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ARTICLE

In vitro propagation of two triploid hybrids of watermelon through adventitious shoot organogenesis and shoot tip culture

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ABSTRACT *In vitro* propagation protocol for two triploid hybrids of watermelon using cotyledon explants and shoot tips was achieved. Five benzyladenine (BA) concentrations were tested using cotyledon and shoot tip explants. Cotyledon explants and shoot tips from 6 and 15-20 days aseptically germinated were cultured on Murashige and Skoog medium (MS) containing test concentration of benzyladenine (2.22, 4.44, 10, 24.61 and 44.4 µM). Adventitious hoot organogenesis was initiated in all induction media and the differences among BA concentration were significant. MS medium containing 4.44, 10 and 24.61 µM BA showed the highest percentage of explants with shoots. The stimulation of axillary-bud development from excised shoot tips by a high cytokinin (BA) was observed. Axillary shoots were obtained from shoot tips of triploid watermelon and the multiplication rate ranged from 2 to 5.6 plants dependence on benzyladenine concentration and genotype. Obtained data showed that variation in regeneration rate was demonstrated. Shoots were excised and elongated in MS medium without hormones. The elongated shoots were rooted in MS medium containing 0.1 µM α-naphthalene acetic acid (NAA). Rooted plants were successfully acclimatized and gradually hardened-off to green-house conditions and subsequently established in soil with a survival rate of 80%.
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KEY WORDS

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Watermelon is an important cucurbitaceous vegetable. It is grown worldwide and ranked sixth in world production of fruit crop. China has been the number one producer of watermelon since 1986 and Egypt is a distant fourth in production of watermelon with 1.45 million metric tons. Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai] is an annual vegetable crop with sweet tasting fruit that is often consumed as a cool dessert. The species originated in tropical Africa (Decoteau 2000). Excessive seed number in watermelon fruit is fast becoming unacceptable in international markets. Seedless watermelon cultivars have been available for over 50 years (Kihara 1951) and are becoming more prevalent (Lucier and Lin 2001). Seedless cultivars are in a high demand by consumers not only because their fruits are seedless but also because they are sweeter than fruits from diploid, seeded cultivars (Marr and Gast 1991). 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 (Andrus et al. 1971). Tetraploid plants commonly sterility and yielded lower number of seeds per fruit (Jaskani et al. 2005). Therefore, the breeding of triploid cultivars and production of triploid seeds is very difficult. Genetic improvement through tissue culture and biotechnology offer potential routes of improving fruit harvest by offering higher quality products, seedless fruit or generating somoclonal variants, with improved resistance to biotic or abiotic stresses (Compton et al. 2004). The use of shoot tip explants for colonal propagation has potential application for the propagation of diploid and tetraploid watermelon breeding lines. Micropropagation of triploid cultivars has potential use in the vegetable industry as a mean of offsetting the high cost and difficulties often encountered when germinating triploid seeds (Elmstrom and Maynard 1992). Techniques to micropropagate triploid and tetraploid watermelon from shoot tips and meristems have been known (Anghel and Rosu 1985). Difficulties with seed germination have retarded the expansion of triploid cultivation. In other cases, the lack of disease resistance was regarded as a serious obstacle (Maynard 1989). High seed cost has generally attributed to difficulties in obtaining a sufficient number of tetraploid plants as they exhibit low fertility and generally required at least 8-10 years of self pollination before enough plants are obtained for commercial triploid seed production (Compton and Gray 1993). Due to high cost of triploid seeds and difficulty in germination, the objective of this study was to propagate two new triploid watermelon hybrids.

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Materials and Methods

The current study was carried out during the years 2006 and 2007 at The Horticulture Department, Faculty of Agriculture at Kafr El-Sheikh.

Plant materials

Two triploid watermelon hybrids (SA100 and SA101) were used as plant materials in this study. These hybrids were selected as the best hybrids from our produced triploid hybrids. This study included two experiments, first was induction of plantlets from cotyledon explants, second, propagation of seedless watermelon via *in vitro* shoot tips culture.

Surface sterilization

Seeds were soaked two hours for ease in removing seed coat. The seeds after removing seed coat were surface disinfected for 20 min in 20% of Clorox plus one drop of tween 20 followed by three times rinses with sterile water and cultured on MS medium (Murashige and Skoog 1962) under dark condition to germinate (Compton 1999).

First experiment

This experiment was conducted to study the effect of BA concentrations on induction of plantlets from cotyledon explants of two triploid hybrids. Explants were obtained from 6 days old seedlings from which the cotyledons were removed by making a cut about 2mm beyond the point of attachment to the hypocotyls. The margins about 1-2 mm were removed and the cotyledons dissected crosswise as described by Compton and Gray (2000). Explants were cultured in petri-dishes that containing 15 ml of test concentration of BA shoot regeneration medium (2.22, 4.44, 10, 24.61 and 44.4 μM). Explants were sub-cultured to fresh medium after 4 weeks and remained on regeneration medium for a total of 8 weeks then the percentage of explants that developed plantlets was determined. Explants with shoots and shoot buds were transferred to MS medium with 30 g l^{-1} sucrose without plant growth regulators to stimulate shoot elongation. After three weeks adventitious shoots were recorded, excised and transferred to rooting medium (MS with 0.1 μM NAA) for two weeks (Compton and Gray 1993).

Second experiment

This experiment was carried out to propagate two triploid hybrids via shoot tip cultures.

Shoot tip (~2 cm) explants were harvested from stem of 15-20 days old aseptically germinated and cultured in glass jars containing 25 ml of MS medium with 30 g l^{-1} sucrose and 8 g^{-1} agar at a pH of 5.7 supplemented with test concentration of BA (2.22, 4.44, 10, 24.61 and 44.4 μM). Five explants were cultured in each jar and 10 replicates for each treatment were used. All cultures were maintained in growth chamber at 27±1°C under 16 h photoperiod for 4 weeks. Meristems developed into masses containing numerous axillary buds which were transferred to MS medium without plant growth regulators to stimulate shoot elongation (Compton 1999). The shoot regeneration frequency rate was averaged over ten jars. Each jar of five explants was treated as a replicate for each treatment. The same procedures for rooting and acclimatization were applied as mentioned in the first experiment. All media contained 3% sucrose and 0.8% agar adjusted to pH 5.7 and autoclaved for 20 min at 121°C. The experiments were conducted twice. The experiments were arranged in a completely randomized design.

Rooting and acclimatization

Elongated shoots (>5 cm in height) were transferred to root induction medium (MS with 0.1 μM NAA) for two weeks (Compton and Gray 1993). After 2 weeks the rooted plants were recorded and plants with good roots were potted in small pots contained autoclaved peat covered with a clear plastic bag and grown under ambient humidity conditions. Acclimatized plants were moved to the greenhouse conditions

Statistical analysis

Data were statistically evaluated by analysis of variance, and LSD test was used for the comparisons among the treatment means.

Results and Discussion

Shoot organogenesis from cotyledon explants

The effect of BA concentrations on adventitious shoot organogenesis was examined using cotyledon explants from two triploid hybrids (SA100 and SA101) seedling.

Data presented in Table 1. show that BA was required to promote shoot organogenesis. Optimum concentration of BA for adventitious shoot regeneration was determined. The adventitious shoot regeneration frequency ranged from 46.2% to 73.6% dependence on the BA concentration and genotype. The ability of cotyledon explants from the two hybrids to form shoots depended on the BA concentrations in the medium. Adventitious shoots were observed by eye from the bottom cut end of the cotyledon explants after 4 weeks of culturing (Fig. 1). The maximum frequency of adventitious shoot regeneration was obtained when cotyledon explants from SA100 hybrid were cultured onto MS medium with 10 μM BA. Han et al. (2004) suggested that only BA considered a crucial factor for the adventitious shoot regeneration of bottle gourd. The same results were reported also with Cucurbita interspecific hybrids (Sarowar et al. 2003). In study of Dong and Jia (1991) it was found that the combination of IAA (0.6-17.1μM) with BA (4.4 – 31.1 μM) improved shoot regeneration efficiency using cotyledon explants of watermelon. Regeneration of adventitious shoots has been reported from a wide range of

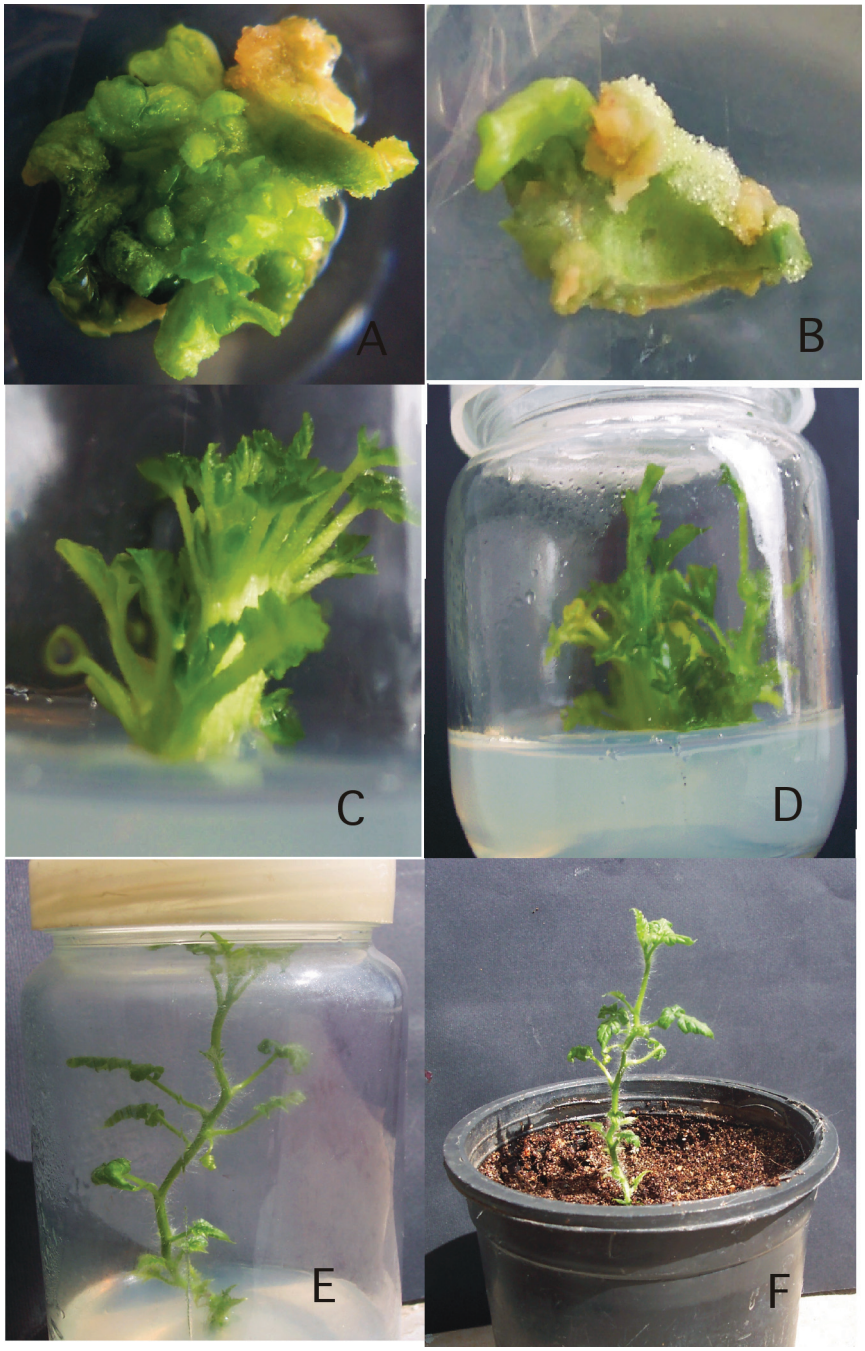

Figure 1. Regeneration of shoots from the cotyledon explants and shoot tip culture of triploid watermelon A and B: adventitious shoot initiation and regeneration from cotyledon explants; C and D: axillary shoot formation from shoot tips; E: elongated shoots cultured on rooting medium; F: acclimatized triploid watermelon plant.

diploid watermelon cultivars (Dong and Jia 1991, Compton and Gray 1993; Zhang et al. 1994; Compton 2000) and from other species like cucumber cotyledon explants (Mohiuddin et al. 1997; Selvaraj et al. 2007) and *Cucumis melo* (Molina and Nuez 1995). Tetraploid and triploid cotyledons generally have lower rates of regeneration and cotyledons of *in vitro* germinated seedlings were rated as the best sources of explants and had high organogenic competence (Compton and Gray 1993). Number of shoots per explants ranged from 2.6 to 5 (Table 1). Percentage of rooted micro-shoots ranged from 50 to 80%. Obtained data showed that watermelon hybrids tested have demonstrated some degree of shoot regeneration

Table 1. Effect of BA concentration on adventitious shoot organogenesis from cotyledon explants of two triploid watermelon hybrids.

Treatments Genotype	BA (µM)	Shoot regeneration frequency (%)	No of shoots per explants	Rooted micro-shoots (%)
SA100	2.22	64.2	3.0	80
	4.44	67.3	4.0	76
	10.0	73.6	5.0	65
	24.61	69.1	2.6	53
	44.4	65.4	2.1	50
SA101	2.22	47.1	4.0	75
	4.44	52.3	4.3	73
	10.0	53.2	5.0	62
	24.61	46.6	3.1	60
	44.4	46.2	3.0	55
LSD P= 0.05		0.39	0.37	1.81
P= 0.01		0.54	0.51	2.47

Table 2. Effect of BA concentration on axillary shoot formation from shoot tips of two triploid watermelon hybrids.

Treatments Genotype	BA (µM)	No of shoot per jar	Multiplication rate	Rooted plantlets (%)
SA100	2.22	10	2.0	80
	4.44	25	5.0	76.9
	10.0	28	5.6	79.2
	24.61	25	5.0	64.4
	44.4	14	2.8	63.3
SA101	2.22	10	2.0	85.8
	4.44	23	4.6	90.0
	10.0	21	4.2	86.7
	24.61	20	4.0	68.9
	44.4	16	3.2	67.8
LSD P= 0.05		1.43	0.83	3.59
P= 0.01		1.94	1.13	5.62

competence. Genotypic variation regeneration rate is common in watermelon (Zhang et al. 1994), in squash (Gonsalves et al. 1995), in muskmelon (Molina and Nuez 1995) and in cucumber (Mohiuddin et al. 1997). Results indicated that addition of BA to medium improved shoot production from watermelon explants but higher concentration of BA are liable to induce vitrified plants, which are usually difficult to root. Compton and Gray (1993) reported that the time frame required from seedling germination to acclimatization of the first plant is about 14 weeks.

Adventitious shoots differentiated directly with no intermediate callus stage and originated from the upper edge of the cotyledon explants (Fig. 1 A and B). These shoots can be easily separated from the original explants and cultured individually in glass jar (Fig.1 E). Adventitious shoots were rooted on MS medium supplemented with 0.1 µM NAA. Regenerated plantlets were acclimatized (Fig.1F) and grew into normal plants in the green house.

Plant regeneration from shoot tip of seedless watermelon

Two hybrids of triploid watermelon were tested for the established a protocol for the *in vitro* propagation of triploid watermelon.

Data presented in Table 2. show the effect of BA concentrations on axillary shoot formation from shoot tip explants of triploid watermelon. Data obtained showed that the differences among treatments were significant. MS medium containing 4.44, 10 and 24.61 µM BA showed the highest axillary shoot formation and multiplication rate (Fig.1C and D). The results indicated that the multiplication rate ranged in this study from 2 to 5.6 and the percentage of rooted micro-shoots ranged from 63.3 to 90%. Higher concentration of BA inhibits root formation. Watermelon plant have been obtained through micro-propagation of shoot tips (Anghel and Rosu 1985), somatic embryogenesis from cotyledons of immature embryos (Compton and Gray 1993) and adventitious shoot regeneration from cotyledon pieces (Dong and Jia 1991; Compton 1999). Compton and Gray (1992) observed that 1 cm shoot tip of triploid genotypes produced two axillary shoots every 21 days when cultured on MS medium containing 1 µM BA.

Rooted plants from both experiments were successfully acclimatized and gradually hardened-off to green-house conditions and subsequently established in soil with a survival rate of 80%.

Regenerated plants showed some phenotypic variations in plant height, internodes length and shoot color. The same results were observed by Thomas et al. (2006). They reported that some extent of dissimilarity in shoot height, inter node length and intensity of leaf were observed with the *in vitro* cultures and such differences were transient in nature and appeared to be related to the positional effect of the micro-cutting on the stock shoot. These modifications, which were not easily quantifiable, appeared to fit in to the description of 'epigenetic variation' (non-heritable variation) observed with the *in vitro* cultures, *i.e.*, phenotypic variability which has no genetic basis (Thomas et al. 2006). Mutations or off-types have not been reported from *in vitro* propagated plants. In this respect, Adelberg et al. (1997) observed that more than 1000 triploid watermelon plants propagated *in vitro* were true to type at maturity in the field.

In conclusion, high frequency regeneration of shoots was achieved using cotyledon explants of triploid watermelon via adventitious shoot organogenesis and shoot tip cultures. BA induced adventitious shoots from cotyledon explants. This regeneration system could be used in the production of transgenic watermelon plants and micro-propagation of elite hybrids of seedless watermelon by shoot tip culture.

References

Adelberg JW, Zhang XP, Rhodes BB (1997) Micropropagation of *Citrullus lanatus* (Thunb.) Matsum. and Nakai (Watermelon). In Y.P.S. Bajaj ed., Biotechnology in Agriculture and Forestry, Vol.39. High-Tech and Micropropagation V. Springer-Verlag Berlin, Heidelberg.

Andrus CF, Seshadri VS, Grimball PC (1971) Production of seedless watermelons. Agric Res Service USDA Technical Bulletin No., 1425.

Anghel I, Rosu A (1985) *In vitro* morphogenesis in diploid, triloid and tetraploid genotypes of watermelon, *Citrullus lanatus* (Thunb.). Mansf Rev Roum Biol.-Biol Végét 30:43-55.

Compton ME (1999) Dark pretreatment improves adventitious shoot organogenesis from cotyledons of diploid watermelon. Plant Cell Tiss Org cult 58:185-188.

Compton ME (2000) Interaction between explant size and cultivar impact shoot organogenesis competence of watermelon cotyledons. HortScience 35:749-750.

Compton ME, Gray DJ (1992) Micropropagation as a means of rapidly propagating triploid and tetraploid watermelon. Proc Fla State Hort Soc 105:352-354.

Compton ME, Gray DJ (1993) Shoot organogenesis and plant regeneration from cotyledon of diploid, triploid and tetraploid watermelon. J Amer Soc Hort Sci 118:151-157.

Compton ME, Gray DJ (2000) Shoot organogenesis from cotyledon explants of watermelon. In Trigiano RN. And DJ Gray, eds., Plant Tissue Culture Concept and Laboratory Exercises. 2nd edition. CRC press LLC.

Compton ME, Gray DJ, Gaba VP (2004) Use of tissue culture and biotechnologyfor the genetic improvement of watermelon. Plant Cell Tiss Org cult 77:231-243.

Decoteau DD (2000) Vegetable crops. Prentice Hall, Upper Saddle River NJ.

Dong JZ, Jia SR (1991) High efficiency plant regeneration from cotyledon of watermelon (*Citrullus vulgaris*). Plant Cell Rep 9:559-562.

Elmstrom GW, Maynard DN (1992) Growing seedless watermelon. Cooperative Extention Service Univ. of Fla. Institute of Food and Agric Sci Bulletin HS 687.

Gonsalves C, Xue B, Gonsalves D (1995) Somatic embryogenesis and regeneration from cotyledon explants of six squash cultivars. HortScience 30(6):1295-1297.

Han JS, Oh DG, Mok IG, Park HG, Kim CK (2004) Efficient plant regeneration from cotyledon explants of bottle gourd (*Legenaria siceraria* Standl.). Plant Cell Rep 23:291-296.

Jaskani MJ, Kwon SW, Kim DH (2005) Comparative study on vegetative, reproduction and qualitative traits of seven diploid and tetraploid watermelon lines. Euphytica 145:259-268.

Kihara H (1951) Triploid watermelon. Proc Amr Soc Hort Sci 58:217-230.

Lucier G, Lin HB (2001) Factors affecting watermelon consumption in the United States. In Annoymous, eds.,Vegetables and Specialties: Situation and outlook. VGS-287 (p. USDAERS pp. 23-29).

Marr CW, Gast KLB (1991) Reaction by consumer in a “farmers” market to price for seedless watermelon and ratings of eating quality. Horttechnology 1:105-106.

Maynard DN (1989) Triploid watermelon seed orientation affects seed coat adherence on emerged cotyledons. HortScience 24:603-604.

Mohiuddin AKM, Chowdhury MKU, Zaliha T, Abdullah ZC, Napis S (1997) Influence of silver nitrate (ethylene inhibitor) on cucumber *in vitro* shoot regeneration. Plant Cell Tiss Org Cult 51:75-78.

Molina RV, Nuez F (1995) Characterization and classification of different genotypes in population of *cucumis melo* based on their ability to regenerate shoots from leaf explants. Plant Cell Tiss Org Cult 43:249-257.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15:473-497.

Sarowar S, Oh HY, Hyung NI, Min BW, Harn CH, Yang SK, Oh SH, Shin JS (2003) *In vitro* micropropagation of a Cucurbita interspecific hybrid cultivar a root stock plant. Plant Cell Tiss Org Cult 75:179-182.

Selvaraj N, Vasudevan A, Manickavasagam M, Kasthurirengan S, Ganapathi A (2007) High frequency shoot regeneration from cotyledon explants of cucumber via organogenesis. Sci Hortic 112:2-8.

Thomas P, Prabhakara BS, Pitchaimuthu M (2006) Cleansing the long-term micropropagated triploid watermelon cultures from covert bacteria and field testing the plants for clonal fidelity and fertility during the 7-10 year period *in vitro*. Plant Cell Tiss Org Cult 85:317–329

Zhang XP, Rhodes BB, Adelberg TW (1994) Shoot regeneration from immature cotyledons of watermelon. Cucurbit Genet Coop Rep 17:111-115.