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Micropropagation of four Banana Cultivars in Micronesia

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Abstract—Four local cultivars of bananas (*Musa* spp.) in Kosrae, Federated States of Micronesia (FSM)—Kufwafwa, Lacatan (dessert bananas), Apat regular and Apat fissuse (cooking bananas)—were subjected to micropropagation experiments at the Micronesia Plant Propagation Research Center (MPPRC), Kosrae during 1999–2000. Protocols for efficient micropropagation of these varieties were developed during the study. Surface sterilization procedures were modified to attain acceptable numbers of aseptic stage I cultures. All varieties studied responded to modified MS medium. Plant growth regulator, 6–Benzylaminopurine (BAP, 3–7 mg L^{-1}) requirements for different varieties were substantially distinct in terms of optimal growth and multiplication. Multiplication rates varied considerably between 20–30 (Apat regular) and 2–3 (Kufwafwa). Longitudinal splitting of the shoot tips improved the multiplication rate in all the varieties.

Introduction

Bananas and plantains are staple food for many millions of people throughout the humid tropics and subtropics (Vuylsteke 1989) and are the fourth most important food crops in the world (Moffat 1999). The low trade volume in contrast, compared to the total production of bananas points to their importance in maintaining food security for millions of resource-poor people in the tropics.

Tropical islands in the Pacific and Atlantic Oceans have rich diversity of banana germplasm. Major diseases like bunchytop of banana, black and yellow Sigatoka, panama wilt and bacterial wilt cause significant production loss worldwide. Bananas have been cultured in vitro through shoot tip culture to provide elite, disease free planting material on a commercial scale (Cronauer & Krikorian 1986, Vuylsteke 1989). Callus culture, cell culture and somatic embryogenesis have been used to apply recombinant DNA technology to the improvement of seedless bananas (Dale 1999, Tenkouano et al. 1999, Becker et al 2000, Ganapathi et al. 2001). Micropropagation and meristem culture is a proven technique for multiplying disease-free banana (Cronauer & Krikorian 1986) and for producing

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plants free from banana bunchytop virus (BBTV) from infected stools (Drew et al. 1992). Bananas are grown in the Federated States of Micronesia (FSM) primarily as a subsistence crop and commercially to a limited extent. Kosrae State alone exported nearly $100,000–worth of bananas in 1994 to nearby islands. A combination of several factors such as pests, diseases and poor management practices has weakened the banana germplasm in Kosrae. Among the rich diversity of banana germplasm, two varieties of bananas, producing upright bunches with unusually high vitamin contents remain unique in Kosrae and the FSM. A tissue culture facility, the Micronesia Plant Propagation Research Center (MPPRC) was established in Kosrae in 1998 to achieve export-oriented commercial production of local dessert bananas through 1) revitalizing banana (*Musa* spp.) germplasm available in Kosrae, 2) providing disease free and uniform planting material to farmers in Kosrae.

Kufwafwa (ABB), Lacatan (AAA), Apat fissuse (BBB/ABB) and Apat regular (AAB) are commercially important banana varieties adapted to Kosrae's environment. Plant genotype and the environment (soil and climate) in which a crop is grown requires a suitable protocol for efficient micropropagation of that crop. The objective of the current research was to develop suitable procedures to efficiently micropropagate banana varieties adapted to Kosrae's environment.

Materials and Methods

Chemicals and supplies

All chemicals used in the experiments were of analytical grade reagents obtained from Sigma Chemical Company, MO, USA, except Clorox, which was purchased locally.

Plant material

Four locally important banana varieties (*Musa* sp.) namely Lacatan (LAC), Kufwafwa (KUF), Apat fissuse (APF) and Apat regular (APR), were used in this study. A landrace of the variety Kufwafwa with larger bunches and fingers was used in this study. Field surveys indicated that this race averages only 1.6 suckers/plant in Kosrae.

Sucker collection and pre-sterilization

Healthy sword suckers, one to two feet long, collected from local farms in Kosrae, served as the source of explants. To ensure higher yield, and good health, suckers were always collected from bearing mother plants showing above average yield and from stools free of *Fusarium* wilt and stem rot. Suckers were collected from November 1999 to July 2000. Roots were carefully removed and corms thoroughly washed under running tap water. Corms showing discoloration or burrows and fissures were discarded to reduce the chance of infection.

Preparation of sterile explants

Approximately 3 cm^3 of corm tissue enclosing the meristem were immersed in undiluted Clorox for 10 minutes for surface sterilization. Fifteen to twenty minutes of incubation in Clorox was necessary for suckers collected during weeks of heavy rain. After two rinses with sterile water, these tissues were trimmed to 1.0 cm^3 pieces enclosing the meristem. These blocks were further sterilized in 0.1% w/v mercuric chloride solution for 4–5 minutes before being rinsed with sterile water four times. Explants consisted of 3–4 mm^3 of sterile tissue enclosing the meristem.

Media and incubation conditions

MS medium (Murashige & Skoog 1962) was used in this study. Concentrations of 6–benzylaminopurine (BAP) and media components, were adjusted to improve culture establishment (Stage I) and multiplication and growth of shoots in vitro (Stage II). All cultures were incubated at 26+1ºC and 4000 lux light under a photoperiod of 8 hours light/16 hour darkness.

Results and Discussion

Stage I – Culture establishment

The rate of explant loss due to contamination was 90% when single step sterilization (15–20 minutes in 25% v/v Clorox) was done during very wet periods, from December 1999 to April 2000. In comparison explant loss was 50% during drier months, from May to June 2000. The two step sterilization procedure reduced explant loss through contamination to 5–8%. The high contamination rate observed during the wet season may have resulted from low temperature and high soil moisture favoring growth and development of fungi and bacteria. In Kosrae, poor agricultural practices including low replanting frequency, lack of artificial nutrition and application of fungicides or pesticides may have favored higher contamination. Hamill et al. (1993) found that a two-step procedure for sterilizing bananas is more effective than a single step process.

Suitability of the basal medium and BAP for stage I culture

MS basal medium supplemented with 30gL^{-1} sucrose, 100 mg L^{-1} ascorbic acid and BAP ranging from 3 mg L^{-1} to 8 mg L^{-1} was used to evaluate suitability of the media for Stage I culture establishment. MS medium containing 4 mg L^{-1} BAP, 5 mg L^{-1} BAP, 6 mg L^{-1} BAP and 7 mg L^{-1} BAP were the most effective media for establishing the varieties APR, APF, LAC and KUF respectively. Larger explants (5–10 mm^3) did not improve establishment rates, and caused more losses due to contamination. The correlation between contamination rate and explant size may have resulted from bacteria and fungi that remained in the tissues around the meristem.

The time interval between the first inoculation of the explant and the beginning of shoot multiplication varied considerably among the varieties studied. APR

started to multiply in about 62 days, APF and LAC in 73 days and KUF in 92 days. This differential response may be attributed to genotypic differences.

Stage II culture – Response and requirements

Stage II cultures of all varieties were subjected to different treatments involving modification of nutrient and BAP levels to optimize shoot multiplication. The varieties APR and APF responded well to low BAP concentration (3–4 mg L^{-1}) while the varieties LAC and KUF needed higher concentration (6–7 mg L^{-1}). MS medium containing BAP is better suited for micropropagation of many banana varieties by Cronauer & Krikorian (1986). Further enhancement of the multiplication rate could be achieved in all varieties by amending the level of monobasic potassium phosphate. Up to 250 mg L^{-1} KH_2PO_4 increased multiplication rate, but higher doses reduced it. Elevated levels of phosphorous and potassium may have increased the multiplication rate. Field experiments have established a higher requirement of potassium for better banana growth. The acidification by phosphate at very high KH_2PO_4 concentrations may have negated the effects of potassium phosphate beyond 250 mg level. The variety KUF needed manipulation of the vitamin thiamin. A ten-fold increase in thiamin concentration resulted in significant increase in the multiplication rate of different varieties. Nandwani et al. (2000) documented the positive effect of thiamin on multiplication of bananas in vitro.

Longitudinal splitting of micro-shoots and culturing thin discs from the activated shoot-tips of the micro-shoots increased the multiplication rate further. The thin disc culture method was attempted only with the variety KUF because other varieties evidenced adequate multiplication rate without it. The thin section procedure is not highly reproducible. The enhancement effect observed with the longitudinal splitting and slicing of active meristem tissue is similar to previous reports on orchids (Prakash et al. 1996) and bananas (Okole & Schultz 1996).

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