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SOMATIC EMBRYONESIS AND SUSPENSION CULTURES FROM MATURE ZYGOTIC EMBRYOS OF DATE PALM *(Phoenix dactylifera L.)*

H.Saka

INRAA, laboratoire de Physiologie Végétale, CRP mahdi Boualem, BP37, Baraki, Alger.

Abstract : *Excised embryos of Deglet Nour, Tagaza and Takerboucht showed a potential of embryogenesis . The picloram, dicamba and 2,4-D were able to induce embryogenic calli. Genotypic differences in embryogenic responses is observed. Friable calli was used to initiate the cell suspension. All the concentrations of the various growth regulators tested allowed an increase in cell fresh weight. as well as the cell suspension growth. However, regeneration from cell suspension cultures was very low .*

Key words *: date palm, picloram, dicamba, 2,4-D, callus, embryogenesis, cell suspension, regeneration.*

Résumé: *Les embryons excisés de la Deglet Nour, Takerboucht et Tagaza ont initiés du cals embryogènes et ce quelque soit les substances de croissance utilisées. Des différences génotypiques sont cependant notées . Le cal globulaire et friable est utilisé pour obtenir des suspensions cellulaires. Les différentes substances de croissance testées ont permis l'établissement et la prolifération des cultures cellulaires . Le taux de germination obtenu qui devait démontrer la totipotencie des cellules s'est avéré faible.*

Mots clé *: Palmier dattier, picloram, dicamba, 2,4-d, cals embryogenése, Suspension cellulaire, régénération.*

INTRODUCTION

Date palm *(Phoenix dactylifera L.)* is hundred by a disease syndrome known as Bayoud , primarily caused by *Fusarium oxysporum* f.sp. albedinis which has already killed 3 millions of trees (DJERBI ,1990) . The most succesfull strategy for Fusarium wilt control in other crops has been the development of resistants varieties (FROMM et al,1986 ; DAN et STEPHEN,1991).The use of somatic cell genetic techniques, such as protoplast regeneration, cell hybridization and cell selection exhibiting resistance to applied stress,has been widely recognized as a tool to improve species (KARP et al ,1990). To explore the ability to grow plant cells in isolation an efficient regeneration system is necessary to genetically transform species . Although mocotyledonous species have been shown to be recalcitrant in in vitro culture, plants can be regenerated from embryogenic cell suspensions, and protoplasts from number of species (VASIL,1887). Few reports dealing with regeneration of date palm using cell suspension culture are available (DAGUIN et LETOUZE,1986) even if numerous papers have been published that described somatic embryogenesis regeneration in date palm using as various explant sources (BENBADISS et al, 1977 ; DRIRA,1984 ; TISSERAT,1979 ; SCARNEC,1992).

The efficiency of tissus culture for competitive cell line selection is hampered by the loss of cell totipotency in a relatively short time . The cell proliferation in prolonged time and the maintainance of cell totipotency is an essential step for cell manipulation. We investigated the establishment of embryogenic suspension cultures of three date palm genotypes . We describe here the first attempt of cell culture calli and maintainance of regenerable embryogenic cultures from zygotic embryos of date palm .

MATERIAL AND METHODS

Three date palm genotypes were used in this experiment : Deglet nour and Tagaza very sensitive varieties to F.o.a., and Takerboucht, a resistant cultivar.

Seeds were surface sterilized by immersion in a fongicide solution (BENLATE ,1g/l) followed by three rinses in sterile distilled water. Seeds were also acid–scarified in 15% H2So4, dipped for a 30 minutes in 1,5% sodium hypochlorite solution and rinsed again .

Callus initiation and maintainance

Excised embryos were placed on a modified MS medium (REYNOLDS et MURASHIGUE,1978) to induce embryogenic calli. This medium is supplemented with 2,4-D, picloram and dicamba plant growth regulators at various concentrations :2,5mg/l, 12,5 mg/l, 25mg/l, and 100mg/l. The pH is ajusted to 5.6 before sterilization. Five embryos each were cultured in Petri dishes at 27+- 2°C. Tewnty replications is done for each treatment. Twenty embryos are placed on germination medium as control to assesss the viability of the embryos excised.

After 18 to 24 weeks of culture, seeds producing compact or friable callus were transferred in a 250 ml flasks in the same basal medium with lower growth regulators were tested to sustain further growth.

Initiation of cell suspension

Suspension cultures were derived by transfer of small portions of friable calli, 1 to 0,5 g/l, to a liquid medium. The cultures were grown on the proliferation media ,in dark at 120 rpm. Cell suspension cultures and cell aggregates were subcultured every 2 weeks at an inoculation rate of 20 to 25%(V/V). At each transfer, biomass is weighed and residual calli removed until a suspension is obtained. Different concentations of growth regulators (12,5 and 2,5 mg/l) were tested to monitor proliferation .

Regeneration

To control the totipotency of the cells , nodules transferred to liquid basal medium for two or four cycles are plated on Petri dishes containing the same medium but solid, devoid of growth regulators and under a 16 hour photoperiod.The suspension is plated and the germination of the embryos noted.

Results – Discussion

Callus initiation

At the end of 6 to 9 month culture, only the calli containing smooth globular structures were recorded as embryogenic callus .Those having a nodular appearence correspond to non embryogenic calli which develop roots only. In all three genotypes formation of nodular and friable callus is obtained. No apparent differences in the callusing frequency were observed between genotypes, and induction media except for the medium containing 100 mg/l of 2,4-D. With this medium no callus was formed and all the embryos germinated .

Table I: Effect of growth regulators on embryogenic calli of three date palm genotypes .
The data are the means of 10 replicates

		DN		TAK		TAG	
Growth Regulator (mg/L)		(1)	(2)	(1)	(2)	(1)	(2)
Pic	2,5	32	32	25	25	32	32
	12,5	58	58	21	21	28	28
Dic	2,5	28	28	50	50	25	25
	12,5	52	52	42	42	28	28
2,4-D	2,5	32	32	25	25	32	32
	12,5	21	21	12	12	25	25
	25	31	31	28	28	25	25
	100	0		0		0	

1-number of embryos showing calli after 6 to 9 months culture.
2-frequency of embryogenic calli (%) with glodular aspect.

Table II: Embryogenesis and regeneration of 6 to 9 –month old primary calli from embryos of three date palm genotypes

		Frequency of calli producting embryos %		
Growth Regulator (mg/1)		DN	TAK	TAG
Pic	2,5	32	15	25
	12,5	24	19	28
Dic	2,5	15	22	25
	12,5	12	36	27
2,4-D	2,5	22	12	21
	12,5	23	32	24
	25	25	28	28
	100	0	0	0

Newly initiated calli consisted of a mixture of morphological distinct tissue as observed in others species (CAI et BUTLER, 1990).this mixture showed a hard ,opaque white callus, a soft watery brownish callus and a nodular hard callus. In concordance with differents reports (TISSERAT, 1978 ; REYNOLDS, 1978 ; SAKA et al, 1997), embryogenic calli have a compact or friable texture, globular shape and opaque white color. Cultures of Tak produce large amounts of phenolic compounds that darkened the medium and the culture underwent necrosis. Decreasing the time between initiation and subculture increased the rate of survival. Genotypic differences in embryogenic responses is observed . The three cultivars tested ranged from 12 to 32% as shown in table1. DN genotype reacted better whith picloram while Tak has higher callus percentage on dicamba medium and Tag reacted invariably to all the growth regulators. However, the concentrations of the various growth regulators tested did not have an effect except for 100 mg/l of 2,4-D. The data suggest that optimal embryo induction medium with regard to auxin type and concentration has to be determined for each genotype.

Genotypic effects have been previously reported in various species (MALMERG ,1979 ; LAZZERI et al,1987) . Since it takes at least 4 months to obtain plants we followed until germination (table II). The germination rate is identical for all types of growth regulators and tested concentrations.

Cell suspension cultures

Attempt is made to adapt the regeneration capability for suspension culture of one genotype DN . Friable calli was used to initiate the cell suspension. From the 5 day in a stirred medium, embryogenic masses started to detach from mother callus tissue . The diameter of the particles varied from 1 to 2 mm. For each initial callus ranging from 1 to 0,5 g/l ,a final weight is obtained. The weight increased 4 times after 4 weeks. All the concentrations of the various growth regulators tested allowed an increase in cell fresh weight (Fig 1.). The best results are obtained with 5 mg/l of picloram . This auxin like has been used with success to maintain callus proliferation and totipotency in sugar cane and date palm (FITCH et MOORE ,1990;

OMAR et NOVAK,1990). Concentrations of 5mg/l and 12,5mg/l of 2,4-D induced cell growth of date palm . This findings is in desagreement with TOUCHET et al (1991) who observed that concentrations of 25 and 50 mg/l of 2,-4D were insufficient to sustain cell growth of oil palm .The auxine – like dicamba showed a cell growth identical to 2,4-D. From 4 to 16 subcultures , the cells sustained growth. Beyond 16 subcultures which correspond to 32 weeks , the cell growth started to decline for all the growth regulators tested

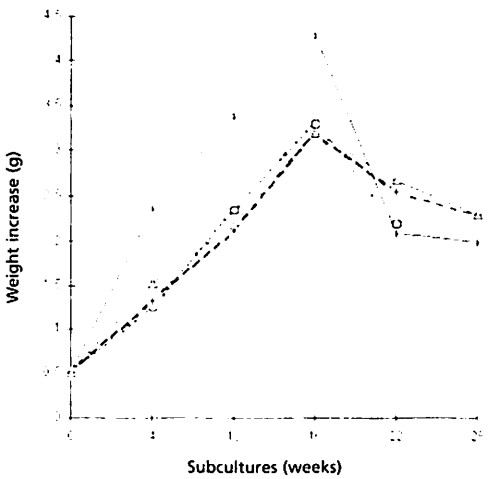

Figure n°1: Weight increase of cells after 28 subcultures for the various growth regulators tested with genotype D.N

Regeneration from cell suspension

The cells were plated at 6 to 22 subcultures on germination medim devoid of growth regulators. The germination occured after 12 weeks and not on all the plates and only a total of 10% of the cells plated showed germination (data not shown). This long time and low germination could be due to the prolonged stay in media with growth regulators and the remaining effect of the growth substances as stated in TOUCHET et al (1991). The cell suspensions contained single cells, cell aggregates, and heavily vitrified structures that could lead to inconsistent regeneration. Regeneration in many monocotyledonous species has been shown to occur from compact callus that can be maintained in long–term cultures and friability results in non-regenerable cultures (MORRISH et al, 1987 ; HUTCHINSON et al, 1997).

CONCLUSION

Excised embryos showed a potential of embryogenesis as in various other species. The picloram and dicamba as well as 2,4-D were able to induce embryogenic calli. Genotypic differences in embryogenic responses is observed which is not specific to date palm. Friable calli was used to initiate the cell suspension. All the concentrations of the various growth regulators tested allowed an increase in cell fresh weight. Dicamba as well picloram auxins-like allowed the initiation of embryogenic calli as well as the cell suspension growth. However, regeneration from cell suspension cultures was very low and could be due to the remaining effect of the growth regulators tested or the heterogeneity of the calli. Histological studies should be performed to assess the totipotency of the cells and improve the method. This paper reported for the first time long-term cell suspension cultures of date palm. It is important to master the cell culture of date palm since information on cultural conditions favoring high frequency maintainance and regeneration through cell cultures is a prerequisite to accelerate application of non conventionnal techniques to date palm improvment.

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