

Karyotype analysis of some indigenous cultivars of *Zingiber officinale* ROSC

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ABSTRACT

Chromosome characterization and Karyotypic analysis of four (4) indigenous cultivars of *Zingiber officinale* Rosc. of Assam, India was carried out. These cultivars are widely cultivated among the farmers of Assam and are rich source of gene pool. Genetic diversity exists in *Zingiber officinale* Rosc. of Assam and all these cultivars have economic importance. Karyotypic analysis is considered as one of the dependable criteria to find out the genetic background and to access the evolutionary status of the species. The present study reports $2n=24$ and $2n=26$ mitotic chromosome number in four (4) cultivars. The cultivars showed differences with respect to their total chromosome length, length of Long and short Arm of chromosome, relative Length percentage, Arm ratio, Centromeric Index etc. Chromosome asymmetry was accessed by considering several asymmetry measures and all four cultivars showed symmetric karyotype but with variable TF% value, Ask%, CV_{CL} index, CV_{CI} index etc. Among the cultivars CV_2 was found to have most symmetric karyotype.

Key words : *Zingiber officinale* Rosc. Indigenous cultivars, Karyotypic analysis, Chromosome asymmetry.

Introduction

Zingiber officinale Rosc. belongs to the family Zingiberaceae is one of the most widely cultivated perennial herb in tropical and sub-tropical regions of the world. India is one of the highest ginger producing countries of the world (Begum *et al.*, 2018). Ginger cultivation of North East India comprises of 49% of India's area and 72% of India's ginger production and thus often projected as "India's Organic Ginger Hub" (Rahman *et al.*, 2009). Ginger is commercially cultivated various districts of Assam are of high production potentiality (Karuppaiyan *et al.*, 2009), and are very rich source of gene pool. Enormous diversity exists in ginger of Assam. Species diversity is prerequisite for survival of the species in the natural habitat and it can be studied by considering Cytological parameters. Cytological study is

also important to understand the genetic background of the species from their natural habitat, to know the evolutionary status of species (Stebbins, 1971) and also could provide valuable information to utilize the plants sustainably near future. It can also play an important role in uncovering the history, distinctiveness and population structure. Although voluminous works have been done on ginger species, but very little is known about the chromosome morphology and chromosome behavior of indigenous ginger cultivars of Assam. Cytological studies on locally available cultivars of ginger can provide us insight on the Chromosome Morphology, possibility of understanding the evolutionary status of this species. Ploidy level study of the cultivars can be correlated to their various morphological and biochemical characteristics near future. Keeping this view in mind the present work has

been undertaken to study the Chromosome Morphology and Karyotype symmetry of four (4) indigenous cultivars of *Zingiber officinale* Rosc. of Assam, India.

Materials and Methods

Four indigenous cultivars of Ginger of Assam were considered for the present investigation. These cultivars were named as CV₁ (mango ginger), CV₂ (Jatiada), CV₃ (Zeng ada) and CV₄ (Moran ada). On initiation of root emergence, the actively growing nascent root tips of 5-10 mm in size of the different cultivars were harvested were pre-treated with 0.008 M 8-Hydroxyquinoline and then placed under refrigeration for 4-6 hrs (Nair, 2016). The root tips were then washed thoroughly with distilled water was then treated with 0.075 M KCl solution for duration of 15-20 minutes (Zatsepina *et al.*, 1998). Then the material was transferred to 70% alcohol. Squash preparation was done in 2% Acetocarmine and 1N-HCl (9:1 (v/v) mixture) and a pinch of Ferric chloride crystals (Khan, 1975). Well separated and evenly spread mitotic metaphase stages were considered for chromosome study under 45 x100 x microscopic magnifications, were photographed and karyotypic analysis was made. Karyotype and Ideogram of the specimen was made by using the software assisted imaging technique. The idiograms of

individual species were drawn on the basis of centromeric indices and lengths of chromosomes. The nomenclature used for determination of the chromosome type and morphology is that proposed by Levan *et al.* (1964). For the characterization of the karyotypes the parameters considered were length of Long arm (LA), Length of Short arm (SA), Length of chromosome (LT), Relative Length (RL), Centromeric Index (CI), Arm Ratio (AR). Total form percentage (TF %) was calculated following Huziwara (1962). Chromosome asymmetry was accessed by considering various measures by following standard methods such as Stebbins' classes A-C by Stebbins (1971), karyotype asymmetry index (Ask) percentage by Arano (1963), Intrachromosomal index (A₁) and Interchromosomal asymmetry index (A₂) by Romero Zarco (1986), degree of karyotype asymmetry (A) by Watanabe *et al.* (1999), Coefficient of variation of the centromeric index (CV_{Cl}); chromosome index (CV_{Cl}) and Asymmetry index (AI) by Paszko (2006).

Results and Discussion

In the present investigation the cultivars showed variations in chromosome number and reported as 2n=26 in CV₁, CV₂ and CV₄. While in CV₃ it was recorded as 2n= 24 (Table 1). The diploid chromosome number 2n=24 was earlier reported in Ginger by

Table 1. Karyotype analysis of four (4) indigenous cultivars of *Z. officinale*.Rosc

<i>Zingiber officinale</i> Rosc	Cv ₁	Cv ₂	Cv ₃	Cv ₄
Chromosome number(2n)	26	26	24	26
Karyotype formula	22 m + 4 Sm	23 m + 3 Sm	20 m + 4 Sm	25 m + 1 Sm
Short arm range(SL)(μ m)	0.71 - 3.68	1.05 -2.56	0.58 -3.08	0.69 -1.90
Long arm range (LA)(μ m)	1.05 - 5.14	1.22 -4.37	1.57 -4.19	1.19 -2.15
Total length (TL) (μ m)	1.69 - 8.30	2.04 - 6.83	2.68 - 7.27	1.80 -3.97
Centromeric index (CI)	31.5 - 50.0	36.4 - 50.0	27.00 -48.20	32.0 - 50.0
Relative length % (RL)	1.21 - 5.96	2.09 - 6.16	2.01 - 6.80	2.45 - 5.41
Arm ratio(LA:SA)	1.30	1.38	1.35	1.28
TF %	42.32	41.93	42.49	43.72
AsK%	56.67	58.06	57.49	56.27
Steb's Type	2C	1B	2B	2B
A ₁	0.331	0.344	0.339	0.215
A ₂	0.209	0.252	0.280	0.203
A	0.435	0.415	0.423	0.437
AI	0.129	0.124	0.148	0.172
CV _{Cl}	12.91	8.37	13.88	13.15
CV _{Cl}	14.07	14.89	17.26	12.43

TF%: Total form %; Ask%: karyotype asymmetry index %; A1: Intrachromosomal asymmetry index; A2: Interchromosomal asymmetry index; A: degree of karyotype asymmetry; AI: Asymmetry index; CV_{Cl}: CV of centromeric index; CV_{Cl} = CV of chromosome index

Kihara *et al.* (1931). Etikawati, Setyawan (2000) also reported similar findings in *Zingiber officinale* Rosc. The karyotype formula of four (4) cultivars were recorded as 22 m + 4 Sm (2n=26), 23 m + 3 Sm (2n=26), 20 m + 4 Sm (2n=24) and 25 m + 1 Sm (2n=26) in cultivar CV₁, Cv₂, Cv₃ and CV₄ respectively (Table 1). In all four (4) cultivars median chromosomes are predominant and few numbers of sub median chromosomes were found. In Cv₃, secondary constriction was found in Chromosome number 19A and 20A (Fig. 1). Chromosomes with Secondary constriction followed by satellite is a crucial entity in chromosome morphology that provides unique identity to the karyotype. Chromosomes with sec-

ondary constriction were earlier reported by Joseph (2010) in *Z. officinale* Rosc. The range of length of short arm varies from 0.71 - 3.68 µm in CV₁, 1.05 - 2.56 mm in CV₂, 0.58 -3.08 mm in CV₃ and 0.69 -1.90 mm in CV₄. Similarly range of length of the long arm was observed as 1.05 - 5.14 mm in CV₁, 1.22 - 4.37 mm in CV₂, 1.57 -4.19 mm in CV₃ and 1.19 -2.15 mm in CV₄. The smallest chromosome, 1.80 -3.97 mm was recorded in CV₄, while in CV₁ the longest chromosomes of 1.69 - 8.30 mm was recorded (Table 1). The arm ratio was recorded maximum as 1.38 in CV₂ and minimum as 1.28 in Cv₄. Range of Relative length percentage was recorded as 1.21 - 5.96 in Cv₁, 2.09 - 6.16 in CV₂, 2.01 - 6.80 in CV₃ and 2.45 - 5.41 in

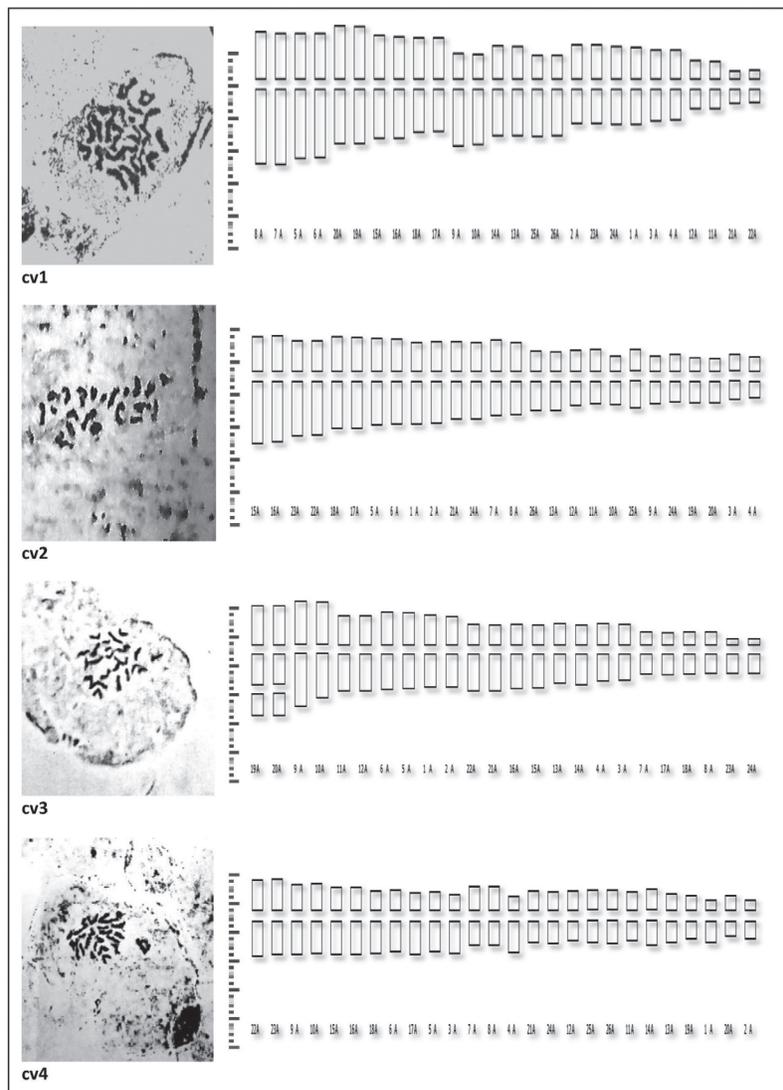


Fig. 1. Karyotype and Ideogram of four (4) indigenous cultivars of *Zingiber officinale*. Rosc. (45 X 100x magnification)

CV₄. Centromeric index in ranging between 31.5 – 50.0 in CV₁, 36.4– 50.0 in CV₂, 27.0 -48.2 in CV₃ and 32.0 – 50.0 CV₄ (Table 1). Ideogram of all four cultivars are presented in Fig. 1. The Total Form percent (TF %) which is the indicator of karyotype symmetry or asymmetry has been calculated to be highest in CV₄ and lowest in CV₂ and was recorded as 43.72 and 41.93 respectively. While In cultivar CV₁ and CV₃ it was found as 43.32 and 42.49 respectively (Table 1). The percentage of karyotype asymmetry index, AsK % was found to be maximum in CV₂ and minimum in CV₄ which was recorded as 58.06 and 56.27 respectively and showed karyotype asymmetry type 1B (CV₂) and 2B (CV₄) according to Stebbins (1971). The result showed no popper correlation of

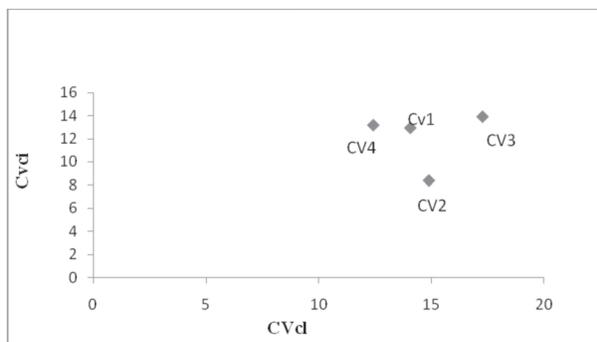


Fig. 2. Scattered diagrams for CV_{CL} against CV_{CI}

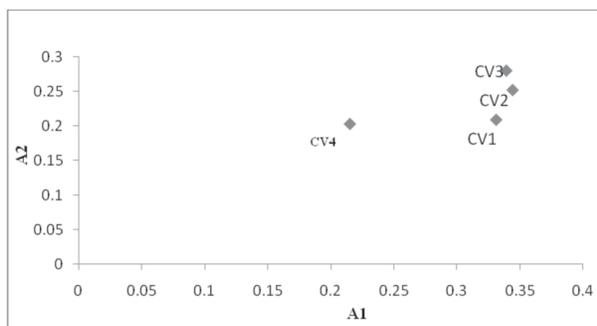


Fig. 3. Scattered diagrams for A₁ against A₂

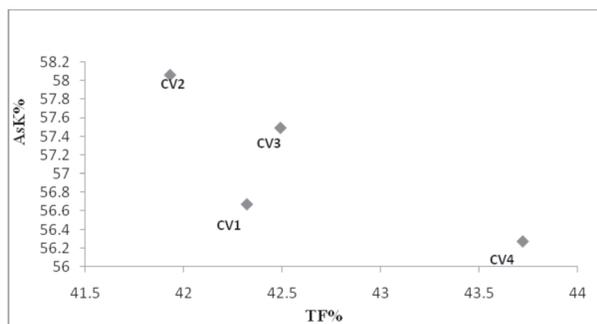


Fig. 4. Scattered diagrams for AsK% against TF%

AsK% with the TF%. Highest value of Intrachromosomal asymmetry index (A₁) was recorded as 0.344 in CV₂ and lowest was 0.215 in CV₄. Interchromosomal asymmetry (A₂) recorded lowest in CV₄ and highest in CV₃ which was 0.203 and 0.280 respectively. Karyotype asymmetry (A) index recorded maximum in CV₄ as 0.437 and minimum in CV₂ as 0.415. Maximum asymmetry index (AI) was found in CV₄ with AI value 0.172 and minimum in CV₂ with AI value 0.124. Coefficient of variation centromeric index (CV_{CI}) ranges from 8.37 -13.88. While chromosome index (CV_{CL}) was recorded as 12.43-17.26. Karyotype asymmetry depends on both the relative variation in chromosome length (CV_{CL}) and the centromeric index (CV_{CI}).

Scattered diagrams for CV_{CL} against CV_{CI}, for A₁ against A₂ and for AsK% against TF% are presented in Fig. 2, 3 and 4, respectively

In the present investigation it was found that there is a small variation in CV_{CI} and CV_{CL} values in karyotypes of four cultivars. (Table 1). In the present study an attempt has been made to find out interrelationship among four indigenous cultivars of *Z. officinale* Rosc. Karyotype asymmetry is most widely used approach in comparative cytogenetics and considered as good expression of karyotype morphology (Peruzzi, Eroglu, 2013). It is an important approach to find out the taxonomic relationship among related species or cultivars of a species. Chromosome morphology is a crucial tool for the characterization of various cultivars and also to deduce the inter-relatedness among them Lavania (1985). Chromosome number of the cultivars showed variation from the normal mitotic chromosome number 2n=22. Increased chromosome number in four (4) cultivars may be due to the occurrence of aneuploidy. In all four cultivars most of the chromosomes are metacentric and secondary constriction was found only in one chromosome of CV₃. Karyomorphic data reveals clear indication towards symmetric karyotype with a high Tf % value in all four cultivars studied. The percentage of karyotype asymmetry index (AsK %) showed karyotype asymmetry type 1B in CV₂, 2B in CV₃ and CV₄ and 1C in CV₁. Intrachromosomal asymmetry index (A₁) showed highest value in CV₂ and positive correlation with Ask%. Relatively lower value of AI in CV₂ reflects karyotypic homogeneity and that indicate towards karyotype symmetry. Lowest value of degree of asymmetry of karyotype (A) was observed as 0.415 in CV₂. Coefficient of variation of chromo-

some index (CV_{CL}) and centromeric index (CV_{CI}) was found to be small and narrow that indicates homogeneity among the cultivars. In present study although perfect interrelationship among the cultivars could not be found but on the basis of certain asymmetry measures it can be concluded that all four cultivars showed symmetric karyotype. Based on Chromosome morphology, predominance of metacentric chromosomes, and other asymmetry measures, the cultivar CV_2 may be considered to be more primitiveness among the cultivars.

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