INDUCTION OF TETRAPLOID YELLOW WATERMELON INBRED BY COLCHICINE

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Abstract

Tetraploid watermelons of diverse genetic background are needed to synthesize hybrid triploid watermelon .We treated germinated seeds of five diploid watermelon (Yamato Cream, GingaMato, Oniku Kodama, Yellow crimson and Desert King cultivars) with a soaked solution of colchicines. A treatment with 400 mg/l of colchicine for 36 hour achieved the highest rates of 24.0%, 25.0 %, 27.0%, 23.0% and 29.3% in "(Yamato Cream, GingaMato, Oniku Kodama, Yellow crimson and Desert King), respectively. The number of chloroplasts per guard cell in tetraploid was 15-25, whereas it was 9-14 for diploid. Content of chlorophyll of leaves tetraploid plants had higher than diploid. We found that the tetraploid pollen grain has four colpi, while diploid pollen grain has three colpi. The leaves and seeds of the former were wider and shorter in tetraploid than those of the latter. Tetraploid watermelon fruits were smaller and the number of seeds was very low (50-70 seeds/fruit) when compared with diploid (250-350). The main objective of this research was to induce tetraploid yellow watermelon for producing seedless watermelon seeds.

Keywords: Watermelon, tetraploid, colchicines, chloroplasts, pollen grain and seeds.

INTRODUCTION

Triploid watermelons are becoming increasingly popular among consumers. Hybrid seed production is one of the most important industries nowadays all over the world. In watermelon, a special importance is paid towards the triploid hybrids (El-Aidy *et al.*, 2002). Triploid watermelons are becoming increasingly popular among consumers. (Omran ,S.A.2006) Tetraploid watermelons of diverse genetic background are needed to synthesize hybrid triploid watermelon (Zhang *et al.*, 1995). Tetraploids can be induced by applying aqueous colchicine solution to the growing apex of diploid seedling (Kihara 1951; Lower and Johnson 1969, and Wang *et al.*, 1993) , or by soaking diploid seeds in colchicine solution (Lower and Johnson 1969).The treatment of germinated seeds by colchicione and dinitroanilines gave the highest rates of tetraploid production compared with dry seeds (El-Aidy *et al.*, 2002 Omran , 2003 & 2006).

Chloroplast numbers per guard cell in tetraploid was higher, compared with diploid (El-Aidy *et al.*, 2002 and Omran 2003 & 2006). The use of chromosomes number to identify tetraploid is extremely time consuming. Therefore, alternative

methods include number of chloroplasts in stomata guard cells. Zhang *et al.* (1994) mentioned that it can be more easily estimated by counting the number of chloroplasts per guard cell pair of leaf epidermal cells. This technique has been used to differentiate between diploid and tetraploid regenerates of melon (Fassuliotis and Nelson 1992). The mean number of chloroplasts in stomata guard cell for tetraploid and diploid watermelon regenerates was 19.1 and 11.2, respectively. Jaejong Noh (2012) found that mean chloroplast counts were 12 and 19 in diploid and tetraploid, respectively. Also, they found that ploidy level from diploid to tetraploid increased the chloroplast density of guard cell pairs by approximately 1.7., Compton *et al.* (1996). Tetraploid watermelons have approximately 10-14 chloroplasts in each side of the guard cell (total in both sides 20-28), whereas diploids have only 5-6 in each side (total in both sides 10-12) (McCuistion and Wehner 2000). Jaskani, *et al.* (2005)found that thenumber of chloroplasts in guard cells ranged 5-7 and 10-12 in diploids and tetraploids, respectively.

The pollen grain surface of tetraploid watermelon was significantly greater than that of diploid watermelon, and pollen grain had four clopi, while pollen grain of the diploid had three clopi (Zhang *et al.*, 1994). Tetraploid pollen grain has four colpi and diploid pollen grain has three colpi (El-Aidy *et al*, 2002). Also, 4 colpi in pollen of tetraploid plants against 3 colpi in pollen of diploid were observed (Jaskani *et al.*, 2005).

The leaves of triploid and tetraploid plants were thicker and darker green than those of diploid of watermelon. Likewise, the leaves of tetraploid watermelon were wider, rounder and thicker than those of diploid plants, leaf margin of tetraploid plants was serration, compared to that of diploid (Kihara, 1951). The leaf lobes of tetraploid plants were rounder and highly overlap. In addition, leaf morphology of tetraploid plants was shorter than those of diploid (Zhang *et al.*, 1994). The leaf length/width ratio was greater of diploids watermelon than those of tetraploid plants (Compton *et al.*, 1996). Tetraploids watermelon plants usually have thicker leaves, slower growth and shorter stems than those of diploids (McCuistion and Wehner 2000). Compton *et al.* (1996), and Omran (2003) found that ovary diameter and both petal and anther diameter of male flowers were greater in tetraploid watermelon plants than those of the diploid plants.

Fruit weight and number of viable seeds formed in tetraploids were always few (Lower and Johmson 1969, Zhang *et al*, 1995, and Omran 2003 &2006) .

The objective of the current study is to produce tetraploid yellow watermelon to use its for produce seedless yellow watermelon hybrids.

MATERIALS AND METHODS

Five diploid (Yamato Cream ,GingaMato, Oniku Kodama, Yellow crimson and Desert King) were used to produce a tetraploid watermelon. The experiments were carried out at 2013 at laboratory and farm Horticulture Research Sakha Station, Agriculture Research Center in Egypt.Before the treatments the seeds (used of 100 seeds for cultivars) were dipped for 20 minutes in *HCl* at 20% and NaOClat 1% for 20 minutes.

Production of tetraploid watermelon:

Colchicine treatments: Germinated diploid seeds were soaked for (24, 36 and 48 hrs) in (400 mg/l colchicine solution).

Estimation of ploidy level:

Cytology: Examination of ploidy plants by cytology to identify tetraploid plants:

Chloroplasts number : The third or fourth expanded leaf from the apex was excised; a strip of epidermis was peeled from the leaf with fine forceps stained with 0.02% silver nitrate solution (Fassuliotis and Nelson 1992) or immersed in one drop of distilled water without staining, covered with a cover slip and examined under the microscope at x 400. Ten guard cell pairs were examined per leaf for a total of 30 per regenerated plants (Compton *et al.*, 1996). Stomata with clearly defined boundaries were measured with the number of chloroplasts within each pair of guard cell was counted and recorded chloroplast density for diploid and tetraploid plants.

Pollen grain: Data on five plants from each plot for 5 flowers per plant(diploid and tetraploid before flowers opening) Pollen grains were stained with a drop of 2% a cetocarmine and microscopically examined for satiability at x 400.

Morphological plant characters investigated:

Data from 5 plants of each genotype were compiled and correlation analysis has identified significant correlation between morphological characteristics and ploidy.

Leaves: Twenty leaves from each genotype and ploidy level were measured for length (L) / width (W).

Fruits characters investigated: Average fruit weight (Kg), No. of seeds/fruit and total soluble solids (T.S.S. % as a measure of sweetness) were determined by a hand Refractometer on at least five fruits from each entry at each picking. (A.O.A.C. 1980 and Maynard) .

The statistical procedure was achieved according to the regular analysis of variance of randomized complete blocks design. Duncan's Multiple Range Test was used for the comparison among the treatments (genotypes) means (Duncan, 1955).

RESULTS AND DISCUSSION

Production of tetraploid watermelon:

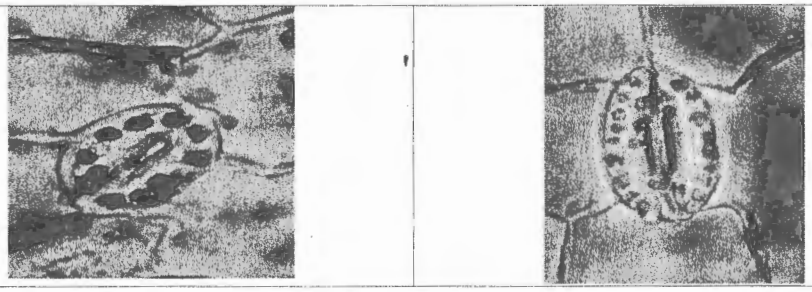
Treatments of colchicine*:* Data in Table 1 show the treatment illustrate that the diploid germinated seeds soaked in colchicine solution of 400 mg/l for 36 hrs. gave the highest rates of tetraploid plants by 24.0%, 25.0 %, 27.0%, 23.0% and 29.3% in Yamato Cream , GingaMato, Oniku Kodama, Yellow crimson and Desert King), respectively.

Identification of tetraploid plants:

Cytology:

Chloroplasts: We determine the chloroplast count per guard cell pair. Tetraploidy was confirmed by determining the chloroplast density of 5 tetraploid plants.

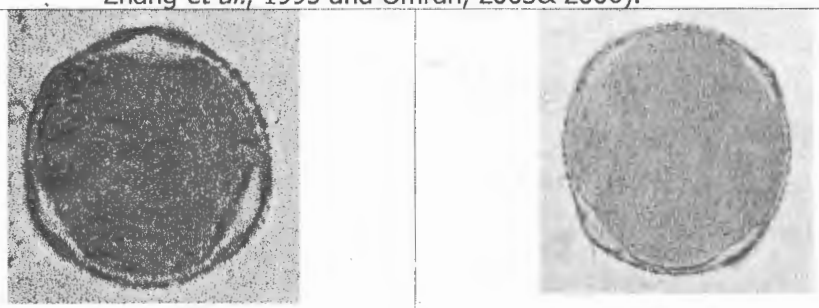
Data in Table 2, illustrated the average of tetraploid lines contained about 15 -25 chloroplast per guard cell pair to plants, compared with diploid plants which was 9-14 chloroplast (Fig.1.). (Zhang *et al.*, 1993 &1996 , Compton 1996 & 1999 and Omran 2003 & 2006) and tetraploid melon (Fassuloitis and Nelson 1992).



A B

Fig.1. chloroplast per guard cell pair diploid (A) and tetraploid (B).

Pollen grains: Fig.1. Show that the pollen grain of diploid had three clopi while the pollen grain of the tetraploid had four colpi(Wang*et al.*, 1993, Zhang *et al.*, 1995 and Omran, 2003& 2006).



A B

Fig.2. : pollen grain of diploid (A) and pollen grain of tetraploid (B) .

Morphological characteristics:

Leaves: The leaf length / width ratio of tetraploids was small by leaves than of diploid .

Table : (2) and Fig. 3.). Concerning of leaf morphology tetraploid plants were shorter ,wider, and rounder and the lobes of tetraploid leaves were rounder and highly overlapped. This result is similar to Zhang *et al.*,1995,Compton *et al.*,1996, and Omran, 2003 & 2006 .

Fruits : Average fruit weight and number of seeds per fruit for tetraploid watermelon were lower value thane diploid.Total soluble solid (T.S.S) of tetraploid was higher thane diploid .This results was similar to Zhang *et al* 1995 and Compton *et al* 1996 , and Omran,2003 &2006 .

Seeds : The seed length / width ratio of tetraploids was small by seeds than of diploid. Fig. 4.

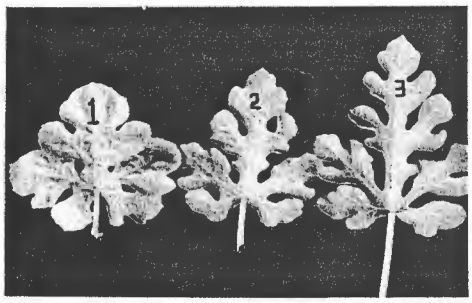
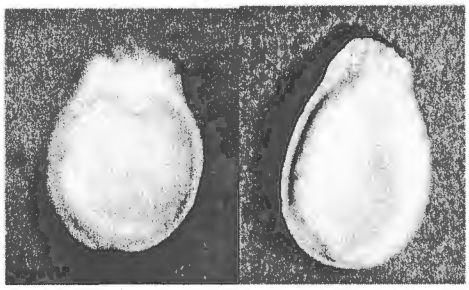


Fig.3. : leaf morphology plants of tetraploid (1) and diploid (2 & 3).



A **B**

Fig.4. : Seed morphology of tetraploid (A) and diploid (B).

Table (1): Show that production of tetraploid watermelon plants % by Colchicine.

Genotypes	Treatments
	Soaking period for germinated seeds 36 hrs. Concentration (400 mg / l)
	Tetraploid plants %
Yamato Cream	24.0
GingaMato	25.0
Oniku Kodama	27.0
Yellow crimson	23.0
Desert King	29.3

We used 100 seeds / line or cultivar

Table 2: Show that number of chloroplasts per guard cell pair, Length / Width ratio of leave, number of seeds/fruit and total soluble solid (T.S.S) of diploid and tetraploid watermelon plants.

Genotype		No. of chloroplasts	Length/width ratio of leave	Average fruit weight (Kg)	No. of seeds/fruit	T.S.S %
Yamato Cream	2x	12.7 b	1.2 a	3.5 a	350.6 a	11.3 b
	4x	24.2 a	0.81 b	1.4 b	60.4 b	12.6 a
GingaMato	2x	12.3 b	1.1 a	2.2 a	320.3 a	11.0 b
	4x	21.3 a	0.90 b	1.2 b	55.4 b	12.2 a
Oniku Kodama	2x	13.3 b	1.09 a	4.1 a	413.7 a	10.7 b
	4x	24.1 a	0.86 b	2.3 b	70.3 b	11.9 a
Yellow crimson	2x	9.6 b	1.02 4a	3.6 a	290.0 a	11.3 b
	4x	23.3 a	0.82 b	1.4 b	60.0 b	12.7 a
Desert King	2x	11.3 b	1.06 a	5.3 a	270.0 a	10.1 b
	4x	23.3 a	0.83 b	2.3 b	55.7 b	11.9 a
F.Test		**	*	**	**	*

Values having the same alphabetical letters within each column are not significantly different at the 5% level, according to Duncan's test.

CONCLUSION

The use of colchicine for chromosome doubling in watermelon .A treatment with 400 mg/l of colchicine for 36 hour. achieved the highest rates of tetraploid plants and use sample way to identify tetraploid by chloroplasts and pollen grain phase .We obtained five tetraploid yellow watermelon ,after identify at tetraploid watermelon. we inbreeding it's for tow generation , It's will use to produce hybrids triploid yellow watermelon .

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إنتاج سلالات بطيخ صفراء رباعية بواسطة الكولشيسين

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الهدف الرئيسي من البحث هو إنتاج سلالات بطيخ صفراء رباعية لتستخدم فى إنتاج البطيخ الاصفرعديم البذور .تمت المعامله للبذور المستنبتة بطول 25ملليمتر ثلاث سلالات يابانيه و صنفان امريكيان بالنقع فى الكولشيسين .أنتجت المعاملة ب 400 ملليجرام /لتر كولشيسين مع النقع للبذور لمده 36ساعة أعلى نسبه مئوية لانتاج النباتات الرباعية من السلالات و الأصناف على التوالى

1- L. Yamato Cream , 2 – L. GingaMato, 3- L. Oniku Kodama, 4- Yellow crimson cv , 5- Desert King cv

تم التعرف على النباتات الرباعية:

سيتولوجيا : كان عدد الكلوروبلاست فى الخلية الحارسة يتراوح من 25 : 15كلوروبلاست للنباتات الرباعية ,و كان يتراوح من 14 : 9كلوروبلاست للثنائية ,شكل حبوب اللقاح في متك الأزهار الرباعية كانت تحتوى على 4فتحات إنبات اى مربعه الشكل بينما فى الثنائية كانت تحتوى على 3فتحات إنبات أي أنها مثلثة الشكل .

مورفولوجيا : (شكل الأوراق فى النباتات الرباعية قصيرة وعريضة) الطول / العرض كان اكبر من الواحد الصحيح (أما في الثنائية) الطول /العرض كان اقل من الواحد الصحيح ، الثمار الرباعية كانت اصغر فى الحجم من الثمار الثنائية ,و كان عدد البذور في الثمار الرباعية اقل جدا من الثمار الثنائية.