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PLANT GENETIC RESOURCES ASSAY FOR ABIOTIC STRESS-TOLERANT TRAITS USING TISSUE CULTURE TECHNIQUES: A REVIEW

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SUMMARY

Considering that identifying the right progenitors among available genetic resources is one of the key factors that determine the efficiency of crop improvement programs, screening germplasm is important. A broad range of different methodological approaches for selection is available. Tissue culture-based *in vitro* selection is one of the most high-throughput, efficient, and cost-effective tools for screening stress-tolerant plants. In the absence of external environmental threats, the testing factor is altered, whereas all other conditions are kept similar. The reduced space requirement for screening a large number of germplasm is also advantageous. This technique has been efficiently used to screen genetic resources in different aspects and for tolerance to abiotic factors, such as drought, temperature, and salt stresses and low nutrient levels. This review summarizes the information on the tested stresses and their levels under *in vitro* conditions, the threshold levels for screening, the types of explants, the compositions of the media, the parameters recorded, the performances of different crops under the stressed conditions, and the selected genotypes with resistant/tolerant traits.

Keywords: Abiotic stress, drought tolerance, *in vitro* screening, nitrogen use efficiency, salt tolerance, temperature tolerance

Key findings: The review summarized the literature on application of the plant tissue culture technique for screening the genetic resources for the abiotic stress-tolerant traits. The technique has successfully and efficiently used for assessing a wide range of genetic resources.

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INTRODUCTION

Abiotic stresses are defined as the negative effects of nonliving factors on living organisms in a specific environment that adversely affect growth and productivity and trigger a series of morphological, physiological, biochemical,

and molecular changes in plants (Sanghera *et al*., 2011). Crops have evolved complex systems to interact with and respond to multiple forms of abiotic stresses, such as soil salinity, drought, extreme temperatures, radiation, and heavy metal toxicity, to complete their life cycles. However, the potential for tolerating or resisting stresses depends on plant genetic makeup (Aazami *et al*., 2010). Therefore, genetic improvement is of utmost importance to enhance the performance of crops. It directly affects the grower by providing the highest potential quality and yield and reducing the input requirement while reducing threats to the existence of fauna and flora, the health of humankind, and the sustainability of the environment.

The continued supply of genetic variability and beneficial traits is a key requirement for successful genetic improvement programs. The modification of the genetic structure of existing crop varieties is one approach to improving crop performance while increasing variations in crop species (Rao *et al*., 2018). The cultivation of superior plants with desirable traits that were identified through selection is another approach that has been practiced since domestication. The continued selection for tolerance to biotic and abiotic stresses has improved crop productivity. Fully and effectively exploiting the genetic variation associated with traits is necessary to enhance crop performance (Lenaerts *et al*., 2019).

Advanced phenotyping methods have been developed in the recent past. However, along with those advancements, equal attention needs to be given to the screening conditions and reliable, rapid, and high-throughput screening techniques for the selection of individuals with significant phenotypic traits. The method for screening genetic resources depends on different factors, such as the availability of the resources and the capability to simulate the stress conditions of plants. A broad range of methodological approaches for selection are available. Screening under field conditions is the simplest and most common method. However, under field conditions, a given variety will perform differently due to its interaction with the environment based on geography and season, leading to high variability (Zhang *et al*., 2013). Furthermore, in the absence of precise technologies, selecting desirable characteristics under field conditions requires many generations and several years and is thus time- and labor-consuming and cost-intensive.

Tissue culture-based *in vitro* selection is one of the most high-throughput, efficient, and cost-effective tools for screening stress-tolerant plants (Rai *et al*., 2011). This system enables the stringent control of the uncontrollable physical environment under field conditions (Maleki *et al*., 2019; Tefera, 2019). The time taken for the selection of desirable traits can be significantly shortened under *in vitro* selection pressure. Monitoring or eliminating plant–environment interaction is possible, and the level of stress can be managed accurately and conveniently. Given that they require a small area, *in vitro* studies are highly cost-effective. As indicated in Figure 1, this technique can be used to evaluate crop performance at early developmental stages that can be utilized for different purposes.

Several excellent reviews on *in vitro* development and selection for abiotic (Maleki *et al*., 2019) stresses based on somaclonal variation or mutation breeding exist. However, reviews on the application of plant tissue culture techniques for screening genetic resources for diverse abiotic stress-tolerant traits are limited. Thus, this review aims to provide an adequate literature review on this aspect.

Drought stress

Drought is one of the prime abiotic stresses that play a crucial role in causing extensive damage to the crops by preventing their optimal performance under field conditions (Khan *et al*., 2015). Agricultural drought refers to the magnitude of drought created by a deficit in plant-available water due to

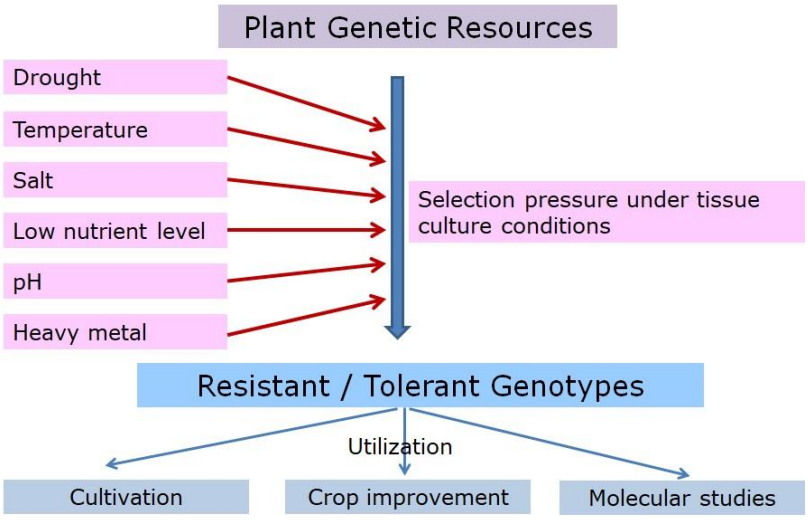

Figure 1. Screening the plant genetic resources through tissue culture techniques and their utilization.

meteorological and hydrological conditions that affect crop yields (Tigkas *et al*., 2017; Sanchez *et al*., 2018; Feng *et al*., 2019). Drought stress affects the performance of crops at different stages from emergence to maturity, including seed germination, vegetative and reproductive development, as well as harvest quality, through different morphological, biological, physiological, biochemical, and metabolic pathways that ultimately impede crop production (Sehgal *et al*., 2018; Kapoor *et al*., 2020).

Water use efficiency (WUE), the amount of carbon that is assimilated as biomass or grain produced per unit of water used by the crop (Hatfield and Dold, 2019), is one of the main factors to be considered when establishing the strategies for overcoming drought stress. Plants regulate water uptake through developmental and environmental responses, including root morphology and architecture, cuticle development, stomatal development, and guard cell movements in response to the environment (Ruggiero *et al*., 2017). In this scenario, opportunities are open to enhancing WUE through crop selection to offset the influence of a changing climate (Hatfield and Dold, 2019).

In vitro selection has been applied as a tool to screen the effect of drought on the early growth of crops or the development of callus lines (Table 1). Drought tolerance is tested on the basis of the measurement of the electro-conductivity of the culture media, wherein an artificial drought condition is created by using different chemicals as osmotic stress agents. Polyethylene glycol (PEG) is the most frequently used simulator of drought conditions (Kacem *et al*., 2017). It is a synthetic polyether with molecular weight < 100 000. Low-molecular-weight PEG (<4000) can be permeable (Verslues *et al*., 1998). By contrast, high-molecular-weight PEG is nonpenetrating (Aazami *et al*., 2010) and is thus nonphytotoxic. Therefore it has been used to induce drought stress conditions for *in vitro*-cultured explants by reducing water potential. PEG withdraws water from the cell and cell wall and thus mimics dry soil more closely than solutions of compounds with low molecular weights (Verslues *et al*., 1998). PEG with the molecular weight of 6000 (PEG 6000) is the widely used polymer for screening different crops (Joshi *et al*. 2011; Mahmood *et al*., 2012; Kacem *et al*., 2017), whereas PEG 8000 (Bundig *et al*., 2017) is seldom used for the purpose.

Table 1. Application of plant tissue culture technique for screening genetic resources for drought tolerance.

Crop	Drought stimulant	Tested levels	Explant	Tolerant/resistant genotypes	Reference
Durum wheat (*Triticum durum*)	PEG	0%, 10%, 20%	Callus	Djenah , Khetifa	Kacem *et al*. 2017
Bread wheat (*Triticum aestivium*)	PEG	0%, 5%, 10%, 15%, 20%	Callus	Genotypes 2, 5, 9, 13	Farshadfar *et al*. 2012
	PEG	0, −0.3, −0.6, −0.9, −1.2 MPa	Callus	GA-2002	Mahmood *et al*. 2012
Potato (*Solanum tuberosum*)	Sorbitol	0%, 2%, 4%, 6%, 8%, 10%	Nodal cuttings	SY-C.28, 52, 56, 53, 31, 54	Albiski *et al*. 2012
	Agar	6, 8, 10, 12, 14 g/l	Sprouted microtubers	IWA-1	Gopal *et al*. 2008
	PEG	4.8%, 9.6%	Nodal cuttings	Clon 37 FB, Coquiao, R91193-1	Barra *et al*. 2013
	Sorbitol	0.1, 0.2, 0.3, 0.4 M	Nodal cuttings	PKHT4, PKHT	Laisina *et al*. 2021
	PEG	118, 197 g/l	Nodal cuttings	Sreedhara, Subala	Sahoo *et al*. 2020
Potato (*Solanum tuberosum, S. chacoense*, *S. tarijense*)	Sorbitol, PEG	Sorbitol (0.1, 0.2, 0.3, 0.4 M) PEG-0%, 4.8%, 9.6%	Shoot tips	Maxi	Bundig *et al*. 2017
Rice (*Oryza sativa*)	PEG	0%, 0.5%, 1.0%, 1.5%, 2.0%	Callus	PR 116	Wani *et al*. 2010
	PEG	0%, 1%, 2%, 3%, 4%	Seeds	Binadhan-10	Akte *et al*. 2016
	PEG	10, 20, 30, 40, 50, 60, 70 g/l	Mature embryos	Narendra 359, Pusa Basmati-1	Joshi *et al*. 2011
Tomato (*Solanum lycopersicom*)	PEG	0, 200, 270, 295 g/l	Seedling, Callus	PS10	Aazami *et al*. 2010
Sorghum (*Sorghum bicolor*)	PEG	0%, 0.5%, 1.0%, 1.5%, 2.0%	Callus	76T1#23, Teshale, Meko, Gambella-1107, Melkam	Yohannes *et al*. 2013
	PEG	0, 10, 20, 40 g/l	Cladodes	Suluhna, Gerao, Limo, Lemats Beles	Mengesha *et al*. 2016
Sugar beet (*Beta vulgaris*)	PEG	0%, 3%, or 5%	Seeds	Genotype 10,1, 4	Putnik-Delic *et al*. 2013
Almond (*Prunus dulcis*)	PEG	0%, 3.5%, 7%	Nodal cuttings	Supernova, GF677	Karimi *et al*. 2012
Iranian almond (*Prunus* L. spp.)	Sorbitol, PEG	Sorbitol:0.1, 0.2, 0.3, 0.4 M PEG: 0.003, 0.006, 0.009, 0.012 M	Immature seeds	*P. arabica, P. glauca, P. scoparia*	Sorkheh *et al*. 2011
Banana (*Musa accuminata*)	Sorbitol	0.09 M sucrose, 0.09 M sucrose + 0.21 M sorbitol	Shoots	Cachaco	Vanhove *et al*. 2012
Chickpea (*Cicer arietinum*)	PEG	0, 20, 35, 50, 60 g/l	Seeds	Binachola-2, Binachola-7	Salma *et al*. 2016
Sugarcane (*Saccharum officinarum*)	PEG	0.0%, 5.0%, 7.5%, 10.0%	Leaf	Isd 35, Isd 38	Begum *et al*. 2011
	PEG	0%, 3%, 6%, 9%	apical meristems	Mex 69-290	Hernández-Pérez *et al*. 2021
Moth Bean *(Vigna aconitifolia)*	PEG	5%, 10%, 15%, 20 %	Seeds	RMO-435, RMM-12-Single, and Poly, CZM-105	Priyanka *et al*. 2011
Forest Red Gum *(Eucalyptus tereticornis)*	Mannitol	0, 250, 500, 750, 1000 mM	Shoot	KE8	Singh *et al*. 2020
Cactus (*Opuntia ficus-indicia*)	PEG	0, 10, 20, 40 g/l	Cladodes	Suluhna, Gerao, Limo, Lemats Beles	Mengesha *et al*. 2016

PEG 6000 has been used to select drought-tolerant genotypes in cereals, such as wheat (Mahmood *et al*., 2012; Farshadfar *et al*., 2012; Kacem *et al*., 2017), rice (Wani *et al*., 2010; Joshi *et al*., 2011; Akte *et al*., 2016), and sorghum (Yohannes *et al*., 2013). It has also been used in tuberous crops, such as potato (Barra *et al*., 2013; Sahoo *et al*., 2020). PEG 6000 has been used to screen vegetable crops, including tomato (Aazami *et al*., 2010; George *et al*., 2013) and sugar beet (Putnik-Delic *et al*., 2013). Legumes and pulses, such as beans (Priyanka *et al*., 2011) and chickpea (Salma *et al*., 2016), have been screened by using PEG 6000. PEG 6000 has also been used for tree crops, for example, almond (Sorkheh *et al*., 2011; Karimi *et al*., 2012), and other crops, including sugarcane (Begum *et al*., 2011; Hernández-Pérez *et al*., 2021) and cactus (Mengesha *et al*., 2016) genotypes, to assess the potential for drought tolerance.

In different crops, PEG-mediated drought stress is created by supplementing different concentrations of PEG. Low concentrations of up to 5% has been used for sugar beet (Putnik-Delic *et al*., 2013) and up to 10% in soybean, almond, potato, and sugarcane (Begum *et al*., 2011; Albiski *et al*., 2012; Karimi *et al*., 2012; Hernández-Pérez *et al*., 2021). PEG 6000 has been tested at concentrations of 60–70 g/l in chickpea and rice (Salma *et al*., 2016) and 295 g/l in tomato (Aazami *et al*., 2010). The high-molecular-weight PEG 8000 has been used in potato at concentrations of 0%–9.6% (Bundig *et al*., 2017). A varying threshold for a significant decline in parameters was reported for different crops. In the media supplemented with PEG 6000, the threshold ranged 3-40% (w/v) (Table 2). In PEG-8000-supplemented media, the highest levels tested, i.e., 4% and 9.6% (w/v), were identified as threshold levels (Bundig *et al*., 2017).

The other potential simulators of drought conditions are sorbitol, mannitol, and agar. Sorbitol-mediated water stress created by using 0.2–0.4 M and 10% solutions has commonly been used to screen potato genotypes (Albiski *et al*., 2012; Bundig *et al*., 2017; Laisina *et al*., 2021). Iranian almond and banana have also been screened by using sorbitol at concentrations of up to 0.4 (Sorkheh *et al*., 2011) and 0.21 M, respectively (Vanhove *et al*., 2012). Water stress induced by mannitol concentrations of up to 1000 mM was used to select drought-tolerant eucalyptus (Singh *et al*., 2020). The increment in agar concentration in the culture medium decreased the water potential of the medium that was used to screen for drought tolerance in potato (Gopal *et al*., 2008). The highest concentration among the tested concentrations (6–14 g/l) was used to differentiate performance.

Table 2. The threshold levels of PEG 6000 that gave a significant decline in the parameters of different crops.

Crop	Threshold level
Durum wheat	20%
Bread wheat	20-40%; 0.75 MPa- 0.9 MPa
Potato	4.8-9.6%; 0.003 M
Rice	1.5-15%
Tomato	4-16%; 0.8 MPa
Sorghum	2.0-20%
Sugar beet	3%
Soybean	6%
Chickpea	6%
Almond	7%
Date palm	5%
Sugarcane	10%
Beans	20%.

Different types of tissues have been used for the *in vitro* screening of germplasm for drought tolerance. *In vitro*-derived seedlings are the most common type. Calli derived from different explants were used to screen sorghum (Yohannes *et al*., 2013), cactus (Mengesha *et al*., 2016), rice (Wani *et al*., 2010), and tomato (Aazami *et al*., 2010). Plants derived from calli were also used as plant material for screening purposes in sorghum (Yohannes *et al*., 2013). For potato and almond, nodal cuttings were used as explants (Albiski *et al*., 2012; Karimi *et al*., 2012; Sahoo *et al*. 2020; Laisina *et al*., 2021).

In majority of the studies, the treatments were applied by supplementing the simulant into the MS medium (Murashige and Skoog, 1962). Specific media developed for particular crops were also used for screening purposes. In most studies, the simulators were directly dissolved into the culture medium (Aazami *et al*., 2010; Wani *et al*., 2010; Joshi *et al*., 2011). The explants were also pretreated in solutions containing the chemical. Certain crops were initially treated with the drought simulator on Whatman filter paper and single- or double-layered paper towels moistened with PEG and then cultured in nutrient medium (George *et al*., 2013).

Drought tolerance has been assessed by using different parameters on the basis of the type of tissue used for the study. For example, in rice, seed germination is the initial response of cultured seeds, and germination rate is the first parameter (Akte *et al*., 2016). Then, growth parameters, such as plantlet height, leaf and root number, fresh and dry weights, and shoot:root ratios, were recorded (Albiski *et al*., 2012; Barra *et al*., 2013; Akte *et al*., 2016). The relative water content and turgid weight are also important parameters for screening drought tolerance in rice (Akte *et al*., 2016). The index of accumulated free proline is another important parameter that has been used to determine tolerance to drought stress (Putnik-Delic *et al*., 2013; Akte *et al*., 2016; Bundig *et al*., 2017). Proline is the most widely distributed metabolite that accumulates under various stress conditions (Putnik-Delic *et al*., 2013). The accumulation of glycine betaine was also used in determining stress-tolerant sugarcane genotypes (Hernández-Pérez *et al*., 2021). The analysis of relative decline and stress-susceptibility index (SSI) was used to determine stress tolerance in potato (Laisina *et al*., 2021). Analysis has also been done at the cellular level. In durum wheat, the cell membrane stability of the callus has been used to determine the degree of tolerance to drought (Kacem *et al*., 2017). Callus multiplication rate has been recorded to determine drought tolerance in wheat (Mahmood *et al*., 2012). The measurement of chlorophyll fluorescence is also a suitable tool for studying changes in the photosynthetic capacity of plants exposed to water stress.

Irrespective of the type of the stimulant and the crop, drought-stress media negatively affect the overall vegetative growth and development of susceptible plants, including their aerial and root parts. A gradual reduction in seedling parameters, such as seed germination percentage, germination rate, survival rate, root and shoot lengths, root and shoot dry weights, and water content is prominent in susceptible plants. Increases in the root/shoot ratio, number of primary and lateral shoots, and dry matter content and the early and rapid elongation of roots are important indications of drought resistance/tolerance. PEG-mediated water stress adversely affects callus growth and *in vitro* regeneration capacity as well. In susceptible genotypes, simulated water stress increases the time required for callus initiation and reduces the number of calli, frequency of embryogenic structures, and number of plants regenerated. Furthermore, in susceptible genotypes, callus fresh weight, callus health, callus growth rate, callus survival, and regeneration percentages have been reported to decrease during the stress period.

In the seedlings and callus explants of varieties that show significant growth under a high level of drought stress (e.g., in tomato), proline content increases with the increase in the level of stress (Aazami *et al*. 2010), indicating that the high accumulation of proline can be used to identify genotypes that tolerate stress well. Some studies have shown that the activities of antioxidant enzymes are significantly elevated under stress conditions. Sahoo *et al*. (2020) reported that induced osmotic stress resulted in the overproduction of reactive oxygen species (ROS), which is considered as a hallmark of plant stress response that protects the plants from the deleterious effects of PEG-mediated osmotic stress. In some studies, reduced chlorophyll content was also identified in susceptible genotypes.

The drought-tolerant genotypes of different crops have been identified through *in vitro* screening. This technique has widely been used to screen cereal genotypes. 'Djenah Khetifa' was identified as the most resistant durum wheat genotype (Kacem *et al*., 2017), and GA-2002 (Mahmood *et al*., 2012) genotypes have been selected as the best performers in bread wheat. The rice genotypes 'PR 116' (Wani *et al*., 2010), 'Binadhan-10' (Akte *et al*., 2016), 'Narendra 359', and 'Pusa Basmati-1' (Joshi *et al*., 2011) have shown high tolerance for induced water stress conditions. Through the analysis of callus performance, the '76T1#23' and 'Teshale' (Yohannes *et al*., 2013) genotypes were identified as the superior sorghum genotypes for water-stressed conditions.

Among tomato accessions, 'EC-620428' × 'Arka Saurabh', 'EC-620360' × 'Arka Saurabh', 'PS10' (Aazami *et al*., 2010), 'Walter', 'Punjab Chuhara', 'Kurihara' (George *et al*., 2013), 'PKHT4', and 'PKHT6' (Laisina *et al*., 2021) showed better performance than other accessions under water stress conditions. Among legumes, 'Binachola-2' and 'Binachola-7' chickpea genotypes (Salma *et al*., 2016) and 'RMM-12-Single', 'RMM-12-Poly', and 'CZM-105' *V. aconitifolia* genotypes (Priyanka *et al*., 2011) were selected as drought-tolerant genotypes under water-stressed conditions simulated by using PEG-supplemented media. 'IWA-1' (Gopal *et al*., 2008), 'Maxi' (Bundig *et al*., 2017), 'Clon 37 FB', 'Coquiao', and 'R91193-1' (Barra *et al*., 2013) genotypes have been identified as drought-tolerant potato genotypes.

This technique has also been used in tree species, including eucalyptus, among which 'KE8' (Singh *et al*., 2020) was selected as a drought-tolerant genotype. *Prunus dulcis* (Mill.) ('Supernova' and 'GF677' genotypes) (Karimi *et al*., 2012)*, Prunus arabica, Prunus glauca*, and *Prunus scoparia* species (Sorkheh *et al*., 2011) of almond demonstrated superior performed under simulator-based drought conditions generated *in vitro*. *In vitro* screening conducted by using sorbitol- and PEG-supplemented media revealed that the banana genotype 'Cachaco' (Vanhove *et al*., 2012) and the sugarcane genotypes 'Isd 35' and 'Isd 38' (Begum *et al*., 2011) also showed high drought stress tolerance.

Temperature stress

Temperature can be adversarial when it is above or below the threshold level, imposing heat or cold stress on the plants. The effect of heat stress is more commonly reported than that of cold stress. Seedlings exposed to high temperatures show growth retardation, necrotic symptoms, decreased photosynthetic activity, reduced productivity, and finally death (Mathur *et al*., 2014). Plants have developed mechanisms to avoid or tolerate high-temperature conditions (Mathur *et al*., 2014). Avoidance occurs through leaf orientation changes, transpirational cooling, stomatal closure, leaf rolling, and early maturation, whereas tolerance occurs through alterations in lipid membrane composition, stress-related protein synthesis by transcriptional control, osmoprotectant accumulation, and detoxification pathways (Mathur *et al*., 2014). Variability in resistance to high

temperatures has the potential for the genetic improvement of a certain trait in crops.

The *in vitro* screening technique has many advantages over *in vivo* controlled environments, such as phytotrons, growth rooms, greenhouses, and water baths, that can be used to provide different temperature levels. Under *in vitro* conditions, the temperature can be precisely maintained by using incubators, growth rooms, and growth chambers. However, due to the high cost of equipment, this technique has not been widely applied. Moreover, *in vitro* screening is mostly applicable only at the early developmental stages of the crops, whereas the reproductive stage is the most sensitive stage for temperature stress (Jagadish *et al*., 2010).

In vitro assays have been performed to screen tolerant genotypes of wheat (Benderradji *et al*., 2012) and potato (Khan *et al*., 2015; Guedes *et al*., 2019) (Table 3). Benderradji *et al*. (2012) screened two wheat genotypes for heat tolerance under four different thermal stress intensities (25 °C, 30 °C, 35 °C, and 40 °C) and assessed callus proliferation, embryonic, and regeneration efficiencies. The genotype 'Mahon–Demias' showed high performance under stress conditions. Khan *et al*. (2015) studied the performance of potato by using three temperature levels, namely, 18 °C, 25 °C, and 32 °C, and found that *in vitro* tuberization decreased with the increase in temperature. The clonal population of LTVR was identified as a heat-tolerant genotype. Guedes *et al*. (2012) studied the heat tolerance of diploid wild *Solanum* species at two temperature levels (19 °C and 25 °C) by evaluating their capability to form microtubers in tissue culture and found that *Solanum kurtzianum* and *Solanum sogarandinum* were more heat resistant than the other tested species. MS medium was used for all the above assays.

Cold stress, i.e., low temperature (>15 °C), chilling (0 °C–15 °C), or freezing (< 0 °C), is a major environmental factor that limits the agricultural productivity of crops grown in hilly or temperate areas by mainly exerting effects on the seedling and reproductive stages, resulting in slow establishment and low seed set (Sanghera *et al*., 2008; Yadav, 2010; Sanghera *et al*., 2011; Ding *et al*., 2019). Under cold stress, crop species of tropical or subtropical origin exhibit various phenotypic symptoms, such as poor germination, chlorosis, necrosis, growth retardation, reduced leaf expansion, and wilting, whereas tolerant crops show increased chlorophyll accumulation; reduced photosynthesis sensitivity; and

Table 3. Application of plant tissue culture technique for screening the genetic resources tolerant of different temperature conditions.

Crop	Tempe-rature stress	Tested temperature levels	Explant	Tolerant/resistant genotypes	Reference
Wheat (*Triticum aestivum*)	Cold	−15 °C	Callus	Partizanka, Centurk, GK Cipo	Kondic *et al*. 2006
	Heat	25 °C, 30 °C, 35 °C, 40 °C	Callus	Mahon-Demias	Benderradji *et al*. 2012
Potato (*Solanum tuberosum*)	Heat	18 °C, 25 °C, 32 °C	Nodal cuttings	LTVR	Khan *et al*. 2015
Wild potato	Heat	19 °C, 25 °C	Nodal cuttings	*S. kurtzianum, S. sogarandinum*	Guedes *et al*. 2019
Strawberry (Fragaria × ananassa Duch.)	Cold	−8 °C to −13 °C	Embryo	Kent × Nida	Rugienius and Stanys 2001

improved germination, pollen fertility, and seed set (Yadav, 2010; Sanghera *et al*., 2011; Ding *et al*., 2019).

A few reports on *in vitro* screening for low temperature tolerance could be found. Sebastiani *et al*. (1996) demonstrated that *in vitro* screening for cold-tolerant cultivars was comparable with *in vivo* selection and suggested that the *in vitro* method is more precise than the *in vivo* method and ensures better environmental control and aseptic conditions than *in vivo* screening. Rugienius and Stanys (2001) screened strawberry plants for cold tolerance at six different temperature levels ranging from −8 °C to −13 °C in MS medium culture. Among the genotypes screened, 'Kent' × 'Nida' showed the best cold resistance under *in vitro* conditions. Kondic *et al*. (2006) screened 12 wheat genotypes for low-temperature tolerance at −15 °C. Isolated zygomatic embryos were inoculated into modified MS medium containing 1.5 mg/l 2,4-D, 0.5 mg/l NAA, and 0.5 mg/l thiamine HCl. The 'Partizanka', 'Centurk' and 'GK Cipo' genotypes were identified as the most tolerant cultivars given that low-temperature treatments did not effect their callus growth. A standard *in situ* test in a cold chamber was used, and the *in vitro* test was concluded to be can be successfully used to screen genotypes for low-temperature tolerance. Sharma and Chaudhary (2007) screened 78 doubled haploid (DH) lines derived from 21 elite and diverse winter × spring wheat F_1 hybrids for cold tolerance along with their parental genotypes *in vitro* by using 2,3,5-triphenyl tetrazolium chloride. One DH, DH 69, was characterized as cold-tolerant, seven DH and five winter wheat parents were considered as moderately tolerant, whereas the rest were susceptible.

Salt stress

Salinity is another major stress condition that affects the growth and performance of crops, including germination, plant vigor, and crop yield. Salt stress causes ion toxicity due to the accumulation of Na^+ ions, water deficit due to osmotic stress created by an excess of Na^+ and Cl^- ions in the soil, oxidative stress due to the increased accumulation of ROS and nutrient imbalances caused by alterations in respiration; photosynthesis; and nucleic acid, protein, and lipid metabolism that result in the inhibition of crop growth and subsequently death (Wang *et al*., 2011; Rasool *et al*., 2013; Mahmood-ur-Rahman *et al*., 2019; Ma *et al*., 2020). Approximately 7% of the total land on earth, 20% of the total arable land, and 33% of irrigated land are affected by salt stress (Rasool *et al*., 2013; Machado and Serralheiro, 2017). These areas can be utilized for crop production if adaptive mechanisms, such as those in haplotypes, are genetically inherent in crops. Salinity tolerance is associated with ion transport by maintaining a balanced cytosolic Na^+/K^+ ratio (Assaha *et al*., 2017; Ketehouli *et al*., 2019). The accumulation of compatible solutes with low molecular weights, such as proline, glycine betaine, sugars, proteins, and polyols, was observed in salt-tolerant crops (Giri, 2011; Hayat *et al*., 2012; Rasool *et al*., 2013).

In vitro screening for salt tolerance has been conducted on many important crops, including potato (Zaman *et al*., 2015; Murshed *et al*., 2018; Rahman *et al*., 2018; Ahmed *et al*., 2020; Rashid *et al*., 2020), rice (Revathi and Pillai, 2015; Kumari, 2017), tomato (Rashed *et al*., 2016; Zaki and Yokoi, 2016), date palm, (Ibraheem *et al*., 2011; Al-Khateeb *et al*., 2020), and maize (Balkrishna and Shankarrao, 2013) (Table 4). Tolerance to salt stress was screened by evaluating the performance of cultures originating from different explants. Single node cuttings containing axillary buds (Campanelli *et al*., 2013; Murshed *et al*., 2018; Rahman *et al*., 2018; Ahmed *et al*., 2020; Raoufi *et al*., 2021) and calli (Patade *et al*., 2008; Garg, 2010; Revathi and Pillai, 2015; Kumari, 2017; Al-Khateeb *et al*., 2020) are the most commonly used explant types. Apical buds (Zaman *et al*., 2015; Zaki and Yokoi, 2016), seedlings (Kumari, 2017), and mature embryos (Balkrishna

Table 4. Application of plant tissue culture technique for screening genetic resources for salt tolerance.

Crop	Salt levels	Explant	Tolerant/resistant genotypes	Reference
Potato (*Solanum tuberosum*)	0.0, 10, 20, 40, 60, 80, 100 mM	Shoot tips	Kroda, Sh-5	Zaman *et al*. 2015
	0, 25, 50, 75, 100, 125, 150, 200 mM	Single node cuttings	Taurus, Sultana	Murshed *et al*. 2018
	0, 2, 4, 6, 9 dS/m	Single node cuttings	CIP 102, CIP 112, CIP 139	Rahman *et al*. 2018
	0, 100, 150, 200, 250 mM	Sprouts	Arun, Ausha	Rashid *et al*. 2020
	0, 50, 100, 150 mM	Single node cuttings	Innovator, Hermes, Kennebec, Slaney	Ahmed *et al*. 2020
Tomato (*Solanum lycopersicm, S. pimpinellifolium*)	100, 200, 300 mM	Shoot apexes	*S. peruvianum* line 0043-1, *S. lycopersicum* 'Rutgers'	Zaki and Yokoi 2016
Tomato (*Solanum lycopersicu*)	0, 50, 100, 200, 250 mM	Germinated seeds	BARI-2, Line BD-7292	Rashed *et al*. 2016
Date palm (*Phoenix dactylifera*)	0, 50, 150, 250, 350 mM	Embryos	Zahdi, Majhool	Ibraheem *et al*. 2011
	0, 100, 200, 300, 400 mM	Callus	Khalas	Al-Khateeb *et al*. 2020
Maize (*Zea mays*)	0.5%, 1%, 1.5%, 2%	Callus	EC 558706	Balkrishna and Shankarrao 2013
Cape periwinkle *(Catharanthus roseus)*	0, 15, 30, 45, 60, 75, 100 mM	Shoots	Rosea	Garg 2010
Sugarcane *(Saccharum officinarum*)	42.8, 85.6, 128.3, 171.1, 213.9, 256.7, 299.5, 342.2 mM	Callus	147 plantlets selected	Patade *et al*. 2008
Alfalfa *(Medicago sativa*)	0, 50, 100, 150, 200 mM	Seeds	*Medicago sativa* L. var. icon	Campanelli *et al*. 2013
Pistachio *(Pistacia vera*)	0, 60, 120 mM	Single node cuttings	Akbari 2	Raoufi *et al*. 2021
Cassava (*Manihot esculenta*)	50, 100, 150, 200, 250 mM	Callus	-	Alaa *et al*. 2016
Rice (*Oryza sativa*)	0.5, 1.0, 1.5, 2.0	Callus	Pokkali	Revathi and Pillai 2015
	0%–2.5%	Seeds	CSR-30, Narendra Usar Dhan-3	Kumari 2017
Citrus (*Citrus macrophyll*)	0, 80 mM	Shoot tips	Alemow	Perez-Jimenez and Perez-Tornero 2020

and Shankarrao, 2013) have also been used to screen the genotypes of different plants.

Stress is created by supplementing the culture medium with stimulants. NaCl is a commonly used stimulant. MS medium containing full-strength minerals is used frequently as the basal medium for several crops. However, a few studies on tomato used the half-strength mineral composition of MS medium (Zaki and Yokoi, 2016). An array of NaCl concentrations were tested in different crops, including 120 mM in pistachio (Raoufi *et al*., 2021) and *Medicago sativa* L. (Campanelli *et al*., 2013); approximately 250 mM in potato (Rashid *et al*. 2020) and cassava (Alaa *et al*., 2016); and 300 mM in tomato (Zaki and Yokoi, 2016), sugarcane (Patade *et al*., 2008), and maize (Balkrishna and Shankarrao, 2013).

The tolerance for saline conditions is evaluated by using different parameters. The early development of the

plants under induced stress is assessed by and roots. Shoot growth and multiplication are assessed by using shoot number, shoot length, node number, internodal distance, and stem thickness (Campanelli *et al*., 2013; Zaman *et al*., 2015; Rashid *et al*., 2020). The leaf is the main plant organ wherein photosynthesis occurs; thus, its performance is also a key factor for assessment. Leaf number and leaf area are the relevant parameters studied (Rahman *et al*., 2018). The function of the leaves in the development of tolerance is assessed on the basis of stomatal conductance. The growth and development of the roots are evaluated by using the parameters of root number, root length, and root diameter (Zaki and Yokoi, 2016). Plant water content is the main factor that is affected by salinity. Therefore, the water content of plants is assessed by measuring the fresh and dry weights of shoots and roots. For root and tubers, microtuberization and stolon growth are used as the parameters (Zaman *et al*., 2015).

The callus induction system can detect variation in the salinity tolerance levels of genotypes. A different set of parameters is applied when calli are used to assess for salinity tolerance. The percentage of callus induction is used when explants are directly cultured on the simulated medium. By contrast, the callus recovery rate is recorded when calli are used as the explants (Patade *et al*., 2008). The relative growth rate of the calli, root formation, root elongation, and regeneration efficiency are also analyzed (Zaki and Yokoi, 2016). Similar to those of plants, the fresh and dry weights of calli are a main parameter when calli are used as the explants (Zaki and Yokoi, 2016).

Salt stress causes alterations in the chemical compositions of plants and calli that consequently affect physiological processes. Therefore, the content of certain compounds is used to screen tolerant genotypes. In plants, the chlorophyll content of the leaves is used to assess photosynthetic ability (Garg, 2010; Raoufi *et al*., 2021). Proline accumulation is common in plants (Raoufi using the growth performance of shoots *et al*., 2021) or tissue (Balkrishna and Shankarrao, 2013) subjected to stress conditions and is thus used as a key parameter. Salt stress mainly affects the ion balance of plants; therefore, concentrations of Na^+ and K^+ in the tissues and their ratio (K^+/Na^+ ratio) are the key parameters used in many studies (Garg, 2010; Raoufi *et al*., 2021). Starch, soluble sugars, malondialdehyde, and enzymes, such as glutathione reductase and glutathione peroxidase, are also measured (Raoufi *et al*., 2021). In pistachio cultivars, increasing salinity levels significantly reduces stem elongation, leaf number, fresh weight, starch, chlorophyll index, and K^+ content. By contrast, the contents of soluble sugars, proline, malondialdehyde, and Na^+ and Cl^- significantly increase with increasing salinity levels in many crops, including *Pistacia vera* L. (Raoufi *et al*., 2021).

Increasing salinity levels significantly reduce the growth performance in explants, plants, and calli. However, the concentration that critically affects growth and development varies across different studies and range from 80 mM to 340 mM. Stress levels of 80–250 mM affects plant development in potato. Ahmed *et al*. (2020) reported that the microtuberization and stolon growth of varieties are completely inhibited at the concentrations of 100 and 150 mM. The highest concentration of 350 mM is lethal for most date palm cultivars (Ibraheem *et al*., 2011). A gradual increase in total phenol, soluble sugar, malondialdehyde, proline, and Na^+ and Cl^- has been reported in different crops, whereas leaf chlorophyll content shows a marked decline (Garg, 2010; Campanelli *et al*., 2013; Raoufi *et al*., 2020).

Salinity-tolerant genotypes have been identified through *in vitro* screening. The potato genotypes 'Kroda', 'Sh-5' (Zaman *et al*., 2015), 'Taurus', 'Sultana' (Murshed *et al*., 2018), 'Arun', 'Ausha' (Rashid *et al*., 2020), 'Innovator', and 'Kennebec' (Ahmed *et al*., 2020) and the lines CIP 102, CIP 112, and CIP 139

(Rahman *et al*., 2018) have been reported as salinity-tolerant genotypes in different countries. 'CSR-30', 'Narendra', 'Usar', and 'Dhan-3' have been selected as salinity-tolerant rice genotypes (Kumari, 2017). Zaki and Yokoi (2016) identified *S. peruvianum* line 0043-1 and *S. lycopersicum* 'Rutgers' as good candidates for inclusion in tomato breeding programs for salt tolerance. Rashed *et al*. (2016) identified 'BARI-2' and 'BD-7292' as salinity-tolerant tomato lines. A total of 147 plantlets of the sugarcane cultivar 'CoC-671' were selected as salt-tolerant under different salt levels (Patade *et al*., 2008). Among citrus (*Citrus macrophylla*) genotypes, 'Alemow' was selected as salt tolerant (Perez-Jimenez and Perez-Tornero, 2020). *Medicago sativa* L. var. 'Icon' (Campanelli *et al*., 2013) and *P. vera* L. genotype 'Akbari 2' (Raoufi *et al*., 2021) were reported to be salt tolerant. The maize line EC 558706 (Balkrishna and Shankarrao, 2013) and the *Catharanthus roseus* cultivar 'Rosea' (Garg, 2010) were identified by screening callus performance.

Nutrient stress

The availability of nutrients in the soil is one of the key requirements for the normal growth and development of plants and their production. Crop yield depends on the efficiency of utilizing available nutrients in the soil. Therefore, nutrient use efficiency is one of the major aspects to be addressed through crop improvement programs. Marginal lands with low nutrient availability can be converted into productive lands by planting crops with high nutrient use efficiency and thereby expanding arable lands. However, the excess use of inorganic fertilizers poses a threat to farmers' economy and causes environmental pollution and health concerns. These adverse effects can be overcome by using improved crops, thus converting the agricultural system into a sustainable and profitable one. Potential genotypes can be selected by screening germplasm for nutrient use efficiency.

Among the three major nutrients, nitrogen (N), phosphorus (P), and potassium (K), N is the most important nutrient that contributes to the formation of many essential structural, genetic, and metabolic compounds in plant cells, such as proteins, hormones, enzymes, chlorophylls, and vitamins (Uchida, 2000). It is essential for improving the quality and quantity of dry matter in leafy vegetables and protein in crops. Plants absorb N in the form of nitrate (NO_3^-) and ammonium (NH_4^+) ions. These ions are easily removed from the soil via leaching, runoff, and erosion or in the form of gas, resulting in the demand for frequent application to the soil. Langholtz *et al*. (2021) reported that increasing nitrogen use efficiency (NUE) by 20% could save $743 m/year and reduce N loadings in freshwaters by 5.7%.

NUE is the most studied nutrient factor under *in vitro* conditions. The potential genotypes for NUE have been identified by altering the medium composition with NH_4NO_3, $(NH4)_2SO_4$, or KNO_3 (Table 5). MS medium was used as the basal medium in all the reported studies. Schum *et al*. (2017) screened 17 potato cultivars to identify genotypic differences in traits associated with N uptake and utilization. N levels of 60, 30, 15, and 7.5 mM were tested. The genotypes 'Euro–starch', 'Euroresa', 'Verdi', 'Tomba', and 'Jasia' were selected as the comparably tolerant genotypes by evaluating N uptake, fresh and dry matter of shoots and roots, and chlorophyll and crude protein contents at the concentration of 7.5 mM. Bachmann-Pfabe and Dehmer (2020) investigated 28 accessions of wild *Solanum* species by using plantlets under optimal and low N supply (30 and 7.5 mmol/L N). Lines GLKS 30177_15, GLKS 30177_20, and GLKS 30160_15 of *S. chacoense* were found to produce the highest shoot and root biomass under N stress. Hajari *et al*. (2015) examined four varieties of sugarcane by exposing rooted plants to 4 and 20 mM N and found that the 'NCo376' and 'N12' varieties displayed the highest NUE. Akinyosoye *et al*. (2018) reported

Table 5. Application of plant tissue culture technique for screening genetic resources tolerant of low nitrogen levels.

Crop	Test nitrogen source	Nitrogen levels	Explant	Tolerant/resistant genotypes	Reference
Potato (*Solanum tuberosum*)	NH_4NO_3, KNO_3	60, 30, 15, 7.5 mmol/l	Shoot tips	Euro-starch, Euroresa, Verdi, Tomba, Jasia	Schum *et al*. 2017
Wild Potato	NH_4NO_3, KNO_3	30, 7.5 mmol l/N	Seeds	GLKS 30177_15, GLKS 30177_20, GLKS 30160_15	Bachmann-Pfabe and Dehmer 2020
Sugarcane (*Saccharum spp.*)	KNO_3, $(NH4)_2SO_4$	4, 20 mM	Shoot	NCo376, N12	Hajari *et al*. 2015
Maize (*Zea mays*)	NH_4NO_3	0.02, 0.01, 0.005, 0 mM	Immature embryos	TZPB Prol C3, LAPOSTA, SEQUIA C6	Akinyosoye *et al*. 2018
Atractylodes lancea, Brassica napus, *Orychophragmus violaceus)*	NH_4NO_3,	0.263–2.663 g/l	Plantlets	*Atractylodes lancea*	Wu *et al*. 2011

that the medium without ammonium nitrate was highly suitable for screening for low N-tolerant maize genotypes. They studied eight maize lines under *in vitro* and *in vivo* conditions. Four concentrations of ammonium nitrate (1650, 825, 412.5, and 0 mg/l) were tested. TZPB Prol C3 and LAPOSTA SEQUIA C6 performed better under *in vitro* conditions, and TZPB Prol C3 performed consistently under *in vitro* and *in vivo* conditions. Wu *et al*. (2011) assessed *Atractylodes lancea, Brassica napus*, and *Orychophragmus violaceus* cultured in medium containing various N concentrations ranging from 0.263–2.663 g/l. The NUE of plantlets was determined from the variation in their biomass and the variation in N content in the medium. The incised leaf-type *A. lancea* had higher NUE than other plants. Valkov and Chiurazzi (2016) evaluated three different *Lotus corniculatus* cultivars in complete medium supplemented with 19 mM KNO_3, 20 mM NH_4NO_3, and P in the form of 1.25 mM KH_2PO_4 against the medium devoid of these nutrients. The cultivar 'Leo' showed higher performance than the other two cultivars.

Other abiotic stresses

Heavy metal pollutants have reached toxic levels in millions of hectares, thus posing a serious risk to agriculture. Phytoremediation is a sustainable environmental technology used for heavy metal decontamination (Nedjimi, 2021). Lonardo *et al*. (2011) demonstrated a useful *in vitro* screening tool for selecting *Populus alba* clones suitable for phytoremediation techniques. Plantlets cultured on Woody plant media (Lloyd and McCown, 1980) supplemented with different concentrations of the four heavy metals, arsenic (Na_2HASO_4), cadmium ($CdSO_4$), and copper ($CuSO_4$) at the concentrations of 0, 5, 50, and 250 µM and zinc ($ZnSO_4$) at the concentrations of 0, 250, 1000, and 2000 µM, were evaluated with biomass production as a key factor reflecting phytoextraction capacity. *Villafranca* was selected as the best clone for phytoremediation among the three clones on the basis of the parameters of root and shoot fresh/dry biomass and metal accumulation.

Soil pH is one important factor that determines nutrient availability in the soil. Finn *et al*. (1991) screened eight blueberry germplasm for high pH tolerance by culturing the plantlets on modified Zimmerman's medium (Zimmerman and Broome, 1980). The germplasm was evaluated at low (5.0) and high (6.0) pH for vitality, height, and dry weight. *Vaccinium angustifolium* was selected as the tolerant genotype for high pH.

Stress due to high ultraviolet radiation (UV-B) causes photoinhibitory and photo-oxidative damages to crops in terms of plant development and physiology and promotes the biosynthesis of UV-absorbing compounds that negatively affect plant production. However, *in vitro* assays are seldom used to screen radiation-tolerant germplasm possibly due to the requirement of advanced technologies. Mosadegh *et al*. (2021) assayed nine ecotypes of basil (*Ocinum basilicum* L. var. *basilicum*) by using an *in vitro–vivo* system. Plants were exposed to a high UV-B dose of 68 kJ/m day through continuous exposure to UV-B lamps with emissions ranging from 280 nm to 400 nm for 4 h min/day. The changes in chlorophyll-*a* fluorescence parameters and the leaf compound rosmarinic acid were assessed in the samples after 0, 4, 24, and 48 h of UV-B exposure. A significant variation in UV-B protection mechanisms among the accessions was observed. Genotype 'OCI160' had an improved photosynthetic capacity associated with the enhanced biosynthesis of UV-absorbing compounds.

CONCLUSIONS

The literature reveals that the plant tissue culture technique is a reliable high-throughput method for screening crop genetic resources for diverse abiotic stress-tolerant traits. It is extensively used in screening genetic resources for drought and salt tolerance but is seldom applied to screen other stresses. This technique has been applied for various crops, including monocots, dicots, and herbaceous and woody plant species. Seedling and calli derived from different explants are the most common materials subjected to selection pressure. The adaptability of the selected genetic resource is demonstrated by comparing the growth and development of the plants and chemical composition under controlled and stressed conditions. The information on the selected genotypes can be utilized for various purposes (as shown in Figure 1), such as the continuation of cultivation with minimal agroinputs, the expansion of cultivation to marginal areas with stress conditions, that utilization of crop improvement programs, and the study of the molecular and physiological faction of the crops for changing adaptability to adverse conditions.

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