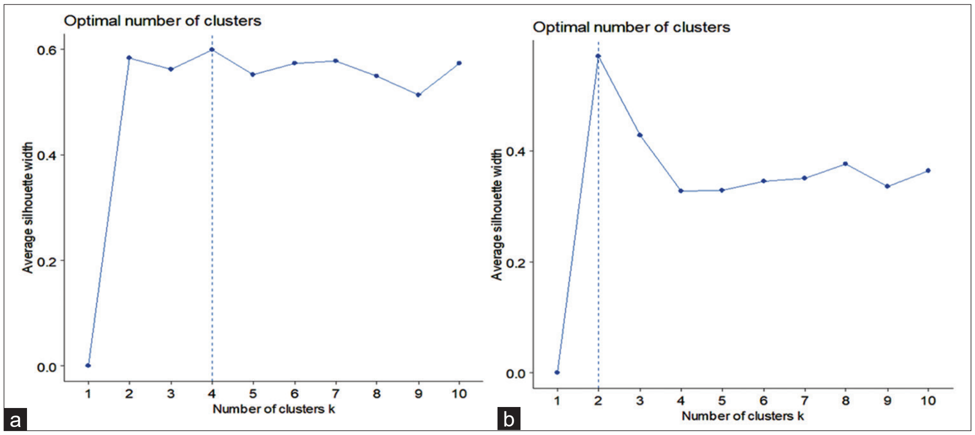


Figure 3: Parallel K-mean survey predicting optimal number of cowpea cultivar clusters by agro-morphological (a) and biochemical (b) features.

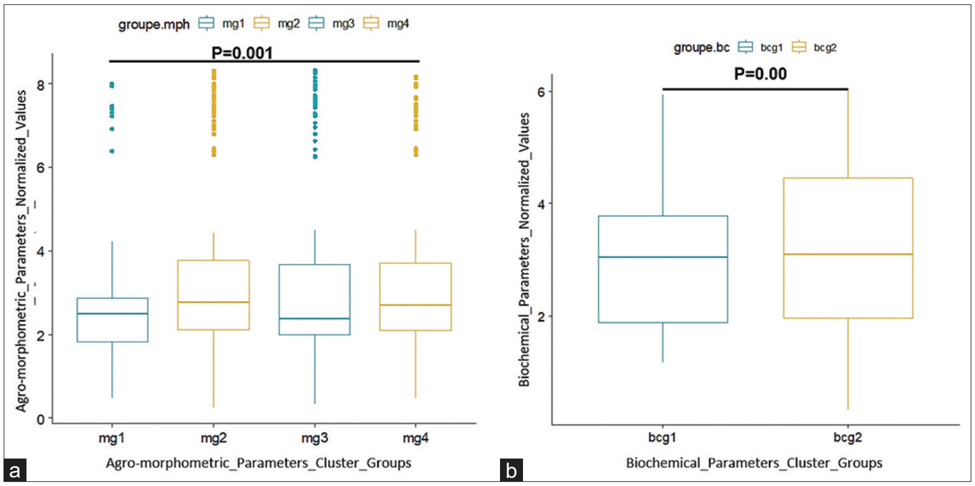


Figure 4: Multivariate boxplot analysis comparing cowpea cultivar agro-morphological (a) and biochemical (b) average value by evaluating variance differences between cluster groups generated by the K-mean parallel analysis. Mg and/or groupe.mph and Bcg and/or groupe.bc acronyms referred respectively to the cluster groups generated by cowpea agro-morphological and biochemical parameters.

Table 2: ANOVA test evaluating variance difference in generated cowpea cluster groups through k-mean survey by processing agro-morphological and biochemical and/or nutritional cowpea cultivar parameters.

K-mean	DF	Sum Sq.	Mean Sq.	F value	Pr (>F)
Cowpea cultivar plants agro-morphological parameters					
Groups	3	4724832	1574944	5.22	0.001
Residual	2556	770854285	301586	–	–
Cowpea cultivar plants biochemical parameters					
Groups	1	438584	438584	33.52	0.00
Residual	766	10021151	13082	–	–

3, and 4 by k-mean analysis by processing cowpea agro-morphological features [Table 3]. Indeed, the criterion for factor extraction is where the eigenvalues generated by random data exceed the eigenvalues produced by the experimental data. So considering cluster groups 1, 2, 3, and 4 generated by the agro-morphological parameter, 2, 4, 2, and 2 eigenvalues generated by random data exceed the eigenvalues shaped by cowpea angiosperm experimental data respectively for the first, second, third, and fourth clustering groups generated by above-mentioned K-mean survey [Table 3]. Horn PA applied to cowpea biochemical parameters suggested two components and/or biochemical factors as enough to explain data variability in the two generated clustering groups by the K-mean analysis. Of note, for processed cowpea cultivar biochemical parameters 2 eigenvalues generated by random data exceed the eigenvalues shaped by cowpea angiosperm experimental data of the two clustering groups generated by the aforementioned K-mean analysis [Table 4]. Comparative analysis of estimated error and/or bias assessing components and/or factor number explaining data variability suggested a good performance of predictive aptitude with regard to cowpea biochemical parameters by contrast to those of agro-morphological features [Tables 3 and 4].

Table 3: Horn parallel analysis in selecting cowpea agro-morphological factors and/or components explaining data variability in cluster groups generated by k-mean survey.

Components	Normalized and/or adjusted eigenvalue	Unadjusted eigenvalue	Estimated error and/or bias
K-mean cluster group 1			
1	1.57	4.69	3.12
2	2.02	4.22	2.2
K-mean cluster group 2			
1	5.76	6.63	0.88
2	2.16	2.83	0.67
3	1.04	1.55	0.51
4	1.06	1.45	0.39
K-mean cluster group 3			
1	5.92	7.13	1.21
2	1.85	2.77	0.91
K-mean cluster group 4			
1	6.11	7.72	1.61
2	1.28	2.45	1.18

Table 4: Horn parallel analysis in selecting cowpea biochemical factors and/or components explaining data variability in cluster groups generated by k-mean survey.

Components	Normalized and/or adjusted eigenvalue	Unadjusted eigenvalue	Estimated error and/or bais
K-mean cluster group 1			
1	2.75	3.57	0.82
2	1.73	2.22	0.48
K-mean cluster group 2			
1	2.55	3.11	0.56
2	2	2.35	0.34

3.6. Statistical Descriptive Analysis of k-mean Survey Generated Clustering Groups for Both Cowpea Biochemical and Agro-morphological Parameters

We embarked here in computing position and dispersion descriptive statistical parameters concerning each analyzed biochemical and agro-morphological parameters obtained clustering groups by the K-mean analysis [Figure 3]. We reported the results of these analyses in Supplementary Tables 1-4 for agro-morphological parameters and Supplementary Tables 5 and 6 for biochemical parameters. Integrative analysis, between Horn PA and descriptive survey of clustering groups obtained by K-mean survey by processing cowpea agro-morphological parameters revealed (i) cowpea plants yield, (ii) cowpea pods number (CPN), (iii) cowpea plant height (CPH) and (iv) weight of 100 grain agro-morphological parameters as potential source of data variability [Table 3 and Supplementary Tables 1-4]. Of note, yield agro-morphological parameter displayed a higher variance coefficient than CPH, CPN, and weight of 100-grain agro-morphological parameters [Supplementary Tables 1-4]. In the same tendency merging Horn parallel results with those of descriptive statistics of cowpea cultivar clustering groups generated by K-mean analysis based on biochemical parameters exhibited glucose and cowpea energetic value (CPEV) as influencing data variability [Table 4 and Supplementary Tables 5 and 6]. It is noteworthy to underline that CPEV exhibited higher variance than glucose biochemical parameter [Supplementary Tables 5 and 6].

3.7. Assessment of Selected Agro-morphological and Biochemical Parameters Impact on Detected Cowpea Clustering Group's Data Variability

Here, we removed (i) cowpea plants yield, (ii) CPN, (iii) CPH and (iv) weight of 100-grain agro-morphological parameters, agro-morphological features supporting data variability as suggested by parallel Horn analysis, from the list of agro-morphological parameters to be analyzed. The same have done for biochemical parameters i.e. (i) glucose and (ii) CPEV, that have been detected by Horn parallel survey as a source of data variability in characterizing cowpea cultivar, basing on their nutritional parameters. Next, we performed an ANOVA test of the new generated matrix. The analysis of the variance in this context showed no significant variance difference between the four cowpea-clustering groups generated by the K-mean analysis for agro-morphological ($P = 0.34$) and biochemical and/or nutritional parameters ($P = 0.9$) [Figure 5]. This result confirmed cowpea plants yield, (ii) CPN, (iii) CPH,and (iv) weight of 100-grain agro-morphological parameters as well as (i) glucose and (ii) CPEV, biochemical features as a source of data variability. In addition, this analysis suggested a relatively high effect of (i) cowpea pods glucose content (CPGC) and (ii) cowpea pods energetic value biochemical parameters, influencing data variability by contrast to the selected agro-morphological parameters (cowpea plants yield, CPN, CPH and weight of 100-grain) [Figure 5].

3.8. Comparison Between Data Matrix Generated by k-mean and Parallel Horn Surveys in Selecting Agro-morphological (Yield, Plant Height, Pods Number and 100 Cowpea Seed Grain Weight) and Biochemical (Cowpea Seed Energetic Value and Glucose Content) Parameters

Assuming that data are normally distributed ($n > 30$) in characterizing cowpeas cultivar by processing above mentioned agro-morphological (yield, plant height, pods number, and 100 cowpea seed grain weight) and biochemical (cowpea seed energetic value and glucose content) parameters, we proceeded to variance homogeneity test between data matrix referred to these two above mentioned parameters typologies. This survey showed a significant difference in terms of variance homogeneity between data matrix referred to selected agro-morphological and biochemical parameters (Bartlett's K-squared = 494.53, df = 1, $P = 0.00$). It is noteworthy to underline that variance homogeneity analysis was carried out on a population of 93 cowpea plants reporting the 6 selected agro-morphological and biochemical variables (number of pods, weight of 100 cowpea grain, CPH, CPGC and energetic value and yield). Z-score analysis evaluating the impact of these parameters in clustering the above-mentioned 93 cowpea cultivar displayed three parameters groups cluster as follows: (i) CPH + weight of 100 cowpea grain, (ii) number of cowpea pods + glucose content of cowpea pods, and (iii) cowpea pods energetic value + cowpea yield [Figure 6]. The same analysis (z-score survey) by computing Euclidean distance parameter between these six considered cowpea cultivar agro-morphological and biochemical features attributed the high variability to cowpea cultivar data to their yield and energetic value component [Figure 6]. Considering as a whole,

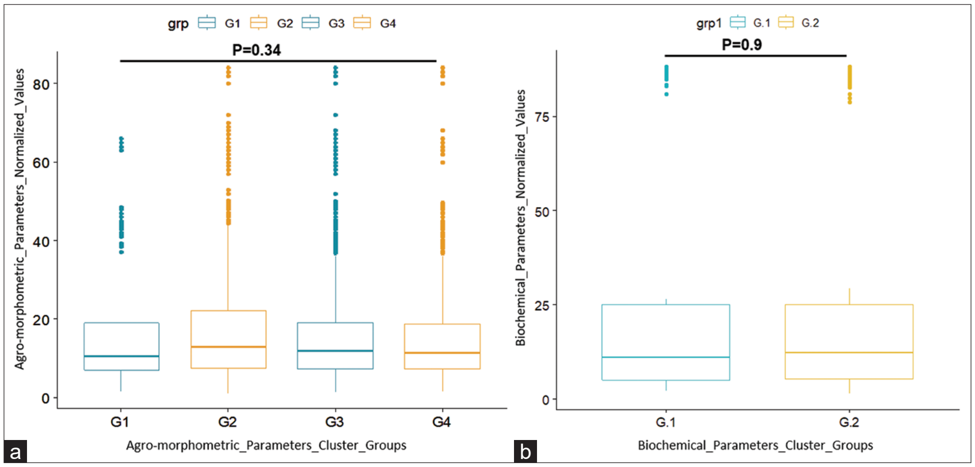


Figure 5: Multivariate boxplot analysis comparing variance difference of cowpea cultivar clustering groups generated by k-mean analysis for agro-morphological (a) and biochemical and/or nutritional (b) parameters by removing potential parameters of data variability source selected by Horn parallel survey.

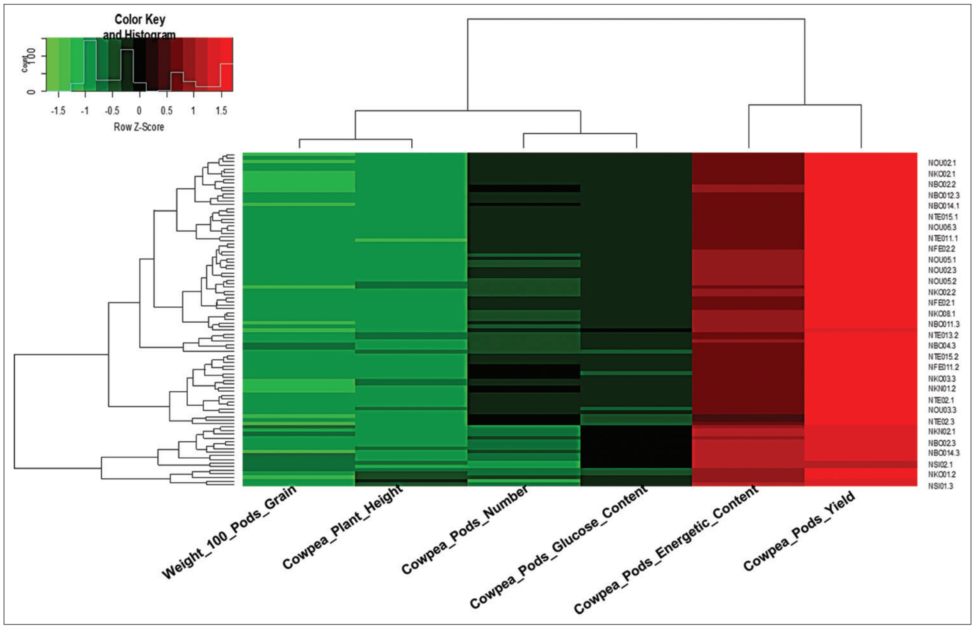


Figure 6: Z-score Euclidean distance analysis clustering cowpea cultivar by merging selected agro-morphological (Weight of 100 Pods Grain (W100PG), Cowpea Plant Height (CPH), CPN and Cowpea Pods Yield [CPY]) and biochemical (Cowpea Pods Energetic and Glucose Content) parameters.

cowpea pods energy content and yield exhibited high performance for characterizing and describing cowpea germplasm and/or phenotype.

3.9. Assessment of the Interaction between Selected Agro-morphological (W100PG, CPH, CPN and CPY) and biochemical (Cowpea Pods Energetic and Glucose Content) Parameters by Pearson Correlation and Tukey Multiple Comparison Test

Pearson correlation survey suggested as expected a strong significant concordance between cowpea yield and CPN parameters [Figure 7a and Table 5]. Interestingly, the same analysis exhibited a significant correlation between agro-morphological parameters i.e. cowpea yield, CPN, and cowpea cultivar glucose content biochemical parameter [Figure 7a and Table 5] and suggested a relatively good clustering between cowpea height and W100PG agro-morphological parameters and cowpea pods energetic content [Figure 7a]. Indeed, the Pearson correlation analysis showed a relatively significant correlation between cowpea height and W100PG agro-morphological parameters ($R^2 = 0.4$). The same investigation suggested a relative concordance in terms of Pearson correlation coefficient value ($R^2 = 0.13$) of the two heterogenic agro-morphological (CPH) and biochemical (cowpea pods energetic value) parameters [Table 5 and Supplementary Table 7]. Of note, CPEV exhibited a significant negative correlation with the cowpea glucose content component ($R^2 = -0.33$) [Table 4 and Supplementary Table 7]. Tukey multiple comparative test clearly showed the significant performance at 95% of confidence level of cowpea yield and energetic value content respectively agro-morphological and biochemical parameters in characterizing cowpea cultivar phenotype as opposed to the other four analyzed parameters (W100PG, CPH, CPN and cowpea glucose content) [Figure 7b]. However, the same survey suggested the high aptitude and/or performance of cowpea yield, agro morphometric parameter with respect to cowpea energetic content component in cowpea cultivar clustering survey [Figure 7b].

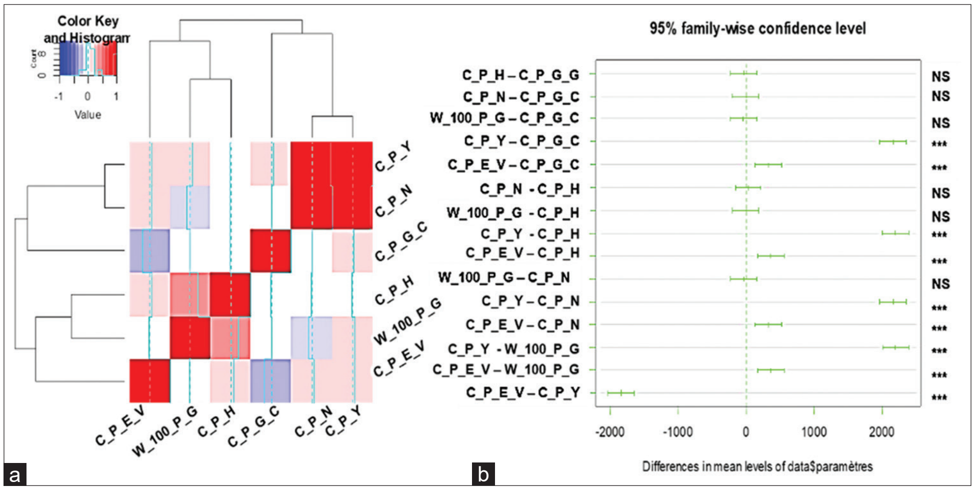


Figure 7: Pearson correlation (a) and Tukey multiple comparison (b) surveys measuring statistical interaction and/or link between cowpea energetic value (CPEV), cowpea pods glucose content (CPGC), Weight of 100 Pods Grains (W100PG), Cowpea Plant Height (CPH), Cowpea Pods Number (CPN) and Cowpea Pods Yield (CPY) biochemical and agro morphometric parameters. NS acronym referrers to non-significant difference ($P > 0.05$), while *** indicates statistical significant difference ($P \leq 0.05$).

Table 5: Pearson correlation between the six selected agro-morphological and biochemical parameters assessing cowpea germplasm performances.

Selected agro-morphological and biochemical parameters	**1**	**2**	**3**	**4**	**5**	**6**
Glucose (1)	--					
Energetic value (2)	−0.33	--				
Cowpea plant height (3)	−0.04	0.13	--			
Pods number (4)	0.03	0.1	−0.05	--		
100 pod grain weight (5)	0.00	−0.03	0.4***	−0.17	--	
Yield (6)	0.2**	0.12	−0.03	0.9***	0.14	--

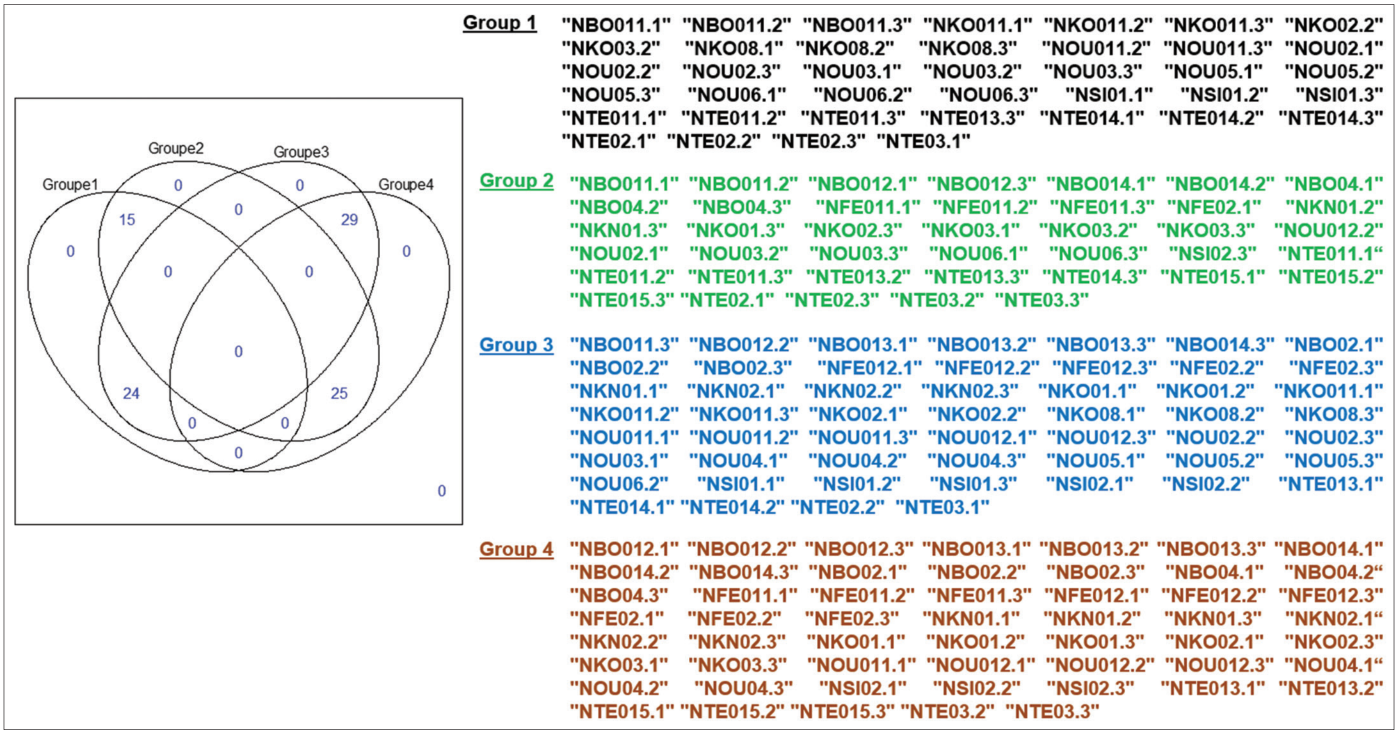
NS: Non-significant correlation (P>0.05) and *** Statistical significant correlation (P≤0.05)

3.10. Cowpea Cultivar Selection Basing on Selected Agro-morphological (CPY) and Biochemical (Energetic Content) Parameters

We generated cowpea-clustering groups based on yield averages and as well energetic average values. Indeed, we clustered cowpea plants basing on their performance in term of average values of yield as well as to energetic average content. Venn diagram clustering analysis suggested 39 cowpea plants (group 1) having yield and energetic content parameters higher to average values [Figure 8]. The same survey revealed 40 cowpea cultivars (group 2) showing energetic value higher than the estimated energetic average value. Of note, 15 plants out those cultivars exhibit high performance by considering (i) yield agro-morphological and (ii) cowpea energetic content average values [Figure 8]. Venn diagram analysis selected 53 cowpea plants (group 3) as exhibiting low performance in terms of agro-morphological yield average parameter. However, 24 cowpea plants of this group exhibit high performance in terms of energetic value content average [Figure 8]. The same analysis suggested 54 cowpea plants referred to as group four as exhibiting low performance in terms of energetic value content average for 29 cowpea cultivar 45 as well as low performance in terms of yield average for 25 cowpea cultivar [Figure 8]. Considering as a whole, Venn diagram clustering analysis suggested a relatively high number of cowpea cultivar exhibiting high performance by combining both yield and energetic average content parameters.

3.11. ROC prediction Model Analysis Assessing Yield Agro-morphological and Energetic Content Biochemical Parameters Performances in Selecting Cowpea Germplasm

We performed ROC prediction model analysis to evaluate the performance of the aforementioned agro-morphological and biochemical parameters in characterizing and/or clustering cowpea cultivars [Figure 9]. Our predicting ROC model suggested a relatively acceptable performance and/or accuracy with regard to agro-morphological yield parameter (yield average) assessing cowpea germplasm [Figure 9b]. ROC predicting model displays biochemical parameter in terms of energetic content average of cowpea cultivar as an excellent and suitable factor in evaluating the latter's germplasm [Figure 9a]. Of note, ROC predicting analysis attributed high accuracy (1) and low cutoff value (0%) to cowpea energetic content average component in characterizing cowpea cultivar germplasm as opposed to yield average component that exhibits accuracy values ranking from 1 to 0.5 and accuracy ranking from 0.41 to 0.435 [Figure 9a and 9b]. Interestingly, ROC predicting model suggested the combination of agro-morphological (i.e., cowpea yield average component) and biochemical (i.e. cowpea energetic content average component) parameters, as a suitable arrangement and/or approach to evaluate and characterize analyzed cowpea cultivar germplasm. Indeed, the merging of these parameters in characterizing cowpea cultivars' performance revealed an accuracy rating between 0.8 and 1 for a cutoff around 0 [Figure 9c]. In other words, the combination of cowpea yield and energetic content average components results to be an excellent model in predicting and selecting cowpea germplasm. Previous results suggested the sensitivity of the combination of cowpea yield and energetic content average components in selecting cowpea cultivars since this combination discriminated the highest number of cowpea cultivar [Figure 9].



Group 1 "NBO011.1" "NBO011.2" "NBO011.3" "NKO011.1" "NKO011.2" "NKO011.3" "NKO02.2" "NKO03.2" "NKO08.1" "NKO08.2" "NKO08.3" "NOU011.2" "NOU011.3" "NOU02.1" "NOU02.2" "NOU02.3" "NOU03.1" "NOU03.2" "NOU03.3" "NOU05.1" "NOU05.2" "NOU05.3" "NOU06.1" "NOU06.2" "NOU06.3" "NSI01.1" "NSI01.2" "NSI01.3" "NTE011.1" "NTE011.2" "NTE011.3" "NTE013.3" "NTE014.1" "NTE014.2" "NTE014.3" "NTE02.1" "NTE02.2" "NTE02.3" "NTE03.1"

Group 2 "NBO011.1" "NBO011.2" "NBO012.1" "NBO012.3" "NBO014.1" "NBO014.2" "NBO04.1" "NBO04.2" "NBO04.3" "NFE011.1" "NFE011.2" "NFE011.3" "NFE02.1" "NKN01.2" "NKN01.3" "NKO01.3" "NKO02.3" "NKO03.1" "NKO03.2" "NKO03.3" "NOU012.2" "NOU02.1" "NOU03.2" "NOU03.3" "NOU06.1" "NOU06.3" "NSI02.3" "NTE011.1“ "NTE011.2" "NTE011.3" "NTE013.2" "NTE013.3" "NTE014.3" "NTE015.1" "NTE015.2" "NTE015.3" "NTE02.1" "NTE02.3" "NTE03.2" "NTE03.3"

Group 3 "NBO011.3" "NBO012.2" "NBO013.1" "NBO013.2" "NBO013.3" "NBO014.3" "NBO02.1" "NBO02.2" "NBO02.3" "NFE012.1" "NFE012.2" "NFE012.3" "NFE02.2" "NFE02.3" "NKN01.1" "NKN02.1" "NKN02.2" "NKN02.3" "NKO01.1" "NKO01.2" "NKO011.1" "NKO011.2" "NKO011.3" "NKO02.1" "NKO02.2" "NKO08.1" "NKO08.2" "NKO08.3" "NOU011.1" "NOU011.2" "NOU011.3" "NOU012.1" "NOU012.3" "NOU02.2" "NOU02.3" "NOU03.1" "NOU04.1" "NOU04.2" "NOU04.3" "NOU05.1" "NOU05.2" "NOU05.3" "NOU06.2" "NSI01.1" "NSI01.2" "NSI01.3" "NSI02.1" "NSI02.2" "NTE013.1" "NTE014.1" "NTE014.2" "NTE02.2" "NTE03.1"

Group 4 "NBO012.1" "NBO012.2" "NBO012.3" "NBO013.1" "NBO013.2" "NBO013.3" "NBO014.1" "NBO014.2" "NBO014.3" "NBO02.1" "NBO02.2" "NBO02.3" "NBO04.1" "NBO04.2“ "NBO04.3" "NFE011.1" "NFE011.2" "NFE011.3" "NFE012.1" "NFE012.2" "NFE012.3" "NFE02.1" "NFE02.2" "NFE02.3" "NKN01.1" "NKN01.2" "NKN01.3" "NKN02.1“ "NKN02.2" "NKN02.3" "NKO01.1" "NKO01.2" "NKO01.3" "NKO02.1" "NKO02.3" "NKO03.1" "NKO03.3" "NOU011.1" "NOU012.1" "NOU012.2" "NOU012.3" "NOU04.1“ "NOU04.2" "NOU04.3" "NSI02.1" "NSI02.2" "NSI02.3" "NTE013.1" "NTE013.2" "NTE015.1" "NTE015.2" "NTE015.3" "NTE03.2" "NTE03.3"

Figure 8: Venn diagram clustering cowpea cultivar basing on selected agro-morphological (average of cowpea yield) and biochemical (average of cowpea plants energetic content) parameters.

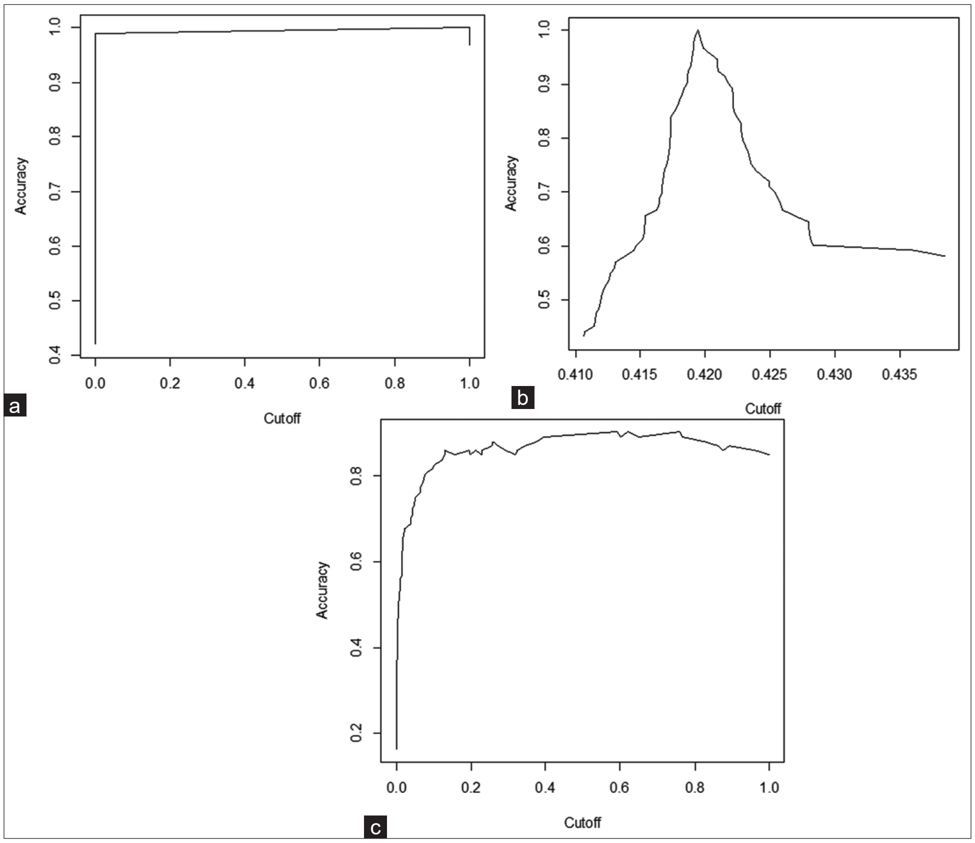


Figure 9: ROC predicting model assessing (a) biochemical parameters (i.e. energetic content average) and (b) agro-morphological parameters (i.e. yield average value) and as well (c) both agro-morphological (i.e. yield average value) and biochemical (i.e. energetic content average) parameters in selecting cowpea germplasm.

4. DISCUSSION

Any rational use of existing cultivars of a plant species in an agrarian area requires prior knowledge of their genetic variability. The identification of this variability on the basis of certain agro-morphological traits is an important component in the initiation of plant breeding programs. Like this, Fraleigh [30] states that characterization provides breeders with the necessary information for their research. The level of genetic diversity of populations, measured at the agro-morphological and molecular (enzyme and gene) levels, depends on the respective actions of four evolutionary forces that can interact with each other: mutation, selection, migration, and drift. These evolutionary pressures are responsible for the structure of genetic diversity and its evolution. Characterization is an approach widely used to understand the structure of plant genetic diversity at different levels for its conservation and use [31]. It thus allows us to understand the structuring of the population based on morphological, agronomic, ecological, biochemical and/or molecular descriptors [32]. Several methods are available for the analysis of genetic diversity in crop plants. These methods have relied on genealogical, morphological, agronomic, and more recently molecular data. For reasonably accurate and unbiased estimates of genetic diversity, adequate attention must be paid to these different methods [11]. Computational statistical approaches play an essential role in the evaluation and processing of biological data [29]. The complexity and large size of these data make computational statistics a crucial tool in bio-statistical approaches with the purpose to estimate fittingly genetic diversity by integrating several statistical computational functions and/or packages. Because of that, we characterized the phenotypic performance of cowpea cultivars in Northern Côte d'Ivoire by developing a computational statistical approach, exclusively in the R programming language. In the other words, we performed a multivariate statistical analysis basing on a computational statistical approach by integrating several scripts and/or functions of descriptive statistic as well as analytical tests. Pearson correlation tests and Z-score clustering analyses revealed significant interactions within each of the morphological and biochemical data types. Nutritional and/or biochemical features exhibited four clustering groups as following: (i) ash and sweet residues, (ii) protein, lipid, and water content, (iii) energetic value, and (iv) glucose and dry mass [Figure 2a]. The same analysis referred to five clustering groups for agro-morphological parameters as follows: (i) yield, (ii) pods number, ripening time, plant width and flowering time, (iii) pods weight, plant leaf width, plant nodes and branches number, cowpea seed length and width, (iv) plant height, plant leaf length and 100-cowpea seeds weight and (v) pods length, pods seed number and seed cell number. This result indicates that the use of a single descriptor within each morphological or biochemical clustering group is sufficient to characterize Cowpea cultivars. Indeed taking correlations into account may reduce the number of descriptors, as the biological information provided by two positively correlated descriptors is similar as reported in the earlier works on Shea tree and coconut [33,34]. The grouping of certain agro-morphological or biochemical traits in the same cluster revealed by Z-score analysis would suggest that the genes responsible for the expression of these traits are located on the same chromosomes. Of note, the processes to retrieve parameters and/or environmental factors explaining plant crop agro-morphometric and genomic data variability is a challenge for biologists. K-mean analysis is a powerful computational statistical algorithm that tries to partition the dataset into K pre-defined distinct non-overlapping subgroups (clusters) where each data point belongs to only one group [27]. Because of that, k-mean survey were used to discriminate cowpea cultivar clustering group numbers basing agro-morphometric and biochemical traits revealed by the z score and Pearson correlation analysis. K-mean revealed four and two cowpea phenotypic clustering groups by processing respectively agro-morphometric and biochemical parameters. Interestingly ANOVA test strongly supported the variance difference between cowpea cultivar groups revealed by the k-mean analysis ($P < 0.05$). Then, we integrated Horn's PA to select number of cowpea agro-morphometric and/or biochemical factors (parameters) provoking phenotypic data variability in the detected cowpea cultivar groups by the k-mean survey. Of note, Horn's PA revealed four agro-morphological (yield, plant height, pods number, and 100 cowpea seed grain weight) and two biochemical (cowpea seed energetic value and glucose content) factors as inducing phenotypic variability between cowpea cultivars. In the other words, findings revealed (i) cowpea pods glucose content, and (ii) energetic value (biochemical parameters) and (i) CPH, (ii) number of pods, (iii) weight of 100 cowpea grain, and (iv) yield (agro-morphological parameters) out the 24 assessed characters as significantly involved in structuring the phenotype of cowpea cultivars into 2 and 4 groups, respectively. Plant height was variable both within and between cultivars. In this study, it varied from 6 cm to 30.9 cm. This high variability in plant height could be of genetic origin grouping cowpea genotypes according to their height into three classes: dwarf (≤ 15 cm), medium (15–25 cm), and tall (> 25 cm). The number of pods per plant varied from 10 to 90 both within and between cowpea cultivars. This variability in the number of pods per plant was also observed in the works of Cobbinah *et al*. [35] in Ghalmi [36] in Algeria. According to Aryeetey and Laing [37], the number of pods per plant in cowpea is a low heritability trait. This would explain that the expression of this trait is strongly influenced by the environment. Thus, improvement of the number of pods per plant by selection is rather difficult. The 100-seed weight is a discriminating morphological trait in cowpea. Sène [38] also reported the high variability of this trait in an F2 hybrid population (9–18.1 g) and Olawale and Bukola [39] in cowpea. The 100-seed weight being a highly heritable trait as reported by Sène [38] indicates that the variability observed among cowpea cultivars evaluated in the current study is of genetic origin. The seed yield per plant per hectare also varied significantly between cultivars. The average yield obtained was 2139.61 kg/ha and ranged from 317.88 to 3855.07 kg/ha. Sarutayophat *et al*. [40] and Stoilova and Berova [41] have reported similar variations previously. In this study, seed yield was positively correlated with leaflet length, leaflet width, and number of pods per plant. Indeed, yield is a complex heritable trait as it is strongly influenced by the environment [42,43]. It is composed of several single heritable traits [44] and its improvement is achieved through the improvement of its different components [42,43,45]. Thus, these traits can be used as selection criteria to improve cowpea yield. The length and width of leaflets that constitute the leaf area could be early traits for selection and yield improvement of cowpea since they are vegetative stage-related traits. In addition, several studies have shown the correlation between leaf area and crop yield [46-48]. Indeed, the green leaf area is the one that fixes light and atmospheric CO_2, which are used for photosynthesis.

Cowpea cultivars seeds physico-chemical analysis revealed glucose content and energy value as the most discriminating biochemical features, confirming cowpea as leguminous plant. Of note, high-level content of carbohydrates in cowpea seeds was reported by Huang and Guo [49], Adebooye and Singh [50], highlighting cowpea as energy food. Interestingly, the average energy value of cowpea reported in our study is similar to those obtained by Koko *et al*. [51] and Chinma *et al.* [52] in the Akidi cultivar in Nigeria.

Tukey multiple comparative test clearly showed the significant performance of cowpea yield and energetic value content, respectively agro-morphological and biochemical parameters in characterizing cowpea cultivar phenotype as opposed to the other four analyzed parameters (W100PG, CPH, CPN, and cowpea glucose content). Correlations analysis between nutritional and agronomic parameters revealed a positive relationship between some agro-morphological parameters i.e. yield and nutritional parameters. Of note, these biochemical parameters, which are difficult to access, could be improved through the agronomic traits linked to them. The identification of elite genotypes in the cowpea collection of Peleforo GON COULIBALY University based on their performance in yield and energy. Interestingly, ROC predicting model supported the high performance of integrating cowpea crop (i) yield (agro-morphological) and (ii) energy content (biochemistry) parameters, for identifying elite genotype in the Peleforo GON COULIBALY University cowpea collation. To the best of our knowledge, this computational statistical scheme resulting in the combination of several descriptive and analytical statistical tests in R programming environment is the first one describing plant agro-morphometric phenotypic differences by merging morphometric and biochemistry features.

5. CONCLUSION

This work made it possible to characterize the phenotypic performance of cowpea cultivars in northern Côte d'Ivoire by developing a computational statistical approach, exclusively in R programming language. This approach and/or model exhibited an excellent aptitude in evaluating, characterizing, and managing agro-morphological and biochemical characteristics of cowpea in recent genetic selection procedures. In perspective, because of its flexibility, our computational statistical model could strongly contribute to characterizing and comparing germplasms diversities of multiple crops basing on their agro-morphological and biochemical features.

6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. FUNDING

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

Data are available at Genetic UPR, Peleforo Gon Coulibaly University, Korhogo, Ivory Coast.

11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

12. PUBLISHER'S NOTE

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