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KARYOTYPE STUDIES ON TEN GENOTYPES OF ONION GROWN IN JOS PLATEAU, NIGERIA

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ABSTRACT

The large number of inter- and intra-specific differences in the genus *Allium* has necessitated karyotype analysis of the cultivated species, in order to describe patterns and directions of chromosomal evolution, which affects morphological differences. In this study, ten genotypes of onion (*Allium cepa* L.) (Ares, Violet de Galmi, Red Creole, "Wase", "Dan Zaria", "Dan Garko", "Dan Giyawa", "Bahaushe", "Bakana" and "Yar Aleiro") were used for karyotype analysis in the Cytogenetics Laboratory of the University of Jos, Nigeria. Fresh root tips (1-1.5 cm long) obtained from each genotype were prepared and stained with aceto-orcein for 12 minutes. Each stained root tip was placed in a drop of water on a slide, covered with a coverslip and squashed. Each prepared slide was mounted on the microscope to observe the different stages of mitotic division. The results showed that the arm ratio, centromeric index, coefficient of variation, total form and disparity index varied with genotypes. Metacentric, sub-metacentric and sub-telocentric chromosomes were also observed. Based on a dendrogram of the phylogenetic relationships, the genotypes were grouped into four clusters. Cluster I comprised of genotypes Ares, "Wase" and "Bakana". Cluster II comprised of genotypes "Dan Garko", Violet de Galmi and "Dan Giyawa". Cluster III consisted of the genotypes Red Creole, "Bahaushe" and "Dan Zaria", while Cluster IV comprised of genotype "Yar Aliero". The principal component analysis revealed that the centromere position and variation in complement length accounted for 92.71% of the total variations among the genotypes. These attributes could be responsible for the differences in fresh bulb size and dry matter yield of onion.

Key Words: *Allium cepa*, chromosome, metacentric, phylogenetic

RÉSUMÉ

Le grand nombre de différences inter et intraspécifiques dans le genre Allium a nécessité une analyse caryotypique des espèces cultivées pour décrire les modèles et les directions de l'évolution chromosomique, ce qui affecte les différences morphologiques. Dans cette étude, dix génotypes

d'oignon (*Allium cepa* L.) (Ares, Violet de Galmi, Créole rouge, «Wase», «Dan Zaria», «Dan Garko», «Dan Giyawa», «Bahaushe», «Bakana» et «Yar Aleiro») ont été utilisés pour l'analyse du caryotype au laboratoire de cytogénétique de l'Université de Jos, au Nigéria. Des extrémités de racines fraîches (de 1 à 1,5 cm de long) obtenues à partir de chaque génotype ont été préparées et tachées à l'acéto-orcéine pendant 12 minutes. Chaque extrémité de racine tachée a été placée dans une goutte d'eau sur une lame, recouverte d'une lamelle couvre-objet et écrasée. Chaque lame préparée a été placée sur le microscope pour observer les différentes étapes de la division mitotique. Les résultats ont montré que le ratio des bras, l'indice centromérique, le coefficient de variation, la forme totale et l'indice de disparité variaient selon les génotypes. Des chromosomes métacentriques, sous-métacentriques et sub-télocentriques ont été observés. Le dendrogramme des relations phylogénétiques a montré que les génotypes étaient regroupés en quatre groupes. Le groupe I comprend les génotypes Ares, «Wase» et «Bakana». Le groupe II comprend les génotypes «Dan Garko», Violet de Galmi et «Dan Giyawa». Le groupe III était constitué des génotypes Red Creole, «Bahaushe» et «Dan Zaria», tandis que le groupe IV était constitué du génotype «Yar Aliero». L'analyse en composantes principales a révélé que la position du centromère et la variation de la longueur du complément représentaient 92,71% du total des variations parmi les génotypes. Ces caractéristiques pourraient être responsables des différences de taille des bulbes frais et de rendement en matière sèche de l'oignon.

Mots Clés: *Allium cepa*, chromosome, métacentrique, phylogénétique

INTRODUCTION

Onion (*Allium cepa* L.) is grown for a variety of purposes, ranging from kitchen to factory-made products and for dehydration. It is valued for its distinct pungent flavour, and is an essential ingredient in cuisine (Singh *et al*., 2013). Onion is also used world wide to treat diseases such as blood sugar, rheumatism, cancer, digestive disorders and prolonged cough (Singh *et al*., 2013).

Onion breeding and seed-production programmes world wide have generated short-day, day-neutral and long-day cultivars. Today, almost 90% of the cultivars used in different parts of the world come from local breeding programmes (Paknia and Karimzadeh, 2010).

The chromosomes of *Allium* have been studied for decades (Fritsch *et al*., 2001; Cui *et al*., 2008) for their diversity in size, structure and number. Variations in karyotypes are common, between and within species (Cui *et al*., 2008). Vijayavalli and Mathew (1990) reported the existence of intraspecific polyploidy within several species of *Allium*. They also asserted that chromosomal differences in terms of number or size are related to morphological differences in some cases. A general phenomenon observed in both the plant and animal kingdoms is that chromosome variation has accompanied evolutionary divergence in the taxa studied (Paknia and Karimzadeh, 2010).

Chromosome identification is essential for biotechnological studies, including genome analysis, somatic hybridisation and ploidy manipulation (Paknia and Karimzadeh, 2010). Chromosomal aberrations are common in varieties of the same plant species, due to multiple translocations, which sometimes involve eight or even ten chromosomes as in the case of garlic.

The cytogenetic characteristics of *Allium* species have been observed to vary with the geographic location of the plants. This has necessitated the several studies of *Allium* species, including *Allium cepa* (Awe and Akpan, 2017). New species are still being documented from different parts of the world, hence the need for continuous cytogenetic studies on them (Oyuntsetsegel *et al*., 2013; Tzanoudakis and Trigas, 2015; Awe and Akpan, 2017). This paper reports the results of the karyotype studies on ten genotypes of onion cultivated in different parts of Nigeria.

MATERIALS AND METHODS

Onion seedlings of the genotypes used in this study were raised in the green house of the Federal College of Forestry, Jos, Plateau State. Topsoil, sharp sand and manure (cow dung) in the ratio of 2:1:1 were thoroughly mixed and set in ten plastic trays, measuring 70 cm in diameter, 23 cm in height and 23 cm in length.

The karyotype study was conducted in the Cytogenetics Laboratory of the University of Jos, in north-central Nigeria. Nine out of the ten onion genotypes (Ares, Violet de Galmi, Red Creole, "Dan Zaria", "Dan Giyawa", "Dan Garko", "Bahaushe", "Bakana" and "Yar Aleiro"); were sourced from the Centre for Pastoral and Agricultural Research, Usmanu Danfodiyo University, Sokoto, Nigeria; while a farmers' variety, 'Wase', was obtained from Wase Local Government Area of Plateau State in Nigeria. The agronomic characteristics of the genotypes used in this study are shown in Table 1.

Karyotype study. Fresh root tips of 1-1.5 cm long were cut from rapidly growing seedlings (3-4 weeks after sowing). The root tips were pre-treated with 0.5 g colchicine, dissolved in 100 ml of distilled water, at room temperature for 3 hr. The roots were washed with distilled water for 5 min, at room temperature; after which they were fixed in Carnoy's fixative (glacial acetic acid: ethanol, 3:1) overnight at room temperature (25 °C). After thorough washing with distilled water, 1N HCl was added using a dropper. The root tips were allowed to stand in 1N HCl for 10 minutes to hydrolyse the cells. The HCl was removed from the plastic Petri dish, using the dropper. The Petri dish was then refilled with distilled water twice. A microscope slide was placed on a paper towel. Three drops of aceto-orcein stain were placed in the centre of the slide. The root tip was transferred from the Petri dish to the stain in the slide using a pair of forceps. It was allowed to remain in the stain for 10-15 minutes, to pick up the stain.

Triplicates of well-spread metaphase plates in terms of clarity and organisation, from different individuals, were processed and analysed for each genotype. The best metaphase plates were photographed using an external camera (Celestron digital microscope imager 2.0) attached to the BX50 Olympus microscope, and scanned at 100-resolution. All measurements were recorded using the software Image J (Abramoff *et al*., 2004). For numerical characterisation, the following parameters were assessed:

Chromosome Arm Ratio (AR). The arm ratio (AR) of each pair was calculated using the formula:

$$AR = \frac{L}{S}$$

Where:

L = Long arm length ; and S = Short arm length

Total chromosome length (TL). The total chromosome length (TL) of each pair was calculated using the formula:

$$TL = L+S$$

Where:

L = Long arm length; and S = Short arm length

Relative value (r- value). The ratio of the shortest to the longest chromosome pair (r-value) was calculated using the formula:

$$r\text{-value} = \frac{S}{L}$$

Where:

TABLE 1. Agronomic characteristics of the karyotype onion genotypes used in the study in Nigeria

Genotype	Agronomic characteristics
Ares	An improved variety with red bulbs
Violet De Galmi	An improved variety with light purple bulb. Tropical and strictly short-day, with flattened bulb and very strong taste. Shows excellent tolerance when kept for several months
Onion Red Creole	An improved onion variety with red bulbs
Wase	A local farmer's variety with red bulbs from Wase Local Government Area of Plateau State
Dan Zaria	A local variety sourced from Zaria, Kaduna State. Characterised by distinct traits, which include early maturity and small bulbs. It is cultivated during the rainy season
Yar Giyawa	A local variety from Gwaranyo, Sokoto State, characterised by small bulbs and early maturity. It is cultivated under irrigation
Dan Garko	A local variety from Kano State characterised by large dark-red bulbs and is cultivated under irrigation
Bahaushe	A local variety obtained from Gwaranyo, Sokoto State and characterised by large bulbs. It is cultivated under irrigation
Bakana	A local variety from Kano State, characterised by late maturity and large bulbs. It is cultivated under irrigation
Yar Aliero	A local variety from Kebbi State, characterised by early maturity with large bulbs. It is cultivated in the rainy season

Sources: 1. Centre for Pastoral and Agricultural Research, Usmanu Dan Fodiyo University, Sokoto (Ares, Violet de Galmi, Red Creole, "Dan Zaria", "Dan Giyawa", "Dan Garko", "Bahaushe", "Bakana" and "Yar Aleiro";
2. Wase Local Government Area, Plateau State ("Wase")

L = Long arm length; and S = Short arm length

Total Form Percentage. The total form percentage (%TF) was calculated using the formula:

$$\%TF = \frac{\sum S}{\sum TL} \times 100$$

Where:

S = Short arm length of chromosome; L = long arm length of chromosome; and TL = Total chromosome length

The karyotype symmetry class of Stebbins (1971), as reported by Awe and Akpan (2017) was quantitatively differentiated into finer karyotype evolutionary dispersions using the centromeric index (CI) and disparity index (DI) as follows:

Centromeric index (CI) and Disparity Index (DI). The centromeric index was calculated as the ratio of the length of the short arm of the chromosome to that of the total chromosome length. The Disparity Index (DI) of a given karyotype was estimated from the following equation:

$$CG = \frac{Sx}{TLx} \times 100$$

Where:

CG = Centromeric gradient; *Sx* = Length of median short arm, and *Lx* = Total length of median chromosome

$$CV = \frac{SD}{X} \times 100$$

Where:

SD = Standard deviation; *X* = Mean chromosome length; and *CV* = Coefficient of variation for chromosome length

Therefore, DI = Propotionate measure of *CG* with respect to *CV*

Karyotype asymmetry. Karyotype asymmetry was estimated using two numerical parameters according to Romero-Zarco (1986) as follows:

$$\frac{\sum_1^n = \frac{Sx}{Lx}}{A_1 = n}$$

Where:

Sx = Mean length of the short arms of each homologue; Lx = Mean length of the long arms of each homologue; n = Number of homologues; and A_1 = Intrachromosomal index

$$A_2 = \frac{S}{X}$$

Where:

S = Standard deviation; *X* = Mean chromosome length; and A_2= Interchromosomal index (Paknia and Karimzadeh, 2010).

Statistical analysis. The data collected on short arm length, long arm length, total length, arm ratio, r- value and centromeric index were analysed with the one-way analysis of variance (ANOVA) test, using the GenStat 64-bit Release 17.1 software. Means were compared using the Least Significant Difference (LSD) test at 5% level of probability.

Cluster analysis was performed using the Cophenetic Correlation Coefficient (CP). Principal component analysis (PCA) was carried out to differentiate the studied populations based on karyotype parameters. Dendrogram and principal component analysis (PCA) were computed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) software.

RESULTS

Short arm length and long arm length. The short arm length varied from 4.8 µm in the genotypes "Bahaushe" and "Dan Giyawa", to 5.8 µm in the genotype "Yar Aliero" (Table 2); but was statistically similar in all the genotypes. The longest chromosome arms were observed in the genotype "Bahaushe" (7.7 µm), although this did not differ significantly from the other genotypes studied.

Total length and arm ratio. The total length was statistically similar in all the genotypes studied (Table 2). The highest arm ratio of 1.62 ìm was observed in the genotype "Bahaushe"; which differed significantly from the genotype "Yar Aliero" with an arm ratio of 1.14 ìm (Table 2). Genotypes "Yar Aliero", "Dan Zaria", "Wase", Red Creole and Violet de Galmi with arm ratios of 1.4, 1.2, 1.3, 1.3 and 1.4, were statistically similar.

r- Value and centromeric index. The ten genotypes of onion studied did not differ significantly ($P<0.05$) in r- value, which ranged from 0.64 ìm in the genotype "Bahaushe" to 0.89 ìm in the genotype "Yar Aliero" (Table

2). The highest centromeric index of 0.47 ìm was observed in genotypes "Yar Aliero" and Red Creole; while the lowest (0.39 ìm) was observed in the genotype "Dan Giyawa" (Table 2).

Coefficient of variation (CV) and total form (%TF). The coefficient of variation (CV) ranged from 10.07% in the genotype "Yar Aliero" to 28.89% in the genotype "Wase" (Table 3). The total form ranged from 46.72% in the genotypes "Yar Aliero " to 38.14% in the genotype "Bahaushe" (Table 3).

Disparity index (DI) and intrachromosomal index (A_1). The highest disparity index of 41.80% was observed in genotype "Bakana"; while the lowest (14.09%) was observed in

TABLE 2. Mean values of short arm length (S), long arm length (L), total length (TL), arm ratio (AR), relative value (r- value) and centromeric index (CI) of some genotypes of onion

Genotype	Short arm length (µm)	Long arm length (µm)	Total length (µm)	Arm ratio (µm)	r- value (µm)	Centromeric index (µm)
Ares	5.142±0.559	7.719±0.615	12.861±1.257	1.501±0.105	0.685±0.064	0.425±0.023
Violet de Galmi	5.391±0.632	7.109±0.629	12.500±1.172	1.319±0.139	0.759±0.062	0.426±0.022
Red Creole	5.450±0.520	7.049±0.553	12.499±1.031	1.293±0.063	0.773±0.037	0.474±0.040
Wase	5.626±0.639	6.876±0.724	12.503±1.276	1.222±0.109	0.829±0.059	0.449±0.019
Dan Zaria	5.739±0.498	6.760±0.494	12.499±0.929	1.178±0.068	0.853±0.046	0.458±0.014
Dan Giyawa	4.801±0.285	7.698±0.815	12.499±0.941	1.603±0.177	0.665±0.064	0.393±0.024
Dan Garko	5.250±0.590	7.250±0.616	12.500±0.049	1.381±0.131	0.735±0.066	0.418±0.022
Bahaushe	4.768±0.598	7.731±0.647	12.499±0.878	1.621±0.341	0.641±0.081	0.379±0.034
Bakana	5.275±0.701	7.225±0.819	12.500±1.264	1.370±0.238	0.751±0.074	0.421±0.028
Yar Aliero	5.840±0.218	6.659±0.316	12.499±0.445	1.140±0.059	0.887±0.040	0.468±0.012
LSD (0.05)	1.537	1.808	2.968	0.468	0.029	0.071

TABLE 3. Coefficient of variation (CV), total form (TF), disparity index (DI), intrachromosomal index (A_1), interchromosomal index (A_2) and karyotype formula (KF) of ten genotypes of onion

Genotypes	CV (%)	TF (%)	DI (%)	A_1	A_2	KF
Ares	28.46	41.14	40.62	0.083	0.29	6m+2sm
Violet de Galmi	25.89	43.13	33.91	0.074	0.26	7m+1sm
Red creole	24.22	43.60	30.51	0.001	0.24	8m
Wase	28.89	45.01	40.22	0.102	0.29	7m+1sm
Dan Zaria	21.04	45.91	28.84	0.115	0.21	8m
Dan Giyawa	21.31	38.41	31.48	0.078	0.21	5m+3sm
Dan Garko	23.75	42.00	34.02	0.091	0.24	5m+3sm
Bahaushe	19.88	38.14	26.46	0.077	0.20	5m+2sm+1st
Bakana	26.59	42.21	41.80	0.091	0.27	7m+1st
YarAliero	10.07	46.72	14.90	0.110	0.10	8m

CV = Coefficient of variation, TF = Total form, DI = Disparity index, A_1 = Intrachromosomal index, A_2 = Interchromosomal index and KF = Karyotype formula

the genotype "Yar Aliero" (Table 2). The intrachromosomal index ranged from 0.001 in the genotype Red Creole to 0.115 in the genotype 'Dan Zaria' (Table 2).

Interchromosomal index (A_2) and karyotype formula (KF). The highest interchromosomal index of 0.29 was observed in the genotypes Ares and "Wase"; while the lowest value of 0.10 was observed in the genotype "Yar Aliero" (Table 3).

All chromosomes in genotypes Red Creole, "Dan Zaria" and "Yar Aliero" were metacentric (8m). In genotypes Violet de Galmi and "Wase", seven chromosomes were metacentric; while one was sub-metacentric (7m+1sm). In genotype Ares, six chromosomes were metacentric and two were sub-metacentric (6m+2sm). In genotypes "Dan Giyawa" and "Dan Garko", five chromosomes were metacentric; while three were sub-metacentric (5m+3sm). In genotype "Bahaushe", five chromosomes were metacentric, two sub-metacentric and one sub-telocentric (5m+2sm+1st). In genotype "Bakana", seven chromosomes were metacentric; while one was sub-telocentric (7m+1st) (Table 3).

Cluster analysis and principal component analysis. The phylogenetic relationship among the genotypes is presented in Figure 2. Grouping was based on Euclidean distance co-efficient using the Pearson's correlation. Cluster I consisted of three genotypes, namely Ares, "Wase" and "Bakana"; while Cluster II consisted of three genotypes: "Dan Garko", Violet de Galmi and "Dan Giyawa"; Cluster III consisted of three genotypes: Red Creole, "Bahaushe" and "Dan Zaria"; while Cluster IV consisted of one genotype ("Yar Aliero").

Principal component analysis (PCA) based on karyotype parameters showed that the first two components (centromere position and variation in complement length) accounted for 92.71% of the variations and these were projected in two-dimensional graphics (Fig. 3).

DISCUSSION

Chromosomes were observed to be metacentric, sub-metacentric or sub-telocentric (Fig. 1; Plate 1), as has been reported by Jalilian and Rahiminejad (2012). The four different formulae observed in this study include 8m, 7m+1sm, 6m+2sm and 5m+3sm (symmetrical). Two other formulae, 5m+2sm+1st and 7m+1st, representing asymmetrical morphology, were also observed. The symmetrical morphology is a reflection of relatively primitive karyotypes of the genotypes in this genus. Davis and Heywood (1973) noted that this view might not be universal since many highly evolved species are also known to show karyotype symmetry. Stebbins (1971) reported that primitive wild species had asymmetrical chromosomes in their karyotypes.

The presence of sub-telocentric chromosome morphology (secondary constriction) in some genotypes ("Bahaushe" and "Bakana") suggests that these genotypes have retained some of their primitive wild traits, as has been reported by Zuo and Yuan (2011). The differences in karyotype among the genotypes of onion used in this study, as evidenced in the relative length of haploid complement of chromosomes, arm ratio, centromeric index and chromosome formula, may have arisen by chromosomal re-arrangements such as translocation, deletion and inversion (Mukherjee and Roy, 2012; Awe and Akpan, 2017). Variations in the number and morphology of chromosomes may also arise due to mutations in the natural populations. The role of the structural alteration of chromosomes in the evolution of new races is evidenced by the detailed analysis of karyotypes (Awe and Akpan, 2017). Differences in karyotype formula (asymmetric karyotypes) may be due to different geographic origins, an evidence that the genus *Allium* has evolved into different strains in different locations over time.

Figure 1. Idiograms of somatic cells of some onion genotypes. V_1 = Ares, V_2 = Violet de Galmi, V_3 = Red Creole, V_4 = Wase, V_5 = Dan Zaria, V_6 = Dan Giyawa, V_7 = Dan Garko, V_8 = Bahaushe, V_9 = Bakana, V_{10} = Yar Aliero

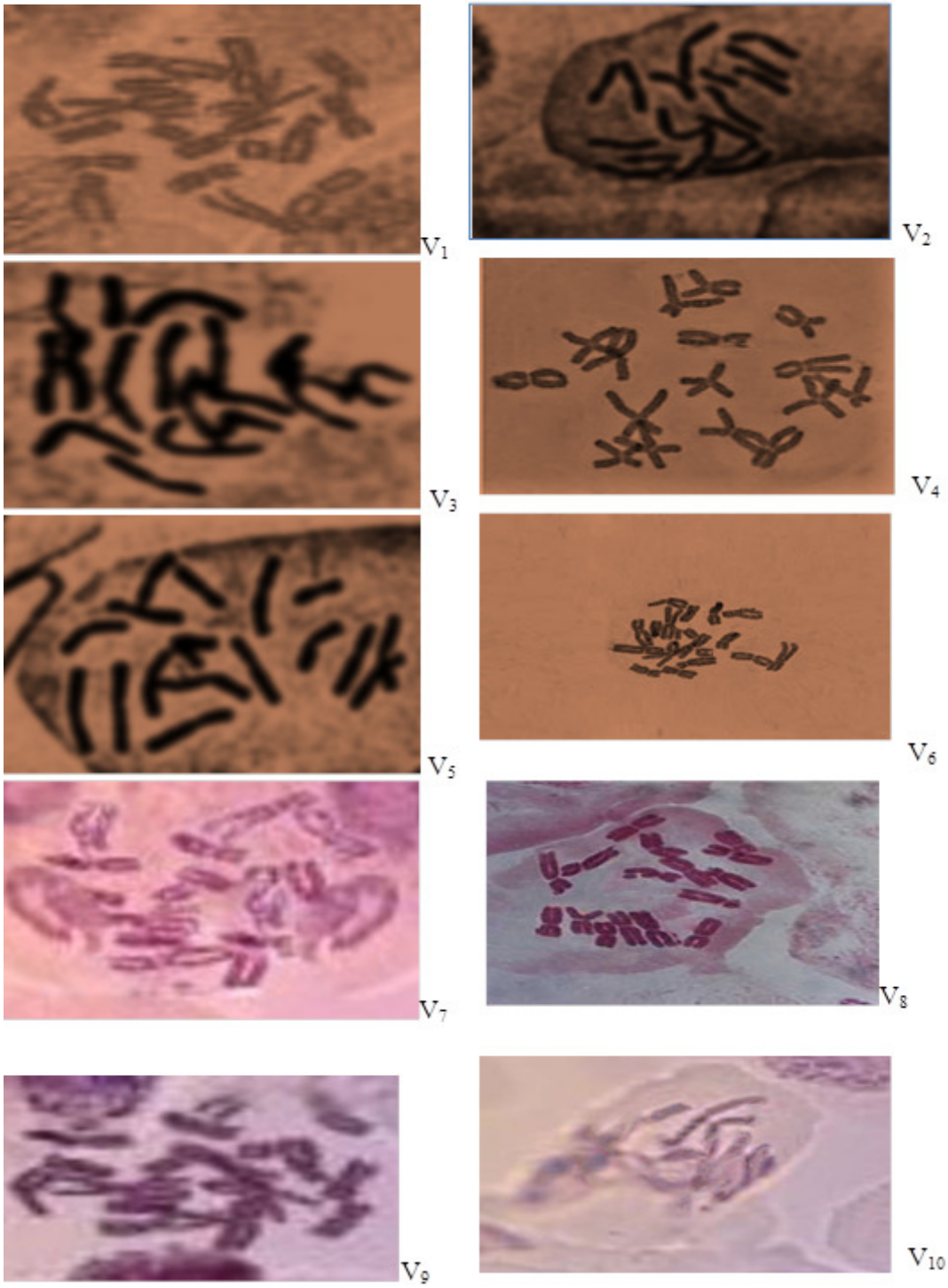

Plate 1. Somatic chromosome of ten genotypes of onion (2n=16) used in the study. Scale bar = 5µmπ. V_1 = Ares, V_2 = Violet De Galmi, V_3 = Red Creole, V_4 = Wase, V_5 = Dan Zaria, V_6 = Dan Giyawa, V_7 = Dan Garko, V_8 = Bahaushe, V_9 = Bakana, V_{10} = Yar Aliero.

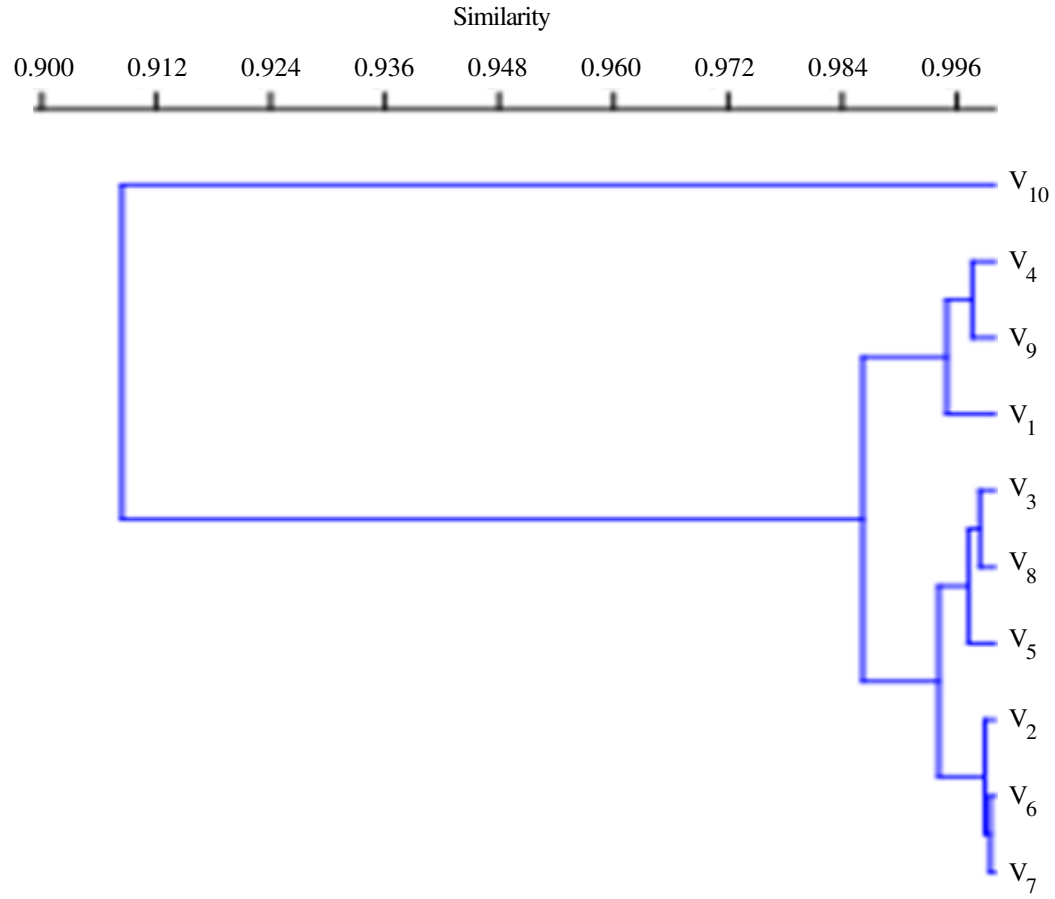

Figure 2. Dendrogram showing the phylogenetic relationships amongst ten genotypes of onion (constructed based on the Ward's method which is the matrix of karyotype distances) Cophenetic Correlation Coefficient (CP) = 0.902032656224554 Key: V_1 = Ares, V_2=Violet De Galmi, V_3 = Red Creole, V_4 = Wase, V_5 = Dan Zaria, V_6 = Dan Giyawa, V_7 = Dan Garko, V_8 = Bahaushe, V_9 = Bakana, V_{10} = Yar Aliero.

The genotypes of onion used in this study showed differences in total form percentage (Table 3), which could be due to chromosomal aberrations (Mukherjee *et al.,* 2012). Variation in chromosome morphologies, as evidenced in the presence of secondary constrictions, could be due to chromosome duplication or translocation between chromosomes with secondary constrictions at the early stages of evolution (Das, 1991; Das *et al.,* 1998; Mohanty *et al*., 2004).

The high value of disparity index in this study (Table 3) indicates a high level of karyotype differentiation, because disparity index (DI) has been reported as a useful tool to differentiate quantitatively and closely related karyotypes belonging to the same class of symmetry (Lavania and Srivastava, 1992).

The genotypes had similar CV values, indicating their phylogenetic relatedness. In other words, they are symmetrical. The coefficient of variability is used to estimate the homology of chromosomes or chromosome arms (Mukherjee and Roy, 2012). It is used to determine the extent of variations among karyotypes. Stebbins (1971) noted that

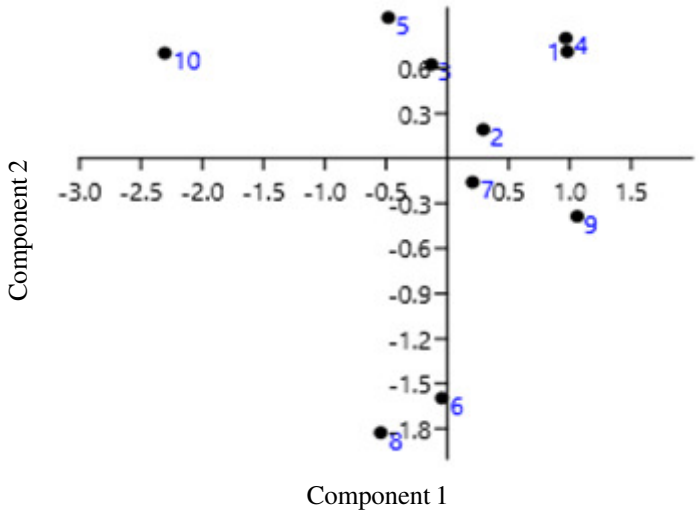

Figure 3. Diagrammatic result of principal component analysis of ten genotypes of onion grown in Jos 1 = Ares, 2 = Violet De Galmi, 3 = Red Creole, 4 = Wase, 5 = Dan Zaria, 6 = Dan Giyawa, 7 = Dan Garko, 8 = Bahaushe, 9 = Bakana, 10 = Yar Aliero.

increased karyotype asymmetry was associated with increased morphological specialisation. Generally, the karyotypes of the onion genotypes used in this study were symmetrical, except for genotypes "Bahaushe" and "Bakana". The intrachromosomal and interchromosomal indices showed slight variations amongst the genotypes. Variations in long and short arm lengths within and between genotypes form the basis of morphological variations amongst different genotypes of onion (Mukherjee and Roy, 2012).

Cluster analysis. The cluster analysis is used to determine the closeness in relationship amongst genotypes. The karyotype parameters were used to calculate genetic association. The results showed that the genotypes were grouped into four different clusters (Fig. 2). In other words, while some were closely related, others were distantly related, perhaps, indicating genetic and/or geographical divergence. Mohanty (2001); Khar *et al.* (2006) reported similar findings in the onion.

In developing a crop improvement programme, a cross between distantly related varieties (a cross between a variety from one cluster group and a variety from another cluster group) could result in a high degree of heterosis (hybrid vigour) in the first filial generation (F_1) and subsequent generations. Ghaderi *et al.* (1984) observed that parents that were distantly related had contrasting alleles located at different loci. These loci recombine in the F_2 or F_3 generation, following crosses between the distantly related parents. Such crosses and recombinations provide opportunities for effective selection and crop improvement. In this study, genotypes in cluster I could be crossed with those in cluster III; while those in cluster II would be crossed with those in cluster IV for maximum genetic recombination and possible hetorotic effect.

Principal component analysis. The principal component analysis (PCA) was used to determine the relative closeness of the genotypes studied. Two principal components, PCA_1 and PCA_2, which were extracted from the original karyotype data had a latent root greater than one and accounted for nearly 92.71% of the total variation amongst the genotypes studied. Characters with high absolute values within the first principal

component are believed to influence clustering more than those with lower absolute value (Chahal and Gosal, 2002). In the present study, the differentiation of the genotypes into different clusters might have resulted from large contributions of few characters rather than small contributions of many characters. The grouping of the genotypes based on two PCAs was in line with the result of the cluster analysis shown in Figure 4. Differences in karyotype formula and asymmetric indices among the onion genotypes in this study suggest that structural chromosomal changes might have contributed to the morphogenetic differences in the genotypes.

Generally, crop improvement techniques such as selection and hybridisation depend, to a large extent, on the existence of variability. Genetic diversity or variations that relate to karyotype forms have been employed as a useful tool in assessing the crop improvement potentials of some crops (Anand and Latha, 2003; Bakhshi *et al.,* 2004; Dhamavanthi, 2005; Oyama *et al.,* 2006).

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