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**Research Article**

# Genetic diversity, discriminant and trait association analyses of *Celosia argentea* accessions

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*Celosia* (*Celosia argentea*), is an important tropical vegetable for households in sub-Saharan Africa. Despite the multifaceted usefulness, available genotypes are low-yielding, and the vegetable faces dangers of genetic erosion due to poor research attention. The magnitude and pattern of variability will guide the choice of breeding methods for improvement. Twenty-one celosia accessions were evaluated in 2018 and 2019 to determine study genetic variability and heterotic patterns among clusters. Accessions and clusters differed significantly ($p \leq 0.05/0.01$) for plant height, number of leaves/plant, stem weight, harvest index and dry matter content. Genotypic coefficients of variation; ranging from 37.89 to 0.12, were lower than phenotypic coefficients of variation which ranged from 114.55 to 0.12, both for number of leaves/plant and harvest index respectively, indicating the importance of environment in the variability. Discriminant analysis indicated low (8.12%) classification error rate, indicating the possibility of heterotic patterns among clusters. Principal component (PC) analysis controlled 73% of the observed variability among accessions and identified all measured traits as important contributors with loadings ranging from 0.30 (in PC 1) to 0.63 (in PC 2) for harvest index and stem weight respectively. Useful levels of association were also observed among measured traits. The study concluded that there was sufficient genetic variability for effective selection. Discriminant and principal component analyses identified plant height, number of leaves/plant and dry matter content as major contributors to variation among accessions. Weight of edible parts of *Celosia* can be simultaneously improved with plant height and number of leaves/plants.

**Keywords:** *Celosia*, cluster, discriminant analysis, genetic diversity, genetic erosion

## 1 Introduction

*Celosia*, Lagos spinach, is an important leaf vegetable for millions of households in sub-saharan Africa because of its multifaceted usefulness. The leaves and tender stem of Plumed cockscomb (*Celosia argentea* L) are consumed as a vegetable and the inflorescence eaten as a herb (Ilodibia et al., 2016; Olawuyi, Bamigbegbin & Bello, 2016). Despite its importance, the vegetable is underutilized and understudied (Olawuyi, Bamigbegbin & Bello, 2016) with little being done towards germplasm improvement. Deficient soil nutrition and plant competition for resources are constraints to production of *Celosia* (Ilodibia et al., 2016; Adeyeye, Ogunwale & Mofikoya, 2013). Most studies have focused on yield and/or nutritional enhancement of the vegetable through plant spacing and soil amendments (Babajide & Olla, 2014; Yagi, Modawei & Mohammed, 2014; Abdulmaliq et al., 2016). Irrigation management also plays a role in optimizing yield (Ewemoje, 2007). The authors did not consider the genetic makeup of *Celosia* where genetic information could serve as guide to making more effective inferences to improve productivity. Selection is a fundamental tool for crop improvement, and it is a function of available genetic variability within a population. The magnitude of available variability guides the choice of breeding for improvement (Oyetunde, Olayiwola & Osho, 2021; Oyetunde & Ariyo, 2015; Ariyo, 1995). 7lant breeders classify germplasm lines into groups of genetically related individuals, across which selection

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can be effective. Cluster analysis is a useful tool for defining individuals in a population into groups that were not defined from theoretical deduction, whereas discriminant analysis is a statistical tool used to classify data into known groups and can be used to assess the adequacy of a classification when members of each group are known. The main objective of discriminant analysis is to develop a set of discriminant functions that are linear combinations of independent variables that will perfectly discriminate between categories of the dependent variable. The accuracy of assigning members into clusters in a dendrogram can be assessed and the separation of less-related individuals enhanced. Studies by Mangaiyarkarasi et al. (2019); Olawuyi, Bamigbegbin & Bello (2016) and Ilodibia et al. (2016) have been concerned with the extent of genetic diversity in available *Celosia* germplasm. Little success has been achieved in yield and agronomic improvement of the crop. There is a need to investigate the extent and pattern of genetic diversity in available germplasm to enhance hybridization and yield improvement. This study was conducted to determine the extent of genetic diversity and relationships among *Celosia* germplasm with a view to providing useful information for future breeding programs.

## 2 Materials and methods

### *2.1 Experimental location and genetic materials used*

The study was conducted during May–July of 2018 and 2019, at the Teaching and Research Farms, Lagos State Polytechnic, Ikorodu, Nigeria. Twenty-one genotypes of *C. argentea* were used, of which 20 were obtained from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria, and the local check was obtained from a farmer in Ikorodu. The check was a genotype adapted to Ikorodu growing conditions.

### *2.2 Field establishment, layout and maintenance*

Land preparation in both years was done manually by making sunken beds 2.0 m long, 1.0 m wide and 0.25 m deep. In the 2018 trial, cured poultry manure was applied at the rate of 10 $t.ha^{-1}$ (Oguntade et al., 2017) at bed preparation. Seeds were sown at the rate of 13 $kg.ha^{-1}$ by drilling. For the 2019 trial, seed were sown in nursery bags filled with soil collected from 0–15 cm depth from the same Teaching and Research Farm used for the 2018 trial. The soil was thoroughly mixed before filling into bags. Seedlings transplanted 3 weeks after sowing, using a spacing of 0.05 × 0.5 m to obtain a population density of 400,000 plants·$ha^{-1}$. Organo-mineral fertilizer (OMF) was applied at the rate of 10 $t.ha^{-1}$. Genotypes were arranged in a randomized complete block design with 3 replications. A 0.5 m alley separated blocks to allow passage during field operations. In both trials, weeding was done manually.

### *2.3 Data collection*

In the field, at marketable stage; determined based on knowledge of local farmer-consumer preferences, data were collected on plant height (PHT), stem diameter (STD), and number of leaves per plant (NOL), from 10 randomly selected and tagged plants per accession per replicate. The soil in each bed was soaked to enhance easy uprooting with minimal loss of root hairs. Subsequently, all plants in each plot were harvested by uprooting. Roots of the plants were rinsed, and plants air-dried briefly to determine the plant fresh biomass weight (data used to determine harvest index). The root of each plant was cut and discarded, leaving the stems and leaves which are the consumable parts of the plant. Stem weight (STWT) was converted to $kg.ha^{-1}$, and harvest index (computed as percent stem weight; with leaves intact, in plant fresh biomass) were determined. Dry matter content (DMC) was estimated in percent of stem weight of each accession after oven-drying in a DHG-9202 Electrothermal Thermostatic Oven at the Environmental Biology Laboratory of Lagos State Polytechnic, Ikorodu, Lagos State, Nigeria. Bags containing each accession per replicate were removed from the oven and weighed. Bags were not removed at the same time and were not returned when a constant weight was obtained twice. Percent dry matter content was determined and reported as grammes per 100 g of stem weight that was oven-dried.

### *2.4 Data analysis*

Data from the 2 trials were pooled for combined analysis of variance (ANOVA) across years, using SAS (ver. 9.3, SAS Inst. Cary, NC). Means, when variation among genotypes was significant by the *F*-test, were separated using Duncan's multiple range test. Genetic, phenotypic and environmental variance estimates were estimated from expected mean squares obtained using PROC VARCOMP in SAS. Genotypic (GCV) and phenotypic coefficients of variation (PCV) were computed according to Singh and Chaudhary (1985). For each trait, broad-sense heritability ($H_B$) was computed according to Falconer (1989); genetic advance (GA), percent of mean, was estimated following Shukla et al. (2006).

Pearson correlation coefficients (from SAS) were used to investigate the level of relationship among characters; loadings from principal component analysis (from SAS) were used to identify traits with significant contributions to variation among accessions. Dendrogram of relatedness among accessions was constructed using

single linkage cluster analysis (SLCA) of means of traits for which accession mean squares were significant. Data for 3 distinct clusters, at 50% level of genetic similarity, were compared using the ANOVA *F*-test with cluster rather than genotype as source of variation, and significantly different cluster means separated using DMRT. The clusters were compared in pairs using the *t*-test in MS Excel. Fisher's discriminant functions as well as clustering error rates were estimated using SAS.

## 3 Results and discussion

Analysis of variance revealed that variation attributable to genotypes, years, and genotype × year interaction was significant ($p \leq 0.05$ or 0.01) for plant height, number of leaves per plant, and stem weight (Table 1). Additionally, variation attributable to genotypes and years was significant for dry matter ($p \leq 0.05$ or 0.01) and stem diameter, while genotype and genotype × year interaction effects were significant ($p \leq 0.01$) for harvest index. The significant genotype effects for all the measured traits except stem diameter indicated native genetic variability among the accessions, which suggested prospect of good progress from selection under the experimental conditions. Also, significant variation attributed to years indicated that the growing conditions in 2018 and 2019 were distinct and adequately discriminated among the *Celosia* genotypes. The significant genotype × year

**Table 1** Mean squares and means of selected traits of *Celosia argentea* accessions evaluated in Ikorodu, Nigeria in 2018 and 2019 ·

| Genotype ID | Genotype name | Plant height (cm) | Number of leaves per plant | Stem diameter (mm) | Stem weight ($t \cdot ha^{-1}$) | Harvest index (%) | Dry matter content ($g.100\ g^{-1}$ stem weight) |
|---|---|---|---|---|---|---|---|
| **G1** | NGB00125 | 20.09ab | 25.68def | 6.87bc | 6.39de | 79.82efg | 2.29a-f |
| **G2** | NGB00126 | 21.87ab | 22.62efg | 6.79bc | 8.60b | 81.68ed | 1.36d-g |
| **G3** | NGB00127 | 20.44ab | 23.85d-g | 7.33bc | 4.21ghi | 85.67cb | 1.39d-g |
| **G4** | NGB00128 | 19.83ab | 24.53def | 6.54bc | 6.63de | 88.56ab | 1.73c-f |
| **G5** | NGB00129 | 25.38a | 30.90b-e | 8.07ab | 5.77def | 76.00hg | 1.75c-f |
| **G6** | NGB00130 | 19.92ab | 25.48def | 6.62bc | 3.05i | 51.36k | 1.14gf |
| **G7** | NGB00133 | 16.19dbc | 18.10fg | 5.87bc | 7.00de | 84.33cd | 1.62c-f |
| **G8** | NGB00137 | 22.27ab | 30.71b-e | 8.31ab | 8.78b | 79.53efg | 2.40a-e |
| **G9** | NGB00151 | 24.78a | 33.77a-d | 7.58abc | 5.83def | 82.72cde | 3.32a |
| **G10** | NGB00155 | 23.59a | 24.56def | 7.08bc | 8.43bc | 79.33efg | 1.93b-f |
| **G11** | NGB00171 | 21.68ab | 35.60abc | 6.00bc | 7.20dc | 82.76cde | 2.44a-d |
| **G12** | NGB00172 | 18.30abc | 23.76d-g | 10.20a | 5.53efg | 79.28efg | 1.24efg |
| **G13** | NGB00173 | 18.43abc | 21.60efg | 6.27bc | 10.83a | 82.47cde | 1.69c-f |
| **G14** | NGB00174 | 19.06abc | 21.21efg | 7.03bc | 4.78fgh | 76.67fgh | 1.70c-f |
| **G15** | NGB00176 | 12.77dc | 14.13g | 4.80c | 2.93i | 57.63j | 0.38g |
| **G16** | NGB00179 | 20.45ab | 38.10ab | 8.06ab | 3.77hi | 80.60def | 1.85c-f |
| **G17** | NGB00180 | 10.84d | 30.17b-d | 6.80bc | 11.23a | 89.37a | 2.59abc |
| **G18** | NGB00181 | 20.72ab | 23.61d-g | 5.91bc | 4.33ghi | 69.33i | 1.32d-g |
| **G19** | NGB00182 | 23.87a | 42.42a | 8.85ab | 6.11def | 74.67h | 2.98ab |
| **G20** | NGB00185 | 18.83abc | 24.08d-g | 7.02bc | 5.98def | 79.67efg | 1.45c-g |
| **G21** | Local check | 18.91abc | 26.44c-f | 7.26bc | 5.83def | 69.19i | 1.75c-f |
| **Source** | DF | | | | | | |
| **Replication** | 2 | 239.45** | 248.78ns | 19.76* | 2529735.00ns | 0.20** | 95.96* |
| **Year (Y)** | 1 | 2582.55** | 11781.74** | 386.33** | 279998726.30** | 0.02ns | 15.14ns |
| **Genotype (G)** | 20 | 445.87* | 309.34** | 6.77ns | 2897860.20* | 0.40** | 34.84* |
| **G × Y** | 20 | 342.07* | 222.45* | 7.14ns | 2513621.11* | 0.20** | 10.79ns |
| **Error** | 82 | 132.61 | 112.56 | 5.57 | 1556936 | 0.01 | 20.71 |

DF – degrees of freedom; ns, *, ** – not significant or significant at 95 and 99% confidence levels respectively

interaction effect for noted traits implied that the accessions were inconsistent in their performance in the two research years but were stable with respect to stem diameter and dry matter content across the two years. The significance of year and genotype × year interaction effects indicated that the growing conditions in the individual years were unique, and the genotypes behaved differently from one environment to another, and genotype selection would be inconsistent from one environment to another. Thus, different genotypes could be identified for possible release and commercialization in the different environments. Olawuyi, Bamigbegbin & Bello (2016) reported similar observations for vegetative and yield characters among 10 genotypes of *Celosia*.

The dendrogram obtained from SLCA, showing the grouping of the accessions is presented in Fig. 1 while the *F*-test levels of significance among clusters are displayed in Table 2. Cluster effect was significant for all the measured traits, except stem diameter, similar to observations earlier made for genotype effect. This is an indication of possibility of improvement through selection across clusters for the respective traits. Accessions in cluster 2 consistently had highest mean performance for all the measured traits, except stem weight, which was highest in cluster 3 accessions. This implied the ability of the clustering procedure to identify clear heterotic groups among the evaluated genotypes and suggested the possibility of genotype selection across clusters for future breeding strategies.

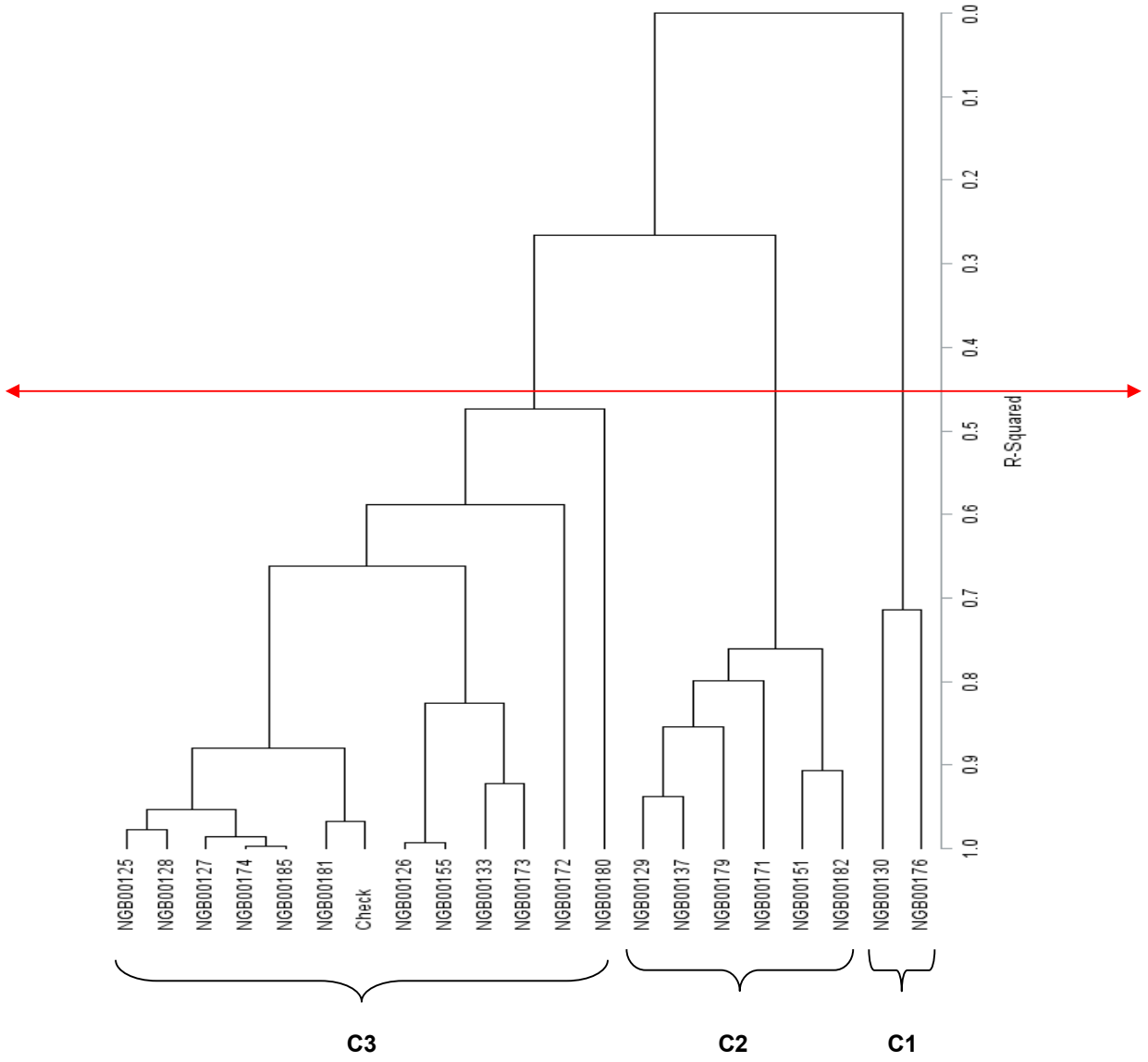


**Figure 1** Dendrogram of relatedness among accessions (X-axis) of *Celosia argentea*, based on genetic similarity (Y-axis) from Single-Linkage Cluster analysis. The red double-arrowed line delineates the accessions into clusters at approximately 55% level of similarity; C1, C2, C3 are clusters 1, 2, and 3, respectively

**Table 2** Level of significance from *F*-test among clusters, with cluster means for measured traits in Ikorodu during 2018 and 2019 growing seasons

| Cluster | Member genotypes | Plant height | Number of leaves per plant | Stem diameter | Stem weight | Harvest index | Dry matter content |
|---|---|---|---|---|---|---|---|
| **1** | G6, G15 | 16.35b | 19.81b | 5.71b | 2.99b | 0.544b | 0.76c |
| **2** | G5, G8, G9, G11, G16, G19 | 23.07a | 35.25a | 7.81a | 6.24a | 0.80a | 2.46a |
| **3** | G1, G2, G3, G4 G7, G10, G12, G13, G14, G17, G18, G20, G21 | 19.01b | 23.86b | 7.00ab | 6.91a | 0.79a | 1.70b |
| **Level of significance of cluster effect** | | ** | ** | ns | ** | ** | ** |

** – significant at 99% confidence level; ns – not significant; values in columns followed by the same letter(s) are not significantly different, $p \leq 0.05$; G1 – G20 described in Table

**Table 3** Level of significance from *T*-test among clusters of *Celosia argentea* accessions

| Cluster pair-wise comparison | Plant height | Number of leaves per plant | Stem diameter | Stem weight | Harvest index | Dry matter content |
|---|---|---|---|---|---|---|
| **Cluster 1 vs Cluster 2** | ** | ** | ** | ** | ** | ** |
| **Cluster 1 vs Cluster 3** | ns | Ns | ns | ** | ** | ** |
| **Cluster 2 vs Cluster 3** | ** | ** | ns | Ns | ns | ** |

ns, ** – not significant or significant at 99% confidence level

The *t*-test among pairs of clusters (Table 3) revealed that cluster 1 was significantly ($p \leq 0.01$) different from cluster 2 for all the traits measured and significantly ($p \leq 0.01$) different from cluster 3 for stem weight, harvest index and dry matter content. Clusters 2 and 3 were found to be significantly ($p \leq 0.01$) different for plant height, number of leaves per plant and dry matter content. Thus, there is possibility of improvement through inter-cluster hybridization.

Estimates of genetic components of *Celosia* accessions are shown in Table 4. The values of genotypic variance ranged from 0.01 for harvest index to 102.56 for number of leaves per plant, while the phenotypic counterparts ranged from 0.01 for harvest index to 937.49 for number of leaves per plant. The differing genotypic and phenotypic values for all measured traits except harvest index are an indication of the influence of environment in the expression of these traits. Thus, offspring of the celosia genotypes will differ from the parents for the measured traits due to environmental variance except for harvest index for which genotypic variance was equal in magnitude to phenotypic variance indicating no influence of the environment (Hallauer and Miranda, 1988). Heritability has been described as the level of correspondence between the phenotype and the breeding value (i.e., the genotype) for a particular trait. Thus, heritability is the percentage of phenotypic variance that is due to genetic variance (Hallauer and Miranda, 1988). In this study, values of broad-sense heritability ranged from low (less than 30% for number of leaves per plant, stem weight and stem diameter) through medium (30–60% for plant height and dry matter content) to high (100% for harvest index). Thus, environmental influence is minimal on number of leaves per plant, stem weight, and stem diameter while plant height and dry matter content had moderate environmental influence with harvest index having no influence of the environment in expression. Comparable reports have been made on *Celosia argentea* by Olawuyi, Bamigbegbin & Bello (2016). The GCVs were low to moderate, with a range from 0.12% for harvest index to 37.89% for number of leaves per plant, while phenotypic coefficients of variation (PCVs) were mostly higher than GCVs (also except for harvest index), and ranged from 0.12% to 114.55% for harvest index and number of leaves per plant respectively. The difference between estimates of genotypic (GCVs) and phenotypic coefficients of variation (PCVs) were observed for almost all the measured traits, indicating the important role of the environment in the phenotypic expression and inheritance of the measured traits except harvest index. Thus, harvest index, plant height and dry matter content will likely be good for selection. These observations are similar to those of Oyetunde & Ariyo (2015) on okra and Mangaiyarkarasi et al. (2019) on *Celosia argentea*. Estimates of genetic advance ranged from 0.25 for harvest index to 44.62 for dry matter content. According to Ogunniyan & Olakojo (2015), high heritability is not always associated with high genetic advance. Thus, the low genetic gain for harvest index is adequately compensated for by the high heritability estimate. The

moderately high estimate of genetic advance for dry matter content further strengthens its usefulness for selection unlike stem diameter and stem weight with low estimates of both heritability and genetic advance.

Selection across groups can only be effective when between-group variability is higher than within-group variability. This can be enhanced through appropriate classification of germplasm lines into subgroups. Discriminant analysis (Table 5) revealed a total of 8.12% error rate in classifying the accessions into the three clusters. Two accessions were appropriately classified into cluster 1 (error rate = 0) while each of clusters 2 (six accessions) and 3 (13 accessions) had one accession misclassified, generating error rates of 16.67 and 7.69%, respectively. The low classification error rates suggested the possibility of improvement through selection across clusters. Values of Fisher's linear discriminant functions showed that traits with significant ($p$ ≤0.05/0.01) contributions to classification were plant height, number of leaves per plant, harvest index and dry matter content. These traits, except harvest index, were also identified by the principal component analysis (Table 6) as major contributors to the observed variation among the accessions.

**Table 6** Loadings from principal component analysis of *Celosia* accessions grown in Ikorodu

| Factor | PC1 | PC2 |
|---|---|---|
| **Plant height** | 0.54 | -0.07 |
| **Number of leaves per plant** | 0.56 | -0.053 |
| **Stem diameter** | 0.51 | -0.12 |
| **Stem weight** | -0.15 | 0.63 |
| **Harvest index** | 0.13 | 0.58 |
| **Dry matter** | 0.30 | 0.50 |
| **Eigen value** | 2.72 | 1.65 |
| **Proportion of variation** | 0.45 | 0.27 |
| **Cumulative variation** | 0.45 | 0.73 |

PC – principal component

**Table 4** Variance component, heritability and genetic advance estimates of *Celosia argentea* accessions for measured traits

| Component | Plant height | Number of leaves per plant | Stem diameter | Stem weight | Harvest index | Dry matter content |
|---|---|---|---|---|---|---|
| **$\sigma^2g$** | 4.02 | 102.56 | 0.24 | 0.93 | 0.01 | 0.25 |
| **$\sigma^2e$** | 8.55 | 834.93 | 1.36 | 4.20 | 0.00 | 0.19 |
| **$\sigma^2p$** | 12.57 | 937.49 | 1.60 | 5.13 | 0.01 | 0.44 |
| **$H_B$ (%)** | 31.98 | 10.94 | 15.00 | 18.13 | 100 | 56.82 |
| **GCV (%)** | 10.07 | 37.89 | 6.92 | 15.22 | 0.12 | 27.49 |
| **PCV (%)** | 17.80 | 114.55 | 17.81 | 35.71 | 0.12 | 36.42 |
| **GA** | 12.25 | 26.94 | 5.78 | 13.94 | 0.25 | 44.62 |

$\sigma^2g$ – genotypic variance; $\sigma^2e$ – environmental variance; $\sigma^2p$ – phenotypic variance; $H_B$ – broad-sense heritability; GCV – genotypic coefficient of variation; PCV – phenotypic coefficient of variation; GA – genetic advance (% of mean)

**Table 5** Linear discriminant function coefficients and classification error count estimates for accessions within clusters

| Function | Cluster | | | Level of significance |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| **Intercept** | -154.92 | -326.10 | -276.90 | |
| **Plant height** | 4.25 | 6.12 | 5.50 | * |
| **Number of leaves** | 2.23 | 3.33 | 2.50 | ** |
| **Stem diameter** | 4.10 | 5.45 | 5.80 | ns |
| **Stem weight** | 0.21 | 0.23 | 0.43 | ns |
| **Harvest index** | 3.31 | 4.69 | 4.51 | ** |
| **Dry matter** | -10.56 | -9.40 | -9.67 | ** |
| | **cluster 1** | **cluster 2** | **cluster 3** | **total** |
| **Error rate (%)** | 0.00 | 16.67 | 7.69 | 8.12 |

ns,*, ** – not significant or significant at 95% and 99% confidence levels, respectively

**Table 7** Pearson correlation coefficients among traits of *Celosia*

| Trait | NOL | STD | SWT | HID | DM |
|---|---|---|---|---|---|
| **PHT** | 0.79** | 0.66** | -0.21* | 0.08ns | 0.34** |
| **NOL** | | 0.72** | -0.25** | 0.13ns | 0.38** |
| **STD** | | | -0.26** | 0.10ns | 0.21* |
| **SWT** | | | | 0.35** | 0.28** |
| **HID** | | | | | 0.37** |

ns, *, ** – not significant or significant at 95 and 99% confidence levels, respectively; PHT – plant height; NOL – number of leaves per plant; STD – stem diameter; SWT – stem weight; HID – harvest index; DM – dry matter content

As displayed in Table 7, useful levels of association were revealed among pairs of measured traits. Plant height had significant ($p \leq 0.01$) and positive association with number of leaves per plant ($r = 0.79$), stem diameter ($r = 0.66$) and dry matter content ($r = 0.34$). Similarly, number of leaves per plant had significant ($p \leq 0.01$) and positive association with stem diameter ($r = 0.72$) and dry matter content ($r = 0.38$). Interestingly, stem weight of celosia had significant, positive/negative association with all measured traits, indicating the possibility of stem weight improvement with the traits. Expectedly, significant and positive relationship was observed in the associations of harvest index with stem weight ($r = 0.35$) and dry matter content ($r = 0.37$) since harvest index and dry matter content were derivative of the stem weight. The significant levels of association observed among the pairs of traits indicated that these traits can be simultaneously improved with stem weight of *Celosia argentea* through selection focused on plant height, number of leaves per plant and stem diameter. Since most farmers in our locality grow the vegetable for the stem, which is harvested and sold as food, this will subsequently increase farmers' income. Significant associations have been reported in for yield (seed and/or stem + leaf) and various other traits of celosia. For instance, Ojo (2001) identified number of leaves as an indicator of yield while Olawuyi, Bamigbegbin & Bello (2016) reported significant relationships among various pairs of traits of celosia.

## 4 Conclusion

The level of genetic diversity within the *Celosia argentea* germplasm was sufficient for selection to be made. Classification of accessions into clusters elucidated useful genetic differences within the germplasm. Plant height, number of leaves per plant and dry matter content were the most important contributors to observed variation among the *Celosia argentea* accessions. Stem weight of *Celosia argentea* can be simultaneously improved with plant height and dry matter content while harvest index is good for selection in *Celosia argentea*.

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