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Observations on Traits Used as Indicators of Ploidy Levels in Plants in Two Varieties of Diploid Tomato

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ABSTRACT

The reliability of indirect method for ploidy level determination in two tomato varieties with the same ploidy level was tested. Observations were made on traits used as indirect indictors of ploidy levels in plants on two varieties of diploid tomato which are characterized by significant differences in their size. The results revealed that all the morphological traits, pollen grain measurements as well as chloroplasts number in stomata guard cells significantly, differ in the two varieties although they are both diploid. In contrary, number of germinal pores in pollen grains and different stomata measurements, like stomata size, guard cell size and stomata frequency in addition to epidermal cell frequency, showed non-significant differences between the tested varieties. Thus, investigated traits, including stomata measurements and number of germinal pores, cannot be used to screen plants of *Solanum Lycopersicum* for ploidy level. Furthermore, the indirect method of ploidy level determination is species-specific and need to be confirmed by other methods.

***Key words*:** *Solanum lycopersicum*, ploidy level, indirect method , stomata measurements

1. INTRODUCTION

Ploidy level determination is a necessary step before breeding programs and/or genetic studies (Bonos *et al*., 2002). Soon after it was proved that polyploidization affects plant cell size, a large scale of traits were used as ploidy level indicators. Overall, ploidy level can be estimated by the following three methods:

(1) flow cytometry, which is rapid and reliable and reduces time and efforts, but it needs experience and equipment,

(2) chromosomes counting, known as the direct method, is accurate, but tedious and time consuming, and
(3) comparing the number of morphological traits, pollen grain measurements, chloroplasts number in stomata guard cells, and other stomata measurements, are known as the indirect method.

Different studies have encouraged the using of indirect method instead of direct chromosome counting. For example, Beck *et al*. (2003) considered chromosomes counting method as a tedious-accurate and time consuming method, especially in case of small chromosomes size, as it was found on *Acacia mearnsii*. They found that the mean of stomatal length was significantly larger in tetraploids compared to diploids, while the frequency of stomata per leaf surface was shown to decrease significantly in tetraploids. They concluded that these two traits are rapid indirect methods to identify ploidy level in black wattle. Almann et al. (1994) found good correlation between the ploidy level and the size of pollen grains in Arabidopsis thaliana and they recommend it as a quik and simple method to test ploidy level in transgenic Arabidopsis plants. In *Citrullus lanatus*, Sari *et al*. (1999) found that the stomata size and number of chloroplasts in guard cells differ significantly in haploid and diploid plants, in addition to the difference in morphological traits, including stem length, stem diameter and leaf area. This technique of indirect method was successfully used and highly recommended for other plant species, such as plantain and banana hybrids (Vandenhout *et al*., 1995).

Tomato, *Solanum lycopersicum* formerly *Lycopersicon esculentum*, is a good model for biology research (Shibata, 2005). It is genetically well known and has relatively small diploid genome (2n = 24). In the present study, we aimed to detect the reliability of the indirect method as a reliable ploidy level indicator.

2. MATERIALS AND METHODS

2.1 Plant materials

We used two diploid tomato varieties: (1) Ailsa Craig (AC), and (2) Micro-Tomato/Micro-Tom (MT), which have the same genetic background. Ailsa Craig is the wild normal size variety (Quinet *et al*., 2006). while the other variety (Micro-Tom) has dwarf phenotype (short stem length ≈10-20cm) and short life cycle ≈ 70-90 days (Shibata, 2005; and Yano *et al*., 2006), which are ascribed to at least two major recessive mutations (Meissner *et al*., 1997; and Martí *et al*., 2006).

2-2 Growth conditions

Seeds of Ailsa Craig (AC) and Micro-Tom (MT) were used in an experiment. They were surface sterilized with 3 % (v/v) sodium hypochlorite for 3 minutes, and then were washed three times with distilled water. After that, they were placed in 9 cm diameter Petri dishes lined with a single layer of filter paper (Scheicher and Schuell No. 595), wetted with distilled water. Germination was observed daily until radical emergence. Seedlings with the same age were transferred into a cups containing peat moss. After they formed the fifth true leaf, they were transferred again into culture pots filled with clay soil.

2.3 Morphological traits

At flowering stage, ten individual plants were randomly selected, and the following morphological traits were recorded: (1) Leaf size, (2) Stem length, (3) Stem diameter, (4) Flower diameter, and (5) Fruit diameter. All these traits were taken, using a metric ruler.

2.4 Pollen grains parameters:

A number of matured flowers was randomly selected. A longitudinal cleavage was made within each androecium, and the pollen grains were collected by tapping the androecium few times on clean-dry slides. Thirty microscopic readings of the pollen grain length and width were measured at 400X, using a light microscope with an ocular micrometer, where each ocular unit of the scale measured 5µm. The pollen size was calculated as a product of length and width. The same slides were also used to calculate the number of germinal pores per pollen grain. Pollen grains were stained with a drop of Aceto-Carmine to show the germinal pores. Thirty readings were recorded from each tomato variety.

2.5 Stomata measurements:

Five leaves were randomly selected from five plants of both Ailsa Craig and Micro-Tom varieties. The middle leaflets were collected and a thin layer of the lower epidermis was peeled using forceps and placed on a microscopic slide for the following stomatal parameters. In order to measure the length and width of thirty stomata and guard cells, the lower epidermis were spread on a drop of distilled water on a slide, and then covered with a cover slide. Measurements were taken using the light microscope under a magnification of 400X with an ocular micrometer. Each ocular unit of the scale measured 5µm. The stomata and guard size was calculated as a product of length and width. The same previous slides were also used to determine the number of stomata and epidermal cells per leaf unit area (stomatal and epidermal cell frequency). Stomata and epidermal cells were counted in fifteen microscopic fields with square micrometer at a magnification of 280X. The guard cells were excluded from the counting of the epidermal cell frequency. In the chloroplasts scoring method another group of lower epidermis slides were prepared and a drop of potassium iodide iodine was used instead of distilled water (Bingham, 1968). Then, the number of chloroplasts was counted in 30 guard cell pairs.

The obtained data were statistically analyzed and the statistical differences between both tomato parameters were determined using two sample t-tests (MINITAB version 12).

3. RESULTS and DISCUSSION

3.1. Morphological traits

The results of the morphological traits are shown in Table (1). It was revealed that the wild variety (Ailsa Craig) showed higher stem length, stem diameter, leaf size, flower diameter and fruit diameter than Micro-Tom, with high statistical significant differences as well (Fig. 1). It is well established and common knowledge that polyploidy plants have thicker and bigger leaves, larger flowers and fruits and as result bigger vegetative volume and larger weight (Yildiz 2013).

In this study all these differences were resulted from the difference in the two recessive mutation: dwarf (d) and miniature (mnt) in Micro-Tom (Meissner et al., 1997). However, some authors reported a number of morphological traits as indirect ploidy level indicators

in many species as results of polyploidy (Sari *et al*., 1999; Bonos *et al*., 2002; and Seidler-Lożykowska, K., 2003). Based on this study results, it is clear that polyploidy is not the only cause of significant difference in plant size or plant parts size within the varieties or species.

Table 1: Comparison between the morphological traits of two tomato varieties (Alisa Craig and Micro-Tom) grown under the same environmental conditions

Traits	Alisa Craig	Micro-Tom	P-value
Stem length (cm)	32.34 ± 1.1	8.48 ± 0.37	0.0000
Stem diameter (cm)	4.4 ± 0.16	3.8 ± 0.19	0.027
Leaf size (cm²)	4.946 ± 0.17	3.038 ± 0.20	0.0000
Flower diameter (mm)	20.1 ± 0.43	13.8 ± 0.13	0.0000
Fruit diameter (cm)	2.83 ± 0.040	1.79 ± 0.074	0.0000

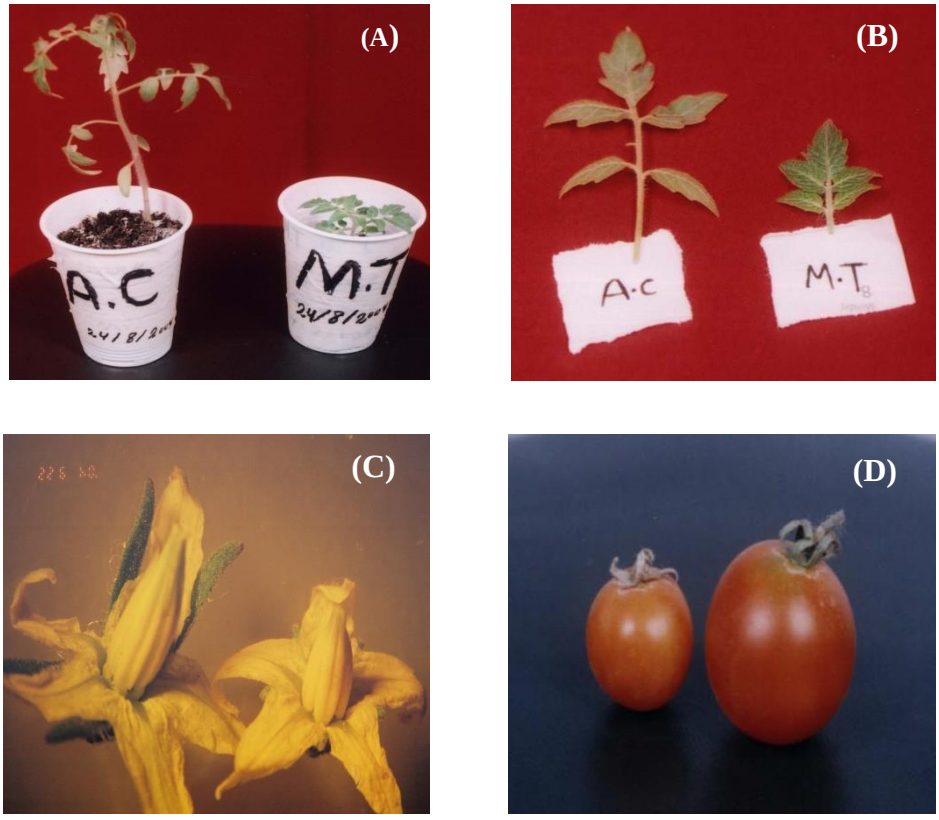

Fig. 1: Morphological differences between two tomato varieties (Alisa Craig and Micro-Tom) in stem length for seedlings have the same age (A), leaf blade size (B), flower diameter (C) and fruit diameter (D).

3.2. Pollen grain parameters

Microscopic examination showed that the wild variety (Ailsa Craig) had larger pollen grains than Micro-Tom, with high statistical significant difference (Table 2 and Fig. 2). On the other hand, the number of germinal pores per pollen grain showed no significant difference where the examined pollen grains of both tomato varieties showed three germinal pores. Early reports on Arabidopsis, by Almann et al., 1994, linked the greater pollen grain size to polyploidy. They concluded that it can be used as a quick and simple test of ploidy level in transgenic Arabidopsis. While recently, another report contradicted Almann report and approved that pollen grain measurements are not recommended for determining ploidy (Jones and Reed 2007). Seidler-Łożykowska (2003) also found that pollen grain diameter is recommended as an indirect polyploidy level determinant. This is in agreement with our finding, which means that this trait can be species-specific and not generally recommended as ploidy indirect indicator.

Table 2: Comparison between pollen grain size and stomatal measurements of two tomato varieties (Alisa Craig and Micro-Tom) grown under the same environmental conditions.

Traits	Alisa Craig	Micro-Tom	P-value
Pollen grain size (µm²)	1955 ± 35	1734 ± 26	0.0000
Chloroplasts number	18.3 ± 0.53	10.03 ± 0.52	0.0000
Stomata size (µm²)	167.5 ± 9.7	162.5 ± 9.5	**0.71**
Guard cell size (µm²)	787.5 ± 7.0	770 ± 11	**0.18**
Stomata frequency	3.067 ± 0.067	3.400 ± 0.16	**0.075**
Epidermal cell frequency	7.067 ± 0.18	7.333 ± 0.23	**0.37**

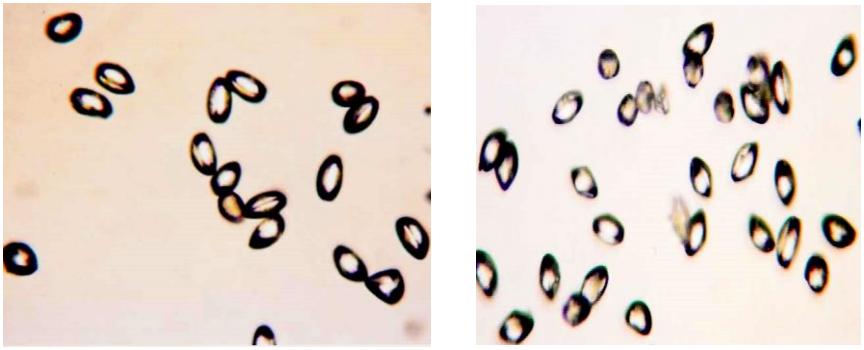

Fig. 2: Differences between two Alisa Craig (A) and Micro-Tom (B) in pollen grain size. Microscopic photos were taken at 400X magnification power. The pollen grain size was calculated as a product of length and width.

3.3. Stomata measurements

The results of stomata measurements are represented in Table (2). It is indicated that the chloroplasts number per stomata guard cell pair was high, significantly differing between the two tomato cultivars (Table 2, Fig. 3). The average stomatal chloroplast number of the wild variety (Ailsa Craig) was 18.3, compared to 10.03 for the other genotype (Micro-Tom). In *Citrullus lanatus* (Sari *et al*., 1999), the chloroplasts number per stomata differs

significantly in haploid and diploid plants. In tetraploid chamomile, Seidler-Łożykowska (2003) found that chloroplasts number per stomata guard cell differs significantly. It was also found, in our study, that the difference is not due to ploidy level. It is clear, then, that this trait and other tested stomatal traits, including, stomata size, guard cell size and stomata frequency, and epidermal cell frequency do not differ between both tomato varieties (Table 2).

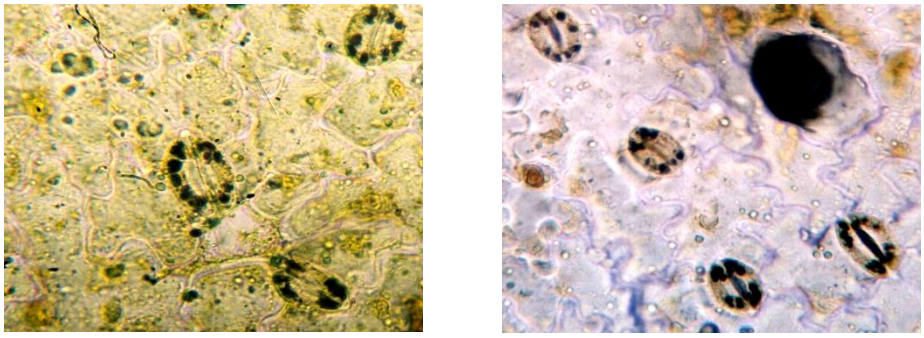

Fig. 3: Differences between Alisa Craig (A) and Micro-Tom (B) in chloroplasts number and guard cell size. Microscopic photos were taken at 400X magnification power.

Moreover, the study results revealed that the chloroplasts number in guard cells cannot serve as an indirect indicator for ploidy level determination. It showed significant difference which is only due to the difference in the two genes of the two varieties. While in an early report on transgenic *tomato* (Jacobs and Yoder, 1989), it was found that in tetraploid tomato, the number of chloroplast in stomatal cells were greater than that in diploid plants. Ploidy level determination is a necessary step before breeding programs and/or genetic studies. In some plant species, like Musa, the determination of ploidy level is essential and sometimes it is needed at early stages of plant development. However, the indirect method can be used in some species and not in others. We strongly recommend dealing with each species after conforming the direct and indirect methods.

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مشاهدات علي صفات تستخدم كمؤشرات علي مستوى التضاعف الكرموسومي في النبات في صنفين من الطماطم ثنائية التضاعف

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ملخص

موثوقية الطريقة الغير مباشرة لتحديد مستوى التضاعف الكرموسومي في النبات اختبرت في صنفين من الطماطم لها نفس مستوى التضاعف تم اختبارها من خلال مشاهدات علي الصفات التي تستخدم كمؤشرات غير مباشرة علي مستوى التضاعف في صنفين مميزين باختلاف معنوي في الحجم. النتائج اظهرت ان الصفات المظهرية وقياسات حبوب اللقاح وكذلك عدد البلاستيدات في الخلايا الحارسة للثغر تختلف معنويا في الصنفين بالرغم من ان مستوى التضاعف واحد وهو ثنائي. وعلي النقيض من ذلك عدد الفتحات في حبة اللقاح والقياسات الخاصة بخلايا الثغر مثل حجم الثغر وحجم الخلية الحارسة وتكرار الثغور اضافة الي تكرار خلايا البشرة اظهرت فروق غير معنوية بين الصنفين. وعليه فان الصفات المدروسة بما فيها القياسات الخاصة بالثغر وفتحات حبوب اللقاح لا يمكن ان تستخدم كمؤشرات غير مباشرة لتحديد التضاعف في الطماطم، والطريقة الغير مباشرة لتحديد التضاعف قد تكون مقيدة بالأنواع النباتية كلا على حده ولابد من تأكيدها بطرق مباشرة

كلمات مفتاحية: *Solanum lycopersicum*، مستوى التضاعف ، طريقة غير مباشرة ، قياسات خلايا الثغر