Importance of Tissue Culture for Orphan Crops

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Abstract

Tissue culture is one of the most basic biotechnological tools available in sub-Saharan Africa (SSA), and its applications are varied and vast. The technique has contributed tremendously to the safeguarding, improvement and distribution of orphan crops, especially the vegetatively produced crops. As a tool, it has been a driver for biotechnological advances made in orphan crops, both for research as well as commercial purposes. Tissue culture is also a vehicle to most efficiently deliver important biotechnological products such as genetically modified orphan crops. Commercial micropropagation, despite potential pitfalls, is essential tool to distribute crops such as cassava and banana to smallholder farmers in SSA.

Keywords: micropropagation, orphan crop, tissue culture

1. Introduction

In sub-Saharan Africa, several orphan crops are essential for food security and income generation of smallholder farmers, ensuring their livelihood. Orphan crops can be organized as fruit crops [including banana and plantain (*Musa* spp.)], root and tuber crops [cassava (*Manihot esculenta*), sweetpotato (*Ipomoea batatas*), yam (*Dioscorea* sp.), enset (*Ensete* spp.), taro (*Colocasia esculenta*) and *Plectranthus* spp.], cereals [pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), fonio (*Digitaria* spp.) and tef (*Eragrostis tef*)], legumes [cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogaea*), grass pea (*Lathyrus sativus*) and bambara groundnut (*Vigna subterranea*)], and oilseed crops [sesame (*Sesamum indicum*) and noug (*Guizotia abyssinica*)].

Some of these orphan crops are relatively better researched than others, and it is therefore dangerous to lump them all into the same category. For example, research into bananas on the continent has been implemented by the International Institute of Tropical Agriculture (IITA) and the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) since decades, and has covered in depth not only upstream issues (pests and diseases, conventional breeding, etc.) but also downstream ones (market pathways, product diversification, etc.). It is equally encouraging to notice that The Alliance for a Green Revolution in Africa (AGRA), a joint venture between the Rockefeller Foundation and the Bill and Melinda Gates Foundation, is taking a proactive interest in some orphan crops like cassava and cowpea [1]. However, all crops have in common that they are largely ignored by the international community and are almost solely investigated by Africa-based international organizations and their national partners.

A large discrepancy exists between the potential role of these crops in improving food security and livelihoods, and the low levels of private or public investment they have received [1, 2]. The reason why these orphan crops receive relatively less attention in sub-Saharan Africa compared to, for example, maize (*Zea mays*) and rice (*Oryza sativa*) is unknown and surprising. For example, sorghum and pearl millet are more important than rice and wheat, both in area and in contribution to diet. Cassava is the third most important source of calories in Africa [3]. In Uganda, the average annual per capita consumption of banana is estimated at 207 kg [4]. Furthermore, many of these orphan crops, such as cassava, are known for their hardiness and tolerance to adverse environmental conditions such as infertile soils [5]. Finally, many of these orphan crops, cultivated mainly in Africa, are less vulnerable to price fluctuations on international markets and act as safe havens when international prices increase.

2. Tissue culture as a research tool for orphan crops

Tissue culture is a wide concept, and involves the culture or maintenance of plant cells or organs in sterile *in vitro* conditions. The technique has been applied to only a number of orphan crops, and most applications included the exchange of breeding material and the production of disease-free germplasm through micropropagation [6]. Nevertheless, applications of tissue culture are varied and vast, and examples from orphan crops are highlighted below.

2.1. Germplasm conservation

Orphan crops are very diverse in terms of their genetic, agroclimatic, and economic niches [7]. This diversity needs to be captured, and tissue culture techniques such as cryopreservation are essential for the conservation of large gene pools of especially vegetatively propagated crops. At IITA, an extensive *in vitro* genebank of cassava and yam is maintained for worldwide distribution [8]. Such genebanks function as a foundation for conventional breeding. As a matter of fact, molecular marker characterization of genetic diversity from genebanks appear to be the most widely used biotechnological technique on orphan crops [6].

2.2. Recombinant proteins and biopharmaceuticals

Plant cells can be cultured commercially inside liquid culture in large bioreactors as a source of secondary products like recombinant proteins or biopharmaceuticals. Examples of such technology among orphan crops are few, although, based on their intrinsic diversity and unexplored potential, the production of secondary metabolites from orphan crops could be worthwhile. For example, Jin *et al*. [9] used the hairy root technique to mass produce recombinant phytase from sesame.

2.3. Conventional breeding

Tissue culture is very important for breeding of clonally propagated orphan crops because it allows movement of sufficient quantities of pathogen-tested plants for safe multi-locational screening, not only internationally but also within large countries.

Several specialized tissue culture techniques exist that are fundamental to breeding efforts in orphan crops. *Anther culture* (androgenesis) generates haploid plants from microspores, and significantly speeds up crosses between distantly related species. If barriers are present that prevent development of interspecific crosses, it is sometimes possible to aseptically culture the plantlets from the embryo in a technique called *embryo rescue*. For example, embryo rescue is an essential technique, used for decades, in banana breeding. Another, more complicated method to cross distantly related species is by *protoplast fusion*. Tissue culture techniques are also being used for production of *dihaploid plants* to reduce breeding cycle by obtaining pure line for further improvement in crops like tef [10]. Finally, using *somaclonal variation* and *in vitro* mutagenesis, plant breeders have actively used tissue culture systems to create variability in their breeding programs.

One of the more recent promising applications of tissue culture is that it allows plant breeders to screen for advantageous characters in cells rather than plants, thereby greatly reducing time and costs. Studying cells opens the way for genomics, and the related fields of proteomics and metabolomics. Ngara et al. [11] produced cell cultures of sorghum (*Sorghum bicolor*), which provided them with a continuous supply of experimental units.

2.4. Recombinant DNA technology

Although genetic transformation can be performed on explants, protoplasts or cell suspensions obtained through tissue culture are usually the starting material for genetic transformation. Somatic embryogenesis followed by regeneration of transgenic plants is often a central component in genetic modification. For example, most transformation protocols for banana use cell suspensions, although systems are being developed that are based on organogenesis from meristematic tissues [12].

Plant regeneration is a prerequisite for successful transformation. However, this tissue culture step is sometimes the most difficult and limiting step in the development of genetic engineering technology for orphan crops such as sorghum and millet [13]. O'Kennedy *et al*. [14] established an improved regeneration system for pearl millet using immature zygotic embryo explants. The lack of an efficient regeneration system had also slowed the improvement of cowpea, an orphan legume crop. However, Diallo *et al*. [15] reported an efficient regeneration method from cotyledonary node explants, which is different from that of other *Vigna* spp.

Plant generation systems are often highly genotype-dependent and therefore problematic especially in orphan crops due to their genetic divergence. Successful millet tissue cultures show strong genotype dependency and rapidly lose their morphogenic capacity after subculturing [13]. In cassava, an efficient regeneration system based on embryogenesis has been developed but it is, however, limited to a few cultivars. Development of regeneration systems that are genotype-independent are important for orphan crops.

Another challenge is the optimization of gene delivery system for orphan crops. Although efficient transformation techniques have established for model crops, the same method can not be applied to other crops including orphan crops. Hence, optimum transformation method has to be investigated for each crop type. This might require special facilities and significantly increase the cost and time of study. Although, cowpea is known to be recalcitrant to transformation, efficient DNA delivery techniques has been recently developed [16].

3. Commercial tissue culture for orphan crops

3.1. Meristem culture: virus elimination

Meristem culture is a technique used to eliminate viruses from virosed plants based on propagation from meristematic tissues. Virus elimination techniques have been used for the last 20 years in South Africa for the production of disease-free planting material for sweet potato. All commercial plantings of sweet potato in South Africa use these types of matrials in order to suppress the level of disease present in the field [17].

3.2. Micropropagation: mass production

Micropropagation uses meristem and shoot culture on stock plants to rapidly produce large numbers of clonal plantlets that are pest- and disease-free. Somatic embryogenesis, in conjunction with genetic transformation, can also be used for mass production. For many plants, healthy seeds are easily produced in great numbers, rendering micropropagation not applicable. However, micropropagation is particularly important for sterile plants (i.e., in the absence of seeds or pollinators to produce seeds), that have low-germinating seeds, do not produce enough seeds or produce seeds that cannot be stored (recalcitrant seeds).

Many of Africa's orphan crops, such as cassava, banana, sweet potato and yam, are vegetatively propagated [8, 18, 19] and are consequently perfect candidates for commercial micropropagation. Since orphan crops are genetically diverse, new cultivars adapted to local conditions can be constantly developed. Efficient distribution channels for these improved cultivars, however, remain a problem [2]. Micropropagation is ideal when it comes to rapidly upscale production and delivery large quantities of superior cultivars and as such a perfect distribution channel for these improved yet underutilized cultivars [6].

3.3. The delicate balance between private and public sector

By 1992, very few countries in sub-Saharan Africa had reached the take-off stage for large-scale micropropagation of important crops. However, several countries had established biotechnological centers of regional or international character specializing in micropropagation of not only cash crops such as coffee (*Coffea* spp.) and vanilla (*Vanilla* spp.), but also orphan crops such as banana, cassava and cowpea [20].

Ultimately, micropropagation for mass production should be carried by the private sector, which fills a niche in sub-Saharan Africa by focusing on commercial production of orphan crops. In West Africa, some successful public-private partnerships have been set up for this purpose. In 1986, CIRAD set up the subsidiary Vitropic (Saint-Mathieu-de-Tréviers, France) to produce disease-free banana plants [19]. Especially banana micropropagation has seen a rapid commercial growth in some countries in East Africa, with a handful of small- and medium-scale enterprises collectively producing in excess of more than 1.5 million banana plants per year in Burundi, Kenya and Uganda. Although the entry barrier is steep, the "tissue culture business" is very lucrative for the entrepreneur who engages in it.

In several countries in Africa, however, tissue culture orphan crops continue to be commercially produced by both the private and public sector, often with their roles blurred. In Zimbabwe, for example, both academic institutions and private organizations have been actively involved in supplying tissue-cultured planting materials to smallholder sweet potato farmers [21]. In Uganda, Kenya and Burundi, private companies compete with universities and research organizations in production of banana. On the other hand, researchable issues are sometimes too lightly transferred to the private sector, under the label of "public-private partnerships" and in vogue with some of the donor's current perceptions. Roles for each should be clearly defined, so that donors and governments can engage in more efficient use of taxpayer money.

The burden for crop improvement will continue to solely fall onto the shoulders of the public sector. Sometimes, the public sector can play a temporary but essential role in micropropagating orphan crops with little commercial value even for local tissue culture laboratories. In South Africa, the Agricultural Research Council (ARC) micropropagated Livingstone potato (*Plectranthus esculentus*), a popular semi-domesticated orphan crop once part of the diet of rural communities but whose planting material became neglected. Tissue culture was used to rapidly produce plantlets and reintroduce them to the Northern Province to benefit resource-poor farmers [22]. Another temporary role of the public sector focuses on the development of proper tissue culture protocols (e.g. optimal growth media, reduction of off-types or reduction of secondary metabolites during multiplication).

3.4. Dangers and bottlenecks of commercial tissue culture

One of the biggest dangers for sustainable commercial tissue culture is the lack of phytosanitary and quarantine conditions, in the form of certified standards, codes, protocols and laws that are regionally harmonized. Such conditions are especially important to avoid spread of viruses, which are easily transmitted through tissue culture. In the case of banana, viruses such as Banana Bunchy Top Virus (BBTV) and Banana Streak Virus (BSV) are widely distributed on the continent [23, 24] yet implementation of harmonized virus indexing schemes are largely absent in East Africa, despite the fact that tissue culture bananas are being moved across borders in ever increasing quantities. Based on the experience from East Africa, where the private sector is outpacing implementations from public phytosanitary and quarantine institutions, it is clear that the capacity to use tissue culture to generate clean planting material must be developed in tandem with efficient virus indexing mechanisms. Elements of such mechanisms include rapid diagnostic kits for detection during import inspections, certification of nursery-propagated materials, establishment of independent institutes that set and implement standards, and properly trained personnel [25, 26].

Physical infrastructure is expensive, while human capacity is often lacking. The costs of establishing a tissue culture laboratory in sub-Saharan Africa is relatively high, since most of the equipment and chemicals are imported at elevated costs [25]. In addition, water and electricity supply is sometimes erratic, further elevating the cost. These elevated production costs are ultimately off-loaded onto costumers.

A danger for a healthy commercial tissue culture sector is the lack of sustainable market pathways to deliver the plants to the farmer. In Burundi, tissue culture plants are bought from the private sector but given for free to farmers by donors. This temporary solution is not sustainable and reduces demand and flow of improved seeds, fertilizers, tools and pesticides [27]. In some cases, commercial supply is trailing demand, which can be caused by private sector players focusing on large orders from donors, rather than small ones from farmers. In Zimbabwe, farmers increased yields and economic returns when growing tissue culture sweet potato compared to conventional material, but they were also constrained by an inadequate supply of improved planting material [21]. To make tissue culture systems sustainable, they often involve propagators as distribution points between the tissue culture laboratories and the farmers [25].

In sub-Saharan Africa, distributing planting material alone will not ensure a good crop. Whereas commercial farmers are skilled in juggling the inputs and effort needed to produce and make profit from crops, small-scale farmers are constrained by factors such as lack of land, capital, access to technology and good marketing infrastructure [2]. Hence, efficient distribution systems need to deliver the tissue cultured plants as part of an agronomic package, including training and access to micro-credit. Most of the current grants awarded by The Alliance for a Green Revolution in Africa (AGRA) address seed development programs [28]. A strong focus is on policy support and market development, and AGRA has set aside significant funds for creating conducive environments for agro-business development, including market development of tissue culture banana in East Africa.

Finally, for reasons of convenience, cost reduction and uniformity, biotechnology involves the passage of germplasm through narrow genetic bottlenecks. This is especially the case for commercial micropropagation [6, 29] and an aggravated danger for orphan crops that are heralded as resilient partly because of their genetic variability.

4. Tissue culture as a driver and a vehicle for biotechnology

4.1. Tissue culture as a driver

Tissue culture is a driver for biotechnology in two ways. First, as is demonstrated above, it lays at the basis of most other biotechnological technologies, from conventional breeding to genetic engineering. Although many countries in sub-Saharan Africa are becoming less hostile to genetically modified foods and many believe that transgenic technologies offer the key to unlocking the full potential of crops like cassava and banana [30], several scientific, legal, economic and political barriers exist to their widespread acceptance. As a result, genetically modified orphan crops, as opposed to tissue cultured ones, are still relatively a long way from routine use by smallholder farmers [8, 31].

Second, simple tissue culture techniques are the initial stepping stone for development of more advanced biotechnological research capacity in Africa because they are the easiest to implement. By focusing on tissue culture, the skills necessary to maintain and manage a biotechnology laboratory can be developed. The second phase is the application of more advanced biotechnological tools, such as molecular marker applications, ultimately leading the way for the third phase, which is the development of capacity to produce transgenic plants [18, 19]. Tissue culture capacity seems to be well present across sub-Saharan Africa. For instance, a recent survey conducted in 12 sub-Saharan countries indicated that national programs in all countries implement tissue culture, but only three of them apply genetic engineering [32].

4.2. Tissue culture as a vehicle

In the vegetatively propagated orphan crops, transgenic technologies might have the significant impact [30]. However, successful application of advanced biotechnologies is conditional on connecting the science to downstream delivery efforts [3]. Unfortunately, many of the laboratories that have the capacity to produce transgenic plants still lack the ability to commercialize the product or ensure that these plants reach the end user, i.e. the African farmer. To bridge this gap, it is necessary to form partnerships with either seed companies, producer organizations or government institutions who can ensure that the sophisticated technology be delivered in the most well known and accepted technology known to farmers: the seed [33]. In the case of the vegetatively produced orphan crops, this often translates in a vibrant commercial micropropagation sector, as detailed above.

5. The need for local capacity

In the future, the genetic and biotechnological improvement of orphan crops is confined to local and specialized research at specific crop centers within Africa [19]. In sub-Saharan Africa, there are many research organizations and universities with well established biotechnology facilities [8]. While some of the technologies from the west might be appropriate, many of the solutions in the future need to come from African research laboratories focused on African constraints [2]. Insights and tools with practical utility for orphan crops can be obtained from research obtained using model species and major crops [3]. However, local capacity in developing countries will still need to be built to address specific local problems [8, 33, 34]. Advanced tissue culture systems such as embryogenesis need to be adapted to suit germplasm, which requires time and resources. In the case of cassava, for example, it is important that development of tissue culture systems required for the transformation of specific cassava varieties be carried out within the respective cassava-growing regions.

Acknowledgements

The author wishes to thank Jim Lorenzen and Leena Tripathi for their critical input.

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