ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Karyotypic analysis in Western Himalayan species of *Berberis* L.

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ABSTRACT

Cytological studies with special reference to karyotaxonomy have been made in two taxa of *Berberis i.e., B.asiatica* and *B. lycium* . Although both the species have a chromosome number of 2*n* = 28, they could be differentiated by their karyotype formula and quantitative parameters of the karyotypes. Phenetic distance and principal component analysis showed that in spite of the differences observed among entities, they can be grouped in clusters that coincide with the taxonomic sections established by Linnaeus and with the life cycle of the species. From an evolutionary point of view, variation in total chromosome length without major changes in the karyotype formula suggests that changes in the amounts of genomic DNA are proportional to the relative length of each chromosome arm .Variation in genome size, however, is congruent with morphological variation.

Keywords: *Berberis*; Concerted evolution; Karyotypes; Variation.

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ISSN: 0975-8585

INTRODUCTION

The genus *Berberis* (family Berberideaceae) has about 200 species of deciduous shrubs distributed all over the world, characterized by high genetic variation and known for edible, medicinal, and ornamental value (Dellu *et al* 1994). In order to differentiate various taxa and to clarify the interspecific phylogenetic relationships, several studies based on DNA markers have been conducted on *Berberis* species. Despite these approaches, the taxonomic limits are not definitely established and some researchers indicate that the phylogeny in family Berberideaceae and the monophyly of genus *Berberis* remain uncertain and need to be reevaluated. The phenotypic and genotypic studies have revealed a very large variation in the morphological, biochemical, and cytogenetic profile in all the species including *B. asiatica* and *B. lycium*. The knowledge of genetic constitution is of basic importance in the context of this extremely large phenotype heterogeneity. The establishment of chromosome numbers, karyotypes, and identification of ploidy levels can be analyzed in relation to phenotypic variability to delimit various inter and intraspscific taxa. In order to have an overall assessment, it is essential that the molecular studies should be integrated with the traditional cytogenetic data. Therefore, the main objectives of this work were the establishment of somatic chromosome number, karyotypic analysis and description, and their representation as idiograms in *B. asiatica* and *B. lycium.*

MATERIALS AND METHODS

A total of 290 metaphases were scored in order to establish the somatic chromosome number in plant material. Because of the difficulties encountered in the processing of plant material and the small size of the chromosomes, 5 metaphases (n = 14) with well-spread and optimally condensed chromosomes were measured for chromosome length and the other cytogenetic parameters in order to construct the karyotypes.

Karyotype details

Chromosome measurements included absolute length of individual chromosomes (CL), long arm length (L), short arm length (S), arm ratio (r = L/S), centromeric index (CI = 100 × S/CL), length of the haploid complement (LHC). Chromosome designation followed Levan's terminology (Levan et al 1964), and the homology was assigned according to similarities in length, morphology, and centromere position, respectively, on the basis of CI and r values.Thus the chromosomes are metacentric when they have a mean arm ratio of up to 1.7 and CI = 37.5–50.0, submetacentric (r = 1.70–2.99, CI = 25.0–37.5), subtelocentric (r = 3.00– 6.99, CI = 12.5–25.0), or telocentric (r = 7.00 and above, CI < 12.5). In karyotypes, the chromosome pairs were grouped in descending order of their length.

Karyotype symmetry/asymmetry

To evaluate the karyotype symmetry/asymmetry, the following indexes were analyzed: TF%, AsI%, A1, A2, and Stebbins indicators (Stebbins 1971).The AsI% index (synonymous with AsK%) (Arano et al 1980 & B.Paszko 2006) represents the ratio of the sum of the long arm lengths of individual chromosomes to the haploid complement length: AsI% = (Σ long arms/haploid complement length) ×100.The total form

percent (TF%) is expressed by the ratio of the total sum of short arm lengths of individual ch romosomes to the haploid complement length (Y Huziwara1962):TF% = (Σ short arms/haploi d complement length) ×100.The intrachromosomal asymmetry index (A1) and the interchromosomal asymmetry index (A2) were calculated according to the following formulae (Paszko 2006 & Romero 1986): A1 = 1 – [Σ(b/B)/n], where b and B are the mean lengths of short and long arms of each pair of homologues, and n is the number of homologues. It measures the average position of the centromere in karyotype and ranges from 0 (completely symmetrical) to 1 (completely asymmetrical). A2 = SCL/XCL, where SCL is the standard deviation of chromosome length and XCL is the mean chromosome length for each genotype. It is defined as a coefficient to evaluate the heterogeneity of chromosome length. Stebbins' indicators (1971), based on the proportion of chromosomes with arm ratio (r) higher than 2 and on the ratio between the lengths of the longest and the shortest chromosome pair in the complement (R), were employed to establish the karyotype symmetry classes. The asymmetry increases from type 1 to type 4 (as the proportion of chromosomes with r > 2 increases) and from type A to type C (in relation to the ratio between the size of the largest and the smallest chromosome pair)

Idiogram construction

An idiogram was drawn based on the average of the mean values calculated for the karyotypes of the 4 analyzed selections (Table 3). Short arms of the chromosomes were placed above the imaginary line representing centromere position. For each parameter, the mean and the standard error of the mean (x– ± SE) were calculated.

RESULTS

The small size of the chromosomes and the low occurrence of well-spread chromosomes in metaphase plates often hampered the cytogenetic investigations. It was somewhat difficult to exactly establish the centromere position, especially for chromosomes smaller than 2 µm where few details are distinguishable .The base chromosome number in the genus *Berberis* is recognized as n = 14, and so the formula in somatic cells of shoot tip meristems of our material is 2n =28. The results of this study reveal a detailed picture of the chromosome features of *Berberis* species and of their pattern of variation in relation to their systematic position and life cycle. The study is based on two species of *Berberis i.e. B. asiatica and B. lycium*, with 2n=28 chromosomes (Figure 1,2 and 3). The karyotype formula as well as respective idiograms (Figure 4) obtained and the parameters analyzed are summarized in Table 2. Both the species exhibited variation in chromosome length. The observed value of mean total chromosome length of complement (Table 2) *in B. asiatica* (4.63 µm) which was found much smaller than that of *B. lycium* (7.28 µm). It was supported by the mean length of long arms of complements , which was studied minimum in *B. asiatica* (3.1 µm) and maximum in *B. lycium* (4.5 µm) and the same pattern was maintained in case of mean length of short arms that showed highest in *B. lycium* (2.81 µm) followed by *B. asiatica* (1.5 µm) whereas *B. asiatica* is observed with higher level of variation (34.17) of centromeric index. Between both the species, the karyotype morphology is rather homogeneous (Table 1, Figure 2). Arm ratio and centromeric index mean values showed that most of the chromosomes are either metacentric (m) or submetacentric (sm). Mean arm ratio/karyotype is 2.03 and 2.36 in *B.asiatica and B.lycium* respectively exceeding the

limit of 1.70, beyond which the chromosomes are included in the submetacentric type. Karyotypic formulae of the chromosome complements for *B.asiatica* and *B.lycium* are K (2n) = 4 m+22 sm+2 st and 8 m+18 sm +2 st respectively. The numerical predominance of small m and sm chromosomes is only one criterion in defining karyotype symmetry. *B. lycium* is more symmetrical than *B. asiatica* due to high number of meta centric chromosomes .The analysis of karyotype asymmetry indicated moderate interspecific uniformity for all specific variables (Truta etal 2013) (Table 2), therefore, the mean chromosome length is x ± SE = 4.63 ± 0.05 µm, with a range of variation from 2.1 ± 0.09µm to 7.33 ±0.07 µm in *B.asiatica* and x ± SE = 7.28 ± 0.85 µm, with a range of variation from 3.2 ± 0.07 µm to 11.2 ± 0.09 µm in *B.lycium.*

Table 1: Average values (X±SE) of cytogenetic parameters of *Berberis asiatica* utilized for ideogram construction (CL=chromosome length, L= long arm length, S= short arm length, r= arm ratio(L/S), CI=centromeric index)

S.No.	Type	CL	L	S	r	CI%
1	sm	7.1±0.04	4.9±2.83	2.2±0.04	2.26±0.01	30.99±0.79
2	sm	7.2±0.09	4.9±2.83	2.3±0.09	2.13±0.07	32.01±0.73
3	sm	7.2±0.04	4.9±2.83	2.3±0.04	2.16±0.01	31.93±0.44
4	sm	7.3±0.07	5.0±2.90	2.2±0.07	2.21±0.03	30.49±1.26
5	sm	5.3±0.07	3.9±2.25	1.4±0.09	2.93±0.07	26.33±2.11
6	sm	5.2±0.07	3.8±2.19	1.4±0.04	2.65±0.02	26.73±0.54
7	m	5.3±0.07	2.9±1.67	2.4±0.04	1.22±0.01	45.01±0.89
8	m	5.2±0.04	2.9±1.71	2.2±0.07	1.32±0.03	42.93±1.15
9	sm	4.3±0.04	2.8±1.65	1.4±0.07	2.08±0.07	33.39±2.04
10	sm	4.4±0.07	3.0±1.75	1.3±0.07	2.13±0.06	30.54±1.13
11	sm	4.4±0.09	3.1±1.79	1.3±0.09	2.27±0.04	29.44±1.50
12	sm	4.2±0.07	2.9±1.71	1.3±0.07	2.09±0.09	32.10±2.05
13	sm	4.3±0.04	2.9±1.71	1.3±0.07	2.21±0.08	31.05±1.94
14	sm	4.3±0.10	2.9±1.67	1.4±0.07	2.01±0.08	33.20±2.14
15	sm	4.4±0.09	2.7±1.56	1.7±0.04	1.59±0.03	38.65±0.96
16	sm	4.2±0.70	2.6±1.50	1.6±0.04	1.59±0.03	37.79±0.85
17	sm	3.3±0.10	2.2±1.27	1.1±0.04	2.00±0.07	33.32±0.49
18	st	3.2±0.07	2.0±1.15	0.8±0.02	3.65±0.61	24.37±5.70
19	sm	3.2±0.07	2.0±1.15	1.1±0.07	1.85±0.01	35.24±2.97
20	sm	3.3±0.09	2.5±1.44	0.8±0.09	2.91±0.20	24.25±3.49
21	st	3.3±0.07	2.5±1.44	0.8±0.01	3.38±0.74	24.06±5.63
22	sm	3.3±0.01	2.3±1.36	0.9±0.07	2.69±0.05	27.87±1.14
23	sm	3.3±0.07	2.1±1.25	1.1±0.09	1.79±0.09	34.06±3.15
24	sm	3.4±0.07	2.4±1.38	1.0±0.09	2.60±0.18	29.27±3.11
25	sm	2.3±0.09	1.7±0.98	0.6±0.09	2.59±0.24	25.98±3.60
26	m	2.3±0.09	1.4±0.80	0.9±0.04	1.50±0.06	38.15±2.21
27	m	2.1±0.09	1.2±0.71	0.8±0.09	1.55±0.07	40.23±4.97
28	sm	2.1±0.07	1.3±0.75	0.8±0.09	1.805±0.18	37.35±5.46

ISSN: 0975-8585

Table 2: Average values (X±SE) of cytogenetic parameters of *Berberis lycium* utilized for ideogram construction (CL=chromosome length, L= long arm length, S= short arm length, r= arm ratio(L/S), CI=centromeric index)

S.No.	Type	CL	L	S	r	CI%
1	m	11.23 ± 0.65	6.65±3.84	4.63 ±0.07	1.46±0.03	41.24±0.65
2	m	11.20 ± 0.47	6.60±3.81	4.53 ±0.07	1.43±0.04	40.47±0.47
3	m	11.23 ±0.12	6.70±3.87	4.46 ±0.02	1.50±0.02	39.76±0.12
4	m	11.20 ± 0.61	6.65±3.84	4.53 ±0.07	1.46±0.03	40.47±0.61
5	sm	7.10 ±0.79	4.85±2.80	2.20 ±0.04	2.15±0.07	30.99±0.79
6	sm	7.20 ±1.73	5.00±2.89	2.30 ±0.09	2.27±0.13	32.01±1.73
7	sm	7.20 ±0.44	4.85±2.80	2.30 ±0.04	2.06±0.60	31.93±0.44
8	sm	7.33 ±1.26	5.15±2.97	2.23 ±0.07	2.39±0.12	30.49±1.26
9	sm	7.10 ±0.79	4.85±2.80	2.20 ±0.04	2.15±0.07	30.99±0.79
10	sm	7.20 ±0.73	5.00±2.89	2.30 ±0.09	2.27±0.13	32.01±0.73
11	sm	7.20 ±0.44	4.95±2.86	2.30 ±0.04	2.10±0.06	31.93±0.44
12	sm	7.33 ±1.26	5.15±2.97	2.23 ±0.07	2.39±0.12	30.49±1.26
13	sm	5.33 ±2.11	3.80±2.19	1.40 ±0.09	2.54±0.27	26.33±2.11
14	sm	5.23 ±0.34	3.85±2.22	1.36 ±0.02	2.85±0.09	26.11±0.34
15	m	5.33 ±0.89	2.85±1.64	2.40 ±0.04	1.16±0.04	45.01±0.89
16	m	5.20 ±1.15	3.05±1.76	2.23 ±0.07	1.41±0.07	42.93±1.15
17	sm	4.30 ±2.04	2.85±1.64	1.43 ±0.07	1.99±0.14	33.39±2.04
18	sm	4.46 ±1.13	3.05±1.76	1.36 ±0.07	2.30±0.17	30.54±1.13
19	sm	4.30 ±1.94	3.05±1.76	1.33 ±0.07	2.44±0.17	31.05±1.94
20	sm	4.33 ±2.14	3.00±1.73	1.43 ±0.07	2.22±0.17	33.20±2.14
21	m	4.40 ±0.96	2.75±1.58	1.70 ±0.04	1.66±0.07	38.65±0.96
22	m	4.23 ±0.85	2.15±1.24	1.60 ±0.04	1.35±0.06	37.79±.85
23	sm	4.30 ±1.63	3.20±1.84	1.30 ±0.09	2.69±0.26	30.16±1.63
24	sm	4.26 ±1.23	3.05±1.76	1.23 ±0.07	2.65±0.20	28.85±1.23
25	ssm	3.30 ±0.49	2.15±1.24	1.20 ±0.05	2.01±0.12	33.32±0.49
26	st	3.23 ±5.70	2.00±1.15	0.80 ±0.20	3.65±0.61	24.37±5.70
27	sm	3.23 ±2.97	2.00±1.15	1.10 ±0.07	1.68±0.13	35.24±2.97
28	st	3.33 ±3.49	2.50±1.44	0.80 ±0.09	3.40±0.52	24.25±3.49

Table 3: Karyotype formulae and asymmetry indexes in karyotypes of Berberis L.species (CI=centromeric index, AsI%=asymmetry, TF%=total form percent, A1=intrachromosomal asymmetry index, A2=interchromosomal asymmetry index)

Species	Karyotypic Formula	CI%	AsI%	TF%	A1	A2	Stebbins class
B.asiatica	4m+22sm+2st	34.17	66.71	32.70	0.4	0.022	2B
B.lycium	8m+18sm+2st	32.75	65.18	34.47	1.11	0.020	2Bs

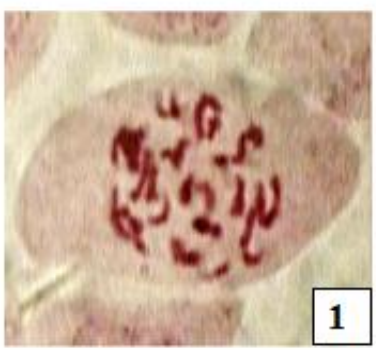
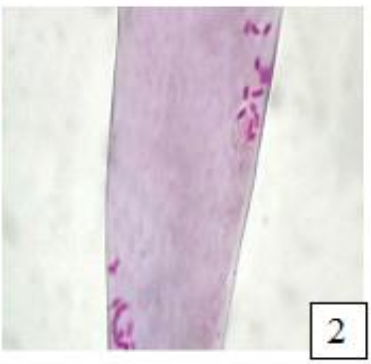


Figure: 1 Metaphase of *Berberis asiatica*



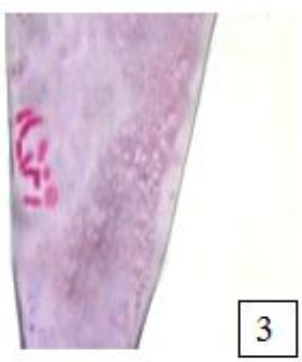


Figure: 2 and 3 Metaphase stage of *Berberis lycium*

ISSN: 0975-8585

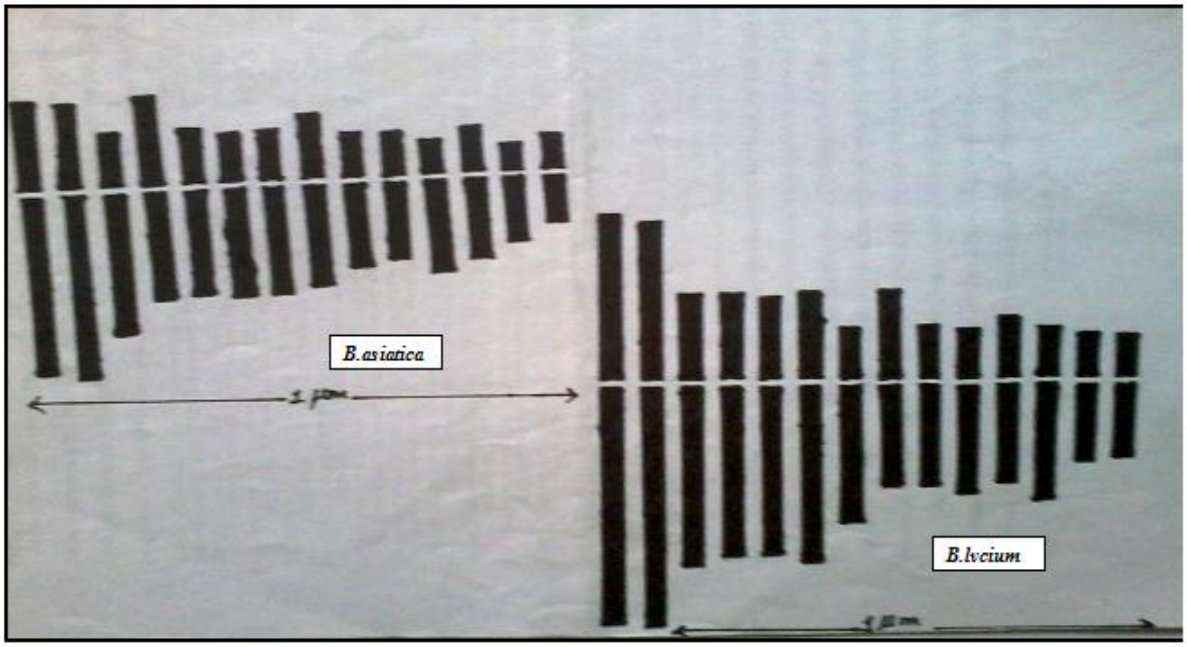


Figure: 4 Idiogram of two species of *Berberis*: (A) *Berberis asiatica* and (B) *Berberis lycium*

DISCUSSION

Results obtained from this research allowed us to compare for the first time the karyotypes of *B. asiatica* with the *B. lycium*. The present work revealed intraka ryotypic similarities in chromosome morphology, mainly submetacentric and metacentric ch romosomes being identified. Regarding the symmetry/asymmetry degree, comparative anal ysis established that, although the differences are small, karyotypes of *B. asiatica* is more as ymmetrical then *B*. *lycium* because they have the highest AsI%, the lowest TF%. Based on these results and according to Stebbin's classification, the karyotypes fall into 1B and 2B categories, considered relatively primitive in this system. The tendency toward karyotypic asymmetry by the increase of the number of telocentric chromosomes in addition to metacentric and submetacentric types represents a progressive step in karyotype evolution and has repercussions on species evolution but in this study only a pair of st chromosomes has been found in both the species. Differences in karyotype formulae and asymmetry indices found in both the species suggest that structural changes may have contributed to the diversification of the genus. On the other hand, the fact that species formed groups that share major karyotypic characteristics may indicate that if the mechanisms of speciation within each group involved chromosome rearrangements, these may not have been large structural mutations, but small or cryptic changes. Alternatively, if speciation has occurred as a consequence of large chromosome modification, these may have been changes that did not modify the karyotype morphology, such as paracentric inversions or reciprocal translocations with segments of equal size (Seijo 2003). The existence of very similar karyotypes in both the species of *Berberis* suggests that chromosome evolution in this section may be constrained to nonrandom changes with particular restrictions for the occurrence or fixation of structural rearrangements. The stability of complements among a group of species was first explained by orthoselection, which considers the occurrence of random chromosome mutation, but with the fixation of a restricted type of rearrangement (White, 1978). An alternative hypothesis was offered by King (1993), who considered the

nonrandom nature of chromosomal evolution. This model contemplates that structural characteristics of the genome restrict the position and number of breaks that could occur and the type of rearrangements that could form. Even though both mechanisms would have similar results, a bulk of molecular and chromosome data is accumulating in favor of the position that claims that chromosomal mutations are not only nonrandom but are constrained by the chromosome structure to the type of change that can be produced (Peters, 1982; Shaw et al., 1983; King, 1993; Narayan, 1988). This pattern of evolution at molecular and sub chromosomal levels suggests that species within each group evolved in a concerted fashion, maintaining the karyotype morphology (Seijo 2003).

Our results suggest that during the speciation and divergence of the genus, cycles toward symmetry and asymmetry may have occurred, as has been pointed out for different groups of angiosperms (Jones, 1970; Stebbins, 1971).Differences in TCL also indicate that during the diversification of the genus, cyclic changes in genome size may have occurred. These facts suggest that the utilization of asymmetry indices for the establishment of the evolutionary relationships in *Berberis* may not be straightforward and the variation in genome size might also not be unidirectional, and that both increments and decreases in genome size may have participated in the evolution and diversification of the genus, even within a related group of species (Seijo 2003). Karyotypic resemblances in both the species are not surprising because the varieties are at low taxonomic levels, a fact implying only small differences between them. The differences in biochemical phenotypes could prove that the genetic changes have occurred at the subchromosomal level(Truta *et a*l 2013). As observed in both the species karyotypes with chromosomes smaller than 4 µm and predominantly of the metacentric and submetacentric types are considered to be primitive and little evolved, because they do not supported significant genetic restructuring and rearrangement during evolution (Truta *e tal* 2013).

Taking into account the previous considerations and based on the results of our research, the section of *Berberis* have symmetrical and relatively few evolved karyotypes because DNA changes are in relation to ecogeographic fluctuations, it is possible that they represent an adaptive response and may also constitute an incipient speciation(Miyashita T *et al* 2011). However, additional molecular assays are required in order to realize the distinction between closely related groups and to elucidate when and how the changes at the genome level took place. On the basis of present karyotypic study due to difference in TCL in both the species it may be justified to treat them as two different species of *Berberis*.

ACKNOWLEDGEMENTS

The author Varsha Srivastava is thankful to Dr. Harsh Singh, Ms.Veena for their support in germplasm collection and, Dr. Shyam Kishore for their help in study.

Funding Information

This work was supported by DST SERB scheme F.No.-SR/SO/PS-2010/09 for the financial assistance.

ISSN: 0975-8585

REFERENCES

[1] Dellu C, Cracium C, Veronica C, and Temas M. Plant Science 1994;96(1-2): 143-150.
[2] Levan A, Fredga A, Sanderberg AA. Hereditas 1964;52: 201–220.
[3] Stebbins GL. Chromosomal Evolution in Higher Plants.Edward Arnold Ltd. London; 1971.
[4] Arano H, Saito H. Kromosomo 1980;2: 471–480.
[5] Paszko B. Plant Syst Evol 2006;258: 39–48.
[6] Huziwara Y. Am J Bot 1962;49:116–119.
[7] Romero Zarco C. Taxon 1986;35: 526–530.
[8] Truta E, Vochita G, Rosu C.M, Zamfirache M.M, Olteanu Z, Oprica L. Turkish J Biol 2013. doi: 10.3906/biy-1205-28.
[9] Seijo JG, and A Ferna´ ndez. Caryologia 2001;66:173–179.
[10] White MJD. 1978. Modes of speciation. WH Freeman and Company.
[11] King M. Species evolution: the role of chromosome change. Cambridge University Press, Cambridge, UK .1993.
[12] Jones, K. Taxon.1970;19: 172–179.
[13] Miyashita T, Araki H, Hoshino Y. J Plant Res 2011;124: 1–9.