

## REVIEW

# Development and Future Application of Transgenic Tall Fescue (*Festuca arundinacea* Schreb.) with Improved Important Forage and Turf Traits

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### Abstract

Tall fescue (*Festuca arundinacea* Schreb.) is a major cool-season perennial grass widely grown for forage, turf, and roadside cover. Tall fescue is an allohexaploid outcrossing species with self-incompatibility; these reproductive characteristics and genetic complexity often make it difficult to improve its agronomic traits by conventional breeding. Genetic transformation allows the direct introduction of foreign genes into target plants to create desired phenotypes and novel variants. Functional analyses of transgenes have been performed to improve important forage and turf traits, namely, abiotic stress tolerance, disease resistance, forage quality, and herbicide resistance. In this review, the development and future application of transgenic tall fescue have been summarized and discussed.

**Discipline:** Biotechnology

**Additional key words:** abiotic stress tolerance, disease resistance, forage quality, herbicide resistance

### Introduction

Tall fescue (*Festuca arundinacea* Schreb.) is a major cool-season perennial grass grown globally for forage, turf, and roadside cover. It is valued for its tolerance to environmental stresses, high persistence, and adaptability to a wide variety of soils. Conventional breeding has enhanced the agronomic performance of tall fescue using natural gene pool from different cultivars or ecotypes. However, tall fescue is an allohexaploid ( $2n = 6x = 42$ ) outcrossing species with self-incompatibility, which complicates trait inheritance. These reproductive characteristics and genetic complexity often make it difficult to improve its agronomic traits. Each tall fescue cultivar is a heterogeneous population with heterozygous genotypes maintained by crossing a variable number of selected individuals to avoid inbreeding depression. Conventional breeding is based on selecting observable phenotypes and uses complicated recurrent selection to accumulate favorable alleles in a population (Vogel & Pedersen 1993). Genetic transformation creates desired phenotypes and novel genetic variants through the

direct introduction of foreign genes into target plants. To complement conventional breeding and facilitate the improvement of tall fescue, transgenic approaches based on both particle bombardment (Cho et al. 2000, Spangenberg et al. 1995) and *Agrobacterium*-mediated transformation (Dong & Qu 2005, Lee et al. 2004, Wang & Ge 2005) have been developed. The genomes of cool-season grasses are poorly characterized compared with those of model plants, but the accumulation of genomic information in other plants has assisted the transgenic approach in tall fescue. Various transgenes introduced to improve important forage and turf traits in tall fescue have been evaluated (Table 1). This review summarizes the development of transgenic tall fescue.

### Abiotic stress tolerance

Abiotic stresses, such as cold, drought, heat, salinity, oxidative damage, and heavy metal toxicity, have adverse effects on plant growth and productivity. The expression of *Agrobacterium tumefaciens* isopenentenyl transferase increased cold tolerance, tillering ability, and chlorophyll

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*a* and *b* levels in transgenic tall fescue, resulting in a more vigorous growth and in plants that remain green at low temperatures longer than the wild type (Hu et al. 2005). Some transcription factors confer abiotic stress tolerance by activating downstream stress-related genes. Transgenic tall fescue carrying the *Arabidopsis thaliana* gene for dehydration-responsive element-binding protein 1A/C-repeat-binding factor 3 under the control of the stress-inducible *rd29A* promoter had increased tolerance to drought stress (Zhao et al. 2007a). The overexpression of a tall fescue A2-type heat stress transcription factor maintained plant growth under heat stress (Wang et al. 2017).

Salt damage is primarily caused by sodium ion accumulation, which leads to water deficit in plant tissues. Na<sup>+</sup>/H<sup>+</sup> antiporters are ubiquitous membrane proteins that are important in the regulation of cellular pH and Na<sup>+</sup> homeostasis. The overexpression of a vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter gene from *Arabidopsis* enhanced salt tolerance in tall fescue (Tian et al. 2006, Zhao et al. 2007b). *Arabidopsis* salt overly sensitive 1 (AtSOS1) is a plasma membrane Na<sup>+</sup>/H<sup>+</sup> antiporter, and AtSOS2 and AtSOS3 regulate AtSOS1 transport activity. The co-expression of *AtSOS1 + AtSOS2 + AtSOS3* genes in tall fescue resulted in the accumulation of less Na<sup>+</sup> and more K<sup>+</sup>, and thus increased salt tolerance (Ma et al. 2014).

As reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, induced by abiotic stresses cause oxidative damage to plant cells, genetic transformation to remove ROS from tall fescue was attempted. The simultaneous expression of both cassava (*Manihot esculenta* Crantz.) copper–zinc superoxide dismutase and pea (*Pisum sativum* L.) ascorbate peroxidase under the control of the oxidative stress-induced sweet potato (*Ipomoea batatas* [L.] Lam.) peroxidase anionic 2 promoter enhanced antioxidant enzyme activities and conferred tolerance to oxidative stress-inducing substances, such as methyl viologen and heavy metals (Lee et al. 2007). The ectopic expression of *Arabidopsis* homeodomain glabrous 11, a homeodomain StAR-related lipid transfer transcription factor, under the control of the cauliflower mosaic virus 35S promoter with four enhancers, resulted in tolerance to drought and salt stress, which was associated with increased ROS scavenging capability (Cao et al. 2009). Transgenic tall fescue overexpressing an *Arabidopsis* 2-cysteine peroxiredoxin, which has both peroxidase and chaperon functions, acquired enhanced tolerance to methyl viologen and heat stresses (Kim et al. 2010). Rice (*Oryza sativa* L.) chloroplast-localized small heat-shock protein 26 that is overexpressed in tall fescue helped maintain photosynthesis under heat and oxidative stresses (Kim et al. 2012). Alfalfa (*Medicago sativa* L.)

mitochondrial small heat-shock protein 23 conferred tolerance to salt and arsenic stresses by lowering the accumulation of hydrogen peroxide in transgenic tall fescue (Lee et al. 2012).

## Disease resistance

Gray leaf spot caused by *Magnaporthe grisea* and brown patch caused by *Rhizoctonia solani* are severe fungal diseases of tall fescue that is maintained as turf. Two genes that encode alfalfa β-1,3-glucanase and bacteriophage T4 lysozyme were separately introduced into tall fescue to degrade fungal cells and conferred resistance to both diseases (Dong et al. 2007, 2008). A truncated frog (*Phyllomedusa sauvagei*) dermaseptin S1, which has antimicrobial activity, enhanced resistance to both the diseases in transgenic tall fescue (Dong et al. 2007). The expression of the shrimp (*Litopenaeus setiferus*) antimicrobial peptide penaeidin 4-1 in tall fescue reduced leaf lesions caused by *R. solani* (Zhou et al. 2016b). Rice *Pi9*, an *R* gene that confers resistance to rice blast disease, was specifically active against *M. grisea* in transgenic tall fescue (Dong et al. 2007). RNAi constructs to silence the expression of *R. solani* genes essential for its growth inside the fungus, encoding RNA polymerase + importin beta-1 subunit and cohesin complex subunit Psm1 + a ubiquitin E3 ligase, were introduced into tall fescue and improved resistance to *R. solani* (Zhou et al. 2016a).

## Forage quality

As tall fescue plants grow and mature, they become fibrous, with secondary cell wall deposition and lignification of sclerenchyma cells, resulting in low forage quality (Chen et al. 2002). Transgenic tall fescue plants carrying either a sense construct for co-suppression or an antisense construct to target the gene for either cinnamyl alcohol dehydrogenase or caffeic acid *O*-methyltransferase, both of which function in the lignin biosynthesis pathway, had reduced lignin content, altered lignin composition, and improved digestibility (Chen et al. 2003, 2004). Rice SECONDARY WALL NAC (NAM ATAF1/2 and CUC2) DOMAIN PROTEIN 2 (OsSWN2) regulates secondary cell wall formation in sclerenchyma cells (Yoshida et al. 2013). The *OsSWN2* chimeric repressor under the control of the *OsSWN1* promoter decreased indigestible fiber content and increased tall fescue forage digestibility by reducing secondary cell wall thickness (Sato et al. 2018).

Lignin–ferulate–arabinoxylan complexes cross-link cell walls and inhibit cell wall degradation. The

expression of *Aspergillus niger* ferulic acid esterase in transgenic tall fescue decreased cell wall feruloylation and increased cell wall digestibility (Buanafina et al. 2008, 2010). Transgenic tall fescue plants were produced to improve the quality of protein by the accumulation of sulfur-rich sunflower (*Helianthus annuus* L.) albumin 8, which contains high levels of methionine and is resistant to degradation *in vitro* (Wang et al. 2001).

## Herbicide resistance

Conferring herbicide resistance facilitates weed elimination in forage production and turf maintenance

because controlling weeds without damaging desirable plants is otherwise difficult. Protoporphyrinogen oxidase is the target enzyme of diphenyl-ether herbicides, which prevent chlorophyll biosynthesis, and thus are phytotoxic. The overexpression of *Myxococcus xanthus* protoporphyrinogen oxidase conferred resistance to two diphenyl-ether herbicides—oxyfluorfen and acifluorfen—in transgenic tall fescue (Lee et al. 2008). Acetolactate synthase (ALS), also known as acetohydroxyacid synthase, is the first common enzyme in the biosynthesis pathways that leads to branched-chain amino acids and is the target enzyme of herbicides such as pyrimidinylcarboxylates. Different types of mutations in ALS confer resistance

**Table 1. Transgenic tall fescue with improved important forage and turf traits**

Target traits	Transgenes	Origin of transgenes	References
Tolerance to cold	<i>ipt</i>	<i>Agrobacterium tumefaciens</i>	Hu et al. 2005
to drought	<i>DREB1A/CBF3</i>	<i>Arabidopsis</i>	Zhao et al. 2007a
to heat	<i>FaHsfA2c</i>	tall fescue	Wang et al. 2017
to salt	<i>AtNHX1</i>	<i>Arabidopsis</i>	Tian et al. 2006
	<i>AtNHX1</i>	<i>Arabidopsis</i>	Zhao et al. 2007b
	<i>SOS1 + SOS2 + SOS3</i>	<i>Arabidopsis</i>	Ma et al. 2014
to some abiotic stresses	<i>CuZnSOD+APX</i>	cassava, pea	Lee et al. 2007
	<i>AtHDG11</i>	<i>Arabidopsis</i>	Cao et al. 2009
	<i>2-Cys Prx</i>	<i>Arabidopsis</i>	Kim et al. 2010
	<i>Oshsp26</i>	rice	Kim et al. 2012
	<i>MsHsp23</i>	alfalfa	Lee et al. 2012
Disease resistance	<i>AGLU1</i>	alfalfa	Dong et al. 2007
	<i>Derm</i>	frog	Dong et al. 2007
	<i>Pi9</i>	rice	Dong et al. 2007
	<i>T4 e</i>	bacteriophage	Dong et al. 2008
	<i>Pen4-1</i>	shrimp	Zhou et al. 2016b
	<i>RNApoly + Imbs</i>	<i>Rhizoctonia solani</i>	Zhou et al. 2016a
	<i>Coh + UbiE3</i>	<i>Rhizoctonia solani</i>	Zhou et al. 2016a
Forage quality	<i>CAD</i>	tall fescue	Chen et al. 2003
	<i>COMT</i>	tall fescue	Chen et al. 2004
	<i>OsSWN2</i>	rice	Sato et al. 2018
	<i>faeA</i>	<i>Aspergillus niger</i>	Buanafina et al. 2008, 2010
	<i>sfa8</i>	sunflower	Wang et al. 2001
Herbicide resistance	<i>MxPPO</i>	<i>Myxococcus xanthus</i>	Lee et al. 2008
	<i>OsALS (dm)</i>	rice	Sato et al. 2009
	<i>OsALS (sm)</i>	rice	Sato et al. 2013

Gene abbreviations: *AGLU1*,  $\beta$ -1,3-glucanase; *APX*, ascorbate peroxidase; *AtHDG11*, homeodomain glabrous 11; *AtNHX1*, vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter; *CAD*, cinnamyl alcohol dehydrogenase; *Coh*, cohesin complex subunit Psm1; *COMT*, caffeic acid *O*-methyltransferase; *CuZnSOD*, copper–zinc superoxide dismutase; *Derm*, truncated dermaseptin SI; *DREB1A/CBF3*, dehydration-responsive element-binding protein 1A/C-repeat-binding factor 3; *faeA*, ferulic acid esterase; *FaHsfA2c*, A2-type heat stress transcription factor; *Imbs*, importin beta-1 subunit; *ipt*, isopentenyl transferase; *MsHsp23*, mitochondrial small heat-shock protein 23; *MxPPO*, protoporphyrinogen oxidase; *OsALS (dm)*, acetolactate synthase (*ALS*) containing double point mutations; *OsALS (sm)*, *ALS* containing a single point mutation; *Oshsp26*, chloroplast-localized small heat-shock protein 26; *OsSWN2*, secondary wall NAC domain protein 2; *Pen4-1*, penaeidin 4-1; *RNApoly*, RNA polymerase; *sfa8*, sulfur-rich albumin 8; *SOS1-3*, salt overly sensitive 1-3; *T4 e*, T4 phage lysozyme; *UbiE3*, ubiquitin E3 ligase; *2-Cys Prx*, 2-cysteine peroxidoredoxin

+ indicates co-expression of transgenes in plants.

to different classes of herbicides (Kawai et al. 2007). Transgenic tall fescue plants expressing the rice *ALS* gene with single or double point mutations at particular positions were resistant to pyriminobac or bispyribac-sodium, respectively (Sato et al. 2009, 2013).

## Future perspectives

By the end of the 21<sup>st</sup> century, the global mean surface temperature is predicted to increase by 0.3°C-4.8°C relative to 1986-2005 (IPCC 2014). Climate change will strongly affect pasture production and turf maintenance. The demand for livestock products is growing owing to world population growth, whereas the worldwide agricultural area, including grasslands, remains static (O'Mara 2012). Extreme temperatures and rainfalls, water shortages, and soil quality changes are expected to limit plant growth; shift production seasons; and change patterns of diseases, insects, and weeds. Forage quality has a direct effect on livestock performance because a small increase in digestion of fiber can greatly increase production of beef or milk. For stable cultivation and high production in grasslands, it is essential to quickly and effectively improve agronomical traits such as tolerance to a wide range of environmental stresses and forage quality. The development of transgenic tall fescue summarized in this review could offer the potential to create novel genetic variants that are useful as breeding materials.

Genetic transformation has been used in various forage and turf grasses (Giri & Praveena 2015, Wang & Ge 2006), whereas there are only a few examples of deregulated transgenic forage crops such as herbicide-resistant alfalfa (Waltz 2011) and creeping bentgrass (*Agrostis stolonifera* L.) (USDA-APHIS 2016). As major cool-season grasses are cross-pollinated anemophilous species, public concerns have focused on the environmental or ecological impacts of pollen-mediated transgene flow (Wang & Brummer 2012). Therefore, transgenic tall fescue with male sterility is useful to prevent transgenic pollen flow, but they can be crossed with sexually compatible wild species.

Genome editing is a promising approach to completely avoid the problem of transgene flow in tall fescue. Because it has facilitated plant improvement without transgenes in the final products. Genome editing technologies, such as the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system (Jinek et al. 2012), allow the insertion, removal, or replacement of DNA at particular positions in the genome. The CRISPR/Cas9 transgene can be segregated out in the progeny by crossing with wild-type plants. Furthermore, DNA-free genome editing, such

as via the introduction of CRISPR/Cas9 ribonucleoprotein (Metje-Sprink et al. 2019) or the *Agrobacterium*-mediated transient CRISPR/Cas9 gene expression system (Chen et al. 2018, Veillet et al. 2019), has been developed.

However, the transformation efficiency of tall fescue is still not enough because selecting genome-edited plants from transgenic plants is necessary. Tall fescue cultivars consist of various genotypes responding differently in tissue culture and transformation. Although responsive genotypes derived from mature seeds may lose their regeneration ability in prolonged callus culture, we can produce transgenic plants at any time with calli induced from shoot tips of *in vitro*-maintained responsive genotypes (Takamizo & Sato 2020). The screening of highly transformable genotypes is an efficient approach for generating large numbers of transgenic plants. Although to the best of our knowledge, genome-edited tall fescue has not yet been reported, genome editing will accelerate the practical application of genetically modified tall fescue.

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