

Short Communication

Somatic embryogenesis in date palm (*Phoenix dactylifera* L.) from apical meristem tissues from 'zebia' and 'loko' landraces

Chukwuemeka R. Eke¹, Peter Akomeah² and Omorefe Asemota^{1*}

¹Physiology Division, Nigerian Institute for Oil Palm Research, P.M.B. 1030, Benin City, Nigeria.

²Botany Department, Ambrose Alli University, Ekpoma, Nigeria.

Accepted February 23, 2005

The shoot apical meristem from young suckers were used as sources of explants for initiation of culture using MS basal medium which contained 2,4-D. This was incubated at 27°C in the dark. Callogenesis was observed as early as the second subculture. Continuous subculture of the callus in the establishment medium at about the third subculture from calls production, resulted in somatic embryo formation. The somatic embryos were then transferred to MS medium without hormones under light where they matured after about two subcultures and developed into shoots. The shoots produced roots when transferred to a medium which contained NAA at 0.1 mg/L.

Key words: somatic embryogenesis, date palm, *in vitro*, micropropagation.

INTRODUCTION

In vitro multiplication has been applied to crop improvement using many methods. In some cases, *in vitro* multiplication is directed at commercial production of planting materials but in other cases, it is aimed at solving problems related to hybridization difficulties, production of disease free plants and germplasm conservation.

The date palm is an out-crossed, perennial monocotyledon which is consequently very heterozygous. In addition to these features, date palm is dioecious, that is with separate male and female palms. Seedlings would therefore be approximately 50% male. Male and female seedlings are not identifiable until flowering. Only

few male palms are required in the plantations as sources of pollen for fruit development. In order to obtain known female planting materials, offshoots could be taken from mother palms for planting. The limitations however, are that the average sucker production per palm per lifetime is low and restricted mainly to the juvenile years and the suckers are difficult to root. Some genotypes also do not produce suckers. In date palm production therefore, *in vitro* multiplication is particularly useful because it provides a means of overcoming difficulties of producing large numbers of relatively homogenous female date palm seedlings.

There have been previous reports of date palm micropropagation through the callus-somatic embryo pathway (Tisserat, 1979, Sharma et al., 1984, Daquin and Letouze, 1988, Letouze et al., 2000) as well as organogenesis (Rhiss et al., 1979, Beauchesne, 1983). However, the protocol has been continuously refined. The experiments of this report were done with this objective of developing reliably repetitive methods of somatic embryogenesis for date palm land races in Nigeria. For this purpose, we have used the sweet purple 'Zebia' and the soft 'Loko' land races.

*Corresponding author. E-mail: efe_asekota@hotmail.com. Tel: 234-52-602485. Fax: 234-52-602486.

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

MATERIALS AND METHODS

Plant material

Female date palms of the 'Zebia' and 'Loko' land races, obtained mostly as offshoots but in a few cases as mature palms were used as sources of explants. These were obtained from the Nigerian Institute for Oil Palm Research (NIFOR) date palm substation at Dutse, Jigawa state, Nigeria. From such palms, the shoot tip apical meristem with a few adjoining leaf bases was taken after carefully eliminating all leaves and other tissues from the offshoot.

Media

The Murashige and Skoog (MS) (1962) medium containing 3% sucrose, 0.85% agar, 0.01% inositol was used as the basal medium. The medium was supplemented with 0.002% aneurine hydrochloride, 100 mg/L 2,4-D and 3 mg/L 2-ip. The pH of the medium was adjusted to 5.8 after which the medium was autoclaved. Explants were always incubated in the dark for callus initiation.

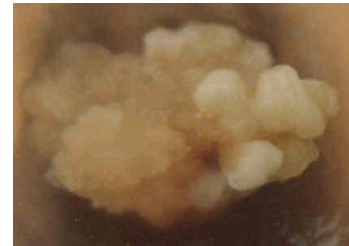
Somatic embryo initiation medium was essentially the same as the establishment medium but contained glutamine at 0.2 g/L. In addition, it was supplemented with 5 mg/L 2-ip and 0.1 mg/L NAA as growth hormones. For shoot regeneration and root development, 30 g/L sucrose, 0.2 g/L glutamine and 0.15% activated charcoal were added to the basal medium. It was further supplemented with 0.05-0.1 mg/L NAA and 1 mg/L 2-ip.

RESULTS AND DISCUSSIONS

The date palm usually does not produce branches and thus has only one growing point. It does produce a few suckers early in its life time. Therefore the number of offshoots and consequently, the number of meristems obtainable as sources of explants from a date palm is usually relatively few. From these, however, callus could be obtained readily and reproducibly, usually at about the second or third subculture, from initiation. With more subcultures, the size of callus also grew (Figure 1a). Initially, the callus appeared watery but the form of the callus grew compact and globular over time with more subcultures just as the colour became milky, usually after two subcultures from initial callus formation.

Several media induced the production of somatic embryos of date palm. In a few cases this occurred even while the callus was still in the callus induction medium. In other cases, it occurred upon the transfer of the callus to a hormone free medium. However, the most reliable medium for the induction of somatic embryogenesis, in our system, was that which was supplemented with 0.05 mg/L NAA and 1 mg/L 2-ip (Al-Baiz et al., 2000). In this medium, many somatic embryos emerged uniformly from the callus mass either during the first or the second subculture of callus into this medium (Figure 1b). This somatic embryo induction could be either in the light or in the dark. Over the next few weeks and months, depending on the maturity of the callus, the somatic embryos increased rapidly in number and in size. The

a



b



c



d

Figure 1. Stages of date palm development *in vitro* (a) callus, (b) somatic embryos, (c) date shoot clusters and (d) isolated shoot in rooting medium.

somatic embryos could develop into shoots in this medium.

As soon as the shoot initials were well formed, subsequent subculture was into a medium which contained 0.1 ppm NAA and 1 ppm 2-ip. This process took place in the presence of light, 14 h light and 10 h dark cycles. Light intensity was initially low but was gradually increased as the somatic embryos began to develop into shoots. Incubation in light brought about the development of green shoot (Figure 1c) and roots. A number of shoots could grow up together which would be subsequently separated. The generation of multiple shoots on agar solidified media have been recorded by other workers including (Tisserat, 1982), Al-Baiz et al. (2000). However, the development of liquid suspension cultures for somatic embryo development facilitates the development of more and relatively homogenous somatic embryos in culture.

In vitro multiplication is quite useful for date palm because of the dioecious nature of the palm which puts limitations on seed propagation for the production of planting materials. The methods used are varied and have been successful in many countries although refinements are in many cases still being made. We have successfully used leaves as sources of explants for date palm *in vitro* somatic embryogenesis which has 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 Director of Nigerian Institute for Oil Palm Research (NIFOR) for supporting this research work and for his permission to publish this article. We are also grateful to Dr. M. McCubbin for useful suggestions. We would like to thank Drs I.B. Omamor and E.C. Okolo as well as the NIFOR staff at Dutse for assistance in providing offshoots.

REFERENCES

- Al-Baiz AA, Mouli CK, Al-Oraini F (2000). Suspension cultures from embryogenesis. In Proceedings of the date palm international symposium, Windhoek, Namibia, pp.36-40.
- Beauchesne G (1983) Vegetative propagation of date palm (*Phoenix dactylifera* L.) by *in vitro* culture. In Proceedings of first symposium on date palm, King Faisal University, Hofuf, Saudi Arabia. pp. 698-699.
- Daquin F, Letouze R (1988). Regeneration of date palm (*Phoenix dactylifera* L.) by somatic embryogenesis, improved effectiveness by dipping in a stirred liquid medium. *Fruits* 43: 191-194.
- Duval Y, Durand-Gasselin T, Konan K, Pannetier C, (1988) *In vitro* vegetative propagation of oil palm (*Elaeis guineensis* Jacq.). *Oleagineux* 43: 39-47.
- Letouze R, Daquin F, Hamama L, Paquier K, Marionnet F, Javouhey M (2000). Mass propagation of date palm (*Phoenix dactylifera* L) through somatic embryogenesis. Histological study of embryo formation and cultivar identification by RAPD markers. In Proceedings of the date palm international symposium, Windhoek, Namibia. pp. 55-64.
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plantarum* 15: 473-495.
- Rhiss A, Poulain C, Beauchesne G (1979) La culture *in vitro* appliquée à la multiplication végétative du palmier dattier (*Phoenix dactylifera* L.). *Fruits* 34: 551-554.
- Sharma DR, Dawra S, Chowdhry JB (1984) Somatic embryogenesis and plant regeneration in date palm (*Phoenix dactylifera* L.) cv. 'Khadrai' through tissue culture. *Ind. J. Exptl. Biol.* 22: 496-598.
- Tisserat B (1979) Propagation of date palm (*Phoenix dactylifera* L.) *in vitro*. *J. Exp. Bot.* 30: 1275-1283.
- Tisserat B (1982) Factors involved in the production of plantlets from date palm callus cultures. *Euphytica* 31: 201-267.
- Verdeil JL, Huet C, Grosdemanges F, Rival A, Buffard-Morel J (1992). Embryogenese somatique du cocotier (*Cocos nucifera* L): obtention de plusieurs clones de vitroplants. *Oleagineux* 47: 465-469.