Secondary Somatic Embryos in Banana cultivar -

A case Study of Nanjangud Rasabale

Dr. K.Y. Pratibha

Maharani's Science college for Women,Bangalore

Abstract

Studies were initiated to develop regeneration protocol through somatic embryogenesis in banana cv. Rasthali. Embryogenic callus and somatic embryos were generated with male flower buds on MS medium supplemented with 2,4-D (18.10 µM), NAA (5.37 µM) and IAA (5.71 µM) with 3% sucrose and 0.2% gelrite. After 6–8 months well developed somatic embryos were sub cultured on to MS supplemented with NAA (1.07 µM), Zeatin (0.23 µM) ,2-ip (0.60 µM) and kinetin (0. 46 µM) for further maturation. Plantlets were obtained when somatic embryos were cultured on MS with Morel vitamin, IAA (11.42 µM)), and BAP (2.22 µM). Formation secondary somatic embryos on the primary embryos were observed if the primary embryos were left in the induction media a little longer (8–9 months). Transparent, fragile, secondary buds emerged either on top of young globular embryos or on cotyledonary region of matured primary somatic embryos. Histological and histochemical studies were carried out to verify the mode of development of secondary somatic embryos on the primary somatic embryos and it revealed that the secondary embryos were formed directly on the epidermis of primary somatic embryos without intervening callus. The study has shown two modes of development of direct secondary embryogenesis. In the first mode, the secondary somatic embryos originated from single epidermal cells of the young globular primary embryos. In the second mode several epidermal cells of matured primary somatic embryo were involved in the formation of secondary somatic embryos. Localization of starch, total insoluble proteins and nucleic acids were seen in the secondary somatic embryos.

Introduction

Successful regeneration of banana plants from somatic embryogenesis is reported by many workers (Novak et al., 1989; Escalant et al., 1994; Ganapathi et al., 1999; Sadik et al., 2007).

Secondary somatic embryogenesis is a phenomenon whereby new embryos are initiated from pre-existing embryos (Vasic et al., 2001). It has compared to primary embryogenesis advantages such as high multiplication rate, independence of an explant source and repeatability. Furthermore, embryogenecity can be maintained for prolonged periods of time by repeated cycles of secondary embryogenesis (Raemakers et al., 1995). Secondary somatic

embryogenesis has potential application for both plant breeding practice and research. Dhed'A et al. (1991) reported secondary embryogenesis from the suspension cultures of scalp derived primary somatic embryos in banana cv. Bluggoe. The present study reports the origin and development secondary somatic embryos in banana cv. Rasthali using male flowerbuds.

MATERIAL AND METHODS

Inflorescences of banana cultivar 'NanjangudRasbale' (Silk gp. Rasthali AAB) bearing male flowers were collected from Nanjungud near Mysore and were used as explants within 24 hours of its excision. The immature male flower clusters from position 0–16 were removed and inoculated on MA1 medium of INIBAP (2003) consisting of Murashige and Skoog medium (MS) supplemented with 18.10 µM 2,4-dichlorophenoxy acetic acid (2,4-D), 5.71 µM indole 3-acetic acid (IAA),5.37 µM naphthalene acetic acid(NAA) and 4.09 µM d-Biotin, 3% sucrose and 0.2% gelrite for callus induction in dark at a temperature of 25±2°C. After 8–9 months, cultures producing embryogenic callus with somatic embryos were selected under a microscope and fixed for histological studies. Well-developed somatic embryos were sub cultured on to MS supplemented with NAA (1.07 µM), Zeatin (0.23 µM), 2-ip (0.60 µM) and kinetin (0.46 µM) for further maturation. Plantlets were obtained when somatic embryos were cultured on MS with Morel vitamin, IAA (11.42 µM) and BAP (2.22 µM). Standard histological studies were conducted during the course of study (Jensen,1962).

RESULTS AND DISCUSSION

Secondary somatic embryos were observed on the primary embryos if the primary embryos were left in the induction medium supplemented with 2,4-D (18.10 µM), NAA (5.37 µM) and IAA (5.71 µM) for 8–9 months. Single induction media with auxins was sufficient to induce secondary somatic embryogenesis. Transparent, fragile, secondary buds emerged either on top of globular primary somatic embryos or on cotyledonary region of matured primary somatic embryos. Histological studies done on the ontogeny of secondary somatic embryogenesis revealed that secondary embryos were formed directly from the epidermis of primary embryos without intervening callus.

The study has shown two modes of direct secondary somatic embryogenesis and the most common mode of development of secondary somatic embryogenesis was through single cell of epidermis. Globular or young primary somatic embryos rich in starch and proteins with dense nucleus and cytoplasm became meristematic at the epidermal region. Single epidermal cell with starch became enlarged and divided obliquely to result in two celled secondary

somatic embryo in turn divided to produce globular secondary somatic embryo. Epidermal cells on all the sides of primary globular embryos produced secondary somatic embryos which in turn formed tertiary globular somatic embryos by repetitive embryogenesis. Somatic embryos were multiplied in chains, each one forming 4 to 5 new embryos which then separated and continued the phenomenon.

In the second mode of embryo development several epidermal cells of matured primary somatic embryo without proper shoot apical meristem undergo cell division over large area on the epidermis and forms compact secondary embryos. Usually several epidermal cells of cotyledonary region of primary somatic embryo with dense nucleus and cytoplasm became meristematic and divided to form 1–4 secondary somatic embryos of different shapes. Multiplication rate by repetitive secondary embryogenesis was limited in this type of developmentalphase.

Starch accumulation in the primary somatic embryos was very intense and such primary embryos only formed secondary somatic embryos which showed the presence of starch even in the epidermal region. Intense localization of total insoluble proteins and nucleic acids were seen in primary embryos which formed secondary somatic embryos in formative stage as well in the developmental stages of secondary somatic embryogenesis.

CONCLUSION

In conclusion, a reliable method to induce primary and secondary somatic embryogenesis from banana cv. Rasthali was established. This system may be highly useful for developing transgenic plants for disease resistance and apparently superior to the previously reported system in banana and other crops.

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