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Review Article

Plant Tissue Culture Technology to Improve Crop Species – A Comprehensive Approach

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Abstract

Plant tissue culture response for callus induction and regeneration is prerequisite for improvement of wheat through Embryo culture, Somatic hybridization, enhancing regeneration capacity and in vitro mutagenesis. Plant tissue culture techniques are being used as a novel tool to assist plant biologists in crop improvements. Different novel tools may be used to either increase the efficiency of breeding process in order to improvement of existing wheat germplasm and to develop new genetic variations for crop improvement. These achievements are not possible through conventional breeding methods. The plant growth regulators, various auxins and cytokinins or their combinations are generally used in MS medium. In this way optimization of regeneration system may be developed to create healthy plants from embryos using interspecies crosses. Genome editing, and mutation techniques are the novel tools nowadays including TILLING for creating new lines of wheat with incorporation of novel traits. To create new lines using such techniques required efficient protocol for callus development using various explants especially mature embryos and further efficient regeneration systems to make the research systems very fast towards development of biotic and abiotic stress resistant crops to cope the climatic changes experiencing worldwide in the present time.

Keywords: Plant Tissue Culture; Regeneration; Mature Embryo; Mutation and Climate Change

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple food crop of the family *Poaceae*. It is the third largest cereal in terms of production in the world after rice and maize and the second most important in terms of calories after rice. It is the source of maximum proteins among cereals (www.wheat.org). Among the food crops, wheat is a common source of energy and proteins for the world population. Worldwide, it is cultivated on approximately 220 million hectares of land with a production of 749 million tons. India it is cultivated on approximately 30.2 million hectares of land with a production of 93.5 million tons (FAOSTAT 2016). A number of environmental factors such as temperature, moisture, soil and light intensity affect the growth and yield of wheat [1]. The plant tissue culture is an integral part of biotechnology breeding and provides an added advantage to crop improvement programmes. The hindrance of different cereal crops to regeneration through callus is a major bottleneck in any crop improvement program including wheat [2]. In 1902 Gottleib Haberlandt proposed the idea of growing individual plant cells on artificial medium. While Haberlandt never realized his idea, the 105 years since has seen the concept evolve into a powerful tool utilized throughout the plant sciences. Plant tissue culture broadly refers to growing plant cells, tissues, organs, seeds, or other plant parts in a sterile environment on a nutrient medium. Tissue culture is being used for an increasing variety of purposes. Originally used largely for fundamental research to study cell division, plant growth, and biochemistry, the technology has grown and is being widely implemented on a more applied scale. In many cases, protocols have been developed and refined so

that they have become a standard and commercially viable practice for propagating many important horticultural crops [3]. The mature embryos are viable alternative to immature tissue derived explants for efficient shoot regeneration in a comparatively genotype independent manner on a simple basal medium containing 2,4-D and TDZ [2]. For this important group, alternative approaches are developed, the techniques of manipulation with somatic tissue: mutation breeding and biotechnology. The applications of plant tissue culture technology considered to shorten the time it takes to bring them to market. A right condition an entire plant, large amounts of new cells or tissue can be regenerate from a single cell, however, in certain situations, the conventional methods have to be supplemented with plant tissue culture techniques either to increase their efficiency and to be able to achieve the objective which is not possible through the conventional breeding methods (Tazeb, 2017). The plant tissue culture has been given the highest priority in plant breeding work since it provides immense potential for crop improvement programs such as selecting disease, insect, or stress resistant plants, regeneration of the novel hybrid technology, rescue the embryos from wide crosses through embryo culture, haploid and dihaploid production within short time frameetc [4]. Applications of Plant Tissue Culture: Recently, plant tissue culture has an indispensable application on both agricultural and industry, through providing plants needed to meet the ever increasing world demand. Therefore, the novel applications of plant tissue culture particularly in the area of plant breeding for the sake of crop improvement are listed below:

Embryo culture

Embryo culture, an *in-vitro* technique, is referred as embryo rescue technique used to save deterioration of hybrids. *In vitro* condition is known as embryo rescue and is widely used for crop improvement. Hanning in 1904 first cultured isolated mature embryo of a cross between *Cochlearia × Raphanus* and successfully obtained the plants. Mature embryos of crucifer were used on a salt medium mixed with sugar. Interspecific hybridization is the specific technique for embryo culture used in plant breeding due to abortion of most of the embryo crosses [5]. Studied the most common explants used for wheat transformation is immature embryo. However, special measures are required to obtain immature embryos and its use is restricted after a while. Therefore, mature embryos are prominent alternative for callus development and somatic embryo formation, which can be used in wheat transformation. Turhan and Baser [6] studied that development of callus in wheat from mature embryos is helpful for crop improvement of wheat through the modern plant tissue culture as well as genetic transformation techniques. Embryos with or without endosperm can be helpful to obtain regeneration of plantlets of mature embryos can also be used for regeneration of plantlets and callus formation. Hanning (1904) obtained viable plant from mature embryos of different species. He conducted experiment on mineral salt containing medium under aseptic conditions. Genotype response towards regeneration and induction of callus were checked to culture mature embryos. Using mature embryo cultures considerable differences in wheat cultivars were recorded in the form of regeneration of plants and effectiveness of callus [7]. Ahmadpour., *et al*. [8] did *in vitro* regeneration of wheat cultivars successfully using mature and immature embryos. In general, the frequency of plant regeneration from immature embryos was slightly higher than mature embryos as they recorded.

Callus induction and Regeneration

Plant tissue culture is considered as an integral part of biotechnology and breeding and provides an added advantage to crop improvement programmes. The hindrance of different cereal crops to regeneration through callus is a major problem in any crop improvement program including wheat [2]. The good and efficient callus induction system in wheat is highly depends on sterilization process, genotypes, type of explants, culture media composition and its pH, concentration of growth hormones, inducers and incubation conditions [9]. Different type of explants have been tested as starting material for wheat callus cultures, such as immature embryos [10], leaf tissue [11], anthers culture [12], immature embryos [13], microspores [14] and mature embryos [2] which showed the different response for callus induction and regeneration. First successful regeneration in wheat was reported by [15]. Kumar., *et al.* [16] reported that optimized media showed good callus induction and regeneration in both mature and immature embryos as explants irrespective of the genotype used. Malik*., et al.* [17] studied this simple MS basal medium containing dicamba and zeatin, maltose (30 gl-1) without any growth additives and agar (8.0 gl-1) was found to be the best for efficient plant regeneration from mature wheat embryos. Shah, 2017 developed a standard protocol for efficient callus induction and its subsequent regeneration.

Somatic Hybridization

The incompatibility barriers in sexual recombination at interspecific or intergeneric levels are also overcome by using proto-

plast fusion technique. It may be due to that (i) many desirable combinations of characters cannot be transmitted through conventional breeding methods of genetic manipulation and (ii) conventional hybridization is also limited to only very closely related species and was total failure for distantly related species as well as in sexually incompatible species. However, by using a protoplast fusion technology, it is now possible to fuse two genetically different species by only protoplast to obtain para-sexual hybrid protoplast. Thus, protoplast fusion is a novel tool for plant biologist and crop improvement by developing interspecific and/or intergeneric unique hybrid plants which cannot be produced by conventional sexual hybridization [18]. The technique involves the fusion of protoplasts of two different genomes of genetically unrelated species followed by the selection of desired somatic hybrid cells and regeneration of hybrid plants [19]. Protoplast fusion technology being the pioneered technology and provides an efficient ways of gene transfer with desired trait from one species to another and thus has significant contributions on national crop improvement program [18]. *In vitro* production of haploids can solve some problems in genetic studies of plants since gene action is readily manifested due to a single allelic gene present in chromosome of entire genome. By doubling their chromosomes number, the plants can be made fertile and resultant plants will be homozygous. Thus, tissue culture techniques enable to produce homozygous plants in relatively short time period through protoplast, anther and microspore cultures instead of conventional breeding methods [20]. Haploids are sterile plants having single set of chromosomes (one-half of the normal number of chromosomes) which are converted into homozygous diploids by spontaneous or induced chromosome doubling [21] since the doubling of chromosomes restores the fertility of plants resulting in production of double haploids with potential to become pure breeding new cultivars [22].

Effect of EMS

EMS is considered as one of the most important chemical mutagenic agents and it is considered as an alkylating agent that has a mutagenic effect on the DNA [23]. Genetic variability apart from conventional breeding can be easily discernible in *in-vitro* regenerated somaclonal variants. Tissue culture techniques through mutation induction can be used to increase the speed or efficiency of breeding programs to get a new diversity of germplasm. In vitro mutation induction can be done by using chemical mutagens and also with use of growth regulators which have high activity [24]. Wheat mutant characterization is a part of the breeding programs to investigate the diversity that may influence the production at a larger level. Bhat., *et al*. [25] studied that there was a gradual decrease in the number of runners and increase in days taken to initiate the runners with increase in EMS concentrations and treatment duration in strawberry. EMS was found to effective to generate higher type and magnitude of variants in strawberry [26]. Qin., *et al*. [27] suggested that these EMS treatment embryos could produce plants with wide variation in chromosome number once budded successfully. Ethyl methane sulfonate (EMS) is a mutagen with the characteristic of high mutation rate and has been successfully used in many crops [28]. Bahar., *et al*. [29] reported that statically determined observations on some traits affected by EMS mutation will be useful when large number of mutants is required for TILLING experiments to identify and confirm function of the candidate genes. For which purpose, the highest dose of EMS treatment (0.3%) is considered to be the best, since it not only produces highest variability but also not detrimental for the survival of the plants. Ahmed., *et al.* [23] investigated that EMS solution with a concentration of 0.07% have a mutagenic effect on the seeds of the tomato plant and cause a positive mutation on tomato plant in the form of fast growth rate and a taller plant and bigger leaves size. Sari., *et al.* [30] also reported that increasing EMS dose and soaking time in embryogenic callus inhibited the growth of callus. The higher EMS dose applied, the less calluses are formed. Sen., *et al.* [31] showed that physical mutagenesis has an effect on generating mutant lines with enhanced drought stress tolerant. *In vivo* types of these mutants may also indicate that the mutations are definite under drought stress and these candidate drought-tolerant mutant lines have the potential to be used as genetic resources in wheat functional genomics studies during plant breeding.

Effect of UV light

The ultra violet radiation (UV) is a part of non-ionizing radiations electromagnetic spectrum the approximately 8-9% of the total solar radiation [32]. The Ultraviolet wavelength (400 – 200 nm) is a small part of the solar radiation reaching the Earth's surface but with significant biological impact on the living organisms, including plants. According to the International Commission on Illumination this wavelength region is divided in to three wavelength range in this UV-A (315 – 400nm), UV-B (280 – 315nm) and UV-C (200 – 280nm). The negative effect of UV radiation increases towards the shorter wavelengths. Therefore, due to its highest energy, UV-C quickly provokes high levels of injuries and it is most detrimental for the living organisms [33]. Teresa., *et al*. [34] showed that UVB irradiation led to increase in content of chlorophyll a and b in regenerated shoots. Bravo., *et al*. [35] reported that UV-B light

in agriculture systems constitutes a promising tool to increase crop production and protection against pests. Tasheva., *et al.* [36] contributed to deeper understanding of influence of UV - rays on plant growth, development and metabolism. Manaf., *et al.* 2016 studied the maximum increase in caffeic acid, total phenols and PAL activity in cell suspension was achieved by 4 h exposure time. Likewise, using 2 UV-B lamps for 2 h was the most effective in creating more biochemical components than the other treatments. Hopkins., *et al.* [37] recorded the reduction in growth of wheat primary leaves was found to be due to an effect of UV-B on the rate and duration of both cell division and elongation. In UV-B-grown plants, the proportion of mitotically active cells was reduced, and the duration of cell division increased. Thus, the supply of cells into the elongation zone was reduced in UV-B-grown plants, which, coupled with a reduction in the rate of elongation, resulted in the observed reduction in leaf growth [38,39].

Conclusion

Plant tissue culture technology has been widely used for improvement of the most important agricultural crops as well as endangered native species. Mutation and somatic hybridization is another way of enhancing variation in crop species using this novel technique. Embryo culture may also help to optimize the media nutrient regime, concentration of growth hormone and developmental stages of a particular plant species. Apart from conventional breeding program, somatic embryogenesis, *in vitro* mutation and molecular techniques have been proved as valuable tools to enhance the genetics of different crop plants. The *in vitro* mutant lines have the potential to be used by plant biologists as genetic resources in wheat functional genomics studies.

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