SYNTHETIC SEEDS : A NOVEL CONCEPT IN SEED BIOTECHNOLOGY

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Progress in biotechnological research during the last two decades has opened up unprecedented opportunities in many areas of basic and applied biological research. Plant tissue culture, which is an important component of plant biotechnology, presents new strategies for the improvement of cereals, legumes, forest trees, plantation crops and ornamental plants. Besides, plant cell cultures provide a good system for many basic studies in plant breeding, plant physiology, genetics and cell biology. Cell manipulations through the sophisticated methods of genetic engineering for plant quality and product improvement has to rely on plant tissue culture for the final goal. Micropropagation is an area of plant tissue culture which has received maximum attention of researchers for its potential commercial applications.

The regeneration of plants through the techniques of plant tissue culture and their subsequent acclimatization and delivery to the field poses many problems to make tissue culture technology a viable alternative proposition. The successful demonstration of encapsulation of tissue culture derived propagules in a nutrient gel has initiated a new line of research on synthetic seeds. Synthetic seeds are basically defined as, "encapsulated somatic embryos which functionally mimic seeds and can develop into seedlings under sterile conditions". In a broader sense, it would also refer to encapsulated buds or any other form of meristems which can develop into plants.

The main thrust idea is to prepare a simple, inexpensive delivery unit of tissue culture propagated plants and a method for direct sowing of encapsulated material in the field. The encapsulating matrix has the ability to incorporate nutrients, biofertlizers, pesticides, nitrogen - fixing bacteria, antibiotics or other essential additives. The direct delivery of encapsulated material will save many subcultures to obtain plants and also eliminate the difficult stage of acclimatization of *in vitro* plants. The uniform and simultaneous production of encapsulated propagules followed by uniform germination could possibly remove many drawbacks associated with natural seeds.

Many plant systems are known to produce abundant number of embryos in culture which share many properties similar to natural embryos including germination leading to plant production. To mimic the natural seeds, embryos from cultures are encapsulated in a nutrient gel containing essential organic/inorganic salts, carbon source, plant hormones and antimicrobial agents and coated completely to protect the embryos from mechanical damages during handling and to allow the development and germination to occur without any undesirable variations. Several agents have been attempted for encapsulation and sodium alginate complexing with calcium chloride is found to be the most suitable. By this method, two types of synthetic seeds are prepared: hydrated and desiccated. Hydrated synthetic seeds consist of embryos individually encapsulated in a hydrogel, whereas in desiccated type the coating mixture is allowed to dry for several hours in a sterile hood.

The Plant Cell Culture Technology Group of Nuclear Agriculture and Biotechnology Division had initiated research on synthetic seeds in the late 1980s working with sandalwood and mulberry. Eventually other crop systems such as banana, cardamom and rice have also been taken up for the production of synthetic seeds.

In general, the method used is as follows : The propagules (embryos / axillary buds / shoot tips) are carefully isolated from aseptic cultures and blot dried on filter paper, and are then mixed in sodium alginate prepared in nutrient medium. The

Fig. I (1-3) : Synthetic seeds and plantlets in mulberry and banana. 1- synthetic seeds of mulberry planted in soil; 2- mulberry synthetic seeds germinating into plantlets in soil; 3-complete plantlets of banana obtained from synthetic seeds (arrow indicates portion of the synthetic seed still attached to the plantlet)

propagules are then picked up manually by forceps and dropped into a solution of calcium chloride for 40 minutes. After the incubation period, the beads (synthetic seeds) are recovered by decanting the calcium chloride solution and washing them in sterile water 3 to 4 times before culturing on nutrient medium or on different substrates such as filter paper, cotton or soil for their growth and conversion to plants.

Results of the research on synthetic seeds in different plants are briefly described here and applications are highlighted in each case (Fig. 1).

Banana

Banana is an economically profitable crop with a large consumption in the country and a considerable export potential. Edible bananas are vegetatively propagated by suckers as viable seeds are generally not produced in these cultivars. New and effective means of propagating banana would be advantageous over conventional use of sucker material for germplasm maintenance, exchange and transportation. Shoot tips excised from the aseptically raised shoot cultures were excised and encapsulated to prepare synthetic seeds. High percent germination of these synthetic seeds was achieved on a very simple nutrient medium. Addition of the extract of blue green algae to the nutrient matrix enhanced germination frequency. A cell mass (callus) initiated from male flower buds produced embryos which have been successfully encapsulated and germinated. Hence, a twin facility is available in banana to either encapsulate shoot apices or embryos.

Cardamom

ardamom referred to as the queen of spices is an important plantation crop with considerable export earnings. It is generally propagated vegetatively as well as through seeds. Since cardamom is highly cross pollinated, seed derived plants exhibit considerable variation. Multiple shoot cultures from elite clones have been established aseptically for rapid micropropagation to generate a large number of plants. Shoot apices from aseptic cultures have been used for making synthetic seeds. Maximum germination of synthetic seeds was achieved and plants were grown successfully in soil.

Sandalwood

Sandalwood is a commercially valuable forest tree of India which, when propagated by seeds, shows variation. Culture of stem segments placed on an appropriate nutrient medium produced an undifferentiated callus which regenerated into a large number of embryos with the inherent potential to develop into a plant. Synthetic seeds were prepared by encapsulating embryos in a nutrient matrix of calcium alginate. Germination of these embryos into plants was possible. Addition of growth promoting substances to the matrix enhanced the germination frequency considerably.

Mulberry

Mulberry is an important plant whose leaves serve as chief source for feeding of silkworms and is therefore an important component of the silk industry. It is a perennial crop propagated by cuttings or by grafting. However, several cultivars of mulberry are difficult to root and this impedes the propagation. Axillary buds from aseptically growing plants were encapsulated to make individual synthetic seeds which look like pearl beads. Such beads could be stored for considerable time without loss of viability. In case of mulberry, only 30 –40 % cuttings survive the time period between pruning, transportation and final transplantation whereas synthetic seeds could be easily packed in bottles and transported, thus limiting space and ensuring increased viability and survival rate.

Rice

Rice is a major staple food crop of the world and has received considerable attention for investigations on genetic manipulations. Research on synthetic seeds can be useful for the large scale propagation of superior hybrids. Methods have been standardized

for embryogenesis, and plant regeneration from indica rice cultivars has been achieved. Somatic embryos were singly encapsulated and were placed on nutrient medium and also on different substrates such as cotton, filter paper and macpeat. Encapsulated embryos developed into plants with varying frequencies. This technique has immense potential as it would permit multiplication of large scale propagation of elite hybrids.

Conclusion

The examples presented above suggest that, by employing synthetic seeds, the tissue culture raised plants can be regenerated on a simplified medium eliminating subcultures, thus reducing the cost of operation. Development of protocols for direct recovery of plants from synthetic seeds under non sterile conditions may have a greater impact. Although large number of plants can be produced in tissue cultures through embryogenesis / multiple shoot cultures, their delivery is cumbersome. Embryos or shoots have to be separated singly and transferred for rooting to achieve root shoot balance, and the plants have to be hardened in the green house before field planting. Direct sowing of synthetic seeds in the soil does not need acclimatization often required for the tissue cultured plants. It thus provides an ideal delivery system enabling easy flexibility in handling and transport as compared to large parcels of seedlings or plants.

For large scale commercialization in synthetic seeds technology, enhanced production of propagules is necessary. Current tissue culture methods do not generate adequate propagules and are not sufficient to meet the demands of commercial exploitation of synthetic seeds technology. Standardization of methods for synchronization of developing propagules followed by automation of the whole process of sorting, harvesting, encapsulation and germination of the coated propagules can enhance the pace in the production of synthetic seeds.

(For further details, please contact the author.)

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