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EDITORIAL

The role of biotechnological tools for mitigating abiotic stress in a changing climate – preface

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The Department of Economic and Social Affairs of the UN has identified 17 goals, the Sustainable Development Goals (SDGs), to maintain the sustainable development and future welfare of mankind. Among them, SDG2 is to end hunger, achieve food security, improve nutrition, and promote sustainable agriculture. In this context, global climate change pressures plants to adapt to changing environments coupling biotic stress from pests and diseases with abiotic stresses, including extreme temperatures, salinity, drought, and heavy metals. Abiotic stress-tolerant plants in economically important crops can be produced through in vitro selection, gene transfer, and new plant breeding tools, such as genome editing, based on molecular biology and recombinant DNA technologies. These tools can potentially accelerate the generation of novel genotypes with attractive crop breeding traits, resulting in better yields, enhanced quality and productivity even under environmental constraints. This SI focuses on research work using these biotechnological approaches to increase tolerance to abiotic stresses in a range of species, and the manuscripts reflect the trends in studies in this domain.

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Zhang et al. studied the expansin gene *SmEXPA13* in the 9901 variety of the willow *Salix matsudana* to examine its link with salt stress defense. Using the *Agrobacterium* leaf disc method, they cloned the corresponding fragment and transformed it into tobacco (*Nicotiana tabacum*) plants. They found that, under salt conditions, in three transformed tobacco lines, the expression of the *SmEXPA13* gene was 10% (in roots) to 30% (in leaves) higher than in the respective tissues of control plants. In addition, the transgenic lines relative electrical conductivity and malondialdehyde content was lower than in the wild-type plants at both 50 mM/L NaCl and 100 mM/L NaCl-induced stress conditions. Na^+ absorption and accumulation were strongly decreased, while that of K^+ was concomitantly increased in the transgenic plants compared to wild-type plants, showing that *SmEXPA13* efficiently contributed to Na^+/K^+ homeostasis.

Working with the multipurpose woody species *Sapindus trifoliatus*, Ashtana et al. performed in vitro selection studies on embryogenic calluses derived from leaf discs exposed to increasing concentrations of up to 250 mM NaCl. A callus line tolerant to 100 mM NaCl was identified after applying a recurrent in vitro selection strategy (i.e., with back-and-forth passages from control to stress medium to eliminate the physiologically adapted tissues and enrich the population in truly tolerant ones). The callus growth, tolerance index, and accumulation of Na^+, K^+, and organic compounds, including proline, glycine betaine, and soluble sugars, were determined. The 100 mM NaCl tolerant callus gave somatic embryos on media with and without NaCl, two-thirds germinated in the salt-free medium and one-third in the NaCl-containing medium. Such plants further survived upon transplant to 0.2 or 0.3% (w/w) NaCl mixed potting mixture and, at six months old, were characterized in terms of their chlorophyll, carotenoid, and L-proline content as well as antioxidative (MDA, SOD, CAT, and APx) activities. The 100 mM NaCl-tolerant plants produced showed all adaptive features toward salt stress.

Tomato (*Lycopersicon esculentum* L) is known for its relative susceptibility to salt stress that increases ethylene accumulation, induces leaf epinasty, and reduces growth and/or viability. Zarei and Ehsanpour examined how protecting tomato plants from ethylene action in vitro by adding silver nitrate (0, 2, 4, mg. L^{-1} $AgNO_3$) and pyrazinamide (0, 2, 4 mg. L^{-1} PZA) protected them against NaCl (0, 100, 150 mM). They observed that at 4 mg. L^{-1} $AgNO_3$ and 4 mg. L^{-1} PZA, in vitro NaCl-stressed plants exhibited increased fresh and dry weight, total chlorophyll, and carotenoids. Further, $AgNO_3$ and PZA reduced H_2O_2 and malondialdehyde (MDA) contents and oxidative damage by enhancing antioxidant enzyme (catalase, superoxide dismutase, and ascorbate peroxidase) activity under salt stress.

Studying stress-related genes, and their regulatory mechanisms are crucial to coping with saline-alkali stress. Hence, based on the transcriptome of *Malus halliana*, Wu et al. identified the *Phytoene synthase 1* (*PSY1*) gene in the carotenoid metabolic pathway. *MhPSY1* expression was significantly down-regulated in the leaves of *M. halliana* under saline-alkali stress conditions. *MhPSY1* was isolated, and its function was studied in *Arabidopsis thaliana* and apple calli. *MhPSY1* had the highest homology and closest genetic relationship with *Pyrus*×*bretschneideri*. Its overexpression reduced the tolerance of *A. thaliana* and apple calli to saline-alkali stress due to significantly lower SOD, POD, and CAT activities and proline content, coupled with a significantly higher content of MDA in overexpressed apple calli and transgenic Arabidopsis compared to the wild type. In addition, saline-alkali stress inhibited the growth of transgenic plants, indicating that the *MhPSY1* gene, involved in the carotenoid metabolic pathway, plays a negative regulatory role in saline-alkali stress tolerance.

Field experiments on evaluating the effects of salinity are very difficult due to the enormous variability in the physical and chemical properties of soils, and strategies to resolve this are needed. With an original approach, Yarte et al. evaluated the salt tolerance of the tree *Handroanthus impetiginosus*, pink lapacho and the effect of inoculation with plant growth-promoting bacteria previously isolated from adult plants. Shoots were induced for three days in a medium with IBA then transferred for 40 days to an auxin-free medium, supplemented with 0, 40, 80, or 160 mM NaCl and inoculated or not with 108 cfu of *Bacillus* sp. L15 or *Sphingobacterium* sp. L22. Bacterization displaced the inhibitory concentration 50 (IC50) to higher salt concentrations (147–160 mM NaCl) relative to controls (109 mM NaCl), while inoculation with L22 improved biometric parameters at 40 mM NaCl. Bacterization and NaCl concentration modified proline, phenolic, and chlorophyll content during the first 15 days of culture, and bacteria inoculation promoted in vitro rooting and alleviated anatomical alterations induced by salt stress.

Sharma et al. examined the modulating effects of NaCl (50–250 mM) on the morphology, physiology, and metabolism of in vitro-grown shoots of *Withania somnifera*. High NaCl strongly affected micro shoots morphology and photosynthetic ability, while the proline, phenol, and sugar contents were highest with 250 mM NaCl. Free radical scavenging activity and malondialdehyde levels were upregulated, and antioxidant enzymatic activities increased linearly. Interestingly, the highest production of flavonoid (49.5%) and withaferin-A (192.9%) was limited only to 150 mM NaCl, and maximum expression of genes related to the accumulation of withanolide occurred with the 150 mM NaCl treatment. Conversely, the expression of photosynthetic-related genes significantly declined after increasing doses of NaCl. The authors conclude that a moderate dose of NaCl can elicit enhanced secondary metabolite production in *W. somnifera* without compromising its growth.

Sacu et al. evaluated the growth and biochemical responses of cotton (Gossypium hirsutum L.) cultivars Carmen and NM-503 during studies on in vitro selection to salt stress of hypocotyl callus on media with 0, 100, 200, or 400 mM NaCl for four weeks. Both cultivars showed similar growth and browning tendencies when exposed to salinity stress, with decreased biomass accumulation and relative growth rate on 200 and 400 mM NaCl. Still, calluses survived up to 200 mM NaCl. Carmen exhibited the lowest photosynthetic pigment content on 400 mM NaCl. In comparison, the NM-503 cultivar did not accumulate proline in response to 200 and 400 mM NaCl treatment and showed a lower lipid peroxidation level than the Carmen cultivar. No significant differences were seen in superoxide dismutase activity between the control and NaCl-treated groups, except for the 400 mM NaCl-treated callus of Carmen. Catalase activity of both cultivars was similar at all NaCl concentrations, and peroxidase and ascorbate peroxidase activities increased in response to increases in NaCl treatment. Conversely, glutathione reductase activity increased dose-dependent in the NM-503 cultivar, while it gradually decreased in Carmen. Differences were apparent in the mechanism of cellular antioxidant protection between the cultivars, and the larger tolerance displayed by the calli of NM-503 against severe salt stress can be attributed to a more efficient cellular antioxidant defence mechanism.

Also exploiting the eliciting role of NaCl to trigger the biosynthesis of flavonoids, Abedi et al. studied the oxidative stress responses of cell suspensions of *Haplophyllum virgatum* var. *virgatum* subjected to NaCl (0, 100, 150, and 200 mM) for various time durations (0, 8, 12, 24, 48, 72, and 168 h). The expression levels of the R2R3-MYB transcription factor and chalcone synthase and chalcone isomerase

(two major enzymes in flavonoids biosynthetic pathway), as well as rutin accumulation in the NaCl-treated cells, were all significantly enhanced. Moreover, they showed a positive correlation between the expression levels of the corresponding genes and rutin content. Results underlined a possible regulation of flavonoid biosynthesis by R2R3-MYB transcription factors and the role of flavonoids in response to NaCl. At the same time, NaCl was a suitable elicitor for flavonoid production in cell suspension culture of *H. virgatum* var. *virgatum*.

With *Brassica nigra*, Zia et al. applied lupeol, a plant triterpenoid, as a salinity stress-mitigating agent in vitro. NaCl stress (25–100 mg/L) decreased the root length, shoot length, number of roots, and dry weight of plants in a dose-dependent manner, while lupeol alleviated salinity stress and enhanced all these traits. Moreover, the free radical scavenging potential and the antioxidative response decreased with lupeol under salt stress, while phenolic and flavonoid concentrations in plant parts (elevated under salt stress alone) also decreased when lupeol was applied along with salt. NaCl increased antioxidative enzymes in shoots and roots, but the level of POD and SOD significantly varied upon applying lupeol in NaCl-augmented media. Lupeol protected the plant from oxidative damage and modulated stress by causing cell redox balance.

Radi et al. examined the morphological and physio-biochemical response of in vitro shoots of cactus pear (*Opuntia ficus indica*) to salt (2.5–10 $g \cdot L^{-1}$ NaCl) and polyethyleneglycol-induced (50–150 $g \cdot L^{-1}$ PEG) osmotic stress over 1–3 weeks of culture. Both stress reduced shoot proliferation, growth, and fresh and dry weights. Still, while NaCl severely decreased the survival rate, drought stress had no such effect and even promoted rhizogenesis. In addition, one week of stress notably decreased the levels of glycine betaine, proline, and carbohydrates while increasing the duration of stress promoted their accumulation, suggesting an adaptation to stress. The total protein and chlorophyll content was also decreased under stress conditions, while chlorophyll b varied randomly.

In an exciting study, the promoter region of *Phoenix dactylifera* (date palm) *PdDREB1G* (*Dehydration Responsive Element Binding 1G*) gene was isolated and characterized by Kodackattumannil et al., who compared the activity of two promoter fragments, 880 bp (DS) and 1.6 kb (DF) of *PdDREB1G* to *AtRD29A*. Histochemical and RT-qPCR analyses confirmed the induction of *β-glucuronidase* (*GUS*) expression in T3 plants of transgenic tobacco subjected to various abiotic stresses. Moreover, compared with the widely used AtRD29A promoter, the relative expression of GUS in leaves was significantly increased by both DS and DF under salt stress and by DS for PEG and abscisic acid (ABA). Under salicylic acid stress, both DF and DS displayed similar expression in leaves. Still, DS induced a significantly increased expression in roots under all four stresses (salt, PEG, ABA, and salicylic acid).

Various physical and chemical elicitors have been shown to modulate the responses of in vitro cultured tissues and plants toward stress, and Đurić et al. examined the potential effects of pre-treating control and PEG8000-induced osmotic-stressed in vitro shoots of *Impatiens walleriana* with increasing contents of methyl jasmonate (0, 5, 50, and 100 µM MejA). At 5 µM, MejA improved the fresh weight, plant height, number of leaves per plant, and proliferation rate of control shoots. Under PEG8000-induced water stress conditions, pretreatment with 5 µM MeJA increased all these growth traits and decreased photosynthetic pigment, proline and total amino acid content, total polyphenol, and DPPH (1,1′-diphenyl-2-picrylhydrazyl) activity, hydrogen peroxide and MDA content. Changes were induced in the superoxide dismutase, peroxidase, and catalase activities, promoting defense-related metabolism. As with other elicitors, the use of MejA beyond the 5 µM optimum mainly suppressed the *I. walleriana* shoots growth under water deficit, combination treatments, or treatment with MeJA alone.

In a study combining osmotic stress and light conditions, Lamara et al. assessed antioxidant activities and growth responses of anise (*Pimpinella anisum* L.) callus to 5% and 7.5% PEG6000 cultured under darkness and light (12 H-photoperiod) for 30 days. Water-stressed calli exhibited morphological and physiological variations in fresh and dry weight. MDA content increased progressively with increasing PEG6000 concentrations and coupled with higher values under dark than in light. The activity of antioxidant enzymes was also affected by PEG-6000, whereby APX and CAT increased gradually with 5% and 7.5% of PEG-6000 and with higher values under light. In comparison, the SOD enzyme showed a slight increase of 5% in light and a higher one under darkness with 7.5%. As for GPOX, the activity consistently decreased with PEG, regardless of the culture conditions.

Two studies by Yadav et al. concerned mitigating several abiotic stresses in chickpeas (*Cicer arietinum*). The first of these works examined the effect of overexpression of the PGPR (plant growth-promoting rhizobacteria) responsive chickpea *miRNA166* targeting *ATHB15* for alleviation of osmotic stress (induced with 300 mM mannitol) in Arabidopsis. The transgenic Arabidopsis lines showed an increase in seed germination and root length compared with stressed wild-type plants. The photosynthesis and transpiration rate, water-use efficiency, and stomatal conductance were better, with lower electrolyte leakage and higher relative water content in treated transgenic lines under inoculated conditions. The enzymatic and non-enzymatic antioxidants were also improved in transgenic lines with less membrane

damage and the highest proline accumulation under inoculation and drought stress. The *miR166* in drought-treated inoculated plants was highly upregulated, while the target *ATHB15* was highly downregulated, indicating that RA-responsive Car-miR166 plays beneficial stress-mitigating roles under drought in transformants. In a spin-off study, these same authors investigated the role of RA-responsive *miR166* for drought mitigation in a multi-stressed chickpea genotype through miR166-directed cleavage of *ATHB15* upon RA inoculation using 5′RLM-RACE analysis. Tissue-specific expression patterns of leaves, shoot, and roots in 15 day old chickpea seedlings exposed to salinity, drought, and abscisic acid at different time points indicated the role of miR166 toward different abiotic stress responses suggesting that miRNAs plus microbial applications could be important for stress management in crop improvement.

In studies on the induction of multi-stress tolerance, Zhang et al. undertook a genome-wide analysis of the gene family *bHLH* (basic Helix-Loop-Helix). They identified the function and involvement of *BrbHLH* in the acquisition of stress tolerance by wucai (*Brassica campestris* L. ssp. *chinensis* var. *rosularis*). Exogenous application of 2, 4-epibrassinolide significantly increased the *bHLH* gene expression at low temperatures, and *bHLH57* was upregulated significantly. A total of 239 *bHLH* genes were identified, and their amino acid physicochemical properties, chromosome location, gene structure, phylogeny, and cis-acting elements were analyzed by bioinformatics. Thus, *bHLH* gene families in *Brassica rapa* and Arabidopsis were divided into six groups, unevenly distributed and hence irregularly mapped in the cultivated *B. rapa* genome. The maximum number of *BrbHLH* genes were mapped on Chr09, with tandem repeats in some genes. Collinearity analysis showed that 152 *AtbHLH* genes and 239 *BrbHLH* protein genes formed 296 collinearity pairs, and all duplicated *BrbHLH* gene pairs had a Ka/Ks ratio of $<<1$, indicating that *bHLH* family genes may have undergone, robust purification and selection during evolution. Cis-acting element analysis showed that the promoter region of *bHLH* family genes in *B. rapa* had more responsive elements related to light, hormones, and abiotic stress. *BcbHLH57* overexpressing Arabidopsis lines subjected to different stress treatments were more tolerant to salt, heat, and drought stress than the control wild-type plants under stress.

In this same context, Stehling et al. undertook a quantitative analysis of the gene expression of *Lippia alba*, a medicinal plant of commercial interest, under abiotic stress. They first evaluated six constitutive reference genes (*NADH*, *CIT*, *G6i*, *TUB*, *RNApol*, and *ELONG*), of which *TUB* emerged as the most stable gene under osmotic stress. At the same time, *NADH* was the most stable reference gene under saline, wounding, cold, and exogenous abscisic acid and/or salicylic acid stress. Therefore, *TUB*, and *NADH* were recommended for reference genes in expression studies with *L. alba*.

Extreme temperatures can devastate the yield and performance of various crops, and two studies in this special issue focused on the induction of tolerance to this abiotic stressor.

Kumar et al. examined the role of the *Multiprotein-bridging factor 1c* (*MBF1c*) gene from *Triticum aestivum* L. on heat tolerance in transgenic *N. tabacum*. They isolated a high heat stress-responsive transcription factor from wheat. The candidate gene (*TaMBF1c*) was cloned in pJET1.2/blunt vector and then further into binary vector followed by *Agrobacterium*-mediated transformation in tobacco (*N. tabacum*). The heat stress tolerance of such transgenic tobacco plants was validated through their RWC, MDA, proline, and chlorophyll content. *TaMBF1c* gene expression in transgenic plants was checked by qRT-PCR, and the 3:1 (resistance: susceptible) segregation inheritance ratio obtained in their progeny followed the Mendelian inheritance pattern.

On the other hand, Li et al. performed the cloning and functional analysis of the *PLkF3H2* promoter in Japanese larch (*Larix kaempferi*). The flavanone 3-hydroxy-lase2 (*F3H2*) gene is one of the key genes in flavonoid biosynthetic pathways in this species, but its transcriptional regulation mechanism in flavonoid metabolism has not been studied. The authors cloned a 2398 bp upstream promoter sequence and tested its transcriptional activity in larch protoplasts by transient transformation. In addition, they also constructed 5' shortened fragments of this sequence into the upstream of the *GUS* gene in the pBI121 expression vector to produce transient and stable transgenic tobacco. GUS staining revealed that the key promoter region was located from -526 bp to -429 bp. PLkF3H2 promoter responded to MeJA, ABA, NaCl, and low temperature in transgenic tobacco. MeJA, ABA, and NaCl regulated *lkF3H2* gene expression via PLkF3H2 promoter elements but was *lkF3H2* negatively regulated by low temperature.

In a study with plantain (*Plantago major* L.), a medicinal plant rich in secondary metabolites, Siadeni et al. examined the effects of oxidative stress induced by drought (0%, 6%, and 12% PEG) and heavy metals (0, 4 and 8 µM $HgCl_2$) on metabolites production by 21-day-old plants in liquid culture. The total phenol, flavonoid, terpene, alkaloid, and anthocyanin content was evaluated and correlated to the expression of crucial genes involved in their biosynthesis, including *phenylalanine ammonia-lyase* (*PAL*), 3-deoxy-Darabino-heptulosonate-7-phosphate-synthase (DAHPS), 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoAR), caffeic acid o-methyl transferase (COMT), and squalene epoxidase (SQE). Phenol, flavonoid, terpene, alkaloid, and anthocyanin content were significantly affected by PEG and

$HgCl_2$ compared to control plants. Gene expression and secondary metabolite production did not always follow the same pattern. Still, the effect of both stresses on increasing the expression of some genes related to phenol and terpenoid pathways was significant.

In an infrequently studied domain, Manokari et al. assessed the effects of using seismic stress to amend several negative morphological traits observed in micropropagated plants of *Dioscorea pentaphylla* L. They observed that plantlets developed under an in vitro seismic stress induced by 60 rpm for 8 min twice a day during 4 weeks had more shoots per explant, an increased shoot length and leaf area, and, overall, larger biomass than the controls, together with an improved rooting, acclimatization and survival rate of plants produced. In contrast, the control plants exhibited leaves with stomatal abnormalities fewer defense trichomes and raphides. These results led the authors to suggest that seismic stress could counter in vitro environment stress.

As a metalloid element, selenium (Se) is not essential for plant nutrition, and, at elevated or prolonged exposure, it produces toxic effects similar to those of heavy metals, but selenium also has a role against oxidative stress. Del Pino et al. assessed the antioxidant effect of Se in suspensions of olive callus cells exposed to hydrogen peroxide. Given the disruptive action of oxidative stress on cytosolic Ca^{2+} homeostasis, Se treatments could be effectively examined with cytosolic Ca^{2+} measurements. Interestingly, Se did not affect cell morphology nor interfere with fluorometric determinations. Moreover, the authors used thapsigargin (TG) which is a known inhibitor of the Ca^{2+}-ATPases of the endoplasmic reticulum, to verify if Se had a specific impact on oxidative stress and found that Se antagonized H_2O_2-mediated perturbations of cytosolic Ca^{2+} but was unable to offset TG-mediated disruptions.

The earlier part of the present century has been marked by frequent adverse environmental constraints, mainly of an abiotic origin, that compelled plants to resist, tolerate or evolve following different strategies. PCTOC can help to understand underlying physiological mechanisms and genetic and epigenetic responses involved and to enhance rapid responses under these changes, among other strategies to preserve gene pools. This Special Issue was designed to compile some answers to these questions and as a means of identifying targets of new ones.