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Conference Paper · March 2021

DOI: 10.17660/ActaHortic.2010.882.87.

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Investigation of Somatic Embryogenesis for In Vitro Cultures of Date Palm (*Phoenix dactylifera* L.)

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Keywords: *Phoenix dactylifera*, in vitro culture, growth regulators, sucrose

Abbreviations: 2,4-D: 2,4-Dichlorophenoxyacetic acid; 2-ip, 6.γ.γ.dimethylallylamino purine; NAA: naphthalene acetic acid.

Abstract

Date palm is increasingly recognised as an important crop in northern Nigeria. Agriculturally, it can sustainably generate incomes for farmers to take families out of the poverty line while at the same time it can be used as a tree crop to check desertification. To achieve these expectations, a national date palm development program involving planting material production and date palm agronomy is currently underway. The somatic embryogenesis method is being currently adopted to produce planting materials. Apical meristem and leaf explants were used to initiate in vitro cultures. A range of media and media conditions were tested and different media induced callus. These media were supplemented with different growth regulators, sucrose at different concentrations and nitrogen in the growth medium. NAA and 2,4-D provoked callus production. Callus could be produced at various sucrose concentration levels but 30 g/L was optimum. Callus generation potential was best from apical meristems, followed by leaf bases and leaves. Similarly different media also induced somatic embryos but the most reliable medium was the one containing 0.05 mg/L NAA and 1 mg/L 2-ip. The somatic embryos readily developed into shoots which could be rooted in NAA and sucrose (45-90 g/L). Some plants are in the nursery while some have been planted in the field undergoing observation.

INTRODUCTION

Date palm is important in the dry regions of northern Nigeria. It has been grown in the region for centuries and is adapted to the ecology of the region. It is an important component of the farming system in the area where it is planted in mixed cropping with arable crops such as maize, cowpea, sorghum, ground nut and millet. It is an important source of income as a few date palm trees provide significant cash earnings while the food crops provide for the dietary needs of the family. Current concerns on enhancing farmers' livelihoods within the context of the millennium development goals and checking desert encroachment in the area have renewed interest in date palm as one of the candidate crops with multiplier benefits that can be used quickly to ameliorate the living standards of the rural poor and at the same time, improve the environment. This project therefore fits into a national date palm development program for Nigeria with the goal of providing planting materials for distribution to farmers. Other components of the program include development of fruit processing techniques and marketing strategies.

In vitro propagation is very important as an option to complement conventional methods of generating planting materials. It is a viable method of producing large numbers of date palm planting materials. The advantages offered by in vitro multiplication include the avenue it provides of multiplying a single productive individual plant. For date palm which is dioecious, this feature is extremely useful. Many published findings are available on date palm in vitro multiplication through somatic embryogenesis

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(Tisserat, 1979; Sharma et al., 1984; Daquin and Letouze, 1988; Letouze et al., 2000) as well as by direct organogenesis (Rhiss et al., 1979; Beauchesne, 1982). However, the importance of the crop, the continuous expansion of cultivation areas into new communities and the preference sometimes, for particular local varieties make it necessary to evolve and refine working protocols. Our current efforts on date palm in vitro multiplication use the somatic embryogenesis method.

Different factors affect the performance of cultures in vitro. These factors include the type and concentration of plant growth regulators added to the basal medium as well as the interaction between auxins and cytokinins (Rao et al., 1973; George and Sherrington, 1984). Other workers have observed however, that some morphogenetic responses were not influenced by growth regulators only, but that growth regulators could interact with media components such as sucrose and nitrogen (Welander, 1976; Wetherall and Dougall, 1976; Jeannin et al., 1995; Ahn et al., 1996). The mechanism of auxin and cytokinin mediated morphogenesis has been the subject of extensive studies using different tissues and organs of various plant species (Murashige, 1974; Paranjothy, 1984; Lioseau et al., 1995). In addition other factors such as explants source and culture conditions affect the performance of in vitro cultures.

This work was therefore aimed at investigating the effects of some of these conditions on *Phoenix dactylifera* leaf explants cultured in vitro.

MATERIALS AND METHODS

Plant Materials

Offshoots of 2-3 years were harvested from mother palms and used as sources of explants for culture initiation. The different explants used were shoot tips, leaf bases and immature leaves.

Media

The basal medium for callus induction was that of Murashige and Skoog (1962) supplemented with 3% sucrose, 0.85% agar and 0.01-0.5% inositol. In addition, the following were also added: 0.002% aneurine hydrochloride and 0.3% activated charcoal. Different concentrations of NAA were initially tried for callus induction. From the preliminary findings, 100 mg/L NAA, or 100 mg/L 2,4-D and 3 mg/L 2-ip (Eke et al., 2005) was adopted for further work. This constituted the establishment medium. The pH of the medium was adjusted to 5.8. Explants were always incubated in the dark for callus induction while subcultures were done monthly.

Somatic embryo induction medium contained 0.05 mg/L NAA and 1 mg/L 2-ip while GA_3 was added at 2 mg/L for shoot elongation, retaining the same basal medium. Somatic embryos and shoots were cultured in 14 hours of light and10 hours of dark daily cycles. For root development, the MS basal medium used was supplemented with 30 g/L sucrose, 0.2 g/L glutamine and 0.15% activated charcoal as well as 0.05-0.1 mg/L NAA.

RESULTS

Callus could be initiated with a wide range of auxin concentrations although the time taken for both initial response and quantities produced varied significantly. From the initial trials on the effect of different auxin concentrations on callus formation, it was found that callus initiation and growth were best stimulated by a relatively high auxin concentration, 100 mg/L NAA or 2,4-D (Fig. 1). Further experiments were carried out in which the media were supplemented with NAA and one cytokinin at a time (Kinetin or 2-ip or BAP). The addition of cytokinin to the culture medium containing near optimal levels of NAA did not promote further noticeable growth at least in terms of quantity. It is conceivable however, that the addition of the cytokinin programmed the callus to cellular reorganisation thus opening the way for successful somatic embryo development. Date palm callus initiation is thus primarily under auxin control.

P. dactylifera callus could be induced at different sucrose concentrations (Fig. 2).

Callus fresh weight increased with sucrose concentration up to 0.1 M sucrose and then declined. The optimum sucrose concentration was found to be 30 g/L (Fig. 2) which is in agreement with Tisserat (1982). On the other hand, while nitrogen in the form of nitrate alone supported moderate callus formation and growth, ammonium alone was not very favourable for callus formation (Table 1). Both sources were needed by the explants for formation of appreciable quantities of callus. Synergy between the two sources of nitrogen is thus demonstrated. Sheat et al. (1959) reported similar effects explaining that NH_4^+ nitrogen can only serve as the sole source of nitrogen in a medium at a pH close to neutrality whereas the media used in this work had pH 5.8.

All three explants sources, meristems, leaf bases and immature leaves produced callus. In order of callogenesis potential, meristems were found to more readily form callus than leaf bases which in turn more readily formed callus than immature leaves. However, the ability to regenerate somatic embryos was much more enhanced in meristem derived callus when compared with the other two. Often, leaf derived callus was blocked at the callus stage and did not develop further. In general, callus could be obtained readily and reproducibly, usually at about the third subculture, from initiation. With more subcultures, the size of callus (Fig. 3a) grew. Initially, the callus appeared watery but the form of the callus became compact and globular over time with more subcultures.

It was possible to induce somatic embryo formation from the callus by using different media. Somatic embryos emerged reproducibly, reliably and uniformly on a medium that was supplemented with 0.05 mg/L NAA and 1 mg/L 2-ip (Al-Baiz et al., 2000). On the other hand, while hormone free medium also produced somatic embryos, this production was erratic. The somatic embryo (Fig. 3b) production could be either in the light or in the dark but normally the cultures are transferred to light once somatic embryos production started. The somatic embryos also developed into shoots in this medium. A number of shoots could grow up together which would be subsequently separated. The generation of multiple shoots (Fig. 3c) on agar solidified media have been recorded by other workers including Tisserat (1982) and Al-Baiz et al. (2000). As soon as the shoots were formed, they were transferred to a medium which contained 2 mg/L GA_3 to produce well formed shoots. This process took place in the presence of light (14 hours light and 10 hours dark cycles). Light intensity was initially low but was gradually increased as the somatic embryos began to develop into shoots. Transfer of the shoots to a medium supplemented with 0.05-0.1 mg/L NAA promoted root formation. Although roots could be formed in media with 30 g/L sucrose, much better roots were formed in media with higher sucrose levels of between 45-90 g/L. Optimum sucrose concentration for root formation with charcoal was 50 g/L. In vitro plants which brought forth roots could be acclimatized and transferred to soil. Some plants are currently in the nursery while some have been planted in the field undergoing observation.

DISCUSSION

In vitro multiplication is quite useful for date palm multiplication because of the dioecious nature of the palm which puts limitations on seed propagation for the production of planting materials. Different methods have been used by different workers many of which have been successful in many countries although refinements are in many cases still being made.

The date palm industry in Nigeria is relatively small but could potentially become very large. Nigeria has a population of over 140 million and has a considerable amount of trade especially in commodities, both within its borders and with the neighbouring countries. Already there is a lot of trade in dried date fruits locally within the country although most of the fruits are imported from North Africa and the Middle East. Consequently, there is huge scope for developing the industry in terms of production and production chain development. This will lead to a significant increase in secondary activities such as development and trade in inputs (planting materials, fertilizers, processing and related equipment, etc.).

We have successfully used meristems, leaves and inflorescences as sources of explants for date palm somatic embryogenesis. Leaves have the advantage of allowing a plant to be sampled without being itself destroyed. This leaf sampling technique is also sometimes applied in the oil palm and coconut (Duval et al., 1988; Verdeil et al., 1992). However, callus production is faster from shoot apices than from leaf explants. The combination of the growing shoot tip and the leaves as sources of explants, if so desired, could significantly multiply the harvest of callus and subsequently of plantlets from the mother plant.

ACKNOWLEDGEMENTS

We are grateful to the Executive Director of NIFOR for funding this research work and for his permission to publish this article and to the Director General, NABDA for travel grant to the conference.

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Table

Table 1. Effect of varying concentrations of organic and inorganic nitrogen on callus initiation and growth measured as callus mean weight (mg).

KNO_3 (mM)	NH_4 (mM)			
	0	10	20	40
0	0	0	0	0
10	0	220±5.3	180±2.6	0
20	316±4.5	570±6.1	145±2.8	50±0.3
40	250±3.6	920±10.3	90±1.1	20±0
60	200±3.6	720±9.1	60±1.0	20±0
80	170±2.6	200±3.1	0	10±0

Figures

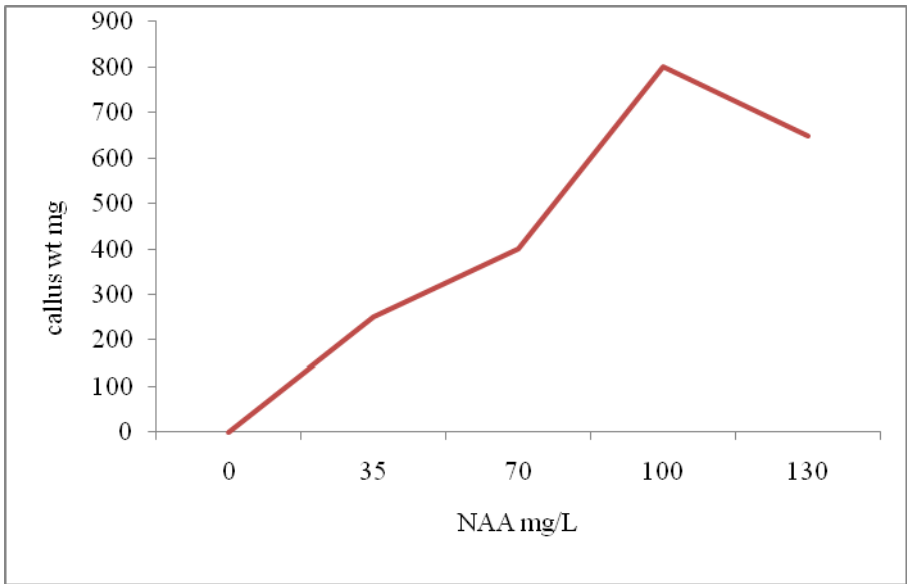

Fig. 1. Effect of combinations of NAA with BAP on date palm explants morphogenesis measured as mean fresh weight of callus produced.

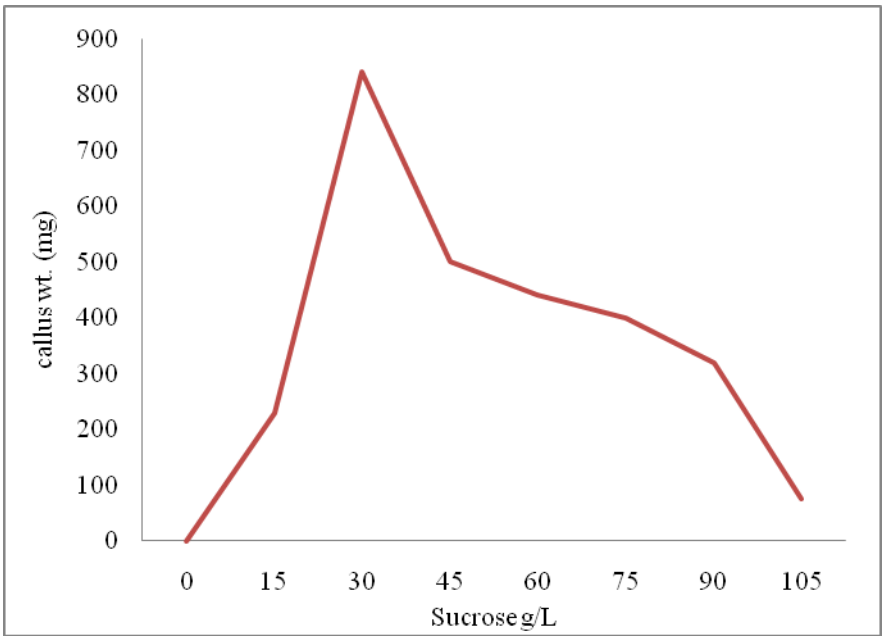

Fig. 2. Effect of sucrose and NAA concentration on callus induction measured as mean weight (mg) of callus obtained per treatment.

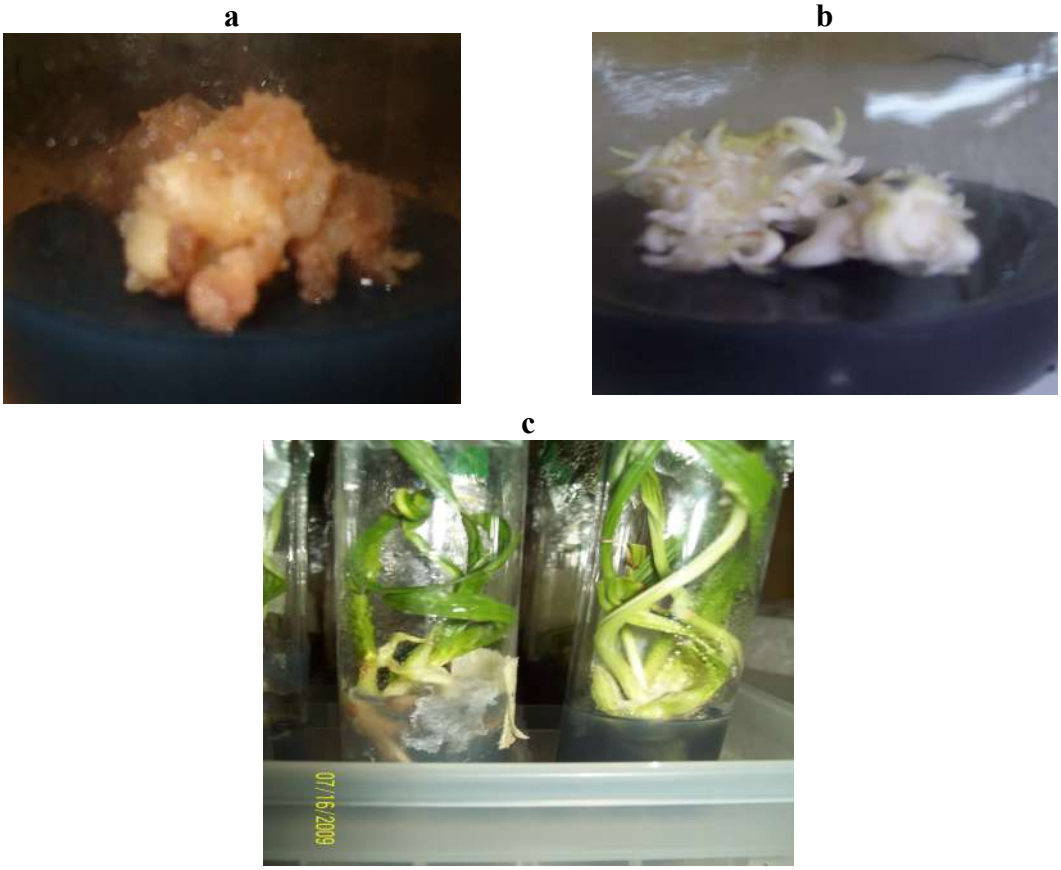

Fig. 3. Date palm development in culture (a) callus, (b) somatic embryos (c) shoots.