



Short Communication

An attempt to induce polyploidy in genotypes of ber (*Zizyphus mauritiana* Lamk)

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Organised efforts to induce various levels of ploidy have been made in crop plants and to use them in developing superior varieties. Transfer of powdery mildew resistance from diploid genotypes of *Zizyphus* to tetraploid cultivars required doubling of chromosomes in resistant genotypes in order to overcome the possible post-fertilization barriers. It was in this context that an attempt has been made to induce polyploidy in some of the resistant diploid genotypes of *Zizyphus* that were confirmed cytologically [1].

Colchicine treatment was initially given to young emerging shoots of (two, four and six months age) bud grafted plants of the four genotypes at six different concentrations. However, there was hardly any response probably due to woody nature of the plant. Therefore apical meristems of seedlings of each of the four genotypes (Darakhi-1, Darakhi-2, Guli and Villaiti) were treated with 0.10, 0.20, 0.40, 0.50, 0.75 and 1.00 per cent colchicine solution for 6h in polyhouse for ten days continuously. Treated portions were washed immediately by distilled water after the period of treatment to make them free from colchicine. Similarly apical buds of the young shoots emerging from the pruned plants of nine months age were treated with higher concentrations (0.75 and 1.00 per cent) of colchicine solution for 12h for ten days continuously. A cotton swab was placed on the apical buds and maintained wet by putting colchicine solution on it by using a dropper. After treatment the buds were washed with distilled water.

Stomatal and chloroplast number studies were conducted three and seven weeks (21 and 45 days after treatment) after treatment. A special procedure developed by Pradeep [2] was followed to obtain an epidermal peel from young and succulent abaxial leaf surface of both the treated and control plants. The peel so obtained was mounted in a drop of water on a glass slide and observed under microscope. Length

Table 1. Induction of polyploidy in diploid genotypes of ber

Name of the genotype	Conc. of colchicine (%)	Period of treatment (h)	No. of plants treated	Response	
				No. of plants survived	No. of suspected polyploid plants
Darakhi-1 (Apical buds of young seedlings)	0.10	6h	10	All	-
	0.20	6h	10	All	-
	0.40	6h	10	All	-
	0.50	6h	10	All	-
	0.75	12h	12	Nil	-
	1.00	12h	12	Nil	-
(Apical buds of bud grafted old plants)	0.75	12h	12	6	6
	1.00	12h	13	7	6
Darakhi-2 (Apical buds of young seedlings)	0.10	6h	10	All	-
	0.20	6h	10	All	-
	0.40	6h	10	All	-
	0.50	6h	10	All	-
	0.75	12h	14	Nil	-
	1.00	12h	15	Nil	-
Apical buds of bud grafted old plants)	0.75	12h	12	8	8
	1.00	12h	15	4	4
Guli (Apical buds of young seedlings only)	0.10	6h	10	All	-
	0.20	6h	10	All	-
	0.40	6h	10	All	-
	0.50	6h	10	All	-
Villaiti (Apical buds of young seedlings only)	0.10	6h	10	All	-
	0.20	6h	10	All	-
	0.40	6h	10	All	-
	0.50	6h	10	All	-

and breadth of 25 random stomata were measured using ocular and stage micrometers and average dimensions were worked out.

Studies on chloroplast number were made as per the method suggested by Jambhale and Nerkar [3] with slight modification. Number of chloroplasts per

Table 2. Stomatal characters in diploid genotypes of *ber* and their suspected polyploids

Name of genotype	Character	Time of observation	diploid		polyploid		't' value	$\frac{4n}{2n} \times 100$
			Mean \pm S.E.	Range	Mean \pm S.E.	Range		
Darakhi-1	Length of stomata (μ)	21 DAT	22.20 \pm 0.36	18.20 - 24.57	32.86 \pm 0.51	29.12 - 36.40	32.08	148.01
		45 DAT	23.20 \pm 0.11	18.20 - 26.39	24.80 \pm 0.49	20.02 - 29.12	11.28	136.26
Darakhi-2	Length of stomata (μ)	21 DAT	22.68 \pm 0.32	20.02 - 27.30	33.48 \pm 0.11	29.12 - 40.04	54.62	147.62
		45 DAT	23.08 \pm 0.26	21.84 - 26.39	25.80 \pm 0.50	23.66 - 30.94	17.04	111.78
Darakhi-1	Breadth of stomata (μ)	21 DAT	18.00 \pm 0.51	14.56 - 21.84	27.05 \pm 0.48	23.66 - 30.94	47.88	150.27
		45 DAT	18.80 \pm 0.36	15.47 - 21.84	20.08 \pm 0.27	15.47 - 23.66	9.94	106.80
Darakhi-2	Breadth of stomata (μ)	21 DAT	18.05 \pm 0.25	15.47 - 20.02	27.02 \pm 0.36	21.84 - 30.94	72.35	149.69
		45 DAT	18.50 \pm 0.31	16.38 - 22.75	22.90 \pm 0.24	21.84 - 25.48	4.83	123.78
Darakhi-1	No. of stomata/microscopic field	21 DAT	33.20 \pm 1.05	28.00 - 36.00	19.60 \pm 0.40	18.00 - 22.00	12.07	59.03
		45 DAT	29.40 \pm 0.72	26.00 - 33.00	26.60 \pm 0.56	25.00 - 30.00	3.29	90.47
Darakhi-2	No. of stomata/microscopic field	21 DAT	31.10 \pm 0.85	28.00 - 34.00	20.00 \pm 0.42	18.00 - 22.00	11.68	64.30
		45 DAT	29.70 \pm 0.71	26.00 - 32.00	26.90 \pm 0.89	24.00 - 32.00	2.45	90.57
Darakhi-1	No. of chloroplasts/two guard cells	21 DAT	14.01 \pm 0.39	11.00 - 18.00	24.68 \pm 0.16	24.00 - 26.00	88.33	175.28
Darakhi-2	No. of chloroplasts/two guard cells	21 DAT	13.96 \pm 0.27	12.00 - 16.00	20.88 \pm 0.18	20.00 - 22.00	74.09	149.57

stomatal apparatus were counted in 25 random stomata and mean values worked out. Observations on chloroplast number at 45 DAT were not taken as the leaf surface in *Zizyphus* is not amenable for easy removal of the epidermal peel.

The results of colchicine treatment are given in Table 1. Colchicine treatment of 0.1, 0.2, 0.4, and 0.5 per cent concentrations both to apical buds of young seedlings and bud grafted old plants had no effect in all the four genotypes. Colchicine treatment of 0.75 and 1.00 per cent concentration appeared to be lethal to the apical buds of young seedlings in the genotypes Darakhi-1 and Darakhi-2. However, the same treatment to the apical buds of bud grafted old plants was found to be effective in inducing polyploidy in both Darakhi-1(50%) and Darakhi-2 (45%) as indicated by 50 - 55 per cent mortality of shoots.

Colchicine treatment of 0.75 and 1.00 per cent to apical buds of old plants affected growth of the buds initially which subsequently produced shoots with larger and thicker leaves. These shoots were treated as suspected polyploids and observed for their various characters (Table 2). The suspected polyploids of Darakhi-1 and Darakhi-2 had significantly more length and breadth of stomata and reduced number of chloroplasts / stomatal apparatus and number of stomata / microscopic field when recorded at 21 days after treatment. The initial gigantism though found to be reduced to some extent for these characters at 45 DAT, their values were still significantly higher than those of normal controls.

The polyploid and diploid cells present in a shoot tip compete with each other and diploid cells may often

out-compete the polyploid ones. Therefore to avoid diplontic selection the suspected polyploid branches were allowed to grow by removing buds underneath the treated portion and were maintained for subsequent observation and utilization. Stomatal size and frequency and pollen size have been used commonly for preliminary identification of ployploids in various crops. Ploidy level variations for these characters in some of the naturally occurring *Zizyphus* genotypes were also reported earlier [4]. Results of the present study also suggested that frequency of stomatal chloroplasts can serve as a reliable criterion for identification of ploidy level in a fruit crops like *ber* in addition to stomatal size and frequency. This technique has much more relevance in the present context where confirmation of suspected polyploids by cytological studies is cumbersome and gets delayed until flowering commences.

References

1. Pradeep T. and Jambhale N. D. 2002. Cytomorphological studies in relation to powdery mildew resistance in *ber* (*Zizyphus mauritiana* Lamk.). Indian J. Genet., **62**: 46-51.
2. Pradeep T. 1997. Cytomorphological and biochemical studies in relation to powdery mildew resistance in *ber* (*Zizyphus mauritiana* Lamk.) Ph.D. thesis, Mahatma Phule Krishi Vidyapeeth, Rahuri.
3. Jambhale N. D. and Nerkar Y. S. 1980. A technique for permanent chloroplast preparations. Curr. Sci., **49**: 150.
4. Pradeep T. and Jambhale N. D. 2000. Ploidy level variations for stomata, chloroplast number, pollen size and sterility in *ber* (*Zizyphus mauritiana* Lamk.). Indian J. Genet., **60**: 519-525.