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Synthetic potato seeds offer the potential to improve the Kenyan seed system

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Abstract

Agricultural production in Kenya is concentrated in areas where the climate is favourable in the densely populated high potential highland areas (1,600 to 2,700 m) by poor farmers in small land holdings less than 1 ha. Less than 5 % of potato farmers have access to imported or local certified seed. For that reason, the seed potato systems in Kenya is dominated by a farmer based informal seed system, which has a large number of cultivars in an apparent substitution for normal seed renovation. Consequently, farmer's seed tubers are highly diseased which is reflected by low yields and high tuber losses during storage. The major components of the formal seed system are split up between the Ministry of Agriculture and the Kenya Agricultural Research Centre (KARI). This formal system has not been able to match cultivars in seed production with cultivars popular among farmers and its commercial marketing system does not reach the majority of the farmers, nor does it produce enough certified seeds, accounting for less than 1 % of the total seed production.

Synthetic potato seeds offer a range of advantages: virus free, genetically identical with the plant from which it is derived, small space requirements due to small size of propagule, ease of handling, storage, and transport. We have developed synthetic seeds of potato by encapsulating *in vitro* derived shoot tips in calcium alginate hollow beads. Germination of synthetic seeds when directly sown in the field was poor. However, results of ongoing research indicate a potential solution to this problem by use of pregerminated synthetic seeds or transplanting of seedlings derived from the synthetic seeds.

Key words: Kenya, potato, seed systems, shoot tips, synthetic seeds

Zusammenfassung

Synthetische Kartoffelsamen haben das Potenzial zur Verbesserung des kenianischen Saatgutsystems

Die landwirtschaftliche Produktion in Kenia konzentriert sich auf die klimatisch begünstigten Gebiete des dicht bevölkerten kenianischen Hochlandes (1.600 bis 2.700 m), wo ressourcenarme Bauern landwirtschaftliche Nutzflächen von weniger als 1 ha bewirtschaften. Weniger als 5 % der Kartoffelbauern steht importiertes oder lokales zertifiziertes Pflanzgut zur Verfügung, weshalb in Kenia ein „informales" Saatgutsystem vorherrscht. Es basiert auf einer größeren Anzahl Sorten, die ohne regelmäßigen Saatgutwechsel von den Bauern nachgebaut werden. Bedingt durch Krankheitsbefall ist dieses Saatgut von schlechter Qualität, die Erträge sind niedrig und die Lagerverluste hoch. Das „formale" Saatgutsystem wird vom Ministerium für Landwirtschaft und dem Kenianischen Forschungszentrum für Landwirtschaft (KARI) getragen. Allerdings sind die vom „formalen" Sektor angebotenen Sorten bei den Bauern wenig populär, das Vermarktungssystem erreicht die meisten Bauern nicht und das bereitgestellte zertifizierte Saatgut macht nur etwa 1 % der gesamten Saatgutproduktion aus.

Synthetische Kartoffelsamen haben eine Reihe von Vorzügen: Virusfreiheit, genetische Identität mit der Spenderpflanze, geringer Platzbedarf wegen der Kleinheit der Samen sowie einfache Handhabung, Lagerung und Transport. Wir haben synthetische Kartoffelsamen durch Verkapselung der Sprossspitzen von *in-vitro*-Pflanzen in Ca-Alginat-Hohlkugeln hergestellt. Unter Feldbedingungen war die Keimung der synthetischen Samen schlecht. Jedoch deuten derzeit laufende Versuche eine Lösung dieser Probleme an, durch Verwendung von vorgekeimten synthetischen Samen oder Verpflanzung von Sämlingen die von synthetischen Samen abstammen.

Schlüsselworte: Kenia, Kartoffel, Saatgutsysteme, Sprossspitzen, synthetische Samen

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1 Introduction

Potato was introduced in Kenya by the end of the 19th century by expatriate farmers and after independence, around the 1960's the Kenyan government took control of production and began co-operative programs with England and Germany to establish a basis for improvement (McArthur-Crissman, 1989). Today potato is an important crop, particularly for large sectors of the population in the Kenyan highlands (Recke et al., 1997). The area devoted to potato production has been expanding steadily for the last several decades, principally by the small-scale farmers, who cultivate potatoes in numerous production areas and utilise a large number of potato cultivars, many of which are of uncertain origin.

This farmer system is characterised by self supply and provides the vast majority of seed used each growing season. Seed renewal is often accomplished by changing cultivars when farmers perceive that a given cultivar has become 'tired'. As farmers change cultivars, volunteer tubers remain from old cultivars and these are used as a reserve should the farmer decide to re-introduce that cultivar later. This changing pattern of seed renewal has produced significant shifts in cultivar distribution and popularity. 'Nyayo', the current most popular farmer cultivar was unknown a decade ago and is of uncertain origin. It is not on the list of the official cultivars. This changing mix of cultivars has led to inconsistencies between the farmer-based seed system and the official potato seed program. Cultivars included in the official seed program are frequently unpopular with farmers and those popular farmer cultivars are frequently not included the seed production program.

On annual basis, certified seed production has never exceeded more than 1% of the seed requirements. This has seriously limited the impact of certified seeds by restricting the potential for informal diffusion of quality seed from those farmers that do purchase certified seed. It is also estimated that less than 5% of potato farmers use or have access to certified seed. Traditional farmer's seed tubers are of poor quality, which is reflected by high tuber loss during storage (Enrique and El-Badewy, 2001).

The intentions of the official seed program in Kenya is to produce true to type seed, judged free of or with tolerable amounts of diseases and pests. Seed programs need access to facilities or institutions capable of handling large volumes of bulky perishable commodity. The slow multiplication rate (averaging a ratio of 10 : 1 of product to seed) places a premium on technologies which can speed up the process, thus the value of rapid multiplication procedures.

2 Potato farmers in Kenya

Kenya with approximately 29 million people covers about 575,000 km^2 of which only 19% is arable. There are four broad geographical regions: coastal plains, arid low plates, the highlands and the lake Victoria basin. Within the highlands, most soils are volcanic and fertile and it is here that potato is produced (Durr and Lorenzl, 1980). In the Kenyan highlands ranging from 1,600 to 2,700 m above sea level, potatoes are found on an estimated half million farms. Most of the farms are small holding with an average of 2 ha (World Bank, 1986) producing for home consumption and local markets. With a large population and one of the worlds highest population growth rates, a high and growing pressure is continually exerted on the limited available land. An average 231 persons per arable km^2 has resulted in continued subdivision of the small holdings to units between 0.1 to 1 ha (Enrique and El-Badewy, 2001). Potatoes also often compete for farmland with coffee, tea, pyrethrum and other subsistence crops hence further limiting the potential for potato production. In Kenya potato farmers are female and despite their dominant role in food production, women have less access to formal channels of information such as farmer training, contact with extention-officers or attendance of agricultural fairs.

The Food and Agriculture Organization of the United Nations (FAO) reported national average potato yields at 7 t ha^{-1} in 1975 increasing to 10 t ha^{-1} in 1987 (FAO, 1991). In the 1990's cultivated area was 47,000 ha with lower tuber yields of 5 t ha^{-1} (FAO, 1997), with Recke et al. (1997) citing the main limiting factors as lack of certified seed, diseases, poor storage facilities, nutrient deficiency, poor soil management, and lack of marketing systems. Currently the total potato production area has increased to 99,310 ha producing 643,909 tons with an average of yield of only 6.5 t ha^{-1} (Enrique and El-Badewy, 2001).

Potato farmers in Kenya have to contend with a wide variety of diseases and pests, mainly bacterial wilt (*Ralstonia solancearum)*, late blight (*Phytophthora infestans*), black scurf (*Rhizoctonia solani*) and several types of viruses, with potato leafroll virus and potato virus Y causing serious damages. Problematic pest include cutworms (*Agrotis* spp.) and the potato tuber moth (*Phtorimea operculata*), a major pest during storage. Losses of up to 50% caused by bacterial wilt and 90% caused by the potato tuber moth have been reported in Kenya (KARI, 1998). Expenditure on chemical control of the pests and diseases is especially expensive, may carry risks to consumers, is also beyond the ability of the farmers, and hence Kenyan farmers carry out periodic replacement of cultivars due to the gradual build-up of diseases. Programs have been set up to train farmer groups and technical staff, to produce and maintain clean seed tuber of established cultivars like

'Tigoni', 'Asante' and 'Dutch Robyjn', and to disseminate more effectively any newly released cultivar through participatory seed multiplication. But due to volunteer potato tubers, infected plant materials and symptomless carrier-weeds, as sources of inoculum, these programs have not been successful (KARI, 1998).

3 The informal seed system

The farmer-based or informal seed system is the only option for obtaining seed for most potato farmers in Kenya. The farmers select and store small tubers from their own production to use as seed and hence do not use clean seed from other sources. In case, seed is not enough or when the crop is badly diseased, farmers purchase new seed from neighbours within the same community and often blame diseases on lack of robustness in the cultivar itself rather than on poor quality. For this reason, farmers tend to change cultivars when they change seed or when yields decline to intolerable levels. Farmers further select seed based on size and not on basis of plant health. Seed storage is done in heaps in the field, heaps indoors (Fig. 1), in pits lined with leaves or straw and in gunny sacks indoor with storage losses reaching up to 19 % (Crissman et al., 1993).

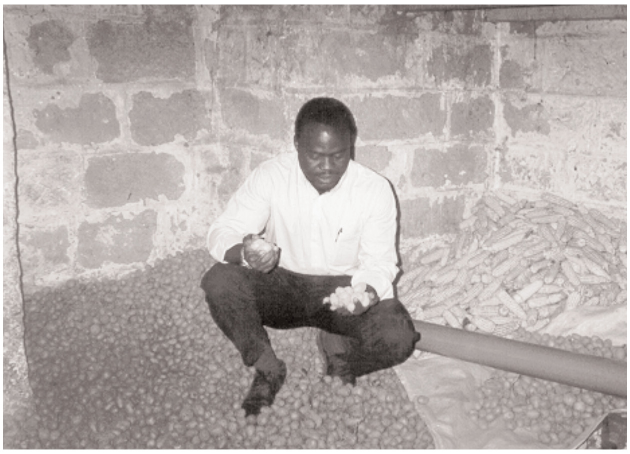

Fig. 1
The first author in a farmer's typical seed store where potato seed is often mixed with other crops and miscellaneous material

Most farmers being poor rarely buy certified seed from the formal institution (Kenya Agricultural Research, KARI), due to the high costs involved and limited availability of seed. Credit is also generally not available. Farmers also complain that certified seed is often not available at designated distribution centres or not available at the proper planting time. And in addition the cultivars for which certified seeds exist do not correspond to the most popular cultivars found in the farmers fields. In Meru district a single cultivar, 'Kerrs Pink', has remained popular throughout the decades and farmers in Meru invest more in potato production. This informal seed sector accounts for approximately 99 % of the total seed potato supply.

4 The formal seed system

The Kenyan formal system consists of the government seed program. The development of the seed program has been assisted by a series of "foreign" funded special projects (Crissmann et al., 1993). Policy studies determined that the supply of quality seed potatoes was limiting production and in response, the government sought to expand the availability of certified seed potatoes (Siror, 1981; Kuto, 1982). With exception of the Agricultural Development Corporation (ADC), the Kenyan government institutions involved with the formal seed potato systems are all member institutions of the Kenya Agricultural Research Institution which is a research organisation that co-ordinates, manages and executes agricultural research. In 1968, the Kenyan government co-operated with the German Technical Assistance Agency (GTZ) in a development project to establish a viable seed potato industry (Homann, 1979). Another special project was earlier initiated in 1969 with the Overseas Development Administration (ODA) of Great Britain, which was to establish a breeding program with an emphasis on late blight resistant cultivars and to provide information for its control and viral diseases (Robertson, 1976). The FAO also provided assistance on bacterial wilt research (Todd, 1972). The National Potato Research Station (NPRS) at Tigoni was started in 1972 (Njoroge, 1986) as a quarantine site for seed multiplication; but it was difficult to maintain seed stocks free of virus there due to presence of aphids. In 1975 NPRS set up sub-centres at high altitude, Njabini, Molo and Meru division (Homann, 1979).

In 1974 GTZ and ODA merged with stations at Nairobi and Tigoni and assisted the implementation of germplasm maintenance, basic and certified seed production with support of a virus testing laboratory. But after this project ended, lack of budget and the loss of staff quickly eroded the ability of the station to continue these tasks. In 1982, the National Plant Quarantine Station (NPQS) at Muguga, together with the International Potato Centre (CIP) at Nairobi, were in agreement to receive imported materials *in vitro* or as botanical seed in addition to tubers and to check and propagate the material for release as seed stock for field trials in Kenya (Wambugu and Murithi, 1982). The NPQS at Muguga provides the NPRS at Tigoni, since 1986, with *in vitro* plantlets for use in the rapid multiplication scheme for pre-basic seed production.

Seed certification in Kenya starts with the registration of seed growers. Currently KARI sub-station NPRS at Tigoni is the sole certified seed grower. Special printed bags are routinely labelled and sealed by the National Seed Quality Control Service (NSQCS) (Siror, 1980). But problems often arise with poor inspection logistics, poor handling and storage, lack of commitment to seed multiplication with seed sold as ware sometimes, frequent rejection of crops grown in marginal areas with high disease presence.

5 National seed production

The KARI sub-station NPRS at Tigoni has the national country mandate to produce disease free planting material (pre-basic seed) of approved cultivars, which it multiplies or distributes for multiplication to selected institutions, farmers or both. These selected institutions include the KARI sub-station at Embu, University of Nairobi, non-governmental organisations like Plan International, Seed of Hope and World Vision, Farmer Training Colleges, and farmers with financial ability to handle seed multiplication. Multiplication is done to give the certified seed, which is then sold to the farmers. The Kenya Plant Health Inspectorate Services (KEPHIS) inspects the multiplied seeds to ensure quality. On average the NPRS at Tigoni produces only 20 t (Table 1) per season, compared to the countries overall requirement of 30,000 tonnes (Kabira, 2002). In 1997 the NPRS at Tigoni produced 8.8 t of seed potato in the long rains period and 10.8 t in the short rains period, and distributed to 13 farmers and six institutions for multiplication (KARI, 1997). The KARI sub-station at Embu produces 3.5 t maximum to distribute to the farmers at the price of 25 Kenya shillings kg^{-1} (0.3 dollars), while other individual selected farmers sell at 40 Kenya shillings kg^{-1} (0.5 US dollars). This formal seed sector accounts for less than 1 % of the total seed potato requirements, the rest of the seed is obtained from the informal farmer-based seed sector.

Table 1
Seed production and distribution by the NPRS at Tigoni for the seasons 1998 to 2000 (Kabira, 2002)

Year	Long rains		Short rains	
	Production (t)	Distributed for multiplication	Production (t)	Distributed for multiplication
1998	17.44	25 farmers 7 institutions	20.96	16 farmers 11 institutions
1999	13.60	39 farmers 11 institutions	19.04	19 farmers 15 institutions
2000	15.05	23 farmers 11 institutions	8.55	27 farmers 7 institutions

KARI and its selected distributors / multipliers cannot produce enough certified seed material, while KEPHIS does not efficiently inspect and monitor seed multiplication and hence farmers don't get enough and clean seed material. This scenario leads to unavailability of clean certified seeds and hence the farmers continue to plant their own poor quality seeds. Recently, the NPRS at Tigoni embarked on the production of mini-tubers in the greenhouse to supplement its seed process for production of pre-basic seeds (KARI, 1997). The target figure was 150,000 minitubers but this could not be attained due to various factors including, high temperatures in the greenhouse, hot weather in the field, breakdown of the water source pump and the soil sterilisation unit, tubermoth damage and virus infestation.

6 The concept of synthetic seed technology

Originating from an idea proposed by Murashige (1977, 1978) the concept of synthetic or artificial seeds has evolved from a futuristic idea into a real field of experimentation and research. His original definition limited synthetic seeds to the encapsulation of somatic embryos (Datta et al., 1999). Apart from Kamada et al. (1988) and Redenbaugh (1991), no consistent embryo to plant conversion had occurred from encapsulated embryos then. Bapat et al. (1987) subsequently proposed the making of synthetic seeds through encapsulation of *in vitro*-derived propagules, different from somatic embryos especially in non-embryogenic species. In mulberry (*Morus indica*), for instance, they proposed the use of encapsulated buds.

A current definition of synthetic seeds is the one given by Aitken-Christie et al. (1995) which considers synthetic seeds as artificially encapsulated somatic embryos, shoots or other tissues which can be used for sowing under *in vitro* or *ex vitro* conditions and hence extends the concept of synthetic seeds to any type of vegetative propagule, characterising it only as a 'seed-like' structure. Encapsulation can be considered an important application of micropropagation to improve the success of *in vitro*-derived plant delivery into field or greenhouse or to contribute to synthetic seed technology (Piccioni and Standardi, 1995). Less attention has been given to the possibility of encapsulating non-embryogenic *in vitro*-derived vegetative propagules. Nonetheless, some researchers have tried to encapsulate shoot tips or axillary buds of different species with promising results (Mathur et al., 1989; Ganapathi et al., 1992; Bapat, 1993). This kind of capsules could be useful in the exchange of sterile material between laboratories, due to the small size and relative ease in handling the structures or in the germplasm conservation, with proper preservation techniques (Fabre and Dereuddre, 1990; Accart et al., 1994) or even in plant propagation and nurseries, if the development of the plant could be properly directed towards proliferation, rooting, elongation etc. (Mathur et al., 1988; Bapat, 1993).

Somatic embryogenesis has been obtained in more than 150 species of important agricultural crops and is a routine procedure in crops like soybean (Ranch et al., 1985; Barwale et al., 1986), grasses and cereals (Gray and Conger 1984; Vasil and Vasil, 1984). The prospect of using plant

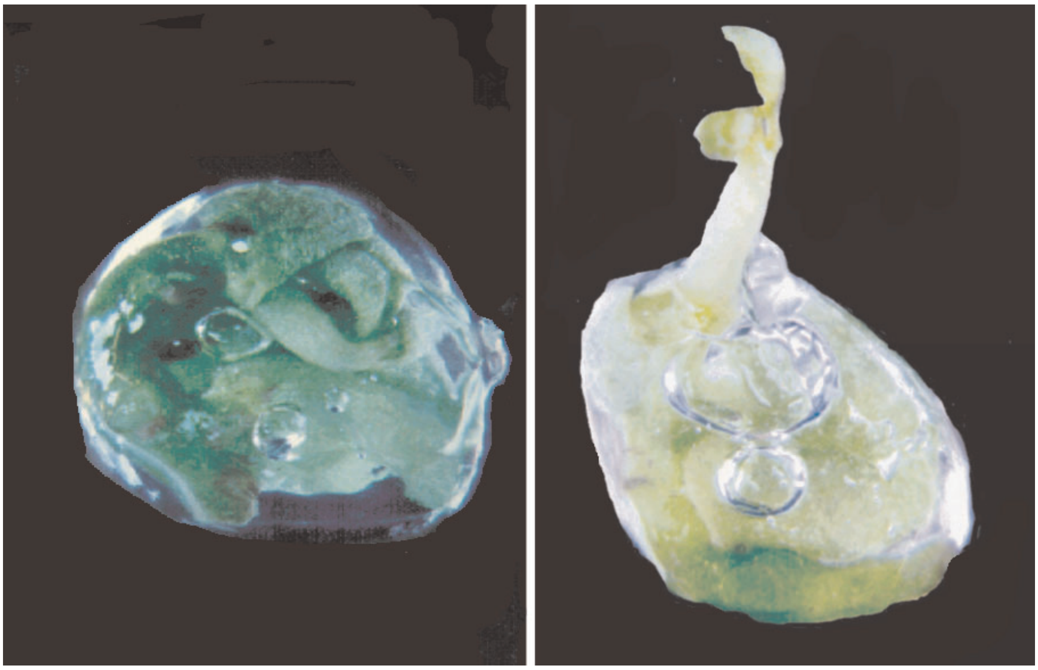

Fig. 2
Synthetic seed of potato, before germination (left) and after germination (right)

somatic embryos produced in tissue culture as synthetic seeds has been a subject of increasing interests (Rogers, 1983; Herman, 1985). The objective of synthetic seeds is to produce a propagule that is genetically, developmentally and morphologically as close as possible to the seed of the plant from which it is derived. The synthetic seed technology could be considered to offer a range of advantages of micropropagation, high production efficiency, perfect sanitary conditions, reduced size of propagule, reduced space requirements, handling ease and storability, sowing, transportability and mechanisation potential.

7 Synthetic seeds in potato

Vegetative propagation of the potato assures the stability of cultivar characteristics over successive planting, but tubers spread disease, hence giving rise to the need and use of *in vitro* culture techniques for vegetative propagation. The use of somatic embryos as synthetic seeds was long felt as a promising alternative to conventional potato propagules (Sarkar and Naik, 1998), and signs of embryonic capacity was first observed by Lam (1975). To date, few studies have involved potato somatic embryogenesis. Petrova and Dedicova (1992) showed somatic embryogenesis in potato cultivar Désirée from immature, zygotic embryo sections. Garcia and Martinez (1995) reported the formation of somatic embryos from potato cells which become incorporated into mature tissue. Here embryoids were formed 6 months after the culture initiation of stem nodal sections but presence of abnormal somatic embryos were found. Plantlets were formed after another 45 days on culture on artificial MS media without growth hormones. Fiegert et al. (2000) elucidated somatic embryogenesis from excised shoot tips of the cultivar ‘Tomensa’, taking 80 days to obtain the embryos in liquid culture. On encapsulation, up to 87 % germination of the synthetic seeds was observed on sterile sand in the growth chamber.

However, somatic embryogenesis has not been common in potato tissue culture and as a result little has been accomplished to develop synthetic seeds in potato. Coupled to this, the problems of abnormal somatic embryos, the long periods required to induce the embryos and the problems of synchronising embryo maturity has greatly discouraged many researchers. Further, as in other crops where somatic embryogenesis is uncommon, attempts have been made to encapsulate *in vitro*-derived vegetative propagules in an appropriate gel as synthetic seeds (Sarkar and Naik, 1998). They showed for the first time that *in vitro*-derived potato nodal segments encapsulated in alginate-MS solution can be used for potato propagule production. As high as 57 % encapsulated nodal segments survived in the soil when not covered and only 3% when covered on soil in the greenhouse after incubation under light for three days and treatment with a rooting hormone at planting time. Patel et al. (2000) first developed synthetic seeds of the potato cultivar ‘Priwal’, using shoot tips encapsulated in calcium alginate hollow beads. After four weeks on MS medium, 13 out of 16 shoot tips had developed into whole plants. They also encapsulated potato calli, with results showing calli growing in 50 % of the capsules.

Currently we have developed synthetic seeds of potato by encapsulating *in vitro*-derived shoot tips of German and Kenyan cultivars in calcium alginate hollow beads (Fig. 2). The synthetic seeds showed 100 % germination on MS media after six months storage at 4 and 10 °C, 100 and 61.8 % germination after 9 and 12 months storage at 4 °C, respectively. On average, for all six cultivars, 93 and 25% germination was obtained in the greenhouse and field, respectively on direct sowing of the synthetic seeds in soil.

The synthetic seed plants grown in the greenhouse were up to the seventh week significantly shorter, had significantly more leafs after six weeks and matured three weeks later than the plants from the seed tuber. No significant differences were observed for the tuber and leaf dry weights, leaf area, net assimilation rate, leaf nitrogen and tuber starch contents. Consistent with the greenhouse results the synthetic seeds matured later, were shorter and no significant differences were also observed in the tuber dry matter yield when grown in the field.

Currently we are researching on how to optimise the production of synthetic seeds of potato, to improve the germination rates and delivery of the synthetic seeds into the field, to assess the regeneration ability of the synthetic seeds after storage for more than one year and to evaluate the growth development of synthetic seeds of potato.

8 Need for further research

Synthetic seed technology seems to represent an alternative massive clonal propagation and also appears to be a potential solution to the problems of seed storage and field propagation, especially with respect to disease transmission and maintenance of cultivar that have been selected for important genetic characteristics. Furthermore, minitubers can also be produced directly from the encapsulated microcuttings to be used in basic seed production, thus eliminating an additional cycle of microtuber production, saving on costs and labour. These advantages might greatly boast the seed systems in Kenya and coupled with the fact that the technology is simple and requires little space and facilities, adoption would not be difficult. Synthetic seeds being tiny, not more than 1 cm in diameter, also offer a practical solution to other related problems of transport and storage of tubers which require special refrigeration facilities and large space. Synthetic seed technology may also offer a solution to the high risks of contamination and labour demands met by commercial breeders who carry out fast clonal propagation *in vitro*.

In spite of the above named advantages, the synthetic seeds technology has various unsolved problems that require careful consideration. Germination according to Kozai et al. (1991), Piccioni et al. (1995), Toutorus and Dunstan (1998), and as indicated in our preliminary research results, is one of the most important aspects of synthetic seed technology that still limits its practical use. One of the major hurdles in the development of synthetic seed technology is the ability of the encapsulated embryo or shoot buds to germinate under non-sterile environmental conditions. Several factors are involved in this process. Shoots and buds do not have root meristems, and they must regenerate roots to be able to convert (Bapat, 1993; Piccioni, 1997). The synthetic seeds should also be able to survive at least short and medium term storage in order to be convenient in commercial practical uses (Piccioni et al., 1996). More so since maintenance of potato germplasm in the field consumes a lot of time, manpower and space aside from diseases and environmental stress, while conservation of *in vitro* cultured potato plants entails high maintenance costs, risks of somaclonal variation and genetic stability, especially when growth retardants are used (Harding, 1991). Preliminary results indicate that synthetic seeds of potato can regenerate after one year of storage (Nyende et al., 2002).

Large gaps between high rate of success *in vitro* and the disappointing realities *in vivo* is primarily due to inefficient delivery systems and poor understanding of hardening processes, that are ultimately reflected in the high mortality rate during acclimatisation (Mathur and Ahuja, 1991). Improving sowing and nursing techniques and the soil mix composition especially in nutrient and water availability will probably increase germination rates up to acceptable standards and an appropriate micro-organism control system (Standardi and Piccioni, 1997), more so since the synthetic seeds are susceptible to micro-organisms attacks.

Seeds represent the ultimate convenience for crop production due to their ease of use and the low cost when compared to the other forms of propagules. In potato production, the major input is seed and approximately 50% of the total input cost is spent on seed. The planting material is also quite bulky, as tubers are used for propagation and a seed rate of 2.0 to 2.5 t ha^{-1} is needed for the crop. In addition to the high cost of the seed, potato propagation is characterised by the low rate of multiplication. Under normal conditions, the rate of multiplication is 4 to 6 times and at best about 15 times under expert supervision in favourable growing. Potatoes are conventionally, clonally propagated by tubers, and this propagation method has been one of the potato's most important disadvantages. As the crop is propagated vegetatively, so are all the tissue-borne viruses, fungi and bacteria that have infected the crop during the previous seasons, which ultimately lead to significant losses in yield and tuber quality. Due to such disease accumulations, potato seed has to be replaced every 3 to 4 years. Traditionally some Kenyan farmers cut tubers and dress the wounds with ash and plant after healing. The cutting can result in infection by bacterial wilt pathogen. Tuber cutting to increase planting material is not recommended (KARI, 1998) unless the farmer can follow the knife sterilisation procedure and only if certified disease-free seed tubers are used.

The primary concern in any potato production system and especially in the Kenyan situation is to obtain initial propagating material which is free of diseases, mainly viruses (Georgakis et al., 1997). Unipolar explants such as shoot tips offer an exciting alternative to somatic embryos for potato synthetic seed production. These synthetic seeds have the potential advantages of virus-free, clonally similar material, easy to handle, store and transport, especially for agricultural production in Kenya, the tropics, and third world countries where high quality disease free certified planting seed material is a major limiting factor.

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