E/ESCWA/ID/89/CONF.1/4

The First Arab Conference on
Perspective of Modern Biotechnologies
in the Arab Countries
27-30 March 1989
Amman - Jordan

INDUCTION AND SELECTION OF SALT TOLERANT DATE PALM BY CELL BIOTECHNOLOGICAL TECHNIQUES

Mostafa M. AboEl-Nil
Saudi Arabia

89-0192

INDUCTION AND SELECTION OF SALT TOLERANT DATE PALM BY CELL BIOTECHNOLOGICAL TECHNIQUES

Mostafa M. AboEl-Nil

USREP/JECOR/AGWAT, P.O. Box 5927
Riyadh, 11432, Saudi Arabia

Breeding new salt tolerant date palm cultivars is important for establishing new plantations in areas of saline soil. Salt tolerance in plants is genetically controlled, since individuals of the species may vary in their tolerance to saline environmental conditions and their tolerance is usually inherited from one generation to the other. Whenever natural-genetic variability in tolerance to salinity is lacking or not accessible by natural hybridization methods, conventional breeding programs cannot be used to develop saline stress tolerant plants. In such cases, cell and tissue culture techniques are the most promising means for genetic improvement which employ modern plant cell biotechnology methods of mutation, in vitro selection, somaclonal variation and protoplast breeding. Tissue explants of date palm were excised from buds and grown on culture medium supplemented with 50 uM NAA for callus induction. High frequency embryogenesis occurred when cell cultures were grown on the same medium but containing 5 uM 2ip. Cell cultures were obtained from one-year-old callus tissue and were cultured on media containing 160 mM EMS (Ethyl-methane-sulphonate). Large numbers of embryoids and cell clusters at different stages of differentiation were plated on the surface of embryogenesis medium containing increased concentrations of sodium chloride of 4.2, 8.5, 17.1, 25.6, 34.2, 42.7 and 51.3 mM. Cells and embryos grew well on increased concentrations of sodium chloride of 4.2 and 8.5 mM. Cell growth decreased gradually and stopped totally at the higher concentrations. In some cultures containing high concentrations, few embryos continued to grow. These embryos were isolated and cultured on a medium devoid of sodium chloride and containing 10 uM NAA and 5 uM 2ip to induce adventive embryogenesis from the salt tolerant clones for micropropagation and further testing on high salt containing media in vitro and in vivo. This is the first report on the successful isolation of salt tolerant clones of date palm by exposing cultured cells to a mutagen followed by selection at embryo level on sodium chloride containing media.

Key Words: Phoenix dactylifera L., tissue culture, Ethyl-methane-sulphonate, salinity tolerance, genetic engineering, micropropagation.

INTRODUCTION

Breeding of date palm (Phoenix dactylifera L.) has been impeded by the biological nature of the plant due to its long generation time, high degree of heterozygosity, being diaecious, and lack of rapid vegetative propagation methods. Genetic improvement of date varieties is important for increasing the efficiency of date production by planting better varieties in many countries where date palm is grown. Due to the above-mentioned impediments, it is apparent that new techniques and approaches, such as biotechnology genetic engineering, have to be tested seriously for genetic improvement of date palm.

Contributions of in vitro culture techniques are well established in plant propagation and breeding. They are contained in two main forms: (1) Manipulation of cells, tissues or organs as a type of in vitro horticulture for use in propagation, in elimination of systemic pathogens, in embryo rescue of some difficult crosses and in germplasm preservation and exchange; and (2) Manipulation of cells and protoplasts to produce genetically novel recombinations by means of spontaneous or induced mutation, haploid culture, protoplast fusion and gene insertion, to enrich diversity of the genetic pool of breeding stock. In this decade, implementation of the techniques, included in the first form, resulted in benefits to the horticultural nursery business of considerable magnitude; while research utilizing techniques, included in the second form, showed great promise for genetic engineering of plants for the future.

Recent advances in cell culture technology led to progress in selection of useful traits at cellular level. Selection of tolerant cell variants was achieved by recovering visible growth of plant cells under selective stressful conditions of salinity, phyototoxin or herbicide (Rangan and Vasil 1983). Intensity of selection is increased by gradual elevation of stressful conditions until a desired level of tolerance is achieved. A clone capable of sustaining growth on a selective medium was probably originated from a mutated single cell.

Advantages of cell culture in vitro selection for mutant induction and detection include:

1) Physiological aspect of the mutated trait can be studied in a simplified cellular level.

2) Culturing cells on rigidly defined media permit uniform and precise treatment with mutagens and stress factors.

3) Cell culture allows the exposure of millions of cells to mutagens and stress factors which enhance the chance for induction and selection of the desired traits at low cost compared to the use of whole plants.

4) Cells in culture seemed to be much more genetically responsive to environmental changes in comparison to cells in the intact plants.

The value of the mutated cell line, for breeding purposes, is evaluated by testing the produced plants. Contribution of mutated plants in breeding is determined by the inheritance pattern through seed generations. For clonally propagated plants, a phenotypically stable mutant of a desired character is readily usable (Widholm, 1978).

The possibility of developing a new clone of date palm identical to a commercial variety incorporating a single additional improved trait, by sexual breeding, is remote without many backcrosses. Somatic variations that may result from somaclonal variation in cultures, induced mutations and gametoclonal variations, offer higher potential than sexual breeding for clonally propagated variety improvement. Schematic representation of integrating tissue and cell culture into a genetic plant improvement program including technologies mentioned above, is presented in Figure 1.

Several reports on tissue culture of date palm have been published since the early attempts of Schroeder in 1970. Regeneration of plants was achieved by two morphogenetic processes: (a) axillary bud proliferation of cultured buds on bud primordia (Poulain et al. 1979; Rhiss et al 1979), and (b) asexual embryo formation from callus of various explant tissues (Reynolds and Murashige 1979; Tisserat 1979; and Tisserat 1982). Embryogenic date palm calli were derived from zygotic embryo, bud, leaf primordium and immature inflorescence explants. Free-living plants were obtained from cultures via embryogenesis (Tisserat 1981; AboEl-Nil 1986) and via organogenesis (Poulain et al. 1979; Beauchesne, 1982; Beauchesne et al. 1986). AboEl-Nil (1987) developed a new technology for high frequency mass propagation of date palm by embryogenesis in cell culture. This recent development made it possible to genetic engineer cells in vitro and regenerate plantlets via embryogenesis. The general strategy in plant cell and tissue culture techniques is to produce cell lines from the plant of interest, challenge these cells with a stress-inducing agent(s), select the surviving cells and, finally, inducing plant regeneration from growing callus tissues. Modifications of this strategy may involve utilizing mutagenic agents before selection, or repeating the selection under increasing stress pressures to achieve higher tolerance levels.

Salinity of agricultural lands, in many arid and semi-arid regions, is a serious problem which deserves concerted efforts to breed saline tolerant crop varieties. Salt tolerance in plants is genetically controlled, since individuals of the species may vary in their tolerance to saline environmental conditions, and that their tolerance is usually inherited from one generation to the other (Norlyn 1980). Breeding of salt tolerance in the vegetatively propagated crops, such as date palm, can be done by selection of cell lines under salinity stressful conditions (Croughan, Stavarek and Rains, 1972). Salt tolerant cell clones were isolated by growing tissue cultures of many plants on media containing salts (Dix and Street 1975; Nabors et al. 1975; Kochba et al. 1978; and Rangan and Vasil 1983).

This study was initiated to utilize tissue and cell culture techniques in developing new salt tolerant date palm cultivars by means of cell genetic engineering.

MATERIALS AND METHODS

Origin of Cultures:

Date palm callus cultures were initiated according to the method described by AboEl-Nil (1986). Buds were cultured on modified MS medium (Marashige and Skoog, 1962) containing 50 uM NAA. Induced callus was transferred into the same medium supplemented with 10 uM 2ip for embryogenic callus induction. Cultures were incubated at 23-25°C in a 16-hour photoperiod with two 40-watt flourescent lamps.

Mutagen Treatment:

A modified EMS (Ethyl-methane-sulphonate) treatment was used as described by (Templeton-Somers et al. 1981). Embryogenic callus clusters were transferred into liquid medium of the same composition without activated charcoal and containing 160 mM EMS . The cultures were incubated on a gyrotary shaker for 60 minutes.

Selection Technique:

Treated callus clusters were plated on to agar geled modified MS medium (AboEl-Nil 1986) containing 0, 4.2, 8.5, 17.1, 25.6, 34.2, 42.7 and 51.3 mM sodium chloride to select embryogenic cell lines that were tolerant to salinity.

Plant Regeneration:

Sodium chloride tolerant embryoids were transferred from selection medium into sodium chloride-free medium for multiplication of embryoids by adventive embryony. Germinated embryos were transferred into modified MS medium (without growth regulators) (AboEl-Nil, 1986) in test tubes for further growth of plantlets.

RESULTS

EMS Concentrations:

Callus regeneration and embryo development were obtained from callus cultures exposed to EMS in liquid suspension of callus at concentrations of up to 160 mM for one hour (Fig. 2). Higher concentrations were detrimental to callus growth.

Callus Regeneration:

Numbers of callus colonies regenerated after EMS treatment was reduced to about one fourth that of the untreated callus.

Growth on Sodium Chloride Medium:

When treated and untreated callus clusters were plated on media containing increased sodium chloride concentrations, colony regeneration from callus clusters was variable depending upon treatment (Table 1).

Number of callus colonies declined with increased sodium chloride concentration in the medium (Table 1, Fig. 3). EMS treatment induced more callus colonies at sodium chloride concentrations of 25.6 to 51.3 mM than the untreated callus. The highest tolerance of the untreated callus was at 17.1 mM sodium chloride.

Embryo and Plantlet Regeneration:

Embryo differentiation occurred in high frequency at sodium chloride concentrations up to 8.5 mM for treated and untreated calli. EMS treated calli regenerated callus colonies and somatic embryos when grown on media containing sodium chloride up to 42.7 mM. Tree embryos regenerated from EMS treated callus grown on 42.7 mM sodium chloride, which germinated readily upon transferring into a medium devoid of sodium chloride.

DISCUSSION

Results, presented above, demonstrated the efficiency of cell and callus culture when coupled with in vitro selection as a powerful genetic breeding tool. Salt tolerant date palm were produced by in vitro selection from EMS treated calli in a very short time in reference to the conventional breeding methods. Seed breeding in date palm has not yet been successful in producing date palm plants superior or equal to their parents (Carpenter and Ream, 1976; Carpenter, 1979). Date palm is the highest salt tolerant crop plant tested (Toutain, 1979). So it was not unusual for its callus to grow well on sodium chloride up to 17.1 mM. The ability of EMS treated callus to grow at the very high salt concentrations up to 42.7 mM indicated the great natural potential for breeding for salt tolerance in date palm. Cell and tissue culture techniques are the best available tools to capture this potential.

REFERENCES

AboEl-Nil, M. M. 1986.
Refining methods for date palm micropropagation. The Second Symposium on Date Palm, King Faisal University, Saudi Arabia

AboEl-Nil, M. M. 1988. Role and importance of plant cell and tissue culture for improving horticultural varieties in: M. M. AboEl-Nil (ed.) Proceedings of the symposium "Importance of Tissue Culture and Its Utilizations in Plant Production" Rabat, 1986. Federation of Arab Scientific Research Councils, Baghdad.

Beauchesne, G. 1982.
Vegetative propagation of date palm (Phoenix dactylifera L.) by in vitro culture. Proceedings of the First Symposium on Date Palm, King Faisal University, Saudi Arabia. Pp. 698-700 .

Beauchesne, G., A Zaid and A. Rhiss. 1986.
Meristematic potentials of bottom of young leaves to rapidly propagate date palm. Proceeding of the Second Symposium on Date Palm, King Faisal University, Saudi Arabia.

Carpenter, J. B. 1979.
Breeding date palms in California. Ann. Rep. Date Grower's Inst. 54:13-14.

Carpenter, J.B. and C.L. Ream. 1976.
Dale palm breeding, a review. Ann. Rep. Date Grower's Inst. 53:25-33.

Croughan, T.P., S.J. Stavarek and D.W. Rains. 1978.
Selection of NaCl-tolerant cell line of cultured alfalfa cells. Crop Science 18:959-963.

Dix, P.J. and H.E. Street. 1975.
Sodium chloride resistant cultured cell lines from Nicatiana Sylvestris and Capsicum annaum. Plant Science Letters 5:231-237.

Kochba, J., P. Spiegel-Roy, S. Saad and H. Newmann. 1978.
Tissue culture studies with citrus. 2. Application of citrus tissue culture for selection of mutants. Alferman, A.W. and E. Reinhard (eds.). In: Production of Natural Compounds by Cell Culture Methods. Proc. Intl. Symp. on Plant Cell Culture. Pp. 229-230.

Murashige, T. and F. Skoog, 1962.
A revised medium for rapid growth and bio-assays with tobacco tissue culture. Physcol. Plant. 15: 473-497.

Nabors, M.W., A. Daniels, L. Nodolny and C. Brown. 1975.
Sodium chloride tolerant lines of tobacco cells. Plant Science Letters 4:155-159.

Norlyn, J. 1980.
Breeding salt-tolerant crop plants. In: Rains, D.W., R.C. Valentine and A. Hollander (eds.). Genetic Engineering of Osmoregulation: Impact on Plant Productivity for Food, Chemicals and Energy. Plenum Press, New York. Pp. 293-309.

Poulain, C., A. Rhiss and G. Beauchesne. 1979.
Multiplication vegetative en culture in vitro du palmier-dattier (Phoenix dactylifera L.). C.R. Acad. Agric. France. 65(13):1151-54.

Rains, D.W. 1972.
Salt tolerance by plants in relation to salinity. Ann. Rev. Plant Physiol. 23:367-388.

Rangan, T.S. and I.K. Vasil. 1983.
Sodium chloride tolerant embryogenic cell lines of Pannisetum aericanum (L.) K. Schum. Annals of Botany 52:59-64.

Reynolds, J. F., and T. Murashige. 1979.
A sexual embryogenesis in callus culture of palms. In vitro. 15(5):383-87.

Rhiss, A., C. Poulain and G. Beauchesne. 1979.
La culture in vitro appliquee a la multiplication vegetative palmier-dattier (Phoenix dactylifera L.). Fruits 34(9):551-54.

Schroeder, C.A. 1970.
Tissue culture of date shoots and seedlings. Annual Report of Date Growers' Institute 47:25-27.

Templeton-Somers, K.M., W.R. Sharp and R.M. Pfister. 1981.
Selection of cold-resistent cell lines of carrot. Z. Pflanzen Physiol. Bd. 103, S.: 139-148.

Tisserat, B. 1979a.
Propagation of date palm (Phoenix dactylifera L.) in vitro, J. Exp. Bot. 30(119):1275-1283.

Tisserat, B. 1979b.
Tissue culture of the date palm. J. Hered. 70(3):221-222.

Tisserat, B. 1982.
Development of new tissue culture technology to aid in cultivation and crop improvement of date palms. Proceeding of the First Symposium on Date Palm, King Faisal University, Saudi Arabia. Pp. 698-700.

Toutain, G. 1979. Elements d'Agronomie Saharienne, cellule des zones arides, Institut National de la Recherche Agronomique, Groupe de Recherche et d'Echanges Technologieques. p. 68.

Widholm, J.M. 1978.
The selection of agriculturally desirable traits with cultured plant cells. Hughes, K.W., R. Henke and M. Constantin (eds.). In: Propagation of higher plants through tissue culture, Technical Information Center, U.S. Dept. Energy. Pp. 189-199.

TABLE 1. Number of callus clusters from treated and untreated cultures growing on modified MS medium containing increased concentrations of sodium chloride.

Sodium Chloride Concentration	Means of number of colonies in three plates per treatment*	
mM	Untreated	EMS Treated
0.0	52.63 ± 2.01a	13.34 ± 3.38c
4.2	61.30 ± 2.35a	15.21 ± 2.59c
8.5	6.35 ± 4.26b	14.73 ± 2.32c
17.1	5.10 ± 4.91b	11.18 ± 2.32c
25.5	2.25 ± 1.41b	6.57 ± 3.72d
34.2	0.0	2.30 ± 1.50d
42.7	0.0	2.00 ± 1.25d
51.3	0.0	0.0

*Means followed by same letter are not significantly different ($p = 0.05$) by Duncan's Multiple Range Test.

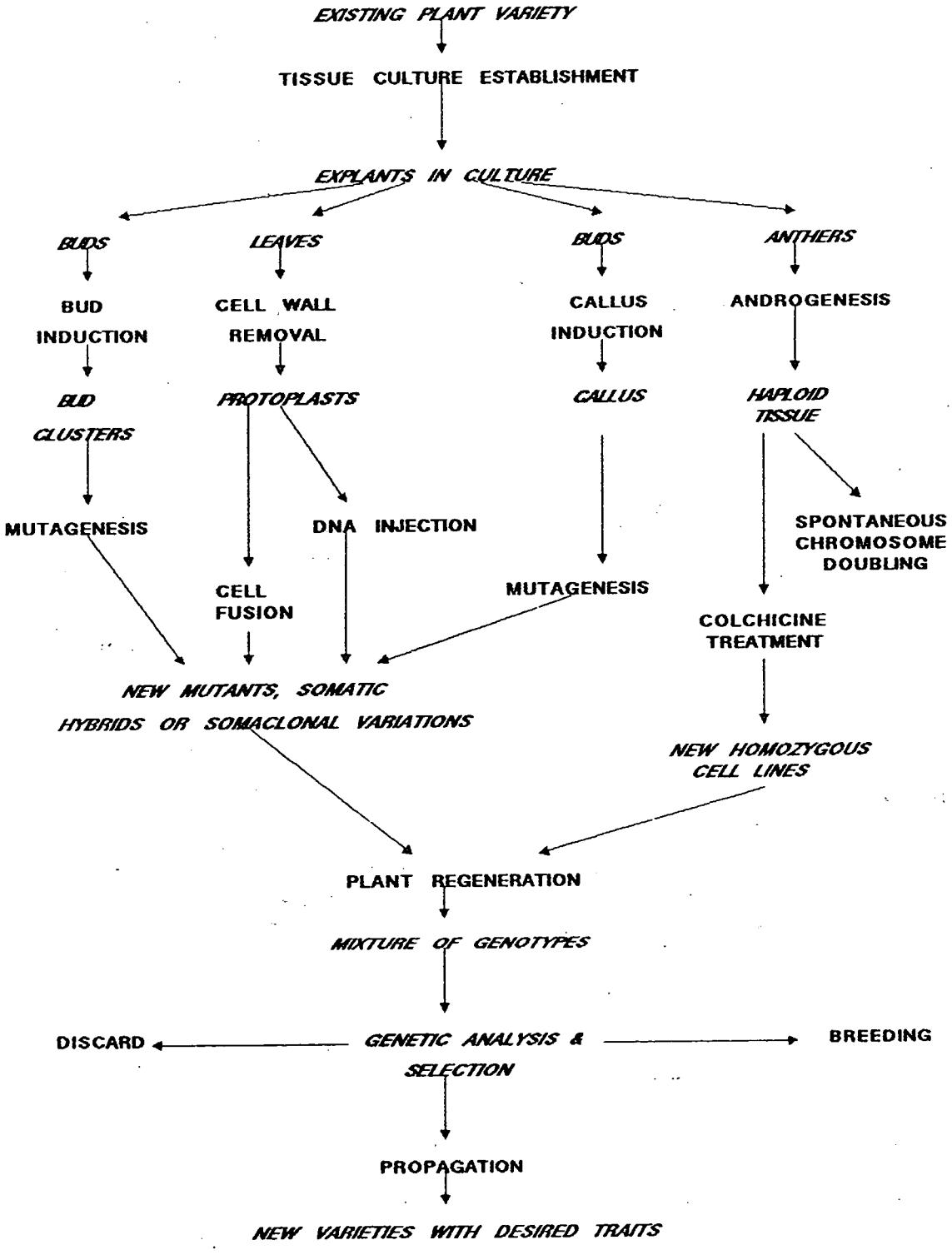

Figure 1: Schematic representation mummarizes logically the sequence of events leading into the development of a new horticultural variety employing methods of tissue, cell and protoplast culture (from AboEl-Nil, et al. 1988).

Figure 2: Regeneration of callus and embryogenesis after EMS treatment:

A. Callus clusters in liquid medium containing 160 mM EMS.
B. Callus colonies regenerated on callusgrowth medium.
C. Embryo regeneration from callus.

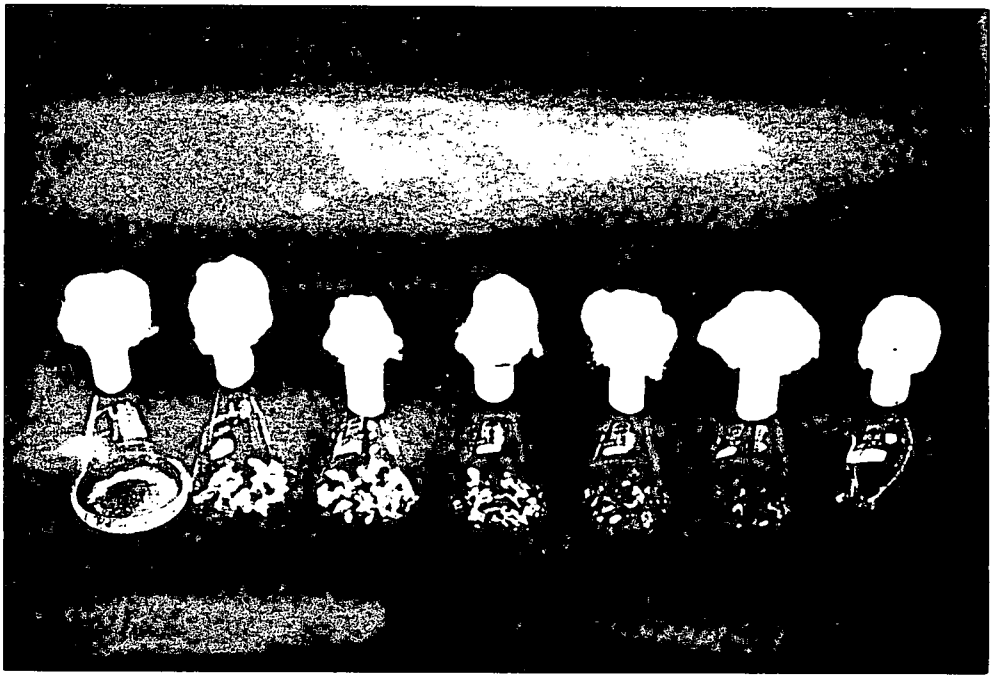

Figure 3: Regeneration of salt tolerant plants after EMS treatment:
A. Embryogenic callus in liquid medium containing 160 mM EMS.
B.- E. Embryo regeneration on callusgrowth medium containing increased concentrations of sodium chloride (B = 0.0, C = 8.5 mM, D = 25.6 mM, E = 42.7 mM).
C. A plantlet resulting from germinated embryos selected on a medium containing 42.7 mM sodium chloride.

تكوين وانتخاب سلالات مقاومة للملوحةمن نخيل التمر باستخدام طرق التقانة الحيوية

مصطفى محمود أبوالنيل
اللجنة السعودية الأمريكية للتعاون الاقتصادى
ص. ب. (٥٩٢٧) - الرياض ١١٤٣٢
المملكة العربية السعودية

ان التوسع فى زراعة نخيل التمر فى الأراضى الملحية ليعتمد على تربية وانتخاب سلالات ذات قدرة عالية على تحمل الملوحة. وقد دلت التجارب على أن عوامل وراثية تتحكم فى مقدرة النباتات على تحمل الاجهادات الفسيولوجية كالجفاف والحرارة والملوحة، حيث أن هذه الصفات تختلف من لآخر داخل الصنف الواحد كما أنها تنتقل من جيل الى جيل حسب قواعد وراثية محددة. وعندما لاتوافر درجة التحمل العالية للاجهاد الفسيولوجى طبيعيا أو أنة لا يمكن نقل هذة الصفة بالتهجين الجنسى فانه يتحتم استعمال طرق التقانة الحيوية الحديثة للحصول على أصناف جديدة ذات قدرات عالية على تحمل الظروف الجوية الصعبة. وتعتمد طرق التقانة الحيوية للخلايا النباتية على التطفر أواحداث الخلافات الوراثية الجسدية أو دمج البروتوبلاستات أو نقل الجينات على هيئة الحامض النووى (د.ن.أ) ثم يلى كل هذه الطرق انتخاب للخلايا والنباتات فى أنابيب الاختبار ثم الزراعة فى حقول التجارب.

تم فى هذا البحث تنمية خلايا نخيل التمر من براعم زرعت على بيئة غذائية تحتوى على ٥٠ ميكرومول نفثالين حامض الخليك وتكونت الأجنة الجسدية عندما زرعت الخلايا على بيئة تحتوى على ٥ ميكرومول من ٢ايزوبنتانيل امينوبيورين. عرض معلق الخلايا الى مركب ايثيل ميثان سلفونات بتركيز ١٦٠ ميليمول لمدة ساعة ثم زرعت على سطح بيئة النمو والتى احتوت على التركيزات الآتية من كلوريد الصوديوم ٤,٥ ، ٨,٥ ، ١٧,١ ، ٢٥,٦ ، ٣٤,٢ ، ٤٢,٧ ، ٥١,٣ ميليمول. فوجد أن الخلايا والأجنة قد نمت فى وجود كلوريد الصوديوم حتى تركيز ٨,٥ مليمول. أما الخلايا والأجنة التى زرعت على تركيزات مرتفعة من كلوريد الصوديوم فقد تناقص نموها تدريجيا ثم توقف تماما. ولكن فى بعض المزارع التى عرضت لمركب ايثيل ميثان سلفونات فان الأجنة قد نمت فى وجود ٤٢,٧ مليمول من كلوريد الصوديوم. وقد عزلت هذه الأجنة على بيئة مناسبة لاكثارها وانتاج عدد من النباتات ليتم تقييمها بعد النمو فى تربة ملحية ثم زراعتها فى البستان لتقييم صفاتها الانتاجية.