Micropropagation of some Pakistani cultivars of date palm (*Phoenix dactylifera* L.) through inflorescence technique

Mushtaque Ahmed Jatoi[1*], Adel Ahmed Abul-Soad[2], Ghulam Sarwar Markhand[1] and Najamuddin Solangi[1]
1 Date Palm Research Institute, Shah Abdul Latif University, Khairpur, Sindh, Pakistan.
2 Horticulture Research Institute, Agricultural Research Center, Cairo, Egypt.
Corresponding author: mushtaqjatoi@gmail.com

ABSTRACT

Pakistan has remarkable position in dates production and export in the world and has very rich date palm varietal structure. Date palm is traditionally propagated by vegetative means because of seeds heterozygosity. Work on propagation of date palm through tissue culture is being done since long back using various explants and techniques. The Date Palm Research Institute (DPRI), Shah Abdul Latif University (SALU), Pakistan has developed an efficient protocol for rapid and large scale in vitro propagation of valuable Pakistani date palm cultivars using Inflorescence explants.

Immature inflorescences of date palm initially washed with low torrent of current tap water and then 30% NaOCl2 solution for surface sterilization, spikelets were cut into the 2-3 cm small pieces and cultured on modified Murashige and Skoog (MS) medium supplemented with 0.1 mg l-1 2, 4-D + 0.1 mg l-1 IAA + 5.0 mg l-1 NAA for initiation and establishment of cultures. Somatic embryos were subjected to multiplication medium involved 0.1 mg l-1 NAA + 0.05 mg l-1BA. Rooting was achieved using quarter strength MS medium containing 0.1 mg l-1 NAA without Activated Charcoal (AC) initiatialy and then with 3 g l-1 AC. Strong rooted plantlets with 2-3 leaves were transferred to pots contained sand and peatmoss mixture (1:1 v/v) with more than 95% success in acclimatization. The acclimatized plants with at least one compound leaf were shifted to the open field conditions. High multiplication efficiency and survival percentage ensure the efficacy of the protocol developed for the production of elite cultivars of date palm of Pakistan.

INTRODUCTION

Pakistan always ranked among top 6 dates producing countries in the world and one of the strongest contenders among the countries claiming place of the date palm origin. Pakistan, particularly Khairpur (Sindh) and Makran (Balochistan) districts have very rich date palm varietal structure. Khairpur district (located in northern Sindh) is famous for its bountiful harvest of dates and often referred to as “city of date palm” and “biodiversity center of dates” having more than 300 varieties (Mahar, 2007; Markhand *et al.,* 2010). Almost 85% of the Sindh dates produced only in Khairpur district (Jatoi *et al.,* 2009).

The Date palm (*Phoenix dactylifera* L.) can be propagated naturally through seeds or offshoots and by Plant tissue culture artificially. Date palm Propagation by seeds always brings Heterozygosity due to its dioecious nature. While using offshoots for commercial propagation facing

limitation of offshoot availability and source of spreading diseases in case if the offshoots taken from infected trees.

A huge number of individual efforts of tissue culture of date palm from both dates producing & non dates producing countries have been reported, particularly from UAE, Egypt, Saudi Arabia, Algeria, Morocco, Tunisia, Pakistan, Iraq, Oman and few from Nigeria, Sudan, Spain, USA etc., but are limited to callus, somatic embryogenesis, multiplication, rooting and only few succeeded to acclimatize plants. At present, a number of public and private sector laboratories concerned with date palm micropropagation on commercial scale such as; Date palm Developments (UK), Al-Rajhi tissue culture laboratory (Saudi Arabia), Al-Ain University date palm tissue culture laboratory (UAE), Marrionet G.F.A (France), Rahan Meristem (Israel), Sapad tissue culture date palm company (Saudi Arabia), Domaine Agricole el bassatine (Morocco), date palm research center (Oman), Green Cost nurseries, Fujairah (UAE), Al-Wathba Marrionet (Iran) producing thousands of tissue cultured plants annually (Zaid *et al.,* 2011; Rajmohan, 2011; Jatoi, 2013).

Usage of the offshoots derived explants in tissue culture of date palm has been practicing since decades. Afterwards, the potential of inflorescence explants have been tested to develop direct (Abul-Soad *et al.,* 2004) and indirect somatic embryos (Drira and Al-Sha'ary, 1993; Abul-Soad *et al.,* 2005) of date palm. Inflorescence explants have many advantages over worldwide frequently used shoot tip explants for date palm micropropagation such as: no or less bacterial contamination, no browning, short production cycle and possibility to produce rare male and elite female cultivars of date palm in case of no offshoots availability (Bhaskaran and Smith, 1992; Abahmane *et al.,* 1999; Feki and Drira, 2007; Zaid *et al.,* 2007; Abul-Soad and Mahdi, 2010; Abul-Soad, 2011; Jatoi, 2013).

The micropropagation of elite local and international marketable varieties in Pakistan is need of the day. The efforts have been made for few decades through dispersed trials in the country to produce date palm plants by tissue culture technology. However, limited success has been achieved and trials weren't fruitful on large scale (Qureshi and Rashid, 1993; Rashid and Qureshi, 1994; Hussain *et al.,* 1995; Qureshi *et al.,* 1997; Hussain *et al.,* 2001; Khan and Bibi, 2012).

The Date Palm Research Institute (DPRI), SALU, Khairpur, Pakistan is working on several aspects of date palm including propagation of local and international varieties through tissue culture using shoot tip and Inflorescence explants. Plant tissue culture laboratory of DPRI has established cultures of many elite varieties of Date Palm in the lab for commercial production range from juvenile to rooting stage and shifted more than 4000 tissue cultured date palm plantlets of various varieties to Glass house (Markhand, 2009; Abul-Soad and Mahdi, 2010; Abul-Soad, 2011). Produced plants were shifted successfully into field conditions for field and fruit evaluation (Jatoi, 2013).

MATERIAL AND METHODS

This work was carried out in the Biotechnology Lab. of Date Palm Research Institute, Shah Abdul Latif University, Khairpur, Sindh, Pakistan in 2007 - 2013. The protocol was done as under:

Plant Source

The immature inflorescences were excised from the mother trees of different date palm cultivars namely Gajar, Kashoo wari and Dedhi (Fig. 1) from Khairpur, Sindh, Pakistan in early spring. The excised inflorescences were kept in clean plastic cover and handled carefully from an open field to the laboratory.

Surface Sterilization & Explant Preparation

The intact spathes were dipped into fungicide solution (2 grams l-1 Topsin M 70) for 30 seconds only without any shaking followed by washing under current tap water for 30-60 seconds only. 30% Sodium Hypochlorite (NaOCl) solution was used as surfactant for 5 minutes and washed three times with sterilized distilled water for 30-60 seconds without shaking.

After sterilization, the outer protective sheath or cover was removed carefully without any damage to the spikes inside. The spikes were cut from their bases and cultured directly if 3-4 cm in length while longer spikelet were cut and divided in to 2-3 cm each of which possessed 2-4 immature florets and laid in such a way that the entire explant is in contact with the surface of nutrient medium.

Cultural Conditions

All cultured explants were incubated in a controlled growth room at 25 ± 2ºC under full darkness and re-cultured about 3-4 weeks on same initiation medium as mentioned in Table 1. Well-responded explants were transferred on to maturation medium for 1-2 re-cultures. Matured and early differentiated explants under darkness were shifted onto differentiation medium under light conditions for 1-2 re-cultures. Subsequently the differentiated cultures were shifted to the multiplication medium to acquire desired number of shoots and then the elongated shoots were detached from multiplication stage and subjected to rooting medium. The individual plantlet with 2-3 leaves and thickened adventitious roots were selected and shifted to the glass house for acclimatization (Fig. 2).

Acclimatization

The acclimatization protocol of date palm was followed as described by Abul-Soad (2011). Plantlets were taken out from test tubes and the roots were gently washed in Luke-warm distilled water to remove any residual gel or medium. Before planting, plantlets were immersed in 0.5% (w/v) fungicide solution for 5 minutes. The plants were placed into 250 mm plastic pots containing soil mixture 1:1 of wash sand: peat moss (v/v) with little amount of Perlite. Plants kept under natural day light and high relative humidity (90-95%) using a cover of white polyethylene sheet for one week and removed gradually to develop the plants under greenhouse conditions. The plants were watered once a week and sprayed with the fungicide if needed.

RESULTS AND DISCUSSION

The current study was conducted to achieve successful large scale micropropagation protocol of date palm using inflorescence explants. No browning and bacterial contamination observed during initiation phase and all spike explants responded well to the starting nutrient medium. Shining globular creamy structures formation was obtained within 2 months through 1-2 re-cultures. Maturation of initial structures occurred within 2-3 months through 2-3 re-cultures. After the differentiation process, three types of cultures were obtained e.g. embryogenic callus, somatic embryos and green shoots. Somatic embryos can be generally divided into two categories. First category is the individual somatic embryos and second is a cluster of embryos (multiple embryos). The growth behavior of the individual embryo is to grow vertically to produce more leaves and roots while the multiple embryos is usually proliferating to additional shoots and somatic embryos which suits the multiplication stage.

In the first subculture of multiplication stage, 110, 24 & 70 jars were having embryogenic callus in cvs. Kashoo wari, Gajar & Dedhi, respectively. While, with 38 jars having multiple embryos cv. Kashoo wari appeared the only variety produced embryos. However, 28, 2 & 17 jars of cvs. Kashoo wari, Gajar & Dedhi were appeared with shoots respectively (Table 2). All of these cultured were transferred onto the proliferation medium (Table 1). The embryogenic callus exposed high morphogenetic potentiality to differentiate to intact somatic embryos. During this process very little callus formation was occurred till subculture 7 of multiplication stage where the callus jars decreased to 5, 0 & 6 jars in cvs. Kashoo wari, Gajar & Dedhi respectively. While, with 111 and 408 jars of embryo and shoot cv. Kashoo wari produced prominent number of cultures as compared to cv. Gajar (44 embryo and 119 shoot jars) and cv. Dedhi (31 embryo and 86 shoots jars). During multiplication stage some shoots were growing up and reached to an appropriate height for rooting stage and subsequently subjected to the rooting medium (Table 1). It was observed that removing the initial roots completely or trimming to 1-2 mm enhanced thicker-white adventitious root formation. Leaving the primary roots without trimming during rooting stage inhibited the adventitious roots formation which is important for the further growth in the acclimatization stage (Abul-Soad and Jatoi, 2014).

Finally, all callus and embryos differentiated into shoots and rooted plantlets on rooting medium. Where number of shoots and plantlet were reached at 419 and 773 in cv. Kashoo wari, 299 and 295 in cv. Gajar, and 50 and 257 in cv. Dedhi, respectively.

The shoot jars increased from 28, 2 & 17 in subculture 1, then 408, 119 & 86 in subculture 7, and 419, 299 & 50 in subculture 12. Each jar maintained 20-30 shoots, 5 of them at least in the size of rooting stage while 1325 plantlets were in rooting stage in sub culture 12. It is quite important to mention that no any study has been conducted on these date palm cultivars before and offshoots were only the source of traditional propagation method in the region.

Rooting quality of the ex vitro plantlets of date palm was the vital factor increased the survival percentage in the greenhouse. Most of the reports indicated low survival percentage 25-35% during acclimatization stage rather than it used to be a big obstacle in the whole micropropagation protocol (Abul-Soad *et al.,* 1999; Hegazy *et al.,* 2006; Taha *et al.,* 2007). But in current study and based on the utilization of high sugar concentration, AC after adventitious roots formation and proper handling for the plant material, the survival percentage reached more than 95%. The used soil bed was a simple mixture of washed sand and peat-moss (1:1 ratio) with little amount of perlite. The acclimatized plants with at least one compound leaf were shifted to the field conditions (Fig. 3-5). High multiplication efficiency and survival percentage ensure the efficacy of the protocol developed for the production of elite cultivars of date palm of Pakistan.

References

Abahmane, L., M. Bougerfaoui and M. Anjarne. 1999. Use of tissue culture techniques for date palm propagation and rehabilitation of palm groves devastated by bayoud disease. Proceeding of International Symposium on Date Palm, Assiut University, Assiut, Egypt. 9-11 Nov. pp. 385- 388.

Abul-Soad, A.A and M.A. Jatoi. 2014. Factors are affecting in vitro rooting of date palm (*Phoenix dactylifera* L.). Pak. J. Agri. Sci., Vol. 51(2). In press.

Abul-Soad, A.A. 2011. Micropropagation of date palm using inflorescence explants. In: Jain, S.M.,

J.M. Al-Khayri and D.V. Johnson (Eds.). pp. 91-118. Date Palm Biotechnology, Springer, Dordrecht.

Abul-Soad, A.A. and M.S. Mahdi. 2010. Commercial production of tissue culture date palm (*Phoenix dactylifera* L.) by inflorescence technique. J. Gen. Eng. Biotech. 8(2):39-44.

Abul-Soad, A.A., I.A. Ibrahim, N.R. El-Sherbeny and S.I. Baker. 1999. In vitro and ex vitro optimization for rooting and acclimatization of date palm. Proceedings of the First International Conference in Egypt on Plant Tissue Culture and Its Application, 12-14 September, Egypt. pp. 227-241.

Abul-Soad, A.A., I.A. Ibrahim, N.R. El-Sherbeny and S.I. Baker. 2004. Improvement and characterization of somatic embryogenesis in date palm (*Phoenix dactylifera* L.). Proceedings of the International Conference of Genetic Engineering & its Applications, The Egyptian Society of Genetics and Suez Canal University, Sharm-El Sheikh City, South Sinai, Egypt. 8-11 April 2004. pp. 359-373.

Abul-Soad, A.A., N.R. El-Sherbeny and S.I. Baker. 2005. Date palm (*Phoenix dactylifera* L. cv. Zaghloul) propagation using somatic embryogenesis of female inflorescence. Proceedings of the 3rd Conference on Recent Technologies in Agriculture, 14-16 November 2005, Cairo University, Egypt. 3: 423-441.

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

Drira, N. and A. Al-Sha'ary. 1993. Analysis of date palm female floral initials potentials by tissue culture. Third Symposium on Date Palm, King Faisal University, Al-Hassa, Saudi Arabia. pp. 161-170.

Feki, L. and N. Drira. 2007. Development of an efficient plant regeneration system for date palm. Fourth Symposium on Date Palm. King Faisal University, Al-Hassa, Saudi Arabia.

Gamborg, O.L., R.A. Miller, and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50: 151-158.

Hegazy, A.E., O.A. Kansowa, A.A. Abul-Soad and M.I. Nasr. 2006. Growing behaviors of ex vitro date palm plants after acclimatization. Proceedings of the 2nd International Conference of Genetic Engineering & Its Applications, 14-17 November 2006, Sharm El-Sheik City, South Sinai, Egypt.

Hussain, I., H. Rashid, A. Muhammad and A. Quraishi. 2001. In vitro multiplication of date palm. Proceedings of Second international conference on Date palm, Al-Ain, UAE. pp. 432-438.

Hussain, I., M. Ahmad and A. Quraishi. 1995. Effect of explant source on in vitro regeneration of plants through tissue proliferation in *Phoenix dactylifera* L. cv. Fusli. Pakistan Journal of Botany, 27(1): 101-104.

Jatoi, M.A. 2013. In vitro rooting and acclimatization of date palm (*Phoenix dactylifera* L.) plantlets. M.Phil Thesis, Dept. of Botany, Shah Abdul Latif University, Sindh, Khairpur, Sindh, Pakistan.

Jatoi, M.A., Z. Markhand and N. Solangi. 2009. Dates in Sindh: facts and figures. Proceedings of the International Dates Seminar, 28 July 2009, Date Palm Research Institute, Shah Abdul Latif University, Khairpur, Pakistan. pp. 59-71.

Khan, S. and T. Bibi, 2012. Direct shoot regeneration system for Date palm (*Phoenix dactylifera* L.) cv. Dhakki as a means of micropropagation. Pak. J. Bot., 44(6): pp. 1965-1971.

Mahar, A.Q. 2007. Post-harvest studies of different varieties of Date Palm (*Phoenix dactylifera* L.*)* fruits, their protection, identification, processing and preservation at district Khairpur, Sindh, Pakistan. Ph.D Dissertation, Date Palm Research Institute, Shah Abdul Latif University, Khairpur, Sindh, Pakistan.

Markhand, G.S. 2009. Activities of Date Palm Research Institute (DPRI). 2009. Proceedings of the International Dates Seminar, 28 July 2009, Date Palm Research Institute, Shah Abdul Latif University, Khairpur, Pakistan. pp. 1-32.

Markhand, G.S., A.A. Abul-Soad, N.A. Kanhar and A.A. Mallah. 2010. Fruit characterization of Pakistani dates. Pakistan Journal of Botany, 42:6. pp. 3715-3722.

Murashige, T. and F.A. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physio. Planta. 15:473-479.

Quraishi, A. and H. Rashid. 1993. Establishment of long term embryogenic cultures in date palm. Pakistan Journal of Agricural Research. vol. 14(4).

Quraishi, A., I. Hussain, M. Ahmed, H. Rashid and M. Latif. 1997. Sustained multiplication of long term embryogenic cultures of date palm and their field performance. Pakistan Journal of Botany, 29:1 pp. 135-141.

Rajmohan, K. 2011. Date palm tissue culture: A pathway to rural development. In: Date Palm Biotechnology. Jain, S.M., Al-Khayri, J.M., Johnson, D.V. (Eds.) Springerlink. Netherland. pp 1-3.

Rashid, H. and A. Quraishi. 1994. Micropropagation of date palm through tissue culture. Pakistan Journal of Agricultural Research. Vol: 15(1).

Taha, H.S., M.M. Hassan and M.K. El-Bahr. 2007. Micropropagation of some Egyptian date palm dry cultivars, 1- Maturation of somatic embryos. Arab J. Biotech., Vol. 10, No.2:333-340.

Zaid, A., B. El-Korchi and H.J. Visser. 2011. Commercial date palm tissue culture procedures and facility establishment. In: Jain. S.M., J. M. Al-Khayri and D.V. Johnson (Eds,). Date Palm Biotechnology, Springer, Dordrecht. pp. 137-180.

Zaid, A., H.H. Al Kaabi, B. El Korchi. 2007. Large scale in vitro propagation of a rare and unique male date palm (*Phoenix dactylifera*) using inflorescences technique. Acta Hort 736, pp. 243-254.

Table 1: Nutrient media composition for Inflorescence protocol and its sequence (Abul-Soad and Mahdi, 2010)

Medium	Composition ($mg\ l^{-1}$)			
	Salts	Additives	Auxins	Cytokinins
Initiation	Macro of B5[z]+ Micro of MS[y]	30000 Suc.[x] + 2200 Agar + 1400 Gel + Vit.[w] of MS + 170 KH_2PO_4 + 100 Glutamine + 40 Ad.[v]	0.1 2,4-D + 0.1 IAA + 5.0 NAA	---
Maturation	Macro of B5+ Micro of MS	30000 Suc. + 2200 Agar + 1400 Gel + Vit. of MS + 170 KH_2PO_4 + 100 Glutamine + 40 Ad. + 1500.0 AC[u]	5.0 2,4-D	1.0 2iP
Differentiation	MS	30000 Suc. + 2200 Agar + 1400 Gel + Vit. of MS	0.1 NAA	0.1 Kinetin
Multiplication	MS	30000 Suc. + 2200 Agar + 1400 Gel + Vit. of MS	0.1 NAA	0.05 BA
Rooting	*¼ MS*	50000 Suc. + 2200 Agar + 1400 Gel + Vit. of MS + 0.1 Ca-panthothianate + with & without 3000.0 AC	0.1 NAA	---

zB5: Gamborg et al. (1968) nutrient medium. yMS: Murashige and Skoog Medium (1962). xSuc.: Sucrose. vVit.: Vitamins. wAC: Activated Charcoal. uAd.: Adenine sulfate.

Table 2: production capacity of three different cultivars of date palm from a single inflorescence after 1, 7, 12 subcultures during the multiplication and rooting stages.

Variety	Sub 1*				Sub 7				Sub 12		
	Callus	Embryo	Shoot	Total	Callus	Embryo	Shoot	Total	Shoot	Plantlet	Total
Kashoo wari	110	38	28	176	5	111	408	524	419	773	1716
Gajar	24	---	2	26	---	44	119	163	299	295	757
Dedhi	70	---	17	87	6	31	86	123	50	257	430
General Total	204	38	47	289	11	186	613	810	768	1325	2903

*Sub 1 considered during multiplication stage.

Each culture vessel (350 ml jar or 250 × 25 mm long tube) contained 1 gram of callus, or 10 embryos or shoots in average. Each long tube contained 1 intact plantlet with shoot-root system.

Gajar | Kashoo wari | Dedhi

Fig. 1 The fruit of studied cultivars used for micropropagation

Fig. 2. Different growth stages of date palm micropropagation using Inflorescence explants in DPRI. A. Inflorescence Spikelets B. Initiation stage C. shoots cluster with somatic embryos D. shoots elongation E. Multiplication stage E. Rooting stage.

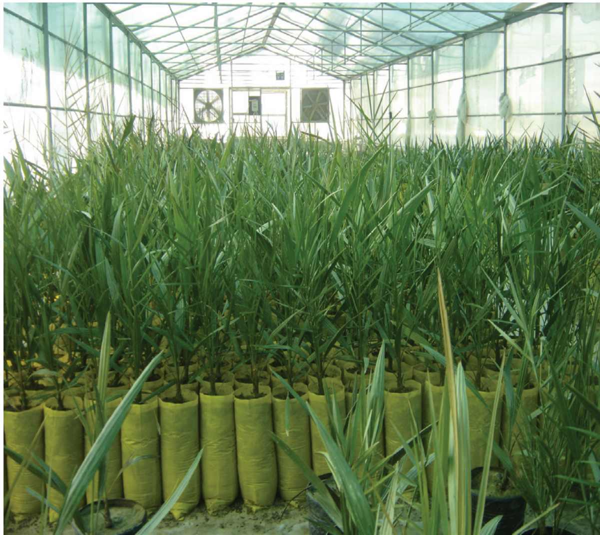

Fig. 3. Date palm plantlets acclimatization process in DPRI Glass house.

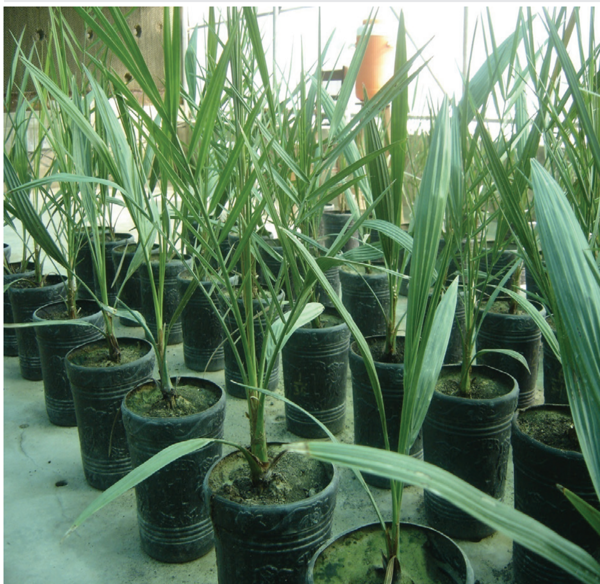

Fig. 4. Tissue cultured date palm with compound leaves ready to be shifted to field conditions.

Fig. 5. Tissue culture derived date palm plants in field conditions, DPRI, SALU, Khairpur, Pakistan.