International Conf. on Water Resources & Arid Environment (2004)

The role of Biotechnology in developing plant resources in deserts environment

Nasser S. Al-Khalifah

King Abdulaziz City for Science &Technology
P o Box 6086 Riyadh 11442, abujawad@kacst.edu.sa

Abstract

Plant tissue culture technique is one of the applications of biotechnology by which plants can be mass multiplied using vegetative and sexual tissues. Micropropagation for plant mass production , rehabitation of destructed vegetation and studying the micro environmental stresses *in vitro* are some of the purposes of tissue culture. Biotechnology also involves the study of the plant biodiversity through DNA fingerprinting and genetic engineering through gene transfer. This paper illustrate some of the present tissue culture studies achieved on some desert plants that are considered as multipurpose and water saving plants in Saudi Arabia. Performance of twelve cultivars of Date palm (*Phoenix dactylifer*a L.) was studied at different stages of *in vitro* conditions. The problems encountered with the culture of 'Ajwah' and 'Nabtat Sultan' were compared to the easy cultures of 'Mosaifah' , 'Barhy' and 'Maktoumy'. DNA fingerprinting was carried out on date palm cultivars produced through field and tissue culture. Tissue culture was also applied on some wild sand dune plants such as Ghada *(Haloxylon persicum)* and Erta (*Calligonum comosum*) for mass production , results indicates that Ghada can be easly produced compared to Erta , however slow growth of both plants are considered as obsticles. Some species of Acacia were also micropropagated. The results showed that some species were easy *in vitro* growing as in the case of Samar *(Acacia tortilis)* Salam (A. *ehrenbergiana)* and Talh (*A. gerrardii*) at the time some other Acacia were difficult to initiate as in the case of Syial (*A. Seyal*) and Sinigal (*A.senegal*). These difficulties were persisted throughout *in vitro* and acclimatisation stages in green house conditions. This study was directly involved in the utilisation of desert plants which require less watering, for planting and landscaping programs in the local deserts of Saudi Arabia and similar conditions.

Introduction

Survival of mankind has always depended upon the use of earth's resources. In the past few decades due to habitat destruction and unsustainable utilization of natural resources many species including plants came under threat and their existence is now into the hands of human beings. Arid lands of the world are facing seriuos problems from scarcity of water, food, fodder, fuelwood and harsh environmental conditions. Fortunately most of the natural plants of these areas are multipurpose and if they are properly utilised they could resolve some of these issues to some extant. The indegenous plants of the region are well adapted to desert conditions and consume less water for the sustanance. This will also help in alleviating desertification threats through fixation of sand, protection and improvement of the soil, amelioration of climate and micro-environment and other conditions suitable for sustainable agriculture

Biotechnology has great potential of solving many problems pertaining to agriculture, industry, environment and health which has direct relevance to sustainable development of desert countries. Since the beginning of domestication and cultivation of plants, human beings are looking for techniques that help to produce maximum number of individuals from the minimum number / quantity of propagules. Tissue culture is the ultimate finding of mankind enquiry towards mass multiplication of plants using minimum quantity of propagules. Some of the advantages of this technique are heterozygous materials may be perpetuated without much alteration, easier, faster, dormancy problem eliminated and juvenile stage reduced. It is also a mean for perpetuating clones that do not produce viable seeds or that do not produce seeds at all.

Tissue culture refers to the aseptic growth of cells, tissues or organs in artificial media. Although the culture of plant cells and tissues has long been a tool of the plant physiologists, this technique is now increasingly used as a means of rapid plant propagation (Janick, 1979). Tissue culture or micropropagation of plants involves a sequence of steps, each of which requires specific set of conditions. Three distinct steps are usually involved: (1) establishment of aseptic culture, (2) multiplication of the propagule, and (3) preparation and establishment of the propagule for an independent existence by hardening and acclimation. These steps involve the use of different chemicals, media and management of light, humidity, temperature etc depending on the plants and objectives of the study..

The objective of this study partly was to employ the micropropagation techniques to some of the economically viable and environment friendly plant species of desert, therefore three criteria were used to select the most appropriate plant species. (1) the selected groups of plants must be most productive, economically viable and suitable for the desert environment. (2) they must be multipurpose in use and less water consuming. (3) Since the 30% of the geographical area of Saudi Arabia is sandy desrts, some of these plants must be able to rehabitate the less productive sand dunes.

Based on the above criteria, twelve cultivars of datepalms (Phoenix dactylifera L.), the most productive and economical crop of Saudi Arabia , eight species of indegenous Acacias of Arabian Peninsula and neighbouring areas and two species of Psammophilous woody plants, which are highly successful in the sandy deserts where the annual rain preciptation is very much minimal were selected for mass multiplication through micropropagation technique.

Various aspects related to the micropropagation of date palms like optimizing methods and media for tissue culture (Tisserat, 1979; Baskaran and Smith,1982; Abo El-Nil, 1986; Al-Khalifah,2000;); eliminating contamination (Sharma et al.,1980); browning of explants (Zaid, 1984; Al-Khalfah,2000) and hyperhydricity (Ching et al., 1999) were studied by the earlier workers. Many species of Acacia have been subjected to micropropagation studies with successful results(Nandavany, 1995; Rout et al., 1995; Xie and Hong, 2001; Hossain et al.,2001; Al-Khalifah and Nasroun, 2002).

Materials and methods

Plant materials

Date palm cultivars- 'Mosaifah', 'Maktomi', 'Barhy', 'Koweria', 'Subbakah', 'Shagra', 'Sukkary', 'Nabtet Ali', Khalas', 'Nabtet Sultan', 'Ajwa' and 'Mobbakkarah'.

Acacias – *Acacia tortilis* (Forssk.) Hayne; *A. ehrenbergiana* Hayne; *A. gerrardii* Benth.; *A.seiberiana* ; *A. nubica* Benth.; *A. seyal* Del.; *A. mellifera*(Vahl.)Benth.; and *Acacia senegal*(L.) Willd.

Psammophilous woody plants- *Calligonum comosum* L'her. and *Haloxylon persicum* Bunge.

Explants

Apical meristems with leaf primordia were extracted from the Off-shoots and used for the date palms. Mature seeds were harvested from the properly identified and healthy Acacia trees and germinated asceptically to get shoot segments. These shoot segments were used as explants for further studies. In order to break dormancy seeds of *Acacia tortilis* were treated with conc. Sulphuric acid for 15 minutes and washed thoroughly before sterilization Seeds of *Calligonum comosum* and *Haloxylon persicum* were collected from the natural populations at different stages of maturity. Isolated embryos from the immature seeds as well as the mature seeds were germinated in the tissue culture media to get callus or organogenesis. Actevely growing shoots from plants that had grown in a greehouse were used as source material for stem cuttings.

Sterilization

All explants were sterilized with 1.0% Sodium hypochlorite solution with one drop of Tween 20/100ml for 20 minutes followed by 4-5 rinses in cold sterilized

distilled water. Then it was immersed in 0.1% mercuric chloride solution for 5 minutes and followed by 4-5 washes in sterilized distilled water. Sterilized explants of date palms were kept in a dilute sterilized solution of Ascorbic and Citric acids (150mgl^{-1}) to aviod browning where as the other materials were kept in sterilized distilled water.

Media

MS medium(Murashige and Skoog,1962) in solid form with 7.0gl^{-1} of agar and supplimented with 2,4-D(100mg^{-1}), NAA(3mg^{-1}), 2iP(3mg^{-1}) and Kinetin(3mg^{-1}) was used for date palms. pH was adjusted to 5.6. Twenty five ml of aliquots of medium were distributed in 25mm culture tubes and the medium was sterilized for 15min. at 121^{o}C and 0.1 MPa. Activated charcoal (1.5gl^{-1}) and a mixture of ascorbic acid and Citric acid (75mg^{-1}) were added to the medium for avoiding browning process. For the induction of callus and embyogenic callus culture were incubated in a growth chamber at 25 $\pm$1 ^{o}C under dark conditions. Embryogenesis and embryo multiplication were carried out in sterile MS medium devoid of growth regulators and incubated under 16-hour photoperiod (2000lux). The embryos obtained to the size of 5-10mm were seperated from the embryogenic callus and cultured in fresh media for multiplication and germination. When the germination of embryos begin light intensity was increased to 3000lux. The regenerated plants were allowed for shoot elongation and then transferred to the MS medium supplimented with 0.1mgl^{-1} NAA for root development. Five embryos were cultured in 40mm culture tubes and 5 tubes were used for each cultivar. Data was recorded at weeky intervals and continued for 28 weeks.

In vitro germination of woody plant seeds or embryos were carried out in sterile MS medium. Cultures were incubated in a growth chamber at 25$\pm$1 ^{o}C under 16-hour photoperiod. Data was recorded daily and period required for initial germination and stabilization were found out. Three centimeter long stem segments obtained from the *in vitro* germinated seedlings of Acacias were transferred to MS medium containing BAP (2mgl^{-1}) and NAA(0.51mgl^{-1}) for shoot proliferation. Shoots measuring 4cm and above were transferred to rooting medium containing IBA (1mgl^{-1}).

Sterilized stem cuttings of *Calligonum comosum* and *Haloxylon persicum* were cultured in MS medium supplimented with IAA (1 mgl^{-1}), IBA (1 mgl^{-1}), GA3 (2 mgl^{-1}) for shoot proliferation. The elongated shoots were then transferred to MS medium containing IBA(1 mgl^{-1}) for root development.

Calligonum comosum and *Haloxylon persicum* multiple shoot formation, shoot proliferation and root development etc were placed for incubation at 25$\pm$1 ^{o}C under 16-hour photoperiod(3000lux). Data was recorded every alternate days and analised using Tukey's HSD test.

Rooted healthy plants were deflasked and washed in running water and dipped in Benlate solution(1g/L) for 5 minutes. Plantlets were transferred to the medium containing Peatmoss, Perlite and coarse sand in the ratio 1:1:1. To provide additional

humidity the plan-lets were kept in transparent containers and coverd with polythene sheets.

RAPD analysis

Total genomic DNA was extracted from the young sprouting leaves off-shoot derived plants and tissue culture derived plant-lets of date palm cultivars. The leaves were first ground into a fine powder in liquid nitrogen and then DNA was extracted following the steps of protocol provided by Dellaporta *et al*.(1983). The quantity and quality of the DNA were determined using flourometer. The stock DNA samples were diluted with sterile TE buffer to make a working solution of 10 ng μl^{-1} for use in PCR analysis. A total of 20 RAPD primers(Operon) were used for PCR amplification. Amplification reactions were performed in volumes of 25µl containing I U of Taq DNA polymerase per reaction in a thermal cycler. The RAPD proucts were seperated by electrophoresis according to their molecular weight on agarose gel submerged in1x TBE buffer and then stained with ethidium bromide solution for 20min. RAPD fragments were observed on a UV transilluminator and documented using Gel Documentation System. Amplification profiles of the 12 cutivars were compared with each other using Diversity Data Base Software package. The data of the selected primers were applied to estimate the similarity on the basis of number of shared amplification products (Nei and Li, 1979). Cluster analysis by the unweighted pair group method of arithmatic means (UPGMA) was also performed with the diversity data base software package.

Results and discussion

Among the 12 cultivars of date palms subjected to micropropagaion studies 'Koweria' produced 100% callus induction and 'Ajwah' produced significantly very low percentage of callus formation (Table-1). The cultivar 'Mobakkarah' seldom produced any callusing. The performance of 'Barhy', 'Sukkay', 'Khalas', 'Nabtet Ali', and 'Maktomi' are above average giving more than 60% callus induction. The production of callus in 'Mosaifah', 'Subbakah', and 'Nabtet Sultan' were almost same and not significant between them but low when compared to the other cultivars except 'Ajwa' and 'Mobakkarah' . Minimum period required for the induction of callus was very low for 'Mosaifah' (4 weeks) and very high for 'Ajwah' (28 weeks). Embryogenesis was observed significantly high in ' Maktomi', 'Koweria', 'Nabtet Ali', 'Shagra' and 'Mosaifah '(Table-2, Plate 1). Maximum embryogenic callii were produced by 'Mosaifah' and 'koweria' but 'Ajwah' and 'Mobakkarah' neither produced embryogenic callus nor embryos. For the production of embryos 'Sukkary' take 43 weeks while 'Barhy' initiated within 8 weeks.

The cultivars 'Nabtet Ali' produced maximum number of embryos followed by 'Mosaifah' (Table-3). Regeneration and rooted plant production were less than 50%

of the embryo produced in 'Nabtet Ali' while 'Mosaifah produced more than 90% Eventhough the embryogenic callus production in 'Nabtet Sultan' was high they did not produce any regeneration . *In vitro* germination of Acacias in tisuue culture medium produced differential growth behavoiur (Table-4, Plate 2). *Acacia tortilis* seeds pretreated with Sulphuric acid provided maximum percentage of germination followed by *Acacia mellifera*. *Acacia ehrenbergiana* and *A.gerrardii* were also provided moderate rate of germination but the other species produced significantly very low percentage of germination. Multiple shoot formation was very high in the cases of *A.tortilis, A.ehrenbergiana and A. gerrardii* (Table-5) while the other species performed poor shooting. Percentages of rooting was also significantly high in *A.tortilis, A.ehrenbergiana and A.gerrardii*.

In vitro responses of Ertaa and Ghada were varying depending on the explants. Mature seeds of *Calligonum comosum* did not germinate in the medium while in *Haloxylon persicum* they provided 58.3% germination (Table-6, Plate 3). Out of the total number of seeds germinated 41% produced direct seedlings and rooted plants. But 8.3% of the total germinated seeds produced callus and another 6.6% showed organogenesis. Isolated embryos from *Calligonum comosum* provided 58.3% germination but failed to produce callus or organs and ultimately died. Isolated embryos from *Haloxylon persicum* produced 80% germination, out of which 43.3% developed in to seedling and produced 28 rooted plants. 20% of the germinated seeds produced callus and another 8.3% showed organogenesis. 45% of the stem cuttings produced callus in Erta and another 35% proliferated in to shoots. In the case of Ghada 33.3% produced callus, 16.6% organs and 53.3% produced multiple shoots. Mean number of multiple shoots produced are high in *Haloxylon persicum* than in *Calligonum.comosum* Root development was also found better in *Haloxylon persicum than Calligonum comosum.*

These results reflects that the slow growth of both Erta and Ghada is of the stable nature of these desert plants while in the case of some of the Acacia like Samar (*Acacia tortills*) the in vitro conditions changed the nature of the plant becoming activly grown like cultivated plants .Tissue culture technique is a promising alternative for the propagation of uniform population of useful plant species of diverse use. Propagation of date palm through tissue culture has been proved a successful replacement over the conventional method of propagation by off shoots. Out of the 12 cultivars of date palms studied, 9 were amenable to the developed protocol and the remaning 3 needs modification of medium and further study. The cultivars 'Nabtet Sultan', 'Ajwah' and 'Mobakkarah exihibited highest in vitro environmental stress and failed to exihibit regeneration. The protocol developed during this study was found suitable for *Acacia tortilis, A.ehrenbergiana and A. gerrardii* but the remaining species needs further study. The main problem encountered with the micropropagation of *Haloxylon persicum* using mature seeds and isolated embryos was the low percentage of organogenesis and lack of elongation of shoots. Steps are being taken to overcome.

this difficulty exihited by the species. The sterilization procedure adopted for eliminating contamination was found very effective and useful**.**

RAPD

Out of the 20 primers screened 14 produced discrete polymorphism (Fig. K). Genetic similarities between genotypes showed upto 88% similarity in the similarity matrix based on Nei and Li's coefficients (Table 7). Cluster analysis by UPGMA showed expected affinities between the material studied (Fig. L). Maximum silmilarity was observed between TC Mosaifah and TC Barhy (0.88). Shishi and Om-Hamam showed second highest(0.87) similarity followed by TC Sukkary and TC Maktomi (0.86). All the five TC derived plants showed similarity within the range of 0.84-0.88 while the off-shoot derived plants showed the similarity in the range of 0.81-0.87. These results are in concurrence with the findings of Al-Khalifah &Askari, 2003 and Al-Khalifah *et al*., 2004.

The application of some of these results appear in production of date palm trees that are alredy cultivated in the field and use of some of the tissue cultured Acacia and Ghada and Erta in a wild and cultivated plantation areas as part of KACST research funded project AR 20 – 81 (Al-Khalifah et al. 2004).

References

Abo El-Nil, M. 1986. Effect of different auxins on callus induction and growth of Date Palm *in vitro*. A structure and function study. *Proc. Second symp. On Palm, KFU, Al-Hassa, Date Palm. pp. 51-57.*

Al-Khalifah,N. S., Khan, F. A., Askary, E. and Hadi, S. 2004. *In vitro* culture and genetic analysis of male and female date palm (*Phoenix dactylifera* L.). *5 th IVCHB Symposium, Debrecen, Hungary, PP. 144, 12-17 September*

Al-Khalifah,N. S. 2000. *In vitro* culture studies in date palm. *Plant Tissue Culture. 10 (1): 1- 8.*

Al-Khalifah, N. S and Nasroun, T. H. 2002. Seed germination of some arid land

Acacias. *Tanta Uni. J. Agric. Sci. 28: 1379-1385*.

Al-Khalifah N. Al-Abdulqader A. Nasroun T & Al-Farhan A. 2004. Rehabutation of some fire wood trees and shrubs in Saudi Arabia (Final technical report of AR 20 – 81 , KACST reserch funded project)

Bhaskaran, S. and Smith, R. H. 1992. Somatic embryogenesis from shoot tip and immature inflorescence of *Phoenix dactylifera* L cv. Barhee. *Plant Cell Report. 12: 22-25.*

Ching, Y. C., Al- Oraini, F. A., Al-Hazmi, A. R. M. and Khan, F. A. 1999. Control of hyperhydricity in Date Palm (*Phoenix dactylifera* L. cv. *Khalas*). *Saudi Biol. Soc. Jeddah. PP*

Dellaporta, S.L., Wood, J and Hicks, J.B. 1983. A plant DNA minipreperation:version II. Plant Molecular Biology Rep.1:19-21.

Hossain, S. N., Rahman, S., Joydhar, A., Islam, S., and Hossain, M. 2001. *In vitro* propagation of A. catechu willd. *Plant Tissue Culture 11 (1): 25-29.*

Janic, J. 1979. Horticultural Science. Purdue University, W. H. Freeman and Company, San Francisco.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiologia Plantarum 15: 473-497.*

Nandawani, D. 1995. In vitro propagation of a tree legume adapted to arid lands *Acacia tortilis* subsp radiana. *Ann. Sci. 52: 183-189.*

Nei, M. and Li, W. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci., USA. 76:5269-5273.

Rout,G. R., samantaray, S., and Das, P. 1995. Somatic embryogenesis and plant regeneration in Acacia catechu- a multiporpuse leguminous tree. *Plant Cell Tissue and Organ Culture 42: 283-285*.

Sharma, D. R., Kumari, R. and Chowdhury, J. B.1980. *Invitro* culture of female Date Palm (*Phoenix dactylifera* L.) tissues. *Euphytica. 29: 169- 174.*

Tisserat, B. H. 1979. Propagation of Date Palm (*P. dactylifera L.) In vitro. Journal of Exp. Bot. 30: 1275-1283*.

Xie, D. and Hong, Y. 2001. In vitro regeneration of Acacia Mangium via organogenesis. *Plant Cell Tissue and Organ Culture 66: 167-173.*

Zaid, A. 1984. *In vitro* browning of tissues and media with special emphasis on Date Palm cultures. A review. *Date Palm J. 3: 369-375.*

Table-1.Callus induction in 12 cultivars of date palms.

Cultivars	Period required to induce callus (Weeks)	No. of cultures produced callus	Percentages of callus induction
Mosaifah	04a	11bc	36.6b
Maktomi	18d	18cd	60.0d
Barhy	16d	21d	70.0c
Koweriah	08b	30e	100.0f
Subbakah	17d	08b	33.3b
Shagra	16d	15c	50.0c
Sukkary	05ab	20d	66.6d
Nabtet Ali	12c	19cd	63.3d
Khalas	14cd	20d	66.6d
Nabtet Sultan	08b	07b	33.3b
Ajwah	24e	02a	06.6a
Mobakkarah	-	0a	0a
HSD	3.99	4.81	7.41

No. of initial cultures = 30 tubes. (n=30). Mean separation within columns by Tukey's HSD at $p \leq 0.05$

Table-2. Embryogenesis in 12 cultivars of date palms.

Cultivars	Period required to induce embryogenesis(weeks)	No. of cultures produced	
		Embryogenic callus	Embryos
Mosaifah	28e	9c	8d
Maktomi	14c	10c	5c
Barhy	08a	7bc	6cd
Koweriah	12bc	10c	7cd
Subbakah	37g	6b	2ab
Shagra	32f	9c	6cd
Sukkary	43h	6b	4bc
Nabtet Ali	20d	10c	4bc
Khalas	34fg	7bc	5c
Nabtet Sultan	32f	7bc	4bc
Ajwah	-	0a	0a
Mobakkarah	-	0a	0a
HSD	3.4	2.08	2.69

Initial culture: 3gms of *in vitro* derived callus were cultured in 10 tubes for each cultivar.(n=10). Mean separation within columns by Tukey's HSD at p≤0.05

Table-3. Multiplication and plant regeneration in 12 cultivars of *in vitro* derived date palm embryos.

Cultivars	No. of embryos produced	No. of plants	
		regenerated	Rooted
Mosaifah	450g	430f	344g
Maktomi	395f	265e	185d
Barhy	365ef	216d	122c
Koweriah	350e	286e	275f
Subbakah	135c	105c	60b
Shagra	138c	100c	70b
Sukkary	189d	125c	86b
Nabtet Ali	492h	229d	204e
Khalas	106b	65b	63b
Nabtet Sultan	6a	0a	0a
Ajwah	0a	0a	0a
Mobakkarah	0a	0a	0a
HSD	32.42	27.98	30.74

Initial culture: 5 embryos were cultured in each tube and 5 replicates were used for each cultivar. Mean separation within columns by Tukey's HSD at p≤0.05

Table-4. *In vitro* germination and seedling- heights of some *Acacia species* .

Name of species	Period of germination (days)	Percentages of Germination	Mean seedling height after 4 weeks(cm)
A.tortilis	2-21	89.3d	5.2c
A.ehrenbergiana	2.14	13.3a	3.5b
A.gerrardii	7-30	50.0b	4.2c
A.seiberiana	4-18	12.0a	10.7e
A.nubica	6-28	4.0a	2.6b
A.seyal	6-21	6.0a	5.4c
A.mellifera	2-21	72.0c	6.6cd
A. Senegal	6-28	10.0a	0.9a
HSD (0.5)		12.62	1.39

Mean separation within columns by Tukey's HSD at p≤0.05

Table-5. Shoot multiplication and root formation in some species of *Acacias.*

Name of species	Mean number of cultures produced multiple shoots	Mean number of shoots/culture	Percentages of rooting
A.tortilis	10c	24b	78.3d
A.ehrenbergiana	10c	21.b2	79.5d
A.gerrardii	8bc	20.8b	73.3d
A.seiberiana	2a	3a	0a
A.nubica	1a	3a	0a
A.seyal	2a	2a	10b
A.mellifera	6b	6a	33.3c
A. Senegal	2a	3a	10b
HSD	2.9	4.73	7.89

Mean separation within columns by Tukey's HSD at $p \leq 0.05$

Table-6. *In vitro* performance of *Calligonum comosum* and *Haloxylon persicum.*

Name of species	Explants	Percentages of development					Mean no. of shoots /culture	Mean no. of rooted plants
		Germination	Direct seedlings	Callus	Organogenesis	Shoot proliferation		
Calligonum comosum	Mature seeds	0	0	0	0	-	-	
	Isolated embryos	53.3	0	0	0	-	-	
	Stem cuttings	-	-	45	0	35	20	50
Haloxylon persicum	Mature seeds	58.3	41.6	8.3	6.6	-	21	
	Isolated embryos	80.0	43.3	20	8.3	-	23	
	Stem cuttings	-	-	33.3	16.6	53.3	27.9	120

Figures and Plates

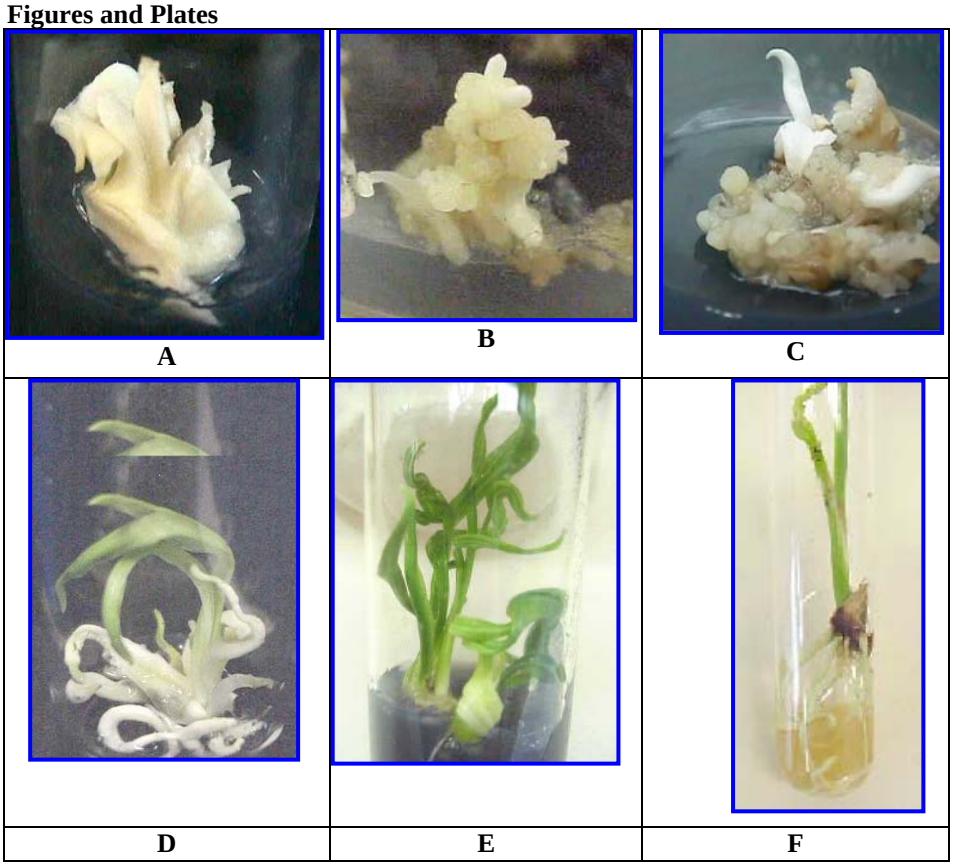

Plate-1. (a-f). *In vitro* regeneration in Date Plam cv Mosaifah (a) Explant; (b) Embryogenic callus; (c) Embryogenesis; (d) Embryo multiplication and germination; (e) Plantlet growth; f) Rooted plant

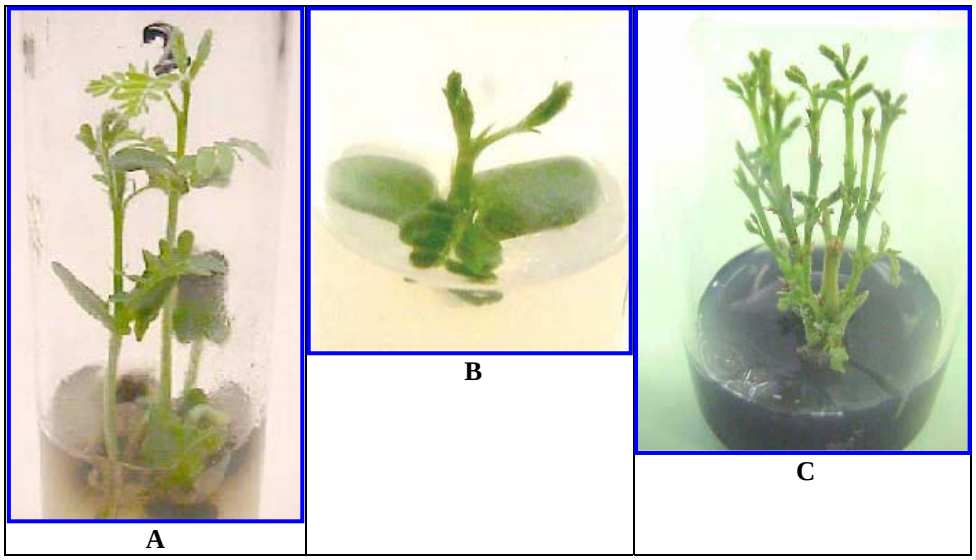

Plate-2. (a-g). *In vitro* regeneration of *Acacia tortilis* (a) Seed germination; (b) stem segment for multiple shoot induction; (c) Induction of multiple shoot (d) Induction of callus and multiple shoot; (f) Rooting stage; (g) *In vitro* derived potted plant

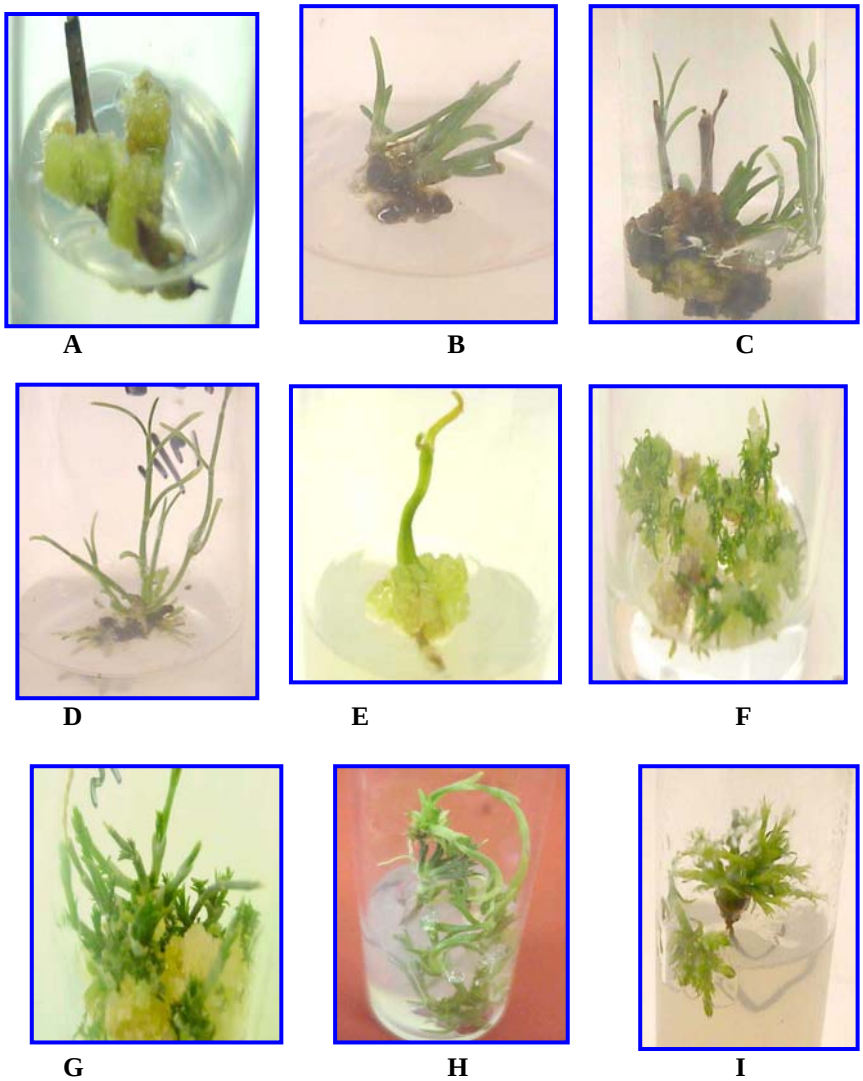

Plate-3. (a-i). *In vitro* regeneration of *Calligonum comosum*(a-e); *Haloxylon persicum* (f-i). (a) Induction of callus; (b) Shoot induction from nodal stem segment; (c) Shoot elongation (d) Shoot elongation and root development; (e) callus induction from invitro germinated young embryo;(f) Shoot induction from callus (organogenesis); (g) Shoot elongation; (h) Development of shoot from nodal stem segment; (i) Root development

.

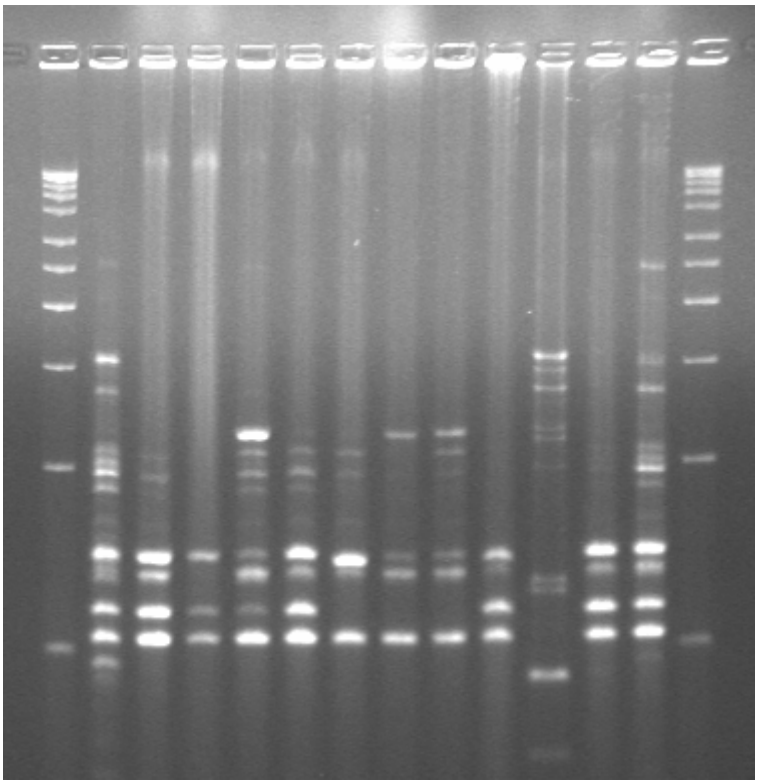

Fig. K. RAPD profile of 7 offshoot derived and 5 tissue cultued derived date palm cultivars using OPA 11 primer. M Molecular weight marker 1 Sukkary Asfar 2 Hilali 3 Sugai 4 Shishi 5 Om Hamam 6 Barhy 7 Nabtet Ali 8 T.C. Sukkary 9 T.C. Nabtet Ali 10 T.C. Maktoomi 11 T.C. Mosaifah 12 T.C. Barhy.

Table-7. Similariry matrix for Nei and Li's coefficients of 10 date palm cultivars obtained from RAPD markers.

		1	2	3	4	5	6	7	8	9	10
Hilali	1	100.0									
Om Hamam	2	81.9	100.0								
Shishi	3	83.2	87.0	100.0							
Sugai	4	78.0	84.1	88.4	100.0						
Sukkary Asfar	5	81.3	75.7	76.8	73.5	100.0					
T.C Barhy	6	75.8	76.5	79.6	76.3	75.3	100.0				
T.C Maktoomi	7	81.3	83.5	82.8	77.6	76.6	79.6	100.0			
T.C Mosaifah	8	75.0	81.6	84.8	73.5	76.6	88.2	80.9	100.0		
T.C Nabtet Ali	9	82.2	82.5	85.5	78.9	70.5	84.6	80.0	83.8	100.0	
T.C Sukkary	10	78.4	81.6	84.0	82.8	71.6	80.9	86.3	77.9	81.1	100.0

Fig.L A dendrogram of phylogenetic relationships among 10 cultivars of date palm based on Nei and Li's similarity coefficient obtained from 14 RAPD primers.