

Evaluation of MM106 and Omara apple rootstocks for salt tolerance in vitro

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Key words: : NaCl , salt tolerance , in vitro , apple rootstocks.

Abbreviations: BA,(Benzyl adenine) –IBA,(Indolebutyric acid) –GA3, (Gibberellic acid) – MS,(Murashige and Skoog (1962)medium) – MM106,(Malling Merton 106)

Abstract

This experiment was conducted at Plant Tissue Culture Laboratory / Agricultural and Biological Research Center / raqi Atomic Energy Commission during 2001-2003 to study the possibility of using in vitro shoot culture in rooting stage to evaluate salt tolerance of two apple rootstocks (MM106 and Omara).Single shoots were cultured on MS medium as a rooting media supplemented with 0, 20, 40, 60, 80 and 100mM NaCl for 8 weeks.

All rooting parameters (rooting percentage, roots number, roots length)and plantlets growth(height of rooted shoot) decreased as salt level increased in culture medium, with the reductions generally greater for Omara than MM106 rootstock. Also NaCl effect resulted in plantlet necrosis and a reduction in total chlorophyll content of both rootstocks.owever, plantlets of MM106 showed less relative root and shoot growth reduction under salt stress compared with Omara, therefore, it appeared to be more salt tolerant in vitro than Omara.

Introduction

Plants are continuously subjected to different biotic and a biotic stresses such as salt, drought, cold and heavy metal (14, 27).Salinity is considered as one of the most important a biotic stresses that limits crop productivity, affecting several aspects of plant metabolism that generally results in the reduction of plant growth in non-halophytes plants (5,8,16).

Salinity tolerance by plants depends primarily on the genotype that determines alterations on processes such as uptake and transport of salts by roots, together with metabolic and physiological events occurring at cellular level (26).Several studies have shown that apple rootstocks possess genetic differences in salt stress tolerance in vivo (15,24) and in vitro (1,21).

In vitro culture techniques, besides its use as a tool for obtaining salt tolerant plants, may offer potential for quick screening of germplasm against salt stress (4). Screening or evaluation methods involving in vitro shoot culture could be a better system for testing salt tolerance (14). With regard to the whole plant, a similar response to salt stress could be expected in plantlets grown through in vitro shoot culture because such explants can be considered mini-copy of a plant with the anatomical organization and ability to root and grow into a whole plant. This evaluation system were used in several horticulture plants such as tomato (4,19) potato (7,28) banana (10) grape (22,23) and mulberry (18) and apple rootstocks (1). In the present paper , we try to select growth and physiological traits to evaluate salt tolerance of two apple rootstocks (MM106 and Omara) through in vitro shoot culture at rooting stage.

Materials and methods

This study was conducted at Plant Tissue Culture Laboratory / Agricultural and Biological Research Center /Iraqi Atomic Energy Commission during 2001-2003.

Salinity tests were conducted on MM106 and Omara Apple rootstocks under in vitro conditions. Shoot cultures of apple MM106 and Omara rootstocks were initiated in vitro as described by AL-Sabiry (2) by culturing sterilized terminal and axillary buds on Murashige and Skoog (MS) medium supplemented with 1mg /L (IBA), 0.2 mg / L (BA) and 0.2 mg /L (GA3). Once cultures were established then transferred to MS medium containing 1mg /L (IBA) and 2 mg / L (BA) for shoots proliferation as reported by Shlash (21) and then shoots were sub cultured three times for further shoots production.

At the end of the third subculture, uniform single shoots(3 cm in length) were excised from proliferating cultures and cultured on plant growth regulators free - MS medium supplemented with (0, 20 , 40 , 60 , 80 or 100 mM) NaCl . Each treatment consist of 20 jars (325 ml) as a replicates, each jar (containing 50 ml solidified media) contained 5 shoots. Cultures were maintained for 8 weeks (as long term salt stress) at the growth room (25±2°C) under a 16 hour photoperiod fluorescent light. Then rooting percentage, number & length of roots per rooted shoot, height of rooted shoot and total chlorophyll content were recorded. Total chlorophyll was calculated according to the method of Hendry and Price (12).

The experiment was set up as a factorial arrangement of two apple rootstocks and six NaCl levels in a completely randomized design. Analysis of variance was performed on the data, and comparisons among treatment means were made via the Least Significant Difference (LSD) test ($\alpha = 0.05$) (6) which was carried out using SAS program (20).

Results and Discussion

Data represented in Fig.1 reveal the negative effect of NaCl salt stress significantly on rooting percentage of both apple rootstocks (MM106 and Omara). It is clear that rooting percentage of the two apple rootstocks decreased with increasing NaCl levels in rooting medium. While the reduction of rooting percentage was greater in Omara rootstock than in MM106 at all NaCl levels (from 20 to 100mM), only 20% of MM106 shoots developed roots at 100mM NaCl but Omara shoots failed in rooting at the same

NaCl level. These results indicated that in vitro rooting ability under salt stress was genotype dependent which is in agreement with the data found in tomato (4) and apple rootstocks (1,21), where a high effect of genotype on rooting ability under NaCl salt stress in vitro. Also, these authors observed that higher concentrations of NaCl caused a significant reduction in rooting percentage of MM106 and Omara apple rootstocks shoots. Similar results were reported for other genotypes of tomato (4) and potato (14) and myrtle (9). The reduction in rooting percentage might be due to the inhibitory effects of salt on the metabolic activities which associated with cell division, differentiation and elongation (3), and these processes companied with adventitious root initiation on the base of microcuttings (11).In addition, salinity has been shown to reduce the endogenous auxin (IAA)(13) and rooting co-factors levels which might lead to reduce root initiation ,and these substances (especially IAA)play an important regulatory role in root formation and their development (1,11).

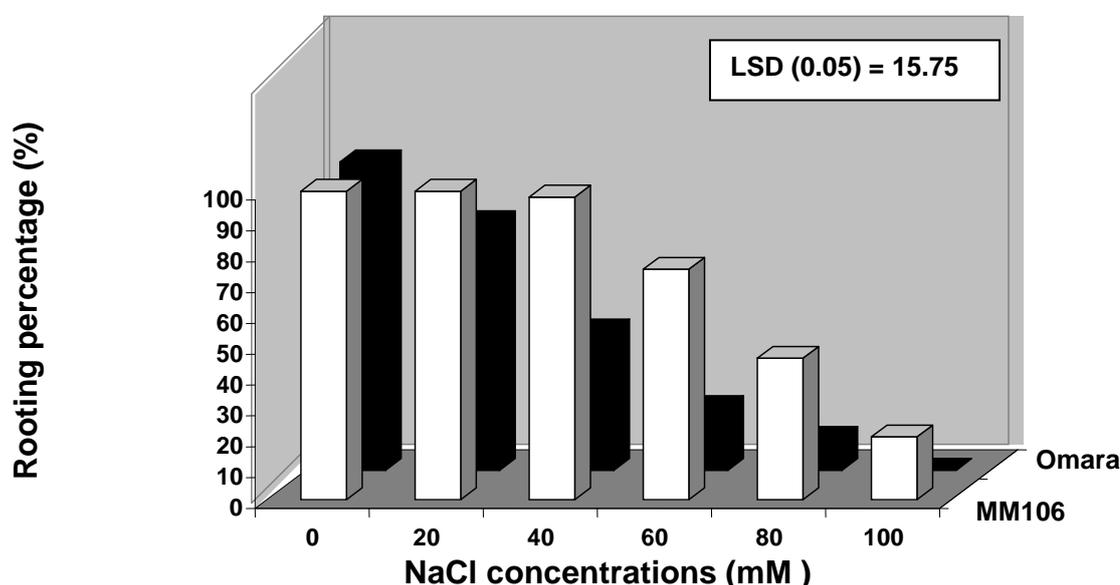


Figure 1. Effect of NaCl on rooting of MM106 and Omara apple shoots in vitro after 8 weeks

In general, the different levels of NaCl significantly decreased number and length of roots when compared to control (0 mM NaCl) for both rootstocks with increasing salinity levels(Table 1).The means root number decreased from 1.41 at 0mM NaCl to 1.12 at 100mM and for root length from 25.3 mm at 0mM NaCl to 7.0 mm at 100mM. The apple rootstock MM106 had the higher significant root number (1.35) and root length (20.1 mm) than Omara rootstock (1.25 and 15.9mm respectively) when tested over all salinity levels. For MM106, the effects of NaCl on root number and length reduction was significant at above 60mM NaCl level, while this effect was significant at above 40 mM for Omara root stock. This result was in line with (1,21) in apple rootstocks, (23) in grape and (10) in banana. The reduction of root number and length caused by high NaCl concentration might be due to the same reasons which reported above in discussion of rooting percentage reduction by salinity.

Table(1) Effect of NaCl concentrations on root characters Of MM106 and Omara apple rootstocks during rooting stage.

NaCl concentrations (mM)	Apple rootstocks		Mean (B)
	MM106	Omara	
Root number per plantlets			
0	1.44	1.38	1.41
20	1.42	1.32	1.37
40	1.37	1.30	1.34
60	1.34	1.25	1.30
80	1.27	1.23	1.25
100	1.24	-----	1.12
Mean (A)	1.35	1.25	
LSD (0.05)	A= 0.04 B= 0.04 AB= 0.08		
Root length per plantlets(mm)			
0	27.0	23.5	25.3
20	26.3	20.0	23.1
40	22.5	17.6	18.6
60	17.3	17.3	17.3
80	14.6	15.9	15.3
100	14.0	-----	7.0
Mean (A)	20.1	15.9	
LSD (0.05)	A=3.8 B=3.7 AB=5.18		

The addition of NaCl to rooting medium caused decrease in height of rooted shoot for both rootstocks(table 2) and a significant differences in height of plantlets was observed between them (44.65 and 31.36 mm for MM106 and Omara respectively). No plantlets of MM106 shows reduction in their height below 60mM NaCl level, while for Omara, the first reduction was observed at 40mM NaCl . These results had been already observed for apple rootstocks (1,21) .The decrease in plantlet growth in response to salinity was the consequence of reduction in the number of new leaves formed on the axis which make the photosynthesis area will be eventually become too low to support continuing growth. Also , salt induced water deficiency, osmotic dehydration and ion toxicity (23) .

Table 2 shows the decrease in the content of total chlorophyll when the NaCl concentration increased in rooting medium. Total chlorophyll content was significantly lower in leaves of salt rooted shoots treated with(60,80 and100mM NaCl) than in leaves of control(0mM NaCl) while, total

Table (2) : Effects of NaCl on height and total chlorophyll content of MM106 and Omara apple rootstocks plantlets after 8 weeks in culture.

NaCl concentrations (mM)	Apple rootstocks		Mean (B)
	MM106	Omara	
Height of the plantlets (mm)			
0	56.03	49.31	52.67
20	53.09	39.68	41.38
40	46.63	33.42	40.02
60	40.15	33.32	36.73
80	36.87	32.43	34.65
100	35.12	-----	17.56
Mean (A)	44.65	31.36	
LSD (0.05)	A=5.42	B=5.42	AB= 11.17
Total chlorophyll (mg/ g F.W.)			
0	0.46	0.42	0.44
20	0.42	0.36	0.37
40	0.37	0.30	0.34
60	0.34	0.26	0.30
80	0.27	0.23	0.25
100	0.24	-----	0.12
Mean (A)	0.33	0.26	
LSD (0.05)	A= 0.06	B= 0.05	AB= 0.12

chlorophyll of rooted shoots treated with 20 and 40 mM NaCl remained unaltered compared to that of control. Rooted shoots of MM106 showed higher total chlorophyll than Omara when tested over all salinity levels used in this study. On the other hand, as shown in table (2), decreases in the total chlorophyll content were more pronounced in treatments of 80 mM and 100 mM NaCl in MM106 rootstock, while, the most inhibiting effect of salt was determined during the 60 mM and 80 mM NaCl treatments for Omara rootstock. Such reduction total chlorophyll under in vitro salt stress conditions was observed in some in other plant species as mentioned by (25) in quince and (23) in grape and (10) in banana. These results might be described to biosynthesis of chlorophyll was generally inhibited by the depressive effect of stress conditions on the absorption of some ions which were involved in the chlorophyll formation, such as (Mg and Fe) which could be expected as a reason for chlorophyll suppression in leaves and / or an increase of some growth inhibitors, such as ethylene or abscisic acid production which enhances senescence that might be occurred under salt stress condition (9).

Conclusions

The results obtained in this study suggest that shoot culture at rooting stage may be useful as a rapid evaluation method for classifying apple cultivars (rootstocks) for salt tolerance in vitro. Although the rooting parameters can be used to give a coefficient in evaluated rootstocks (MM106 and Omara), further research is needed to investigate effects of salinity under field conditions for MM106 and Omara rootstocks, where the problem is more complicate. Also, further research is needed to study the effect of NaCl for other rootstocks in vitro.

References

- 1-Abdel-Hussein M.A-A. (2004) Application of in vitro mutagenesis technique for improvement the tolerance of MM106 and Omara apple rootstocks to sodium chloride. Ph.D. Thesis. College of Agriculture, Baghdad University, Iraq.
- 2-AL-Sabiry H. A-R. (1996) Comparative study of apple (*Malus domestica* Borkh.) cultivars propagated in vitro. Ph.D.Thesis.College of Science, Baghdad University, Iraq.
- 3-Binh D-Q. ; Heszky, L. E.; Gyulai, G. and A. Csillag (1992) Plant regeneration of NaCl-pretreated cells from long-term suspension culture of rice (*Oryza sativa* L.) in high saline conditions. *Plant Cell Tissue Organ Culture*, 29: 75-82.
- 4-Cano E.A, F. Pérez-Alfocea, V. Moreno, M. Caro and M.C. Bolarin (1998) Evaluation of salt tolerance in cultivated and wild tomato species through in vitro shoot apex culture. *Plant Cell, Tissue Organ Culture*, 53:19-26.
- 5-Chinnusamy V., A. Jagendorf and J-K. Zhu (2005) Understanding and improving salt tolerance in plants. *Crop Science*, 45:437–448.
- 6-Compton M.C. (1996) Statical analysis of plant tissue culture data. In: Trigiano R.N. and D.J.Gray (eds.)*Plant Tissue Culture :Concepts and Laboratory Exercises*.(pp.47- 60).CRC.Press.
- 7-Farhatullah R. Mahmood and Raziuddin (2002) In vitro Effect of salt on the vigor of potato (*Solanum tuberosm* L.) plantlets. *Biotechnology*, 1 (2-4) 73-77.
- 8-Flowers T.J. (2004) Improving crop salt tolerance. *Journal of Experimental Botany*, 55(396) 307-319.
- 9-Hanafy Ahmed, A.H. ; Hassan, H.M. ; Gad, M.M.A. and M.A.Amin (2002) Micropropagation of *Myrtus communis* and increasing its tolerance to salinity stress by using polyamines. *Proceedings of the 2nd Congress on Recent Technologies in Agriculture, Faculty of Agriculture, Cairo University 28 – 30 October*. Pp.995- 1011.
- 10-Harb E.M.; O.M. El – Shihy; A. H. Hanafy Ahmed and N.A. Mualla (2002) Using plant tissue culture technique for rapid propagation and salt tolerance in banana plant. *Proceedings of the 2nd Congress on Recent Technologies in Agriculture, Faculty of Agriculture, Cairo University 28 – 30 October*. Pp.979-994.
- 11-Hartmann, H. J ; D. E. Kester ; R.L. Geneve and Jr. F . T. Davies (1997). *Plant Propagation: Principles and Practices*. (6th edn.) Prentice - Hall Inc., New Jersey, USA.
- 12-Hendry G.A.F. and A.H. Price (1993) Stress indications: chlorophylls and carotenoids. In: Hendry G.A.F. and J.P. Grime (Eds.) *Methods in Comparative Plant Ecology*. pp 148-52. Chapman & Hall.
- 13-Khan M.I. ; Khan M.A. and T. Khizar (1976) Plant growth regulators from species differing in salt tolerance as affected by soil salinity .*Plant and Soil* , 45 : 267-271.

- 14-Martinez C.A. ; M. Maesin and E.G. Lam (1996) In vitro salt tolerance and proline accumulation in Andean potato (*Solanum spp.*) Differing in frost resistance. *Plant Science*, 116: 177-184.
- 15-Motosugi H. ; A. Sugiura and T. Tomana (1987) Salt tolerance of various apple rootstock cultivars. *Journal Japanese Society Horticulture Science*, 56(2) 135 – 141.
- 16-Munns R. and A. Termaat (1986) Whole plant response to salinity. *Australian Journal of Plant Physiology*, 13: 143-160.
- 17-Orcutt D.M. and E.T. Nilsen (2000) *The Physiology of Plants Under Stress: Soil and Biotic Factors*. John Wiley & Sons, Inc.: USA.
- 18-Poontariga H. ; S. Srisunaka ; S. Pitukchaisopola and R. Charoensatab (2000) Mechanisms of adaptation to increasing salinity of mulberry: Proline content and ascorbate peroxidase activity in leaves of multiple shoots. *Science Asia* 26: 207-211.
- 19-Sancho–Carrascosa M.A. ; S. Jimenez–Bermudez. ; R. Peran–Quesada ; F. Peliego–Alfaro and M. Quesada (2000) Assessment of in vitro growth of apical stem sections tolerance in tomato. *Plant Cell, Tissue Organ Culture*, 62: 101–6.
- 20-SAS Institute (1992). *SAS/STAT user's guide*, Vol. 1; Release 6.03, ed. SAS Institute Inc. Cary, NC. USA.
- 21-Shlash J.S.(1999)Tolerance of in vitro propagated apple rootstocks to salinity(NaCl).Ph.D. Thesis. College of Agriculture, Baghdad University, Iraq.
- 22-Singh S.K. ; H.C. Sharma ; A.M. Goswami ; S.P. Singh and S.P. Datta (2000) In vitro and leaf composition of grapevine cultivars as affected by sodium chloride. *Biologia Plantarum*, 43(2)283-286.
- 23-Sivritepe N. and A. Eris (1999) Determination of Salt Tolerance in Some Grapevine Cultivars (*Vitis vinifera L.*) under in vitro conditions. *Turkish Journal of Biology*, 23: 473–485.
- 24-Therios I. N. and N.D. Misopolinos (1989) Differences in tolerance to sodium chloride salinity between three commercial apple rootstocks. *Soiless Culture*, 5(1) : 55-71.
- 25-Vitaglino C. ; A. Mensuali-Sodi and F. Blando(1992) Effect of NaCl on quince (*Cydonia oblonga*) tissue culture. *Acta Horticulture*, 300: 347-352.
- 26-Winicov L. (1993) Gen expression in relation to salt stress. In Basara A.S.(ed.)*Stress-induced Gene Expression in Plants*. Harwood Academic Publishers, Langhorne, Pa., USA. pp 61-130.
- 27-Xiong L. ; K.S. Schumaker and J.-K. Zhu (2002) Cell Signaling during Cold, Drought, and Salt Stress. *The Plant Cell*, 14: 165–183.
- 28-Zhang Y.L. ; D.J. Donnelly and Y.L. Zhang (1997) In vitro bioassays for salinity tolerance screening of potato. *Potato Research*, 40: 285- 295.

تقييم تحمل أصلي التفاح MM106 و عماره للملوحة خارج الجسم الحي

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الخلاصة

أجري هذا البحث في مختبرات زراعة الأنسجة النباتية في دائرة البحوث الزراعية و البيولوجية في منظمة الطاقة الذرية العراقية للمدة 2001-2003 بهدف دراسة إمكانية استخدام تقنية الزراعة خارج الجسم الحي في تقييم تحمل أصلين من أصول التفاح MM106 و عماره لملح كلوريد الصوديوم NaCl في مرحلة التجذير. فصلت الأفرع المتضاعفة وزرعت بصورة منفردة على وسط MS كوسط لتجذيرها والمجهز بـ 0 ، 20 ، 40 ، 60 ، 80 أو 100 ملي مول ملح كلوريد الصوديوم NaCl لمدة 8 أسابيع.

إن جميع مؤشرات التجذير (نسبة التجذير وعدد وطول الجذور) ومؤشرات النمو الخضري للنباتات (ارتفاع الأفرع المجذرة وعدد الأوراق الحديثة المتكونة عليها) قد إنخفضت بزيادة مستويات الملح في الوسط الغذائي مع إختزال أعلى في أصل التفاح عمارة مقارنة مع الأصل MM106. كما أظهر الملح تأثيراً في إصفرار النباتات مع إختزال في محتوى أصلي التفاح من الكلوروفيل الكلي. على أية حال فإن الأصل MM106 قد أظهر أقل أضراراً للملوحة وأقل إختزلاً في نمو الجذور والفروع تحت الإجهاد الملحي لذا يمكن اعتباره أكثر تحملاً للملوحة خارج الجسم الحي.