

# Characterization of M1 Generation Of Polyploids in Watermelon Variety 'Sugar Baby'

**T. Pradeepkumar**

Associate Professor, Department Of Olericulture, College Of Horticulture, Vellanikkara, P.O. Kau, TRICHUR, KERALA, INDIA 680656 pradeepkau@gmail.com

Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai] exhibit great variation in seed morphology, particularly in seed size and shape, and in seed coat tissue texture, and color. However, in recent years, seedless watermelon are more acceptable to consumers, and seed companies make great efforts in breeding for high quality seedless watermelon varieties. Kihara (1951) produced triploid seedless hybrid watermelon by crossing tetraploid (4n) and diploid (2n) plants. A large number of triploid varieties have been developed in Asia and North America. However, in contrast with the USA, seedless watermelons have not been popular in India. There is a need to develop tetraploid breeding lines suitable for consumer needs in India. Breeding stable tetraploid line with adequate fertility is a major challenge in triploid watermelon production (Mohr, 1986).

Compton *et al.* (1996) developed an easy technique for the early identification of putative tetraploid watermelon plants. In tetraploid plants, the guard cells of the stomata contain a high number of chloroplasts compared with these in diploid plants. Here, a procedure has been optimized to induce polyploidy in the commercial watermelon cultivar Sugarbaby, and the resultant M1 generation plants were morphologically characterized.

## Materials and Methods

Polyploidy was induced in the seeds and seedlings of the watermelon variety Sugarbaby using colchicine solution (0.1 % and 0.5%). The seeds of Sugarbaby were soaked in clean water for 6 hrs and then soaked for 24 hrs in a solution containing 0.1, 0.5, 1, 1.5, or 2.0% colchicine. The seeds were briefly rinsed before sowing in polybags filled with sand. For seedling treatment seeds were sown in polyhouse. Once seedlings emerged, a drop of colchicine (0.1 or 0.5%) was applied to the shoot apex between the cotyledons. The chemical solution was applied to the growing point during morning hours for three consecutive days. The plants derived from seed and seedling treatments were examined with respect size of stomata guard cells and number of chloroplasts in each guard cell.

## Results and Discussion

Seeds failed to germinate when exposed for 24 hours to a concentration of 1, 1.5 and 2 % colchicine. Variable response to high concentration of colchicine was reported by Jaskani (2005). Giant guard cell was observed in plants generated through 0.1% colchicine seed treatment and 0.5% colchicine seedling treatment (Table 1). Polyploid plants had a higher chloroplast number, ranging from 17-22, while the diploid (control) plants had about 12 chloroplasts in the stomata guard cells. Chloroplast counting is an efficient indicator of polyploidy during early phase of plant growth (Jaskani *et al.*, 2005) and a higher chloroplast number is an indicator for higher ploidy. In general, polyploids took more days to form male and female flowers (Table 2). Slower growth rate and delayed appearance of shoots and flowers were observed in colchicine treated seedlings. The same trend was also reflected in the days taken to harvest.

Plants generated following treatment of seedlings with 0.5 % colchicine produced large pollen grains (40.35  $\mu$ ). Consequently, the polyploidy plants produced smaller fruits with a small number of seeds (Table 3). The number of seeds produced in plants treated with 0.5 % colchicine (15-46 seeds) is significantly lower than that produced in the diploid control plants (430-530 seeds). In general, plants regenerated from seeds treated with 0.1 % colchicine or from seedlings treated with 0.5 % colchicine exhibited polyploidy characters. On the other hand, seedling treated with 0.1 % colchicine produced chimeral growth and normal fruits with high seed numbers. Variability in colchicine tolerance by watermelon lines was observed in a previous study (Jaskani *et al.*, 2004). The variable response of genotypes to colchicine indicates that optimal colchicine concentrations may vary in treating watermelon genotypes.

Although tetraploid watermelon plants are morphologically distinct, they may not be stable and produce mixoploid vines on the same plant. Rose *et al.* (2000) reported that mixoploid plants in which more than 40% of the cells were tetraploid could be mistaken for full tetraploids but that mixoploids with 10-30% tetraploid

cells usually resembled diploid plants. However, chloroplast counts and other morphological features could be helpful in successive generations to develop pure line. The results of this study underscore the need for continual observation for distinguishing polyploids from diploid plants and presence of mixoploids calls for a cautious approach in breeding polyploid lines. Overall, ploidy level can be determined by examining epidermal tissue from the lower leaf surface, without the requirements of specific equipment and high expenditure.

## Literature Cited

- Compton, M.E, D.J. Gray, and G.M. Elmstrom. 1996. Identification of tetraploid regenerants from cotyledons of diploid watermelon cultures *in vitro*. *Euphytica* 87:165-172.
- Jaskani, M.J., W.K. Sung, and H.K. Dae. 2005. Flow cytometry of DNA contents of colchicines treated watermelon as a ploidy screening method at m1 stage. *Pakistan Journal of Botany* 37: 685-696.
- Jaskani, M.J., S.W. Kwon, G.C. Koh, Y.B. Huh and B.R. Ko. 2004. Induction and characterization of tetraploid watermelon. *Journal of Korean Society of Horticultural Science* 45: 60-65.
- Kihara, H. 1951. Triploid Watermelons. *Proceedings of American Society of Horticulture Science* 58 : 217-230.
- Mohr, H.C. 1986. *Breeding Vegetable Crops* AVI Publishing Company, Westport, 584<sub>pp</sub>
- Rose, J.B., J. Kubba and K.R. Tobutt. 2000. Chromosome doubling in sterile *Syringa vulgaris* x *S.pinnatifolia* hybrids by *in vitro* culture of nodal explants. *Plant Cell Tissue and Organ Culture* 63: 127-132.

Sl no	Treatment	Leaf Guard cell			
		Length (Micron)	Width (Micron)	No.of chloroplast/cell	
1	0.1% seed	1	29.55±1.2	21.21±1.7	19±2.0
		2	28.82±1.5	22.99±1.9	19±1.2
		3	26.99±1.3	22.79±1.5	18±1.1
		4	29.49±1.7	22.58±1.3	19±1.2
2	0.1% Seedling	1	29.85±1.3	21.17±1.3	18±0.5
		2	30.55±1.2	22.70±1.1	17±0.3
		3	27.06±1.2	20.47±1.2	17±1.0
		4	25.19±1.1	21.50±1.2	17±1.2
3	0.5% Seedling	1	28.76±1.2	21.48±1.3	22±0.7
		2	26.59±1.2	21.67±1.2	22±1.0
		3	29.05±1.2	21.61±1.3	22±0.7
		4	29.44±1.2	23.96±1.3	22±0.6
4	Control	1	20.52±1.4	15.86±1.0	12±0.3

**Table 1 : Leaf characters of plants produced from seeds or seedlings treated with colchicine versus untreated control plants. (values are mean±SD , n=12)**

Sl no	Treatment	Size of pollen grain (Micron)	Days taken to open first male flower	Days taken to open first female flower	Days taken to harvest	
1	0.1 seed	1	35.0±0.2	57	69	137
		2	34.54±0.3	57	69	137
		3	35.09±0.4	52	69	131
		4	33.07±0.2	52	69	131
2	0.1 seedling	1	32.69±0.2	56	60	145
		2	31.26±0.1	50	62	153
		3	32.20±0.1	58	63	140
		4	32.00±0.2	63	65	142
3	0.5 seedling	1	37.46±1.0	57	63	116
		2	33.05±1.1	60	68	153
		3	38.04±1.0	60	68	153
		4	40.35±1.1	61	61	166
4	Control	1	31.74±1.0	29	32	82

**Table 2 : Biometric characters of plants produced from seeds or seedlings treated with colchicine versus untreated control plants. (values are mean±SD , n=12)**

Sl no	Treatment	Fruit						
		Rind thickness (cm)	Wt (Kg)	Perimeter (cm)	TSS	Seed no	Avg.wt of 10 seeds	
1	0.1% seed	1	2	1.51	45	7	32	0.2
		2	1.5	1.21	34	7	51	0.2
		3	1.5	2.64	54	8.3	506	0.3
		4	1.5	1.50	40	8	15	0.2
2	0.1% seedling	1	1.5	4.73	65	9.3	596	0.3
		2	1.5	1.50	45	7	323	0.3
		3	1.5	1.81	49	7.8	521	0.3
		4	1.5	2.51	55	7.8	340	0.3
3	0.5 % Seedling	1	1	0.50	30	7.5	34	0.25
		2	1.4	0.96	40	8	18	0.3
		3	1	0.52	33	8	15	0.3
		4	1	1.10	38	10.3	46	0.4
4	Control	1	1.5	3.20	55	7	530	0.3

**Table 3: Fruit characters of plants produced from seeds or seedlings treated with colchicine versus untreated control plants.**