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DIFFERENTIAL DISPLAY OF *IN VITRO* TRANSLATION AND SUBTRACTIVE ENRICHED cDNA FOR mRNAs FROM GRAFTED AND UNGRAFTED F1 MELON HYPRID ON *Lagenaria siceraria* ROOTSTOCKS

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ABSTRACT

The four grafted melon genotypes; Ismaillawi, Ananas El-Dokki, PI 124111 and the F1 (Ismaillawi PI124111), on *Lagenaria siceraria* rootstocks were found to have higher mean values of all studied developmental characters than the respective values of ungrafted genotypes. The differential increase in developmental characters in different scion genotypes revealed that the PI124111 followed by the F_1 (Ismaillawi x PI124111) were the best combination with this rootstock under irrigation conditions using under-ground water of EC 1.7 m.mhos.

In vitro translated mRNAs isolated from leaves of dark-treated grafted and ungrafted melon genotypes on *Lagenaria siceraria* rootstock, showed that after one week of grafting date, mRNA samples of F_1 (Ismaillawi x PI 124111) expressed two more protein bands (108.4 and 41.7KD) than those of ungrafted plants. After two weeks of grafting date, mRNAs of leaves of dark-treated grafted F_1 plants showed to have the transcript of additional third protein band with a molecular weight of 67KD which was also found to be absent of *in vitro* translated mRNAs of respective ungrafted plants.

The differential display of *in vitro* translated mRNAs of roots and leaves of both scion and rootstock of dark-untreated plants showed that the two unique protein bands (108.6, 42KD) which were also appeared in *L. siceraria* roots found in the *in vitro* translated mRNA samples of leaves of F1 melon scion grafted on *L. siceraria* rootstock. The results suggest that both the two transcripts of mRNAs either might be migrated from the roots of *L. siceraria* rootstock to scion leaves or stimulated to be transcribed in scion leaves as a response of signals originated in roots of the rootstock.

Positive subtractive enriched cDNA fragment, after two rounds of PCR amplification, was observed with a size of about 200bp which will be used as a probe for fishing a complete cDNA involved in enhancing the developmental characters of grafted melon plants on *L. siceraria* rootstocks.

Keywords: *cDNA, Cucumis melo, Grafting, in vitro* translation*, Lagenaria siceraria; mRNA, PCR,* Salinity, Subtractive enrichment.

INTRODUCTION

Grafted genotypes showed to have an induced systematic response and protection against some stresses through the rootstock. This induced systematic response of scion through the rootstock is considered as a source of signals of some proteins (Tiedemann and Carstens 1994).

In grafting studies, the associated phloem-specific proteins are recently considered as useful markers for investigating the long-distance trafficing of macromolecules in plants either for proteins (Lucas *et al.,* 1996; Tiedemann

and Carstens, 1994; Golecki *et al.,* 1998 and 1999) or both RNA and proteins (Smith and Murakishi, 1993; Xoconostle *et al.,* 1999).

Grafting of vegetable crops has been applied on different rootstocks according to the characteristics which can be offered to the scions through the rootstocks. These rootstocks may enhance earliness, growth and production (Chen *et al.,* 1989; Canizares and Goto, 1998) or offer scions the resistance either to some soil diseases (Weng *et al.,* 1993) or to low temperatures (Jebari, 1994; Gao *et al.,* 1998).

Moreover, using F_1 hybrids as scions for different rootstocks were also adopted to enhance the performance of growth and yield of these F_1 genotypes by Tsambanakis (1984).

In this work, an attempt was carried out to study the performance of some developmental characters in grafted and ungrafted plants of four melon genotypes under salinity conditions of underground water. The differential display of *in vitro* translation and subtractive enrichment of cDNA for mRNAs isolated from different tissues of grafted and ungrafted hybrid melon plants as scions and of *Lagenaria siceraria* as a rootstock were also studied.

MATERIALS AND METHODS

Four melon (*Cucumis melo* L.) genotypes were used in this work to study the performance of grafted melon plants as scions on the bottle gourd (*Lagenaria siceraria* L.) as a rootstock and to detect both the differential display of *in vitro* translated mRNAs and a subtractive small cDNA fragment. The four melon genotypes were the two local varieties Ismaillawi and Ananas El-Dokki, the P.I. 124111 which is kindly provided from the North Central Regional Plant Introduction Station, Iowa State University and the F_1 hybrid P.I. 124111 x Ismaillawi.

A cross between Ismaillawi x P.I. 124111 using P.I. 124111 as a female parent was achieved during the early summer season of 1998 in the Experimental Farm at El-Kassasein Horticultural Research Station. Plants of each of the other three genotypes were intercrossed to avoid the inbreeding depression and to multiply their seeds. In the season of 1999, seeds of the four genotypes were sown in 1kg. plastic pots. Seeds of *L. siceraria* were also sown in same-sized pots after one week of melon sowing date. After two weeks, *L. siceraria* seedlings were used as root stocks and melon ones as scions using the tongue grafting approach. At least twenty-five grafted plants from each genotype were achieved. Then grafted and ungrafted plants of each of the four genotypes were transferred to the field. The analysis of underground water used in irrigation was, EC 1.7 m.mhos (1088 ppm), pH 7.6 and the meq/L for the following ions were K^+ = 0.21, Na^+ = 12.4, Mg^{2+}= 0.78, Ca^{2+}= 3.3, CO_3^{2-} = 3.2, Cl^-= 8.47, SO_4^{2-} = 4.86 and HCO_3^- = 3.28.

Growth and developmental characters were measured for both grafted and ungrafted plants on five plants of each entry over three replications. Plant height, growth rate, fresh weight and dry weight were determined. Leaf area was measured using the leaf area meter (CI-203 Area Meter, CID, Inc., USA). Data were collected and variances were analysed according to Zar (1974).

a. RNA Isolation:

RNA of leaf samples were isolated from both dark-treated and untreated plants. RNA of dark-treated grafted and ungrafted F_1 hybrid plants was isolated from leaves twice, once after one week and the other after two weeks of grafting date. Dark treatment was applied using a two layers of black plastic sheets for 48 hours just before RNA isolation. Single-step RNA isolation procedure (Sambrook *et al.,* 1989) based on the original method of Chomczynski and Sacchi (1978) was applied. All solutions and plastic-ware were all DEPC (diethyl pyrocarbonate) treated to inhibit Rnase activity (Chirgwin *et al.,* 1979).

b. mRNA Isolation:

mRNA fraction was isolated from all other nucleic acids using a biotinylated oligo (dT) primer to hybridize the 3' poly (A) region utilizing the PolyAtract mRNA isolation system III (Promega, Z5300). The hybrids were captured and washed using streptavidin coupled to paramagnetic particles and a magnetic stand and the pure intact mRNA fraction was eluted in DEPC-treated water according to Promega manual instructions.

c. *In Vitro* Translation, SDS-PAGE, Electrobloting and Non- Radioactive Detection of Translated Proteins:

The Transcend Non-Radioactive Translation Detection System (Promega, L 5070) coupled with the Wheat Germ Extract (Promega, L 4380) as a cell-free system contains the cellular components necessary for protein synthesis were used. The translation reaction were mixed according to the direction of the manufacturer (Promega) with wheat germ extract, complete amino acid mixture minus leucine and similar volume of that minus methionine, ribonuclease inhibitor, mRNA template in nuclease-free water and a precharged labelled biotinylated lysine-tRNA complex (Transcend tRNA). Then the mixture was incubated at 25^{o}C for 2 hours (Sambrook *et al.,* 1989).

The samples were directly loaded in a 12.5% SDS-polyacrylamide-Bis gel using a prestained SDS-PAGE molecular weight standard (Sigma, SDS-7B). The buffer system and SDS-PAGE procedures were applied according to Laemmli (1970) using a BioRad Protean II 20x20cm Vertical Slab Cell.

Electroblotting was achieved on nitrocellulose membrane (Boehringer Mannheim) using BioRad Mini Trans-Blot cell according to Salinovich and Montelaro (1986) and Sambrook *et al.,* (1989). Proteins were transferred to nitrocellulose membrame at 14 volts for 12 hours.

Membrane preparation, blocking, streptavidin-alkaline phosphatase binding, color development and reagents components were applied according to manufacturer instructions. RNA and mRNA isolation, *in vitro* translation, electropheresis, electroblotting and detection were carried out in the Biotechnology Lab El-Kassasein Horticulture Research Station.

Dried nitrocellulose membranes were photographed and blotted protein bands were analysed and matched using the Phoretix ID gel analysis software version 4.01, Phoretix-International UK, at the Biocomputation Lab.,

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d. Subtractive Enrichment:

1. First strand synthesis.

It was achieved using mRNA templates from grafted and ungrafted melon plants, and the anchored oligo-dT primer (oligo-dT_{12} A/C/G, A/C/G/T) synthesised by Operon Technologies Inc. USA, according to Oh *et al.,* (1995).

2. Second Strand Synthesis and PCR Amplification:

A 25ul of the first strand reaction was mixed with 10ul second strand 10X buffer, 3.5ul DNA polymerase I, 1ul Rnase H, 5 ul 100uM mixed dNTP, 5ul 20uM anchor oligo (dT) primer and 49.5 ul DEPC-treated water. The mixture was incubated for 3 hours at 14^{o}C. Using a random hexamer primar (Promega), PCR reaction was constructed and run at 94^{o}C for 3 min., 40 cycle of 94^{o}C for 30 sec., 42^{o}C for 1min, 72^{o}C for 40 sec. followed by one cycle of 94^{o}C for 2min, 42^{o}C for 1.5 min and 72^{o}C for 5 minuts (Sambrook *et al.,* 1989).

3. Restriction Digestion and *Eco* RI Adapter Ligation:

The cDNA of grafted and ungrafted leaf samples were digested with *Rsa* I (Promega, USA). Then the digested cDNAs were ligated to *Eco* RI adapters (Promega) and used as testers while the cDNA of ungrafted leaves were left as a driver without *Eco* RI adapters. The ligation reaction, was incubated at 15^{o}C overnight. Enzymes were heat inactivated at 70^{o}C for 10 minutes. Then, both adapter-ligated and non-ligated cDNAs were phenol/Chloroform purified, precipitated and resuspended in DEPC-water (Sambrook *et al.,* 1989).

4. Subtractive Hybridization:

Subtractive hybridization and subsequent polymerase chain reaction amplification were conducted as discribed by Wang and Brown (1991). For enrichment of up-regulated genes, cDNA from the ungrafted melon leaves was used as the “driver”, and cDNA from the leaves of grafted melon plants was used as tester. Two rounds of hybridization between testers and drivers were achieved and PCR were done using the primer 5’AAT, TCC, GTT, GCT, GTC, G3’ for the enrichment of up-regulaled genes in tester cDNA. The PCR cycling was as follows; 75^{o}C for 5 min, 94^{o}C for 4min, 40 cycles of 94^{o}C for 40 sec, 50^{o}C for 1 min, 72^{o}C for 1.5 min and one extension cycle of 94^{o}C for 2 min, 48^{o}C for 1.5 min and 72^{o}C for 5 minutes. A mixture of unsubtracted tester and driver also PCR amplified and used as unsubtracted control.

PCR amplified enriched cDNA either subtracted or unsubtracted controls were electrophoresed in 2% agarose containing 0.5ug/ml ethidium bromide in 1x TAE mixture at 72 volts and photographed using a UVP Transilluminator and a 35mm custom Camera.

RESULTS AND DISCUSSION

One. Performance of Grafted Melon Genotypes on *Lagenaria siceraria* rootstock;

Grafted melon plants showed higher mean values in all the studied developmental characters over the four genotypes (Table 1). Ananas El-Dokki showed to have the highest values of plant height for both grafted and ungrafted genotypes. However, grafted plants of the PI124111 showed wider difference (27.7cm) in plant height values, than those of ungrafted plants followed by F_1 (Ismaillawi x P.I. 124111) which had a difference of 25.3 cm. Moreover, the differences between grafted and ungrafted melon plants were the highest in the P.I. 124111 followed by the F_1 (Ismaillawi x P.I.124111) for plant growth rate and leaf area. Greater plant height and growth rate values in grafted plants were also, observed by Tsambanakis (1984), Park and Chung (1989), Chen *et al.,* (1989), Weng *et al.,* (1993), Canizares and Goto (1998) and Gao *et al.,* (1998). Moreover, higher values of fresh and dry weight for each of root, stem and leaves were, also observed in all grafted plants of the four genotypes. Jebari (1994) reached the same conclusion using a hybrid of *Cucurbita moschata* x *C. maxima* as a rootstock.

These results were also confirmed by those obtained from the analysis of variance for the five characters in Table (2). Significant mean squares were observed between grafted and ungrafted melon plants over the four genotypes, indicating that grafting of melon plants of these genotypes is clearly effective in increasing the performance of the studied growth and developmental characters.

However, highly significant mean square value among the four genotypes was only observed for plant height. This clearly suggests that the genetic achitecture of this character interferes the effect of grafting effect. The differential increase in growth characters in different scion genotypes were greater than those of corresponding self-rooted controls (Table 1). This indicates that the best combination with the bottle gourd rootstock, under irrigation with underground water of 1088 ppm salinity, was P.I. 124111 followed by the F_1 (Ismaillawi x P.I. 124111). Similar results were observed for hybrid melons by Leoni *et al.,* (1991).

Two. Differential Display of *in vitro* Translated mRNA Isolated of Dark-Treated Grafted and Ungrafted F1 Plants;

The electroblotted *in vitro* translated proteins showed three unique protein bands to be *in vitro* expressed by mRNA samples from leaves of grafted dark-treated F1 melon plants after two weeks of grafting date. These three bands were absent from ungrafted ones (Fig.1). Meanwhile, mRNA samples isolated after one week of grafting date of F1 melon scions on bottle gourd rootstocks showed only to be differed from ungrafted plants with only two out the three forementioned protein bands. Results in Table (3) based on the molecular weight and Rf matches using the Phoretix matching PC program, showed that the three *in vitro* translated unique protein bands which were found to be expressed by scion leaf mRNA after two-weeks of grafting date,

Table 3: Presence (1) and absence (o) of protein bands of *in vitro* translated mRNA samples from leaves of grafted (Gr.) and ungrafted (Ungr.) F1 melon plants after seven and fifteen days of grafting date.

Molecular Weight	Rf	Seven days		Fifteen days	
(KD)		Ungr.	Gr.	Ungr.	Gr.
180	0.076	0	0	0	0
116	0.136	0	0	0	0
108.4*	0.155**	1	1	0	1
	0.235	1	1	1	1
	0.263	1	1	1	1
84	0.216	1	1	1	1
67.0*	0.288**	1	1	0	1
58	0.326	0	0	0	0
48.5	0.386	1	1	1	1
	0.436	1	1	1	1
41.7*	0.530**	0	1	0	1
	0.564	1	1	1	1
36.5	0.640	1	1	1	1
	0.695	1	1	1	1
26.6	0.767	1	1	1	1
	0.801	1	1	1	1
	0.860	1	1	1	1
	0.915	1	1	1	1
	0.987	1	1	1	1

***, **; Calculated molecular weight and Rf values, respectively.**

have the molecular weights of 108.4, 67 and 41.7 KD. Meanwhile, those of one week mRNA samples expressed only two more protein bands than those of ungrafted F_1 melon plants. These two protein bands had the molecular weights of 108.4 and 41.7 KD. Nine additional proteins were observed by Golecki *et al.,* (1998) in SDS-PAGE gels of scion (cucumber) exudate, after 9-11 days of grafting on *Cucurbita* rootstock.

c. Differential Display of *in vitro* Translated mRNAs Isolated from Roots and Leaves of Scion and Rootstock;

Results of polyacrylamide gel electrophoresis were confirmed with the electroblotted *in vitro* translated mRNAs which were matched using the Phoretix matching PC program (Fig. 2). These results showed, almost, similar *in vitro* translated protein profiles for mRNAs isolated from roots and leaves.

However, two unique protein bands were found to be expressed from mRNA transcripts isolated from leaves of both grafted melon and bottle gourd plants. These two unique bands had the molecular weights of 108.6KD and 42.0 KD. In addition, another band of molecular weight 75.2 KD was observed in the profiles of *in vitro* translated proteins of mRNAs from bottle gourd roots but it was absent from the respective samples of either roots or leaves from grafted melon plants (Table 4).

Table 4: Presence (1) and absence (o) of protein bands of *in vitro* translated mRNA samples from, roots of melon (Rm), roots of bottle gourd (Rb), leaves of melon (Lm), leaves of bottle gourd (Lb), leaves of ungrafted melon (Lun) and leaves of grafted melon (Lgr.) and their molecular weights and relative mobilities (Rf.).

Molecular weight	Rf.	Root tissues		Leaf tissues			
(KD)		Rm	Rb	Lm	Lb	Lun	Lgr.
180	0.046	0	0	0	0	0	0
116	0.117	0	0	0	0	0	0
108.6*	0.135**	0	1	0	1	0	1
	0.163	1	1	1	1	1	1
	0.170	1	1	1	1	1	1
84	0.195	0	0	0	0	0	0
75.2*	0.230**	0	1	1	1	1	1
	0.258	1	1	1	1	1	1
	0.286	1	1	1	1	1	1
58	0.298	0	0	0	0	0	0
	0.343	1	1	1	1	1	1
48.5	0.379	0	0	0	0	0	0
	0.430	1	1	1	1	1	1
42.0*	0.484**	1	1	0	1	0	1
	0.519	1	1	1	1	1	1
	0.572	1	1	1	1	1	1
36.5	0.589	0	0	0	0	0	0
	0.643	1	1	1	1	1	1
26.6	0.739	1	1	1	1	1	1
	0.802	1	1	1	1	1	1
	0.845	1	1	1	1	1	1
	0.929	1	1	1	1	1	1
	0.993	1	1	1	1	1	1

***, **; Calculated molecular weight and Rf values, respectively.**

The results showed that the two unique bands appeared in translated mRNAs of bottle gourd roots which were used as rootstock, were, also, observed in those isolated of leaves from grafted melon plants. These results clearly suggest that the mRNAs, which expressed both proteins, might be migrated from roots of bottle gourd rootstocks to leaves of melon scions, especially both were also observed in leaves of dark-treated grafted melon plants (Fig. 1 and Table 3). In this concern, Smith and Murakishi (1993) observed that, in reciprocal grafts, the transport of virus from inoculated rootstock was limited with resistant material as scion or rootstock. The possibility of some kind of migration of proteins across the graft union via the connecting phloem was observed by Tiedemann and Carstens (1994). Moreover, the results also suggest the possibility that these unique mRNAs might be induced in leaves of scion melon grafted on bottle gourd rootstocks as a result to some signals from these rootstocks. In this concern, Lucas *et al.,* (1996) reported that grafted transgenic plants expressing the movement protein of tobacco mosaic virus (TMV-MP) analysed under tissue-specific

promoters indicated that the mesophyll cells may be the site of TMV-MP action in addition to the evidence that plasmodesmata are capable of trafficing macromolecules. They proposed that plasmodesmata within the leaf, establish a special communication network and input-output signals between the companion cells (CC) and the mesophyll which are involved in regulating photosynthesis in mesophyll. This could result in a shift in biomass partitioning and carbon allocation.

However, Golecki *et al.,* (1998 and 1999) reported that the appearance of additional proteins in scions; either structural or precursors, are followed by their translocation across the newly established phloem bridge at the graft union region. Meanwhile, Xoconostle *et al.,* (1999), in grafting studies, reported that the CmPP16 protein moves from cell to cell, mediates the transport of sense and antisense RNA and moves together with its mRNA into the sieve element (SE) of scion tissue. They, also, reported that the CmPP protein has the characteristics required to mediate RNA delivery into the long-distance translocation stream. They concluded that RNA may move within the phloem as a component of a plant "information superhighway".

Three. Subtractive Enriched cDNA Fragment from Grafted Leaf-mRNA:

To obtain small enriched cDNA fragment to be used as a probe for fishing a whole length of cDNAs of growth enhancing genes, polymerase chain reaction (PCR)-based subtractive hybridization method was applied. This method enriches condionally expressed genes (Wang and Brown, 1991). Starting material consisted of cDNA prepared from control leaf mRNA (ungrafted) and mRNA isolated from leaves of grafted F1 melon plants on *L. Siceraria* rootstock. The enrichment process consisted of two rounds of subtraction and PCR amplification, resulted in the suppression of common ungrafted leaf cDNA and the indirect enrichment of grafted-specific cDNAs.

Sub-populations of the total mRNA pool of both grafted (tester) and ungrafted (driver) were utilized as template for representative cDNA synthesis by reverse transcription with one of twelve possible "anchor" primers, 5'T_{12} (A/C/G) (A/C/G/T). The twelve cDNA sub-populations (together representing the total mRNA pool of either grafted or ungrafted F1 melon plants) were then PCR-amplified with the same anchor primer which employed in cDNA synthesis.

Figure (3) showed that cDNA digestion with *Rsa* I, *Eco* RI adapter ligation to grafted cDNA fragments and two rounds of hybridization between tester and driver cDNAs followed by PCR amplification ended with a fragment of a size about 200 bp. This fragment will be used as a probe in fishing a complete length of cDNA involved in enhancing growth in grafted melon.

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العرض المتباين للترجمه فى الأنبوبه والطرح المكثر لـ د ن أ المكمل لـ ر ن أ الرسول المعزول من هجين الجيل الأول للشمام المطعم والغير مطعم على أصول جذرية من لاجيناريا سيسراريا

عادل أبسخرون جرجس

معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية – جامعة المنوفية

أوضحت الدراسة أن الأربعة تراكيب الوراثية من الشمام (اسماعيلاوى وأناناس الدقى والمدخل النباتى 124111 وهجين الجيل الأول "اسماعيلاوى X المدخل النباتى 124111") والتى طعمت على أصول جذرية من اللاجيناريا سيسراريا يكون لها متوسطات أعلى لكل صفات النمو موضع الدراسة وذلك من متوسطات نظيرتها الغير مطعومة، وقد أوضح التباين فى تلك الزيادة فى صفات النمو فى التراكيب الوراثية الممثلة كطعم أن المدخل النباتى 124111 ويليه هجين الجيل الأول "إسماعيلاوى X المدخل النباتى 124111" يعتبران أفضل توليفة مع هذا الأصل الجذرى تحت ظروف الرى باستخدام المياه الجوفية والتى لها قيمة توصيل كهربى 7ر1 ملليموز (1088 جزء فى المليون).

وقد أظهرت عينات الحمض النووى الريبوزى الرسول (ر ن أ الرسول) والمترجم فى الأنبوبة والمعزول من أوراق التراكيب الوراثية لنباتات الشمام المطعمة والغير مطعمة على الاصول الجذرية للاجيناريا سيسراريا والتى تعرضت للمعاملة بالاظلام أنه بعد أسبوع واحد من تاريخ التطعيم عبرت عينات " ر ن أ " الرسول لهجين الجيل الأول "إسماعيلاوى X المدخل النباتى 124111" عن حزمتين من حزم البروتين (4ر108، 7ر41 كيلو دالتون) أكثر مما ظهر فى النباتات الغير مطعمة من حزم بروتينية، بينما أظهرت ترجمة ر ن أ الرسول المعزول من أوراق نباتات الجيل الأول المطعومة والمعاملة بالإظلام بعد أسبوعين من تاريخ التطعيم أنها تمتلك نسخة لحزمة بروتينية ثالثة بالإضافة للحزمتين الأخريين وهى ذات وزن جزيئى 67 كيلو دالتون والتى وجد أنها تغيب جميعها من عينات ر ن أ الرسول المترجم فى الأنبوبة والمعزول من نظيرتها من النباتات الغير مطعمة.

وقد أوضح العرض المتباين لترجمة الأنابيب للـ ر ن أ الرسول المعزول من جذور وأوراق من كل من الطعم والأصل الجذرى من نباتات لم تعرض للإظلام أن حزمتى البروتين الفريدتين (6ر108 ، 42 كيلو دالتون) والتى ظهرت كلاهما فى جذور الأصل الجذرى لاجيناريا سيسراريا قد وجدتا أيضا فى عينات ر ن أ الرسول المترجمه فى الأنبوبة والمأخوذة من أوراق طعم نباتات الجيل الأول للشمام المطعومة على اصول جذرية للاجيناريا سيسراريا، وتقترح تلك النتائج أن كلا من تلك النسختين الـ ر ن أ الرسول إما أن تكون قد هاجرتا من جذور الأصل الجذرى لاجيناريا سيسراريا الى أوراق الطعم أو تم نسخ كلتاهما فى أوراق الطعم كاستجابة لإشارات نشأت أساسا فى جذور الأصل الجذرى.

لوحظ وجود شظيه من د ن أ المكمل المكثر بعد الطرح واجراء دورتين من الاكثار بتقنين تفاعل البلمرة المتسلسل وكانت الشظية بحجم يقرب من 200 زوج من القواعد والتى ستستخدم كمجس لاصطياد د ن أ مكمل كامل الطول والذى يشترك فى احداث زيادة فى صفات النمو فى نباتات الشمام المطعم على اصول جذرية من لاجيناريا سيسراريا.

Table 1: Mean values of five developmental characters in grafted (Gr.) and ungrafted (Ungr) plants of four melon genotypes on *Lagenaria sceraria* rootstock.

Genotype	Plant height (cm.)		Plant growth rate		Leaf area (cm^2)		Fresh weight (gm)				Dry weight (gm)			
			cm/day				Stem & Root		Leaves		Root & Stem		Leaves	
	Gr.	Ungr.	Gr.	Ungr.	Gr.	Ungr.	Gr.	Ungr	Gr.	Ungr.	Gr.	Ungr.	Gr.	Ungr
F1 (Ismaillawi x PI124111)	59.3	34.0	1.06	0.46	51.9	30.0	10.4	4.11	4.10	1.95	1.21	0.39	0.52	0.28
Ismaillawi	57.3	39.0	0.86	0.43	52.5	33.8	13.2	3.75	5.70	1.85	1.73	0.32	0.70	0.27
P.I. 124111	62.7	35.0	1.28	0.65	55.8	29.4	16.6	5.86	9.68	4.33	2.05	0.72	1.02	0.46
Ananas El-Dokki	77.3	54.0	0.74	0.48	45.2	26.0	12.9	8.43	6.68	5.00	1.51	0.87	0.68	0.56
Average	64.2	40.5	0.99	0.51	51.4	29.8	13.3	5.54	6.54	3.28	1.63	0.58	0.73	0.39

Table 2: Mean squares for five developmental characters in four grafted (Gr.) and ungrafted (Ungr.) melon genotypes on *Lagenaria siceraria* rootstock.

Source of Variations	Plant Height	Growth rate	Leaf area	Fresh weight		Dry weight	
				Root & Stem	Leaves	Root & Stem	Leaves
Replications	37.8	0.032	24.37	2.71	1.21	0.011	0.012
Genotypes	1854.9**	0.151	71.66	20.7	17.96	0.37	0.14
Gr. vs Ungr.	5193.5**	1.392**	2791.6**	357.2**	45.65*	6.6**	0.69*
Error	42.3	0.041	25.31	19.1	7.36	0.37	0.09

***, **; Significant at 5% and 1% levels of probability, respectively.**