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KARYOTYPE ANALYSIS AND CHROMOSOMAL CHARACTERIZATION OF *HIBISCUS CANNABINUS* L.

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Abstract

Seven germplasm of *Hibiscus cannabinus* L. (Acc. No. 95, 4652, 4654, 4655, 4656, 4657, and 4810) were investigated for their karyotype analysis and chromosomal characterization. These germplasm of *H. cannabinus* were found to possess 2n = 36 chromosomes. Symmetric nature of karyotype was observed in Acc. No. 4654. In contrast, metacentric and submetacentric chromosomes were present in other six accessions revealing the asymmetric nature of karyotype. Based on karyotypic features Acc. No. 4654 was considered relatively primitive and Acc. No. 4652 was relatively advanced among the seven germplasm. Moreover, karyomorphological study proved their divergence in relation to different morphological feature of the chromosomes.

Introduction

Hibiscus is a genus under Malvaceae family possessing about 300 species that grow in different regions throughout the world (Barssum 1966, Akpan 2000, Anderson and Pharis 2003). Different members of this genus are economically very important as sources of food, fibre, beverage, medicines and decorative purpose (Wilson and Menzel 1964, Mohamad *et al*. 2005, Bolade *et al.* 2009). *Hibiscus cannabinus* L. (Kenaf) is one of the most important bast fibre yielding cultivated species. The various members of this species are considered as the main renewable source of raw materials for production of paper-pulp (Andrew and Peters 1980). Kenaf originated from eastern Africa and through Egypt disseminated to India. Now it is cultivated in several country of the world for its fibre like Bangladesh, Indonesia, Malaysia, South Africa, Vietnam, Thailand, African country, and to a little extent in south-east Europe etc.

A good number *H. cannabinus* germplasm are available in the gene bank of Bangladesh Jute Research Institution. These germplasm were collected randomly from different region and stored without proper identification and characterization. These were only characterized on the basis of their phenotypic features which are not always reliable. It is well known that cytogenetical feature is very important for identification and screening of different germplasm.

In view of this, the present study assisted at investigating different cytogenetical features for proper identification and characterization of different germplasm of *H. cannabinus* in Bangladesh.

Materials and Methods

Seven germplasm belonging to *Hibiscus cannabinus* species were investigated during this study. The seeds of various accessions of *H. cannabinus* were collected from Bangladesh Jute Research Institute (BJRI), Dhaka which were maintained in the Botanical Garden, Department of Botany, Jahangirnagar University. Healthy roots were collected from the maintained area. The optimum time of root collection was 12.00-12.30 pm. The collected root was pretreated with 0.002M 8-hydroxyquinoline for 30 min at room temperature (28-30°C) followed by 15 min fixation in 45% acetic acid at 4°C. These were then hydrolyzed in a mixture of 1N HCl and 45%

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acetic-acid (2:1) at 60°C for 45 sec. Then the hydrolyzed root were soaked on a filter paper and taken on a clean slide. The meristematic region was cut with a fine blade. A drop of 1% aceto-orcein was added to the material. A clean cover glass was placed on the material. Then the materials were tapped gently by a tooth pick and then squashed by placing thumbs. Finally, the slides were observed under microscope (Cmos EU 2050890) with the magnification of 100× at auto mode. For measuring the magnification, at first the magnification was calibrated to 100× scale with the measuring tool software of the camera. At least 20 clear metaphase cells were counted for each species for chromosome study.

Results and Discussion

In this investigation, all the germplasm of *H. cannabinus* (Figs 1-7) were found to possess same somatic chromosome number (2n = 36) with basic number of x = 18. Menzel and Wilson (1963) also reported a basic 18 for this species. Presence of different somatic chromosome numbers (2n = 20, 36 and 72) in different germplasm of *H. cannabinus* were recorded (Table 1). These different 2n chromosome numbers have created confusion regarding the basic chromosome number. According to Darlington and Wylie 1956, *Hibiscus* contain larger haploid numbers and a range of basic numbers varying from x = 7 to x = 39.

Table 1. Chromosome count database of *Hibiscus cannabinus.*

Species	Chromosome number	References
H. cannabinus L.	2n=20	Chen *et al.* 2003.
	2n=36	Menzel and Wilson 1961, 1963, Wilson and Menzel 1967, Renard *et al*. 1983, Akpan and Hossain 1998**,** Hiron *et al.* 2006, Islam and Alam 2011.
	2n=72	Rao 1935, Menzel and Wilson 1961, 1963.

Menzel and Wilson 1963 reported a basic number of x = 18. According to previous reports, *H. cannabinus* could be considered as a species with multiple basic chromosome numbers. There are much evidence about diploid, tetraploid, hexaploid, octaploid and decaploid members of *Hibiscus.* The results of the present investigation only correlates with the previous reports of 2n = 36. However, the above data indicated that different cell division abnormalities and chromosomal aberration played important role in the evolution of a series of new basic chromosome numbers, accompanied with the diversification of different germplasm within the species *H. cannabinus*.

In the present study, the total length of 2n chromosome complement was lowest (37.41±1.95 µm) for 4652 and highest (56.17±1.85 µm) for 4655 was observed in *H. cannabinus* (Table 2). The total chromosomal length of other five germplasm was reported as 46.88±1.13 µm for 95, 42.44±2.25 µm for 4654, 48.31± 2.39 µm for 4656, 53.15±2.09 µm for 4657 and 52.85±2.14 µm for 4810 (Table 2). Islam and Alam (2011) reported 52.94 µm for Acc. No. 95 and around 56 µm for other two germplasm of *H. cannabinus* which are nearer to present investigated germplasm.

Among the germplasm of *H. cannabinus*, range of individual chromosomal length was almost nearer. Smallest 0.62 ± 0.02 µm and highest 2.11 ± 0.08 µm both values were found in case of Acc. No. 4810. Average chromosomal length ranged from 1.04 to 1.56 µm. Lowest average chromosomal length was found in 4652 and highest in 4655 (Table 2). According to Dematteis

(1998), Dematteis and Fernandez (1998), the more primitive species have larger chromosome than more derived species. In this regard, a part of present findings agree with them.

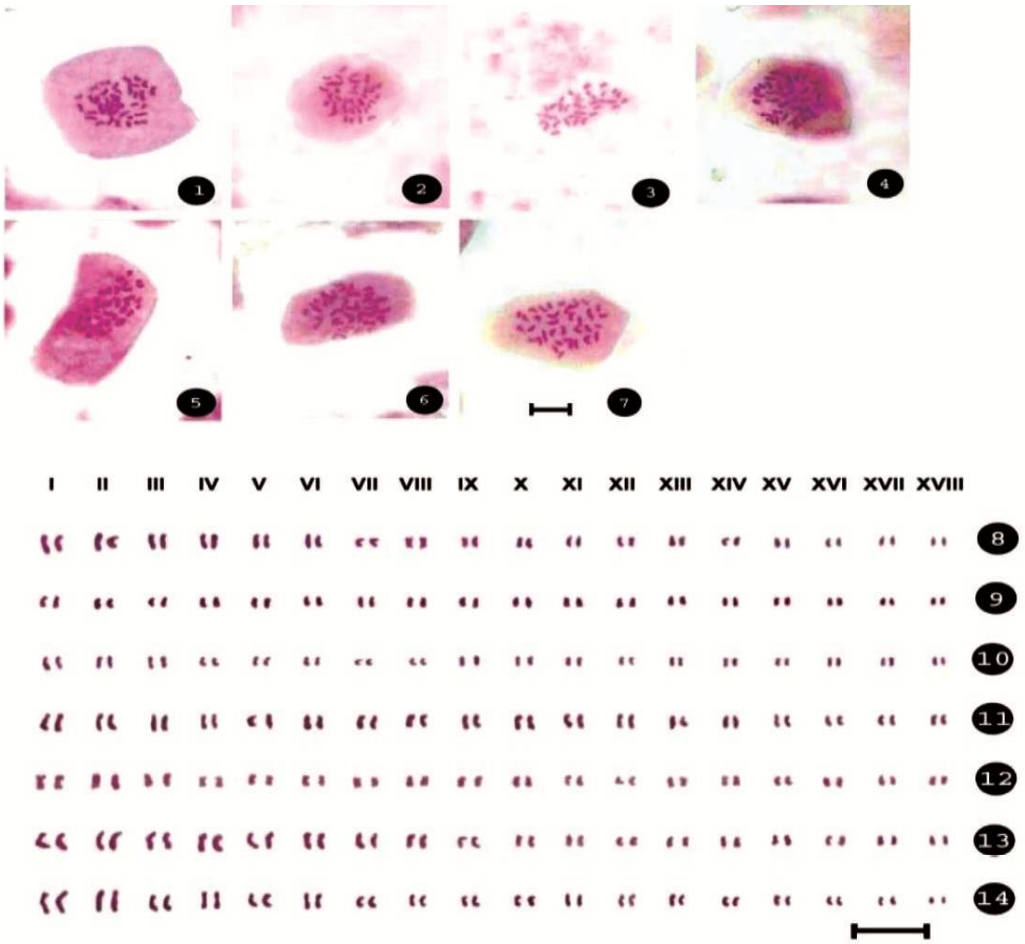
Table 2. Comparative orcein-stained karyotype analysis of seven germplasm of *Hibiscus cannabinus.*

Accession No/Identity	2n	RCL(μm) ($\bar{x} \pm SD$)	TCL(μm)	ACL (μm)	RRL	DRL	TF%	Sy%	AsK%	CF	Nature of karyotype
95	36	0.81 ± 0.04 –2.00 ± 0.05	46.88 ± 1.13	1.30	0.02-0.04	0.02	41.92%	72.37	58.08	26b+10sm	RA
4652	36	0.73 ± 0.04 –1.53 ± 0.10	46.88 ± 1.13	1.04	0.02-0.04	0.02	38.41	60.50	61.59	12m+24sm	RA
4654	36	0.82 ± 0.07 –1.65 ± 0.10	37.41 ± 1.95	1.18	0.02-0.04	0.02	47.10	88.89	53.04	36m	S
4655	36	1.08 + 0.04 –2.03 + 0.08	42.44 + 2.25	1.56	0.02-0.04	0.02	40.16	67.74	59.84	20m+16sm	RA
4656	36	0.98 + 0.05 –1.95 + 0.10	56.17 + 1.85	1.34	0.02-0.04	0.02	42.04	71.79	57.96	22m+14sm	RA
4657	36	0.85 ± 0.05 –2.06 ± 0.10	48.31 ± 2.39	1.48	0.02-0.04	0.02	41.98	72.09	58.02	28m+8sm	RA
4810	36	0.62 ± 0.02 –2.11 ± 0.08	53.15 ± 2.09	1.47	0.01-0.04	0.03	42.10	72.94	57.88	26m+10sm	RA

The relative length of all germplasm ranged from 0.01 to 0.04 (Table 2). Six germplasm of *H. cannabinus* showed range of relative length of 0.02 to 0.04. Whereas, only 4810 showed 0.01 to 0.04. In respect of this karyotypic parameter more or less similarity observed among the studied germplasm.

In seven germplasm of *H. cannabinus*, the range of chromosomal length i.e. distance between small and large chromosomes was almost negligible. Out of seven germplasm, all metacentric chromosomes were found in 4654 (Fig 10, Table 2). Presence of all metacentric chromosomes is a feature of symmetric karyotype (Stebbins 1971). Therefore, 4654 was found to possess homogeneous karyotype, representing strictly symmetric nature (Fig 10, Table 2). In contrast, few metacentric and submetacentric chromosomes were observed the rest six germplasm of *H. cannabinus*. This feature indicated moderately symmetric or relatively asymmetric nature of their karyotype (Figs 8, 9, 11, 12, 13 and 14, Table 2). Islam and Alam (2011) found both meta and submetacentric chromosomes in 95 which is correlate with the present study. They also reported all metacentric chromosomes in other two germplasm which were not included in the present studied materials. Hiron *et al.* 2006 also reported all metacentric chromosome in *H. cannabinus*. Both the meta and submetacentric chromosome were observed in the present investigation. The submetacentric chromosome might be originated from metacentric chromosomes by some chromosomal aberration viz. terminal deletion, pericentric inversion, nonreciprocal or unequal translocation between fragments of chromosomes within different germplasm of *H. cannabinus.* In the present study, a combination of strictly symmetric and moderately symmetric or relatively asymmetric karyotype were found in different germplasm of *H. cannabinus*. Stebbins (1971) mentioned that the symmetric karyotypes were primitive character. Therefore, among the seven germplasm of *H. cannabinus* 4654 was comparatively primitive than the other six germplasm.

Total form percentage (TF%) is an indicator of plant species for the symmetric and asymmetric nature of karyotype or advanced and primitive nature of a specimen. The TF% value decreased with increasing asymmetry. So more symmetric karyotype is related to higher TF% (a maximum of 50%), while lower TF% values indicate asymmetric mainly caused by displacement of the centromere on some of the chromosomes (Lombello and Forni-Martins 1998). In the present study, TF% was varying from 38.41% to 47.10%. Highest TF% was found in 4654 representing strictly symmetric nature of karyotype which was correlated with it's chromosomal formula (all metacentric). In contrast, lowest TF% (38.41%) was found in 4652 representing comparatively asymmetric nature of karyotype which was correlated with it's chromosomal formula (12m + 24sm). The value of TF% of other five germplasm indicates their moderately symmetric or relatively asymmetric nature of karyotype. These karyotypic features were not found in available literatures and internet sources in any germplasm of *H. cannabinus.* So, the above features are very important for characterization of these germplasm.



Figs. 1-14. Orcein stained mitotic metaphase chromosome and karyotype of seven germplasm of *Hibiscus cannabinus* L. 1. Metaphase of Acc. No. 95, 2. Metaphase of Acc. No. 4652, 3. Metaphase of Acc. No. 4654, 4. Metaphase of Acc. No. 4655, 5. Metaphase of Acc. No. 4656, 6. Metaphase of Acc. No. 4657, 7. Metaphase of Acc. No. 4810, 8. Karyotype of Acc. No. 95, 9. Karyotype of Acc. No. 4652, 10. Karyotype of Acc. No. 4654, 11. Karyotype of Acc. No. 4655, 12. Karyotype of Acc. No. 4656, 13. Karyotype of Acc. No. 4657, 14. Karyotype of Acc. No. 4810. Scale bar=5µm.

Karyotype symmetry index (Syi%) was highest (88.89%) in case of Acc. No. 4654. On the other hand, smallest karyotype symmetric index value (62.50%) was found in 4652 (Table 2). Karyotype symmetry index values decreased with increasing asymmetry. On the other hand, karyotype asymmetry index (AsK%) was lowest (53.04%) for 4654 and highest (61.59%) for 4652 was observed in *H. cannabinus* (Table 2). The value of asymmetric index (AsK%) increased with the increasing asymmetry.

Thus the above findings indicating the symmetric nature of 4654 among other six germplasm of *H. cannabinus* which correlated with its chromosomal formula (36m) and highest TF% (47.10%) value. However, no report is available on these cytogenetical parameters in Bangladesh even abroad. These features are so much important for characterization of these germplasm and considered as a salient features of *H. cannabinus*.

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