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Production of Synthetic Seed in Cassava (*Manihot esculenta* Crantz)

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Abstract

Cassava is an important source of energy in the diet of most tropical countries of the world. It has enormous potential in India for food security and industrial uses due to its ability to grow in marginal and waste lands where other crops could not survive. Commercial planting of cassava is done from stem cuttings and because of the low multiplication rate, bulkiness of seed material and difficulty in transporting the planting materials to distant places, the crop could not make much impact either as food crop or industrial crop in the country. These limitations can be overcome by the development of synthetic seed technology. Synthetic seeds have multiple advantages including mass propagation of elite plant, ease of handling, long-term storage and low cost of production. In this study, different concentrations of sodium alginate and calcium chloride solutions were tested in order to optimize the shape, texture and germination frequency of synthetic seeds of cassava. The nodes containing axillary buds from *in vitro* grown cassava variety, H-226 were encapsulated with 2%, 3% and 4% sodium alginate (w/v) along with Murashige and Skoog (MS) salts without calcium salt and exposed to 75mM, 100mM and 125 mM calcium chloride solution ($CaCl_2.2H_2O$). Round and sufficiently hard beads/ synthetic seeds were observed by the encapsulation with sodium alginate 3% and exposed to 100 mM $CaCl_2.2H_2O$ combination. It showed that encapsulation at 2% and 3% sodium alginate concentrations and exposed to $CaCl_2.2H_2O$ at 75mM and 100mM concentrations, gave high germination frequency under *in vitro* condition. The synthetic seeds have the possibility of being an efficient way for germplasm storage, exchange and propagation of pathogen-free planting material.

Key words: Synthetic seed, artificial seed, encapsulation, cassava

Introduction

Cassava (*Manihot esculenta* Crantz) is the fifth most important food crop in the world and which was initially adopted as a popular famine reserve crop as it provided a more reliable source of food during drought and hunger. In recent times it has emerged as both staple food and profitable cash crop of industrial significance in the world economy (Aerni, 2006). Cassava has enormous potential in India for poverty alleviation, food security and industrial uses due to its ability to grow and yield well in marginal and wastelands. The crop is commercially propagated through vegetative by stem cuttings. The slow multiplication rate (1:8) under clonal multiplication, difficulty in transporting the planting materials to distant places due to bulkiness of seed material and the cassava mosaic disease (CMD) infection are the major hindrance which prevent the spread of the crop in non-traditional areas of the country. In addition, distribution and exchange of germplasm is difficult because of the vegetative propagation of the crop and more risks of transfer of disease through planting material. Cassava can also be propagated through seed. The major barriers in the commercial adoption of true seed are high level of heterozygosity and heterogeneity of the crop.

Synthetic seed production is an applied technology which allows rapid multiplication of elite plant. Synthetic seed

refers to artificially encapsulated explants such as shoot tips, axillary buds, somatic embryos or other tissues and that can be developed into a plant under *in vitro* or *ex vitro* conditions. The encapsulation (coating) protects the explants from mechanical damage and drying of plant propagule during handling and storage. It behaves like true seed and sprout into seedlings under suitable conditions. The alginate coat protects the micropropagules and thus has practical application in germplasm exchange and conservation.

Germplasm conservation is necessary for sustainable crop improvement and as a means of maintaining crop diversity to prevent genetic erosion. The propagation and conservation of cassava traditionally take place by cuttings and field conservation. The encapsulation technique and cryogenic procedures may be reliable methods for long-term storage of plant genetic resources without risk of genetic instability using minimum space, lower labour and less maintenance cost. Additional benefits of synthetic seeds include low production costs, short and long-term storability, facilitation of germplasm exchange between laboratories, easy handling, transportation of propagules to distant places and subsequent propagation (Parveen and Shahzad, 2014).

In the present study, investigations were made for the first time to produce synthetic seeds in cassava by using nodal segments. The aim of this study was to determine the optimum concentration of sodium alginate solution and calcium chloride solution (encapsulation matrix) to optimize the size, shape and texture of alginate beads for maximum germination. Studies were also carried out on the effect of different storage durations on the conversion of encapsulated somatic embryos.

Materials and Methods

In vitro grown, 30 to 35 days old popular cassava plants, cv, H226 cultured on MS basal medium (Murashige and Skoog, 1962) were used to excise 2 to 5 mm apical and axillary buds for encapsulation.

For synthetic seed production, 2, 3 and 4% sodium alginate solution with and without MS nutrients, free from calcium salt were tested. For complexation (the formation of insoluble calcium alginate after ion exchange reaction between Na^+ and Ca^+), 75, 100 and 125 mM of calcium chloride solutions were used (Mathur et al., 1989). Both the gel matrix and complexing agent were sterilized by autoclave at 15 lb pressure at 121°C for 15 min.

Encapsulation was accomplished by mixing the explants into the sodium alginate solution and dropping these into the calcium chloride solution. Explants along with alginate solutions were pipetted using pipette with tip cut off and drop wise into the calcium solution and maintained for at least 30 min to polymerize the beads. The drops set as white bead when allowed in calcium chloride solution for 30-35 min. When sodium alginate drops come in contact with calcium chloride solution, surface complexion begins and firm round beads are formed; each bead contains one explant. The beads containing the entrapped explants were retrieved from calcium chloride solution and washed 2-3 times with sterilized distilled water.

Encapsulated apical and axillary buds were cultured on MS basal medium supplemented with 3% sucrose and 0.3% gelrite. The pH of the MS medium was adjusted to 5.8 and sterilization was done by autoclaving at 121°C for 15 min. All cultures were maintained in the culture room at 25 ± 1°C under 16 h photoperiod provided by cool white fluorescent lamps to observe the germination ability of the cassava synthetic seed. Studies were also carried out on the effect of different storage durations on germination ability of the encapsulated somatic embryos.

Physical characteristics of the synthetic seed like shape, texture, germination percentage and days to germination were recorded after *in vitro* culture. Two replications were used in each treatment and experiment was repeated thrice. The per cent data transformed using angular transformation and analyzed following completely randomized design (CRD) to test for statistical significance.

Results and Discussion

Cassava synthetic seeds were produced by encapsulating apical and axillary buds taken from *in vitro* grown, 30 to 35 days old plants. The most commonly used plant material for synthetic seed production is somatic embryos, because they easily develop roots and shoots uniformly (Gantait et al., 2015; Redenbaugh et al., 1986). In cassava, somatic embryogenesis is highly depends on the genotype, explant and growth regulators used. In general, the rates of somatic embryo induction are also low (Anuradha et al., 2015). In addition to somatic embryos, other explants

such as shoots with apical bud and nodes with axillary bud have been used in synthetic seed production (Gantait et al., 2015). These explants are easier to handle and cheaper to produce synthetic seeds as compared with somatic embryos. Hence, the possibilities of encapsulating alternative materials such as cassava nodal segments and shoot tips have been explored.

The cassava synthetic seeds differed morphologically in respect to texture and shape with different combinations of sodium alginate and calcium chloride ($CaCl_2.2H_2O$) solutions. With the complexing time fixed at 30 to 35 min, the assessment of beads by various concentrations of sodium alginate (2 to 4%) and calcium chloride (75 to 125 mM) is presented in Table 1. The exposure of at least 30 min was required to achieve complete complexation during synthetic seed production (Ipekci and Gozukirmizi, 2003). Different shape and texture of beads were observed and they were classified as irregular, slightly round, round and fragile, soft, firm, hard respectively. An encapsulated matrix of 3% sodium alginate with 100 mM calcium chloride was found most suitable for the formation of ideal beads (Fig. 1 and Table 1) which produced round and optimally firm synthetic seeds. Lower concentration of sodium alginate (2%) was not suitable for encapsulation because the resulting beads were of undefined shape and too soft to handle, whereas at higher concentration the beads were uniform and round, but hard enough to cause considerable delay in sprouting and reduce germination. Nor-Asmah et al. (2011) and Sharma and Shahzad (2012) observed significant difference in the shape and texture of the beads when used low to high percentage of sodium alginate.

Germination of cassava synthetic seed was determined when the expanded leaves and growing roots of the explant

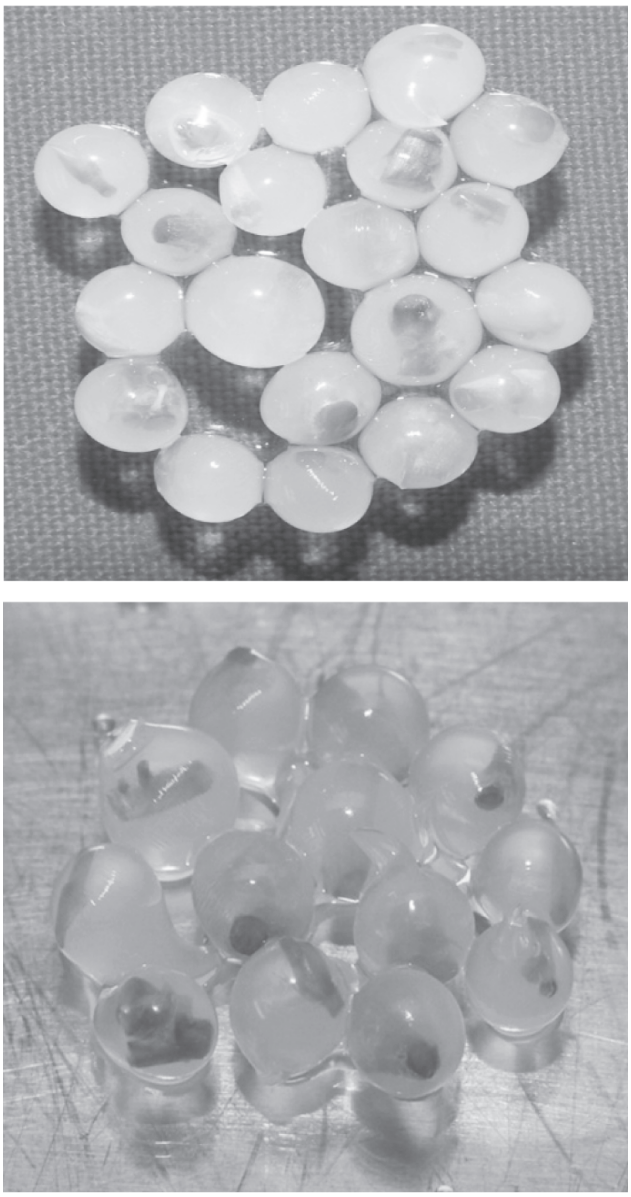

Fig.1. Cassava synthetic seeds using 3% sodium alginate and 100 mM calcium chloride

Table 1. Physical characteristics of synthetic seed by the encapsulation with different concentrations of sodium alginate and calcium chloride

Sodium alginate (%)	MS salts	Calcium chloride (mM)		
		75	100	125
2	0	Irregular, fragile	Irregular, soft	Irregular, soft
3	0	Slightly round, firm	Round, firm	Round, firm
4	0	Slightly round, hard	Round, hard	Round, hard
2	1	Irregular, fragile	Irregular, soft	Irregular, soft
3	1	Slightly round, firm	Round, firm	Round, firm
4	1	Slightly round, hard	Round, hard	Round, hard

appeared and break the gel. The highest growth speed (6.80 days) and germination percentage (96.67%) was obtained combining 2 % sodium alginate with 75 and 100mM calcium chloride and with or without MS salts. However, 2 % sodium alginate produced irregular, fragile capsules, prone to breakage and difficult to handle. The combination of 3 % sodium alginate with 100 mM calcium chloride produced the best overall performance, producing optimally uniform, isodiametric capsules (Fig. 1), with good results in terms of germination percentage (93.33%) and days to germinate (8.11 days) (Table 2, Fig. 2). The encapsulation with 3% sodium alginate polymerized in 100 mM calcium chloride was the optimal concentration to produce synthetic seeds in cassava. Similar reports were given in other crops by Daud et al. (2008); Geetha et al. (2009); Sarmah et al. (2010); Nor-Asmah et al. (2011); Saha et al. (2015) and Ghanbarali et al. (2016).

The encapsulated explants started to germinate after sixth day of culture on the MS medium. Generally, the germination rate for all combinations was within 60.0% and 96.7% in the duration of 6 to 16 days. The lowest germination observed was 60.0% when 4% sodium alginate with 125mM calcium chloride was use for encapsulation (Table 2). Daud et al. (2008) also observed less germination when sodium alginate concentration was high and reported that this probably suppressed the ability of shoots and roots to emerge. Ghanbarali et al. (2016) revealed that, alginate concentrations above 3 % produced too hard capsules and caused considerable delay in germination and reduced growth speed. The concentration of the complexing agent, calcium chloride, also affected the germination of the encapsulated explants. Addition of MS salts in the alginate solution increased the germination, but the result was not significantly different (Table 2). Cassava synthetic seed storage study was conducted at different durations under room temperature and germination of seed was tested. Drastic reduction of sprouting of encapsulated cassava explants were observed after 21 days of storage under room temperature (Table 3).

Conclusion

For the first time, optimized a procedure for encapsulation of apical and axillary buds of cassava in 3 % sodium alginate with 100 mM calcium chloride. This protocol can be considered a promising alternative to propagation of cassava. The method described hereby is a simple, quick, stable and highly cost-effective. This study not only gives the conditions for short, midterm storage of encapsulated explants, but which can considerably benefit

Table 2. Effect of sodium alginate, calcium chloride and MS salts on germination of cassava synthetic seed

Treatments	Germination %	Days to germination	
		Mean	Range
2% Na alginte + 75 mM CaCl2	86.67 (9.37 ±0.06)	6.89±0.09	6-9
2% Na alginte + 100 mM CaCl2	83.33 (9.18 ±0.02)	7.44±0.03	6-10
2% Na alginte + 125 mM CaCl2	80.00 (9.00 ±0.01)	7.50±0.02	6-10
3% Na alginte + 75 mM CaCl2	86.67 (9.36 ±0.13)	7.78±0.22	6-10
3% Na alginte + 100 mM CaCl2	83.33 (9.17 ±0.47)	8.50±0.87	6-12
3% Na alginte + 125 mM CaCl2	80.00 (8.99 ±0.44)	9.25±0.91	7-12
4% Na alginte + 75 mM CaCl2	66.67 (8.23 ±0.04)	10.50±0.10	8-14
4% Na alginte + 100 mM CaCl2	63.33 (8.02 ±0.20)	12.40±0.63	10-16
4% Na alginte + 125 mM CaCl2	60.00 (7.81 ±0.13)	12.67±0.40	10-16
2% Na alginte + 75 mM CaCl2 + MS	96.67 (9.88 ±0.06)	6.80±0.08	6-9
2% Na alginte + 100 mM CaCl2 + MS	96.67 (9.88 ±0.04)	7.44±0.06	6-10
2% Na alginte + 125 mM CaCl2 + MS	90.00 (9.54 ±0.01)	7.67±0.02	6-10
3% Na alginte + 75 mM CaCl2 + MS	96.67 (9.89 ±0.20)	7.40±0.15	6-10
3% Na alginte + 100 mM CaCl2 + MS	93.33 (9.58 ±0.05)	8.11±0.09	6-12
3% Na alginte + 125 mM CaCl2 + MS	90.00 (9.54 ±0.06)	9.22±0.13	6-12
4% Na alginte + 75 mM CaCl2 + MS	73.33 (8.62 ±0.12)	9.67±0.28	8-12
4% Na alginte + 100 mM CaCl2 + MS	70.00 (8.43 ±0.01)	10.00±0.01	8-12
4% Na alginte + 125 mM CaCl2 + MS	66.67 (8.23 ±0.01)	10.67±0.01	8-14
C.D.	0.51	1.08	
SE(m)	0.17	0.36	

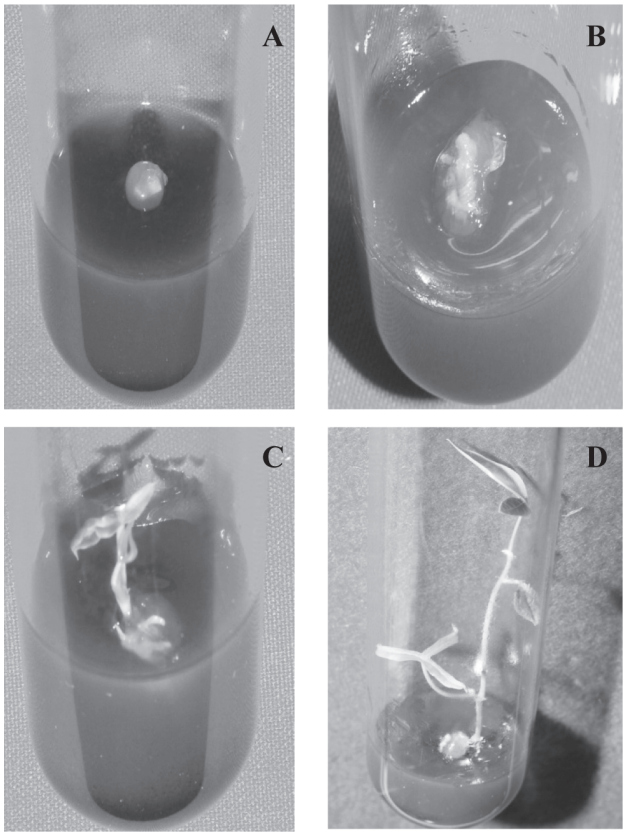

Fig. 2. A. *In vitro* sown synthetic seed, B and C. germinating synthetic seed under *in vitro*, D. plant with well-developed shoot and roots

Table 3. Germination of synthetic seeds of cassava after being stored at different durations under room temperature

Storage period (days)	Germination (%)
0	93.33 (9.71±0.06)
7	73.33 (8.62 ±0.02)
14	46.67 (6.90 ±0.01)
21	16.67 (4.20 ±0.06)
28	6.67 (2.77 ±0.12)
35	0.00 (1.00 ±0.00)
C.D.	0.22
SE(m)	0.06

in germplasm exchange and long-term conservation programs by standardizing cryo-preservation techniques using cassava synthetic seed. However, further research is ongoing aiming to direct field sowing of cassava synthetic seed without *in vitro* germination.

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