MORPHOLOGICAL STUDIES ON TETRAPLOID AND DIPLOID WATERMELON (CITRULLUS LANATUS THUNB)

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Abstract: Polyploidy plays an important role in plant morphology and is an important tool to create genetic and morphological variations. Tetraploid breeding lines have been created for use in producing triploid hybrids. The lack of suitable tetraploid breeding parents has, however, hampered progress towards the development of a wide range of quality seedless cultivars. The idea of this investigation was to differentiate the tetraploid and diploid watermelon morphologically. Three diploid varieties (Arka Muthu, Sugar Baby, IIHR-14) were treated with different concentrations of colchicine (0.2%, 0.3% and 0.4%). Morphological variations were observed between the induced tetraploids and diploids. In the present study, morphological parameters observed include number of seeds per fruit, leaf area, seed parameters, days to first male flower appearance and days to first female flower appearance. Results revealed that number of seeds per fruit, leaf area, seed length and seed width were found to be maximum in tetraploids than in diploids and number of days to first female and male flower appearance were more in tetraploids than in diploids.

Keywords: colchicines, morphology, polyploids, tetraploid and Watermelon.

Introduction: Excessive seed number in watermelon (*Citrullus lanatus* Thunb.) fruit is becoming unacceptable in international markets. Seedless watermelon cultivars are in high demand made by consumers not only because their fruits are seedless but also because they are sweeter than the fruits from diploid and seeded cultivars (7). Seedless watermelon are triploid (2n=3x=33) hybrids. The use of interploid hybridization between tetraploid (female) and diploid (male) plants has been the most effective method to obtain triploid progeny (2). Traditionally, tetraploid parents have been obtained by treating newly emerged diploid seedlings with colchicine (11). However, this treatment produces only a limited number of tetraploids and they are identified based on the cytological characters by counting the number of chloroplasts per guard cell of fully expanded leaves (8). Tetraploid plants generally produce leaves that are broader than diploids. Seed size and shape may also be used to identify polyploids (5). Hence the present investigation was conducted to differentiate the tetraploids and diploids morphologically.

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, Karnataka, India. Geographically, the experimental site was located at 130 N latitude and 170 37'E longitude at an altitude of 890 m above mean sea level. Soils are red loam with a pH ranging from 5.2 to 6.4. The present research project was planned to study the characterization of tetraploid and morphological dissimilarity between tetraploid and diploid Watermelon (*Citrullus lanatus* Thunb.). The seedlings were treated with different treatments 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. The treated seedlings were transplanted to the main field at 2-true leaf stage after one month of sowing. Transplanting was done onto raised and mulched beds with drip irrigation at spacing of 60 cm between plants and 2 m between rows. Standard cultural practices and plant protection measures were followed during the entire crop period. Putative tetraploids were identified based on and chloroplast count in the guard cells and observing stomatal density per microscopic area. Thus identified putative tetraploids were self pollinated to obtain M1 seeds.

Number of seeds per fruit: Self pollinated fruits from putative tetraploid plants were harvested at mature stage. The number of seeds present in each fruit were counted and expressed as average number of seeds per fruit for tetraploids in each variety.

Seed length (mm): Length of ten randomly selected seeds per tetraploid line were recorded using digital vernier calliper and expressed as average seed length.

Seed width (mm): Width of ten randomly selected seeds per tetraploid line were recorded using digital vernier calliper and expressed as average seed width.

Leaf area (mm2): The image of the fully developed leaf at the seventh node was captured using digital camera for five randomly selected plants per tetraploid line. Later, the leaf area was calculated using Image J software (http://rsb.info.nih.gov/ijl download.html).

Number of days to 1st male flower opening: The number of days taken from sowing to the onset of first male flower was recorded for five randomly selected plants per tetraploid line.

Number of days to 1st female flower opening: The number of days taken from sowing to the onset of first female flower was recorded for five randomly

selected plants per tetraploid line.

Results and Discussion:

Number of seeds per fruit: Seed yield of tetraploid lines in the early generation was often only 50-100 seeds per fruit compared to 200-800 in diploids (4). Even in the current investigation, there was a significant difference between tetraploids and diploids in all the three varieties and the seed yield in tetraploids was less than the diploids (Table 1) Among the tetraploids of four varieties, maximum number of seeds were recorded in the variety Sugar Baby (131.54), followed by Arka Muthu (94.31) and IIHR-14 (51.00). Physiological factors were also responsible for the meager fruit set and seed setting in tetraploids (10). He observed that as a result of polyploidy, the number of ovules per ovary was considerably reduced in some plants and this was correlated with low seed set.

Seed length: Significant difference was observed between the tetraploid and diploid seed length in all the varieties studied (Table 1). Tetraploids had a coarse appearance with increased seed length. Among the tetraploids of three varieties, maximum seed length was observed in the variety Arka Muthu (11.91 mm), followed by IIHR-14 (9.10 mm) and Sugar Baby (7.57 mm) and diploid seed length of variety Arka Muthu (6.21 mm), IIHR-14 (6.20 mm) and Sugar Baby (5.64 mm). Hence, it was concluded that there is a consistent significant difference between the diploids and tetraploids with respect to seed length among three varieties. Hence it can be used as a diagnostic trait for tetraploidy. Further, the seeds of diploids were oblong whereas the tetraploids were varied from oblong to round. Diploid seeds were completely filled but tetraploids showed cavity at the chalazal end of the seed (5).

Seed width: Significant difference was observed between the tetraploid and diploid seed width in all the varieties studied (Table 1). Tetraploid seeds were wider compared to diploids, and among the tetraploids of three varieties, Arka Muthu seeds had maximum seed width (6.81 mm) followed by IIHR-14 (5.61 mm) and Sugar Baby (4.58 mm). Bigger seed size was related to higher ploidy level in watermelon (4).

Leaf Area: The leaf area of diploid and tetraploid plants of the three varieties are compared in Table 2. Maximum leaf area was noticed in the variety IIHR-14 (17800.3 mm^2), followed by Sugar Baby (15547.8 mm^2) and Arka Muthu (14898.2 mm^2). Significant differences were observed between tetraploids and diploids in all the three varieties. Leaf area was high in tetraploids compared to diploids. There was a significant difference between the leaf area of tetraploid and diploid lines. The tetraploids are vigorous in growth with greater biomass and leaf area (9).

Days to first male flower appearance: Variation in days to first male flower appearance was found among the three varieties in Table 2. Arka Muthu has taken 42.80 days for the opening of the first male flower, whereas Sugar Baby and IIHR-14 the flowers appeared 35.77 and 35.50 days after sowing respectively. Significant differences were observed between tetraploids and diploids in all the three varieties. Slow growth in tetraploids leads to delay in male flower appearance.

Days to first female flower appearance: Days to first female flower appearance was a good measure of the earliness of the variety in watermelon. The tetraploids in Cv. Arka Muthu has taken 48.12 days for the appearance of the first female flower where as Sugar Baby and IIHR-14 female flower appeared in 43.70 and 41.51 days after sowing. Significant difference was observed between tetraploids and diploids (1) in all the three varieties in Table 2.

Conclusion: A conclusion section is not required. Hence, it was concluded that there is a consistent significant difference between the diploids and tetraploids with respect to number of seeds per fruit, seed length, seed width, leaf area, days to first male and female flower appearance among three varieties. Hence these characters can be used as a diagnostic trait for tetraploidy. The investigation conclusively proved the morphological dissimilarities present between the tetraploids and diploids which can be used in the identification of those polyploidy plants at the early stage of the plant growth.

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Table 1. Variation in number of seeds per fruit seed length and seed width among tetraploid, mixoploid and diploid lines watermelon varieties

Variety / Ploidy	Number of seeds per fruit			Seed length (mm)			Seed width (mm)		
	Arka Muthu	Sugar Baby	IIHR-14	Arka Muthu	Sugar Baby	IIHR-14	Arka Muthu	Sugar Baby	IIHR-14
Tetraploid	94.31	131.54	51.00	11.91	7.57	9.10	6.81	4.58	5.61
Mixoploid	65.80	85.3 3	66.00	11.60	7.12	9.10	6.48	4.56	5.23
Diploid	315 .00	261.60	156.62	6.21	5.64	6.20	2.52	2.90	2.21
S.E(m)±	24.9	33.7	18.2	0.5	0.05	0.05	0.33	0.07	0.05
CD (P=0.05)	74.7	101.2	54.7	1.5	0.17	0.15	1.00	0.23	0.16
CV (%)	37.07	33.40	34.80	9.8	2.9	2.1	12.3	6.8	4.3

Table 2. Variation in leaf area, days to first male and female flower appearance among tetraploid, mixoploid and diploid lines watermelon varieties

Variety / Ploidy	Leaf area (mm^2)			Days to first male flower appearance			Days to first female flower appearance		
	Arka Muthu	Sugar Baby	IIHR-14	Arka Muthu	Sugar Baby	IIHR-14	Arka Muthu	Sugar Baby	IIHR-14
Tetraploid	14898.2	15547.8	17800.3	42.80	35.77	35.50	48.12	43.70	41.51
Mixoploid	12456.8	14558.2	18252.5	42.66	36.81	35.01	46.76	41.52	41.25
Diploid	10222.8	10921.6	11.226	35.53	33.00	30.06	35.55	33.02	32.00
S.E(m)±	852.2	1036.3	1786.5	0.46	0.76	0.86	1.16	0.76	0.86
CD (P=0.05)	2556.7	3109.0	5359.5	1.4	2.3	2.6	3.5	2.3	2.6
CV (%)	14.48	10.24	14.7	1.7	3.1	3.4	3.8	2.4	3.4