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PLANT TISSUE CULTURE AS A TOOL FOR DEVELOPING DROUGHT-TOLERANT CROPS: ADVANCES IN IN VITRO SELECTION AND SOMACLONAL VARIATION

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ABSTRACT

Drought stands out as a significant environmental factor that not only caps crop productivity but also poses a risk to food security worldwide, especially in a changing climate. Efficient approaches are needed that can complement conventional breeding for developing drought-tolerant crops. Plant tissue culture offers a rapid and controlled environment for the production, screening, and propagation of stress-tolerant plant material. This review highlights recent utilization of the tissue culture system to enhance drought tolerance based on the somaclonal variation, in vitro screening, osmotic stress screening, and combination of in vitro selection with molecular techniques. Under in vitro conditions, it is possible to develop drought-resistant crops and aid in controlling water-deficit stress by using osmotic agents like polyethylene glycol (PEG), sorbitol, and mannitol, and to select tolerant cells, calli, or regenerated plants at an early developmental stage. The somaclonal variation that occurs during tissue culture can also generate extra genetic and epigenetic variability from which drought-tolerant variants can be selected. The approaches have been shown to be effective in enhancing drought-related physiological, biochemical, and growth traits in plants such as wheat, rice, sugarcane, banana, peanut, maize, and Tagetes minuta. The use of tissue culture, molecular markers, and genetic engineering further improves the identification and fixation of desirable traits. In conclusion, tissue culture-based approaches provide a fast, controlled, and complementary solution for the development of crops more resilient to drought conditions and aids to sustainable agriculture production, as water availability and climate change become more and more critical.

Keywords: Drought Tolerance, in Vitro Screening, Osmotic Stress, Somaclonal Variation, Plant Tissue Culture

INTRODUCTION

Drought is one of the most limiting abiotic stresses for crop growth and productivity, especially in agricultural systems that rely on rainfall to grow crops or have limited irrigation. Its effects on plants depend on stress intensity and duration, developmental stage, soil conditions, and genotype. At the plant level, water deficit may cause decreases in cell growth, photosynthesis, and biomass production and induce plant responses, including stomatal regulation, osmotic adjustment, and antioxidant activity. The improvement of crop performance under water-limited conditions is therefore an important goal of plant breeding, but drought tolerance is a complex trait resulting from various physiological and genetic mechanisms Cattivelli et al. (2008). Conventional breeding is still required, but it is difficult to assess drought responses in different environments and to combine desirable traits. Complementary approaches that aid in the early detection of potentially valuable genetic variation could thus be useful in crop improvement.

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Plant tissue culture is a method used to grow plant cells, tissues, and organs in a controlled environment under a nutrient medium and is widely utilized in micropropagation, regeneration, and plant biotechnology George et al. (2008). One application is in vitro selection, where stresses are applied to tissues in culture, and the response is determined to find material that may be tolerant. Osmotic stress has been imposed in culture media by polyethylene glycol (PEG) and mannitol, but the response to these agents does not necessarily mimic that of field drought that involves soil properties, root development, atmospheric demand, and changing water availability. Therefore, in vitro selection should be considered as a preliminary screening or a measure of drought tolerance rather than evidence of field-level drought tolerance Rai et al. (2011). Its value is dependent on the plant species and genotype, the tissue or explant used, the culture conditions, and the selection regime. The selected material should then be regenerated and tested in greenhouse and field conditions.

A related approach is somaclonal variation, which includes genetic and epigenetic variation that occurs during in vitro culture of plant tissue. The variation can offer an extra level of diversity for selection but can also generate undesirable and/or unstable characteristics, necessitating evaluation of candidate plants for trait stability, inheritance, and agronomic performance. Experimental research shows that such a combination is possible, and it is possible to reap some benefits from the in vitro selection process, the regeneration, and subsequent evaluation of the plants. For instance, Mohamed et al. (2000) grew and selected a drought-tolerant clone of Tagetes minuta using culture conditions involving mannitol and characterized the selected man. Rahayu and Sudarsono (2015) used 15% PEG 6000 for selecting somatic embryos in peanut (Arachis hypogaea L.) and regenerated plants to test their progeny under limited watering in a greenhouse. According to the authors, 44% of the plants evaluated were PEG-tolerant somatic embryos that were drought-tolerant in their greenhouse experiments. The result is encouraging for the approach in that experimental system but should not be extrapolated to other crops or considered a test of field performance.

Tissue culture can also be used for genetic improvement by allowing the regeneration of plants after genome editing or genetic transformation. However, this process does not mean that tissue culture itself ensures that the developed plant is drought tolerant: it is dependent on the nature of the genetic change or variation that is achieved, the ability to regenerate plants, and the proof of the appropriate phenotype. Moreover, the responses that are observed in vitro may not always be representative of whole-plant responses, and tissue culture may be genotype-dependent and necessitate specialized facilities, expertise, and optimization George et al. (2008), Rai et al. (2011). Therefore, a beneficial crop-improvement strategy would involve in vitro screening along with regeneration, physiological characterization and molecular characterization if applicable, and greenhouse or field testing under pertinent conditions.

This review describes the use of in vitro selection and somaclonal variation in identifying and breeding drought-tolerant plant material and focuses on these two techniques. The main steps of developing drought-tolerant plants by tissue culture methods are shown in Figure 1, which includes explant selection and in vitro culture, selection under stress conditions, plant regeneration, and validation. The review evaluates the use and restriction of osmotic stress agents and examples of crop-specific selection and validation, as well as difficulties in transferring laboratory responses to valuable agronomic traits. The review is meant to differentiate between preliminary in vitro tolerance and established plant performance and to elucidate the contributions tissue culture can make to the enhancement of drought tolerance and the need for additional testing prior to being selected for crop development.

Figure 1

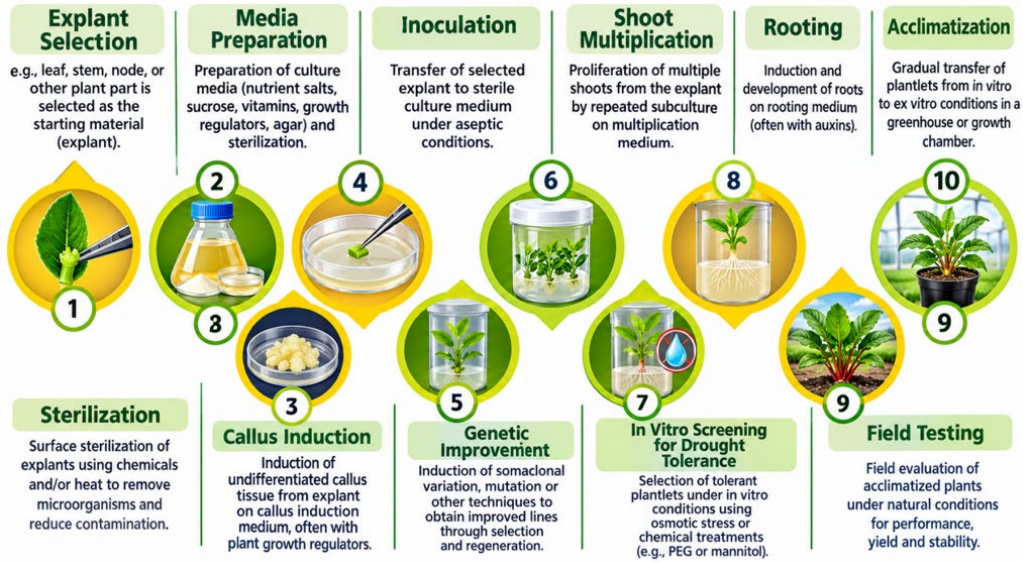


Figure 1 Workflow for Producing Drought-Tolerant Plants Via Tissue Culture Techniques.

SOMACLONAL VARIATION FOR DROUGHT TOLERANCE

Somaclonal variation is the genetic and epigenetic variation that occurs between plants regenerated from cells, tissues, or organs cultured in vitro. This phenomenon has been suggested as a potential source of useful variability for plant improvement by Larkin and Scowcroft (1981) and may occur in several ways, such as chromosome number changes, structural changes in the chromosomes, mutations in DNA sequences, activation of transposable elements, and changes in DNA methylation. The level and frequency of variation are dependent on the genotype of the plant, the origin of the explants, culture period, plant growth regulators, and the pathway for regeneration Larkin and Scowcroft (1981), Jain (2001), Bairu et al. (2011). Somaclonal variation can thus increase the variety available for crop development without the deliberate transfer of foreign genes. But it does not necessarily generate desirable traits, and, if it does, these desirable variants will need to be selected by systematic screening and then assessed.

Culturing cells or tissues under controlled selection conditions (in vitro selection) and somaclonal variation can be used together to enhance drought tolerance. PEG and mannitol are two commonly used chemicals to impose osmotic stress by lowering the water potential of culture media. Any tissues that still develop or regenerate under such conditions can be used for further investigation Rai et al. (2011). However, being able to survive or grow in vitro does not prove that a regenerated plant will withstand drying soil and have field yield. The selection pressure should be properly optimized, as excessive stress can reduce regeneration, and lack of stress can make it difficult to differentiate between sensitive and responsive material. The results also are dependent upon the genotype, explant, culture medium, stress intensity, and duration of stress.

An example of this type of crop-specific study is the study of rice (Oryza sativa L.), cultivar PR113, by Verma et al. (2013). Rice tissues in vitro were selected by PEG 6000 at 30, 50, and 70 g L^{-1}, and then the regenerated somaclone plants were assessed in limited irrigation conditions in field trials. Several reported vegetative and yield-related traits reported showed better performance for the 30 g L^{-1} selected lines as compared to the 50 g L^{-1} selected lines. In the first field evaluation, for example, the authors reported plant heights of 87.33 cm and 75.67 cm, respectively, and grain yields per plant of 17.56 g and 9.03 g. The same study also showed that the performance of 30 g L^{-1} selected lines was improved in some yield components. The results show that in vitro selection can generate candidate material that is useful to test under water-limited conditions, but these findings may not be applicable to all rice cultivars or other crops without testing.

Characterization is important after selection because of the genetic and epigenetic basis of variation. Simple sequence repeat (SSR) markers, single-nucleotide polymorphism (SNP) markers, and DNA methylation analysis will be useful in estimating genetic differences, genetic identity, and regenerated line stability. Markers are useful in drought-tolerance information only if there is a link between the marker and the drought-tolerant trait, but a difference in the marker does not necessarily indicate an improvement in drought tolerance. Phenotypic measurement should thus continue to play a key role in screening, and molecular characterization should be employed to explain variation, confirm identity, and aid in selection when appropriate, based on associations of the markers with the plants' traits Jain, (2001), Bairu et al. (2011), Hu and Xiong (2014).

Selected lines may show differences in performance, which may be explained by physiological and biochemical measurements. Various parameters such as relative water content, leaf water status, stomatal conductance, photosynthetic performance, root and shoot growth, proline accumulation, membrane stability, and antioxidant enzyme activity can be useful depending on the crop and experimental goals. These are signs of plant response, and not all drought-tolerant plants nor all of the measures individually determine drought tolerance. For instance, the higher accumulation of proline does not necessarily mean that the plant is better tolerated, should be used as such, and should only be regarded as such if backed with growth and recover yield data. Similarly, exogenous abscisic acid (ABA) could affect stomatal regulation and drought-response signalling but is a plant hormone and not an osmotic-stress agent such as PEG or mannitol. ABA treatments can prove beneficial in certain experiments; however, this is dose-dependent and will vary based on the developmental stage and the experimental context.

Although somaclonal variation has potential, there are important limitations. Abnormal morphology, low growth, low fertility, or low agronomic performance are traits of some of these variants, which are undesirable. Other changes may be temporary or not be passed on to subsequent generations. These risks are of special importance when using callus survival as a selection criterion because cellular responses might not be retained at the plant regeneration stage. The plant-level testing and assessment of trait stability and inheritance, if applicable, should be repeated, and the lines should be tested in the greenhouse and field under both control and water-limiting conditions. The yield, stress recovery, and performance under more than one environment are particularly important when developing cultivars for agricultural purposes Jain, (2001), Bairu et al. (2011), Rai et al. (2011).

In general, somaclonal variation is considered a source of potentially useful genetic variation, and in vitro selection is considered a screening method for selecting candidate plant material for drought tolerance. The in vitro evidence for drought tolerance in selected and somaclonal variants for specific crops is summarized in Table 1. The pairing can help contribute to drought-tolerant improvement if coupled with better plant regeneration techniques, phenotypic evaluation, molecular characterization, and testing under realistic growing conditions. The best evidence is provided by studies that go beyond survival to show consistent plant performance when water is limiting.

Table 1

Table 1 Crop-Specific Evidence for in Vitro Selection and Somaclonal Variation Associated with Drought Tolerance

Plant species	In vitro selection method	Traits measured	Evidence of drought tolerance	References
Rice (Oryza sativa L.)	PEG 6000 (30–70 g L^{-1})	Growth, roots, tillers, yield	Lines selected at 30 g L^{-1} performed better under limited irrigation	Verma et al. (2013)
Peanut (Arachis hypogaea L.)	Somatic embryos selected with 15% PEG 6000	Biomass and pod yield	44% of evaluated R_2 plants showed drought tolerance under greenhouse conditions	Rahayu and Sudarsono (2015)
Wild marigold (Tagetes minuta L.)	Mannitol selection (60 mM)	Biomass, water potential, proline, sugars	Selected clone showed better growth under greenhouse drought conditions	Mohamed et al. (2000), Mohamed et al. (2002)
Durum wheat (Triticum durum Desf.)	PEG-based selection across generations	Membrane stability, photosynthesis, stomatal conductance	Improvements in several drought-related traits persisted across R_2–R_4 generations	Bajji et al. (2004)
Bread wheat (Triticum aestivum L.)	Immature-embryo callus selected with PEG 6000	Leaf water potential and leaf rolling	Selected R_1 somaclones maintained higher leaf water potential under stress	Mahmood et al. (2012)
Sugarcane (Saccharum spp.)	PEG 6000 in callus medium	Callus growth, regeneration, proline, sugars	CoH 119 showed greater in vitro tolerance than CoJ 83; whole-plant tolerance remains unconfirmed	Manchanda et al. (2014)

GENETIC ENGINEERING FOR DROUGHT TOLERANCE VIA TISSUE CULTURE

The introduction or modification of genes associated with drought responses can be done through genetic engineering and plant tissue culture. In contrast to conventional breeding, genetic engineering can be used to manipulate individual genes or regulatory pathways in response to water deficiency. The process can be aided by tissue culture techniques which allow the transformation of suitable plant cells or explants, the selection of transformed cells and the regeneration of whole plants. But drought-tolerant plants cannot be obtained by successful transformation alone; the regenerated plants need to be genetically characterised and physiologically and agronomically evaluated Kudo et al. (2017), Muthurajan et al. (2021).

There are several candidate genes that could be linked to drought tolerance, such as transcription factors, osmoprotectant-biosynthesis genes and genes related to cellular protection and stress signalling. The DREB/CBF family of transcription factors activates downstream stress-responsive genes and may affect osmotic adjustment, membrane stability, etc. during dehydration. They are dependent on the gene, promoter, plant species and level of expression. Kudo et al. (2017) showed improved drought-stress tolerance when both DREB and PIF transcription factors were co-overexpressed in experimental plants. This is a step towards the possibility of tuning the networks of genes involved in stress response and is not intended to imply that the same combination of genes will enhance yield in every type of crop.

As an example, transgenic indica rice (Oryza sativa L.) expressing the gene AtDREB1A of Arabidopsis thaliana under the control of a stress-inducible promoter has been developed for the crop. Ravikumar et al. (2014) introduced the gene into the rice cultivar Samba Mahsuri by Agrobacterium-mediated transformation. The transgene was identified and observed in the following generations. Selected transgenic lines exhibited higher relative water contents and chlorophyll and decreased ion leakage and proline accumulation under water stress compared to non-transgenic controls. The study also showed that the grain yield and spikelet fertility of selected lines were improved under drought stress. These results suggest that a stress-inducible transcription factor can enhance drought responses, but additional independent and multi-environment tests are required to extrapolate the results to other cultivars of rice.

It is important to consider promoter choice for functional assays, as they may impact normal growth and stress responses. Lee et al. (2003) demonstrated in tomato that the expression of AtCBF1 from an ABA/stress-inducible promoter resulted in plants that were more tolerant to water deficit, chilling and salinity without affecting growth and yield under normal conditions. The authors compared this result with previous studies which had employed a constitutive promoter, resulting in increased stress tolerance and decreased growth and yield. This demonstrates the importance of the function of the candidate gene and also the where and when of expression in engineering strategies.

Another is the use of genes which are involved in osmoprotectant synthesis. Proline can play a role in the osmotic adjustment as well as may play a role in protecting cellular structures under stress conditions. The enzyme Δ^1-pyrroline-5-carboxylate synthetase (P5CS) is involved in proline biosynthesis. Recently, Sellamuthu et al. (2024) have introduced the gene PgP5CS from pearl millet into tobacco. The transgenic plants exhibited higher levels of proline, chlorophyll and relative water content and lower malondialdehyde content under drought and heat stress conditions when compared with the wild-type plants. The results indicate the role of P5CS in stress response, but not in improved crop yield under field drought in tobacco.

The general procedure for generating genetically engineered plant varieties by tissue culture with drought resistance is shown in Figure 2. It consists of candidate gene selection, design of an expression cassette, transformation of plant cells, isolation of transformed tissue, regeneration of plants and analysis of the presence and expression of the introduced gene. The presence, the insertion properties and the expression of transgenes can be assessed by molecular analysis including PCR, Southern blotting and quantitative reverse-transcription PCR (qRT-PCR). Regenerated plants should then be compared with appropriate non-transgenic controls under conditions of adequate and limited water availability, using assessments of plant water status, growth, recovery, fertility and yield. If genome editing is employed in lieu of the introduction of transgenes, the results of genome editing and unintended changes should also be assessed.

Figure 2

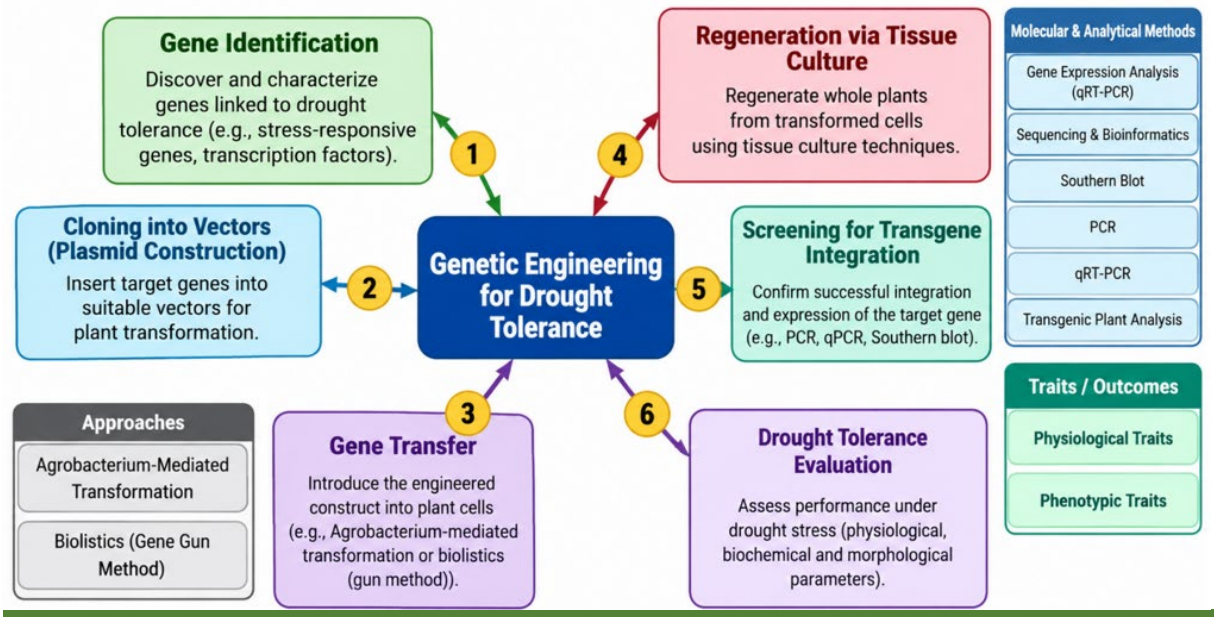


Figure 2 Schematic Representation of the Genetic Engineering Process for Developing Drought-Tolerant Plants Via Tissue Culture

Despite its potential, genetic engineering remains limited by genotype-dependent regeneration, transformation efficiency, time and cost, biosafety requirements, and regulatory approval. Representative genes engineered to improve drought tolerance in plants are summarized in Table 2. Stress tolerance measured in controlled experiments may not translate into consistent yield advantages across environments, particularly when drought coincides with heat or other stresses. Therefore, tissue culture-assisted genetic engineering should be viewed as one component of crop improvement, complementing conventional breeding and field validation rather than replacing them.

Table 2

Table 2 Representative Genes Engineered to Improve Drought Tolerance in Plants

Gene	Function	Plant Species	Transformation Method	Reported drought-tolerance improvement	References
AtDREB1A	Activates stress-responsive genes	Arabidopsis, rice	Agrobacterium-mediated	Improved water-deficit tolerance; increased proline and relative water content	Kasuga et al. (1999), Lata and Prasad (2011)
OsDREB1G, OsDREB2B	Drought-responsive transcription factors	Rice	Agrobacterium-mediated	Improved tolerance to water deficit	Cattivelli et al. (2008)

SNAC1	NAC-family transcriptional regulation	Rice	Agrobacterium-mediated	Improved drought tolerance and seed setting under stress	Hu et al. (2006)
OsTPS1	Trehalose biosynthesis	Rice		Increased drought tolerance and accumulation of trehalose and proline	Esmaeili et al. (2022)
CBF3/DREB1A	Activates cold- and drought-responsive genes	Rice	Agrobacterium-mediated	Enhanced drought and salinity tolerance; growth effects depend on expression	Oh et al. (2005)
AtDREB1A	Stress-responsive transcriptional regulation	Indica rice	Agrobacterium-mediated	Improved drought tolerance at vegetative and reproductive stages; higher yield in tested lines	Ravikumar et al. (2014)

IN VITRO SCREENING AND SELECTION OF DROUGHT-TOLERANT PLANTS

In vitro screening is one of the plant tissue culture methods used to select plant genotypes or regenerated lines that can still grow under controlled water-deficit-related stress and remain regenerative. It provides the ability to compare several different genotypes under the same conditions and can be extremely useful during early-stage selection. Most culture-medium experiments, however, are better termed 'osmotic-stress screening' than 'direct simulation of field drought' because they do not simulate the full effects of drying soil and/or changing environmental conditions, plus interactions with the root system Rai et al. (2011), Kylyshbayeva et al. (2024).

The process is usually done by lowering the water potential of the culture medium by adding PEG, mannitol, or sorbitol. PEG 6000 is a highly popular agent due to its ability to cause osmotic stress without being a conventional nutrient, but the effects of PEG depend on molecular weight, concentration, culture conditions, and exposure time. Callus growth and plant regeneration may be reduced, and oxygen diffusion inhibited, at high PEG concentrations. Mannitol and sorbitol also have to be taken with care, as they may be taken up and metabolized differently between different species. Hence, rather than general stress thresholds, stress should be optimized for each crop and genotype Rai et al. (2011), Claeys et al. (2014), Kylyshbayeva et al. (2024).

Crop-specific studies show the value of this approach. In rice (Oryza sativa L.), Verma et al. (2013) selected somaclonal lines of cultivar PR113 using PEG 6000 at 30, 50, and 70 g L^{-1}. Under limited irrigation, the authors found improved performance of the 30 g L^{-1}-selected lines compared to the 50 g L^{-1}-selected lines for various vegetative and yield-related characteristics. This is just one example of why regenerated plants should be tested following the initial culture stage—more selection pressure in vitro does not necessarily lead to a better-performing plant.

Rahayu and Sudarsono (2015) also isolated peanut (Arachis hypogaea L.) somatic embryos after six months of culture in embryogenic callus medium after adding 15% PEG 6000. The plants were regenerated and advanced to the R_2 generation and tested at 50% of the watering in a greenhouse. When evaluated, 44% of the R_2 plants generated from PEG-tolerant embryos were drought tolerant under the greenhouse conditions. The proportion represents the success rate of the peanut/other crop and is not necessarily a standard rate of success for peanut or other crops.

Mohamed et al. (2000) selected one clone of Tagetes minuta using mannitol, which demonstrated higher growth under mannitol stress and improved growth at 40% of soil field capacity in a greenhouse. Also under the reported conditions, the selected clone contained more proline and soluble sugar. Kacem et al. (2017) investigated three durum wheat (Triticum durum Desf.) genotypes with 0%, 10%, and 20% PEG 6000 in the callus induction and plant regeneration. The genotype Djenah Khetifa was the most responsive under the in vitro conditions tested, with Oued Zenati and Waha in second and third place, respectively, as reported under field conditions. The examples demonstrate the feasibility of in vitro screening as a preliminary selection tool and indicate that genotype and experimental protocols influence the results.

Several complementary indicators should be used to make the selection, not just one. During the culture stage, the induction of callus, callus fresh weight, callus dry weight, formation of the embryogenic callus, shoot regeneration, and survival can be recorded. After the regeneration, beneficial parameters can be root growth, RWC, chlorophyll content, membrane stability, proline accumulation, and antioxidant enzyme activity. The selection of the indicators should be appropriate to the crop and research purpose. For instance, increased proline accumulation may reflect an osmotic stress response but should not necessarily be used as a drought tolerance indicator alone and should be considered in conjunction with growth, recovery, and yield data.

ABA is not an osmotic agent like PEG or mannitol. But it can be used in some experiments to study hormone sensitivity or drought-response signaling. A list of commonly used osmotic agents for in vitro drought-tolerance screening is provided in Table 3. Similarly, molecular markers may be used in combination with phenotype screening when they can be used to identify validated

alleles or marker–trait association. They do not necessarily confer drought tolerance, and the value for drought stress depends on the genetic background and the characteristics being examined. A workflow thus involves a highly effective in vitro screening methodology, plant regeneration, phenotypic and, if appropriate, molecular characterization, and greenhouse and field testing in relevant water-limited settings.

Table 3

Table 3 Commonly Used Osmotic Agents in in Vitro Drought Tolerance Screening.

Osmotic Agent	Concentration reported in studies	Crops Tested	Observed Effects	References
PEG 6000	10% and 20% (w/v)	Durum wheat	Reduced callus growth; differences among genotypes helped identify candidates for drought tolerance.	Kacem et al. (2017)
PEG 6000	15% (w/v)	Peanut (Arachis hypogaea)	Selected stress-tolerant somatic embryos; 44% of evaluated R_2 plants showed drought tolerance under greenhouse conditions.	Rahayu and Sudarsono (2015)
PEG	0, 30, 50 and 70 g L^{-1}	Rice	Increasing PEG reduced callus growth and proliferation; selected lines were subsequently evaluated under limited irrigation.	Verma et al. (2013)
Mannitol	60–80 mM during selection; tolerance tested up to 90 mM	Wild marigold (Tagetes minuta)	A selected clone maintained growth at 90 mM mannitol and performed better under greenhouse water deficit than comparison plants.	Mohamed et al. (2000)
Mannitol	100 and 200 mM	Chrysanthemum (Chrysanthemum hybridum)	Reduced growth; proline and soluble protein responses varied with concentration and stress exposure.	Samarina et al. (2017)
Mannitol	100–400 mM	Chrysanthemum (Chrysanthemum morifolium)	Reduced shoot growth and proliferation; responses differed among cultivars.	Shibli et al. (1992)
Sorbitol	100–400 mM	Chrysanthemum (Chrysanthemum morifolium)	Reduced shoot length and proliferation; responses differed among cultivars.	Shibli et al. (1992)

Overall, in vitro screening can help narrow large collections of genotypes or regenerated lines to a smaller set of candidates for further testing. Its strongest contribution is early, controlled selection but not the replacement of field trials. Validation under soil-based water deficit is essential before a line can be considered agronomically drought-tolerant.

ADVANCES IN TISSUE CULTURE TECHNIQUES FOR DROUGHT TOLERANCE

The use of plant tissue culture to assist in the improvement of drought tolerance involves controlled screening of plant tissues, the use of somaclonal variation, the regeneration of selected plants, and the multiplication of promising lines. The efficiency is attributed to the selection protocol and the regenerated plants' evaluation. In vitro screening can be very valuable for screening out many candidate lines, but tolerance to an artificial osmotic agent does not necessarily correlate with performance under soil drought. Hence, tissue-culture selection needs to be followed by greenhouse and field testing Rai et al. (2011), Claeys et al. (2014).

1) Optimization of Tissue Culture Media for Drought Tolerance

Optimization of culture media is crucial for consistent selection and regeneration. Callus growth, embryogenic potential, shoot regeneration, and survival may be affected by the basal nutrient composition, carbon source, plant growth regulators, gelling agent, and osmotic potential. PEG, especially high molecular weight PEG, is frequently used to decrease the water potential of culture media and to impose osmotic stress. The PEG treatment, however, is an approximation of water deficit, not the actual field drought, and the concentration and exposure duration need to be optimized for every crop and genotype Claeys et al. (2014), Kylyshbayeva et al. (2024).

The selection pressure must be high enough to be able to differentiate sensitive from relatively tolerant tissue but not to kill out all the regenerable tissue. In wheat cv., for instance, immature embryo-derived calli (IEC) of GA-2002 were selected under osmotic

stress induced with PEG 6000 (−0.9 MPa) for 4 weeks. In these special conditions, the survival of the callus was 26.62%, and regeneration was 21.50%. The regenerated R_1 somaclones exhibited higher leaf water potential than the parent under simulated water stress, and further studies of the selected lines are warranted Mahmood et al. (2012). These are protocol-specific values, and they shouldn't be used to set selection points anywhere.

The use of plant growth regulators must also be carefully optimized. The induction of callus, somatic embryogenesis, and shoot and root regeneration are primarily under the control of auxins and cytokinins, which by themselves do not induce drought tolerance. There is also a plant hormone called ABA, which is related to stress and might be important in stress responses, but culture media supplemented with abscisic acid do not mimic the entire drought stress in soil. Optimizing media for selection pressure and healthy plant regeneration should be followed by tests of physiological traits of media, including relative water content, leaf water potential, membrane stability, and biomass in well-controlled water deficit.

2) Use of Bioreactors in Mass Propagation of Selected Plants

Bioreactors (such as temporary immersion systems (TIS)) are useful for optimizing plantlet and somatic embryo multiplication by regulating the provision of liquid medium, immersion frequency, and culture conditions. Appropriately designed temporary immersion systems can be used to achieve shoot proliferation, somatic embryogenesis, nutrient uptake, and acclimatization, albeit with some variation depending on species and protocol Etienne and Berthouly (2002).

It is worth noting that bioreactors are more suited as a scale-up tool after promising lines have been selected and validated for drought tolerance breeding and not as a direct tool to create drought tolerance. The duration of immersion, aeration, volume of the medium, and culture density should be optimized to reduce issues like hyperhydricity and inadequate culture acclimatization. Selected plants with regeneration should exhibit the same characteristics and should be tested for water stress before being widely planted. There is no evidence that a bioreactor alone results in drought-tolerant plantlets.

3) Cryopreservation for Long-Term Conservation of Selected Germplasm

Cryopreserved plant genetic resources are held at ultra-low temperatures, typically in liquid nitrogen at around -196°C, which stops the cellular metabolism and division of cells, complements the work done with tissue culture crop improvement, and facilitates the preservation of the accessions over long periods of time. This is especially useful for vegetatively propagated crops, elite genotypes, and material that has limited ability to be maintained as seed Panis et al. (2001), Nagel et al. (2024).

Vitrification and droplet vitrification involve using the dehydration and cryoprotectants to minimize the formation of damaging ice crystals during cooling. Protocols should be modified for the species, the type of explant, the water content, and the recovery needs, and the survival of thawed tissues and successful regeneration should be evaluated. Drought tolerance is not inherent in cryopreservation, nor is it any better after the process. Rather, it maintains some drought-tolerant lines and other valuable germplasm to be evaluated, bred, and distributed in the future.

Figure 3

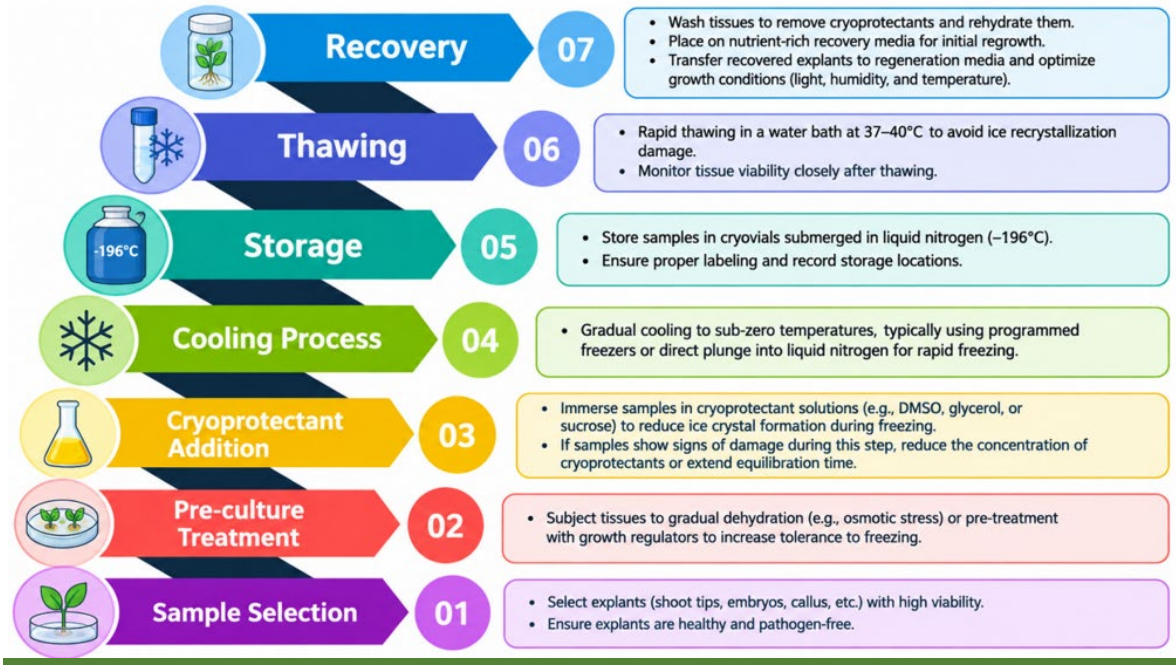


Figure 3 Schematic Representation of the Major Steps Involved in Plant Tissue Cryopreservation for Long-Term Conservation of Plant Genetic Resources

Table 4

Table 4 Cryopreservation Techniques for Long-Term Conservation of Plant Germplasm

Cryopreservation method	Crops studied	Reported survival or regrowth	Key benefits and limitations	References
Vitrification	African violet	Up to 100% survival; 90% regrowth in a specific protocol	Reduces ice-crystal damage	Moges et al. (2004)
Encapsulation–dehydration	African violet, potato, sugarcane	African violet: up to 100% survival; 75% regrowth	Beads protect explants during dehydration and freezing	Moges et al. (2004), Engelmann (2011)
Droplet-vitrification	Potato	About 46–94% regrowth, depending on accession and protocol	Rapid cooling and warming	Niino and Arizaga (2015), Roque-Borda et al. (2021)
Encapsulation–vitrification	African violet, potato	African violet: 85% survival; 80% regrowth in one protocol	Combines encapsulation and vitrification	Moges et al. (2004), Niino and Arizaga (2015)
Droplet freezing	Potato	Recovery varies by accession; around 46% regeneration reported in a reviewed collection	Suitable for vegetatively propagated germplasm	Niino and Arizaga (2015)
Encapsulation–dehydration	Pear, grape, cassava, coffee, carrot	Protocol-dependent; no common rate	Conserves diverse plant genetic resources	Engelmann (2011)

CONCLUSION

Plant tissue culture is a useful tool for identifying, selecting, regenerating, and conserving plant tissue that might be drought tolerant. The combination of in vitro selection using osmotic agents, e.g., PEG, and somaclonal variation has given better results in different crops such as Tagetes minuta, wheat, rice, sugarcane, banana, and peanut. Some studies, however, show that under greenhouse and limited-irrigation conditions, regenerated plants exhibit better responses than controls, while in cultured tissues, the effect is limited to tolerance of osmotic stress. Genetic engineering can also be applied to enhance drought tolerance by introducing or altering stress-responsive genes; bioreactors are used to propagate and amplify on a large scale, and cryopreservation can be used to conserve germplasm for longer periods of time. These methods thus complement each other and do not necessarily ensure drought-resistant varieties. Future research should focus on stable regeneration protocols, genetic and phenotypic stability for selected lines, and multi-environment greenhouse and field evaluation of selected lines. Combining tissue culture with molecular characterization and traditional breeding can boost crop improvement programs and help build more resilient farming systems in a changing climate and under water constraints.

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