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Studies on tetraploidization in watermelon, *Citrullus lanatus* Thunb

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ABSTRACT

Seedless watermelon cultivars are in high demand by consumers not only because their fruits are seedless but also because they are sweeter than the fruits from diploid and seeded cultivars. Diploid watermelon varieties were treated with the chemical mutagen colchicine to develop tetraploid seed parents. Seedlings of different genetic backgrounds (Arka Manik, Arka Muthu, Sugar Baby and IIHR-14) were raised and injected with different treatment combinations of colchicine (0.2, 0.3 and 0.4%) with and without 0.5 per cent PEG. The varieties responded differently for different treatment combinations of colchicine. The treated seedlings were evaluated for the tetraploidy induction on the basis of chloroplast count. The results revealed that the variety Arka Manik was sensitive to the colchicine treatments and among the four varieties maximum number of tetraploids were identified in the variety Arka Muthu at 0.2 per cent colchicine whereas 0.3 per cent colchicine + PEG was found to be the best for variety Arka Manik and 0.4 per cent colchicine + PEG and 0.4 per cent colchicine treatments were found to be best for the Sugar Baby and IIHR-14 respectively. The efficiency rate of tetraploid induction levels in the present experiment varied with the varieties selected even though they belong to same crop.

Keywords: Watermelon; colchicine; tetraploid; polyploids; ploidy level

INTRODUCTION

Watermelon, *Citrullus lanatus* Thunb is a major cucurbit and an important crop in India. It is cultivated in an area of 76000 ha with an annual production of 1.70 MT in our country (Anon 2012). As a popular summer fruit and dessert watermelon had been investigated continually to improve its eating quality. Its fruits contain plenty of seeds impairing the flesh quality. The presence of numerous seeds in the flesh disturbs the palate and charm of enjoying its sweet taste and granular texture. Seedless watermelon provides an opportunity to satisfy our sense

of taste without fear of swallowing the seeds.

Due to excessive seed number in watermelon the fruit is becoming unacceptable in international markets. Seedless watermelon cultivars are in high demand by consumers not only because their fruits are seedless but also because they are sweeter than the fruits from diploid and seeded cultivars (Marr and Gast 1991). The use of interploid hybridization between tetraploid and diploid plants has been the most effective method to obtain triploid seedless progeny.

Seedless watermelons have special virtues such as extra hard rind and firmer flesh which contribute to less damage in shipment and longer shelf-life for distant markets along with deep colour and highly stable soluble solids content (Kihara and Nishiyama 1951). Traditionally tetraploid parents have been obtained by treating the newly emerged diploid seedlings with colchicine (Suying et al 1995) and they were identified by counting the number of chloroplasts per guard cell pair of fully expanded leaves (McCuistion and Elmstrom 2002). However lack of suitable tetraploid breeding parents has hampered progress towards the development of wide range of quality seedless cultivars (Andrus et al 1971). Hence the present research was undertaken to standardize the colchicine concentration to induce of tetraploidy in four genetic backgrounds (Arka Manik, Arka Muthu, Sugar Baby and IIHR-14).

MATERIAL and METHODS

The present investigation was carried out during Rabi and summer seasons of 2012-2013 at the experimental plots of the Division of Vegetable Crops, Indian Institute of Horticultural Research (IIHR), Bangalore. Soils of the experimental area were red sandy loam with a pH ranging from 5.2 to 6.4. Four varieties of watermelon (Arka Manik, Arka Muthu, Sugar Baby and IIHR-14) possessing diverse agronomic and quality characters were selected for the study. The experiment was laid out in a factorial RBD with 32 treatment combinations replicated thrice with 100 plants per replication. The seedlings were treated with different concentrations of colchicine at cotyledonary stage before the true leaf appeared. 20 µl of each treatment was applied to the growing tips of the seedlings for six consecutive days in the evening hours. Putative tetraploids were identified based on the chloroplast count in the guard cells and observing stomatal density per microscopic area as suggested by Gursoz et al (1991). Three concentrations of 0.2, 0.3 and 0.4 per cent of colchicine were tested for their efficacy in induction of tetraploidy. Poly ethylene glycol (PEG) is used as an adjuvant and expected to increase the permeability of colchicine into the cell wall. Hence the PEG

at a concentration of 0.5 per cent was also used along with concentrations of colchicine. The details of colchicine treatments and their preparations are mentioned in Table 1.

RESULTS and DISCUSSION

Four varieties of watermelon were subjected to different treatments of colchicine as described in the material and methods. The ploidy in watermelon was assessed by counting the number of chloroplasts per guard cell and observing stomatal density per microscopic area. Jaskani et al (2005) reported that the number of chloroplasts in each of guard cell pair ranged from 5 to 7 in diploids and 10-14 in tetraploids. In certain cases the number of chloroplasts varied from 8 to 9 in each guard cell pair which might be due to mixoploidy. Based on this among the four varieties highest grand mean of tetraploids (14.16%) was identified in the variety Arka Muthu (Table 2) followed by Arka Manik (10.90%), Sugar Baby (10.62%) and IIHR-14 (9.76%). Rhodes and Zhang (2000) reported that chloroplast count could be a useful tool to determine ploidy as variability in the number of chloroplasts within the guard cells is genetically related to the variability in the meristamatic cells.

The percentage of induction of tetraploids in different varieties was affected significantly at various concentrations of colchicine (with and without PEG). Maximum percentage of tetraploids (17.73%) in the variety Arka Manik and 15.45 per cent in Sugar Baby was obtained when treated with 0.3 per cent colchicine solution whereas the induction percentage was 21.71 in the variety Arka Muthu at 0.2 per cent colchicine and for IIHR-14 it was 14.30 at 0.4 per cent colchicine. Similarly Suying et al (1995) also recorded higher number of tetraploids at 0.2 per cent of colchicines. These results are similar to that of Jaskani et al (2005).

Table 1. Colchicine treatments and their preparations

Concentration	Description
0.2% colchicine	0.2 g of colchicine in 100 ml of distilled water
0.2% colchicine + 0.5% PEG	0.2 g of colchicine + 0.5 g of PEG in 100 ml of distilled water
0.3% colchicine	0.3 g of colchicine in 100 ml of distilled water
0.3% colchicine + 0.5% PEG	0.3 g of colchicine + 0.5 g of PEG in 100 ml of distilled water
0.4% colchicine	0.4 g of colchicine in 100 ml of distilled water
0.4% colchicine + 0.5% PEG	0.4 g of colchicine + 0.5g of PEG in 100 ml of distilled water
Distilled water	100 ml of distilled water
0.5% PEG	0.5 g of PEG in 100 ml of distilled water

Table 2. Effect of colchicine concentrations on induction of tetraploidization among four varieties of watermelon

Treatment	Variety (V)											
	Arka Manik (V_1)			Arka Muthu (V_2)			Sugar Baby (V_3)			IIHR-14 (V_4)		
	P_0	P_1 (0.5%)	Mean	P_0	P_1 (0.5%)	Mean	P_0	P_1 (0.5%)	Mean	P_0	P_1 (0.5%)	Mean
C_0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(0.28)	(0.28)	(0.28)	(0.28)	(0.28)	(0.28)	(0.28)	(0.28)	(0.28)	(0.28)	(0.28)	(0.28)
C_1 (0.2%)	11.70	12.46	12.08	38.60	4.82	21.71	10.60	14.71	12.65	14.41	6.60	10.50
	(19.9)	(20.6)	(20.25)	(38.3)	(12.3)	(25.3)	(18.9)	(22.5)	(20.7)	(22.2)	(14.7)	(18.45)
C_2 (0.3%)	17.40	18.06	17.73	14.91	25.80	20.35	16.27	14.72	15.45	15.10	13.22	14.15
	(24.6)	(25.1)	(24.85)	(22.7)	(30.5)	(26.6)	(23.7)	(22.5)	(23.1)	(22.9)	(21.2)	(22.05)
C_3 (0.4%)	14.66	13.46	14.06	9.80	19.43	14.60	5.41	23.32	14.35	17.60	11.02	14.3
	(22.5)	(21.5)	(22.0)	(18.2)	(26.1)	(22.15)	(13.4)	(28.8)	(21.1)	(24.7)	(19.3)	(22,0)
Mean	10.94 (16.82)	10.99 (16.87)	10.90	15.82 (19.87)	12.5 (17.29)	14.16	8.05 (14.07)	13.17 (18.52)	10.62	11.77 (17.52)	7.7 (13.87)	9.76

Figures in parenthesis indicate arc sin transformed values

	SEm±	$CD_{0.05}$
Variety (V)	0.074	0.223
Colchicine (C)	0.074	0.223
PEG (P)	0.052	0.158
V x C	0.148	0.446
C x P	0.105	0.315
V x P	0.105	0.315
V x C x P	0.21	0.630

Table 3. Variation in stomatal density in polyploids of watermelon

Ploidy	Arka Manik	Arka Muthu	Sugar Baby	IIHR-14
Tetraploid	43.06	42.30	41.13	44.41
Mixoplid	44.21	44.53	45.51	47.90
Diploid (control)	62.21	63.30	64.32	62.10
$CD_{0.01}$	3.9	4.6	3.8	2.7
SEm±	1.3	1.5	1.2	0.9
CV (%)	11.02	13.3	10.7	10.99

Significantly higher value of tetraploids (38.60%) was recorded in the variety Arka Muthu when treated at 0.2 per cent colchicines without PEG, 18.06 per cent in Arka Manik at 0.3 per cent colchicine + PEG, 23.32 per cent in Sugar Baby at 0.4 per cent colchicine + PEG and 17.60 per cent in IIHR-14 (Table 2) when treated with 0.4 per cent colchicine. The efficiency rate of tetraploid induction levels in the present experiment varied with the varieties selected.

These identified polyploids were subjected for stomatal count per microscopic area. It was observed that the number of stomata per microscopic area was significantly fewer in tetraploids than in diploids in all the varieties studied. Among the tetraploids the maximum number of stomata per microscopic area was observed in the variety IIHR-14 (44.41) followed by Arka Manik (43.06), Arka Muthu (42.30) and Sugar Baby (41.13). The stomatal density was found to be high in diploid plants (control) compared to mixoploid and tetraploids. The diploids of IIHR-14, Arka Manik, Arka Muthu and Sugar Baby were 62.10, 62.21, 63.30 and 64.32 respectively (Table 3). Besides this it was generally observed that the stomata were bigger in tetraploids when compared to the diploids. Nigel et al (2007) reported that increasing ploidy often resulted in increased cell size and reduced frequency of stomata per unit leaf area in tetraploids when compared to the diploids.

CONCLUSION

Based on the findings of the present investigation it can be concluded that variety Arka Manik was sensitive to the colchicine treatments and among the four varieties maximum number of tetraploids were identified in the variety Arka Muthu at 0.2 per cent colchicine whereas 0.3 per cent colchicine + PEG was best for variety Arka Manik and 0.4 per cent colchicine + PEG, 0.4 per cent colchicine treatments were found to be best for the Sugar Baby and IIHR-14 respectively.

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