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Effect of colchicine ($C_{22}H_{25}NO_6$) in *Oryza sativa*

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Abstract

Oryza sativa L. is a main cereal crops in Bangladesh. It is cultivated all over the world but mainly in Asia. In This Paper Three Varieties of *Oryza Sativa* found in Bangladesh; BRR-39, BINA-7 and Shorna were studied cytogenetically. The aim of this study was to determinate the similarities and differences of karyotype and other cytological parameter like nuclear phenotype (mitotic index, nuclear volume and interphase chromosome value) among the three varieties of rice, cytological studies have shown that all the three varieties were found to possess 2n=24 chromosomes. The karyotype formula of BRRI-39, BINA-7 and shorna were respectively $M^m + M^{sm} + 4S_1^m + 3S_1^{sm} + 2S_1^{st} + S_2^{sm}$, $M^m + 4S_1^m + 2S_1^{sm} + 4S_2^m + S_2^{sm}$, $M^m + 2M^{sm} + 2S_1^m + 3S_1^{sm} + 4S_2^{sm}$. Karyotype analysis among three varieties shows that BRRI-39 is little difference from BINA-7 and shorna; because of the presence of sub-terminal chromosome in BRRI-39.This show its advanceness. This study reveled that BRRI-39 is advance than other two varieties. Therefore each variety could be characterized on the basis of nuclear phenotype and karyotype analysis.

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Introduction

Oryza sativa L. seeds of the poaceae family. Rice is the seed of the grass species *Oryza sativa* (Asian rice) or *O. glaberrima* (African rice).This plant height is about 30-180 cm and the parts of this plant may be divided into roots, stem, leaves, reproductive organs (panicle, spikelet, grain). As a cereal crop, it is a staple food that is most commonly consumed Human population in the world, particularly in Asia. In Asia in particular rice comprises a basic component of the daily diet. As expected, Asia is by far the main producer and China the most representative country, producing 200 million tons of rice and paddy in 2010 (The Statistics Division of the Food and Agriculture Organization of the United Nations). About 40,000 varieties of rice are cultivated in the world. Some varieties are Ambemohar, Atop, Basmati rice. Champaa Rice, Clearfield Rice, Dubraj rice, Gandhasala, Hasan Serai, Joha Rice, Kamini Rice, Katta Sambar, Jyothi, Patna Rice, Annapoornna, Hansraj, HMT Rice, Pusa Rice, Surekha, Aizon Rice, Jyothi,Bhut Muri, Gobindobhog, Sona Masuri etc. Sometimes rice affected by many disease such as sheath blight, rice ragged stunt (vector: BPH), rice blast (caused by the fungus Magnaporthe grisea) and tungro (vector: Nephotettix spp). Rice can be grown in different environments, depending upon water availability. It refers a mean annual rainfall in the range 1500-2000 mm, but tolerates 1000-4000 mm. refers a pH in the range 5.5-7, but tolerating 4.5-9. White rice is about 90 % carbohydrate, 2 % fat and 8 % protein. It is a good source of magnesium (Mg), iron (Fe), manganese (Mn), folic acid, selenium, phosphorus (P), thiamine and niacin. In golden rice contains vitamin A. For many country *O. sativa* L. has been cultivated all over the world, mainly Bangladesh, Vietnam, India, the Philippines, China, Indonesia, Thailand, Burma, Cambodia, Pakistan etc. Every year many *O. sativa* L. is cultivated in Bangladesh because the main food of the people here is rice. In Bangladesh many varieties rice are cultivated, they are Katarivog, Boro, Aush, Amon, Birui, Ajoldigha, Shorna, Sorshori, BINA-7, Sadavaula, BRRI-39 etc. New improved seed with higher yield potentiality be development and cultivated to meet the needs of the people. Through this research I have tried to know the nuclear phenotype and karyotype germinated root of rice. For the sake of convenience of securing well spread metaphase chromosome, karyotype studies are mostly confined to root apical meristem. Thus the present investigation was undertaken to study the cytological situation in root tips of *Oryza sativa* L. due to nuclear phenotype and karyotype.

Materials and methods

Materials

Oryza sativa L. belonging to the family poaceae were used as experimental materials in this cytological study. It is a self or cross pollinated and annual crop. Its chromosome number is 2n=2x=24. Colchicine is a chemical compound with the molecular/chemical formula of $C_{22}H_{25}NO_6$. Molecular weight: 399.443 g/mol Colchicine is used for broken down the spindle fibre or making for chromosome number doubling.

Methods

Mitotic index (MI)

Mitotic index value were expressed in percentage as follows:

$$MI = MI = \frac{No.of\ dividing\ cells}{Total\ number\ of\ cells} \times 100.$$

Nuclear volume (NV)

In order to determine nuclear volume of cells, the cells were measured by (oculometer) and converted into micrometer (µm) by a stage micrometer. The nuclear volume (NV) was calculated using the formula:

$$NV = \frac{4}{3}\pi r^3.$$

Karyotype analysis

For the karyotype analysis at first, we collected the young anthers. Then staining the mature anthers and prepare the desire slide to perceive the photomicrography for chromosome to be study. There after measurement the length of chromosome, arm ratio, centromere position and the length of chromatin. Photomicrographs of well spread metaphase chromosomes of three varieties of rice cells were taken by camera photomicroscope.

Photomicrographs were used in the determination of the position of the centromere and length of chromosomes. Chromosomes were measured from photomicrographs of metaphase plates with the help of a divider and a millimeter scale. The values for chromosome size (mm) were converted into mili-micron (µm) with the help of stage micrometer. For making the analysis chromosome were classified according to the position of the centromere. Arm ratios were calculated by dividing the lengths of the short arm by that of the long arm. Ideograms of chromosome pairs were prepared side by side according to their length (from longer to shorter) keeping the short arm in each case pointing upwards and the centromere at the same plant. The total frequency (TF %) was calculated by using this formula of Huziwara, (1962).

$$TF\% = \frac{Total\ sum\ of\ short\ arm}{Total\ sum\ of\ chromosome\ length} \times 100.$$

Results

In the present investigation mitotic index, nuclear volume and interphase chromosome volume were determined from meristematic cells of root tips of three varieties of Rice Fig-2: (A-C). The root tips were stained with the help of Haematoxylin method. The results recorded on mitotic index, nuclear volume and interphase chromosome volumes are presented in Table 1. The interphase chromosome volume was obtained by using the formula ICV= NV/2n. The highest interphase volume was found to be 0.60 ± 0.057µ^3 in BINA-7 and lowest interphase chromosome volume was found to be 0.38 ± 0.020µ^3 in Shorna.

Table 1. Mitotic index, Nuclear volume and Interphase chromosome volume in three varieties of Rice.

Name of variety	Total No. of cells	No. of dividing cells	Mitotic index (MI) %	Nuclear volume (NV) µ^3	Interphase chromosome volume(ICV) µ^3
BRRI-39	150	7	6	11.71 ± 0.14	0.48 ± 0.032
BINA-7	150	9	4.66	14.42 ± 0.12	0.60 ± 0.057
Shorna	150	3	2	9.28 ± 0.190	0.38 ± 0.020

Table 2. Chromosome length, Arm ratio, Centromeric position, Chromosome type and karyotypic formula in *Oryza sativa* (BRRI-39).

Name of the variety	Number of chromosome	Chromosome pairs	i	Ii	Iii	iv	v	Vi	vii	viii	ix	x	xi	xii
		Long arm(µm)	1.53	1.18	1.2	1.18	1.27	1.22	1.13	1.51	1.07	1.49	1.02	1.47
		Short arm(µm)	1	0.64	0.96	0.67	0.64	0.98	0.93	0.87	0.84	0.64	0.82	0.8
BRRI-39	24													
		Total length	2.53	1.82	2.16	1.85	1.91	2.2	2.06	2.38	1.91	2.13	1.84	2.27
		Arm ratio	0.653	0.542	0.803	0.567	0.503	0.803	0.823	0.57	0.78	0.42	0.80	0.54
		SA/LA												4
		Centromeric position	sm	sm	m	sm	st	M	m	sm	m	st	m	sm
		Type	M	S_2	S_1	S_1	S_1	S_1	S_1	S_1	S_1	S_1	M	S_1

Karyotypic formula = $M^m + M^{sm} + 4S_1^m + 3S_1^{sm} + 2S_1^{st} + S_2^{sm}$.

Karyotype analysis

Karyotype analysis was made from the root tip cells (RTCs) of three varieties of Rice and the results are presented in tables (2-4). It was obtained that the root tips treated with colchicine for 1 hour results well spread metaphase chromosomes. Photomicrographs were taken from desired metaphase plate. Chromosomes were measured also from camera-lucida drawings. Idiogram of chromosome complement were prepared.

Photomicrographs of metaphase plates were also used for measuring the chromosomes (Fig-3: A (i- iii)) along with the camera-lucida drawings (Fig-3: B (i-iii)). Idiogram of chromosome complement of three varieties of Rice are shown in (Fig- 4).

Table 3. Chromosome length, Arm ratio, Centromeric position, Chromosome type and karyotypic formula in *Oryza sativa* (BINA-7).

Name of the variety	Number of chromosome	Chromosome pairs	i	ii	iii	iv	v	Vi	vii	viii	ix	x	xi	xii
BINA-7	24	Long arm(µm)	0.8	0.93	1.16	1.22	1.27	0.96	0.91	1.2	1.4	1.13	1.09	1.18
		Short arm(µm)	0.67	0.89	0.89	0.91	0.91	0.82	0.84	1.11	1.11	0.67	0.91	0.93
		Total length	1.47	1.82	2.05	2.13	2.18	1.78	1.75	2.31	2.51	1.8	2	2.11
		Arm ratio SA/LA	0.83	0.95	0.76	0.74	0.71	0.85	0.92	0.92	0.79	0.59	0.83	0.78
		Centromeric position	m	m	m	sm	sm	M	m	m	m	sm	m	m
		Type	S2	S2	S1	S1	S1	S2	S2	S1	M	S2	S1	S1

Karyotypic formula = $M^{m} + 4S1^{m} + 2S1^{sm} + 4S2^{m} + S2^{sm}$.

Table 4. Chromosome length, Arm ratio, Centromeric position, Chromosome type and karyotypic formula in *Oryza sativa* (Shorna).

Name of the variety	Number of chromosome	Chromosome pairs	i	ii	iii	iv	v	Vi	vii	viii	ix	x	xi	xii
Shorna	24	Long arm(µm)	1.11	1.04	1.6	1.29	1.13	1.16	1.4	1.4	1.2	1.53	1.56	1.8
		Short arm(µm)	0.69	0.64	1.33	0.89	0.69	0.96	0.98	0.96	1.02	1.04	0.91	1.09
		Total length	1.80	1.68	2.93	2.18	1.82	2.12	2.38	2.36	2.22	2.57	2.47	1.8
		Arm ratio SA/LA	0.62	0.61	0.83	0.68	0.61	0.82	0.7	0.68	0.85	0.67	0.58	0.60
		Centromeric position	sm	sm	m	sm	sm	M	sm	sm	m	sm	sm	sm
		Type	S2	S2	M	S1	S2	S1	S1	S1	S1	M	M	S2

Karyotypic formula = $M^{m} + 2M^{sm} + 2S_1^{m} + 3S_1^{sm} + 4S_2^{sm}$.

For karyotype analysis chromosomes were classified according to the position of their centromere and their arm length ratios pair wise. Data regarding total length of individual chromosome, centromeric position, arm ratio and types the chromosome pairs are given in tables (2-4).

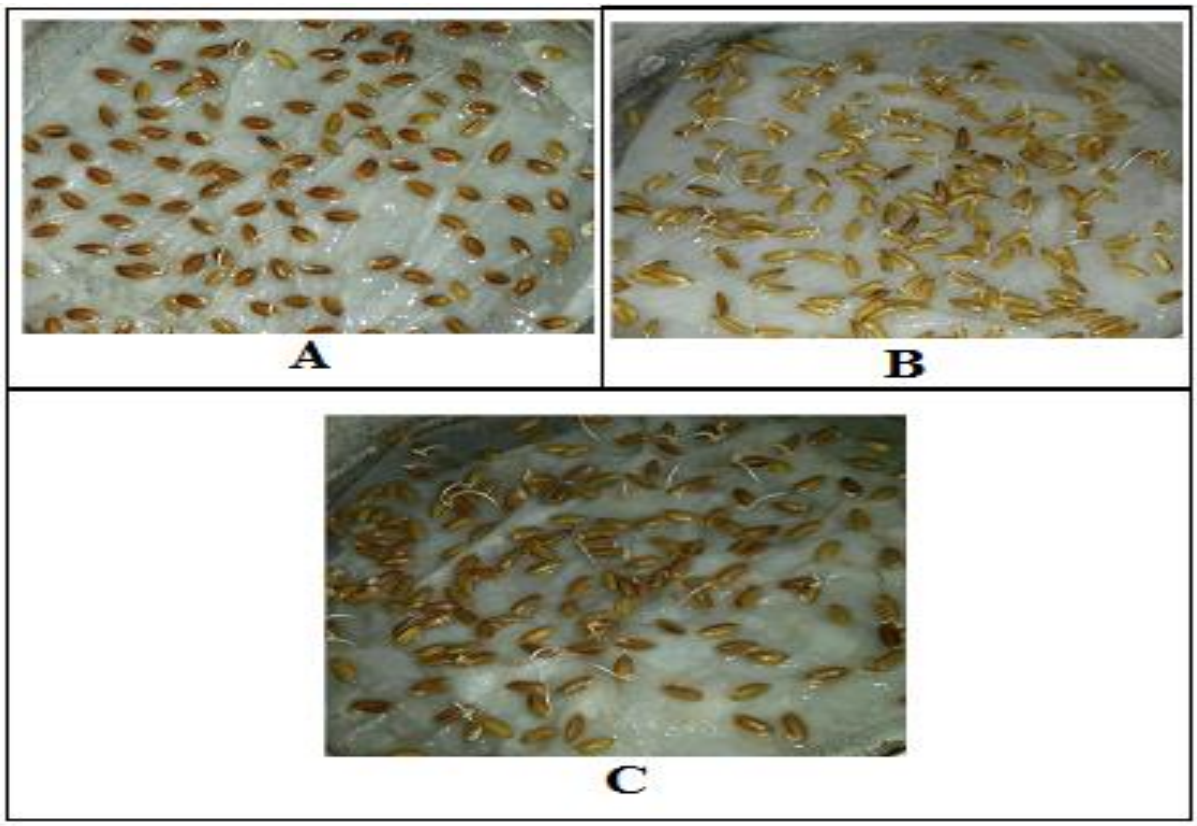

Fig. 1. Germinated root of different varieties of Rice (A) BRRI-39, (B) BINA-7 and (C) Shorna.

Discussion

Lafontania (1974) stated that the structural organization in plant cell nuclei are two types, chromocentric and reticulate. In the present study interphase nuclei of meristematic cells of three varieties of Rice were found to be reticulate. A more plausible view is that the reticulate organization of interphase nuclei in plants with high DNA content is related to the nature of the DNA.

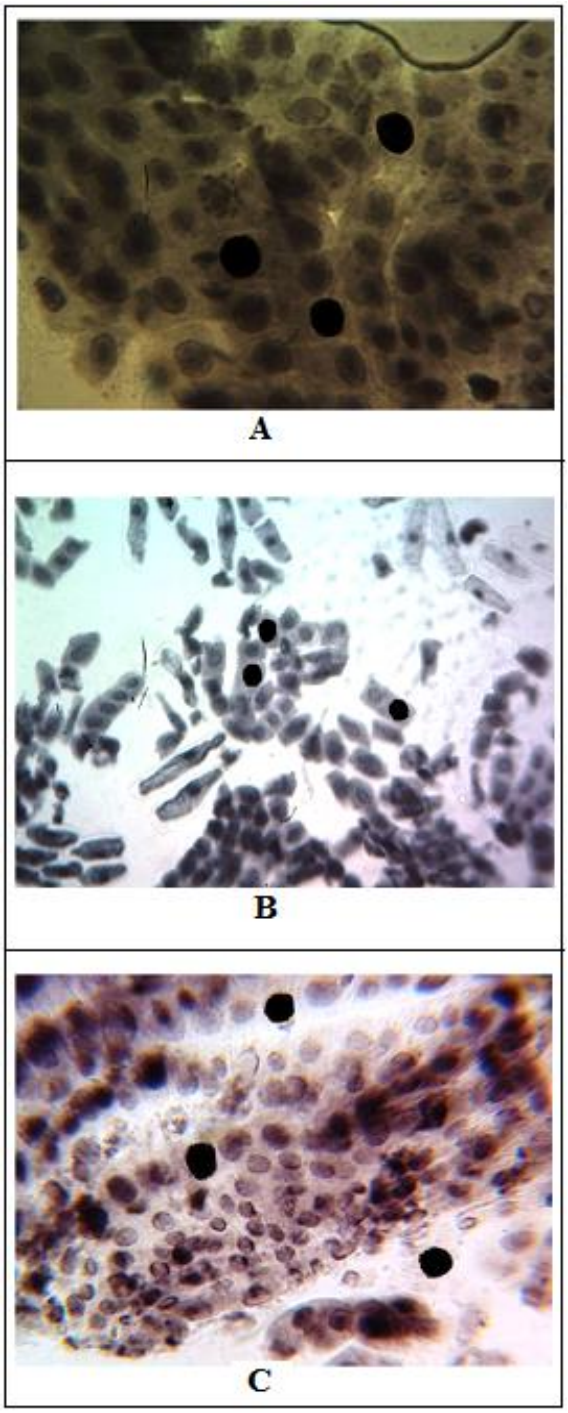

Fig. 2. (A-C): Interphase nuclear phenotype of (A) BRRI-39, (B) BINA-7 and (C) Shorna.

The rice varieties became more clear and distinct after disruption of euchromatin by 50% HCL indicating their high degree of repetitive or to certain characteristics of the protein complexed with it (Nadeau *et al.*, 1974). It is stated that there exists a good enough relation between the average length of mitotic chromosomes and degree of organization of the interphase nuclei. Plants with pro-chromosomal interphase nucleus have thus been noted to process relatively short chromosomes were as varieties of the reticulate type have longer chromosomes (Harish Busch, 1974). In terms of mitotic index (MI), nuclear volume (NV) and interphase chromosome volume (ICV) in three varieties of Rice in the present investigation were found to vary among them. Mean values of mitotic index (6) was highest in BRRI-39 and 28 lowest (2) in Shorna. Mean value of nuclear volume (14.42 μ3) was highest in BINA-7 and lowest (9.28 μ3) in Shorna. Mean value for NV and ICV in Shorna were lowest. The interphase chromosome volume (ICV) was found to be lowest in Shorna (0.387 ± 0.020 μ^3) and highest (0.600 ± 0.057 μ^3) in BINA-7 rice in meristematic cells. Among the different species in the present study ICV of varieties 1 and 2 were found to be more or less similar. Variation of ICV among these three species was remarkable. However, nuclear volume (NV) and interphase chromosome volume (ICV) were found to be dependent proportionally on the number and size of diploid chromosomes in cells.

Study of karyotype is an important field of investigation for understanding generic or specific interrelationships and evolutionary trends (Bhatt and Das gupta (1976); Mehra and Choda (1978) and Faden (1980). In order to carry out the karyotypic analysis of three varieties of Rice root tips were treated with saturated solution of Colchicine for 1 hour and it exhibited good spreading of metaphase chromosome. Numerous rice karyotypes based on mitotic prometaphase chromosomes or meiotic pachytene chromosomes have been reported since the identification of the rice chromosomes number as 2n=24 (Kuwada 1910). However, the accuracy of the measurements of chromosome size and arm ratios in

these previous efforts has been disputed because of the potential misidentification of the centromeric locations in pachytene chromosomes and the large variation of the measurements. In addition, different researchers used their won nomenclature syetems in karyotying. Thus, significant discrepancies exist among the previously published karyotypes. More importantly, none of these karyotypes are fully integrated with the two most saturated rice genetic linkage maps constructed by Causes *et al* (1994) and by Harushima *et al* (1998). Based on this result, it is possible to accumulate large numbers of metaphase chromosomes from rice root tip meristemic cells.

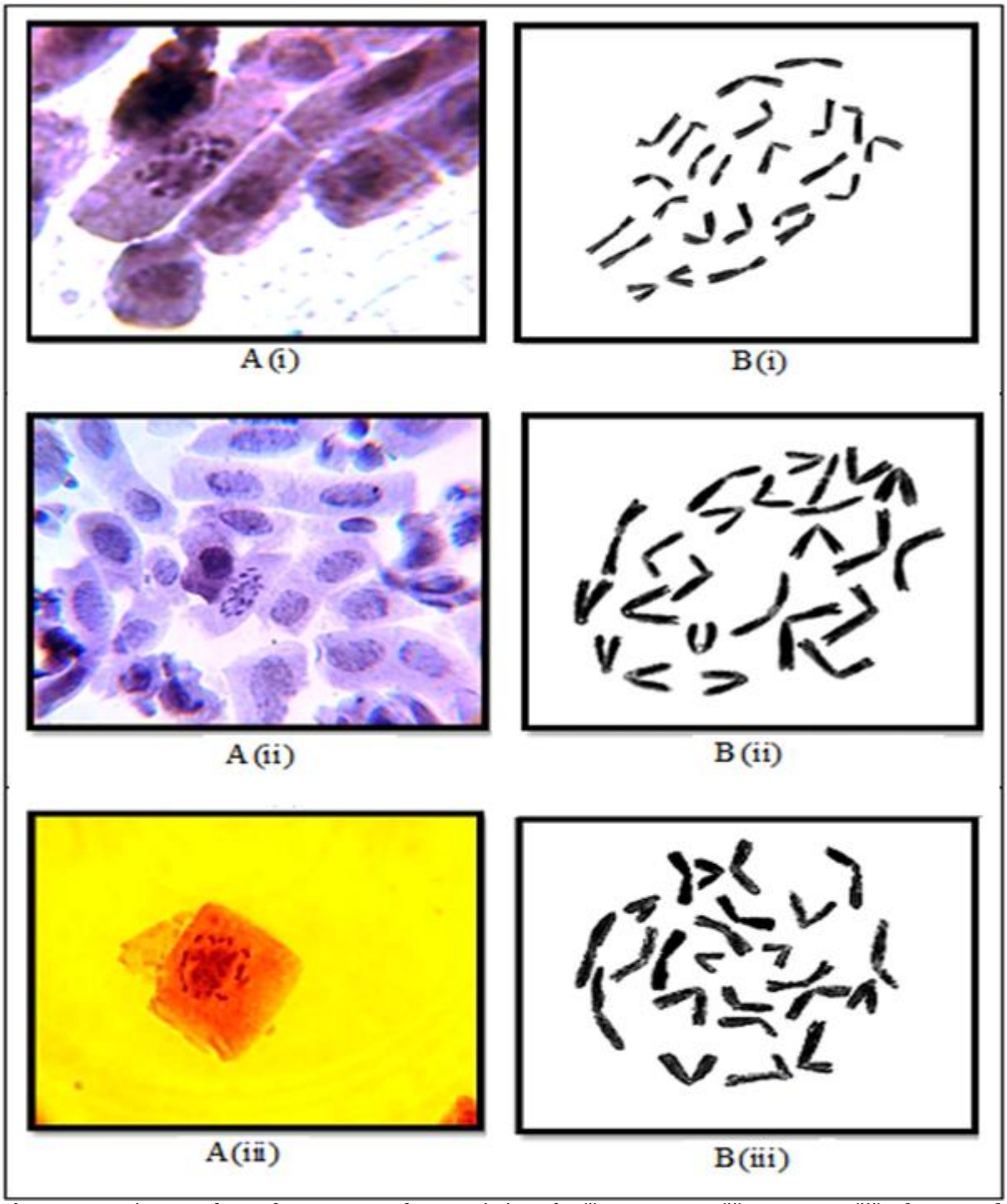

Fig. 3. Somatic metaphase chromosomes three varieties of A(i) BRRI-39, A(ii) BINA-7, A(iii) Shorna and Camera-lucida drawings of three varieties of B(i) BRRI-39, B(ii) BINA-7, B(iii) Shorna.

The reported procedures have been successfully used to sort high quality and quantity metaphase chromosomes. These sorted chromosomes should allow the construction of rice chromosome-specific libraries.

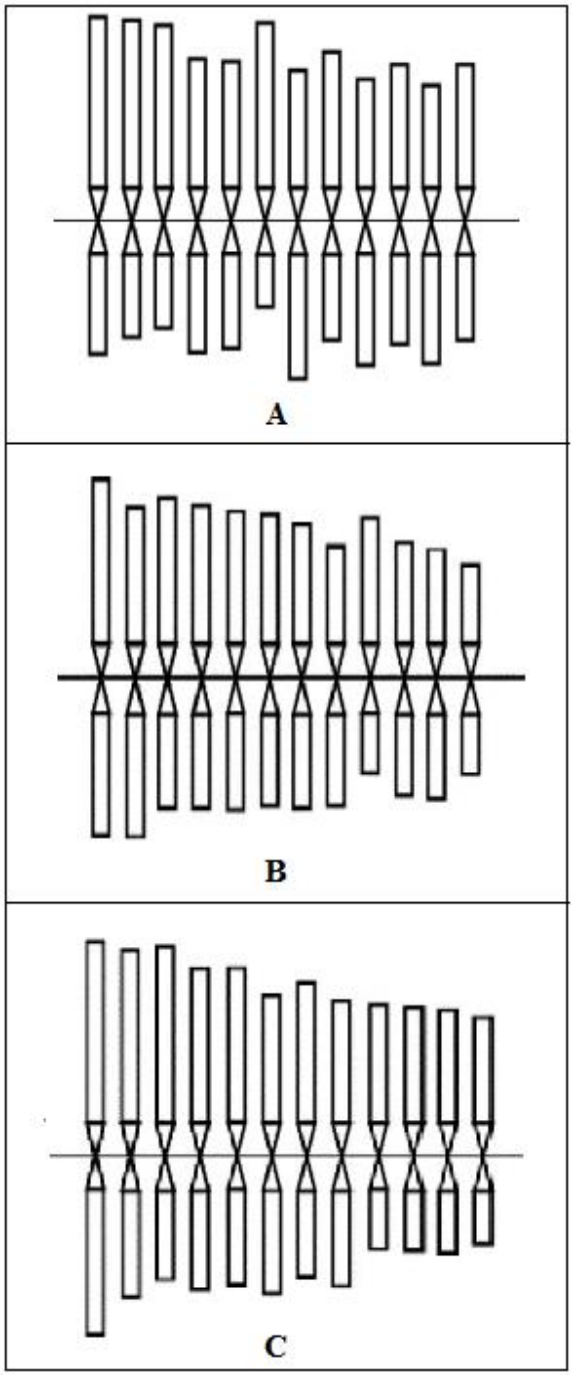

Fig. 4. (A-C): Idiogram of the somatic metaphase chromosome pairs of (A) BRRI- 39, (B) BINA-7 and (C) Shorna.

In present study karyotypic formula determined for three varieties of rice were as follows: Karyotypic formula (BRRI-39) = M^m+ M^{sm}+ $4S1^m$ + $3S_1^{sm}$+ $2S_1^{st}$+ S_2^{sm}, Karyotypic formula (BINA-7) = M^m + $4S1^m$ + $2S1^{sm}$ + $4S2^m$ + $S2^{sm}$, Karyotypic formula (Shorna) = M^m + $2M^{sm}$ + $2S_1^m$+ $3S_1^{sm}$ + $4S_2^{sm}$. Among the three varieties BRRI-39 contain 2 sub terminal chromosome. Due to the presence of sub-terminal chromosomes it shows it advanceness and the process of undergoing structural evolution. From this studies it may be said that BRRI-39 is advanced and BINA-7, Shorna are primitive.

Summary

The present investigation was carried out to study mitotic index (MI), nuclear volume (NV), interphase chromosome volume (ICV) and karyotype analysis of Rice. Nuclear phenotype was studied at interphase of the root tip cells of three varieties of Rice. Mitotic index (MI), nuclear volume (NV) and interphase chromosome volume (ICV) were determined from the meristematic cells of root tips.

The lowest (2%) mitotic index was recorded of Shorna and the highest (6%) mitotic index recorded of BrRRI-39 of the experiment. The lowest (9.28 ± 0.190 μ^3) nuclear volume was found of Shorna and highest (14.42 ± 0.12 μ^3) nuclear volume of BINA-7. Interphase chromosome volume (ICV) was found to range from 0.38 ± 0.020 μ^3 to 0.60 ± 0.057 μ^3 (root tips). Interphase chromosome volume in meristematic cells of BINA-7 was found to be highest (0.60 ± 0.057μ^3) and lowest (0.38 ± 0.020 μ^3) of Shorna. Karyotype analysis studies revealed the diploid chromosome number to be 2n= 24 in all three varieties of Rice. The largest chromosome (2.93 µm) was found in BRRI-39 and shortest (1.47 µm) chromosome in BINA-7. Differences of mitotic index (MI), nuclear volume (NV), interphase chromosome volume (ICV) and karyotype of difference varieties of rice may occur due to effect of the colchicine.

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