1 INTRODUCTION

"Seeds represent the most important product of a plant´s life cycle; and seed germ constitutes the evolutionary continuum of the plant species. Human beings have long recorded the essential role of seeds in crop establishment and improvement. As prime dispersers seeds can be stored for long periods of time in a suspended metabolic state without loss of essential genetic attributes of the crop. Considering the vital role of agriculture, the continuing development of improved seeds of crop plants has taken vital importance. Today seed industries and technologies are being evolved to ensure the continuous supply of high quality seeds of crop plants to farmers" (Desai et al., 1997).

1.1 Potato production

Potato is the fourth most important food crop in the world, and is grown in about 140 countries, more than 100 of which are located in the tropical and sub-tropical zones, with annual production approaching 300 million t (CIP, 2002). World wide in 1997 the production area was 18 million ha of which 1.8 million ha were located in Africa and South America, 6 million ha were located in Asia with average tuber yields in Africa, Asia, and South America about one half yields in Europe (Manrique, 2000). Potato is an ideal food for supplementing cereal-based diets and due to its starch value it provides 3-4 times more calories per unit area as compared to cereals (Khan, 1993). Potato also contains high quality protein, substantial amounts of essential vitamins, minerals, trace elements (Khan, 1993), and very low fat content (CIP, 2002).

Potato is grown under a wide variety of conditions in Sub-Saharan Africa, but production is concentrated in niches where the climate is favourable and markets are available. Approximately 450,000 ha are reported by the FAO (Ewell and El-Bedewy, 1999 unpublished) to be under cultivation and over the past 35 years, production has quadrupled increasing consistently and at a rate significantly faster than population growth, which has increased only two and a half times over the same period. This record is an exception among food crops, production overall having fallen behind growth in population and reflects the growing importance of potato as a food in the rapidly expanding urban areas of Africa.

Potatoes in Kenya were introduced in the 19th century by European settlers (Manrique, 2000) and have been grown in the Kenyan highlands between 1600 m to 3000 m above sea level (Fig. 1). Between 1917 and 1918 the crop was introduced into small scale farming among the African farmers who grew it for consumption and later for export to neighbouring countries. Organised potato breeding and cultivar selection in Kenya started in 1943 (Todd,

1969), but these cultivars degenerated rapidly mainly due to their lack of resistance to late blight (*Phytophthora infestans*) and to bacterial wilt (*Ralstonia solanacearum*) (Wabule, 1982). In 1965 and 1966, 16 new cultivars were introduced from Germany (Homann, 1979) with Maritta, Anett and Feldeslohn being the most successful. Over 50 potato cultivars have been grown by the Kenyan farmers (KARI, 1988; McArther-Crissman, 1989). FAO reported national average potato yields of 7 t ha^{-1} in 1975 increasing to 10 t ha^{-1} in 1987 (FAO, 1991). In the 1990s cultivated area was 47,000 ha with low tuber yields of only 5 t ha^{-1} (FAO, 1997). Recke et al. (1997) cited 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 t with an average yield of 6.5 t ha^{-1} (Enrique and El-Badewy, 2001). The majority of the small-scale farmers select and store small tubers from their own production to use as seed, and hence have to contend with a variety of diseases and pests (KARI, 1998; Nyende et al., 2002).

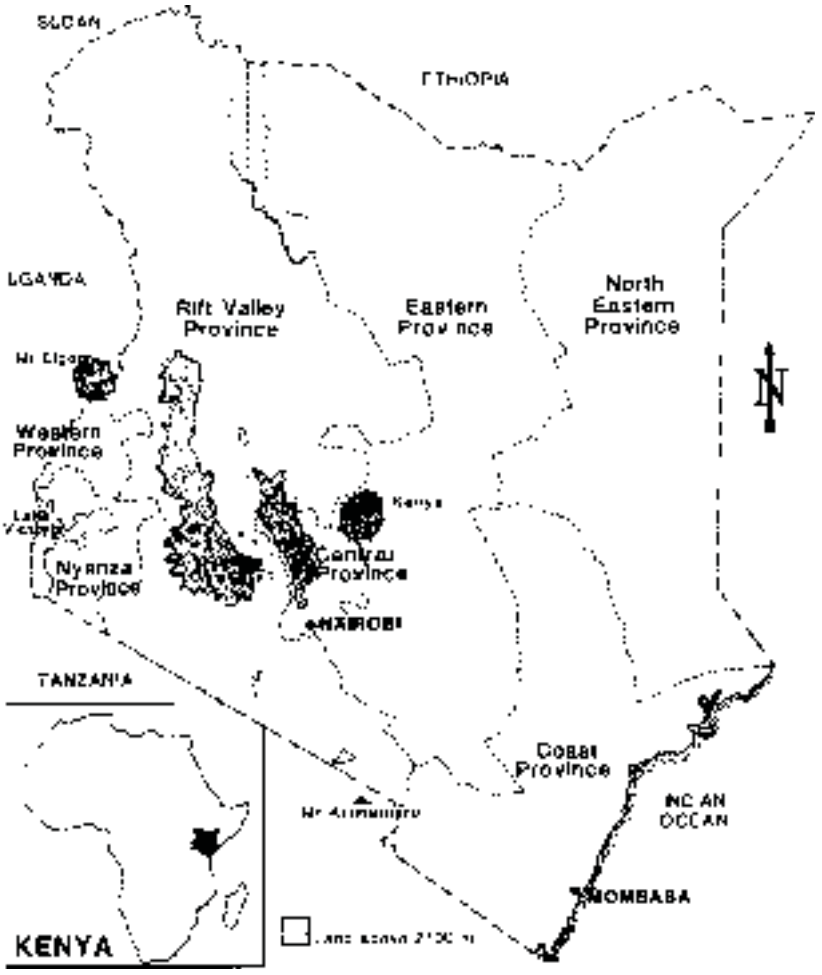

Fig. 1. Map of Kenya with the shaded part showing areas of concentrated potato production in the highland regions.

1.2 The concept of synthetic seeds

Early efforts for development of artificial seeds were by Keith Walker, at Monsanto company, working with alfalfa (*Medicago sativa*) and by Robert Lawrence at Union Carbide working with celery (*Apium graveolens*) and lettuce (*Lactuca sativa*) (Redenbaugh et al., 1991). Originating in 1977 from an idea proposed by Murashige (1977, 1978) the concept of synthetic seeds or artificial seeds has evolved from a futuristic idea into a real field of research. Murashige's original definition was that of "a single somatic embryo", a clonal product that could be handled and used as a real seed for storage and sowing. This definition limited artificial seeds to the encapsulation of only somatic embryos (Kamada, 1985). Kamada (1985) broadened the scope of this technique by suggesting that an artificial seed comprised a capsule prepared by artificially coating a cultured matter, like a plant tissue piece or an organ, which could grow into a complete plant. Bapat et al. (1987) subsequently proposed the making of synthetic seeds through encapsulation of *in vitro*-derived propagules, different from somatic embryos. Little attention was given to the possibility of encapsulating these non-embryogenic *in vitro*-derived vegetative propagules but where tried, promising results were achieved in different species (Ganapathi et al., 1992; Bapat, 1993).

Later the definition given by Aitken-Christie et al. (1995), considered synthetic seeds as "artificially encapsulated somatic embryos, shoots or other tissues which could be used for sowing under *in vitro* or *ex vitro* conditions", and hence extended the concept of synthetic seeds to any type of vegetative propagule. Piccioni (1997) then emphasised that the propagule must be able to convert and grow into a plantlet after sowing. Redenbaugh (1993) described conversion as the development of a green plant with a normal phenotype. According to Standardi and Piccioni (1998), germination which is a property of plant seeds, zygotic and apomictic seeds should be avoided when talking about synthetic seeds. They proposed that the general term "regrowth" should be used to indicate any vegetative development that is performed by an encapsulated propagule after sowing and that the regrowth should be specified as sprouting, rooting or shoot elongation accordingly.

Presently, synthetic seeds are defined as artificially encapsulated somatic embryos, shoot buds, cell aggregates, or any other tissue that can be used for sowing as seeds and that possess the ability to convert into plants under *in vitro* or *ex vitro* conditions, and most importantly retain this potential also after storage (Capuano et al., 1998; Hussain et al., 2000). Besides, the encapsulated materials could also be useful in the exchange of sterile material between laboratories, due to the small size and relative ease in handling of the structures, or in the germplasm conservation, with proper preservation techniques (Fabre and Dereuddre,

1990; Niino et al., 1992; Accart et al., 1994; Na and Kondo, 1996; Niino et al., 2000) or even in plant nurseries, if the development of the plant could be properly directed towards proliferation, rooting, and elongation (Mathur et al., 1989; Bapat, 1993). Today, the synthetic seed technology has been extended to a large number of plant species belonging to both dicotyledonous and monocotyledonous species (Table 1).

Table 1. Plant species from which synthetic seeds have been produced (after Mathur and Ahuja, 1991b).

Plant species	Common name	Encapsulated propagule
Apium graveolens	Celery	Somatic embryos
Daucus carota	Carrot	Somatic embryos
Gossypium hirsutum	Cotton	Somatic embryos
Lactuca sativa	Lettuce	Somatic embryos
Medicago sativa	Alfalfa	Somatic embryos
Dactylis glomerata	Orchard grass	Somatic embryos
Morus indica	Mulberry	Axillary buds
Santalum album	Sandal wood	Somatic embryos
Valeriana wallichii	Mushkbala	Axillary buds, Apical buds
Dioscorea floribunda	No common name	Axillary buds
Hyoscyamus muticus	Henbane	Adventitious buds
Picrorhiza kurroa	Picrorhiza	Adventitious buds, Apical buds
Salvia sclarea	Clary	Adventitious buds
Rheum emodi	Rhubarb	Adventitious buds
Pogostemon cablin	Patchouli-plant	Adventitious buds

Since somatic embryogenesis has been possible in more than 150 species of important agricultural crops (Tissert et al., 1979), 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 somatic embryos as synthetic seeds has been a subject of increasing interest (Rogers, 1983; Herman, 1985). The application of somatic embryogenesis to synthetic seed production requires many embryos per amount of callus cultured in suspension and the formation of individualised embryos (Cantliffe, 1992). Encapsulation of somatic embryos has widely been reported to produce synthetic seeds, however the number of converted morphologically normal plantlets is often low (Ghosh and Sen, 1994; Bazinet et al., 1996). Due to the low conversion frequencies of the somatic embryos, micropropagated buds have been considered as an alternative to use for synthetic seed production (Piccioni and Standardi, 1995).

The objective of the development 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. Synthetic seeds could have particular relevance in the propagation of hand-pollinated hybrids, elite germplasms, genetically engineered hybrids with sterility or unstable genotype complications (Standardi and Piccioni, 1998), and would be essential for genetically engineered crop plants that do not breed true due to the incorporation of meiotically unstable foreign genes, and the then intentionally introduced meiotic instability would become an alternative to hybrid seed for commercialising propriety germplasm (Desai et al., 1997).

1.3 Encapsulation concept

Since somatic embryos are sensitive to desiccation on exposure, it was envisaged that to improve the success of planting it would be necessary to require some protective coating. Encapsulation in a protective bead was conceived to solve this problem and the encapsulation material, a biodegradable synthetic polymer coating that acts as an artificial seed coat, should be non-damaging to the somatic embryo or shoot tip and durable enough to allow ordinary handling during storage, transportation and planting. For many purposes the synthetic coat will need to contain and deliver nutrients, developmental control agents and other components necessary for germination and conversion. In the last few years several methods for the entrapment of living and growing cells have been developed. A preparation that provides extremely mild immobilisation conditions is the entrapment within ionotropic gels such as calcium alginate (Vorlop and Steinert, 1987). Other important encapsulating agents, which have been tried out and shown success, include agar, agarose, carrageenan, gelrite and polyacrylamide. A variety of hydrogels have been employed (Table 2) in synthetic seed production but however, according to Redenbaugh et al. (1987a, 1991) sodium alginate was chosen as the best encapsulation matrix because of its gelation properties (complexes easily with calcium chloride through an ionic exchange reaction), ease of use, non-toxicity, biodegradable, universally available and low cost. Alginate also dissolves easily and remains stable at room temperature (Redenbaugh et al., 1993; Tsai and Saunders, 1999), while its gels also have low solution spinnability.

Capsules made for most synthetic seed production research have been produced by mixing the embryos in sodium alginate followed by dropping into a calcium salt solution producing whole beads (Fig. 2), whereby the embryos protrude from the beads or are located near the surface so that complete protection of the embryo as in natural seed is not ensured (Patel et al., 2000). Initially hollow beads (Fig. 2) were successfully used to encapsulate

highly sensitive animal cell cultures (Spiekermann et al., 1987) and entomopathogenic nematodes (Patel and Vorlop, 1994) and later in plant tissues (Patel et al., 2000).

Table 2. Various hydrogels used for artificial seed production (after Redenbaugh et al.1986, 1987a).

Hydrogel	Working concentration	Complexing agent	Working concentration
	w/v (%)		mM
Sodium alginate	0.5-6.0	Calcium chloride	30-100
Sodium alginate + gelatin	2.0-4.0 5.0-6.0	Calcium chloride	30-100
Carrageenan + Locust bean gum	0.2-0.8 0.4-0.8	Potassium chloride or Ammonium chloride	500
Gelrite	0.25-0.40	Lowering of temperature	

Various advantages are associated with these hollow beads over whole beads and botanical seeds (Table 3). Synthetic seed capsules are basically of two types, hydrated or desiccated. Hydrated synthetic seed capsules consist of propagules individually entrapped in a hydrogel, while the desiccated synthetic seed capsules, on the other hand are produced by mixing propagules with polyoxyethylene glycol and then dispensing this mixture on a teflon surface in the form of wafers. These are left to dry for several hours under sterile conditions.

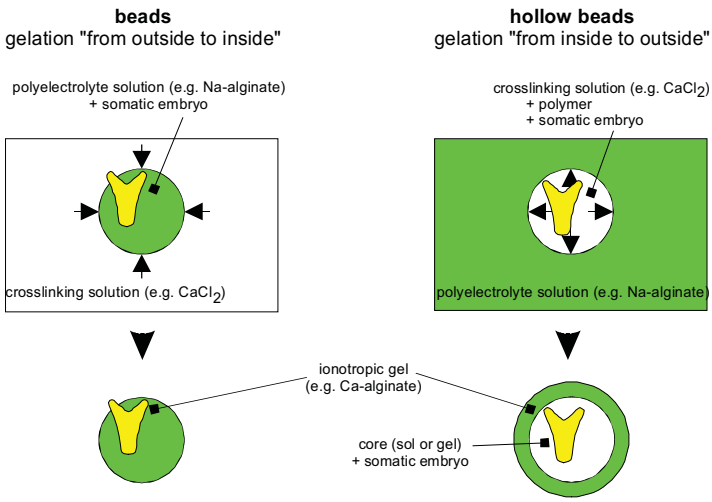

Fig. 2. Formation process of whole and hollow bead capsules for synthetic seed production (Patel et al., 2000).