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Intra and Inter-specific Karyotype Variability of the Genus *Zephyranthes* Herb.

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ABSTRACT

Karyotype analysis of three species of *Zephyranthes* Herb. showed extensive variation in chromosome numbers. Basic chromosome number was reported as x = 6. Somatic chromosome number 2n = 66, 72, 92 in *Z. candida* (Lindl.) Herb. and 2n = 14, 24, 32, 44 in *Z. carinata* Herb. were reported. While *Z. tubispatha* (L'Her.) Herb. showed 2n = 44 and 2n = 75 chromosome numbers. Chromosomes in these three species of *Zephyranthes* Herb. differed in terms of their chromosome length (CL), centromeric index (CI), relative length percentage (RL%), etc. Chromosomes with secondary constrictions were found. Asymmetric indices like TF%, Ask%, A_1, A_2, A, CV_{CL}, CV_{CI} and AI were calculated and an attempt was made to infer the evolutionary status of the species.

Key words: *Zephyranthes* Herb., chromosome morphology, numerical chromosomal variation, karyotype asymmetry

INTRODUCTION

The genus *Zephyranthes* Herb. belonging to the family Amaryllidaceae, commonly known as rain lilies, exhibits wide species variability. The genera comprise of about 90 species according to World checklist of selected plant families (Katoch and Singh, 2015) and is native to diverse areas of the new world including Argentina, the Caribbean, Mexico and North America. The genus *Zephyranthes* is naturalized and cultivated in different parts of the world like India, Hawaii, Indonesia, Thailand, etc. (Afroz *et al.,* 2018). *Zephyranthes* is a perennial herb, scapose with underground or semi-underground bulbs. Flower colour ranges from various shades of white, pink and yellow. Taxonomically, it is a diverse genus which shows poorly distinct morphological demarcation among species (Dash *et al.,* 2020). Amaryllidaceae alkaloids (AAs) which are a specialised kind of secondary metabolites of immense chemotaxonomic importance are abundant in the genera. These are obtained from the members of family Amaryllidaceae and are well known for different pharmacological properties (Prakash and Vedanayaki, 2019). The genus *Zephyranthes* has been used in traditional medicine in many regions of the world for the treatment of simple health complications like cough and cold, headache, boils, etc. to very complicated health issues like tuberculosis, rheumatism, tumours and breast cancer. The major economic importance of this genus lies in their showy flowers. *Zephyranthes* is a karyologically variable genera with different chromosome numbers (Dash *et al*, 2020). The variation in chromosome number and chromosome morphology was found even within the same species acts as an obstacle in determining the basic chromosome number in the genera. Species-wise variation in the number and form of chromosomes is very helpful for biosystematic and breeding studies (Maryam *et al.*, 2021). Karyotype analyses are also useful to characterize the species and to investigate the relationships among species. Occurrence of wide range of chromosome variability, chromosome polymorphism and polyploidy series in the genus provides a scope for further investigation on the genus to know more about the genetic background of the species. The present investigation was undertaken to study the variability and chromosome symmetry/ asymmetry of three species of *Zephyranthes* Herb. available in Assam, India by considering cytological parameters and to draw out probable evolutionary status of the species.

MATERIALS AND METHODS

Bulbs in three *Zephyranthes* species viz., *Zephyranthes candida* (Lindl.) Herb.,

Zephyranthes carinata Herb. and *Zephyranthes tubispatha* (L'Her.) Herb. were collected in the month of November, 2021 and planted in the departmental garden, Botany, Cotton University, Assam, India.

For chromosome counting, actively growing root tips (5-7 mm) of three species of *Zephyranthes* were collected and treated with 8-Hydroxyquinoline solution for 3 h. After 3 h of treatment, roots were washed thoroughly with distilled water and subsequently fixed in 3:1 Carnoy's I fixative (ethanol: glacial acetic acid) for 24 h. Squash preparation of roots was made by following the standard procedures. The slides were observed under microscope and well separated metaphase stages were photographed at magnification of 100X × 45X and proceeded with karyotyping using the software assisted imaging application. Length of long arm (L), length of short arm (S), length of chromosome (CL), total chromosome length of diploid complement (TCL), arm ratio (AR), relative length percentage (RL%) and centromeric index (CI) were considered for the characterization of the karyotypes. The nomenclature of the chromosome type and morphology was done following the standard system proposed.

Chromosome asymmetry was measured by considering standard indices like Huziwara's total form percentage (TF%), Stebbins' classes A-C, Arano's index of karyotype asymmetry (Ask%), Zarco's intrachromosomal asymmetry index (A_1) and interchromosomal asymmetry index (A_2), degree of karyotype asymmetry (A), co-efficient of variation of chromosome length (CV_{CL}) and co-efficient of variation of centromeric index (CV_{CI}) and asymmetry index (AI).

RESULTS AND DISCUSSION

Mitotic cells of all the three species showed variation in the chromosome number as well as chromosome morphology (Table 1). Being an evolutionary dynamic genera *Zephyranthes* showed extensive variation in chromosome number starting from 2n=10 to 2n=200. Under the present study, the species *Z. candida* (Lindl.) Herb. showed 2n=66, 2n=72 and 2n = 92 chromosome numbers (Table 1; Figs. 1 and 4). In *Z. carinata* Herb, chromosome number were found as 2n=14, 2n=24, 2n=32 and 2n=44 (Table 1; Figs. 3 and 6). However, *Z. tubispatha*

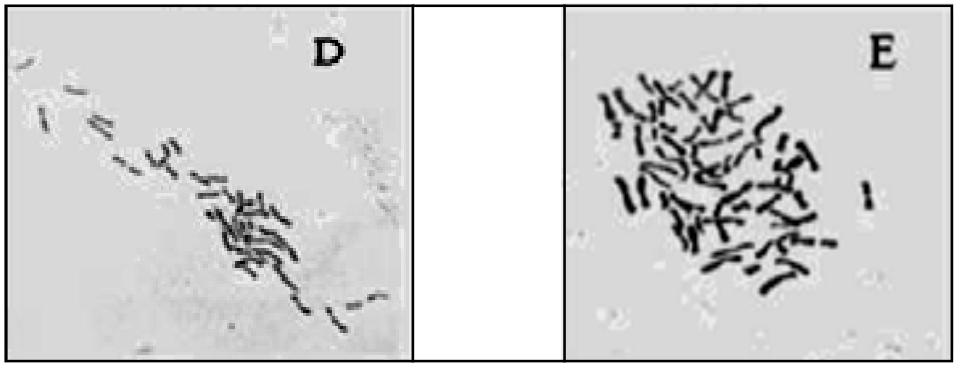
Table 1. Karyotype details of three species of *Zephyranthes* Herb.

Species	Chromosome number	Karyotype formula	Length of short arm (µm)	Length of long arm (µm)	Total chromosome length (TCL) (µm)	Relative length of chromosome (%)	Arm ratio (AR)	Centromeric index (CI)
Z. candida	2n=66	4M+28m+24sm+10st	0.21-1.92	0.54-3.63	153.82	1.27-6.94	1.00-5.25	0.16-0.50
	2n=72	13M+45m+12sm+2st	0.21-2.11	0.27-2.60	146.35	0.71-5.17	1.00-3.11	0.24-0.50
	2n=92	3M+40m+43sm+6st	0.27-1.90	0.38-2.66	221.57	0.63-3.74	1.00-4.80	0.17-0.50
Z. carinata	2n=14	8m+4sm+2st	1.13-3.25	1.95-5.14	69.46	9.67-24.18	1.20-3.57	0.22-0.45
	2n=24	8m+14sm+2st	0.55-2.21	0.94-3.38	83.34	3.59-11.32	1.10-3.69	0.21-0.48
	2n=32	2M+19m+5sm+6st	0.49-2.82	0.81-4.55	115.74	2.55-10.04	1.00-4.94	0.17-0.50
	2n=44	4M+29m+11sm	0.43-2.16	0.60-3.30	124.18	1.68-8.53	1.00-2.81	0.26-0.50
Z. tubispatha	2n=44	2M+24m+10sm+8st	0.47-2.32	0.69-3.11	135.50	1.71-8.71	1.00-4.67	0.17-0.50
	2n=75	2M+35m+26sm+12st	0.47-2.43	0.79-4.33	242.04	1.08-4.38	1.00-5.31	0.15-0.50

(L'Her.) Herb showed 2n=44 and 2n=75 chromosome number (Table 1; Figs. 2 and 5). Numerical variation in somatic chromosome was observed in root tips of many angiosperm species (Gutiérrez-Flores *et al.* 2018; Winterfeld *et al.*, 2015, 2020). Many workers considered *Zephyranthes* as a polybasic genera with x=5, 6 and 7 chromosome number (Dash *et al.*, 2020). The present findings with 2n=24 (tetraploid) in *Z. carinata* Herb., 2n=66 (endecaploid) and 2n=72 (dodecaploid) in *Z. candida* (Lindl.) Herb. also supported x=6 as basic chromosome number for the genus. However, chromosome number 2n=92 (15x+1+1) in *Z. candida* (Lindl.) Herb, 2n=14 (2x+2), 2n = 32 (5x+1+1) and 2n = 44 (7x+1+1) in *Z. carinata* Herb. and 2n=44 (7x+2) in *Z. tubispatha* (L'Her.) Herb. indicated polyploids with upward disploidy (Rahman *et al.*, 2021). The present study revealed that aneusomaty was common in all the three species. Endomitotic replication or non-disjunction may be the primary reason for these irregularities in chromosome complement within the same

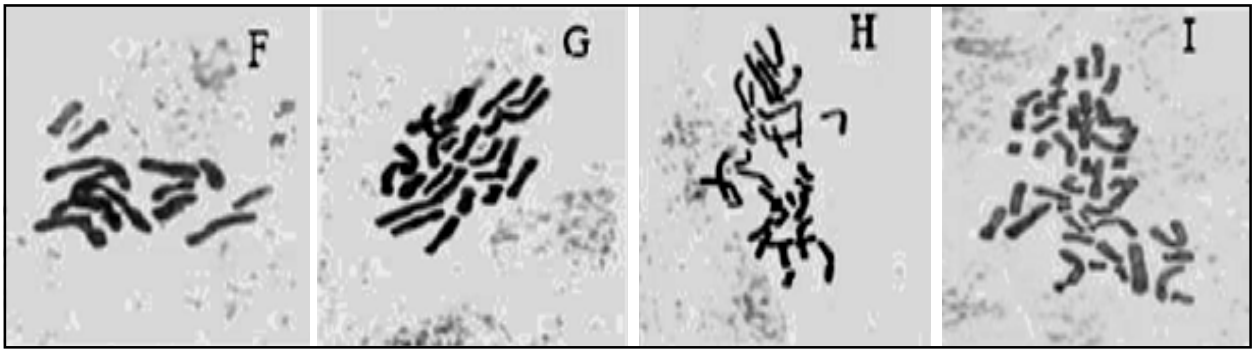
2n=66 **2n=72** **2n= 92**

Fig. 1. Mitotic metaphase chromosome of *Zephyranthes candida* (45 x 100 X).



2n=44 **2n=75**

Fig. 2. Mitotic metaphase chromosome of *Zephyranthes tubispatha* (45 x 100 X).



2n=14 **2n=24** **2n= 32** **2n=44**

Fig. 3. Mitotic metaphase chromosome of *Zephyranthes carinata* (45 x 100 X).

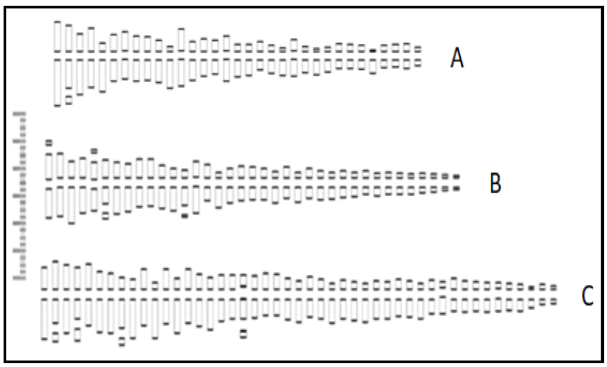


Fig. 4. Idiogram of mitotic metaphase chromosome of *Zephyranthes candida* : A, B and C.

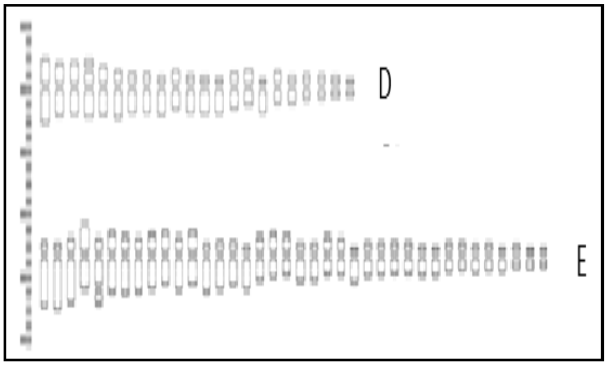


Fig. 5. Idiogram of mitotic metaphase chromosome of *Z. tubispatha* : D and E.

individual. Several reports suggest the occurrence of endomitotic replication in differentiated tissues of biological materials that produces hyper-aneuploid cells. Moreover, mitotic abnormalities such as laggard, bridges, split anaphase and tripolar spindles may be one of the possible reasons for chromosomal irregularity. The present study also showed the clear laggard and unequal separation in mitotic cells of *Z. candida* (Lindl.) Herb. Development of abnormal embryo sacs due to non-division of a nucleus at an early stage was reported in *Z. chlorosolen* D. Dietr. by Crane (2019). The variant karyotypes once produced transmit either through the sexual cycle or by the means of vegetative propagation through bulb. Vegetative reproduction hindered the rectification of genetic error. The karyotype in all the three species under the present investigation showed more number of metacentric to sub-metacentric chromosomes. The lowest chromosome number 2n=14 in *Z. carinata* Herb. showed longest chromosome ranging from 3.57-8.39 µm among all the karyotypes. The highest chromosome number 2n=92 of *Z. candida* (Lindl.) Herb. showed short chromosome length ranging from 0.87-4.17 µm (Table 1).

The total chromosome length (TCL) was found highest in *Z. tubispatha* with 2n=75 and lowest in *Z. carinata* with 2n = 14 which was recorded as 242.04 and 69.46 µm, respectively (Table 1). Studies suggested that there was a general decrease in chromosome size with increase in chromosome number but this correlation was not perfect. One of the crucial observations in case of karyotype analysis lied in the chromosome with secondary constrictions. Except 2n=24 of *Z. carinata* Herb., chromosomes with secondary constrictions were found in the rest. The secondary constrictions were also earlier reported in *Zephyranthes* species.

Karyotype asymmetry indices of all the eight karyotypes found in three *Zephyranthes* Herb. species are presented in Table 2. The scattered diagrams for A_1 against A_2, CV_{CL}, against CV_{CI} and TF% against Ask% are presented in Figs. 7, 8 and 9. The A_1 showed almost negative correlation with A_2. Similarly, the TF% and Ask% showed perfect negative correlation. Except 2n=72 chromosome number from *Z. candida* (Lindl.) Herb., the TF% studied in all the complements lied in the range of 34.22-39.02 which indicated asymmetric karyotype. The lowest value of

Table 2. Karyotype asymmetry indices of three *Zephyranthes* Herb. species

Species	*Z. candida*			*Z. carinata*				*Z. tubispatha*	
Chromosome number	2n=66	2n=72	2n=92	2n=14	2n=24	2n=32	2n=44	2n=44	2n=75
TF%	36.50	41.68	36.42	35.41	34.22	35.95	39.02	37.52	34.27
AsK%	63.49	58.32	63.58	64.59	65.78	64.05	60.98	62.48	65.73
Stebbins category	2C	2C	2C	2B	2B	2C	2C	1C	1C
Intrachromosomal asymmetry (A_1)	0.38	0.25	0.40	0.41	0.45	0.36	0.32	0.36	0.41
Interchromosomal asymmetry (A_2)	0.46	0.48	0.37	0.39	0.30	0.46	0.45	0.35	0.36
Karyotype asymmetry (A)	0.27	0.16	0.27	0.27	0.30	0.25	0.20	0.25	0.29
CV_{CL}	45.87	47.99	37.10	39.32	30.15	46.14	45.44	35.39	36.60
CV_{CI}	26.13	17.08	21.51	24.44	21.77	26.07	14.04	27.28	26.85
AI	11.98	8.20	7.98	9.60	6.56	12.03	6.38	9.65	9.82

TF% was recorded as 34.22 in *Z. carinata* Herb. The TF% value in *Z. candida* (Lindl.) Herb. was recorded as 41.68 that gave indication towards the karyotype symmetry. The higher value of CV_{CL} and CV_{CI} was evident for the heterogenous karyotype. All the three chromosome complements i.e. 2n=66, 2n=72 and 2n=92 of *Z. candida* (Lindl.) Herb. along with 2n=32 and 2n=44 of *Z. carinata* Herb fell under Stebbin's 2C category. The other two chromosome complements (2n=14 and 2n=24) from *Z. carinata* Herb. fell under Stebbin's 2B category. The 2n=44 and 2n=75 of *Z. tubispatha* (L'Her.) Herb. belonged to 1C category of Stebbin's classification.

Karyotype asymmetry index (AI) is one of the latest and well represented. Higher value of AI indicated higher level of karyotype heterogeneity. In the present study, among all the chromosome complements from three studied species, 2n=32 of *Z. carinata* Herb. showed the highest AI (12.03) which indicated more heterogenous karyotype. Whereas the lowest AI was found in 2n=44 (6.38) from the same species. Such variable data of particular asymmetric indices in different chromosome complements of a species created a hindrance in comparative study for karyotype evolution with related taxa and also to draw out phylogenetic relationship solely based on asymmetry indices.

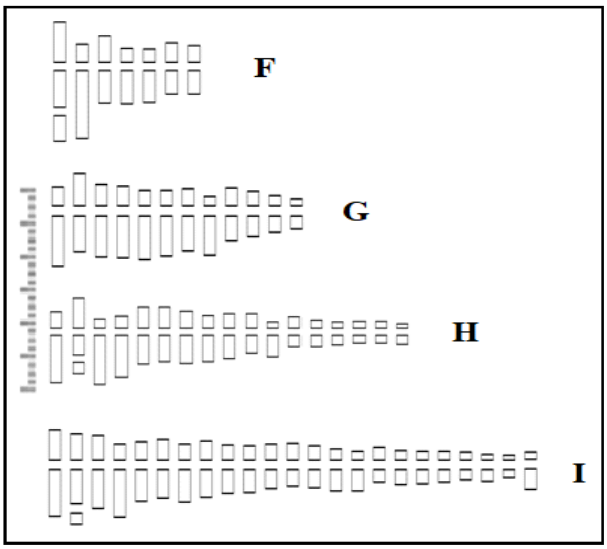


Fig. 6. Idiogram of mitotic metaphase chromosome of *Z. carinata* : F, G, H and I.

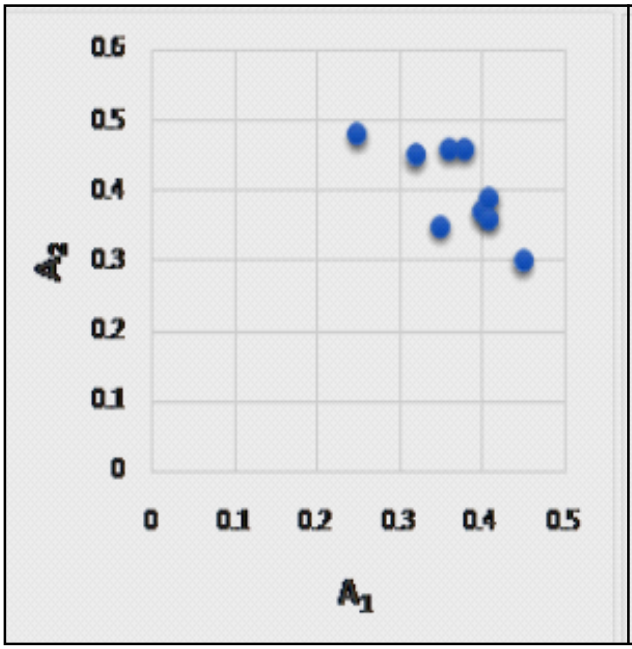


Fig. 7. Scatttered diagram for A_1 against A_2.

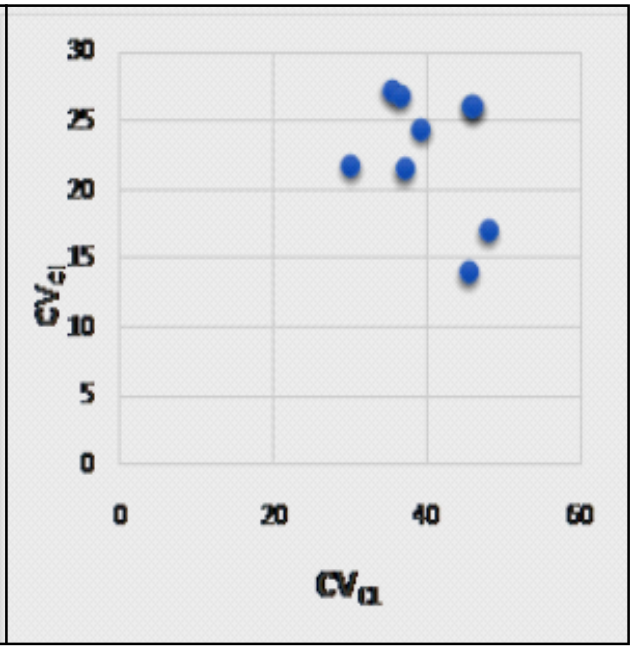


Fig. 8. Scattered diagram for CV_{cl} against CV_{ci}.

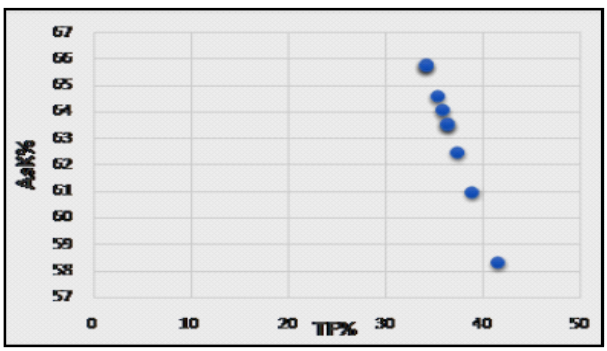


Fig. 9. Scattered diagram for TF % against ASK %.

CONCLUSION

All the three species of the genus *Zephyranthes* under the present study showed variation in chromosome number and chromosome morphology. The basic chromosome number was reported as x=6. Majority of the karyotypes investigated fell under Stebbin's 2C category that indicated towards asymmetry. Other karyotype asymmetry indices calculated for all three species can explain individual karyotype but cannot be considered to find out the evolutionary status of the species due to the occurrence of dynamicity in chromosome number and karyotype heterogeneity. Karyotype heterogeneity of three species of *Zephyranthes* Herb under the present study gave clear indication of occurrence of polyploidy and aneuploidy which might be the cause of rapid evolution of the genus. Ploidy change may also have great impact of the phenotype of the species and the forces that cause the genome change to affect the selection and evolution of the species. Chromosomal instability of three species under investigation provided a scope for further investigation of the phenomena.

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