

# Medium Supplements and Support Matrices for Better In Vitro Growth of Date Palm (*Phoenix dactylifera* L.)

N.S. Al-Khalifah<sup>a</sup>, F.A. Khan, E. Askari, S. Hadi and A.E. Shanavaskhan  
National Center for Agriculture Technology  
King Abdulaziz City for Science and Technology  
PO Box 6086, Riyadh-11442  
Saudi Arabia

**Keywords:** polyurethane foam, support matrix, date palm, tissue culture, micropropagation, activated charcoal, phenol

## Abstract

Despite its extensive use as gelling agent for tissue culture media, agar possesses many shortcomings including impurities, scarcity and high cost in local markets and sensitivity to the harsh local environment. In view of the need to find alternative gelling agents or solid support matrices, growth and development of date palm (*Phoenix dactylifera* L.) cultures were evaluated on polyurethane (PU) foam discs in comparison with agar-gelled medium. Incorporating activated charcoal in tissue culture media has been shown to affect growth and development of various organisms. It plays a critical role in the micropropagation of date palms by adsorbing inhibitory compounds in media and decreasing toxic metabolites, phenolic exudation and brown exudate accumulation. However, in some cases activated charcoal adsorbs hormones required for the callus growth and shoot development thereby retarding active growth. A comparative study of in vitro growth responses of two date palm cultivars in the medium containing charcoal and no charcoal showed significant differences in all the growth parameters. Date palm cultures growing on PU foam showed significantly superior rates of shoot multiplication and shoot elongation as compared to cultures in agar-gelled media. The rooting response of cultures on PU foam and agar-gelled media was nearly similar. It is argued that enhanced aeration and better suited physical characteristics of the material may be the reasons for superior performance of PU foam as support matrix in comparison with agar. It is therefore suggested that a polyurethane matrix can be used satisfactorily for micropropagation of date palms. The poor response of date palm cultures in the charcoal containing medium may be attributed to the lowering of pH of medium during autoclaving as reported by earlier workers thereby inhibiting the uptake of required growth regulators. Polyurethane can also be used for special applications where low pH of culture medium is required, and its composition and resulting physical properties may be precisely modified during manufacture to suit specific culture requirements. At the same time the matrix is very cheap as compared to agar even in a single use cycle.

## INTRODUCTION

Different types of media supplements and support matrices are used in plant tissue culture to enhance growth and development of explants. Activated charcoal has been used in tissue culture media to improve culture growth and promote morphogenesis in a wide variety of species (Wann et al., 1997). Activated charcoal is often used in plant tissue culture to improve cell growth and development (Pan and van Staden, 1998). It plays a critical role in the micropropagation of date palms by adsorbing inhibitory compounds in media and decreasing toxic metabolites, phenolic exudation and brown exudate accumulation. However, there are reports that, in addition to adsorbing unwanted substances, it may also adsorb needed hormones (Ebert and Taylor, 1990; Ebert et al., 1993; Nissen and Sutter, 1990) vitamins (Weatherhead et al., 1979; Pan and van Staden,

---

<sup>a</sup> abujawad@kacst.edu.sa

1998), or metal ions such as  $\text{Cu}^{++}$  and  $\text{Zn}^{++}$  (Van Winkle et al., 2003).

In vitro cultures require a surface to grow on. For this reason agar is widely used to gel plant tissue culture media and provide a physical support to the growing cultures. Agar possesses many peculiar properties that suit its use as gelling agent in tissue culture media. It is chemically inert in the medium and is not digested by plant enzymes (Henderson and Kinnersley, 1988). It also forms a uniform gel that remains stable over the range of pH, temperature, and light conditions maintained during incubation.

However, despite the above characteristics, performance of agar as a gelling agent is not always consistent. The quality of agar and corresponding performance of cultures has been found to vary from brand to brand presumably due to varying levels of impurities (Scholten and Pierik, 1998; Nairn et al., 1995; Debergh, 1983). Agar also hinders aeration in the medium and may curtail availability of oxygen to the cultures (Newell et al., 2003; Anon., 1988).

Besides its qualitative shortcomings, agar constitutes the single costliest component of tissue culture media (Puchooa et al., 1999; Bhattacharya et al., 1994). There might be variation in the price of agar of different brands at different locations, but the predominance of its cost would remain overbearing.

Due to its qualitative deficiencies and high cost several attempts have been made to find cheaper alternatives to agar that should put up better or similar performance of cultures as well. Starches from cassava (Maliro and Lameck, 2004; Gerbe and Sathyanarayana, 2001), corn (Puchooa et al., 1999; Hendersen and Kinnersley, 1988), sago (Bhattacharya et al., 1994), and potato (Calleberg et al., 1989), etc. have proved variable efficiencies as gelling agents in culture media. Some gums like gellan gum (Puchooa et al., 1999; Calleberg et al., 1989), gum-katira obtained from bark of *Cochlospermum religiosum* have also been found to impart good gel strength to the medium (Jain and Babbar, 2002). Among other natural products, microcrystal cellulose (Gorivnova et al., 1993), parenchymatic solidifier from apple (Titel et al., 1987), an agar-like polysaccharide obtained from *Pseudomonas* (Kang et al., 1982), husk of *Plantago ovata* seed (Babbar and Jain, 1998; Bhattacharya et al., 1994) have been tried. These materials have ample potential for use as gelling agent and are comparatively cheaper. But their performance has been inconsistent, perhaps due to lack of standardization and presence of impurities.

Synthetic solid matrices offer some distinct advantages over agar and other gelling agents. Their quality and properties can be precisely controlled for consistent performance; they are comparatively very cheap, can be reused and are more convenient to handle. Glass beads and glass beads with filter paper (Puchooa et al., 1999) have shown limited superiority over agar. While some synthetic matrices like glass wool cloth, nylon cloth, polystyrene foam (Bhattacharya et al., 1994), polyester-acetate membrane (Matsumoto and Yamaguchi, 1989), polypropylene membrane (Hew et al., 1990; Tanny et al., 1993; Desamero et al., 1993; Adelberg et al., 1992), and polyurethane foam (Conner and Meredith, 1984) have shown promising results.

This study was designed to test the utility of polyurethane foam as support matrix during two standard procedures of in vitro plant production, viz., callus mediated somatic embryogenesis and shoot bud proliferation in date palm (*Phoenix dactylifera* L.). An attempt was also made to compare the efficacy of media with or without charcoal in the somatic embryogenesis and further growth of date palms.

## MATERIALS AND METHODS

### Explant Preparation and Sterilization

Two elite cultivars of date palm, 'Sukkary' and 'Mosaifah' were used for the experiment. Tissue from the apical region of 2-3-year-old offshoot was used as explant. The top 8-10 cm portion was excised and was treated twice with 1.0% solution of sodium hypochlorite for 20 and 10 min respectively followed by treatment with 0.2% solution of mercuric chloride for 5 min. Tween-20 (2-3 drops per 100 ml) was added to both the

sterilizing solutions as surfactant. After rinsing thrice with sterilized distilled water the block was cut into small pieces of 5-8 mm for plating.

### **Nutrient Media**

Medium for date palm cultures consisted of MS (Murashige and Skoog, 1962) salts supplemented with 170 mg L<sup>-1</sup> mono-sodium phosphate, 200 mg L<sup>-1</sup> glutamine, 125 mg L<sup>-1</sup> meso-inositol, 3.0 mg L<sup>-1</sup> glycine, 0.1 mg L<sup>-1</sup> thiamine HCl, 1.0 mg L<sup>-1</sup> pyridoxine HCl, 1.0 mg L<sup>-1</sup> nicotinic acid and 30 g L<sup>-1</sup> sucrose.

Plant growth regulators in the media were added according to the genotype and stage of the cultures as described in the following sections. All media were brought to pH of 5.8 before autoclaving. To prevent browning of cultures at initial stages, 1.5 g L<sup>-1</sup> activated charcoal, 75 mg L<sup>-1</sup> ascorbic acid and 75 mg L<sup>-1</sup> citric acid were added to both the media.

### **Physical Support Systems in the Media**

In date palm, callus induction medium was solidified with 8 g L<sup>-1</sup> agar (Hi Media). At subsequent stages of date palm cultures physical support in the medium was provided by polyurethane (PU) foam discs and agar (8 g L<sup>-1</sup>) in two separate sets of cultures for the purpose of comparison. 1.5 g L<sup>-1</sup> activated charcoal was added to the callus induction medium and somatic embryogenesis medium along with agar. Another set of cultures maintained with agar and without charcoal served as control.

Circular discs of polyurethane foam, fitting the inner diameter of the culture jars were cut out from commercially available sheets of 1.5 cm thickness. Material of the foam was confirmed as linear aliphatic polyurethane by mass spectrometry. Density of the foam was estimated to be 0.0142 g cm<sup>-3</sup> by weight-volume ratio.

The discs were washed with mild detergent, rinsed thoroughly with distilled water, and dried before use. As the discs would gradually absorb medium and sink, enough volume of medium to reach approximately 2/3 the height of the discs was poured in the jars. This level was maintained by 60 ml of medium in the jars used in this study. In the case of agar-gelled media, the same volume was dispensed in each jar.

### **Culture Procedures**

For callus initiation in date palm, small pieces of soft meristematic tissue from the shoot tip zone were implanted on agar-gelled (8 g L<sup>-1</sup>) modified MS medium containing 450 µM 2,4-dichlorophenoxyacetic acid (2,4-D), 15 µM 6-(γ,γ-dimethylallylamino) purine (2-iP), and 15 µM 6-furfurylaminopurine (kinetin). The explants were sub-cultured in the same medium, after 2 weeks and subsequently, at an interval of 4 weeks. After 4-5 sub-cultures white granular portions of the callus were transferred to another agar-gelled medium containing 15 µM α-naphthalene acetic acid (NAA), 15 µM 2-iP and 15 µM kinetin for the proliferation of embryogenic callus.

After 4 weeks in the proliferation medium granular embryogenic callus was transferred to hormone-free medium for the development and germination of embryos. Further this stage, physical support in the medium was provided by polyurethane foam discs and agar in two separate experimental sets of cultures. Young somatic embryos were sub-cultured twice on this medium at an interval of 4 weeks each before starting collection of data on their rate of multiplication and germination.

For shoot growth, germinating somatic embryos were transferred to fresh hormone-free medium. For root induction, 5-6 cm long shoots were transferred to foam-bearing liquid and corresponding agar-gelled media containing 0.5 µM NAA (Fig. 3). From the callus induction stage to embryo germination stage two sets of cultures; agar with charcoal and agar without charcoal, were maintained.

### **Data Collection and Analysis**

Data was recorded concurrently on cultures on PU foam and in agar-gelled media. Efficiency of the two physical support systems was compared on the basis of parameters

representing growth of somatic embryos, shoots and roots. In the case of date palm, rate of multiplication of somatic embryos was assessed as percent increase in number of these structures in a 4-week period and frequency of germination of somatic embryos was recorded for the same period, while rate of shoot elongation was evaluated as percent gain in shoot length during a 6-week period.

Rooting response in date palm was assessed as days to initiate rooting in 50% cultures. Number of roots per plant was recorded four weeks after initiation. Average length of root after four weeks of initiation was also recorded in date palm cultures. For all parameters, data were recorded on at least 25 cultures in five replicates separated temporally or spatially. The experiment was laid out in completely randomized design and analysis of variance was done by the students 't' test to compare the independent influence of the support matrices and the influence of activated charcoal.

## RESULTS

Callusing started early in the explants cultured on media without charcoal, but due to severe browning most of the cultures died (Table 1). Within the next two months somatic embryos started proliferating in granular embryogenic calli. Multiplication of somatic embryos and their rate of germination were found significantly higher in the media without charcoal (Fig. 1). The duration required for different morphogenesis was also found significantly decreased in the media without charcoal (Table 2).

The rate of multiplication of somatic embryos in a 4-week period was significantly higher on PU foam (mean 106%) as compared to 82.6% in agar-gelled medium (Table 3). No significant difference was noticed between responses of the two cultivars on the respective support systems.

Frequency of germination of somatic embryos in 4 weeks was also noted to be superior on PU foam (37.2%) than in agar-gelled media (25.2%). Significantly greater shoot elongation in a 6-week period was recorded on PU foam (55.1%) as compared to agar (36.8%). Germination of somatic embryos on PU foam was better in 'Mosaifah' (38.7%) as compared to 'Sukkary' (35.7%), whereas in agar-gelled medium the two cultivars performed at par with each other, leading to an overall similar response. Elongation of shoots was greater in 'Mosaifah' than in 'Sukkary' on both types of support systems reflecting significantly higher mean for 'Mosaifah' (57.7%) as compared to 'sukkary' (34.2%).

Initiation of rooting took nearly the same time both on PU foam (14.8 days) and agar-gelled media (15.4 days) as shown in Table 2. However, 'Sukkary' stroked root significantly earlier on PU foam (14.4 days) as compared to agar (16.7 days), while 'Mosaifah' took the same period of time on both surfaces. In agar-gelled medium, 'Mosaifah' showed roots earlier (14.0 days) than 'Sukkary' (16.7 days).

Means of the two cultivars after 4 weeks of root initiation in 50% of cultures indicate that fewer roots per plant were formed on PU foam matrix (4.1) as compared to agar-gelled medium (5.3). 'Mosaifah' developed more roots (5.9) than 'Sukkary' (4.6) in agar-gelled medium but on PU foam it formed a similar number of roots as 'Sukkary'.

Based on the means, it was noticed that longer roots were formed on PU foam (8.4 cm) than on agar-gelled medium (Fig. 2). Means of the treatments showed greater root length for the cultivar 'Mosaifah' (8.2 cm) because it formed longer roots on both the surfaces as compared to 'Sukkary' (7.0 cm).

## DISCUSSION

The study was conducted with two genotypes to take into account the genotypic influence and its interaction with the support matrix and media supplements. Not many cultivars of date palm could be induced to produce callus and somatic embryos because of the comparatively recalcitrant nature of the species. A considerable delay or failure of morphogenesis was observed in the different stages of micropropagation of date palm, when activated charcoal was used. Constantin et al. (1977) observed that the hormones required for the callus growth and shoot development in 'Wincosin-38' tobacco are

adsorbed by activated charcoal, thereby inhibiting callus growth and prohibiting shoot development. In some cases activated charcoal prevented development of callus from embryos in the embryo culture (Nguyen et al., 2007).

During micropropagation the exudation of phenol is very common and it often influences the result. Thomas (2008) compiled some recent reports on application of activated charcoal in plant tissue culture of 105 crops. In 90 cases it was found positive, 12 cases found negative and in 3 cases reported as positive and negative. Two important negative aspects of activated charcoal are catalyzed hydrolysis of sucrose into fructose and glucose (Druart and De Wulf, 1993) and drastic reduction of pH after autoclaving (Wann et al., 1997). Phenolic exudation is a matter of serious concern in the micropropagation of date palms. In the explant culture for callus induction incorporation of activated charcoal in the medium is inevitable; otherwise it may lead to considerable loss. On the other hand, as this study indicates, activated charcoal delays morphogenesis and reduces success rate. Alternative use of antioxidants and peroxidases may help to eradicate the problems associated with phenolic exudation in date palm tissue culture and provide the full efficacy of growth hormones and vitamins used in the medium.

In general date palm cultures growing on PU foam showed significantly superior proliferation and germination of somatic embryos and also shoot elongation as compared to cultures in agar-gelled media. Several studies have shown appreciable variation in growth responses of cultures on different solid matrices in comparison with agar. Prasad and Gupta (2006) working with three types of support systems found that shoot production in *Gladiolus* cultures was higher on membrane raft as compared to agar while, shoot elongation was highest on 'duroplast' foam matrix.

Newell et al. (2003) found that compared to agar, rooting response was superior on porous agar, sand, in vitro soil system (IVS) and aerated IVS. They inferred that a higher level of oxygen in aerated systems may have been the cause of early initiation and better growth of roots. Puchooa et al. (1999) have also shown that shoot growth of tobacco cultures in agitated liquid medium allowing enhanced aeration is superior as compared to static liquid medium. Tanny et al. (1994, 1993) have illustrated that cultures growing on a synthetic membrane raft floating on liquid medium put up significantly higher growth as compared to cultures maintained on agar and have suggested aeration and superior water pickup potential of the rafts to be the possible reason for better growth.

In our study somatic embryos and germinated shoots of date palm showing superior growth were placed above the PU foam discs where they received abundant aeration. Gangopadhyay et al. (2004) used coir and 'luffa sponge', the dried vascular net of *Luffa egypitica* fruit for in vitro root induction in *Philodendron* cultures and showed that root initiation was delayed on luffa sponge and coir as compared to agar but the highest number of roots was produced on luffa followed by coir and agar. They have surmised that superior performance of coir and luffa sponge might be due to some purely physical phenomenon. Earlier, they tested coir in comparison with jute and paddy straw as support matrix in media for multiplication of several species and found coir to be the best presumably because of its better water retention capacity (Gangopadhyay et al., 2002). On the other hand, Bhattacharya et al. (1994) experimenting with filter paper, glass wool cloth, nylon cloth and polystyrene foam for micropropagation of chrysanthemum cultures found that generally, these matrices performed at par with agar except that cultures on polystyrene foam produced longer shoots and fewer but longer roots, similar to the findings of the present study. Matsumoto and Yamaguchi (1989) used nonwoven materials like different grades of polyester (100%), polyester-acetate (50:50), acetate (100%), and absorbent cotton as support matrices for culturing protocorm-like bodies (PLBs) of banana. They have noted that gain in fresh weight of PLBs on all nonwoven matrices except a particular type of polyester was significantly superior to agar. Of the physical properties noted, this particular polyester has significantly lower water content (0.6-0.7%) at 20°C-95% RH as compared to all other matrices (10-27%). Tanny et al. (1994, 1993) have suggested that water pickup potential of the material used as support matrix may be one of the physical properties determining availability of water and solvents to the

cultures.

Generally, presence of impurities, inadequate diffusion of nutrients and lack of aeration are arguably considered the major factors responsible for inconsistent and slower growth of cultures on agar (Puchooa et al., 1999; Debergh, 1983). However, there are indications that some physical phenomena may also be playing a role in the variable response of cultures on agar. Like other polysaccharides, agar is a viscoelastic material simultaneously possessing solid and liquid properties with quantifiable moduli of elasticity and viscosity allowing measurement of predominance of solid or liquid characteristics through rheological analysis. On the basis of this analysis Pereira et al. (2007) have shown that elasticity and viscosity differ widely in different brands of agar. Moreover these characteristics change randomly during storage of media and incubation of cultures. Changing moduli influence the interaction between water molecules, media constituents and agar and hence availability of water and nutrition to the cultures. This seems to be a more plausible explanation for inferior performance of agar as compared to the synthetic support system where interaction between water molecules, media components and material of the matrix would remain stable over a long period of time.

Genotypic influence was mild in date palm cultures. It only reflected in greater shoot elongation and root length of cultivar 'Mosaifah'. As an indication of cultivar-matrix interaction, 'Mosaifah' showed higher germination of somatic embryos on PU foam and a greater number of roots in agar. This study shows that PU foam is a satisfactory support material for in vitro cultures. Conner and Meredith (1984) have also successfully used PU foam for monitoring the growth of callus cultures; we suggest that it can be equally beneficial for micro propagation as well. The matrix would be of special use in applications where media are maintained at such pH which would not allow gelling of agar or where in situ change of medium is required. Chemical composition and resulting physical properties of the material may be precisely modified during manufacture to suit specific culture requirements. At the same time the matrix is very cheap as compared to agar even in a single use cycle.

## ACKNOWLEDGEMENTS

Authors express thanks to King Abdulaziz City for Science and Technology, Riyadh for providing facilities.

## Literature Cited

- Adelberg, J., Desamero, N.V., Hale, A. and Young, R. 1992. Orchid micropropagation on polypropylene membranes. Amer. Orchid Soc. Bull. July:688-695.
- Anon. 1988. New system for plant tissue culturing. Genet. Eng. Biotechnol. Moni. (UNIDO) 23:73.
- Babbar, S.B. and Jain, N. 1998. 'Isabgol' as an alternative gelling agent in plant tissue culture media. Plant Cell Rep. 17:318-322.
- Bhattacharya, P., Dey, S. and Bhattacharyya, B.C. 1994. Use of low-cost gelling agents and support matrices for industrial scale plant tissue culture. Plant Cell Tissue Org. Cult. 37:15-23.
- Calleberg, E.K., Kristjansdottir, I.S. and Johansson, L.B. 1989. Anther cultures of tetraploid *Solanum* genotypes: the influence of gelling agents and correlations between incubation temperature and pollen germination temperature. Plant Cell Tissue Org. Cult. 19:189-197.
- Conner, A.J. and Meredith, C.P. 1984. An improved polyurethane support system for monitoring growth in plant cell cultures. Plant Cell Tissue Org. Cult. 3:59-68.
- Constantin, M.J., Henke, R.R. and Mansur, M.A. 1977. Effect of activated charcoal on callus growth and shoot organogenesis in tobacco. In Vitro 13:293-287.
- Debergh, P.C. 1983. Effects of agar brand and concentration on the tissue culture medium. Physiologia Plant. 59:270-276.
- Desamero, N.V., Adelberg, J.W., Hale, A., Young, R.E. and Rhodes, B.B. 1993. Nutrient utilization in liquid/membrane system for watermelon micropropagation. Plant Cell

- Tissue Org. Cult. 33:265-271.
- Druart, P. and De Wulf, O. 1993. Activated charcoal catalyses sucrose hydrolysis during autoclaving. *Plant Cell Tissue Org. Cult.* 32:97-99.
- Ebert, A. and Taylor, H.F. 1990. Assessment of the changes of 2,4-dichlorophenoxyacetic acid concentrations in plant tissue culture in the presence of activated charcoal. *Plant Cell Tissue Org. Cult.* 20:165-172.
- Ebert, A., Taylor, F. and Blake, J. 1993. Changes of 6-benzylaminopurine and 2,4-dichlorophenoxyacetic acid concentration in the plant tissue culture media in the presence of activated charcoal. *Plant Cell Tissue Org. Cult.* 33:157-163.
- Gangopadhyay, G., Bandopadhyay, T., Gangopadhyay, S.B. and Mukherji, K.K. 2004. Luffa sponge - unique matrix for tissue culture of *Philodendron*. *Curr. Sci.* 86:315-319.
- Gangopadhyay, G., Das, S., Mitra, S.K., Poddar, R., Modak, B. and Mukherjee, K.K. 2002. Enhancement rate of multiplication and rooting through the use of coir in aseptic liquid culture media. *Plant Cell Tissue Org. Cult.* 68:301-310.
- Gerbe, E. and Sathyanarayana, B.N. 2001. Cassava - a new and cheaper alternative to agar for direct in vitro regeneration and microtuber production from nodal cultures of potato. *African Crop Sci. J.* 9:1-8.
- Gorivnova, N., Atanasov, A., Alexandrova, K., Velkova, R. and Kasachka, A. 1993. Comparison of agar and microcrystal cellulose as gelling agent for in vitro culture of *Nicotiana tabacum* stem explants. *Biol. Plant.* 35:217-221.
- Henderson, W.E. and Kennersley, A.M. 1988. Corn starch as an alternative gelling agent for plant tissue culture. *Plant Cell Tissue Org. Cult.* 15:17-22.
- Hew, C.S., Chan, Y.S., Lee, Y.K. and Chia, T.F. 1990. Culture of orchid tissue on polypropylene membrane. *Malayan Orchid Rev.* 24:78-81.
- Jain, N. and Babbar, S.B. 2002. Gum Katira - a cheap gelling agent for plant tissue culture media. *Plant Cell Tissue Org. Cult.* 71:223-229.
- Kang, K.S., Veeder, G.T., Mirasoul, P.J., Kaneko, T. and Cottrell, I.W. 1982. Agar-like polysaccharide produced by a *Pseudomonas* species: production and basic properties. *Appl. Environ. Microbiol.* 43:1086-1091.
- Maliro, M.F.A. and Lameck, G. 2004. Potential of cassava flour as a gelling agent in media for plant tissue cultures. *African J. Biotechnol.* 3:244-247.
- Matsumoto, K. and Yamaguchi, H. 1989. Nonwoven materials as a supporting agent for in vitro culture of banana protocorm-like bodies. *Trop. Agric. (Trinidad)* 66:8-10.
- Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant.* 15:473-497.
- Nairn, B.J., Furneaux, R.H. and Stevenson, T.T. 1995. Identification of an agar constituent responsible for hydric control in micropropagation of radiata pine. *Plant Cell Tissue Org. Cult.* 43:1-11.
- Newell, C., Grown, D. and McComb, J. 2003. The influence of medium aeration on in vitro rooting of Australian plant micro-cuttings. *Plant Cell Tissue Org. Cult.* 75:131-142.
- Nguyen, T.V., Thu, T.T., Claeys, M. and Angenon, G. 2007. Agrobacterium mediated transformation of sorghum (*Sorghum bicolor* (L.) Moench) using an improved in vitro regeneration system. *Plant Cell Tissue Org. Cult.* 91:155-64.
- Nissen, S.J. and Sutter, E.G. 1990. Stability of IAA and IBA in nutrient media to several tissue culture procedures. *Hort. Science* 25:800-802.
- Pan, M.J. and van Staden, J. 1998. The use of charcoal in in vitro culture - review. *Plant Growth Regul.* 26:155-63.
- Pereira-Netto, A.B., Petkowicz, C.L.O., Cruz-Siha, C.T.A., Gazzoni, M.T., Mello, A.F.P. and Silveira, L.M. 2007. Differential performance of Marubakaido apple rootstock shoots grown in culture media containing different agar brands: dynamic rheological analysis. *In Vitro Cell Dev. Biol. Plant* 43:356-363.
- Prasad, V.S.S. and Gupta, S.D. 2006. In vitro shoot regeneration of gladiolus in semi-solid agar versus liquid cultures with support systems. *Plant Cell Tissue Org. Cult.* 87:263-271.

- Puchooa, D., Purseramen, P.N. and Rujbally, B.R. 1999. Effects of medium support and gelling agent in the tissue culture of tobacco (*Nicotiana tabacum*). Sci. Technol. Res. J. 3:129-144.
- Scholten, H.J. and Pierik, R.L.M. 1998. Agar as gelling agent: chemical and physical analysis. Plant Cell Rep. 17:230-235.
- Tanny, G.B., Greenwald, J., Greenwald, S., Watad, S.S. and Gaba, V. 1994. Controlling the performance of an interfacial membrane raft in plant micropropagation. [www.osmotek.com/lit.htm?ref=s0d.org](http://www.osmotek.com/lit.htm?ref=s0d.org).
- Tanny, G.B., Watad, A.A., Kochba, M. and Gaba, V. 1993. Synthetic membranes for use in plant tissue culture. In Vitro 29 A:61.
- Thomas, D.T. 2008. The role of activated charcoal in plant tissue culture. Biotechn. Adv. 26:618-631.
- Titel, C., Ehwald, R., Zoglauer, K. and Hellmig, A. 1987. A parenchymatic medium solidifier for plant in vitro culture. Plant Cell Rep. 6:473-475.
- Van Winkle, S., Johnson, S. and Pullman, G.S. 2003. The impact of gelrite and activated carbon on the elemental composition of tissue culture media. Plant Cell Rep. 21:1175-1182.
- Wann, S.R., Veazey, R.L. and Kaphammer, J. 1997. Activated charcoal does not catalyze sucrose hydrolysis in tissue culture media during autoclaving. Plant Cell Tissue Org. Cult. 50:221-224.
- Weatherhead, M.A., Burdon, J. and Henshaw, G.G. 1979. Effects of activated charcoal as an additive plant tissue culture media. Z. Pflanzenphysiol. 94:399-405.



## Tables

Table 1. Response of activated charcoal in the micro-propagation of date palms.

Cultivar	Growth parameters								
	Callus induction (%)			Multiplication of somatic embryos (numbers)			Germination of somatic embryos		
	AC	WAC	t-value	AC	WAC	t-value	AC	WAC	t-value
Sukkary	36.6**	11.71**	21.85	189*	208*	2.73	125**	176**	9.42
Mosaifah	66.6**	29.97**	17.02	450**	512**	3.38	430**	486**	5.28
t-value	21.43	9.14		13.75	57.45		28.9	52.62	

AC - activated charcoal; WAC - without activated charcoal. \*Significant at  $P>0.05$ ; \*\* significant at  $p<0.01$ .

Table 2. Duration of morphogenesis under the influence of activated charcoal.

Cultivar	Number of weeks taken to initiate								
	Callus induction			Somatic embryogenesis			Germination of somatic embryos		
	AC	WAC	t-value	AC	WAC	t-value	AC	WAC	t-value
Sukkary	10	8	0.77	43**	24**	5.89	12	10*	1.55
Mosaifah	8	6	1.22	28**	16**	5.04	9*	6*	2.60
t-value	0.77	1.22		6.3	2.48		1.96	4.9	

AC - activated charcoal; WAC - without activated charcoal. \*Significant at  $P>0.05$ ; \*\* significant at  $p<0.01$ .

Table 3. Shoot growth response of date palm cultivars on PU foam in comparison with agar.

Cultivar	Growth parameters								
	Multiplication of somatic embryos			Germination of somatic embryos			Elongation of shoots		
	Agar (%)	PU foam (%)	t-value	Agar (%)	PU foam (%)	t-value	Agar (%)	PU foam (%)	t-value
Sukkary	88.0**	101.3**	6.02	25.7**	35.7**	4.96	24.0**	44.4**	9.16
Mosaifah	77.3**	110.6**	6.96	24.6**	38.7**	3.50	49.7**	65.8**	5.57
t-value	4.02	2.04		0.55	0.74		9.87	8.37	

\*Significant at  $P>0.05$ ; \*\* significant at  $p<0.01$ .

Table 4. Rooting response of date palm cultivars on PU foam in comparison with agar.

Cultivar	Rooting parameters								
	Days to initiation in 50% cultures			Roots per plant			Mean root length		
	Agar	PU foam	t-value	Agar	PU foam	t-value	Agar	PU foam	t-value
Sukkary	16.7*	14.4	2.19	4.6	4.4	0.55	6.2	7.8	1.93
Mosaifah	14.0	15.2	1.19	5.9*	3.7*	3.24	7.4*	9.1*	2.46
t-value	3.11	0.66		1.93	1.87		2.38	0.97	

\*Significant at  $P>0.05$ ; \*\* significant at  $p<0.01$ .

## Figures

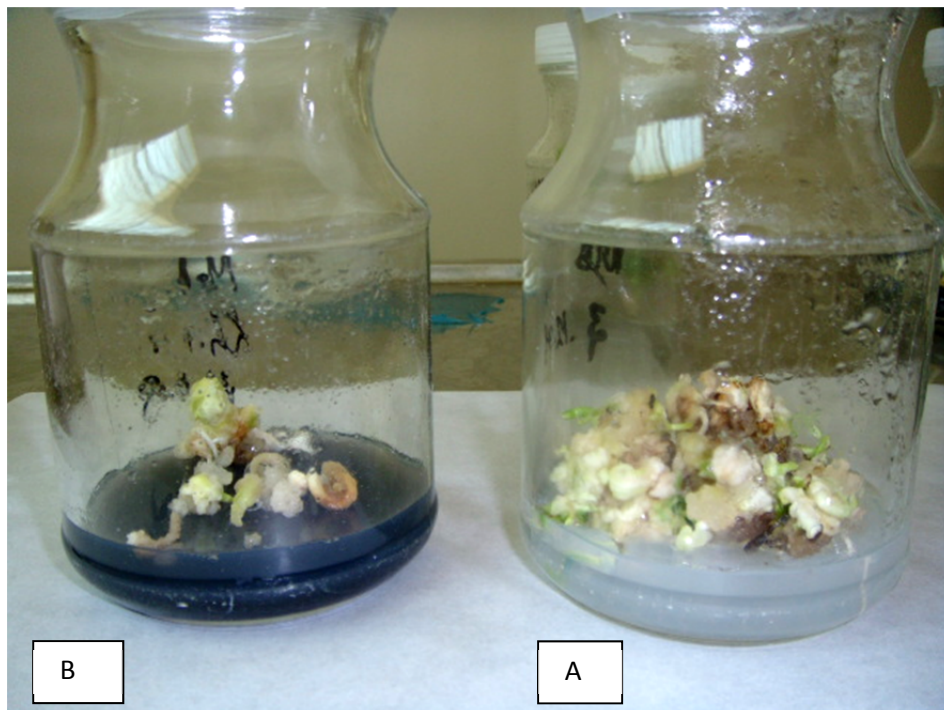


Fig. 1. More somatic embryos and embryo germination on media without charcoal (A) compared to media with charcoal (B).

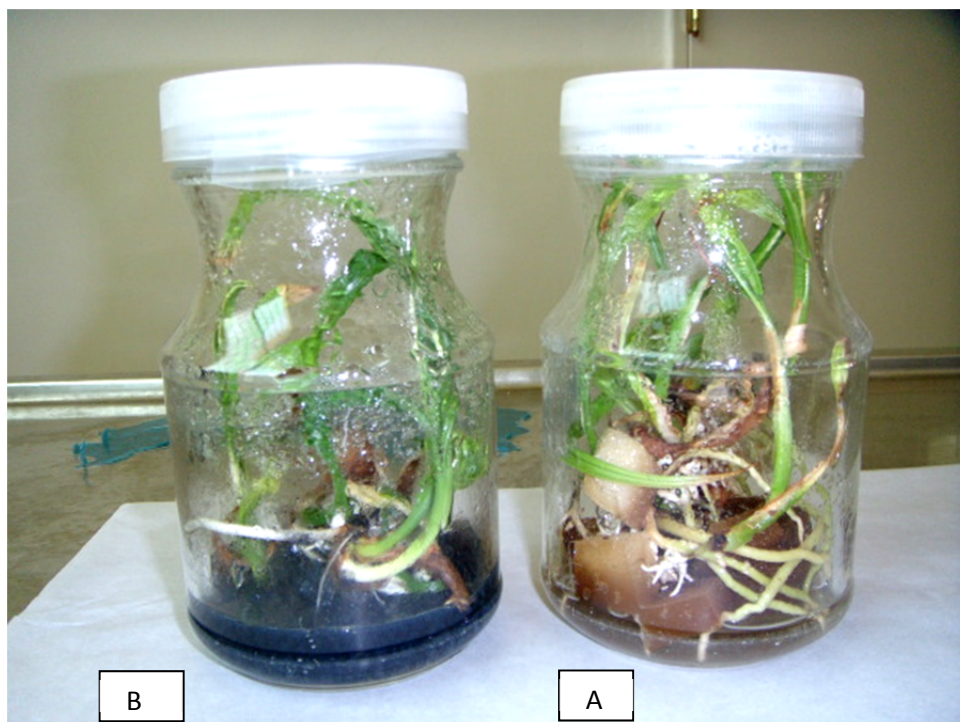


Fig. 2. Profuse and longer rooting in PU foam containing medium (A) compared to agar gelled medium (B).

