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In Vitro Induction of Polyploids in Watermelon and Estimation Based on DNA Content

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ABSTRACT

Polyploids are desired to produce seedless fruits in watermelon. *In vitro* techniques pave way towards production of tetraploids by culturing explants on media containing colchicine. Cotyledon, embryonic end of seed, epicotyl and hypocotyl explants were cultured on MS media supplemented with BA (1 mg L^{-1}). Shoot proliferation response was maximum in cotyledonary explants cultured on 0.01% colchicine level for four days. The DNA content of regenerated plants was estimated using spectrophotometery. DNA content was found double in tetraploids (4.41µg mL^{-1}) than diploids (2.18 µg mL^{-1}) in unit gram of sample. Cotyledon explants cultured on colchicine supplemented medium (0.01%) for four days resulted in maximum tetraploids than plantlets regenerated from epicotyl and hypocotyl explants. Embryonic ends and cotyledon as explants produced aneuploids when cultured on the same media for seven days.

Key Words: Polyploids; Colchicine; Watermelon; *In vitro*; Ploidy estimation

INTRODUCTION

Watermelon (*Citrullus lanatus* Thunb) is an important Cucurbetaceous vegetable. In Pakistan, it is grown over an area of 19 thousand hectares with a production of 420 thousand MT (FAO, 2002). Seedlessness is an important and desirable breeding objective in horticultural crops. In watermelon polyploidy is utilized to produce seedless fruits. Seedless watermelons are triploids (3x=33) and result from crossing a tetraploid (2n=4x=44) seed parent with a diploid (2n=22) pollen parent (Andrus *et al*., 1971; Kihara, 1951). Seedless (triploid) watermelons are preferred by consumers because of high fruit quality and absence of seeds; even consumers are willing to pay 50% more per pound (Marr & Gast, 1991). Polyploid watermelons are also found to be resistant to watermelon fruit blotch (*Acidivorax avenae* subsp. *citrulli*) and nematodes (Montalvo & Esnard, 1994; Garret *et al.*, 1995). Moreover, in triploid watermelons orange flesh turns into deeper orange color as it ripens and flavor can even improve after harvesting. Also, their tough sunburn-resistant rind makes them excellent for long-distance shipping.

However, production of seedless watermelons have been hampered by high seed cost ($150-$200/1000 seeds) and poor seed germination. High seed cost has generally attributed to difficulties in obtaining a sufficient number of tetraploid plants as they exhibit low fertility and generally require at least 8-10 years of self pollination before enough plants are obtained for commercial triploid seed production (Compton & Gray, 1992). Moreover, *in vivo* treatment of colchicine results in a mixed population of diploid, tetraploid, aneuploids and sectoral and periclinal chimeras. The induction of tetraploid *in vitro* offers an alternative method to obtain tetraploid plants because of reduction in number of aberrant plants produced and also reduce the time span required for triploid seed production from 10 years to approximately 1-2 years and also allows use of male sterile lines of tetraploids without cumbersome maintenance lines (Compton & Gray, 1992; Compton *et al*., 1993).

The objective of the study was to produce watermelon polyploids *in vitro* for breeding seedless watermelons. As in advanced countries people are willing to pay 50% more for seedless watermelon than seeded watermelons, there is a potential for seed companies and growers to export seedless watermelon which in turn will be good source of foreign exchange earning.

MATERIALS AND METHODS

Seeds of diploid watermelon (*Citrullus lanatus* Thunb.) cultivar ‘Sugar Baby’ were sterilized in 2.5% NaOCl plus one drop 100 mL^{-1} Tween 20 for 30 min followed by three rinses with autoclaved double distilled water. The seeds were soaked for 5 h for ease in removing seed coat. The embryos were surface disinfected for 20 minutes in 1.25% NaOCl plus one drop 100 mL^{-1} of surfactant Tween 20 prior to three rinses with sterile double distilled water and were cultured on MS medium supplemented with Benzyl Adenine (BA; 0, 1 or 5 µM) to test the effect on seed germination and regeneration. Transversly cut epicotyl (E_1), transversly cut hypocotyl (E_2), cotyledons (E_3) and the embryonic end (E_4) of *in vitro* grown seedlings were used as explants. Later the best level of BA in the medium was used for explants culturing on following media formulations for 4 days (D_1) and 7 days (D_2).

M_o = MS + 1 µM BA (control)
M_1 = MS + 1 µM BA (0.01% colchicine)
M_2 = MS + 1 µM BA (0.05% colchicine)
M_3 = MS + 1 µM BA (0.1% colchicine)

After 4 and 7 days culturing on colchicine supplemented media, the explants were subcultured on MS + 1 µM BA. Each experiment was laid out in CRD with factorial arrangement and data was analyzed according to Steel and Torrie (1980). Comparisons of means were made by applying DMR test. Data were summarized as percent of explant producing shoots, number of shoots per explant, percent of explants producing roots and number of roots per explant. Estimation of ploidy level was made with spectrophotometery. DNA content of polyploid cell (tetraploid, hexaploid etc) is higher as compared to diploid. DNA content increase proportionately with increase of ploidy level, as in polyploidy chromosome number increases hence the DNA content would also increase accordingly. The unit mass of tissue from the diploid and polyploid would have different DNA content which can be determined by spectrophotometery, selecting 260 nm wavelength. The DNA of regenerated plants was extracted by the standard procedures.

RESULTS AND DISCUSSION

Per cent of explants producing callus. Explants producing callus was the highest (90.5 & 90.25%) for E_2 transversely cut hypocotyls (E_2) on medium devoid of colchicine (control) cultured for 4 days and 7 days, respectively and were non-significantly different form each other. The least results were on explants E_1 (14.5%) and E_4 (12.75%) cultured for seven days on the media containing 0.1% colchicine. Among colchicine added media, medium with 0.01% colchicine (M_1) gave the best results (57.81%) followed by medium with 0.05% colchicine (M_2) in which explants were cultured for four days (54.0%). The medium with 0.1% colchicine (M_3) on which explants are cultured for four days and the medium with 0.01% colchicine (M_1) on which explants were cultured for seven days occupied the same position with 44.94 and 45.31% explants, respectively. While the medium containing 0.05% colchicine (M_2) and the medium with 0.1% colchicine (M_3) on which explants were cultured for seven days fall in descending order giving 40.75 and 33.31% explants responding, respectively.

Transversely cut hypocotyls explants yielded the highest (83.63%) callus induction cultured for four days on colchicine containing media while the same explant cultured for seven days and cotyledons cultured for four days on colchicine added media held second position (75.13 & 75.88%, respectively, Table I). It is suggested to use transversely cut hypocotyls and culture for four days on lower concentration of colchicine in the media if maximum callusing is required in minimum time. The results of present studies are supported by the results of Lower and Johnson (1969), Perry and Lyrene (1984), BugaenKo *et al*. (1988), Tan-Suying *et al*. (1993) and Gao *et al*. (1996).

Per cent of explants producing shoots. Among colchicine supplemented media the percent of explants producing shoots was significantly maximum (17.56%) on media containing 0.01% colchicine (four days treatment) when we study duration and media interaction. These results show that the highest percentage of explants producing shoots were on control for both the intervals. This is actually not desired according to research objectives. The lowest (0.01%) concentration of colchicine tested for smaller interval of time (four days) regenerated the best. This indicates that both the concentration and incubation duration are equally important for shoot regeneration as lower concentration of colchicine for longer duration has the same results than that of higher (0.05%) concentration of colchicine for smaller intervals. The least percent of explants producing shoots were at the highest level (0.1%) of colchicine solution tested for seven days but the same concentration showed slightly greater per cent of explants producing shoots for D_1 it indicates that the high level of colchicine and longer durations are toxic for healthy plants. However, if higher levels and longer intervals produce greater percent of polyploid plants then their other harmful effects on regeneration can be ignored.

A glance at the interaction of duration and explant presented in the Table II indicates that percent explants producing shoots were statistically the greatest (38.94%) for explants E_3 (cotyledons) cultured on colchicine media for 4 days followed by the same explant cultured for seven days on colchicine supplemented media with 27.56% explants producing shoots. The explants E_4 (embryonic end) cultured on colchicine added media for four days showed the second

Table I. Effect of explant, medium and treatment duration on callus induction (%)

	4 days culture on colchicine (D_1)						7 days culture on colchicine (D_2)			
Media	**E_1**	**E_2**	**E_3**	**E_4**	**Mean (DxM)**	**Mean (DxM)**	**E_1**	**E_2**	**E_3**	**E_4**
M_o 0% colchicine	40.25 c	90.5 a	84.75 b	34.5 m	62.5 A	60.06 B	45.5 k	90.25 a	75.5 e	29.0 n
M_1 0.01% colchicine	34.75 m	85.25 b	80.75 c	30.5 n	57.81 C	45.31 E	29.75 n	75.5 e	55.5 I	20.5 p
M_2 0.05% colchicine	30.5 n	79.5 cd	77.5 de	28.5 n	54.0 D	40.75 F	24.5 o	69.5 f	49.25 j	19.75 p
M_3 0.1% colchicine	20.25 p	79.25 cd	60.5 h	19.75 p	44.94 E	33.31 G	14.5 q	65.25 g	40.75 l	12.75 q
Mean (DxE)	31.44 D	83.63 A	75.88 B	28.31 E			28.56 E	75.13 B	55.25 C	20.5 F
	Mean (D_1) 54.81 a						Mean (D_2) 44.86 b			

E_1= Transversely cut epicotyl, E_2=Transversely cut hypocotyl; E_3=Cotyledons, E_4= Embryonic end of seeds

best results (16.56%). The other two types of explants produced no shoots at any duration of colchicine treatment.

The interaction of the three factors studied i.e., explant, media and duration of colchicine treatment is also shown in the Table II. The highest (55.5 & 54.75%) of explants producing shoot was observed on the explant E_3 (cotyledons) cultured for both durations on colchicine lacking media (M_0). They were followed by the same explant cultured on M_1 (media with 0.01% colchicine) for four days duration showing 50.25% explants with shoots. The explant E_4 (embryonic end) showed lesser response than E_3, while the explants E_1 (transversely cut epicotyl) and E_2 (transversely cut hypocotyl) showed least (0%) response on the all concentrations of colchicine in the media for both the durations of culture. Our results are favored by the studies of Lower and Johnson (1969), Perry and Lyrene (1984), BugaenKo *et al*. (1988), SuYing *et al*. (1993) and Gao *et al*. (1996).

Number of shoots per explant. Both the durations of cultures were statistically similar to each other producing 4.54 and 4.53 shoots per explant, respectively. The medium with 0.01% colchicine (M_1) produced 3.94 shoots at D_1 but significantly differed at D_2 with 3.65 shoots per explant. The lowest number of shoots (2.01) was induced with combination of M_3 at treatment duration of seven days. The overall interaction of the three factors studied is also presented in the Table III. It can be observed that the maximum (9.63 & 9.6, respectively) number of shoots were formed on embryonic end explant cultured on colchicine lacking media for both durations of colchicine treatment. It was lagged behind by cotyledons cultured on the similar media for both the duration with 8.53 shoots per explant. These two explants on higher levels of colchicine on both the durations gave intermediate results. The explants E_1 (transversely cut epicotyl) and E_2 (transversely cut hypocotyl) produced no shoot at any media and duration of treatment.

The results obviously show that the greatest number of shoots per explant was formed on colchicine devoid media at both treatment durations. As the duration of culture increased from 4 to 7 days the number of shoots on each explant decreased in all the media tested. Further it was noted that the number of shoots was more when explants were cultured for four days than the explants cultured on similar medium but with longer duration. Similar results were observed with the amount of colchicine in the medium

Table II. Effect of explant, medium and treatment duration on shoot induction (%)

Media	4 days culture on colchicines (D_1)						7 days culture on colchicines (D_2)			
	E_1	E_2	E_3	E_4	Mean (DxM)	Mean (DxM)	E_1	E_2	E_3	E_4
M_0 0% colchicine	0 i	0 i	55.5 a	25.25 d	20.19 A	18.75 B	0 i	0 i	54.75 a	20.25 e
M_1 0.01% colchicine	0 i	0 i	50.25 b	20 e	17.56 C	10.19 D	0 i	0 i	30.25 c	10.50 g
M_2 0.05% colchicine	0 i	0 i	29.75 c	11 g	10.19 D	4.94 F	0 i	0 i	14.75 f	5.0 h
M_3 0.1% colchicine	0 i	0 i	20.25 e	10 g	7.56 E	3.69 G	0 i	0 i	10.50 g	4.25 h
Mean (DxE)	0 E	0 E	38.94 A	16.56 C			0 E	0 E	27.56 B	10.0 D
	Mean (D_1) 13.88 a						Mean (D_2) 9.39 b			

E_1= Transversely cut epicotyl, E_2=Transversely cut hypocotyl; E_3=Cotyledons, E_4= Embryonic end of seeds

Table III. Effect of explant, medium and treatment duration on number of shoots per explant

Media	4 days culture on colchicines (D_1)						7 days culture on colchicines (D_2)			
	E_1	E_2	E_3	E_4	Mean (DxM)	Mean (DxM)	E_1	E_2	E_3	E_4
M_0 0% colchicine	0 k	0 k	8.53 b	9.63 a	4.54 A	4.53 A	0 k	0 k	8.53 b	9.6 a
M_1 0.01% colchicine	0 k	0 k	7.20 b	8.55 d	3.94 B	3.65 C	0 k	0 k	6.58 e	8.03 c
M_2 0.05% colchicine	0 k	0 k	5.45 g	6.43 e	2.97 D	2.78 E	0 k	0 k	5.1 f	6.03 e
M_3 0.1% colchicine	0 k	0 k	5.15 h	5.10 h	2.56 F	2.01 G	0 k	0 k	4.5 i	3.55 g
Mean (DxE)	0 E	0 E	6.58 C	7.43 A			0 E	0 E	6.18 D	6.8 B
	Mean (D_1) 3.5 a						Mean (D_2) 3.24 b			

E_1= Transversely cut epicotyl, E_2=Transversely cut hypocotyl; E_3=Cotyledons, E_4= Embryonic end of seeds

Table IV. Effect of explant, medium and treatment duration on root induction (%)

Media	4 days culture on colchicines (D_1)						7 days culture on colchicines (D_2)			
	E_1	E_2	E_3	E_4	Mean (DxM)	Mean (DxM)	E_1	E_2	E_3	E_4
M_0 0% colchicine	0.5 i	24.75 b	14.75 d	100 a	35.0 B	36.25 A	0.5 i	24.75 b	19.75 c	100 a
M_1 0.01% colchicine	0.25 i	15.25 d	11.5 e	100 a	31.5 C	30.44 D	0.25 i	11.75 e	11 ef	100 a
M_2 0.05% colchicine	0 i	9.75 f	10.5 ef	100 a	30.31 DE	29.69 E	0 i	10.5 ef	7 g	100 a
M_3 0.1% colchicine	0 i	4.75 h	5 h	100 a	27.44 F	27.69 F	0 i	5.75 gh	5 h	100 a
Mean (DxE)	0.19	13.63	10.44	100			0.19	13.19	10.69	100
	Mean^{ns} (D_1) 31.06						Mean^{ns} (D_2) 31.02			

E_1= Transversely cut epicotyl, E_2=Transversely cut hypocotyl; E_3=Cotyledons, E_4= Embryonic end of seeds

Table V. Effect of explant, medium and treatment duration on number of roots per explant

Media	4 days culture on colchicines (D_1)						7 days culture on colchicines (D_2)			
	E_1	E_2	E_3	E_4	Mean (DxM)	Mean (DxM)	E_1	E_2	E_3	E_4
M_o 0% colchicine	1 g	2 ef	2.3 ef	4.45 a	2.44 AB	2.56 A	1 g	2.65 de	2 ef	4.6 a
M_1 0.01% colchicine	0.5 gh	2 ef	2.18 ef	4.15 ab	2.21 B	1.78 CD	0.5 gh	2 ef	1.08 g	3.55 bc
M_2 0.05% colchicine	0 h	2 ef	2.08 ef	3.55 c	1.91 C	1.57 D	0 h	1.9 f	1 g	3.38 c
M_3 0.1% colchicine	0 h	1.95 ef	2.13 ef	2.03 ef	1.53 D	1.48 D	0 h	1.85 f	0.98 g	3.08 cd
Mean (DxE)	0.38 D	1.98 B	2.17 B	3.55 A			0.38 D	2.1 B	1.26 C	3.65 A
	Mean (D_1) 2.02 a						Mean (D_2) 1.85 b			

E_1= Transversely cut epicotyl, E_2=Transversely cut hypocotyls; E_3=Cotyledons, E_4= Embryonic end of seeds

and number of shoots per explant. It can be stated that both the longer duration of culture on colchicine supplemented media and its higher levels of in the media were limiting factors for shoot proliferation. In other words, an inverse relation was observed for length of culture and duration with the number of shoots per explant. It can be surely said that the number of shoots per explant is dependent on the concentration of colchicine in the medium as well as the duration of culture on these media. These findings were also obtained by Lower and Johnson (1969), Perry and Lyrene (1984), BugaenKo *et al*. (1988), SuYing *et al*. (1993) and Gao *et al*. (1996).

Per cent of explants producing roots. When we compare the means of culture duration on colchicine added media and culture duration it is clear from the Table IV that the percent of explants forming roots was the highest (36.25%) on explants cultured on media lacking colchicine (M_o; control) cultured for seven days and this was followed by the explants cultured on the similar medium (control) for four days. The mean of medium with 0.05% colchicine (M_2) on which explants were cultured for four days was statistically non-significant with the lower and higher colchicine concentration but was statistically similar when cultured for seven days. The explants cultured on media with 0.1% colchicine for 4 and 7 days were at par from other means but non-significant from each other giving 27.44 and 27.67% response, respectively.

Table IV also shows the interaction of explants, media and duration of culture on colchicine media. It is clearly shown that the explant E_4 (embryonic end) had the same response to rooting on all the colchicine levels in the medium for both intervals, i.e. 100% explants giving rise to roots and thus forming a one large group statistically. The response of explant E_2 (transversely cut hypocotyl) was same on colchicine devoid medium cultured for the both durations. The explants E_1 (transversely cut epicotyl) showed unaffected (0-0.5%) response on the different amounts of colchicine in the medium and duration of culture on that medium. All the other means occupied intermediate position.

The results for the interaction of explant, concentrations of colchicine in the media and duration of culture showed that the percentage of explants producing roots was not much affected by media type and culture duration on embryonic end (E_4) while the other explants showed the inverse relation of colchicine concentration and incubation duration with present of explants producing shoot. Hence, the hindrance of colchicine for regeneration of *in vitro* grown plants is again confirmed. The results obtained after these studies confirms the results of Lower and Johnson (1969), Perry and Lyrene (1984), BugaenKo *et al*. (1988), SuYing *et al*. (1993) and Gao *et al*. (1996).

Number of roots per explant. The interaction of duration of culture and media type gave highly significant results (Table V). The largest (2.56 and 2.44) number of roots were formed on control medium at the both 7 and 4 days culture durations, respectively. Thus, constituting statistically same group. The Table V also shows the comparison of means of the interaction of duration of culture of explants on colchicine media and the type of explants used. Maximum number of roots were formed on explants E_4 (embryonic end) which was 3.65 for 7 days duration and 3.55 on 4 days duration and were statistically alike. The explant E_2 (transversely cut hypocotyl) was the next coming explant forming 1.99 and 2.1 roots on each explant cultured for 4 and 7days on colchicine containing media, respectively. Statistically these constitute same group along with the explant E_3 (cotyledons) which had 2.17 roots per explant when cultured for four days on colchicine containing media. The group with least number of roots per explant was formed by explant E_1 (transversely cut epicotyl) giving 0.38 roots per explant for both durations of culture.

The interaction of the three factors under study is shown in Table V. It can be easily seen that the maximum (4.6 & 4.45) roots were produced on explant E_4 (embryonic

Table VI. DNA content of regenerated seedlings of watermelons as measured by spectrophotometer

Treatment	DNA content per gm Sample (µg/ml)	Ploidy
E_3- D_1 (Control)	2.245	2n
E_3- D_1 (Control)	1.992	2n
E_3- D_2 (Control)	2.456	2n
E_4- D_1 (Control)	2.031	2n
E_4- D_1 (Control)	2.186	2n
E_4- D_2 (Control)	2.179	2n
E_3-D_1 (0.01% Colchicine)	4.182	4n
E_3- D_1 (0.01% Colchicine)	4.221	4n
E_4- D_1 (0.01% Colchicine)	4.08	4n
E_4- D_1 (0.01% Colchicine)	4.264	4n
E_4- D_2 (0.01% Colchicine)	4.814	Aneuploid
E_3-D_2 (0.01% Colchicine)	4.923	Aneuploid

end) cultured for 7 and 4 days, respectively on control. The embryonic ends cultured for 4 days on 0.01% colchicine containing media (M_1) yielded 4.15 roots and were statistically non-significant with the previous two. It also differs non-significantly with the same explant cultured on 0.01% colchicine supplemented media (M_1) but cultured for seven days. The later is at par from the explants cultured on control media. The least (0-1) roots were produced by the explant E_1 (transversely cut epicotyl). Its regenerative ability seemed to be affected by the colchicine concentration in the medium but not by the duration.

The results again described that the different explants had differential response of regeneration (rooting) and all the explants exhibited a decreased root proliferation with increasing level of colchicine in the media. The duration of culture on that media was equally important as the colchicine amount in the media. However in this case, number of roots per explant, this effect was not remarkable. Our results are in alliance with Lower and Johnson (1969), Perry and Lyrene (1984), Bugaenko *et al.* (1988), SuYing *et al.* (1993) and Gao *et al.* (1996).

DNA concentration studies. DNA content was found to be double in shoots regenerated on the explants E_3 (cotyledons) and E_4 (embryonic end) cultured for 4 days on 0.01% colchicine added media. These explants E_3 and E_4 when cultured on similar medium for seven days resulted in aneuploid. All the other treatments did not regenerate tetraploid shoots (Table VI).

The results proved superiority of 0.01% colchicine concentration in the media when explants were cultured for four days. The longer duration of culture i.e., seven days, resulted in mix ploids, so it proved to be inferior to four days incubation. It appeared that after formation of shoot primordia they were immediately killed due to higher levels of colchicine in the media, as it had already observed that polyploid shoot initials/buds were killed in colchicine (Colijn-Hooymans *et al.* 1994; Gao *et al.* 1996; Griesbach & Bhat, 1990).

DNA content is a good indicator of ploidy so when we measured the optical density of plant after the DNA extraction, the spectrophotometer gives it optical density. The optical density is then used to calculate ploidy level. Our results are also supported by the studies regarding DNA content as a indicator of ploidy level of plants carried out by Colijn-Hooymans *et al.* (1994) and Gao *et al.* (1996). This method also reduces the time to determine ploidy of *in vitro* regenerated plants as there is no requirement of hardening them off.

CONCLUSIONS

The present studies conclude that lower levels of colchicine in the media gave better induction of polyploidy in watermelon. Cotyledon and embryonic ends are better explants as they directly give rise to shoots. Polyploids of watermelon *in vitro* can be produced and identified in a shorter time span (1-2 years) than *in vivo* (8-10 years).

REFERENCES

Andrus, C.F., V.S. Seshadri and P.C. Grimball, 1971. Production of seedless watermelon. *Agric. Res. Service, US Deptt. of Agri. Technical Bull. No.* 1425

FAO, 2002. *FAO Production Year Book.* Food & Agriculture Organization of the United Nations, Rome, Italy, 54: 162

BugaenKo, L.A., O.A. Davydova, V.S. Rodov and S.M. Gladan, 1988. Producing polyploid plants of mint using *in vitro* culture. *Bilogiya kul tiviruemykh Kletok i biotekhnologiya.,* 1: 91

Colijn-Hooymans, C.M., J.C. Hakkert, J. Jamien and J.B.M. Custers, 1994. Competence for regeneration of cucumber cotyledons is restricted to specific developmental stages. *Plant Cell Tissue Organ Culture*, 39: 211–7

Compton, M.E. and D.J. Gray, 1992. Micro propagation as a mean of rapidly propagating triploid and tetraploid watermelon. *Proc. Fla. State Hort. Soc.*, 105: 352–4

Compton, M.E., D.J. Gray and G.W. Elmstrom, 1993. A simple protocol for micropropagating diploid and tetraploid watermelon using shoot-tip explants. *Plant Cell Tissue and Organ Culture*, 33: 211–7

Gao, S.L., D.N. Zhu, Z.H. Cai and D.R. Xu, 1996. Autotetraploid plants from Colchicine-treated and culture of *Salvia miltiorrhiza* Bge. *Plant Cell Tissue & Organ Culture*, 43: 73–7

Garret, J.J., B.B. Rhodes and Z. Xing-Ping, 1995. Triploid watermelons resist fruit blotch organism. Rpt. Cucurbits Genet. *Crop.*, 18: 56–7

Griesbach, R.J. and R.N. Bhat, 1990. Colchicine induced polyploidy in *Eustoma grandiflorum*. *HortSci.*, 25: 1284–6

Kihara, H., 1951. Triploid watermelons. *J. American Soc. Hort. Sci.*, 58: 217–30

Lower, R.L. and K.W. Johnson, 1969. Obervations on sterility of induced autotetraploid watermelons. *J. American Soc. Hort. Sci.*, 94: 367–9

Marr, C. and K.L.B. Gast, 1991. Reaction of consumers in a farmers market to prices for seedless watermelon and ratings of eating quality. *HortTechnol.*, 1: 105–6

Montalvo, A.E. and J. Esnard, 1994. Reaction of ten cultivars of watermelon (*Citrullus lanatus*) to a puerto rican population of *Meloidogyne incognita*. *J. Nematology*, 26: 640–3

Perry, J.L. and P.M. Lyrene, 1984. *In vitro* induction of tetraploidy in *Vaccinum darrowi, V. ellioti andV. darrowi x V. elliotti* with Colchicine treatment. *J. American Soc. Hort. Sci.*, 109: 4–6

Steel, R.G.D. and H.J. Torrie, 1980. *Principles and Procedures of Statistics. A Biometrical Approach.* McGraw Hill Book Company, New York.

SuYing, T., H. XiuQiang, L. JiWei and L. WenGe, 1993. Raising the frequency of inducing tetraploid watermelon by treating of Colchicine. *Acta Horticulturae*, 402: 18–22

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