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Full Length Research Paper

Morphological distinctiveness between *Solanum aethiopicum* Shum group and its progenitor

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Use of morphological markers offers an alternative in germplasm discrimination of research-neglected crop species. A collection of 25 accessions including five wild progenitors was evaluated in screen house to identify the morphological difference between *Solanum aethiopicum* Shum and *Solanum anguivi*. An Unweighted Pair Group Method with Arithmetic mean hierarchical clustering revealed presence of moderate structure with a cophenetic correlation coefficient of 0.73. Five distinct clusters were produced; the progenitor accessions for the *S. aethiopicum* Shum were grouped in their own cluster. The Richness, Shannon-Weaver and Simpson indices were also different among qualitative variable categories. A 'prcomp' function based Principal component analysis (PCA) in R on quantitative variables indicated that days to germination and emergence, cotyledonous leaf length, cotyledonous leaf width, shoot biomass, plant height, petiole length, days to first flowering opening, plant width, plant branching, and number of leaves per plant are the major drivers of variability in the study accessions. Further, results from canonical discriminant analysis to discern between the *S. aethiopicum* and its progenitor accession groups showed that the days to germination and emergence provide the best separation; with the former emerging earlier than the latter. The mean values for flowering time, leaves per plant, number of branches per plant and plant height were more favorable for the Shum than its wild progenitor accessions. The study revealed that morphological markers are useful in distinguishing between the *S. aethiopicum* Shum and its progenitor accessions.

Key words: African indigenous vegetable species, genetic diversity, reordered hierarchical clustering, Principal component analysis (PCA), linear discriminant analysis.

INTRODUCTION

African indigenous vegetable species (AIVS) require genetic improvement in order to address constraints that curtail the crops' productivity and contribution to household income and food security in sub-Saharan

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Africa (Abukutsa-Onyango, 2014; Cernansky, 2015). *Solanum aethiopicum* and *S*. *anguivi* are some of the major AIVS that are research-neglected. The *S*. *aethiopicum* is morphologically diverse with four recognized groups, of which Shum is a leafy type (https://avrdc.org/african-eggplant-solanum-aethiopicum/). The *S*. *aethiopicum* evolved from *S*. *anquivi* (Ebert, 2014; Sękara et al., 2007). Although the two species are domesticated, *S*. *anguivi* is grown for its fruits only. The *S*. *anguivi* exists both in the wild and at farmers' fields; indicating its environment robustness. The availability of germplasm of known diversity is important in variety improvement efforts (Gramazio et al., 2016). Morphological markers are cheap can be used for diversity analyses (Kouassi et al., 2014; Kubie, 2013). However, the usefulness of morphological traits in accession discrimination for the Shum and *S*. *anguivi* had not yet been investigated.

The most commonly used morpho-agronomic traits for genetic diversity studies in *Solanum* spp. have been published (Adeniji et al., 2013; Gramazio et al., 2016). Multivariate statistical methods such as multidimensional scaling (MDS), linear (canonical) discriminant analysis (LDA), cluster and principal component analysis are suited for use in understanding genetic diversity (Harding and Payne, 2012). MDS is a form of non-linear dimensionality reduction for visualizing the level of similarity of individual cases of a data set (information) that is contained in a distance matrix. Cluster analysis and PCA are the two most commonly used methods of genetic diversity analysis; the former handles both quantitative and qualitative variables while the latter is powerful and sensible with quantitative data sets (Zimisuhara et al., 2015). Clustering employs either the Ward's or "average" (Unweighted Pair Group Method with Arithmetic mean; UPGMA) method algorithms (Odong et al., 2011). Although both methods rely on coefficients such as cophenetic correlation coefficient (CPCC) to judge the strength (reliability) of subgroup differentiation, UPGMA is the most commonly used (Odong et al., 2011; Zimisuhara et al., 2015).

Further, diversity indices such as Richness, Shannon-Weaver, and Simpson can reveal groupings of accessions per level of qualitative variable (Altaye, 2015). A diversity index is a quantitative measure that reflects how many different types (in this case the levels of qualitative variables) there are in the dataset (community), and simultaneously takes into account how evenly the basic entities (such as individuals) are distributed among those types (Zimisuhara et al., 2015). On the other hand, the PCA serves to identify how different variables work together to reduce dimensionality and redundancy; thus helping to reveal hidden structure (Coghlan, 2017; Zimisuhara et al., 2015). LDA is aimed at finding linear combinations of original variables that gives the best possible separation among study groups (Coghlan, 2017; Harding and Payne, 2012). This study aimed to identify: hierarchical groups existing in the study accessions, major drivers of variability in collected data set for the study accessions, and variables that account for the greatest separation between the Shum and *S*. *anguivi* progenitors.

MATERIALS AND METHODS

Study site and germplasm

The study was carried out in screen house at West Africa Centre for Crop Improvement (WACCI) research farm, University of Ghana, Greater Accra, Ghana. During the experiment (October 2016 to April 2017), temperature ranged between 21-26°C (morning), 31-43°C (afternoon) and 29-35°C (evening hours). Relative humidity ranged between 74-81% (morning), 57-75% (afternoon) and 62-69% (evening hours). The germplasm was obtained from Department of Agricultural and Biological Sciences, Uganda Christian University, Bishop Tucker Road, Mukono, Uganda. The list of study accessions is as shown (Table 1).

Design

A total of 25 accessions which comprised 20 entries of *S*. *aethiopicum* Shum group and five of *S*. *anguivi* were used. Twelve plants of each accession were established in individual plastic pots of 5-L size in a completely randomized block design. Three seeds of an accession were directly sown in a pot on 10th Oct., 2016 followed by thinning to one plant per pot at 4-leaf stage (seedling stage) on 8th Nov., 2016. The potting soil was clay-loam. Optimum watering with uniform quantities of water on a daily basis, appropriate fertilizer application with NPK 17:17:17 at 4 g per pot on a fort-nightly basis and preventive pesticide sprays using mancozeb and dimethoate once every 2 weeks was carried out.

Data collection

Data was collected on; number of days to emergence, cotyledonous leaf length, cotyledonous leaf width, seedling leaf length, seedling leaf width, seedling fresh weight and seedling dry weight were recorded. Additional morphological traits were collected at flowering stage using a modified standard IBPGR *Solanum* species characterization manual.

Cluster analysis

The raw data was summarized in Excel to obtain means for different quantitative variables and cleaning up of the qualitative data followed by subsequent analysis in R (Everitt and Hothorn, 2014). A text delimited data frame of the mean values was imported into R followed by converting of traits to appropriate ordered (qualitative), nominal (qualitative) and numeric/integer (quantitative) variables. The target variables were then selected followed by installing and loading an R package cluster for cluster analysis. Because the data included both quantitative and qualitative variables, a function daisy() was used to group accessions based on a general coefficient of dissimilarity that combines and processes different types of variables according to their own mathematical type (Grum and Atieno, 2007; Zimisuhara et al., 2015). The hierarchical clustering was carried out using average (UPGMA) algorithm. A "re-ordered" dendrogram was plotted so that an accession in one cluster that has the smallest distance to accessions in the next cluster is the accession that is placed adjacent to the next cluster. In order to examine how well the

Table 1. List of accessions used in this study.

Entry	Code	Name (Pedigree)	Species name
1	168G	SAS168/G/2015	*S. aethiopicum* Shum
2	183G	SAS183/G/2015	*S. aethiopicum* Shum
3	163	SAS163/2015	*S. aethiopicum* Shum
4	163P	SAS/163/P/2015	*S. aethiopicum* Shum
5	157P	SAS/157/P/2015	*S. aethiopicum* Shum
6	160	SAS160/2015	*S. aethiopicum* Shum
7	163G	SAS163/G/2015	*S. aethiopicum* Shum
8	183P	SAS183/P/2015	*S. aethiopicum* Shum
9	108	SAS108/2015	*S. aethiopicum* Shum
10	157G	SAS157/G/2015	*S. aethiopicum* Shum
11	148	SAS/148/2015	*S. aethiopicum* Shum
12	145	SAS145/2015	*S. aethiopicum* Shum
13	168P	SAS/168/P/2015	*S. aethiopicum* Shum
14	184G	SAS184/G/2015	*S. aethiopicum* Shum
15	137	SAS137/2015	*S. aethiopicum* Shum
16	184P	SAS184/P/2015	*S. aethiopicum* Shum
17	141	SAS141/2015	*S. aethiopicum* Shum
18	108P	SAS108/P/2015	*S. aethiopicum* Shum
19	185G	SAS185/G/2017	*S. aethiopicum* Shum
20	185P	SAS185/P/2015	*S. aethiopicum* Shum
21	146	SAN146/2015	*S. anguivi*
22	177	SAN177/2015	*S. anguivi*
23	163W	SAN163/W/2015	*S. anguivi*
24	163C	SAN163/C/2015	*S. anguivi*
25	114	SAN114/2015	*S. anguivi*

distance (dissimilarity) matrix is represented graphically, a Mantel test that gives a cophenetic correlation coefficient (CPCC) was used. It measures the relationships between the original (true) pair-wise distance between accessions and pair-wise distances between accessions predicted using the dendogram. The CPCC is defined as a product-moment correlation coefficient between cophenetic distances and input distance matrix from the data; and the cophenetic distance between two accessions is the distance at which two accessions are first clustered together in a dendrogram going from bottom to top (Odong et al., 2011).

In order to ascertain that an optimum number of clusters was generated in the hierarchical tree, a Kelly-Gadner-Scutcliffe (1996) penalty function for pruning was calculated using a function kgs(). A function table() was then used to categorize the number of accessions in each cluster per level of qualitative variable. Diversity indices namely Richness, Shannon-Weaver and Simpson were also calculated. The Richness index defined here as the number of clusters represented in each qualitative variable was calculated using a function specnumber() that is dependent on (contained in) packages permute, lattice and vegan. The Shannon-Weaver index (swi) that combines a measure of richness with a measure of evenness was computed using a function diversity() whose default in R is set for the swi; otherwise an alternative index “simpson” that measures the evenness of group membership (Harding and Payne, 2012) was specified.

Principal component analysis

Principal component analysis (PCA) complements the cluster analysis in a way that the former helps to interrogate the data so as to understand the contribution of each variable to the existing diversity among accessions (Zimisuhara et al., 2015). The PCA procedure was performed in R on quantitative traits of the data frame using a base function prcomp() (Coghlan, 2017; Everitt and Hothorn, 2014). By default, the function prcomp() centres the variable to have mean equals to zero. Thus, the standard deviations were also set to 1 with the parameter scale=T in order to normalize the variables. The mean (center) and standard deviations (scale) of each variable, the principal component loadings (rotation) that constitute the rotation matrix which contains the principal component (PC) loading vector, and the matrix x that contains the PC score vectors were generated. A facility in the function prcomp() enables calculation of the standard deviation (sdev) of each PC (Coghlan, 2017). The variance (var) of each PC was then computed by squaring the sdev. The proportion of variance explained by each principal component (prop_varex) was calculated by dividing variance by total variance (that is, prop_varex= var/sum(var). In order to show the components that explain most of the variability in the data, a biplot and scree plots were used to plot the first two PCs, proportion of variance explained by each PC, and the cumulative proportion of variance explained.

Linear discriminant analysis

A multivariate analysis of variance (MANOVA) was conducted to identify variables that are significantly different between the two groups; *S. aethiopicum* Shum and *S. anguivi* at 99% confidence

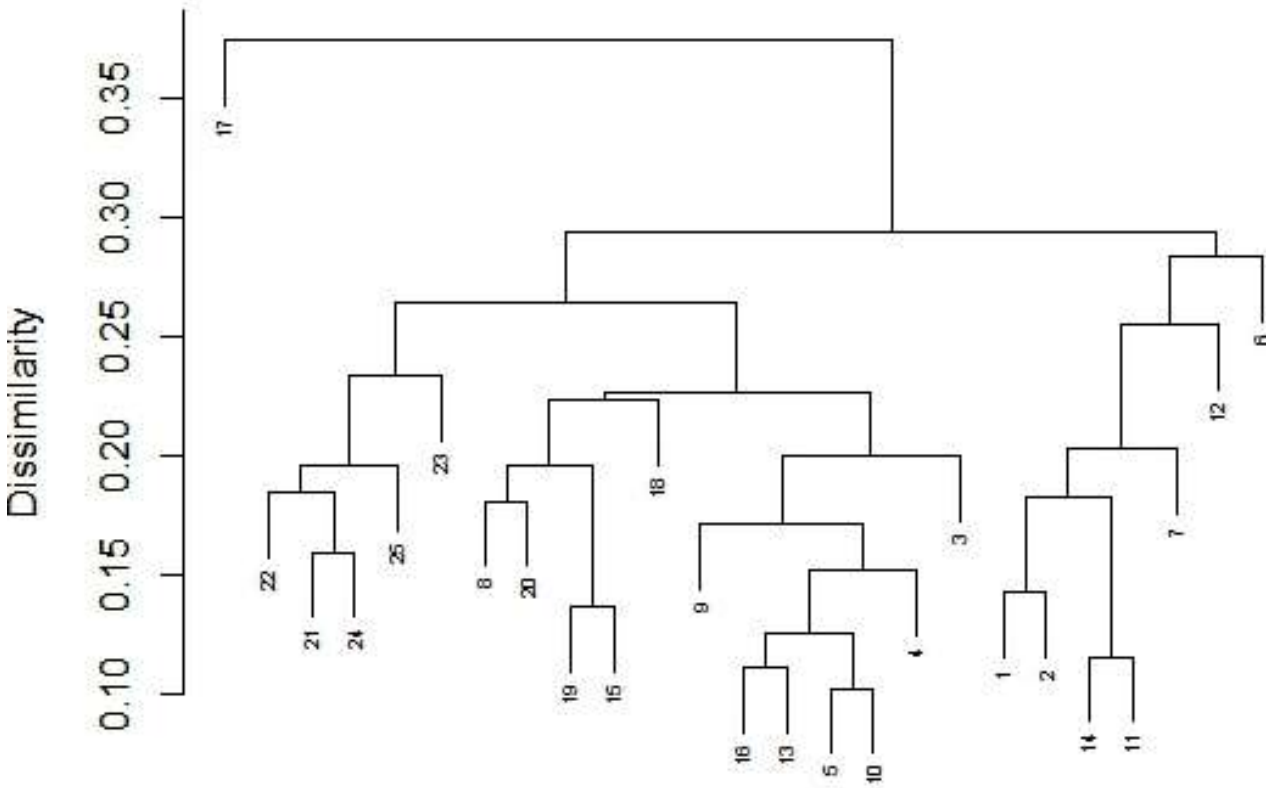

Figure 1. A re-ordered cluster dendrogram for the study accessions using the UPGMA method of agglomeration. The labels are the different study entries: 1-20 and 21-25 are the Shum and *S*. *anguivi* accessions, respectively.

level using GenStat Release 12.1 (VSN International Ltd). In MANOVA, the independent variables are the groups and the dependent variables are the predictors (Coghlan, 2017; Li and Wang, 2014). However, in LDA, the independent variables are the predictors and the dependent variables are the groups. The LDA was performed on data variables with significantly different means between the groups. Canonical vector loadings of discriminant function, correlations between data variates, and the correlations between the variates and discriminant function were generated. The maximum number of discriminant functions will be equal to the degrees of freedom, or the number of variables in the analysis, whichever is smaller (Coghlan, 2017); in this case the degrees of freedom is the smaller at 1 (that is, number of groups minus one). Thus, in this analysis, only one discriminant function was possible. The canonical loadings (standardized beta coefficients) were used to define the discriminant function (Harding and Payne, 2012). The larger the loading, the greater is the unique contribution of the respective variable to the discrimination between groups – without necessarily specifying the groups that the function discriminates (Coghlan, 2017). It is notable that in this case we are dealing with two groups; thus the loadings should give a reliable indication of the canonical data variable(s). Otherwise, a factor structure would be used to determine which variables define the discriminant function. The factor structure coefficients are the correlations between the data variates and the discriminant function; that denote the simple correlations between variables and the discriminant function (Harding and Payne, 2012). The pearsonian correlation coefficients between significant variables; and Mahalanobis (*D*-squared) intergroup distance (Harding and Payne, 2012; Zimisuhara et al., 2015) are also reported.

RESULTS

Clustering

Hierarchical clustering of the accessions based on the “average” (UPGMA) method produced five clusters (Figure 1). To test the goodness of the dendrogram by telling how well the distance (dissimilarity matrix) is represented graphically, the Mantel test revealed that the clusters were significantly distinct ($p<0.01$) with a cophenetic correlation coefficient of 0.73. On whether to prune the hierarchical cluster tree, a Kelly-Gardner Sutcliffe penalty function that compares the mean across all clusters with the mean within clusters of the dissimilarity measure further showed that the optimum number of clusters was five (Figure 2). The first and fifth clusters contained one accession each. The second, third and fourth cluster had five, twelve and six members, respectively. A summary of the cluster groups and their members are shown in Table 2. Entry 17 which comprises the first cluster was the only accession with spines. Table 3 shows how different qualitative traits are spread over the different clusters.

The Richness index (RI) was at maximum (at 5; the total number of clusters in the hierarchical tree) for

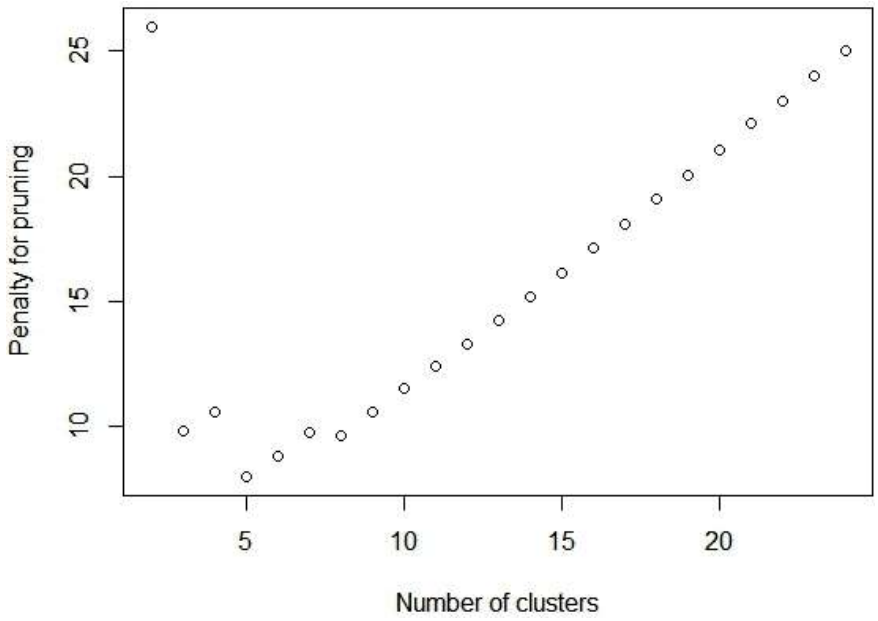

Figure 2. Kelley-Gardner-Sutcliffe penalty function for the cluster data showing that the optimum number of accessions is five.

Table 2. List of accessions per cluster.

Cluster name	Member (Entry)
1	17
2	22, 21, 24, 25 and 23 (all the *S*. *anguivi* accessions)
3	8, 20, 19, 15, 18, 19, 16, 13, 5, 10, 4 and 3
4	1, 2, 14, 11, 7 and 12
5	6

greenish white cotyledonous leaf color, poor seedling vigor, and acute leaf tip angle. The lowest RI of 1 (which implies that all accessions belonged to only one of the 5 clusters) was observed for many spines on stem, sparse stem pubescence, green stem color, green petiole color, very many prickles on lower leaf surface, very many prickles on upper leaf surface, many petiole prickles, weak leaf lobbing, very strong leaf lobbing, and purple leaf mid-rib color. Shannon-Weaver index (swi), a measure of richness and evenness, all clusters contained greenish white accessions based on cotyledonous leaf color (that is, swi is maximum at 1.27). The most diverse (in terms of number of clusters captured of out the total) and abundant (in terms of number of accessions represented) for spines on stem, seedling vigor, plant growth habit, stem pubescence, stem color, petiole color, leaf tip angle, leaf prickles was glabrous (swi=1.15), poor vigor (1.55), prostrate (1.33), medium (0.69), purple (0.95), acute (1.38), and glabrous (1.15). Generally, variable levels with high swi also had high values for the Simpson index (Table 4).

Principal component analysis

Sixteen principal components (PCs) were generated; the first two and ten PCs accounting for up to 51.53 and 96.83% of variation, respectively. The first PC that had higher loadings for days to germination (emergence; DG), leaf blade width (LBW), leaves per plant (LPP), leaf blade length (LBL) and leaf blade width (LBW) than the rest of the variables, accounted for 28.46% of variation. The second PC (23.07%) had high loadings for cotyledonous leaf length (CLBL), seedling fresh weight (SDFW), seedling leaf blade length (SLBL), seedling leaf blade width (SLBW) and days for first flower opening (FLW, Table 5). When represented on a scaled biplot such that the longer the arrows the higher the contribution to variation, it was shown that CLBL, LPP, DG, LBW, FLW, SDFW, SLBW, LBL, and plant width (PW) were shown to contribute to the highest variation among the study accessions (Figure 3). A scree plot showed that the first 10 PCs account for most of the variation at up to ~97% (Figure 4). Going by one variable per PC based on

Table 3. Number of members per cluster in the different levels of qualitative variables.

Variable / Levels	No. of accessions per cluster					Variable / Levels	No. of accessions per cluster				
	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5		Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Cotyledonous leaf color											
Greenish white	1	5	12	6	1						
Spines on stem						**Petiole prickles**					
Glabrous	0	5	12	6	1	Glabrous	0	5	12	6	1
Many	1	0	0	0	0	Many	1	0	0	0	0
Visual seedling vigor						**Leaf blade lobbing**					
Intermediate	0	2	4	2	0	Weak	0	0	0	1	0
Poor vigor	1	2	1	2	1	Intermediate	0	0	6	3	0
Very poor vigor	0	1	5	1	0	Strong	1	5	6	2	0
Very vigorous	0	0	1	1	0	Very strong	0	0	0	0	1
Vigorous	0	0	1	0	0						
Plant growing habit						**Leaf blade color**					
Intermediate	0	3	5	1	1	Green	0	0	0	6	1
Prostrate	1	2	1	1	0	Pale purple	0	5	11	0	0
Upright	0	0	3	2	0	Purple	1	0	1	0	0
Very upright	0	0	3	2	0						
Stem pubescence						**Leaf midrib color**					
Glabrous	0	0	11	5	0	Green	0	0	0	6	1
Sparse	0	0	1	0	0	Pale purple	0	5	12	0	0
Medium	1	0	0	0	1	Purple	1	0	0	0	0
Dense	0	5	0	1	0						
Stem color						**Leaf pubescence on upper surface**					
Green	0	0	0	6	0	Glabrous	1	0	10	5	0
Pale purple	0	4	9	0	1	Sparse	0	1	2	0	1
Purple	1	1	3	0	0	Dense	0	4	0	1	0
Petiole color						**Leaf pubescence on lower surface**					
Green	0	0	0	6	0	Dense	0	4	0	1	0
Pale purple	0	5	10	0	1	Glabrous	1	0	10	5	0
Purple	1	0	2	0	0	Sparse	0	1	2	0	1
Leaf tip angle						**Leaf vein pigmentation**					
Acute	1	4	7	4	1	Green	0	0	0	6	1
Intermediate	0	1	5	2	0	Pale purple	1	5	12	0	0
Leaf prickles on lower surface						**Leaf prickles on upper surface**					
Glabrous	0	5	12	6	1	Glabrous	0	5	12	6	1
Very many	1	0	0	0	0	Very many	1	0	0	0	0

Diversity indices: Richness, Shannon-Weaver and Simpson.

Table 4. Richness, Shannon-Weaver (swi) and Simpson indices for the different variable levels.

Variable / levels	Richness	swi	Simpson	Variable / levels	Richness	swi	Simpson
Cotyledonous leaf color							
Greenish White	5	1.27	0.67				
Spines on stem				**Petiole prickles**			
Glabrous	4	1.15	0.64	Glabrous	4	1.15	0.64
Many	1	0.00	0.00	Many	1	0.00	0.00
Visual seedling vigor				**Leaf blade lobbing**			
Intermediate	3	1.04	0.63	Weak	1	0.00	0.00
Poor Vigor	5	1.55	0.78	Intermediate	2	0.64	0.44
Very Poor Vigor	3	0.80	0.45	Strong	4	1.20	0.66
Very Vigorous	2	0.69	0.50	Very strong	1	0.00	0.00
Vigorous	1	0.00	0.00				
Plant growing habit				**Leaf blade color**			
Intermediate	4	1.17	0.64	Green	2	0.41	0.24
Prostrate	4	1.33	0.72	Pale purple	2	0.62	0.43
Upright	2	0.67	0.48	Purple	2	0.69	0.50
Very Upright	2	0.67	0.48				
Stem pubescence				**Leaf midrib color**			
Glabrous	2	0.62	0.43	Green	2	0.41	0.24
Sparse	1	0.00	0.00	Pale purple	2	0.61	0.42
Medium	2	0.69	0.50	Purple	1	0.00	0.00
Dense	2	0.45	0.28				
Stem color				**Leaf pubescence (upper surface)**			
Green	1	0.00	0.00	Glabrous	3	0.83	0.51
Pale Purple	3	0.83	0.50	Sparse	3	1.04	0.63
Purple	3	0.95	0.56	Dense	2	0.50	0.32
Petiole color				**Leaf pubescence (lower surface)**			
Green	1	0.00	0.00	Dense	2	0.50	0.32
Pale Purple	3	0.83	0.51	Glabrous	3	0.83	0.51
Purple	2	0.64	0.44	Sparse	3	1.04	0.63
Leaf tip angle				**Leaf vein pigmentation**			
Acute	5	1.38	0.71	Green	2	0.41	0.24
Intermediate	3	0.90	0.53	Pale purple	3	0.79	0.48
Leaf prickles (lower surface)				**Leaf prickles (upper surface)**			
Glabrous	4	1.15	0.64	Glabrous	4	1.15	0.64
Very Many	1	0.00	0.00	Very many	1	0.00	0.00

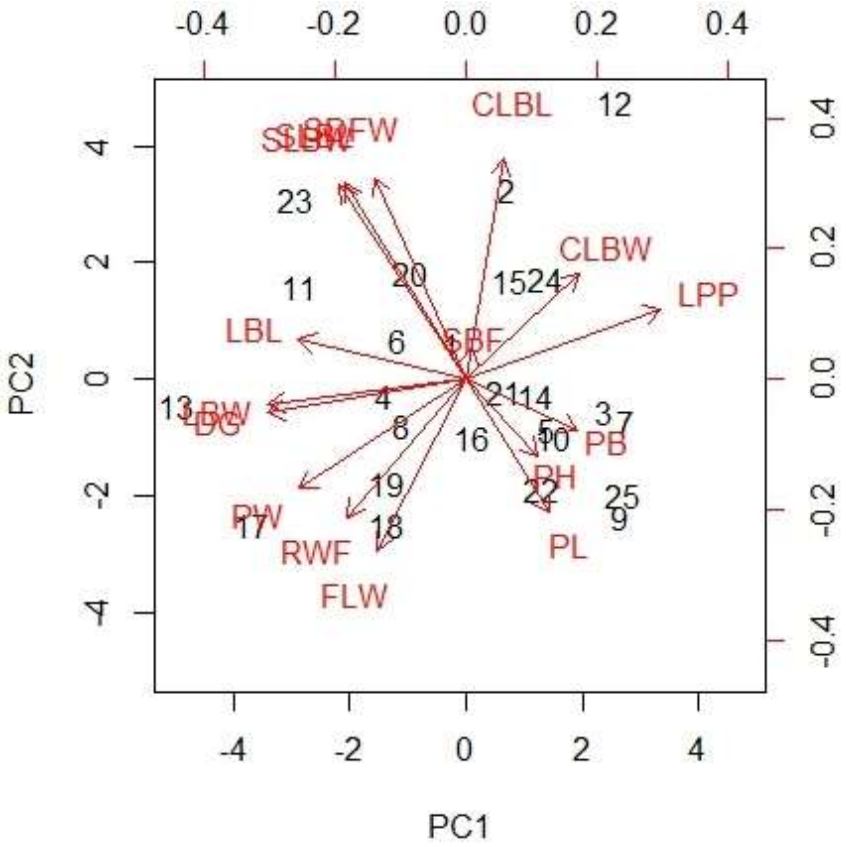

Figure 3. Biplot of the first two principal components of variation scaled by loadings (arrow length) to show contribution to variation. DG, days to germination and emergence; CLBL, cotyledonous leaf blade length (mm); CLBW, cotyledonous leaf blade width (mm); SLBL, seedling leaf blade length (mm); SLBW, seedling leaf blade width (mm); SDFW, seedling fresh weight (grams, g); LPP, number of leaves per plant; PH, plant height (cm); PB, number of branches per plant; PW, plant canopy width (cm); PL, petiole length (mm); LBL, leaf blade length (cm); LBW, leaf blade width (cm); SBF, shoot fresh biomass (g); RWF, root fresh weight (g); FLW, days to first flower appearance.

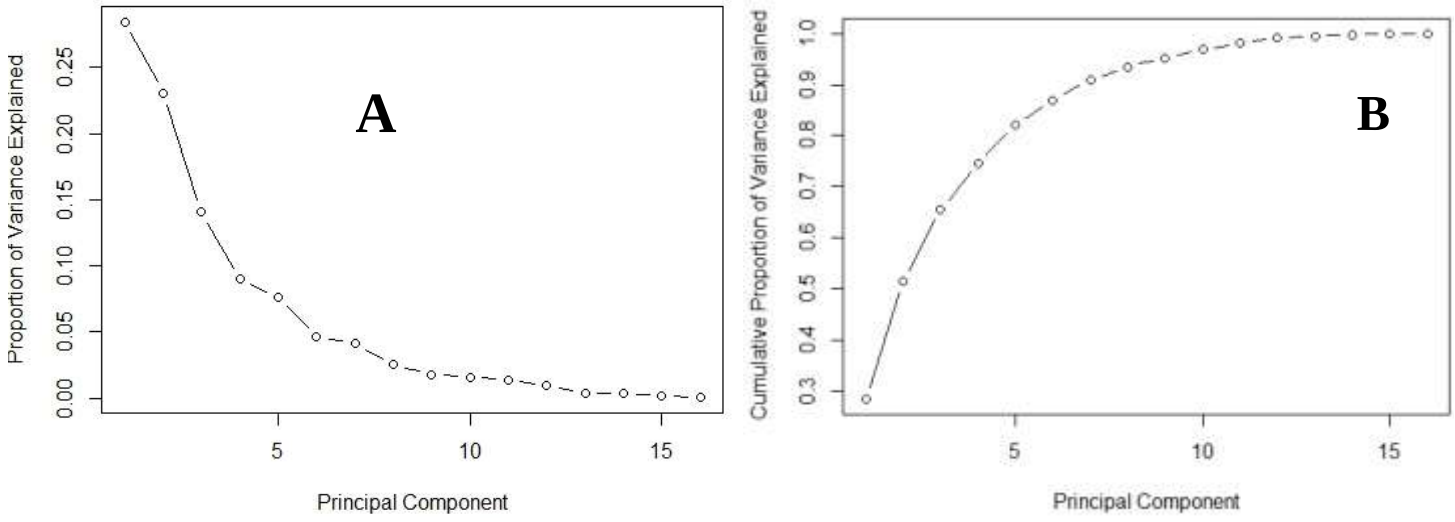

Figure 4. Scree plots for proportion (A) and cumulative proportion (B) of variance explained.

Table 5. The loadings and proportion of variation explained by each of 16 principal components among study accessions.

Variable	Mean	StDev	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16
DG	7.60	3.24	-0.3802	-0.0649	0.1189	-0.0298	-0.2758	-0.3073	0.0391	-0.1807	0.5055	-0.0347	0.3806	0.2527	-0.1469	0.1179	-0.1958	-0.3037
CLBL	13.09	1.70	0.0718	0.4242	-0.1834	-0.1941	-0.1780	0.1842	0.0179	0.0618	0.0653	-0.4994	-0.3483	0.3363	-0.3531	0.2351	0.0527	-0.0554
CLBW	5.11	0.94	0.2164	0.2025	-0.4189	-0.2328	0.1742	0.0203	-0.2412	-0.0955	0.4711	0.0660	-0.0737	-0.1408	0.1404	-0.4985	-0.1373	-0.2141
SLBL	26.62	10.34	-0.2317	0.3756	0.2616	0.1378	0.1257	0.0673	-0.0281	-0.0915	0.0491	-0.0314	0.0928	-0.2125	-0.0303	-0.1388	0.7176	-0.3099
SLBW	19.00	7.20	-0.2422	0.3712	0.2708	0.1251	0.0677	0.0418	0.0356	-0.1101	0.2223	0.0164	-0.0167	0.0626	-0.0858	-0.2999	-0.1693	0.7176
SDFW	0.41	0.28	-0.1762	0.3860	0.2391	0.2278	0.1345	0.2222	0.0112	0.2223	-0.0263	0.0775	-0.1625	-0.1233	0.3157	0.2129	-0.5182	-0.3633
LPP	39.81	9.15	0.3737	0.1339	0.2214	0.0347	-0.0312	-0.0832	-0.1724	0.0100	0.2001	0.6794	-0.2243	0.3301	-0.1455	0.2261	0.1449	-0.0151
PH	29.55	8.38	0.1369	-0.1468	0.3035	-0.1712	0.6417	0.1245	0.1990	0.0689	-0.0611	-0.1270	0.1762	0.4422	-0.1697	-0.2286	-0.0859	-0.1784
PB	10.19	1.38	0.2147	-0.0966	0.3484	-0.4402	-0.1154	0.1760	0.2129	0.3861	0.3812	-0.0590	0.0940	-0.4420	-0.0546	0.1484	0.0391	0.1057
PW	48.58	4.15	-0.3191	-0.2092	-0.0100	0.0697	-0.2062	0.1804	-0.3888	0.6636	-0.0034	0.0733	-0.0522	0.2082	-0.1155	-0.3340	0.0731	-0.0340
PL	58.56	14.47	0.1597	-0.2546	0.2412	0.1610	-0.0203	0.4863	-0.5791	-0.3659	0.1457	-0.2477	0.0927	-0.0075	0.0521	0.1517	-0.0325	0.0380
LBL	20.95	2.80	-0.3212	0.0767	-0.2074	-0.4545	0.1737	0.1949	-0.0853	0.0161	0.0249	0.1414	0.1768	0.2743	0.5027	0.3236	0.2024	0.2007
LBW	14.59	2.42	-0.3789	-0.0488	-0.0983	-0.3190	0.1678	0.2150	-0.1135	-0.2280	-0.1862	0.3260	-0.0918	-0.2978	-0.5706	0.0902	-0.1660	-0.0632
SBF	137.75	32.75	0.0113	0.0620	0.4011	-0.4707	-0.4294	-0.0373	-0.0423	-0.2551	-0.3451	0.0187	-0.1650	0.1085	0.2274	-0.3536	-0.0807	-0.1384
RWF	51.26	12.23	-0.2292	-0.2637	0.1944	-0.0989	0.3108	-0.4619	-0.2100	-0.0047	0.1748	-0.2139	-0.6010	-0.0819	0.1136	0.1173	0.0657	0.0322
FLW	37.79	6.44	-0.1697	-0.3285	-0.0751	0.1611	-0.1229	0.4406	0.5202	-0.1973	0.2654	0.1325	-0.4043	0.1152	0.1187	-0.1275	0.1111	-0.0749
Standard deviation			2.1339	1.9211	1.5004	1.2013	1.1024	0.8597	0.8148	0.6329	0.5310	0.5034	0.4642	0.3834	0.2363	0.2281	0.1750	0.0809
Variance			4.5534	3.6908	2.2511	1.4430	1.2153	0.7390	0.6638	0.4006	0.2820	0.2534	0.2155	0.1470	0.0558	0.0520	0.0306	0.0065
Proportion			28.4589	23.0676	14.0696	9.0190	7.5954	4.6187	4.1490	2.5035	1.7625	1.5837	1.3470	0.9188	0.3489	0.3251	0.1915	0.0409
Cumulative			28.4589	51.5265	65.5962	74.6151	82.2105	86.8293	90.9783	93.4818	95.2442	96.8279	98.1749	99.0937	99.4426	99.7677	99.9591	100.00

DG, days to germination and emergence; CLBL, cotyledonous leaf blade length (mm); CLBW, cotyledonous leaf blade width (mm); SLBL, seedling leaf blade length (mm); SLBW, seedling leaf blade width (mm); SDFW, seedling fresh weight (grams, g); LPP, number of leaves per plant; PH, plant height (cm); PB, number of branches per plant; PW, plant canopy width (cm); PL, petiole length (mm); LBL, leaf blade length (cm); LBW, leaf blade width (cm); SBF, shoot fresh biomass (g); RWF, root fresh weight (g); FLW, days to first flower appearance.

scores (loadings), the ten principal components that account for the greatest differences among the study accessions include DG, CLBL, CLBW, shoot fresh biomass (SBF), plant height (PH), petiole length (PL), FLW, PW, PB, and LPP.

Discriminant analysis

There were significant differences (p<0.01) between the two groups of accessions; *S. aethiopicum* Shum, and *S. anguivi*) for CLBL, CLBW, DG, FLW, LPP, RWD, and SBD. The differences between the groups were however, non-significant (p>0.01) for LA, LBL, LBW, PB, PH, PL, PW, SDDW, SDFW, SLBL, SLBW and TBD (Table 6). Thus, subsequent linear discriminant analysis (LDA) was carried out on the seven significantly different variates between groups as predictors for the groups. The vector loadings (scores) that indicate unique contribution of each trait led to the following discriminant function: $Group = 0.0586CLBL - 0.2609CLBW + 0.6321DG + 0.0727FLW + 0.0627LPP + 0.0310RWD + 0.0200SBD$. In addition, simple correlations between variates and the discriminant function were generated. The variates with the highest loadings (0.6321 and -0.2609) and the highest correlations between the variates and discriminant function (0.7891 and -0.3491) were DG and CLBW, respectively (Table 7). The mean DG was 6.25 and 13.00 for *S. aethiopicum* Shum and *S. anguivi* accessions, respectively.

Table 6. Mean squares and probability values for rejecting a hypothesis of no difference (α=1%) between *S. aethiopicum* Shum and *S. anguivi* accessions

Variate	Mean for SAN	Mean for SAS	Between Groups MS	Within Groups MS	F.pr	Variate	Mean for SAN	Mean for SAS	Between Groups MS	Within Groups MS	F.pr
CLBL	12.10	13.34	51.028	3.527	<0.001	PH	27.41	30.09	163.89	81.64	0.163
CLBW	4.10	5.36	42.447	0.7524	<0.001	PL	60.67	58.04	524.1	339.2	0.22
DG	13.00	6.25	989.908	3.104	<0.001	PW	51.51	47.84	204.67	33.95	0.018
FLW	42.33	36.65	849.97	43.18	<0.001	RWD	13.60	9.84	313.76	29.88	0.002
LA	358.94	303.17	37735	23466	0.211	SBD	23.07	19.14	491.20	43.05	0.001
LBL	21.90	20.71	8.38	19.17	0.511	SDDW	0.04	0.04	0.00076	0.00168	0.505
LBW	16.23	14.18	85.45	14.19	0.018	SLBL	30.60	25.63	341.4	113.1	0.088
LPP	34.25	41.20	839.8	110.7	0.008	SLBW	23.20	17.95	385.54	53.55	0.01
PB	10.07	10.21	2.846	2.864	0.324						

SAN, *S. anguivi*; SAS, *S. aethiopicum*. Range for days to germination and emergence (DG) was 4-11 and 12-15 for SAS and SAN, respectively. CLBL, cotyledonous leaf blade length (mm); CLBW, cotyledonous leaf blade width (mm); LPP, number of leaves per plant; PB, number of branches per plant; LBL, leaf blade length (cm); LBW, leaf blade width (cmFLW, days to first flower appearance.

Table 7. Discriminant function (DF) and *r* for the correlations between variates and the DF.

Variate	Scores for DF	Coefficients for correlations between variates and the DF (*r*)
CLBL	0.0586	-0.1251
CLBW	-0.2609	-0.3491
DG	0.6321	0.7891
FLW	0.0727	0.1971
LPP	0.0627	-0.1378
RWD	0.031	0.15
SBD	0.02	0.1561

DG, days to germination and emergence; CLBL, cotyledonous leaf blade length (mm); CLBW, cotyledonous leaf blade width (mm); LPP, number of leaves per plant; SBD, shoot dry biomass (g); RWD, root dry weight (g); FLW, days to first flower appearance.

The Mahalanobis' intergroup distance (D^2) between the two groups was estimated to be 24.51. The discriminant scores for the group means for SAN and SAS were 3.864 and -1.087, respectively (Figure 5).

DISCUSSION

A moderately high CPCC obtained indicates presence of structure or strong group (subgroup) differentiation among the study accessions. It is notable however, that the higher the CPCC value up to over 0.9, the better the usefulness of dendrograms especially for taxonomic purposes (Coghlan, 2017; Odong et al., 2011). The clustering was unbalanced considering that one of the groups contained only one member compared to twelve in one of the four remaining groups. The dendrogram produced was the most appropriate with the data used considering that five clusters were shown; in concordance with optimum number read from the Kelley-Gardner-Sutcliffe penalty function (Grum and Atieno, 2007; Kelley et al., 1996). The UPGMA typically produces unbalanced dendrograms, leading to exposure of outliers (Grum and Atieno, 2007; Odong et al., 2011) like entry 17 that had leaf prickles. Leaf prickles are not a common attribute within the *S. aethiopicum* Shum and its progenitor *S. anquivi* (Adeniji et al., 2012, 2013). As a leafy vegetable, the *S. aethiopicum* Shum need not possess leaf spines unless prickliness is a marker associated with a yield, quality or other desired attribute like tolerance to a major productivity constraint.

The Richness index of 1 for leaf prickles further indicates that only one cluster of the five clusters was represented; implying that apart from entry 17, the rest of the 24 accessions spread in the 2-5th cluster did not have spines. For the Shannon-Weaver index (swi), the higher the value the higher the diversity and abundance of a certain category of qualitative variable. It is thus suggested that poor seedling vigor, acute leaf tip angle, prostrate plant growth habit, greenish white cotyledonous

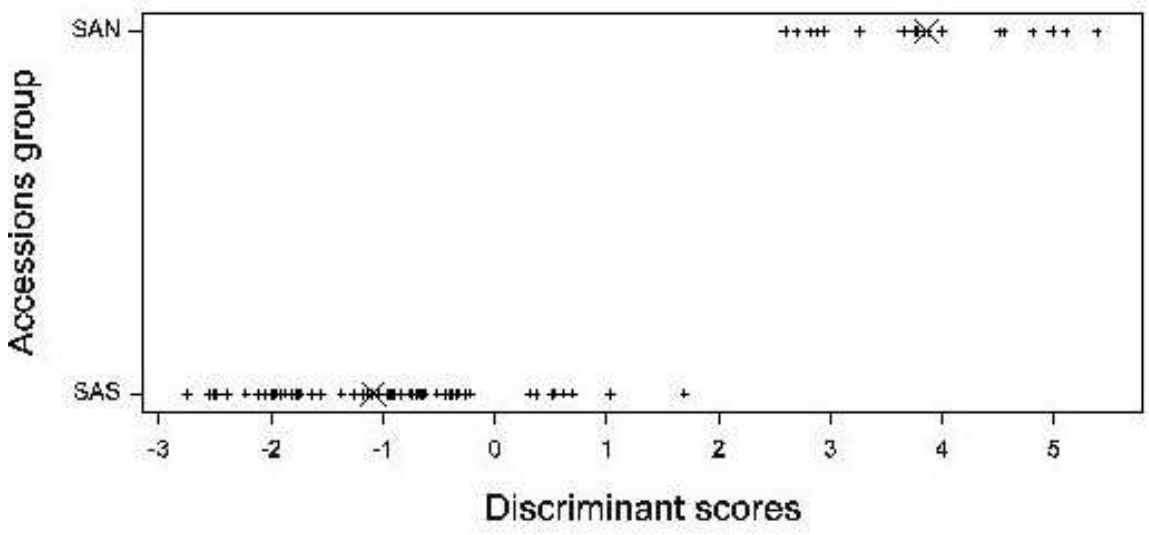

Figure 5. The discriminant scores for the two groups; SAS (*S. aethiopicum* Shum) and SAN (*S. anguivi*) accessions. The position of a group mean score is marked with an 'X'.

leaf color, strong leaf blade lobbing, glabrous stem prickliness, glabrous petiole prickliness, sparse leaf pubescence, and glabrous leaf prickliness are the most diverse and abundant attributes among the study accessions. The poor seedling vigor category had the highest Simpson index; suggesting a higher abundance of the accessions with poor vigor. The other variable categories that are highly abundant include prostrate plant growth habit, acute leaf tip angle, and greenish white cotyledonous leaf color. The Simpson index is a measure of evenness of group membership or the likelihood of two randomly selected accessions being different from each other (Coghlan, 2017; Harding and Payne, 2012).

The PCA indicated that by just selecting the variables leading for loadings in the first two PCs; at least half of the drivers of diversity among the accessions are captured. To this end, it is suggested that cotydonous leaf blade length (CLBL), number of leaves per plant (LPP), days to germination and emergence (DG), leaf blade width (LBW), days to first flower opening (FLW), seedling fresh weight (SDFW), seedling leaf blade width (SLBW), leaf blade length (LBL), and plant width (PW) greatly contribute to the variation captured by the first two PCs. Because the aim of conducting PCA is two pronged; reduce redundancy and retaining variables that explain as much variation as possible (Coghlan, 2017), a further scrutiny with help of the scree plots guided that the first 10 PCs explain up to ~97% of the diversity as revealed by hierarchical clustering. It is notable however, that the PCA was based on quantitative variables only. Therefore, DG, CLBL, CLBW, shoot fresh biomass (SBF), plant height (PH), petiole length (PL), FLW, PW, plant branching (PB), and LPP account for much of diversity (say ~97%) in the study accessions based on quantitative traits.

Based on group means for discriminant scores and Mahalanobis' intergroup distance (Harding and Payne, 2012), the morphological data clearly classified the Shum and *S. anguivi* accession groups as distinct. The two species can generally be distinguished based on variates; CLBL, CLBW, DG, FLW, LPP, RWD, and SBD in a discriminant function: $Group = 0.0586CLBL - 0.26CLBW + 0.63DG + 0.073FLW + 0.063LPP + 0.031RWD + 0.02SBD$. It is thus suggested that a clear distinction between the Shum and its progenitor can be made at seedling (CLBL, CLBW and DG), flowering (FLW) and harvest maturity (LPP, SBD and RWD). A correlation between the variates and discriminant function suggested that the DG, having a strong Pearsonian correlation coefficient (*r*) is a major canonical discriminant variate. The *r* obtained for DG was positive and strong; though a direction of the correlation would not affect interpretation in this case. It is thus suggested that a screening out of non-Shum genotypes can be done if germination and emergence exceeds eleven (min., 4; max., 11; mean, 6.25) days from the time of sowing; under conditions similar to those used in this study. Whereas morphological markers provide a good distinction among *S. aethiopicum* and between the Shum and *S. anguivi* accessions as reported earlier (Kouassi et al., 2014), a follow-up validation study with molecular markers could be complementary.

Conclusion

There is significant structure within the *S. aethiopicum*

Shum and *S*. *anguivi* accessions studied; with distinct clusters. Qualitative variables put aside, the principal component analysis revealed that diversity is mainly contributed by differences in days to germination and emergence, cotyledonous leaf length, cotyledonous leaf width, shoot fresh biomass, plant height, petiole length, days to flowering, plant width, plant branching, and number of leaves per plant. It was further revealed that the days to germination and emergence provide the greatest separation between the Shum and *S*. *anguivi* progenitors; with the former emerging earlier than the latter. Other traits which were more favorable among the Shum than the *S*. *anguivi* accessions include number of leaves per plant, number of branches per plant and plant height. This information is useful in suggesting germplasm conservation and breeding approaches for development of improved varieties of *S*. *aethiopicum* Shum.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Introgresion of the SH3 gene resistant to rust (*Hemileia vastatrix*) in improved lines of CASTILLO® variety (*Coffea arabica* L.)

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The genetic improvement at Cenicafé has developed varieties with resistance to Coffee Leaf Rust (CLR) (*Hemileia vastatrix*) derived from the Timor Hybrid. These varieties have durable rust resistance, especially against race II, the predominant race. In Colombia, these varieties have been cultivated for three decades and still show resistance. Recently, new rust races have appeared attacking these varieties. Consequently incorporation of different resistance genes into new varieties is being sought. The present study aims to determine the presence of the SH3 resistance gene using the SCAR marker BA-124-12K-f and to evaluate its effect in F1 and F2 progenies derived from crosses of 4 lines of the Castillo® variety (*C. arabica*) with the varieties S288/23 and BA-2 introgressed with the SH3 gene. Eight F1 populations were inoculated using the detached leaf method and evaluated by the incubation and the latency periods (IP and LP). Plants 9 months old from the F2 populations were inoculated in the greenhouse and compared for sporulation presence. All F1 and F2 populations whose progenitor contained the SH3 gene-marker were resistant. However, rust resistant plants that did not present the band in the F2 progenies derived from BA-2 progenitor also had the rust resistance SH2 gene and, some plants derived from S.288 progenitor, in addition to the SH3 gene, also had the SH1 gene. The resistant F2 plants were planted in the field to be evaluated for agronomic traits and to continue the breeding process. The best progenies will be the basis future varieties aimed for durable resistance to CLR.

Key words: *Coffea arabica*, *Hemileia vastatrix*, *Coffea liberica*, SH3 gene, marker assisted selection.

INTRODUCTION

According to the International Coffee Organization (ICO, 2015), Colombia is currently the third largest producer of coffee in the world. The coffee crop contributes to the economy of millions of families around the world and approximately 529,035 in Colombia, which is responsible for a production that represents between 16 and 17% of

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National Gross Domestic product (GDP), with an annual average of 11 million bags of 60 kg (Federación Nacional de Cafeteros de Colombia FNC, 2015).

Coffee leaf rust (CLR) (*Hemileia vastatrix*) affects productivity causing losses of up to 30% in the accumulated production of a four year cycle (Rivillas et al., 2011) in susceptible varieties such as Caturra. It is now known that there are more than 50 races of the fungus which have been identified mainly in the Research Center of the Coffee Rust (CIFC) in Oeiras, Portugal (Rodrigues et al., 1975; Talhinhas et al., 2017). In Colombia, it is believed that there are more than 10 races of the fungus, with race II being the predominant one (Rivillas et al., 2011).

In order to deal with the imminent outbreaks of rust in Colombia, the National Federation of Coffee Growers of Colombia launched different varieties. The Colombia variety was launched in the 1980s (Castillo and Moreno, 1987). Later in 2000, the Tabi variety (Moreno, 2002) and more recently in 2005, the Castillo® variety and its regional components (Alvarado et al., 2005), all of them with high resistance to the disease coming from different crosses between Caturra and the Timor Hybrid. This is the genetic resource most widely used as a donor of resistance to CLR, derived from a natural interspecific cross between *C. arabica* and *C. canephora* found on the island of Timor (Bettencourt, 1973). The Colombia variety has been cultivated in the field for more than 30 years in this country. However, the cultivation of these resistant varieties in extensive areas and for very long periods favors a high selection pressure that increases the probability of occurrence of new races of the fungus. As a consequence, in the next years the gradual appearance of compatible biotypes, capable of breaking the genetic resistance displayed in the field, is expected. This is a major challenge for the genetic improvement program of the FNC (Moreno and Alvarado, 2000).

In order to offer alternatives to this problem, in the last five years Cenicafé undertook a plan to introgress the CLR-resistant SH3 gene derived from *C. liberica* (Lashermes et al., 2010) in genotypes of the Castillo® variety. First, those accessions of the Colombian Coffee Collection (CCC) bearing the gene were identified and then crossed with a group of elite lines of Castillo® with excellent agronomic characteristics (González et al., 2009).

Nevertheless, the development of resistant lines takes approximately 25 years of continuous genealogical selection based on field evaluations, including resistance to rust. To assist the selection for CLR resistance there is a marker identified by Mahé et al. (2008), linked to the SH3 gene. This marker was used to select resistant progenies in this work, allowing an earlier and a more specific selection, to incorporate the SH3 gene in new elite varieties of coffee, maintaining a durability scheme of resistance, but without sacrificing the good agronomic and productive characteristics that distinguish the current varieties.

The present study was aimed to determining the presence of the SH3 gene in genotypes derived from crosses with advanced lines of Castillo® variety, and to establish its effect on genetic resistance to rust.

MATERIALS AND METHODS

Vegetal material

F1 populations

Eight F1 populations were developed using as male parents S288/23 and BA-2 (plants 1628 and 1621) which are *C. arabica* genotypes selected in India and introgressed with the SH3 gene. As female parents, four progenies of the Castillo variety, CX2848, CX2178, CU1843 and CU1852 selected for showing rust in the field were used. It should be annotated that these four progenitors possess genes SH6, SH7, SH8 and SH9 individually or in combination and the races compatible with those are already present in the field in Colombia but are resistant to race II.

F2 populations

When the F1 populations reached reproductive maturity, were collected seeds from each of the crosses to propagate 30 F2 plants per each one. The plants selected were:

(CX2848 x S288/23) plant 7 and (CX2848 x BA-2) plant 7
(CX2178 x S288/23) plant 2 and (CX2178 x BA-2) plant 1
(CU1843 x S288/23) plant 7 and (CU1843 x BA-2) plant 1
(CU1852 x S288/23) plant 9 and (CU1852 x BA-2) plant 5

As susceptible controls, Caturra variety and the female parents were used. As a resistant control, the differential for the SH3 rust gene HW35 was used.

Molecular marker

A sequence characterized amplified region (SCAR) derived from Bacterial artificial chromosome (BAC) sequences, known as the BA-124-12K-f marker and found linked to the SH3 gene (Mahé et al., 2008), and validated by González et al. (2009) was used to identify the plants that contain the resistance gene.

Inoculum of rust

The rust inoculum used for the assembly of the different detached leaf and nursery experiments was collected in each of the four female progenitors CX2848, CX2178, CU1843 and CU1852 growing in the field. These inoculums belong to new races emerging in the field in Colombia.

DNA extraction

DNA extraction from F1 plants was performed by the method of Bernatzky and Tanksley (1986).

For the DNA extraction of the genotypes of the F2 population, the Protocol Dnaeasy Plant Mini Kit® of Qiagen was used, since the plants were in seedling stage and hence little leaf tissue was available.

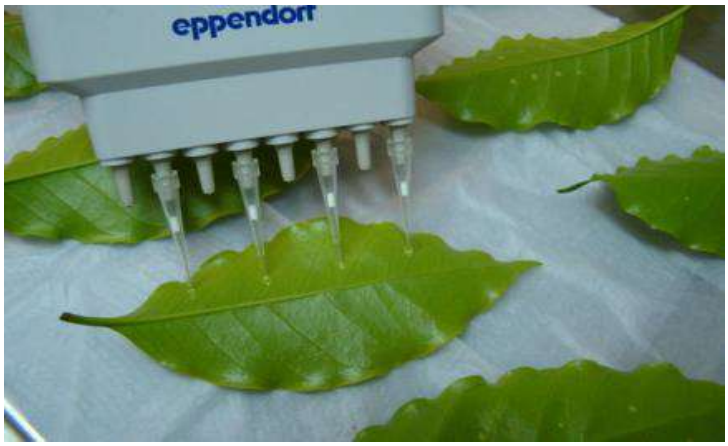

Figure 1. Inoculation of *Hemileia vastatrix.*

plants were in seedling stage and hence little leaf tissue was available.

DNA amplification

Amplification using the BA124-12K-f marker was done as described by Chen et al. (1997), with the following modifications in the PCR profile: initial denaturation was done at 94°C for 2 min, denaturalization at 94°C for 45 s, annealing at 60°C for 60 s, extension at 72°C for 75 s; then repeat the cycle from 2 to 4 for 35 cycles; next denaturation is done at 90°C for 45 s, annealing at 55°C for 60 s, extension at 72°C for 75 s; this second cycle is repeated for 30 times and terminated with an extension at 72°C for 8 min.

Marker band detection

All amplicons were evaluated on silver-stained polyacrylamide denaturing gels. Samples were prepared by mixing 10 μl of sequence loading buffer (95% formamide, 100-mM NaOH, 20-mM EDTA, 0.5 mg/mL of bromophenol blue, 0.5 mg/mL of xylene cyanol FF and deionized water) with 5 μL of amplicon, and the mix was denatured at 95°C for 5 min. For each sample, 6 μl was loaded per lane in a 4% polyacrylamide gel, and run at 2000 V, 75 W, 50 mA and 45^{o}C, maintained constant throughout the runs. Later the gels were stained with silver to visualize the bands. Gel images were scanned at high resolution and then scored by presence of the band linked to the SH3 gene.

Evaluation of rust resistance in the field

Field rust evaluations were carried out during the years 2013 to 2014, following the Eskes and Braghini scale (1981). They were done during a two years period in order to take into account the annual variation in the degree of rust attack. The evaluations were carried out in the following dates: October, 2nd, 2013, Feb.10th, July, 17th and December, 2014.

Detached leaf experiment (F1 plants):

It was carried out in the laboratory, at an average temperature of 25°C and a humidity of 80%. The light is alternated every 12 h. by means of a timer. A completely randomized experimental design was used. The experimental unit was the box, the sample unit the leaf and the number of replicates was three. From each of the plants, healthy young leaves from the second and third pair of each of the fourth upper branches exhibiting complete development were collected and immersed in a solution of DETEX® 2 ml/lt. which is a neutral liquid detergent, for 5 min and then were washed in distilled water. Eight leaves of the same genotype were placed in a pre-sterilized clear box, over a sterile paper napkin, moistened with 40 mL of sterile distilled water. The boxes were arranged randomly on a table under laboratory conditions which were measured with a thermo-hygrograph equipment. The temperature was 25°C ± 0.5 and relative humidity of 80% ±1.0. The inoculation solution with 50 mg of spores in 100 mL of sterile distilled water was applied to the underside of the leaves, depositing four drops with 10 μL of inoculum on each side of the central vein (Figure 1).

Evaluation of Incubation Period (IP) and Latency Period (LP) in each of the leaves was done according to Leguizamón (1983). Two evaluations were performed per week, starting 20 days after inoculation and for 45 days, totaling 13 evaluations.

Greenhouse experiment:

It was done for the F2 population, using 30 plants per population, 10 plants per repetition with three replicates and controls of 20 plants of Caturra and next to each crossing 6 plants belonging to the mother (the susceptible female progenitor). This method was used because the plants were only 9 months old and did not have enough mature leaves for the assembly of detached leaves; in addition the amount of genotypes to evaluate was very high to do it in the laboratory.

The inoculation was done by the spray method. The inoculum was prepared at a concentration of 50 mg of spores per 100 mL of sterile distilled water. A hose atomizer connected to a compressor was used at a distance of approximately 10 cm, to make the spray under 40 kg.m^{-2} of pressure. Eight leaves per plant were inoculated due to inoculum amount limitations in the field and the large number of plants to be assayed.

After inoculation, the plants were kept in a wet room, under dark for 48 h. A Bahnson humidifier was used to guarantee saturated atmosphere (100% relative humidity) necessary for rust infection. Subsequently, the plants were moved to the greenhouse, and organized under a randomized block design with tree repetitions. Each block or repetition was set with a row with 10 plants of each genotype, one row with 10 plants of the female parent and each

Figure 2. Distribution of blocks (lines) on the greenhouse experiment with F2 populations. Among the blocks there is a line of the Caturra variety susceptible to CLR (arrows).

block separated of the following one with a row of Caturra variety (Figure 2).

In order to identify the resistant and susceptible plants, they were evaluated twice a week until 90 days after the inoculation date, for the appearance of the rust symptoms (presence or absence of chlorosis and spores). Susceptible plants presented spores, otherwise there were resistant.

RESULTS

F1 populations

Verification of the SH3 gene-marker in the F1 populations

Molecular evaluations in three plants of each population allowed establishing the presence of the SH3 gene-marker in the F1 populations (Table 1). However, plant number 7 of the CX2178xBA-2 No.1621 cross did not present the marker, which was explained by the fact that there was no hybridization. All plants of the CU1843xBA-2 cross did not present the SH3 gene-marker. They had the BA-2 No. 1628 which was determined by molecular evaluation of the different plants from the BA-2 accession; this allowed us to conclude that the BA-2 No.1628 did not contain the SH3 gene-marker. Therefore, all of the crosses made using this plant did not present the SH3 gene-marker. On the other hand, the crosses made with the plant BA-2 No.1621 that was the progenitor that owns the gene SH3 did exhibit it. It was also observed that the susceptible controls, Caturra and females of the crosses, did not present the marker (3, a, f, g, h, i).

Detached leaves experiments

The F1 populations derived from the resistant progenitor S288/23 did not present incubation nor latency periods. They presented hypersensitivity reaction.

As expected, the control genotypes, Caturra variety and the susceptible female parents showed incubation and latency periods and the HW35 which is a differential for the SH3 gene did not present any symptoms.

The F1 populations derived from the resistant progenitor BA-2 1621 did not show incubation and latency periods with exception of one plant from the cross with CX2178. This result was explained before by the fact that there was no hybridization and it agrees with the results of the molecular analyses in which the SH3 gene-marker was not present in this plant (Figure 3j).

For the F1 population derived from the cross between CU1843 and BA-2 No. 1628 (Table 2) the plant “P1” presented incubation and latency periods; in contrast, the plants, “P2” and “P4” did not show them. As expected, none of these plants showed the SH3 gene-marker in the molecular analysis (Figure 3, c, d, e).

All of the F1 populations showing the band corresponding to the SH3 gene did not present IP and LP. For the CU1843xBA-2 No. 1628 cross, plant 1 showed susceptibility in detached leave tests and did not possess the SH3 gene; plants 2 and 4, belonging to the same cross did not have the SH3 gene band but were

Table 1. F1 Population, presence (+) and absence (-) of SH3 gene-marker.

F1 population	BA124-12K-f Gene- marker Present	BA124-12K-f SH3 gene-marker Absent
CX- 2848 x S-288/23	3	0
CU-1843 x S-288/23	3	0
CX-2178 x S-288/23	3	0
CU-1852 x S-288/23	3	0
CX-2848 x BA-2 (1621)	3	0
CU-1852 x BA-2 (1621)	3	0
CX-2178 x BA-2 (1628)	2	1
CU-1843 x BA-2 (1628)*	0	3
CX-2848 female progenitor	0	1
CU-1843 female progenitor	0	1
CX-2178 female progenitor	0	1
CU-1852 female progenitor	0	1
Caturra susceptible control	0	1
BA-2 1628* male progenitor	0	1
BA-2 1621 male progenitor	1	0
S288 male progenitor	1	0
HW35 differential of SH3 gene resistant control	1	0

*Highlighted and underlined are the crosses CX2178 and CU1843 by the BA-2 progenitor. They did not contain the SH3 gene-marker.

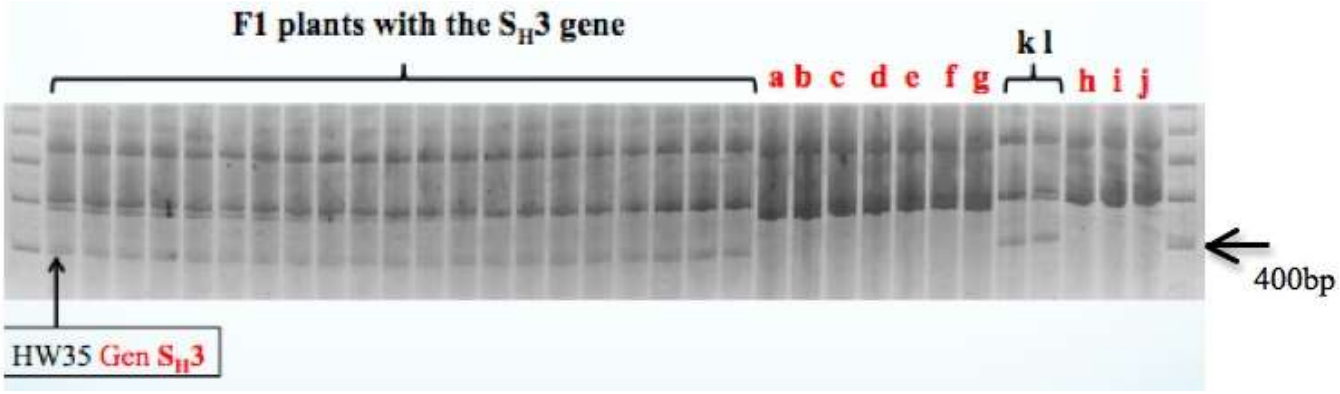

Figure 3. Image of the gel of DNA electrophoresis revealing the presence of the BA124-12K-f marker associated with the SH3 gene in F1 populations and negative controls for the SH3 gene in coffee genotypes. In order on the gel: HW35 differential that contains the band associated with the gene SH3, plants F1 with the marker; a: Caturra, b: CX2178xBA-2 P7, c: CU1843xBA-2 P1, d: CU1843xBA-2 P2, e: CU1843xBA-2 P4, f: CX2848, g: CX2178, h: CU1843, i: CU1852, j:BA-2 (1628) (Genotypes without the SH3 gene); K: BA-2 (1621) and l: S288 / 23 (progenitors with SH3 gene).

resistant. This result can be explained if the SH2 gene present in the BA-2 is in heterozygous form, and therefore, it produced resistant and susceptible plants (Castillo et al., 1976).

Plants 2 and 4 of crosses by BA-2 No. 1628 did not have the gene SH3, but they possessed the gene SH2, which also confers resistance to rust. The plant No. 1 of hybrid CU1843xBA-2 No. 1628 did not possess the SH3 gene, nor did the SH2 gene as explained above and showed IP and LP (Table 2).

The results of the field evaluations coincided with the detached leaves evaluations. Only plant 1, belonging to the cross of CU1843xBA-2 No. 1628 showed the disease, which is explained by the absence of the SH3 and SH2 genes. The rest of the plants so far are still unaffected by rust (*H. vastatrix*).

Table 2. Incubation and latency periods for populations of the CU1843 x BA-2 No. 1628 cross.

Genotype	Days to			SH3 gene-marker presence
	Box	IP	LP	
CU1843XBA-2 P1	1	30	41	-
CU1843XBA-2 P1	2	43	48	-
CU1843XBA-2 P1	3	42	43	-
CU1843XBA-2 P2	1	75*	75*	-
CU1843XBA-2 P2	2	75*	75*	-
CU1843XBA-2 P2	3	75*	75*	-
CU1843XBA-2 P4	1	75*	75*	-
CU1843XBA-2 P4	2	75*	75*	-
CU1843XBA-2 P4	3	75*	75*	-
HW35	1	75*	75*	+
HW35	2	75*	75*	+
HW35	3	75*	75*	+

*When the number of days for IP and LP is 75, it indicates that there was no incubation and latency periods. Presence (+), absence (-) of SH3 gene.

F2 populations

Greenhouse experiment

After DNA extraction, the SH3 gene was verified by the specific molecular marker BA124-12K-f. Thirty F2 plants of each of the 8 crosses of each of the female parents CX2848, CX2178, CU1843 and CU1852 by each of the male parents (BA-2 and S288 / 23) for a total of 240 plants, were evaluated for the presence or absence of the SH3-marker and for the presence or absence of rust symptoms in F2 populations (Table 3).

CX2848XS288/23 and CX2848xBA-2

For the 30 plants of the CX2848xS288/23 cross, 26 plants were resistant in the greenhouse tests and 15 of them showed the band corresponding to SH3 gene-marker.

In the case of the plants of the CX2848xBA-2 (1621) cross, 27 plants were resistant and 7 presented the band for the SH3 gene-marker.

CX2178XS288/23 and CX2178xBA-2

For the 30 plants of the CX2178xS288/23 cross, 23 plants did not present rust and from them 4 did not present the band associated to the SH3 gene. For the 27 plants of the CX2178 xBA-2 cross, 18 plants were resistant and 6 had the band linked to the SH3 gene.

CU1843xBA-2 and CU1843XS288 / 23

According to the results of Table 3, for the 30 plants of the cross CU1843x BA-2 none had the SH3 gene-marker. However there were 15 resistant plants. Looking at the 30 plants of the cross CU1843xS288/23, 23 plants showed resistance to CLR and 18 had the band associated to the SH3 gen.

CU1852xBA-2 and CU1852xS288 / 23

Of the 26 plants of CU1852xBA-2 cross, 25 presented resistance and 20 plants contained the band associated with the SH3 gene. For the 30 plants of the cross CU1852xS288/23 there were 29 resistant plants and 23 of them showed the band for the SH3 gene.

DISCUSSION

F1 populations

Evaluations of resistance to rust on detached leaf experiment

In the present study, 240 genotypes belonging to improved lines of Castillo® introgressed with *C. liberica* were evaluated molecularly using the BA-124-K-f marker associated to the SH3-gene that confers resistance to CLR, which was corroborated by detached leaves experiment and field evaluations (data not presented). CX2178xBA-2 No.1621 cross did not present the marker, which was explained by the fact that there was no hybridization. None of the plants from the cross CU1843 x BA-2 (1628) showed the SH3 gene-marker.

Molecular evaluations in three plants of each population allowed establishing the presence of SH3 gene in F1 populations. However, plant number 7 of the this could be

Table 3. Evaluation of the marker linked to the SH3 gene and its reaction to CLR in eight progenies F2.

F2 progeny	Marker linked to SH3 gene			
	Absence		Presence	
	Without rust	With rust	Without rust	With rust
CX2848xS288/23	11	4	15	0
CX2848xBA-2	20	3	7	0
CX2178xS288/23	4	7	19	0
CX2178xBA-2	12	9	6	0
CU1843xBA-2	15	15	0	0
CU1843xS288/23	5	7	18	0
CU1852xBA-2	5	1	20	0
CU1852xS288	6	1	23	0

explained by molecular evaluation of different plants of BA-2 accession, which allowed us to establish that the BA-2 No. 1628 did not contain the SH3 gene-marker. Therefore, all of the crosses made using this plant did not present the SH3 gene-marker. On the other hand, the crosses made with the plant BA-2 No.1621 that was the progenitor that owns the gene SH3 did exhibit it.

As mentioned, F1 populations were obtained from crosses with male parents carrying the SH3 resistance gene from crosses carried out by the breeding program of India. Specifically the male progenitor S288/23 was selected after a process of successive self-fertilization from the accession S.26 (*C. arabica* x *C. liberica*). The male progenitor BA-2, was derived from the F1 of S288/23 x Kent variety (Prakash et al., 2004). The molecular marker (BA-124-12K-f) was reported by Mahé et al. (2008) as a marker linked to the SH3 gene. Gonzalez et al. (2009) used it to identify the gene band in our F1 populations. The presence of the SH3-marker was confirmed by rust resistance observed in all plants of our F1 population.

The detached leaves evaluations coincided with the molecular evaluations, with the exception of crosses by CU1843xBA-2 (1628) plants 2 and 4 that did not possess the SH3 gene and yet did not develop a latency period, which is explained by the presence of the SH2 gene in the BA-2 parent (Castillo et al., 1976).

The remaining crosses that presented the band corresponding to the SH3 gene did not develop incubation period and latency period, indicating their resistance to rust. Similar results were obtained by Prakash et al. (2011) when he did MAS using the same marker in F2 progenies of crosses between *C. arabica* and the S. 795 donor of the SH3 gene.

The results of field evaluations were consistent with molecular and detached leaves evaluations. Those that presented the band linked to the SH3 gene and were resistant in the laboratory, also showed resistance in the field. This indicates that the races compatible with this resistance gene did not yet exist in the field.

In addition, the results for two plants of CU1843xBA-2 that did not possess the SH3 gene and yet did not develop a latency period was explained by the resistance conferred by the SH2 gene that is part of the genetic composition of the BA-2 (Castillo et al., 1976).

Therefore it is important to use the SH2 gene as a new source of resistance to rust, pyramided together with the SH3 gene, which may allow the achievement of more durable resistance.

F2 populations

Evaluations of resistance to rust on greenhouse experiment

In the evaluation, segregation of resistant and susceptible plants was observed, which is to be expected in this generation. In F2 populations the two alleles codifying for each trait are segregated during gamete production. This means that each gamete will contain a single allele for each gene allowing the maternal and paternal alleles to recombine to ensure variation in their offspring.

The fact that the susceptible control and the female progenitors showed CLR, guarantee that the inoculation was efficient and the inoculum used was not of race II. Because of that the results on the F2 plants is reliable. On the other hand the evaluation was done using very unequivocal variables such as presence or absence of chlorosis and spores twice a week for three months. Besides, the progenies in the greenhouse were organized on blocks with three repetitions. All of these factors allow the obtention of reliable results.

The plants that showed the SH3 gene-marker were resistant to rust (Table 3). This suggests that races that recognize it have not yet developed in Colombia. Besides, some plants that did not present the band associated to the SH3 gene showed resistance, which implies presence of other resistance genes.

In the case of crosses with the BA-2 progenitor, 52 resistant plants did not show the SH3 gene-marker, which is explained because, in addition to the SH3 gene,

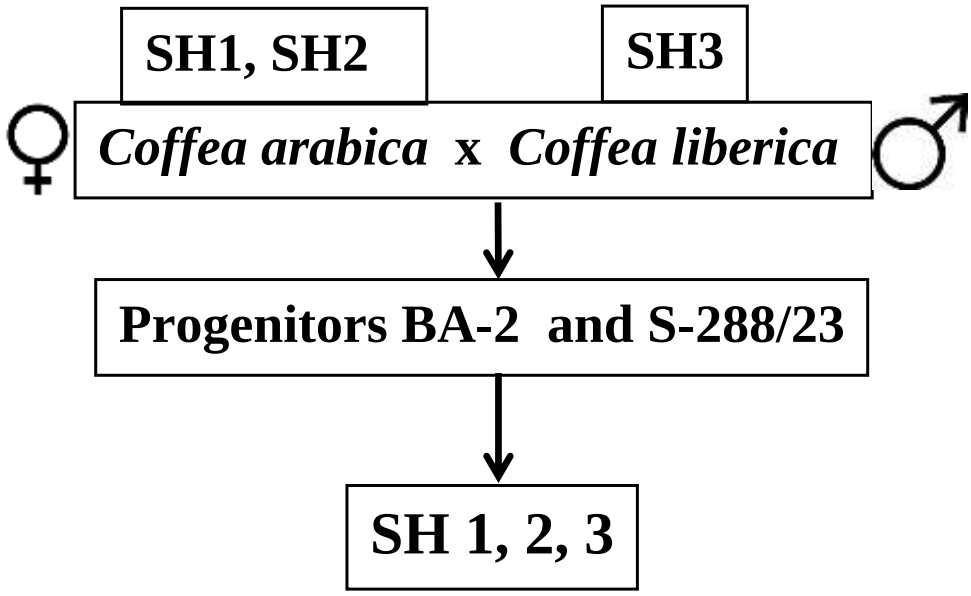

Figure 4. Genealogy of male progenitors BA-2 and S288/23 used to obtain F2 populations.

they also contain the SH2 gene (Castillo et al., 1976). Resistance in the 26 plants derived from crosses with the S.288 progenitor that did not have the presence of the SH3 gene, was probably due to the SH1 gene that according to Ramachandran and Srinivasan (1979) is also carried by this progenitor.

Comparing greenhouse and detached leaf evaluations, it can be said that both have advantages and disadvantages.

In the lab less time is required to obtain the results of inoculation, while in the greenhouse the experiment should be kept for longer periods of time to assure that there is enough time for the symptoms to appear. The inoculation is easier by aspersion in the greenhouse, although the amount of inoculum required is higher. From the point of view of plant breeding, the greenhouse evaluation is more convenient, given that during the breeding process, it is necessary to have the plants in greenhouse before planting them in the field. Furthermore, there is no limitation of infrastructure as there is in the detached leaf experiment.

So far, the resistance genes that have been used, have provided resistance with significant durability, but over time the rust races have evolved to the point of developing disease. For this reason, it is also important to use the plants that showed resistance in these evaluations, because they contained different rust resistance genes besides the SH3 (Figure 4). The genes SH1, 2, 3, 4 and 5 do not provide durable resistance because they have been used individually as mentioned by Prakash et al. (2005) in India. The use of those genes in mixture with the ones derive from Timor hybrid, in a multiline variety will allow durable resistance, as have been demonstrated with the Colombia variety, in which this strategy has been used and after 30 years is still resistant. In general, this strategy consist in utilize the higher number of resistance genes as possible as a diversity strategy (Browning and Frey, 1969). Van der Vossen (2005) also states that durable resistance is related to the effect of a combination of genes, either by stacking (pyramiding) of major genes, or by the accumulation of several minor genes. This implies that the CLR needs to have much more mutations for the variety to become susceptible.

In studies conducted by Hiroshi et al. (2007), SH1, SH2 and SH4 genes from *C. arabica* in combination have provided lasting resistance. Therefore, if the resistance of the genotypes belonging to the F2 population, which do not carry the SH3 gene, comes from some combination of SH1, and SH2, it increases the variability and durability of rust resistance (*Hemileia vastatrix*). Therefore, derived progenies containing all these resistance genes should be maintained in the successive selection cycles evaluating them for agronomic traits in order to select the best, and advance to the F3 generation.

According to Browning (1974), the equilibrium observed on natural ecosystems is based on combination of different mechanisms of resistance, which include general and specific resistance, tolerance and antagonisms between pathogens and non-pathogens. In Colombia, based on those principles, the breeding program has as strategy, namely genetic diversity, to develop resistant varieties.

That is to say that the varieties are composed of several lines with different combination of genes. This strategy have proved successful because after thirty year of the varieties liberation, they are still resistant. Indeed the combination of different sources of resistance (SH1,

SH2 and SH3) with resistance lines that have combinations of resistant genes from Timor Hybrid, will be of great value in the breeding program.

Unlike the field evaluations that have a response to the race or races prevalent in the field, the methodologies used for the evaluation of resistance allow to choose the inoculum from the race or races that need to be evaluated.

Conclusion

This work confirms that the SH3 gene from *C. liberica*, provides genetic resistance to rust to the genotypes derived from crosses with advanced lines of the Castillo® variety. The absence of rust in the progenies F1 and F2 evaluated, which contain the SH3 gene from *C. liberica*, indicates that the rust gene Vr3 compatible with this gene (SH3), is not yet recognized by the host plant, and consequently, at the moment there are no races that contain this gene in the field. The implementation of the BA-124-12K-f marker in selection of resistant plants that contain the SH3 gene allows doing earlier selection.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Agronomic performance evaluation of cowpea [*Vigna unguiculata* (L.) Walp] varieties in Abergelle District, Northern Ethiopia

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Cowpea (*Vigna unguiculata* L.) is one of the most important grain legumes in the semi-arid regions of many African countries including Ethiopia. However, it is less cultivated and scarcely distributed pulse crop in Abergelle due to a lack of improved varieties. A field experiment was conducted during the 2014 and 2015 main cropping seasons using a randomized complete block design with three replications to evaluate seven cowpea varieties for yield and yield related traits under rain-fed conditions at Abergelle Agricultural Research Center on station. Analysis of variance of data showed significantly varietal differences at P< 0.05 for days to 50% flowering; pod filling period, 90% physiological maturity, pod length and plant height, number of seeds per pod, seed yield, grain yield, biomass yield and thousand seed weight. However, no significant varietal difference was observed for harvest index. Bekur had the highest seed yield (14.85 qt.ha^{-1}) followed by Bole (13.57 qt.ha^{-1}), while the lowest seed yield was observed from BEB (6.71 qt.ha^{-1}). Overall, Bekur and Bole had better performance compared to the other varieties for yield and the yield related traits. As compared to the rest, these two varieties were therefore recommended as promising varieties to the farmers of Abergelle area and other districts having the same agro-ecologies based on their optimal performance for adoption.

Key words: Optimal performance, yield, yield related traits, *Vigna unguiculata* L.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is a leguminous plant belonging to the Fabaceae family. It is an important grain legume in drier regions and marginal areas of the tropics and sub-tropics. The grains are good source of human protein, while the haulms are valuable source of livestock protein (Dereje et al., 1995; Fatokum, 2002). It is the second most important food grain legume crop in tropical Africa, next to *Phaseolus vulgaris*, the common bean (Arnon, 1972). Nigeria, Niger, Burkina Faso, Uganda and Senegal grow cowpeas for the market, but they are widely grown as a subsistence crop for human use in nearly all sub-Sahara African countries.

Cowpea is a warm-weather crop with somewhat higher temperature requirement than maize. Cowpea is drought

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tolerant annual crop in condition where moisture deficiency has less effect on seed formation. It grows with less rainfall and under more adverse condition than haricot bean. Like other leguminous crops, it is also grown for improving soil fertility (Tesema and Eshetayehu, 2003). Cowpea withstands heat better than most other legumes do. Cowpea can be grown in a wide variety of soils, but yields better on well-drained soils with medium fertility (Arnon, 1972).

Cowpea is a principal food legume in many African countries including Ethiopia, where tender leaves, fresh pods and grains are consumed. In Ethiopia, cowpea seed is mostly used in the form of food dishes *'nifro', 'shiro'* etc. Cowpea provides feed, forage, hay, and silage for livestock, and green manure or maintaining the productivity of soils. When intercropped with cereals, it compensates for the loss of nitrogen absorbed by cereals through nitrogen fixation. It is also a good cover crop that limits soil erosion (Onwueme and Sinha, 1991).

Early maturity and moderate degree of drought tolerance led the crop's vital role in farmers' strategies for risk aversion in drought prone lowland areas of the country (Fikru, 2007). However, yield per unit area is very low especially in Abergelle, Northern Ethiopia. The major constraints that limit the production of cowpea are low productivity of the crop at the farmer's level, moisture stress, absence of improved high yielding varieties, low soil fertility, losses due to insect pests and disease. Hence, this study was intended to select the best performing and suitable improved variety/varieties of cowpea for their yielding ability in the moisture stressed areas of Abergelle district.

MATERIALS AND METHODS

Description of the study area

The field experiment was carried out under rain-fed conditions at Abergelle Agricultural Research Center on station during the 2014 and 2015 main cropping seasons. Abergelle is located in the central zone of Tigray region at about of 903 km north of Addis Ababa and 120 km south west of 'Mekelle' and situated at 13°14'06"N latitude and 38°58'50"E longitude. The area is agro-ecologically characterized as hot warm sub-moist lowland (SMI-4b) located at an altitude of 1450 masl. Plains, hills and river valley, characterize the topography of the district and it is highly exposed to soil erosion. Most soils of the district are dominated by sandy textured with poor water holding capacity and less fertile hence most crops failed to produce good yield (Dereje et al., 2007).

The dominant soil types of the study area are fine-grained ones called 'walka', 'bahkel', 'hutsa', and 'mekayih'. The average annual rainfall varies from 350 to 650 mm and the temperature ranges from 18 to 42°C. The distribution of rainfall is erratic and variable, which results in strong variation in crop yields (Dereje et al., 2007). The rainfall distribution is unimodal, concentrated during the summer (July to August) leading to one cropping season per year (Belay and Meresa, 2017).

Experimental design and crop management

Six cowpea varieties, sourced from Melkasa Agricultural Research Center (MARC) and one local check from Abergelle area, were evaluated in this study (Table 1).

These varieties were planted under field conditions in a randomized complete block design (RCBD) replicated thrice. The plot size was 4 × 3.2 m (12.8 m^2) having 8 rows with harvestable plot area of 1.6 × 4 m (6.4 m^2) with four rows and spacing 40 cm between rows and 10 cm between plants was maintained. The spacing between replication, blocks and plots within each block was 1.50, 1 and 0.50 m, respectively. Di-ammonium phosphate (DAP) fertilizer was applied at a rate of 100 kg ha^{-1} at planting. Livestock were excluded by fencing. No irrigation was applied. Weeds were controlled periodically by hand weeding and other management practices like pest or disease-control was done as required.

Data collection and sampling techniques

Phenological data such as days to flowering, pod filling period and days to maturity; growth traits (plant height and pod length) were recorded. Days to flowering was recorded as the number of days form emergence to when 50% of the plants had flowered in a plot. Seed filling period was recorded as days from flowering to maturity. Days to 90% maturity is the number of days from emergence to the stage when 90% of the plants in a plot have reached physiological maturity. At pod setting stage five plants were randomly selected from each plot and carefully tagged. These plants were used to measure traits like plant height and pod length. Data were also collected on seed yield and yield related traits such as number of pods per plant, number of seeds per pod, 1000 seed weight, grain yield, biomass yield and harvest index. The four central rows of each plot were harvested for grain yield. Five plants were randomly selected from the four central rows to determine the yield related traits, which consisted of number of pods per plant, number of seeds per pod. Number of pods per plant was determined by counting pods of the five randomly selected plants averaged. To determine the number of seeds per pod, total number of seeds in a pod was counted on five randomly sampled pods taken from the five randomly selected plants average for each. Thousand seed weight was recorded as the weight of one thousand seeds randomly picked and weighted. Seed yield: central four rows were threshed from each plot and seeds obtained from them was adjusted to standard moisture level (10%) per plot in grams and converted into quintal per hectare. Biomass yield: The weight in grams of sun dried above ground parts of the plants was recorded from the central four rows. Harvest index: was calculated as the ratio of seed yield to total above ground biomass yield (biological yield).

Data analysis

The collected data was subjected to combined analysis of variance (ANOVA) using the Statistical Analysis System (SAS) software version 9.1 program (SAS Institute, 2004). Means were separated using Fisher's Least Significant Difference (LSD) test at 5% level of probability as stated in Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Phenological and growth traits

The combined analysis of variance showed that varieties performed significantly differently for all the phenological and growth traits (days to 50% flowering, pod filling period, 50% maturity, plant height and pod length (Table 2).

Bekur recorded the tallest plants with an average height of 59.92 cm followed by the variety WWT with a height of

Table 1. Description of the experimental materials.

Genotype	Year of release	Seed size	Status	Source
BEB	1976	Medium	Released variety	Melkasa ARC
Bekur	2001	Medium	Released variety	Sirinka ARC
Kenkety	2012	Small	Released variety	Melkasa ARC
WWT	1976	Small	Released variety	Melkasa ARC
TVU1977OD-1	1978	Small	Released variety	Melkasa ARC
Bole	2005	Medium	Released variety	Melkasa ARC
Adengur		Small	Local cultivar	Abergelle area

ARC = Agricultural Research Center.

Table 2. Mean values of morphological traits of cowpea varieties tested at Abergelle.

Variety	DF	SFP	DM	PH	PL
BEB	45^{d}	29^{a}	74^{ab}	51.17^{ab}	15.62^{a}
Bekur	54^{ab}	19^{bcd}	73^{b}	59.92^{a}	15.43^{a}
Kenkenty	50^{c}	22^{bc}	72^{b}	37.46^{c}	11.47^{b}
WWT	55^{ab}	20^{bcd}	75^{a}	58.42^{a}	15.47^{a}
TVU	53^{bc}	22^{bc}	75^{a}	57.58^{a}	12.93^{b}
Bole	57^{a}	18^{d}	75^{ab}	51.54^{ab}	15.30^{a}
Adengur	55^{ab}	18^{d}	74^{ab}	45.91^{b}	13.07^{b}
LSD	3.4	3.6	2.54	8.14	2.05
CV (%)	3.7	3.5	1.98	9.29	8.25

Means with the same letters within the columns are not significantly different at P <0.05. DF = Days to flowering, SFP = Seed filling period (DM- DF), DM = Days to maturity, PH = Plant height (cm) PL = Pod length (cm); LSD = Least significance difference, CV (%) = Coefficient of variation in percent.

58.42 cm, while Kenkety recorded the least plant height of 37.46 cm. BEB and Kenkety had the longest (15.62 cm) and the shortest (11.47 cm) pods, respectively (Table 2). The good performance of the cowpea in the study area with sandy loam soil is in conformity with the findings of Rayar (1986) and Futuless and Bake (2010) who reported that legumes can do well on sandy loam soils which can help in producing an extensive root system that extracts moisture from lower depth of soil. The obtained result was also in line with the works of Bilatu et al (2012) and Sisay (2015). The longest days to 50% flowering (57, 55 and 55) were observed for the varieties Bole, WWT and Adengur (local), respectively. Bekure and Bole filled their seeds in a short period of time (18). Kenkety matured earlier than the other varieties thus making it more adaptable in drought prone areas. However, no single variety of cowpea can be suitable for all conditions, it differed from region to region and the maturity, growth habit and photosensitivity requirement depends upon the cropping systems (Ndaeyo et al., 1995).

Yield and yield related traits

Significant (P≤0.05) varietal difference was observed for yield and all yield related traits except harvest index (Table 3). The current variations in yield and yield related traits among varieties consent with previous reports of Daniel et al. (2014) and Teame et al. (2017) in common bean, Tekle (2014) in cowpea. The results from the analysis of variance for number of seeds per pod and thousand seed weight showed very highly significant (P<0.001) difference (Table 4). The number of seeds per pod ranged 10 to 14. The highest number of seeds per pod was obtained from Bekur followed by Kenkenty, while the lowest number of seeds per pod was obtained at BEB. However, the highest thousand seed weight was recorded from BEB variety (130 g) followed by Bole (118) and the lowest thousand seed weight recorded from local cultivar (67 g). Bekur and Bole had the highest seed yield of 14.85 and 13.57 qt.ha^{-1}, respectively, while BEB had the lowest yield of 6.71 qt.ha^{-1}. Finally, the highest biomass yield was observed in Bole (33.93 qt.ha^{-1}) followed by Bekur (32.72 qt.ha^{-1}), whereas the lowest biomass yield was recorded from BEB (17.97 qt.ha^{-1}).

Based on the result from the present study, cowpea yield in the study area is low. The adoption of early maturing varieties, as done in this study, can help in minimizing this problem. Early maturing varieties have been shown to yield better than the late maturing varieties.

Table 3. Mean values of yield and yield components of cowpea varieties tested at Abergelle.

Variety	NSPP	BY	SY	HI	TSW
BEB	10[b]	17.97[c]	6.71[c]	0.38[a]	130[a]
Bekur	14[a]	32.72[a]	14.85[a]	4.45[a]	106[ab]
Kenkenty	14[a]	21.82[c]	8.47[bc]	0.39[a]	85[bc]
WWT	13[a]	23.40[bc]	9.83[bc]	0.28[a]	86[b]
TVU	13[a]	22.64[c]	7.99[bc]	0.36[a]	67[d]
Bole	11[ab]	33.93[a]	13.57[a]	0.40[a]	118[a]
Small seed	12[ab]	23.24[bc]	7.93[bc]	0.35[a]	67[d]
LSD	2.88	7.27	3.31	ns	13.78
CV (%)	13.63	15.88	20.34	294.63	8.84

Means with the same letters within the columns are not significantly different at P <0.05. ns = non-significant; NSPP = number of seeds per pod, BY = Biomass yield (qt.ha-1), SY = Seed yield (qt.ha-1), HI = Harvest index, TSW = Thousand seed weight (g); LSD = Least significance difference, CV (%) = Coefficient of variation in percent.

Table 4. Mean squares of yield and other traits from analysis of variance of seven cowpea varieties grown at Abergelle area (on station).

Traits	Mean square			Mean
	Replication (DF=2)	Treatments (DF=11)	Error (DF=44)	
Days to flowering	847	48.71**	10	53
Pod filling period	128.57	44.42***	5.24	21
Days to maturity	302.29	40.0*	3.95	74
Plant height	603.57	19195**	27.57	51.71
Panicle length	0.014	8.35***	0.014	14.18
Number of seeds per pod	10.32	6.86***	0.33	12.43
Thousand seed weight	8989	1809.4***	209.8	94.14
Seed yield	24.55	28.86**	4.83	9.91
Biomass yield	52.76	105.01*	30.24	25.10
Harvest index	0.0088	0.0082	0.0052	0.373

*, ** and ***, Significant at P≤0.05, P≤0.01 P≤0.001, respectively, DF = Degrees of freedom.

Besides, they are more suitable to areas with unreliable rainfall in terms of total amount, distribution and duration where crop failure is often attributed to early cessation of rains and thereby making it adaptive to different agro ecological environments.

Conclusion

Analysis of variance of data showed significant varietal differences at P≤0.05 for days to 50% flowering; pod filling period, 90% maturity, and plant height, number of seeds per pod, grain yield, biomass yield and thousand seed weight. However, no significant varietal difference was observed for harvest index. The combined analysis result indicated that the early maturing cowpea varieties Bekur and Bole had the highest yield of 14.85 and 13.57 qt.ha^{-1} respectively. The earliness traits (days to flowering, seed filling period and days to physiological maturity) enables them to flower, pod fill and mature early, therefore escaping from moisture stress, the most important drought factors that results in reduced yield. As compared to the others, Bekur and Bole were therefore recommended as promising varieties to the farmers of Abergelle area and other districts having the same agro-ecologies based on their optimal performance for adoption.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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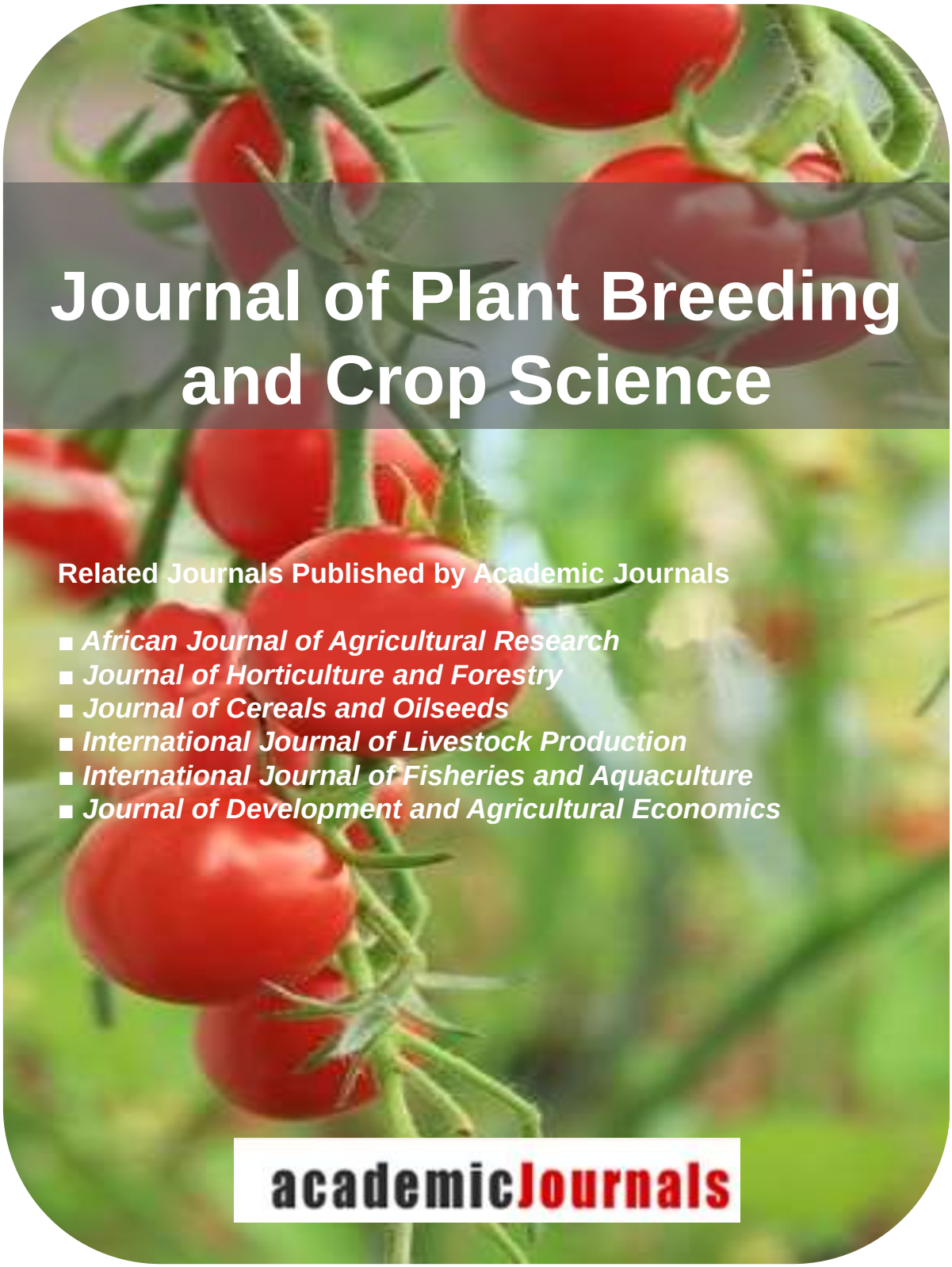

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