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MICROPROPAGATION OF DATE PALM (*PHOENIX DACTYLIFERA* L.) CV KHADRAWY USING TISSUE CULTURE TECHNIQUE

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ABSTRACT

Small segment of explant (1 cm) from tip of young offshoot (1-2 year) of date palm were cultured on modified Murashige and Skoog (1962) medium. Significant reductions in the time required for callus formation (two weeks) and development of plantlets were observed in the modified nutrient medium. Developed plantlets were transferred on a modified MS medium containing 2 mg/l each of naphthylacetic acid, naphthoxyacetic acid and benzyl adenine for successful hardening and then transferred to pots. Potted plants became ready for field transfer within twomonths.

INTRODUCTION

Date palm, a dioecious tree, is commercially propagated by offshoots. The production of offshoots from mother plant is limited (Pareek, 1984) and consequently, clonal multiplication rate is slow. Extensive efforts to propagate female date palm through tissue culture are under way (Sharma et al., 1984; Dass et al., 1989). In this communication a modified method is described to regenerate whole plantlets of female date palm through somatic embryogenesis by using meristematic tissues from the tip of offshoot as the explant.

MATERIAL AND METHODS

The culture medium of Murashige and Skoog (1962) was modified to contain the following constituents (mg/l): $NaH_2PO_4 2H_2O$ 170.0, glycine 2.0, 2,4-dichlorophenoxy acetic acid (2,4-D) 100.0. benzyl adenine (BA) 3.0; besides it also contained activated charcoal 0.3% sucrose 3% and agar (BDH) 0.8%. The pH of the medium was adjusted to 5.8.

One-cv to two-year old offshoot of female date palm (*Phoenix dactylifera* L.) Khadrawy, growing in the Horticulture field of Central Arid Zone Research Institute, Jodhpur, was detached from mother plant in the month of September and was washed thoroughly with tap water to remove sand particles. The leaves, along with leaf sheaths, were removed acropetally till the tender portion was reached. It was further trimmed to completely remove the woody tissues, keeping the succulent shoot tip intact. The tip thus obtained was kept in an anti-oxidant solution (citric acid 100 mg/l

of double distilled water) and then treated with 0.5% carbendazim solution for five minutes. The tip was then sterilized with 0.1% $HgCl_2$ for 4-5 minutes followed by washing with sterile distilled water 5-6 times under aseptic conditions.

The explant was trimmed to about 1 cm length and was cut into 10-15 small segments. These were initially cultured on the modified MS nutrient medium. These cultures were incubated at 28 ± 2°C in dark for callus production. The callus was transferred to the modified hormone-free MS medium (devoid of 2,4-D and BA) for the initiation of somatic embryos. Such cultures were maintained under light (3000 lux) at 28 ± 2°C for two to three weeks.

Somatic embryos were then subcultured on another modified MS medium containing 2 mg/l each of naphthoxyacetic acid (NOA), naphthylacetic acid (NAA) and benzyl adenine (BA), to separate single embryos for the devlopment of complete plantlets with shoot and root.

After three weeks the fully developed plantlets were kept for hardening by increasing the temperature from 28° to 32°C for two weeks.

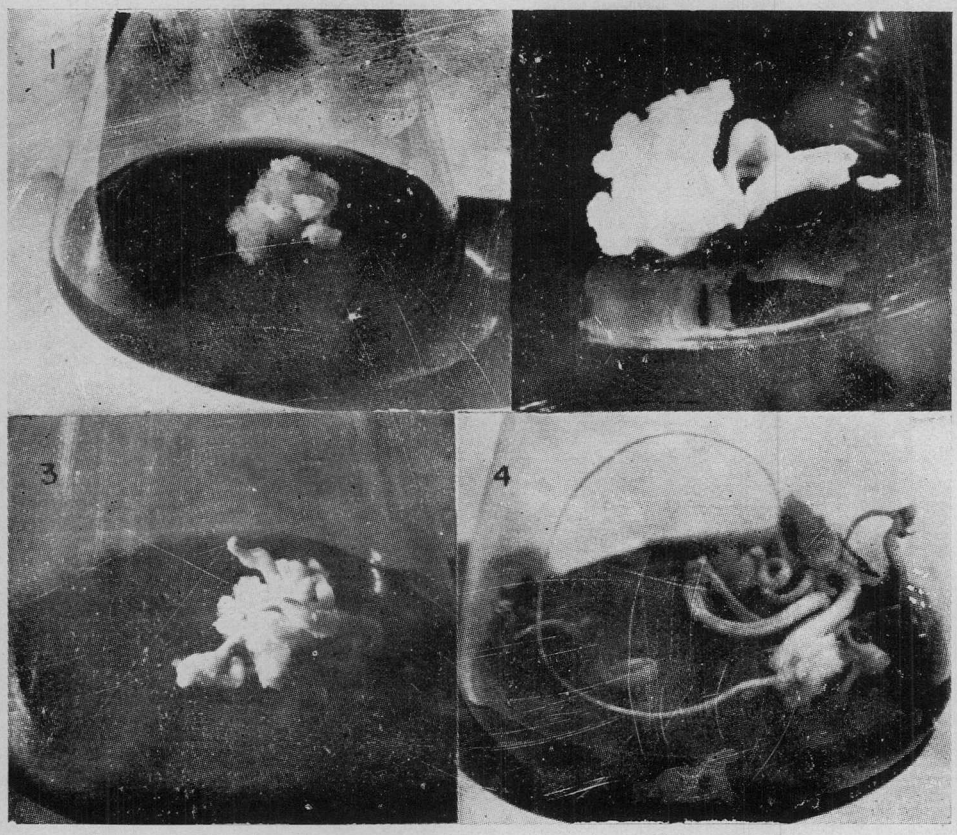
After hardening, the plantlets were removed from the culture tubes, washed thoroughly with tap water, treated with carbendazim (O.2%) and streptocycline (0.1%) each for 30 seconds, and again washed thoroughly with sterile distilled water before planting in the earthen pots (30x15x10 cm) containing equal proportions of dune sand, FYM and clay.

RESULTS AND DISCUSSION

Callus formation on the modified MS medium containing activated charcoal, 2,4-D and BA could be initated from the explant in just two weeks. The period of about 6-8 weeks for initiation of callus reported by others (Sharma et al., 1986; Dass et al., 1989) could thus be significantly reduced to advantage by modification of the MS Medium as suggested by us. Younger age of the offshoot (1-2 years) and size of explant (1 cm only) may also be the other factors favouring early initiation of the callus in our study.

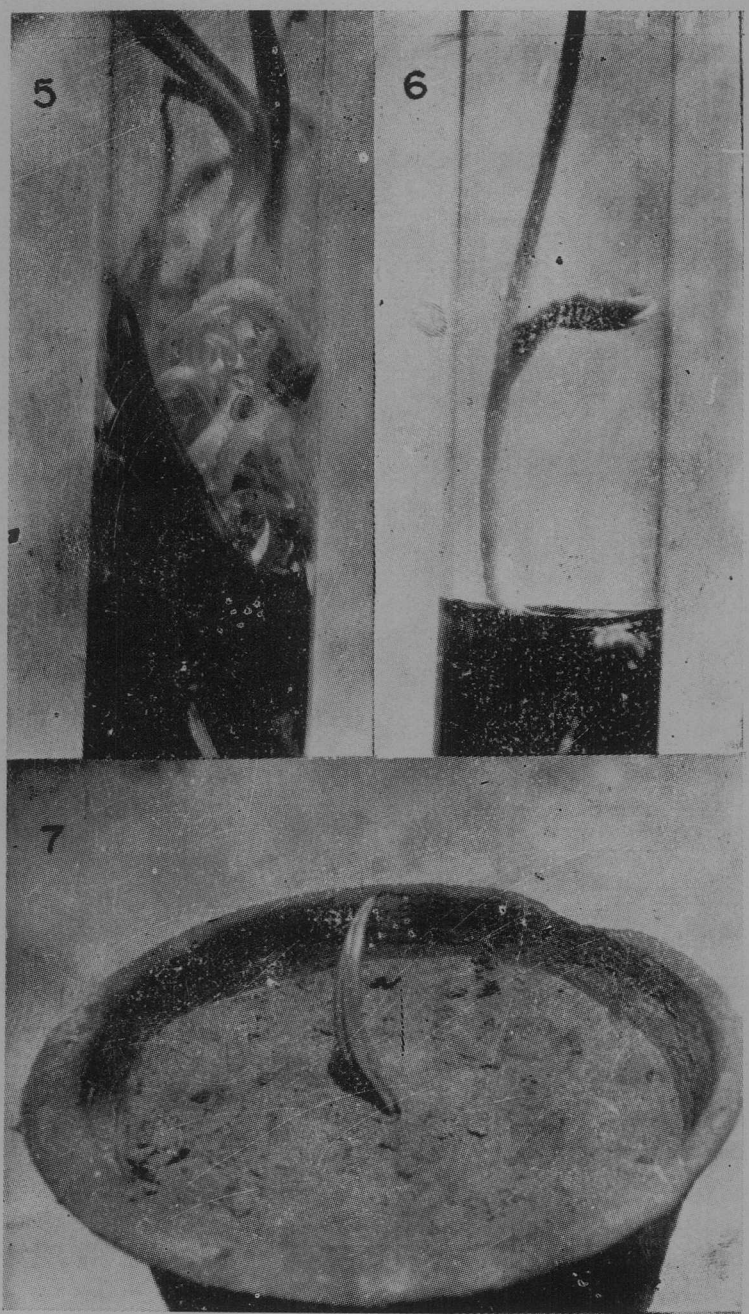
This callus could be maintained by subculturing on fresh medium after every two to theree weeks (Fig. 1).

Differentiation of callus into embryogenic nodules could be initiated by transferring 3-week old callus to hormone-free modified MS medium and incubating it under light intensity of 3000 lux for 16 hours/day and within two weeks period, the callus differentiated into numerous somatic embryos (Fig. 2). These somatic embryos were subcultured on the same medium for 'germination' and subsequent growth (Fig. 3).



Figures : 1. Callus developed from shoot tip on medium containing high levels of auxin (100 mg / l)
2. Proliferation of embryogenic callus and development of embryogenic nodules on hormone-free medium
3. Germination of embryogenic nodules on hormone - free medium for further development
4. Plantlets on hormone-free medium having long slender roots and small shoots

Plantlets on hormone free medium developed very long slender root and small shoot (Fig 4). Transfer of such plantlets to the modified MS medium containing NOA, NAA and BA resulted in further growth to give full plantlets (Fig.5). To avoid overcrowding and clumping of the plantlets, subculturing was necessary to separate the individual plantlets (Fig. 6). Adequate hardening of these plantlets could be obtained by raising the temperature to 32°C for two weeks. Before transferring the plantlets into earthen pots, it was considered appropriate to treat them for 30 seconds with 0.2% carbendazim and 0.1% streptocycline each to rule out possibilities of fungal and bacterial infections. For new leaf to appear and the plant growth to continue the earthen pots were initially covered with polythene bags and kept in a net house for 2



Figures : 5. Developing plantlets in medium containing auxins and cytokinin
6. Plantlet showing distinct shoot, root and cotyledon on medium containing auxins and cytokinin
7. Plantlet growing in pot kept in net house

months to maintain them at proper humidity (70-90%) and temperature (32-35°C) under field conditions. Thereafter the plants attain satisfactory growth without further protection (Fig. 7). After two months the potted plants become ready for transfer to field.

Thus, a modification in MS medium and selection of explant material as suggested by us holds promise in significantly reducing the time required for the tissue culture of date palm plantlets.

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