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PROSPECTS OF PLANT TISSUE CULTURE IN CROP IMPROVEMENT

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Tissue culture is the aseptic culture of cell, tissue, organ or total plant under-regulated nutritional and environmental conditions (Thorpe, 2007) which is utilized frequently to generate the clones of plants *in vitro* conditions. The clones produced are the true-to-type to the genotypes of the selected plants. The regulated environment provides the culture of an environment conducive for their favorable growth and multiplication. These controlled conditions include proper supply of nutrients, pH medium, suitable temperature, proper gaseous and liquid environment. Plant tissue culture technology is being extensively used for large scale plant multiplication. Apart from its use as a research tool, plant tissue culture techniques in the recent years have become important for use in the areas of propagation of plants, disease or insect reduction, crop improvement and secondary metabolites production. The explant is being utilized to produce hundreds or even thousands of plants in an uninterrupted manner. Explant can be multiplied to produce several thousand plants in a very limited period of time and even requires less space under a controlled environment, irrespective of the season and weather throughout the year (Akin-Idowu *et al.*, 2009). Rare species have been successfully grown and conserved by plant tissue culture technique because of the high rate of multiplication and limited requirements of the number of initial plants material and space. In addition to this, plant tissue culture is contemplated as the most effective technique for improving crops by the utilization of somaclonal and gametoclonal variations. The micropropagation technique has a huge capacity to develop genotypes of superior quality, isolation of desirable variants with high yielding capacity, enhanced disease and stress tolerance capabilities. Callus cultures may give rise to the clones that have heritable behaviour differing from those of parent plant due to the probability of

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induction of somaclonal variation, which leads to the production of commercially important improved cultivars.

Basics of plant tissue culture

Plant cells, tissues or organs are grown *in vitro conditions* on suitable artificial media, under aseptic and controlled environmental conditions. This technique relies mainly on the ability of totipotency of plant cells that means the capacity of any single cell which can express the whole genome by the division of its cells. Along with this totipotency capacity, the capacity of plant cells to alter their growth, development and metabolism is also equally decisive for the regeneration of whole plant form. Tissue culture media generally contains some or all of these constituents: macronutrients, micronutrients, amino acids, vitamins or source (s) of carbon, nitrogen supplements, growth regulators, organic supplements, and solidifying agents. Murashige and Skoog medium (MS medium) is the most widely used media for vegetatively propagating most of the plant species *in vitro* conditions. The adjustment of the pH of media is very important which affects the growth and working of plant growth regulators. It is adjusted to the range between 5.4 - 5.8. Plant growth regulators (PGRs) are necessary for plant tissue culture since they play important roles in tropism, stem elongation and apical dominance. They are generally auxins, gibberellins, cytokinins and abscisic acid (ABA). However, the ratio of auxins to cytokinins decides the type and extent of organogenesis in plant tissue cultures. The high level of auxins is responsible for root formation, however, the high level of cytokinins favours shoot formation. An equal proportion of both cytokinin and auxin leads to the formation of an undifferentiated mass of cells (called callus). Cytokinins stimulate cell division, induces axillary shoot proliferation, shoot formation and retard root formation. Gibberellins inhibit later stages of embryo development and also enhance the proliferation of shoots.

Genetic transformation

It is the recent aspect of plant tissue culture technique that provides the means to transfer genes with desirable trait into host plants and recovery of desirable transgenic plants. This technique has a great scope for genetic improvement of various crop plants by integrating various plant biotechnological and breeding programmes. It has wide and promising utility for the introduction of agronomically important traits such as enhanced yield, improved quality and increased resistance to pests and diseases. In plants, genetic

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transformation can be attained by either indirect gene transfer or direct gene transfer method. Among vector-mediated or indirect gene transfer methods, *Agrobacterium*-mediated gene transformation is the widely used method for the expression of foreign genes in plant cells. The successful introduction of agronomic traits in plants can be achieved through the use of root explants for the genetic transformation. virus-based vector methods offer an alternative way of rapid and transient protein expression in plant cells and thus, provides an efficient way of recombinant protein production on a huge scale. Recently successfully generated transgenic plants of Jatropha were obtained by direct DNA delivery to the mature seed-derived shoot apices through particle bombardment method (Purkayastha *et al.*, 2010). This technology has been found efficient in the reduction of toxic substances in seeds and thus, overcoming various obstacles of seed utilization in different industrial sectors. Regeneration of disease and viral resistant plants can now be achieved by employing genetic transformation technique. Scientists have succeeded in developing transgenic plants of potato, resistant to potato virus Y (PVY) which was a major threat to potato crop worldwide.

Somatic hybridization

A somatic hybridization is a useful tool for the production of inter-specific and inter-generic hybrids. This method involves the fusion of protoplasts of two diverse species or genomes followed by the selection of superior hybrids and their regeneration to form hybrid plants. It provides a useful method of gene transfer with the desired attribute from one species to another and has a remarkable impact on crop improvement. Protoplast fusion opens up a method of developing unique hybrid plants by overcoming various barriers of sexual incompatibility. This method is applicable in the horticultural sector to create new hybrids with enhanced fruit yield and better resistance to diseases and pests. The potentiality of somatic hybridization in plants is best illustrated by the production of inter-generic hybrid plants among the family Brassicaceae. To solve the problems of chromosome loss and decreased regeneration capacity, various protocols have been developed for the production of somatic hybrid plants by using two unlike types of wheat protoplast as recipient and protoplast of *Haynaldia villosa* as a donor for fusion. It has also been used as an important gene source for Improvement of wheat.

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Haploid production

Through plant tissue, culture techniques such as protoplast, anther and microspore cultures facilitate the production of homozygous plants in a comparatively short interval of time. In general, haploids are sterile which are changed into homozygous diploids by an impulsive or induced doubling of chromosomes. Doubling helps to fix the fertility of plants results in the creation of doubled haploids. At the present, the haploid technique has come out as a vital part of plant breeding technique by speedy up the development of inbred lines and hence overcome the limitation of non-viability of the embryo and seed dormancy. This method used in genetic renovation by the production of haploid plants with additional resistance to various stresses. For example in wheat for drought tolerance, Introduction of genes with desirable attribute at haploid phase after that doubling of a chromosome leads to the development of double haploids inbred lines and also drought tolerant genotypes.

Somatic embryogenesis

It is an *in vitro* method of plant rejuvenation which is widely used as major biotechnological tools for clonal propagation (Park *et al.*, 1998). It is a method by which somatic cell or tissues evolved into differentiated embryos. These embryos can develop into complete plants without undergoing sexual fertilization. The somatic embryogenesis can be directly evolved from the explants or indirectly by callus. Plant regeneration by means of somatic embryogenesis arises by the initiation of embryogenic cultures from leaf, zygotic seed, stem parts and further development of embryos. The full-grown embryos are then cultured for germination and further development and lastly transferred to soil.

Conclusion

As an emerging technology, tissue culture has a great influence on both industry and agriculture, by providing a large number of plants that are needed to meet the ever-increasing food demand. It has made important contributions to the advancement of agricultural sciences in recent times and today they form a necessary tool in modern agriculture. Tissue culture offers the production and propagation of genetically homogeneous, disease-free plant materials. It is a useful tool for the induction of somaclonal variations. Genetic variability induced by plant tissue culture can be used as a source of variation to obtain new favourable genotypes. *In vitro* cultures of mature or immature zygotic embryos are applied to recover

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plants obtained from wide crosses that do not produce fertile seeds. Genetic engineering can make it feasible to improve crop varieties with high yield potential and even resistance to pests. Genetic transformation technique relies on the various technicalities of plant tissue culture and molecular biology for the production of improved crop varieties, disease-free plants (virus), production of secondary metabolites, genetic transformation, production of varieties tolerant to drought, salinity and heat stresses.

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