In Vitro Multiplication Of Date Palm

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ABSTRACT

In vitro multiplication of three popular Pakistan cultivars was studied. Cultivars tested were Zaidi, Hussain, and Asil. Shoot tips of 4-6 mm excised from field grown suckers were cultured on M.S medium supplemented with IBA and BAP. After three weeks these cultures were shifted on multiplication medium. The multiplication medium was M.S basal with different concentrations of TDZ, 2,iP. Maximum multiplication was achieved after six weeks when medium contained 0.5 mg/l TDZ 1.0 mg/l 2, iP and was found genotypic dependent.

KEY WORDS: *In vitro* multiplication, date palm and Thidiazuran (TDZ).

INTRODUCTION

Date palm being dioecious crop is conventionally propagated through its offshoots or suckers. Propagation through offshoots is a slow & laborious because only a limited number of suckers or offshoots are produced from date palm tree during its life cycle. Commercial scale production of superior cultivars of date palm is therefore in the verge of diminishing due to slow asexual propagation, which is the only mean of propagation for date palm. Rapid clonal propagation of date palm on mass scale is a pre-requisite for commercial production.

Date palm is considered as one of the most important cash crop in the world especially in Middle East. About 90% of the total world production is produced from this region. Luckily the Pakistan is blessed with multiclimatic environmental conditions & date palm is also thrive well in different parts of Pakistan.

AREA & PRODUCTION OF DATES IN PAKISTAN

Year	Punjab	Sindh	Balochistan	NWFP	Pakistan
		(AREA 000 HECTARES)			
1995-96	11.1	19.7	42.2	0.9	73.9
1996-97	11.1	20.1	42.4	0.9	74.5
1997-98	11.1	20.6	42.4	1.0	75.1
1998-99	11.1	20.5	42.6	1.0	75.5
		(PRODUCTION 000 TONES)			
1995-96	91.5	31.5	403.6	5.9	532.5
1996-97	92.2	32.1	404.1	6.0	534.5
1997-98	93.7	34.0	403.5	6.3	537.5
1998-99	95.4	215.0	404.3	6.5	721.6

SOURCE: AGRICULTURAL STATISTICS OF PAKISTAN 1999-00

In all four provinces of Pakistan date palm is grown and peoples are getting economic advantages from its cultivation and production. The Baluchistan province is major contributor in date palm production followed by Sindh, Punjab and N.W.F.P. respectively. The area under date palm cultivation is stagnent due to unavailability of planting material and Pakistan is deprived of its actual potential. The tissue culture technology has potential to produce maximum number of plants in limited time and space which are true to type and agronomically equal or superior to conventionally propagated plants. A numbers of reports so for has been published in this regard (Tisserat 1979, 1981, 1984, Zaid & Tisserat 1983, Sharma et al., 1984, 1986, Matter 1986. Hussian et al., 1995, Quraishi et al. 1997, Veramendi & Navarro 1996, 1997. Daguin and Letouze 1992). Tissue culture technologies are now extensively used as a biological tool for clonal propagation, disease elimination & mass propagation of several horticulture crops and as well for date palm..

This paper describes the practical application of tissue culture technology for *in vitro* multiplication of date palm cv. Hussaini, Zaidi & Asil, the famous varieties in Pakistan.

Methodology

Three different varieties were taken from farmers fields of D.I .Khan. NWFP Pakistan for tissue culture responses. The suckers of the age 3-4 years were detached from mother date palm trees. Apical buds of (6-8 mm)

were excised under aseptic conditions and soaked in the antioxidant solution (100mg/l ascorbic acid & 150 mg/l citric acid) for over night.

Explants were surface sterilized with commercial bleach solution (containing 50% of 5.25% NaOCl) for 30 min containing few drops of Tween-20. Then explants were washed three times continuously with sterilize distilled water for 20 minutes each.. Final trimming of the explant was administered to get a desirable size of shoot tips i.e. (4-6 mm) after pealing off surrounding leaves & tissue.

Basal medium:

Murashige & Skoog (M.S., 1962) Micro + macro nutrient + 20 mg/l adenine sulphate .3g/l activated charcoal, myoinositol 100 mg/l & thiamine HCl 0.4 mg/l.

The basal medium used for shoot initiation was supplemented with IBA (0.1-6.0 mg/l) and BAP (1.0 mg/l) + 30 g/l sucrose and medium was solidified with 2 g/l gelrite.

Cultures were kept under complete darkness at 250oC $\pm$ 2oC. After completion of shoot initiation stage the explants wee subjected to the multiplication media containing 2iP 1.0 mg/l & TDZ. 0.1mg/l – 0.5 mg/l. (Table-3).

RESULTS & DISCUSSION

As the suckers were taken from field grown plants contamination rate was high (40%). Shoot tips is most appropriate explant used for date palm *in vitro* multiplication (Tisserat, 1979, 1984, Zaid & Tisserat, 1983, Reuveni 1979, Matter, 1986, Omar, 1988, Kacker et al. 1989, Showky & Mahmoud 1998). Therefore in this study shoot tips of 4-6 mm were used for culture initiation. A number of media were tested, but M.S basal medium supplemented with IBA 4.0 mg/l BAP @ 1.0 mg/l showed maximum culture initiation (Table 1). The color of tissues changed from creamy white to whitish due to incubation in the dark. The response was quite fast as compared to the previous reports in which NAA and 2,ip were used for culture initiation (Hussain et al., 1995, Quraishi et al., 1997, 1999 & Rashid et al., 1991 and 1994). Explants started growth after one week of incubation and first leaf primordia opened. After three weeks the size of explant increased from 6mm – 15 mm. Initiation response was also genotypic dependent as Asil showed maximum response (60%) followed by Hussaini

(25%) and Zaidi (15%) respectively as given in table –1, similar reports are given by (Beauchesne 1982, Veramendi & Navarro, 1996,1997). When explants showed growth and enlargement in size (after 3-4 weeks), these were shifted on multiplication media. Multiplication medium was M.S. basal supplemented with TDZ and 2,ip at different concentrations (table 2). To control the phenolic secretions, explants were treated with antioxidants prior to culturing and AdSO4 and activated charcoal was added in the medium.

After shifting on multiplication media cultures were kept under 16 hr photo period (2000 lux). The color of explant changed from whitish to green due to light after two weeks. Proliferation of axillary bud started after four weeks of culturing. After eight weeks, 10-15 shoots were formed from single explant. Maximum multiplication was achieved when media contained 0.5mg/l TDZ (Thiodiazuran) and 1.0mg/l 2,ip. Again multiplication rate was also genotypic dependent. (Table 2). Cultivar Asil showed the maximum number of shoots followed by Hussaini and Zaidi respectively. Similar results were observed during initiation of cultures. Results show that cultures better initiated respond better for multiplication. It was observed *in-vivo* that behavior of date palm is also reflected *in vitro* e.g. cultivars producing more suckers in the field, show higher rate of multiplication *in vitro* (Krikorian & Cronauer, 1984). TDZ plays an important role in multiplication due to enhancing the horizontal growth of cultures rather than vertical.

Conclusion

In vitro multiplication behaviors of date palm are genotypic dependent TDZ Plays an important role in multiplication of date palm.

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Table 1. Shoot initiation response of asil, zaidi & hussaini on different media

B.M.	IBA(mg/l)	BAP(mg/l)	ASIL	HUSSAINI	ZAIDI
BM-0	0.0	0.0	0.0	0.0	0.0
BMI-1	0-0	1.0	0.0	0.0	0.0
BMI-2	0.1	1.0	2.0	0.0	0.0
BMI-3	0.5	1.0	2.0	1.0	0.0
BMI-4	1.0	1.0	15.0	4.0	1.0
BMI-5	2.0	1.0	33.0	8.0	5.0
BMI-6	4.0	1.0	60.0	25.0	15.0
BMI-7	6.0	1.0	55.0	25.0	13.0

Table 2. *In vitro* shoot multiplication in date palm. (basal media +)

2ip (mg/l)	TDZ (mg/l)	Asil	Hussaini	Zaidi
0.0	0.0	1	1	1
1.0	0.1	3-4	1-2	1-2
1.0	0.2	5-8	1-2	1-2
1.0	0.3	5-11	2-3	1-4
1.0	0.4	10-12	2-3	2-4
1.0	0.5	10-15*	3-5*	2-7*
1.0	0.6	10-13	3-4	3-5
1.0	0.7	10-13	2-3	2-5

* Maximum multiplication

Results are average of 20 replicates.