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Genetically modified poplars with improved abiotic stress resistance – recent accomplishments. A review

Genetycznie modyfikowane topole o zwiększonej odporności na stresy abiotyczne – najnowsze osiągnięcia. Praca przeglądowa

Summary. Great progress has been made within the last few years in terms of abiotic stress resistance improvement of forest trees. The application of genetic modification techniques allows developing trees displaying superior growth under various abiotic stresses such as high salinity, drought, low temperature, and environmental pollution. Poplar, which is an important agroforestry crop, is especially responsive to improvement through genetic engineering. With the view of imminent environmental changes, the application of transformation techniques to forestry breeding seems to be an attractive perspective. Here, we review the most recent achievements of researchers developing genetically modified (GM) poplar trees displaying enhanced performance under unfavorable experimental conditions.

Key words: GM trees, transformation, abiotic stress, poplar

INTRODUCTION

In the view of climate changes, the improvement of trees' adaptability to unfavorable environmental conditions and the development of highly resistant and high-yielding varieties is essential. Conventional tree breeding, however, relies on limited sources of resistance genes and its major bottleneck is the long generation cycle of the forestry tree species. Genetic improvement of forestry trees in terms of abiotic stress resistance might be accomplished with the aid of genetic engineering [Häggman et al. 2016, Cortés et al. 2020]. The first successful attempt at the development and regeneration of a transgenic tree (*Populus* hybrid NC-5339 overexpressing *aroA* gene) was reported by Fillatti et al. in 1987. Since then, great progress has been made in forest biotechnology – efficient transformation methods of woody plants have been developed, regeneration protocols for distinct tree species have been optimized, genes involved in stress response have been

identified and characterized [Nagle et al. 2018, Thakur et al. 2021]. As the accumulation of reactive oxygen species (ROS) occurring during extreme abiotic conditions causes oxidative stress, one of the crucial mechanisms of stress resistance involves the efficient induction of the antioxidant system [Rajput et al. 2021]. Of special interest is also the regulation of the transcriptional activity of genes involved in stress response and the role of the transcription factors (TFs). TFs are proteins that regulate the expression of downstream target genes, thus participating in cell response to various stimuli, including both abiotic and biotic stresses [He et al. 2020, Xin et al. 2021]. A vast body of literature shows a variety of both exogenous and endogenous genes that have been overexpressed in different plant species in order to improve their performance under unfavorable environmental conditions [Bakhsh and Hussain 2015, Nguyen et al. 2018, Anwar and Kim 2020]. Poplars became model organisms among forest trees due to their several practical advantages involving small genome, ease of genetic transformation and vegetative propagation as well as relatively fast growth rate [Yadav et al. 2017]. Therefore here we present a review of the most recent accomplishments aiming at the enhancement of abiotic stress resistance of *Populus* species.

DROUGHT TOLERANCE

Out of all abiotic stressing factors, drought can cause particularly serious harm to both agricultural and forest plants. It has the most detrimental effect on plant growth, productivity, and survival [Brodribb et al. 2020]. Therefore, plants developed various complex mechanisms for alleviating the negative effects of water deprivation. Among them are alternations of morphological structures, modifications in stomatal conductance, osmotic adjustments, and other strategies aiming at either improving water uptake or decreasing water loss. Of special interest is the abscisic acid (ABA) signaling pathway, which plays a crucial role in plant's response to water deficit. Identification of the drought-induced genes has contributed to the development of GM trees exhibiting drought-tolerant phenotype, which might prove essential in a water-limited environment [Moran et al. 2017, Polle et al. 2019].

The positive function of *PeSHN1* in plant drought stress tolerance was reported by Meng et al. [2019]. The regulation of water-use efficiency (WUE) observed in transgenic 84K poplars occurred by upregulation of wax biosynthesis, change in its composition, and reduced transpiration. The *PtXERICO* (a RING zinc-finger E3 ubiquitin ligase) was proven to be another positive regulator of drought tolerance in poplars. It was found to be involved in the abscisic acid-dependent stress response pathway. The abscisic acid (ABA) is recognized as the major signaling molecule controlling plant physiological and biochemical responses to water shortage. Under the experimental conditions, transgenic poplars demonstrated substantial upregulation of genes involved in ABA homeostasis as well as enhanced water retention capacity and reduced ion leakage [Kim et al. 2020]. Increased drought resistance that could be linked to ABA biosynthesis regulation was also reported by Bian et al. [2021]. The overexpression of the yellowhorn *XsAGL22* TF gene resulted in increased ABA content. The elevated ABA level subsequently induced stomatal closure, which reduced transpiration. What is more, the *XsAGL22* transgenic poplars performed better under water deficit conditions and displayed increased photosynthetic rate and plant biomass accumulation. Similar results were obtained by He et al. [2018] who generated

transgenic poplar 84K overexpressing *PeCHYR1*, a ubiquitin E3 ligase cloned from *Populus euphratica*. Although transgenic poplars exhibited reduced stomatal conductance, no negative effect on photosynthesis and biomass production during dehydration treatment was observed.

In a study by Wang et al. [2022], overexpression of *PtaERF194* (ERF family) in poplar 717 hybrids (*Populus tremula* × *Populus alba*) enhanced water-use efficiency (WUE) and limited water loss by reducing stomatal conductance and transpiration rate. However, the phenotypic analysis revealed that the overexpression lines displayed inhibited growth. A reduction in plant height and leaf area was observed. Nonetheless, suppressed growth of transgenic poplars and modulation of their physiology contributed directly to their drought tolerance. In another study, the *PdGNC*, which belongs to the GATA TF family and is preferentially expressed in adult leaves, was found to contribute to water loss prevention through the reduction of stomatal apertures [Shen et al. 2021]. Likewise, modulation of stomatal conductance leading to enhanced drought tolerance was a result of the overexpression of *PeABF3*, an ABA-responsive element binding factor [Yang et al. 2020]. The overexpression of *PuC3H35*, the CCCH type TF from *Populus ussuriensis*, contributed to drought tolerance via increased lignification and upregulation of proanthocyanidin biosynthesis in roots, which consequently led to enhanced ROS-scavenging under water deficit [Li et al. 2022].

SALINITY TOLERANCE

Soil salinization is a major concern in many coastal and inland areas because it severely constrains crop yield and wood production. Salinity stress is also experienced by trees growing in the cities or along the roads during winter season. Not only does high salinity result in ionic toxicity and hypertonic pressure, but it also brings about some secondary stresses, such as oxidative stress [Llanes et al. 2021, Zhang et al. 2021]. Plants have developed intricate salt tolerance mechanisms which help them survive in the harmful environment. These mechanisms involve numerous genes which participate in the regulation of physiological and biochemical processes (e.g. maintenance of ion homeostasis or antioxidant defense) as well as developmental and morphological changes [He et al. 2020, Llanes et al. 2021]. Some of the previously identified salt stress-related genes have been subsequently used with the aim of developing salt-resistant trees via a transgenic approach. For instance, the study by Geng et al. [2020] showed that the overexpression of *NsNHX1* gene from halophytic shrub *Nitraria sibirica* greatly improved salt tolerance of transgenic poplar. The *NsNHX1* encodes a tonoplast Na^+/H^+ antiporter which participates in compartmentalization of Na^+ in the vacuole and consequently helps to maintain intracellular ion balance. Likewise, the introduction of *PeHKT1;1* to *Populus davidiana* × *Populus bolleana* resulted in its better performance under salt stress conditions. The HKTs are high-affinity K^+ transporters which protect plants from salinity toxicity by contributing to Na^+ exclusion from roots and shoots [Xu et al. 2018]. Overexpression of *PtAnnexin1* from *Populus trichocarpa* also proved to be beneficial in terms of increasing the salinity tolerance of poplars. In comparison to wild-type plants, transgenic poplars subjected to salt stress demonstrated enhanced growth, increased chlorophyll and proline concentration, and significantly lower MDA level (a measure of lipid peroxidation) [Wang et al. 2020]. Furthermore, the heterologous overexpression of *AtSRK2.8* (sucrose non-fermenting-1 related protein kinase), which is involved in the osmotic stress signaling pathways

in *Arabidopsis thaliana*, also improved the growth of transgenic poplars under high salt stress [Wei et al. 2019].

Among the TFs which form the regulatory network involved in the salt stress response, the *GbLWD1-like* from *Ginkgo biloba*, which belongs to the WD40 family, improved the salt tolerance of transgenic aspen hybrid (*P. davidiana* × *P. bolleana*). Clear phenotypic differences, as well as differential expression of some salt tolerance genes (*NAC130* and *bZIP60*), were noticed between transgenic and control trees subjected to salt stress [Xin et al. 2021]. Another TF originating from *G. biloba* that was overexpressed in *P. davidiana* × *P. bolleana* is *GbBBX25*, which belongs to a subfamily of zinc finger proteins (ZFP). Salt treatment led to increased accumulation of the soluble sugar in the transgenic lines in addition to higher peroxidase (POD) activity and upregulation of several genes (*RabE1b*, *ERF76*, *bZIP60*, *WRKY106*, *NAC130*) related to salt stress [Huang et al. 2021].

The important role of *PsnHDZ63* in poplar salt tolerance was revealed by Guo et al. [2021]. The *PsnHDZ63* is a member of the HD-Zip transcription factor family and was found to be significantly upregulated in *Populus simonii* × *Populus nigra* in response to high salinity. The overexpression of *PsnHDZ63* improved the growth of transgenic poplars under salt stress as well as their reactive oxygen species (ROS) scavenging ability. Reduced accumulation of ROS under salt treatment was also reported in poplar 84K (*Populus alba* × *Populus glandulosa*) overexpressing *PeSTZ1* – a SALT TOLERANCE ZINC FINGER1 transcription factor from *Populus euphratica* [He et al. 2020]. It was concomitant with increased biomass accumulation resulting from precise K⁺/Na⁺ homeostasis regulation in photosynthetic organs which allowed for alleviation of salt toxicity during NaCl stress. Likewise, enhanced salt tolerance that could be associated with maintaining ROS balance was reported by Zhao et al. [2018] who introduced the *LbDREB* TF gene [the ethylene response factor (ERF) family] from *Limonium bicolor* into *Populus ussuriensis*. The genetically modified (GM) poplars exhibited higher superoxide dismutase and peroxidase activities and their gene expression profiles were consistent with the expression of the transgene. Furthermore, the comparison of several physiological and biochemical indexes revealed more severe salt injury symptoms, weaker photosynthetic capacity and lower proline and relative water content in non-transgenic trees than in transgenic lines. The study performed on another salt-inducible ERF transcription factor (*ERF38*) also revealed the superior performance of the transgenic poplars over control plants under NaCl treatment [Cheng et al. 2019].

LOW TEMPERATURE TOLERANCE

Low temperature is a major environmental factor affecting plant species' geographic distribution. It has an especially detrimental effect on young plant's leaves and apical meristems. Injuries sustained due to the chilling (0–15°C) or freezing (<0°C) stress might lead to stunted growth, reduced productivity, or even pose a threat to a plant's survival. Therefore plants evolved a special cold-signaling network, which modulates the expression of cold-responsive (COR) genes and subsequently triggers proper response at a molecular level, such as accumulation of protective compounds (e.g. soluble sugars, proline) or activation of an antioxidant system [Sharma et al. 2020].

The *PeSTZ1*, C2H2-type zinc finger transcription factor from *P. euphratica*, was used to develop transgenic poplars with significantly enhanced freezing tolerance. The *PeSTZ1*

was found to substantially upregulate ascorbate peroxidase 2 (*PeAPX2*) expression, which promoted the scavenging of ROS, reduced the oxidative damage, and subsequently helped to withstand the freezing conditions. Moreover, the transgenic poplars had increased soluble sugar content and maintained higher photosynthetic activity when subjected to freezing stress than the wild-type controls [He et al. 2019].

The research of Wang et al. [2021] demonstrated that *PsnICE1*, encoding the inducer of CBF expression 1 in *Populus simonii* × *Populus nigra*, acts as a positive regulator of cold tolerance by modulating the expression of cold stress-responsive (COR) genes. Among them were stress-related genes involved in maintaining ROS homeostasis, such as *POD*, *SOD* and *GST*. Their induction resulted in enhanced activity of the antioxidant enzymes which in turn mitigated the negative effect of ROS on cellular membranes and improved cold tolerance of transgenic trees.

POLLUTANTS TOLERANCE

Industrial activities, mining, transportation and agricultural practices are the main sources of environmental pollutants such as heavy metals, pesticides, waste products and an excessive amount of nutrient minerals. They have a detrimental effects on living organisms and pose a threat to ecological balance. Reduced seed germination, impaired nutrient uptake, increased ROS accumulation leading to oxidative damage, decrease in photosynthetic activity and finally yield reduction can all be the results of water, air and/or soil pollution. Plants have developed numerous protective mechanisms to cope with exposure to various contaminants. Increased knowledge regarding genes involved in these mechanisms led to the development of many transgenic plants with improved tolerance toward environmental contaminants. Among them of special interest are GM trees which not only are able to withstand the exposure to contaminants better, but also display enhanced accumulation capabilities and can therefore be used in the phytoremediation of heavily contaminated areas [Sun et al. 2018, Rai et al 2020, Ozyigit et al. 2021].

With regard to heavy metals, a study by Wu et al. [2022] revealed the important role of *PyWRKY75* TF in the regulation of poplar tolerance to cadmium (Cd) stress. Not only did overexpression of *PyWRKY75* promoted the absorption and accumulation of Cd in poplar tissues, but it also activated efficient protective mechanisms in transgenic lines. This included increased chlorophyll and carotenoid synthesis, enhanced accumulation of Cd chelating agents (such as reduced glutathione and phytochelatins), and modulation of antioxidant enzymes activity. Neri et al. [2020] tested the role of aquaporin (*aqual*) in Cd tolerance of *Populus alba* ‘Villafranca’ clone. The overexpression of *aqual* resulted in faster uptake and improved Cd translocation to aerial parts, which is advantageous in terms of Cd phytoremediation. Even though the transgenic lines accumulated significantly more Cd in their leaves, they displayed no visible symptoms of toxicity. Enhanced tolerance and accumulation of another heavy metal, mercury (Hg), was reported in transgenic poplar overexpressing an ATP-binding cassette (ABC) transporter gene *PtABCC1*. In addition to increased Hg accumulation, the transgenic poplars displayed a lower level of foliar damage in comparison to untransformed controls. Although the detoxification mechanism of *PtABCC1* still remains to be elucidated, its potential involvement in vacuolar compartmentalization is suggested [Sun et al. 2018].

The role of *Saccharomyces cerevisiae* vacuolar transporter *ZRC1* in conferring zinc (Zn) tolerance was studied by DalCorso et al. [2021]. In spite of being an essential micro-nutrient, Zn can have a toxic effect when present in excess. The *ScZRC1*-expressing white poplars were characterized by enhanced tolerance and accumulation of Zn when grown in the presence of high metal concentration. Additionally to the lack of metal toxicity symptoms in transgenic lines, increased SOD activity was noted. Another study on Zn stress tolerance indicated that the heat shock transcription factor A4a (*PuHSFA4a*) participated in regulating Zn homeostasis in *Populus ussuriensis*. Performed analyses revealed that the overexpression of *PuHSFA4a* activated the antioxidant system and root development-related genes which subsequently contributed to stronger development of the root system of transgenic poplars in spite of excessive Zn levels [Zhang et al. 2019].

Worth mentioning is also one of the main pollutants of agricultural origin – nitrogen (N). On the one hand, N is an essential macronutrient required for plant growth and development. On the other hand, the excessive use of N fertilization in agriculture leads to nitrate pollution of ground and surface waters [Lebedev et al. 2021]. The prospect of using transgenic poplars overexpressing *GS1a* (cytosolic glutamine synthetase) for potential phytoremediation of nitrate pollution was discussed by Castro-Rodríguez et al. [2016]. The study showed that *GS1a*-transgenic poplars subjected to high N levels displayed greater nitrogen-use efficiency, accumulated enhanced levels of cellulose, and exhibited increased biomass production in comparison to non-transgenic plants growing in the same conditions. Obtained results confirmed that transgenic poplars could be of interest not only for biofuels or paper production but also for environmental remediation of agricultural pollutants.

CONCLUDING REMARKS

Abiotic stresses, such as drought, high salinity, low and high temperature, or heavy metal contamination, have a negative impact on tree growth and development. Plants evolved a myriad of response mechanisms in order to mitigate or overcome abiotic stresses. Their growth and survival depend on the modulation efficacy of many physiological and biochemical processes including photosynthesis, transpiration, nutrient uptake, metabolites biosynthesis, osmotic regulation, and changes in antioxidant profile [Nowicka et al. 2018, dos Santos et al. 2022].

In view of upcoming environmental changes, the breeding of stress-tolerant trees has become a necessity. In order to survive and tolerate the harsh conditions, woody plants need to possess efficient coping mechanisms. The adaptation mechanisms developed by various species allowing them to withstand unfavorable conditions might be successfully transferred to trees via a transgenic approach. The development of stress-resistant trees with the aid of genetic engineering might be accomplished in a relatively short period compared to conventional breeding [Yadav et al. 2017, Chang et al. 2018, Wei et al. 2019]. All of the above-mentioned studies corroborate that the genetic transformation of poplar trees is a valuable approach for abiotic stress resistance improvement and can serve as an excellent tool for sustaining biomass production under changing environmental conditions.

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