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Cytogenetic research of *Allium cepa* (Onion) and *Allium sativum* (Ginger) for genetic variability in Owerri, Southeastern Nigeria

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Abstract Cytogenetic research on newly discovered species is imperative due to the ongoing discovery of new species across the globe. Chromosome studies of two *allium* species evaluated the chromosome number, structure and sizes in *Allium cepa(onion)* and *Allium sativuum (garlic)*. The diploid chromosome number of *(Allium cepa*) and (*Allium sativum*) were found to be 2n=16 representing 2 sets of chromosomes of *Allium cepa* which were larger in size than those of *A. sativum*. Metaphase plates with a well-dispersed chromosome were counted and each of the chromosome numbers were determined. The chromosome structures in *Allium cepa* were dominated by metacentrics (X=8.8) and Sub-metacentrics (X=4.2). Acrocentrics scores had a mean of (X=3 .0) and Telocentric had a mean of (X=0.6). In *A. sativum,* the structures were metacentrics (X=9.2), Sub-metacentrics (X=5.4) and Acrocentrics (X=0.8). No Telocentric was identified in *A. sativum*. Plants with greater number of acrocentric chromosomes were more advanced than those with greater number of metacentric and sub-metacentric chromosomes. Also, variations in the morphological characters seen in the species could result in distinct geographic origins, supporting the idea that genus *Allium* is developed into distinct strains in various locations over time, as evidenced by differences in chromosome structures.

Keywords: Chromosome structure, Metaphase, Chromosome number, Variation, *Allium* spp.

Introduction

Allium consists of 1006 accepted species, making it one of the largest monocotyledonous genera (KewScience, 2021). Within the *Amarylidaceae* family, the genus *Allium* is the largest with over 800 species of

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monocotyledonous perennial flowering plants, the majority of which are bulbous (Fritsch *et al.,* 2010). Polyploidy, the frequent presence of B chromosomes (Bs), and species-specific variations in ploidy levels are characteristics of the genus *Allium'*s chromosomal numbers. Bs have been detected in 97 species, according to Vujošević *et al*. (2013). Ten of these species also have polyploid forms of Bs. the chromosome. The characteristics of *Allium* can be divided into two groups, i.e.: (i) onion group had satellite chromosomes, and (ii) garlic group had secondary constrictions on their chromosomes.

The number of chromosomes is an essential characteristic for plant evolutionary studies, as it provides information regarding polyploidy and genomic changes. These investigations generate useful information for systematic comparisons of geographical and taxonomic plant groups. The morphology and number of chromosomes are useful in plant systematics for clarifying the origin and evolutionary relationships of plants. Based on the principles of symmetry and asymmetry, the centromere position and relative chromosome length are the most significant karyotypic characteristics utilized to determine chromosomal affinity (Lavania and Srivastava, 1992).

The onion is a staple food that is prized for its unique, strong flavor (Singh *et al.,* 2013). According to Gruben and Denton (2004), salads containing raw onions that have been sliced may be less contaminated with bacteria, protozoa, and helminths. Onion is also utilized globally for the treatment of conditions like blood sugar, rheumatism, cancer, digestive issues, and protracted cough. (Singh *et al*., 2013).

The garlic, *Allium sativum* L.*,* is an old, cultivated plant, valued since ancient times as a vegetable and a spice. It occurs in a great variety of morphologic types; all completely seed sterile. Garlic can be used as both food and medicine in many cultures for thousands of years. It is a wonderful seasoning to add aroma, taste and added nutrition to dishes. Various products, such as pickled garlic, fried garlic, shallots, pickled shallots and pickled onions, are processed from several species of this genus (Pholhiamhan *et al.,* 2018).

In general, all portions of the *Allium* plant are used by humans as spices, vegetables, medicinal herbs and ornamentals, as well as being used as animal feed (Oroji *et al*., 2019) *Allium species* with "antibacterial" or "anticancer" chemicals have attracted interest including *A. ascalonicum, A. ampeloprasum, and A. hirtifolium* (Miri and Roughani, 2018). Currently, shallot, onion and garlic are found in several markets in the world especially in supermarkets, local markets and home gardens in villages (Pholhiamhan *et al.,* 2018). However, their morphology is similar but differs in size, which is confusing due to the use of the common name or local name in each area in the world such as shallot and red onion (Donsakul and Phornphisutthimas, 2010).

For many years, researchers have examined the variation in size, structure, and number of *Allium* chromosomes (Fritsch *et al.,* 2001; Cui *et al*., 2008). It was reported by Vijayanalli and Mathew (1990) that several *Allium species* exhibit intraspecific polyploidy. They also stated that in certain instances, morphological variations are associated with chromosomal number or size. Varieties within the same plant species frequently exhibit chromosomal aberrations as a result of numerous translocations, which can occasionally involve eight or even ten chromosomes, as in the case of garlic. Although favorable cytological characteristics makes species from the genus *Allium* attractive subjects for study, chromosome numbers are known for only about one-third of them and detailed cytological data are very limited (Ramesh, 2015). Although x = 8 is the most frequent basic chromosomal number in *Allium,* additional, numbers (x = 7, 9, 10 and 11) as well as variations in ploidy levels such as diploidy (2n = 2x = 16), triploidy (2n = 3x = 24) and tetraploidy (2n = 4x = 32) have been noted for *Allium* (Sayadi *et al*., 2020; Akhavan *et al*., 2015).

Geographical location of plants has been found to influence the cytogenetic features of *Allium species*. This has prompted numerous investigations into *Allium species*, including that of *Allium cepa* (Awe and Akpan, 2017). Heterochromatin variation in *A. pulchellum* with higher altitude plants are found to have an increased heterochromatin content (Vosa, 1996). In spite of the advantages of *Allium sativum* for chromosome work, only little of such work has been done, especially in comparison with the classical chromosome material of *Allium cepa.* This compelled numerous research of *Allium species,* including *A. cepa.,* according to Oyuntestseg *et al.* (2013) and Tzanoudakis and Trigas (2015).

Cytogenetic research on newly discovered species is imperative due to the ongoing discovery of new species across the globe. These, like other plant species the world over, need to be continually documented and studied. There is the need for cytogenetic studies to establish the phylogenetic relationships of such species (Paknia and Karimzadeh, 2011). Data generated from such studies could be used to characterize and improve the crop species. This research was therefore, conducted to ascertain the genetic variability in *Allium cepa* (onion) and *Allium sativuum* (garlic) in terms of chromosome number, structure, sizes and morphology.

Materials and methods

The study was carried out at the Cytogenetics Laboratory of the Clifford University Owerrinta campus, Abia State, Nigeria. The onion and garlic bulbs used for the study were obtained from Owerri main market in Imo state, Nigeria. They were planted in fine saw dust saturated with water before dispensing in perforated plastic bowls. The germinated bulbs were carefully removed from the saw dust and the young root tips of the bulbs were

harvested at 6-7 days after planting, washed and about 3cm root-tips were cut between 6am-12pm (1hour interval) and pre-treated in 0.02% colchicine for slide preparation. The procedures for slice preparations were as follows:

Pre-treatment

Pre-treatment breaks the spindle apparatus and aids preparation and concentration of the chromosome to make it countable and measured. Harvested root-tips of about 3cm-4cm from the tips were cut between 9am-11pm (1hour interval) and pre-treated in 0.02% colchicine or 8-hydroxyquinoline concentration for 3-4hours.

Fixation

The pre-treated root-tips were washed 3-4 times in distilled water and fixed in acetic alcohol (3 parts of acetic alcohol to 1 part of glacial acetic acid). The fixed root-tips were then kept in the refrigerator (fixative) for about 4-6°C for at least 6 hours. This was carried out to kill the cells while the content of the cell was retained in a normal state.

Hydrolysis

The root- tips were removed from the fixative and washed 3 times in distilled water, then it was transferred into a test tube containing 0.1N HCL in water bath (thermostatic bath) and was left for 7 minutes at 60°C (acid temperature). This was carried out to soften the tissues.

Squashing and staining

The hydrolyzed root-tips were removed from the test tubes with a pair of forceps after been hydrolyzed and washed thrice in distilled water. This was carefully carried out to avoid cutting off the root-tip end. Each of the root-tip was placed on a grease free slide and the meristematic region (milky colour) about 1mm was squashed in a drop of aceto-orcein stain with the aid of a dissecting knife. After squashing, a cover-slip (grease free) was carefully placed over the squashed material gently avoiding the attraction of air bubbles. The slide was placed over two strips of filter paper and a hard thumb pressed applied to further spread out the cells and chromosomes, which also absorbed excess stain.

Data analysis

The slides were studied using binocular microscope under ×10 magnification objective lens. Cells that displayed the different mitotic stages

and well spread out chromosomes at metaphase stage were further studied under higher magnification ×40 and ×100 oil immersion objective lens. Photographs of the stages were captured under oil immersion objective lens (×100) at the Veterinary Pathology laboratory of the Department at the Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

Results

The morphological traits of the genotypes that were used for this investigation is displayed in Table 1. It revealed that the in *Allium cepa*, the colour of the leaf sheath is dark red when young and mature and has a linear with tubular leaf shape and has no cloves (Plate A) while in *Allium sativum*, the colour of the leaf sheath is white when young and mature and has a broadly linear to linear-lanceolate leaf shape with small cloves (Plate B).

Table 1. Morphological characteristics of *Allium species* used in the study

Species	Colour of Leaf Sheath	Leaf shape	Cloves
***Allium cepa* L. (Onions)**	Dark red when young and mature	Linear with tubular	No Cloves
***Allium sativum* L. (Garlic)**	White when young	Broadly linear to linear-lanceolate	Cloves

Plate A. ***Allium cepa* (Onion)**

Plate B. ***Allium sativum* (Garlic)**

Figure 1. Morphology of bulbs from the *Allium* species used in the study (Plate A and B)

Determination of chromosomes structures

Chromosomes were scored at metaphase for structure; i.e. Telocentric (centromere at the end), Metacentric (when is centrally situated), Sub-metacentric (when the centromere is oriented towards the center) and Acrocentric (when the chromosome is oriented towards the terminal position) are shown in Figure 2.

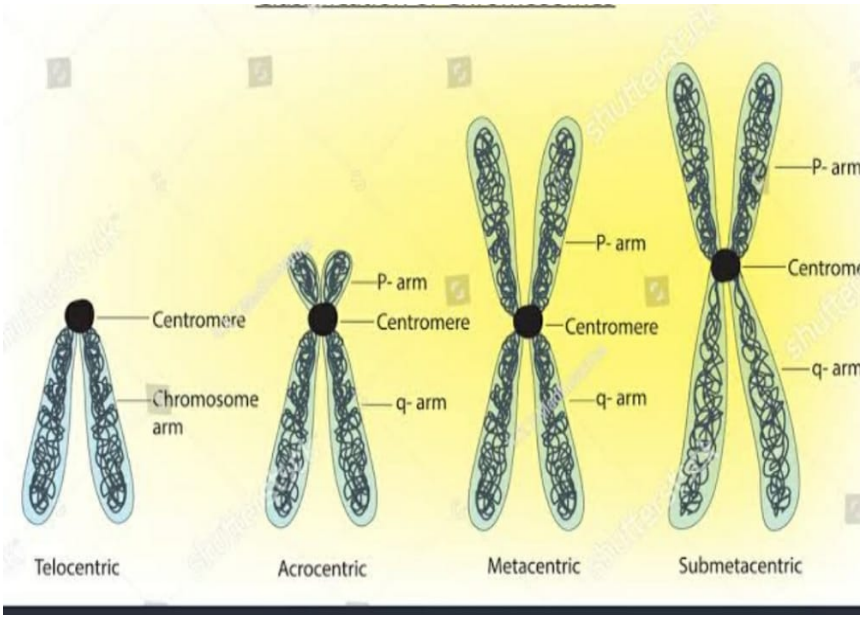


Figure 2. Chromosome structures of *Allium cepa* and *Allium sativum*

Determination of chromosome number

Metaphase plates with a well-dispersed chromosome from various individuals were counted for clarity and organization and the chromosome number of each of the two crops were determined. The chromosome under each crop was taken to be the most frequently occurring number (Table 2 and 3).

Table 2. Chromosome counts in metaphase plates of *Allium cepa*

Metaphase Plate	Number of chromosomes
1	16
2	16
3	18
4	18
5	16
6	16
7	16
8	16

*Most likely chromosome number is 16

Table 3. Chromosome counts in metaphase plates of *Allium sativum*

Metaphase plate	Number of chromosomes
1	16
2	16
3	16
4	18
5	16

*Most likely chromosome number is 16

Determination of chromosome size and structures in Allium cepa and Allium sativum

The different chromosome structural types in *Allium cepa* and *Allium sativum* are revealed in Tables 4 and 5 respectively. The dominant structures in *Allium cepa* are metacentric (×=8.8) followed by sub-metacentric (×=4.2) acrocentric (×=3.0) and telocentric is less than 1.

The dominant structures in *Allium sativum* are metacentric (×=9.2) followed by sub-metacentric (×=5.4) acrocentric is less than 1and Telocentric was not found. The dominant structures in *Allium cepa* are metacentric (×=8.8) followed by sub-metacentric (×=4.2) acrocentric (×=3.0) and telocentric is less than 1 (Tables 4 and 5).

The different mitotic stages in *Allium cepa* are revealed in Figure 3 while Figure 4. It showed different mitotic stages in *Allium sativum*. Both Figures had magnification of x100. Consequently, the sizes of chromosomes in *Allium cepa* are larger than those of *Allium sativum*.

Table 4. Summary of chromosome structures of six metaphase plates of *Allium cepa*

Metaphase Plates	
1	1Telocentric+2Acrocentric + 3 Sub-metacentric +10 Metacentric
2	4 Acrocentric + 9 Metacentric + 3 Sub-metacentric
3	9 Metacentri+1 Telocentric +4 Acrocentric + 4 Sub-metacentric
4	1 Telocentric + 4Sub-metacentric +3 Acrocentric + 8 Metacentric
5	10 Metacentric + 5 Sub-metacentric +1 Acrocentric
6	7 Metacentric + 6 Sub-metacentric +1 Telocentric + 4 Acrocentric

Chromosome Type	Total	Mean
Telocentric	4	0.6
Acrocentric	18	3.0
Submetacentric	25	4.2
Metacentric	53	8.8

Table 5. Summary of chromosome structures of *Allium sativum*

Metaphase plates	
1	1 Acrocentric + 5 Submetacentric+10 Metacentric
2	11 Metacentric + 4 Sub-metacentric + 3 Acrocentric
3	1 Acrocentric + 9 Sub-metacentric + 6 metacentric
4	10 Metacentric + 5 Sub-metacentric + 1 Acrocentric
5	9 Metacentric + 4 Sub-metacentric + 3 Acrocentric

Chromosome Type	Total	Mean
Telocentric	0	0
Acrocentric	9	0.8
Submetacentric	27	5.4
Metacentric	46	9.2

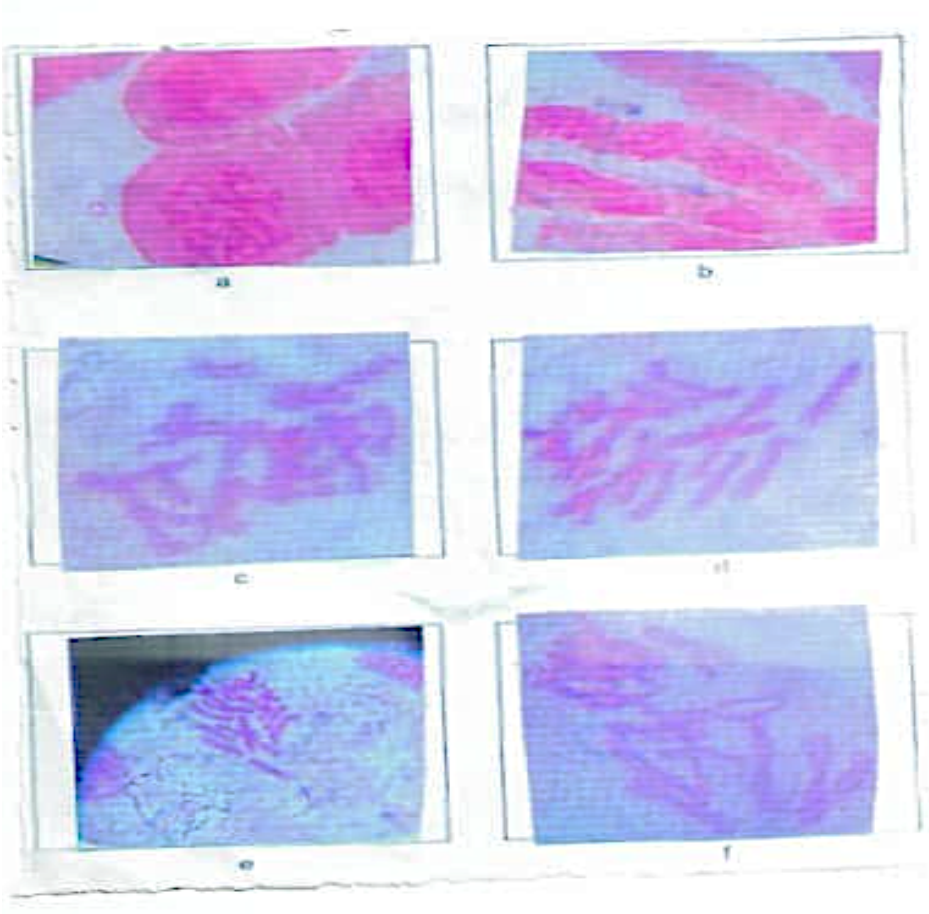


Figure 3. Mitosis in *Allium cepa* root tips (onion): (a) prophase; (b-f), metaphase plate (x1000)

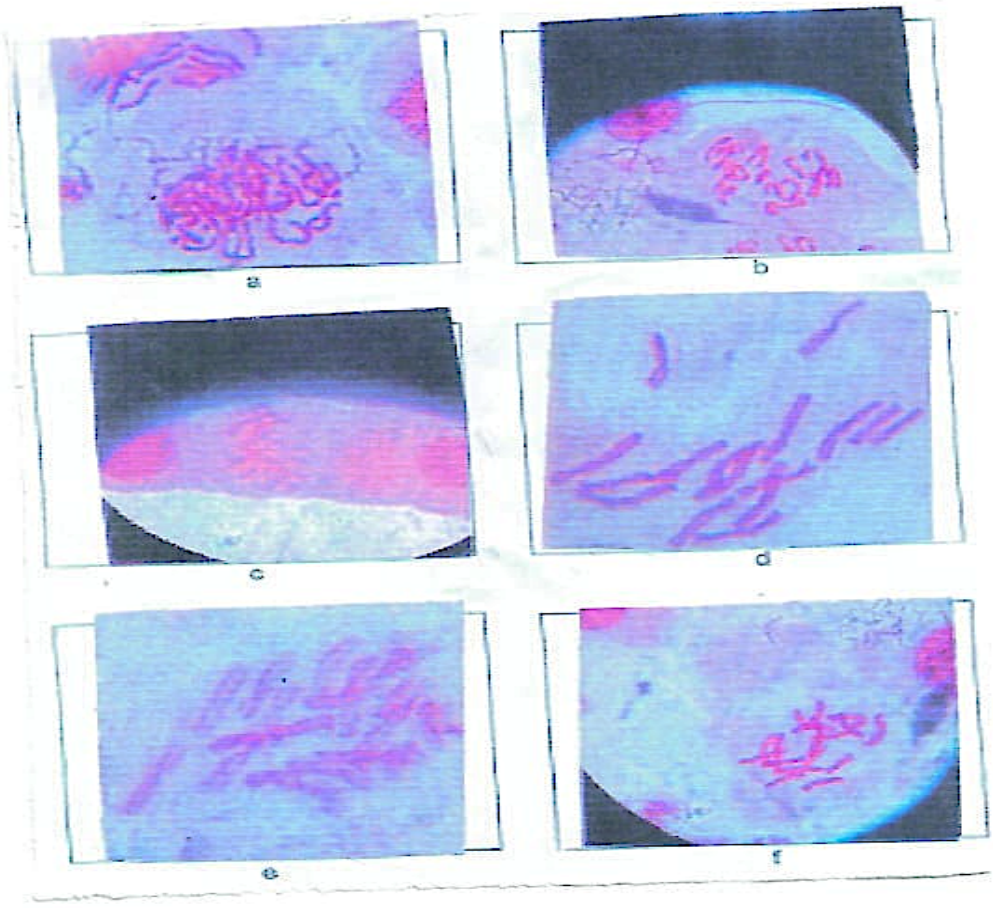
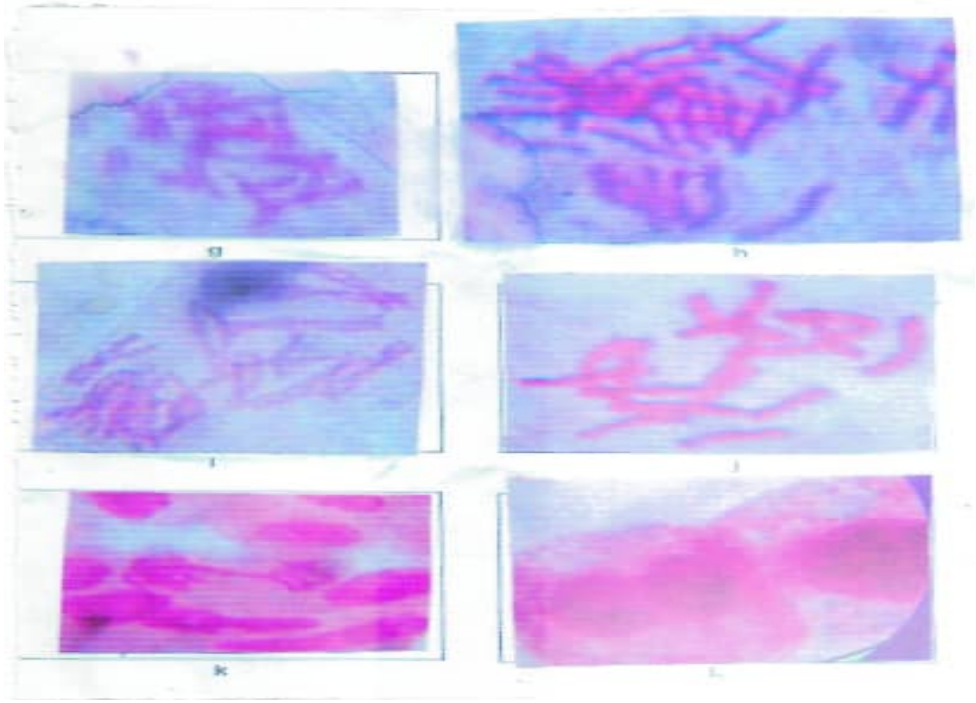
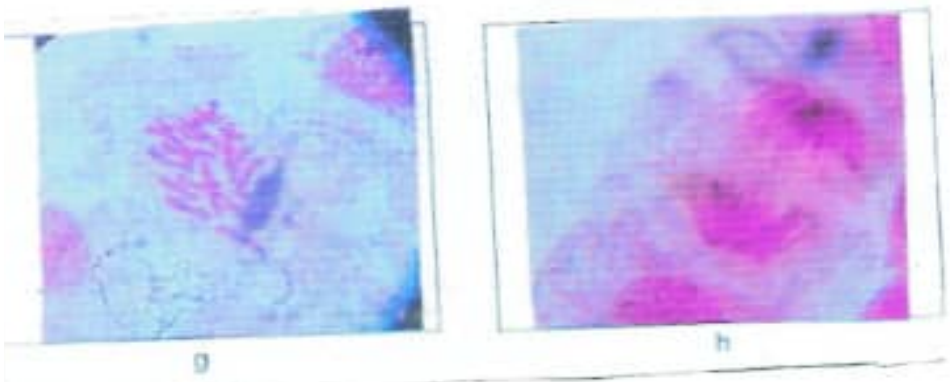


Figure 4. Mitosis in *Allium sativum* root tips (garlic): (a) prophase stage (b) late prophase; (c) metaphase (without pretreatment); (d-f) metaphase (pretreated) (x1000)



G-j Metaphase; J: Late anaphase (noted delayed – disjunction of one chromosome); L: Telophase



(G) Metaphase (pretreated); (h) anaphase (x1000)

Figure 5. Delayed disjunction of one chromosome; G-H pretreated (x1000)

Discussion

The best metaphase plates were scanned at 100 resolutions after being captured on camera with an external camera (Celestron digital microscope imager 2.0) mounted on the BX50 Olympus microscope. The program Image J was used to record each measurement (Abramoff *et al.,* 2004). According to Jalilian and Rahiminejad (2012), chromosomes were found to be metacentric, sub-metacentric, or sub-telocentric.

Somatic chromosome count, base number and ploidy level of species form the primary basis of cytotaxonomy. Chromosome number might be used in systematic characteristics. The closest relationship between two plants can be known from similar number of chromosomes among them. Somatic metaphases showed that the chromosome number of *Allium cepa* and *Allium sativum* was found to be 2n=16. This finding agrees with Akhavan *et al*. (2015), who determined a basic chromosome number of x = 8 in *A. przewalskianum* and 10 species of *Allium (sect. Acanthoprason*). Contrary to this findings Han *et al*., found chromosomal counts of 2n = 16, 24, 32, 40, and 48 in various *A. ampeloperasum* subspecies. The chromosomes in *Amerillidaceae* showed great variability in length. Plants with greater number of acrocentric chromosomes are more advanced than those with greater number of metacentric and Sub-metacentric chromosomes (Levan *et al*., 1964; Wajahatullah and Vahidy (1990).

According to Zuo and Yuan (2011), the *Allium* species under study exhibited sub-telocentric chromosome morphology, or secondary constriction, which implies that these genotypes have preserved some of their ancestral wild features. According to Mukherjee and Roy (2012), variations in the number of chromosomes could be the consequence of chromosomal aberrations or mutations that occurred in natural populations.

The comprehensive review of karyotypes demonstrates the significance of chromosomal structural modifications in the emergence of novel races (Awe and Akpan, 2017). Early in evolution, chromosome duplication or translocation between chromosomes with secondary constrictions may have caused variation in chromosomal morphology, as shown by the presence of secondary constrictions (Das *et al.,* 1998; Mohanty *et al*., 2004).

Investigations into the chromosomes aid in the solution of challenging taxonomic puzzles and offer hints regarding the basic processes of plant evolution and genome structure. Consequently, variations in the number of cloves, leaf size, leaf shape, and leaf sheath color seen in the species may also be the result of distinct geographic origins, supporting the idea that the genus *Allium* has developed into distinct strains in various locations over time, as evidenced by differences in chromosome structures.

Variability is generally necessary for crop improvement methods like hybridization and selection to work. In order to evaluate the potentials for

crop improvement in certain crops, genetic diversity or variations related karyotype forms have been used. All native populations have altered genetic makeup due to habitat-related genetic processing, which may have an impact on physiological and morphological processes. The environment may have an impact on a species' genotype and potentially alter the likelihood of chromosomal differentiation. Furthermore, the similarities and variations exhibited in the two *allium species* studied demonstrated that they can be used as potential genotypes for evaluating inter-generic classification in plant breeding programs.

Conflicts of interest

The authors declare no conflict of interest.

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