

## A NEW METHOD FOR PLANT BREEDING THROUGH THE USE OF PLANT TISSUE CULTURE

KHALIL M<sup>1\*</sup>, SARWAR MS<sup>2</sup>, TARIQ HM<sup>2</sup>, FAROOQ MU<sup>2</sup>, ULLAH MZ<sup>2</sup>

<sup>1</sup>Department of Plant Breeding and Genetics, Faculty of Agricultural Sciences, University of the Punjab  
P.O.Box.54590, Lahore, Pakistan

<sup>2</sup>Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, P.O BOX. 54590,  
Lahore, Pakistan

\*Correspondence author email address: [khalilmalik8585@gmail.com](mailto:khalilmalik8585@gmail.com)

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**Abstract** Plant tissue culture techniques have been created as a novel tool to support plant breeders in crop improvement perspectives as an enabling and developing technology. To improve the accessibility of currently available germplasm, generate new genetic variation for crop improvement, and accomplish goals that are not achievable through traditional breeding methods, these innovative tools can be used to either speed up or increase the efficiency of the breeding process. These include eliminating pathogens from planting materials, removing sexual incompatibility using an embryo rescue technique, producing haploids via anther culture, utilizing protoplast technology for somatic hybridization, utilizing gene transformation in transgenic technology, and, above all, inducing new genetic variability through somaclonal variation and the selection of desired agronomic traits. Therefore, the plant tissue culture method holds great promise for producing exceptional quality plants and selecting beneficial variants in highly productive genotypes that are well-adapted and have improved stress tolerance and disease resistance capacity.

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### Introduction

The world's population is growing, there is a decline in agricultural resources like land and water, and the yield curves of staple crops appear to be plateauing (Lobell et al., 2009). As a result, plant breeders need to develop new approaches to crop improvement technologies to overcome these challenges (Morris and Bellon, 2004). A great deal of research has led to the creation of new fields of plant breeding called "plant biotechnology" and "genetic engineering," which are based on cellular totipotency or the capacity to regenerate entire flowering plants (Ranabhat et al.). It might take years for some trees and plants to blossom and produce seeds, which makes improving them challenging (Karp and Shield, 2008). A few crops, such as sugarcane, bananas, apples, cassava, and yams, reproduce vegetatively, particularly the entirely sterile ones that lack seeds. Other strategies, such as somatic tissue mutation breeding and manipulation procedures, had to be devised for this significant group (Auxilia and Shabha, 2017). To help breeders in this area, scientists have thus established the science and art of plant tissue culture (Trigiano and Gray, 1999). It is well known that creating new crop varieties through traditional plant

breeding is labor-intensive and can take seven to ten years (da Silva Dias, 2015). However, the time it takes to market products has significantly decreased thanks to applications of plant tissue culture technologies (García-González et al., 2010).

Given the correct conditions, a single cell can regenerate into many new tissue cells in an entire plant (Mironova and Xu, 2019). Furthermore, in some circumstances, plant tissue culture techniques must be added to conventional procedures to improve their efficiency or accomplish the goal that conventional breeding methods cannot do. As a result, using plant tissue culture is currently the most promising field, providing hope and greater attention for future science-based agricultural research (Henkhaus et al., 2020).

Consequently, it has emerged as the most effective technique for crop improvement programs by generating novel genetic variations, a prerequisite for any work done to improve crops (Takeda and Matsuoka, 2008). This method has many potential uses, including producing higher-quality plants and discovering beneficial variants in highly productive genotypes better adapted to biotic and abiotic stress

(Younis et al., 2020). Because somaclonal variability can generate enhanced varieties that are less important commercially, these somaclonal variants were generated through tissue culture procedures (Jain et al., 1998). Give rise to clones with inheritable features that differ from the parent plants. Furthermore, since the production of mutant lines is highly desirable for plant breeding efforts, the tissue culture procedure has also created many somaclonal and gametoclonal variants with the potential to boost crop yields (Jain et al., 1998). Consequently, this work aims to demonstrate some of the many innovative ways plant tissue culture is used in plant breeding (Trigiano and Gray, 1999). These include the preservation of germplasm, haploid production, protoplast fusion, embryo rescue, pathogen-free plant material generation, synthetic seed production, somaclonal variation, and haploid production (Tazeb, 2017).

#### **An Overview of Plant Tissue Culture History**

The science of cultivating plant cells, tissues, organs, or other parts separated from the mother plant in artificial media under aseptic circumstances is known as tissue culture (Bhojwani and Dantu, 2013b). Plant tissue culture science originates in groundbreaking biological studies such as the discovery of the cell and the development of cell theory 30 years ago with the work of Gauthier, Nobecourt, and White, who discovered and used auxins. These and other scientists identified the dietary and hormonal needs of the cultivated plant tissues (Gaspar et al., 1996). In the past several years, there have been significant advancements in various plant tissues (Hussain et al., 2012). Tissue developed in an enriched media (Phillips and Garda, 2019). Despite establishing the groundwork for tissue culture technology, he is recognized as the originator of plant tissue culture (Collin and Dix, 1990). The second phase of plant tissue culture began in more culture areas, and plant tissue culture methods have been widely used in industry and agriculture (Thorpe, 2007). Relationship between Leaf Structure and Carbon Isotope Discrimination in Field Grown Barley (Araus et al., 1997).

#### **Fundamentals of Tissue Culture in Plants**

The explants are then typically placed on top of a solid culture media, but occasionally, they are placed straight into a liquid medium; this is especially true when it is wanted to create cell suspension cultures (Bhojwani and Dantu, 2013b). The principle of totipotency, which describes a single plant cell's capacity to express its entire genome through cell division and/or to proliferate and differentiate into a fully formed plant, is the foundation of plant tissue culture (de Almeida et al., 2015). Apart from the totipotent potential of plant cells, the ability of individual cells to modify their growth, development, and metabolism is also crucial for the regeneration of the entire plant (Bidabadi and Jain, 2020). The culture media contains all the nutrients plants need to grow and develop normally (Landis et al., 1990). A solid

culture medium primarily comprises certain gelling agents, vitamins, amino acids, plant growth regulators, macro and micronutrients, and carbon sources (Mehetre and Aher, 2004). Since the pH of the media influences both plant growth and the activity of plant growth regulators, it is also very important and must be adjusted to the critical value (Bhojwani and Dantu, 2013a). The composition of the culture media, particularly the nitrogen supply and plant growth regulators, significantly impacts how the first explant responds (Gaspar et al., 1996). various tissue culture methods exist, each with applications (Neumann et al., 2009). Since plant tissue culture offers enormous potential for crop improvement programs like choosing disease/insect or stress, it has recently received the greatest priority in plant breeding studies (Bharadwaj, 2016). Resistant plants, creating new hybrids through protoplast technology, preserving embryos from extensive crossings through embryo culture, quickly synthesising haploid and diploid plants, etc. Callus cultures, cell suspension cultures, meristem cultures, ovule cultures, anther/pollen cultures, root cultures, endosperm cultures, ovule cultures, embryo cultures, seed cultures, and so on are some of the techniques used (Gosal and Wani, 2018).

#### **New Uses for Plant Tissue Culture in Plant Breeding**

Plant tissue culture is becoming increasingly important in agriculture and business, providing the necessary plants to meet the growing global demand (Chandran et al., 2020). It has recently significantly advanced agriculture as well, and these days, they serve as innovative tools to help current plant breeders in their tasks (Acquaah, 2009). Thus, the innovative uses of plant tissue culture, especially for plant breeding to enhance crop quality, are summarized below.

- Embryonic rescue
- Fusion somatic hybridization of protoplasts
- Production of haploids
- Creation of plant material devoid of pathogens
- Production of synthetic seeds
- Somaclonal variation
- Germplasm conservation

#### **Embryonic rescue**

Small shrunken seeds from wide hybridization crosses can indicate that fertilization has occurred but cannot grow further (Cooper and Brink, 1940). Wide hybridizations typically do not result in normal sexual reproduction; hence, embryo rescue can help avoid this issue (Shivanna and Bahadur, 2015). Among the first and most effective tissue culture techniques, embryo rescue helps develop plant embryos that may not live to become viable plants (Bridgen, 1994). It is primarily used to develop interspecific and intergeneric crosses that would otherwise result in abandoned seeds (Posselt, 2009). Creating numerous interspecific and intergeneric crop hybrids is one-way

embryo rescue contributes significantly to modern plant breeding (Rogo et al., 2023). Apart from other factors, the primary cause of interspecific incompatibility in plants is thought to be embryonic abortion (Mehetre and Aher, 2004). Consequently, the embryo culture approach has effectively resolved low seed set, seed dormancy, and slow seed germination. Therefore, a breeder may generally successfully make extensive crosses with a larger number of related species of wild plants and have access to a much wider variety of genes that can be employed for crop plant genetic improvement thanks to the embryo cultivation approach (Brown and Thorpe, 1995).

#### **Main Uses of Embryo Culture Techniques: Preventing Embryo Abortion in a Broad Cross**

Incompatibility barriers frequently impede proper seed development and hybrid generation in interspecific and intergeneric hybridization programs (Eeckhaut et al., 2006). Shrunken seeds are the result of embryo abortion, even though some incompatible crosses may fertilize normally (Bridgen, 1994). Embryo malnutrition and subsequent abortion were caused by poor and aberrant endosperm development (Amiteye, 2023). Thus, these potent post-zygotic barriers may be avoided by isolating hybrid immature embryos before an abortion and cultivating them in a system. The most beneficial and common use of embryo cultures is the rescue of incompatible cross embryos to produce uncommon hybrids (Mehetre and Aher, 2004). Breaking through Seed Dormancy and Reducing Breeding Cycle Breeding efforts are delayed by long seed dormancy, particularly in horticultural and agricultural species (Sohindji et al., 2020). These plants' breeding cycles can be reduced by using embryo culture methods (Rogo et al., 2023).

#### **Breaking Through Seed Dormancy and Slashing Breeding Cycle**

Breeding efforts are delayed by long seed dormancy, particularly in horticultural and agricultural species (Sohindji et al., 2020). These plants' breeding cycles can be reduced by using embryo culture procedures (Bridgen, 1994). For example, the iris life cycle was shortened from two to three years to just one year. Two generations of flowering could be obtained in the Rosa species in opposition to one. Particularly during the dormant phase, the germination of an excised embryo is thought to be a more accurate test for quickly determining the viability of seeds (Bradbeer, 2013).

#### **Getting Past Seed Sterility**

Due to the immaturity of their embryos, seeds from early-ripening fruit varieties do not germinate (Bareke, 2018). By using the embryo culture method, seedlings of early-ripening stone fruits, such as peaches, apricots, and plums, can be raised from sterile seeds (Sallom et al., 2021).

#### **Creation of Haploid/Monoploid**

Barley monoploids have been produced using an embryo culture (Devaux and Kasha, 2009). Fertilization proceeds normally with the cross

*Hordeum vulgare*; however, after that, the chromosomes of *H. bulbosum* are eliminated, resulting in the production of a monoploid *H. vulgare* embryo that can be saved via embryo cultures (Zhang, 2000).

#### **Protoplast Fusion Hybridization**

Protoplast fusion is a novel tool for crop improvement and plant breeding because it allows for the development of unique, interspecific, intergeneric hybrid plants that cannot be produced through traditional sexual hybridization (Shuro, 2018). The process involves fusing the protoplasts of two different genomes of genetically unrelated species, selecting desired somatic hybrid cells, and then regenerating hybrid plants (Shuro, 2018). Protoplast fusion technology is one of the pioneering gene transfer methods with the desired results (Davey et al., 2005). One of the first methods to effectively transfer genes with desirable traits from one species to another is protoplast fusion technology, which has substantially contributed to the national crop development program (Davey et al., 2005). Many crop species have recently separated their protoplasts, including barley, carrot, cassava, cotton, pea, and soybean. However, the tomato was one of the first uses of this technology (Grevich and Daniell, 2005). Furthermore, intergeneric protoplast fusion has also been observed in soybean x barley, maize x sorghum, and carrot x petunia. More recently, protoplast fusion technology has opened up means of creating unique hybrid plants by solving semi-incompatibility problems. It is highly applicable in the horticultural industry to develop new hybrids with increased fruit yield and better resistance to biotic and abiotic stress (Helaly, 2017). It has also been reported that successful, viable hybrid plants were developed when protoplasts were fused with other related cross-species recommendations (Eeckhaut et al., 2013). However, there are several limitations and considerations with this technology, such as the inability to perform intergeneric crosses between sexually incompatible and closely related plants, the removal of chromosomes from hybrid cells in some wide crosses, the extremely low percentage of fusion between two different parental protoplasts in protoplast fusion experiments, and the lack of an optimized standard method for hybrid identification, selection, and isolation at the culture level.

#### **Production of Haploids**

Certain genetic shade issues in plants can be resolved through the in vitro creation of haploids because the angle allelic gene in every chromosome of the genome rapidly manifests its effects (Duesberg and Rasnick, 2000). The plants can be rendered fertile, and the resulting plants will be homozygous by doubling the amount of u chromosomes (Wijnker et al., 2012). Therefore, instead of traditional breeding approaches, tissue culture techniques allow for the comparatively quick production of homozygous plants using

protoplast, anther, and microspore cultures ([Wijerathna-Yapa et al., 2022](#)).

Sterile plants with a single set of chromosomes (one-half of the typical number) are haploids ([Choo et al., 1985](#)). Spontaneous or artificial chromosomal doubling transforms haploids into homozygous diploids ([Humphreys and Knox, 2015](#)). Plants become more fertile when their chromosomes double, producing double haploids that may eventually become pure and be used to produce new cultivars ([Murovec and Bohanec, 2011](#)). The approach has demonstrated a remarkable mechanistic ability in genetic transformation by creating haploid plants with induced tolerance to various biotic and abiotic stresses ([Anwar and Kim, 2020](#)). Furthermore, it was suggested that the successful generation of double haploid inbred wheat and drought-tolerant plants resulted from introducing genes containing the desired feature during the haploid state, followed by chromosome doubling ([Tadesse et al., 2012](#)).

#### **Production of Plant Material Free of Pathogens**

Utilizing the meristem culture technique, one of the most intriguing applications of stem plant tissue culture is in the bulk propagation, maintenance, and taming of certain pathogen-free plants ([Amin et al., 2022](#)). This method produced pathogen free plants and was initially developed by Morel and Martin to eradicate viruses on dahlia ([Bhojwani and Dantu, 2013b](#)). Recently, meristem culture has proven to effectively eliminate viruses from several plants, including potatoes, sugarcane, and strawberry ([Mori, 1971](#)). It is frequently employed to eradicate numerous viral illnesses from plant materials ([Jassim and Naji, 2003](#)). Plant diseases, including nematodes, fungi, bacteria, and viruses, can spread from diseased plants to healthy ones. Plastic vascular and non-vascular plants were the only two pages in Gamed Plant ([Jordan, 1999](#)). Not every one of the apical dome and the initial young primordial leaf are five viruses in the macerates of facts and shoots of the infected plant; other times, they are rich in tobacco rattle virus potter and potato virus X (PVX). The precise reason behind this is unknown, although it's thought that cot in each of the ensuing elements is in charge ([Hopwood, 2013](#)).

#### **High metabolic activity**

Viruses spread by commandeering the metabolic pathways of their hosts ([Storm and Muller, 2012](#)). These cells have a high metabolic activity, which prevents viruses from taking over the host's biosynthetic machinery ([Halldorson, 2012](#)).

#### **Lack of vascular system**

Within the vascular system, viruses proliferate quickly ([Gilbertson and Lucas, 1996](#)). The absence of cell differentiation allows phloem-restricted viruses to infiltrate the meristematic tissues. Viruses that impact non-vascular tissues separate from cells to call through the plasmodesmata in the meristematic region ([Xie et al., 2014](#)). Because of this sluggish process, I find it

challenging to completely infect the cancerous dividing cells ([Preston-Martin et al., 1990](#)).

#### **High auxin concentration**

Compared to tissue from the other region, plant meristematic tissues have a higher concentration of auxin produced ([Aloni, 2004](#)).

#### **Production of synthetic seeds**

The term "synthetic seeds" refers to artificially encapsulated somatic embryos, shoot buds, cell aggregates, or any other tissue that can be sown as a seed that retains this potential even after storage ([Magray et al., 2017](#)). The technology behind synthetic seeds was developed to successfully use somatic embryos and/or other micropropagules as seed analogues in the field or greenhouse and their mechanical planting at a commercial level ([Suprasanna et al., 2006](#)). The technology offers methods for preparing synthetic seeds, also known as artificial seeds, from micropropagule such as somatic embryos, axillary shoot buds, apical shoot tips, embryogenic calli, as well as protocorm or protocorm-like bodies on the synthesis of synthetic seeds in various plant species. The generation, development, maturation, and subsequent conversion of the micropropagules into plantlets under in vitro or ex vitro circumstances present constraints that have prevented the complete practical deployment of the technology, notwithstanding these research investigations ([Niedz](#)). But, creating synthetic seeds has opened up new avenues for plant biotechnology ([Roy and Tulsiram, 2013](#)). Synthetic seed technology aims to combine the benefits of seed propagation and storage with those of clonal propagation ([Garg and Maheshwari, 2023](#)).

#### **Soma-clonal variation**

In nature, recombination processes produce genetic heterogeneity and diversity within a population ([Spencer et al., 2006](#)). Natural selection, mutation, migration, and population size vary depending on genetic variability ([Amos and Harwood, 1998](#)). On the other hand, somaclonal variation is the name given to genetic variation resulting from plant tissue culture. Variation has been noted in several kinds of plants originating from diverse explants utilizing various tissue culture methods ([Bairu et al., 2011](#)). Crop improvement is a multidisciplinary endeavor that focuses on optimizing genetic characteristics while considering environmental factors and genetic material limits ([Kholová et al., 2021](#)). To regain elite crops, conventional breeding takes advantage of the natural variety present in plant populations ([Begna, 2021](#)). However, one of the barriers to crop improvement is the amount of genetic variety present in gene pools. In its initial attempts to create a plant phenotype, conventional breeding takes advantage of this innate variety in the base ([Van de Wiel et al., 2010](#)). The past ten years have seen an explosion in the practical uses of in vitro culture, which may be attributed to the development of the theoretical aspect ([Mather and Roberts, 1998](#)). Originally employed for

the clonal propagation of plants, it subsequently introduced a number of novel possibilities, including the removal of sexual incompatibility through embryo rescue techniques, somatic hybridization via protoplast technology, genetic engineering to create transgenic plants, anther culture to produce haploids, and, most significantly in the context of the selection of desired qualities such as pest resistance, disease resistance, and salt tolerance as well as the introduction of novel genetic variability. Thus, the once inviolable *in vitro* culture has become a biotechnological instrument for expanding the germplasm basis (Shiva, 2014). Genetic diversity can be abundant in *in vitro* culture (Croll et al., 2008). The greatest accessible germplasm undergoes regenerants selected for the superiority of one or more qualities while keeping all original characteristics in a tissue culture cycle with or without selection pressure. Thus, little improvements in desired features may eventually result in the spontaneous generation of novel alleles *in vitro* (Maluszynski et al., 1995). The bottom line is that the genetic variability recovered from tame culture regenerated plants should ultimately result in an agriculturally useful phenotype (Pathirana and Carimi, 2022). Consequently, tissue culture techniques leading to somaclonal variation could be capitalized upon to accelerate progress in conventional breeding (Tazeb, 2017). Additionally, it has performed best in crops with restricted genetic bases and/or limited genetic systems, where it can quickly supply variety for crop improvement (Sharma et al., 2002).

#### **Germplasm Conservation**

Another method for preserving endangered genotypes and/or species is through the use of plant tissue culture techniques (Pence, 2010), because plant species are disappearing at a rapid rate, and countries need to protect their floristic heritage more than ever, conserving germplasm is turning into a critical task (Volis, 2018). When the goal is to preserve clones rather than seeds, tissue culture procedures can be utilized to preserve vegetative tissues, preserve a crop's genetic heritage, and prevent the loss of conserved patrimony as a result of biotic or abiotic stressors from natural catastrophes (Suman, 2017). Using *in vitro* procedures, plant species that are seedless or whose seeds are "recalcitrant" and cannot be stored for an extended time can be effectively preserved (González-Arno et al., 2017).

Cryopreservation technology is required for the long-term *in vitro* preservation of crucial biological material and genetic resources (Engelmann, 2000). It involves the cryopreservation of *in vitro* cells or tissues in liquid nitrogen, which exposes the tissues to chemical and physical stressors and causes cryo injury. Successful cryopreservation is frequently determined by the survival of cells and tissues and their capacity to proliferate, regenerate, and create new colonies (Whaley et al., 2021). After cryopreservation, evaluating the recovered

germplasm's genetic integrity is desirable to ascertain whether it is "true-to-type" (Harding, 2004). The information that recovered plants' fidelity may be evaluated at the phenotypic, histological, cytological, biochemical, and molecular levels; nevertheless, each method for evaluating genetic stability has benefits and drawbacks (Biswas and Kumar, 2023).

#### **Conclusions**

Utilizing plant tissue culture technology, it has become possible to improve both endangered native species and the most significant crops (García-González et al., 2010). Another method of increasing crop species variation is somatic hybridization, which involves introducing genes or even entire chromosomes from unrelated species that are not closely related enough for typical sexual crossover (Stebbins, 1959). While somatic hybridization shares goals with traditional hybridization, it takes a more radical technological approach (Holmes, 2018). Anther culture, which involves the *in vitro* growth of immature anthers, can be used to make haploid plants (Germana, 2011). The plants produced from pollen are haploid as well because the pollen grains are haploid (Maheshwari et al., 1982). Colchicine therapy was originally used in the 1960s to generate doubled haploid plants (Eng and Ho, 2019). Ovule culture can also result in the production of doubled haploids. Plants with doubled haploid DNA are referred to as this results in a significant reduction of the time needed following a conventional hybridization to select pure lines carrying the necessary character recombination (Murovec and Bohanec, 2011). The costs associated with the equipment and labor required to generate and test large populations of doubled haploids prevent this technology from being applied to plant breeding (Dwivedi et al., 2015). Plant breeders often manipulate plants to create sterile types from wide crosses that are difficult to propagate (Bradshaw, 2017). Breeders may purposefully design this characteristic if they believe it to be beneficial. One of the most crucial technologies for creating successful interspecific hybrids is chromosomal doubling during embryo culture (Niemann et al., 2015). Large hybrid plants frequently have sterile seeds, making seed propagation impossible (Wright, 1980). This is because stable chromosomal pairing during meiosis is prevented by variances in chromosome sets inherited from genetically distinct parental species (Soares et al., 2021). On the other hand, if the number of chromosomes is purposefully doubled, the hybrid might be fruitful if it can generate viable pollen and eggs (Frankel and Galun, 2012). Somaclonal alterations in cultivated plant cells may offer plant breeders a significant new tool for producing genetic diversity (Ferreira et al., 2023). It has been possible to modify features in crop species such as drought and salt tolerance, nutritional value, insect resistance, and disease resistance by somaclonal mutagenesis (Jain, 2010).

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#### Declarations

#### Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

#### Ethics approval and consent to participate

Not applicable

#### Consent for publication

Not applicable

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#### Conflict of Interest

Regarding conflicts of interest, the authors state that their research was carried out independently without any affiliations or financial ties that could raise concerns about biases.



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