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ASSESSING ECONOMIC ANALYSIS AND MORPHOLOGICAL TRAITS OF TOMATO TISSUE CULTURE PROPAGATION

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ABSTRACT: In order to assess the ability of establishment of tomato tissue culture protocol and economic analysis an experiment was done at Plant Tissue Culture Laboratory, Genetic Department, Faculty of Agriculture, Zagazig University, Egypt. To produce a healthy transplants of different tomato hybrids (Ace 55 VF-Q, Marglobe IMP-VF, Earl Pearson-USA/Q and Marmand-VF), the experiment divided into four stages (seed germination, multiplication, rooting and acclimatization). Shoot number of tomato hybrids gradually increased as number of sub-culture was repeated from initiation, sub-culture one to sub-culture two. In multiplication stage, nodals about 2-3 mm from the previously achieved seedlings were cultured on MS supplemented with 1.5 mg/l of BA + 1mg/l of kinetin. This treatment significantly increased shoots number per explant, shoot length and leaf number per shoot compared to control. The highest values of these traits were noticed on Ace 55 VF-Q hybrid compared to the other hybrids under study. IAA at 1mg/l and NAA at 1mg/l in rooting stage produced the maximum roots number per plantlet and longest roots. Ace 55 VF-Q hybrid recorded the longest root and the higher number of roots per plantlet. In acclimatization stage, the highest transplants survival percentage was achieved with peat moss + vermiculite at 1: 1 (V/V). From economic analyses, the total cost of one transplanting in the tissue culture lab was 0.789 Egyptian pounds, while the total cost of one seedling in the traditional nursery in the summer season was 2.50 Egyptian pounds and in the winter season 1.25 Egyptian pounds.

Key words: Tomato, hybrids, multiplication, rooting, acclimatization, economic

INTRODUCTION

A member of the Solanaceae family, the tomato (*Solanum lycopersicum* Mill.) is a popular, significant, and nutrient-dense food that is grown throughout Egypt in all seasons (**Haque *et al.*, 1999**). The top five tomato producers are China, India, the United States, Turkey, and Egypt, accounting for over 60% of global production. Tomatoes currently rank third in terms of vegetable production worldwide, after potatoes and sweet potatoes (**FAO, 2014**). The tomato crop is a vital component of Egypt's vegetable crop production, both economically and nutritionally (**Bediwy and El-Habbaq, 2016**). About 25% of Egypt's total vegetable cultivated area is used for tomatoes each year, making it one of the most important food commodities and one of the main vegetable crops in terms of cultivated area. More than half of which are on recently reclaimed ground that might yield greater quality and productivity if the marketing mechanism is enhanced. It is anticipated that increasing marketing effectiveness will increase farmers' profits (**Attia and El-Sayed, 2023**). Effective manufacturing of this widely used product in Egypt ensures higher quality and fair prices for both producers and consumers by stabilizing market supply and limiting price swings (**Bhatia and Ashwath, 2004**).

The tomato responds very well to *in vitro* cultures and is especially susceptible to tissue culture. Tomatoes are cultivated in vitro for a

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range of biotechnological purposes, such as producing virus-free plants and researching how tomato cultivars are affected by plant growth regulators and variety (**Praveen and Swamy, 2011**). It has been discovered that genotype, explant, and plant growth regulators utilized in the culture media significantly influence the response of tomato propagation at tissue culture (**Mogadam *et al*., 2015**).

Different elements of the culture media have an impact on the morphogenetic responses of cultured plants *in vitro*, and it's critical to assess how these elements affect plant propagation (**Gubis *et al*., 2004**). There have been several reports of tomato shoot multiplication stages employing various explants with various plant growth regulators, such as benzyl adenine (BA) and kinetin (Kin), both separately and in combination (**Sherbeni *et al*., 2019**).

MS medium containing 16.8 µm kinetin produced a high number of tomato shoots. Micro-shoots were placed on MS media supplemented with 0.5 mg/l IAA and IBA in order to root tomatoes *in vitro*. The plantlets were then acclimated in the culture room and kept in the greenhouse (**Vikram *et al*., 2012**).

Therefore, the purpose of this study was to determine the economic values of specific tomato hybrid seeds that were expensive and propagate them using tissue culture technique.

MATERIALS AND METHODS

In two consecutive seasons of 2022 and 2023, at Plant Tissue Culture Lab., Genetic Department, Fac. Agric., Zagazig Univ., Egypt, carried out this effort to create transplants of four tomato hybrids (Ace 55 VF-Q, Marglobe IMP-VF, Earl Pearson-USA/Q and Marmand-VF) utilizing tissue culture technique. The Alain Seeds Nursery in Abou Hammad, Sharkia Governorate, Egypt, provided the seeds for four tomato hybrids.

For an hour, the obtained seeds were cleaned under running water. Five minutes of soaking in a soap solution were followed by surface sterilization for 60 seconds with 75% aqueous ethanol, 15 to 20 minutes of sodium hypochlorite of 15% solution ($NaClO_4$) containing 1% (V/V) tween 20 as a wetting agent, four rinses with sterile distilled water, and placing on sterilized filter paper (in the culture cabinet) to remove any remaining water. Seeds of four hybrids sterilized and placed on sterilized filter paper then seeds cultured in jars containing MS basal medium without hormones.

Seeds Germination

To obtain sterilized seedlings for the multiplication stage, two seeds of four tomato hybrids were cultivated in jars containing MS basal medium without hormones (**Cortina and Culiáñez-Macià, 2004**). The jars were then left for 25 days. The MS medium was solidified with 0.7% agar and supplemented with 30 g sucrose/l and 0.1 g myoinistol/l. Depending on the goal of each stage, more tested growth regulators were added to the chosen medium. After adjusting the pH to 5.8, the media were autoclaved for 20 minutes at 121 ℃ to sanitize them. Every culture, regardless of stage, was kept in a growth chamber at 25 ± 2℃ for 16 hours with a photoperiod at 2000 watts of cool white fluorescent light. Approximately 3000 Lux was throughout the acclimatization stage and during the germination, multiplication and rooting stages.

Multiplication Stage

Nodals that were roughly 2-3 mm from the previously acquired seedlings were cultivated on MS basal medium that was supplemented with 1.5 mg/l of benzyl adenine (BA) and 1 mg/l of kinetin (Kin). The cultures were incubated for two weeks, with the MS basal medium serving as a control treatment. The number of leaves per shoot, shoot length (cm), and number of shoots per explant were measured.

Rooting Stage

Four tomato hybrids' multiplied branches were removed and cultivated on MS medium enhanced with rooting growth regulators, specifically naphthalene acetic acid (NAA) at 1 mg/l and indole acetic acid (IAA) at 1 mg/l, in comparison to the control treatment. Four weeks after culture, the length of the roots (cm) and the number of roots per plantlet were measured.

Acclimatization Stage

Adapting tomato plantlets before moving them to the wide field was the objective of this stage. After being removed from the containers, the roots were cleaned with sterile distilled

water and then immersed in Rhizolex solution (1g/l) for ten minutes to disinfect them. Plantlets were placed in plastic containers that contained three different types of media: peat moss and sand at a ratio of 1:1, peat moss and vermiculite at a ratio of 1:1 (V/V), or peat moss by itself. To keep the humidity levels surrounding the plantlets high (70 to 80%), cups were covered with polyethylene bags. After seven days, each cup received weekly fertilization with an equal amount of complete fertilizer. Six weeks later, the acclimatization stage was assessed to determine the survival percentage, and plantlet length, number of leaves/plantlet. The cups were maintained in a growth room with 3000 lux of light intensity and a temperature of 25 ± 2°C.

Statistical Analysis:

In accordance with **Snedecor and Cochran (1980)**, statistical analysis was performed on the collected data. It is likely that the **Duncan** (**1955**) multiple range test was used to compare the means at 0.05.

RESULTS AND DISCUSSION

Multiplication Stage

Results in Tables 1 and 2 reveal that the highest shoots number per explant were obtained with Ace 55 VF-Q hybrid which gave (11.50 shoots) at initiation culture, (36.50 shoots) at sub-culture 1 and (109.50 shoots) at sub-culture 2 compared to the other hybrids under study. In contrast, the lowest values in this connection, (8.00 shoots) at initiation culture, (24.50 shoots) at sub-culture 1 and (73.50 shoots) at sub-culture 2 were achieved with Earl Pearson-USA/Q hybrid. In general, their no significant differences were noticed between Marmand-VF and Marglobe IMP-VF hybrids regard shoots number per explant of tomato explants, in most cases. Moreover, shoot length of tomato hybrids significantly affected with BA and Kin at different multiplication periods. Again the longest shoots were obtained from Ace 55 VF-Q hybrid compared to the other hydrides under study (Tables 3 and 4 as well as photo 1). Earl Pearson-USA/Q hybrid recorded the lowest shoots length which were (7.75 cm) at start explant, (9.25 cm) initiation culture, (10.50 cm) at sub-culture 1 and (10.75 cm) at sub-culture 2 in comparison with the other hybrids under study.

In addition, Tables 5 and 6 shows that Ace 55 VF-Q hybrid had more leaves per shoot (7.50, 7.75, 8.00 and 8.00 shoots) at start point, initiation culture, sub-culture 1 and sub-culture 2, respectively. At sub-culture 2 there no significant difference between Ace 55 VF-Q and Marmand-VF hybrids concerning leaves number per shoot of tomato plant. Similar findings were reached by **Arkita *et al*. (2013)** about tomatoes, who found that combining BA with kinetin resulted in an average number of shoots. However, the maximum value of shoots per explant was obtained with BA at 1.5 mg/l. **Sherbeni *et al.* (2019)** reached similar conclusions in this regard. Using the tissue culture approach, they demonstrated how the BA levels were linked to an increase in the quantity and length of tomato shoots.

Rooting Stage

Data recorded in Table 7 and 8 demonstrate that Ace 55 VF-Q hybrid gave the highest number of roots/ plantlet (11.52 roots) and average root length (7.67 cm) compared to the other hybrids. Whereas, the lowest values in this connection were obtained from Earl Pearson-USA/Q hybrid. **Deklerk *et al.* (1999)** observed that auxins have a promotive influence on rooting initials, which may account for these results. Likewise, supplementing NAA at 1 mg/l + IAA at 1 mg/l to MS media were the best treatment for Ace 55 VF-Q, Marglobe IMP-VF, Earl Pearson-USA/Q and Marmand-VF tomato hybrids rooting stage (**Sherbeni *et al.*, 2019**)

Acclimatization Stage

Ace 55 VF-Q hybrid gave the highest values for survival (%) and transplant length compared to the other hybrids (Table 9, 10 and photo 2). However, there no significant differences were noticed between Marmand-VF and Marglobe IMP-VF hybrids concern survival percentage and transplant length of tomato . Furthermore, the lowest values in this connection were achieved with Earl Pearson-USA/Q hybrid. From the foregoing results, it could be concluded that the agriculture media for acclimatization of Ace 55 VF-Q, Marglobe IMP-VF, Earl Pearson-USA/Q and Marmand-VF tomato hybrids produced from tissue culture technique were vermiculite + peat moss at 1:1 (*V/V*).

Table 1. Analysis of variance of shoot number of tomato hybrids under study at through initiation culture, sub-culture 1 and sub-culture 2 during multiplication stage

S.V.	Initiation culture			
	D.F.	MS	$F_{calculated}$	$F_{tabulated}$
Replication	3	0.50000	1.5	(0.05)= 3.86
Treatments	3	9.16667	27.5**	
Error	9	0.33333		(0.01)= 6.99
Total	15			
		Sub-culture 1		
Replication	3	2.833	0.37	(0.05)= 3.86
Treatments	3	107.333	14.31**	
Error	9	7.500		(0.01)= 6.99
Total	15	2.833		
		Sub-culture 2		
Replication	3	25.500	0.37	(0.05)= 3.86
Treatments	3	966.000	14.31**	
Error	9	67.500		(0.01)= 6.99
Total	15			

SV=source of variation, df=degree of freedom, MS=mean sums of square, * = Significant at P <0.05, ** = Significant at P < 0.01, Values are means of four replicates.

Table 2. Means of shoots number per explant of tomato hybrids through initiation culture, sub-culture 1 and sub-culture 2 during multiplication stage

Hybrids	Initiation culture	Sub-culture 1	Sub-culture 2
Ace 55 VF-Q	11.50[a]	36.50[a]	109.50[a]
Marmand-VF	9.75[b]	30.00[b]	90.00[b]
Marglobe IMP-VF	8.75[c]	27.00[bc]	81.00[bc]
Earl Pearson-USA/Q	8.00[c]	24.50[c]	73.50[c]
LSD at 5 %	**0.92**	**4.38**	**13.14**

Table 3. Analysis of variance of shoot length of tomato hybrids under study at through initiation culture, sub-culture 1 and sub-culture 2 during multiplication stage

S.V.	Explants			
	D.F.	MS	$F_{calculated}$	$F_{tabulated}$
Replication	3	0.7500	0.40	(0.05)= 3.86
Treatments	3	48.9167**	26.28	
Error	9	1.8611		(0.01)= 6.99
Total	15			
	Initiation culture			
Replication	3	2.0000	1.71	(0.05)= 3.86
Treatments	3	39.8333**	34.14	
Error	9	1.1667		(0.01)= 6.99
Total	15			
	Sub-culture 1			
Replication	3	2.7500	3.41	(0.05)= 3.86
Treatments	3	30.0833**	37.34	
Error	9	0.8056		(0.01)= 6.99
Total	15			
	Sub-culture 2			
Replication	3	1.5625	3.46	(0.05)= 3.86
Treatments	3	34.3958**	76.20	
Error	9	0.4514		(0.01)= 6.99
Total	15			

SV=source of variation, df=degree of freedom, MS=mean sums of square, * = Significant at P <0.05, ** = Significant at P < 0.01, Values are means of four replicates.

Table 4. Means of shoot length (cm) of tomato hybrids through initiation culture, sub-culture 1 and sub-culture 2 during multiplication stage

Hybrids	Explants	Initiation culture	Sub-culture 1	Sub-culture 2
Ace 55 VF-Q	15.75^{a}	16.25^{a}	16.75^{a}	17.50^{a}
Marmand-VF	11.75^{b}	12.50^{b}	12.75^{b}	13.50^{b}
Marglobe IMP-VF	9.25^{c}	10.00^{c}	11.50^{bc}	12.00^{c}
Earl Pearson-USA/Q	7.75^{c}	9.25^{c}	10.50^{c}	10.75^{d}
LSD at 5 %	**2.18**	**1.73**	**1.4**	**1.07**

Table 5. Analysis of variance of number of leaves per shoot of tomato hybrids under study at through initiation culture, sub-culture 1 and sub-culture 2 during multiplication stage

S.V.	Explants			
	D.F.	MS	$F_{calculated}$	$F_{tabulated}$
Replication	3	0.91667	2.19	(0.05)= 3.86
Treatments	3	5.08333**	12.20	
Error	9	0.41667		(0.01)= 6.99
Total	15			
	Initiation culture			
Replication	3	0.56250	1.42	(0.05)= 3.86
Treatments	3	6.39583**	16.16	
Error	9	0.39583		(0.01)= 6.99
Total	15			
	Sub-culture 1			
Replication	3	0.75000	2.07	(0.05)= 3.86
Treatments	3	4.08333**	11.31	
Error	9	0.36111		(0.01)= 6.99
Total	15			
	Sub-culture 2			
Replication	3	0.72917	1.84	(0.05)= 3.86
Treatments	3	9.39583**	23.74	
Error	9	0. 39583		(0.01)= 6.99
Total	15			

SV=source of variation, df=degree of freedom, MS=mean sums of square, * = Significant at P <0.05, ** = Significant at P < 0.01, Values are means of four replicates.

Table 6. Means of number of leaves per shoot of tomato hybrids through initiation culture, sub-culture 1 and sub-culture 2 during multiplication stage

Hybrids	Explants	Initiation culture	Sub-culture1	Sub-culture 2
Ace 55 VF-Q	7.50[a]	7.75[a]	8.00[a]	8.00[a]
Marmand-VF	5.75[b]	6.50[b]	6.75[b]	7.25[a]
Marglobe IMP-VF	5.25[b]	5.75[bc]	6.00[bc]	6.00[b]
Earl Pearson-USA/Q	5.00[b]	4.75[c]	5.75[c]	4.50[c]
LSD at 5 %	**1.03**	**1.01**	**0.96**	**1.01**

Initiation Culture Sub-culture1 Sub-culture 2

Ace 55 VF-Q Hybrid

Photo. 1. Growth of plantlets of Ace 55 VF-Q Hybrid at intiation culture, sub-culture 1 and sub-culture 2 during multiplication stage

Table 7. Analysis of variance of number of roots per plantlet and root length of tomato hybrids under study during rooting stage

S.V.	D.F.	MS	$F_{calculated}$	$F_{tabulated}$
		Number of roots per plantlet		
Replication	3	0.0788	0.406	(0.05)= 3.86
Treatments	3	14.2416**	73.43	
Error	9	0.1939		(0.01)= 6.99
Total	15			
		Root length		
Replication	3	0.4356	2.37	(0.05)= 3.86
Treatments	3	3.0973**	16.84	
Error	9	0.1840		(0.01)= 6.99
Total	15			

Table 8. Means of number of roots per plantlet and root length (cm) of tomato hybrids during rooting stage

Hybrids	Number of roots per plantlet	Root length (cm)
Ace 55 VF-Q	11.52[a]	7.67[a]
Marmand-VF	9.63[b]	6.53[b]
Marglobe IMP-VF	8.82[b]	6.37[bc]
Earl Pearson-USA/Q	6.98[c]	5.43[c]
LSD at 5 %	**0.90**	**1.03**

Table 9. Analysis of variance of survival percentage and transplant length of tomato hybrids under study during acclimatization stage

S.V.	D.F.	MS	$F_{calculated}$	$F_{tabulated}$
		Survival percentage		
Replication	3	0.138	0.139	(0.05)= 3.86
Treatments	3	215.169**	216.62	
Error	9	0.993		(0.01)= 6.99
Total	15			
		Transplant length		
Replication	3	3.000	2.08	(0.05)= 3.86
Treatments	3	107.000**	74.08	
Error	9	1.444		(0.01)= 6.99
Total	15			

Table 10. Means of survival percentage and transplant length (cm) of tomato hybrids during acclimatization stage

Hybrids	Survival (%)	transplant length (cm)
Ace 55 VF-Q	79.74[a]	35.33[a]
Marmand-VF	76.50[b]	29.00[b]
Marglobe IMP-VF	74.51[b]	27.33[bc]
Earl Pearson-USA/Q	62.98[c]	23.67[c]
LSD at 5 %	**2.35**	**4.26**

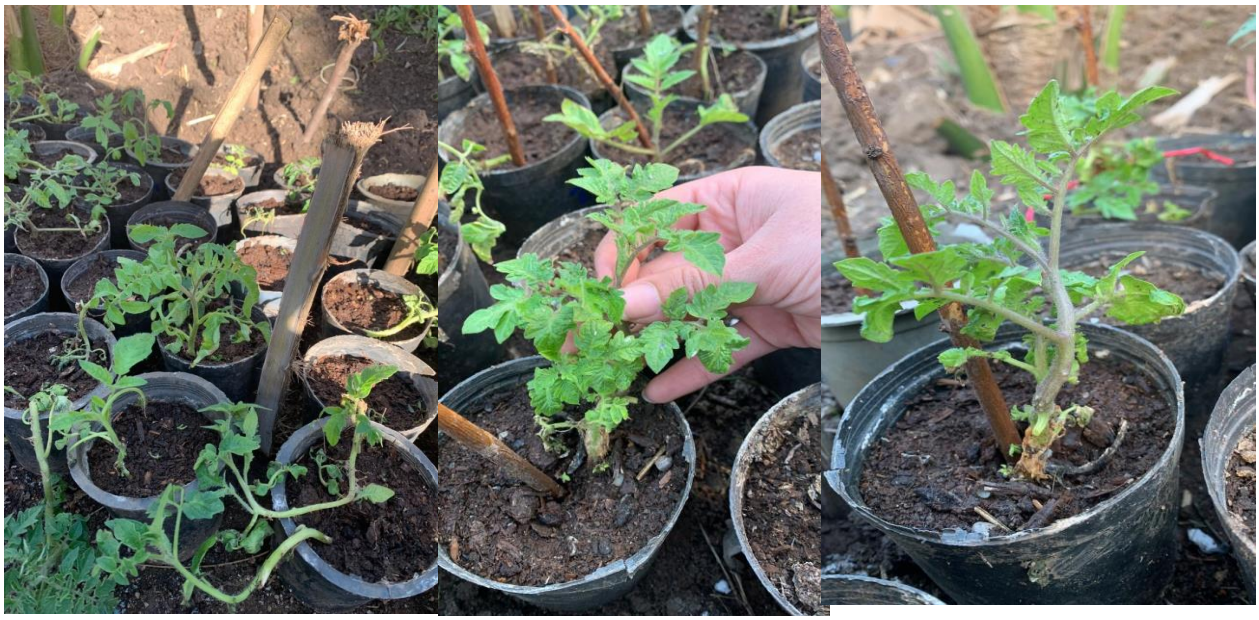

Photo 2. Growth of seedlings of Ace 55 VF-Q tomato hybrid during acclimatization stage

Economic Analysis

From the economic analysis it is clear that the total cost of one transplanting in tissue culture lab was 0.789 Egyptian pounds, while the total cost of one seedling in the traditional nursery in the summer season was 2.50 Egyptian pounds and in the winter season 1.25 Egyptian pounds (Table 11). Through this study, it was found that the price of a package containing 5,000 seeds in the summer season is 7,000 Egyptian pounds. Each jar cultured with 2 seeds, each seed produced 90 seedlings. Each of agar, MS and sugar can be used four times in tissue culture technique. BA and Kin utilized two times per production cycle. In the meantime, NAA and IAA used one time only. The rent of the laboratory for three months is 5,000 Egyptian pounds (production cycle). The laboratory capacity is 10,000 seedlings. The cost of one tray is 500 pounds in the summer season and 250 pounds in the winter season, while one tray produces 200 seedlings.

Table 11. Cost of producing Ace 55 VF-Q tomato hybrid seedlings in tissue culture laboratory compared to traditional seedlings

Statement	Unit	Price	Value (pound/jar)	Value (pound/seed)	Plantlet number /seed	Price of one seedling
Seeds	5000	7000	1.40	1.40	90	0.016
Agar	500	1650	26.4	0.66	90	0.029
MS	50	1550	136.4	3.41	90	0.152
Sugar	1000	35	1.05	0.03	90	0.001
BA	1	650	65.0	1.63	90	0.036
Kin	1	800	80.0	2.0	90	0.044
IAA	5	750	15.0	0.38	90	0.004
NAA	5	750	15.0	0.38	90	0.044
Factory Rent	Pound	10000	-	-	-	-
Jars	1	11	-	-	-	-
Total cost of one seedling in the tissue culture laboratory						**0.789**
Total cost of one seedling in traditional nursery during summer season						**2.50**
Total cost of one seedling in traditional nursery during winter season						**1.25**

CONCLUSION

The maximum number of shoots/explant of Ace 55 VF-Q hybrid was obtained by supplementing MS media with BA at a rate of 1.5 mg/l plus 1 mg/l of kinetin. Additionally, the optimal treatments for the same tomato hybrid's rooting stage were adding 1 mg/l of IAA and 1 mg/l of NAA to MS media. Peat moss and vermiculite at a ratio of 1:1 (V/V) were the most effective agricultural media for acclimatizing plantlets of the Ace 55 VF-Q hybrid tomato hybrid created using the tissue culture technique. Lastly, compared to traditional nurseries and the high cost of imported seeds, the tissue culture technique yielded the lowest pricing for tomato transplants.

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تقييم الجدوى الإقتصادية والصفات المورفولوجية لإكثار هجن الطماطم عن طريق زراعة الأنسجة

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أجريت تجربة في معمل زراعة الأنسجة النباتية، قسم الوراثة، كلية الزراعة، جامعة الزقازيق، مصر وذلك لتقييم القدرة على إنشاء بروتوكول زراعة أنسجة لبعض هجن الطماطم والتقييم الاقتصادي لها. لإنتاج شتلات سليمة من هجن الطماطم المختلفة (Ace 55 VF-Q و Marglobe IMP-VF و Earl Pearson-USA/Q وMarmand-VF) تم تقسيم التجربة إلى أربع مراحل (إنبات البذور والتضاعف والتجذير والأقلمة). زاد عدد براعم الهجن الطماطم تدريجيا مع تكرار عدد من مرات إعادة الزراعة منذ البداية، إعادة الزراعة للمرة الأولى إلى إعادة الزراعة للمرة الثانية. في مرحلة التضاعف، تم زراعة السلاميات بطول حوالي 2-3 مم من الشتلات التي تم الحصول عليها سابقًا على بيئة موراشيج وسكوج مضافًا إليها 1.5 مللجم / لتر من + BA البنزيل أدنين + 1مللجم / لتر من الكينيتين. أدت هذه المعاملة إلى زيادة معنوية في عدد الأفرخ لكل منفصل نباتي وطول الأفرخ وعدد الأوراق لكل فرخ مقارنةً بالكنترول. سُجِّلت أعلى القيم لتلك الصفات في هجين Ace 55 VF-Q مقارنةً بالهجن الأخرى قيد الدراسة. أدي استخدام إندول حمض الخليك بتركيز 1 مللجم/لتر وحمض النفثالين أسيتك بتركيز 1 مللجم/ لتر في مرحلة التجذير أقصى عدد جذور للشتلة، وأطول جذور. سجل الهجين Ace 55 VF-Q أطول جذر وأعلى عدد من الجذور لكل نبيتة. وفي مرحلة الأقلمة، تحققت أعلى نسبة بقاء للشتلات باستخدام البيت موس + الفيرميكوليت بنسبة 1:1 (حجم/حجم). وأظهرت التحليلات الاقتصادية أن التكلفة الإجمالية لإنتاج شتلة طماطم واحدة في معمل زراعة الأنسجة بلغت 0.789 جنية مصري، بينما بلغت التكلفة الإجمالية لشتلة واحدة في المشتل التقليدي في فصل الصيف 2.50 جنية مصرى، وفي فصل الشتاء 1.25 جنية مصري.

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