IN VITRO PROPAGATION AND STRESS TOLERANCE OF SOME STONE FRUIT ROOTSTOCKS

By

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B.Sc. Agric. Sci. (Horticulture), Fac. Agric., Cairo Univ., 1995
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APPROVAL SHEET

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3 أ. د. نادية محمد منصور الفاكهة المتساقطة معهد بحوث مركز البحوث

البساتين

الزراعية

4 ----------

5 – مستخلص الرسالة (Abstract)

5 – 1 باللغة العربية : بشرط ألا يزيد عن 7 أسطر

(**الكلمات الدالة :** فاكهة ذات النواة الحجرية، أصول، زراعة الأنسجة، الملوحة، الجفاف، الدنا)

تضمن هذا البحث ثلاثة تجارب رئيسية. أجريت الأولى بهدف الوصول إلى تقنية زراعة الأنسجة المثلى لإكثار بعض أصول الفاكهة ذات النواة الحجرية وهى ميت غمر، أوكيناوا، نيماجارد، نيمارد (خوخ) و GF677 (هجين خوخ مع لوز) وتيترا (برقوق). والثانية لتقييم مدى تحمل تلك الأصول للملوحة والجفاف. والثالثة لتعريف تلك الأصول على المستوى الجزيئي، الكشف عن حدوث التباينات الجسدية وتحديد كاشفات جزيئية مرتبطة بمقاومة نيماتودا تعقد الجذور. وبصفة عامة فإن أصل التيترا هو الأسهل فى الإكثار، التجذير والأقلمة كما أنه الأكثر تحملاً للملوحة بينما أصل GF677 هو الأكثر تحملاً للجفاف. النباتات المنتجة معملياً طبقاً للبروتوكول المتبع تتمتع بثبات وراثى.

5 – 2 باللغة الأجنبية : بشرط ألا يزيد عن 7 أسطر

(Key Words : Stone fruit, rootstocks, tissue culture, salinity, drought, DNA **)**

This research was conducted in three main experiments. The first aimed to optimize tissue culture techniques for propagation of some stone fruit rootstocks; Meet-Ghamr, Okinawa, Nemaguard, Nemared (peach), GF677 (peach X almond) and Tetra (plum). The second was evaluating their salinity and drought tolerance. The third was molecular identification, detection of somaclonal variations and detection of molecular markers related to root-knot nematode resistance. Generally, Tetra rootstock was the simplest rootstock to propagate, root and acclimatize and is more tolerant to salinity stress, while GF677 rootstock is more tolerant to drought stress. The studied regenerates according to the used protocol possess high genetic stability.

6 – <u>أهم النتائج التطبيقية التى تم التوصل إليها</u> :

(لا تزيد عن سطرين لكل منها)

6 – 1 أصل التيترا هو الأسهل فى الإكثار، التجذير والأقلمة مقارنة بالأصول الأخرى محل الدراسة.

6 – 2 أصل التيترا هو الأكثر تحملاً للملوحة بينما أصل GF677 هو الأكثر تحملاً للجفاف مقارنة بالأصول الأخرى محل الدراسة.

6 – 3 النباتات المنتجة معملياً طبقاً للبروتوكول المتبع تتمتع بثبات وراثى.

6 – 4 صلاحية تقنيات RAPD وISSR لتحديد البصمة الوراثية والعلاقات بين أنواع الفاكهة ذات النواة الحجرية.

7 – ما هى الجهات التى يمكن أن تستفيد من هذا البحث :

(اذكر هذه الجهات مع شرح أهمية البحث لهذه الجهة بما لا يزيد عن أربعة سطور لكل جهة)

7 – 1 مراكز البحوث والمشاتل التابعة لها.

7 – 2 المشاتل المعتمدة.

3 – 7

4 – 7

8 – هل توجد علاقة قائمة بإحدى هذا الجهات : نعم ✓ ☐

فى حالة نعم اذكر هذه الجهات :

8 – 1 مركز البحوث الزراعية والمشاتل التابعة له.

2 – 8

3 – 8

ما هى طبيعة العلاقة :

مشروع بحثى ☑

تعاون أكاديمى ☐

مشروع ممول من جهة ثالثة (اذكر ما هى : ☐)

أخرى (تذكر ☐)

9 – <u>هل توافق على التعاون مع جهات مستفيدة من خلال الجامعة :</u>

(لماذا

)

✓

(I) لتطبيق البحث : ✓

(II) لاستكمال البـ ✓

(ج) أخرى (**تذكر**

)

10 – <u>هل تم نشر بحوث مستخرجة من الرسالة فى مجلات أو مؤتمرات علمية</u>

(تذكر مع جهة النشر و المكان و التاريخ)

1 – 10 Journal of Biological Chemistry and Environmental Sciences, 2008, 3 (3): 359- 383.

لا

2 – 10

3 – 10

11 – <u>هل سبق التقدم لتسجيل براءات اختراع</u> (تذكر مع الجهة و المكان و التاريخ)

لا

12 – <u>هل توافق على إعطاء البيانات المذكورة فى هذه الاستمارة لجهات أخرى</u>

نعم ✓

توقيع الطالب : توقيع المشرفين :

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– د. محمد حلمي عبد الظاهر

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وكيل الكلية (المعهد) للدراسات العليا و البحوث :

التاريخ

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Title of Thesis: *In vitro* Propagation and Stress Tolerance of Some Stone Fruit Rootstocks
Supervisors: Dr. Mohamed Ahmed Fayek
Dr. Mohamed Helmy Abd El-Zaher
Dr. Nadia Mohamed Mansour
Department: Pomology **Approval:** 10/ 10/ 2009

ABSTRACT

This research was conducted in three main experiments. The first aimed to optimize tissue culture techniques for propagation of some stone fruit rootstocks; Meet-Ghamr, Okinawa, Nemaguard, Nemared (peach), GF677 (peach X almond) and Tetra (plum). The second was evaluating their salinity and drought tolerance. The third was molecular identification, detection of somaclonal variations and detection of molecular markers related to root-knot nematode resistance. Two explant types (shoot tip & nodal cutting) of each rootstock were used. The explants were quick immersed in 70% ethyl alcohol, then soaked in commercial bleach "Clorox" at 10 % for 20 min, for successful surface sterilization. The prepared explants were cultured on MS (Murashige and Skoog) medium supplemented with 0.01 mg/L IBA (Indolebutyric acid) and 0.2 mg/L BA (6- Benzyl adenine), as an establishment medium. During multiplication, BA at 3.0 mg/L was used only for the first two subsequent subcultures to avoid vitrification; then at 0.5 to 3.0 mg/L in the following subcultures. The rooting treatment of 2 mg/L IBA without AC gave the highest rooting % and average root length besides sufficient number of roots during the rooting stage for all rootstocks under study which was suitable for successful acclimatization. Generally, Tetra rootstock proved to be the simplest rootstock to propagate, root and acclimatize. Three kinds of salts; NaCl, Na_2So_4 or $CaCl_2$ were separetely used to induce salt stress and mannitol was used as an osmoticum to induce drought stress. Tetra rootstock seems to be more tolerant to salinity stress while GF677 rootstock is more sensitive compared to other rootstocks under investigation. On contrast, the peach-almond hybrid GF677 rootstock is more tolerant to drought stress whereas Tetra plum rootstock is more sensitive compared to other tested rootstocks. Some of stress-induced proteins, as revealed by SDS-PAGE, were synthesized for the particular stress; the 23.276 KDa protein which associated with inclusion of highest sodium salts (NaCl and Na_2SO_4) concentration in Meet-Ghamr, Okinawa, Nemaguard and GF677 rootstocks. However, certain proteins were common and synthesized under more than one type of stress; the 67.173 and 30.155 KDa proteins in Okinawa rootstock and 61.122 and 17.097 KDa proteins in Tetra rootstock which appeared as a response to both salinity and drought stresses. On the other hand, most of the disappeared proteins in stressed-Tetra rootstock were at the highest concentrations of mannitol which consider the most harmful stress to this rootstock. Thirty of the total RAPD and ISSR markers were found to be rootstock specific including both of positive and negative markers. All of the six stone fruit rootstocks had rootstock-specific markers. No detectable differences were found between the DNA fingerprints of mother plants and their analogous *ex vitro* plants after 3 months acclimatization, indicating that the studied regenerates possess high genetic stability and that the protocol used seems to be suitable for maintaining the integrity of the genome. By using SSR, STS and SCAR markers, it was suggested that, Tetra rootstock as a RKN resistant European plum (*Prunus domestica*) belongs to the subgenera Prunophora and the other tested resistant Amygdalus (peaches) might possess two different resistance systems. Therefore, more work is required for the detection of major genes involved in the RKN resistance of the Amygdalus sources.

Keywords: Stone fruit, rootstocks, tissue culture, salinity, drought, DNA.

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