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Screening of tetraploidy induction methods using anti-microtubule agent colchicine in watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai)

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Abstract

Three distinct watermelon genotypes (Sugar Baby, California Sweet and 5255-1-3-1) were subjected to anti-microtubule agent, colchicine with three different doses (0.1%, 0.2% and 0.3%) to induce tetraploidy using three methods viz. shoot apex, seed soaking and inverted hypocotyl. The experiment was carried out at Vegetable Research Farm and Laboratory, Department of Vegetable Science, Punjab Agricultural University, Ludhiana during the period 2013-2015. The observations were recorded on chloroplast count, palynological and phenotypic traits to confirm the tetraploidy. California Sweet showed the highest rate of efficiency of generating putative tetraploids (6.12%). Amongst the various methods and concentrations of colchicine for induction of tetraploids, 0.2% and 0.3% concentrations of colchicine by inverted hypocotyl and shoot apex method was comparatively more effective. The highest frequency of putative tetraploids (11.55%) was recorded with inverted hypocotyl method @ 0.3% colchicine.

Keywords: Watermelon, Tetraploid, Induction, Colchicine, Chloroplast count

Introduction

The genus *Citrullus* of the family *Cucurbitaceae* encompasses four diploid species that thrive in Africa, Asia and the Mediterranean (Levi et al. 2001). Among these, *Citrullus lanatus* (Thunb.) Matsum &Nakai is commercially exploited. Presently, seedless watermelons are becoming more preferred because of sweetness and absence of hard seeds (Marr and Gast 1991). Kihara (1951) pioneered the production of triploid seedless hybrid watermelon by crossing a tetraploid (4n) and a diploid (2n) line. To produce the triploid watermelons, development of stable tetraploid breeding line(s) with adequate fertility is a pre requisite (Mohr 1986). The tetraploidy can be induced variously by applying aqueous colchicine solution to the growing apex of diploid seedlings or by soaking diploid seeds in colchicine solution prior to germination (Jaskani et al. 2007, Gaikwad et al 2007, Pradeepkumar 2010-2011) or at hypocotyl portion of diploid germinating seeds (Noh et al.2012, Sheikh et al 2013).The techniques generally used in determining the ploidy level are chromosome count, chloroplast count and phenotypic traits identification. Chromosome counting is difficult in watermelon due to its small chromosome size. The alternative method of counting the number of chloroplasts per guard cell pair of fully expanded leaves using a leaf peel under the microscope (Fassuliotis and Nelson 1992) has been successfully reported in watermelon (McCuistion and Elmstrom 1993). The plant morphological traits such as leaf and flower size, size of the pollen grains and the number of colpi (4 versus 3) are also good indicators for the characterization of ploidy (Rhodes and Zhang 1999). Colchicine treatments induce only 4-6% pure tetraploids in watermelon (Jaskani et al.2004). The major limiting factors in the induction of tetraploids are selection of suitable diploid varieties and induction method with high frequency percentage. Keeping in view, the present study was planned to evaluate the different methods and concentrations of colchicine treatments for tetraploid plants induction in diverse genotypes.

Materials and Methods

The distinct watermelon genotypes *viz.* Sugar Baby (SB), California Sweet (CS) and one advanced breeding line 5255-1-3-1 (5255) were selected to induce tetraploid lines with the different concentrations of colchicine (0.1%, 0.2% and 0.3%) with shoot apex (SA), seed soaking (SS) and inverted hypocotyl (IH) methods. For the shoot apex method (SA 0.1%, SA 0.2% and SA 0.3%), the seeds of each genotype were planted in the polythene bags having mixture of soil and farmyard

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manure in 1:1. One drop of each colchicine solution was applied to the shoot apex of seedlings at the true leaf stage for three consecutive days, in the morning and evening hours. In the seed soaking method (SS 0.1%, SS 0.2% and SS 0.3%), firstly seeds were soaked in water for 24 hours to soften the seed coat. After that, the seeds were soaked in the respective colchicine solution and kept in the dark for 24 hours at room temperature. The treated seeds were rinsed gently in clean water twice and then sown in the polythene bags with similar ratio of soil and farmyard manure. In the Inverted hypocotyl method (IH 0.1%, IH 0.2% and IH 0.3%), seeds of each genotype were kept in petri dishes for germination in incubator at 28^0 C. Germinated seeds were then placed at inverted angle position when radicle became 2 cm long in glass tubes. All the respective colchicine solution was applied to the hypocotyl portion. Colchicine treated seeds were covered tightly with thin plastic film and kept in dark for 15 hours inside an incubator at 28^0 C (Noh et al. 2012). After treatments, the seeds were rinsed gently in clean water 2 times and planted in polythene bags having soil and farmyard manure. This experiment was carried out in randomized complete block design with three replications during 2013-2015 at Department of Vegetable Science, PAU, Ludhiana.

The observations were recorded for the chloroplast count, palyonological (pollen colpi and pollen viability), vegetative (leaf length, leaf width, internode length), flowering (number of days to first female flower), fruiting (number of days to first picking, total soluble solids, fruit weight, rind thickness) and seed (seed number per fruit, developed seeds per fruit, undeveloped seeds per fruit, seed length, seed width, seed thickness, 100 seed weight) traits. Chloroplast count in each side of guard cells of stomata was calculated at 3-5 true leaves stage. The lower epidermis was removed by piercing the leaf with hand and placed on the glass slide after putting a one drop of distilled water and stained with (1%) of Gram's Iodine solution (Fassuliotis and Nelson 1992). The number of chloroplasts was scored and photographed under the light microscope, Leica (LEC Image Analyser) at 40x10 magnifications. Ten microscopic fields of guard cell pairs were examined per leaf from each plant. The chloroplasts per guard cell pairs thus identified, were grouped as diploid (d" 8), tetraploid (11-12) and higher ploidy level (> 12) (McCuistion and Elmstrom 1993). Among the palyonological observations, pollen colpi was calculated in the diploid and tetraploid plants from ten freshly opened staminate flowers, placed on a drop of water on a glass slide. Pollen grains were observed and photographed under the microscope Leica (LEC Image Analyser) at 40x10 magnifications. Several random microscopic fields all over the slide were counted and average worked out. The pollen viability (%) was recorded by dusting pollen on a slide and stained with acetocarmine (1%) under the light microscope, Leica (LEC Image Analyser) magnified by 10 x 10. The averages of ten freshly opened staminate flowers per replication were taken in the morning time. Those pollen grains which were not stained and appeared to have no content were regarded as being abortive. For determining the pollen viability, the number of stained and well filled pollen grains and unstained shriveled pollen grains per unit area were counted in five microscopic fields taken at random and from the average, pollen viability was calculated. Vegetative traits such as leaf length (cm) and leaf width (cm) was recorded from the longitudinal distance from leaf base to leaf apex and the horizontal distance at the widest ends of the leaf by taking three leaves from 7th, 9th and 11th node of five plants per replication using hand held measuring scale, respectively. The first three internodes were taken for the measurement of internode length (cm) from the main vine of five plants per replication using hand held measuring scale. In addition to this, the number of days taken from date of transplanting to date of appearance of first female flower was counted on five plants per replication. Among the different fruiting traits, the number of days taken by the fruit to mature from the date of transplanting was recorded from five plants per replication to determine the days to first picking. The TSS content (^{0}brix) of fruits was determined by putting a drop of juice from five fruits per replication on the hand held refractometer and the freshly harvested fruits were weighed on an electronic balance to measure the fruit weight (kg). The rind thickness (cm) was measured at the middle portion of the fruit after making longitudinal section into two equal halves using Verniers' Calliper. The average rind thickness was calculated from five fruits per replication. After that, seeds were extracted manually and counted with seed counter. The average seed number per fruit, developed seeds per fruit (%) and undeveloped seeds per fruit (%) was taken from five fruits per replication. Seed length (mm) and seed width (mm) was calculated from the longitudinal distance and horizontal distance at widest ends of ten well filled and fully mature seeds taken at random from five fruits per replication, using digital Vernier's Caliper (Mitutoyo Inc., Japan), respectively. Also, the seed thickness (mm) was measured at mid portion of seeds using digital Verniers' Caliper (Mitutoyo Inc., Japan) on the same ten well filled and fully mature seeds selected randomly per replication. 100 seed weight (g) was recorded from 100 well developed seeds counted with

seed counter from five fruits per replication by using an electronic balance and average was taken. Analysis of variance was conducted for various quantitative traits by using WINDOWSTAT 9.3 software and the percentage data were arc sine-transformed.

Results and Discussion

Chloroplast count and palyonological traits: The tetraploids were identified on the basis of number of chloroplasts per guard cell pair, they were grouped as diploid (d" 8.00), tetraploid (11.00-12.00) and higher ploidy level (> 12.00) as described in earlier reports (McCuistion and Elmstrom 1993, Jaskani et al.2005a). The data in Table 1 exhibited that California Sweet had the highest number of chloroplasts per guard cell pair (8.20) and at par with 5255-1-3-1 (8.10), which was significantly different from Sugar Baby. The variation in number of chloroplasts might be due to their genetic make-up. Compton et al. (1996) found genotypic variation with respect to chloroplast guard cells that Royal Sweet had the highest number of chloroplasts per guard cell pair (11.30) and at par with Mickylee (11.00) of watermelon. Among the different methods of colchicine application, treatments SA 0.3%, SA 0.2%, IH 0.3% and SS 0.2% revealed presented significant increase in number of chloroplasts per guard cell pair as compared to other treatments. The interaction between the genotypes and methods of colchicine application revealed that the highest number of chloroplasts (12.00) was significantly recorded in California Sweet with SA 0.2% and statistically at par with California Sweet IH 0.3% and SA 0.3%, Sugar Baby with SA 0.3% and IH 0.3% and 5255-1-3-1 with SA 0.3%, SA 0.2% and SS 0.2% treatments and were putative tetraploids. The rest of the treatments as well as control had significantly lower number of chloroplasts per guard cell pair. Similar results were corroborated by Noh et al.(2012), who treated the five diploid inbred lines of watermelon by applying two concentrations of colchicine with three different methods of application and reported that mean chloroplast count was (18.00) or (19.00) in tetraploids and (12.00) in diploids. The increase in chloroplast count might be due to the fact that cells with a larger complement of chromosomes grow larger to maintain a constant ratio of cytoplasmic to nuclear volume. This increase in size may translate to an increase in plant and its organs (Amiri et al. 2010).

The palyonological observations (pollen colpi and pollen viability) were also recorded (Table 1) to distinguish the effect of different doses of colchicine on watermelon genotypes. The data pertaining to pollen colpi in showed that number of pollen colpi (4.00) were at par in putative tetraploids namely Sugar Baby with SA 0.3% and IH 0.3%, California Sweet with SA 0.2%, SA 0.3% and IH 0.3%, 5255-1-3-1 with SA 0.2%, SA 0.3% and SS 0.2% treatments. These results agreed with the work of Jaskani et al. (2005a) that tetraploids had (4.00) and diploids had (3.00) pollen colpi in watermelon. The increase in number of pollen colpi might be due to the increased cell size. However, pollen viability was the highest in Sugar Baby (75.37%) and highly significant over the other genotypes. Similarly, genotypic variations were studied by Gok et al.(2007) that the highest pollen viability rates (97.40% and 97.36%) in accessions 70 and 71, and the lowest rates (49.65% and 61.08%) were observed in accessions 25 and 24 in the 2,3,5-Triphenyl Tetrazolium Chloride (TTC) test in watermelon. In case of different methods of colchicine application, the

Table 1: Effect of different doses of colchicine on chloroplast count and palynological traits (i.e. pollen colpi and pollen viability) of watermelon genotypes

Treatments (T)		Chloroplast count				Pollen colpi				Pollen viability (%)			
		Genotypes (G)				Genotypes (G)				Genotypes (G)			
		SB	CS	5255	Mean	SB	CS	5255	Mean	SB	CS	5255	Mean
SA	0.1%	6.67	7.00	7.00	6.89	3.00	3.00	3.00	3.00	78.92	79.32	79.73	79.32
	0.2%	7.00	12.00	11.33	10.11	3.00	4.00	4.00	3.67	79.70	57.62	49.28	62.20
	0.3%	11.67	11.33	11.67	11.56	4.00	4.00	4.00	4.00	58.19	58.19	53.85	56.75
SS	0.1%	6.00	6.00	6.33	6.11	3.00	3.00	3.00	3.00	79.73	79.43	79.72	79.63
	0.2%	6.33	6.67	11.33	8.11	3.00	3.00	4.00	3.33	79.79	78.99	58.79	72.53
	0.3%	6.33	6.33	6.00	6.22	3.00	3.00	3.00	3.00	79.57	79.46	79.85	79.63
IH	0.1%	7.00	7.00	7.00	7.00	3.00	3.00	3.00	3.00	79.79	79.36	79.59	79.58
	0.2%	7.00	7.00	6.67	6.89	3.00	3.00	3.00	3.00	79.87	79.30	79.76	79.65
	0.3%	11.67	11.67	7.00	10.11	4.00	4.00	3.00	3.67	58.24	55.09	79.93	64.42
Diploid		7.00	7.00	6.67	6.89	3.00	3.00	3.00	3.00	79.87	79.76	79.95	79.86
Mean		7.67	8.20	8.10	7.99	3.20	3.30	3.30	3.27	75.37	72.65	72.04	73.36
C.D. 5%		G-0.42	T- 0.77	GxT- 1.33		G- 0.03	T- 0.06	GxT- 0.10		G- 0.54	T- 0.99	GxT- 1.71	

treatments SS 0.2% (72.53%), IH 0.3% (64.42%), SA 0.2% (62.20%) and SA 0.3% (56.75%) showed significant decrease in pollen viability as compared to other treatments and control. It is conspicuous from interaction that the lowest pollen viability was recorded in putative tetraploids of 5255-1-3-1 (49.28%) with SA 0.2%, followed by SA 0.3% while the highest pollen viability was recorded in diploids of 5255-1-3-1. Sheikh et al. (2013) also observed that pollen dust was noticeably more abundant in diploids than in tetraploids in the five cultivars of watermelon. It might be due to various factors such as the instability of chromosome number during an abnormal meiosis (Evans and Rahman 1990) or laggards and bridges due to the higher number of multivalent formation at metaphase I (Darlington 1965).

Vegetative and flowering traits: Slow growth rate and delayed emergence of shoots with rosette-like appearance of first leaves was observed in colchicine treated seedlings of watermelon. Earlier studies by Suying et al.(1995) in watermelon and Jaskani et al.(1996) in citrus, reported that colchicine application had a negative effect on the regeneration of plants. The effect of different doses of colchicine on the vegetative and flowering traits of watermelon cultivars was recorded and is presented in (Table 2). Leaf length, leaf width and internodal length showed significant differences among genotypes and ploidy. Among the different genotypes, California Sweet produced significantly the highest leaf length (13.98 cm) and leaf width (14.96 cm) over the other genotypes. The different methods of colchicine application showed wide range in leaf length and leaf width. The treatments SS 0.2%, SA 0.2%, IH 0.3% and SA 0.3% exhibited significantly more leaf length and width than other treatments and control. The highest leaf length (14.67 cm) and leaf width (16.11 cm) was significantly recorded in the treatment SA 0.3%. It is vivid from the interaction that the highest leaf length (16.67cm) and leaf width (19.97 cm) was recorded in California Sweet with IH 0.3% and at par with SA 0.3% and SA 0.2% treatments. The increase in size of leaf size of tetraploids was the result of increase in the size of cells and the stomata. Similar trend of increase was recorded by Jaskani et al. (2005b) that tetraploid plants had more leaf area (298.90 cm^2) than diploid plants (208.40 cm^2) of watermelon.

However, the data presented in (Table 2) revealed that internode length was the longest in Sugar Baby (5.54 cm) and significantly better than other genotypes. The variation in internode length might be due to their genetic differences. Also, Jaskani et al. (2005b) reported that the highest internode length (119.70 mm) in SS-11 while the lowest (97.20 mm) in NH3 tetaploid line of watermelon. Among different methods of colchicine application, the treatments SS 0.2%, SA 0.2%, IH 0.3% and SA 0.3% attainted shorter internode length as compared to other treatments and control. The shortest internode length (4.08 cm) was significantly recorded in the treatment SA 0.3%. It is vivid from interaction that the shortest internode length (4.00 cm) was found in tetraploids of Sugar Baby with IH 0.3% and statistically at par with SA 0.3%. Sheikh et al. (2013) also reported that internode length was shorter in tetraploids (6.40 cm) than in diploids (6.50 cm) of five cultivars of watermelon treated with three different methods of colchicine application. The decrease in internode length of putative tetraploids might be due to the slow cell divisions of larger cells with more chromosomes (Noggle 1946). Besides this, first female flower appeared late in tetraploids of California Sweet with SA 0.2% (37.33 number of days) and statistically at par with SA 0.3% and IH 0.3% treatments (Table 2). Similarly, delayed flowering was observed by Chopra and Swaminathan (1959) in Asahi Yamato and Farrukhabadi tetraploids of watermelon by 10-15 days. It might be due to various factors such as prolonged vegetative phase, or relatively lower transport of manufactured food from the site of production to the place of utilization (Biswas 1998).

Fruit traits: Similarly, tetraploids had significantly the longest period to first picking (72.50 days) in California Sweet with IH 0.3% followed by SA 0.2% and SA 0.3% treatments (Table 3). Similarly the tetraploids of Sugar Baby had longer period to first picking with IH 0.3% and SA 0.3% and in 5255-1-3-1 with SA 0.3%, SS 0.3% and SA 0.2% treatments. Pradeepkumar (2010-2011) reported the (134.00) and (147.00) days to harvest in plants of Sugar Baby of watermelon with 0.1% colchicine seed and 0.5% colchicine seedling treatment method, respectively and (82.00) days in diploids. It might be due to the slow cell divisions of larger cells with more chromosomes (Noggle 1946). Variation in TSS content of fruits was recorded in different cultivars of watermelon. The perusal of data in (Table 3) recorded the highest TSS content (11.00^0 brix) in diploids of Sugar Baby while the lowest (8.87^0 brix) in tetraploids of 5255-1-3-1 and Sugar Baby with SS 0.2% and SA 0.3%, respectively. However, the TSS content (9.93° brix) of tetraploids in California Sweet with IH 0.3% was statistically at par with diploid fruits when harvested on delayed maturity (*i.e.* 72.50 days after sowing). Chopra and Swaminathan (1959) reported that TSS content was (8.00%) in the fruits of both diploid and tetraploids of Asahi Yamato and Farrukhabadi of watermelon. The

Table 2: Effect of different doses of colchicine on vegetative and flowering traits of watermelon genotypes

Treatments (T)		Leaf length (cm)				Leaf width (cm)				Internode length (cm)				No. of days to first female flower			
		Genotypes (G)				Genotypes (G)				Genotypes (G)				Genotypes (G)			
		SB	CS	5255	Mean	SB	CS	5255	Mean	SB	CS	5255	Mean	SB	CS	5255	Mean
S A	0.1%	11.47	12.93	12.00	12.13	11.73	13.43	11.37	12.18	5.94	5.35	5.62	5.64	23.33	26.67	21.33	23.78
	0.2%	11.40	16.18	13.60	13.73	11.73	18.17	15.30	15.07	5.92	4.15	4.05	4.71	23.33	37.33	29.00	29.89
	0.3%	13.77	16.43	13.80	14.67	14.50	18.17	15.67	16.11	4.07	4.14	4.03	4.08	31.66	36.67	30.00	32.78
S S	0.1%	11.41	13.00	12.13	12.18	11.67	13.37	11.43	12.16	5.91	5.34	5.58	5.61	22.67	26.67	21.67	23.67
	0.2%	11.40	12.93	14.73	13.02	11.60	13.23	15.87	13.57	5.95	5.32	4.52	5.26	23.00	26.00	29.00	26.00
	0.3%	11.43	12.90	11.97	12.10	11.70	13.20	11.40	12.10	5.90	5.33	5.52	5.58	22.00	26.33	21.33	23.22
IH	0.1%	11.47	12.90	12.00	12.12	11.73	13.27	11.43	12.14	5.92	5.36	5.53	5.60	22.33	26.67	21.67	23.56
	0.2%	11.43	12.93	11.90	12.09	11.90	13.23	11.43	12.19	5.90	5.34	5.55	5.59	22.00	27.67	21.33	23.67
	0.3%	13.80	16.67	11.93	14.13	14.93	19.97	11.47	15.46	4.00	4.02	5.62	4.55	31.00	37.00	21.33	29.78
Diploid		11.43	12.97	12.00	12.13	11.90	13.57	11.43	12.30	5.95	5.39	5.64	5.66	23.00	27.00	22.00	24.00
Mean		11.90	13.98	12.61	12.83	12.34	14.96	12.68	13.33	5.54	4.97	5.16	5.23	24.43	29.800	23.867	26.03
C.D. 5%		G- 0.27 T-0.48 GxT- 0.84				G- 0.29 T-0.53 GxT- 0.91				G-0.12 T-0.22 GxT- 0.39				G- 0.55 T-1.00 GxT- 1.74			

Table 3: Effect of different doses of colchicine on fruit traits of watermelon genotypes

Treatments (T)		No. of days to first picking				TSS (^{0}brix)				Fruit weight (kg)				Rind thickness (cm)			
		Genotypes (G)				Genotypes (G)				Genotypes (G)				Genotypes (G)			
		SB	CS	5255	Mean	SB	CS	5255	Mean	SB	CS	5255	Mean	SB	CS	5255	Mean
S A	0.1%	63.00	66.67	63.33	64.33	10.97	10.17	10.60	10.57	2.24	2.26	2.14	2.22	0.90	0.87	0.89	0.89
	0.2%	63.33	69.67	69.67	67.56	11.00	9.37	8.99	9.79	2.21	0.88	1.03	1.37	0.89	1.02	1.02	0.97
	0.3%	69.67	70.00	70.33	70.00	8.87	9.17	8.90	8.98	0.88	0.93	0.95	0.92	0.99	0.99	0.99	0.99
SS	0.1%	62.00	63.67	63.67	63.11	10.97	10.15	10.63	10.58	2.21	2.26	2.13	2.20	0.89	0.86	0.87	0.87
	0.2%	63.33	64.00	69.33	65.56	10.33	10.15	8.87	9.98	2.19	2.25	0.78	1.74	0.87	0.86	0.97	0.90
	0.3%	62.00	63.00	63.33	62.78	11.00	10.15	10.60	10.58	2.22	2.25	2.13	2.19	0.87	0.85	0.88	0.87
IH	0.1%	62.33	64.67	64.00	63.67	10.97	10.16	10.60	10.57	2.23	2.23	2.123	2.19	0.90	0.87	0.88	0.88
	0.2%	63.67	64.33	63.33	63.78	10.67	10.14	10.57	10.46	2.24	2.23	2.12	2.19	0.87	0.88	0.88	0.88
	0.3%	69.33	72.50	63.67	68.50	8.95	9.93	10.60	9.83	0.86	0.88	2.13	1.29	1.02	1.20	0.89	1.03
Diploid		63.00	64.00	64.00	63.67	11.00	10.17	10.70	10.62	2.26	2.27	2.15	2.23	0.91	0.88	0.89	0.89
Mean		64.17	66.25	65.47	65.29	10.53	9.95	10.11	10.19	1.96	1.84	1.77	1.86	0.91	0.93	0.91	0.92
C.D. 5%		G- 0.42 T-0.76 GxT- 1.32				G- 0.20 T-0.37 GxT 0.64				G- 0.04 T- 0.07 GxT- 0.12				G- 0.04 T-0.07 GxT- 0.11			

lower activity of metabolites (Joshi and Verma 2004) and vigorous increase of vegetative growth in the later stages of development (Talukdar 2010) decreases the TSS content of tetraploid fruits. Also, the lowest fruit weight (0.79 kg) was significantly obtained from the putative tetraploids of 5255-1-3-1 with SS 0.2% followed by SA 0.2% and SA 0.3% treatments (Table 3). The analysis of variance presented significant differences for rind thickness in ploidy but non-significant among genotypes. Tetraploids had significantly the highest rind thickness (1.20 cm) in California Sweet with IH 0.3%, followed by SA 0.2% and SA 0.3% treatments (Table 3).

Seed traits: Seed number accounted in both ploidy fruits differed significantly. It is vivid from (Table 4) that tetraploid fruits of all the three cultivars recorded in SA 0.2%, SA 0.3%, SS 0.2% and IH 0.3% treatments produced lower seed number per fruit as compared to other treatments and control. The lowest seed number per fruit (127.67) was recorded in putative tetraploids of Sugar Baby with SA 0.3%. However, as a percentage the number of developed seeds in tetraploid fruits was much lower than the percentage of developed seeds in the diploid fruits (Table 4). The lowest percentage of developed seeds (52.72%) was significantly recorded in Sugar Baby with SA 0.3%. This indicates that tetraploid plants had lower fertility due to the unbalanced number of chromosomes during meiosis. Sheikh et al. (2013) reported that tetraploid fruits had less developed seeds (51.50) than the diploid fruits (110.00) in watermelon. Although the tetraploid fruits inherited larger seeds over their diploids. The highest seed length (11.59 mm) and seed width (7.15 mm) was recorded in the putative tetraploids of California Sweet with SA 0.3% and SA 0.2% treatments, respectively (Table 5). Similarly, the highest seed thickness (2.17 mm) and 100 seed weight (8.30 g) was observed in California Sweet with SA 0.3% (Table 5). Kumari et al. (2014) reported that tetraploid watermelon had the highest seed width in three varieties, Arka Muthu (6.81 mm) followed by IIHR-14 (5.61 mm) and Sugar Baby (4.58 mm) as compared to their respective control.

Table 4: Effect of different doses of colchicine on seed traits of watermelon genotypes

Treatments (T)		Seed number per fruit				Developed seeds per fruit (%)				Undeveloped seeds per fruit (%)			
		Genotypes (G)				Genotypes (G)				Genotypes (G)			
		SB	CS	5255	Mean	SB	CS	5255	Mean	SB	CS	5255	Mean
SA	0.1%	539.67	603.00	455.00	532.56	81.17	80.05	80.39	80.54	8.83	9.95	9.60	9.46
	0.2%	539.00	171.30	144.33	284.89	81.29	55.45	55.19	63.98	8.71	34.54	34.81	26.02
	0.3%	127.67	177.00	148.33	151.00	52.72	56.45	57.45	55.54	37.27	33.55	32.55	34.46
SS	0.1%	539.67	602.67	455.33	532.56	81.40	79.87	80.66	80.65	8.59	10.13	9.34	9.35
	0.2%	538.00	603.00	151.67	430.89	81.27	79.96	55.90	72.38	8.72	10.04	34.09	17.62
	0.3%	539.00	602.33	454.33	531.89	81.07	79.77	80.39	80.41	8.93	10.23	9.61	9.59
IH	0.1%	538.67	602.67	454.67	532.00	81.28	79.77	80.52	80.53	8.72	10.22	9.48	9.47
	0.2%	539.00	603.33	455.00	532.44	81.07	79.87	80.52	80.49	8.93	10.13	9.48	9.51
	0.3%	148.67	173.33	455.33	259.11	55.25	55.01	80.66	63.64	34.74	34.99	9.34	26.36
Diploid		540.33	603.67	456.00	533.33	81.07	79.88	80.28	80.41	8.92	10.12	9.72	9.59
Mean		458.97	474.23	363.00	432.07	75.76	72.61	73.19	73.86	14.24	17.39	16.80	16.14
C.D. 5%		G- 11.98	T-21.87	GxT- 37.88		G-0.25	T- 0.46	GxT- 0.79		G-0.25	T- 0.46	GxT- 0.79	

Table 5: Effect of different doses of colchicine on seed traits of watermelon genotypes

Treatments (T)		Seed length (mm)				Seed width (mm)				Seed thickness (mm)				100 Seed weight (g)			
		Genotypes (G)				Genotypes (G)				Genotypes (G)				Genotypes (G)			
		SB	CS	5255	Mean	SB	CS	5255	Mean	SB	CS	5255	Mean	SB	CS	5255	Mean
SA	0.1%	8.24	10.42	7.98	8.88	4.85	5.19	4.66	4.90	1.76	1.65	1.72	1.71	4.62	6.12	4.29	5.01
	0.2%	8.22	11.53	8.81	9.52	4.84	7.15	5.76	5.92	1.78	1.95	2.06	1.93	4.62	7.05	4.98	5.55
	0.3%	8.92	11.59	8.67	9.73	5.65	6.98	5.54	6.05	2.01	2.17	2.03	2.07	5.13	8.30	5.01	6.14
SS	0.1%	8.20	10.43	7.97	8.87	4.83	5.19	4.65	4.89	1.78	1.64	1.72	1.71	4.60	6.10	4.29	5.00
	0.2%	8.22	10.41	8.85	9.16	4.84	5.18	6.05	5.35	1.76	1.67	2.16	1.86	4.62	6.09	5.00	5.24
	0.3%	8.21	10.42	8.01	8.88	4.82	5.19	4.67	4.89	1.76	1.65	1.72	1.71	4.60	6.09	4.27	4.98
IH	0.1%	8.20	10.44	7.97	8.87	4.84	5.17	4.65	4.89	1.78	1.64	1.73	1.72	4.63	6.12	4.28	5.01
	0.2%	8.21	10.43	8.02	8.88	4.83	5.18	4.67	4.89	1.78	1.65	1.72	1.72	4.62	6.12	4.29	5.01
	0.3%	8.88	11.42	7.97	9.42	5.69	7.09	4.65	5.81	2.33	2.25	1.71	2.10	5.11	7.85	4.28	5.75
Diploid		8.23	10.45	8.01	8.89	4.84	5.20	4.66	4.90	1.77	1.66	1.72	1.72	4.63	6.13	4.30	5.02
Mean		8.35	10.75	8.23	9.11	5.01	5.75	4.99	5.25	1.85	1.79	1.83	1.82	4.72	6.59	4.50	5.27
C.D. 5%		G-0.14 T- 0.25 GxT- 0.43				G- 0.09 T-0.16 GxT- 0.29				G- 0.03 T- 0.06 GxT- 0.10				G- 0.09 T-0.17 GxT- 0.29			

Putative tetraploids (%): The present results (Table 6) illustrate that California Sweet had the highest efficiency of generating putative tetraploids (6.12%) and was significantly better over the other genotypes. The variable response of genotypes to colchicine indicated that optimal colchicine concentrations may vary in treating diverse watermelon genotypes. Among the various methods of colchicine application, the treatments SA 0.2%, SA 0.3%, SS 0.3% and IH 0.3% resulted into the putative tetraploids as compared to other treatments. However, the treatment IH 0.3% had the highest frequency of generating putative tetaploids (11.55%) and SS method was least effective. The highest percentage of putative tetraploids (16.43%) was recorded in the Sugar Baby with IH 0.3% treatment. Similarly, the California sweet had higher efficiency of putative tetraploids (14.17 %) with IH 0.3% treatment.

Table 6: Effect of different doses of colchicine on the percentage of putative tetraploids

Treatments (T)		Putative tetraploids (%)			
		Genotypes (G)			
		SB	CS	5255	Pooled
SA	0.1%	4.05	4.05	4.05	4.05
	0.2%	4.05	10.51	5.73	6.76
	0.3%	6.96	8.13	6.96	7.35
SS	0.1%	4.05	4.05	4.05	4.05
	0.2%	4.05	4.05	5.73	4.61
	0.3%	4.05	4.05	4.05	4.05
IH	0.1%	4.05	4.05	4.05	4.05
	0.2%	4.05	4.05	4.05	4.05
	0.3%	16.43	14.17	4.05	11.55
Diploid		4.05	4.05	4.05	4.05
Mean		5.58	6.12	4.68	5.462
C.D. 5%		G- 0.16 T- 0.30 GxT- 0.52			

सारांश

तरबूज में त्रिगुणित (टिट्राप्लोइडी) प्रेरणा के लिए एक उपयुक्त विधि की छँटनी करने के लिए, तीन अलग–अलग प्रभेदों (शुगर बेबी, कैलिफ्रोर्निया स्वीट और 5255–1–3–1) का मूल्यांकन अघुलनशील प्रतिकारक–कोल्चीसिन का प्रयोग तीन अलग मात्रा (0.1 प्रतिशत, 0.2 प्रतिशत और 0.3 प्रतिशत) और विधियाँ (प्ररोह शीर्ष, बीज भिगोना और उलटा बीजपत्राधार प्ररोह) के साथ कियाग या। यह प्रयोग वर्ष 2013–15 के दौरान सब्जी अनुसंधान और प्रयोगशाला वनस्पति

विज्ञान विभाग, पंजाब कृषि विश्वविद्यालय, लुधियाना (पंजाब) में किया गया त्रिगुणिता की पुष्टि करने के लिए हरितलवक गणना, परागाणु और प्ररूपी लक्षणों पर अवलोकन दर्ज किया गये। तरबूज के विभिन्न प्रभेदों कैलिफ्रोर्निया स्वीट में अनुमानित त्रिगुणित उत्पादन क्षमता (6.12 प्रतिशत) पायी गयी। त्रिगुणिता प्रेषित करने के लिए कोल्चीसिन के विभिन्न तरीकों और सांद्रता में उल्टा बीजपत्राधर और प्ररोह शीर्ष विधि द्वारा कोल्चीसिन की 0.2 प्रतिशत और 0.3 प्रतिशत सांद्रता तुलनात्मक रूप से अधिक प्रभावी पाया गया। अनुमानित त्रिगुणित (11.55 प्रतिशत) की उच्चतम आवृत्ति उल्टा बीजपत्राधर 0.3 प्रतिशत के साथ प्राप्त हुई।

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