Minireview

Propagation of Ensete in vitro: a review

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Received 12 March 2004, accepted in revised form 25 May 2004

The in vitro propagation of Ensete, an important food crop in parts of east Africa, has been investigated in a number of previous studies. These studies have reported on the culture of Ensete species using a variety of methods, including zygotic embryo germination, shoot tip culture, adventitious shoot regeneration and multiple shoot development, and somatic embryo cultures. Various problems, such as extensive blackening of explants, necrosis and the formation of unwanted callus have been observed. E. ventricosum, in particular, is a very valuable food crop in Ethiopia and micropropagation techniques are important for clonal multiplication and germplasm conservation. This review brings together various aspects related to the in vitro micropropagation of Ensete.

Introduction

The genera Ensete and Musa, belonging to the Musaceae, are monocotyledons. The genus Ensete consists of six species, E. gilletii, E. homblei, E. perrieri and E. ventricosum with an African distribution, and E. glaucum and E. superbum with an Asian distribution (Simmonds 1960). E. ventricosum is widespread in the wild in Africa from Cameroon to east Africa and is cultivated as a staple or co-staple food crop in Ethiopia (Bezuneh and Feleke 1966). In Ethiopia, E. ventricosum is known as ‘enset’ in the Amharic language. The use of tissue culture techniques to propagate plants in vitro is an extension of conventional propagation methods. Some aspects of in vitro propagation of E. ventricosum and E. superbum were previously investigated and can be used for the genetic improvement and conservation of Ensete. This review examines the available information on the propagation of Ensete in vitro.

Importance of Ensete

Enset (E. ventricosum) is a source of food, high in carbohydrates, which is grown and consumed by about 15 million Ethiopians (Spring 1996). A mixture of scraped leaf sheath and pulverised corms, after fermentation in a pit, results in the production of ‘kocho’, the main product of enset which is used to make a pancake-like food. ‘Bulla’ is another important food product from enset. It is produced from solidified liquid after dehydrating a fresh mixture of scraped leaf sheath and pulverised corms. ‘Bulla’ is consumed mainly as porridge, in gruel and in a crumbled form. Corms of some clones are cooked and consumed in a similar way to roots and tubers of other food crops. Parts of some clones are also used in traditional medicine. A novel phenylphenalenone was detected and isolated from E. ventricosum (Holscher and Schneider 1998). A starch that can be used for paper, textile and adhesive industries is produced from enset (ESTC 2003). There is also a potential to use enset starch in binding and disintegrating of compressed tablets (Gebre and Nikolayev 1993). Fibre, a by-product of enset in food processing, is a versatile raw material for household usage and as a raw material for local fibre factories. Enset is a valuable security crop as it tolerates transient drought and can be harvested throughout the year at different growth stages to buffer seasonal food deficits. A large leaf area, closure of canopy and litter from enset cultivation. Therefore, enset contributes to sustainable agriculture and food security in Africa.

Potential use of tissue culture of Ensete

Enset is a subsistence crop that can easily be propagated using conventional vegetative propagation methods. The importance of micropropagation would therefore be to propagate new genotypes and/or specific clones tolerant to pathogens, for example, Xanthomonas campestris cv. musacearum, that causes enset wilt, a destructive disease...
(Ashagari 1985, Quimio and Tessera 1996). Enset root mealy bug, the root lesion nematode Pratylenchus goodeyi and the root knot nematode Meloidogyne sp., mosaic and chlorotic streaks, both of viral nature, are widely distributed in association with enset (Quimio and Tessera 1996). Tissue culture techniques can be used to produce pathogen-free plant material. A procedure for in vitro screening of Musa spp. for resistance to burrowing nematode (Radopholus similis) was developed (Eisen et al. 2002). Therefore, in vitro techniques can also be used to screen enset genotypes against nematodes. Shoot tip culture can supplement a field genebank in conserving enset genetic resources using in vitro slow-growth conditions and/or cryopreservation (Negash et al. 2001). Genetic variability can be created using callus culture and in vitro mutation and important variants can be selected. Somatic hybridisation and recombinant DNA techniques can potentially be used in enset improvement. However, since enset is fertile and produces viable embryos, the use of existing variability through conventional breeding and selection would also be practically possible. In the breeding process the poor germination of intact seeds of enset can be overcome by germination of excised embryos in vitro. Therefore, tissue culture techniques can contribute to clonal multiplication, germplasm conservation and in developing enset genotypes with desirable traits.

**Propagation of Ensete in vitro**

Plant propagation in vitro can be achieved by zygotic embryo culture, organogenesis, or somatic embryogenesis. Organogenesis involves regeneration of organs from existing meristems or from de novo meristems. Various combinations of nutrients, plant growth regulators and environmental factors for different species or genotypes may stimulate the micropropagation of plants. Manipulation of these factors may enable plant breeders and propagators to control plant cell morphogenesis and to develop reliable cells to plant regeneration systems. Information on the type of explant, medium composition and culture environment used in the propagation of Ensete in vitro is summarised in Table 1. Different pathways for the regeneration of whole plants from excised enset plant parts are discussed. From the literature cited with respect to the micropropagation of Ensete it is quite clear that the voluminous literature on the in vitro culture of its fellow genus Musa has contributed positively to our present understanding of the biotechnology surrounding Ensete (Israeli et al. 1995, Zeweldu 1997, Diro Chimsa 2003) and will no doubt continue to do so in future.

**Zygotic embryo culture**

In this technique, mature or immature seed embryos are cultured in vitro to produce seedlings. Embryo cultures have been used to explore the nutritional and physical requirements for embryonic development to bypass seed dormancy which may shorten the breeding cycle, to test seed viability, to provide microcloning source material and to rescue immature hybrid embryos from incomparable crosses (Hu and Wang 1986, Pierik 1987). Mature embryos, in general, require only inorganic salts supplemented with sucrose, whereas immature embryos have an additional requirement for vitamins, amino acids, growth regulators and sometimes endosperm extract. Germination of intact seeds of the Musaceae (Ensete and Musa) is very poor. In vitro germination of excised zygotic embryos was used as an alternative pathway to regenerate plants from seeds of Ensete (Bezuneh 1980, Negash et al. 2000, Diro and Van Staden 2003) and Musa (Cox et al. 1980, Afele and De Langhe 1991, Asif et al. 2001). Bezuneh (1980) regenerated plants from mature embryos of Ensete on a semi-solid medium modified from Murashige and Skoog (MS) (1962) and Cox et al. (1980), supplemented with 5g l⁻¹ sucrose and agar. When the embryos were preincubated for 15–20min in 4mg l⁻¹ giberrellic acid (GA₃) additional swelling and additional elongation (10%) was observed. Negash et al. (2000) cultured mature enset embryos on benzyladenine (BA) and indole-3-acetic acid (IAA) containing MS medium. Recently the procedure for embryo culture of Ensete was further optimised (Diro and Van Staden 2003).

**Shoot tip culture**

Shoot tip culture is the use of a lateral or main shoot apex (apical dome plus a few subjacent leaf primordia), which may be up to 20mm in length, to produce multiple shoots (George and Sherrington 1984, Nehra and Kartha 1994). Murashige (1974) suggested three sequential stages of micropropagation; since then Stages 0 and 4 have been added (George and Sherrington 1984). Thus, the 5 Stages of micropropagation are: 1) preparation of the mother plants, 2) initiation of an aseptic culture, 3) multiplication of propagules, 4) regeneration of whole plants, and 5) hardening-off for subsequent field planting.

In previous studies on the micropropagation of Ensete, mother plants were grown in greenhouses and shoot tips (corm tissues with some leaf primordia) were used (Afza et al. 1996, Morpurgo et al. 1996, Zeweldu 1997, Negash et al. 2000). In vitro germinated seedlings have also been used as a source of explants (Negash et al. 2000). Zeweldu (1997) used 2cm² corm tissues of greenhouse-grown Ensete. These were decontaminated in 1.5% (w/v) sodium hypochlorite solution with some drops of Tween 20 for 10–15min, before rinsing three times with sterile distilled water. However, some endogenous contaminants were observed. Initiation of sterile shoot tips of E. ventricosum was difficult due to blackening of the explants. As a result swelling and callusing explants were obtained (Afza et al. 1996, Morpurgo et al. 1996, Zeweldu 1997). Shoot tips of E. superbum from a botanical garden were decontaminated with 0.1% mercuric chloride solution for 5min followed by three washings with sterile water (Mathew and Philip 1996). The shoot tips were incubated in liquid medium supplemented with 1.5mg l⁻¹ BA and 1mg l⁻¹ kinetin.

In the culture of E. ventricosum, 2.25–5mg l⁻¹ BA + 0.1–0.5mg l⁻¹ IAA was used for the multiplication of propagules (Afza et al. 1996, Zeweldu 1997, Negash et al. 2000). In E. superbum, 1.5mg l⁻¹ BA + 3mg l⁻¹ IBA was used for multiplication (Mathew and Philip 1996). The plantlets from
<table>
<thead>
<tr>
<th>Genus/species</th>
<th>Initial explant</th>
<th>Culture medium</th>
<th>Plant growth regulators (mg l⁻¹)</th>
<th>Culture conditions</th>
<th>Plant regeneration</th>
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<tr>
<td><em>Ensete</em> sp.</td>
<td>Zygotic embryos</td>
<td>Modified MS, Cox</td>
<td>Pre-incubation in 4 GA₃ for 15–20 min</td>
<td>–</td>
<td>20–60% germinated, callusing and swelling embryos in 8 weeks</td>
<td>Bezuneh 1980</td>
</tr>
<tr>
<td><em>E. ventricosum</em> (Genotypes Choro, Ketano and Nobo)</td>
<td>Zygotic embryos</td>
<td>MS</td>
<td>Germination: 0.45 BA + 0.17 IAA Rooting: 1.02 IBA</td>
<td>25°C in the dark</td>
<td>14–71% <em>in vitro</em> germination of the embryos in 10–12 days</td>
<td>Negash <em>et al.</em> 2000</td>
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<tr>
<td><em>E. ventricosum</em></td>
<td>Shoot tips of greenhouse-grown suckers</td>
<td>MS</td>
<td>2.7 BA + 0.5 IAA</td>
<td>–</td>
<td>A few plants were regenerated; extensive explant blackening occurred</td>
<td>Afza <em>et al.</em> 1996, Morpurgo <em>et al.</em> 1996</td>
</tr>
<tr>
<td><em>E. ventricosum</em></td>
<td>Corm explants</td>
<td>MS</td>
<td>9 BA</td>
<td>27°C and 3000µmol m⁻² s⁻¹</td>
<td>Callus was induced in six weeks; adventitious shoots regenerated from callus and plants from somatic embryos</td>
<td>Afza <em>et al.</em> 1996, Morpurgo <em>et al.</em> 1996</td>
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<td><em>E. superbum</em></td>
<td>Shoot tips with 2–3 leaf primordia with small portion of corm from six-month-old greenhouse-grown plants</td>
<td>MS</td>
<td>Initiation: 5 BA + 0.1 IAA and transferred to 5 BA + 1 TDZ</td>
<td>27°C and 16h photoperiod</td>
<td>Swelling and callusing explants after 3–4 months</td>
<td>Zeweldu 1997</td>
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<tr>
<td><em>Ensete</em> sp. and <em>E. ventricosum</em></td>
<td>Swelling and callusing explants</td>
<td>MS</td>
<td>4.5 BA + 0.017 IAA or 0.19 NAA</td>
<td>–</td>
<td>Adventitious shoots</td>
<td>Zeweldu 1997</td>
</tr>
<tr>
<td><em>E. ventricosum</em> (Genotypes Choro, Ketano and Nobo)</td>
<td>5mm long corm and 2cm long leaf explants from 5-month-old greenhouse-grown plants or from <em>in vitro</em> germinated zygotic seedlings</td>
<td>MS</td>
<td>Initiation: 2.25 BA + 0.17 IAA Multiplication: 2.25 or 4.5 BA + 0.17 IAA; Rooting: 1.02 IBA + 0.225 BA + 1000 AC</td>
<td>25°C, 16h photoperiod</td>
<td>About 30 shoots per explant in four months</td>
<td>Negash <em>et al.</em> 2000</td>
</tr>
<tr>
<td><em>E. ventricosum</em></td>
<td>Shoot tips</td>
<td>MS with 1000 or 2000mM mannitol</td>
<td>2.25 BA</td>
<td>At 15°C or 18°C for slow growth</td>
<td>Shoots were stored for six months and regenerated</td>
<td>Negash <em>et al.</em> 2001</td>
</tr>
<tr>
<td><em>E. superbum</em></td>
<td>Shoot tips 10–15mm in length from two-three-year-old plants grown in a botanical garden</td>
<td>MS</td>
<td>Initiation: 1.5 BA + 1 Kinetin; Multiplication: 1.5 BA + 3 IBA; Rooting: 3 IBA + 0.1 BA</td>
<td>Initiation: 24 ± 2°C in the dark; Multiplication: 12h photo-period with an irradiance of 30µmol m⁻² s⁻¹</td>
<td>84 shoots per corm in three months</td>
<td>Mathew and Philip 1996</td>
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<tr>
<td><em>E. superbum</em></td>
<td>About 10mm shoot tips from field grown plants</td>
<td>MS</td>
<td>10 NAA + 2.5 Kinetin 2 NAA + 2.5 Kinetin for plant regeneration</td>
<td>–</td>
<td>A proliferating callus in 120 days after first inoculation; shoots with roots were regenerated after 60 days of subculture</td>
<td>Mathew <em>et al.</em> 2000</td>
</tr>
<tr>
<td><em>E. superbum</em></td>
<td>Shoot tips from plants regenerated and maintained in <em>in vitro</em></td>
<td>MS</td>
<td>Embryogenic callus induction: 2.24-24 D + 1.5 BA; Somatic embryo production: hormone free MS</td>
<td>–</td>
<td>Embryogenic callus in 90 days and somatic embryos after 40 days</td>
<td>Mathew <em>et al.</em> 2000</td>
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<td><em>E. superbum</em></td>
<td>Shoot tips from plants regenerated and maintained in <em>in vitro</em></td>
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<td>Embryogenic callus induction: 2.24-24 D + 1.5 BA; Somatic embryo production: hormone free MS</td>
<td>–</td>
<td>Somatic embryos were compared with zygotic embryos and similarities were found between their ontogenetic stages</td>
<td>Mathew and Philip 2003</td>
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</tbody>
</table>
Ensete showed high rooting capacity on hormone-free MS medium (Zeweldu 1997), while 1.02mg l⁻¹ IBA + 0.22mg l⁻¹ BA + 1g l⁻¹ activated charcoal for rooting of Ensete ventricosum (Negash et al. 2000) and 3mg l⁻¹ IBA + 0.1mg l⁻¹ BA for Ensete superbum (Mathew and Philip 1996) were used. Rooted in vitro plants of Ensete superbum (Mathew and Philip 1996) were planted in plastic cups containing compost and acclimatised in a mist chamber.

Bezuneh (1980) observed that blackening of explants, formation of unwanted callus and a low rate of multiplication in vitro were associated with shoot tip culture. The blackening of cultures and callusing of explants inhibited formation of roots from zygotic embryos of Ensete in vitro. Shoot tips of Ensete ventricosum at the initiation stages turned brown/black and became necrotic, which resulted in failure of initiation of the explants in vitro (Afza et al. 1996, Morpurgo et al. 1996, Zeweldu 1997). Maintenance of initiating shoot tips of Ensete superbum in the same medium for more than four weeks resulted in blackening and necrosis of the explants (Mathew and Philip 1996). Activated charcoal and frequent subculturing were used to reduce the problem of explant blackening. Swelling and callusing of shoot tip explants of Ensete at the initiation stage was also a problem (Afza et al. 1996, Zeweldu 1997).

The indirect production of adventitious shoots from explants via a callus stage may increase the problem of genetic instability of the progeny. It is suggested that the more organised the explant, the less the variation in progeny; and the less organised the starting material, the more the variation (Krikorian 1989). Using this view, shoot tips would progressively generate the most varied plantlets. The more organised the starting material, the less variation (Krikorian 1989). Using this view, shoot tips would progressively generate the most varied plantlets. This is possibly due to a monopodial corm morphology of Ensete species is important, given the value of this crop as a staple food in many African countries.

Callus culture and somatic embryogenesis

Callus is a coherent and amorphous tissue formed when plant cells multiply in a disorganised way (George and Sherrington 1984). Root formation generally takes place in a medium with a relatively high auxin and low cytokinin concentration while formation of adventitious shoots takes place if there is a low auxin and high cytokinin concentration (Pierik 1987). Mostly, whole plant regeneration from cultured cells may occur either through shoot-bud differentiation (organogenesis) or somatic embryogenesis. Organogenic events of Ensete callus are influenced by the ratios of auxins and cytokinins as well as the light regime (Zeweldu 1997). Under the same hormonal treatment, light promoted differentiation of callus with subsequent organ formation, whereas the callus that was maintained in darkness usually grew without further differentiation. The greatest number of adventitious shoots was observed with 4.5mg l⁻¹ BA in combination with either 0.017mg l⁻¹ IAA or 0.19mg l⁻¹ NAA (Zeweldu 1997). Morpurgo et al. (1996) established callus cultures from corm explants and adventitious buds of Ensete ventricosum.

Somatic embryos of Ensete ventricosum could be obtained by lowering the level of cytokinins in the medium (Afza et al. 1996, Morpurgo et al. 1996). In Ensete superbum, the shoot tips from in vivo conditions cultured on MS medium supplemented with 10mg l⁻¹ NAA, 2.5mg l⁻¹ kinetin and 1g l⁻¹ L-glutamine produced a proliferating callus within 120 days of culture from which adventitious shoots were regenerated but somatic embryos were not formed (Mathew et al. 2000). The presence of 2mg l⁻¹ 2,4-D and 1.5mg l⁻¹ BA was important for induction of a proliferating embryogenic callus of Ensete superbum from the corn tissue at the base of in vitro generated plants. However, the differentiation and maturation of somatic embryos happened only in hormone-free medium (Mathew and Philip 2003).

Conclusions

The agricultural importance of Ensete is not widely known. As a result, only limited in vitro investigations have been carried out in the past. Studies using shoot tip explants of Ensete ventricosum have shown low levels of multiple shoot formation. This is possibly due to a monopodial corm morphology and complete apical dominance. The use of cytokinins does not result in a large amount of multiple shoot formation from the shoot tip explants in Ensete ventricosum. Moreover, extensive blackening, as a result of phenolic oxidation creates unfavourable culture environments and causes high rates of tissue mortality. Further in vitro investigation on the propagation of Ensete species is important, given the value of this crop as a staple food in many African countries.

Acknowledgements — The financial support of the Agricultural Research and Training Project of Ethiopian Agricultural Research Organization and University of KwaZulu-Natal is appreciated.

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