TETRAPLOID WATERMELON INDUCTION OF EGYPTIAN CULTIVARS BY DINITROANILINE AND COLCHICINE

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ABSTRACT

Hybrid seed production is one of the most important industry nowadays all over the world. In watermelon, a special importance is paid towards the triploid hybrids. The main objective of this research was to induce tetraploid watermelon from the Egyptian varieties for use in triploid seed production, by treating dry and germinated seeds ones of diploid with soaked solution of dinitroaniline and colchicine . The treatemnt with 15 mg/l dinitroaniline for 48 hrs gave the highest rates of tetraploid production in "Giza 1" and "Giza 21",the percentage was 22 % and 24 % of dry seeds, respectively. In germinated seeds, the treatment with 12 mg/l solution for 36 hrs gave the highest rates in "Giza 1" and "Giza 21", 50.% and 51%, respectively. The treatment of dry seeds with 500 mg/l colchicine for 60 hrs gave the highest rates of tetraploid production, 21.2% and 22% in "Giza 1" and "Giza 21", respectively. In germinated seeds, the treatment with 500mg/l solution for 48 hrs gave the highest rates, 26.2% and 28% in "Giza 1" and "Giza 21", respectively. Chloroplast numbers per guard cell in tetraploid was 17.-26, whereas those of diploid was 9-16. Tetraploid pollen grain has four colpi and diploid pollen grain has three colpi. Size of pollen grain of tetraploid was bigger than that of diploid. Tetraploid leaves were wider and shorter than diploid . Tetraploid watermelon fruits were smaller than diploid, and seeds numbers per fruit were very low than those in diploid. Progenies were obtained from self-pollination of fertile tetraploid for using induction of F1 seedless watermelon.

INTRODUCTION

Tetraploid watermelon of diverse genetic background are needed to synthesize hybrid triploid watermelon (**Zhang *et al.*, 1994**). Tetraploids can be induced by applying aqueaus colchicine sloution 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**). However, the frequency of tetraploids in the treated population is low(less than 5%) in most cases (**Lower and Johnson, 1969**). Dinitroanilines have been used to double chromosome

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numbers, and their effectiveness has previously been compared with other crops. However, tetraploid watermelon can be induced by colchicione and dinitroanilines (**YingLi *et al.*, 1999**). Fruit weight and number of viable seeds formed in tetraploids were always few. (**Lower and Johmson 1969,Ezura 1993 and Zhang *et al* 1994**). Recently, due to the environmental concern, more and more scientists are using colchicine alternates for chromosome dubling with cell or microspor culture and used tissue culture. Uses of tissue culture to induction tetraploid or triploid are very extremely time consuming. The objectives of the current study is to produce an Egyptian triploid hybrid , easily germinated and decreases the cost of its production by attempted to develop a new simple method for induction of tetraploid watermelon .

MATERIAL AND METHODS

A total of two diploid local varieties (Giza1 and Giza 21) were used to produce a tetraploid to line in producing triploid watermelon. Seeds were dipped for 20 minutes in *Hcl* at 20% and *Naocl* at 1% for 20 minutes before the treatments.

1- Induction of tetraploid watermelon

1.1- Dinitroanilines treatments

1.1.1-The first method

Dry diploid seeds were soaked(for 12, 24, 36, 48 and 60 hrs) in dinitroanilines solution (1.5, 3, 6, 12 and 15 mg/l).

1.1.2-The second method

Germinated diploid seeds were dipped(for 12,24,36 ,48 and 60 hrs) in dinitroaniline solution(1.5,3,6,12 and 15mg/l).

1.2- Colchicine treatments

1.2.1- The first method

Dry diploid seeds were soaked (for 12, 24, 36 , 48 and 60 hrs) in colchicine solution (50,100,200,400, 500 and1000 mg/l).

1.2.2-The second method

Germinated diploid seeds were soaked (for 12, 24,36,48 and 60 hrs) in colchicine solution (50, 100, 200, 400 , 500 and 1000 mg/l).

2- Estimation of ploidy level

2.1- Cytology

Examination of ploidy plants by cytology to identify tetraploid plants:

2.1.1- Chromosome number

Chromosome number was used to identify tetraploid and diploid plants according to **(Trivedi and Ray1970)**

2.1.2- Chloroplasts number

The use of chromosome number to identify tetraploid is extremely time consuming. Therefore, alternative methods have been used for watermelon **(Zhang *et al* 1994)**. 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 and the number of chloroplasts within each pair of guard cell was counted and recorded chloroplast density for diploid and tetraploid.

2.1.3-Pollen grain

Pollen shape and size were determined by microscopic examination after staining with a cetocarmine **(Zhang *et al* 1994)**. Pollen grains (diploid and tetraploid) were stained with a drop of 2% a cetocarmine and microscopically examined for stainability and size. Pollen diameter was measured with a micrometer eyepiece.**(Lower *et al* 1969)**.

3- Data Recorded at diploid and tetraploid

3.1. Morphological plant characters investigated

Data on 5 plants from each line were compiled and correlation analysis was done to identify the significant correlation between the morphological characteristics and ploidy.

3.1.1- Leaves

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

3.1.2. Flowers

3.1.2.1. Male flowers

Data on anthesis of the first flower, counted in days after the date of transplanting into the field.

Diameter of petal (cm), Length and width of anther(cm) were measured on the morning of the first day of the flower anthesis(Abd-EL-Hafez,1969).

3.1.2.2. Female flowers

Diameter of petal (cm) , diameter of overy (cm) and length, and width of ovary, were measured on the morning of the first day of the flower antithesis(Abd-EL-Hafez,1969).

3.1.3. Fruits characters investigated

Length and width (cm), weight (kg.), rind thickness (cm) , and number of seeds/fruit (Only the mature seeds were counted in each fruit.).

RESULTS & DISCUSSION

1- Dinitroaniline treatments

Data in Fig.(1) indicate that the diploid dry seeds soaked in dinitroainliline solution of 15 mg/l for 48 hrs gave the highest induced tetraploid watermelon, the precentage was 22% and 24% for Giza 1 & Giza 21, respectively.

Data in Fig. (2) show that the diploid germinated seeds soaked in dinitroaniline solution of 12 mg/l for 36 hrs gave the highest induced tetraploid ,the precentage was 50% and 51 % for Giza 1& Giza 21, respectively.

2- Colchicine treatments

Data in Fig.(3)illustrate that the diploid dry seeds soaked in colchicine solution of 500 mg/l for 60 hrs gave the highest induced tetraploid .The results were 21.2 % and 22% for Giza1& Giza 21 ,respectively. Data in Fig.(4)show that the diploid germinated seeds soaked in colchicine solution of 500 mg/l for 48 hrs gave the highest induced tetraplaid. The values were 26.2% and 28% for Giza 1 & Giza 21,respectively.

Identification of tetraploid plants

1- Chromosome counts

Fig. (5) show the chromosome counts from root tip for diploid (2n = 22x) and tetraploid (4n = 44 x).

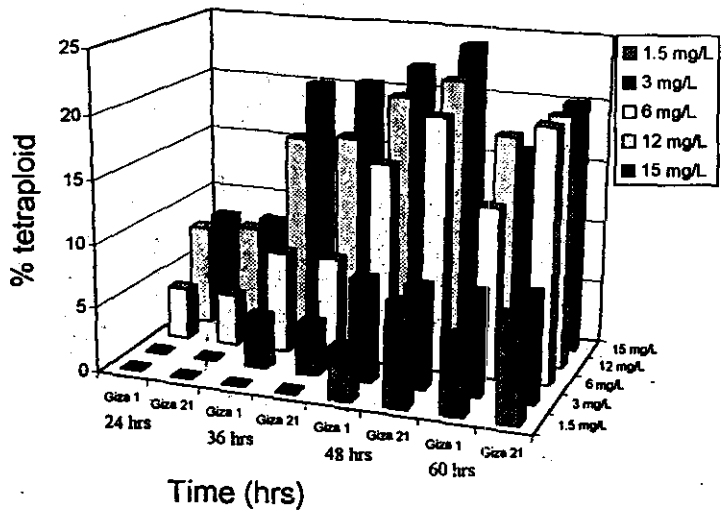


Fig. (1): Effect of treating dry seeds with soaked dinitroaniline solution.

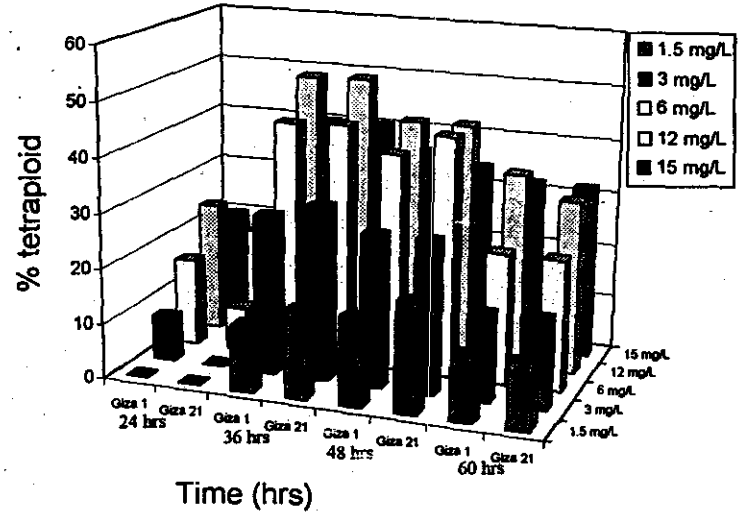


Fig. (2): Effect of treating germination seeds with soaked dinitroaniline solution.

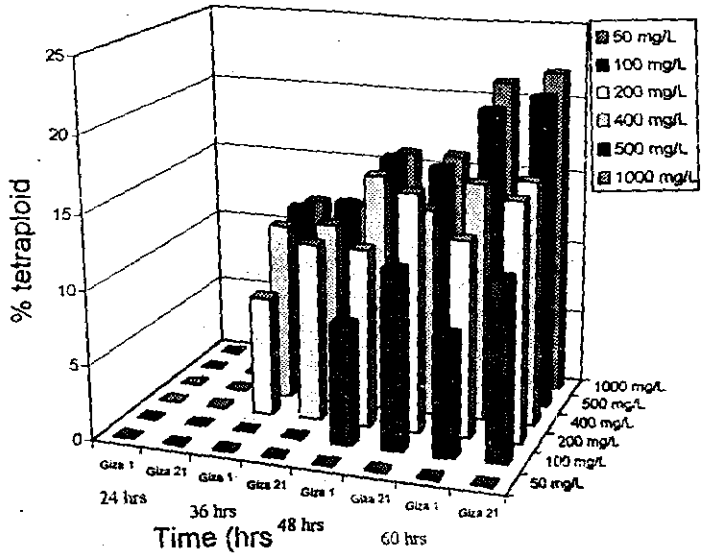


Fig. (3): Effect of treating dry seeds with soaked colchicine solution.

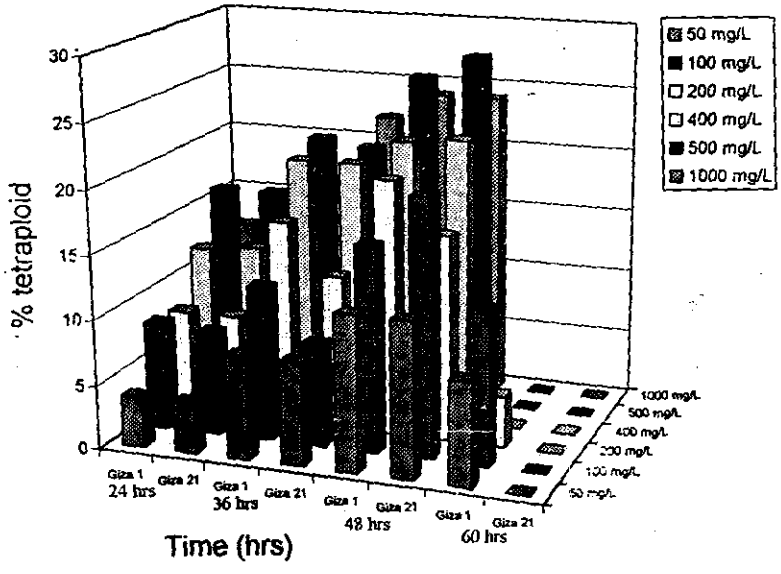


Fig. (4): Effect of treating germination seeds with soaked colchicine solution.

2- Chloroplasts

Identifcation in was done by counting the number of chloroplast per guard cell pair. Tetraploidy was confirmed by determining the chloroplast density of two line of tetraploid.

Data in Table (1) and Fig. (6) illustrate that average of tetraploid lines contained about 17-26 chloroplast per guard cell pair to plants, compared with diploid plants which was 9-16 chloroplasts. There was no significant difference between the lines.

Table(1) show that average length of stomata for tetraploid was enlarged stomatas with more chloroplasts in the guard cells (40.0 um), compared with the diploid which had smaller stomatas (33.7 um for both cultivars).These results are similar to that reported by identification of tetraploid watermelon (**Zhang *et al.*, 1994 and Compton 1996**) and tetraploid melon (**Fassuloitis and Nelson 1992**)..

3- **Pollen grains**

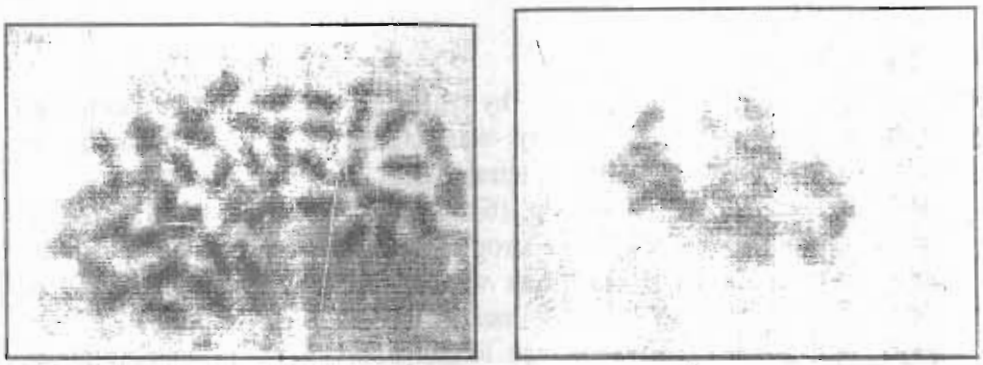
Pollen grain diameter of diploid and tetraploid, were measured.Data in Table (1) show that pollen grains from all tetrapliods were larger than those diploid. The pollen grain diameter of tetraploids (43.0 um) was significantly greater than that of diploid (29.3 um) of both varieties (Giza.1 & Giza.21).

This differences were in agreements with data reported by (**Kihara 1951, Lower and Jonson, 1969, and Zhang et *al* 1994**).In addition, the pollen grain of diploid had three clopi while the pollen grain of the tetraploid had four colpi Fig (7) pollen grain with four colpi were Common in tetraploid watermelons which were distinct from three colpi pollen grain of diploid watermelon and this may be due to their cubical shape (**Fassuliatis and Nelson 1992, Adelberg *et al* 1993, 1994 and Zhang *et al* 1994**).

4- Morphological characteristics

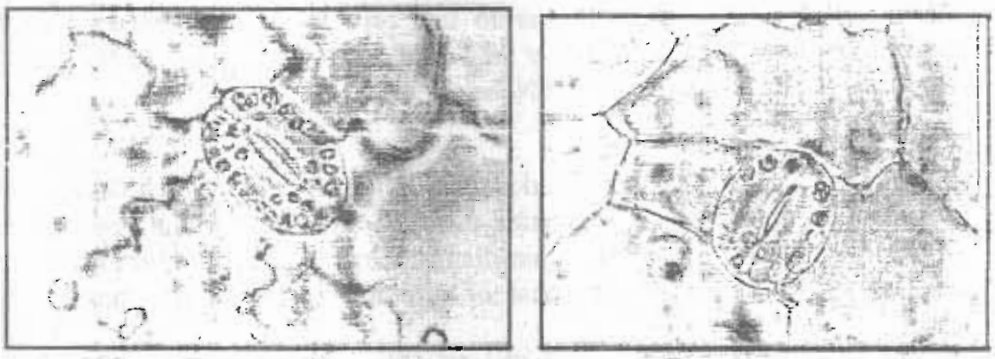
Data in Table (2) and Fig. (9&10) indicate that average petal diameter, ovary length and average diameter for female flowers of tetraploid had higher values than diploid flowers. Also tetraploid male flowers(petal diameter and anther diameter) were larger, compared with diploid flowers. There was significant different between tow lines in both tetraploid and diploid plants for all characters.

The leaf length/width ratio was greater for diploids than tetraploids. The leaf length/width ratio of tetraploids (0.756) was significantly less than of diploid (1.084), Table (2) and Fig (8). In addition, leaves tetraploid



A B

Fig. (5): Chromosomes (A) 4n, and (B) 2n of water melon.

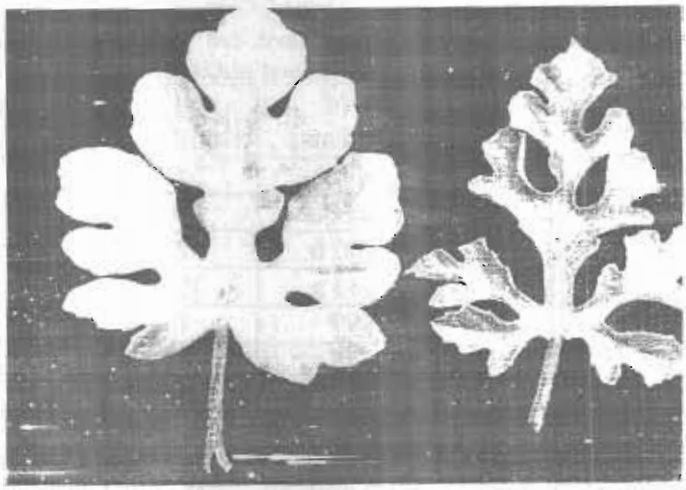


A B

Fig. (6): Guard cell chloroplasts from (A) tetraploid and (B) diploids watermelon plants.

A B

Fig. (7): Pollen grain morphology of tetraploid (A) and diploid watermelon (B). Pollen grains were from field grown plants.

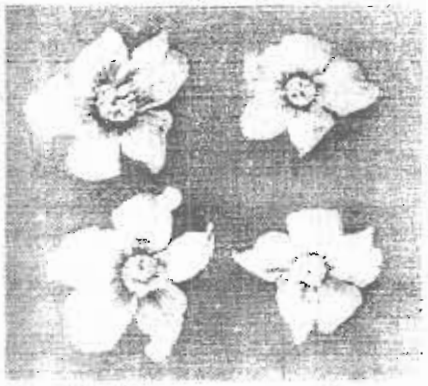


A B

Fig. (8): Leaf morphology of tetraploid (A) and diploid (B) of watermelons after 4 weeks.

A. B

Fig. (9): Morphology characteristcs of watermelon female flower tetraploid (A) and diploid (B).



A B

Fig. (10): Morphology characteristics of watermelon male flower tetraploid (A) and diploid (B).

plants were shorter and wider, rounder and the lobes of tetraploid leaves were rounder and highly overlapped . These results are similar to (**Zhang *et al.*, 1994 and Compton *et al.*, 1996).**

Table (1): Number of chloroplasts per guard cell pair of diploid and tetraploid watermelon plants from leaves and poiien grains diameter.

Genotyp		No. of chloroplasts	Average of stomata length	Pollen grains diameter um
Giza 1	2x	10.600 b	35.367 c	29.3 b
	4x	25.667 a	40.433 a	43.0 a
Giza 21	2x	10.300 b	32.267 d	29.2 b
	4x	25.233 a	39.167 b	42.0 a
L.S.D. 5%		1.497	0.658	1.835
L.S.D. 1%		2.673	0.911	2.542

Table (2): Morphological characteristics of flowers and leaves from diploid and tetraplaid watermelon plants.

Genotype		Female flowers			Male fiowers		Leaves		
		Petal diameter (cm)	Overy length (cm)	Overy diameter (cm)	Petal diameter (cm)	Another diameter (cm) .	Length (cm)	Width (cm)	L/W ratio
Giza 1	2x	2.973	1.010	0.750	2.523	0.580	9.137	8.417	1.090
	4x	3.920	1.860	1.230	3.720	0.780	9.117	11.687	0.763
Giza 21	2x	3.000	1.200	0.830	3.300	0.610	9.203	8.500	1.080
	4x	3.700	2.010	1.310	3.830	0.850	9.147	12.230	0.757
L.S.D.5%		0.155	0.065	0.074	0.959	0.042	0.029	0.402	0.015
L.S.D.1%		0.225	0.094	0.107	1.394	0.061	0.042	0.585	0.022

Table (3): Number of seeds, weight, Rind thickness and diameter of fruits (2 x and 4 x).

Genatyp		No. of fruits tested	No. of average seeds per fruit	Average weight of fruit (kg)	Average . rind thickness of fruit	Average diameter of fruit
Giza1	2x	5	407.95 a	4.275 a	1.2 a	24.3 a
	4x	5	81.55 b	2.125 b	0.6 b	12.5 b
Giza21	2x	5	420.63 a	3.900 a	1.3 a	25 a
	4x	5	82.73 b	1.900 b	0.6 b	12.1 b
L.S.D.5%			59.6	0.2	0.6	0.8
L.S.D.1%			82.5	0.3	1.2	1.2

Data in Table (3) indicate that average fruit weight (2.125 & 1.900 kg) and number of seeds (81.55&82.73) per fruit for two line tetraploid watermelon had lower values thane those diploid of fruit weight (4.235.1&3.900 420.63)and number of seeds (407.95& 420.63)obtaned from Giza 1& Giza 21 ,respectively .These results are similar to **Nugent and Ray 1992, Ezuro *et al* 1993, Zhang *et al* 1994 and Compton *et al* 1996.**

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الملخص العربى

إنتاج البطيخ الرباعى من الأصناف المصرية بواسطة الداى نيتروأنيلين والكولشيسين

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إنتاج بذور الهجن هامة جداً فى العالم خاصة فى البطيخ وبخاصة إنتاج البطيخ اللابذرى ، الهـدف الرئيسى من هذا البحث إنتاج البطيخ الرباعى من الأصناف المصرية لإستخدامها فى أنتاج بذور هجن البطيخ اللابذرى (الثلاثى).

- ***المعاملة بالداى نيتروأنيلين:***

- أعطت معاملة البذور الجافة بالنقع بتركيز ١٥ ملجرام/لتر لمدة ٤٨ ساعة أعلى نسبة نباتات رباعية فى صنفى جيزة ١ وجيزة ٢١ وكانت النسبة ٢٢% ، ٢٤% على الترتيب.
- أعطـت معاملـة البذور الملسنة بالنقع بتركيز ١٢ ملجرام/لتر لمدة ٣٦ ساعة أعلى نسبة نباتات رباعية فى صنفى جيزة ١، جيزة ٢١ وكانت النسبة ٥٠% ، ٥١% على الترتيب.

- ***المعاملة بالكولشيسين:***

- أعطـت معاملـة البذور الجافة بالنقع بتركيز ٥٠٠ ملجرام/لتر لمدة ٦٠ ساعة أعلى نسبة نباتات رباعية فى صنفى جيزة ١ وجيزة ٢١ وكانت النسبة ٢١,٢% ، ٢٢% على الترتيب.
- أعطت معاملة البذور الملسنة بالنقع فى تركيز ٥٠٠ ملجرام/لتر لمدة ٤٨ ساعة أعلى نسبة نباتات رباعية فى صنفى جيزة ١ وجيزة ٢١ وكانت النسبة ٢٦,٢% ، ٢٨% على الترتيب.

- ***فحصت النباتات الرباعية ووجد أن :-***

- عدد الكلوروبلاست فى الخلايا الحارسة للنباتات الرباعية كان عددها يتراوح من ١٧ – ٢٦ كلوروبلاست وفى النباتات الثنائية كان عددها من ٩ – ١٦.
- شـكل حبوب اللقاح الرباعية كانت مربعة الشكل ولها ٤ فتحات إنبات أما فى الثنائية كانت مثلثة الشكل ولها ٣ فتحات إنبات ، علاوة على أن حبوب اللقاح الرباعية كانت أكبر حجماً من الثنائية.
- الأوراق الرباعية كانت أقصر وأعرض ومستديرة عن الأوراق الثنائية .
- الثمار الرباعية كانت أقل فى الوزن وعدد البذور بالمقارنة من الثمار الثنائية.
- تم التلقيح الذاتى للنباتات الخصبة لإستخدامها فى إنتاج هجن البطيخ اللابذرى (الثلاثى).