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THE KARYOTYPIC STUDIES OF THREE LOCAL SHALLOT OF MANIPUR, INDIA

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ABSTRACT

The shallot, the bunching onion of Manipur is not studied cytologically besides its medicinal values. The three different indigenous cultivars of Manipur were collected from three different habitats and compared with a brown onion from the market. The meristematic cells of root tips showed identical diploid count of chromosomes of 16. When chromosomes were arranged according to their lengths, the karyotypes from three different habitats and one exotic consisted of 2 metacentric; 12 submetacentric and 2subtelocentric chromosomes. Broadly the four onions showed rather consistent karyotypes. Only variation is the chromosome numbers where one is subtelocentric in chromosome number V in three onion specimens while it is submetacentric in Khurai specimen which could be brought about by pericentric inversion. Another importance of onion chromosomes is application for genotoxic/cytotoxic test of variant chemicals. Regarding this extra perspective, indigenous shallot is much advantageous over brown because in this brown onion showed chromosomal abnormalities like Anaphase Bridge, disturbed anaphaseetc. So the genotoxic/cytotoxic test using onion chromosomes should take precaution about the type of the onion. Hence our indigenous onion variety is much advantageous than the brown onion for the any genotoxic/cytotoxic test.

KEYWORDS: Karyotypic Studies of Three Local Shallot of Manipur

INTRODUCTION

Shallots are favourite ingredient of Manipuri society. They are used as a food and medicinal ingredient for certain diseases. The onion is the most studies vegetable of the genus *Allium* which is again one of the largest in the world flora with nearly seven hundred species (Friesen et al., 2005). *Allium*species particularly *A. ascalonicum* are usually known for their anti-microbial (Amin et al., 2009 a), antimycobactorial (Amin et al., 2009 b), anticancer and anti-inflammatory (Mohammadi-Motlagh et al., 2015) and many more of the species. From Manipur only the aromatic compounds were reported from *A. ascalonicum* (Debala et al., 2014) so far to our knowledge.

Ramesh (2005) report the diploid count of 16 chromosomes from Ujjain and Kashmir, India and laid emphasis on understanding of number, morphology of chromosomes which are useful in cyto-taxonomy and also beneficial for further research in cytogenetics The cytogenetic studies of the *A. ascalonicum*isscarce from Manipur.

Another emerging aspect of study is genotoxic/cytotoxic test using *Allium* as test material. *Allium cepa*assay is undoubtedly a rapid and sensitive first tier assay for the cytological screening of toxic effluents and chemicals (Fiskesjö, 1993). Keeping all these in our consideration we investigate the chromosome compliments of *A. ascalonicum*from three localities of Manipur and compared with on exotic *Allium* species from local market. Further we investigate viability of the indigenous shallot for the test material for the genotoxic/cytotoxic test in our future works.

MATERIALS AND METHODS

Plant Materials: The bulbs of *Allium ascalonicum*L from three different localities namely Mayang Imphal, Khurai and Heinoubok were collected in the month of September, 2015 from the local farmers. One specimen of *Allium cepa* designated as Brown onion Figure. 1 was obtain from local market to compare with our specimens.

Plant Material Used to Study the Mitosis: The local shallot, *Allium ascalonicum*L. Figure 1 were planted in the laboratory in BOD incubator at 28^0C on a petri-plate with water and after 24 hours, root tips of average 0.5 cm were fixed in fixative (3:1 ethanol glacial acetic acid by volume) for 24 hours and preserve in 70% ethanol. Preserved root tips were used for slide preparation. Some of root tips were pretreated with 0.5% Colchicine (Himedia RM342 10g) solution for four hours at room temperature for the study of metaphase chromosomes for ascertaining the diploid count.

Slide Preparation: In a test tube, 1 ml of Acetocarmine (2%, Merck, India- C. I. No. 75470, S. No. 1381)mixed with 20µl each of 45% glacial acetic acid and 1NHCl were taken along with seven root tips of *Allium* specimens and were warm for 10 minutes over sprit lamp. Meristematic cells from the soften root tips were used for micro slides preparation by squashed method to obtain the different stages of mitotic cells. Fifty cell plates were used for each stage and photographs of best 5 were selected for each stages starting from Interphase, Prophase, Metaphase, Anaphase and Telophase. The chromosome morphology and types are according to Levan et al., (1964).

RESULTS

The normal mitosis stages were studied from 50 cells of each stages and 50 metaphase plates were studied for diploid count and 10 well spread metaphase plates were taken photographs for karyotyping. Out of 50 cells of anaphase plates 5 of cells showed abnormal chromosome arrangement (Figure 2 A - E), and 3 cells in 50 anaphase cells in Brown onion. Only abnormal rather specific to shallot is cornered anaphase stage and faster anaphase chromosomes (Figure 2 H and I). The polar views were rarely seen and more frequently in Brown onion than local shallot (Figure 2 E, F and G).

The karyotypes of the three indigenous shallot/onion specimens compared with one exotic species are quite identical. The diploid count in all well spread metaphase plates of each specimens are 16 as reported else with Fundamental Numbers 58. The karyotype consists of 2n = 2 metacentric (M) + 12 Submetacentric (SM) + 2 Subtelocentric chromosomes (ST). Chromosome number VII is metacentric in all; I to V, VIII aresubmetacentric and VI are subtelocentric in Brown, Mayang Imphal and Heinoubok except chromosome V are submetacentric so that chromosome number VI is submetacentric in Khurai Figure 3 A –H.

DISCUSSIONS

The chromosome studied of different species are still an utmost for any downstream works like mutation research or plant breeding. The indigenous shallot or bunching onions of Manipur is rarely studied through chromosomes. In other parts of the world the shallots are regarded as anti-microbial (Amin et al., 2009 a), anti-mycobactorial (Amin et al., 2009 b), anticancer and anti-inflammatory (Mohammadi-Motlagh et al., 2015). From Manipur only the aromatic compounds were reported from *A. ascalonicum*(Debala et al., 2014) which emphases the importance this kind of onion.

Guignard (1884) introduced the *Allium cepa* for studying the mitosis from root meristem for the first time; still now this is the easiest material to allover the world for studying cell division in practical classes. There are many reasons like easily available; easy to collect a lot of meristematic cells in one or two days, they have large chromosomes etc.,

another very important factor might be Hydrochloric acid and Acetic acid soluble cell wall components because paddy or maize are not easily digestible cell wall components with Hydrochloric acid or Acetic Acid. The diploid count in all well spread metaphase plates of each specimens are 16 as reported else with Fundamental Numbers 58 (Ramesh, 2015).Chromosome number VII is metacentric in all; I to V, VIII are submetacentric and VI are subtelocentric in Brown, Mayang Imphal and Heinoubok except chromosome V are submetacentric so that chromosome number VI is submetacentric in Khurai (Figure 3 A – H). The karyotypic changes in onion and local species might be due to pericentric inversion but the actual mechanism could have come into light if we study the centromeric bandings.

The emerging prospective of *Allium* cytology is involvement in the genotoxic/cytotoxic test. *Allium cepa*assay is undoubtedly a rapid and sensitive first tier assay for the cytological screening of toxic effluents and chemicals (Fiskesjö, 1993), clastogenic (Rank and Nielsen, 1997) mutation due to high density brine (Vidakovic-Cifrek et al., 2002). In our endemic species which is characterised by long prophases (data not shown) and only abnormalities is the occurrence of fast moving subtelocentric chromosomes in one of the specimens (Heinoubok Figure H) and complete separation of chromosomes into two domains at anaphase stage (Figure I). But abnormalities occurred in Brown onion cells are disturbed anaphase, anaphase bridges from early anaphases to late anaphases at the rate of six in 100 anaphases count. Such abnormalities are reported in *A. cepa*on treatment of Chloroquine (Nwangburuka and Oyelana, 2011), Magnesium Sulphate (Bhatta and Sakya, 2008), Gama irradiation (RochanabanthitandJompuk, 2014). So such features are really observed in our normal untreated Brown onion or whether the imported onions were treated with chemicals as preservatives is yet to be confirmed.

At the end our conclusions are the endemic shallots display consistent diploid count of 16 as reported and these shallots are more viable candidate for screening genotoxic/cytotoxic or clastogenic test material for their conservative nature of mitosis cell division.

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REFERENCES

1. Amin Mansour, EffatAbasiMontazeri, Mohammad Ali Mashhadizadeh3, Ahmad Farajzadeh Sheikh (2009 a) Characterization of Shallot, AnAntimicrobial Extract Of*Alliumascalonicum*. Pak J Med Sci 2009 Vol. 25 No. 6 www.pjms.com.pk
2. Amin Mansour, SepidehSegatoleslami, Mohammad Hashemzadeh(2009 b). Antimycobacterial activity of partial purified extract of *Allium ascalonicum*. *Jundishapur Journal of Microbiology* 2(4): 144-147.
3. DebalaA. Devi, O. Ibeton Devi, T. Chand Singh and E. J. Singh (2014). A Study of Aromatic Plant Species Especially in Thoubal District, Manipur, North East India. *International Journal of Scientific and Research Publications,* 4 (6): 2250-3153.
4. Friesen N., Fritsch R.M. and Blattener F.R.,(2005). Phylogeny and new intergeneric classification of *Allium* L. (Alliaceae) based on nuclear ribosomal DNA ITS sequences, *Aliso,* 22: 372-395.

5. Fiskesjö, G. (1993). The *Allium* test in wastewater monitoring. *Environmental Toxicology and Water Quality* **8:** 291-298.

6. Guignard L. (1884). Recherchessu la Structure at la Division du MoyauCellulaire chez vegetaux, *Ann,des Sci. Nat. bot*., Ser. VI, 17: 5 – 59.

7. Levan Albert, Karl Fredga and Avery A. Sandberg (1964). Nomenclature for Centromeric position on chromosomes

8. Mohammadi-MotlaghHamid-Reza, Ali Mostafaie, Kamran Mansouri (2011).Anticancer and anti-inflammatory activities of shallot (*Allium ascalonicum*) extract. *Arch Med Sci.* 1: 39 –

9. PrabhakarBhattaandS.R. Sakya (2008). Study of mitotic activity and chromosomal behaviour in root meristem of *Allium cepa*l. treated with magnesium sulphate.*Ecoprint*. 15: 83-88.

10. Ecological Society (ECOS), Nepal www.ecosnepal.com

11. Ramesh Ahirwar**(2015)** Karyotypic analysis in three species of *Allium* and their someVarieties. *sInt. Res. J. Biological Sci.*4 (9), 1-9.

12. Rank J. and M.H. Nielsen (1997). *Allium cepa*anaphase–telophase root tip chromosome aberrationassay on *N*-methyl-*N*-nitrosourea, maleic hydrazide, sodiumazide, and ethyl methanesulfonate. *Mutation Research*. 390: 121–127.

13. RochanabanthitPechladaandPeeranuchJompuk (2014). Effects of Chronic Gamma Irradiation on shallot Chromosomes (*Allium ascalonicumLinn*). ARPN Journal of Agricultural and Biological Science. 9 (11); 367 – 374.

14. Vidakovic-Cifrek Z., M. Pavlica, I. Regula, D. Papes (2002). Cytogenetic Damage in Shallot (*Allium cepa*) Root Meristems Induced by OilIndustry High-Density Brines. *Arch. Environ. Contam. Toxicol*. 43: 284–29. DOI: 10.1007/s00244-002-1223-2.

APPENDICES

Table 1: The Karyotypes of the Four Specimens

Chromosome Numbers	Localities and Types of Chromosomes			
	Brown	Mayang Imphal	Khurai	Heinoubok
I	SM	SM	SM	SM
II	SM	SM	SM	SM
III	SM	SM	SM	SM
IV	SM	SM	SM	SM
V	SM	SM	ST	SM
VI	ST	ST	SM	ST
VII	M	M	M	M
VIII	SM	SM	SM	SM

Table 2: The Comparative Chromosomal Morphological Features

Localities/Chromosome nos.	#1	#2	#3	#4	#5	#6	#7	#8
Brown onion								
Total length, c	11	10	10	9	8	8	8	7
Short arm, s	5	4	4	4	4	2	4	3
Longer arm, l	6	6	6	5	5	6	4	4
Arm ratios=l/s	1.2	1.5	1.5	1.25	1.25	3	1	1.33
Centromeric index, i=100 Xs/c	45.4 5	40	40	44.4 4	50	25	50	42.8 5
Mayang Imphal								
Total length, c	13	12	12	12	11	9	8	7
Short arm, s	6	6	5	4	4	2	4	3
Longer arm, l	7	6	7	8	7	7	4	4
Arm ratios=l/s	1.16	1	1.4	2	1.75	3.5	1	1.33
Centromeric index, i=100 Xs/c	46.1 5	50	41.6 6	33.3 3	36.3 6	18.1 8	50	42.8 5
Khurai								
Total length, c	12	11	11	10	9	8	8	8
Short arm, s	5.5	5	4	3	2	4	3	3
Longer arm, l	6.5	6	7	7	7	4	5	5
Arm ratios=l/s	1.18	1.2	1.75	2.33	3.5	1	1.66	1.66
Centromeric index, i=100 Xs/c	45.8 3	45.4 5	36.3 6	30	22.2 2	50	37.5	62.5
Heinoubok								
Total length, c	11	10	10	10	10	9	8	7
Short arm, s	5	4	4	4	4	3	4	3
Longer arm, l	6	6	6	5	6	6	4	4
Arm ratios=l/s	1.2	1.5	1.5	1.25	1.5	2	1	1.33
Centromeric index, i=100 Xs/c	45.4 5	40	40	40	40	33.3 3	50	42.8 5

Table 3: The Arm Ratios of the Chromosomes of the Four Onions

Brown onion	Arm ratios=l/s	1.2	1.5	1.5	1.25	1.25	3	1	1.33
Mayang Imphal	Arm ratios=l/s	1.16	1	1.4	2	1.75	3.5	1	1.33
Khurai	Arm ratios=l/s	1.18	1.2	1.75	2.33	3.5	1	1.66	1.66
Heinoubok	Arm ratios=l/s	1.2	1.5	1.5	1.25	1.5	2	1	1.33

Figure 1: The Onion Specimens Used in the Study Brown Onion (A), Heinoubok (B), Khurai (C) and Mayang Imphal (D)

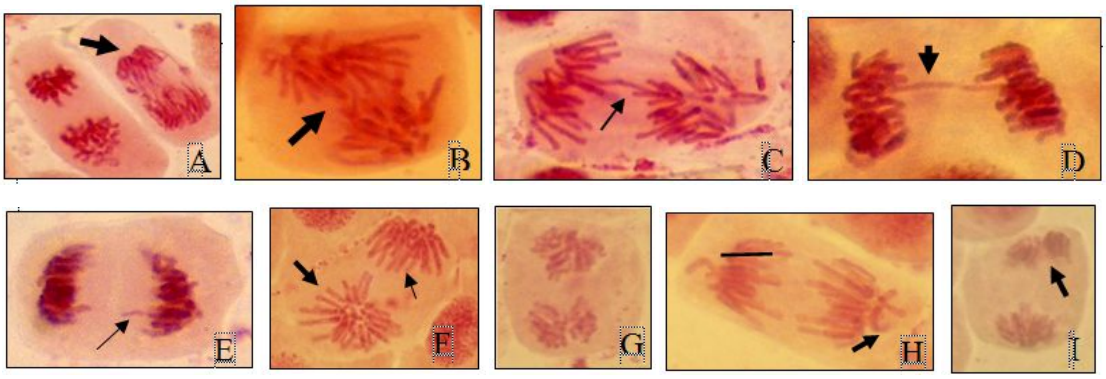

Figure 2: The Abnormalities Observed in the Brown Type A- Huge Anaphase Bridge and Fragmentation, B- Distorted Anaphase, C, D, and E – Single Anaphase Bridge, F- Polar Views (Bold Arrow) and Lateral View (Thin Arrow) of Brown Onion, Polar Views of Local Shallot of Heinoubok- G, Faster Anaphase Chromosomes (Arrowed) Probably the 3rd Submetacentric Chromosomes- H, Two Domains in Local Shallot- I the Bar Represents 10 µm