

MITOTIC REGULARITY AND CHROMOSOME NUMERICAL STATUS IN SOME VARIETIES OF *CAPSICUM* CROPS CULTIVATED IN THE NORTH-EAST SUB-REGION OF NIGERIA

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ABSTRACT

Chromosome studies were conducted on 14 varieties of *Capsicum* crops in cultivation within the six states of north-eastern Nigeria. These varieties belonging to three species namely: *Capsicum chinense*, *Capsicum frutescens*, and *Capsicum annuum* were investigated in order to compare their morphology and numerical status and to ascertain if observed variations in fruit morphology had any numerical support. Seeds were collected from locations across the sub-region and processed for planting and subsequent field morphological characterization. Ripe fruits were harvested, seeds extracted and germinated in the Laboratory. A cytological investigation was carried out using germinating root tips. Results showed all 14 Karyotypes had remarkable homogeneity in chromosome morphology. The array of fruit morphological formations existing at the phenotypical level did not reflect in karyotype constitutions, thus suggesting a common origin for the species which had resulted in the formation of a uniform congregation.

KEYWORDS: Capsicum, Crops, Morphological & Karyotype

INTRODUCTION

The *Capsicum* peppers, wild or domesticated, belong to the genus *Capsicum*. The genus makes up one of the 85 genera situated in the family *Solanaceae* (the nightshades) (Gill, 1988). The family comprises of many economically important food and industrial crops such as potato, tobacco, tomato, garden egg, petunia and pepper (*Capsicum*). Recent workers in the field of plant taxonomy have pegged the list of species in the genus to between thirty to thirty-one species (Eduardo *et al.*, 1996; Oyama *et al.*, 2006). Among this vast number of species, five species are generally recognized as domesticated (Heiser and Smith, 1953; IBPGR, 1983; Kochlar, 1986; Bosland, 1996). The domesticated species are:

- *Capsicum annuum* L. 1753
- *Capsicum frutescens* L. 1753
- *Capsicum chinense* Jacq. 1776
- *Capsicum pubescens* Ruiz and Pav. 1797
- *Capsicum baccatum* L. 1767-1771

Though relatively recent in Africa, having been introduced after 1500 AD (Okigbo, 1994), peppers have been part of the Human diet since 7500 BC, with Tehuacan, Mexico as the center of origin (Pickersgill, 1971; Heiser, 1969). In Nigeria, three of the five domesticated species, namely, *Capsicum annum* L. (Tattasi group), *Capsicum frutescens* L. (Borkunu group) and *Capsicum chinense* Jacq. (Attarugu group) grow well in many communities. In the North-eastern sub-region. The peppers are grown by almost all local communities and it constitutes an important seasoning spice for most adult foods. Due to its high capsaicin content and composition of vital vitamins such as vitamins A and C, *Capsicum* fruits are used in the home as condiments. Pepper fruits have also been put to other medicinal, industrial and environmental uses.

Generally, each species of an organism has its own characteristic chromosome complement i.e. its karyotype. Though it is reasonable to expect every species to have a constant number of chromosomes, but some intervening variables such as polyploidy, the presence of B chromosomes and even errors in methodology tend to lend this constant number as controversial in some cases Darlington and Wylie (1955). Different chromosome numbers have been reported by different workers for the *Capsicum* plant. Some have reported $2n = 24$ as the chromosome number (Dixit, 1931; Benerji, 1932; Raghavand and Venkatanban, 1940; Pal *et al.*, 1941; Kumar *et al.*, 1987; Eduardo, 1990; Morakinyo and Falusi; 1992; Kumar and Raja, 2004; Oyama *et al.*, 2006; Araceli *et al.*, 2009). Others have documented $2n = 36$ and $2n = 48$ as the chromosome numbers (Darlington and Wylie, 1955; Datta, 1968).

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 intraspecific relationships in the genus has remained insufficient. Though, crossability potentials amongst some taxa in the genus have been explored (Egawa and Masatake, 1989; Eshbaugh *et al.*, 1983; Kumar *et al.*, 1987), but most of the work had concentrated on western and Asian varieties. Indigenous African varieties have received scanty attention (Nwankiti, 1976, Morakinyo and Falusi, 1992; Malgwiet *et al.*, 2000).

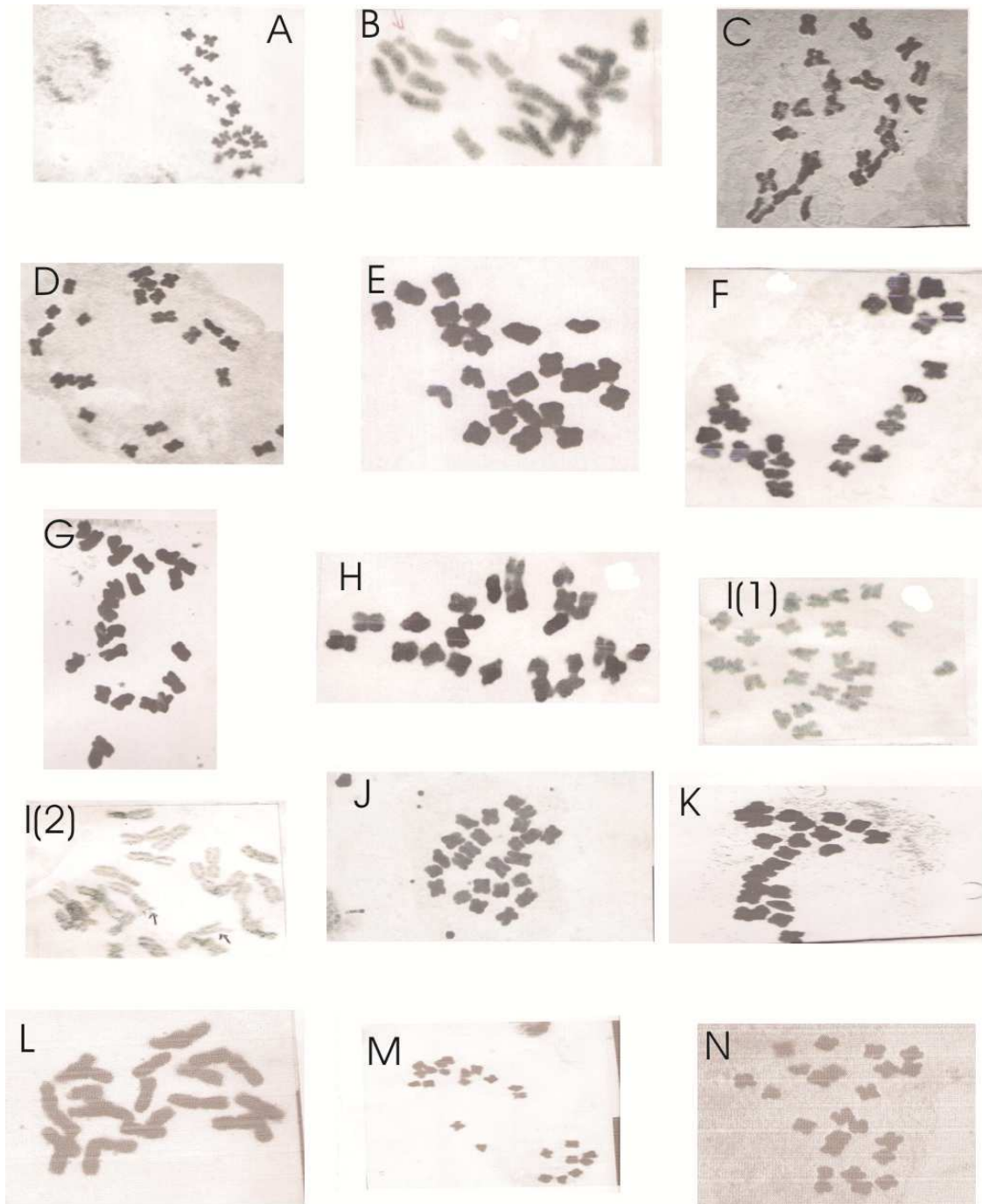
MATERIALS AND METHODS

The Northeast sub-region of Nigeria is an area located between latitudes 6° and 14° N of the equator and longitudes 8° and 15° E of the Greenwich Meridian. The region covers a distance of approximately 690 Kilometers north to south and 480 kilometers east to west. Four varieties were selected from the *Capsicum chinense* species, reputed for producing the most pungent, aromatic and spicy of all *Capsicum* varieties, with a persistent pungency when eaten. Five varieties were also selected from the *Capsicum frutescens* species. The last five varieties were selected from the *Capsicum annum* species.

The chromosomes were accessed through the root tip axis. Fruits were first collected from twelve localities across the six states of the sub-region. The fruits were sorted into varieties based on morphology and processed for cytological investigations. Air-dried seeds were germinated in the laboratory using wet cotton wool and young root tips pretreated in 0.05% colchicine solution for 5 hours and fixed in acetic alcohol 1:3v/v for 24 hours. Before storing them in 70% alcohol, the root tips were first hardened in absolute alcohol for 1 hour and washed in 30% alcohol for 3 hours. Root tips were later hydrolyzed in at 60°C in 1N HCl for 8 – 10 minutes in a water bath. Staining was done in 1 drop of 2% acetic orcein. The stained root tips were then squashed, mounted and viewed under an optical binocular research microscope fitted with a digital camera. Chromosome count was carried out in good metaphase cells and later confirmed in photomicrographs

RESULTS

The Chromosome status of all fourteen accessions was determined. All had $2n=24$ diploid chromosomes. In all accessions, the chromosome formula was the same, which is: $0M + 9m + 2sm + 1st$. The karyotypes of all fourteen varieties are shown in Plate 1.



(A) red habanero (B) red rokoto (C) yellow habanero pt (D) yellow habanero rt
 (E) thai dragon (F) deArbol (G) pequin (H) new mexico
 (I[1]) nardello Full complement (I[2]) nardello satellites (J) paprika (K) northstar
 (L) mariachi (M) pimiento (N) pepperoncini

Plate 1: Karyotypes of all Fourteen Varieties

DISCUSSIONS

The basic structure of the chromosomes was similar in all varieties. As revealed in the chromosome formula, there are no mega chromosomes, nine metacentrics, two submetacentric and one subtelocentric chromosome within the haploid complement. The chromosome status of all varieties investigated in this study did not vary. There was no polymorphism in number as all showed $2n = 24$ chromosomes at metaphase (Plate 1 [A-N]). This result is in agreement with reported chromosome counts in the genus *Capsicum* (Marisa, *et al.*, 2006; Araceli *et al.*, 2009; Eduardo, 1990; Eduardo, *et al.*, 2004, Stiefkens and Bernardello, 2005; Bosland and Eric, 2000; Lamaye and Patil, 1989 and Ohta, 1962). Uniformity in chromosome numbers in these varieties, despite a vast array of morphological forms, is a clear indication of their common origin which had resulted in the formation of a uniform congregation. This has allured to the assumption that their putative parents must have lived in the very remote past. In a study of the phylogenetic relationships of some *Solanaceous* species, Wu and Tanskley (2010) estimated the age of their most recent common ancestors (MRCAs) using both plastid and nuclear sequences. Their report of molecular dating showed that 19.6 million years (MYA) have elapsed since the MRCA of tomato, potato, eggplant, and pepper lived; thus confirming the hypothesis that *Capsicum* had existed as a distinct genus for a very long time, hence the stability in the chromosome status of species in the genus.

This stability in status may have accounted for the regularity observed in mitosis, as no mitotic abnormalities were recorded in all fourteen varieties. This leads to the suggestion that a balanced genetic system is in operation in the genus so that abnormal combinations do not survive and the species' ecological aptitude increased successfully.

Reports are available of the existence of a diploid chromosome number of $2n = 26$, $n = 13$ in the genus. Bosland and Eric, (2000) observed that the non-pungent species and varieties were inclined towards $2n = 26$. The present study however includes variety Northstar which is known to have zero pungency. As with the other thirteen varieties, the somatic chromosome count was $2n = 24$ (Plate 1K). This indicated that the single mutation which had caused the loss of the ability to produce capsaicinoids in some bell peppers as reported by Ana-Garceet *et al.*, (2007) was not accompanied by an increase in the number of chromosomes in the nucleus of the Northstar variety cultivated in north-east Nigeria. The lack of pungency in this variety could have been as a result of minor rearrangements such as non-deleterious deletions which were modified by the influence of the environment.

However, diploid chromosome number of $2n = 26$ had been reported to be prevalent among Brazillian species of *Capsicum* (Marisa *et al.*, 2006). With the observed prevalence of $2n = 24$ among the Nigerian varieties investigated presently, the theory of multiple evolutionary lines as proposed by Bosland, (1996) in the genus is thus reinforced. Relying on the evidence of lack of Telomeric sequences in the ectopic localizations in $2n = 24$ species, Eduardo *et al.*, (2004) proposed that $n = 13$ Karyotypes were derived from $n = 12$. Based on this, it could be concluded that the varieties of pepper in this investigation may probably belong to the ancestral *Capsicum* genepool.

CONCLUSIONS

In investigating the karyology of these fourteen varieties of *Capsicum* sampled from three out of the five domesticated species, an absence of numerical polymorphism was evident. In all accessions, the diploid metaphase chromosome count was $2n = 24$. Thus, suggesting a balanced genetic system with regard to chromosome status. Also, the absence of mitotic abnormalities in all the varieties reinforces the conclusion that regularity of separation during division exist in the *Capsicum* species.

REFERENCES

1. Ana Garces, C; Ramiro, G; Ana, A and Maria, S (2007). Inheritance of Capsaicin And Dihydrocapsaicin, determined by HPLC – ESI/MS, in intraspecific cross of *Capsicum annum* L. *Journal of Agricultural and Food Chemistry*, **55** (17): 6951 – 6957
2. Araceli, A; Peter, M; Mikeal, R and Seung, C (2009). Genetic diversity and structure in semiwild and domesticated chiles(*Capsicum annum*; *solanaceae*) from Mexico. *American Journal of Botany*, **96**:1190 – 1202.
3. Benerji, A.R., (1932). Cytology of capsicum. *Procedure of 19th Indian Science congress*: 315-316.
4. Bosland, P.W (1996). *Capsicums: Innovative uses of an ancient crop*. 479 – 487. In: J. Janick (ed.), *Progress in new crops*, ASHS press, Arlington, VA.
5. Bosland P.W and Eric, V (2000). *Peppers: vegetable and spice Capsicum*. <http://books.google.com.ng/books?Id=SAWTPZeFL8QC&pg=PA16&dq>.
6. Darlington, C.D and Wylie, A.P (1955). *Chromosome atlas of flowering plants*. Allen and Unwin Ltd, London. 155-156.
7. Datta, P. C (1968). Karyology of India varieties of *Capsicum annum* Linn. (*Solanaceae*). *Caryologia*, **21**(2): 121-126.
8. Dixit, P.D; (1931). A cytological study of *Capsicum annum*. *Indian Journal of Agricultural Science*, **1**:419-433.
9. Eduardo, E.A (1990). Chromosome studies in *Capsicum (Solanaceae)*, I. Karyotype analysis in *C. chacoense*. *Brittonia*, **42**(2): 147-154.
10. Eduardo, E.A; Lambrou, M and Ehrendorfe, F (1996). Fluorescence Chromosome banding in the Cultivated Species of *Capsicum (Solanaceae)*, *Plant Systematics and Evolution*, **202**: 37 – 63.
11. Eduardo, E.A. Scaldaferrro, M.A; Gabelle, M; Cecchini, N.M; Sanchez, Y; Jarret, R. Davina, J.R; Ducasse, D.A; Barboza, G.E and Ehrendorfer, F (2004). The evolution of chilli peppers (*Capsicum - Solanaceae*). A Cytogenetic perspective. <http://www.actahort.org/members/showpdt/booknrarnr=7453>.
12. Egawa, Y and Masatake, T (1989). Cytogenetical study of the interspecific hybrid between *Capsicum annum* and *Capsicum baccatum*, *Japan Journal of Breeding*, **36**: 16 – 21.
13. Eshbaugh, W.H; Smith, P.G and Nickrent, D.L (1983) *Capsicum toverri (Solanaceae)*, a new species of pepper from peru *Brittonia*, **35** (1) 55 – 60.
14. Gill, L.S (1988). *Taxonomy of Flowering Plants*, African-Fep Publ.. Ltd. Ibadan, 225 – 226.
15. Heiser, C.B (1969). Systematic and Origin of cultivated plants. *Taxon*, **19**: 214 – 217.
16. Heiser, C.B and Smith, P.G (1953). The Cultivated *Capsicum* pepper. *Economic Botany*, **7**: 214 – 227.
17. IBRGR, (1983). Genetic resources of *Capsicum* Annual publication (1983) International board for plant genetic resources, Rome. 285
18. Kochlar, S.L (1986). *Tropical Crops- a textbook of economic botany*, Macmillan publs. Ltd; London. 259 – 60.

19. Kumar, A.O and Raja, K.G (2004) Cytomorphological Studies in Gamma ray induced Autotriploid *Capsicum annuum* L., *Cytologia***68**. (No. 1); 45 – 50.
20. Kumar, A.O Ramesh, P.C and Raja K.G (1987). Cytogenetic Studies of the FI Hybrids of *Capsicum annuum* and *C. chinense* and *C. baccatum*, *Theoretical and applied genetics*,**74**(2): 242 – 246..
21. Kumar, A. O; Sape, S.T and Kanda, G.R (2006). Cytogenetics of a Spontaneous Fasciated Stem Mutant of Chilli Pepper (*Capsicum annuum* L.), *Cytologia*, **71**, No. 3: 321 – 324.
22. Lamaye, V.A and patil, V.P (1989). Karyomorphological Studies in the genus *Capsicum* Linn. *Cytologia*,**54** (3): 455 – 463..
23. Malgwi, M.M; Kibikiwa, T and Adelanwa,M (2000).Sex chromosomes and synapsis in local chilli pepper, *the nucleus*, **43**(1,2): 43-44.
24. Marisa, T.P; Maria, T.S and Lucia, D (2006). Chromosome numbers in wild and semi-domesticated Brazilian *Capsicum* L. (*Solanaceae*) species. *Botanical journal of the Linnean society*, **151**: 131-146.
25. Morakinyo, J.A and Falusi O.A (1992). Chromosome behavior in *Capsicum annum*, *C. frutescens*and their intra and interspecific hybrids *Nigerian Journal of Botany*, **5**: 135 – 138.
26. Nwankiti, O.C (1976). Cytogenetics of some Nigerian peppers, genus *Capsicum*: hybrids origin of *C. frutescens* OS/Un/19 and its Implication. *Nigeria Journal of science*.**10** : 560 – 75.
27. Ohta, Y. (1962). Karyotype analysis of *Capsicum* species (Japanese with English summary) *SeikenZiho*, **13**, 93 – 99.
28. Okigbo, B.N (1994). Conservation and use of African traditional Agriculture and land use system, In: putter, A (1998) (ed.) *Safeguarding the genetic basis of African traditional crops*,CTA,the Netherlands, 14 – 38..
29. Oyama, K; Sergio, H; Carlo, S; Antonio, G; Pedro, S; Jose, A and Alejandro, C (2006). Genetic Structure of wild and domesticated populations of *Capsicum annuum*.*Genetic Resources and Crop Evolution*,**00**: 1 – 10.
30. Pal, B.P; Ramanujan, S; and Joshi, A.B (1941). Colchicine induced polyploidy in crop plants II Chilli (*capsicum annuum* L.). *Indian Journal of Genetics and Plant Breeding*,**1**:28-40.
31. Pickersgill, C.B (1971). Relationship between weedy and cultivated farms in some species of chilli peppers (genus *capsicum*). *Evolution*,**25**: 683-691.
32. Raghavand,T.S; and Venkatanban,K.R (1940). Studies in the south Indian Chillies, *Proceedings of Indian Academy of Science*, **12B**: 29-49.
33. Stiefkens, L and Bernardello, G (2005) Karyotype studies in *Lycium* sections *schistocaly* X and *sclerocarpellum*(*Solanaceae*). *Edinburgh Journal of Botany*.**62**: 50 – 61.
34. Wu, F and Tanksley, S.D (2010) Chromosome evolution in the plant family *Solanaceae* Biomedcentral genomics, **11**:182.