International Journal of Agricultural Technology 2021Vol. 17(3):959-976
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Potential role of somatic embryo-generated synthetic seed production on mass propagation of recalcitrant grain legume crops in Sub-Saharan Africa – A review article

Mangena, P.[*]

Department of Biodiversity, School of Molecular and Life Sciences, Faculty of Science and Agriculture, University of Limpopo, Private Bag X1106, Sovenga, 0727, Republic of South Africa.

Mangena, P. (2021). Potential role of somatic embryo-generated synthetic seed production on mass propagation of recalcitrant grain legume crops in Sub-Saharan Africa. International Journal of Agricultural Technology 17(3):959-976.

Abstract This paper reviews the potential role of synthetic seed production as another avenue for obtaining genetic variability, averting recalcitrance and fleeting seed viability challenges in legume species. Synthetic seeds, also known as synseeds or artificial seeds produced via somatic embryogenesis are also crucial for successful plant establishment and an important determinant of plant productivity, especially in recalcitrant legume species. Recalcitrant seeds are commonly characterised by a rapid moisture loss and reduced seed longevity, which further impose physiological/biochemical changes, that reduce seed viability, germinability and seedling vigour. The production of these kinds of seeds would perform a fundamental role in ensuring cheap and rapid supply of genetic resources to breeders and seed propagules for farmers in Sub-Saharan Africa. Furthermore, this approach would ultimately maximise crop yield and offer a tremendous potential for micropropagation and germplasm preservation of recalcitrant legume species in this region. This review analyses such a variety of applications and benefits of plant biotechnology in the African context, and highlights important advantages such as genetic uniformity, simplified handling, storage, efficient distribution etc. that could be of immeasurable benefit to the seed value chain in agriculture, particularly for rural communities in developing countries.

Keywords: Agriculture, Recalcitrant seeds, Somatic embryogenesis, Sub-Saharan Africa, Synthetic seeds

Introduction

Mass propagation by plant tissue culture is one of the most interesting and promising areas of applied and commercial research in plant biotechnology. This approach presents the opportunity to establish and maintain plant organs (shoots, roots, stems, embryos, flowers etc.) and tissues (cells, callus and

---

[*] **Corresponding Author:** Mangena, P.; **Email:** mangena.phetole@gmail.com/ Phetole.Mangena@ul.ac.za

protoplasts) under aseptic culture conditions for mass propagation. The system additionally exerts a high degree of culture control over each aspect of *in vitro* regeneration, especially through the control of environmental culture conditions to accelerate and successfully achieve clonal propagation in many commercially viable and recalcitrant plant species. Among the techniques used to regenerate plants via tissue culture, the use of synthetic seeds (also called synseed or artificial seeds) may serve as a valuable alternative technology for preservation and micropropagation of important agroeconomic legume crops (Rihan *et al*., 2017). Synthetic seeds contain somatic embryos that are directly encapsulated in artificial seed coats or embryos that are partially dehydrated before being encapsulated (Bhatia, 2015). The seeds can then be sown for germination like traditional seeds, which is a way of sowing somatic embryos for mass propagation.

Synthetic seeds may be used for clonal propagation to replace traditional practices that include natural seed propagation and hand-pollinated hybrids. The technique may also lead to the development of drought tolerant varieties and disease-free or resistant plants. Thus, this technique can be fast-tracked for commercial applications such as crop improvement and various other strategies for inducing genetic interference. Therefore, this procedure holds considerable potential for major biotechnological applications that are highly required to counteract seed recalcitrance of many food legume crops grown in Sub-Saharan Africa where unemployment, poverty and hunger are rife. Sub-Saharan African countries import an increasingly high number of agricultural products than most countries and the population still experiences the highest prevalence of undernourishment (Shimeles *et al*., 2013). The agricultural sector's productivity in this region still lags far behind other regions. This is primarily due to the lack of innovation in agriculture and limited adoption of improved seed varieties. Modernisation of agriculture by the inclusion of biotechnological approaches and modern breeding tools, as well as the adoption of genetically improved varieties remain a major priority, and a prerequisite for achieving increased crop yield and quality.

Failure of implementation of agricultural policies, water scarcity, lack of significant financial investment and creative innovation in agriculture are some of the major persistent problems that are ever experienced in this region (Gashu *et al*., 2019). However, production of high quality seeds and other propagating materials will positively influence potential yield, distribution, processing and consumption of many recalcitrant legume crops. Legumes such as peanut, common bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.), lentil (*Lens culinaris*), mung bean (*Vigna radiata* L.), faba bean (*Vicia faba* L), cowpea (*Vigna unguiculata* L.), pigeon pea (*Cajanus cajan* L.) and soybean (*Glycine* *max* L.) belonging to the *Fabaceae* family, play a critical role in human nutrition (Shi *et al*., 2016; Jarpa-Parra, 2017; Solomon *et al*., 2017; Celmeli *et al*., 2018; Etemadi *et al*., 2019; Lu *et al*., 2019; Mangena, 2020a). Interest in these legumes as functional food and chemical ingredients immensely grew in the past decades. This prompted many studies focussing on their nutritional composition, agronomy and medicinal value (Shi *et al*., 2016; Etemadi *et al*., 2019). Furthermore, the global significance of these crops emanated from their relatively high amounts of quality proteins, carbohydrates, unsaturated fats, starch, phytochemicals and a high content of mineral elements (Mangena, 2020b).

Legume seeds contain lesser amounts of saturated fats and have no cholesterol. The seeds contain high amounts of oil comparable to that of cottonseed (*Gossypium hirsutum* L.) and rape/canola seeds (*Brassica napus*) (Imran and Nadeem, 2015). It is therefore, due to the above-mentioned benefits that, legumes showing recalcitrance to germination or genetic improvement be tested via direct or indirect somatic embryogenesis for mass propagation. Important factors that determine the application of tissue culture based protocols include the level of vigour/viability of seeds and explants, as well as the inefficiencies of *in vitro* regeneration protocols, when cultures are established under nutrient improved medium. This situation demands scientists to explore the potential role of synthetic seeds on clonal propagation of important recalcitrant legume seed crops to complement conventional seed propagation. The main objectives of this paper were to analyse the status of biotechnology in Sub-Saharan Africa, review the role of plant tissue culture and its specialised techniques such as synthetic seed technology in agriculture, and strengthen the debate around the use of mass propagation via synthetic seeds to reach commercial applications in this region.

Recalcitrant seeds in legume species

Almost all grain legume crops remain well known for their highest level of recalcitrance to both *in vitro* regeneration and plant genetic improvement. Recalcitrance means that, particular grain legume species may have the lowest responsiveness to *in vitro* regeneration and genetic transformation compared to their counterparts, for example; forage legumes and cereal grain crops (Hnatuszko-Konka *et al*., 2014). This obstacle continues to hamper progress in micropropagation and subsequent attainment of genetic variability, as observed in the most widely cultivated crop species including soybean, common bean, lentil and pigeon pea (Table 1). These crops already serve as important food legumes for human consumption in several Sub-Saharan African countries.

Recalcitrant legumes like chickpea, common bean, cowpea and groundnut form part of a daily diet in countries like Ethiopia, Kenya and Tanzania (Van Loon *et al*., 2018). Similarly, cowpea has also been indicated to form part of the staple diet for most rural populations in this region, especially in East and West African countries (Hnatuszko-Konka *et al*., 2014; Van Loon *et al*., 2018). Among these, a wide range of grain legume crops and varieties as indicated on Table 1 exist that exhibit different vegetative growths and yield characteristics, accompanied with the greatest potential niche on agriculture in Sub-Saharan Africa.

Table 1. List of some of the most recalcitrant grain legume crop species found in Sub-Saharan Africa

Species name	**Common name**
Phaseolus vulgaris	Common bean/ dry bean
Cicer arietinum L.	Chickpea
Vigna unguiculata (L.) Walp	Cowpea
Arachis hypogaea	Groundnut
Cajanus cajan (L.) Huth	Pigeon pea
Pisum sativum	Pea
Lens culinaris	Lentil
Phaseolus lunatus	Lima bean
Phaseolus coccineus	Scarlet runner bean
Phaseolus acutifolius	Tepary bean
Vigna angularis	Adzuki bean
Vigna radiata	Mungo bean
Vicia faba	Faba bean
Vigna subterranea	Bambara bean
Glycine max (L.) Merrill **	Soybean
Sphenostylis stenocarpa *	African yam bean
Mucuna pruriens *	Velvet bean
Canavalia ensiformis *	Jack bean

N.B. Legume species with asterisk show minor (*) or major (**) relevance at international level (Finch-Savage and Bassel, 2016; Snapp *et al*., 2018)

However, crop productivity remains un-intensified due to poor quality of seeds, lack of innovative breeding strategies for mass propagation of superior genotypes, lack of effective conservation strategies, frequent and severe droughts, as well as limited adoption of genetically improved varieties. These challenges will persist as long as certain physical seed characteristics, which have implications on legume seed germination, regeneration and amenability for genetic improvement, remain unachieved. These factors/characteristics will have further implications on the economic value of seeds, production, distribution, and conservation of threatened/endangered legume species. Extinction threats are genuine also for short-lived legume species whose seeds cannot undergo long-term storage and with well-maintained long-term seed viability. The long-term viability of legume seeds is critical for maintaining genetic diversity, particularly to enable the plants to survive unfavourable biotic and abiotic growth conditions (Molnar *et al*., 2015; Finch-Savage and Bassel, 2016; Snapp *et al*., 2018; Mangena and Mokwala, 2019).

Several key features required for successful germination and plant establishment under *in vitro* or *in vivo* conditions, as well in the field are presented on Table 2. Plant seeds are very critical and central to crop production, human nutrition and food security. Their genetic, physiological and physical characteristics define their ability to germinate and rapidly establish uniform seedlings robustly across diverse environmental conditions as echoed above. Seed banks established around the world contain cultivars and landraces of crops, but with only a small portion of them falling under wild type species of legumes used as crops. These seed collections have storage conditions favourable for maintaining seed viability (Molnar *et al*., 2015), but reports indicate that improvements of the conditions are still necessary for the conservation of some specialised recalcitrant legume species. Moreover, the number of neglected and underutilised grain legumes identified based on their domestication, adaptation and utilisation coupled with their general abandonment in research is increasing. Such neglected crops must be improved, explored and their utilisation awareness reawakened to reduce the over-dependency on major staple crops (Popoola *et al*., 2019). Therefore, the improvement of recalcitrant legumes using plant tissue culture, seed technology, omics technologies and recombinant DNA technology need to be advanced in order to save these crops from overexploitation and possible extinction.

Table 2. Vital characteristics of good quality seeds, which may differ according to specification of a variety

Seed trait
Genetic purity
Physical quality/ purity (for certification)
Shape, size and colour
Seed weight
Seed viability
Seedling vigour
Optimum seed moisture content (for storage)
Seed longevity
Physiological quality
Germination rate/ germinability
Good market value

Data source: Finch-Savage and Bassel (2016); Snapp *et al*. (2018) and Mangena (2020a).

Somatic embryogenic cultures and production of synthetic seeds

The plant embryo, also known as the seed embryo is the part of a seed or bud that contains the earliest multicellular stage of a plant's root, stem and leaf. An embryo that develops from sexual recombination of male and female gametes is termed a zygotic embryo. Although, there is familiarity with zygotic embryo development from seeds, which function as an intermediate stage in the transition between the gamete forming plants to spore producing plants. Plant embryos are also produced apoptically (without sexual reproduction) from somatic cells in plant tissue culture through a direct or indirect somatic embryogenesis (Gray, 2005; Rihan *et al*., 2017). This *in vitro* regeneration system also serve as a prerequisite tool for genetic improvement of horticultural crops, especially through techniques such as recombinant DNA technology, mutagenesis and gene editing methods like the clustered regularly interspaced short palindromic repeats and associated protein 9 (CRISPR-Cas 9). These technologies allow the genetic materials of plants to be altered, added or removed at particular loci in the targeted host plant tissues without the formation of undesirable gene combinations or chimeras (Raza *et al*., 2020).

Studies showing high rate direct somatic embryogenesis using immature cotyledons, leaf tissue explant or indirect embryogenesis from isolated mesophyll protoplasts were reported in legume species such as soybean (*Glycine max* L.) and barrel medic (*Medicago truncatula*) (Rose, 2019; Raza *et al*., 2020). In common bean (*Phaseolus vulgaris* L.), somatic embryogenesis was induced using cytokinin (benzyladenine) coupled with osmotic stress adaptation instead of supplementing the culture medium with an auxin-cytokinin hormone combination. The study reported pro-embryogenic cell masses derived from apical meristems and cotyledonary zones of zygotic embryos from which secondary somatic embryos were indirectly initiated (Cabrera-Ponce *et al*., 2018). Somatic embryos (non-zygotic) that are derived from vegetative cells, reproductive tissues or callus were recently reported to be functionally equivalent to zygotic embryos (Figure 1).

Moreover, non-zygotic somatic embryos from callus (Figure 1) developed from nutrients enhanced culture media, nutritionally similar to zygotic embryos that typically developed from nutritive seed tissues and were connected to the mother cells via a suspensor for further nourishment (Gray, 2005). Callus induced somatic embryos; including embryos from other explant types share the common trait of being able to be manipulated via *in vitro* culture. Such embryogenic culture systems form the basis for plant improvement using *in vitro* plant regeneration protocols, coupled with genetic improvement techniques as indicated above (Rose, 2019; Raza *et al*., 2020).

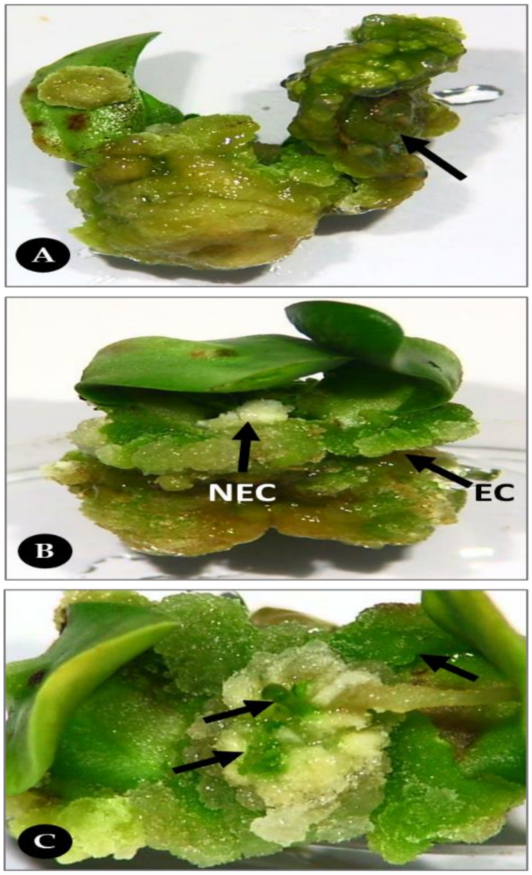

Figure 1. Somatic embryogenesis in soybean (*Glycine max* L.) using matured cotyledonary node explants, showing vitrified embryogenic callus cells (A), (B) non-embryogenic and embryogenic callus (B) and development of non-zygotic embryo from callus (C)

Somatic embryogenesis approaches can be used to bypass many barriers associated with sexual reproduction during plant propagation or genetic improvement, including preventing the inherent unwanted consequences of conventional breeding technology. Because of these many benefits and commonalities exhibited by these embryo types as well as the various methods available to study and manipulate plants regenerated from them, synthetic seeds emerged as a useful commercial plant propagation tool, which is efficiently served via direct non-zygotic somatic embryogenesis. Synthetic seeds, also known as artificial seeds, are encapsulated somatic embryos, and are produced from other explants such as shoot tips, axillary buds as well as zygotic tissue for deriving secondary embryos (Ravi and Anand, 2012). Synthetic seeds produced by encapsulation of somatic embryos in a matrix consisting of various concentrations of sodium alginate were reported using leaf explants cultured on Murashige and Skoog medium (MS).

Leaf explants cultured on MS medium containing thidiazuron (1.4-5.0 mg/L), dichlorophenoxy acetic acid (1.5 mg/L) and benzylaminopurine (2.0 mg/L) in African violet (*Saintpaulia ionantha* Wendl.) were reported (Taha *et al*., 2009). Synthetic seed technology was also used for germplasm preservation of transgenes and transgenic materials in alfalfa (*Medicago sativa*). Alfalfa somatic embryos were first transformed using *Agrobacterium* strain containing DNA construct with a *uid* reporter gene coding for *β-glucuronidase* (GUS) driven by a 35S promoter or a tCUP promoter. After desiccation, reported artificial seeds contained about 12-15% water content, equivalent to the normal seeds and exhibited high degree of viability as well as normal germination upon rehydration (Liu *et al*., 2013).

Requirement for synthetic seeds

Reports indicated that recalcitrant legume seeds exhibiting momentary decrease in seed viability and seedling vigour owe their response to the lack of suitable protective mechanisms or processes conferring tolerance to dehydration. The reduction on seed performance results from consequence variability of the responses to stresses, particularly factors imposing severe dehydration on the mature legume seeds. In cucumber (*Cucumis sativa*) and pea (*Pisum sativum*), dehydration of seeds induced imbalanced metabolism causing the loss of membrane integrity because of acetaldehyde and ethanol emission from pro-embryo or embryo tissues (Leprince *et al*., 2000; Awosanmi *et al*., 2020). Meanwhile in *Inga* (*Inga vera*) and other legumes, failure to reconstitute damaged microtubular cytoskeleton, short storability and altered cell cycle progression are directly associated with decreased germination and recalcitrance of the seeds (Faria, 2006). The biochemical and physiological processes, followed by morphological changes during seed formation and seed filling were also strongly related to seed longevity/viability and to the survival rate and vegetative growth of seedlings, which consequently affected yield and quality of seeds produced. Generally, metabolic "switch-off" and intracellular dedifferentiation are crucial mechanisms lacking in recalcitrant seeds, contributing significantly to their desiccation sensitivity (Berjak and Pammenter, 2013).

For instance, upon rehydration, orthodox seeds "switch-on" their germination program, resuming metabolic activities that leads to seedling formation. The expression of thousands of genes form part of this rapid swift from the quiescent state to vegetatively growing seedlings (Faria, 2006).

Additionally, when the developmental program of the individual mature ovules is switched over from embryogenesis to germination, various cell activities change on the level of gene expression, especially when seed embryos are subjected to unfavourable environmental stress. The germinating seed embryos induce the synthesis of anti-stress proteins to protect themselves and continue with seedling development (Azarkovich, 2016). Gene expression is responsible for the accumulation of dehydrin proteins as a result of drought, chilling, salinity and heat shock proteins as well as chaperones. General-stress-responsive (GSR) genes and heat-shock-proteins (HSP) genes including *hspA*, *groES*, *groELI*, *groEL2*, *dnaJ*, *htpG*, *dnak2*, *clpBI* and *htrA* are directly related to the molecular mechanisms providing abiotic stress tolerance (Zhang *et al*., 2015; Liu *et al*., 2019).

Abiotic stress often leads to excessive accumulation of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radical (O^{2-}) and hydrogen peroxide (H_2O_2). ROS causes oxidative stress damage to the DNA, protein and lipids in which these effects ultimately lead to the failure in seed formation, seed germination and seedling development. As ROS are implicated in the occurrence of various cellular events associated with the inhibition of seed germination, reports indicated that they also promote protection of seed embryos from pathogens and play a critical physiological role in breaking seed dormancy (Zhange *et al*., 2015; Azarkovich, 2016; Liu *et al*., 2019; Bailly, 2019). H_2O_2 for example, is considered a stable form of ROS involved in cellular signalling during germination (Petrov and Van Breusegem, 2012; Bailly, 2019). This ROS species was found to safeguard seeds from biotic stress attack by directly affecting pathogens or indirectly inducing programmed cell death (PCD) of the local infected cells. Therefore, considering the advantageous nature of the evidence presented above, it is my view that the production of synthetic seeds via direct or indirect somatic embryogenesis may serve as a proficient alternative to preserve and propagate recalcitrant legume species. Furthermore, the system may provide tight controls on the production and accumulation of ROS, while ensuring seed survival and maintenance of seed viability for longer periods.

Requirement for synthetic seeds in Sub-Saharan Africa

The production of synthetic seeds and consideration of the above-mentioned characteristics will contribute in the use of the produced seeds for germplasm preservation, breeding and the diverse utilisation of recalcitrant seed legumes in Sub-Saharan Africa. This region remains one of the most underdeveloped and highly densely populated regions around the world, yet it is still experiencing low crop yield compared to other regions globally (Figure 2 A). Sub-Saharan Africa is currently faced with a myriad of economic, health, social and food security crisis among others. The seed system and its economic value chain are under a massive strain due to inadequate functions resulting from the current narrow germplasm. The lack of organised innovative strategies to deal with adverse conditions (such as drought) and limited adoption of genetically improved cultivars that override internal or external inhibitions, preventing immediate germination also are implicated.

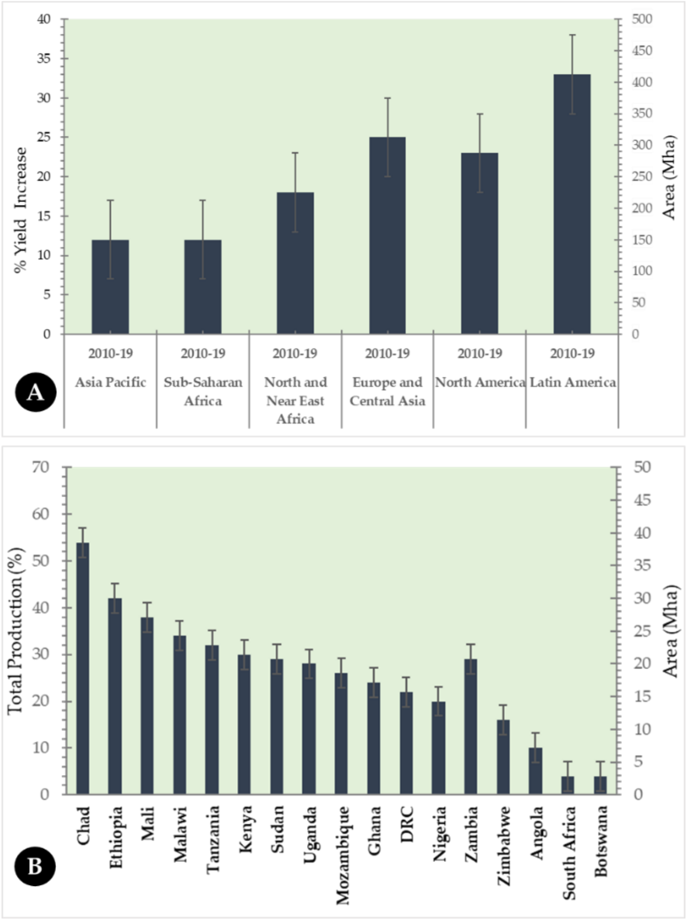

Figure 2. OECD-FAO estimates of agricultural production based on arable land available for agriculture globally: (A) Total crop yield per region (B) Average gross agricultural value in Sub-Saharan Africa from 2010-2016 (OECD-FAO, 2019)

Studies emphasised that, the necessary investments in agriculture, particularly those that would improve the low crop productivity as demonstrated in Figure 2 B, requires seeds that immediately respond to dormancy-breaking stimuli and germination cues under appropriate conditions (Leprince *et al*., 2000; Faria, 2006; Berjak and Pammenter, 2013; Azarkovich, 2016). Even though seed research has evolved and productivity/ improvement outcomes greatly varies across countries (Figure 2), renewed interest in the use of agricultural biotechnology applications in Sub-Saharan Africa is significant. Thus, synthetic seed formation and maturation suited for efficient rapid multiplication, growth and yield even under stress conditions must be developed to sustain life and maintain adequate food supply for poor rural communities in this region.

Potential role of synthetic seeds in Sub-Saharan African Agriculture

Generally, seeds are classified into recalcitrant and orthodox seed types based on their ability to germinate following rehydration under optimum growth conditions (Kumar *et al*., 2015). For orthodox seeds, known as desiccation tolerant, rapid changes on moisture content and metabolism contribute to high seed viability, as well as the high rate of germination upon subsequent receiving of adequate moisture (Kumar *et al*., 2015; Azarkovich, 2016). Unlike orthodox seeds, legume seed desiccation is directly associated with seed deterioration on both quality and germinability. This phenomenon makes seeds of legume species to be desiccation sensitive and prone to oxidative stress that leads to the loss of seed viability during seed storage. However, the use of synthetic seeds produced through plant tissue culture-based somatic embryogenesis as alternative for natural seeds may prolong longevity and ageing challenges faced by recalcitrant species. Recent studies have shown that seed deterioration naturally results from several factors including the genotype, growing environment, harvesting conditions, handling and storing conditions (Kumar *et al*., 2015; Berjak and Pammenter, 2013; Barbedo, 2018; Bereke, 2018). Meanwhile, synthetic seeds provide ease of handling, genetic uniformity, low cost of production and direct transplanting without acclimatisation into soil (Figure 3). Synthetic seeds produced from recalcitrant species would not require special procedures for cryopreservation or specialised conditions to improve amenability for plant genetic improvement (Figure 3).

This form of new seeds will have the ability to maintain high moisture content, metabolic readiness and ability for rapid germination without suffering desiccation-induced damage, which is frequently observed in natural recalcitrant seeds. The inability of recalcitrant seeds to survive partial post-harvest drying, exhibition of short life span and failure to survive in long-term storage in the form of active or baseline seed banks (Obroucheva *et al.*, 2016), make it impossible for farming systems to rely primarily on biological processes for the production of agro-ecologically based foods. The use of legume crops is considered the only alternative to the use of synthetic fertilisers. This may include minimal levels of CO_2/gases emissions, land degradation, tilling and other agricultural practices that are not eco-friendly.

There are seriously reported environmental concerns associated with the unsustainable agricultural practices, primarily including increased occurrence of invasive plant species, resistance to herbicides, impaired water quality and severe health effects, often affecting farmworkers, consumers and the general public (Snapp *et al.*, 2018). It is therefore important to also note that agricultural operations, involving the purchasing of seeds are also very expensive for farmers in Africa, a continent with almost no capacity to produce its own genetically improved varieties of legume crops. Therefore, implementation of synthetic seed technology that is not limited to the use of somatic embryos, but includes shoot buds, cell aggregates or any other plant tissue that can be explored for sowing as seeds and regenerate into a mature plant under *in vitro* or *ex vitro* conditions must be explored.

In addition to the abovementioned properties, the seeds must possess the ability to retain high seed viability after short-term or long-term storage, and maintain high seedling vigour for successful plant establishment even under abiotic stress conditions like drought (Figure 3). Apart from encapsulated somatic embryos, other induced somatic embryos can be proliferated for rapid and efficient large-scale *in vitro* multiplication of elite and desirable plants. The system can be effectively used in a number of micropropagation procedures when mass propagation from recalcitrant legume species, as well as when specific genetic barriers need to be overcome.

The plant tissue culture system, together with the synthetic seed technology could be used to efficiently produce planting materials transplanted into soil in large-scale commercial farming. Crops of other types like bread wheat (*Triticum aestivum*), corn (*Zea mays* L.), sorghum (*Sorghum bicolar*) and rice (*Oryza sativa* L.) were also reported to have been propagated using *in vitro* regeneration for mass propagation in commercial farming (Baskaran and Jayabalan, 2005; Yasmin *et al.*, 2009; Vorpsi *et al.*, 2012; Islam *et al.*, 2014; Dreger *et al.*, 2019). The use of legume crops undoubtedly provides greater opportunities relevant to farmers, consumers, countries' economies and uplifting the socio-economic status of the general population. Governments and their research entities need to give important consideration to address the backlog in the use of synthetic seed technology in Sub-Saharan African agriculture, preferably those that are farmer-relevant and showing the understanding of the region's demand for food and raw materials.

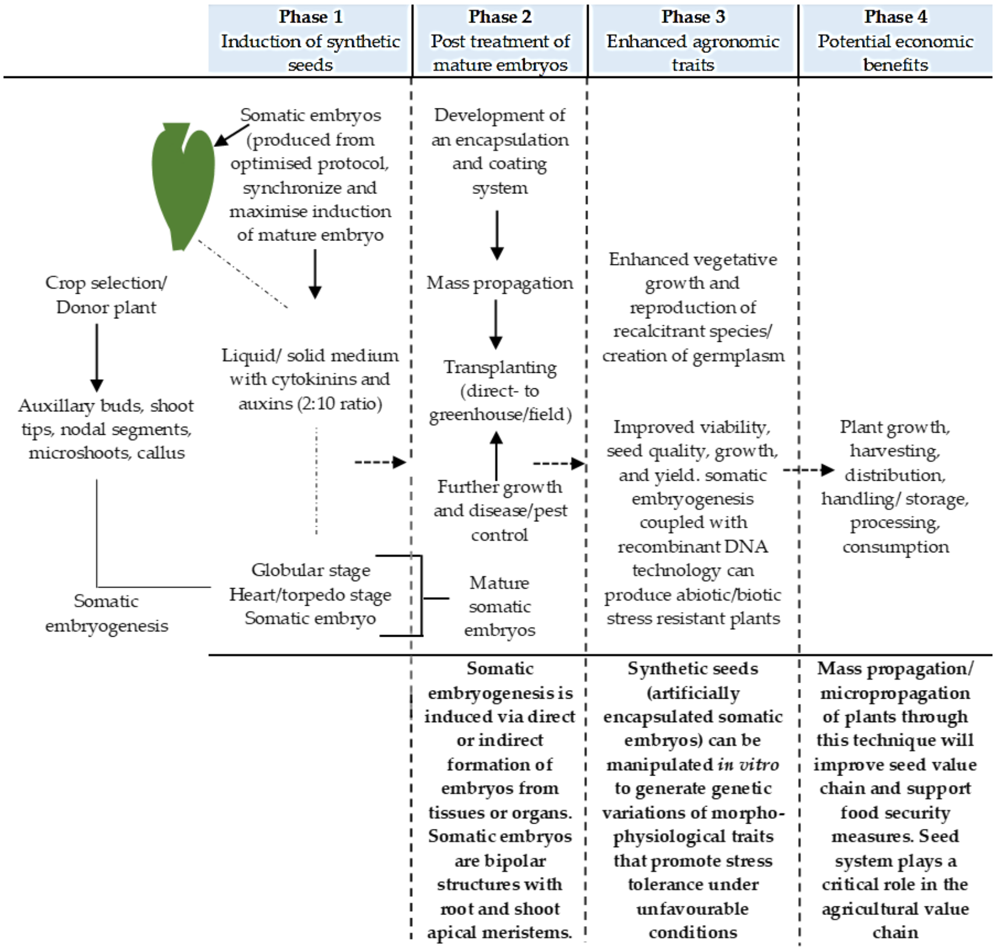

Figure 3. Schematic representation highlighting stages involved in the commercial production of synthetic seeds and their potential role in agriculture and the economic value chain of seeds in Sub-Saharan Africa

Application of plant tissue culture and biotechnology for agricultural sustainability in Sub-Saharan Africa

The barriers to the adoption of plant tissue culture and plant biotechnology-based applications in Sub-Saharan Africa involves the lack of investments in agricultural technology for plant production, especially for human consumption, animal feed and processing industries. These poor financial and research investments in applications such as the synthetic seed technology lead to massive losses of income, poor GDP growth and the lack of trade returns from agricultural commodity exports. Far along, these losses have an influence on investments in other sectors, the agricultural sector itself and contributions to food security. As previously indicated, the African continent is marked by very low economic growths, high rates of unemployment, diseases, high mortality rates (especially in children) and food insecurity (Leprince *et al*., 2000; Shimeles *et al*., 2013; Van Loon *et al*., 2018; Snapp *et al*., 2018; Gashu *et al*., 2019). There are further challenges in this region, beyond developing efficient and profitable agricultural technologies. Sub-Saharan Africa desperately needs research to develop comprehensive approaches in dealing with complex problems facing agriculture, including the foreseeable major impacts of climate change. A key challenge for Sub-Saharan Africa is the frequent occurrence of extreme temperatures that subsequently lead to both short-term and long-term drought in many parts of the continent.

Given the stated drought conditions, many countries rely heavily on rain-fed agriculture with very low use of irrigation systems (Shimeles *et al*., 2013). Under rain-fed agriculture, crops must be provided with sufficient water supply through irrigation, especially when rainfall is not sufficient. An adequate water supply is important for plant growth and productivity, and furthermore, ensures that the effects of climate change and drought stress have very minimum influence on crop yield and grain quality (Mangena, 2020a,b). Certain countries in Sub-Saharan Africa have high potential for agriculture, especially the cultivation and production of common bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), chickpea (*Cicer arietinum*) and pigeonpea (*Cajanus cajan*), with the additions of Bambara groundnut (*Vigna subterranea*) and groundnut (*Arachis hypogaea*). These crops serve as stable legume food crops for the millions of people living in rural communities (Snapp *et al*., 2018). Thus, the increasing importance of these legume species in the region is evident in their increased exploration for food ingredients, medicinal supplements, biofuel manufacturing and several other products in the textile as well as timber industry. Despite these efforts, the main barriers in the productivity and utilisation of legume crops like cowpea, common bean and pigeonpea is their susceptibility to diseases and pests, in addition to salinity, chilling and drought stress. These forms of abiotic and biotic stresses were also reported in grains such as rice (Islam *et al*., 2014), soybean (Mangena, 2020a), and forage crops such as sorghum (Baskaran and Jayabalan, 2005), annual/ perennial ryegrass (*Lolium* spp.), trefoil (*Lotus corniculatus*), medics (*Medicago* spp.) and clover (*Trifolium* spp.) (Capstuff and Miller, 2018).

All these reported crops had a greater impact on agriculture globally in a number of unique ways, particularly when plant propagation by seeds or cuttings was coupled with *in vitro* tissue culture and biotechnology. For this reason, mass propagation of these crop plants via synthetic seeds can be used not for clonal multiplication purposes only, but as a tool for plant breeders to enhance genetic improvement and then simplify mechanisms to regenerate mature plants from recalcitrant legume species. If plant tissue culture is widely used in Sub-Saharan Africa, mass propagation under industrialised conditions at low cost per unit would allow clonal propagation that replaces traditional use of seeds for propagation, replacing production of hybrid plants through hand-pollination and then serving as a reliable source of preferred biochemical, epigenetic or genetic variations (Hartmann *et al*., 2014). Since *in vitro* regeneration serves as one of the important standard propagation systems in common horticultural crops, techniques involved in these systems still need to be optimised for efficient and increased frequency of mass propagation or genetic recombination of desirable traits. The biotechnological development of clonal propagation, pathogen-free plants, drought tolerant plants, fertile and nutritionally enhanced cultivars, year-round nursery production and germplasm preservation are fundamental prerequisites for the Sub-Saharan African economy and the population's livelihoods.

Conclusion

The mass production of important leguminous crops that have proved recalcitrant for many generations could also be achieved through the production of synthetic seeds via somatic embryogenesis. This demand for micropropagated plant materials is high in Sub-Saharan Africa where the need for economic emancipation, nutrition and health-promoting solutions is continuously expanding due to the increase in population and climate change. However, lack of political stability, implementation of progressive agricultural policies and biotechnological solutions are a reflection of the ineffective seed value chain that do not translate into better economic returns despite the large diversity of germplasm that this region contains. Therefore, tools such as synthetic seed technology have the potential to increase crop plant propagation rates, improve crop market value, make propagation of recalcitrant crops possible and play a critical role in germplasm preservation. Furthermore, this seed system will facilitate the distribution of important and commercially viable legume plant materials, assisting this region in meeting its food security obligation.

Acknowledgements

The Author would like to thank the Department of Research Administration and Development, University of Limpopo, Republic of South Africa for funding the article publication charge.

References

Awosanmi, F. E., Ajayi, S. A. and Baffoe, E. E. (2020). Influence of seed moisture content on short term storage of cowpea (*Vigna unguiculata* L. Walp) seeds. Agriculturae Conspectus Scientificus, 85:37-42.

Azarkovich, M. I. (2016). Stress-induced proteins in recalcitrant seeds during deep dormancy and early germination. In Biotic and Biotic Stress in Plants- Recent Advances and Future Perspectives., Shanker, A. and Shanker, C., ed., IntechOpen, United States, London, pp.317-335.

Bailly, C. (2019). The signalling role of ROS in the regulation of seed germination and dormancy. Biochemistry Journal, 476:3019-3032.

Barbedo, C. J. (2018) A new approach towards the so-called recalcitrant seeds. Journal of Seed Sciences, 40:221-236.

Baskaran, P. and Jayabalan, N. (2005) *In vitro* plant regeneration and mass propagation system for *Sorghum bicolor*- A valuable major cereal crop. Journal of Agricultural Technology, 1:345-364.

Bereke, T. (2018). Biology of seed development and germination physiology. Advances in Plants and Agricultural Research, 8:336-346.

Berjak, P. and Pammenter, N. W. (2013). Implications of the lack of desiccation tolerance in recalcitrant seeds. Frontiers in Plant Sciences, 478:1-9.

Bhatia, S. (2015). Application of plant biotechnology. In Modern Applications of Plant Biotechnology in Pharmaceutical Sciences, eds., Bhatia, S., Sharma, K., Dahiya, R. and Bera, T., Academic Press, pp.157-207.

Cabrera-Ponce, J. L., Gonzalez-Gomez, I., Leon-Ramirez, C., Sanchez-Arrequin, J. A. and yGarfias, A. E. J. (2018). Somatic embryogenesis in common bean *Phaseolus vulgaris* L. In Plant Cell Culture Protocols- Methods in Molecular Biology., Loyola-Vergas, V. and Ochoa-Alejo, N., ed., Humana Press, United States, New York, Volume 1815, pp. 189-203.

Capstuff, N. M. and Miller, A. J. (2018). Improving the yield and nutritional quality of forage crops. Frontiers in Plant Science, 9:535.

Celmeli, T., Sari, H., Adak, H. C. D. S. A. and Eker, T. (2018). The nutritional content of common bean (*Phaseolus vulgaris* L.) landraces in comparison to modern varieties. Agronomy, 8:166.

Dreger, M., Mol, R., Deja, A., Raj, E., Mankowska, G. and Wielgus, K. (2019). Improved plant regeneration in callus cultures of *Sorghum bicolor* (L.) Moench. *In Vitro* Cellular and Developmental Biology- Plant, 55:190-198.

Etemadi, F., Hashemic, M., Barker, A. V., Zandvakili, O. R. and Liu, X. (2019). Agronomy, nutritional value and medical application of faba bean (*Vicia faba* L.). Horticultural Plant Journal, 5:170-182.

Faria, J. M. R. (2006) Desiccation tolerance and sensitivity in *Medicago truncatula* and *Inga vera* seeds. (PhD Thesis). Wageningen University, Netherlands.

Finch-Savage, W. E. and Bassel, G. W. (2016). Seed vigour and crop establishment: Extending performance beyond adaptation. Journal of Experimental Botany, 67:567-591.

Gashu, D., Demment, M. W. and Stoecket, B. J. (2019). Challenges and opportunities to the African agriculture and food systems. African Journal of Food, Agriculture, Nutrition and Development, 19:1490-14217.

Gray, D. J. (2005). Propagation from non-meristematic tissues: Non-zygotic embryogenesis. In Plant Development and Biotechnology., Trigiano, R.N. and Gray, D.J., CRC Press, United States, New York, pp.187-200.

Hartmann, H. T., Kester, D. E., Davies, F. T. and Geneve, R. L. (2014). Hartmann & Kerster's Plant Propagation Principles and Practices, 8th ed.; Pearson Education Limited, United States, New York, pp.670-724.

Hnatuszko-Konka, K., Kowalczyk, T., Gerszberg, A., Wiktorek-Smagur, A. and Kononowicz, A. (2014). *Phaseolus vulgaris*- Recalcitrant potential. Biotechnology Advances, 34:1205-1215.

Imran, M. and Nadeem, M. (2015). Triacylglycerol composition, physico-chemical characteristics and oxidative stability of interestified canola oil and fully hydrogenated cottonseed oil blends. Lipids in Health and Diseases, 14:138.

Islam, M., Roly, Z. Y., Lee, Y. and Khalekuzzaman, Md. (2014). *In vitro* propagation and genetic transformation system using immature embryos in elite rice (*Oryza sativa* L.) cultivars. Plant Breeding and Biotechnology, 2:88-96.

Jarpa-Parra, M. (2017). Lentil protein: A review of functional properties and food application. An overview of lentil protein functionality. International Journal of Food Science + Technology, 53:892-903.

Kumar, S. P. J., Prasad, S. R., Banerjee, R. and Thammineni, C. (2015). Seed birth to death: dual functions of reactive oxygen species in seed physiology. Annals of Botany, 116:663-668.

Leprince, O., Harren, F. J. M., Buitink, J., Alberda, M. and Hoekstra, F. A. (2000). Metabolic dysfunction and unabated respiration precede the loss of membrane integrity during dehydration of germinating radicles. Plant Physiology, 122:597-608.

Liu, L., Li, J., Zhu, Y., Jones, A., Rose, R. J. and Song, Y. (2019). Heat stress in legume seed setting: Effects, causes, and future prospects. Frontiers in Plant Sciences, 10:938.

Liu, W., Liang, Z., Wang, X., Sibbald, S., Hunter, D. and Tian, L. (2013). Presentation and faithful expression of transgene via artificial seeds in alfalfa. PLoS One 8:e56699.

Lu, Z. K., He, J. F., Zhang, Y. C. and Ding, D. J. (2019). Composition, phytochemical properties of pea protein and its application in functional foods. Critical Reviews in Food Sciences and Nutrition, pp.1-13.

Mangena, P. (2020a). Effect of hormonal seed priming on germination, growth, yield and biomass allocation in soybean grown under induced drought stress. Indian Journal of Agricultural Research, 2020:1-7.

Mangena, P. (2020b). *In vivo* and *in vitro* application of colchicine on germination and shoot proliferation in soybean [*Glycine max* (L.) Merr.]. Asian Journal of Crop Sciences, 12:34-42.

Mangena, P. and Mokwala, P. W. (2019). The influence of seed viability on the germination and *in vitro* multiple shoot regeneration of soybean (*Glycine max* L.). Agriculture, 9:35.

Molnar, A. V., Sonkoly, J., Lovas-Kiss, A., Fekete, R., Takacs, A., Somlyay, L. and Terok, P. (2015). Seed of the threatened annual legume, Astragalus comtortuplicatus, can survive 130 years of dry storage. Preslia, 87:319-328.

Obroucheva, N., Sinkevich, I. and Lityagina, S. (2016). Physiological aspects of seed recalcitrance: A case study on the tree *Aesculus hippocastanum*. Tree Physiology, 36:1127-1150.

Organisation for Economic Co-operation and Development (OECD) and the Food and Agriculture Organisation (FAO) of the United Nations. (2019) OECD-FAO Agricultural Outlook 2019-2018. OECD-FAO, pp.1-140.

Petrov, V. D. and Van Breusegem, F. (2012). Hydrogen-peroxide- a central hub for information flow in plant cells. AoB PLANTS 014.

Popoola, J., Ojuederies, O., Omonhinmin, C. and Adegbite, A. (2019). Neglected and underutilised legume crops: Importance and future prospects. In Recent Advances in Grain Crops Research, eds., Shah, F., Khan, Z., Iqbal, A., Turan, M. and Olgun. M., Intech Open, United Kingdom, London, pp.1-23.

Ravi, D. and Anand, P. (2012). Production and application of artificial seeds: A review. International Research Journal of Biological Sciences, 1:74-78.

Raza, G., Singh, M. B. and Bhalla, P. L. (2020). Somatic embryogenesis and plant regeneration from commercial soybean cultivars. Plants, 9:38.

Rihan, H. Z., Kareen, F., El-Mahrouk, M. E and Fuller, M. P. (2017). Artificial seeds (Principle, aspects and applications). Agronomy, 17:71.

Rose, R. J. (2019). Somatic embryogenesis in the *Medicago truncatula* model: Cellular and molecular mechanisms. Frontiers in Plant Sciences, 10:267.

Shi, Z., Yao, Y., Zhu, Y. and Ren, G. (2016). Nutritional composition and antioxidant activity of twenty mung bean cultivars in China. Crop Journal, 4:398-406.

Shimeles, A., Verdier-Chouchane, A. and Boly, A. (2013). Introduction: Understanding the challenges of the agricultural sector in Sub-Saharan Africa. In Building a Resilient and Sustainable Agriculture in Sub-Saharan Africa by Shimeles, A., Verdier-Chouchene, A. and Boly, A., Palgrave Macmillan, Cham, pp.1-12.

Snapp, S., Rahmanian, M. and Batello, C. (2018). Pulse crops for sustainable farms in Sub-Saharan Africa. FAO, Rome, pp.1-60.

Solomon, S. G., Okomodu, V. T. and Oda, S. O. (2017). Nutritional value of toasted pigeon pea, *Cajanus cajan* seed and its utilisation in the diet of *Clarias gariepinus* (Burcell, 1822) fingerlings. Aquaculture Reports, 7:34-39.

Taha, R. M., Daud, N., Hasbullah, N. A. and Awal, A. (2009). Somatic embryogenesis and production of artificial seeds in *Saintpaulia ionantha* Wendl. Acta Horticulturae, 829:331-336.

Van Loon, M. P., Deng, N., Grassini, P., Edreira, J. I. R., Wolde-meskel, E., Baijukya, F., Marrou, H. and van Ittersum, M. K. (2018). Prospect for increasing grain legume crop production in East Africa. European Journal of Agronomy, 101:140-148.

Vorpsi, V., Rama, P. and Hodaj, B. (2012). The use of corn starch on *in vitro* propagation of plum. Albanian Journal of Agricultural Sciences, 11:169-172.

Yasmin, S., Khan, I. A., Khatri, A., Seema, N., Nizamani, G. S. and Arain, M. A. (2009). *In vitro* plant regeneration in bread wheat (*Triticum aestivum* L.). Pakistan Journal of Botany, 41:2869-2876.

Zhang, L., Zhao, H. K., Dong, Q. L., Zhang, Y. Y., Wang, Y. M., Li, H. Y., Xing, G. J., Li, Q. Y. and Dong, Y. S. (2015). Genome-wide analysis and expression profiling under heat and drought treatments of HSP70 gene family in soybean (*Glycine max* L.). Frontiers in Plant Sciences, 6:773.

(Received: 11 August 2020, accepted: 28 March 2021)