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Characterization of *Celosia argentea* Linn. germplasm using ISSR markers

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Abstract. *Celosia argentea* is an annual leafy vegetable popularly known for its dietary and medicinal values. Hence, it is important to preserve and further improve this vegetable to enhance its numerous benefits. This study therefore investigated the genetic variability among different genotypes of *C. argentea* using ISSR primers. A total of 15 *C. argentea* genotypes were sourced from National Centre for Genetic Resources and Biotechnology (NACGRAB) in Ibadan, Nigeria and 10 genotypes sourced from different markets. The open field experiment was set up in a completely randomized design. Seeds of each cultivar were grown and seedlings transplanted. Fresh young apical leaves were harvested. DNA was extracted from young frozen apical leaves. Six ISSR primers were optimized and used in PCR with a touch-down procedure in a thermocycler. Agarose gel electrophoresis was performed, and bands were visualized. Molecular data was analyzed for total gene diversity, while morphological data was analyzed using ANOVA. The genotypes of NGB recorded the highest mean performance for plant height, leaf biomass and seed weight, while the A00 genotypes were observed to have higher values of leaf length, leaf area and root biomass. The principal component analysis showed that the first component accounted

for 42% of the total variation. The correlation matrix for growth, agronomic and yield characters show highly significant positive relationship among the growth characters at $P<0.05$. Primer UBC-866$(CTC)_6$ was highly polymorphic. Genotype A005 performed best for growth characters while NGB00182 performed best for yield characters. Genetic assessment and improvements in *C. argenta* germplasm play key role in future studies and improvements of vegetable crop.

Keywords: *Celosia argentea*, germplasm, genomic DNA, ISSR primers.

Introduction

Celosia argentea L. is an annual leafy vegetable of the genus *Celosia*, order Caryophyllales and family Amaranthaceae which shares features with members of the genus Amaranthus (Thorat, 2018). It is one of the leading vegetables in South-Western Nigeria, it is propagated by seed and grows up to 200 cm (6.5 feet) in height. The plant produces globular fruits, black seeds, and simple, spirally arranged leaves. It also frequently exhibits pink or white flowers (Ejoh *et al.,* 2021).

Celosia argentea is a tetraploid species (2n=36), though some varieties were found to be octaploid (Olawuyi *et al.,* 2016; Hussain *et al.*, 2024). The commonly cultivated *C. argentea* are the green broad-leaved cultivars (soko green), the broad-leaved cultivars with anthocyanin pigmentation of the leaf blades and part of the stem (soko pupa-red soko) and cultivars with deep green narrow leaves with a hard texture and early flowering (Grubben and Denton, 2004; Falodun *et al.,* 2022). The leaves and stems are prepared as soups, sauces or stew which could be consumed with food items such as maize, rice, yam and cassava (Bamigbegbin *et al.,* 2016). Medicinally, the stems and leaves are applied as poultice smeared in honey as treatment for infected sores, wounds and abscesses. Leaf concussions are used to relieve gastrointestinal disorders. Finely powdered or decocted seeds are considered antidiarrhoeal or aphrodisiac. Furthermore, the plant root is used for abdominal colic, gonorrhea and eczema while the whole plant serves as antidote for snake poison (Nahida *et al.,* 2012; Stuart, 2016).

Molecular markers are used to assess the influence of various factors on genetic diversity (Zhao *et al.,* 2023). Inter-Simple Sequence Repeats (ISSRs) are DNA fragments about 100 - 3000 bp in length which are located between flanking microsatellite regions. The ISSR technique is a PCR based technique involving the amplification of DNA segments present between two identical microsatellite regions that are oppositely oriented to each other. The technique uses single microsatellite primers ISSRs of different sizes usually 16-25 bp long amplifying

in a PCR reaction to target multiple genomic loci. Thus, fragments of several loci are generated at once, separated by gel electrophoresis and scored for presence or absence (Omondi *et al.,* 2016; Conţescu and Anton, 2023). In spite of this, there are limited information on the characterization of *C. argentea* using ISSR primers. Therefore the study was carried out to investigate the molecular characterization of *C. argentea* genotypes.

Materials and methods

Sample collection and study location

Twenty-five genotypes of *Celosia argentea* were used for this research. The *C. argentea* genotypes were sourced from National Centre for Genetic Resources and Biotechnology (NACGRAB) in Ibadan and ten (10) selected markets from ten local governments in Ibadan, Nigeria shown in Table 1. An open field experiment was conducted between June and September 2018 at the research farm of the Department of Botany, University of Ibadan, Ibadan (7.4417° N; 3.9000°E) located in the rainforest area of Southwestern Nigeria (Table 1). The molecular studies were carried out at the Department of Virology, University College Hospital, Ibadan.

Experimental design, method of planting, cultural practices and storage

A total of one hundred perforated polythene bags were each filled with 8 kg of sandy-loamy soil and arranged in a completely randomized design, with four replicates, spaced at 0.75 m within the row and column. The genotypes were raised for 2 weeks in the nursery bags before transplanting into polythene bags. The crop was raised following good agronomic practices according to standard procedures of FAO (2004). The weeds were removed manually weekly whenever it appears and watering was done on daily basis till the rainy period started. Two plants were transferred into polythene bags and thinned to a plant per bag after two weeks. Fresh young apical leaves were collected randomly for each genotype early in the morning, preserved in ice bags before being transported to the laboratory where it was stored in the refrigerator at -20°C prior to molecular studies.

Data collection

A total of 20 morphological characters comprising 15 quantitative and 5 qualitative traits were evaluated on the cultivated *C. argentea* genotypes according to the method described by IPGRI (2006). Data collection on growth characters of *C. argentea* genotypes commenced after transplanting at four weeks after planting

(WAP). This was carried out continuously every week till the 13th week after planting. Data collected were: growth habit, leaf shape, leaf color, color of flower, petiole pigmentation, plant height (cm), leaf length (cm), leaf width (cm), leaf area (cm^2), petiole length (cm), number of internodes on stem, number of leaves per plant, plant height at flowering (cm), number of days to flowering, fruit length at maturity (cm), number of flowers per plant. Harvesting was done at fourteen weeks after planting and data collected on yield related characters were: leaf biomass (g), root biomass (g), seed weight (g) and shoot weight (g).

Table 1. *Celosia argentea* genotypes and their location

S/N	Genotype name	Location	Local government
1	A001	Mapo	Ibadan South East
2	A002	Agbowo	Ibadan North West
3	A003	Bodija	Ibadan North
4	A004	Iwo road	Egbeda
5	A005	New garage	Oluyole
6	A006	Apata	Ido
7	A007	Olodo	Lagelu
8	A008	Oja Oba	Ibadan South West
9	A009	Amuloko	Ona ara
10	A0010	Moniya	Akinyele
11	NGB00126	NACGRAB	Ibadan
12	NGB00128	NACGRAB	Ibadan
13	NGB00129	NACGRAB	Ibadan
14	NGB00133	NACGRAB	Ibadan
15	NGB00136	NACGRAB	Ibadan
16	NGB00137	NACGRAB	Ibadan
17	NGB00138	NACGRAB	Ibadan
18	NGB00151	NACGRAB	Ibadan
19	NGB00155	NACGRAB	Ibadan
20	NGB00170	NACGRAB	Ibadan
21	NGB00172	NACGRAB	Ibadan
22	NGB00177	NACGRAB	Ibadan
23	NGB00179	NACGRAB	Ibadan
24	NGB00182	NACGRAB	Ibadan
25	NGB00183	NACGRAB	Ibadan

DNA extraction

DNA was extracted from young frozen apical leaves of twenty-five *C. argentea* samples using the ISSR technique following the protocol outlined in the Jena Bioscience Plant DNA extraction kit. Fresh frozen tissue was macerated in a mortar and homogenized with a pestle, followed by the addition of 1 ml of phosphate buffer saline (PBS). In a 2 ml Eppendorf tube, 300 µl of cell lysis solution was added, followed by 500 µl of ground sample. The solution was then incubated at 65°C for 60 minutes, with occasional inversion during incubation, and subsequently allowed to cool at room temperature (25°C). After the addition of 100 µl of protein precipitation solution, the cell lysate was vortexed, and the solution was centrifuged at 15,000 rpm for three minutes. The DNA-containing supernatant was carefully transferred into a clean 2 ml Eppendorf tube containing 300 µl of isopropanol. The mixture was vortexed for 2 seconds and then centrifuged at 15,000 rpm for 1 minute. The supernatant was discarded, and the tube was drained on a clean absorbent paper. Subsequently, 500 µl of buffer was added, and the tube was inverted multiple times to create the DNA pellet. The solution was then centrifuged for one minute at 15,000 rpm, and the supernatant was gently discarded. The DNA pellets were air-dried at room temperature. Finally, 50-100 µl of DNA hydration solution was added to the dried pellets, and the samples were stored in the refrigerator for PCR analysis.

DNA amplification

Six Inter Simple Sequence Repeat (ISSR) primers were optimized and utilized in Polymerase Chain Reactions (PCR) (Table 2). Each locus was amplified within a 10 µl PCR cocktail reaction mixture comprising 2 µl of each primer, 1 µl of PCR buffer, 0.8 mM dNTPs, 0.4 mM MgCl2, 0.06 units of Taq polymerase, 0.8 µl of DMSO, 3 µl of PCR-grade H2O, and 2 µl of total genomic DNA. DNA amplification was conducted using a touch-down PCR procedure in a thermocycler. The PCR protocol included the initial denaturation at 95°C for 2 minutes, followed by 10 cycles of touch-down cycling comprising of denaturation at 95°C for 30 seconds, annealing starting at 65°C and decreasing by 1°C each cycle until reaching 55°C, for 30 seconds per cycle, and extension at 72°C for 1 minute. This was followed by 25 cycles of standard PCR with denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. The final extension step lasted five minutes at 72°C, followed by indefinite maintenance at 4°C until further analysis.

Table 2. Oligonucleotide primers and their sequences

S/N	Primers	Nucleotide sequence 5'-3'
1.	UBC-866 $(CTC)_6$	CTC CTC CTC CTC CTC CTC
2.	ISSCR-2 $(CA)_8$ AG	CA CA CA CA CA CA CA CA AG
3.	ISSCR-3 $(CA)_8$ CG	CA CA CA CA CA CA CA CA CG
4.	ISSCR- 4 $(CT)_8$ TG	CA CA CA CA CA CA CA CA TG
5.	ISSCR- 5 $(CA)_8$ AC	CA CA CA CA CA CA CA CA AC
6.	ISLA- $(AGC)_4$ G	AGC AGC AGC AGC G

Agarose gel electrophoresis

Agarose gel electrophoresis was performed to detect the presence of DNA amplicons after PCR. To prepare the gel, 1 g of agarose was dissolved in 100 mL of 0.5×TBE buffer using a microwave for 3 minutes. The solution was then allowed to cool before casting the gel. Once solidified, the gel was placed in the electrophoresis tank and covered with 0.5×TBE buffer. Each DNA sample was mixed with loading dye and then loaded into the wells of the prepared gel in the electrophoresis tank. The electrophoresis was run for 30 minutes at 100 volts. A negative control, lacking a DNA template, was included. After electrophoresis, the gel was stained with SYBR Green and the separated amplified fragments were visualized under UV transilluminator light to observe the formation of bands.

Statistical analysis and data interpretation

Morphological data was subjected to Analysis of Variance (ANOVA) using SAS 9.1 software (2003 version), with differences in means determined through the Duncan Multiple Range Test (DMRT). Relationships among growth and yield characteristics were assessed using dendrograms, Pearson correlation coefficients, and Principal Component Analysis (PCA).

Molecular data was analyzed to elucidate total gene diversity using the NTSYS-pc version 2.02e package and PowerMarker version 3.25 software. Amplified fragments were scored as 1 for present and 0 for absent according to Liu and Muse (2005). These scores were then utilized to construct a dendrogram employing the Unweighted Pair Group Method with Arithmetic Average (UPGMA) cluster analysis, as outlined by Sneath and Sokal (1973), to elucidate the genetic relationships among *C. argentea* genotypes.

Results

The results obtained in this study indicate great morphological and molecular variability among the investigated genotypes. Due to the high polymorphism level detected by the primers, the study validates ISSR markers as useful tools to assess the *C. argente*ga genotypes.

Mean square effect of location and growth stages on growth characters of C. argentea

The analysis of variance of morphological traits (Table 3) shows that there is significant variation in all the samples of *C. argentea* for growth characters (P<0.05). The growth characters of *C. argentea* are significantly affected by the age of the plant (weeks).

Table 3. Mean square effect of genotypes and growth stages on growth characters of *Celosia argentea*

SOV	DF	PH (cm)	LL (cm)	LW (cm)	LA (cm^2)	PL (cm)	NOI	NOLPP	PHF (cm)
Genotypes	24	1561.32**	30.35**	24.80**	7635.73**	10.62**	405.48**	2887.96**	430.20**
Weeks	9	92474.68**	275.29**	87.22**	37756.66**	132.91**	10897.30**	33312.93**	39110.86**
Replicates	3	82.13^{ns}	8.64*	6.04**	1449.31*	3.83^{ns}	103.07^{ns}	891.96*	10.06^{ns}
Error	923	129.28	2.87	0.96	391.46	1.48	42.76	174.86	107.67
Corrected total	959	996819	5835.42	2260.07	872953	2799.02	147652	527048	454761

Note: ** P<0.01 highly significant, * P<0.05 significant, ns=not significant; KEYS: SOV: sources of variation, DF: degree of freedom, PH: plant height, LL: leaf length, LW: leaf width, LA: leaf area, PL: petiole length, NOI: no. of internodes on main stem, NOLPP: no. of leaves per plant, PHF: plant height at flowering.

Mean square effect of genotypes and growth stages on yield characters of C. argentea

The results in Table 4 show that the agronomic and yield characters of *C. argentea* highly vary with genotypes, being significantly affected by the age of the plant (weeks), but the observed variation among replicates for these characters are not statistically significant.

Table 4. Mean square effect of genotypes and growth stages on yield characters of *Celosia argentea*

SOV	DF	NDF	FLM (cm)	NFPP	LB (g)	RB (g)	SW (g)	SHW (g)
Genotypes	24	429.33**	3.99**	175.84**	328.72*	367.80*	43.81**	1948.20*
Weeks	9	32381.24**	205.96**	5238.92**	37807.57**	23251.55**	1075.47**	124956.35**
Replicates	3	7.62^{ns}	0.05^{ns}	19.14^{ns}	243.22^{ns}	188.10^{ns}	10.88^{ns}	203.68^{ns}
Error	923	97.78	1.08	59.17	191.43	176.19	15.29	821.77
Corrected total	959	38398.3	2889.74	104279	525531	377789	24668.1	1904906

Note: ** P<0.01 highly significant, * P<0.05 significant, ns=not significant; KEYS: SOV: sources of variation, DF: degree of freedom, NDF: no. of days to flowering, FLM: fruit length at maturity, NFPP: no. of flowers per plant, LB: leaf biomass, RB: root biomass, SW: Seed weight, SHW: shoot weight.

Mean square effect of genotypes on growth and yield of C. argentea

Plant height ranged from 35.22 cm in NGB00133 to 62.08 cm in NGB00151. The height of 50.22 cm recorded in A001 did not differ significantly from genotypes A002, A003, A010, NGB00137, NGB00155, NGB00170, NGB00172 and NGB00182, with respective heights of 46.10, 51.24, 50.12, 47.50, 47.13, 51.24, 51.68 and 49.3 cm. Similarly, genotypes NGB00126, NGB00128, NGB00138, NGB00151, NGB00177 and NGB00183 with heights 61.30, 57.85, 62.08, 56.68, 61.15 and 58.76 cm did not vary significantly from one another, but they were significantly taller than all other genotypes studied (Table 5).

The leaf parameters (leaf length, leaf width and leaf area) recorded the greatest values in A005, NGB00177 and A005 respectively, while the lowest values were recorded in NGB00151, NGB00183 and NGB00183 respectively (Table 6). Plant height at flowering, number of days to flowering and the root biomass did not differ significantly across all the genotypes, except for A001, with a value of 0.00 in each character state. Also, NGB00182 produced the highest leaf biomass. Generally, it was observed that the highest mean performance for plant height, leaf biomass and seed weight are found in the NGB genotypes while the A00 genotypes were observed to have remarkably higher values in terms of leaf length, leaf area and root biomass.

Table 5. Mean square effect of genotypes on growth and yield characters of *C. argentea*

GN	PH (cm)	LL (cm)	LW (cm)	LA(cm^2)	PL (cm)	NOI
A001	50.22^{efgh}	10.85^{efghi}	5.80^{hij}	66.08^{efg}	4.99^{cde}	16.33^{defg}
A002	46.1^{ghi}	11.85^{bc}	6.46^{cde}	79.79^{cd}	5.80^{ab}	18.67^{defgh}
A003	51.24^{defg}	11.53^{bcde}	6.40^{cdef}	75.91^{cd}	5.36^{abcd}	22.31^{abc}
A004	44.53^{i}	10.12^{hijk}	5.67^{hij}	60.55^{ghi}	4.66^{cde}	14.75^{lm}
A005	45.07^{hi}	12.89^{a}	6.85^{bc}	94.79^{a}	5.49^{abcd}	21.64^{bcde}
A006	54.52^{cde}	11.90^{b}	7.03^{ab}	84.21^{bc}	5.95^{a}	20.19^{cdefg}
A007	38.12^{k}	10.24^{ghijk}	5.83^{ghij}	63.11^{fghi}	5.51^{abc}	14.86^{klm}
A008	42.43^{j}	10.35^{ghijk}	5.78^{hij}	61.96^{ghi}	5.96^{a}	12.08^{m}
A009	55.06^{bede}	11.02^{cdefg}	6.35^{def}	72.81^{fe}	5.51^{abc}	18.28^{fghij}
A010	50.12^{efgh}	10.07^{ijk}	5.54^{ij}	59.46^{ghi}	4.89^{cde}	17.78^{ghijk}
NGB00126	61.30^{a}	12.18^{b}	6.60^{cd}	83.82^{c}	5.43^{abcd}	23.70^{ab}
NGB00128	57.85^{abc}	10.92^{defgh}	6.11^{efgh}	68.78^{efg}	5.12^{cde}	20.90^{bcdef}
NGB00129	54.85^{bcde}	9.97^{jk}	5.68^{hij}	58.73^{hi}	5.13^{cde}	15.08^{jklm}
NGB00133	35.22^{k}	10.49^{ghij}	5.54^{j}	63.64^{fgh}	5.11^{cde}	13.60^{lm}
NGB00136	52.32^{def}	10.47^{ghijk}	5.57^{ij}	61.12^{ghi}	4.97^{cde}	21.85^{abcd}
NGB00137	47.50^{fghi}	9.77^{jk}	5.55^{ij}	54.95^{i}	4.60^{e}	16.20^{ijkl}
NGB00138	62.08^{a}	10.69^{fghi}	5.90^{ghij}	65.64^{fg}	4.84^{de}	20.50^{bcdefg}
NGB00151	56.68^{abcd}	10.69^{efghi}	6.00^{efghi}	66.54^{efg}	5.31^{abcd}	19.23^{cdefgh}
NGB00155	47.13^{ghi}	9.57^{k}	5.45^{j}	52.81^{i}	4.97^{cde}	17.25^{hijk}
NGB00170	51.24^{efg}	11.02^{defg}	5.98^{fghij}	67.40^{efg}	5.46^{abcd}	17.90^{fghijk}
NGB00172	51.68^{defg}	10.33^{ghijk}	5.65^{ij}	63.03^{ghi}	5.53^{abc}	18.48^{fghi}
NGB00177	61.15^{a}	11.98^{b}	7.30^{a}	90.11^{b}	5.36^{abcd}	20.33^{cdefg}
NGB00179	56.57^{bcd}	11.37^{cdef}	6.31^{defg}	74.95^{de}	5.80^{ab}	24.28^{a}
NGB00182	49.32^{fgh}	11.61^{bcd}	6.16^{defgh}	73.26^{def}	5.22^{cde}	19.00^{defgh}
NGB00183	58.76^{ab}	9.60^{k}	3.06^{k}	28.85^{j}	5.63^{f}	13.40^{m}

Note: Mean with the same letter in the same column are not significantly different at P≥0.05 according to DMRT; KEYS: GN: genotype, PH: plant height, LL: leaf length, LW: leaf width, LA: leaf area, PL: petiole length, NOI: no. of internodes on main stem.

Table 6. Genotypic effect on agronomic and yield characters of *Celosia argentea*

GN	NOLPP	PHF (cm)	NDF	FLM (cm)	NFPP	LB (g)	RB (g)	SW (g)	SHW (g)
A001	34.86^{defg}	0.00^{b}	0.00^{b}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{c}
A002	42.33^{c}	7.41^{a}	7.08^{a}	0.54^{abcd}	3.08^{abcd}	4.69^{bcd}	3.53^{ab}	0.81^{c}	11.08^{bc}
A003	49.33^{a}	5.72^{a}	5.89^{a}	0.64^{abc}	4.06^{abcd}	2.19^{d}	3.11^{ab}	1.89^{bc}	7.22^{bc}
A004	36.69^{def}	5.75^{a}	6.50^{a}	0.41^{bcd}	1.83^{bcd}	5.92^{abcd}	6.67^{ab}	0.67^{c}	15.14^{abc}
A005	47.92^{ab}	4.64^{ab}	5.58^{a}	0.92^{a}	4.25^{abcd}	3.0^{cd}	3.08^{ab}	2.17^{abc}	9.72^{bc}
A006	33.72^{efgh}	4.95^{ab}	7.14^{a}	0.63^{abc}	1.81^{bcd}	7.11^{abcd}	8.28^{a}	0.61^{c}	16.33^{ab}
A007	22.50^{jkl}	8.62^{a}	7.34^{a}	0.56^{abcd}	2.69^{abcd}	6.28^{abcd}	8.75^{a}	1.00^{c}	16.92^{ab}
A008	22.47^{kl}	7.38^{a}	7.53^{a}	0.64^{abc}	20.3^{bcd}	3.83^{bcd}	2.72^{ab}	0.70^{c}	9.72^{bc}
A009	28.78^{hijk}	7.43^{a}	7.47^{a}	0.44^{abcd}	4.94^{ab}	2.58^{cd}	3.31^{ab}	1.03^{bc}	13.44^{abc}
A010	30.97^{ghi}	7.40^{a}	6.75^{a}	0.51^{abcd}	3.97^{abcd}	3.56^{cd}	3.83^{ab}	1.36^{bc}	11.81^{abc}
NGB00126	38.83^{cde}	6.11^{a}	5.90^{a}	0.64^{abc}	2.10^{bcd}	2.23^{cd}	1.75^{b}	0.09^{c}	6.95^{c}
NGB00128	32.90^{fghi}	8.47^{a}	6.95^{a}	0.84^{ab}	4.78^{abc}	9.91^{abc}	8.16^{a}	2.17^{bc}	15.82^{abc}
NGB00129	26.33^{jk}	8.87^{a}	6.30^{a}	0.54^{abcd}	3.93^{abcd}	7.57^{abc}	7.52^{a}	3.07^{ab}	15.52^{abc}
NGB00133	22.25^{l}	8.39^{a}	6.75^{a}	0.54^{abcd}	3.90^{abcd}	9.53^{abc}	5.61^{ab}	1.29^{bc}	14.70^{abc}
NGB00136	38.33^{cde}	4.20^{b}	5.95^{a}	0.15^{d}	0.48^{d}	6.71^{abcd}	3.13^{ab}	0.11^{c}	9.71^{bc}
NGB00137	26.50^{ijk}	7.72^{a}	7.13^{a}	0.54^{abcd}	2.38^{bcd}	8.23^{abc}	8.38^{a}	0.58^{c}	12.70^{abc}
NGB00138	37.98^{cde}	6.74^{a}	7.08^{a}	0.28^{cd}	1.43^{bcd}	7.26^{abcd}	4.74^{ab}	0.55^{c}	9.72^{bc}
NGB00151	29.30^{ghij}	7.46^{a}	4.98^{a}	0.80^{ab}	6.68^{a}	5.91^{abcd}	3.98^{ab}	4.07^{a}	12.09^{abc}
NGB00155	27.73^{ijk}	8.33^{a}	7.10^{a}	0.63^{abc}	2.90^{abcd}	9.20^{abc}	7.56^{a}	0.76^{c}	16.12^{abc}
NGB00170	26.45^{ijk}	7.96^{a}	8.03^{a}	0.43^{abcd}	0.73^{cd}	5.92^{abcd}	5.49^{ab}	0.26^{c}	9.85^{bc}
NGB00172	40.13^{cd}	8.56^{a}	8.18^{a}	0.42^{abcd}	0.60^{cd}	6.32^{abcd}	3.96^{ab}	0.28^{c}	10.23^{bc}
NGB00177	38.53^{cde}	7.40^{a}	6.85^{a}	0.46^{abcd}	1.93^{bcd}	7.96^{abc}	5.50^{ab}	1.28^{bc}	15.32^{abc}
NGB00179	46.58^{b}	7.83^{a}	5.98^{a}	0.71^{abc}	2.56^{bcd}	10.78^{ab}	8.06^{a}	1.20^{bc}	13.54^{abc}
NGB00182	30.98^{fghi}	7.55^{a}	6.45^{a}	0.54^{abcd}	4.05^{abcd}	11.74^{a}	7.29^{ab}	1.72^{bc}	26.79^{a}
NGB00183	19.53^{l}	8.34^{a}	8.00^{a}	0.49^{abcd}	1.20^{bcd}	6.69^{abcd}	7.33^{ab}	0.56^{c}	13.18^{abc}

Note: Mean with the same letter in the same column are not significantly different at P≥0.05 according to DMRT; KEYS: GN: Genotype, NOLPP: no. of leaves per plant, PHF: plant height at flowering, NDF: no. of days to flowering, FLM: fruit length at maturity, NFPP: no. of flowers per plant, LB: leaf biomass, RB: root biomass, SW: Seed weight, SHW: shoot weight.

PCA of growth and yield characters of C. argentea

The result of the PCA analysis reveals that the quantitative characters of *C. argentea* are delineated into nine different principal component axes (Table 7). 78% of the total variation is explained by the first three components (prin 1, prin 2 and prin 3) with Eigen values 6.3, 3.89 and 1.49 respectively. The first component, prin 1, is a measure of the agronomic and yield characters of *C. argentea*. It captures 42% of the total variation and shows that plant height (0.37) at flowering is closely related with number of days to flowering (0.36), fruit length at maturity (0.36), number of flowers per plant (0.34), leaf biomass (0.35), root biomass (0.35), seed weight (0.31) and shoot weight (0.37). It is an indication that these eight characters vary together.

Table 7. PCA of growth and yield characters of *C. argentea*

Characters	Prin 1	Prin 2	Prin 3	Prin 4	Prin 5	Prin 6	Prin 7	Prin 8	Prin 9
PH	-0.02	0.23	0.5	0.1	0.77	0.15	0.25	0.1	0.04
LL	-0.07	0.43	-0.26	0.03	0.14	0.03	-0.25	0.19	-0.69
LW	-0.07	0.44	-0.27	0	0.1	0	-0.16	-0.21	0.64
LA	-0.06	0.46	-0.26	0.03	0.09	0.03	-0.28	-0.01	0.08
PL	-0.05	0.38	-0.22	-0.04	-0.27	0.09	0.85	0.08	-0.04
NOI	0.03	0.33	0.5	-0.11	-0.27	-0.01	-0.05	0.7	-0.2
NOLPP	-0.01	0.32	0.49	-0.13	-0.39	-0.22	-0.17	0.61	0.18
PHF	0.37	0.02	0	-0.1	0.03	0.44	-0.03	0.05	0.1
NDF	0.36	0.02	0	-0.18	-0.04	0.53	-0.08	0.05	0.03
FLM	0.36	0.05	0.03	0.17	-0.15	0.31	-0.07	0.08	-0.06
NFPP	0.34	0.06	0	0.48	-0.06	-0.16	-0.01	-0.12	-0.05
LB	0.35	0.04	-0.08	-0.35	0.12	-0.32	0.04	0.02	-0.04
RB	0.35	0.02	-0.07	-0.32	0.14	-0.32	0.08	0	0
SW	0.31	0.06	0	0.63	-0.04	-0.23	0.06	0.06	0.08
SHW	0.37	0.03	-0.06	-0.2	0.13	-0.27	0.03	-0.03	-0.03
Eigen value	6.3	3.89	1.49	0.81	0.64	0.48	0.43	0.27	0.21
Proportion	0.42	0.68	0.78	0.83	0.88	0.91	0.94	0.95	0.97

Prin 2, accounting for a little above a quarter of the total variation, gives a measure of the growth characters of *C. argentea*. It shows the closeness of leaf length (0.43), leaf width (0.44) and leaf area (0.46) and number of leaves per plant (0.49) while petiole length (0.38), number of internodes on stem (0.33) and number of leaves per plant (0.32) are related to one another. This means genotypes with large leaf length are likely to have large leaf width and leaf area while genotypes with petiole length have similar characteristics with number of internodes on main stem and number of leaves per plant. The third component shows that plants height (0.50) is similar to number of internodes on main stem (0.50). This implies that closeness of these traits could be used as a predictor for the other. Overall, the observation from the PCA analysis confirms the result of the correlation matrix which shows that the growth characters of *C. argentea* are not significantly associated its agronomic and yield characters.

Correlation matrix among growth and yield characters of C. argentea

Correlation matrix for growth, agronomic and yield characters (Table 8) show a highly significant positive relationship among the growth characters; although, no significant correlation existed between the growth characters and any of the agronomic and yield characters, it was observed that the agronomic and yield characters are well related to one another. This suggests that the variation in the growth characters does not affect the agronomic and yield characters.

Plant height had a positive correlation with leaf width ($r = 0.5$) and a strong positive association with leaf length ($r = 0.63$), leaf area ($r = 0.62$), petiole length ($r = 0.61$), number of internodes on main stem ($r = 0.85$), number of leaves per plant ($r = 0.79$) at $p<0.01$. Leaf length is positive and strongly related to leaf width ($r = 0.86$), leaf area ($r = 0.94$), petiole length ($r = 0.77$), number of internodes on main stem ($r = 0.65$), number of leaves per plant ($r = 0.64$). Likewise, leaf width is positive and strongly correlated with respect to leaf area ($r = 0.95$), petiole length ($r = 0.77$), number of internodes on main stem ($r = 0.61$) and number of leaves per plant ($r = 0.60$). A positive and strong correlation exist between leaf area and petiole length ($r = 0.79$), number of internodes on main stem ($r = 0.66$) and number of leaves per plant ($r = 0.68$). Petiole length has a strong positive correlation with number of internodes on main stem ($r = 0.66$) and number of leaves per plant ($r = 0.64$). A positive and strong association also exists between number of internodes on main stem and number of leaves per plant ($r = 0.68$). Furthermore, plant height at flowering has strong positive correlation with number of days to flowering ($r = 0.97$), fruit length at maturity ($r = 0.90$), number of flowers per plant (0.77), leaf biomass ($r = 0.82$), root biomass

(r = 0.81), seed weight (r = 0.69) and shoot weight (r = 0.85). A strong positive correlation was observed for number of days to flowering with fruit length at maturity (r = 0.89), number of flowers per plant (r = 0.72), leaf biomass (r = 0.88), root biomass (r = 0.81), seed weight (r = 0.63) and shoot weight (r = 0.83). Fruit length at maturity had strong and positive relationship with number of flowers per plant (r = 0.84), leaf biomass (r = 0.75), root biomass (r = 0.76), seed weight (0.78) and shoot weight (r = 0.81). Number of flowers per plant had strong and positive relationship with leaf biomass (r = 0.69), root biomass (r = 0.70), seed weight (r = 0.89) and shoot weight (r = 0.77). Leaf biomass had strong and positive correlation with root biomass (r = 0.92), seed weight (r = 0.61) and shoot weight (r = 0.93). Root biomass also had strong and positive relationship with seed weight (r = 0.62) and shoot weight (r = 0.92) while strong and positive association existed between seed weight and shoot weight (r = 0.69) (Table 8).

Frequency, diversity of alleles and PIC of C. argentea genotypes using ISSR markers

A total of six polymorphic primers of ISSR markers were used to investigate the genetic diversity and molecular relationship of *C. argentea* genotypes (Figure 1, Table 2). The percentage gene diversity recorded 80% while the polymorphism in the population was diverse at 78%. The number of allele ranges from 4.0 to 15.0 with a mean of 10.50. Major allele frequency ranges from 0.24 to 0.48, with a mean of 0.35. Gene diversity and PIC varied from 0.67 to 0.87 and 0.61 to 0.85 with means of 0.80 and 0.78 respectively. There was variation in major allele frequency, number of alleles, gene diversity and PIC. Primer ISSCR3 had the highest major allele frequency at 0.48 and primer UBC866 had the lowest major allele frequency at 0.24. Primers ISLA, ISSCR-2, ISSCR-4, and ISSCR-5 had major allele frequencies of 0.28, 0.32, 0.36 and 0.44 respectively. Primer ISSCR2 had the highest number of alleles at 15.0, primer ISSCR5 had the lowest number of alleles at 4.0, primers ISLA and ISSCR3 had the same numbers of allele at 12.0, while primers UBC866 and ISSCR4 had allele numbers of 11.0 and 9.0 respectively. Primer UBC866 had the highest value for gene diversity at 0.87, the lowest value of gene diversity was found in primer ISSCR5, primers ISSCR2 and ISLA had gene diversity values of 0.86 while primers ISSCR3 and ISSCR4 had gene diversity values of 0.74 and 0.81 respectively. PIC was highest in primers UBC866 with 85.40% followed by ISLA with 85.22% and ISSCR2 with a value 85.21%. Primer ISSCR5 had the lowest PIC value at 60.61%, while primers ISSCR4 and ISSCR3 had PIC values of 77.67 and 72.45 respectively (Table 9).

Table 8. Correlation matrix among growth and yield characters of *Celosia argentea*

Character	PH (cm)	LL (cm)	LW (cm)	LA (cm^2)	PL (cm)	NOI	NOLPP	PHF (cm)	NDF	FLM (cm)	NFPP	LB (g)	RB (g)	SW (g)	SHW (g)	ACC	WK
PH																	
LL	0.63**																
LW	0.57*	0.86**															
LA	0.62**	0.94**	0.95**														
PL	0.61**	0.77**	0.77**	0.79**													
NOI	0.85**	0.65**	0.61**	0.66**	0.66**												
NOLPP	0.79**	0.64**	0.60**	0.65**	0.64**	0.88**											
PHF	0.45ns	0.22ns	0.19ns	0.23ns	0.26ns	0.45ns	0.36ns										
NDF	0.46ns	0.24ns	0.19ns	0.24ns	0.26ns	0.46ns	0.37ns	0.97**									
FLM	0.43ns	0.25ns	0.20ns	0.26ns	0.27ns	0.46ns	0.39ns	0.90**	0.89**								
NFPP	0.37ns	0.22ns	0.19ns	0.24ns	0.22ns	0.39ns	0.31ns	0.77**	0.72**	0.84**							
LB	0.38ns	0.22ns	0.19ns	0.22ns	0.23ns	0.39ns	0.31ns	0.82**	0.80**	0.75**	0.69**						
RB	0.37ns	0.19ns	0.16ns	0.19ns	0.22ns	0.37ns	0.30ns	0.81**	0.80**	0.76**	0.70**	0.92**					
SW	0.35ns	0.21ns	0.18ns	0.22ns	0.22ns	0.34ns	0.29ns	0.69**	0.63**	0.78**	0.89**	0.61**	0.62**				
SHW	0.40ns	0.22ns	0.19ns	0.22ns	0.23ns	0.39ns	0.32ns	0.85**	0.83**	0.81**	0.77**	0.93**	0.92**	0.69**			
ACC	0.08ns	-0.08ns	-0.16ns	-0.14ns	-0.08ns	0.02ns	-0.08ns	0.05ns	0.03ns	0.00ns	-0.02ns	0.08ns	0.05ns	0.01ns	0.04ns		
WK	0.92**	0.61**	0.54*	0.59*	0.62**	0.81**	0.74**	0.50*	0.51*	0.48ns	0.40ns	0.43ns	0.42ns	0.36ns	0.44ns	0.07ns	
REP	0.00ns	0.05ns	0.08ns	0.06ns	-0.04ns	-0.00ns	-0.07ns	0.01ns	-0.00ns	-0.00ns	0.01ns	-0.02ns	-0.03ns	0.00ns	-0.01ns	-0.00ns	-0.00ns

Note: * P<0.05 significant, ** P<0.01 highly significant, ns=not significant; KEYS: PH: Plant Height, LL: leaf length, LW: leaf width, LA: leaf area, PL: petiole length, NOI: no. of internodes on main stem, NOLPP: no. of leaves per plant, PHF: plant height at flowering, NDF: no. of days to flowering, FLM: fruit length at maturity, NFPP: no. of flowers per plant, LB: leaf biomass, RB: root biomass, SW: Seed weight, SHW: shoot weight, SOV: sources of variation, ACC: Genotypes, WK: Weeks, REP: Replicates.

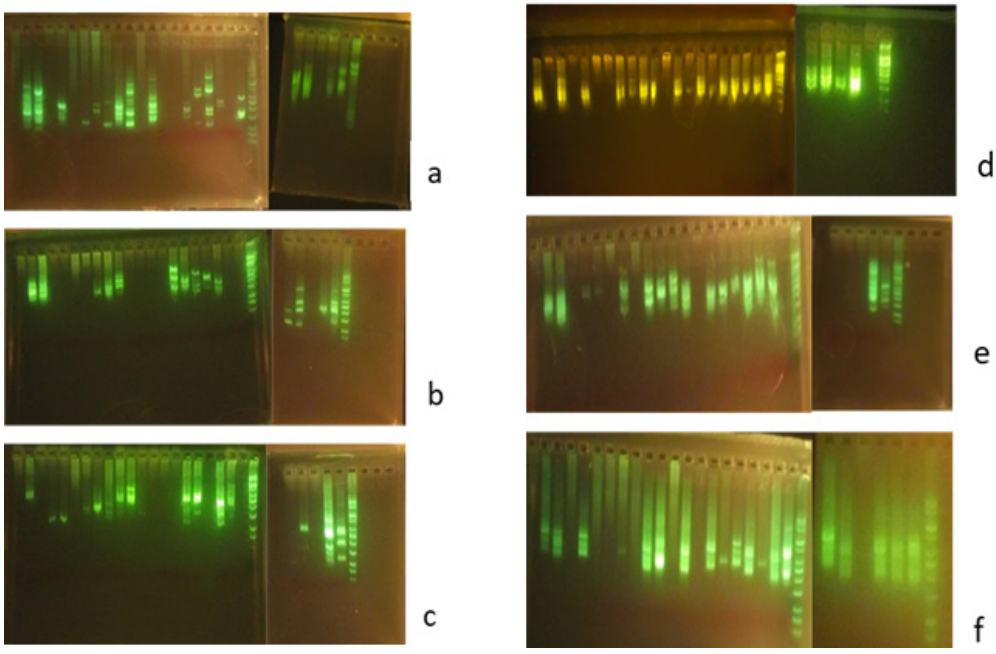

Figure 1: Gel Photograph showing 25 *Celosia argentea* genotypes with primers: a. (ISSCR-2 $(CA)_8$ AG), b. (ISSCR-3 $(CA)_8$ CG), c. (ISSCR- 4 $(CT)_8$ TG), d. (ISSCR- 5 $(CA)_8$ AC), e. (ISLA- $(AGC)_4$ G) and f. (UBC-866 $(CTC)_6$).

Note: Order of loading gel from well 1-27: NGB00129, A006, NGB00155, NGB00182, A005, NGB00128, NGB00183, NGB00179, NGB00151, NGB00138, A003, NGB00126, A002, NGB00172, A001, NGB00170, A004, A008, NGB00136, NGB00137, control, ladder, A007, A010, NGB00177, A009, NGB00133 and ladder.

Table 9. Frequency, diversity of alleles and PIC of *Celosia argentea* genotypes using ISSR markers

Marker	Major allele frequency	No. of observations	Allele No	Gene diversity	PIC (%)
UBC-866	0.24	25.00	11.00	0.87	85.40
ISSCR-2	0.32	25.00	15.00	0.86	85.21
ISLA	0.28	25.00	12.00	0.86	85.22
ISSCR-3	0.48	25.00	12.00	0.74	72.45
ISSCR-4	0.36	25.00	9.00	0.81	78.67
ISSCR-5	0.44	25.00	4.00	0.67	60.61
Mean	0.35	25.00	10.50	0.80	77.93

Dendogram showing qualitative characters of C. argentea genotypes

The result of the cluster analysis in Figure 2 shows that *C. argentea* genotypes clustered into six main groups based on their qualitative characters. Groups 1, 4 and 6 are groups comprising of single genotypes each namely NGB00128, NGB00179 and NGB00126 respectively. Group 2 composed of A010, NGB00136, A005, A006, A007, A008, NGB00183, A004, NGB00137, A001 and A003. Group 3 contains NGB00177, NGB00182, NGB00138, NGB00170, A009, NGB00172, and A002 while group 5 contains the remaining genotypes. The clustering indicates that NGB00128, NGB00179 and NGB00126 are more distantly related to each other than any of the other NGB genotypes but are more related to the market accessions.

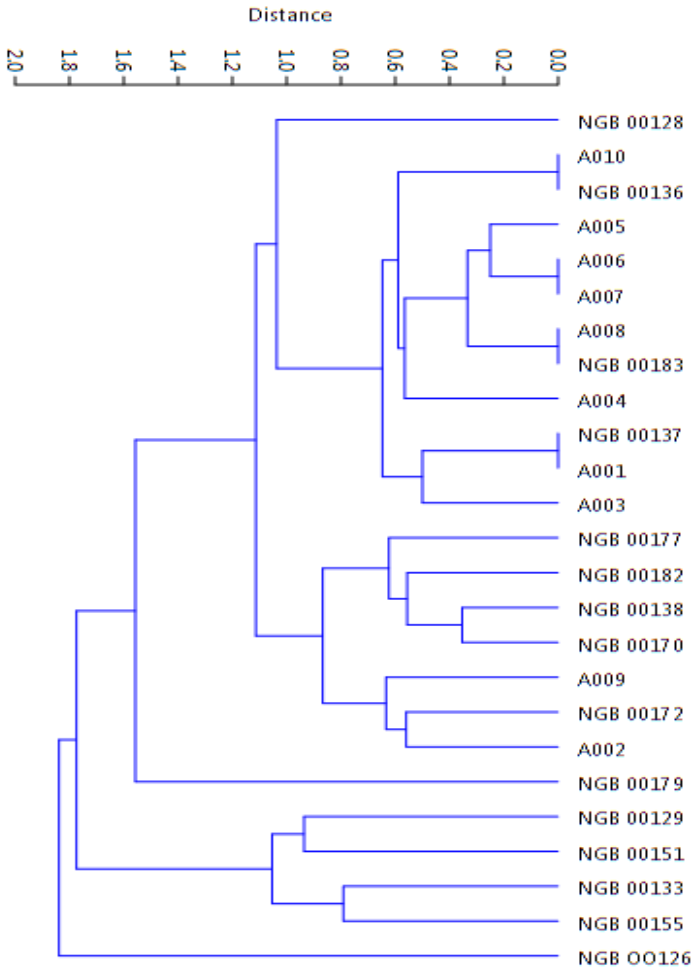

Figure 2. Dendogram showing qualitative characters of *Celosia argentea* genotypes

Dendogram showing genetic relatedness of C. argentea genotypes

The dendogram in Figure 3 consist of five clusters and two monolifolious groups (A003 and A005). Cluster 1 had the highest number of genotypes (12), while clusters 2 and 3 had each at least 2 genotypes. NGB00182 and NGB00172, as well as NGB00128 and NGB00177 are closely related. Also, NGB00126 and NGB00137 are genetically related as compared to NGB00179. Meanwhile NGB00151 and NGB00136 are related and A004 and A010 are closely related in

cluster 2 and 3. In cluster 4, A002 and NGB00156 are closely related to each other while in cluster 5, A006 and A007 are closely related to each other, and also A001 and NGB00137 (Figure 3). The result in Figure 4 depicts that the growth habit, leaf shape and leaf color are more closely related than petiole pigmentation and colour of flowers across the qualitative character studied in all twenty-five genotypes of *C. argentea.*

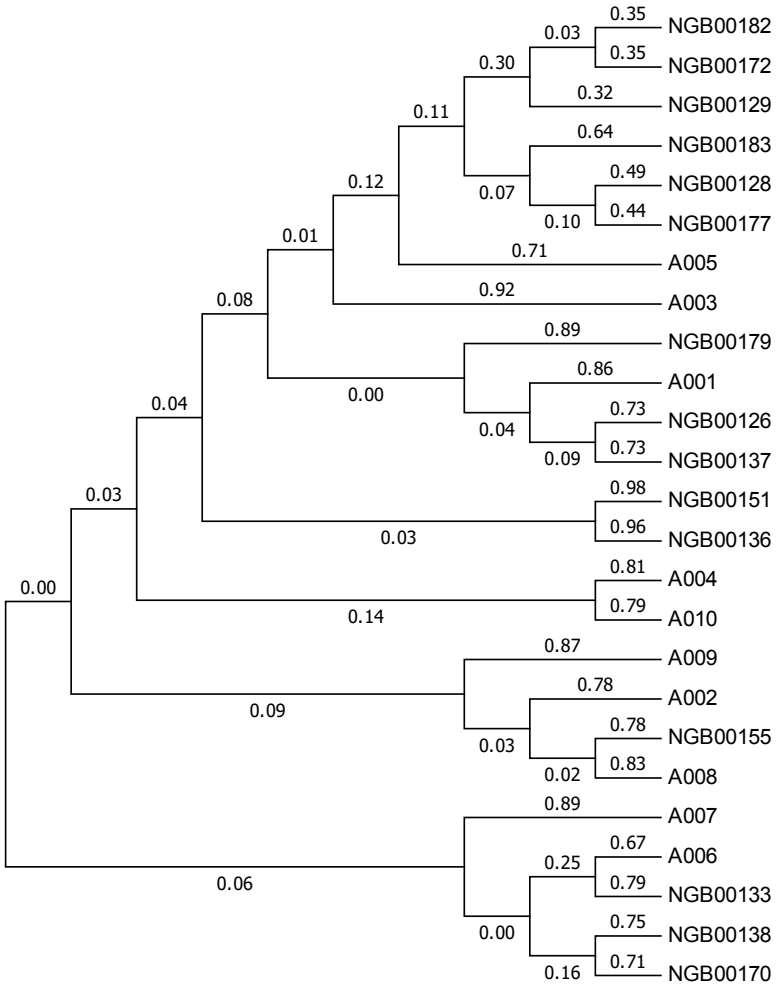

Figure 3. Dendogram showing genetic relatedness of *Celosia argentea* genotypes

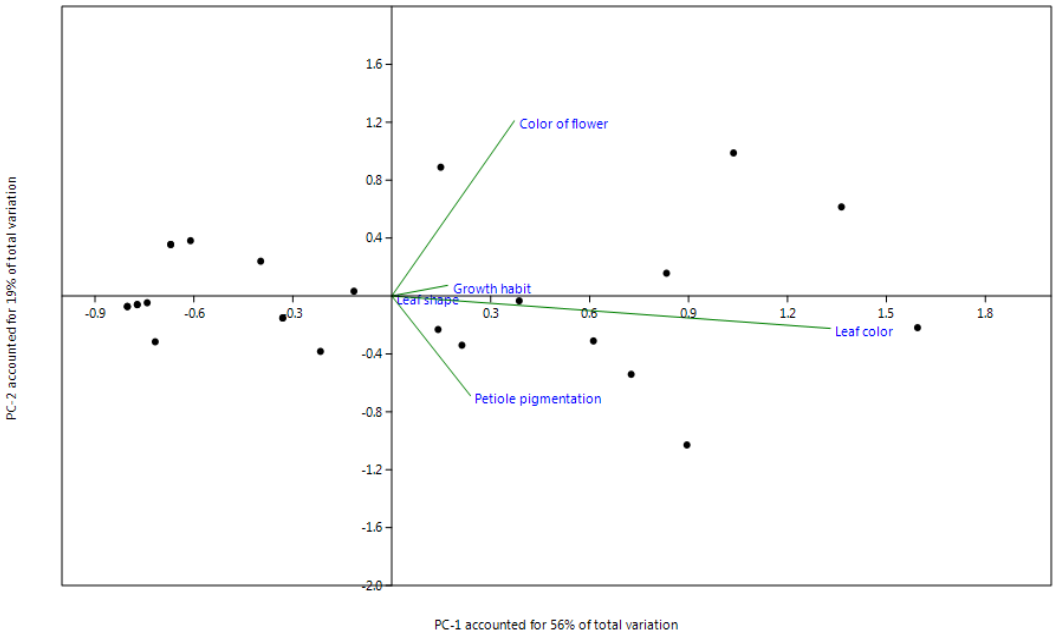

Figure 4. Scattered diagram of qualitative characters of *Celosia argentea* genotypes

Discussion

The information on genetic variability of morphological characters is of economic importance and pre-requisite for studies on any plant species (Olawuyi and Fawole 2005; Begna and Begna, 2021), it as well enables effective conservation and utilization of crop germplasm. Variations observed in the mean performance of the growth and yield characters of *C. argentea* across the accessions are in line with the findings of Olawuyi *et al.,* (2015). The plant height among growth characters exhibited positive and significant relationship with other characters studied as similarly observed by Nwangburuka *et al.,* (2012). However, genotype A005 from Oluyole Local government performed best for growth and NGB00182 performed best for yield related characters. The findings from correlation matrix shows that plant height shows a highly significant positive relationship among the growth characters as similarly observed by Nwangburuka *et al.,* (2012) and Olawuyi *et al.,* (2014). The correlation between plant height and growth characters is an implication that selection based on

plant height will favour the growth characters and does not affect those of the agronomic and yield characters (Balbaa *et al*., 2022). Likewise, plant height at flowering shows a positive and significant correlation with number of days to flowering, fruit length at maturity, number of flowers per plant, leaf biomass, root biomass, seed weight and shoot weight.

The PCA from Prin.1 which accounted for the highest variation conformed to the findings made by Olawuyi *et al.,* (2016), previously observed by Olakojo *et al.,* (2005), and Olowe *et al.,* (2013). The principal component analysis reveals the variation patterns among the assessed characters and primarily accounts for the variation within a group of entries (Aremu *et al.,* 2007). This method supplements the insights gained from cluster analysis techniques as it provides more detailed information regarding distances among major groups (Taran *et al*., 2005). This suggests that significant characters converging in specific components, contributing to variability, tend to be associated together. This presents an opportunity for their utilization in crop improvement strategies. Overall, the observation from the PCA analysis confirms the result of the correlation matrix and shows that the growth characters of *C. argentea* are not significantly correlated to its yield characters. In this study, cluster analysis and dendrogram show that cluster groups consist of genotype from different geographical locations with diverse variability, these may be due to cultivation approach and genetic makeup of individual genotype. This agrees with the findings by Ganapathy *et al.,* (2011) who accounted that wide adaptability of different genotype has been attributed to population genetic architecture, selection history and approach under domestic cultivation and developmental traits. The relationships that exist among the genotypes in the clusters show that there were genetic similarities which were similarly reported by Bamgbegbin *et al.,* (2016). This indicates these genotypes could be useful as breeding material in the improvement of this crop.

Christopoulos *et al.* (2010) demonstrated the utility of ISSR markers across various domains including genetic diversity assessment, phylogenetic studies, gene tagging, genome mapping, and evolutionary biology in numerous plant species. Moreover, Mariana *et al.* (2012) highlighted that ISSR markers effectively delineate high genetic variation among genotypes, facilitating their unambiguous identification. In this study, ISSR markers were employed to evaluate the level and distribution of genetic diversity in 25 accessions of Celosia argentea. The markers revealed over 85% polymorphism, indicating extensive genetic variability. The substantial polymorphism observed with the utilized primers aligns with the findings of Rakoczy-Trojanowska and Bolibok (2004), who noted a similarly highly polymorphic pattern when employing microsatellite

sequence-based reaction primers in plants. Also, amplification with the ISSR primers yielded highly informative patterns supporting the reports of Basel (2011). The differences in major allele frequency, number of allele and gene diversity accounted for variations in the population. This is supported by the reports of Denton (2004), Bamigbegbin *et al.,* (2016) and Oduwaye *et al.* (2014).

Conclusion and recommendation

Genetic evaluation and enhancement of *Celosia argentea* germplasm will be pivotal in future studies and the improvement of vegetable crops. The findings indicate that certain traits, including plant height, leaf length, leaf width, leaf area, petiole length, number of internodes on the main stem, and number of leaves per plant, exhibit strong linear relationships and could serve as selection criteria for enhancing other vegetable crops. Genotype A005 displayed superior performance for growth traits, whereas NGB00182 excelled in yield-related characteristics. The primer UBC-866 $(CTC)_6$ demonstrated high polymorphism and gene diversity. Generally, all A00 market genotypes exhibited early emergence, while NGB genotypes showed elevated values for plant height, leaf biomass, and seed weight. Conversely, A00 genotypes exhibited notably higher values in terms of leaf length, leaf area, and root biomass.

Competing interests: The authors declared no competing interests.

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