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# IN VITRO CULTURE OF FEMALE DATE PALM (*PHOENIX DACTYLIFERA* L.) TISSUES

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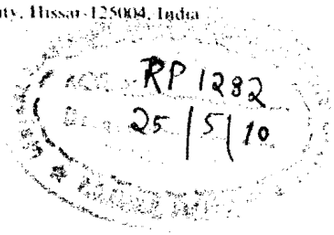


INDEX WORDS

Female date palm, tissue culture, embryo, nutrient media

SUMMARY

Roots, leaf petioles, shoot tips and immature fruits of female date palm formed the source material for in vitro cultures. Isolated embryos excised from mature seeds were also cultured. Of all the tissues, some of the leaf petioles and fruit mesocarp gave rise to calluses in the media of STARITSKY (1970) and LILIEN (1976). Isolated embryos germinated to produce seedlings in MS (MURASHIGE & SKOOG, 1962) medium. Calluses were established from seedling sections but the growth conditions propitious for explants of seedlings were not suitable for female date palm tissues. The main problem in establishing long term tissue cultures has been the browning of the tissues and media. This problem was, at least partially, overcome by adding activated charcoal to the liquid media and cysteine to the solid media. So far, shoot regeneration in the calluses could not be induced.

## INTRODUCTION

Though tissue culture techniques have made it feasible to vegetatively propagate several plant species (MURASHIGE, 1974; HUSSEY, 1978), the success in culturing woody species in general (KING, 1974) and monocotyledonous plants in particular, has been limited to only a few cases (STARITSKY, 1970; BHOJWANI et al., 1977). Nevertheless, the feasibility of producing large numbers of plants from small tissues has prompted the use of such techniques in exploring ways to multiply elite trees. Date palm is one of these cases requiring serious attention for quick vegetative propagation (REUVENI & LILIEN-KIPNIS, 1974). Date palm orchards can be raised either from seeds or from offshoots. When plants are grown from seeds, about half of the palms turn out to be males, which can be identified only at the time of flowering. At this stage it would be difficult to remove these pollen producers and fill in the gaps. Moreover, the plants obtained through seeds are genetically heterogeneous. Consequently, for uniformity of the orchards, date palms are propagated through offshoots only. Since offshoots are limited in quantity (REUVENI et al., 1972; ANONYMOUS, 1976), extensive efforts are being made to propagate female date palms through tissue culture. This communication deals with our observations on various parts of female date palms cultured in vitro.

## MATERIALS AND METHODS

Several parts of female date palms served as the source tissue. Offshoots were dug out and older leaves were removed. Succulent leaves with perioles and shoot tip were kept

Table 1. Comparative effectiveness of different sterilants and their duration (in minutes) on various date palm tissues.

| Tissue | Ethanol (90% v) | Polar liquid bleach (30% v) | $HgCl_2$ (0.1% w) | $KMnO_4$ (1% w) | Effectiveness* |
|---|---|---|---|---|---|
| Leaf pieces | 1 | 20 | | | + + + |
| Leaf pieces | 1 | | 10 | | + + + |
| Shoot tips | 1 | 20 | | | 0 |
| Shoot tips | 2 | 20 | | | + + |
| Shoot tips | 2 | | 10 | | + |
| Shoot tips | 2 | | 20 | | + + |
| Shoot tips | 2 | | | 10 | + + + |
| Immature fruits | 1 | 20 | | | + + + |
| Immature fruits | 1 | | 10 | | + + + |
| Seeds | 2 | | 20 | | + + |
| Seeds | 2 | | | 15 | + + + |

*0 : 100 per cent contamination; + : 50–99 per cent contamination; + + : 25–49 per cent contamination; + + + : Less than 25 per cent contamination

intact for use. Apart from offshoots, mesocarp and ovules of immature fruits as well as segments of seedlings raised from isolated embryos in test tubes, formed the material.

*Surface sterilization.* Since incidence of contamination varied with the material, several sterilants, viz., potassium permanganate, mercuric chloride, 'POLAR' liquid bleach and ethanol, singly or in combinations were used. Their comparative effectiveness for the materials under study has been given in Table 1. Addition of 1–2 drops of 'Teepol' improved the efficiency of the sterilant. The tissue was then washed several times in sterile distilled water and dead ends removed before culturing. Seeds were soaked in water for 10–15 days under aseptic conditions before excising their embryos.

*Nutrient media.* Both liquid and agar media were tested. Filter paper wicks supported the tissue wherever liquid media were used. pH was adjusted to 5.8 in all cases. Sterilization was achieved either by autoclaving at about 1 kg/cm$^2$ pressure (120 °C) for 15 minutes or by cold filtration using 'Maxflow' membranes of 0.3 µm porosity.

MS (MURASHIGE & SKOOG, 1962), NN (NITSCH & NITSCH, 1969), SH (SCHENK & HILDEBRANDT, 1972), S (STARITSKY, 1970), AB (AMMAR & BENBADIS, 1977) and Y3 (EEUWENS, 1976) were used. They were supplemented with growth regulators like IAA, NAA, 2,4-D, kinetin, 6BAP etc. These growth regulators were applied either alone or in combinations. In some cases natural adjuvants as coconut water, casein hydrolysate and malt extract were also added. Only those combinations in which explants responded favourably, have been described (Table 2 and 3).

Since browning of the tissue and medium was a major problem for establishing cultures, ascorbic acid and activated charcoal were added to the liquid media whereas cysteine was added to agar media. Cultures were incubated at 28 ± 1 °C in dark.

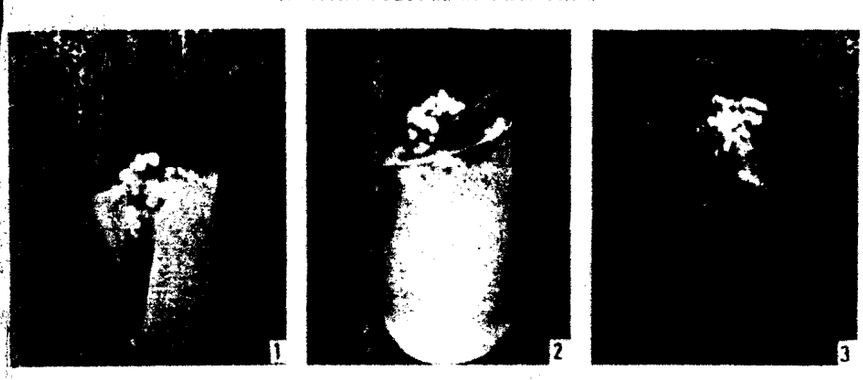

Fig. 1. Formation of callus from petiolar tissue.
Fig. 2. Initiation of roots from petiolar callus.
Fig. 3. Formation of lateral roots

## RESULTS

The first observation after culturing the tissue, irrespective of the medium, was browning of cut ends which were in contact with the medium. Later the pigment exuded into the medium rendering it light brown, dark brown and subsequently black. With roots it occurred within few hours, whereas leaf petioles, stem segments, mesocarp and ovules took 2–20 days. Use of 10 mg l cysteine in the solid, and one per cent activated charcoal in the liquid media proved to be effective in preventing browning for the first 10–15 days. During this period a few cell layers were formed on the cut ends of the explants of leaf petioles and mesocarp tissues. If the tissue was left in the same medium for a prolonged period, pigmentation started developing. This problem, however, was altogether eliminated after 3–4 subcultures at an interval of 10 days in all cases except when roots were the material.

Table 2. Composition of the modified Staritzky medium.

| Macro elements | | Micro elements | | Organic additions | |
|---|---|---|---|---|---|
| component | concentration (mg/l) | component | concentration (mg/l) | component | concentration (mg/l) |
| $Ca(NO_3)_2.4H_2O$ | 347 | $MnSO_4.4H_2O$ | 4.4 | sucrose | 30000 |
| $KNO_3$ | 1000 | $ZnSO_4.7H_2O$ | 1.5 | glycine | 2.0 |
| $NH_4NO_3$ | 1000 | $H_3BO_3$ | 1.6 | cysteine | 10.0 |
| $KH_2PO_4.2H_2O$ | 300 | KI | 0.8 | meso inositol | 100 |
| $MgSO_4.7H_2O$ | 35 | FeEDTA | 32 | casein hydrolysate | 500 |
| KCl | 65 | | | thiamine HCl | 1.0 |
| | | | | adenine | 1.0 |
| | | | | 6BAP | 0.1 |
| | | | | NAA | 5.0 |

Table 3. Composition of the modified Y3 medium

| Macro elements | | Micro elements | | Organic additions | |
|---|---|---|---|---|---|
| component | concentration (mg/l) | component | concentration (mg/l) | component | concentration (mg/l) |
| $KNO_3$ | 2020 | FeEDTA | 32.5 | sucrose | 45000 |
| KCl | 1492 | $MnSO_4.4H_2O$ | 11.2 | meso inositol | 100 |
| $NH_4Cl$ | 535 | KI | 8.3 | thiamine HCl | 0.5 |
| $NaH_2PO_4.2H_2O$ | 312 | $ZnSO_4.7H_2O$ | 7.2 | pyridoxine HCl | 0.05 |
| $CaCl_2.2H_2O$ | 294 | $H_3BO_4$ | 3.1 | calcium pantothenate | 0.05 |
| $MgSO_4.7H_2O$ | 247 | $CoCl_2.6H_2O$ | 0.24 | nicotinic acid | 0.05 |
| | | $Na_2MoO_4.2H_2O$ | 0.24 | biotin | 0.05 |
| | | $CuSO_4.5H_2O$ | 0.25 | 6BAP | 1.0 |
| | | $NiCl_2.6H_2O$ | 0.024 | gibberellic acid | 0.038 |
| | | | | NAA | 5.0 |

The newly formed cell layers stopped growing further in all the media except in medium S (Table 2) and medium Y3 (Table 3) where a few cultures proliferated further to form callus (Fig. 1). These cell masses, however, grew rather slowly. All efforts, in terms of media and culture conditions, to induce luxuriant growth proved futile. After about 6 weeks, protuberances appeared in some of these calluses, which subsequently produced roots (Fig. 2). Once the roots differentiated, they grew rapidly giving rise to lateral roots also (Fig. 3). Out of all the combinations of media and growth regulators, medium S containing 5 mg/l NAA, 0.1 mg/l 6BAP and 500 mg/l casein hydrolysate was found to be best. About 10 per cent cultures proved to grow in it (Table 4). This medium was followed by Y3 containing 5 mg/l NAA and 1 mg/l 6BAP, in which about 2–5 percent explants produced calluses (Table 4). All other combinations along with 15 per cent coconut water, 0.1 per cent malt extract and varying concentrations of casein hydrolysate did not support growth of the calluses. However, the majority of the explants survived over a period of 3 months in most of these media.

When isolated embryos were cultured, there was no browning. Within six weeks seedlings were formed. Callus cultures have successfully been established from the segments of these seedlings on Y3 medium containing 0.047 mg/l NAA and 1.13 mg/l 6BAP. So far, shoot regeneration has not been observed in these calluses.

Table 4. Per cent callus formation in shoot tips and fruit mesocarp tissue of female date palm cultured in Staritzky (S) and Y3 medium.

| Source of explant | Medium | Number of explants | Number of cultures with sustained growth | Per cent of growing cultures |
|---|---|---|---|---|
| Shoot tips | S | 240 | 25 | 10.4 |
| Shoot tips | Y3 | 360 | 19 | 5.2 |
| Fruit mesocarp | S | 430 | 41 | 9.5 |
| Fruit mesocarp | Y3 | 280 | 6 | 2.1 |



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## DISCUSSION

A few unsuccessful attempts were made in the past to clone female date palms through tissue culture (SCHROEDER, 1972; REUVENI & LILIEN-KIPNIS, 1974). So far only seedling segments have been successfully cultured to obtain calluses (REUVENI & LILIEN-KIPNIS, 1974; EEUWENS, 1976, 1978) and even whole plants (AMMAR & BENBADIS, 1977), whereas other tissues have not responded favourably to culture conditions. Since our primary aim remains to clone but female trees, seedling segments can be used only to study the nutrient and other requirements. This information, hopefully, may be used for culturing female plant tissues. However, our observations, along with those of REUVENI & LILIEN-KIPNIS (1974) make it clear that such an extrapolation is not valid and nutrient requirements vary from tissue to tissue even in the same plant. Therefore, methodology developed for cloning seedlings may not, per se, be applicable for multiplying female date palms.

Because of the absence of a lateral cambial layer in this material, shoot tips which consist of over 100 primordial leaves and growing roots seem to be the ideal material for culturing. A serious handicap with roots is the browning of the medium, and therefore, shoot tips are a better alternative. Browning of the tissue and the medium is assumed to be due to the oxidation of polyphenols and formation of quinones which are toxic to the tissues (REUVENI & LILIEN-KIPNIS, 1974). Between the two antioxidants used, only cysteine had a favourable effect, whereas ascorbic acid proved ineffective. Use of activated charcoal is preferable because cysteine at higher concentrations can be toxic to the plant tissues (unpublished data).

Present observations have clearly shown that succulent petiolar tissue of female date palm is capable of dedifferentiating to form callus, which in turn, retains the potential to form roots. However, it was not clear why only a few cultures from the same offshoot produced calluses, whereas others failed to do so under identical conditions. This point, along with the conditions conducive to shoot regeneration, need intensive investigation before any breakthrough is achieved in propagating female date palms.

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