UNIVERSITI PUTRA MALAYSIA

GROWTH AND DEVELOPMENT OF TOMATO (*Lycopersicon esculentum* Mill.) CULTIVARS *IN VITRO* UNDER SALINITY CONDITIONN

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By

ABDALMAJID NASHER AHMED MOHAMED

Thesis Submitted to the School of Graduate Studies, University Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor Philosophy

August 2012

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَىٰ وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ ﴾

"My Lord, enable me to be grateful for Your favor which You have bestowed upon me and upon my parents and to do righteousness of which You approve. And admit me by Your mercy into [the ranks of] Your righteous servants."

صدق الله العظيم
سورة النمل-الاية(19)

DEDICATION

Dedicated to my parents,

To my wife,

To my kids,

And to all my brothers and sisters.

Abstract of thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

GOWTH AND DEVELOPMENT OF TOMATO (*Lycopersicon esculentum*. Mill) CULTIVARS *IN VITRO* UNDER SALINITY CONDITION

By

ABDALMAJID NASHER AHMED MOHAMED

August 2012

Chairman: Professor Mohd Razi Bin Ismail, PhD

Faculty: Agriculture

The salinity of soil and irrigation water present one of the greatest environmental strains affecting plant growth and development. Salinity influences agricultural production in most parts on the world. The tissue culture technique provides a unique chance for studying many aspects of plant growth and development, and studying the physiological and biochemical processes under controlled environment, which contribute to salinity tolerance. In addition, it offers greater control than *in vivo* growth conditions and has the benefit of small scale with clear visibility for observation of shoot and root responses in the presence of enforced stress.

This study was carried out in the Agrobiotechnology laboratory- Faculty of Agriculture and plant physiology in ITA-MTDC, UPM, and achieved the following objectives: Establishment of a protocol for shoot regeneration from

hypocotyl and cotyledon explants by different concentrations of BAP hormone, response hypocotyl and cotyledon explants to shoot formation under NaCl concentration, study of the changes in organic and inorganic solutes in shoots grown from hypocotyl, and cotyledon explants under NaCl stress and increase for NaCl tolerance by shoot apices sub-culture under different levels of NaCl stress.

In vitro, response of hypocotyl and cotyledon explants to shoots regeneration in MS medium supplemented different levels of 6-benzylaminopurine (0. 1, 2, 4) mg/l was studied. The cultures harvested after six weeks from culture explants. Different responses were recorded in tomato explants under different levels of BAP. The formation of roots from hypocotyl explants found in the control treatment, whereas the cotyledons did not show any response. The media supplemented with 4 mg/l BAP resulted in callus in both explants. The medium supplemented 2 mg/l BAP was most effective for the induction of adventitious shoots for both the hypocotyl, and cotyledon explants in tomato cultivars; where the number of shoots/explant was 4 and 4.5 in hypocotyls and cotyledons Pearl cultivar and 2.25 and 3 shoots/explants in hypocotyls and cotyledons Beril cultivars respectively. The best shoots length achieved by hypocotyl explants in Pearl cultivar; where it was 1.62 cm in medium was supplemented 2 mg/l BAP.

In vitro, evaluations of sodium chloride (NaCl) effects on two tomato cultivars (Pearl and Beril) were investigated with four NaCl levels (0, 25, 50 and 75 mM) using hypocotyl and cotyledon explants. The explants were cultured in MS media having 2.0 mg/l BAP (the suitable medium from the previous experiment) along with different concentrations of NaCl. The cultures harvested after eight weeks from culture explants. The growth characteristics (shoots number, shoots length, fresh weight and dry weight) decreased with increasing NaCl in the growth medium. The reduction percentage in shoots length was 85% in high level NaCl (75 mM) in comparison with control; whereas the reduction in shoots number, fresh weight and dry weight was 65, 64 and 54% in high level NaCl. In addition, there was greater degradation of chlorophyll *b* than chlorophyll *a* and different responses of tomato cultivars and explants for salt tolerance *in vitro*; where the reduction percentage in chlorophyll *a* and *b* was 69%, 76% in high level NaCl. The growth characteristics and chlorophyll content were superior in the Beril compared to the Pearl cultivar, and the shoots induced from cotyledon explants showed best in growth characteristics and chlorophyll content than the shoots induced from hypocotyl explants under NaCl stress. The findings may contribute to best multiplication from cotyledon explants in Beril cultivar to raise a salt-tolerant line for future use.

Changes of carbohydrate, soluble protein, proline and ions content (Na^+, K^+, Ca^{2+}, and Mg^{2+}) were studied in shoots induced from hypocotyl, and cotyledon explants of Pearl and Beril cultivars *in vitro* under salt stress. The explants were cultured in MS+2 mg BAP supplemented different levels NaCl (0, 25, 50 and 75 mM). The cultures harvested after eight weeks from culture explants. The accumulation of organic and inorganic solutes is significantly affected by NaCl stress. An increase of NaCl in the growth medium led to increased accumulation of carbohydrates, proline and Na^+ in the shoots induced from hypocotyl and cotyledon explants, whereas the accumulation of protein, K^+, Ca^{+2} and Mg^{+2} decreased by the increase of NaCl in the growth medium. The high amount of carbohydrate and proline (20.2 mg/g and 13.69 μmoles/g) achieved by shoots induced from cotyledon explant in Beril cultivar in high level NaCl. The content of potassium in high level NaCl reached to 2.46 and 2.43 mg/g by shoots induced from cotyledon explants in both Beril and Pearl cultivars respectively. Different responses were seen between the tomato cultivars for the accumulation of organic and inorganic solutes in shoots induced from hypocotyl, and cotyledon explants *in vitro* under salt stress. The Beril cultivars and cotyledon explant were better than Pearl and hypocotyl explant in almost all organic and inorganic solutes.

In vitro, response of shoot apices subculture of Pearl and Beril tomato cultivars under different concentration NaCl (0, 25, 50 and 75 mM) to

enhance of salt tolerance in tomato plantlets was studied. The shoot apices were cultured in MS+0.1 mg/l IBA medium for four weeks, and the shoot apices isolated and subculture in the same medium for four weeks. The growth characteristics were evaluated in both first and subculture shoot apices included (Shoots length, roots length, plantlets fresh and dry weight, shoots and roots carbohydrates contents, shoots and roots protein contents, shoots and roots proline contents and shoots and roots (Na^+, K^+) ions contents were recorded. In respect of the growth of tomato plantlets grown from shoot apices explants affected by NaCl stress, an increase in NaCl in the growth medium led to a decrease of the growth characteristics of tomato plantlets. The shoots length increased after shoot apices subculture to 5.38 and 5.78 cm, while it was 4.30 and 4.50 cm in first culture in both Pearl and Beril cultivar in high level NaCl, and the same trend shown in roots length, fresh and dry weight characteristics. The accumulation of carbohydrates, proline and Na^+ in the roots and shoots of tomato plantlets increased with increased salinity in both the first and subculture, whereas the protein and K^+ content decreased. Shoot apices subculture enhanced the accumulation of organic solutes and K^+ in the roots and shoots, as well as a decrease of Na^+ after the shoot apices subculture. The accumulation of carbohydrate folded two times in roots and shoots after subculture of shoot apices. The high carbohydrate and proline content achieved in roots and shoots of Beril cultivar, where it was the carbohydrate content 18.5 and 14.7 (mg/g. FW)

and the proline content 16.02 and 13.85 (μmoles/g. FW) after shoot apices subculture. The results suggest that the tissue culture technique is an effective tool for developing a new strategy to improve the salt tolerance of tomato plants for regions that are affected by the salinity of soil and water irrigation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PERTUMBUHAN DAN PERKEMBANGAN KULTIVAR TOMATO (*Lycopersicon esculentum* Mill.) SECARA IN-VITRO DI BAWAH KONDISI SALINITI

Oleh

ABDALMAJID NASHER AHMED MOHAMED

Ogos 2012

Pengerusi: Profesor Mohd Razi Bin Ismail, PhD

Fakulti: Pertanian

Saliniti pada tanah dan pengairan merupakan antara faktor kekangan alam sekitar yang sangat mempengaruhi pertumbuhan dan perkembangan tumbuhan. Saliniti mempengaruhi pengeluaran pertanian di kebanyakan tempat di dunia. Teknik kultur tisu merupakan medium terbaik untuk mengkaji pelbagai aspek berkaitan pertumbuhan dan perkembangan, dan proses fisiologi dan biokimia tumbuhan di bawah persekitaran terkawal yang menyumbang kepada sifat toleran saliniti. Di samping itu, skala yang lebih kecil memberikan kawalan dan pemerhatian yang menyeluruh ke atas tindakbalas pucuk dan akar terhadap tekanan persekitaran berbanding kajian dalam persekitaran in-vivo. Kajian ini dijalankan di Makmal Agrobiotechnology, Fakulti Pertanian dan Makmal Fisiologi Tumbuhan di ITA-MTDC, UPM. Kajian ini telah mencapai objektif seperti berikut:

penentuan protokol untuk pembentukan semula pucuk daripada eksplan hipokotil dan kotiledon pada kepekatan hormon BAP yang berlainan, tindakbalas eksplan hipokotil dan kotiledon dalam pembentukan pucuk di bawah persekitaran NaCl, kajian perubahan kimia dan biokimia yang berlaku semasa proses pertumbuhan pucuk dari eksplan hipokotil dan kotiledon di bawah persekitaran NaCl dan peningkatan toleransi subkultur apical pucuk terhadap NaCl pada kepekatan NaCl berlainan.

Sebagai tanggapan vitro dari hipokotil dan kotiledon eksplan untuk tunas regenerasi dalam media MS ditambah berbagai tingkat 6-benzylaminopurine (0 1, 2, 4) mg/l dipelajari. Kultur dipanen setelah enam minggu dari eksplan budaya. Tindakbalas yang berbeza telah direkodkan pada eksplan tomato di bawah kepekatan BAP yang berbeza. Pada rawatan kawalan, pembentukan akar dari eksplan hipokotil telah dicatatkan, manakala tiada sebarang tindakbalas pada eksplan kotiledon. Media yang ditambah dengan 4mg/l BAP menghasilkan kalus pada kedua-dua eksplan. Media yang diberikan rawatan 2 mg/l BAP sangat efektif dalam merangsang pembentukan pucuk adventitus pada kedua-dua sumber eksplan dalam kultivar tomato; mana bilangan pucuk/eksplan adalah 4 dan 4.5 dalam hypocotyls dan kotiledon kultivar Pearl dan 2.25 dan 3 pucuk/eksplant dalam hypocotyls dan kultivar Beril kotiledon masing-masing. Panjang pucuk terbaik dicapai oleh eksplant

hypocotyl dalam kultivar Pearl, di mana ia telah 1.62 cm dalam medium ditambah 2 mg / l BAP.

Dalam evaluasi vitro efek natrium klorida (NaCl) pada dua kultivar tomat (Pearl dan Beril) diselidiki dengan empat tingkat NaCl (0, 25, 50 dan 75 mM) dengan menggunakan eksplan hypocotyl dan kotiledon. Eksplan dikultur dalam media MS memiliki 2 mg/l BAP (media yang sesuai dari percobaan sebelumnya) bersama dengan konsentrasi yang berbeda NaCl. Kultur dipanen setelah delapan minggu dari eksplan budaya. Sifat-sifat pertumbuhan (pucuk nombor, panjang pucuk, berat badan yang segar dan berat kering) menurun dengan peningkatan kepekatan NaCl di dalam media. Peratusan pengurangan panjang pucuk adalah 85% di peringkat tinggi NaCl (75 mM) dalam perbandingan dengan kawalan; manakala pengurangan dalam bilangan pucuk, berat segar dan berat kering adalah 65, 64 dan 54% di peringkat tinggi NaCl. Di samping itu, terdapat kemerosotan yang lebih besar b klorofil daripada klorofil dan jawapan yang berbeza kultivar tomato dan eksplant untuk toleransi garam dalam vitro. di mana peratusan pengurangan dalam klorofil a dan b ialah 69%, 76% di peringkat tinggi NaCl. Dalam keadaan bergaram, sifat-sifat pertumbuhan dan kandungan klorofil sangat tinggi pada kultivar Beril berbanding kultivar Pearl manakala eksplan kotiledon juga menunjukkan pembentukan pucuk dan kandungan klorofil yang tinggi berbanding eksplan hypocotyl. Temuan dapat menyebabkan multiplikasi yang lebih baik dari eksplan kotiledon

dalam kultivar Beril untuk meningkatkan garis garam toleran untuk penggunaan masa depan.

Perubahan dari karbohidrat, protein terlarut, prolin dan konten ion (Na^+, K^+, Ca^{2+}, dan Mg^{2+}) yang belajar di pucuk diinduksi dari eksplan hypocotyl dan kotiledon dari Pearl dan kultivar Beril in vitro di bawah tekanan garam. Eksplan dikultur dalam MS+2 mg BAP dilengkapi tingkat yang berbeda NaCl (0, 25, 50 dan 75 mM). Kultur dipanen setelah delapan minggu dari eksplan budaya. Akumulasi bahan larut organik dan bukan organik dipengaruhi secara signifikan oleh kepekatan NaCl. Peningkatan kepekatan NaCl dalam media pertumbuhan yang membawa kepada peningkatan akumulasi karbohidrat, proline dan Na^+ di dalam pucuk yang terbentuk dari eksplan hypocotyl dan kotiledon, manakala akumulasi protein, K^+, Ca^{2+} dan Mg^{2+} menurun dengan peningkatan NaCl dalam media. Jumlah yang tinggi karbohidrat dan prolin (20.2 mg/g dan 13.69 μmoles/g) yang dicapai oleh pucuk disebabkan daripada eksplan kotiledon dalam kultivar Beril di peringkat tinggi NaCl. Kandungan kalium di peringkat tinggi NaCl mencapai 2.46 dan 2.43 mg/g oleh pucuk disebabkan dari eksplant kotiledon Beril dan kultivar Mutiara masing-masing. Di bawah persekitaran bersaliniti, terdapat perbezaan kadar akumulasi bahan larut organik dan tidak organik pada pucuk yang terbentuk dari eksplan hypocotyl dan kotiledon yang diperolehi daripada kultivar tomato yang berlainan.

Di Dalam tindak balas vitro menembak apices cabang kebudayaan Pearl dan kultivar tomato Beril bawah berbeza kepekatan NaCl (0, 25, 50 dan 75 mM) untuk meningkatkan toleransi garam dalam anak pokok tomato telah dikaji. The apices menembak telah berbudaya dalam MS+0.1 mg/l sederhana IBA selama empat minggu, dan menembak apices diasingkan dan cabang kebudayaan dalam medium yang sama selama empat minggu. The ciri-ciri pertumbuhan telah dinilai dalam kedua-dua apices dan cabang kebudayaan menembak dimasukkan (panjang Pucuk, panjang akar, berat anak pokok segar dan kering, pucuk dan akar karbohidrat kandungan, pucuk dan kandungan akar protein, pucuk dan akar kandungan prolin dan pucuk dan akar (Na^+, K^+) ion kandungan telah direkodkan. Berhubung pertumbuhan anak pokok tomato ditanam dari eksplant apices menembak terjejas oleh tekanan NaCl, satu peningkatan dalam NaCl dalam medium pertumbuhan yang membawa kepada penurunan itu pertumbuhan sifat-sifat tomato anak pokok. The panjang pucuk meningkat selepas apices menembak cabang kebudayaan kepada 5.38 dan 5.78 cm, manakala ia adalah 4.30 dan 4.50 cm dalam budaya pertama dalam kedua-dua Pearl dan kultivar Beril di peringkat tinggi NaCl, dan trend yang sama yang ditunjukkan dalam panjang akar, segar dan ciri-ciri berat kering. pengumpulan karbohidrat, prolin dan Na^+ yang akar dan pucuk anak pokok tomato meningkat dengan kemasinan meningkat dalam apices kedua-dua pertama dan cabang kebudayaan, sedangkan yang protein dan K^+ kandungan menurun. Tembak

cabang kebudayaan dipertingkatkan pengumpulan larutan organik dan K^+ akar dan pucuk, serta penurunan Na^+ selepas yang cabang kebudayaan apices menembak Pengumpulan karbohidrat dilipat dua kali dalam akar dan pucuk selepas cabang kebudayaan apices menembak. The karbohidrat yang tinggi dan kandungan proline dicapai dalam akar dan pucuk kultivar Beril, di mana ia adalah yang kandungan karbohidrat 18.5 dan 14.7 mg/g. FW dan kandungan proline 16.02 dan 13.85 μmoles/g.FW selepas apices cabang kebudayaan menembak. Keputusan mencadangkan bahawa teknik kultur tisu adalah satu alat yang berkesan untuk membangunkan satu strategi baru untuk meningkatkan toleransi garam tanaman tomato untuk kawasan yang dipengaruhi oleh kemasinan pengairan tanah dan air.

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First and foremost, Alhamdulillah for giving me the strength, patience, and determination in completing this work. All grace and thanks belongs to Almighty **ALLAH.**

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Last but of course not least, this work not have been achieved without the support and understanding of my wife and my kids. They have always supported me in everyway I have chosen in life.

I certify that an Examination Committee has met on 14 August 2012 of viva voce to conduct the final examination of Abdalmajid Nasher Ahmed Mohamed on his degree thesis entitled "Growth and development of tomato (*Lycopersicon esculentum*. Mill) cultivars *in vitro* under salinity condition" in accordance with University Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981.The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

Members of the Examination Committee were as follows:

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Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of type of degree. The members of the Supervisory Committee were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University Putra Malaysia or at any other institution.

ABDALMAJID NASHER AHMED MOHAMED

Date: 14 August 2012

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