EXPLANT AND CULTIVAR RESPONSE TO IN VITRO CLONAL PROPAGATION OF FEMALE DATEPALM (*Phoenix dactylifera* L.)

Neelam R. Yadav, Ram C. Yadav, V.K. Chowdhury and J.B. Chowdhury*

Department of Biotechnology and Molecular Biology
CCS Haryana Agricultrural University, Hisar -125004, India
*** Present Address: Vice Chancellor, G.B. Pant University of Agriculture and Technology, Pant Nagar, Uttarakhand, India.**

ABSTRACT

In vitro clonal propagation of female date palm cultivars Khadrawy, Medjool, and Halawy was carried out using shoot tip explants and axillary bud explants. The explants were cultured on MS Modified medium containing NaH2PO 4 170 mg/l, 2,4-D 100 mg/l, 2iP Smg/.l, Adenine 80 mg/l, Activated Charcoal 3% (w/v) and Agar 0.8% (w/v). The initiated calli were transferred to hormone free MS medium to induce somatic embryogenesis. The developing somatic embryos germinated on the same medium and developed into plantlets. Rooting was enhanced using Met in combination with NAA. The plantlets were established in the Research area of CCS Haryana Agricultural University, Hisar. The regenerated plants flowered and set fruits earlier than the control plants. To avoid dependence on offshoots, a simple regeneration system has also been developed using leaf bases of regenerated plants while these are still in culture flasks. The somatic embryogenesis induced in callus on solid medium or in suspension led to somatic embryo formation and plant formation.

Additional Index words:

Date palm, callus, somatic embryogenesis, regeneration, shoot formation

INTRODUCTION

The date palm [*Phoenix dactylifera* L. (2n=36)] is an important horticultural crop grown mainly in Middle East. Its dioecious nature makes seed progenies, heterogenous resulting about half of the progeny

as males. Datepalms are traditionally propagated by the use of offshoots which are produced on the trunk of parent plant.

This provides a limited clonal propagation as the number of offshoots produced are few in number and their cost is high (Branton and Black, 1989). North- Western belt of India is a potential datepalm growing area comprising 'Haryana, Gujrat and Rajasthan. Clonal propagation of desired cultivars using in vitro approaches has a 2 commercial value in mass multiplication of dates (Tisserat, 1982; Zaid and Tisserat, 1984; Sharma et at., 1988; Yadav et at., 1998). Somatic embryogenesis is an efficient and reproducible procedure for obtaining homogeneity in propagated date plants (Tisserat, 1982; Zaid and Tisserat, 1984; Sharma et at., 1988; Yadav et at., 1998).

The present paper describes the somatic embryogensis in four datepalm cultivars viz. Medjool, Khadrawy and Halawy.

MATERIAL AND METHODS

Plant Material

Plant material (offshoots) was obtained from Horticulture Farm Area ofCCS Haryana Agricultural University, Hisar, India.

Female offshoots of Phoenix dactylifera cvs. Medjool, Khadvawy and Halawy were dug out and were dissected acropetally and other mature leaves were removed.

The shoot tips and axillary buds were collected in water containing antioxidant citric acid 200 mg/l (w/v). The explants were thoroughly washed in running tap water.

These were treated with Tween-20 containing water and shaken several times followed by rinsing. Finally the explants were surface sterilized using 0.1% mercuric chloride for 15 minutes in laminar air flow station. The explants were rinsed 3 times with, sterilized double distilled water. Shoot tip~ were trimmed 0.5 -1.0 cm size and cut into several (20-25) pieces longitudinally. The explants were cultured on MS medium (Murashige and Skoog, 1962) containing loo mg/l 2,4-D, Glycine 2 mg/l, sodium dihydrogen orthophophate 170 mg/l, Adenine 80 mg/l, 2iP 5 mg/l activated charcoal 0.3% (w/v), sucrose 3.0% (w/v) and Agar 0.8% (w/v).(Table1). pH of medium was adjusted to 5.8. The cultured explants

were incubated at 25:1:2°C under a photoperiod of 16 hr light (2000 Lux) and 8 hour dark.

Table 1 Composition of media used for date palm somatic embryogenesis

Callus Induction	MS Basal medium + 2,4-D 100 mg/1 + 2ip 3-5 mg/1 + Adenine 80 mg/l Activated Charcoal 0.3% (w/v) + Agar Agar 0.8% (w/v).
Embryogenic callus Multiplication Medium	MS Basal medium + NaH2PO4.2H2O 170 mg/1 + Activated charcoal 0.3% (w/v) + Agar Agar 0.8% (w/v).
Rooting Medium	MS Basal medium + NAA 0.1 mg/1 +Activated charcoal 0.3% (w/v) + Agar Agar 0.8%(w/v).
Suspension Culture Medium	MS Basal medium + NAA 0.1 mg/l.

The shoot tip cultures were subcultured every 4-5 weeks and were kept in the dark. For induction of somatic embryogenesis, shoot tip cultures were transferred to hormone free MS medium (only 2,4-D and 2iP were omitted) and cultures were kept under light (16 hr. light / 8 hr. dark) at 25::1:2°C.

The embryogenic cell suspensions were initiated by transferring about one gram of callus to 40 ml MS liquid medium containing 0.1 mg/1 NAA. The cultures were shaken on gyratory shaker at 100 rpm under the diffused light. The suspension was plated at low density on MS medium without growth regulators as in case of embryogenic callus cultures and were subjected to light conditions as described above.

Somatic embryos developed and germinated on the same medium

RESULTS AND DISCUSSION

The cultures were observed periodically for bacterial and fungal contamination and it could be reduced suitably to 2-3 per cent by following the sterilizing procedure described in material and methods. Some workers have recommended dipping of explants in sterilizing agent

overnight which was not found necessary in the present study (Kuwari et al., 1998). Initiation of callus was observed from primordial leaves in 3-4 weeks. The callus was creamish white and friable in texture (Fig.1). The three cultivars showed variable response in terms of days to callus induction and percent response. Cultivar medjool was found the best responding followed by Khadrawy cultivar. Halawy cultivar showed poor response than the two other cultivars (Table 2). In all the cultivars, explants enlarged in size and initiated callus while other explants turned brown and died eventually. After a subculture on the callus induction medium, the calli were transferred to hormone free MS medium with activated charcoal for the induction of somatic embryogenesis under light conditions. The growth and development of nodular structures took place on this medium and somatic embryos were visible within 5-6 weeks in all the three cultivars. Bhaskaran and Smith (1992) observed that callus formation took minimum of six months. However, we found in this study that somatic embryogenesis was achieved within 3-4 months from the time of initial culture. Callus growth a~d embryogenesis was very fast in cv. Medjool. Cell multiplication and formation of somatic embryogenesis took place on the same medium and did not require a rooting medium for embryo germination.

Table 2: Response of different explants for callus induction and embryogenesis in different cultures of datepalm:

Explants	Khadrawy		Medjool		Halawy	
	a	b	a	b	a	b
Axillary buds	33-3	40-45	22-7	50-60	20.0	30-40
Shoot apices	60.0	75-80	58.6	80-90	65.0	70-80
Leaf bases	53.3	50-60	48.6	70-80	--	--
Roots	40.0	30-40	44.1	50-60	--	--

a: % response
b: % embryogenic callus

Plantlets and older embryos were removed from time to time to allow further embryoid development (Fig. 2). The plantlets with roots were taken out and roots were trimmed before their transfer to the rooting medium. Replacement of NAA with Met (Multiple effect trizole; 0.25 mg/l) produced thicker roots which were more in number. Plants for rooting were transferred to 250 ml flasks for better development.

Out of several explants cultured, shoot tip explants showed better response than any other explant in the three cultivars under study. The response of leaf bases and primary roots was very poor for callus

induction. Secondary roots produced calli but in lower frequency. Cultures once established showed fairly good potential for plant regeneration for three years. Serial subcultrue at 3-4 weeks interval enabled embryogenic mass to retain the morphogenetic potential. An average of 200:J::20 embryos were present in one gram fresh weight of embryogenic mass. The embryo yield started declining after three years and it was reduced to 70-80 embryos in 4 year old cultures. Retention of embryogenic potential for 36 months has also been reported in Eucalyptus citriodora by Muralidharan et al. (1989). Tisserat (1982, 1984) attributed this decline to lower embryo germination while Sharma et al. (1988) suggested that the cause may be the vitrification of cultures.

Considering decline in embryogenesis and regeneration potential with age, we used in vitro regenerated leaf bases and root explants (Fig. 3,4) to initiate the callus cultures. Our results show that these explants can be effectively used for establishing new cultures for clonal propagation. This will allow a non-destructive harvest of explants and to minimize avoidable use of costly offshoots for initiating fresh callus cultures to economize the cost of micropropagation.

Use of suspension culture for a short period (3-4 weeks) can help further in synchronization of somatic embryos and to increased the production of plantlets.

The suspension was plated in low density on hormone free MS medium (Yadav et at., 1998). The cells were actively dividing, densely cytoplasmic with starch grains (Fig.5). Different embryogenic stages like globular to torpedo were found in the suspension culture (Fig.6). One round of suspension culture makes system efficient for embryo germination and plant regeneration and may help in bringing automation in datepalm propagation. This may be due to the fact that suspended embryos ensure uniform germination ofplantlets (Letouze et al.,1998) and complete plant formation (Fig.7).

Adventitons root formation and plant transfer to soil was fairly successful using procedure developed in our laboratory (Shrama et at., 1990).Plants acclimatized in the greenhouse were transferred to the farm area of the CCS Haryana Agricultural University, Hisar. The plants regenerated from cultivar rnedjool showed first flowering in the fourth year and bore fruits (Fig.8)

During the last several years, this laboratory has developed datepalm clonal propagation through somatic embryogenesis. The regeneration system

developed could be exploited for improving this fruit crop through genetic engineering.

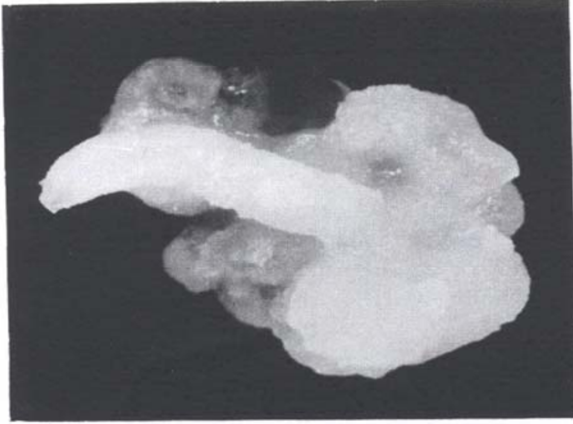
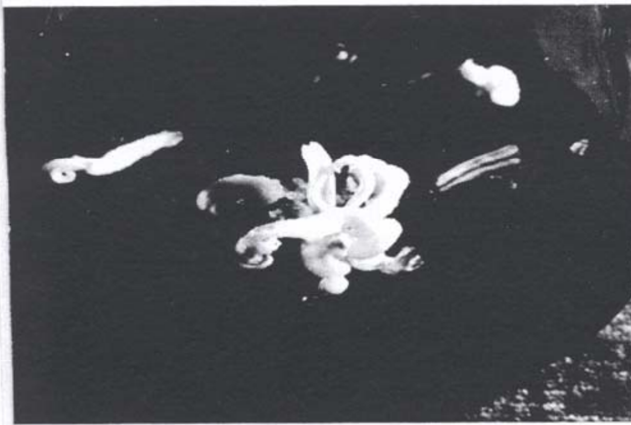


Fig. 1

Fig. 2



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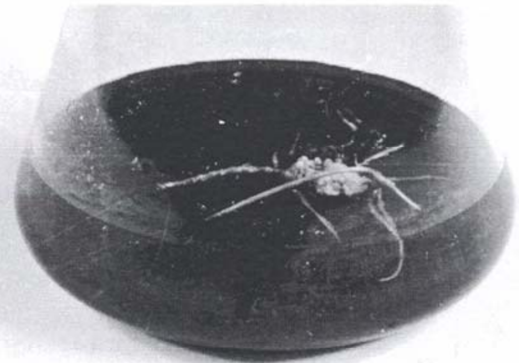
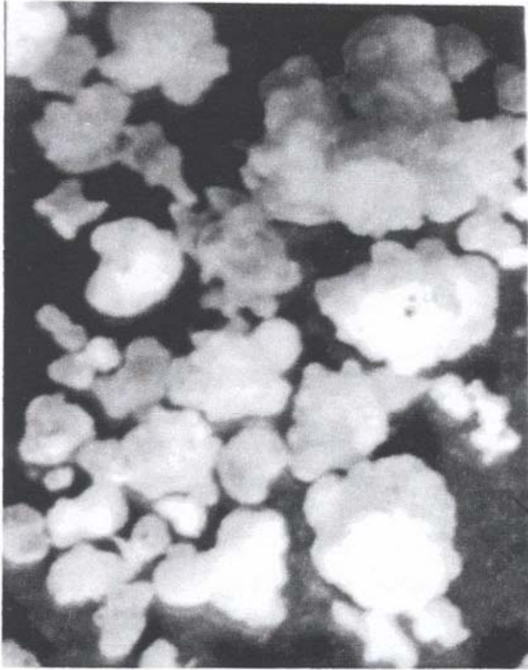


Fig. 4

Fig.l: Callus formation in cultured explants ofPhenix dactylifera L.
Fig.2: Different stages of somatic embryos in Phoenix dactylifera L.
Fig.3: Callus induction in cultured leafbases.
Fig.4 Callus induction in cultured roots.

496



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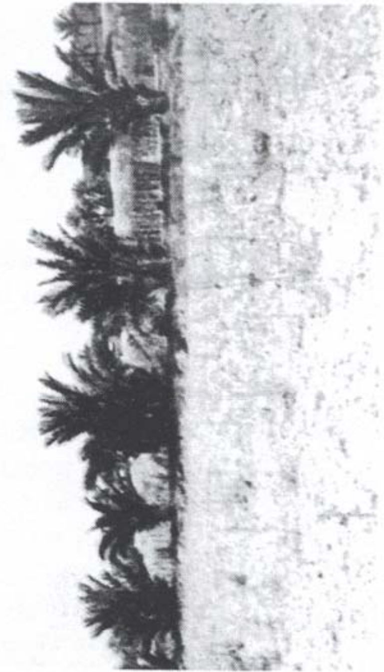

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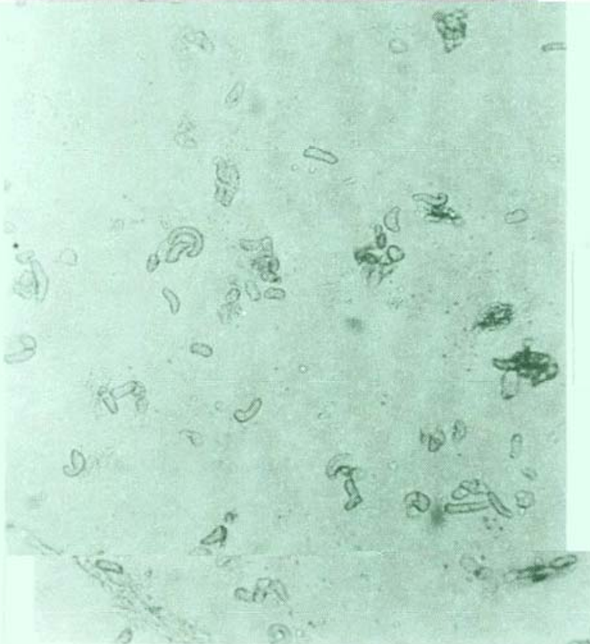

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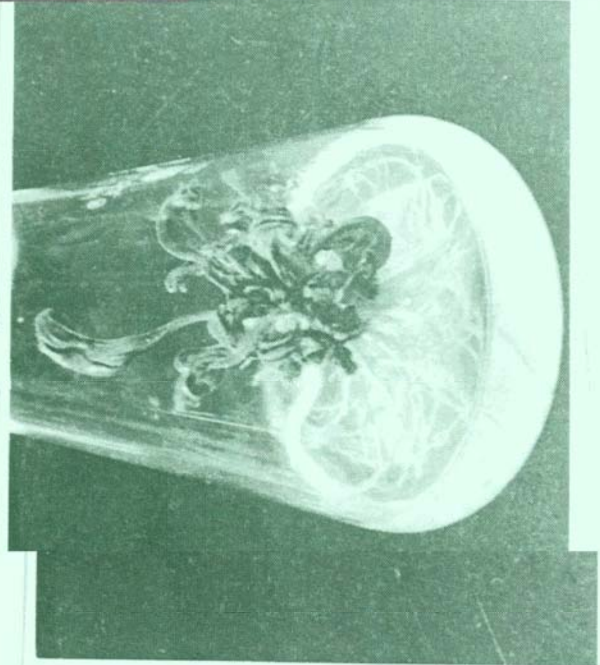

Fig. 7

Fig.5: Suspension cells
Fig,6: Development of somatic embryos in suspension culture.
Fig.7: Plant reneration
Fig.8: A row of regenerated plants in farm area.

497

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