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Cytomorphological studies in relation to powdery mildew resistance in *ber* (*Zizhyphus mauritiana* Lamk.)

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Abstract

Pattern of morphological and cytological variation in relation to powdery mildew resistance in 80 germplasm accessions of *ber* (*Zizhyphus mauritiana* Lamk.) is reported. Metroglyph and index score analyses showed wide morphological variation in the germplasm. Eight distinct groups were recognized on the basis of variation in 23 morphological and fruit characters however, within group variations were of a low order. Meiotic studies revealed an array of forms ranging from diploid to triploid, tetra, penta and octaploid types. Our study suggested that the groupings based on morphological attributes have cytological foundation and a few diploid accessions and an octaploid genotype formed the basic source of immunity/resistance for powdery mildew disease.

Key words : *Z. mauritiana*, *ber*, metroglyph, meiotic behaviour, ploidy, powdery mildew resistance.

Introduction

Ber (*Zizhyphus mauritiana* Lamk.) is an important xerophytic fruit crop of arid and semi arid tropics. Powdery mildew of *ber* caused by *Oidium erysiphoides* f. *Zizhyphi* Fr. has become epiphytotic for the past 10-12 years in Maharashtra state. Similar reports are also available from Haryana, Rajasthan, Punjab and Gujarat states. Incidence of powdery mildew not only reduces yield substantially but also affects the fruit quality. Intensive efforts under germplasm screening programme at Mahatma Phule Krishi Vidyapeeth, Rahuri has led to identification of local *ber* genotypes viz., Darakhi-1, Darakhi-2, Guli, Villaiti and Seedless as resistant/immune to powdery mildew under natural [1] and artificially created epiphytotic conditions [2]. Attempts to incorporate PM resistance from local wild *ber* into cultivated varieties met with failure due to incompatibility barrier as evidenced by fruit drop 20-30 days after pollination. Breeding for resistance to PM in *ber* thus required information on the pattern of morphological and cytological variation in the germplasm and particularly the ploidy levels in cultivars and donors. In the present study metroglyph and index score analyses were carried out on PM resistant, immune and susceptible germplasm accessions of *ber* with a view to find out whether they form a single complex or otherwise. Cytological status of these lines was also studied.

Materials and methods

The experimental material consisted of 80 germplasm accessions of *ber* that are being maintained in the germplasm block of All India Coordinated Research Project of Arid Zone Fruits, Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri. Each of the accession is a bud graft with the scion of a specific genotype budded on seed derived root stock of local *ber* (*Zizhyphus mauritiana* Lamk.). The bud grafts were made on one year old local *ber* stock plants, in situ growing in *murum* soil during 1987-88 and were strictly grown under rainfed conditions. Out of the 80 germplasm accessions 78 were only scored for their morphological and fruit characters. Two genotypes i.e., Sanaur-1 and *Z. rugosa* which did not fruit were excluded from this study. Twentyfive randomly selected leaves and fruits from all sides of the tree were used for recording the observations on nine each of the morphological and fruit characters.

Metroglyph and index score analyses were carried out as per the method suggested by Anderson [3]. Index scores were constructed by dividing the range of variation for each character into three groups, *viz.*, low, medium and high. The index values 1, 2 and 3 were assigned to these three different groups. The sum of index values with regard to all the characters allotted to an individual was the indication of an individual's worth. This index score was constructed for each genotype. In the scatter diagram each of the tetraploid genotype was represented by an open circle while

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diploids, triploids, pentaploid and octaploids by solid circle. X-coordinate for each circle represented fruit weight (g) and Y-coordinate leaf area (cm). Other eighteen characters were represented by rays at different positions on the glyph and range by the length of ray (Table 1).

number determination along with Sanaur-1 and an accession of *Z. rugosa*. Flower buds of proper size were fixed in carnoy's fluid for 24 h and were transferred to 3:1 acetic alcohol fixative for 24 h. After 24 h fixation the material was preserved in 70% alcohol. Anthers were squashed in one per cent aceto-carmine stain.

Table 1. Index score

Sl.No.	Character	Range of mean	Score-1 value & less than	Sign	Score-2 value from to	Sign	Score-3 value above	Sign	Score-4	Sign
1	2	3	4	5	6	7	8	9	10	11
1	Petiole length (cm)	1.01 to 2.32	1.25 & less		1.26 to 1.75		above 1.75		-	-
2	Leaf length (cm)	4.46 to 9.48	5.00 & less		5.01 to 7.50		above 7.50		-	-
3	Leaf breadth (cm)	3.60 to 6.42	4.00 & less		4.01 to 5.50		above 5.50		-	-
4	Leaf area (cm^2)	12.77 to 44.75	22.00 & less		22.01 to 35.50		above 35.50		-	-
5	Leaf thickness (mm)	0.25 to 0.41	0.30 & less		0.31 to 0.35		above 0.35		-	-
6	Spine length (cm)	0.25 to 1.81	0.50 & less		0.51 to 1.25		above 1.25		-	-
7	Mean shoot length (m)	1.77 to 3.95	2.25 & less		2.26 to 3.25		above 3.25		-	-
8	Leaf shape	Oval, Obovate, Oblong, Cordate	Oval		Obovate		Oblong		Cordate	
9	Plant habit	Erect, Semierect & Spreading	Erect		Semierect		Spreading		-	-
10	Pollen sterility (%)	4.30 to 91.65	25.00 & less		25.01 to 50.00		above 50.00		-	-
11	Fruit length (cm)	0.60 to 4.61	2.30 & less		2.31 to 3.50		above 3.50		-	-
12	Fruit diameter (cm)	0.40 to 3.16	1.00 & less		1.01 to 2.00		above 2.00		-	-
13	Fruit weight (g)	2.40 to 20.00	7.00 & less		7.01 to 15.00		above 15.01		-	-
14	Pulp per cent	83.59 to 96.73	85.00 & less		85.01 to 90.00		above 90.00		-	-
15	Stone weight (g)	0.20 to 1.26	0.40 & less		0.41 to 0.75		above 0.75		-	-
16	Fruit shape	Round, Oval, Elliptical & Oblong	Round		Oval		Elliptical		Oblong	-
17	Leaf colour	Light green, Green, Dark Green	Light green		Green		Dark green		-	-
18	Fruit apex	Round, Obtuse	Round, Obtuse		Obtuse		Pointed			-

Cytological studies were conducted on meiotic preparations of pollen mother cells. All the 78 genotypes that were scored for their morphological characters were studied for their meiotic behaviour and chromosome

Depending on the availability about 15-25 well spread pollen mother cells were scored for chromosome number determination and association at diakinesis or metaphase-I.

Table 2. Meiotic studies in *Zizhyphus*

Name of the genotype	Chromosome no. (2n)	Chromosome association				Pollen sterility (%)
		I	II	III	IV	
1	2	3	4	5	6	7
1) Akhrota 2) Badami 3) Bagwadi 5) Banarasi 8) Betawadi 12) BS- 75-2 13) BS-75-3 14) Chinese 20) Desi Alwari 21) Foliso Alwari 22) Glory 26) Golar 30) Illaichi Jhajjar 31) Jhajjar selection 34) Jallundhary 36) Kaithli 37) Kakrolagola 38) Kalagola 39) Katha 40) Kathaphal 41) Kharkhi-1 43) MPKV local 51) Narikali 52) Narma 54) Nazuk 55) Nehru Mandal 56) Noki 58) Ponda 63) Safeda Rohtaki 64) Safarchandi (Hathed) 66) Sanaur-4 67) Sanaur-5 and 74) Surti	48	0-4 [0.22-2.19]	18-24 [79.90-95.51]	0-4 [0.74-12.50]	0-3 [1.04-19.74]	Range 6.5-46.88
4) Bahadurgarhia 9) Betawadi (Hathed) 32) Jhajjar Special 33) Jogia 35) Karaka 42) Ladu 49) Muria Mehrun 50) Nalgarhi 53) Naruk 57) Pathani 59) Popular gola 60) Safarchandi 65) Sanaur-1 68) Sanaur-6 69) Sandhura Narnaul 71) Shamber 73) Seo 75) Tasbataso 76) Umran 78) ZG-2 and 79) ZG-3	48	0-2 [0.15-1.60]	20-24 [90.08-99.16]	0-2 [0.45-3.57]	0-4 [0.84-9.92]	6.06- 23.46
6) Banarasi Karaka	48	- -	11-24 [75.69]	- -	2-6 [23.61]	10.20
7) Banarasi Pewandi	48	-	16-20 [50.00]	- -	4-12 [50.00]	20.76
10) Bhavanagari	48	0-4 [1.54]	18-22 [81.14]	0-2 [1.97]	2-3 [15.35]	39.73
11) BS-75-1	48	-	14-24 [79.82]	-	1-5 [20.18]	14.12
15) Chhuhara	48	- -	6-24 [76.31]	- -	2-9 [23.69]	23.31
16) Chonchal	48	1-4 [1.14]	16-20 [75.00]	0-1 [1.14]	2-4 [22.35]	42.75
23) Gobindgarh Special	48	- -	16-24 [81.06]	2-4 [7.57]	1-3 [11.36]	34.00
24) Gola	48	- -	16-24 [83.33]	- -	2-4 [16.67]	16.00
25) Gola Gurgaon	48	- -	12-24 [87.12]	- -	1-6 [12.88]	15.12
27) Gorfa	48	- -	18-22 [84.45]	-	1-3 [15.35]	9.84
48) Mundia	48	- -	16-24 [88.88]	- -	1-4 [11.12]	15.72
61) Safeda	48	-	16-24 [82.22]	-	2-4 [17.78]	17.55
62) Safeda Selection	48	0-4 [1.67]	16-24 [82.50]	-	2-4 [15.83]	26.72
72) Shamber [Hathed]	48	0-1 [0.19]	16-24 [93.56]	0-1 [0.57]	1-4 [6.06]	16.67
26) Golar	48	- -	18-24 [85.90]	- -	2-3 [10.10]	11.37
18) Darakhi-1	24	- -	12.00 [100.00]	- -	- -	4.30
19) Darakhi-2	24	- -	12.00 [100.00]	- -	- -	4.90

Table 2. Meiotic studies in Zizhyphus

1	2	3	4	5	6	7
		-	-	-	-	
28) Guli	24	-	12.00	-	-	10.70
		-	[100.10]	-	-	
45) Mehrun	24	-	12-00	-	-	6.10
		-	[100.10]	-	-	
46) Mehrun Khedi	24	-	12-00 -	-	-	
		-	[100.00]	-	-	
77) Villaiti	24	-	12.00	-	-	4.86
			[100.0]			
80) Z. rugosa	24	-	12.00	-	-	4.20
			[100.0]			
44) Manukhi	36	-	13-18	-	0-2	10.00
		-	[93.75]	-	[6.25]	
47) Mirchia	36	-	8-18	0-4	1-3	14.14
		-	[86.88]	[6.12]	[5.00]	
17) Dandan	60	(1-6)	10-26	1-6	1-4	13.25
		[1.08]	[77.67]	[3.75]	[17.33]	
29) Illaichi Jhajjar	96	-	12-48	3-6	4-18	91.65
		-	[70.13]	[6.50]	[23.37]	
70) Seedless	96	8-16	6-45	2-8	2-20	81.85
		[2.50]	[58.75]	[11.25]	[28.05]	

Note: Figures in parenthesis indicate percent configuration

Pollen fertility was studied from temporary smears of pollen grains of freshly opened flowers in a drop of one per cent aceto- carmine. Pollen grains from 50 random microscopic fields were scored and the percentage of pollen fertility was determined.

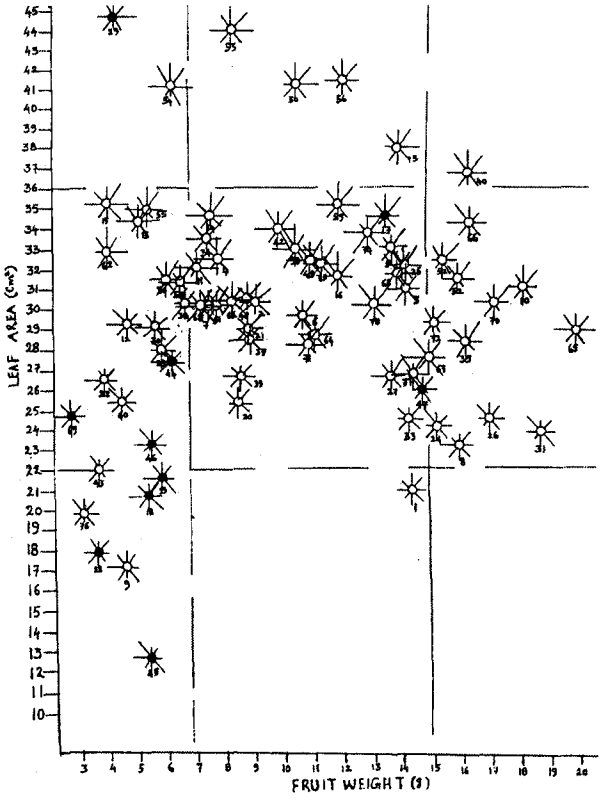

Fig. 1. Scatter Diagram-1

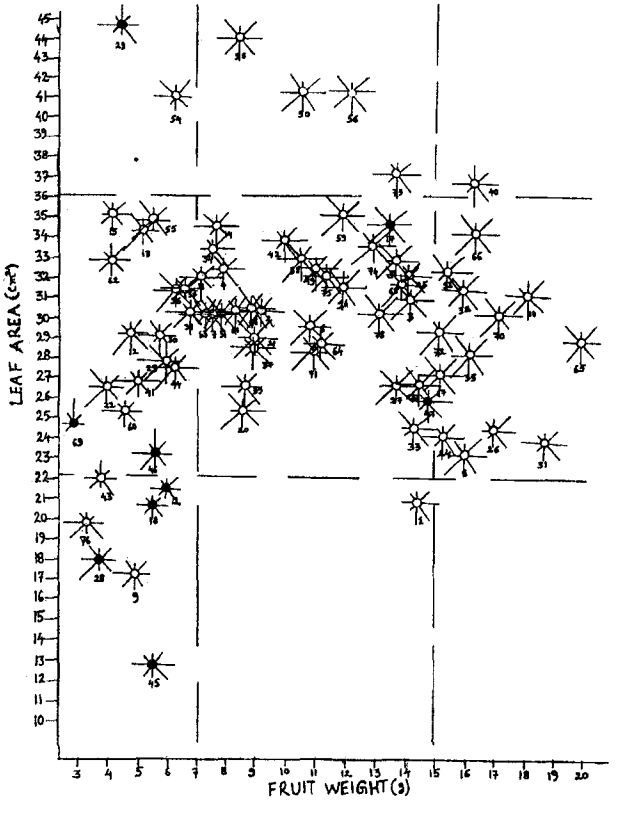

Fig. 2. Scatter Diagram-2

Results and discussion

Morphological characters : An examination of the scatter diagram (Fig. 1 & 2) revealed that 8 groups could be distinguished on the basis of variation in morphological and fruit characters. The first group was represented by seven genotypes having minimum leaf area and fruit weight. The second group consisted of only one genotype i.e., Akhrota characterized by minimum leaf area and optimum fruit weight. The third group comprised of 16 genotypes represented by optimum leaf area and minimum fruit weight. In the fourth group there were 35 genotypes and was characterized by optimum leaf area and optimum fruit weight. Twelve germplasm accessions were sorted out in the fifth group which was represented by optimum leaf area and maximum fruit weight. There were only two genotypes included

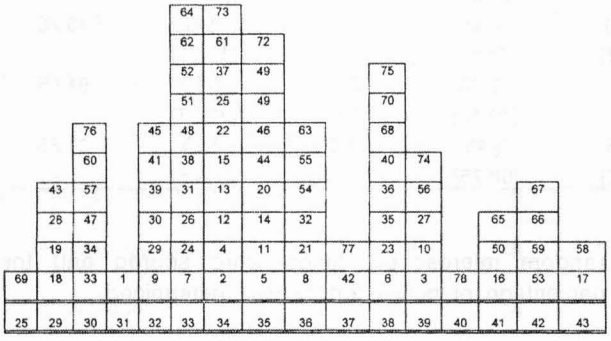

Fig.3. Frequency diagram

in the sixth group representing maximum leaf area and minimum fruit weight. The seventh group consisted of four genotypes and was characterized by maximum leaf area and optimum fruit weight. The last group had only one accession and was represented by maximum leaf area and fruit weight.

It was further observed that with respect to leaf area and fruit weight within group variability in the group five was of a low order. The frequency diagram (Fig. 3) shows index score values of all the characters under study. The range of index scores was from 25 to 43. Both the extreme scores were secured by the genotypes with optimum leaf area and fruit weight. Nearly half of the genotypes had an index score between 32 to 38 and the maximum frequency occurred around the index scores of 33, 34 & 35. Index score and scatter diagram indicated that the pattern of morphological variation was specific for the genotypes having similar ploidy level and followed a definite pattern except for the two triploids (Manukhi & Mirchia) and two octaploids (Illaichi & Seedless) which were found in two different groups. The seperation of two each of the triploids and octaploids in different groups and scattered positions of tetraploids in almost every group could be attributed to the wide variation that was observed for almost all the characters that were scored. Thus it could be concluded that the groupings based on morphological attributes seems to have cytological

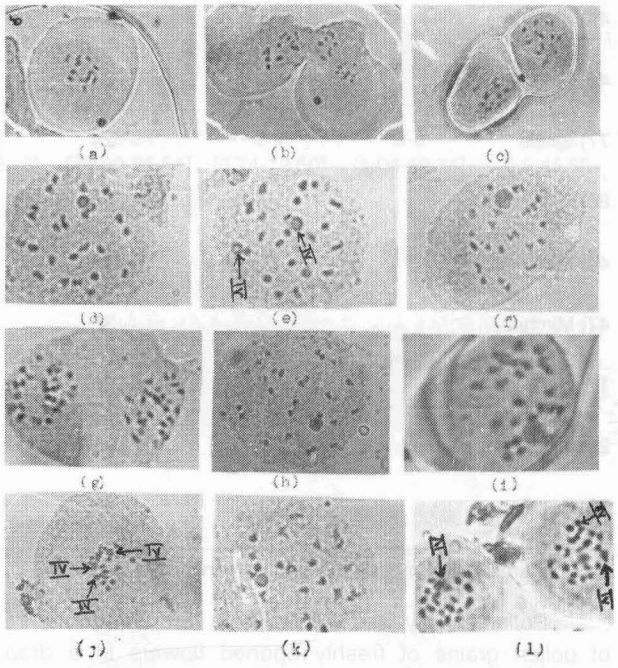

Fig. 4. (a-l) Meiosis in some of the *ber* genotypes with different ploidy levels. (X, 1000) — (a) Darakhi-1 - 12II (Metaphase), (b) Darakhi-2 - 12II (Metaphase), (c) Z. rugosa - 12II (Metaphase), (d) Gola Gurgaon-24II (Diakinesis), (e) Jogia - 20 II + 21V (Metaphase), (f) Safeda Rohtaki-24II (Diakinesis), (g) Banarasi Pewandi - Equal seperation of 24 chromosomes at Anaphase-I, (h) Katha-24II (Metaphase), (i) Narma-24II (Metaphase), (j) Popular Gola - 18II + 3IV (Metaphase), (k) Sanaur-4 - 24II (Metaphase) and (l) Kharki-1 - 20II + 2IV (Metaphase)

foundations. Similar observations were made by Tekale *et al.*, (4) in *Abelmoschus.*

Cytological studies

Cytological studies (Table 2) indicated that a majority of the accessions (68) were tetraploids with 2n = 4x = 48 chromosome number. The powdery mildew resistant genotypes viz., Darakhi-1, Darakhi-2, Guli, Villaiti and *Z. rugosa* were found to be diploids (2n = 24) while Seedless an immune type appeared to be an octaploid with 2n = 8x = 96 chromosomes (Fig. 4).

Besides, the other two diploids viz., Mehrun and Mehrunkhedi, triploids (Manukhi and Mirchia), one pentaploid (Dandan) and octaploid (Illaichi) were susceptible in their reaction to the powdery mildew disease (Fig. 5).

Chromosome association at diakinesis or metaphase-I was regular in all the diploids, triploids

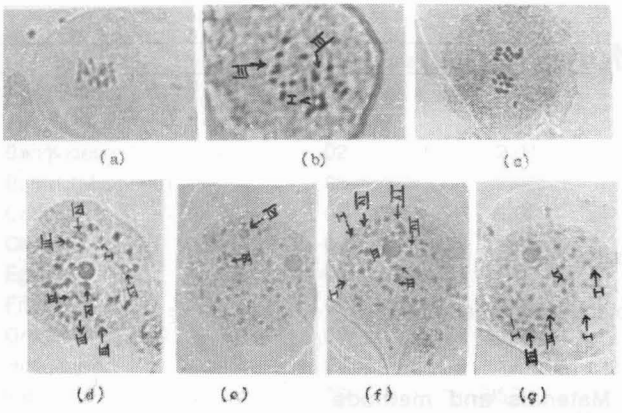

Fig. 5. (a-g): Meiosis in some of the *ber* genotypes with differnt ploidy levels. (X, 1000) — (a) Mehrun khedi - 12 II (Metaphase), (b) Manukhi - 2I + 14 II + 2 III (Metaphase), (c) Mirchia - 18 II (Metaphase), (d) Dandan - 4 I + 16 II + 4 III + 3 IV (Diakinesis), (e) Seedless - 45 II + 1 ring + 1 chain of quadrivalents (Diakinesis), (f) Seedless - 40 II + 4 III + 4 I (Diakinesis) and (g) Seedless - 36 II + 3 III + 3 I + 2 IV (Diakinesis)

and most of the tetraploids forming 'n' bivalents. The number of bivalents per cell in these types ranged from 80 to 100 per cent. However, a few genotypes, *viz.*, Banarasi Pewandi (50% II and 50% IV), B.S. 75-1 (79.82% II and 20.18% IV), Chhuhara (76.31% II and 23.69% IV), Chonchal (77.65% II and 22.35% IV), Illaichi (70.13% II and 23.37% IV) and Seedless (2.50% I, 58.75% II, 11.25% III and 28.05% IV) had exhibited multivalents in more than 20% of the cells observed.

The findings on cytological aspects of *ber* could be analogous to segmental allopolyploids as described by Stebbins [6]. In the present case, in about 60 genotypes chromosomes behaviour is almost regular, forming mostly 'n' bivalents and a small number of multivalents. A wide range of morphological variation observed among different genotypes of higher ploidy levels, their high fertility status and the ability of maintaining themselves under natural conditions clearly indicate the stability of these segmental allopolyploids. Improved fertility in majority of the genotypes except in Illaichi and Seedless and a nearly normal behaviour could be due to disappearance of multivalent associations as a consequence of diploidization.

Morinaga *et al.*, [5] were also of the opinion that the Indian jujube (*Z. jujuba*) contained an array of forms ranging from diploid to tetra, penta, hexa and octa types of which tetraploidy was the most predominant. On the contrary Khoshoo and Singh [7] were of the view that the total absence of triploids indicated that diploids are not common in India. However, in the present study diploids, triploids and a pentaploid is reported. The triploids could be product of genetic exchange between diploids and tetraploids. But the presence of a lone pentaploid could not be substantiated in the absence of hexaploids. Pentaploid could have been evolved through genetic exchange between tetra and hexaploids occurring in different natural habitat [7].

Pollen sterility of the accessions ranged from 4.36 to 91.65% with maximum amount of sterility occurring in Illaichi (91.65) and Seedless (81.85%), the two octaploids. However, the magnitude of sterility in each of the accession was well in accordance with the cytological status of the respective genotypes. Pollen sterility ranged from 6.50% (Sanaur-4) to 46.88% (Kathaphal) in a group of accessions that had 18-24 bivalents. Similarly it ranged from 6.06% (Safarchandi) to 23.46% (Sandhura Narnaul) in other group which contained 20-24 bivalents in their maximum number of cells. Higher percentage of sterility in some of the genotypes could be attributed to the increased frequency of multivalents and occasional univalents.

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