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Review

Tissue Culture—A Sustainable Approach to Explore Plant Stresses

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Abstract: Plants are constantly faced with biotic or abiotic stress, which affects their growth and development. Yield reduction due to biotic and abiotic stresses on economically important crop species causes substantial economic loss at a global level. Breeding for stress tolerance to create elite and superior genotypes has been a common practice for many decades, and plant tissue culture can be an efficient and cost-effective method. Tissue culture is a valuable tool to develop stress tolerance, screen stress tolerance, and elucidate physiological and biochemical changes during stress. In vitro selection carried out under controlled environment conditions in confined spaces is highly effective and cheaper to maintain. This review emphasizes the relevance of plant tissue culture for screening major abiotic stresses, drought, and salinity, and the development of disease resistance. Further emphasis is given to screening metal hyperaccumulators and transgenic technological applications for stress tolerance.

Keywords: micropropagation; tissue culture; germplasm; molecular breeding; biotechnology

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1. Introduction

Plant tissue culture satisfies large-scale plant propagation needs and is an essential tool facilitating other biotechnology applications in plant improvement space [1–3]. In addition, its importance as a tool and direct application in fundamental studies relating to plant biology, biochemistry, and molecular biology is well recognized. Today, the world population has reached an alarming 8 billion people. Providing food and products, of which the majority is plant-based, has become one of the biggest challenges in front of the human race in this era, while the unprecedented climate change challenges are bigger than ever before [4–6]. Various climatic changes as a result of global warming greatly influence agriculture systems around the globe. This includes severe environmental pressures, drought, extreme heat or cold climate, floods, salinity, and exposure to toxic compounds [4]. Human activities in the current production-based economy also contribute toward changes in soil and environmental conditions due to the accumulation of toxins and chemical elutes released to the environment in production processors [7]. The flip side of this is the reduction in agricultural production, flagging food security due to the decrease in plant growth and development due to various environmental stress factors and the decline and scarcity of suitable agricultural land.

The focus on plant stress-related research has gained substantial momentum over the last four decades, especially on the impact of stress factors such as water deficiency, extreme temperatures, salinity, exposure to toxic compounds, inadequate or extreme radiation, plant infection with pathogens, and pest outbreaks [8,9]. Screening plants for various biotic and abiotic stress conditions is vital in breeding and selecting elite varieties. Most plant screening trials for stresses are conducted under field conditions, yet very challenging to

manage, both physically and economically, and subject to various risks due to dynamic external environments.

Plant tissue culture provides an effective, efficient, and comparatively economical platform to screen plants for biotic and abiotic stresses. Plant cell and tissue culture, also known as in vitro culture, is based on the cell theory of Schwann and Schleiden (1838) and the ideas of Gottlieb Haberlandt at the beginning of the 20th century [10]. In vitro plant tissue culture is based on cells' "totipotency" or "total potential". Theoretically, every cell can become a fully grown plant when provided with suitable conditions. Totipotency has been better described as the ability of any fully functional components of plants to undergo dedifferentiation and redifferentiate to form an organized tissue, structure, and eventually a whole organism [11,12]. Based on this phenomenon, whole plants develop when plant cells or tissues are provided with specific nutrients and optimal growth conditions under an in vitro sterile environment. In vitro culture of plants under defined culture media composition with the ability to impose variables with no external environmental influence while maintaining control environment parameters offers the opportunity for more efficient screening for desirable characteristics. This is also applied to test tolerance to selective agents such as toxins and antibiotics. Utilization of in vitro selection can considerably shorten the time and cost of the selection process under selection pressure with minimal environmental interaction. Conducting in vitro screening for stress will not replace but complement field selection resulting in better insight and outcome. Moreover, it can be used as an early evaluation platform to understand and provide direction with justification for the further need for field evaluation. As with any technique or procedure, in vitro screening for stress tolerance has its challenges. The biggest challenge is the requirement for reliable and established tissue culture protocols for specific plant species. Another issue is the lack of correlation between the mechanisms of tolerance operating at the cellular or tissue level in cultured cells to those of whole plants. Epigenetic adaptation can also interfere with the results as non-tolerant cells may have an epigenetic adaptation during in vitro culture process [13–15]. This type of epigenetic adaptation can be overcome using a short-term or one-step in vitro selection process.

Studies on plant biodiversity, especially crop species, have increased tremendously over the years in search of better and more resilient crops despite breeding attempts. The research focused on plant improvement and selection has taken a massive hype for searching elite species for new and better chemicals, disease resistance, productivity, and consumer preference. This review elaborates on how in vitro culture is utilized to screen plant species for different biotic and abiotic stress evaluation studies with examples of a broad variety of crop species. The focus is on in vitro screening for the main stresses: drought, salinity, and disease resistance. This review also highlights other uses of in vitro screening for the identification of metal accumulators and stress-tolerant transgenic plant development with further discussion on the pros and cons of specific studies and the effectiveness of the in vitro culture tools utilized as fast and cost-effective alternative in plant screening.

2. In Vitro Screening of Drought Tolerance

Drought affects plant growth and development, reflecting plants' productivity [16,17]. Drought stress impacts crop performance at several phases in the plant's life cycle, from emergence to maturity, including seed germination, vegetative growth, and reproductive development, ultimately affecting the quality and quantity of the harvest. Drought, especially in arid and semi-arid regions, causes significant agricultural losses [18]. Under drought stress, several molecular, biochemical, physiological, morphological, and ecological characteristics and processes of plants are affected due to the triggering of stress-responsive factors [19]. As a result, the productivity and quality of plants diminish in water-deficient situations. Growth stages, age, plant species, drought intensity, and duration of drought period are the primary determinants for the responses elicited by the plant in response to drought stimuli.

Plants process various mechanisms to adopt, tolerate, or resist drought conditions. However, the ability and the level of tolerance/resistance differ among and within plant species, especially when genetic variability exists due to outcrossing or natural mutation [16,19–21]. Drought responses are governed by activating signal transduction pathways linked with molecular networks to elicit survival or adaptation mechanisms [20]. In general, drought causes a reduction in soil moisture and results in reduced water potential in root cells [17]. For many decades, breeding and selection for drought tolerance or resistance have been major research areas for many crop species i.e., rice, maize, and sorghum [22–27]. However, screening for drought in field conditions requires a substantial amount of resources (land, labor, and energy), which is costly, and is associated with challenges in relying on nature for stable environmental conditions to efficiently and effectively replicate data in expressing exact genotype [21].

In vitro applications for drought screening can be a smart and easy method compared to field studies. The strategy to apply drought conditions in vitro is to impose similar conditions created at cellular levels when plants are subjected to drought in the field environment. Under abiotic stress, plants accumulate solutes or osmolytes within the cell due to less water availability. Therefore, high molecular weight solutes such as sucrose, sorbitol, mannitol, and poly-ethylene glycol (PEG) are suitable candidates to impose physiological drought under in vitro conditions [28–32]. In addition, these osmolytes stabilize proteins and cell membranes' structure during dehydration stress conditions [33,34].

Contrary to drought stress, which induces osmotic stress in plants, accumulating these chemicals reduces osmotic potential, preserving cellular turgor and enhancing water absorption [20,35]. Moreover, they play a crucial function in protecting plant cells from oxidative stress by removing reactive oxygen species [20,32,35]. It has been shown that sucrose accumulates in plant tissues under drought stress [36–38]. PEG, sucrose, mannitol, and sorbitol have been the main chemicals for imposing osmotic pressure in vitro. PEG has reportedly been used to impose physiological drought in plants [28,29,39,40]. This high molecular weight chemical is an inert, non-penetrating osmoticum that decreases the water potential of nutritional solutions without being taken up by the plant or phytotoxic. Since PEG does not reach the apoplast, it drives water from the cell wall and interior. Therefore, PEG solutions resemble dry soil more closely than low molecular weight chemicals, which permeate the cell wall with solute. PEG is not used in the cellular metabolism of plants, but it does induce water stress by lowering the water potential of nutrient solutions, hence inhibiting plant development in vitro [28,39,41]. It has no harmful or toxic effects on the plant; nonetheless, it restricts plant development by lowering the water potential of the culture medium, such as water deficit soil, preventing cultured explants from absorbing water [42]. Mannitol and sorbitol have been usually used as osmotic pressure regulators in plant in vitro cultures while utilized as a carbon source [43]. Several studies have applied PEG, mannitol, and sorbitol for in vitro drought screening studies (Table 1).

Table 1. Application of different chemicals of in vitro screening for drought tolerance.

Plant Species	Marial Screened under In Vitro Conditions	In Vitro Screening Method	Reference
Soybean (*Glycine max*) *Cultivars: B 3731, MLG 2999, MSC 8606,Tidar*	Immature cotyledons were used as explant/Somatic embryogenesis	Subjected to 15% PEG 6000. The application of PEG was terminated after the plants were 28 days old.	[39]
Soybean (*Glycine max*) *Cultivars:* JS335, JS9305	Calli/cell clumps/embryoids rose from the immature and mature embryonic axis and cotyledons	Subjected to discontinuous exposure to a lethal dose of 20% PEG6000	[40]

Table 1. *Cont.*

Plant Species	Marial Screened under In Vitro Conditions	In Vitro Screening Method	Reference
Durum wheat (*Triticum durum*) *Cultivars:* Waha, Oued Zenati, Djenah Khetifa	Immature embryo-derived calli	Subjected to 10% and 20% PEG 6000	[44]
Durum wheat (*Triticum durum Desf.*) *Cultivars:* Karim, Sebou, Ourigh, Anouar	Immature embryo-derived calli	Subjected to 10% and 20% PEG 10000	[45]
Sorghum (*Sorghum bicolor* L. *Moench*)	Embryogenic callus	Subjected to a range of 0–15% PEG 8000	[46]
Wheat (*Triticum aestivum*) *Cultivar:* GA-2002	Immature embryo-derived calli	Subjected to a range of PEG 6000	[47]
Wheat (*Triticum aestivum*) *Cultivars:* Sakha 8, Sakha 69, Giza 157, Sids 1, West bred, Falke, Hahn/Turaco, and Kauz/Gen	Immature embryo-derived calli	Subjected to a range of Mannitol	[48]
Tagetes (*Tagetes minuta*)	Calli-derived from cotyledon explants	Subjected to a range of Mannitol	[49]
Sweet leaf (*Stevia rebaudiana*)	Nodal shoot/micro propagated shoots	Subjected to a range of PEG 6000	[50]
Potato (*Solanum tuberosum*) 27 CIP different cultivars	Nodal cuttings	Subjected to a range of Sorbitol	[51]
Rice (*Oryza sativa*) *Cultivars: PA U 201 and PR 116*	Embryogenic calli	Subjected to a range of (0–2%) PEG 6000	[52]
Potato (*Solanum tuberosum*) *Cultivars:* IWA-1, IWA-3, IWA-5	Well-sprouted microtubers	Subjected to a range of Sorbitol and PEG 6000	[30]
Rice (*Oryza sativa*) *Cultivars: IR 18351-229-3, IR 3185-6-3-3-2, SR 26-B, Nona Bokra, and C 14-8*	Seed-derived calli	Subjected to a range of PEG 6000	[53]
Ground nut (*Arachis hypogaea*) *Cultivars: TMV2, JL24*	Hypocotyl-derived calli	Subjected to a range of PEG (0.0, 0.4, 0.6, 0.8 and 1.0 MPa)	[54]
Brown mustard (*Brassica juncea Czern*) *Cultivars: RW-85-59*	Cotyledon-derived calli	Subjected to a range of Mannitol	[55]
Coconut (*Cocos nucifera*) *Variety: Sri Lanka tall*	Embryo cultures	Subjected to a range of PEG 6000 (1–5%)	[56]
Sugarcane (*Saccharum* sp.) *Cultivars: R570 and CP59-73*	Calli cultures	Subjected to a range of Mannitol	[57]

3. In Vitro Screening of Salinity Tolerance

Salinity in soil and water is the most critical constraint that affects plant growth and development [58,59]. Due to osmotic or ionic stress or nutritional imbalance, salinity stress

has a deleterious effect on plant development [60–62]. Arid and semi-arid environments are characterized by accumulating large quantities of salts in the soil [63]. Although many remedial and management procedures are utilized to make salt-affected soils suitable for agriculture, they are exceedingly costly and do not offer lasting answers to the salinity problem. Therefore, salinity stress has gained considerable traction over the past few decades due to the vast experimental evidence from what has occurred in nature regarding the evolution of highly salt-tolerant ecotypes of various plant species [64–66], as well as the remarkable progress made in improving various agronomic traits through artificial selection [67]. Plant tissue culture is the most efficient method for enhancing and producing salt tolerance in plants. By utilizing plant cell and tissue culture, it is possible to focus on the physiological and biochemical processes crucial to the cell and contribute to the alterations brought about by salt stress. Using two in vitro culture methods, salt-tolerant plants have been obtained through cell and tissue culture procedures. The first method involves selecting mutant cell lines from cultivated cells, followed by plant regeneration using these cells (somaclones). The second method is the in vitro screening of plant germplasm for salt tolerance, which has been successfully used in durum wheat [68]. Doubled haploid lines generated from pollen culture of salt-tolerant F1 hybrid parents have the potential to enhance salt tolerance [69,70]. Somaclonal variation and in vitro-induced mutagenesis can create variability from which crop plants can be improved. Examples of other in vitro selections for increased resistance to salt stresses are shown in Table 2. Enhancing resistance to both hyper-osmotic stress and ion toxicity may also be accomplished by molecular breeding of salt-tolerant plants employing molecular markers or genetic engineering.

Table 2. In vitro selection for increased resistance to salt stresses.

Plant Species	Marial Screened under In Vitro Conditions	In Vitro Screening/ Mutagenesis Method	Reference
Limnophila aromatica (*Lamk.*) *Merr.*	In vitro organogenesis from nodal explant	Nodal explants subjected to callus formation on a series of NaCl concentrations 0–100 mM	[71]
Bacopa monnieri (L.) *Wettst.*	In vitro organogenesis from nodal explant	Nodal explants subjected to callus formation on a series of NaCl concentrations 0–100 mM	[71]
Aubergine (*Solanum melongena*) *Cultivar: Bonica*	Leaf segment-derived calli	Callus formation on a series of NaCl concentrations 40–120 mM	[72]
Sweet potato (*Ipomoea batatas*) Cultivar: Shiroyutaka	Embryogenic calli	Callus formation on a series of NaCl concentrations 25–200 mM	[73]
Potato (*Solanum tuberosum*) Cultivars: Kennebec, Norchip, Red Pontiac, Russet Burbank, Russet Norkotah, and Superior	Stem cuttings, Leaf rachis originated callus-derived cell cultures	Callus formation on a series of NaCl concentrations 0.25–0.5 M	[74]
Canola (*Brassica napus*) Cultivars: Bingo Torpe, Conny and Siberian.	Hypocotyls and Cotyledonary-derived calli	Callus formation on a series of NaCl concentrations 4000, 8000, 12,000, and 16,000 ppm	[75]
Tomato (*Solanum lycopersicom*) Cultivars: Nora, PS-10, Peto, Roma	Hypocotyl-derived calli	Callus formation on a series of NaCl concentrations 5, 50, 75, and 100 mM	[76]
Chrysanthemum (*Chrysanthemum morifolium Ramat.*) Cultivar: Maghi Yellow	Ray floret-derived calli	Callus formation on a series of NaCl concentrations 50, 75 and 100 mM	[77]

Table 2. *Cont.*

Plant Species	Marial Screened under In Vitro Conditions	In Vitro Screening/ Mutagenesis Method	Reference
Sour Orange (*Citrus aurantium* L.)	Embryogenic calli	Callus formation on a series of NaCl concentrations 100–300 mM	[78]
Sugarcane (*Saccharum sp.*) Cultivar: CP65-357	Young leaf-derived callus	Callus formation on a 68 mM NaCl concentration	[79]
Bamboo (*Dendrocalamus strictus Nees*)	Embryogenic calli	Callus formation on a series of NaCl concentrations 50, 100, 150, 200, and 250 mM	[80]
Carrot (*Daucus carota subsp. sativus* L.) Cultivars: Dolanka and two Iranian landraces (DAL and NL)	Protoplasts	Protoplast culture in a series of NaCl concentrations 10–400 mM	[81]
Rice (*Oryza sativa* L.) Cultivars: KDML and LPT	Embryogenic calli	Callus formation on a series of NaCl concentrations 1–2%	[82]
Durum wheat (*Triticum turgidum* var. *durum*)	Immature embryogenic calli	Callus formation on a series of NaCl concentrations 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, and 2.1% *w*/*v*	[68]

4. In Vitro Screening of Disease Resistance

Plant diseases cause substantial revenue loss in agriculture due to lost or poor performing plants when infected. Thus, breeding and selection for disease resistance are at the forefront of crop science. Various air, soil, and water-borne fungal, bacterial, viral, and mycoplasma diseases affect commercial crops, especially in monoculture and chemically fertilized environments. Therefore, studying and screening for disease tolerance and resistance are routine operations. Often such investigations require specialized conditions and highly controlled setups to minimize the risk of an unintentional spread of diseases to the outside environment causing disease outbreaks for major agricultural crops. In vitro selection using pathogenesis-related proteins, antifungal peptides, or phytoalexin production can help select elite-resistant varieties. This method is simpler and cheaper than generating plants through transgenic technology, which is costly, time-consuming, and more challenging to commercialize due to policy and social acceptance barriers. Exposing organogenic or embryogenic calli, shoots, somatic embryos, or cell suspensions to pathogen toxins, culture filtrate, or the direct pathogen can effectively screen plant samples for pathogen resistance in vitro. In Table 3, such in vitro screening studies are listed for various crop species.

Table 3. In vitro selection for increased resistance to biotic stresses.

Plant Species	Marial Screened under In Vitro Conditions	In Vitro Screening/Investigation Method	Reference
Lycoris radiata	Callus induced from meristem tissue of dried bulbs	Wounding stress imposed to analyse the accumulation of galantamine content as a measure of abiotic stress response in plants	[83]
Ground nut (*Arachis hypogaea*) *Cultivars: VRI-2, TMV-7*	Immature leaf-derived calli	Culture filtrate of *Cercosporidium personation*	[84]

Table 3. *Cont.*

Plant Species	Marial Screened under In Vitro Conditions	In Vitro Screening/Investigation Method	Reference
Femminello' lemon (*Citrus limon* L.) *Burro. f.* *Tarocco' orange* (*Citrus sinensis* L. *Osb.*)	Nucellar calli	Culture filtrate and toxin of *Phoma tracheiphila*	[85]
Turmeric (*Curcuma longa*) *Cultivar: Suguna*	Non-embryogenic propagule-derived calli	Culture filtrate of *Pythium graminicolum*	[86]
Cotton (*Gossypium hirsutum* L.) Cultivar: SVPR 2	Hypercotyl-derived somatic embryos	Culture filtrate of *Fusarium oxysporum* and *Alternaria macrospora*	[87]
Sugarcane (*Saccharum sp.*) Cultivars: CoJ 88 and CoJ 64	Apical spindle tips-derived calli	Culture filtrate of *Colletotrichum falcatum*	[88]
Wheat (*Triticum aestivum*) Varieties: Sumai 3 (P1), Mianyang 11 (P2), and their reciprocal F1 hybrids	Embryo-derived calli	Deoxynivalenol	[89]
grapevine (*Vitis vinifera* L.) *Cultivar: Chardonnay*	Proembryogenic masses derived calli	Culture filtrate of *Elsinoe ampelina*	[90]
Populus nigra × *trichocarpa*	Internode-derived callus, Somaclonal selection	Culture filtrate of *Septoria musiva*	[91]
Sweet orange (*Citrus sinensis Osbeck*)	embryogenic callus mutagenesis with EMS by somaclones tolerant	Culture filtrate of *Xanthomonas citri subsp. citri*	[92]
Peach (*Prunus persica*) Cultivars: Sunhigh, Redhaven	Embryo-derived calli	Culture filtrate of *Xanthomonas campestris pv. pruni*	[93]

Some research suggests that rather than the success of screening, somaclonal variation occurring during the tissue culture process is a probable factor in disease-resistant behavoir [91–94]. A combination of a chance mutation in vitro with in vitro selection pressure appears to delay and confound the later examination of plants produced by these methods. In such an attempt by Vos et al. (2000), they grew tens of thousands of standard seedlings in culture and screened in vitro for resistance to guava wilt disease [95]. This is the most impressive example of such in vitro screening study for disease resistance. This accelerated the discovery of potentially resistant plants, saving the South African guava industry.

5. In Vitro Screening of Metal Hyperaccumulators

Phytoremediation is a novel and cost-effective method for removing hazardous heavy metals (Pb, Cd, Cu, Zn, etc.) and organic contaminants from water and soil [7]. There are now accessible biotechnologies for better comprehending plants' mechanism of heavy metal absorption and examining their potential for remediation enhancement [96]. On the other hand, metal accumulators are highly beneficial and trendy as food supplements or for recouping rare and expensive metal elements for cosmetics and other uses [97]. When studying the tolerance of plant cells to hazardous substances, in vitro cultures provide several advantages [98]. In this regard, in vitro screening is a preliminary technique for assessing woody plant materials since it reduces the time required for growth and treatment and the amount of space necessary for the tests. Since it is conducted under controlled conditions, plant tissue culture is one of the most dependable procedures used in fundamental research to establish the metabolic capacity of plants [99]. Research on

phytoremediation typically uses several plant tissue cultures as model plant systems. Some examples of these cultures are calli, cell suspensions, and hairy roots. When it comes to research on the inherent metabolic capacities of plant cells and their ability to tolerate toxicity, in vitro cultures provide several benefits to the otherwise unavailable experimentation process. In the quest for fundamental information about plants, the capacity to determine the specific contributions that plant cells make to the process of pollutant absorption and detoxification in the absence of interference by microbes is of special value. However, the final objective of such studies is to develop a realistic phytoremediation technology. In that case, it is necessary to understand the inherent limitations in using in vitro cultures as a representative of entire plants in the field. It is highly likely that the bioavailability of contaminants and the processes of pollutant uptake and metabolite distribution will be significantly different in the two systems. This can lead to qualitative and quantitative differences in metabolic profiles and tolerance characteristics. In order to gain complete understanding or to identify an effective species in phytoremediation through chemical accumulation, it is necessary to use intact rooted plantlets in tissue culture conditions for screening studies. However, several studies have shown that plant tissue culture is a handy tool in the field of phytoremediation surveys (Table 4). The findings obtained from tissue cultures may be utilized to make predictions regarding the reactions of plants to environmental toxins, as well as to enhance the design of future traditional whole plant tests, which in turn helps to lower their overall costs.

Table 4. In vitro selection for increased resistance to hyperaccumulators.

Plant Species	Tissue Culture Method	Stress	Reference
Potato (*Solanum tuberosum* L.) Cultivar: Iwa	Micropropagation in vitro cell line selection	Cadmium	[100]
Foxtail millet (*Setaria Italica*)	Leaf base and mesocotyl explant-derived calli	Zinc	[101]
Rice (*Oryza sativa* L.)	Embryo-derived calli	Aluminium	[102–104]
Brassica campestris cv. M27 *Brassica juncea cv. Pusabold*	Cotyledon-derived calli	Zinc	[105]
Jungle rice (*Echinochloa colona*)	Leaf base-derived calli	Chromium, Nickel	[106]
Tobacco (*Nicotiana tabacum* L.) *Cultivar: Xanthi*	Leaf-derived calli	Copper	[107]
Mustard (*Brassica juncea*)	Hypocotyl-derived calli	Cadmium	[108,109]
Silver poplar (*Populus alba*)	Microshoot cultures	Cadmium, Copper	[110]
Prunella vulgaris	Shoot-tip explants	Cadmium, Zinc	[111]
Downy *oak* (*Quercus pubescens*)	Seedlings	Cadmium, Copper	[112]
Silver poplar (*Populus alba*)	Microshoots culture	Arsenic, Copper, Cadmium, Zinc	[113]
Flax (*Linum usitatissimum* L.)	Cell culture/Calli/shoot-tip culture	Cadmium	[114–116]

6. Stress Tolerance through Transgenic Technology

Genetic modification technology for agriculturally important plant species has achieved major advances in the last decade. The development of transgenic plants with desirable

characteristics, such as tolerance to biotic and abiotic stress, is a reality. Plant characteristics are altered much faster than ever by utilizing a wide variety of approaches through gene transfer and gene editing [2,4,117]. The technique for tissue regeneration through tissue culture is a prerequisite in such processes, and it is vital.

Genetic transformation has been proposed for several decades as a quick way to modify the morphological characteristics of an organism. Plant genetic transformation techniques can be classified as direct and vector-based, introducing transgenic DNA to the host organism. Direct genetic transformation refers to the direct introduction of transgenic DNA to a plant cell. The most used techniques are biolistics and biological vectors that use *Agrobacterium tumefaciens*-mediated transformation [2].

Genetic transformation through biolistics can transform any totipotent plant cell, from which cell lines, tissues, or whole plants can be created. However, it has the disadvantage that the transformation can be transient and generate chimeric plants (non-transformed cells within the plant), in addition to the requirement of expensive equipment and low transformation efficiency [118]. On the other hand, the most studied and used vector-based transformation method is through infection of *Agrobacterium tumefaciens*, a natural plant pathogenic bacterium capable of incorporating a DNA region (Ti plasmid of the *Agrobacterium tumefaciens*) into the plant genome. This system does not require specialized equipment and thus is inexpensive, and the number of transformation events per cell is limited. However, limitations exist due to plant regeneration challenges from the transformed callus cells [119–121].

The methods of introducing foreign DNA or changing plant genomes have been updated for the use of more defined transformation systems such as the "Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) -associated protein 9 (Cas9) systems". This system is powerful to allow specific genetic edits to change genomes according to the need. The CRISPR/Cas9 system is based on the immune system of bacteria adapted to eukaryotic systems, including plants [122,123], whose principle is the RNA–DNA interaction to search for the sequence genomics [124,125].

Not all plant tissue types and species are conducive to the above transformation methods. An explant can be a variety of tissues, depending on the particular plant species and its regenerative ability. Table 5 below lists several studies conducted to achieve abiotic and biotic resistance through genetic transformation. Identification of a suitable tissue culture approach to maximize the transformation efficiency with higher regeneration of transformed cells is critical. Different approaches are illustrated in Figure 1.

Table 5. Examples of attempts to improve abiotic stress tolerance in crop plants through genetic transformation.

Crop	Trait	Trasformation Tissue Type	Reference
Apple (*Malus domestica Borkh.*) Cultivar Royal Gala.	Herbicide resistance	Callus/Organogenesis	[126]
Apple (*Malus domestica*) Cultivar: Mailing 26	Resistance to Erwínía amylovora	Callus/Organogenesis	[127]
Apricot (*Prunus armeniaca*) Cultivar: Kecskemeter	Plum pox virus resistance	Callus/Organogenesis	[128]
European plum (*Prunus domestica*) Cultivar: Stanley	Papaya ringspot virus resistance	Callus/Organogenesis	[129,130]
Maize (*Zea mays*)	Salt resistance	Embryo culture/Organogenesis	[131]

Table 5. *Cont.*

Crop	Trait	Trasformation Tissue Type	Reference
Rice (*Oryza sativa*)	Salt resistance	Callus/Organogenesis	[132]
Chickpea (*Cicer arietinum*) Cultivar: C 235 (desi type)	Drought resistance	Callus/Direct organogenesis	[133]
Colt cherry (*Prunus avium* × *pseudocerasus*)	Salt and drought resistance	Protoplast	[134]
Pistachio (*Pistacia vera*) Cultivar: Sarakhs	Drought resistance	Somatic embryogenesis	[135]

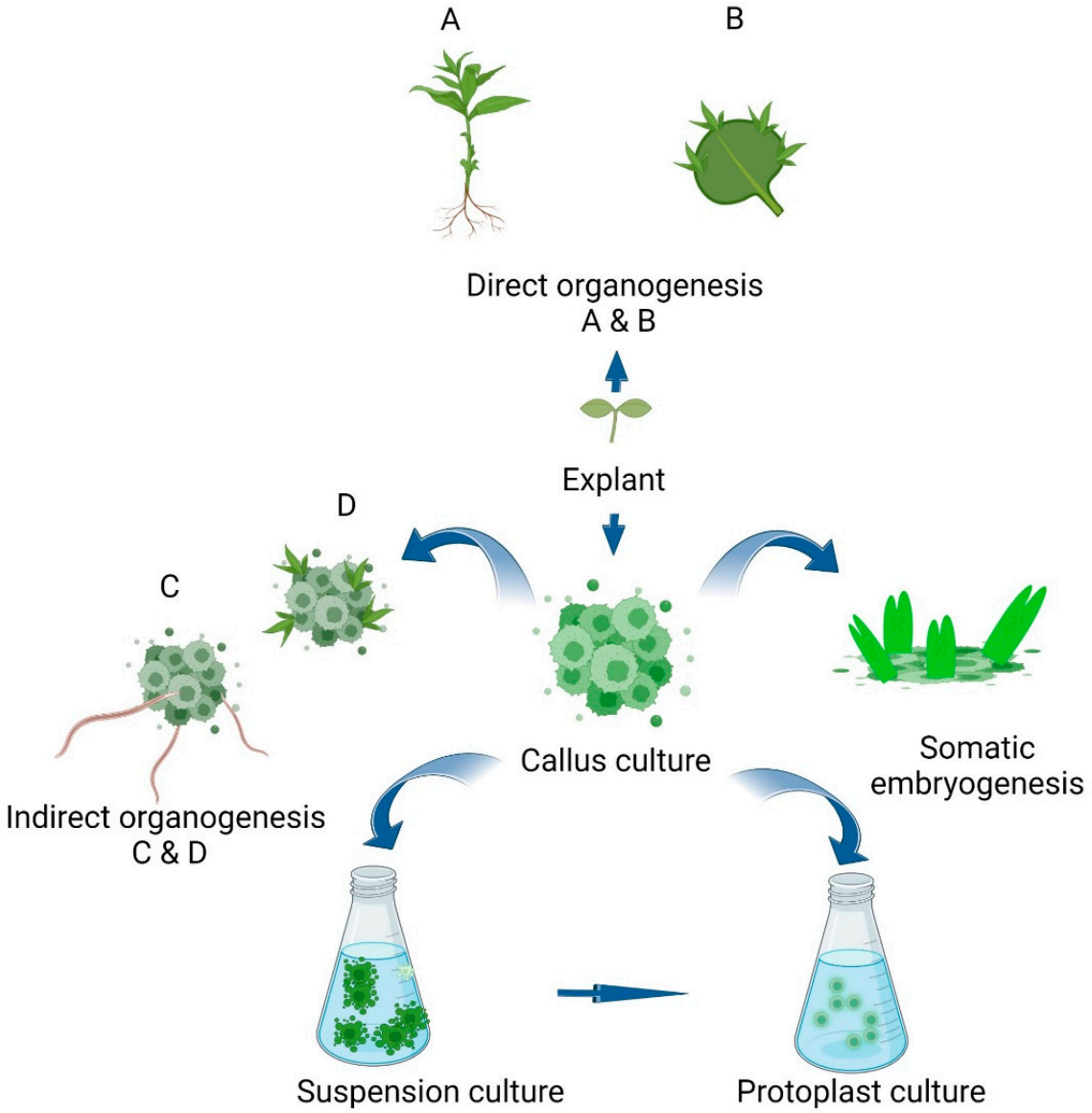

Figure 1. Different techniques in tissue culture for plant regeneration can be utilized for the selection and genetic transformation of plants. Starting from explants under selection mediums, direct organogenesis can be achieved (A and B) or indirect organogenesis (C and D) through an intermediate callus phase. Further, callus can be used to form intact plantlets through an embryonic pathway or in suspension culture directly or via protoplast culture techniques in genetic transformation attempts.

Different combinations of culture type and transformation protocol are used depending on the plant species and cultivar. In some species, various culture types and regeneration methods can be used, which enables a wide variety of transformation protocols to be utilized. However, there is no choice over culture type and regeneration method in other species, limiting the applicable transformation protocols [136].

7. Summary

Salinity, drought, water logging, heat, frost, and mineral toxicities limit commercial agricultural productivity. Biotechnology can bring in solutions to increase crop productivity. Tissue culture-based in vitro selection and mutagenesis have become a viable and affordable method for stress-tolerant plant development. Current research supports the notion that in vitro screening is an alternative and a support platform for stress tolerance screening for drought, salinity, and chemical toxicity. Further, in vitro culture permits accurate modification and assessment of stress variables, identifying stress-tolerance genes and metabolic pathways. Expanding research into this area of science will ensure more efficient and effective screening methodologies for the future of sustainable agriculture.

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