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New reports of somatic chromosome number and symmetric or asymmetric karyotype estimation of *Sechium edule* (Jacq.) Sw. (Cucurbitaceae)

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Abstract. *Sechium edule* (Jacq.) Sw. distributed well in Eastern Himalayan region, fourteen genotypes collected randomly from Kigwema village, Kohima (Nagaland) at an average altitude of 1538 masl (meter above sea level) with 25.61°N latitude and 94.35°E longitudes. Somatic chromosome numbers are diploid in nature. Chromosome numbers are in agreement and comparable with earlier reports except 2n=2x=22. The chromosome number 2n=2x=22 could not be traced out in the present material collected for study. Two (2) new chromosome numbers 2n=2x=32 and 2n=4x=52 were recorded and expected to be diploid and tetraploid in nature respectively. It was expected that tetraploid (2n=4x=52) number of chromosome might be originated from the earlier reports of diploid chromosome number 2n=2x=26. Both chromosome numbers 2n=2x=32 and 2n=4x=52 are reported for the first time by the authors in the present study. Range of chromosome length was recorded in between 0.501 – 1.343. The range of chromosome length are in agreement with earlier reports of 0.700 – 0.900 approximately. Range of chromosome length suggested minute (<1μm) and small (1-3μm) size of chromosomes with small differences and variations in chromosome length (CV_{CL}). Inter-chromosomal indices (A_2 and Rec), intra-chromosomal index (particularly, Stebbin's classification) and both inter and intra chromosomal indices (DI and GI) estimated symmetric nature of the karyotype.

Keywords: *Sechium edule* (Jacq.) Sw., mitosis, somatic chromosome number, karyotype, inter and intra chromosomal symmetry/asymmetry estimation.

INTRODUCTION

Genus *Sechium* P. Browne was first published in a monograph on Cucurbitaceae in 1881. Literature survey on historical background suggested that it was a monospecific genus with a single species and represented as *Sechium edule* (Jacq.) Sw. (Cogniaux 1881; DeDonato and Cequea 1994). Historically, genus *Sechium* was originally recorded from Jamaica (Browne 1756). Genus *Sechium* had been recorded as *Sicyos edulis* and *Chocho edulis* simultaneously in initial classification (Adanson 1763). Jacquin (1788) changed the genus

Chocho into *Chayota* and re-designated as *Chayota edulis*. Later on, *Chayota edulis* re-designated as *Sechium edule* by Swartz (1800). At present, the genus is known as a combination of both Jacquin and Swartz i.e. *Sechium edule* (Jacq.) Sw. The most accepted term for *Sechium* is 'Chayote' worldwide.

The presence of single species for the genus (monospecific genus) had worn-out, when more species were reported for the genus by various authors from different regions during 1900s and some of them are *S. edule* sub spp. *edule*, *S. edule* sub spp. *sylvestre*, *S. chinatlense*, *S. compositum* and *S. hintonii* (Goldblatt1990; Singh 1990; Mercado et al. 1993; Mercado and Lira 1994). Recently, a new species called *Sicyos angulatus* L. for Indian flora and *Sechium mexicana* for Mexico have been reported respectively (Thakur 2016; Lira and Nee 1999). The reported species were morphologically very similar and never verified for the presence of a new species in the genus.

Cytologically, genus *Sechium* was attempted and studied for the presence of some more species, if any. Many authors reported different chromosome numbers for the genus *Sechium* with base chromosome number x=12, 13, 14, and 15. The base chromosome number x=11 has also been reported for the genus by Singh (1990). Genus *Sechium* categorized into *S. edule* sub spp. *edule*, *S. edule* sub spp. *sylvestre*, *S. chinatlense*, *S. compositum*, *S. hintonii*, *S. mexicana* and *Sicyos angulatus* respectively on the basis of earlier reports of base chromosome number. The base chromosome numbers suggest that it remains unresolved and needs thorough examination cytologically. So, the present aim of the paper is to attempt and extend the information of new chromosome number count to the genus *Sechium*, if any.

MATERIALS AND METHODS

Genus *Sechium* is a shrub climber of Cucurbitaceae family. Fruit samples of genus were collected randomly from Kigwema village, Kohima, Nagaland (India) at an average altitude of 1538 meter above sea level (masl), latitude (25.61°N) and longitude (94.35°E). Mitosis was studied from the secondary root tips of germinating fruits. Root tips of 2-3 cm in length were pre-treated with α-bromonaphthalene at 6±2 °C for 3-4 h followed by overnight fixation (3:1 ethanol-acetic acid) and preservation (70% ethanol). The root tips were hydrolyzed with 1 N HCl for 10-15 min at about 50-60 °C. The root tips were squashed in 2% acetocarmine. Three somatic chromosome preparations under 100x (emersion oil) were photographed using digital Motic BA 210 microscope and recorded for further analysis.

Statistical Analysis

Total chromosome length (µm) were measured for the genotypes with the scale bar of 10µm using ImageJ software and further computation was attempted through windows MS-Excel and with the help of standard formulas for inter and intra chromosomal differences among the chromosome complement of genotypes (see Box 1).

RESULTS AND DISCUSSION

The genotypes are diploid in nature in somatic chromosome count except genotype 2 (Fig. 1D). Two diploids with different somatic chromosome number 2n=2x=26 (Fig. 1B) and 2n=2x=32 (Fig.1C) and a tetraploid 2n=4x=52 (Fig.1D) were recorded for the genotype. The chromosome numbers 2n=2x=32 (diploid) and 2n=4x=52 (tetraploid) are the first report for the *Sechium edule*. Other somatic chromosome numbers are in agreement and comparable with earlier reports except 2n=2x=22 which could not be traced out in the present study materials (Sugiura 1940; Sobti and Singh 1961; Giusti et al. 1978; Mercado and Lira 1994).

The presence of differences, if any, in cultivated or wild forms of the *Sechium*, possibly could have been originated through the chromosomal evolutionary factors in due course of time with the help of primary, secondary, agmatoploidy, symploidy, dysploidy or pseudoaneuploidy evolutionary factors and needs to be verified through cytological and molecular techniques.

Similarly, *Sechium edule* suggested ploidy nature 2n=4x=52 (genotype 2) of the species. Ploidy is not reported earlier in the *Sechium edule* and hence, the first report for the species. The findings of ploidy nature in *Sechium* suggested towards the whole genome content change, diversification, evolutionary changes and speciation in the genus. It could be correlated that a new species might be established from the pre-existing species through reproductive or genetic isolation from the progenitors. The speciation through evolution and diversification required various events of primary (deletion, duplication, inversion, and translocation), secondary (fusion, fission, rearrangements) and disploid (ascending or descending) alterations of chromosome numbers. In the present paper, origin, diversification, genetic isolation or possibility of interbreeding between and among *Sechium* needed to be explored (Fig. 1A – P).

In past, few reports are available on the origin and evolution of cultivated cucurbits and suggested the Mexico, Central America and Guatemala as the centre of

Rec index = $\frac{Total \sum length\ of\ each chromosome \div Longest chromosome}{Total number of chromosomes}$ x 100 (Greilhuber and Speta 1976)

A_2 index = $\frac{Standard deviation of chromosome length}{Mean chromosome length}$ (Romero – Zarco1986)

Coefficient of Variation (CV_{CL}) = $\frac{Standard deviation of chromosome length}{Mean chromosome length}$ x 100 (Lavania and Srivastava 1999; Paszko 2006)

Disparity Index (DI) = $\frac{Longest chromosome - Shortest chromosome}{Longest chromosome + Shortest chromosome}$ x 100 (Mohanty et al. 1991)

Value of Relative Chromatin (VRC) = ∑ Total Length of chromosome / n (Dutta and Bandyopadhyaya 2014) where n=somatic chromosome count

Gradient Index (GI) = $\frac{Shortest chromosome}{Longest chromosome}$ x 100 (Lavania and Srivastava 1992)

Chromosome volume = $\pi r^2 h$ where h = total length of chromosome (Toijam et al. 2013)

Box 1

variation for the crop. Earlier, *Sechium edule* was considered mono-specific (genus with single species) and native to New World, but now it includes as many as eight species and cultivated throughout tropical and subtropical regions of the world but not explored extensively (Newstrom 1990). At present, first report on new chromosome number gives a hope for the presence of some more species in the genus, *Sechium*.

Statistical analysis results on genotype chromosomes presented in Table 1. Total chromosome length (ΣTCL) or chromosome volume (CV) was recorded maximum for the genotype 3 (29.618) which is very close to the genotype 2 with 2n=4x=52 (28.884) but the somatic chromosome number differs in both suggested the differences in the size of the chromosomes (Martonfiova, 2013).

Chromosome length range (CLR) was recorded in between 0.501 – 1.343 for the present genotypes. Seven genotypes have chromosome length more than 1 µm which indicates the heterogeneity of chromosome length for chromosome complement. Two types, minute and small size chromosomes were recorded based on the classification minute (<1µm), small (1-3µm), medium (3-5µm) and large (>5µm) suggested by Kutarekar and Wanjari (1983). Earlier, range of chromosome length was reported in between 0.700 – 0.900 for *Sechium edule*. The range of chromosome length are in agreement with earlier reports approximately and comparable (Sanjappa 1979; Cadena- iniguez et al. 2007).

Value of relative chromatin (VRC) was recorded high and indicated towards the heterochromatic nature of genotypes. High heterochromatic nature could be correlated with the less advanced type of karyotype with more number of metacentric chromosomes alongwith small differences in size of largest and smallest chromosome of karyotype (Beevy and Kuriachan 1996).

Coefficient of variation of chromosome length (CV_{CL}) was recorded high for each chromosome and hence Σ CV_{CL} for genotypes indicated variation in the chromosome length of karyotype complement (Thakur and Sinha 1973).

A_2 value was computed for each chromosome and recorded near to zero and summation value ΣA_2 for each genotype presented. A_2 value close to zero indicates the conservation of chromosome size in the karyotype with low variation among the chromosome length and asymmetry remains the constant i.e. A_2, approximately, indicated towards the symmetric nature of the karyotype (Carvalheira et al.1991).

Similarly, Rec index value ranges from 0-100. The value was recorded for individual chromosome in karyotype and summation value Σ Rec for the each genotype presented is high. High value for Rec index suggested the maximum resemblance among the chromosomes with symmetric nature of the karyotype. Rec index value measures the resemblance between the chromosomes and the average degree of symmetry over the whole karyotype (Huziwara 1962).

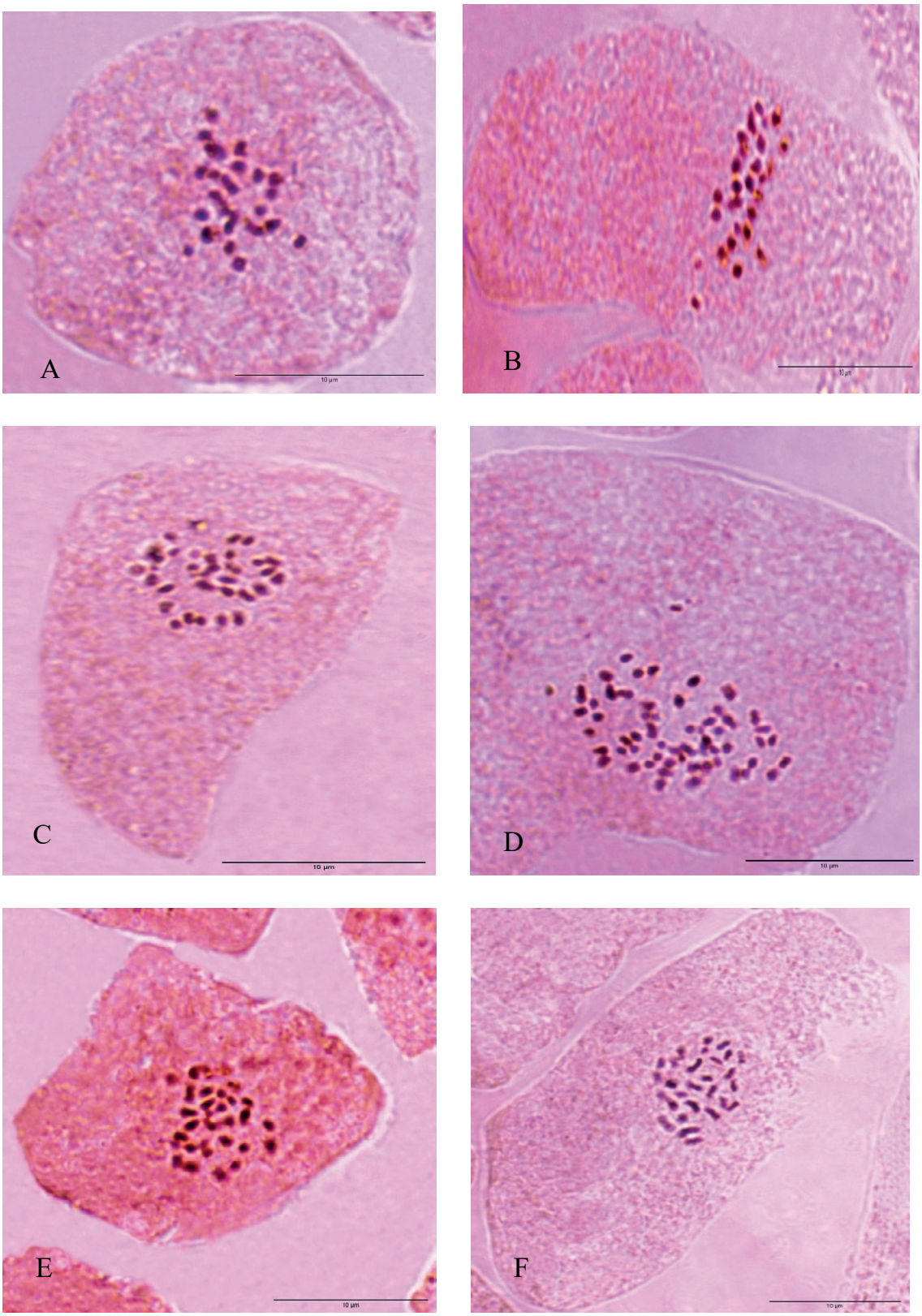

Figure 1. Somatic chromosome count, *Sechium edule* (Jacq.) Sw.; A) G1, 2n=2x=26; B) G2, 2n=2x=26; C) G2, 2n=2x=32; D) G2, 2n=4x=52; E) G3, 2n=2x=30; F) G4, 2n=2x=26.

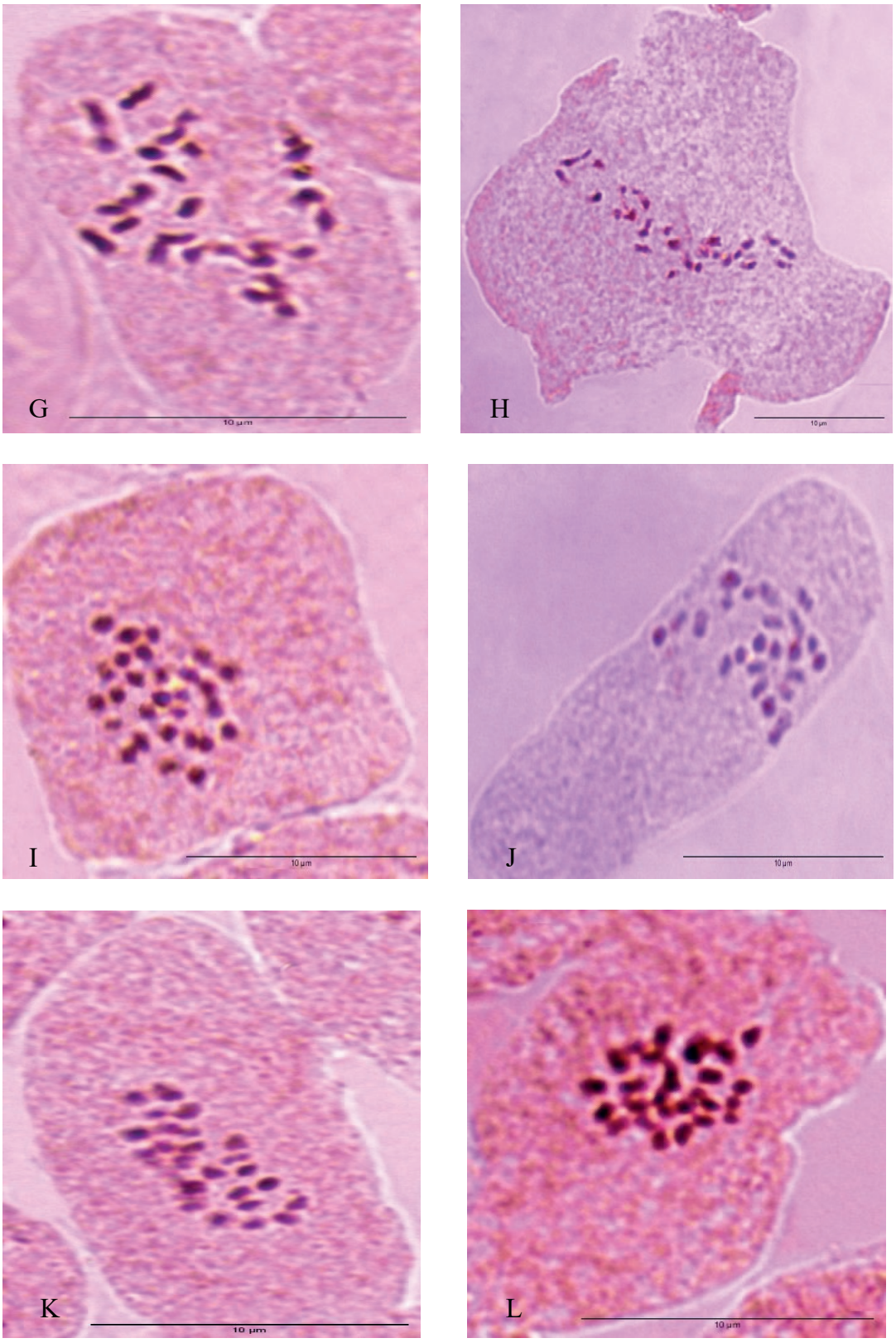

Figure 1 (continued). Somatic chromosome count, *Sechium edule* (Jacq.)Sw.; G) G5, 2n=2x=28; H) G6, 2n=2x=28; I) G7, 2n=2x=28; J) G8, 2n=4x=24; K) G9, 2n=2x=24; L) G10, 2n=2x=28.

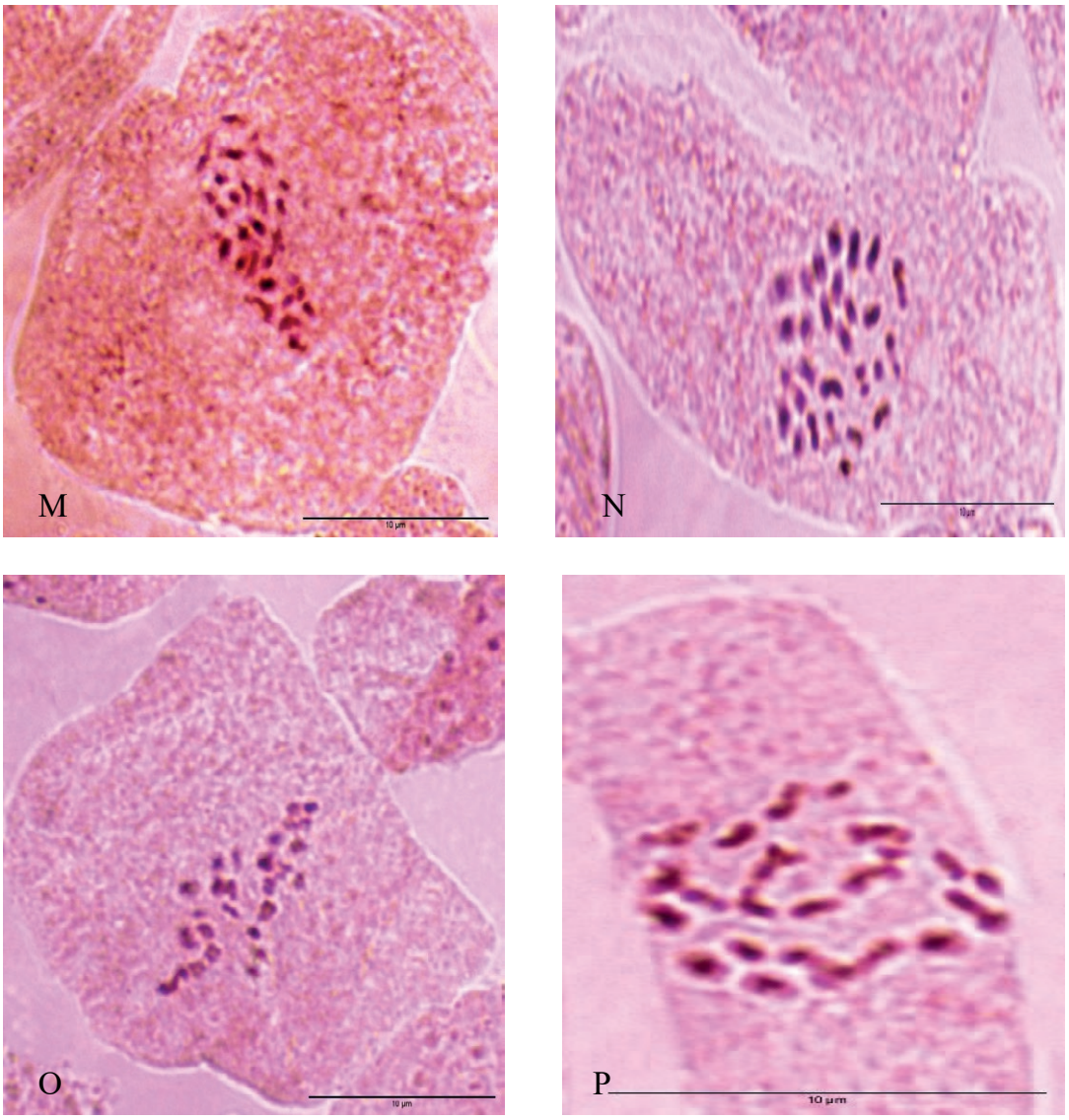

Figure 1 (continued). Somatic chromosome count, *Sechium edule* (Jacq.) Sw.; M) G11, 2n=2x=26; N) G12, 2n=2x=30; O) G13, 2n=2x=26; P) G14, 2n=2x=28.

Intra chromosomal asymmetry index could contain more number of acrocentric or telocentric chromosomes than the metacentric and submetacentric chromosome which could be the result of change in position of centromere. The change in centromere position brings the rearrangement in the chromosomes and may lead to increase in karyotype asymmetry percent.

Intra chromosomal asymmetry depends on exact identification of the centromere and the chromosomal morphology but not only the chromosome size. The extreme symmetry (ideal karyotype A) or asymmetry (ideal karyotype C) of karyotype is meager in nature (Stebbin 1971).

However,the present analysis indicates an extreme symmetric karyotype (1A) among the genotypes except genotypes G2 and G11 and may be classified as ideal karyotype B of Stebbin's classification and suggested that karyotypes of the two genotypes deviated from symmetric to asymmetric and are in agreement with the hypothesis of Stebbins classification (1971). According to the hypothesis asymmetric karyotypes are being originated from the symmetrical karyotypes over a period of time and due course of evolution. Similar work has been reported earlier and in agreement that primitive members with symmetrical karyotypes give rise to advance members with the asymmetrical karyotype (Levitzky 1931; Kumar and Kumar 2014).

Table 1 Karyotype symmetry/asymmetry estimation of *Sechiumedule*.

Genotype (s)	BCN (2x)	ΣTCL or CV	Interchromosomal index					Intrachromosomal index			Inter+Intra chromosomal index	
			CLR	VRC	ΣCV_{CL}	ΣA_2	ΣRec	Largest/Smallest Chromosome ratio	Arm ratio proportion	Stebbin's classification	DI	GI
G1	2n=2X=26	16.733	0.501-0.764	0.643	815.648	3.145	84.225	1.52	< 2:1	1A	20.79	65.575
G2	2n=2X=26	20.919	0.583-1.043	0.804	414.048	4.132	77.128	1.78	< 2:1	1A	28.29	55.896
G2	2n=2X=32	18.099	0.295-0.855	0.565	752.794	7.517	66.138	3.13	> 2:1	1B	70.289	31.929
G2	2n=4X=52	28.884	0.342-0.803	0.555	802.814	8.850	69.098	2.34	> 2:1	1A	40.174	42.643
G3	2n=2X=30	29.618	0.696-1.343	0.987	467.969	4.669	73.496	1.92	< 2:1	1A	31.731	51.824
G4	2n=2X=26	22.890	0.642-1.113	0.880	417.482	4.162	79.029	1.73	< 2:1	1A	26.837	57.681
G5	2n=2X=28	23.470	0.628-1.086	0.838	408.486	4.071	77.177	1.72	< 2:1	1A	26.721	57.826
G6	2n=2X=28	26.090	0.651-1.209	0.931	447.191	4.458	77.043	1.85	< 2:1	1A	30.00	53.846
G7	2n=2X=28	20.320	0.583-0.876	0.725	312.523	3.115	82.819	1.50	< 2:1	1A	20.082	66.552
G8	2n=2X=24	16.274	0.521-0.904	0.678	305.383	3.042	76.735	1.73	< 2:1	1A	26.877	57.632
G9	2n=2X=24	16.899	0.541-0.863	0.704	272.606	2.713	80.907	1.54	< 2:1	1A	22.934	62.688
G10	2n=2X=28	19.276	0.591-0.805	0.688	279.181	3.612	85.503	1.36	< 2:1	1A	15.329	73.416
G11	2n=2X=26	23.784	0.600-1.202	0.914	454.258	4.528	76.089	2.00	> 2:1	1B	33.407	49.916
G12	2n=2X=30	26.747	0.620-1.097	0.891	484.374	4.829	81.258	1.76	< 2:1	1A	27.781	56.517
G13	2n=2X=26	20.009	0.568-0.946	0.769	291.900	2.901	81.349	1.66	< 2:1	1A	24.966	87.925
G14	2n=2X=28	21.956	0.603-0.939	0.784	322.102	4.983	83.46	1.55	< 2:1	1A	21.789	64.217

BCN, Basic Chromosome Number; ΣTCL= Summation of total chromosome length; CV= chromosome volume; CLR=Chromosome length range; VRC=value of relative chromatin; ΣCV_{CL}=Summation coefficient of variation in chromosome length; DI=dispersion index; GI=gradient index.

The presence of asymmetric karyotype could be the result of chromosome structural changes particularly centric fusion or fission which leads to symploid or agmatoploid chromosome rearrangements in due course of plant species evolution. The centric fusion and fission could also be suggested as cause of frequent disploidy or pseudoaneuploidy among plant species (Eroglu et al. 2013).

Both dispersion index (DI) and gradient index (GI) are considered as combination of inter-intra chromosomal index and used for the evaluation of karyotype symmetry. Both represents the nature of evolutionary process occurring or occurred in genus or species and indicates the trend of evolution had taken place in genus, species or cytotypes (Lavania and Srivastava 1992).

Comparatively, lower DI value especially below 30 and higher GI value more than 30 suggested symmetrical nature of the karyotype for the genotypes except G2 with 2n=32 and 52 and supports Stebbins hypothesis. Both DI and GI showed high degree of symmetry which may lead to the lesser degree of chromosomal variation and evolution (Stebbins 1971).

At present, 2n=2x=32 and 2n=4x=52 chromosome numbers were not reported earlier and, hence first report in the present paper. New chromosome number may suggest towards the whole genome content change, diversification, evolution and speciation in the genus.

A very few or negligible reports are available on genus *Sechium* from India (Sanwal et al. 2008; Kapoor et al. 2014; Jain et al. 2015; Jain et al. 2017). Genus *Sehium* remains very poorly known cytologically, therefore, proper chromosome count is important for understanding the interrelationship among different *Sechium* species.

CONCLUSION

Somatic chromosome number and karyomorphometric estimations are in the agreement of earlier reports except two new reports of chromosome number in genotype 2 of the *Sechium edule*.

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