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A Study of Pathogen-free Melons for the Most Suitable Age of Tissue Culture Seedlings to Transplant for Commercial Production

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Abstract

To determine the most suitable age for in vitro melon seedlings to transplant for growing pathogen-free melons, we designed an RCBD experiment with the two factors being cultivar (Galia and Orange Man) and seedling age at the time of transplant (10, 15, and 20 days compared to 14-day-old control seedlings that were grown conventionally), with 10 specimens per treatment. Measuring mature melon plants at 42 days, we found that for the parameters of plant height, stem circumference, leaf width, and leaf length, the factors of cultivar and seedling age at the time of transplant had a statistically significant combined influence, with plants from 15-day-old Galia seedings exhibiting the greatest average plant height at 232.50±2.44 cm and plants from 15-day-old Orange Man seedlings exhibiting the greatest average stem circumference, leaf width, and leaf length at 1.02±0.05, 20.89±0.33 and 20.56±0.29 cm, respectively. For fruit quality, the melons from 15-day-old Galia seedlings had the greatest average flesh thickness and flesh firmness at 2.56±0.28 cm and 34.86±1.80 Newtons. The age of seedlings at the time of transplant did not have a statistically significant effect on flesh color, rind color, total soluble solids or pH. The most suitable age for transplanting pathogen-free melons raised in tissue culture is 15 days.

Discipline: Plant Science.

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1 Introduction

Melon (*Cucumis melo L.*) is an economic plant in the Cucurbitaceae family, the same family as watermelon, musk melon and cucumbers (Pitrat, 2008). India is the most likely center of origin. Melons grow well in both semi-tropical and tropical climates (Jantaku et al., 2016). They are naturally cross-pollinated through insect and wind pollination, but some varieties with perfect flowers can also be self-pollinated. Melons are economically important crops in several countries because they are prized for their good flavor, sweetness and pleasant fragrance .Many different cultivars have been developed that are particularly liked by consumers. They contain a high level of beta-carotene, which when converted to vitamin A in the body is a powerful anti-oxidant (Villanueva et al., 2004). They are relatively quick to cultivate and harvest and tend to give good returns to growers. There is high demand for melons in the Asian and European markets (David, 2015), especially in Japan, Singapore and Thailand. There is good potential for further growth in the domestic melon market in Thailand because it is a fruit that is popular with consumers and at present, the supply is insufficient to meet the demand. Melon cultivation has expanded all over the country, and in 2016 there were 972.2 hectares under cultivation, with production reaching 9,547.17 tons, or an average of 12,719 kg per hectare (Department of Agriculture Extension, 2017).

Growers in Thailand tend to prefer two groups of melon cultivars, *C. melo* var. reticulatus Naudin, with net patterns on the rind, known as Net melons, and *C. melo* var. indorous Naudin, with smooth rinds, also known as the Honeydew group. In general, most melons are round or egg-shaped, with either smooth rinds, rough rinds, or sometimes indents, grooves, or net-like striations. The flesh may be yellowish, brownish, or greenish, and the flesh color ranges from orange to green or whitish, depending on the cultivar (Niphon, 2001; Jani and Miho, 2015). Most modern cultivars can be successfully grown in Thailand, but it is not easy to produce good quality melons that are safe for consumers, because melons are susceptible to many pests and adverse environmental conditions. Even when growers try to care for them assiduously, they often face problems with damage from insect pests and diseases that can occur at any stage of plant growth. Pests may cause serious damage to young seedlings and growing leaves, and the end result is usually a drop in productivity (Napier, 2009). This is especially the case with thrips and aphids, which are often vectors of the most common diseases that cause significant damage to melon crops (Palumbo et al., 2000). A report on serological and biomolecular assays of melons with unusual yellowing by Lecoq et al., (2003) found that Papaya Ringspot Virus (PRSV) and Zucchini Yellow Mosaic Virus (ZYMV) were the most prevalent causes, while Peng et al., (2011) found that Melon Yellow Spot Virus (MYSV) also caused significant damage in the form of misshapen and discolored fruits. These viruses are usually transmitted by insects.

There have been numerous reports of viruses causing damage to melon crops, partly because most farmers tend to grow melons on the same plots crop after crop, year after year. This is conducive to the spread of viruses and results in lower yield and lower fruit quality. It is necessary

to find suitable approaches to solving this problem, one of which could be providing sources of pathogen-free plant material by raising large numbers of seedlings using sterile tissue culture techniques. This is a way to provide large numbers of pathogen-free uniform seedlings in a short time. We were interested in studying the most suitable age of tissue-culture-raised plantlets to transplant and acclimatize for commercial melon production. In time, this can be promoted as a system for quality melon production so that farmers can have a sustainable source of standard quality plants.

2 Materials and Methods

2.1 Surface Sterilization

Seeds of Galia and Orange Man melon were agitated in 0.1% Hg_2Cl_2 solution with 3 drops of Tween20 surfactant for 2 minutes before being surface sterilized in 10% Clorox (1.4% sodium hypochlorite) solution for 15 minutes, and then rinsed with distilled water 3 times for 5 minutes each time. The seeds were then dried with sterilized paper before being placed on a semi-solid MS medium (Murashige and Skoog, 1962) with 30 grams per liter sucrose and 2.5 grams per liter gelrite added, which was adjusted to pH 5.7 before autoclaving. The tissue culture vessels were kept in the lab at 25±2 °C with light for 16 hours a day averaging 60±5 $mM/m^2/s$ from fluorescent bulbs (TLD 36W/84 3350 Im Philips Thailand) and observed after 72 hours to record germination rate and 7 days to record both germination rate and contamination rate.

2.2 Virus Detection

Seven days after sterilization, the sprouted melon seedlings from sterilized seeds had true leaves. Before the step of propagating them in tissue culture to test the most suitable age for transplantation, a leaf tip of each plant was sampled to test for Cucumber Mosaic Virus (CMV). We used the ImmunoStrip® for CMV by Agdia, USA. Following the manufacturer's instructions, we ground 0.15 g-pieces of leaf tips in the solution until dissolved and the solution turned to light green, then dipped the ImmunoStrip in the solution and left it to sit for 30 minutes at room temperature. After the data were recorded and each seedling was found to be pathogen-free, it could be used for *in vitro* propagation (Figure 1)

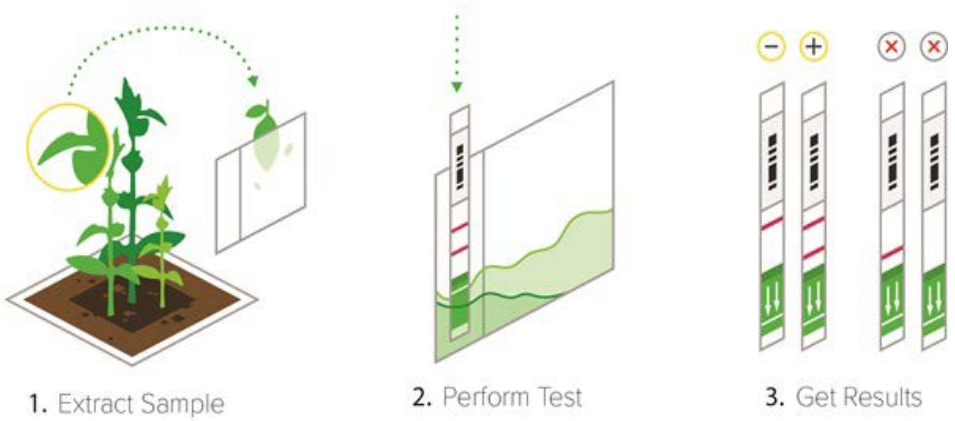

Figure 1: Detection method for Cucumber Mosaic Virus (CMV) by ImmunoStrip® test

2.3 Root Induction before Transplantation/Acclimatization

To induce roots, the tops of the seedlings in tissue culture were aseptically cut and transferred to a semi-solid MS medium containing 0.1 mg per liter naphthalene acetic acid (NAA), 30 grams per liter sucrose and 2.5 grams per liter gelrite (adjusted to pH 5.7 before autoclaving). They were kept in the same tissue culture conditions described above (25±2 °C with light 16 hours a day averaging 60±5 $mM/m^2/s$ from fluorescent bulbs) until roots emerged and they were ready for the transplantation step.

2.4 Transplantation, Acclimatization, and Shade House Growing

Galia and Orange Man melon seedlings of different ages with sufficient root systems were taken out of the tissue culture vessels on a set day for the experiment for each group (10, 15, and 20 days), and the tissue culture medium was thoroughly washed off before the seedlings were planted in planting medium that was pre-sterilized through steam treatment. The planting medium consisted of peat moss:vermiculite: perlite at the ratio of 1:1:1. After watering, for acclimatization, the seedlings were kept in closed plastic boxes to retain moisture for one week, after which the box lids were removed. The temperature and lighting were the same as in the tissue culture lab (25±2°C with light 16 hours a day averaging 60±5 $mM/m^2/s$ from fluorescent bulbs). After acclimatization, seedlings were transplanted from the boxes to 8x13-inch bags filled with the same planting material (one seedling per bag) and kept under shade house conditions. Two weeks after transplantation, the first to eighth side stems were removed, leaving the ninth to twelfth side stems. When flowers formed on the ninth side stem upwards, they were observed and on the day they started to bloom, they were hand pollinated at 06:00-10:00 in the morning. If pollination was successful, the ends of side stems were removed, leaving two leaves on the offshoots. Only one melon fruit per plant was kept and it was tied with a cord to support its weight. Water was provided through drip fertigation at the rate of 1.5 liters per plant per day when they were 14-45 days old, 2.5 liters per plant per day when they were 45-75 days old and reduced to 1 liter per plant per day from day 76 until melon fruits were harvested.

2.5 Data Collection on Growth Parameters and Fruit Quality to Compare Seedlings of Different Ages at Time of Transplantation

To compare the two cultivars and the influence of the factor of seedling age at the time of transplant, we designed an RCBD experiment with the first factor being cultivar (A1=Galia and A2=Orange Man) and the second-factor being seedling age (B1=control [14 days non-tissue culture seedlings raised in a conventional way], B2=10 days old tissue culture seedlings, B3=15 days tissue culture seedlings, and B4=20 days old tissue culture seedlings), with 10 plants per treatment. The parameters recorded for comparing plant growth at 42 days were plant height, stem circumference, number of leaves, leaf width, leaf length, and at harvest time, fruit weight and fruit size. The data collected for comparing fruit quality comprised 1) rind color and flesh color, using Chroma meter CR400 to collect L^*, a^* and b^* values; 2) flesh thickness and rind thickness; 3) flesh firmness using a

firmness tester with 0.3 cm diameter point; 4) total soluble solids (TSS in degrees Brix) using a hand refractometer; 5) titratable acidity by titrating melon juice in 0.1N NaOH and using 0.1% phenolphthalein as the indicator; and 6) pH. Statistical analysis of data was done using SAS version 9.1 and means were compared using Duncan's New Multiple Range Test at 95% confidence.

3 Results and Discussion

3.1 Germination Rate and Virus Testing of the 2 Cultivars

After the Galia and Orange Man seeds were surface sterilized and incubated in tissue culture conditions for 7 days, it was observed that the 150 Galia seeds had a germination rate of 64% while the 150 Orange Man seeds had a germination rate of 26.67%, which is considered very low. This may be because the tissue culture medium used was not optimal for the germination of the Orange Man cultivar. In a study published in 2017, Houngtongkam et al., (2017) tested 3 different tissue culture media for germinating orchid seeds-Vacin and Went (VW) medium, Murashige and Skoog (MS) medium and New Dogashima Medium (NDM), and found that orchid seeds raised in NDM had the highest germination rate and those raised in MS had the lowest germination rate and germinated more slowly because the medium lacked the appropriate substances to promote germination . Another study on melon (Probowati and Daryono, 2018) reported that adding benzyl amino purine (BAP) at the rate of 2 mg per liter to MS medium helped increase the germination rate and callus formation by 90%.

Table 1: Effect of surface sterilization and seed germination rate of Galia and Orange Man cultivars

Cultivar	Number of seeds	Contaminated (seeds)	No contaminated (seeds)	Sprouted (seeds)	%Germination
Galia	150	0	150	96	64.00
Orange Man	150	3	147	40	26.67

Figure 2: Result of pathogen detection by the Cucumber Mosaic Virus (CMV) ImmunoStrip® test: Galia melon (A) and Orange Man melon (B).

After the melon seeds had sprouted and grown true leaves, leaf tip samples were taken to test for Cucumber Mosaic Virus using the CMV ImmunoStrip® test, which is based on serology principles and uses antiserum linkage, or IgG bonding of the virus to colloidal gold particles, a reaction which is easily visualized by a change to reddish-orange color on the test strip. If the sample tested is virus-free, there will be only one reddish-orange band on the test strip, and if the

sample has the virus, then there will be two reddish-orange bands .After testing all the melon seedlings, they were all found to be free of CMV (Figure 2). Due to the use of aseptic techniques in the tissue culture process, we could be assured that all the seedlings used in the experiment were pathogen free.

3.2 Effect of Seedling Age at Time of Transplant on Growth Parameters of Galia and Orange Man Cultivar Melons 42 Days after Transfer to the Shade House

3.2.1 Plant Height and Stem Circumference

After 42 days of growing in the shade house, Galia cultivar melon plants were on average taller than Orange Man cultivar melon plants, a difference that was highly statistically significant. When comparing seedlings of different ages at the time of transplanting, there was no statistically significant difference in their height at 42 days. Average plant height was in the range of 177.05-192.32 cm. At the same time, the combined influence of the factors of cultivar and seedling age at the time of transplanting resulted in a highly statistically significant difference in plant height. Galia cultivar seedlings that were 15 days old at the time of transplant had the highest average height at 232.50±2.44 cm. Analysis of stem circumference at 42 days revealed that Orange Man cultivar seedlings had a greater average stem circumference than Galia cultivar seedlings to a statistically significant degree. Looking at the factor of seedling age at the time of transplant, the 20-day-old seedlings had the greatest average stem circumference at 42 days of 0.94±0.08 cm. The combined influence of cultivar and seedling age at the time of transplanting also resulted in a highly statistically significant difference in stem circumference, with the 15-day-old seedlings of the Orange Man cultivar having the greatest stem circumference at 1.02±0.05 cm, which was not statistically significantly different from the 20-day-old Orange Man seedlings (Table 2, Figure 3).

3.2.2 Number of Leaves, Leaf Width and Leaf Length

Comparing the number of leaves of the two cultivars, after 42 days of growing in the shade house, Galia cultivar melon plants on average had more leaves than Orange Man cultivar melon plants, a difference that was highly statistically significant. When comparing the data from the two cultivars and seedlings of different ages at the time of transplanting, there was also a statistically significant difference in the number of leaves at 42 days. Galia cultivar seedlings from the control group had the highest number of leaves at 27.00±0.93, followed by Galia cultivar seedlings that were 20 days old at the time of transplant, which had an average of 26.33±0.61 leaves. As for the data on leaf width and leaf length, seedlings of the Orange Man cultivar had wider and longer leaves than seedlings of the Galia cultivar to a highly statistically significant degree. Seedling age at the time of transplant also had a statistically significant effect on leaf width and leaf length. Analysis showed that the factors of cultivar and seedling age also had a combined effect-15-day-old Orange Man seedlings had statistically significantly larger leaves, with average width and length of 20.89±0.33

cm and 20.56±0.29 cm, respectively. The sizes were not statistically significantly different from either 10-day-old or 20-day-old Orange Man seedlings, however (Table 2, Figure 3). Our results confirm the finding that each cultivar of melon has its own distinct growth characteristics and size traits that tend to be uniform for that cultivar (Hamid et al., 2002). Our data on the stem circumference and leaf width and length of seedlings transplanted at different ages are consistent with the work of Preecha (n.d.), who reported that 20-day-old seedlings are strong enough to transfer from the tissue culture lab to the shade house.

Table 2: Effect of seedling age on the growth rate of Galia and Orange Man melon cultivated in the shade house at 42 days

Factor		Plant height (cm)	Stem circumference (cm)	Number of leaves per seedling	Leaf width (cm)	Leaf length (cm)
Cultivar (A)						
Galia		218.92±6.78	0.85±0.04b	26.14±0.99a	18.62±0.41b	18.62±0.39b
Orange Man		153.03±4.45b	0.94±0.05a	23.38±1.14b	20.40±0.48a	20.19±0.46a
F-test		**	**	**	**	**
CV (%)		5.41	8.76	7.60	4.03	3.86
Age of seedling (days) (B)						
Control		177.05±16.84	0.91±0.03ab	24.55±1.63	18.95±0.46b	18.95±0.50b
10		184.00±17.57	0.86±0.02b	25.11±1.05	19.56±0.75ab	19.36±0.69ab
15		192.32±24.67	0.89±0.08ab	24.84±1.29	19.68±0.73ab	19.47±0.66ab
20		187.42±18.31	0.94±0.04a	24.42±1.23	19.97±0.61a	19.95±0.48a
F-test		ns	*	ns	*	*
CV (%)		18.83	9.69	9.53	5.87	5.38
Cultivar (A)	Age of seedling (days) (B)					
Galia	Control	205.80±1..96c	0.93±0.03b	27₀00±0.93a	18.50±0.39cd	18v50±0.39c
	10	217.25±4.48b	0.83±0.02d	25.50±0.87abc	18.25±0.38d	18.13±0.19c
	15	232.50±2.44a	0.76±0.03e	25.60±1.16abc	18.60±0.28cd	18.50±0.29c
	20	219.89±5.22b	0.88±0.01c	26.33±0.61ab	19.11±0.43bc	19.33±0.27b
Orange Man	Control	148.30±3.49e	0.88±0.01c	22.10±0.66e	19.40±0.38b	19.40±0.46b
	10	157.40±2.95d	0.88±0.01c	24.80±1.15abc	20.60±0.28a	20.35±0.32a
	15	147.67±3.90e	1.02±0.05a	24.00±1.25cd	20.89±0₀33a	20.56±0.29a
	20	158.20±3.37d	1.00±0.01a	22.70±0.69de	20.75±0.35a	20.50±0.39a
F-test		**	**	**	**	**
CV (%)		3.52	4.93	6.99	3.32	3.18

Means in the same column followed by the same letter are not significantly different by DMRT at P=0.05.

**,* Significant at 0.01and 0.05 probability levels, respectively., ns non-significant

Control is 14 days seedling from seed

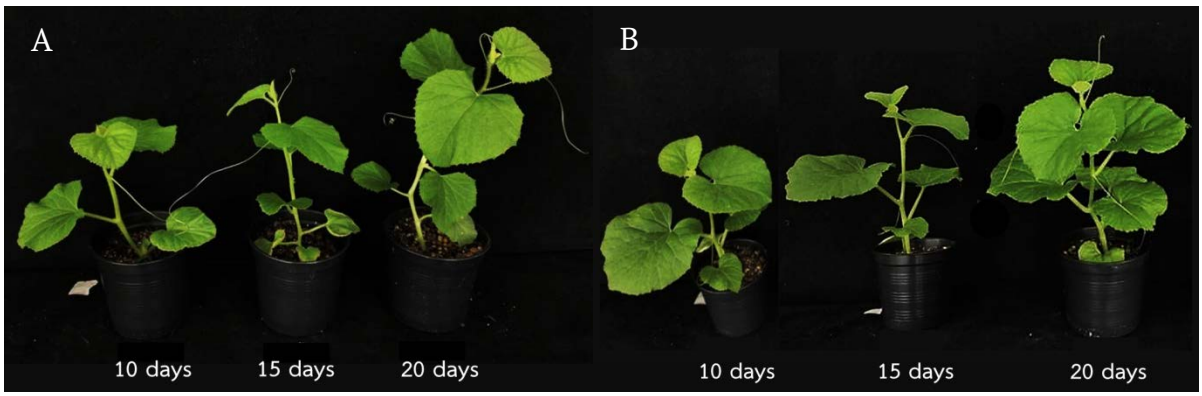

Figure 3: Tissue culture seedlings with different ages at 10, 15 and 20 days of Galia (A) and Orange Man (B)

3.3 Effect of Seedling Age at Time of Transplant on Fruit Quality Parameters of Galia and Orange Man Cultivar melons at Harvest

3.3.1 Fruit Weight, Fruit Width, Fruit Length, Flesh Firmness, Flesh Thickness and Rind Thickness

The data on fruit weight, fruit width, fruit length, flesh firmness, flesh thickness and rind thickness of ripe Galia and Orange Man melons (Table 3) was collected from plants grown from 10-day-old, 15-day-old and 20-day-old seedlings transplanted from tissue culture showed that for some of the parameters, all 3 of the treatments had statistically significantly different average values when compared to the control, which was 14-day-old seedlings grown using conventional methods. The melons from control plants were the heaviest, followed by pathogen-free tissue culture seedlings of both cultivars that were 15 days old at the time of transplantation .Overall, Galia had an average fruit weight of 1.93±0.11 kg (for control) and 1.80±0.17 kg (for 15-day-old at time of transplant) and Orange Man had an average fruit weight of 1.99±0.10 kg (for control) and 1.90±0.17 kg (for 15-day-old at time of transplant). No statistically significant differences were observed in fruit width, and fruit width ranged from 20.80-22.79 cm. Fruit length was greater in the control group and in the 15-days-old at the time of transplant group than in the 10 days old at the time of transplant group and the 20-days-old at the time of transplant group, but was always similar to fruit width, so the fruit shape was round for both cultivars (Figures 4 and 5).

When the figures from measuring flesh firmness, flesh thickness and rind thickness were analyzed, it was found that for the Galia cultivar, the age of the seedling at the time of transplant had no effect on rind thickness, as all Galia fruits had a thin rind measuring less than 1 cm, but the age of seedling at time of transplant did have an effect on flesh thickness. Those that were from plants that were 15 days old at the time of transplant had the thickest flesh and the firmest flesh at 2.56±0.28 cm and 34.86±1.80 Newtons, respectively. For both Galia and Orange Man cultivars, the control seedlings had the lowest flesh firmness. For Orange Man, melons from tissue culture seedlings that were 10 and 15 days old at the time of transplant had the greatest flesh firmness at 26.15±1.80 and 25.25±0.53 Newtons, respectively. These treatments also had thicker rinds. From these results, you can see that the parameters of fruit weight, fruit size, flesh thickness and rind thickness are related .Melons that are larger and heavier tend to be so because the flesh inside is thicker. On the other hand, the combination of thick flesh and thick rind could contribute to larger and heavier fruit overall. Whether the rind thickness or the flesh thickness contributes more, or if the combination is more important, probably depends on the cultivar. In general, greater fruit weight is not always correlated with greater flesh firmness. In the present study, fruits from control seedlings that were raised the conventional way were larger and heavier, but they had less firm flesh than the tissue culture seedlings that were 15 days old at the time of transplantation.

The age of tissue culture seedlings at the time of transplantation affected the fruit quality parameters of the two cultivars in different ways. The tissue culture seedlings were all somewhat

smaller and lighter than the control melons. This could be because the control seedlings tended to have stronger root systems that were less susceptible to damage from handling during transplantation and were capable of absorbing more nutrients from the soil. This probably had a lasting effect on the plant's ability to develop fruits (Higashi, 1999). Still, compared to seedlings that were 10 days old or 20 days old at the time of transplant, the pathogen-free tissue culture seedlings that were 15-days-old at the time of transplant performed well in terms of the parameters that are most important for fruit quality when the melons are consumed, namely a large amount of flesh, firm flesh, and size and weight that were only slightly less than the control melons.

3.3.2 Total Soluble Solids, Titratable Acidity, and pH

Comparing the tissue culture seedlings at different ages at the time of transplant of both cultivars with the control plants, there were no statistically significant differences in TSS or pH . Fruits of the Galia cultivar had TSS ranging from 13.13±0.05 to 14.50±0.24° Brix and fruits of the Orange Man cultivar had TSS ranging from 8.17±0.14 to 9.90±0.40° Brix, and fruits of both cultivars all had pH of close to 6. We can conclude that the age of the seedling at the time of transplant has no significant effect on the sweetness or pH of both these cultivars. The level of sweetness depends on the dominant genes of each cultivar (Akrami and Arzani, 2019). A previous report noted that Galia melons from Japan had TSS of 12-13°Brix (Schultheis et al., 2002). In the present study, there were statistically significant differences in titratable acidity, however. In both cultivars, melons from seedlings that were 20 days old at the time of transplant had the lowest titratable acidity. . Sweetness and acidity are both characteristics that are very important to consumers. Most consumers expressed the greatest satisfaction with melon cultivars that are sweet and fragrant (Apiratikorn et al., 2019). In general, melon flesh that has a TSS of at least 9° Brix is acceptable to consumers (Burger et al., 2006).

Figure 4: Mature fruit of Galia melon and fruit cut open showing flesh color harvested from tissue culture seedlings of different ages: fruit from 14-day-old seedling (control) (A), fruit from 10-day-old tissue culture seedling (B), fruit from 15-day-old tissue culture seedling (C), and fruit from 20-day-old tissue culture seedling (D)

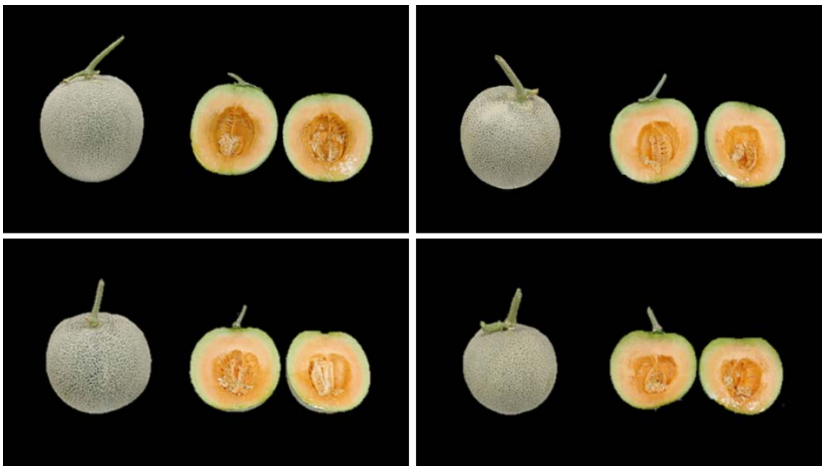

Figure 5: Mature fruit of Orange Man melon and fruit cut open showing flesh color harvested from tissue culture seedlings of different ages: fruit from 14-day-old seedling (control) (A), fruit from 10-day-old tissue culture seedling (B), fruit from 15-day-old tissue culture seedling (C), and fruit from 20-day-old tissue culture seedling (D)

Table 3: Quality of mature fruit of Galia and Orange Man melons harvested from tissue culture seedlings with different ages at the time of transplant (10, 15 and 20 days)

Factor		Fruit weight (kg)	Fruit size (cm)		Fruit firmness (N)	Flesh thickness (cm)	Rind thickness (cm)	TSS (° Brix)	TA (%)	pH
			Width (cm)	length (cm)						
Cultivar (A)										
Galia		1.81±0.13a	22.25±0.69	22.07±0.63a	25.24± 3.7	2.40±0.26	0.60±0.14 b	13.73±0.36a	3.79±0.35a	6.88±0.12a
Orange Man		1.79±0.16a	21.58±0.79	19.83±0.82b	23.60±2.11	2.17±0.33	1.00±0.24a	9.08±0.74b	2.91±0.36b	6.51±0.55a
F-test		ns	ns	**	ns	ns	**	**	*	ns
CV (%)		14.73	6.13	6.41	22.45	15.02	26.92	9.26	19.18	10.79
Age of seedling (days) (B)										
control		1.96±0.11a	22.46±0.50	21.31±0.83	21.90±1.76bc	2.40±0.29a	0.80±0.22	11.33±1.84	3.72±0.43	6.59± 0.70
10		1.70±0.09ab	21.83±0.64	21.17±0.97	25.63±1.60ab	2.43±0.29a	0.70±0.21	11.75±1.43	3.00±0.47	6.69±0.07
15		1.78±0.19ab	21.80±0.98	20.20±0.77	30.06±3.08a	2.37±0.35a	0.95±0.25	11.58±1.02	3.70±0.35	6.54±0.09
20		1.63±0.11b	21.00±0.80	20.13±1.13	20.10±1.76c	1.93±0.16b	0.75±0.21	10.93±1.40	2.98±0.25	6.26±0.02
F-test		*	ns	ns	*	*	ns	ns	ns	ns
CV (%)		12.77	5.91	8.22	16.59	13.57	37.10	24.18	21.81	9.99
Cultivar (A)	Age of seedling (days) (B)									
Galia	control	1.93±0.11a	22.79±0.37	22.43±0.42a	19.57±1.34d	2.53±0.24ab	0.60±0.12c	14.50±0.24a	4.17±0.47a	6.67±0.07
	10	1.65±0.09ab	22.00±0.58	20.00±0.47bc	25.11±1.31bc	2.53±0.17ab	0.56±0.15c	14.00±0.01a	3.77±0.22ab	6.69± 0.09
	15	1.80±0.17ab	22.00±1.16	21.33±0.58ab	34.86±1.80a	2.56±0.28a	0.60±0.12c	13.27±0.22a	3.97±0.07a	6.40± 0.06
	20	1.60±0.05ab	21.33±0.49	19.75±0.97bc	21.42±1.60bcd	1.97±0.14bc	0.63±0.15c	13.13±0.05a	3.27±0.26abc	6.28± 0.02
Orange Man	control	1.99±0.10a	21.93±0.54	22.00±1.16ab	18.78±1.58d	2.27±0.31abc	1.00±0.24ab	8.17±0.14b	3.43±0.44ab	6.91±0.84
	10	1.73±0.09ab	21.75±0.66	20.75±0.75abc	26.15±1.80b	2.17±0.38abc	0.83±0.24bc	9.50±0.85b	2.23±0.07c	6.69±0.01
	15	1.90±0.17ab	21.67±0.83	22.00±0.01ab	25.25±0.53bc	2.33±0.36abc	1.30±0.17a	9.90±0.40b	3.27±0.12abc	6.68±0.03
	20	1.52±0.10b	20.80±0.92	18.75±0.72c	24.23±0.91bcd	1.90±0.17c	0.87±0.23bc	8.73±0.84b	2.70±0.09c	6.25±0.02
F-test		*	ns	*	**	*	**	**	**	ns
CV (%)		12.47	6.23	6.40	12.33	13.44	24.34	8.61	16.73	9.54

Means in the same column followed by the same letter are not significantly different by DMRT at P=0.05.

**,* Significant at 0.01and 0.05 probability levels, respectively. , ns non-significant, Control: 14 days seedling from seed

3.3.3 Fruit Shape, Flesh Color and Rind Color

The fruit shape of both Galia and Orange Man cultivars was round or circular .There were obviously visible differences in the rind color of the two cultivars. CIELAB testing of the L^*, a^* and b^* values (Table 4) showed that Galia, which has net-type rind ornamentation, had L^* and b^* values close to 100 and the lowest a^*, which translates as "bright yellow". As for the flesh inside, the a^* value was negative, reflecting that it is bright green (Figure 4). For the Orange Man cultivar, the inside flesh is orange, living up to its name, and the L^*, $a^*$and b^*were all positive, but for the rind, a^* value was negative, meaning that it is green (Figure 5). It also had net-type ornamentation where

each band was interwoven closer together than that of Galia .From the data on the shape and color of the melons, you can see that the tissue culture seedlings of both cultivars that were 10, 15 and 20 days at the time of transplantation produced melons that were similar to the control seedlings for that cultivar. The shapes, flesh color and rind color were comparable and no different from the controls grown from seed with no tissue culture treatment. So, the factor of seedling age at the time of transplantation had no effect on the shape, flesh color and rind color, but these parameters still could be impacted by environmental factors such as sunlight, temperature, or the nutrients available, such as in the findings of Ferrante et al., (2008), who reported that the L*value of the flesh of *Cucumis melo* L. Var. Reticulatus cv .Prodigio increased when the plants were given fertilizer with more nitrogen .Another factor that can change the shape or color of melons is genetic variations from cross-pollination or intentional breeding efforts (Chayut et al., 2015). In the present study, we did not find any evidence of damage from insect pests or diseases on the leaves or rinds of either cultivar during the growing stage or at harvest.

Table 4: Rind and flesh color of mature fruit of Galia melon and Orange Man melon harvested from tissue culture seedlings with different ages (10, 15 and 20 days at the time of transplant)

Strains	Age of seedling (Days)	Flesh color			Rind color		
		L^*	a^*	b^*	L^*	a^*	b^*
Galia	control	68.23±1.39	-4.30±0.35	17.47±1.27	69.03±0v47	4.50±0.29	45.33±2.65
	10	67.70±1.78	-4.37±0.19	16.30±0.25	67.47±1.11	4.30±0.35	45.63±3.05
	15	66.83±0.71	-5.30±0.49	18.90±0.19	68.73±0.73	4.47±0.40	44.30±2.25
	20	67.23±2.75	-5.10±0.12	17.57±0.47	67.00±0.37	5.33±0.31	44.77±2.20
Orange Man	control	60.10±1.02	9.70±0.38	30.83±0.67	58.73±1.12	-0.75±0.22	10.53±0.53
	10	61.67±0.72	10..37±0.54	32.10±0.59	57.83±1.96	-0.70±0.25	15.50±0.61
	15	61.93±0.75	10.23±0.24	36.27±1.16	59.13±1.75	-0.73±0.17	14.90±1.00
	20	62.73±0.27	10.20±2.41	31.20±1.98	59.97±0.42	-0.73±0.28	16.63±0.45

Control: 14 days seedling from seed

4 Conclusion

It is possible to produce CMV-free melon seedlings using a tissue culture system .This technique can be used to produce a large number of uniform-sized seedlings in a short time. In our study of Galia and Orange Man cultivars, we found that seedling age at the time of transplantation affected some aspects of plant growth and fruit quality .Using seedlings that were 15 days old at the time of transplantation resulted in plants with larger stem circumference, more leaves and larger leaves. All these factors influence important plant functions like photosynthesis, nutrient accumulation, shoot tip development, growth of new leaves and ultimately, fruit development. The seedling age at the time of transplantation was related to the final qualities of ripe fruit at the time of harvest, with 15-day-old seedlings producing fruit with greater firmness and flesh thickness. Their size and weight were also almost as great as the fruits from control seedlings that were grown conventionally from seed .However, seedling age at the time of transplantation did not have any effect on the levels of sweetness or titratable acidity of the ripe fruit. Pathogen-free tissue culture melon seedlings that are transplanted on day 15 are suitable for use in producing melons

commercially. They are comparable to conventional seed-grown melons in all important aspects and are likely to remain disease-free and produce standard, high-quality melons. They can help expand the potential of melon growers in the future.

5 Availability of Data and Material

Data can be made available by contacting the corresponding author.

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