RESEARCH ARTICLE

European Journal of Biology and Biotechnology
www.ejbio.org

Comparative Karyomorphological Analysis of *In vivo* and *In vitro* Grown Plants of *Plumbago zeylanica:* An Important Medicinal Plant

Sweety Majumder, Animesh Biswas and Md. Mahbubur Rahman

ABSTRACT

The present report describes the comparative cytological analysis of *in vivo* and *in vitro* grown plant species of *Plumbago zeylanica*. Somatic chromosome number of *in vivo* and micro-propagated plants was confirmed to be 2n=28. Individual chromosome length of mother plant and micro-propagated plant was ranged from 2.26 to 5.54 µm and 2.26 to 5.46 µm, respectively. The total length of the haploid complement of *in vivo* and *in vitro* grown plants was 49.35 and 52.98 µm. The total form percent (TF%) of mother and *in vitro* grown plants was 45.11% and 45.58%, respectively and according to Stebbins classification (1971) both plants karyotype was fell into 2B symmetric type. The centromeric formula was for *in vivo* and *in vitro* grown plants were 3sm + 11m.

Keywords: Chromosome number, *in vitro, in vivo,* Karyotype, *Plumbago zeylanica.*

Published Online: April 13, 2022

ISSN: 2684-5199

DOI : 10.24018/ejbio.2022.3.2.333

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I. Introduction

Plumbago zeylanica is a medicinal plant which commonly known as "White leadwort" or Chitra or "Chitrak". It belongs to the Plumbaginaceae family and a perennial herb. The family Plumbaginaceae consists of 10 genera and 280 species. More than 1000 plant species of Bangladesh are considered to have medicinal properties and about 455-747 have been described with their therapeutic uses for different diseases (Mia 1990, Ghani 2003 and Yousuf et al. 2009). This plant used in traditional system of medicine in Bangladesh. The genus *Plumbago* includes 3 species, namely *Plumbago indica* (*P. rosea*) *Plumbago capensis* and *Plumbago zeylanica*, which are distributed in several parts of Asia subcontinent. Among these species, *P. zeylanica* (Chromosome no=28) grows in different parts of Bangladesh. Leaves of *P. zeylanica* are dark green in colour and are simple, elliptical with hairy margins along with alternate placement on the stem with the distance of up to 3 inches and thickness of 1.5 inches. Petioles are thin and with an approximate length of 0.5 mm and native stipules are present (Kapoor 1990). These roots are usually very strong having a bitter taste and a distinct odor with acrid (Kumar et al. 2009). The plant contains various bioactive compounds like alkaloids, flavonoids, napthoquinones, glycoside, saponins, steroids (Ghani 2003). *P. zeylanica* has been reported to possess wide range of pharmacological activities like anti-inflammatory, anti-diabetic, memory inducing, lipid metabolism, anti-malarial, allergic and modulatory, anti-fertility, anti-bacterial, anti-viral, anti-cancer, anti-oxidant, larvicidial.

In the course of *In vitro* culture, the genetic materials of cells may undergo mutation in their interaction with the different chemical compositions of the media and such type of change may lead to form variation at morphological or at biochemical level. According to Larkin and Scowcroft (1981) such variation referred to as somaclonal variation because somatic cells are involved here. Recently, Somaclonal variations are easily detected by the Cytological studies (Chromosome analysis or karyotyping). The results of chromosomal studies may be also useful in plant taxonomy and phylogenetic analysis. However, there are only a few reports of chromosome studies in this plant species. Karyotypic analysis of *in vivo* and *in vitro* grown *P. zeylanica* describe in details.

II. Materials and Methods

The roots of *in vivo* grown plants were collected from the medicinal plant block, Department of Botany, University of Chittagong, Bangladesh and *in vitro* grown plants roots were collected from growth chamber of the Plant tissue culture Laboratory of the same department and roots were collected when they become 1-1.5 cm in size at 9.30 to 10.30 a.m.

First growing healthy roots were collected and pretreated with saturated solution of Para-dichlorobenzene (PDB) for 3 h at room temperature (28-30 ºC). The roots were then preserved in 70% (v/v) alcohol for long time preservation at 4 ºC in refrigerator. The pretreated Roots were hydrolyzed in a mixture of 1 N HCl for 10 seconds at 60 ºC. After thorough washing with distilled, roots were immersed into 2% (w/v) aqueous solution of iron alum for 5-10 minutes. Then the roots were further washed with distilled water for 3-4 times. Before squashed in a drop of 0.5% (w/v) acetocarmine, roots were stained in 0.5% (w/v) aqueous solution of haematoxylin for 15-20 minutes and after that the hydrolyzed root tips were soaked on a filter paper and taken on a glass slide. After that the meristematic region of roots was cut with a fine blade. A drop of 1% aceto-orcein was added to the material and kept in an acetic acid chamber for 30–40 min. A clean cover glass was placed on the material then the prepared slides were then examined under a Optica Vison Pro microscope at a magnification of 1000x. In terms of characterization of chromosomes and determination of karyotype asymmetry the following parameters were considered: (1) shortest (s) and longest (l) chromosome length; (2) Arm ratio (l/s); (3) total length of chromosomes (CL); (5) proportion of chromosomes with arm ratio more than 2:1 (6) Centromeric indexs (CI= length of short arm/total length of chromosome x 100) (7) Total form percent (TF%)(Huziwara, 1962); (8)According to Stebbins (1971) qualitative classification for the determination of asymmetry (9) karyotypic formula. Classification of chromosomes was done on the basis of the nomenclature of Levan (1964). Several karyomorphological parameters were estimate by KaryoType software (Karyotype XY).

III. Results and Discussion

A. Karyotype of in vivo Grown Plants of Plumbago zeylanica

In vivo grown *P. zeylanica* had 2n = 28 chromosomes in the somatic cells and the length of chromosome ranges from 2.26 to 5.54 µm (Table I). Among the 14 basic chromosomes, three were submetacentric and 11 were metacentric. The centromeric formula was 3sm + 11m (Fig.1a). Of the three submetacentric chromosomes, two were long and one was medium. On the other hand, of the 11 metacentric chromosomes, one was large, two were medium and eight were small. 2Lsm + 1Lm + 1Msm + 2Mm + 8Sm (Fig. 1c) Karyotypic formula could be assigned for this chromosome characteristic. The total length of the haploid complement was 49.35 and TF% was 45.11. The total length of the long arms was 27.09 µm and that of short arms was 22.26 µm. The ratio of the longest and the shortest chromosome was more than 2:1 and the frequency of chromosomes with arm ratio more than 2:1 was 0.07. Therefore this karyotype fell in 2B symmetrical type. Similar type of observation was noted in some other plants by few scientists such as Zhou et al. 2009 (*Liriope spicata*) and Bhadra and Bandyopadhyay (2015) reported same result in some species of Zingiberaceae family.

B. Karyotype of in vitro Grown Plants of Plumbago zeylanica

Chromosomal analysis of *in vitro* plants revealed the presence of 2n = 28 chromosomes in the somatic cells. The chromosome length ranged from 2.26 to 5.46 µm (Table II). In this genome, three were submetacentric and 11 were metacentric (Fig.1b). The centromeric formula 3sm + 11m could be assigned for this chromosome constitution. Two of the three submetacentric chromosomes two were long and one was medium. Among the 11 metacentric chromosomes, one was long, four were medium and the remaining six were small. This chromosome characteristic could be represented by 2Lsm +1Lm + 1Msm+ 4Mm + 6Sm (Fig.1d). The total length of the haploid complement was 52.98 and TF% was 45.58. The total length of the long arms and that of the short arms were 28.85 µm and 24.13 µm, respectively. The ratio between the length of the longest and that of shortest chromosome was more than 2:1. And the frequency of chromosome having arm ratio more than 2:1 was 0.07. Therefore this karyotype fell also in 2B symmetrical type.

C. Comparative karyotype analysis of in vivo and in vitro grown plants of P. zeylanica

The somatic cells of both *in vivo* and *in vitro* raised plants had 28 chromosomes each. The total length of haploid complement of *in vivo* and *in vitro* grown plants was 49.35 µm and 52.98 µm, respectively. The chromosome length of mother plants ranged from 2.26 to 5.54 µm long with a gradual decrease in length from the longest to the shortest chromosome. On the other hand, the length of chromosome of *in vitro* raised plants ranged from 2.26 to 5.46 µm long with a gradual decrease in length from the longest to the shortest chromosome. The total lengths of long arms in haploid complements of *in vivo* and *in vitro* grown plants were 27.09 µm and 28.85 µm, respectively, whereas the total lengths of short arms chromosome of both plants were 22.26 µm and 24.13 µm, respectively.

The centromeric index of mother plants and *in vitro* raised plants was 31.77 to 50.00 and 34.42 to 50.00, respectively. The total form percent (TF%) of mother and *in vitro* grown plant was 45.11% and 45.58%, respectively. The centromeric formula for *in vivo* and *in vitro* plants was 3sm + 11m (Table-3). The karyotype formula of *in vivo* grown plants was 2n = 28 = 4Lsm + 2Lm + 2Msm + 4Mm + 16Sm, whereas that of the *in vitro* grown plants was 2n = 28 = 4Lsm + 2Lm + 2Msm + 8Mm + 12Sm, that is shown in Fig. 1c and 1d. No satellite was found in chromosomes of the both plants. The result also revealed that the ratio of the longest and the shortest chromosomes of mother plants and *in vitro* grown plants were 2.45:1 and 2.42:1, respectively. The frequency of the chromosome having arm more than 2:1 was same for the both plants that was 0.07. Thus, the karyotype of both plants falls into 2B symmetrical type. The detailed information on the chromosome characteristics of *in vivo* and *in vitro* grown medicinal plants is very limited. Karyomorphological studies of some medicinal plant species, however, carried out at times by previous researchers (Huziwara 1962; Zhou et al. 2009; Aikpokpodion et al. 2011; Chaudhari and Chaudhary 2012; Samaddar et al. 2012).

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TABLE I: LENGTH, ARM RATIO, RELATIVE LENGTH (RL), CENTROMERIC INDEX(CI), CENTROMERIC TYPE (CT), CHROMOSOME TYPE, TF% AND CENTROMERIC FORMULA OF MITOTIC METAPHASE CHROMOSOME OF *IN VIVO* GROWN PLANTS OF *P. ZEYLANICA*

Chromosome pair	Long arm(l) µm	Short arm(s) µm	Total length(T) µm	Arm ratio(l/s)	RL (%)	CI (%)	CT	Chromosome type	TF%	Centromeric formula
1	3.78	1.76	5.54	2.15	11.19	31.77	sm	L	45.11	3sm+11m
2	2.71	2.71	5.42	1.00	10.95	50.00	m	L		
3	3.46	1.91	5.37	1.81	10.85	35.54	sm	L		
4	2.74	1.48	4.22	1.85	8.53	35.13	sm	M		
5	2.09	2.09	4.18	1.00	8.44	50.00	m	M		
6	1.74	1.74	3.48	1.00	7.03	50.00	m	M		
7	1.57	1.57	3.14	1.00	6.34	50.00	m	S		
8	1.55	1.55	3.10	1.00	6.26	50.00	m	S		
9	1.47	1.47	2.94	1.00	5.94	50.00	m	S		
10	1.30	1.30	2.60	1.00	5.25	50.00	m	S		
11	1.24	1.24	2.48	1.00	5.01	50.00	m	S		
12	1.16	1.16	2.32	1.00	4.69	50.00	m	S		
13	1.15	1.15	2.3	1.00	4.65	50.00	m	S		
14	1.13	1.13	2.26	1.00	4.57	50.00	m	S		

TABLE II: LENGTH, ARM RATIO, RELATIVE LENGTH (RL), CENTROMERIC INDEX(CI), CENTROMERIC TYPE (CT), CHROMOSOME TYPE, TF% AND CENTROMERIC FORMULA OF MITOTIC METAPHASE CHROMOSOME OF *IN VITRO* GROWN PLANTS OF *P. ZEYLANICA*

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio (l/s)	RL (%)	CI (%)	CT	Chromosome type	TF%	Centromeric formula
1	3.58	1.88	5.46	1.90	11.50	34.42	sm	L	45.58	3sm+11m
2	3.60	1.79	5.39	2.01	10.96	33.48	sm	L		
3	2.54	2.54	5.08	1.00	10.82	50.00	m	L		
4	2.27	2.27	4.54	1.00	8.51	50.00	m	M		
5	2.16	2.16	4.32	1.00	8.45	50.00	m	M		
6	2.06	2.06	4.12	1.00	7.04	50.00	m	M		
7	2.57	1.36	3.93	1.89	6.28	34.68	sm	M		
8	1.73	1.73	3.46	1.00	6.28	50.00	m	M		
9	1.61	1.61	3.22	1.00	5.93	50.00	m	S		
10	1.58	1.58	3.16	1.00	5.25	50.00	m	S		
11	1.56	1.56	3.12	1.00	5.00	50.00	m	S		
12	1.33	1.33	2.66	1.00	4.70	50.00	m	S		
13	1.13	1.13	2.26	1.00	4.64	50.00	m	S		
14	1.13	1.13	2.26	1.00	4.58	50.00	m	S		

TABLE III: COMPARISON OF CHROMOSOME CHARACTERS BETWEEN *IN VIVO* AND *IN VITRO* GROWN PLANTS OF *P. ZEYLANICA*

Chromosome Characters		*In vivo* raised plant	*In vitro* grown plant
Somatic chromosome number		2n=28	2n=28
Total Haploid Chromatin Length (TCL) (µm)		49.35	52.98
Range of Individual Chromosome Length (µm)		2.26 – 5.54	2.26 – 5.46
Total length of haploid complement	Long arm (µm)	27.09	28.85
	Short arm (µm)	22.26	24.13
Range of relative length (µm)		11.49 – 4.57	11.50 – 4.58
Centromeric index (CI)		31.77 - 50.00	34.42 - 50.00
Ratio of longest/ smallest chromosome		2.45:1	2.42:1
The total form percent (TF%)		45.11	45.58
Karyotypic formula	n=14	$2L^{sm} + 1L^{m} + 1M^{sm} + 2M^{m} + 8S^{m}$	$2L^{sm} + 1L^{m} + 1M^{sm} + 4M^{m} + 6S^{m}$
	2n=28	$4L^{sm} + 2L^{m} + 2M^{sm} + 4M^{m} + 16S^{m}$	$4L^{sm} + 2L^{m} + 2M^{sm} + 8M^{m} + 12S^{m}$

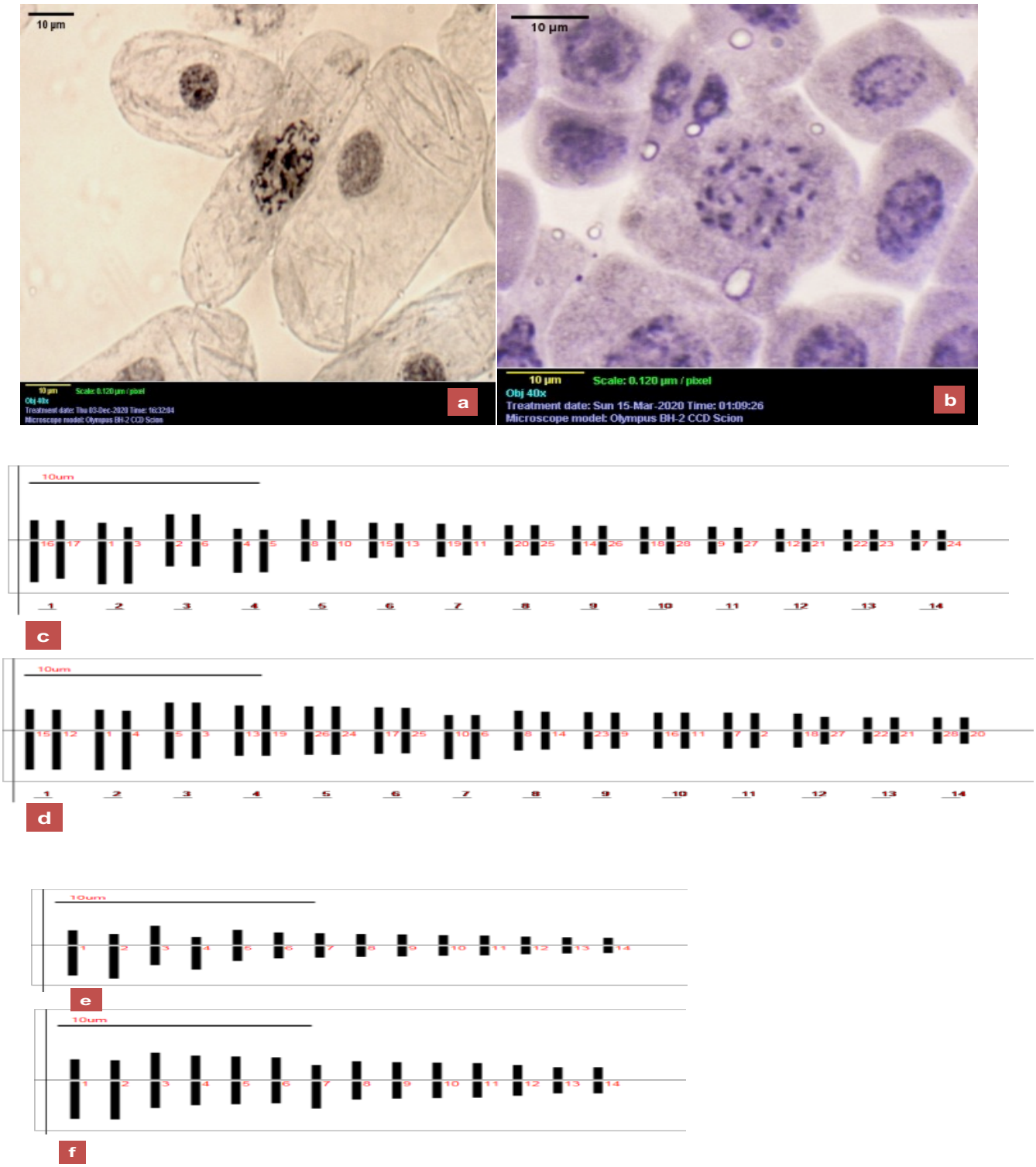

Fig. 1. Microscopic photograph of somatic metaphase chromosomes of *in vivo* grown plant of *P. zeylanica*; b) Microscopic photograph of somatic metaphase chromosomes of *in vitro* grown plant of *P. zeylanica*; c- Ideogram of *in vivo* grown plant (2n=28) of *P. zeylanica*; d- Ideogram of *in vitro* grown plant (2n=28) of *P. zeylanica*; e- Ideogram of *in vivo* grown plant (n=14) of *P. zeylanica*; f- Ideogram of *in vitro* grown plant (n=14) of *P. zeylanica*.

IV. Conclusion

The results from the comparative cytological analysis revealed that mother plants and *in vitro* grown plants of *P. zeylanica* plants had the same number of chromosomes. However, there were very few karyotypic variations observed between *in vivo* and *in vitro* grown plants. These studies in cytological studies will be beneficial to realize the dissimilarities, and arrangement of chromosomes which are useful for future research.

V. Conflict of Interest

Authors declare that they do not have any conflict of interest.

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