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PLOIDY LEVEL VARIATIONS FOR STOMATA, CHLOROPLAST NUMBER, POLLEN SIZE AND STERILITY IN BER (*ZIZHYPHUS MAURITIANA* LAMK.)

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ABSTRACT

Stomata and chloroplast number studies in *Zizhyphus* species indicated significant differences in the stomatal dimensions, frequency of their occurrence and chloroplast number per two guard cells of the stomata among different ploidy levels. However, non significant differences were observed between pentaploid and octaploid cultivars for these attributes, indicating the deleterious effects of high gene dose beyond pentaploid level. In general, there were non significant differences among the genotypes of diploids with respect to number of chloroplasts in the stomatal guard cells while significant differences were apparent among some of the tetraploid cultivars for these attributes suggesting the role of heterozygosity in the success of spontaneous polyploids. Similarly considerable differences were also observed in respect of pollen diameter of the genotypes with different ploidy levels.

Key Words : Ploidy, *Zizhyphus*, stomata, chloroplast.

Stomata size and frequency and pollen size have been used commonly in identification of polyploids in various crops. Frequency of chloroplasts of the stomatal guard cells significantly increased with increase in ploidy level[1-4]. In the present study an attempt has been made to work out the applicability of this technique in some of the naturally occurring *ber* polyploids that were cytologically confirmed[5].

MATERIALS AND METHODS

Stomatal studies : A special procedure developed by Pradeep [5] was followed to obtain an epidermal peel from the abaxial surface. The peel so obtained was mounted in a drop of water on a glass slide and observed under microscope. Length and breadth of 25 random stomata were measured using occular and stage micrometers. Average dimensions were worked out in 5 diploid, 23 tetraploid, one pentaploid and two octaploid types. For determining the frequency of stomata, five random microscopic fields were scored and mean values worked out.

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Chloroplast number studies : Number of chloroplasts per stomatal apparatus was determined by the method suggested by Jambhale and Nerkar[4] with slight modification. The mean data on these attributes were analysed statistically by using the group block design to assess the variation between and within ploidy levels[6].

Pollen grain studies : Studies of pollen sterility were made by sampling 25 flowers in each genotype. Pollen diameter was measured in 4 diploid, 10 tetraploid, one pentaploid and two octaploid cultivars for comparison. Temporary smears of pollen grains were prepared by dusting pollen grains of freshly opened flowers in a drop of 1 per cent aceto-carmine. Deeply stained pollen grains were taken as fertile and unstained and shrivelled ones as sterile. Pollen grains from fifty random microscopic fields were scored, from which the percentage of pollen fertility was determined.

Diameter of 50 random pollen grains per slide was measured in terms of microns (μ).

RESULTS AND DISCUSSION

Analysis of variance had shown significant differences among the ploidy levels for stomata length, stomatal breadth and the number of chloroplasts per two guard cells of the stomatal (Table 1) except between pentaploid and octaploids (Fig. 1).

Table 1. Mean data for stomatal characters and chloroplast number (between ploidy levels)

S. No.	Ploidy level	Stomatal length (μ)	Stomatal breadth (μ)	Frequency/ microscopic field	No. of chloroplast
A	Diploid	23.07	17.92	36.40	13.96
B	Tetraploid	25.06	19.06	28.86	17.82
C	Pentaploid	27.15	20.38	30.00	18.52
D	Octaploid	28.73	21.03	27.30	19.92
SED	(A & B)	0.31	0.20	0.83	0.24
	(A & C)	0.69	0.45	1.66	0.53
	(A & D)	0.52	0.35	1.27	0.40
	(B & C)	0.64	0.42	1.59	0.49
	(B & D)	0.46	0.31	1.17	0.36
	(C & D)	0.77	0.51	1.86	1.17
Mean	$\overline{X}$	25.05	19.04	30.84	17.35

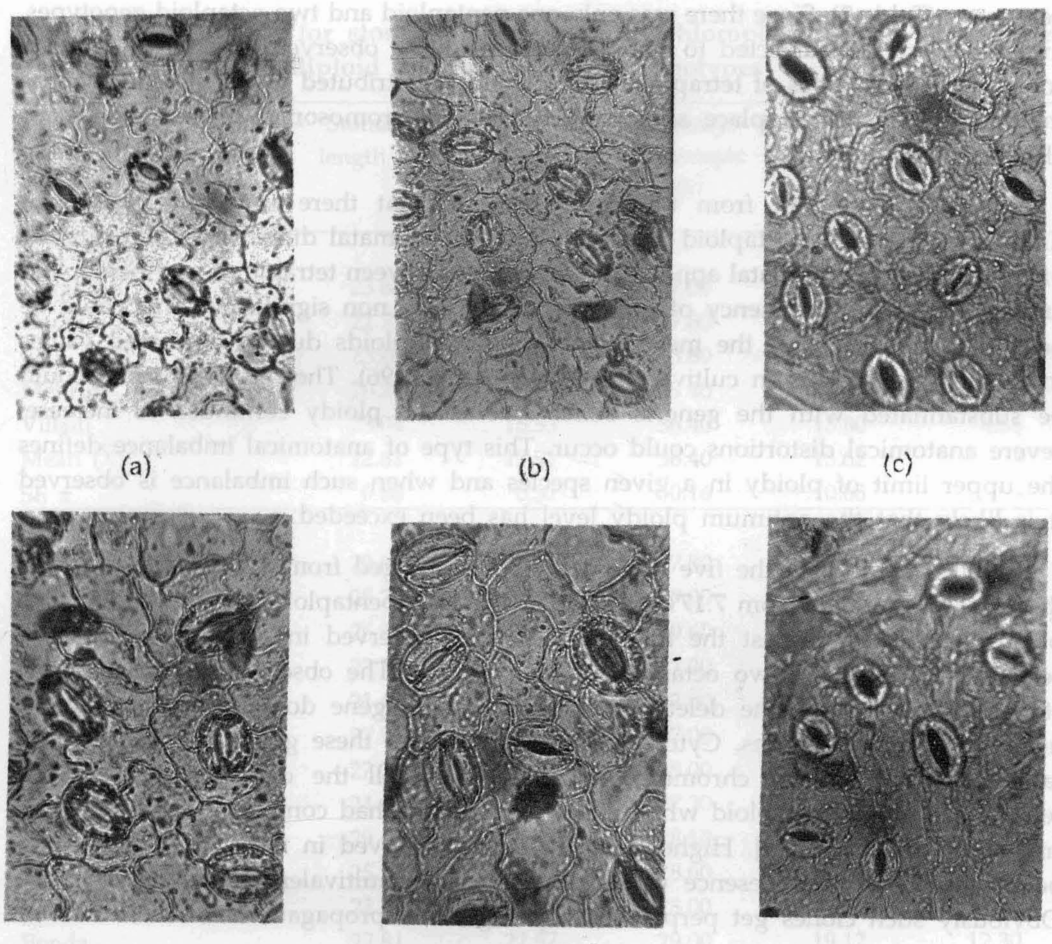

Fig. 1. Variation in stomata size of ber genotypes with different ploidy levels. (a) Diploid (Darakhi-1), (b) Triploid (Manukhi), (c) Tetraploid (Umaran), (d) Pentaploid (Dandan), (e) Octaploid (Seedless), (f) Octaploid (Illaichi, resembling diploid in stomata size)

Differences between tetraploids and pentaploid and octaploids for frequency of occurrence of stomata/microscopic field were not significant and significant differences were also not observed for all these attributes among the genotypes within the diploid. On the contrary, five genotypes differed significantly for stomatal length (Nazuk, Jallundhury, Ponda, MPKV local, Mundia, Seo and Tikri) and only one for chloroplast number (MPKV local) within the tetraploid group. However, no significant differences were observed for frequency of occurrence of stomata among the tetraploid genotypes (Table 2). Since there was only one pentaploid and two octaploid genotypes, the data were not subjected to statistical analysis. The observed differences between some of the genotypes of tetraploid group could be attributed to the genetic changes that might have taken place as a consequence of chromosomal rearrangements or allopolyploidy.

It is also evident from the data (Table 1) that there were non significant differences between pentaploid and octaploids for stomatal dimensions and number of chloroplasts per stomatal apparatus. Differences between tetraploids and pentaploid and octaploids for frequency of occurrence were also non significant. This could be because of reduction in the mean value of the octaploids due to decreased values for all these attributes in cultivar Illaichi ($2n = 8x = 96$). The observed trend could be substantiated with the general belief that as the ploidy continues to increase, severe anatomical distortions could occur. This type of anatomical imbalance defines the upper limit of ploidy in a given species and when such imbalance is observed it is likely that the optimum ploidy level has been exceeded.

Pollen sterility of the five diploid accessions ranged from 4.30% to 10.70% and that of 33 tetraploids from 7.17% to 40.35%. The lone pentaploid Dandan had 13.25% pollen sterility as against the maximum sterility observed in Illaichi (91.65%) and seedless (81.85%), the two octaploids (Table 2 & 3). The observed trend on pollen sterility also confirms the deleterious effects of high gene dose beyond pentaploid level in *Zizhyphus* species. Cytological observations of these genotypes also by and large indicated regular chromosome behaviour in all the diploids, most of the tetraploids and a pentaploid while the two octaploids had comparatively a very less frequency of bivalents[5]. Higher pollen sterility observed in these genotypes could be attributed to the presence of more number of multivalents and univalents[5]. Obviously such clones get perpetuated by vegetative propagation.

Considerable differences in respect of pollen diameter of the genotypes with different ploidy levels were apparent (Table 3). The mean values for this character ranged from 18.95 μ to 21.66 μ for diploids, 22.45 μ to 32.80 μ for tetraploids and 21.06 μ to 30.42 μ for octaploids. The range of variation in all the diploids, one each in tetraploid (Chinese), Pentaploid (Dandan) and octaploid (Illaichi) was comparatively less. The pollen diameter was reduced in the genotypes of higher ploidy levels viz., Dandan (Pentaploid) and Illaichi (octaploid). Findings of this paper clearly suggest that stomatal dimensions and their frequency of occurrence and number of chloroplasts/stomatal apparatus alongwith pollen size could be of great help in preliminary identification of *ber* polyploids.

Table 2. Mean data for stomatal characters and chloroplast number and pollen sterility in diploid and tetraploid *Ber* genotypes

Genotype	Stomatal length (μ)	Stomatal breadth (μ)	Frequency/ microscopic field	No. of chloroplasts/ 2 guard cells	Pollen sterility (%)
A. Diploids					
Darakhi-1	23.66	19.30	37.00	13.40	4.30
Darakhi-2	22.73	17.86	37.60	14.04	4.90
Guli	23.40	18.34	35.60	14.44	10.70
Mehrun	20.20	18.53	35.40	14.12	6.10
Villaiti	23.04	15.53	36.40	13.80	4.86
Mean ($\overline{X}$)	22.61	17.91	36.40	13.62	-
SE ±	0.86	0.31	0.16	0.68	-
B. Tetraploids					
BS-75-1	22.67	16.74	27.80	17.08	14.12
Chinese	25.73	19.44	27.00	18.08	23.00
Jallundhary	26.86	18.78	29.60	17.72	8.90
Kalagola	23.73	17.84	27.20	18.02	11.37
Kaithli	21.47	16.60	32.00	16.37	12.58
MPKV local	27.95	20.64	27.00	21.64	40.35
Mundia	27.22	20.85	28.00	17.86	15.72
Muria Mehrun	24.68	19.69	27.30	17.00	8.32
Nazuk	29.48	23.33	28.10	18.90	14.38
Nehru mandal	25.22	18.42	28.60	15.20	24.37
Pathani	22.93	16.71	28.00	17.20	14.16
Ponda	27.81	22.57	29.00	19.12	12.30
Shamber	23.95	18.31	28.00	17.30	16.16
Shamber (Hathed)	23.37	17.94	27.80	17.00	16.67
Sanaur-5	22.13	15.58	28.40	17.52	10.52
Sanaur-6	24.68	18.52	27.80	17.90	9.83
Sandhura Narnaul	25.62	20.35	26.80	18.00	23.46
Seo	23.95	18.75	27.80	16.80	7.17
Surti	24.90	18.85	29.60	17.70	22.95
Tasbataso	26.13	19.16	28.60	18.60	7.56
Tikri	26.39	20.75	28.80	18.70	-
Umaran	24.86	18.74	29.20	16.00	13.23
ZG-2	24.42	18.71	30.00	19.70	8.00
Mean ($\overline{X}$)	25.07	19.06	29.70	17.78	-
SE ±	0.60	0.18	0.82	0.92	-

Table 3. Pollen diameter in different *Ber* genotypes

Genotype	Ploidy level	Pollen diameter (μ)		Pollen sterility (%)
		Mean ± S.E.	Range	
Darakhi-1	diploid	18.95 ± 0.38	16.40 - 19.36	4.30
Darakhi-2	diploid	20.60 ± 0.54	17.90 - 21.46	4.90
Guli	diploid	19.48 ± 0.30	18.04 - 19.98	10.70
Villaiti	diploid	21.66 ± 0.28	18.86 - 24.02	4.86
Chhuhara	tetraploid	29.68 ± 0.68	22.80 - 38.80	23.31
Chinese	tetraploid	22.45 ± 0.31	21.10 - 24.60	23.00
Gola	tetraploid	27.90 ± 0.46	23.66 - 32.60	16.00
Kadaka	tetraploid	30.40 ± 0.75	24.20 - 38.48	9.08
Ponda	tetraploid	29.60 ± 0.76	22.80 - 34.20	12.30
Sanaur-1	tetraploid	27.05 ± 0.42	22.60 - 33.10	8.86
Sanaur-5	tetraploid	26.50 ± 0.66	22.60 - 32.20	10.52
Sanaur-6	tetraploid	27.02 ± 0.38	22.60 - 34.10	9.83
Saramber	tetraploid	30.15 ± 0.86	23.40 - 36.20	16.16
Umran	tetraploid	32.80 ± 0.14	26.40 - 38.60	13.23
Dandan	pentaploid	20.80 ± 0.19	19.92 - 22.90	13.25
Illaichi	octaploid	21.06 ± 0.48	19.68 - 24.46	91.65
Seedless	octaploid	30.42 ± 0.86	28.60 - 34.38	81.85

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