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In Vitro Propagation and Conservation of Tropical RTBs

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Staple clonally propagated crops such as root, tuber, banana and plantain (RTBs) crops are subjected to genetic erosion and variety loss under the pressure of many factors. The combination of these includes habitat loss and climate change including increased pest and diseases incidence. Safeguarding genetic resources of those food security crops for future use is critical through ensuring their sustainable conservation and use, for global food and nutrition security.

The first conservation system for RTBs crop diversity is *in situ* especially for crop wild relatives. But *in situ* conservation of these world important food crop genepools is highly under-resourced and inadequately managed. Therefore, *ex situ* conservation is key to holding materials in genebanks or seed banks (conserved as seeds, field plants, vegetative shoots *in vitro* or cryopreserved materials) that are also backed-up in another location.

In general, clonally propagated crops (produce very few seeds, are vegetatively propagated for breeding reasons and/or species that require a long life cycle to generate breeding and/or planting materials) such as RTBs, essentially, are not conserved as orthodox seeds. The germplasm is either conserved as live plants in fields, as potted plants in enclosed structures or different plant parts are conserved into *in vitro*, either in slow growth (medium term) or cryopreservation (long-term). Traditionally, the conservation and maintenance of root and tuber crops is done in field conditions. Many technical guidelines and training manuals exist for the management of RTBs germplasm collections held in field genebanks (Reed *et al.*, 2004a; Geburek and Turok, 2005). However, major challenges of field conservation of RTBs are long reproductive cycles, associated with low multiplication rate, high cost, pest and diseases, mislabelling and duplication leading to germplasm losses. These disadvantages are magnified by climatic factors.

In vitro biotechnological approaches are reliable, complementary and/or alternative system to support multiplication, safer and longer conservation RTBs genetic diversity, and their sustainable utilisation. They have the potential to address future, technical, scientific, economical and environmental demands on RTBs (Pilatti *et al.*, 2011).

In vitro Propagation of RTB Crops

The major advantage of *in vitro* propagation of RTBs material is the potential of large multiplication for the seed system, e.g. as an efficient and cost-effective propagation system (Asiedu *et al.*, 1998; Quin, 1998; Thro *et al.*, 1999). The latter authors report two projects in Latin America that used cassava in *vitro* culture to address priorities of small-scale cassava farmers. Cassava propagation is generally done using lignified stem cuttings (Thro *et al.*, 1999). The multiplication rate is as low as 1:10 compared to at least 1: 100 in some cereals, creating a bottleneck for transfer and adoption of new varieties.

Tissue culture techniques have been used for RTB multiplication *via* organogenesis, mainly from younger and vigorous mother plants that might allow higher micropropagation rate (Mitchell *et al*., 2006). For yams, pre-formed meristems (Malaurie *et al*., 1995a, b), shoot organogenesis from immature leaves (Kohmura *et al*., 1995), roots (Twyford and Mantell, 1996) shoot/nodes culture and microtuber formation (Balogun *et al.*; 2006; Ovono, 2007; Salazar and Hoyos, 2007) have been used to initiate *in vitro* multiplication. When the culture medium is supplemented with gibberellin inhibitors, shoots from nodal explants in (Poornima *et al.,* 2007) and numerous axillary nodes (Bimbaun *et al*., 2002; Balogun, 2005) were reported in many yam species.

Among the various *in vitro* propagation techniques for RTBs, synthetic seeds (Standardi and Piccioni, 1998)

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can be cited and recently somatic embryogenesis. Somatic embryos were induced from leaf tissues of *D. rotundata* in culture medium containing 2.4-D and incubated in darkness. However, low induction frequencies (<30%) were recorded and protocols need to be optimized. In *D. alata* and *D. opposita*, embryogenic cell masses were induced from root explants in liquid MS supplemented with 2.4-D and cultured in light (Twyford and Mantell, 1996; Nagasawa and Finer, 1989). Germination of somatic embryos of *D. alata* increased in the presence of GA_3 (Deng and Cornu, 1992; Twyford and Mantell, 1996). Plantlet recovery from somatic embryos of *D. rotundata* was enhanced at 4.5% sucrose but not affected by benzylaminopurine (Okezie *et al*., 1994; Pandro *et al*., 2011). These reports pointed out probable genotype-dependent protocol for yam embryogenesis.

Photoautotrophic propagation (PAP) is another system that should be used for RTBs. In PAP system, explants are directed towards autotrophy while in culture by reducing or completely substituting sucrose with carbon dioxide. Plantlet growth is enhanced in photoautotrophic more than heterotrophic conditions if environmental control is adequate (Hazarika *et al*., 2003; Xiao, 2004, Afreen, 2005). However, after about four weeks, plants may stop responding to carbon dioxide and growth is reduced due to slight closing of stomata on the bottom of the leaves as plants sense high CO_2. However, use of CO_2 enrichment for two weeks, then a week off intermittently will ensure higher yields as the plant continuously seeks as much CO_2 as possible (Andrew, 2002). In potatoes, much work has been done on PAP and photo-mixotrophic propagation (Mohamed and Alsadon, 2010; Santana *et al*., 2012).

The use of temporary immersion bioreactor systems (TIS) allowed handling of the culture by robotization, while optimizing multiplication rate of plantlet and microtuber production and sprouting (Cabrera *et al*., 2011; Watt, 2012). In TIS, growth is enhanced (Escalona, 2006) since there is lack of continuous immersion in liquid/semi-solid culture medium; contamination is controlled while aeration is timed. In addition, microtubers from TIS can be grown on the field and used in original seed production programmes. Enhancement of growth in TIS can to the exploration of medicinal secondary metabolites production, like yam steroidal diosgenins (Raju and Rao, 2012). Shoots grown in TISs had enhanced growth and the leaves had higher photosynthetic pigment content than other techniques (Jova *et al*., 2011, 2012; Cabrera *et al*., 2011).

In Vitro Slow Growth Conservation and Cryopreservation

Slow growth conservation leads to the reduction of loss risks associated with the field banks, and constitute a viable alternative to complement and reduce the large size required for field banks. RTBs genebanks around the world have *in vitro* tissue culture facilities as a complementary conservation system, giving the possibility to clean the germplasm from diseases and pest via meristem culture and/or coupled with other cleaning methods. This conservation method requires technical expertise, facilities and operating budget. Though, they are generally more economical and less risky in a long-term perspective; as compared to field collections. Plant tissue culture is a powerful tool for safer and faster way to multiply large quantity of material for distribution, duplication in other genebanks and international exchange (easier plant material transport); and also for breeding purposes. Slow growth storage is however for short to medium term conservation, after which the plantlets are subcultured when signs of deterioration/necrosis are visible (Balogun, 2009). The conservation of RTBs needs small quantity of material and allows longer duration between two regenerations or subcultures, using slow growth storage. The principle is to place the *in vitro* plantlets under slow growth conditions, through adaptation to physical factors (light, temperature, culture medium, growth retardants). According to Ng and Ng (1997), 47 countries were holding cassava collections but only 12 maintained *in vitro* facilities for conservation. Many of these laboratories combine *in vitro* techniques target for pathogen cleaning with rapid multiplication and genebank conservation. *In vitro* conservation of cassava is still far less common than field conservation. The largest national *in vitro* collections are held in Brazil and Argentina. There appear to be very few RTBs *in vitro* genebanks in Africa. International collections are held at CIAT, CIP, Bioversity International and IITA, while all other *in vitro* genebanks have a national or regional focus.

Cryopreservation, almost systematically associated with *in vitro* conservation, is another conservation method for RTBs germplasm. It allows maintenance of plant material at ultra-low temperature (in liquid

nitrogen at -196°C) using cryogenic techniques. At such low temperature, plant cell biological activities and metabolism are stopped, eliminating the need to regularly rejuvenate or regenerate the plant. It is currently a supplementary tool to improve conservation of germplasm in a longer-term perspective. Cryopreservation is the most reliable technique for long-term storage of plant genetic resources (Popov *et al.*, 2005). It avoids the disadvantages of irreversible loss of totipotent competencies caused by *in vitro* ageing process (Benson, 2008), time and labour consumption. Many studies confirmed that it's economically more competitive compared to other conservation systems (Harvengt *et al.*, 2004; Reed *et al.*, 2004a; Keller *et al.*, 2008). Cryopreservation helps to overcome many of *in vitro* maintenance disadvantages such as labour-intensive sub-culturing, potential elimination of pathogens and somaclonal variation related to multiple subcultures. It also ensures the safe long-term conservation of genetic resources. Thus, cryopreservation techniques have been increasingly used for long-term storage. In the last 25 years, several cryogenic techniques have been developed, especially those based on vitrification method (the transition of water directly from the liquid phase into an amorphous or "glassy" phase, whilst avoiding the formation of crystalline ice) such as encapsulation-dehydration, preculture-dehydration, and encapsulation/vitrification. Therefore, the main requirement for using cryopreservation method is that it should be simple, economical, reproducible and should allow relatively high regrowth rate Leunufna and Keller (2003).

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