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WELLNESS

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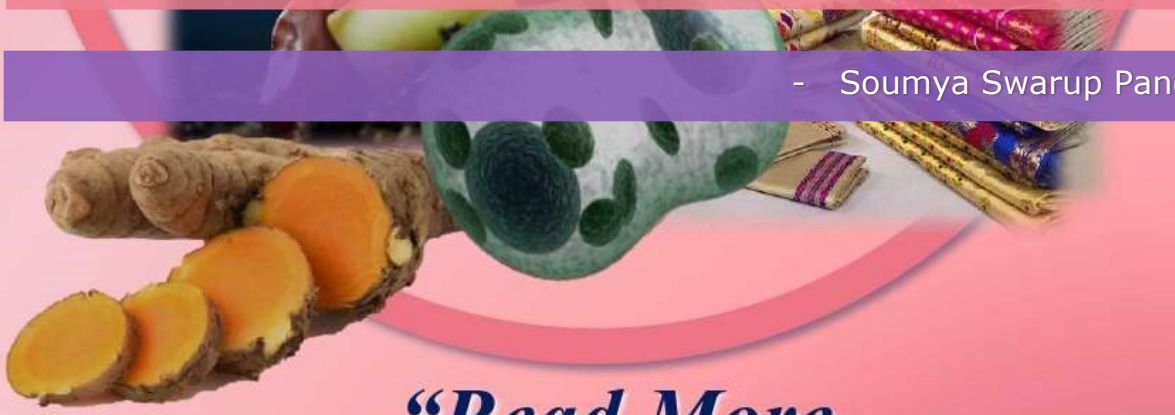

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ASSESSMENT OF PROMISING PROTOPLAST CULTURES FOR PLANT REGENERATION AND ARTIFICIAL SEED TECHNOLOGY

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ABSTRACT

Cell without cell wall is known as protoplast, Prepared usually by digestion with help of enzymes or by mechanical methods. Various nutrients medium are used to isolate and maintain the protoplast to enhance their efficiency. The isolation and culture media used vary with the species and with the type of tissue from which the protoplasts were isolated. Protoplasts are used in a number of ways for research and for plant improvement. Actually the lack of cell wall property of a protoplast provides unique kinds of experimentation and opportunities for observation of living processes in cells. Protoplast isolation is a preliminary step before proceeding to more complex stage such as protoplast regeneration and protoplast hybridization. Likewise nowadays biotechnology starts focusing on artificial seeds to enhance the capabilities as compare to a naturally produced seed and to avoid or sustained different biotic and abiotic stress. Artificial seeds are artificially encapsulated mainly somatic embryos or other vegetative parts such as shoot buds, cell aggregates, auxiliary buds, or any other micropropagules which can be sown as a seed and converted into a plant under in vitro or in vivo conditions with higher efficiency than a conventional seed. this article discusses the plant protoplast isolation, culture and necessity of synthetic/artificial seed production.

Keywords: Protoplast; Artificial Seeds; Genetic transformation; Encapsulation; Regeneration.

INTRODUCTION

Protoplasts culture used to overcome the germination difficulty and development hurdles. It also helps in investigate a broad range of physiological problems reaching from the nutrient uptake to mechanisms related to the synthesis of the cell wall and efficient in production of stress free plant by some alternation in genetic level or by hardening during culture. Likewise synthetic seed technique is a rapid tool of plant regeneration because of its wide use in conservation and delivery of tissue cultured plants. Protocols of encapsulation were already optimized for various plant species. By manipulations in the composition of synthetic endosperm, explant size, media composition, change in the formulation of medium and type of medium, optimization of growth regulators and addition of other additives to the synthetic endosperm are required to enhance the germination frequency of encapsulated propagules and yield of plants.

CULTURE OF PROTOPLASTS:

The very first step in protoplast culture is the development of a cell wall around the membrane of the protoplast. This is followed by the cell divisions that give rise to a small colony. With suitable manipulations of nutritional and physiological conditions, the cell colonies may be grown continuously as cultures or regenerated to whole plants. Protoplasts are cultured either in semisolid agar or liquid medium. Sometimes, protoplasts are first allowed to develop cell wall in liquid medium, and then transferred to agar medium. Agar culture where agarose is the most frequently used to solidify the culture media. The concentration of the agar should be such that it forms a soft agar gel when mixed with the protoplast suspension. The plating of protoplasts is carried out by Bergmann's cell plating technique .In agar cultures, the protoplasts remain in a fixed position, divide and form cell clones. (Sheng et al., 2011; Chen et al., 2004) The advantage with agar culture is that clumping of protoplasts is avoided. And another is liquid culture where this culture is the preferred method for protoplast cultivation for the following reasons like It is easy to dilute and transfer, Density of the cells can be manipulated as desired, For some plant species, the cells cannot divide in agar medium, therefore liquid medium is the only choice and Osmotic pressure of liquid medium can be altered as desired.(Kim et al., 2005; Wang et al., 2008)

FEATURES OF PROTOPLAST CULTURE MEDIA:

The culture media with regard to nutritional components and osmoticum are briefly describe here, as the nutritional requirements of protoplasts are similar to those of cultured plant cells (callus and suspension cultures). Mostly, MS and B5 media with suitable modifications are used. Some of the special features of protoplast culture media are (a) The medium should be devoid of ammonium, and the quantities of iron and zinc should be less. (b) The concentration of calcium should be 2-4-times higher than used for cell cultures. This is needed for membrane stability. (c) High auxin/kinetin ratio is suitable to induce cell divisions while high kinetin/auxin ratio is required for regeneration. (d) Glucose is the preferred carbon source by protoplasts although a combination of sugars (glucose and sucrose) can be used. (e) The vitamins used for protoplast cultures are the same as used in standard tissue culture media. (Yamagishi et al., 2008)

PROTOPLAST CULTURE METHODS

The culture techniques of protoplasts are almost the same that are used for cell culture with suitable modifications. Some important aspects are briefly discussed here. (a) Feeder layer technique: For culture of protoplasts at low density feeder layer technique is preferred. This method is also important for selection of specific mutant or hybrid cells on plates. The technique consists of exposing protoplast cell suspensions to X-rays (to inhibit cell division with good metabolic activity) and then plating them on agar plates. (b) Co-culture of protoplasts: Protoplasts of two different plant species (one slow growing and another fast growing) can be co- cultured. This type of culture is advantageous since the growing species provide the growth factors and other chemicals which help in the generation of cell wall and cell division. The co-culture method is generally used if the two types of

protoplasts are morphologically distinct. (c) Micro drop culture: Specially designed dishes namely cuprak dishes with outer and inner chambers are used for micro drop culture. The inner chamber carries several wells wherein the individual protoplasts in droplets of nutrient medium can be added. The outer chamber is filled with water to maintain humidity. This method allows the culture of fewer protoplasts for droplet of the medium. (Dambier et al., 2011)

REGENERATION OF PROTOPLASTS

Protoplast regeneration which may also be regarded as protoplast development occurs in two stages one is formation of cell wall and other is development of callus/whole plant. Formation of cell wall, the process of cell wall formation in cultured protoplasts starts within a few hours after isolation that may take two to several days under suitable conditions. As the cell wall development occurs, the protoplasts lose their characteristic spherical shape. The newly developed cell wall by protoplasts can be identified by using calcofluor white fluorescent stain. The freshly formed cell wall is composed of loosely bound micro fibrils which get organized to form a typical cell wall. This process of cell wall development requires continuous supply of nutrients, particularly a readily metabolized carbon source (e.g. sucrose). (Cassells and Curry, 2001) Cell wall development is found to be improper in the presence of ionic osmotic stabilizers in the medium. The protoplasts with proper cell wall development undergo normal cell division. On the other hand, protoplasts with poorly regenerated cell wall show budding and fail to undergo normal mitosis. Development of callus/whole plant, As the cell wall formation around protoplasts is complete, the cells increase in size, and the first division generally occurs within 2-7 days. Subsequent divisions result in small colonies, and by the end of third week, visible colonies (macroscopic colonies) are formed. These colonies are then transferred to an osmotic-free (mannitol or sorbitol-free) medium for further development to form callus. With induction and appropriate manipulations, the callus can undergo organogenic or embryo genic differentiation to finally form the whole plant. Plant regeneration can be done from the callus obtained either from protoplasts or from the culture of plant organs. There are however, certain differences in these two calluses. The callus derived from plant organs carries preformed buds or organized structures, while the callus from protoplast culture does not have such structures. The first success of regeneration of plants from protoplast cultures of Nicotianatabacum was achieved by Takebe and team. Since then, several species of plants have been regenerated by using protoplasts. (Xu et al. 2013)

SUB-PROTOPLASTS

The fragments derived from protoplasts that do not contain all the contents of plant cells are referred to as sub-protoplasts. It is possible to experimentally induce fragmentation of protoplasts to form sub-protoplasts. This can be done by application of different centrifugal forces created by discontinuous gradients during centrifugation. Exposure of protoplasts to cytochalasin B in association with centrifugation is a better approach for fragmentation of protoplasts. (Grosser and Gmitter, 2005) Fragmentation of Protoplast to Form Sub-Protoplasts like, (a) Mini-protoplasts:These are also called as karyoplasts and contain the nucleus. Mini-

protoplasts can divide and are capable of regeneration into plants. (b) Cytoplasts:These are sub-protoplasts containing the original cytoplasmic material (in part or full) but lack nucleus. Thus, cytoplasts are nuclear-free sub-protoplasts which cannot divide, but they can be used for cybridization. (c) Micro-protoplasts:Thisterm was suggested for sub-protoplasts that contain not all but a few chromosomes.

ARTIFICIAL SEED

Synthetic seeds are defined as artificially encapsulated somatic embryos, shoot buds, cell aggregates, or any other tissue that can be used for sowing as seed & that posses the ability to convert into plant under in vitro and ex vitro condition. Synthetic seeds has great potential of large scale production at low cost an alternative to true seed. Kitto and Janick in 1982 first coated carrot embryoids with polyoxethylene to develop artificial seeds. (Kitto and Janick, 1982; Gray, 1987) In 1984 Redenbaugh developed a technique for encapsulation of individual SE of alfalfa by using hydrogels like sodium alginate. (Redenbaugh et al., 1991) In alfalfa desiccation-tolerance of SE's embryos was induced by exogenous application of ABA by Seneratna, Mckersie & Bowley.

METHOD FOR PRODUCTION OF ARTIFICIAL SEED

Establishment of Callus culture, Induction of somatic Embryogenesis in callus culture, Maturation of SE, Synchronization & singulation of SE, Mass Production of SE, Standardization and Encapsulation of SE, Mass production of synthetic seeds, Test for embryoids to plant conversion and Green house & field planting. (Fujii et al., 1992)

METHODS USED FOR ENCAPSULATION AND TYPES OF ARTIFICIAL SEEDS:

Mainly two types one Gel complexation via a dropping procedure Isolated somatic embryos are mixed with 0.5 to 5% (W/V) sodium alginate and dropped into 30-100µM calcium nitrate solution. Surface complexation begins immediately and the drops are gelled completely within 30 minutes and other one is Molding Isolated somatic embryos are mixed in a temperature dependent gel such as Gel-rite and placed in the well of a microtitre plate and it forms gel when the temperature is cooled down. Likewise artificial seeds are mainly two types (a) Desiccated seed where SE's is initialy hardened to withstand desiccation & then are encapsulated in suitable coding material. SEs may be hardened either by treating/coating mature SE with suitable polymer followed by treated with ABA(improve germination of SE(Ara et al., 1999) (b) Hydrated artificial seeds here Somatic embryos are enclosed in gels, which remain hydrated. Calcium alginate is most suitable. Hydrated artificial seeds are sticky & difficult to handle on a large scale &are dry rapidly in the open air. This problems can be solved by providing a waxy coating over the bead. (Redenbaugh et al., 1991) However, hydrated artificial seeds have to be planted soon after they are produce. hydrated artificial seeds by mixing SE of alfalfa, celery & cauliflower with sodium alginate followed by dropping into a solution of calcium chloride/nitrate to form calcium-alginate.

POTENTIAL USE OF ARTIFICIAL SEEDS

Artificial seeds make a promising techniques for propagation of plants, non

seed producing plants, polyploids with elite traits and plants line with problems in seed propagation. Being clonal in nature the techniques cuts short laborious selection procedure of conventional recombination breeding and it also reduced cost of transplanting, Carrier of adjuvant such as microorganism availivility, Plant growth regulators, pesticides, fungicides can be blend, Enhanced nutrients & antibiotics, Large scale monoculture and conceivably handling can be done, Production of large no of identical embryos possible, Determination of role of endosperm in embryo development & germination and study of somaclonal variation can be performed. (Mishra et al., 2011; Lata et al., 2011)

CONCLUSION

Somatic hybrid plants developed from post-fused protoplast cultures with desired modifications in genetic level to gives a resistance against biotic and abiotic stresses. Even protoplast culture helps in enhance the efficiency, overcome the germination hurdles and germplasm conservation. Apart from some limitations of artificial seeds like, Limited production of viable micropropagation, Lack of stress tolerance in SE and Improper maturation of SE that makes them inefficient for germination and conversion into normal plants. artificial seeds preferable in some case as it has some advantages like, High volume, large scale propagation, Maintains genetic uniformity of plants, Direct delivery of propagules to the field, thus eliminating transplants, Rapid multiplication of plants and germplasm conservation.

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