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The type of explant plays a determining role in the micropropagation of *Ensete ventricosum*

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Three to five shoots per shoot tip were produced when *in vitro*-grown zygotic seedlings of enset genotype Oniya were used as donor plants and the shoot tips split longitudinally through the apex into two and cultured on a basal Murashige and Skoog (MS) medium supplemented with 11µM BA and 6µM IAA. Only one shoot per shoot tip of greenhouse-grown suckers was regenerated from about 60% of the explants on a similar medium, while the remainder died, presumably due to blackening and necrosis of the tissues. Increasing the BA concentration from 11µM to 22µM or to 44µM did not increase to the number of shoots from the halved shoot tips. On MS medium supplemented with 11µM BA and 6µM IAA or 5µM NAA, the intact shoot tip gave rise to a single shoot that originated from the apical bud. From the intact shoot tips that were grown *in vitro* on medium with 44µM, 89µM, 178µM, 266µM, 355µM and 444µM BA, 72% gave rise to a single shoot/bud from the apical bud without induction of any lateral buds, while the rest produced one or two lateral buds that were mainly hyperhydric. Therefore, wounding the apical dome by splitting appears necessary to release lateral buds from apical dominance of the tip of the monopodial corm of enset during micropropagation. The greater response in production of multiple shoots by zygotic seedlings cultured *in vitro* could be due to the absence of blackening and the juvenile nature of the explants.

Abbreviations: AC = activated charcoal, BA = benzyladenine, IAA = indole-3-acetic acid, MS = Murashige and Skoog medium (1962), NAA = naphthaleneacetic acid

Introduction

Enset (*Ensete ventricosum* Welw.) (Cheesman 1947) constitutes a sustainable agricultural system in southern and south-western Ethiopia, where it is a staple and co-staple food for millions of people. Products from enset are used in different forms in traditional medicine. A starch for textile, adhesive and paper industries is being produced. However, cultivation of enset is constrained by various diseases such as enset wilt, caused by *Xanthomonas campestris* pv. *musacearum*, and by insect pests and abiotic factors. Enset germplasm is currently maintained in a field genebank but is at risk of diseases, pests and adverse environmental conditions. Tissue culture techniques can be used to enhance propagation, conservation of genetic resources and in breeding. Micropropagation through shoot tip culture may offer advantages over conventional propagation methods, such as higher rates of multiplication and production of clean or disease-free plant material. It has been reported that plantlets of micropropagated banana and plantain establish more quickly, grow more vigorously and taller, have a shorter and more uniform production cycle and produce higher yields than conventional propagules (Drew and Smith 1990, Robinson *et al*. 1993, Vuylsteke 1998).

During enset micropropagation, it proved difficult to initiate shoot tips *in vitro* from greenhouse-grown suckers because of extensive blackening and unwanted callus formation (Afza *et al*. 1996, Morpurgo *et al*. 1996, Zeweldu 1997). The involvement of a callus phase in the micropropagation process may result in a greater incidence of somaclonal variation (George and Sherrington 1984). *In vitro*-germinated zygotic seedlings of enset were used as a source of explants along with greenhouse-grown material (Negash *et al*. 2000). However, advantages of using the *in vitro*-grown seedlings to avoid or reduce difficulties encountered with the greenhouse material were not elaborated. Benzyladenine was used to reduce apical dominance of enset corms and induce growth of multiple shoots (Negash *et al*. 2000). However, the monopodial nature of the enset corm needs to be considered. In the

present study, problems of blackening and callusing of explants *in vitro* were addressed by manipulation of seedlings grown *in vitro*. A protocol for more rapid *in vitro* multiplication of *E. ventricosum* from shoot tips was developed and effects of plant growth regulators to release lateral buds from apical dominance documented.

Materials and Methods

Plant material and culture conditions

Seedlings and suckers of *E. ventricosum* genotype Oniya were used. The seedlings were aseptically germinated from zygotic embryos *in vitro.* The suckers were vegetatively propagated in the greenhouse. The experiments were conducted at a multiplication stage and the explants were subcultured for 3–4 weeks onto MS medium and, to reduce carry-over effects, devoid of plant growth regulators prior to use for the experiments. Murashige and Skoog (MS) (1962) medium was used in all the experiments with 90mM sucrose and 7gl^{-1} activated charcoal (Sigma). The pH of the medium was adjusted with KOH/HCl to 5.8 prior to autoclaving. The gelling property of agar decreased in the presence of AC and as a result 11gl^{-1} agar (agar-agar powder, Associated Chemical Enterprises, Glenvista, RSA) was used. The medium was autoclaved at 121°C and 103kPa for 20min. Based on explant size, test tubes with 10ml medium or jars with 30ml medium were used. The explants were inoculated onto the medium under aseptic conditions. Cultures were maintained in a growth room at 26 ± 1°C under a 16-h photoperiod. Light was provided by Osram Cool White L 40W/20S lamps at PAR of about 40µmol m^{-2} s^{-1}. A completely randomised design was used throughout. The cultures were subcultured on the same medium one month after inoculation. After another month, regenerating shoots and buds were subcultured on MS medium without hormones.

Multiplication of shoots in vitro *from different sources of shoot tip explants*

Shoot tips from two sources, *in vitro*-grown seedlings and greenhouse-grown suckers, were compared for their rate of multiplication *in vitro*. The shoot tips from suckers were first initiated *in vitro* for five weeks and used in the experiment at a multiplication stage. For the initiation experiments, six-month-old suckers were uprooted from the greenhouse and trimmed to 2–3cm-long shoot tips. The shoot tips were decontaminated for 15min in 3.5% sodium hypochlorite containing two drops of Tween 20 and rinsed three times with sterile distilled water. They were then reduced to 8–10mm in length and inoculated intact onto MS medium supplemented with 11µM BA + 6µM IAA. Two weeks later, shoots were subcultured onto MS medium containing AC without cytokinin and auxin. After three weeks of subculture (five weeks after inoculation), shoot tips were used to carry out experimentation. The shoots from both sources (*in vitro*-grown seedlings and greenhouse-grown suckers) were shortened to 10–12mm and were split longitudinally through the apex. The halved shoot tips were inoculated separately onto MS medium supplemented with 11µM BA + 6µM IAA. Sixteen test tubes per treatment and one explant per test tube were used.

Multiplication of shoots in vitro *from intact and split shoot tips*

Shoot tips from *in vitro*-regenerated seedlings of zygotic embryos were used. The shoot tips were trimmed to about 10mm and randomly divided into two groups. Those in the first group were used intact, without injuring the apical domes. The ones in the second group were split longitudinally through the apex in two. The explants were inoculated onto MS medium supplemented with either 11µM BA + 6µM IAA or with 11µM BA + 5µM NAA. Eight test tubes per treatment and one explant per test tube were used.

Multiplication of shoots in vitro *from split shoot tips using different plant growth regulators*

Shoot tips of *in vitro*-germinated seedlings were cut to about 10mm in length and were then split longitudinally through the apex. The halved shoot tips were inoculated onto 12 compositions of medium. These were: MS medium without growth regulators; MS medium supplemented with (µM): 11 BA; 22 BA; 44 BA; 6 IAA; 11 BA + 6 IAA; 22 BA + 6 IAA; 44 BA + 6 IAA; 5 NAA; 11 BA + 5 NAA; 22 BA + 5 NAA; or 44 BA + 5 NAA. Ten test tubes per treatment and one explant per test tube were used.

Effect of benzyladenine on in vitro *multiplication of shoots from intact shoot tips*

Shoot tips of *in vitro*-regenerated seedlings were prepared in such a way that the apical domes were not mechanically injured. About 10mm-long intact shoot tips were excised and inoculated onto MS medium supplemented with seven concentrations (µM) of BA (0, 44, 89, 178, 266, 355 or 444). With all BA concentrations tested, 6µM IAA was included. Ten test tubes per treatment and one explant per test tube were used.

Data collection and statistical analysis

Data on the number of shoots produced per explant were collected. For split shoot tips, the number of shoots from the two halves was used to indicate the rate of multiplication per shoot tip. A regenerated organ from the explant was considered a shoot when it produced a rolled leaf. Lengths of the longest shoot and of buds were measured. Buds per explant, leaves per shoot and roots per explant were counted. Blackening was recorded based on a blackening score: 0 = no blackening; 1 = slight; 2 = moderate; and 3 = extensive. Usually, both explant and medium become black. However, because of the presence of AC, blackening was recorded only for the explants. Data collected two months after inoculation of shoot tips onto gelled medium was used for statistical analysis. When the number of treatments was two, the data were subjected to a two-sample t-test to generate statistical evidence of treatment effect. When the number of the treatments was more than two, the data were subjected

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