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SUSTAINED MULTIPLICATION OF LONG TERM EMBRYOGENIC CULTURES OF DATE PALM AND THEIR FIELD PERFORMANCE

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Abstract

Sustained embryogenesis in data palm (*Phoenix dactylifera* L. cv. Dhakki) were induced which were then induced to form plants. These plants after acclimatization were transferred to field in D.I. Khan where they have shown good performance.

Introduction

Agricultural importance of palms has been well documented. Dates have been used as staple food since long (Nixon, 1951), because of high energy food due to high sugar contents. Date palms, being monocotyledonous are restricted to axillary bud outgrowths during their juvenile life cycle as the only means of vegetative propagation. Progress in breeding, genetics, crop improvement and expansion of commercial planting of date-palm has thus been restricted by slow asexual reproduction which employs only the clonal offshoots derived from axillary buds on the palm (Hussain *et al.*, 1995). Propagation by seeds is unsatisfactory because this species is dioecious and completely heterozygous and hence not true to type. Seeds may produce more than 50% male or female plants. By using only offshoots, new or scarce varieties, and the disease or pest resistant varieties of date palm cannot be propagated quickly and distributed on a large scale (Oppenheimer & Reuveni, 1972; Lilien & Kipnis, 1969). Pakistan, which produces one of the best quality dates hinges on the tissue culture technology for mass production and its ultimate commercialization. There has been considerable success in regenerating date palm plants using tissue culture techniques, either through the process of somatic embryogenesis or axillary shoot multiplication (Beauchesne, 1982; Mater, 1986; Tisserat, 1982, 1984, Zaid & Tisserat, 1983, Strive & Mosev, 1984, Shakib *et al.*, 1994). However, there is a need to devise media suitable for our date varieties eg., Dhakki, Khudrawi, Begum Jhungi, Hallawi etc. There are only few reports which describe the establishment of long term embryogenic cultures in date palm. The present study highlights a sustained maintenance of embryogenic culture in *Phoenix dactylifera* L. cv. Dhakki.

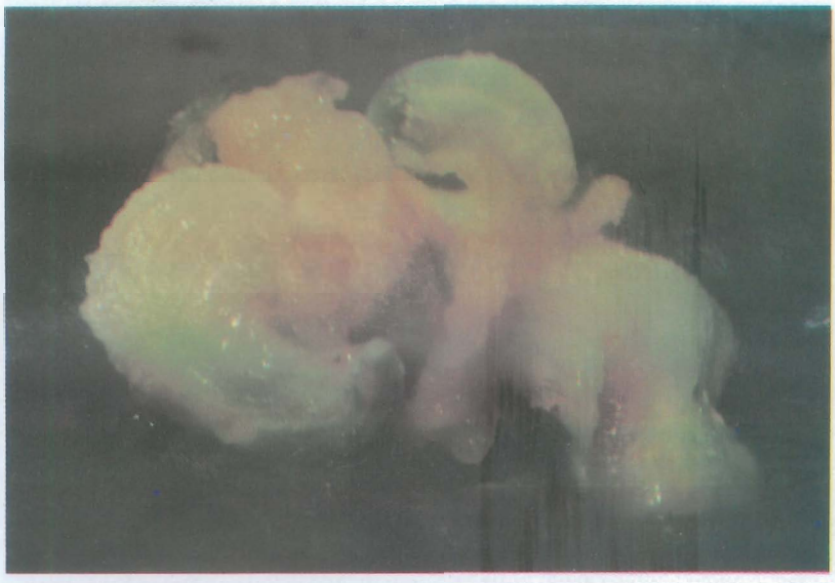

Fig. 1. Callus formation under complete obscurity.

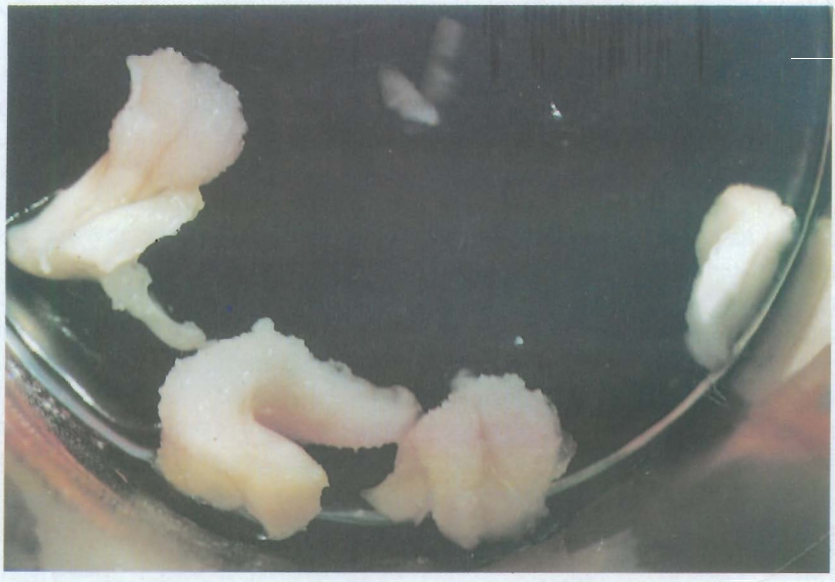

Fig. 2. Somatic embryogenesis.

Materials and Methods

The shoot tip, axillary buds, leaf sheaths and meristematic tissues were used as explant source for this study. The suckers were reextracted, carefully defoliated so as to expose the inner soft tissues. The apical shoot (meristem with one or two layers of thin sheaths), and lateral buds were carefully removed and dipped immediately in antioxidant solution containing 100 mg/l Ascorbic acid and 150 mg/l Citric acid which controlled browning to a considerable extent. The buds were disinfected by a quick dip in 70% ethanol followed by a 15-20 minutes sterilization in (2.65%) Sodium hypochlorite, to which a few drops of Tween 20 were added. A final washing with autoclaved distilled water at least 3 times at an interval of 15-20 minutes each, preceded aseptic preparation of tissues. The tissue was maintained on MS (1962) medium with 30.0g/l sucrose, 0.4 mg/l thiamine HCl, 100 mg/l Myoinsitol, 3.0 g/l activated charcoal and 8.0 g/l agar. The growth hormones added were Dichlorophenoxy acetic acid (2,4-D), Naphthalene acetic acid (NAA), Benzyl amino purine (BAP) and Isopentenyl adenine (2-iP). pH was adjusted at 5.8 prior to autoclaving. The medium was dispensed in standard size glass tubes and flasks capped with cotton plug and sterilized by autoclaving for 15 minutes at 121°C at 15 psi.

Fig.3. Regeneration from callus.

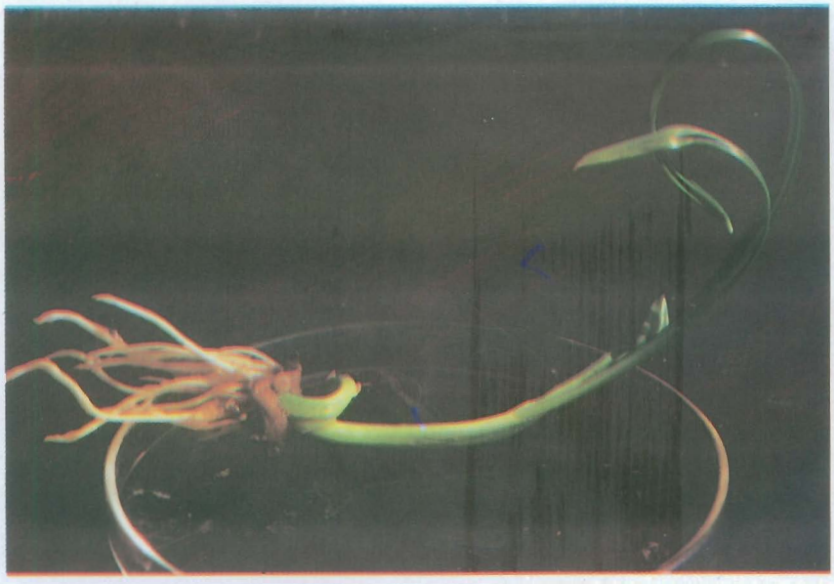

Fig.4. Plant with complete shoot and roots.

Fig.5. Plantlets established in Green House.

Results and Discussion

Apical buds cultured on MS (1962) medium containing 5.0 mg/l 2,4-D showed (30-40%) proliferation after six weeks of incubation, while tips of lateral buds turned green and exhibited slight swelling on the same medium. Good tissue proliferation was observed only from apical buds cultured. Callus initiation was noticed within 8 to 10 weeks of culture (Fig.1). For a sustained maintenance and multiplication, the cultures were regularly subcultured after every 6 to 8 weeks 3 to 4 times on the fresh medium containing the same ingredients of the medium. The callus produced the nodules of somatic embryos (Fig.2) when transferred to MS medium supplemented with GA_3 (0.1-1.0 mg/l) and 2i-P (0.1-2.0 mg/l). After subsequent subcultures on the fresh medium a large number of embryoid regenerated from the proliferating tissues. Our results are similar to the observation of Tisserat (1982) who obtained embryogenic callus from

Fig.6. Plant established in field.

Fig.7. Fruiting of tissue culture raised plant.

lateral buds in date palm on MS medium containing CPA, NAA and 2,4-D which ultimately produced the plantlets.

From callus embryoids were formed, which should then form plantlets. So there is no need of regeneration. Embryoids give rise to plants directly. Only 39% of the cultures survived and showed complete shoot formation (Fig.3) while the remaining cultures turned brown or died due to necrosis. Roots were induced on MS medium containing NAA 0.1 mg/l. This combination favoured thick whitish root growth which appeared within one month of culture (Fig.4). Survival percentage was 70-80% when well rooted plants 8-12 cm in length were used for transfer. Plantlets have been established under screen house conditions and are maintained carefully for a period of two to three months or more unless new leaves appeared (Fig.5). After successful establishment in screen house the plants were transferred to the field in Dera Ismail Khan in August 1991.

The plantlets (Fig.6) successfully established in Dera Ismail Khan (North West Frontier Province of Pakistan), showed profuse growth with 100% survival percentage. The juvenility conferred in tissue culture raised data palm plantlets enhanced vigorous vegetative growth and is an appropriate option for multiplication of date palm. It was also observed that more than 60% of the tissue culture raised plantlets produced suckers much earlier as compared to the control which were observed in third year after establishment and fruiting was noted in 50% of the plants in the fifth year after establishment in the field (Fig.7).

The study provides useful information on somatic embryogenesis, its maintenance and regeneration in one of the best date palm varieties Dhakki which has a good export potential. Apical buds prove to be the best explant source to achieve the objective.

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