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PACHYTENE CHROMOSOME ANALYSES IN BLUE PANIC GRASS

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ABSTRACT

Ten different accessions of Blue Panic Grass, *P. anitodotale* were subjected to detailed pachytene karyotype analyses for estimating genetic variability within this wild species. All the investigated accessions had somatic chromosome count 2n=18 and basic chromosome count x=9. While exploring the pachytene karyotype, the pachytene chromosome complements of different accessions differed in the length of the long arms, short arms, and total chromosomes of their constituent chromosomes. Further, pachytene chromosomes within the same complement also differed in the position of centromeres based on the arm's ratio. Pachytene chromosomes also differ from each other within the same complement and between the complements of different accessions in the pattern of distribution and amount of chromomere on long arms and short arms. In several accessions of presently studied species the portions around the centromeres in both the arms were positively heteropycnotic. All the accessions had symmetrical or slightly asymmetrical karyotypes.

***Key words*:** Pachytene karyotype; millets; chromomere; heteropycnotic; Panicoideae; *Panicum antidotale*.

Introduction

Millets are some of the oldest cultivated crops. Millets are generally considered minor crops except in parts of Asia, Africa, China, and the Soviet Union. Minor crop species that are currently underutilized were very much used in the past. *Still, these neglected crops are of high economic value in developing countries, particularly in Africa.* Millets remain a suitable answer to the climate crisis that the world is facing due to their capability to withstand higher temperature regimes. These crops can be cultivated in low rainfall regions receiving between 200-500 mm rainfall annually without irrigation. Millets are the storehouse of dozens of nutrients that can save a large proportion of the human population from malnutrition. The genus *Panicum*, includes several important wild cereals in dry areas of the southwest United States, the interior of Australia and sub-Saharan Africa. The genus *Panicum* is one of the largest genera of grasses, including more than 400 species distributed primarily in the tropics and subtropics and also warmer parts of temperate zones (Roshevits, 1980). It belongs to the tribe Paniceae and subfamily Panicoideae. Within this large genus, two species are of economic importance; proso millet (*Panicum miliaceum* L.) and to a lesser extent little millet (*P. sumatrense* L.). Significant weed species are *P. antidotale*, *P. barbipulvinatum*, *P. bisulcatum*, *P. brevifolium*, *P. capillare*, *P. dichotomoflorum*, *P. gattingeri*, *P. laevifolium*, *P. maximum*, *P. miliaceum*, *P. natalense*, *P. obtusum*, *P. repens*, *P. sarmentosum*, *P. trichoides*, *P. turgidum*, *P. virgatum*, etc. The genus *Panicum* is poorly represented in drier tropics, apart from a few more or less xerophytic species (Cobley, 1976). Chromosome base number is x=7, 9, and 10 and 2n=18 (seemingly rarely), 36, 37, 54 or 72.

Blue panic grass, *Panicum antidotale* Retz., also known as blue panic, giant panic grass and giant panic is native to temperate and tropical Asia. It grows on sand dunes and dry river beds in northwest Pakistan, India, Afghanistan, Iran, and Yemen, tolerating temporary flooding (Grin 2000; FAO 2002). It is perennial ascending 30-180 cm high. *P. antidotale* (2n=18, 36) reproduces by seeds and rhizomes. Inflorescence is a panicle up to 30 cm long, dense, with 3 mm-long acute spikelets, the lower glume half as long as the spikelet. Blue panic is ready to graze when well established and needs heavy intermittent grazing to keep it at a nutritious stage. It requires 25-30 cm of stubble left after cutting or grazing. Stems rapidly become hard and woody and should be grazed or cut before flowering. The grass often grows too fast for the cattle; surplus should be made into hay. Göhl (1975) recorded fresh early pasture with 18.8 percent crude protein in the dry matter, and fresh mature material with as low as 8.4 percent crude protein in Pakistan (Gohl, 1975). In India figures of 7.3 percent crude protein, 40.5 percent crude fiber, 7.9 percent ash, 1.2 percent ether extract and 43.1 percent nitrogen-free extract were recorded (Sen and Ray, 1964).

Millets are considered orphan crops or lost crops as not traded internationally. The term orphan is usually used to describe crops that receive little scientific research or funding despite their significance for food security in the world's poorest regions (Naylor *et al.*, 2004). These under-researched crops are nutritious, valued culturally, adapted to harsh environments, and diverse in terms of their genetic, agro climatic and economic niches. Advances in crop genomics have resulted in a more unified understanding of the biology of the entire plant kingdom, as well as a powerful set of molecular and bioinformatics tools and methods. Such advances provide an opportunity for the efficient transfer of information systems from model species and major crops to orphan crops (Naylor *et al*., 2004). As a result, relatively small investments in the transfer of advanced science from major crops to larger sets of orphan crops may potentially result in disproportionately high payoffs in terms of crop production, yield stability, and food security in least-developed countries. It is important to emphasize that investment in genomics for a given species is only likely to be useful if a strong conventional breeding effort exists.

Materials and Methods

All ten accessions of *P. antidotale* were collected locally. Laboratory codes (P-1 to P-10) were given to these accessions for the sake of convenience and for maintaining genetic stocks. The seeds of these accessions were cultivated and multiplied in uniform conditions. Young inflorescence was collected from the different accessions and fixed in Carnoy's fluid II (6:3:1: absolute ethanol: chloroform: glacial acetic acid) for 24 hours and stored in 70% ethanol in the refrigerator. Anthers were smeared and squashed one at a time in 1.5% acetocarmine. The pachytene chromosomes were analyzed from the photomicrographs using a computerized Nikon Image-capturing system. Chromosomes were measured manually on enlarged prints and these measured values were further changed into corresponding micrometer values. The following parameters were used for the pachytene karyotype analyses.

a) Total length of the chromosome of a complement (TLCC),

b) Length of long and short arms and the whole chromosome,

c) Arm's ratio (AR),

d) Total length of all short arms (TLSA),

e) Total length of all long arms (TLLA),

f) Centromeric index (ci),

g) Gradient index (GI),

h) Symmetry index (SI),

i) Total chromatin length (TCl%),

j) Relative length of the chromosome of a complement with the longest chromosome of the cell, and

k) Percent chromomere per chromosome (CPC%).

Arm's ratio, ci, GI, SI, TCl% and relative length were calculated using the above-mentioned formulae. CPC % was worked out by using the following formula.

$$\text{CPC \%} = \frac{\text{Total amount of chromomere in long and short arm}}{\text{Total length of long and short arm}} \times 100$$

The pachytene chromosomes of the complement were arranged based on the total length into four types, A-E (A >75.00 mm, B >60.00-75.00 m, C >45.00-60.00 mm, D >30.00-45.00 mm, E <30.00 mm). These chromosomes were further assorted into different types M= Median, m= metacentric, sm= submetacentric, st= subtelocentric, t= telocentric based on the arm's ratio as per Levan *et al*., (1964). Further, pachytene karyotypes were classified into various categories as per Stebbins (1958). The parameters used for categorizing the karyotypes are

(i) the Proportion of chromosomes with arm's ratio >2:1 and

(ii) the Ratio between the largest vs. smallest chromosome of the complement.

Results and Discussion

The detailed data based on the length of pachytene chromosome complements are given in Table 1 along with corresponding karyotype formulae. To obtain the karyotype formula for a particular accession, the pachytene chromosomes were categorized into five categories A-E based on total length. These were arranged in descending order: longest (chromosome 1) to shortest (chromosome 9). Further, pachytene chromosomes within the same complement also differed in the position of

Table 1: Data related to length (µm) of pachytene chromosome.

Acc.	Parameters				LA	S A	Total	l/s	ci	A	B*	TCL
P-1	TLCC	715.40	µm	**C(m)**	37.55	22.09	59.64	1.70	37.04	1.00	0.71	16.67
	TLSA	275.98	µm	**C(m)**	28.02	25.58	53.59	1.10	47.72	0.90	0.64	14.98
	TLLA	439.43	µm	**C(m)**	29.18	18.02	47.20	1.62	38.18	0.79	0.56	13.19
	GI	43.66		**D(m)**	23.48	16.62	40.11	1.41	41.45	0.67	0.48	11.21
	SI	62.80		**D(m)**	22.44	15.69	38.13	1.43	41.16	0.64	0.46	10.66
	KF= 6C(m)+4D(m)+ 2D(sm)+2E(st)+4E(m)			**D(sm)**	24.88	11.74	36.62	2.12	32.06	0.61	0.44	10.24
				E(st)	23.25	5.12	28.37	4.55	18.03	0.48	0.34	7.93
				E(m)	16.39	11.63	28.02	1.41	41.49	0.47	0.33	7.83
				E(m)	14.53	11.51	26.04	1.26	44.20	0.44	0.31	7.28
P-2	TLCC	999.75	µm	**A(M)**	41.85	41.85	83.70	1.00	50.00	1.00	1.00	16.74
	TLSA	372.70	µm	**A(m)**	46.85	34.53	81.38	1.36	42.43	0.97	0.97	16.28
	TLLA	627.05	µm	**C(sm)**	39.18	16.16	55.34	2.42	29.20	0.66	0.66	11.07
	GI	44.31		**C(sm)**	33.25	19.07	52.31	1.74	36.44	0.63	0.63	10.47
	SI	59.44		**C(sm)**	33.25	18.60	51.85	1.79	35.87	0.62	0.62	10.37
	KF= A(m)+2A(M)+2 C(m)+6C(sm)+2C(st)+ 2D(m)+2D(st)			**C(st)**	39.29	11.86	51.15	3.31	23.18	0.61	0.61	10.23
				C(m)	25.58	20.93	46.50	1.22	45.00	0.56	0.56	9.30
				D(st)	33.60	6.98	40.57	4.82	17.19	0.48	0.48	8.12
				D(m)	20.69	16.39	37.08	1.26	44.20	0.44	0.44	7.42
P-3	TLCC	847.00	µm	**B(st)**	46.97	15.00	61.96	3.13	24.20	1.00	0.74	14.63
	TLSA	261.10	µm	**B(sm)**	44.06	16.39	60.45	2.69	27.12	0.98	0.72	14.27
	TLLA	585.90	µm	**C(st)**	44.06	10.23	56.73	4.31	18.03	0.92	0.68	13.40
	GI	48.78		**C(sm)**	39.87	14.18	54.06	2.81	26.24	0.87	0.65	12.76
	SI	44.56		**C(st)**	40.22	12.67	50.45	3.17	25.12	0.81	0.60	11.91
	KF= 2B(sm)+2B(st)+ 2C(sm)+4C(st)+8D(m)			**D(m)**	23.95	18.83	42.78	1.27	44.02	0.69	0.51	10.10
				D(m)	19.07	16.97	36.04	1.12	47.10	0.58	0.43	8.51
				D(m)	18.95	11.86	30.81	1.60	38.49	0.50	0.37	7.27
				D(m)	15.81	14.42	30.23	1.10	47.69	0.49	0.36	7.14
P-4	TLCC	656.58	µm	**C(m)**	25.11	23.48	48.59	1.07	48.33	1.00	0.58	14.80
	TLSA	227.62	µm	**D(st)**	32.90	9.18	42.08	3.58	21.82	0.87	0.50	12.82
	TLLA	428.96	µm	**D(sm)**	25.69	14.18	39.87	1.81	35.57	0.82	0.48	12.15
	GI	60.29		**D(sm)**	28.25	9.77	38.01	2.89	25.69	0.78	0.45	11.58
	SI	53.06		**D(st)**	28.13	7.67	35.81	3.67	21.43	0.74	0.43	10.91
	KF= 2C(m)+4D(m)+ 6D(sm)+4D(st)+2E(m)			**D(m)**	19.65	14.18	33.83	1.39	41.92	0.70	0.40	10.30
				D(m)	18.83	11.74	30.57	1.60	38.40	0.63	0.37	9.31
				D(sm)	20.93	9.30	30.23	2.25	30.77	0.62	0.36	9.21
				E(m)	15.00	14.30	29.30	1.05	48.81	0.60	0.35	8.92
P-5	TLCC	613.57	µm	**C(sm)**	33.60	15.11	48.71	2.22	31.03	1.00	0.58	15.88
	TLSA	208.09	µm	**D(m)**	28.02	16.97	44.99	1.65	37.73	0.92	0.54	14.66
	TLLA	405.48	µm	**D(m)**	23.37	14.07	37.43	1.66	37.58	0.77	0.45	12.20
	GI	38.90		**D(sm)**	24.88	11.86	36.74	2.10	32.28	0.75	0.44	11.97
	SI	51.32		**D(sm)**	25.34	10.00	35.34	2.53	28.29	0.73	0.42	11.52
	KF= 2C(sm)+4D(m)+6 D(sm)+4E(m)+2E(sm)			**D(sm)**	23.13	9.53	32.67	2.43	29.18	0.67	0.39	10.65
				E(m)	15.11	11.74	26.85	1.29	43.72	0.55	0.32	8.75
				E(m)	15.35	9.77	25.11	1.57	38.89	0.52	0.30	8.18
				E(sm)	13.95	5.00	18.95	2.79	26.38	0.39	0.23	6.18
P-6	TLCC	571.25	µm	**C(m)**	30.34	23.48	53.82	1.29	43.63	1.00	0.64	18.84
	TLSA	229.01	µm	**D(m)**	23.83	14.65	38.48	1.63	38.07	0.71	0.46	13.47
	TLLA	342.24	µm	**D(m)**	22.09	15.46	37.55	1.43	41.18	0.70	0.45	13.15
	GI	43.41		**E(sm)**	19.30	10.00	29.30	1.93	34.13	0.54	0.35	10.26

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	SI	66.92		**E(M)**	14.18	14.18	28.37	1.00	50.00	0.53	0.34	9.93
	KF= 2C(m)+4D(m)+ 2E(M)+6E(m)+4E(sm)			**E(m)**	16.04	11.04	27.09	1.45	40.77	0.50	0.32	9.48
				E(m)	14.53	9.77	24.30	1.49	40.19	0.45	0.29	8.51
				E(sm)	16.39	6.98	23.37	2.35	29.85	0.43	0.28	8.18
				E(m)	14.42	8.95	23.37	1.61	38.31	0.43	0.28	8.18
P-7	TLCC	664.02	µm	**C(st)**	38.36	11.74	50.10	3.27	23.43	1.00	0.60	15.09
	TLSA	253.19	µm	**C(sm)**	33.13	14.42	47.55	2.30	30.32	0.95	0.57	14.32
	TLLA	410.83	µm	**C(m)**	23.60	23.13	46.73	1.02	49.50	0.93	0.56	14.08
	GI	43.85		**D(m)**	21.04	18.48	39.53	1.14	46.76	0.79	0.47	11.90
	SI	61.63		**D(m)**	19.53	14.42	33.95	1.35	42.47	0.68	0.41	10.22
	KF= 2C(m)+2C(sm)+ 2C(st)+6D(m)+2E(m)+ 4E(sm)			**D(m)**	17.21	16.51	33.71	1.04	48.97	0.67	0.40	10.15
				E(sm)	20.00	9.53	29.53	2.10	32.28	0.59	0.35	8.89
				E(sm)	20.11	8.84	28.95	2.28	30.52	0.58	0.35	8.72
				E(m)	12.44	9.53	21.97	1.30	43.39	0.44	0.26	6.62
P-8	TLCC	700.52	µm	**B(m)**	39.76	23.48	63.24	1.69	37.13	1.00	0.76	18.06
	TLSA	267.84	µm	**C(st)**	38.60	10.81	49.41	3.57	21.88	0.78	0.59	14.11
	TLLA	432.68	µm	**D(m)**	22.20	20.81	43.01	1.07	48.38	0.68	0.51	12.28
	GI	45.59		**D(m)**	23.48	13.95	37.43	1.68	37.27	0.59	0.45	10.69
	SI	61.90		**D(m)**	19.07	16.28	35.34	1.17	46.05	0.56	0.42	10.09
	KF= 2B(m)+2C(st)+ 8D(m)+2D(sm)+4E(m)			**D(sm)**	23.95	9.77	33.71	2.45	28.97	0.53	0.40	9.62
				D(m)	16.04	14.42	30.46	1.11	47.33	0.48	0.36	8.70
				E(m)	16.28	12.56	28.83	1.30	43.55	0.46	0.34	8.23
				E(m)	16.97	11.86	28.83	1.43	41.13	0.46	0.34	8.23
P-9	TLCC	785.15	µm	**B(st)**	50.80	14.18	64.98	3.58	21.82	1.00	0.78	16.55
	TLSA	255.52	µm	**C(m)**	33.36	20.00	53.36	1.67	37.47	0.82	0.64	13.59
	TLLA	529.64	µm	**C(m)**	28.83	23.60	52.43	1.22	45.01	0.81	0.63	13.36
	GI	42.75		**C(sm)**	37.08	14.30	51.38	2.59	27.83	0.79	0.61	13.09
	SI	48.24		**D(st)**	30.23	9.42	39.64	3.21	23.75	0.61	0.47	10.10
	KF= 2B(st)+4C(m)+2 C(sm)+4D(m)+2D(st)+ 4E(sm)			**D(m)**	21.27	16.16	37.43	1.32	43.17	0.58	0.45	9.54
				D(m)	22.09	14.53	36.62	1.52	39.68	0.56	0.44	9.33
				E(sm)	20.34	8.60	28.95	2.36	29.72	0.45	0.35	7.37
				E(sm)	20.81	6.98	27.78	2.98	25.10	0.43	0.33	7.08
P-10	TLCC	768.41	µm	**C(st)**	49.29	10.46	59.75	4.71	17.51	1.00	0.71	15.55
	TLSA	287.37	µm	**C(sm)**	40.92	14.30	55.22	2.86	25.89	0.92	0.66	14.37
	TLLA	481.04	µm	**C(sm)**	31.04	16.28	47.31	1.91	34.40	0.79	0.57	12.31
	GI	48.25		**C(m)**	25.81	20.69	46.50	1.25	44.50	0.78	0.56	12.10
	SI	59.74		**D(m)**	20.58	19.76	40.34	1.04	48.99	0.68	0.48	10.50
	KF= 2C(m)+4C(sm)+ 2C(st)+8D(m)+2E(m)			**D(m)**	20.93	18.83	39.76	1.11	47.37	0.67	0.48	10.35
				D(m)	18.60	17.32	35.92	1.07	48.22	0.60	0.43	9.35
				D(m)	18.83	11.74	30.57	1.60	38.40	0.51	0.37	7.96
				E(m)	14.53	14.30	28.83	1.02	49.60	0.48	0.34	7.50

centromeres based on the arm's ratio. Remarkable differences were observed within the same complement and between the complements on this basis. For instance, in the accession P-1, the first 6 chromosomes of complement were lying in the C category, the next 6 were in the D category and the last 6 were lying in the E category. Moreover, C category chromosomes were of m type while out of six chromosomes of the D category, the first four were of m type, the next two were of sm type and first two chromosomes of the E category were of st type and the last four were of m type. Out of 10 accessions of *P. antidotale*, the largest chromosome of the pachytene complement belongs to the C category (P-1, P-4, P-5, P-6, P-7, P-10). Most of the pachytene chromosomes were of the 'm' type in the various analyzed accessions of *P. antidotale*.

In pachytene chromosomes, the patterns of the chromomere dispersals were as follows.

a) Densely distributed throughout the whole chromosome,

b) Concentrated around the centromeric region,

Table 2: Data related to the amount of chromomeres in pachytene chromosomes.

Acc.	Chro.	Chromomere length (µm)						CPC
		LA	%	S A	%	Total	%	%
P-1	C(m)	9.53	25.39	7.67	34.76	17.21	28.86	28.85
	C(m)	18.95	67.64	17.32	67.75	36.27	67.68	67.68
	C(m)	15.93	54.58	11.86	65.83	27.78	58.88	58.87
	D(m)	14.42	61.40	8.60	51.77	23.02	57.40	57.39
	D(m)	9.30	41.45	9.07	57.82	18.37	48.17	48.17
	D(sm)	11.86	47.68	7.67	65.44	19.53	53.37	53.33
	E(st)	12.79	55.00	1.86	36.67	14.65	51.64	51.64
	E(m)	9.88	60.34	6.28	54.04	16.16	57.71	57.68
	E(m)	9.18	63.21	1.86	16.18	11.04	42.42	42.41
P-2	B(st)	17.32	36.88	7.79	51.97	25.11	40.53	40.53
	B(sm)	11.16	25.33	9.42	57.50	20.58	34.04	34.04
	C(st)	11.16	25.33	6.63	49.46	17.44	30.80	30.74
	C(sm)	9.77	24.49	5.12	36.15	14.88	27.54	27.53
	C(st)	11.39	28.33	5.00	54.40	16.97	33.63	33.64
	D(m)	13.02	54.38	5.12	27.20	18.14	42.39	42.39
	D(m)	9.07	47.58	6.28	37.02	15.35	42.59	42.58
	D(m)	7.91	41.73	7.21	60.85	15.11	49.09	49.06
	D(m)	5.23	33.15	5.70	39.54	10.93	36.15	36.15
P-3	C(m)	3.02	12.05	13.25	56.46	16.28	33.50	33.49
	D(st)	9.53	28.99	6.98	76.05	16.51	39.23	39.23
	D(sm)	10.00	38.94	8.49	59.84	18.48	46.37	46.36
	D(sm)	13.14	46.51	1.63	16.62	14.76	38.83	38.84
	D(st)	10.93	38.86	4.88	63.79	15.81	44.18	44.16
	D(m)	11.86	60.44	8.60	60.65	20.46	60.51	60.48
	D(m)	9.53	50.65	5.93	50.62	15.46	50.57	50.57
	D(sm)	16.16	77.25	3.72	40.00	19.88	65.78	65.77
	E(m)	9.88	65.93	6.86	48.01	16.74	57.13	57.14
P-4	A(M)	16.97	40.56	10.93	26.11	27.90	33.33	33.33
	A(m)	25.69	54.84	7.21	20.87	32.90	40.43	40.43
	C(sm)	12.56	32.05	7.21	44.62	19.76	35.71	35.71
	C(sm)	9.53	28.68	11.63	61.01	21.16	40.46	40.44
	C(sm)	5.12	15.39	6.39	34.38	11.51	22.20	22.20
	C(st)	11.04	28.11	5.00	42.12	16.04	31.37	31.36
	C(m)	8.25	32.27	6.28	30.01	14.53	31.26	31.25
	D(st)	10.00	29.76	4.77	68.33	14.76	36.39	36.39
	D(m)	6.39	30.96	10.58	64.59	16.97	45.80	45.77
P-5	B(st)	20.93	41.19	8.25	58.20	29.18	44.90	44.90
	C(m)	9.53	28.59	8.60	43.04	18.14	33.99	33.99
	C(m)	16.16	56.04	13.02	55.19	29.18	55.65	55.65
	C(sm)	11.16	30.09	8.72	60.97	19.88	38.69	38.69
	D(st)	14.07	46.54	2.44	25.95	16.51	41.64	41.64
	D(m)	10.58	49.70	9.53	59.00	20.11	53.72	53.73
	D(m)	17.44	78.99	6.74	46.42	24.18	66.06	66.03
	E(sm)	8.60	42.28	5.00	58.27	13.60	46.98	46.99
	E(sm)	8.60	41.35	4.65	66.82	13.25	47.72	47.70
P-6	C(m)	19.76	65.13	16.39	69.85	36.15	67.18	67.17
	D(m)	12.79	53.68	5.81	39.71	18.60	48.35	48.34
	D(m)	9.65	43.67	11.39	73.71	21.04	56.04	56.04
	E(sm)	10.46	54.27	6.74	67.63	17.21	58.78	58.73
	E(M)	9.53	67.29	5.81	40.99	15.35	54.11	54.10
	E(m)	11.51	71.78	5.70	51.77	17.21	63.59	63.52
	E(m)	10.81	74.49	5.93	60.73	16.74	68.94	68.90
	E(sm)	7.67	46.87	4.88	70.15	12.56	53.74	53.73
	E(m)	5.81	40.31	6.86	76.68	12.67	54.25	54.23
P-7	C(st)	14.30	37.27	8.14	69.44	22.44	44.79	44.78
	C(sm)	13.95	42.11	2.09	14.48	16.04	33.74	33.74
	C(m)	10.46	46.89	6.51	28.14	16.97	36.83	36.32
	D(m)	11.39	54.18	14.18	76.73	25.58	64.72	64.71
	D(m)	7.79	39.92	9.53	66.15	17.32	51.03	51.03
	D(m)	11.74	71.23	10.46	60.82	22.20	65.87	65.86
	E(sm)	7.67	38.38	5.81	60.95	13.49	45.67	45.67
	E(sm)	10.35	51.48	6.05	67.74	16.39	56.45	56.63
	E(m)	9.65	77.58	5.00	52.61	14.65	66.67	66.67
P-8	B(m)	14.42	36.27	11.97	51.04	26.39	41.74	41.73
	C(st)	18.60	48.20	6.63	61.54	25.23	51.07	51.06
	D(m)	7.67	34.55	5.81	27.95	13.49	31.35	31.35
	D(m)	11.16	47.54	6.16	44.17	17.32	46.27	46.27
	D(m)	11.51	60.39	14.18	87.18	25.69	72.71	72.70
	D(sm)	14.76	61.68	2.56	26.22	17.32	51.41	51.38
	D(m)	7.09	44.20	8.14	56.46	15.23	50.01	50.00
	E(m)	8.60	52.88	7.91	63.01	16.51	57.29	57.26
	E(m)	8.37	49.34	7.21	60.81	15.58	54.05	54.03
P-9	C(sm)	13.25	39.45	4.88	32.34	18.14	37.24	37.23
	D(m)	14.76	52.70	10.11	59.57	24.88	55.30	55.30
	D(m)	10.35	44.28	8.95	63.66	19.30	51.56	51.55
	D(sm)	9.65	38.81	3.49	29.38	13.14	35.77	35.76
	D(sm)	11.63	45.87	6.98	69.81	18.60	52.62	52.63
	D(sm)	6.86	29.64	2.79	29.32	9.65	29.54	29.54
	E(m)	7.91	52.34	3.49	29.79	11.39	42.47	42.42
	E(m)	10.70	69.83	3.49	35.81	14.18	56.53	56.48
	E(sm)	9.42	67.50	1.86	38.03	11.28	59.53	59.51
P-10	C(st)	21.51	43.63	7.44	71.61	28.95	48.46	48.44
	C(sm)	17.67	43.19	5.00	35.00	22.67	41.06	41.05
	C(sm)	21.16	68.18	8.14	50.04	29.30	61.94	61.92
	C(m)	12.32	47.75	8.14	39.32	20.46	44.00	44.00
	D(m)	13.02	63.28	11.74	59.43	24.76	61.39	61.38
	D(m)	11.86	56.67	12.90	68.54	24.76	62.28	62.28
	D(m)	6.74	36.26	10.23	59.05	16.97	47.24	47.25
	D(m)	13.02	69.17	3.72	31.72	16.74	54.76	54.75
	E(m)	8.37	58.58	8.60	59.20	16.97	58.89	58.87

c) Concentrated on the distal ends of chromosome arms, and

d) Sparsely scattered throughout the chromosomes.

The pattern of distribution (worked out as the total length of chromomeres/chromosome) and the amount of the chromomeres (worked out as percent chromomere/chromosome) present in an individual pachytene chromosome of different accessions are presented in Table 2. Idiograms of pachytene chromosome complements are presented in Fig. 1 and photomicrographs are shown in Fig. 2. Among the various accessions of *P. antidotale*, the length of total chromomere in the long arm, short arm and whole pachytene chromosome ranged from 3.02-25.69 µm, 1.63-17.32 µm and 9.65-37.20 µm, respectively. The percent chromomere in long arm, short arm and whole chromosome ranged from 12.05-78.99 %, 14.48-76.68 % and 22.20-68.94 %, respectively. The pachytene chromosome complements (Table 1) of different accessions as a whole differed from each other in the

Table 3: Categorization of Pachytene Karyotype as per Stebbins.

Acc.	Category	Acc.	Category
P-1	2B	P-6	2B
P-2	3B	P-7	2B
P-3	2A	P-8	2B
P-4	2B	P-9	3B
P-5	3B	P-10	2B

total length of all long arms (TLLA), all short arms (TLSA), and the whole chromosome complement (TLCC), arm's ratio, total chromatin length (TCL%), centromeric index (ci), gradient index (GI), symmetry index (SI). Among the various accessions, accession P-2 exhibited the highest value for a total length of whole chromosome complement while accession P-6 had a minimum value for this. The gradient index was highest in the accession P-4. Out of ten accessions one belonged to the 2A category, six belonged to the 2B category and the rest belonged to the 3B category as per Stebbins (Table 3). These structural changes at the chromosome level might have contributed to the diversification and ultimately could lead to genotypic variability. A comparison of the karyotype data indicated that there was an appreciable difference within and among various accessions at the chromosome complements level.

The word pachytene was coined by Winiwarter in 1900; later on, Gregoire (1907) termed it pachynema. It is the stage of meiosis I, in which the complete pairing of chromosomes and lining up of the chromomeres takes place and synapsis is at its climax. The pairing of homologous chromosomes refers to the recognition and alignment of chromosomes, while synapsis means the physical connection between them by the formation of a tripartite proteinaceous structure called the synaptonemal complex (Page and Hawley, 2004). Pachytene studies have an edge over somatic karyology for chromosome identification. This is because at this stage chromosomes are relatively less condensed and therefore reveal their structural landmarks more easily. Pachytene chromosome analyses are especially effective in plants with small chromosomes (Fransz *et al.*, 2000). Development of trisomic series in tomato (Rick and Barton, 1954), identification of *Arabidopsis* chromosomes (Ross *et al.*, 1996), and examination of chromosome aberrations in tomato (Havekes *et al.*, 1994), etc. were all performed using pachytene chromosomes. On account of these advantages, pachytene chromosomes have been employed for identification in a variety of angiosperms. In several accessions of presently studied species, the portions around the centromeres in both arms were positively heteropycnotic, evidencing the aggregation of chromomeres around the centromeres in both arms. Islam-Faridi *et al.*, (2002) and Kim *et al.*, (2005) describe the 'heteropycnotic aggregation of chromomere around the centromeres' as 'pericentromeric regions of heterochromatin' and the distal regions of euchromatin. A similar arrangement of heterochromatin and euchromatin is found in most of the chromosomes of tomato, *Medicago truncatula* and rice (Peterson *et al.*,

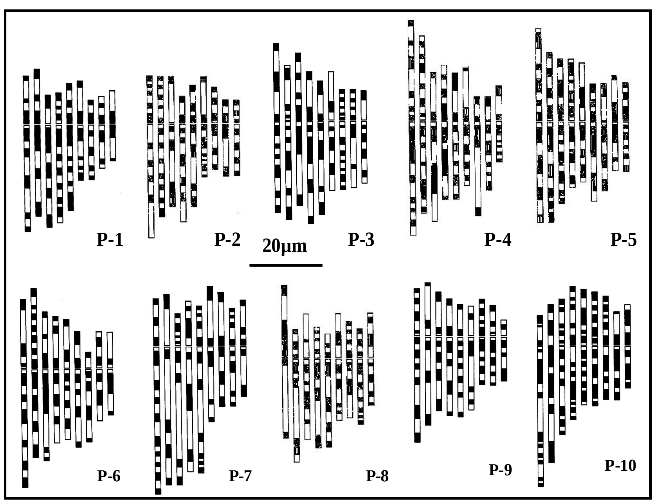

Fig. 1: Idiograms of the Pachytene Chromosome of different accessions.

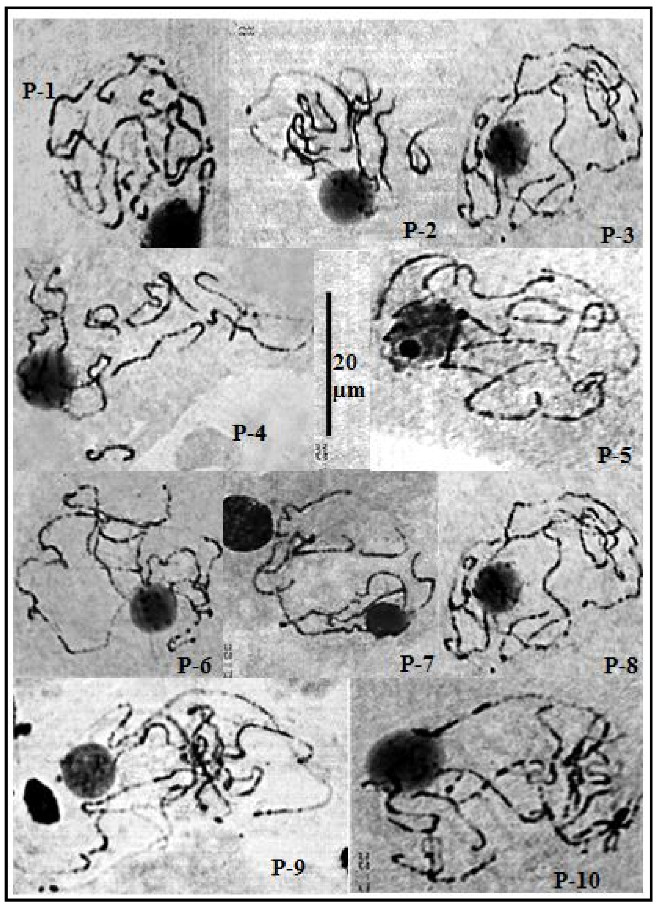

Fig. 2: Photomicrographs showing Pachytene Chromosomes in different accessions.

1999; Kulikova *et al*., 2001; Cheng *et al*., 2001). Genetic resources of a species include its wild relatives, landraces and primitive cultivars as well as advanced breeding lines and modern cultivars (Hoyt, 1988). These groups represent the species gene pool containing all its genetic variation. Landraces and wild populations may contribute valuable traits and genes for breeding. The genetic diversity within a crop gene pool is moreover of intrinsic value as a cultural heritage that needs to be maintained *ex-situ* and *in situ*, when possible, for current and future needs.

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