

Cytological and Karyotypic Studies in Four *Beta* Species

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ABSTRACT

The karyotype and its components have been used to decipher karyo-evolutionary trends within taxa. With this in mind, chromosomal characteristics of four species of the genus *Beta* were studied to find karyotype relationships, *vis-à-vis* meiotic features, and to ascertain the feasibility of using these species for development of inter-specific hybrids and polyploids in sugarbeet (*B. vulgaris* L.) breeding programs in subtropical India. Four species of *Beta* were studied; *B. vulgaris* L., *B. vulgaris* ssp. *maritima*, *B. vulgaris* ssp. *orientalis*, and *B. lomantogona*; all with chromosome numbers of $2n=18$. Their karyotypes were generally asymmetric. Total haploid chromatin length ranged from 17.92 to 24.17 μm , whereas individual chromosome size ranged from 1.47 to 3.15 μm . According to Stebbin's classification of asymmetry, these species ranged from 2A to 4A, thereby confirming an evolutionary trend among the karyotypes. The karyotype of *B. vulgaris* L. var LS-6 was most advanced and was classified as 4A. The karyotypes of *B. vulgaris* ssp. *maritima* and ssp. *orientalis* were classified as 3A. To further quantify gradations within a class of asymmetry, a chromosome Dispersion Index (DI) was calculated. A DI value of 0.479 for *B. vulgaris* L. var LS-6 confirmed its high degree of karyotypic specialization. Meiotically, all the species formed predominantly open bivalents with distal chiasma localization. Chiasma formation per bivalent decreased as the length of pairing blocks increased. This suggested a species specific chromosome condensation gradient because sugarbeet karyotypes are relatively constant during somatic and meiotic phases.

Additional Key Words: chiasma, chromosome, interspecific hybrids, karyotype, meiosis, sugarbeet.

The desirability of enhancing genetic variability in sugarbeet through interspecific hybridization for Indian subtropical conditions was suggested by Kapur *et al.* (1987). Since then, several genetically diverse stocks of *Beta* have been evaluated under subtropical Indian conditions to determine genetic variability for yield and quality characters (Srivastava *et al.*, 1998).

The meiotic chromosome pairing and segregation of parent species is affected by the environment and plays an important role in determining the gametic genotype of progeny. In addition, chromosome size and form are species specific and changes in chromosome morphology affect pairing patterns by altering chiasmatic associations (Srivastava *et al.*, 1992).

With this background, chromosomal characteristics of five accessions belonging to four *Beta* species were studied to determine: (i) the karyotype and its various components, (ii) chiasmatic association patterns, and (iii) relationships between mitotic chromosome characteristics and meiotic behavior among *Beta* species.

MATERIAL AND METHODS

Five accessions of *Beta* belonging to four species with chromosome numbers of $2n = 18$; *B. vulgaris* ssp. *orientalis*, *B. vulgaris* ssp. *maritima*, *B. lomantogona*, and *B. vulgaris* L. (var. LS-6 and R-06); were observed for their karyomorphology, *vis-à-vis* meiotic chromosome features. For analysis of somatic chromosomes, 1 to 2 cm long healthy root-tips from germinating seeds were excised, pretreated, and fixed (Srivastava and Lavania, 1987). Root-tips were stained in 2% Aceto-Orcein — 1N HCl (9: 1) for 3 to 4 hours and squashed in a drop of 45% Acetic acid. At least 5 to 10 well spread plates were used for chromosomal measurements.

For constructing karyotypes, the chromosomes were arranged in order of decreasing size and increasing asymmetry. Based on their size, the chromosomes were grouped in three classes: A= chromosome length greater than $2\mu\text{m}$ and up to $3\mu\text{m}$, B= greater than $1\mu\text{m}$ and up to $2\mu\text{m}$, and C= up to $1\mu\text{m}$. Chromosomal designation was according to Srivastava and Lavania (1987); sm - for chromosomes with submedian centromeres, st - with sub-terminal centromeres and s - for SAT chromosomes.

For meiotic analysis, sporocytes from approximately 0.1 mm long anthers were fixed in Carnoy's fixative (6 alcohol : 3 chloroform : 1 acetic

acid) and squashed in 2% aceto-carmine. Chromosome pairing pattern and chiasma frequency were recorded at diplotene and diakinesis from 5 to 10 anthers with 40 to 60 pollen mother cells in each anther. Frequency estimates of meiotic configurations and chiasmatic associations were made according to Sybenga (1975); where, frequency of ring bivalents, $r = a*b$, and frequency of open bivalents, $o = a+b - 2a*b$. Values of a (chiasma frequency of the long arm) and b (chiasma frequency of the short arm) were determined using the quadratic equation;

$$a, b = \frac{(a+b) \pm \sqrt{(a+b)^2 - 4a*b}}{2}$$

RESULTS AND DISCUSSION

Karyomorphology

The karyotypic details are summarized in Table 1 and the somatic chromosome morphology of each species, in the form of idiograms, is depicted in Figure 1. The species are arranged in decreasing order of haploid chromatin length, *vis-a-vis* increased order of karyotype asymmetry. The haploid chromatin length of these four species ranged from 17.92 μm for *B. vulgaris* L. var. R-06 to 24.17 μm for *B. vulgaris* ssp. *orientalis*. Average chromosome lengths ranged from 1.99 μm (*B. vulgaris* L. var. R-06) to 2.69 μm (*B. vulgaris* ssp. *orientalis*) (Table 1). Total form percentage (TF%), indicating position of the centromere, was calculated as described by Huziwara (1962) and ranged from 32.68 to 38.84 %. Karyotypes of all species were moderately asymmetrical consisting of either submedian or subterminal chromosomes. According to Stebbins' (1958) classification of asymmetry these species ranged from class 2A to 4A, thereby confirming an evolutionary trend among karyotypes. The karyotypes of *B. vulgaris* ssp. *orientalis* and *B. vulgaris* ssp. *maritima*, and *B. vulgaris* L. (var. LS-6 and R-06) were grouped in asymmetry classes 3A and 4A, respectively. To determine gradations within a class of asymmetry, a chromosome dispersion index was calculated (Lavana and Srivastava, 1992). The dispersion index (DI) was used to differentiate closely related taxa. Higher DI values indicate higher levels of karyotypic specialization. Accordingly, subspecies *maritima* (DI=0.396) is more advanced than ssp. *orientalis* (DI=0.360) and var. LS-6 is karyotypically the most specialized with a DI value of 0.479 in class 4A. Based on these measurements, chromosome evolution among these species is associated with diminution in chromosome size, *vis-a-vis* enhanced order of karyotypic asymmetry from *B. vulgaris* ssp. *orientalis* to *B. vulgaris* L. var. LS-6. A decrease in total

Table 1. Karyomorphological characteristics of four species of *Beta*.

Species	Haploid chromatin length (μm)	Mean chromosome length (μm)	TF%	Class of asymmetry	Dispersion index	Karyotype formula
<i>B. vulgaris</i> ssp <i>orientalis</i>	24.17	2.69	37.19	3A	0.360	1smA + 3stA + 5smB
<i>B. lomantogona</i>	21.33	2.37	32.68	2A	0.529	1stA + 1smB + 6stB + 1smC
<i>B. vulgaris</i> ssp <i>maritima</i>	20.50	2.28	36.39	3A	0.396	5smB + 3stB + 1smC
<i>B. vulgaris</i> L. var LS-6	17.99	2.00	38.72	4A	0.479	1sm _s B + 3smB + 5smC
<i>B. vulgaris</i> L. var R-06	17.92	1.99	38.84	4A	0.452	1sm _s B + 3smB + 5smC

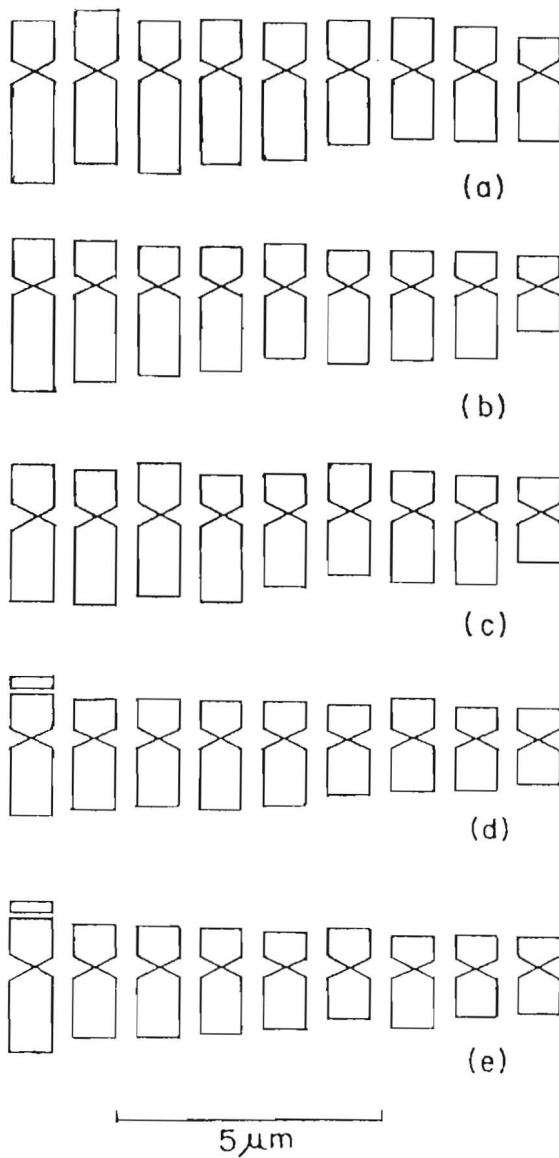


Figure 1. Idiograms of (a) *B. vulgaris* ssp. *orientalis*, (b) *B. Lomantogonia*, (c) *B. vulgaris* ssp. *maritima*, (d) *B. vulgaris* L. var LS-6, and (e) *B. vulgaris* L. var. R-06.

chromatin length is associated with the degree of evolution of a species. This decrease appears to be due to erosion of chromatin segments during evolution of these *Beta* species, since the $2n$ chromosome numbers have remained constant.

Meiotic behavior

The low frequency of univalents that occur normally in all *Beta* species during meiosis does not affect fertility, as distribution at anaphase is regular. All the species exhibited predominantly open bivalent formation with a few ring bivalents. At metaphase, chiasmata were distally localized and interstitial chiasmata were occasionally formed. For a uniform comparison of meiotic associations, the data on type of configurations was expressed as frequency of chiasma of the long arm (a) and short arm (b), for all species. The equations of Sybenga (1975) were used for this purpose. The estimates derived from the data are in Table 2. Weighted averages of chiasma frequency predict a similar probability of association in all species studied. However, the chiasmatic association frequency of one arm was higher, suggesting a strong interference for chiasma formation. Species having submetacentric/subtelocentric chromosomes form frequent open bivalents because, with a reasonable chiasma frequency, interference across the centromere is expected (Srivastava *et. al.*, 1992). The measurement most directly associated with interference is the ratio between arm association frequency (a/b). A positive correlation between open bivalents and the a/b ratio was observed (Table 2). This interference increases the frequency of open bivalents in *Beta*. When viewed in the context of chromatin length, chiasma frequency per bivalent tended to decrease (from 1.26 to 1.15 per bivalent, Table 2) as the length of pairing blocks increased. This is inconsistent with the assumption that the mean number of chiasmata per bivalent and per arm are approximately proportional to the length of the pairing block (Kostoff, 1938). Since sugarbeet karyotypes are relatively constant at somatic and meiotic phases (Nakamura and Tsuchiya, 1982), a species specific chromosome condensation gradient may be occurring in *Beta*. Hence, determining DNA content per cell / per bivalent and chiasma frequency for a constant DNA amount may be of value in establishing a pattern of bound arm association in *Beta*.

Table 2. Chromosome pairing and chiasma frequency in four species of *Beta*.

Species	Meiotic configuration			Chiasma frequency			
	Bivalents		Univalent pairs	Per bivalent	Long arm (a)	Short arm (b)	a/b
	Ring	Open					
<i>B. vulgaris</i> ssp. <i>orientalis</i>	0.2071	0.7237	0.0691	1.150	0.911	0.228	4.00
<i>B. lomantogona</i>	0.1613	0.8005	0.0382	1.167	0.954	0.169	5.65
<i>B. vulgaris</i> ssp. <i>maritima</i>	0.1775	0.8110	0.0115	1.179	0.986	0.180	5.47
<i>B. vulgaris</i> L. var LS-6	0.1810	0.7876	0.0300	1.190	0.961	0.188	5.11
<i>B. vulgaris</i> L. var R-06	0.3294	0.5973	0.0734	1.260	0.883	0.373	2.37

ACKNOWLEDGEMENT

The authors are grateful to the Director, IISR, Lucknow for providing the necessary facilities during the course of these investigations.

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