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Review Article

Somatic Embryogenesis and Production of Artificial Seeds as a Tool for Micropropagation in 'Guava' (*Psidium guajava* L.)

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A B S T R A C T

Guava (*Psidium guajava* L.) is a fruit that belongs to family Myrtaceae and is native to tropical America. Propagation of guava is generally carried out by seeds but late flowering is observed in the seedlings obtained. However, early flowering is observed in plants propagated through somatic embryogenesis. Seeds are the convenient form of propagation for the crop production and it would be beneficial to combine the efficiency aspects of seed with clonal plant production via in vitro somatic embryogenesis. Complex breeding programs are not suitable for producing true breeding seed due to genetic barriers to selfing and long generation time of guava crop. Artificial seed technology is the use of somatic embryos as functional seed. Somatic embryos are developmentally similar to zygotic embryos. The final goal of all the types of artificial seeds is to efficiently deliver the somatic embryos to the germination environment and to recover the whole plants from these embryos. Artificial seeds are produced by mixing somatic embryos with 2% sodium alginate. This have a number of applications such as large scale propagation, germplasm conservation, uniformity etc., However, despite the advantages of artificial seeds, further research is required in order to improve the scope of artificial seed cultivation under non-sterilized conditions.

Keywords

Bio-efficacy, *Ferrisia virgata*, Custard apple

Introduction

Psidium guajava L. is a fruit native to tropical America, which is commonly known as 'guava' and belongs to the family Myrtaceae. It is a fruit with high nutritive value and is also widely distributed all over the equatorial regions of tropical and sub-tropical countries. It is the fourth most important fruit crop in terms of area and production following mango, banana and citrus. Guava fruit is highly palatable in nature because of its intense fruity aroma. It has high medicinal value and considered as economically valuable crop around the globe. Generally, propagation of guava is carried out by seeds

but the seedling trees we obtain are genetically different from their parent population and also late flowering is observed (3-4 years after plantation). However, early flowering (2-3 years) is observed in plants propagated through somatic embryogenesis.

Ideal phenotype of guava with high fruit quality, high yield, disease resistance, long shelf-life, intense aroma, attractive skin, fresh colour and seedlessness cannot be achieved by conventional breeding methods.

Seeds are the convenient form of propagule for crop production when compared to any other propagation methods due to their ease of use, low cost and allowing simplified handling and storage. However, it would be beneficial to combine the efficiency aspects of seed with clonal plant production via in vitro somatic embryogenesis for a number of applications.

Somatic embryos are structurally similar to zygotic embryos found in seeds and possess many of their useful features. Somatic embryos develop from somatic cells unlike zygotic embryos which are the result of sexual reproduction. The use of somatic embryos as functional seed is termed as "synthetic seed technology". Synthetic seeds are functionally defined as somatic embryos engineered to be use in commercial production. This artificially produced seeds have great potential for large scale production of plants as an alternative to true seeds.

Process of somatic embryogenesis

Somatic embryos can be produced from various parts of plants. Unorganized cell clusters or callus can be induced from any part of plant by the use of plant hormones and specific nutrients. Additional hormone treatments will further induce callus to form somatic embryos. These are developmentally and biochemically similar to zygotic embryos. Development of somatic embryos in guava completed in 5-6 weeks, while maturation continued until 8-weeks of culture initiation (Fig. 1).

The need for artificial seed

Basically, seeds are zygotic embryos with enhanced nutritive tissues covered by several protective layers. They are desiccation tolerant, durable and quiescent due to protective coat. Complex breeding programs from which inbred parental lines are developed are used to produce uniform hybrid progeny when crossed.

The main problem associated with such progeny is, it is not possible to produce a true-breeding seed from two parents due to genetic barriers to selfing. The generation time for fruit crops is also too long to achieve rationally an inbred breeding program. This is the major disadvantage of zygotic seeds. Therefore vegetative propagation by layering or low quality open pollinated seed came into use.

After the discovery of somatic embryogenesis, it has become an alternative of conventional zygotic seeds. Somatic embryos are true clones, since they arise from the somatic cells of a single parent. The structural complexity of synthetic seeds depends on requirements of the specific crop application. So, depending on its usage a functional artificial seed may or may not require a synthetic seed coat, be hydrated or dehydrated, quiescent or non-quiescent. Thus, artificial seeds are defined as artificially encapsulated somatic embryos that can be used for sowing as a seed and those possess the ability to convert into a plant under in vitro or ex vitro conditions and retain that potential even after storage.

Types of artificial seeds

There are four types of artificial seed systems ranging from hydrated embryos in a gel capsule or a fluid drilling matrix, to desiccated embryos in a dried wafer. Each system has specific advantages and disadvantages.

The hydrated embryo encapsulation system, consisting of a single somatic embryo in a gel capsule, may be most useful for delivery of single embryos in crops that need to be precision planted. However, the singulation and encapsulation process needs to be automated and this may pose a technical hurdle.

Fluid drilling allows for the mass handling of large quantities of embryos, yet fluid drilling is unsuitable for precision planting and special fluid drilling planters must be used. Desiccated somatic seeds allow for ease of storage and transport but it requires additional steps for desiccation.

The physical appearance of each artificial seed system may differ but the ultimate goal is to efficiently deliver the somatic embryos to the germination environment and to recover the whole plants from these embryos.

The research is required in somatic embryogenesis, embryo coating materials and recovery of plants in soil etc., to achieve the goal of artificial seeds. Integration of all aspects of artificial seeds is crucial if a commercially feasible product is to be obtained (Fig. 2).

Procedures for the production of artificial seeds

Hydrated artificial seeds are most commonly used for the guava crop. It includes the encapsulation of meristematic tissue, such as somatic embryos in hydrated gels. After a large number of tests, it was found that the most useful gel for the encapsulation of somatic embryos was sodium alginate, a common food thickener made from sea algae.

Artificial seeds are produced by mixing somatic embryos with 2% sodium alginate. The alginate is then dropped into a solution of calcium nitrate to complex the drops of alginate. After approximately 20 minutes, a rigid bead of complexed alginate is formed around the somatic embryo.

The alginate bead provides a protective barrier for the fragile embryo while also facilitating the handling of the small embryos. A hydrophobic membrane coating makes the capsules less tacky and increases the flowability.

It is important for interfacing with automated seeding machinery where precision placement of the encapsulated embryo is required. The cultivation of encapsulated somatic embryos of guava in MS medium supplemented with 3% sucrose showed a high conversion rate assessed to be 91.6%.

Storage

The artificial seed's storage capability reported that a high concentration of sucrose or ABA could be useful for short-term conservation of guava (*Psidium guajava* L.) because of their temporary inhibition in encapsulated somatic embryos germination.

Suitable temperature (usually 4°C), suitable capsulation materials, and optimal storage conditions (reduced heat, light, oxygen, etc.) are to be considered while short term storage. Long storage can be achieved by using dehydration or cryopreservation techniques.

Fig.1 Schematic representation of the somatic embryogenesis process for guava

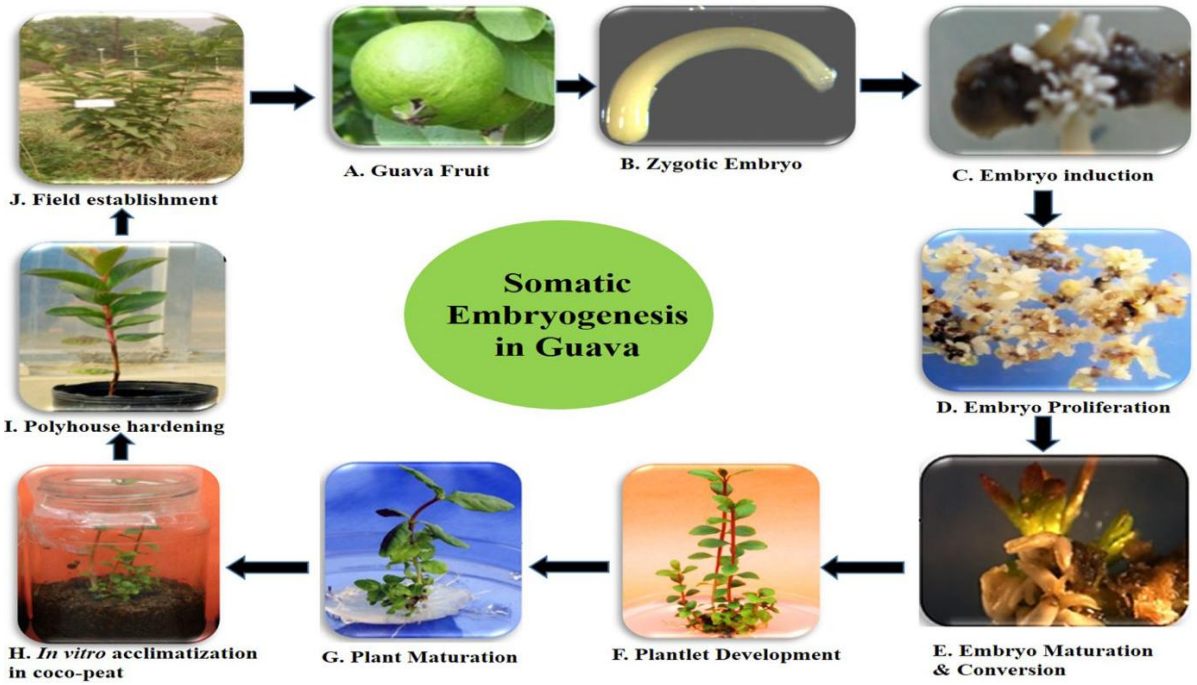
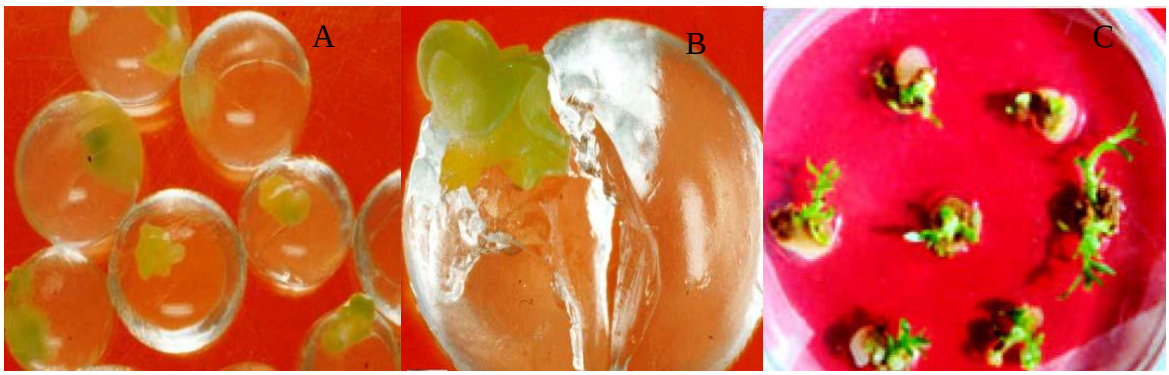


Fig.2 (A) Artificial seeds encapsulated with sodium alginate; (B) Germination of somatic embryo; (C) Ruptured beads showing sprouting of shoots



Advantages

Merits of using artificial seed are listed below.

Synthetic seeds are very much suitable for large scale propagation of the crop.

They play an important role in germplasm conservation.

Synthetic seeds are easy to handle, store, transport and planting as they are small in size.

Seed viability and storage life of synthetic seeds remains for longer period.

The synthetic seeds are in uniform size as they are produced from somatic embryos.

The technology of synthetic seeds production is independent of environmental conditions as these are prepared inside the laboratory.

Basic steps involved in the synthetic seed technology involves seed coat formation, function of endosperm in embryo development and seed germination, somaclonal variation provides wide open facility for study.

This technology can be used for production of hybrids which have unstable genotypes or show seed sterility such as not susceptible towards infection.

Disadvantages

Practical implementation of the artificial seed technology is limited due to the following main reasons.

Production and storage of synthetic seed is cost effective hence the production technique itself becomes costlier.

Production of viable micropopagules useful in synthetic seed production is less.

Anomalous and asynchronous development of somatic embryos.

All the embryos cannot mature at a time hence that makes them inefficient for germination and conversion in to normal plants.

Seed dormancy is considered as a problem but due to lack of dormancy and stress tolerance in somatic embryos the storage of synthetic seeds are limited.

The technology of synthetic seed is limited due to poor conversion of even apparently normally matured somatic embryos and other micropropagules into plantlets.

Applications and limitations

Artificial seeds are the solution for many species that are sterile and produce no seeds. The alternative for these plants is somatic embryogenesis with respect to the cuttings for the propagation. Majority of fruit species are propagated by vegetative means because of the presence of self-incompatibility and breeding cycles very long.

Although the use of somatic embryos has been widely reported for artificial seed production, there are some major challenges that need to be solved to improve the efficiency of protocols. The difficulties in sowing of artificial seeds directly in soil under non-sterile condition are one of the main limitation.

The synthetic seeds should be placed invitro for shoot and root development and need to transfer again to greenhouse for acclimatization. Some experiments were shown that very moist soils and dry soils are extremely detrimental to the embryo survival, causing embryo desiccation and death.

Artificial seed technology is a promising technique for conservation and transport of transgenic plants, non-seed producing plants and plant lines with problems in seed propagation. However, despite the advantages of artificial seeds, further research is required in order to improve the root formation of non-embryogenic artificial seeds.

The scope of artificial seed cultivation under non-sterilized conditions needs to be improved by further research. This could be improved by the use of suitable types and concentrations of anti-diseases and antibiotics and the cryopreservation capacity of artificial seed is also needed to improve by further research.

Research focus on the understanding of plant biology coupled with optimization of delivery and planting methods will push the artificial seed system closer towards commercialization.

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