IN VITRO REGENERATION OF DATE PALM PLANTLETS

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ABSTRACT

Embryogenic cultures were induced from shoot tips of date palm (cv. Muscat) on nutrient medium containing MS basal salts along with NAA, 2,4-D, BAP and PVP under dark condition at 28 C. Cellular embryos further differentiated into well-developed nodular-shaped embryos on medium supplemented with NaH_2PO_4 and Kn and on increasing KH_2PO_4 to 200 mg l concentration. Somatic embryos germinated into complete plantlets by first initiating root and then shoot at 0.1 mg/l NAA.

INTRODUCTION

DATE palm, *Phoenix dactylifera* L. is clonally propagated by offshoots. The production of offshoots from mother plants is limited. Being heterozygous in nature, date palm propagation by seeds offers little promise as half of the progeny will be males and half females. In India, date palm cultivation has excellent scope around Indira Gandhi Canal in districts of Jaisalmer and Bikaner where enough heat summation units are available and precipitation is also minimal and thus date palm fruits are not spoiled[1]. Major constraint, however, for extending date palm cultivation is non-availability of offshoots and hence quick multiplication of offshoots through tissue culture technique offers great promise. Many attempts have been made to employ tissue culture technique for propagation of date palm[1-3]. Some investigators have reported success in producing date palm plantlets from embryogenic callus[4-6]. In this communication, results of techniques tried for induction of somatic embryos and plantlet production are presented.

MATERIALS AND METHODS

Young offshoots of date palm cv. Muscat were detached from the mother plant in February. The offshoots were dissected acropetally to expose shoot tips consisting of apical dome and soft young inner leaves. The shoot tips were trimmed to about 1 cm length and soaked for 1 h in an antioxidant solution of 150 mg/l citric acid and 200 mg/l ascorbic acid. These were then sterilized with 0.1% $HgCl_2$ for 5–7 min followed by 3–4 rinsings with sterilized distilled water and then transferred to 0.1% dicrysticin solution for 15 min and finally kept in sterilized distilled water for inoculation.

For induction of callus and embryogenesis the explants were cultured on three media referred as MMS-I, MMS-II and MMS-III. The composition of the media was as follows:

Medium MMS-I

Murashige and Skoog basal salts[7] plus the following (mg/l): α-naphthalene acetic acid (NAA), 1; 2,4-dichlorophenoxy acetic acid (2,4-D), 2.5: 6-benzyl aminopurine (BAP), 0.1 and polyvinyl pyrrolidone (PVP), 2000.

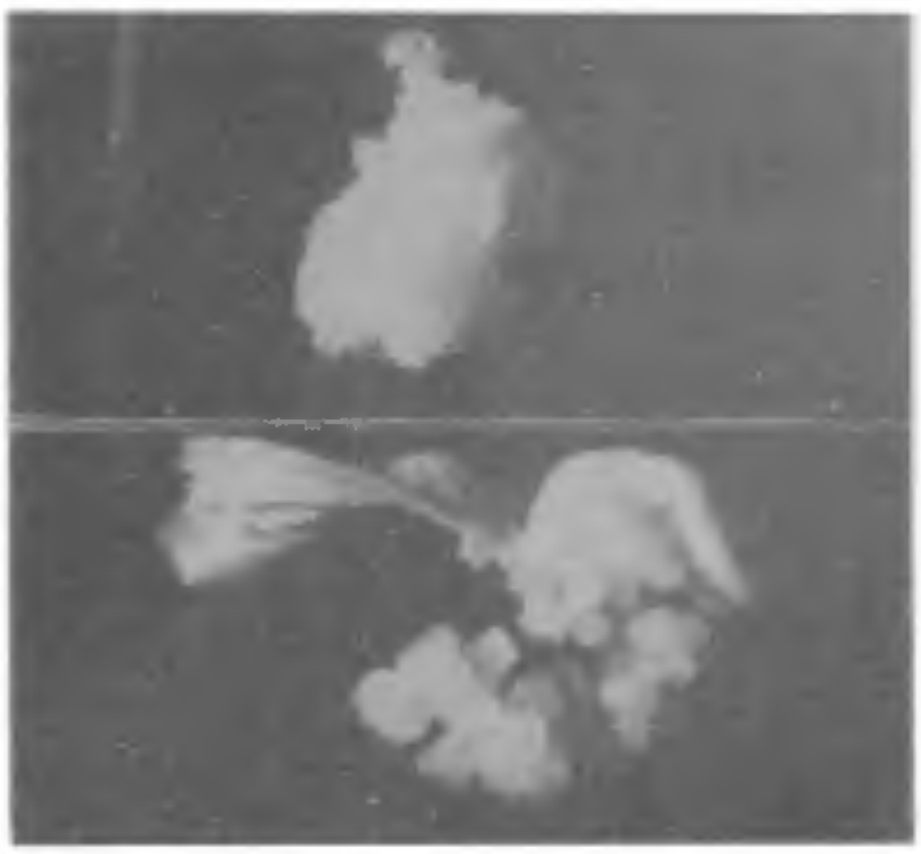
Medium MMS-II

Murashige and Skoog basal salts plus the following (mg/l): napthoxy acetic acid (NOA), 3; NAA, 5; indole acetic acid (IAA), 1; Kinetin, 0.1; BAP, 5; 2,4-D, 0.1 and PVP, 2000.

Medium MMS-III

Murashige and Skoog basal salts except for NaH_2PO_4 and KH_2PO_4 which were added @ 170 and 200 mg/l respectively plus the following (mg/l): Kinetin, 2; BAP, 5 and charcoal, 3000. NAA was included in the medium at 0, 0.1, 1, 10 and 100 mg/l concentrations.

The following constituents (mg/l) were common to all the three media: pyridoxine HCl, 1; thiamine HCl, 1; glycine, 2; nicotinic acid, 1; mesoinositol, 100; calcium pentothenate, 1; glutamine, 200, biotin, 0.01; adenine, 30; agar agar, 7000 and sucrose, 30000. The pH of media was adjusted to 5.7 before adding agar. Thirty ml of medium was dispensed in



Figures 1–7. **1.** Callus initiation from apical dome; **2.** Proliferation of embryogenic callus; **3.** Development of embryogenic nodules; **4.** Elongation and further development of embryos; **5.** Various stages of embryo germination; **6.** Secondary embryos on developing seedling, and **7.** Regenerated complete plantlet.

25 × 200 mm culture tubes or 125 ml Erlenmeyer flasks. After plugging with cotton, culture vessels were sterilized at 1.1 kg cm^2 pressure for 15 min. For callus initiation cultures were kept in dark and for embryogenesis a 16 h light period (10000 lux) was given. All the cultures were kept at 27 ± 2°C and subculturing was done at an interval of 25 days.

For induction of callus, the explants were initially cultured on media MMS-I. Half of the forty-days-old cultures were then transferred to medium MMS-II and remaining half were continued on medium MMS-I. For embryogenesis, eighty-days-old caulogenic explants were transferred to the medium MMS-III.

RESULTS AND DISCUSSION

Establishment of callus cultures

Initiation of callus was first recorded 40 days after the initial transfer of the explant. Subculturing of half of the explants on medium MMS-I induced 7.1% explants to produce callus. Transfer of remaining explants to medium MMS-II caused 14.2% explants to develop callus. Further, callus growth was better on medium MMS-II. The calli produced in both the media were friable and light yellow in colour (figures 1 and 2). Explants that failed to produce callus usually turned brown and died later.

Establishment of embryogenic cultures

In the beginning, transfer of caulogenic explant to medium MMS-III indicated continued growth of callus at 1, 10 and 100 mg/l NAA. The response was, however, more for higher concentrations of NAA (i.e. 10 and 100 mg/l). Following repeated subculturing, initiation of white nodular embryogenic callus was recorded in 0.1 and 1 mg/l NAA about 2 months after the transfer to MMS-III. The nodular white callus consisted of aggregates of small nodules (figure 3). The small nodular aggregates could be multiplied and further differentiated on fresh medium. Production of embryogenic nodules on friable callus was more at 0.1 mg/l NAA. Embryogenic nodules elongated on repeated subculturing and later on developed into plantlets by first emergence of roots and then shoots (figures 4, 5 and 7). Maturation of nodules and their further differentiation into plantlets was more at 0.1 mg/l NAA. Multiple shoots were also observed by induced secondary embryogenesis. Production of secondary embryos were observed either on primary embryos or on developing seedlings (figures 4 and 6). The growth and germination of nodules in medium having 1 mg/l NAA was considerably delayed. Calli, however, failed to respond for embryogenesis at 0, 10 and 100 mg/l NAA. Mater[3] also reported better development and differentiation of embryogenic nodules with 0.1 mg/l NAA. Sharma *et al*[8] on the other hand, reported production of plantlets in modified MS media completely devoid of growth regulators. Our investigations, however, indicated media MMS-III containing 0.1 mg/l NAA to be the best for production of multiple date palm plantlets.

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