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FLOW CYTOMETRY OF DNA CONTENTS OF COLCHICINE TREATED WATERMELON AS A PLOIDY SCREENING METHOD AT M1 STAGE

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Abstract

Flow cytometric analysis of DNA content in watermelon was conducted to identify tetraploids among colchicine treated seedlings and to compare flow cytometry with other methods of ploidy screening such as chloroplast number in stomata guard cells, chromosome counts, leaf area, flower size, pollen colpi and seed characteristics. Flow cytometric analysis proved rapid and convenient to detect tetraploids compared with chloroplast counts. DNA index and chloroplast number were highly correlated. Number of chloroplasts in guard cells ranged from 5-7 and 10-12 in diploids and tetraploids, respectively but it was impossible to differentiate chimeras by chloroplast counts. Chromosome counts were tedious while other methods needed more time to identify tetraploids. Higher leaf area and larger flower size, and 4 colpi in pollen of tetraploid plants against 3 colpi in pollen of diploid were observed. Tetraploid seed showed larger size and thicker seedcoat and partially filled seed cavity due to less developed cotyledons as compared to diploids.

Introduction

Excessive seed number in watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai] 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 & Lin, 2001). Seedless watermelon cultivars are in high demand by consumers not only because their fruit are seedless but also because they are sweeter than fruit from diploid and seeded cultivars (Marr & Gast, 1991). Seedless watermelons are triploid (2n=3x=33) hybrids (Kihara, 1951). 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 breeding lines have been created for use in producing triploid hybrids (Andrus *et al.*, 1971). The lack of suitable tetraploid breeding parents has, however, hampered progress towards the development of a wide range of quality seedless cultivars.

Traditionally, tetraploid parents have been obtained by treating newly emerged diploid seedlings with colchicine (Suying *et al*., 1995). However, this treatment produces a limited number of tetraploids and mostly chimeric seedlings that possess vines of mixed ploidy (Compton *et al*., 1994; Jaskani *et al*., 2004). The production of polyploid regenerants from tissue culture colchiploids has been reported for many plant species (Jaskani *et al*., 1996; Veilleux & Johnson, 1998) and has potential application to establish a large number of new watermelon tetraploid breeding lines (Zhang *et al*., 1994).

The identification of tetraploid plants may require morphological, isozyme, cytological and even molecular techniques. Chromosome counting is the usual method to determine ploidy (Sari *et al*., 1999; Jaskani & Khan, 2000) but this is difficult in watermelon due to small chromosome size. The method is not practical for non-dividing

cells in differentiated tissues, such as leaves (Fahleson *et al*., 1988), since only root-tip meristems (representing the LIII layer) are usually used for chromosomal analysis. In watermelon, induced tetraploid plants are identified by counting the number of chloroplasts per guard cell pair of fully expanded leaves (McCuistion & Elmstrom, 1993). Studies have shown that diploid and tetraploid watermelon plants possess variable number of chloroplasts in guard cells of stomata (Koh, 2000). Ploidy can be estimated by examining plant morphological traits such as leaf and flower size (Rhodes & Zhang, 1999). Tetraploid plants generally produce leaves that are broader than diploids. Confirmation of tetraploidy can be obtained by comparing the size of the pollen grains (about 1.44 X larger than diploid pollen), and the number of colpi (4 versus 3) (Rhodes & Zhang, 1999). Seed size and shape may also be used to identify polyploids, as tetraploid seeds are generally larger and thicker than diploid seeds of the same genotype (Kihara, 1951).

Although plant and seed morphological traits are used as ploidy screening methods, they vary with genotype and are less reliable than root tip chromosome or chloroplast counts (Compton *et al*., 1996). These methods, used to determine ploidy in some species have been found to be non reproducible (van Denhout *et al*., 1995; Tenkouano *et al*., 1998). Moreover, in watermelon colchicine treatments induce only 4-6% pure tetraploids (Jaskani *et al*., 2004) and growing large number of treated seedlings up to flowering or harvest for ploidy characterization by conventional methods is impractical. This necessitates a method for early and rapid screening of tetraploids to save time and space. Flow cytometry is used for ploidy analyses and is regarded as the most accurate tool for ploidy determination (Dolezel, 1998) and can be used to estimate plant ploidy in watermelon. This method requires only a small amount of tissue and is therefore non-destructive, and has the ability to analyze large populations of cells where mixoploidy or aneuploidy exist (Dolezel, 1997).

The aim of the present investigation was to develop an efficient system for the rapid detection of tetraploidy in watermelon induced by colchicine application *in vivo*. The treated plants were characterized by DNA flow cytometry and compared its usefulness with chloroplast counts and morphological characteristics and confirmed by microscopic studies.

Materials and Methods

Colchicine application: Nine diploid watermelon lines 920532, SS1, SS5, SS7, SS8, NH1, NH2, NH3 and M174 were tested to induce tetraploids. A total of 1000 seeds, 100 of each line were germinated at 30°C in an incubator for 48 hours, then planted in pots and placed in greenhouse under high humidity until seedling emergence. Ninety seedlings of each line were treated with three concentrations of colchicine, 0.2, 0.4 and 0.6% concentration and 10 seedlings of each line were grown as control. Colchicine solution (2-3 drops) was injected with 1 ml syringe in the meristem of seedlings at true leaf emergence stage twice daily for three consecutive days (Jaskani *et al*., 2004). Data were analyzed as a 9x3 factorial (lines and colchicine treatment) design with three replications, 30 seedlings in each replication. Data were subjected to an analysis of variance and means were separated by $LSD_{0.05}$.

Chloroplast counts in stomata guard cells: The first ploidy evaluation of seedlings was made by number of chloroplasts in each side of guard cells of stomata at 3-5 true leaves

emergence stage. The lower epidermis was removed by piercing leaf with hand and placed on the glass slide after addition of one drop of distilled water. The number of chloroplasts was scored under the microscope at 10x10 or 10x40 magnification. Five stomata per leaf were observed. On the basis of this evaluation, the putative tetraploid plants were transplanted in the greenhouse.

Flow cytometric analysis: To reconfirm the ploidy of already screened out plants on the basis of chloroplast counts, flow cytometric analysis was made using PA-1 (Partec, Germany) flow cytometer. Leaf tissue (0.5 cm^2) each of the 70 selected plants was chopped with a sharp razor blade in a 50x12 mm plastic Petri dish with 500 µl nucleus-isolation buffer. The sample was filtered through a 30-µm nylon mesh and 1 ml DNA staining solution added for nuclear DNA content estimation. To determine the standard peak of diploid cells (2C DNA), at least 10 leaves were collected from diploid (2n=2x=22) plants grown in the greenhouse. The standard peak was programmed to appear at about channel 50 of relative fluorescent intensity. This setting was kept constant and readjustments were made frequently. In each sample, 40,000-80,000 nuclei were analyzed. The relationship between number of chloroplasts and DNA index was also explored in 30 samples. DNA content of tetraploid watermelon sample from Honam University, Korea (HUK) was used to calculate DNA index of samples. Relative DNA content of individual plants was expressed using a DNA index (DI) calculated according to the following formula:

$$DI = \frac{\text{Mean of the relative DNA content of the } G_1/G_2 \text{ or } G_2/G_3 \text{ nuclei of the sample}}{\text{Mean of the relative DNA content of the } G_1/G_2 \text{ nuclei of HUK tetraploid}}$$

Leaf, flower, pollen and seed observations: The leaf area of diploid and tetraploid plants was also measured. Three leaves from 7^{th}, 9^{th} and 11^{th} node of 10 plants were selected and measured by Li-Cor LI-3100 area meter. The difference in diploid and tetraploid flower size was recorded under Camscope with IT Pro Image Tracer software. The number of colpi in pollen of diploid and tetraploid plants was investigated under the light microscope. Pollen was placed on a drop of water on a glass slide and observed under the microscope magnified by 10x40. The differences in seed morphology of diploids and tetraploids were observed as seed size, thickness, seed coat thickness and embryo development.

Chromosome counts: For chromosome counts, apical meristems were harvested at 9:30 a.m. from greenhouse-grown plants and then 1-2 mm samples excised with a razor blade for better chemical penetration. Samples were prepared as prefixed in saturated P-dichlorobenzene (1.5%) at 15ºC for 2½ hours using Eppendorf microcentrifuge tubes, washed in distilled water, transferred to 3:1 fixative (95% ethanol:glacial acetic acid) for 48 hours at 30ºC, hydrolyzed in 1N HCl for 10 minutes at 60ºC after washing with distilled water, stained in Schiff's reagent for 3 hours in the dark at room temperature, placed in ice cold water for 20 minutes, incubated in pectinase for 1½ hours at 30ºC, transferred to 70% ethanol and stored in refrigerator. Chromosome counts were made under the microscope at magnification 10x100 after squashing the meristem in a drop of 0.5% aceto-carmine stain on glass slide.

Results

Slower growth rate and delayed appearance of shoots was observed in colchicine treated seedlings. The application of higher colchicine concentrations made the first leaves emerge with rosette-like growth and at the same time increased the mortality rates. Mortality rate in treated seedlings indicated that higher colchicine concentration (0.6%) was toxic and showed overall 50% mortality (Table 1). However, watermelon lines showed variable tolerance against colchicine treatments. Lines SS-7 and SS-8 showed low mortality but higher polyploids induction. It was further noted that the increase in colchicine concentration reduced the number of polyploids and it was 49.6% and 37.9% at 0.4% and 0.6% colchicine, respectively (Table 1).

Chloroplast counts: The first screening of colchicine treated seedlings was made by the chloroplast counts in each side of stomata guard cell pair (Table 2). In diploids, number of chloroplasts ranged from 5 to 7 (Fig. 1a) and tetraploids yielded 10-12 chloroplasts (Fig. 1b) in each side of guard cell pair.

Table 1. Percent mortality and polyploids induced by injecting colchicine solution into apical meristem of watermelon seedlings.

Lines	Mortality[z] (%)			Polyploidy[y] (%)		
	0.2%	0.4%	0.6%	0.2%	0.4%	0.6%
920532	20.0 ijk [x]	30.0 h	40.0 ef	73.9 b	56.0 e	57.1 e
SS-1	15.0 l	30.0 h	35.0 g	47.3 fgh	25.0 jk	13.1 n
SS-5	17.3 kl	21.7 ij	65.2 b	43.7 h	36.4 i	35.0 i
SS-7	23.8 i	28.6 h	52.4 c	83.1 a	75.0 b	65.2 d
SS-8	18.2 j	31.4 gh	44.2 de	76.5 b	66.7 d	50.0 f
NH1	28.6 h	42.8 ef	50.9 d	33.3 i	27.6 j	21.4 kl
NH2	31.2 gh	43.7 def	81.5 a	60.0 e	47.6 fg	18.3 lm
NH3	5.1 n	47.9 d	61.9 b	72.0 bc	69.5 cd	67.0 d
M174	9.2 m	14.3 k	19.0 ijk	56.2 e	42.8 h	14.3 mn
Mean	**18.7 c**	**32.3 b**	**50.0 a**	**60.6 a**	**49.6 b**	**37.9 c**

[z]Mortality was recorded after 3 weeks of colchicine treatment.

[y]Ploidy was investigated by chloroplast count at 3-5 true leaf stages.

[x]Any two means within treatments not followed by the same letter are significantly different at $P \leq 0.05$

Table 2. Number of chloroplasts in each side of stomata guard cells and differences in male flower and seed size of diploid and tetraploid watermelon.

Observations	Diploid	Tetraploid
No. of chloroplasts	5-7	10-12
Flower diameter (mm)	36.7 ± 3.76	41.2 ± 1.95
Leaf area (cm^2)	140.02 ± 28.9 [y]	182.09 ± 35.1
Seed length (mm)	8.1 ± 0.51[x]	9.1 ± 0.59
Seed width (mm)	5.2 ± 0.31	6.3 ± 0.30
Seed thickness (mm)	2.2 ± 0.16	2.8 ± 0.51
Seed coat thickness (mm)	0.5 ± 0.05	0.6 ± 0.10

[z]Mean ± Standard deviation of ten flowers; [y]7, 9 and 11th leaf of ten plants and [x]fifteen seeds of each genotype.

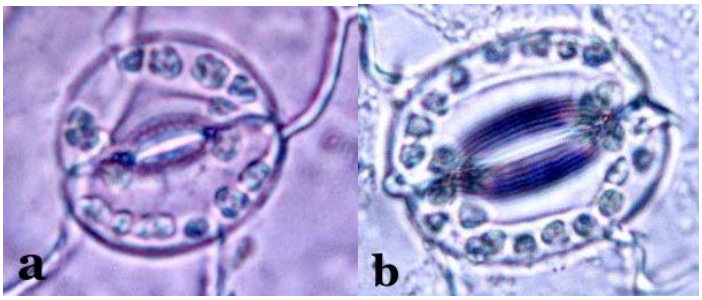

Fig. 1. Number of chloroplasts in stomata guard cells of a) diploid and b) tetraploid leaf of watermelon.

Flow cytometric analysis: The putative tetraploid watermelon seedlings selected on the basis of number of chloroplasts were then transplanted in the greenhouse for further investigations and seed production. Reconfirmation of ploidy level of these plants was made by flow cytometric analysis. The histograms of different ploidy levels of the plants are shown in Fig. 2. These plants were classified into exact ploidy level (2n or 4n) and mixoploids (2n+4n, 4n+6n or 2n+4n+6n). The nuclei released from a diploid plantlet appeared at channel 50 for G_1/G_2 phase as shown in Fig. 2a. Plantlets with double chromosomes (Fig. 2b) showed the G_1 peak at channel 100. Mixoploid plants consisted of both diploid and tetraploid or diploid, tetraploid and hexaploid cells (Fig. 2c,d). Over exposure of watermelon lines to colchicine might have resulted in ploidy levels higher than tetraploid. Figure 2d shows the histogram of higher ploidy with peak G_2 at channel 150. Flow cytometric analysis indicated that among the earlier screened out seedlings on the basis of chloroplast count as putative tetraploids, only 54% were tetraploid. Others were mixoploids (34%) and diploids (12%).

Relationship of DNA index and number of chloroplasts: It was further noted that the G_1/G_2 peaks in diploids had a coefficient of variation (CV) equal to 6.65% whereas the DNA index for tetraploids had CV 4.33%. The DNA content of plants, expressed as the DNA index (Fig. 3), was correlated with number of chloroplasts in each guard cell pair (r^2=0.80). However, DNA indices of samples overlapped sometimes even for the same number of chloroplasts. This may be due to the variable genome size in different watermelon lines or due to instrument drift and/or zero offset error. Moreover, there was doubt about the ploidy of plants with 8 or 9 chloroplasts in guard cells and presumably these were mixoploids.

Leaf size: The leaf area of diploid and tetraploid plants of different watermelon lines was also compared (Fig. 4a,b). It was 140±28.9 and 182.1±35.1 cm^2 in diploid and tetraploid, respectively (Table 2).

Flower and pollen morphology: The size of male flowers was also recorded to characterize diploid and tetraploids. Tetraploid flowers had larger size compared to diploids (Table 2). The flower diameter was 41.2 mm in tetraploids (Fig. 4c) and 36.7 mm in diploids (Fig. 4d). The pollen grains investigation showed 3 colpi in diploids (Fig. 4e) and 4 colpi in tetraploids (Fig. 4f). Pollens with both 3 and 4 colpi were observed in mixoploid plant flower (Fig. 4g).

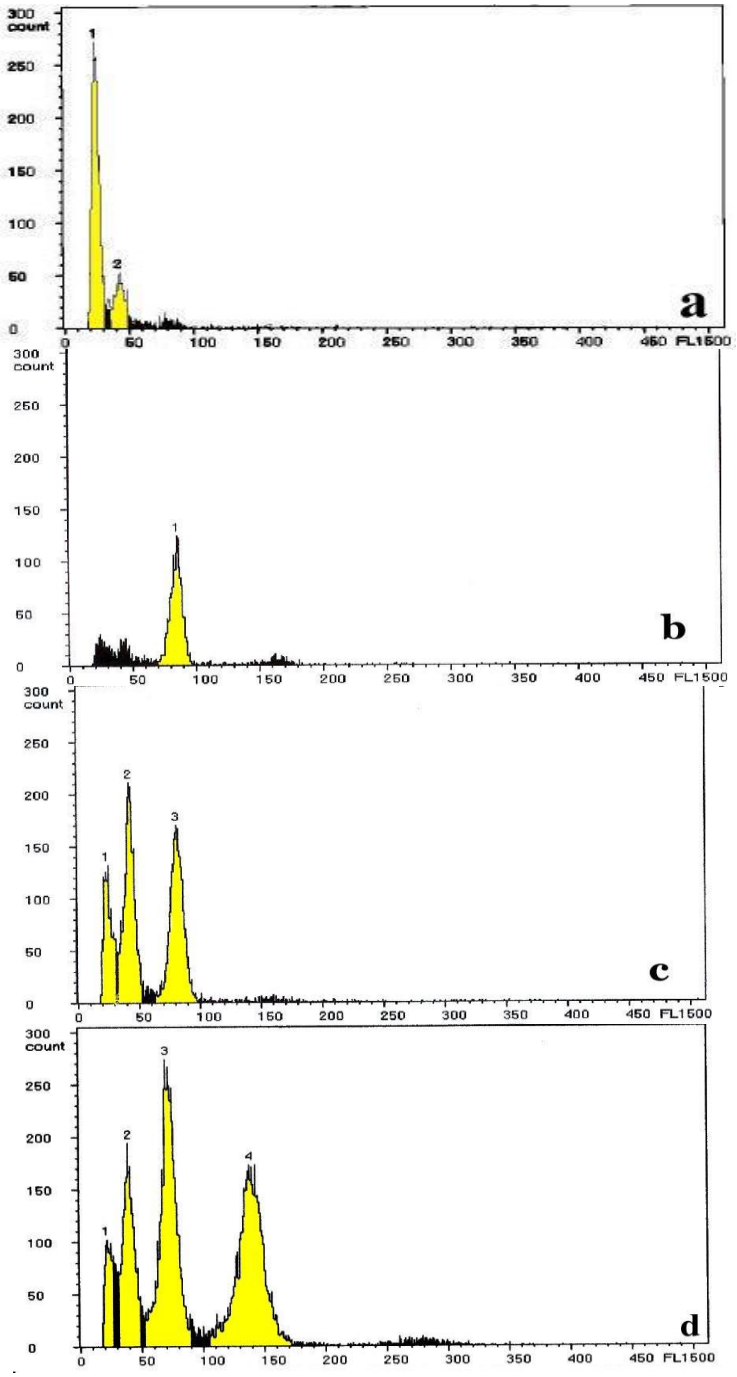

Fig. 2. Histogram of DNA content obtained from flow cytometric analysis of standard diploid and induced watermelon plants from *in vivo* colchicine treatment. (a) Diploid control; (b) induced tetraploid; (c) mixoploid plant with 2n+4n ploidy; (d) mixoploid plant with 2n+4n+>6n ploidy.

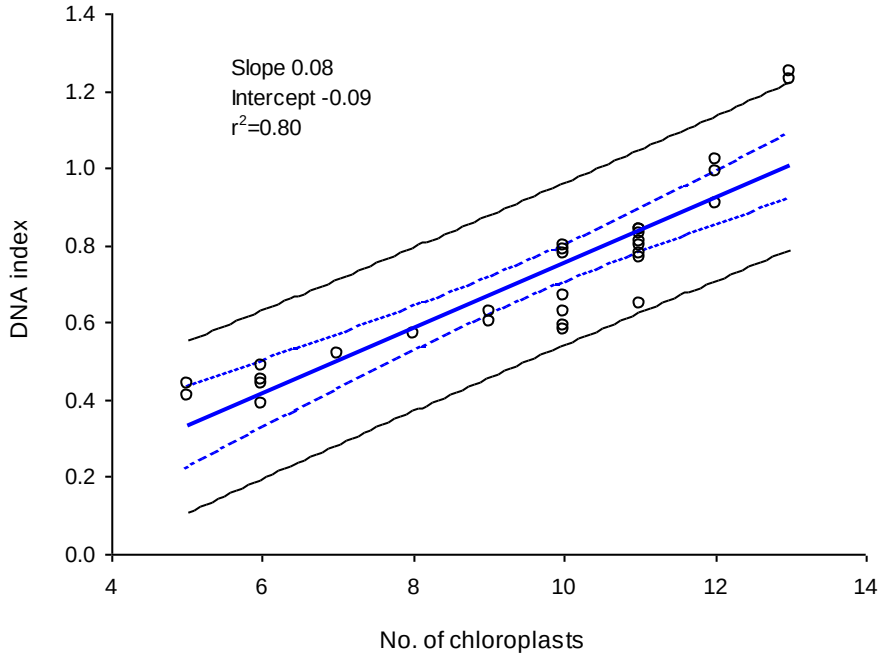

Fig. 3. Relationship between the DNA index and number of chloroplasts in each side of stomata guard cells. (Confidence limits 0.06-0.10 at 95% probability level).

Seed morphology: Differences in seed morphology of diploid and tetraploid were recorded (Fig. 5). The seeds of diploids (Fig. 5a) were oblong whereas the tetraploids (Fig. 5b) varied from oblong to round. Tetraploids had a coarse appearance with increased seed length (9.1±0.59 mm), width (6.3±0.30 mm) and thickness (2.8±0.51 mm) (Table 2). Diploid seeds showed completely filled cavity (Fig. 5c) with cotyledon and embryo but tetraploids showed some empty cavity at the chalazal end of the seed (Fig. 5d). Seedcoat thickness (Table 2) was recorded lower in diploids (Fig. 5e) than tetraploid seed (Fig. 5f).

Chromosome counts: The results of chromosome scoring in plant meristem showed diploid (2n=2x=22) and tetraploid (2n=4x=44). However, counting 44 chromosomes in tetraploids was very tedious (Fig. 5h,i).

Discussion

Colchicine application caused injury and late shoot emergence as reported earlier in watermelon (Suying *et al.*, 1995) and citrus (Jaskani *et al*., 1996). However, some variability in colchicine tolerance by watermelon lines was observed (Jaskani *et al*., 2004). The variable response of genotypes to different concentrations of colchicine support the need for making specific determination of colchicine concentrations suited for a given cultivar.

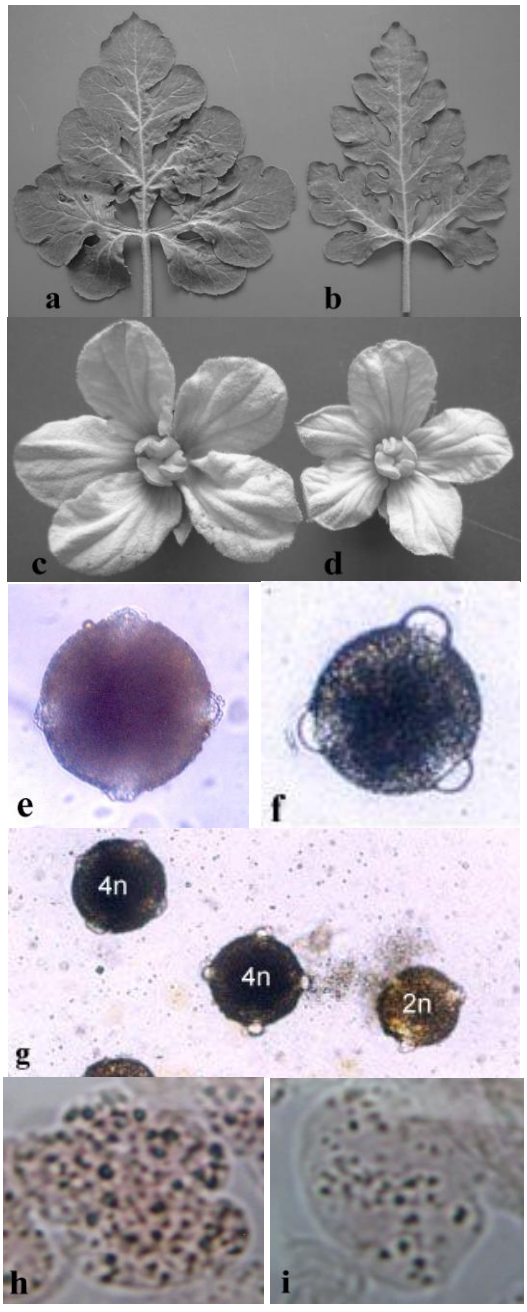

Fig. 4. Parameters of ploidy differences in diploid and tetraploid watermelon. a) leaf size of 7th true leaf on main vine of tetraploid and b) diploid plant; c) different sized tetraploid and d) diploid male flowers; e) tetraploid pollen with 4 colpi; f) diploid pollen with 3 colpi; g) mixoploid plant flower with 3 and 4 colpi pollens; h) tetraploid with 2n=4x=44 chromosomes; i) diploid with 2n=2x=22 chromosomes.

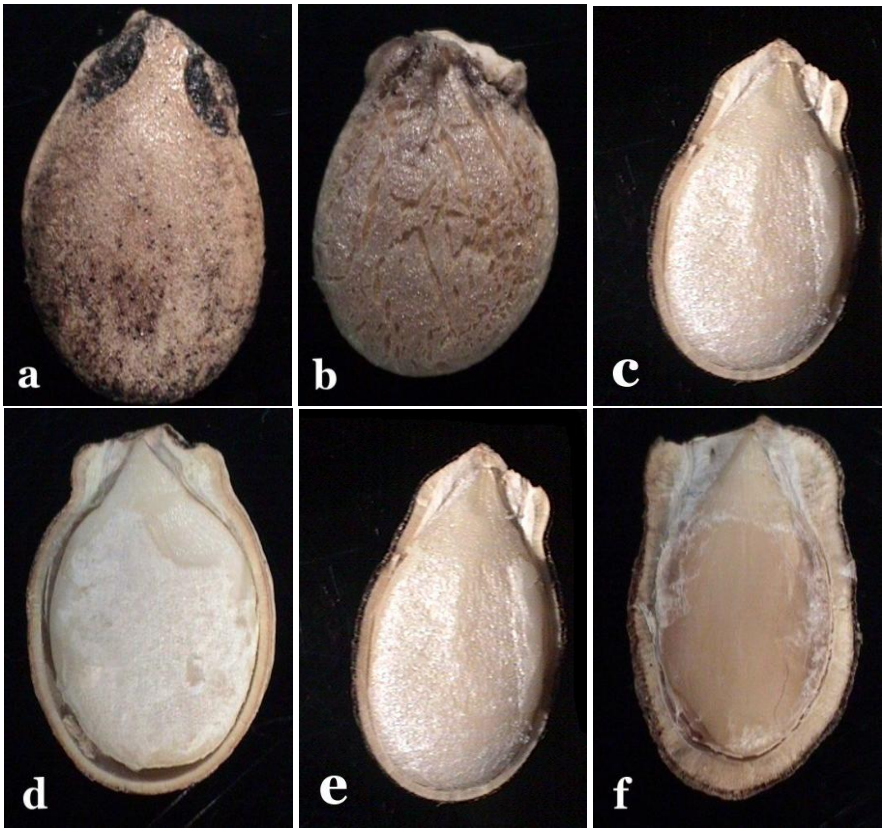

Fig. 5. Comparison of diploid and tetraploid seed in watermelon. a) oblong shape and smooth surface in diploids; b) oblong to round shape and fissures on tetraploid seed surface; c) full seed cavity in diploids; d) some empty cavity at chalazal end in tetraploids; e) thin seedcoat in diploids; f) thick seedcoat in tetraploids.

Chloroplast number in stomata guard cells (Fig. 1) could be a useful tool to determine ploidy as in Brussels sprout (Dore, 1986), carrot (Rode & Dumas de Vaulx, 1987), tomato (Jacobs & Yoder, 1989), sugarbeet (Brown *et al*., 1991), cucurbits (McCuistion & Elstrom, 1993), pepper (Abak *et al*., 1998) and citrus (Jaskani & Khan, 2000). Compton *et al*. (1999) also reported that leaves from diploid and tetraploid shoot cultures of watermelon had 9.7 and 17.8 chloroplasts per guard cell pair, respectively. In certain cases the number of chloroplasts varied up to 8 or 13-16 in each guard cell pair. This might be due to mixoploid nature of leaf tissues (Jaskani *et al*., 2004) and flow cytometry confirmed these as mixoploids (Fig. 2). Hence, chloroplast counting is an efficient indicator during early phase of screening for ploidy changes which must be followed by more certain methods of determination of ploidy level changes.

DNA content estimated using flow cytometry in the watermelon plants obtained after colchicine treatment did not fully agree with the results obtained by chloroplast counting. The proportion of tetraploid plants among the pre-screened plants on the basis of chloroplast count was 54%. The results also indicate that chloroplast counts is not accurate to screen out pure tetraploid seedlings at M1 stage because offsprings of the

treated seedlings are mostly chimeras and produce only 4-6% pure tetraploids (Jaskani *et al*., 2004). Similar results were also reported by van Duren *et al*. (1996) that the number of pure tetraploids obtained from *Musa* by selection based on the morphological features followed by flow cytometric analysis was about half that of the total pre-selected population. Although tetraploid watermelon plants could be distinguished by their large leaves and flowers along with thick stem but the presence of mixoploids tend to confuse the situation. Rose *et al*. (2000) reported that mixoploid plants in which more than 40% of the cells were tetraploid could be mistaken for full tetraploids but that mixoploids with 10–30% tetraploid cells usually resembled diploid plants. However, chloroplast counts and other morphological features could be helpful in successive generations to develop pure line.

To detect ploidy using flow cytometry, it is necessary to eliminate the variation between individual measurements and to establish the number of observations required to reach reproducible classification. Our results indicate a correlation between the chloroplast number and relative nuclear DNA content in watermelon (Fig. 3). However, the fit of chloroplast number appeared to become less precise with an increase in the DNA index. This could be due to mixoploid nature of samples which could not be identified by chloroplast count or variable genome size of tested watermelon lines because the flow cytometric detection of ploidy is based on a simplified assumption that all the chromosomes have the same DNA content (Roux *et al*., 2001), which may not be the case in watermelon. Thus, in contrast to chloroplast counting, the flow cytometric assay seems to be a more precise technique for detecting changes at M1 stage.

Considerable morphological variation (Fig. 4,5) was observed among diploid and tetraploid plants following colchicine treatment. Berdahl & Ries **(**1997) observed higher vigour and longer leaf length in tetraploids of Russian wild rye. Jaskani *et al*., (1996) found tetraploid citrus as less vigorous showing greater leaf breadth. Differences also have been reported in leaf area (Koh, 2002), flower size (Sari *et al*., 1999), 3 colpi in pollen of diploids verses 4 in tetraploids (Rhodes & Zhang, 1999) and seed morphology (Kihara, 1951) but it would be space and time wasting to grow a large population of treated plants until harvest. By using flow cytometry, mixoploids could be rejected and evaluation could then be limited to a lower number of plants as colchicine treatments induce low number of pure tetraploids (4-6%).

Chromosome counting is best performed at the mitotic metaphase stage when the chromosomes are fully contracted. This prerequisite limits the choice of material for chromosome counting to tissues with a high mitotic activity. Because of accessibility and ease of preparation, root tips are the tissue of choice when available. However, watermelon has small chromosomes, and their counting is laborious. Furthermore, this method is difficult to establish the ploidy of non-dividing cells in differentiated tissues, such as leaves (Roux *et al*., 2001). However, Jaskani (1998) established chromosome counts in young leaves of citrus. Alternative methods for ploidy screening based on stomata size and density, and size of pollen have been found to be slow and unreliable (Adniya & Ardian, 1994).

The present results illustrate the usefulness of flow cytometry for accurate detection of tetraploids in watermelon especially at M1 stage. Compared to conventional chromosome counting, flow cytometry is more rapid and convenient whereas chloroplast counts did not distinguish mixoploids and other morphological characteristics are equally applicable but in successive generations during pure line development.

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References

Abak, K., N. Comlekcioglu, S. Buyukalaca and N. Sari. 1998. Use of stomatal characteristics to estimate ploidy level of haploid and dihaploid pepper plants. *Tenth EUCARPIA Meeting Capsicum and Eggplant*, 7-11 September 1998, Avignon, France, pp. 179-182.

Adniya, S. and Ardian. 1994. A new method for selecting cytochimeras by the maximum number of nucleoli per cell in *Allium wakegi* Araki and *A. fistulosum* L. *Euphytica*, 79: 5-12.

Andrus, C.F., V.S. Seshadri and P.C. Grimball. 1971. Production of seedless watermelons. *Agricultural Research Service, USDA Technical Bulletin No*., 1425.

Berdahl, J.D. and R.E. Ries, 1997. Development and vigor of diploid and tetraploid Russian wild rye seedlings. *J. Range Management*, 50: 80-84.

Brown, S.C., P. Devaux, D. Marie, C. Bergounioux and P.X. Petit. 1991. Flow cytometry: Application in vegetables for ploidy analysis (in French). *Biofuture*, 105: 2-16.

Compton, M.E., N. Barnett and D.J. Gray. 1999. Use of fluorescein diacetate (FDA) to determine ploidy of *in vitro* watermelon shoots. *Plant Cell Tiss. Org. Cult.*, 58: 199-203.

Compton, M.E., D.J. Gray and G.W. Elmstrom. 1994. *Regeneration of tetraploid plants from cotyledons of diploid watermelon*. *Proc. Fla. State Hort. Soc*., 107: 107-109.

Compton, M.E., D.J. Gray and G.W. Elmstrom. 1996. Identification of tetraploid regenerants from cotyledons of diploid watermelon cultured *in vitro*. *Euphytica*, 87: 165-172.

Dolezel, J. 1997. Application of flow cytometry for the study of plant genomes. *J. Applied Genet*., 38: 285-302.

Dolezel, J. 1998. Flow cytometry, its application and potential for plant breeding, p. 80-90. In: *Current topics in plant cytogenetics related to plant improvement*. (Ed.): T. Lelley. Universitätsverlag, Vienna.

Dore, C. 1986. Evaluation of ploidy level of Brussels sprout (*Brassica oleracea* L. sp. *Gemmifera*) population originated from pollen (in French). *Agronomy*, 6: 797-801.

Fahleson, J., J. Dixelius, E. Sundberg and K. Glimelius. 1988. Correlation between flow cytometric determination of nuclear DNA content and chromosome number in somatic hybrids within *Brassiceae*. *Plant Cell Rep*., 7: 74-77.

Jacobs, J.P. and J.I. Yoder. 1989. Ploidy levels of transgenic tomato plants determined by chloroplast number. *Plant Cell Rep*., 7: 662-664.

Jaskani, M.J. 1998. *Interploid hybridization and regeneration of Kinnow mandarin*. Ph.D. Diss., Department of Horticulture, University of Agriculture, Faisalabad, Pakistan.

Jaskani, M.J. and I.A. Khan. 2000. Characterization of interploid hybrids of Kinnow mandarin. *Proc. Intl. Soc. Citriculture*, 1: 165-166.

Jaskani, M.J., I.A. Khan and S. Husnain. 1996. Morphological description of citrus colchiploids. *Proc. Intl. Soc. Citriculture*, 1: 130-132.

Jaskani, M. J., S.W. Kwon, G.C. Koh, Y.C. Huh and B.R. Ko. 2004. Induction and characterization of tetraploid watermelon. *J. Kor. Soc. Hort. Sci*., 45: 60-65.

Kihara, H. 1951. Triploid watermelons. *Proc. Amer. Soc. Hort. Sci*., 58: 217-230.

Koh, G.C. 2002. Tetraploid production of Moodeungsan watermelon. *J. Kor. Soc. Hort. Sci*., 43: 671-676.

Lucier, G. and B.H. Lin. 2001. Factors affecting watermelon consumption in the United States, pp. 23-29. In: *Vegetables and Specialties: Situation and Outlook, VGS-287*. Extension and Research Services USDA.

Marr, C.W. and K.L.B. Gast. 1991. Reactions by consumers in a farmers market to prices for seedless watermelon and ratings of eating quality. *HortTechnology*, 1: 105-106.

McCuistion, F. and G.W. Elmstrom. 1993. Identifying polyploids of various cucurbits by various guard cell chloroplast number. *Proc. Fla. State Hort. Soc*., 106: 155-157.

Rhodes, B.B. and X.P. Zhang. 1999. Hybrid seed production in watermelon. *J. New Seeds*, 1: 69-88.

Rode, J.C. and R. Dumas De Vaulx. 1987. Obtaining haploid plants of carrot (*Daucus carota* L.) from induced parthenogenesis in situ by pollen irradiation and *in vitro* immature seed culture (in French). *Rept. Acad. Sci. Paris, Series III*, 305: 225-229.

Rose, J.B., J. Kubba and K.R. Tobutt. 2000. Chromosome doubling in sterile *Syringa vulgaris* x *S. pinnatifolia* hybrids by *in vitro* culture of nodal explants. *Plant Cell Tiss. Org. Cult*., 63: 127-132.

Roux, N., J. Dolezel, R. Swennen and F.J. Zapata-Arias. 2001. Effectiveness of three micropropagation techniques to dissociate cytochimeras in *Musa* sp. *Plant Cell Tiss. Org. Cult*., 66: 189-197.

Sari, N., K. Abak and M. Pitrat. 1999. Comparison of ploidy level screening methods in watermelon: *Citrullus lanatus* (Thunb.) Matsum. and Nakai. *Sci. Hortic*., 82: 265-277.

Suying, T., H. Xiuqiang, L. Jiwei and L. Wenge. 1995. Raising the frequency of inducing tetraploid watermelon by treating with colchicine. *Acta Hort.*, 402: 18-22.

Tenkouano, A., J.H. Crouch, H.K. Crouch and D. Vuylsteke. 1998. Ploidy determination in *Musa* germplasm using pollen and chloroplast characteristics. *HortScience*, 33: 889-890.

van Denhout, H., R. Ortiz, D.R. Vuylsteke, R. Swennen and K.V. Bai. 1995. Effect of ploidy on stomatal and other quantitative traits in plantain and banana hybrids. *Euphytica*, 839: 117-122.

van Duren, M., R. Morpurgo, J. Dolezel and R. Afza. 1996. Induction and verification of autotetraploids in diploid banana *(Musa acuminata)* by *in vitro* techniques. *Euphytica*, 88: 25-34.

Veilleux, R.E. and A.A.T. Johnson. 1998. Somaclonal variation: molecular analysis, transformation interaction, and utilization. pp. 229-268. In: *Plant Breeding Reviews*. (Ed.): J. Janick. John Wiley & Sons, Inc., New York.

Zhang, X.P., B.B. Rhodes and J.W. Adelberg. 1994. Shoot regeneration from immature cotyledons of watermelon. *Cucurbit Genet. Coop. Rep*., 17: 111-115.

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