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Tissue Culture: New Opportunities for Developing Biotic and Abiotic Tolerance Crop Varieties to Meet Global Food Security

Layla A. Almutairi

Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia

*Corresponding author. E-mail: laAlmutairy@pnu.edu.sa

ABSTRACT

In plants, stress is one of the major constraints affecting plant growth and yield, leading to a decrease in crop productivity. The stress can be abiotic or biotic stress, or both can affect crops. Biotic stress involves crop damage caused by living organisms, including insects, parasites, bacteria, fungi, and viruses, and affects crop yield. Abiotic stresses such as drought, salinity, heat, water logging, mineral toxicity and frost limit crop productivity. The development of *in vitro* drought- and salt-tolerant crops, such as vegetables, cereals, fruits and other commercial plants, has contributed to food production. Worldwide, wheat and rice are the major crops and are affected by biotic and abiotic stresses. Conventional breeding techniques and several agronomic methods have been applied for the management of newly developed stresses. Moreover, most of the implemented methods were found to be less successful and undesirable for use in field trials or in greenhouses. Recently, the tissue culture method has proven to be a more powerful and cost-effective approach for the development of stress tolerance in plants. The *in vitro* plant tissue culture method requires less time and space, and experimental trials are performed under controlled environmental conditions, with high potential for the development of various stress-tolerant crop plants. This method allows a good understanding of the biochemistry and physiology of plants cultured under environmental stress *in vitro*. The tissue culture technique allows the development of various stress-tolerant crops in the laboratory and has improved tolerance to both abiotic and biotic stresses and improved yield. Therefore, *in vitro* plant tissue culture methods provide new opportunities for developing stress tolerance in crop plants for environmental sustainability.

Keywords: tissue culture, abiotic stress, biotic stress, crop development, food security.

INTRODUCTION

Plant tissue culture is an important tool facilitating biotechnology applications and enables large-scale plant propagation in agriculture. In addition, tissue culture is an essential tool, and its direct use in fundamental studies related to biochemistry, plant biology, and molecular biology is well documented. Currently, the world population has reached 8 billion and is alarming. The supply of food and food is the basic need for all these populations, and the majority of people depend on plant-based food, which has emerged as one of the challenges faced by humans in the 21st century. To meet the global food demand, adequate water supplies, fertilizers, cultivable lands and suitable seasons are required to manage food production. However, global warming has induced several climatic changes that affect food production more than they did earlier (Wijerathna-Yapa et al., 2022a; Wijerathna-Yapa et al., 2022b). Global warming affects entire agricultural systems throughout the world and affects crop yield. These include drought, severe environmental pressure, cold climates or extreme heat, salinity, floods, pesticides and exposure to toxic inorganic compounds (Gosai et al., 2024). The anthropogenic activities associated with intensive food production cause changes in environmental conditions, resulting in the toxicity of pesticides and chemicals discharged directly into the environment. Chemical contaminants affect agricultural production are unsuitable for consumption, flagging food security due to the sudden decrease in plant growth due to several environmental stress conditions and the scarcity of fertile cultivable land. Abiotic and biotic stress are major factors affecting plant

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growth and yield. By developing biotic or abiotic stress-tolerant plants *via* tissue culture methods, it is possible to improve yield. The focus of plant stress-related research has gained much more attention over the past five decades, especially with respect to the development of stress-tolerant plants, such as those susceptible to salinity, extreme temperature, extreme or inadequate radiation, pest outbreaks, and phytopathogen attack (Trono and Pecchioni, 2022). The screening of stress (abiotic and biotic stress)-tolerant varieties is vital in selecting and breeding elite varieties. However, most screening methods are employed under natural environmental conditions but are highly challenging to manage, and field trials are associated with various risks due to variations in uncontrolled climatic conditions. However, the plant tissue culture method is an efficient, effective and economical way to screen for stress-tolerant plants under both biotic and abiotic stresses. Plant cell and tissue culture is an *in vitro* culture method, and this method was based on the theory proposed earlier by Schwann and Schleiden (1838); later, this idea was expanded by Gottlieb Haberlandt in the 20th century (Thorpe, 2007). *In vitro* plant tissue culture is based on the type of "totipotent" cells. Under optimum *in vitro* or laboratory conditions, the cells can become fully grown plants. The totipotent cells undergo dedifferentiation and redifferentiate, form a functional component of plants and form an organized tissue, structure, and whole plant (Fehér, 2019; Su et al., 2021). In the tissue culture method, whole plants develop when plant tissues or cells are cultured under laboratory conditions with adequate nutritional and physical conditions. In the tissue culture method, cultivation is performed in the laboratory under a controlled environment, no external environmental conditions are affected, and unique media are provided to improve plant growth. This method is also used to analyse the tolerance of selective substances such as antibiotics and toxins. The *in vitro* assay methods considerably reduce the cost and time of the selection process under selection, and environmental interactions are minimal. The laboratory *in vitro* tissue engineering-based screening method is a preliminary screening method, and field trials are recommended after laboratory observations. Moreover, analysing any plant species for stress tolerance is challenging, and the development of a unique tissue culture protocol is challenging. The tissue culture method is rapid, and a one-step or short-term selection process allows the identification of epigenetic adaptations (Smýkal et al., 2007; Miguel and Marum, 2011). Research on plant biodiversity especially that of crop species, has enormous potential over the years in the search for more resilient plants despite breeding methods.

Crop breeding programs have led to steep increases in crop yields over the past few decades. Moreover, the rate of crop yield varies widely (Grassini et al., 2013). Wheat is one of the major crops, and an increase in yield of <1% is not sufficient to meet global food requirements. High-yielding crops constitute an important approach to meet global demand (Ray et al., 2013). Genetic improvement was achieved through sexual hybridization between closely related plant species, resulting in several cultivars with superior yields and good agronomic importance. In the conventional method, crop improvement can be achieved *via* a cytogenetic method, which is the primary method of crop improvement, especially for cereal crops (Salina et al., 2015). Cereal grains dominate the human diet and have emerged as a major target for crop improvement *via* genetic transformation. Genetic transformation methods have been used for the past three decades. In cereals, genetic transformation is achieved by the introduction of DNA into protoplasts and further callus production for the development of plants. The prospects and applications of plant tissue culture and genetic engineering methods for introducing resistance against biotic and abiotic constraints and improving crop yield are reviewed in this paper. Traditionally, plant breeders have used various gene pools to achieve genetic variation, and this method is time consuming and costly. However, tissue culture methods and plant breeding programs offer advancements

for increasing genetic diversity among crop species. Plant tissue culture includes the culture of anthers, protoplasts, microspores, embryos, ovaries and ovules, which results in epigenetic and genetic variation in the breeding samples. These *in vitro* culture methods reduce the overall time period of plant breeding to develop resistant and tolerant genotypes (Slama et al., 2021). Generally, *in vitro* plant tissue culture is applied in self-pollinated crops and vegetatively propagated crops, especially those with narrower genetic bases. The *in vitro* plant tissue culture method is useful for manipulating the desired trait and improving the genetic basis (Bednarek and Orłowska, 2020). Recently, plant tissue culture and *Agrobacterium* transformation have been considered alternative approaches for the genetic manipulation of crop species. The traditional method requires a few years to achieve the desired goal, whereas the application of CRISPR/Cas9 nuclease-mediated genome editing reduces the time needed to achieve the goal (Kumar et al., 2019).

MATERIAL AND METHODS

In the present review, we used the Embase, Web of Science, PubMed, and Google Scholar databases to retrieve the most popular and updated research articles. Review papers were not considered. The keywords used were "biotic stress", "abiotic stress", "plant biotechnology", "Mendelian Laws", "traditional plant breeding", "hybridization", "regeneration methods", "transgenesis", "*Agrobacterium tumefaciens* Ti plasmid", "biolistic", "transgenesis", "genetically modified crops", "biofortification", "edible vaccines", "Zinc Finger Nucleases", "Clustered Regularly Interspaced Short Palindromic Repeats", "Crispr associated protein", "gene knockout", and "amylase rich rice". All these keywords were used in combination with "*in vitro* tissue culture". Only full-text research articles in English were considered, and other language articles were not considered.

Plant biotechnology: simple breeding to genome editing

Over thousands of years, humans have developed plant varieties that select phenotypes with traits to adapt to the culture environment or improve agronomic performance. The phenotypic variations linked with several adaptations under local pressure are called "domestication syndrome" (Meyer and Purugganan, 2013). Later, in the beginning of the 19th century, Mendelian laws were introduced, thus improving the understanding of plant sciences, and the first revolution started in plant science. Earlier cultivation and breeding techniques have improved yield and the development of biotic and abiotic stress-tolerant plants. In the continuation of crop development, the hybridization method was introduced in the late 19th century by Vilmorin and Wilhelm Rimpau (Bonjean and Angus, 2001). The cross-breeding method was introduced in the 19th century. Different methods of crossing increase the degree of genetic variability, which is helpful for determining the desired traits of suitable cultivars, leading to the development of economically important crops (Briggs, 1938). In the middle of the 19th century, especially between 1950 and 1960, dwarf and semidwarf genes were identified from wheat varieties, and this is one of the major achievements in the green revolution (Borlaug, 1983). The common way of increasing the genetic variability of plants is to cross more parents with different genotypes. However, this practice may be involved in the loss of biodiversity. Genetic variability is a method to determine new beneficial traits resulting from gene mutations in genomes, either induced or naturally. The concept of mutagenesis was introduced between the 1920s and 1930s, and plants were developed on the basis of spontaneous mutations. This method allows plant breeders to carry out random mutation by using physical or chemical mutation agents (Stadler, 1928; Leitao, 2012, Mba et al., 2012).

In the 1950s, plant breeders used mutagenesis and developed more than 3350 varieties, including 1500 cereal varieties. The discovery of DNA in 1953 by Watson and

Crick, the introduction of a genetic code in 1968, the introduction of restriction enzymes in the 1970s by Nathan, and the introduction of recombinant DNA technology in 1973 by Cohen and Boyer provided a strong foundation for the development of modern molecular breeding and plant biotechnology (Bhatia and Goli, 2018). In the second half of the 19th century, several achievements were made in plant breeding. The introduction of regeneration techniques and tissue culture techniques allows the application of genetic engineering technology, including transgenesis and chromosome engineering, between distantly related species. The *Agrobacterium tumefaciens* Ti plasmid was used to combine foreign DNA and introduce it into the plant genome (Zambryski et al., 1982; Herrera-Estrella et al., 1983; De Block et al., 1984). The direct gene transfer method was subsequently introduced and is known as biolistic in monocot plants, especially cereals (Klein et al., 1987). Genetic manipulation was proven to be a novel method for introducing functional genetic material into plant protoplasts involving plant physiology mechanisms. Later, transgenesis was widely used in plant breeding programs, as it allows introgression of genes or any DNA sequence from other plants and allows specific editing of plant genetic material to improve genetic variability.

In the late 1990s, genetically modified (GM) crops were produced commercially. A total of 32 GM crop species have been introduced, and more than 44 countries have approved these plants for cultivation, including rice, maize and wheat plants. Several important traits have been identified in GM crops, including herbicide tolerance in soybean by Monsanto, the development of biotic and abiotic stress-tolerant soybean and maize plants by introducing Bt toxin, improved plant growth and yield, improved crop quality (Kramer and Redenbaugh, 1994), biofortification (e.g., Golden Rice) (Ye et al., 2000), the development of pharmaceutical agents and the development of edible vaccines and phytoremediation (Bizily, 2000). In the 1990s, targeted plant selection was performed *via* molecular markers, since various genetic markers were associated with loci controlling traits of agronomic importance. The discovery of next-generation sequencing methods allows for genomic selection, which facilitates simultaneous selection for several available markers. Genomic selection combined with high-throughput phenotyping is considered a powerful tool for the selection of appropriate traits and is associated with quantitative traits. Several resistance genes have been isolated from barley, wheat, potato and rice (Wang et al., 2008; Bhullar et al., 2010; Ramkumar et al., 2011; Wang et al., 2014). In the 2000s, the targeting-induced local lesions in genomes approach were introduced to determine mutant genotypes in genes of interest. This method allows plant biologists to obtain nontransgenic, and disease-resistant bread wheat varieties (Acevedo-Garcia et al., 2017).

Recently, new breeding techniques have been introduced, and these methods enable targeted, precise, and reliable editing of the genome and do not create multiple, unintended mutations or unknown mutations, unlike radiation-induced or chemical mutagenesis. Genome-editing methods have several advantages, and zinc finger nucleases and transcription activator-like effector nucleases are considered the major editing tools. The introduction of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein (Cas) improved genome editing. CRISPR technologies have improved editing efficiency, ease of usage, and multiplex editing capacity and have been adopted for several genome-targeting purposes. To date, several monocots and dicots have been subjected to genome editing to improve their agronomic traits and nutritional and health benefits (de Sousa et al., 2016). To improve grain number and size, genome editing was performed in rice plants, and knockout mutations were carried out in wheat plants (Li et al., 2016; Sestili et al., 2019). Genome editing tools are useful for generating abiotic and biotic stress-resistant plants. Genome editing was performed to knock out polyphenol oxidase-encoding genes in high-amylase-rich rice, waxy maize, low-immunogenicity food, and herbicide-resistant crop species (Waltz, 2016; Sun et al., 2017; León et al., 2018; Li et al., 2018; Camerlengo et al., 2020).

Analysis of biotic and abiotic stresses in plants using tissue culture method

Biotic and abiotic stresses are highly complex, and much research has focused on understanding plant responses to biotic and abiotic stresses (Qin et al., 2011). Abiotic stress is a major constraint on agriculture and affects plant productivity and growth worldwide. In addition, yield loss will be increased in the future by increasing chemical contamination, increasing global warming, decreasing the availability of fertile land, and decreasing the shortage of water. Research on abiotic and biotic stress tolerance mechanisms is important and will help in the development of abiotic and biotic stress management strategies. In the natural environment, several abiotic factors influence crop production. However, one or more abiotic stresses are interconnected and affect osmotic mechanisms and plant cell homeostasis. The focus of stress-related work has gained much more attention over the years, especially with respect to the effects of stress factors such as extreme temperature, water deficiency, exposure to toxic heavy metals, salinity, extreme radiation, pest outbreaks, and phytopathogens (Fahad et al., 2015; Pessarakli, 2019). The screening of plants for stress tolerance is one of the major tasks in the breeding and selection of good varieties. Most of the common stress screening trials are performed under greenhouse or field trials; however, these methods have several challenges to manage, both economically and physically, due to uncontrolled environmental conditions. The plant tissue culture technique is an efficient, effective and economical method for screening plants for abiotic and biotic stresses. This method is considered an *in vitro* tissue culture method based on the cell theory of Schwann and Schleiden (1838) and the concept of Gottlieb Haberlandt proposed earlier in the 20th century (Thorpe, 2007). *In vitro* screening methods are also useful for determining tolerance to antibiotics, heavy metals and toxins. The tissue culture method can be used for early evaluation of the stress response in plants (Figure 1). Moreover, a suitable standard protocol is needed for the culture of new plants, and differences in the mechanism of action of stresses in cultured cells and whole plants are needed.

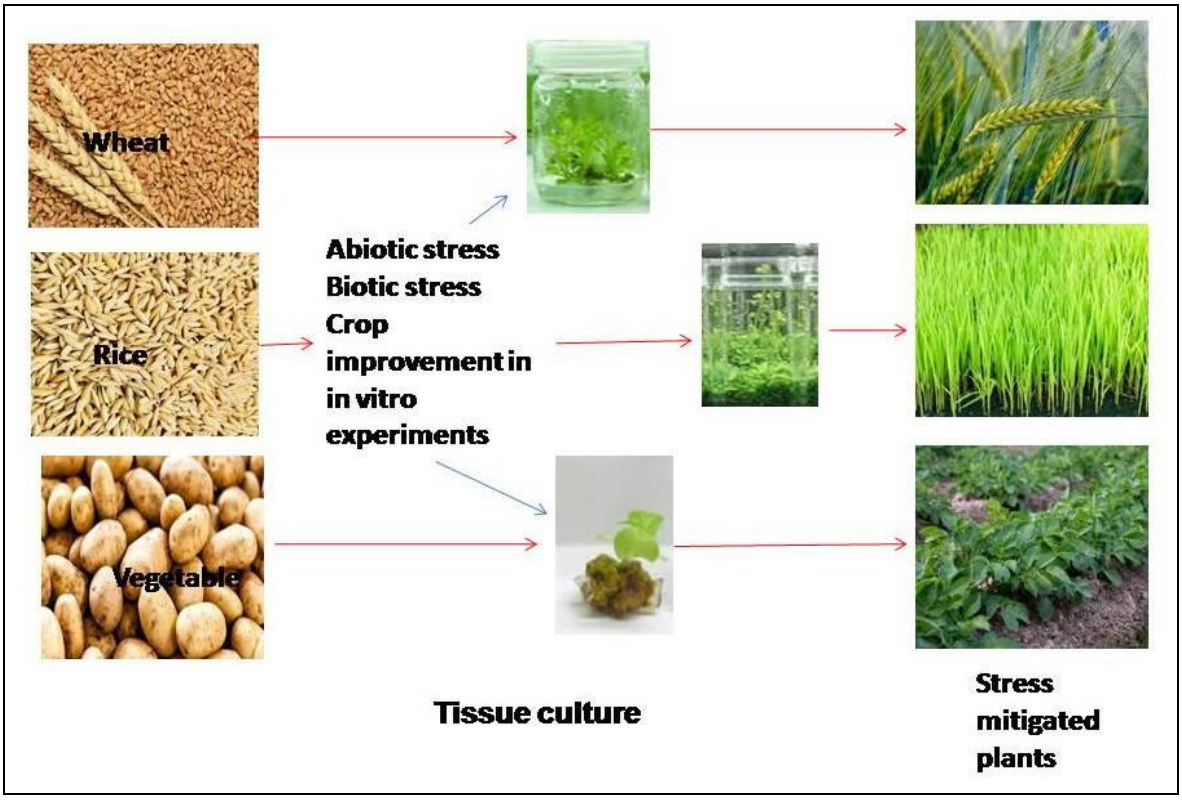

Figure 1. Overview of crop development via the tissue culture technique

Screening of the disease resistance of plants *via* tissue culture

Plant disease is one of the major burdens on agriculture because of severe revenue loss due to poor performance or complete loss during infection. Hence, the selection of disease-resistant plants is a major task in agriculture. *In vitro* screening assays are used for the development of disease-resistant crops (Table 1). In commercial crops, several soil, air, bacterial, waterborne bacterial, fungal, viral and protozoan diseases affect yield (Baazeem et al., 2021a). The disease severity is severe in monocultures and artificial fertilized environments. Hence, screening and selection of disease-resistant strains are widely recommended procedures, especially for crop plants. This method requires a controlled environment and specialized conditions to reduce environmental risk (Malar et al., 2020; Valan Arasu et al., 2023). *In vitro* selection methods analyse the production of antibacterial and antifungal compounds, the expression of pathogenesis-related proteins or phytoalexin production to support the selection of disease-tolerant strains. The *in vitro* assay culture method is less expensive than the production of plant saplings through transgenic technology, which is time-consuming, costly and highly challenging. The culture and maintenance of somatic embryos, embryonic shoots, calli, or somatic embryos to culture filtrates, toxins or pathogenic organisms can screen plant samples for disease or pathogen resistance under *in vitro* culture conditions (Baazeem et al., 2021b). An *in vitro* screening method has been developed for screening rice germplasm for resistance to brown spot disease *via* the use of phytotoxin *via* tissue culture methods (Ling et al., 1985). The tissue culture method is widely used to screen disease-resistant plants, including virus-resistant transgenic potato plants (Russo and Slack, 1998). Phytotoxins are used frequently, and their application in disease resistance has been reported (Slavov, 2005). These phytotoxins improved disease resistance and improved crop productivity. A disease-free ginger variety was produced *via* a tissue culture method. The developed plants present improved genetic traits, and cytological, agronomic, and molecular characterization of the newly developed variety has been reported (Zhao et al., 2022). In addition, the tissue culture screening method effectively promoted the development of disease resistance (blight resistance) in potato plants (Wang et al., 2020). An *in vitro* approach was used to develop bacterial wilt disease-resistant eggplants *via* the tissue culture method (Namisy et al., 2019). A micropropagation method was used to screen cork oak, and the developed plant was resistant to Phytophthora cinnamomi (Martínez et al., 2023).

Table 1. Screening and in vitro development of biotic stress free economically important crop species

Plants	Parts	Pathogen	Reference
Arachis hypogaea	Immature	*Cercosporidium personatum*	
	leaf expiants	and its culture filtrates	Venkatachalam and Jayabalam (1997)
Gossypium hirsutum L. cv. SVPR2	Embryogenic callus	*Fusarium oxysporum* culture filtrate	Ganesan and Jayabalan (2006)
Cucumis sativus lv.	Calli	*Fusarium oxysporum* culture filtrate	El-Kazzaz and Ashour (2004)
Musa spp.	Tissue culture banana	Banana weevil *Cosmopolites sordidus*	
	plantlets	Nematode *Radopholus similis*	Paparu et al. (2008)
Solanum tuberosum	diploid inbred potato	Blight disease causing Phytophthora	
	line B101	infestans	Wang et al. (2020)
Potato	Potato germplasm f	*Globodera pallida*	Mwangi et al. (2019)
	Calli of genotypes	Pathogen culture filtrate of	
Sugarcane	CoJ 88 and CoJ 64	Colletotrichum falcatum Went	Sengar et al. (2009)
	Calli	Phomopsis sp. (Diaporthe sp.) brown	
Sunflower		gray stem spot	Masirevec et al. (1988)
Musa balbisiana	Calli	Banana *Xanthomonas* wilt disease	Tripathi et al. (2008)

Screening of drought - resistant plants

Drought has been found to be responsible for relatively low yields of most crops in dry areas worldwide. To improve the development of crop cultivars in response to drought, biotechnological methods may play a major role, and the selection of suitable cultivars may increase crop production in drought areas (Hemon, 2010). Plant tissue, cell, and organ culture methods are highly suitable for analysing the mechanisms involved in the development of highly drought-resistant plants (Table 2). The *in vitro* culture method neglects environmental and nutritional fluctuations because of the use of preparative culture media, the homogeneity of physical factors and the controlled environment. This method allows the study of large plant populations and the application of a number of stress treatments in a short span of time and in a limited space (Sakthivelu et al., 2008). The *in vitro* culture method was suitable for screening resistant somaclones or tolerant or mutant plants for biotic and abiotic stress, and potential plants were applied as donors of specific drought resistance gene(s) to improve crop productivity (Carlson, 1973). Variation was reported during cell division and differentiation under laboratory conditions (D'Amato and Bayliss, 1985). The germ lines are responsible for these genetic changes, and in the normal life cycle of a plant, the mutant somatic cells are generally completely removed at the time of sexual reproduction and are not transferred to subsequent generations. Moreover, such mutant cells divide continuously *in vitro*. The selective pressure on developing cells can induce the growth of mutant cells, and mutant cell lines can be recovered from the plant. This method is applied for the development of strains that are tolerant or resistant to various fungi and bacterial toxins, drought, salt, herbicides, and heavy metals (Mishra et al., 2021).

Table 2. Screening and in vitro selection of economically important plants for drought stress

Plants	References
Arachis hypogaea (groundnut)	Purushotham et al. (1998)
Brassica juncea (Indian mustard)	Gangopadhyay et al. (1997)
Daucus carota (carrot)	Fallon and Phillips (1989)
Oryza sativa (rice)	Biswas et al. (2002)
	Roy and Mandal (2005)
Prunus avium (colt cherry)	Ochatt and Power (1989)
Saccharum sp. (sugarcane)	Errabii et al. (2006)
Solanum tuberosum (potato)	Sabbah and Tal (1990)
Sorghum bicolor (sorghum)	Duncan et al. (1995)
Tagetes minuta (Mexican marigold)	Mohamed et al. (2000)
Triticum aestivum (wheat)	Barakat and Abdel-Latif (1995)
Beta vulgaris subsp. vulgaris L.)	Putnik-Delić et al. (2012)
Musa	Rukundo et al. (2012)
Triticum aestivum L.	Khakwani et al. (2011)
Zea mays L.	Okafor et al. (2022)
Solanum tuberosum L.	Albiski et al. (2012)
Triticum aestivum L.	Ahmad et al. (2022)

Screening of salinity - resistant plants

Salinity is a prominent abiotic stress that affects more than 1000 million hectares of land throughout the world and is one of the key challenges for agriculture (Qureshi et al., 2019). The increased salt in the environment affects biochemical and physiological functions, resulting in problems such as mineral deficiency, ion imbalance, ion toxicity, osmotic stress, and oxidative stress (Zhu, 2002). Generally, salt stress generates reactive oxygen species, induces oxidative stress and reduces yield (Arzani and Ashraf, 2016). Plants with good antioxidant potential can resist salt-contaminated environments and are resistant to salt-induced damage.

Earlier studies on salt susceptibility or tolerance and overall developmental analysis were unable to differentiate systemic from cellular salinity tolerance processes (Gu et al., 2004; Negrão et al. 2017). Recently, the tissue culture method has been considered an essential tool for analysing the cellular mechanisms involved in mitigating salt stress in plants under *in vitro* conditions (Table 3). In several cop species, cell lines showing increased tolerance to salt stress have been reported, and various biochemical processes are involved in the management of salt stress and adaptation to survive in salt-contaminated environments (Davenport et al., 2003; Lutts et al., 2004; Queiros et al., 2007; Ghane et al., 2014; Al-Khateeb et al., 2020). However, the adoption of the tissue culture method is a reliable tool for screening salt-resistant cell lines, and this method involves the regeneration of salt-tolerant plants (Shankhdhar et al., 2000; Miki et al., 2001; Anwar et al., 2008; Nikam et al., 2014; Santangeli et al., 2019). Somatic variation is a useful strategy and a natural source for various desirable unintended effects in *in vitro* cultured plants in the laboratory. The term "somatic variation" was initially coined by Larkin and Scowcroft to describe the genetic variation that occurs in plant cells or tissue culture (Larkin and Scowcroft, 1981). In addition, the term "somaclonal variation" also refers to the variation that occurs in clonally propagated plants from a single donor and represents a combination of biochemical, morphological, cytological, and genetic variations (Haque et al., 2017). In some cases, somaclonal variation may not be expressed at the phenotypic level and may be expressed at the epigenetic or genetic level. The genetic changes are mainly at the chromosomal level (alterations in chromosome number) or structure (duplications, deletions, and insertions) or at the DNA sequence level, especially characteristics of point mutations. The prevailing epigenetic alterations are modifications of normal metylation patterns of DNA or variations in gene amplification and changes in histones (Bhatia et al., 2015). Hence, to determine the true-to-type replicants obtained from *in vitro* propagation of a genotype, it is important to apply a standard method for analysing somaclonal variation (Shavrukov, 2013). Many factors are involved in the development of somaclonal variation, including nutritional deficit, drought stress, and osmotic stress (Filipecki and Malepszy, 2006).

Table 3. Screening and *in vitro* selection of some economically important plants for salt stress

Plants	References
Brassica juncea (Indian mustard)	Jain et al. (1990)
Brassica napus (rapeseed)	Rahman et al. (1995)
Brassica oleracea (cauliflower)	Elavumoottil et al. (2003)
Citrus aurantium (sour orange)	Koc et al. (2009)
Cynodon transvaalensis × C. dactylon	Lu et al. (2007)
Diplachne fusca (kallar grass)	Nanakorn et al. (2003)
Linum usitatissimum (flax)	McHughen (1987)
Glycine max (soya bean)	Liu and Van Staden (2000)
Ipomoea batatas (sweet potato)	He et al. (2009)
Nicotiana tabacum (tobacco)	Rout et al. (2008)
Morus sp. (mulberry)	Vijayan et al. (2003)
Saccharum sp. (sugarcane)	Gandonou et al. (2006)
Solanum tuberosum (potato)	Queiros et al. (2007)
Triticum aestivum (wheat)	Zair et al. (2003)
Vigna radiata (mungbean)	Hassan et al. (2008)
Oryza sativa L.	Yeo et al. (1990)
Glaux maritima	Freipica and Ievinsh (2010)
Helianthus annuus L.	Chen et al. (2024)
Phoenix dactylifera L.	Benaceur et al. (2024)

Development of flood - resistant plants

Rice is one of the major crops, and more than 50% of the population uses rice. The development of flood-resistant rice plants is one of the major objectives of improving rice yields under flood stress conditions. The development of flood-resistant rice plants is very important because the persistence of floods for a few days can completely damage crops, and yields are completely lost (Mishra and Rao, 2016; Oladosu et al., 2020). The development of flood-resistant rice allows a long period of time under water and elongation of stems, with variations in the structure of plants and metabolic processes (Mishra and Rao, 2016). In rice, the quantitative trait locus Sub1 (QTL) identified on rice chromosome 9 is involved in flood tolerance. The determination of Sub1 in rice plants was a major breakthrough in flood tolerance research (Haque et al., 2023). In addition, two loci associated with flood tolerance (SNORKEL 1 and SNORKEL 2) were identified on chromosome 12. Plants harboring these two flood-tolerant strains exhibit increased plant growth and coleoptile elongation during development under stressful conditions, which is called the "tube effect" (Bailey-Serres et al., 2010). This type of adaptation mechanism has been reported in rice plants, where rapid shoot elongation allows the plant to increase the height of the submerged plant in response to the floodwater level. The genes encoding ethylene sensitivity factors (SK1 and SK2) detected in deep-sea rice varieties are involved in improving plant height during flooding by lengthening the internodes of rice shoots (Bailey-Serres et al., 2010). The flood resistance genes of plants have been reported in Asia. Hence, it is important to isolate and transfer flood resistance genes into the germplasms of other varieties. The combination of classical breeding techniques with biotechnological approaches has allowed the development of flood-resistant strains within a short period of time (Savenko et al., 2020). Most rice-growing countries worldwide have cultivated anthers on synthetic culture media to achieve this goal. This method is useful for obtaining homozygous plants that are resistant to various biotic and abiotic stresses, including soil salinity, drought, temperature and plant diseases (Ilyushko and Romashova, 2021). Rice haploids and homozygous dihaploids were obtained by growing anthers on artificial media for 1-2 years. The Federal State Budget Scientific Institution and the Primorsky Research Institute performed a series of studies and developed several rice varieties *via* anthers (Ilyushko and Romashova, 2019; Savenko et al., 2022). The *in vitro* culture method allows the culture of thousands of anther lines *in vitro* and is useful for screening the regeneration of many green buds and plants in each hybrid combination (Pattnaik et al., 2020).

Development of cold - tolerant plants

Cold stress, such as freezing (<°C) and chilling (0-15°C), is among the most important abiotic stresses that adversely affect crop productivity (Guo et al., 2018). Generally, several methods are applied for the analysis of cold stress-mediated plant death, including electrolyte leakage (Murray et al., 1989). In addition, 2,3,5-triphenyl tetrazolium chloride (TTC) reduction and regrowth tests are recommended for analysing the cold tolerance of plants (Kim, 2006). Under cold stress conditions, plants lose membrane permeability and induce electrolyte leakage (Petcu and Atanasiu, 1994). Evan's blue assay was previously used to determine plant cell death due to heavy metal stress (Gonzalez-Mendoza et al., 2009). Cold tolerance studies have been performed on fruits and crops such as barley, wheat, grape, pear, and peach (Petcu et al., 2000; Hong et al., 2003; Lim et al., 2005; Kwon et al., 2006; Seo et al., 2010). Under cold stress conditions, the protoplast membrane of plants is damaged and killed by the increased development of ice crystals due to water freezing within the plant cells, and extracellular freezing causes dehydration of the protoplasm. The cold-regulated genes (CORs) are associated with the synthesis of hydrophilic polypeptides to protect against

freeze-induced injury. These polypeptides stabilize membranes against freeze-induced damage and support freezing acclimation. Plant tissue culture provides enough opportunities for the production of plants with desirable properties, including cold and drought tolerance. Moreover, in plant tissue culture, tissue and cell cultures grown *in vitro* have been extensively studied to produce cold-resistant plants for the production of agricultural products. Plant tissue culture methods include embryo culture, micropropagation, protoplast culture, another culture and somatic hybridization. All these methods are used for the development of cold-resistant plants *in vitro*.

In vitro culture of plants for heavy metal stress mitigation

The accumulation of various heavy metals in the environment is an increasing problem worldwide. Heavy metal accumulation in plants from water and terrestrial environments transfers heavy metals to the human food chain (Rai et al., 2019; Oladoye et al., 2022). The use of plants for the removal of heavy metals has been reported previously, and various studies have reported the application of plants (Salt et al., 1998; Shen et al., 2022). In recent decades, several methods have been proposed for herbaceous plants, fast-growing woody plant species have been applied, and heavy metal stress/accumulation mechanisms have been reported (Capuana, 2011; Leguizamo et al., 2017; Sorrentino et al., 2018; Baker et al., 2020). The tissue culture method has several advantages, and this method can be used for analysing the physiological process of pollutant uptake and degradation/bioaccumulation. The tissue culture method allows state-of-the-art design of heavy metal biosorption studies, and all experiments can be performed in a controlled environment *in vitro* (Kruglova and Zinatulina, 2022). The highly complex nature of the various abiotic stresses that can effectively interact under greenhouse or field conditions can complicate the understanding of the response of plants to these abiotic factors. The application of *in vitro* tissue culture methods is an important tool for understanding the molecular physiology of plants cultured under various stress conditions (Harms, 1992; Castiglione et al., 2007; Benderradji et al., 2012). Compared with greenhouse or field experiments, the tissue culture method uses organ or cell culture for good handling and better data retrieval (Misra et al., 2002; Golan-Goldhirsh et al., 2004).

Application of tissue culture in genome editing and crop management

Food security is one of the most prominent challenges, and people in all countries face this challenge. By 2050, the requirement for food may increase the amount of food produced today by approximately nine billion people. Tissue culture is used for the development of improved abiotic and biotic resistant crops, and the scheme (laboratory to field) is represented in Figure 2. Crop breeding programs are useful for improving crop management and production *via* the use of existing fertile land (Grassini et al., 2013). The marginal increase in grain yield is not sufficient to meet the global demand. Hence, improving the performance of crop plants *via* a tissue engineering approach is a suitable approach (Ray et al., 2013). In breeding programs, the maintenance of genetic diversity is a prominent task to ensure sustainable food production. Plant breeders use different methods, including improvements in genetic variation, to improve the genetic diversity of plants. Hence, there is an urgent need to regularize such genetic variation and to implement an alternate, cost-effective method for genetic enrichment of the gene pool to minimize uniform or narrow genetic variation (Kazi et al., 2017). Physical mutagens, chemical mutagens, epigenetic agents such as histone deacetylase inhibitors, DNA demethylases, and *in vitro* methods are frequently applied for genetic variation and epigenetic alterations through the induction of mutations, histone and DNA methylation, and histone acetylation (Bridgen et al., 2018; Niazian and Shariatpanahi, 2020).

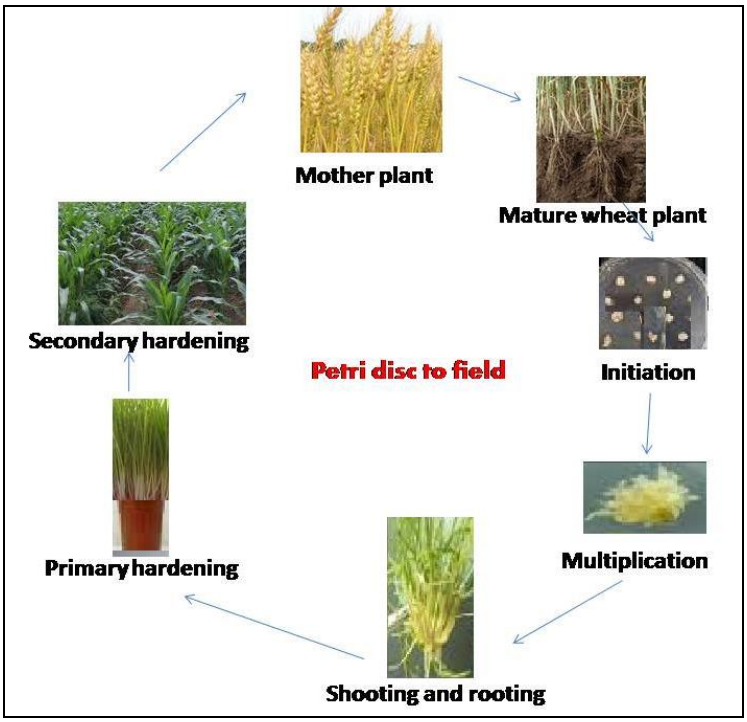

Figure 2. Scheme of *in vitro* tissue culture and the development of biotic and abiotic resistant crops

Genome editing is defined as a collection of various molecular biology tools that involve efficient, precise and targeted modifications at genomic loci. In addition, the genome editing approach, plant tissue culture and transformation of genes *via Agrobacterium*, has emerged as an alternative approach for the genetic manipulation of plants (Borisjuk et al., 2019). To prepare a genetically engineered plant, a new gene must be introduced into the growing plant cells that make up the plant. Two types of methods used to prepare genetically manipulated plants. The first method is highly popular, and the success rate is very high. In this method, transfection takes place in a tissue piece or callus in the tissue culture, and transformed cells containing plants are regenerated. The tissue culture method is very prominent for the production of recombinants. The other method is called planta transformation, and in this method, foreign genes are directly introduced into germ cells during the growth of plants, and genes of interest are obtained in the next generation. This technique is highly complicated, and the commonly used method is the floral dip method (Clough and Bent, 1998). Genetic engineering is a useful method that allows precision breeding that enables desired variation in plants to generate new plants. Various genetic engineering techniques have been applied to improve crop plants. In transgenesis, recombination of genetic elements is performed from a sexually incompatible gene pool. In this method, genetic elements such as genes, promoters, and terminators are inserted. In the cisgenesis method, recombination is performed within a sexually compatible gene pool. In this method, genetic elements such as genes, promoters, and terminators are inserted. Intragenes are hybrid genes and are composed of one or more components of recombinant genetic elements from various genes within a sexually compatible gene pool that are commonly inserted into the desired crop. Recently, several gene editing tools, including genome editing *via* zinc-finger nucleases, have allowed biologists to simply introduce site-specific modifications into target DNA sequences. These selected *in vitro* methods have several advantages over conventional breeding techniques, and genome editing tools have potential applications in new crop improvement (Lowder et al., 2015; Rodríguez-Leal et al., 2017; Zhang et al., 2018).

Clustered Regularly Interspaced Short Palindromic Repeats-Cas (CRISPR/Cas) and Crop Improvement

Genome editing is an important gene manipulation tool that allows the editing of genes at specific regions of DNA fragments *via* highly specific nucleases. The nucleases form a double-stranded break on the target DNA, and the breaks are repaired *via* nonhomologous end joining or homologous-directed recombination pathways, ultimately producing deletions, substitutions or insertions of the base in the specific location of the DNA. Moreover, the production of new genome-edited crops requires approval from the government and is associated with consumption issues. The number of ethical issues related to genome-edited crops is greater than that related to genetically modified crops (Waltz, 2015). In the 1900s, the first-generation genome editing method was introduced, and it involved the use of zinc finger nucleases and FoK1 endonuclease to make double-stranded breaks in DNA (Pabo et al., 2001). This method is useful for the development of new crops, such as maize, tobacco and soybean (Bonawitz et al., 2019). Later, transcription activator-like effector nucleases were applied and considered alternatives to zinc finger nucleases. These methods have several advantages over the use of zinc finger nucleases and have been applied to initiate nonhomologous mutations in crops (Tzfira and White, 2005), including tobacco, rice and Arabidopsis (Zhang et al., 2010; Cermak et al., 2011; Khandagale and Nadaf, 2016). Genome editing, especially editing with the CRISPR/Cas9 tool, has several advantages over other methods in plant breeding research, enabling potential application in the development of new crop plants (Ansari et al., 2020). *Escherichia coli* strains were the first bacteria and were considered model organisms to be applied *via* the CRISPR/Cas9 system before the past few decades (Ishino et al., 1987; Mojica et al., 1993). Cas proteins associated with CRISPR contribute to DNA repair, forming an adaptive immune system guided by RNA, and the whole process is regulated by CRISPR RNA with either class 2 or class 1 Cas proteins (Jansen et al., 2002; Makarova et al., 2015). The CRISPR/Cas9 gene editing strategy has been widely used to develop disease-free crop varieties and improve their resistance to various abiotic stressors. Shan et al. (2013) used rice protoplasts for rice genes associated with abiotic stresses *via* the CRISPR-Cas9 method, including mitogen-activated protein kinase (OsMPK2), betaine aldehyde dehydrogenase (OsBADH2), and phytoene desaturase (OsPDS). The genome editing of OsERF922 was performed in rice plants *via* the CRISPR/Cas9-mediated tool, and the developed plant was highly resistant to the pathogen Magnaporthe oryzae. This pathogen is causative of blast disease in rice (Liu et al., 2012). CRISPR TaMLO knockout in wheat *via* gene editing revealed disease resistance against powdery mildew caused by *Blumeriagraminis* f. sp. Tritici (Btg). Dehydration-responsive element binding protein 2 (TaDREB2) and wheat ethylene-responsive factor 3 (TaERF3) are expressed in 70% of wheat protoplasts and contribute to crop development (Kim et al., 2018). Wang et al. (2019) performed CRISPR/Cas9-based target genome editing, and three phylogeny-related Arabidopsis genes, namely, gibberellic acid insensitive, brassinosteroid insensitive1, and jasmonate-zim-domain protein 1, were analysed in successive generations. In japonica rice, improvements were made in the Wx gene *via* the use of CRISPR/Cas9 tools, and the newly developed rice plants presented improved amylose content in the grains (5-12%). In a previous study, Xu et al. (2019) knocked out the genes R2R3-MYB and DcMYB7 in solid purple carrots, resulting in the development of yellow roots. Most World Trade Organization (WTO) members are actively using gene editing tools and supporting the development of new crop varieties *via* gene editing methods, and this approach would be useful for improving food productivity throughout the world (Liu et al., 2021).

CONCLUSIONS

The world is facing severe challenges because of increasing pollution, effluent, global warming and production loss due to climatic changes in several parts of the world.

To meet the global challenge, increased attention must be given to innovative technologies to obtain results within a shorter time frame to achieve goals. Farmers must adapt and implement the latest technologies to improve crop yield and adapt to rapidly increasing climate change. Drought, salinity, temperature, flooding, mineral toxicity and frost affect commercial crop production. The development of biotechnology can provide real-time solutions to improve crop yield and productivity. Tissue culture-based mutagenesis has become an affordable and *via*ble method for the determination of stress-tolerant plant development. The present review highlights that *in vitro* screening by tissue culture is an alternative approach for screening the salinity, chemical toxicity, drought, heat and flood tolerance of plants. This method allows better assessment of stress variables and the determination of stress tolerance genes and metabolic pathways. The development of new genotypes with new strains is of great interest in agronomic studies and can be developed in *in vitro* cultivated plant species by artificially inducing mutations in their genetic materials. To perform various gene editing applications and broaden their applications, suitable tissue culture systems should be implemented. DNA mutation results in genetic variability, which is a basic mechanism for evolution and adaptation to environmental variations. Genome editing allows investigators to understand gene mutations in plants, generating adequate mutations to improve yield and the preparation of various new phenotypes for breeding within a single generation. The application of these biotechniques in agriculture can effectively improve food security by increasing crop resistance to pathogens and adverse soil and weather conditions, improving the adaptability of crops to various climatic conditions, and improving yields. In the coming decades, biotechnology could lead to classic technological changes and engineering to express resistance proteins that can identify phytopathogens or improve hormone signals to improve crop yield. It is also anticipated to cause disease resistance in crops, including against viral, bacterial, and fungal pathogens. Continuous global climate change has led engineers to develop plants that are relatively resilient to biotic and abiotic stresses. This approach enables farmers to obtain the maximum crop yield while decreasing the use of pesticides and water.

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