KARYOLOGICAL STUDIES IN (CAPSICUM CHINENSES JACQS. VAR. ATTARUGU), GIREI LGA, ADAMAWA STATE

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ABSTRACT

The genus Capsicum of the Solanaceae family, which are found in tropical and temperate regions worldwide, are valued as spices or vegetables by many different cultures. The research was undertaken at the Faculty of Life Sciences, Department of Plant Science, Modibbo Adama University, Yola. The objective of this research was to develop the Karyotype of Capsicum chinenses Jacqs. var. Attarugu variety collected from farmers in Girei LGA, Adamawa State and to understand possibilities of its use in genetic improvement of Capsicum genus. Seeds were germinated on moist filter paper in Petri dishes. Root-tips were pretreated in 0.05% colchicines for 5 hours and fixed in acetic ethanol (1:3v/v). The pre-fixed roots were rinsed in running tap water for 1 minute and then fixed directly into Carnoy's Fluid (Glacial acetic acid, chloroform, and absolute ethanol in a 6:3:1 ratio) solution for 24 hours. The roots were then removed and stored in 70% ethanol until required for cytological analysis. Squashing technique was used in 2% acetic-orcein stain for 3 minutes. Light microscope with (MOTIC Camera Version 2.0) for microphotograph and (IDIEOKAR software Version 2.7) for measuring ideogram and other parameters. The results revealed that there was 2n = 2x = 24 diploids chromosome number, total form of chromosomes was 33.07, coefficient of variation in chromosomes length was 15.24, coefficient of variation in Centromere index was 23.75 and symmetrical tendency of the variety were observed. Karyotype formula (KF) was 7M+15SM+ 2St, respectively.

KEYWORDS: *Capsicum chinense, Karyotype, Root tips, diploid, symmetrical, Cytogenetics*

1. INTRODUCTION

The genus Capsicum of the Solanaceae family, which are found in tropical and temperate regions worldwide, are valued as spices or vegetables by many different cultures. The genus has significant economic importance for the national and international condiment, seasoning and canning markets, and it is cultivated at scales ranging from family production to industrial systems [1]. Capsicum species are immensely valued not only because of their economic importance but also for their rich nutritional value. Besides the nutritional benefits of pepper and their use as food additives, the hot Capsicum species (due to their capsaicin content) have a significant role in pharmacy and are currently used for different therapeutic purposes [2]. Approximately, the genus Capsicum consists of 35 species out of which five are widely domesticated. These are C. annuum L., C. chinenses Jacqs., C. frutescens L., C. pubescens R. and C. baccatum L. [3]. In Nigeria, three out of the five domesticated species namely: Capsicum annuum L. (Tattasi group), Capsicum frutescens L. (Borkunu group) and Capsicum chinense Jacq. (Attarugu group) grow well in many communities and in the North-eastern sub-region [4].

Studies on Capsicum species have shown that they contain 24 chromosomes (2n=2x=24), similar to many species of Solanaceae family. There are two distinct groups present in the genus; some species have 24 chromosomes (2n=2x=24) while other species have 26 chromosomes (2n=2x=26). The most common chromosome number in the genus is x=12 [5]. The species with 24 chromosomes have symmetrical Karyotype. They generally have one pair of acro-centrics and the rest of the chromosomes are metacentric. In contrast with, the species with 26 chromosomes display more asymmetrical complements, with more sub-metacentric (sub-telocentric) chromosomes and often one telo-centric chromosome [6]; [7]; [8].

Cytogenetics provides a valuable and irreplaceable source of information to address taxonomic, evolutionary and applied problems [9]. Despite the many uses to which the Capsicum fruits have been put on the African continent and their enormous potentials as important culinary, medicinal and industrial raw materials, cytological work in the genus is still inadequate, as the cytogenetic data necessary for a proper understanding of the interspecific and interspecific relationships in the genus has remained insufficient [4]**.** The objective of this experiment was to count the number of chromosomes in the commercially important pepper variety (C. chinenses Jacqs. var. Attarugu) collected from Girei LGA, Adamawa State and to understand possibilities of its use in genetic improvement of Capsicum genus.

2. Materials and Methods

2.1. Experimental Site

The research was undertaken at the Faculty of Life Sciences, Department of Plant Science, Modibbo Adama University, Yola. The research covers chromosomes number and Karyotype studies in (*C. chinenses* Jacqs. var. Attarugu) varieties.

2.2. Collection of Plant Samples

The seeds of obtained directly from the farmers in Girei Local Government Area. The plant samples were moved down to the Department of Plant Science for identification using the preserved specimen voucher. The identified sample was *C. chinenses* Jacqs. var. Attarugu.

2.3. Germination of Plant Samples

The Seeds of fruits were extracted, air-dried and processed for chromosomal investigation. The seeds of the plant samples were immersed and washed with tap water to remove the contamination from the outer surface prior to planting of the seeds. Seeds were germinated on moist filter paper in Petri dishes. Prior to root harvesting the set up was not watered for 24 hours.

2.4. Preparation of Root Tips

The technique according to Oroji [10] was used as follows: Root tips were collected when the length of the emerged *C. chinenses* Jacqs. var. Attarugu root tips were 1- 1.5 cm. The harvested roots were washed thoroughly in running tap water so as to remove debris of soil. Prior to root harvesting the set up was not watered for 24 hours.

2.5. Pre-treatment

The technique described by Hosseini [11] was used as follows: Root tips that have bulgy and creamy root tips were selected approximately 1 – 1.5 cm from the root tip was excised. The excised roots were immersed in 0.05 % Colchicine for 5 hours and fixed in acetic ethanol (1:3v/v). The set up was aerated at 30 min intervals using battery operated aerator bubble so as to replenish loss of oxygen.

2.6. Fixation

The technique described by Rasha and Helmey, [12] were used as follows: The pre-fixed roots were rinsed in running tap water for 1 minute and then fixed directly into Carnoy's Fluid ("Glacial acetic acid, chloroform, and absolute ethanol" in a 6:3:1 ratio) solution for 24 hours. The roots were then removed and stored in 70 % ethanol until required for cytological analysis.

2.7. Hydrolysis and Squash Method

A technique by Rasha and Helmey [12] were used as follows: The root tips were then washed in distilled water at room temperature, transferred into 1N Hydrochloric acid (HC l) at 60º C in a water bath for 8-10 minutes. The root tips were then washed with distilled water in the same test tube at room temperature. First 2-3 mm from the root cap was cut and the rest parts were discarded. 2% Aceto-orcein stain was added to the root tips for 3 minutes. The tips were then squashed in the Aceto-orcein stain. The slides were then covered with a cover slip and a blunt end of a pen was used to press gently over the cover before examining under the light microscope with (MOTIC Camera Version 2.0).

2.8. Preparation of Karyotype:

At least five excellent metaphase cell plates were utilized for Karyotype analysis. From photomicrographs, photo-ideograms were created by cutting out individual chromosomes, organizing them in descending order of their length, and matching them based on their Centromere position. The software for measuring chromosomes produced the ideogram and other parameters (IDIEOKAR software 2.7). Six to eight well-separated mitotic plates were utilized for the Karyotype examination. Levan et al [13] created the now employed chromosomal nomenclature. The CVCI parameter measured the relative variation in centromeres location of chromosomes in complement, whereas the CVCL parameter evaluated the relative variation in chromosomal length [14]. The Stebbins classification and [15] asymmetry index (AI) were used to evaluate the Karyotype overall asymmetries.

3. RESULTS AND DISCUSSION

3.1. Results

Metaphase chromosomes Karyotype and ideograms of somatic metaphase chromosomes of each species are shown in (Figure 1a, b.), with detailed parameters listed in (Table 1 Table 2 and Table 3). Brief descriptions of the cytological features are as follows.

Somatic chromosomes number (2n), Long arm (LA), Short arm (SA), Total length (TL)

Cytological investigation of *Capsicum chinenses* Jacqs., var. Attarugu was undertaken in order to generate cytological information on its karyological profile which could be factored into decisions pertaining to appropriate crop improvement regimes for the crop. A somatic chromosome number was 2n = 24. The somatic diploid chromosome number was twenty four, long arm length ranges from 21-47 µm, short arm length ranged from 8-25 µm, and total length ranges from 32-65 µm (Table 1; Figure 1) respectively.

Arm ratio (AR), Mean value (r-value), Relative length (RL), Form percentage (F%), Centro-metric index (CI) and Chromosomes type (CT)

The Arm ratio, mean value (r-value), relative length, form percentage, Centro-mere index and chromosomes type were measured. The Arm ratio ranges from 1.00-3.88 µm, mean value (r-value) ranged from 0.31-1.00, form percentage ranged from 0.69-2.16 %, Centro-mere index 0.21-0.45 µm and chromosomes type submetacentric were predominant and then followed by metacentric chromosomes (Table 2; Figure 1) respectively.

Total form (TF), Gradient index (Ask%), Symmetry Percentage (S %), Arm ratio mean (A), Coefficient of variation in chromosomes length (CVcl), Coefficient of variation in Centromere index (CVci), Disparity index (AI), Stebbins Classification and Karyotype formula (KF)

The total form of chromosomes was 33.07, percentage asymmetry was 66.93, symmetry percentage was 49.23, arm ratio mean was 0.34, coefficient of variation in chromosomes length was 15.24, coefficient of variation in Centromere index was 23.75 and based on stebbins classification the chromosomes were classified as 1B, The somatic complement has seven pairs with median centromeres, fifteen pairs with sub median centromeres and two pairs with sub terminal centromeres. Karyotype formula (KF) was 7M+15SM+ 2St, (Table 3 Figure 1), respectively.

Table 1. Somatic chromosomes number (2n), Long arm (LA), Short arm (SA), Chromosomes length (CL)

(2n)	LA (µm)	SA (µm)	CL (µm)
1	21	11	32
2	29	18	47
3	34	13	47
4	26	19	45
5	32	16	48
6	38	19	57
7	33	15	48
8	35	12	47
9	37	15	52
10	32	22	54
11	31	14	45
12	29	9	38
13	39	13	52
14	34	25	59
15	21	21	42
16	31	8	39
17	34	13	47
18	21	17	38
19	33	16	49
20	30	25	55
21	37	13	50
22	47	18	65
23	34	12	46
24	35	18	53

Table 2. Arm ratio (AR), Mean value (r-value), Relative length (RL), Form percentage (F %), Centro-metric index (CI) and Chromosomes type (CT)

AR (µm)	r-Value (µm)	RL (%)	F (%)	CI (µm)	CT
1.91	0.52	2.77	0.95	0.34	Sm
1.61	0.62	4.07	1.56	0.38	M
2.62	0.38	4.07	1.13	0.28	Sm
1.37	0.73	3.90	1.65	0.42	M
2.00	0.50	4.16	1.39	0.33	Sm
2.00	0.50	4.94	1.65	0.33	Sm
2.20	0.45	4.16	1.30	0.31	Sm
2.92	0.34	4.07	1.04	0.26	Sm
2.47	0.41	4.50	1.30	0.29	Sm
1.45	0.69	4.68	1.90	0.41	M
2.21	0.45	3.90	1.21	0.31	Sm
3.22	0.31	3.29	0.78	0.24	St
3.00	0.33	4.50	1.13	0.25	Sm
1.36	0.74	5.11	2.16	0.42	M
1.00	1.00	3.64	1.82	0.50	M
3.88	0.26	3.38	0.69	0.21	St
2.62	0.38	4.07	1.13	0.28	Sm
1.24	0.81	3.29	1.47	0.45	M
2.06	0.48	4.24	1.39	0.33	Sm
1.20	0.83	4.76	2.16	0.45	M
2.85	0.35	4.33	1.13	0.26	Sm
2.61	0.38	5.63	1.56	0.28	Sm
2.83	0.35	3.98	1.04	0.26	Sm
1.94	0.51	4.59	1.56	0.34	Sm

Table 3. Total form (TF), Gradient index (Ask%), Symmetry index (S%), Asymmetry index (A), Coefficient of variation in chromosomes length (CVcl), Coefficient of variation in Centromere index (CVci), Disparity index (AI), Stebbins Classification and Karyotype formula (KF)

TF	AsK (%)	S (%)	A (µm)	CVcl (µm)	CVci (µm)	AI	Stebbins Class	KF
33.07	66.93	49.23	0.34	15.24	23.75	64.16	1B	7M+15SM+ 2St

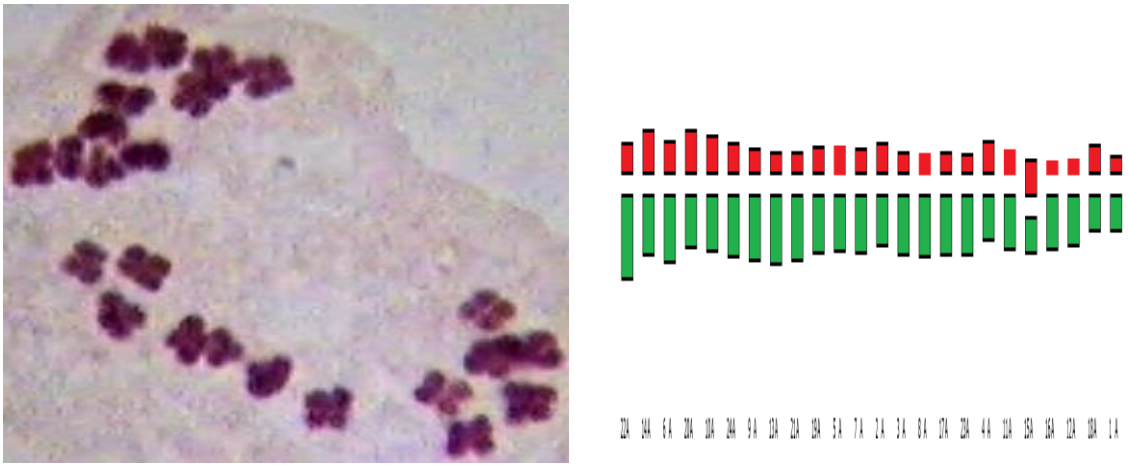

Figure 1. Microphotograph of *Capsicum chinenses* Jacqs., var. Attarugu (**a).** Ideogram of *Capsicum chinenses* Jacqs., var. Attarugu (**b).**

4.2. Discussions

The importance of Karyotype analysis in distinguishing plant species is well known. Each plant species or variety is characterized by its Karyotype. Karyomorphology and chromosome analysis of a variety of a species are useful in its identification as also in establishing the relationship among the species.

Chromosomes number

Cytogenetic provides a valuable and irreplaceable source of information to address taxonomic, evolutionary and applied problems [16]. Based on this present study the results obtained revealed that twenty four somatic chromosomes number were observed. This agreed with the findings of Sousa *et al*. [1]. The basic chromosome number of *C. chinenses* was observed as 2n = 2x = 24. In general, normal plant has two pairs of chromosomes called diploid (2n = 2x) in somatic cells, but some plants have more than two pairs of chromosomes as the potatoes which has four pairs chromosome or tetraploid (2n = 4x) and wheat bread (2n = 6x) [17]; [18]. Wadt *et al*. [19] reported that although most of *Capsicum* species are 2n = 24 and present high similarity in chromosome morphology, the genus possesses high intraspecific and interspecific Karyotype variability. Also, this result is in agreement with reported chromosome counts in the genus *Capsicum* [20; 21].

Chromosomes length long and short arms

The long arm length ranges from 21-47 µm, short arm length ranged from 8-25 µm, and total length ranges from 32-65 µm. The highest value for the long arm length of the chromosome was observed to be 47 µm and the lowest observed was 21 µm respectively. Karyotypes in different species with 24 chromosomes are very similar with each other. The species with 24 chromosomes have symmetrical Karyotype. However, the chromosomes arms length reported in the literature conflict with those observed in the present study. This is because of a variety of parameters such as pretreatment time and chromosome preparation process, the entire or arm length of homologous chromosomes in the same or separate cells might be extremely different [22]; [23]

Karyotype formula arms ratio and Symmetry Index

The Karyotype formula reported in the literature conflict with those observed in the present study. Guerra [25] reported the Karyotype formula 11M + 1A for a number of Venezuelan accessions of *C. chinenses* using Giemsa stain. Souza *et al*. [26] also observed the formula 11M + 1A through conventional Cytogenetics in *C. chinense* accessions from different states of Brazil. Sousa et al. [26] analyzed the evolutionary patterns across species of *Capsicum* by chromosome banding and observed the Karyotype formulas of 11M + 1SM + 1A and 11M + 1A for *C. frutescens*, respectively. This observation is contrary to the results of the variety studied in this work. Here, in this present study Karyotype formula (KF) was 7M+15SM+ 2St, respectively. The total form of chromosomes was 33.07, percentage

asymmetry was 66.93, symmetry percentage was 49.23 and arms ratio mean was 0.34, respectively. However, the Karyotype formulas reported here in this present study conflict with those observed in other literatures. The differences that exist in Karyotype formula and asymmetric indices suggest that structural chromosomal changes might have contributed to the morphogenetic slight differences in the genotypes [27].

Centromeres position, coefficient of variation and Stebbins classification

Based on this present study, metacentric dominated the Karyotype while remaining were submetacentric and subtelocentrics. Also, this present investigation revealed that coefficient of variation in chromosomes length was 15. 24 and coefficient of variation in centromere index was 23.75. This is closely related to the findings of Kelechukwu and Chinenye [4], which revealed that a coefficient of variation value of 10.08 length credence to the inference that ample Karyotype variability exists in this crop to ensure positive response to selection and/or intra-varietal hybridization methods of crop improvement. The classification of Stebbins is the most frequently used qualitative method for assessing karyotype symmetry and asymmetry conditions and describing the karyotypic relationship between different species [28]; [14]. Based on this present study, the chromosomes were classified as 1B according to Stebbins classification and the somatic complement has seven pairs with median centromeres, fifteen pairs with sub median centromeres and two pairs with sub terminal centromeres. They generally have one pair of acro-centrics and the rest of the chromosomes are metacentric [29]; [8]. The results obtained through Karyotype studies are helpful in the development of new and improved forms of the economically important plants. Chromosome study is also used to correlate differences in chromosome number or morphology with morphological differentiation of the species [30], [31]. In the present investigation the metacentric, submetacentric chromosomes and nearly subtelocentrics with negligible variation in their size were observed. It concurs with the findings of [32], [33] that *Capsicum* genus studied were predominantly the chromosomes were metacentric and submetacentric based on their research. However, according to [34], the presence of sub-telocentric chromosomal shape shows that these genotypes have kept some of their original wild features.

4. CONCLUSION

The results obtained from the *Capsicum chinenses* Jacqs., var. Attarugu was similar to the data reported for populations in other natural habitats. Karyotype and chromosome morphology analysis indicated that *Capsicum variety* studied here has metacentric and submetacentric chromosomes were predominantly which indicated symmetric Karyotype. The symmetrical morphology is a reflection of the relatively primitive Karyotype of the variety in this genus. The coefficient of variability was used to estimate the homology of chromosomes or chromosome arms. The karyological characterization of *Capsicum chinense* var. Jacqs., Attarugu, was undertaken in this study, has revealed that data generated from this studies could be used to characterize and improve the *Capsicum* genus. The study revealed that a diploid chromosomes number (2n=2x=24) were observed, a total form (TF) of 33.07 % and coefficient of variation in chromosomes length 15.24 µm, coefficient of variation in centromere index 23.75 µm were also observed. Based on Stebbins classification the chromosomes were classified as 1B. The Karyotype formula (KF) was 7M+15SM+2St, respectively. Further analysis should be carried out using other methods to make a more accurate reconstruction of the genus *Capsicum* and to study the presence of tetraploidy.

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