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Polypeptide variability among the coconut zygotic embryos in two culture conditions

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ABSTRACT

The growth of matured zygotic embryos of coconut in two types of media was studied. Twenty embryos of each West Coast Tall (WCT) and Chowghat Orange Dwarf (COD) were inoculated individually in Y3 liquid medium and solid medium. After 15 days the embryos in liquid medium was transferred to solid medium (liquid-solid medium). The germination percentage was recorded. Polypeptide pattern among the embryos in two different media was analyzed using 12 % SDS –PAGE. In WCT the plantlet recovery was 34 per cent for the liquid-solid culture after 90 days. In COD the germination was 63 % under liquid-solid media, where as only 40 % under solid medium alone. The embryos recorded higher germination percentage and balanced root and growth under liquid-solid medium. Equal amount of protein from all the cultures separated using SDS –PAGE. Proteins from freshly extracted embryos served as control. Fresh embryos of both COD and WCT had many polypeptides ranging from 90 kDa to 12 kDa. Control embryos of COD and WCT had high amount of polypeptide at 40 kDa, which could not be detected in germinated embryos. In comparison with COD and WCT, a polypeptide at 44 kDa was present only in WCT. The abnormal embryo of WCT had higher accumulation of 55 kDa polypeptide. A 45 kDa polypeptide in COD showed variability between embryos cultured in two different media. It is present only in controls and embryos cultured in solid media. It showed that, embryos cultured in solid media have not initiated the germination process and the reserve proteins are present without hydrolysis.

KEY WORDS: Coconut, Embryo culture, Polypeptide variability

INTRODUCTION

It is widely accepted that the movement of coconut germplasm should be through embryo culture because of phytosanitary requirements and for easy transport. Following a series of experiments, a protocol for coconut embryo culture was developed at CPCRI. Its different components include direct field collection of the coconut embryos of 8-11 months, short-term storage (Karun and Sajini 1994a), *in vitro* retrieval (Karun et al. 1993) and *ex vitro* establishment (Karun and Sajini 1994b). The protocol was further tested during 1994 by bringing 86 embryos of six coconut accessions maintained at World Coconut Germplasm Center (WCGC), Andamans to CPCRI, Kasaragod and their subsequent *in vitro* retrieval (Karun, 1998). It has also been successfully utilized during a germplasm expedition in the Indian Ocean Island during 1997. All the collections were made in that expedition in the form of embryos and this is the first attempt of its kind (Kumaran, 1998). Short-term storage of embryos, rescue of immature embryos and the higher rate of acclimatization of seedlings retrieved *in vitro* are the distinct characteristics of the protocol developed at CPCRI. Usually for culturing the coconut embryos solid medium was used. However, Karun *et al.,* (1993) attempted to use liquid and solid media for culturing. In the present paper polypeptide variability was studied in two different culture conditions.

MATERIAL AND METHODS

Coconut zygotic embryos from 11 month old nuts from West Coast Tall (WCT) and Chowghat Orange Dwarf (COD) were used for conducting the experiments. Twenty embryos of each of WCT and COD were used for the study. Surface sterilization of embryos was carried out by treating with 50 % chlorine water for 20 minutes and thereafter washing in sterile water 3 -4 times. First set of embryos were cultured first in liquid medium (Eeuwen's Y3) supplemented with sucrose and charcoal, and later transferred to Eeuwen's Y3 solid medium (liquid - solid media). Another set of embryos were inoculated on to solid medium and continued in the solid media itself for subsequent subcultures. The embryos were kept in the laboratory under ambient conditions (temperature 27±1 ºC, relative humidity 85%) with a photoperiod of 16hr. Subculturing was done in every 25-30 days. Well-developed root system was induced by supplementing the medium with IBA 5 ppm and NAA 1 ppm in both the experiments after about 4 months of inoculation.

Extraction of proteins from coconut embryo

Total soluble proteins were extracted by grinding the cultured embryos individually using liquid nitrogen. Poly vinyl poly pyrrolidone (PVPP) was mixed to the powdered tissue to remove the phenolic contents. The

powder was transferred to 1.5 ml 0.1 M Tris / Hcl buffer, pH 8.0. The homogenate was centrifuged at 12,000 rpm for 15 min at 4°C and the supernatant was separated. The supernatant was again centrifuged at 12,000 rpm for 10 min at 4 °C. Protein content was determined spectrophotometrically. For control, embryos excised from freshly harvested nuts were used.

Sodium dodecyl sulfate poly acrylamide gel electrophoresis of polypeptides

The SDS – PAGE of proteins was done in a discontinuous system as described by Laemmli (1990). Equal amounts of proteins from the samples were taken mixed with 3ml of Bromo Phenol Blue tracking dye. The proteins were denatured by keeping at boiling water bath for 10 min. The samples were loaded on the wells and electrophresis was done with a constant current of 15 mA till the dye front crossed the stacking gel. Then the current was increased to 32 mA and maintained till the run was completed. The gel was stained using Coomassie Brilliant Blue R 250. Protein extraction and gel electrophoresis was repeated thrice to study the protein pattern.

RESULTS AND DISCUSSION

Growth of embryos

In both varieties, West Coast Tall (WCT) and Chowghat Orange Dwarf (COD) the initial growth and size increment of embryos was fast in liquid medium. Initial germination percentage was high in solid medium (84 %) when compared to liquid - solid media (76 %) in case of WCT. But later the embryos cultured in the liquid - solid medium showed vigorous growth leading to the production of more number of leaves and active primary and secondary roots. This in turn led to 34 % final recovery of plantlets as compared to 14 % in case of embryos in solid media. COD embryos also showed similar type of development. In WCT some of the embryos developed abnormalities showing enlargement of embryos and hampered growth.

Polypeptide variability

Fresh embryos of both COD and WCT had many polypeptides ranging from 90 kDa to 12 kDa. Control embryos of COD and WCT had high amount of polypeptide at 40 kDa, which could not be detected in germinated embryos which may serve as reserve protein for the developing embryo. In comparison with COD and WCT, a polypeptide at 44 kDa was present only in WCT. The abnormal embryo of WCT had higher accumulation of 55 kDa polypeptide. Balen *et al.,* (2002) observed spontaneous callusing of *in vitro* cultures of *Mammillaria gracillis*. They detected a 42 kDa protein in habituated callus, hyperhydric regenerants and tumor tissues.

In COD, a 45 kDa polypeptide showed variability between embryos cultured in two different media, which is present only in controls and embryos cultured in solid media. It showed that, embryos cultured in solid media have not completed the germination process and the reserve proteins are present without hydrolysis. Balen *et al.,* (2002) studied the protein and glycoprotein patterns with the intension to detect some developments specific proteins in *Mammillaria sp* and reported tumor specific extra cellular proteins of 22, 23 and 33 kDa. Our results showed that the culture conditions have influenced the growth of coconut zygotic embryos and variability is present among the embryos cultured in liquid -solid and solid media.

REFERENCES

Balen B.; Milosevic, J. and Krsnik-Rasol, M. 2002. Protein and glycoprotein patterns related to morphogenesis in Mammillaria gracillils Pfeiff. tissue culture. *Food Technol. Biotechnol*. **40**: 275-280

Karun, A.; Upadhyay, A. and Parthasarathy, V.A. 1998. Status of research on coconut embryo culture and acclimatization techniques in India: In: Coconut Embryo Culture *in vitro* Culture. Proc. *of first workshop on Embryo culture* held at Banao, Guinobatan, Albay, Philippines during 27-31 October 1997, pp. 29-36.

Karun, A. and Sajini, K.K. 1994a. Coconut embryo culture: *In vitro* germination and *ex vitro* establishment. Paper presented at *National Development in Plant Molecular Biology and Genetic Engineering*, 29l-31 December 1993, TNAU, Coimbatore, Tamil Nadu, India, pp. 23.

Karun, A. and Sajini, K.K. 1994b. Short-term storage of coconut embryos in sterile water. *Curr. Sci*. **67**: 118-20.

Karun, A.; Shivashankar, S.; Sajini, K.K. and Saji, K.V. 1993. Field collection and in vitro germination of coconut embryos. *J. Plantation Crops.* **21** (suppl): 291-94.

Kumaran, P.M.; Koshy, P.K. and Karun, A. 1998. Germplasm exchange through embryo cultures. *Proc. 7th Swadeshi Science Congress*, 6-8 November 1997 held at CPCRI, Kasaragod, Kerala, India, pp. 35-39.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature. **227**: 680-685.

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