

BREEDING FORAGE CROPS FOR IMPROVED ABIOTIC STRESS TOLERANCE-A REVIEW

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SUMMARY

Forage crop production is largely limited by abiotic stress such as drought, salinity, temperature and other edaphic stresses because most forages are grown in marginal agricultural lands that have even poorer soil and land management system featured with low water holding capacity, infrequent irrigation, limited fertility or high salt content. Conventional and genetic engineering approaches have been used to improve stress tolerance of forage grasses and legumes. Modern conventional plant breeding is undergoing revolutionary changes that embrace new marker technologies and more profound understanding of the mechanisms that constitute complex traits. Traditionally, major gene and polygenic variation has been analyzed in different ways, but the use of new DNA markers and techniques of QTL analysis now allow to more integrated approaches in dissecting complex traits and assessing gene effects. Useful information on the genetic basis of abiotic stress tolerance has been obtained by moving genes between plants of the same or closely related species. Gene introgression achieved by conventional cross pollination means that created a range of genetic variation available to understand and manipulate genetic adaptation to environmental change is greatly enhanced. Drought and cold tolerance has been improved within the *Lolium* / *Festuca* species complex. A link was found between drought tolerance and enhanced deeper root growth under water limiting conditions in tall fescue and alfalfa. The differences in the level of freezing tolerance between non-hardy and hardy alfalfa cultivars was found to be related to the capacity of the plants to accumulate raffinose and stachyose in their roots and crowns other than the capacity to accumulate sucrose earlier than non-dormant plants. Proline content in alfalfa leaves and roots increased dramatically when plants were subjected to drought and two genes controlling the transcriptional regulation of key proline cycle enzymes in alfalfa have been identified and cloned. Wide hybridization with relative species followed by chromosome and / or chromosome fragment introgression has been considered an efficient way to transfer drought, salt and other stress tolerance gene(s) to the target species to widen the gene pool. Intergeneric hybrids between *Lolium* (Ryegrass) and *Festuca* (Fescue) species have received much attention by forage breeders. Enhanced drought tolerance in cowpea is accompanied with (i) better water- use efficiency and tolerance to water –deficiency and extreme heat conditions, (ii) better recovery of plants after drought is removed i.e., on re-watering. Both types of drought tolerance are dominant traits controlled by a single dominant gene *Rds1* and *Rds2* respectively. In white clover drought tolerance improvement programme, introgression has also been used as a route to transfer the morphological or physiological traits from its related wild species that show more drought tolerance or have better persistence. Endophyte-infected grasses are better adapted than non-infected grasses to abiotic stresses i.e., drought and marginal soil conditions due to direct changes affecting water status in shoots and indirect changes in root morphology and function.

Key words : Abiotic stress, tolerance, forage crops, breeding, intergeneric hybridization, endophyte

In agricultural context, stress has been defined as the conditions in which plants are prevented from fully expressing their genetic potential for growth, development, reproduction and ultimately the crop productivity (Levitt, 1980). Abiotic stresses adversely affect the livelihoods of farmers and their families, sustainability of livestock as well as national economies and food security. Forages are normally

referred to as plants and plant parts that are consumed by domestic livestock such as dairy and beef cattle, sheep, goats, horses and a wide range of other animals (Barnes and Baylor, 1995). Forage plays a key role in ruminant livestock production and environment protection. In addition to serving as the major sources of feed nutrients for domestic and wild animals, forages contribute to human well-being through many

other ways including: (i) protection and conservation of soil and water resources; (ii) improvement of soil structure and fertility; (iii) providing habitat for wildlife; (iv) improvement and protection of the environment from pollution such as sediment, wind blowing soil and some toxic substances.

In soil-plant-animal biological system, forage are in heavy demand from livestock. On the other hand, they bear severe growth limitations from soil and environment because most forages are grown in marginal agricultural areas. An inevitable increase of human population during the next several decades will force the production of more food, meat and dairy products, requiring more land area for food and fiber crops. We may face the fact forcing forage cultivation in far marginal areas that have even poorer soil and land management system featured with low water holding capacity, infrequent irrigation, limited fertility or high salt content (Sanderson *et al.*, 1997). Water resources available for irrigation are becoming scarce and this trend may increase drastically in future with the likely scenario of global warming (Breshears *et al.*, 2005; Munjal and Dhanda, 2016). For perennial forages and natural vegetations, the ability to survive periods of environmental constraint is an essential characteristic for success. To improve yield and sustainability in forage production, a survival strategy may be more important than a growth strategy, particularly in more severe and variable environments (Eagles *et al.*, 1997).

Mechanism of Abiotic Stress Tolerance

Climate and soil determine many adaptations in plants and the eco-geographical distribution of species and ecotypes demonstrate differences in physiology and developmental patterns that provide good evidence of adaptive mechanisms. Plants respond to environmental changes as individuals through phenotypic plasticity and in population through selection and associated evolutionary processes. It is not always easy to determine the genetics underlying adaptive processes because environmental factors may be complex or not clearly defined. However, extreme environmental pressures such as heavy metal contamination of soils (Humphreys and Bradshaw, 1977) or severe winter conditions (Helgadottir *et al.*, 2001) may produce detectable genetic shifts. Several genes may be responsible for a response to a given factor or the same gene(s) may be involved in different adaptive responses. Specific gene interactions may be in a state of flux or become fixed, restricting

opportunities for further evolution. Phenotypic plasticity serves as a buffer to avoid excessive genetic flux in response to short term changes.

Breeding for Improved Stress Tolerance

Forages are the backbone of sustainable agriculture; they are often grown in less favorable areas and thus require sophisticated protective mechanism to withstand severe environmental conditions (Wang, *et al.*, 2001). Because forage grasses and forage legumes often grow in marginal areas, stress tolerance is one of the most important traits in forage cultivar development. Conventional and genetic engineering approaches have been used to improve stress tolerance of forage grasses and legumes. Although technologies such as tissue culture to create somatic hybrids or gene transformation systems may become effective procedures for improving plant adaptation to abiotic stresses, conventional breeding through sexual hybridization is still the principal route for the development of stress-resistant varieties. However, modern conventional plant breeding is undergoing revolutionary changes that embrace new marker technologies and more profound understanding of the mechanisms that constitute complex traits. Genetic markers are assigned to the genes of interest to enable their selection in subsequent generation.

A need to broaden the gene pools of our major crop species and to use adaptations that have evolved in their wild relatives has led to major efforts in inter-generic and inter-specific plant breeding. Related wild species may extend the range of variation that can be used in crop improvement programmes. Many germplasm have been characterized with one or several specific traits that can be used to improve yield under drought. However, breeders have a number of good reasons not to introduce these so called unadapted parents into their breeding programmes due to the risk of rebuilding combinations of these drought genes and their neighbor genes to act together with other good genes (Richards, 1996). Genetic transformation offers a solution to overcome this issue. Transgenic approaches are expected to complement or accelerate conventional breeding, since they offer the opportunity to generate unique genetic variation that is either absent or has very low heritability. GISH can determine the location of alien *Festuca* gene and genetic marker ascribed to the targeted sequence (Humphreys *et al.*, 1998). *Lolium* lines carrying different *Festuca* genes that convey a range of adaptations to abiotic stresses are currently being developed alongside breeder's toolkits to aid their commercial exploitation. The most advanced introgression line is a *Lolium multiflorum* genotype with

genes on chromosome 2 for drought resistance derived from *Festuca arundinacea* (Humphreys and Pasakinskiene, 1996).

Genetics of Abiotic Stress Tolerance

Successful breeding depends on a broad understanding of the genetic architecture of relevant traits. Genes with major effects and genes contributing to the expression of quantitative traits both have a role in controlling abiotic stress tolerance. Traditionally, major gene and polygenic variation has been analyzed in different ways, but the use of new DNA markers and techniques of OTL analysis now allow to more integrated approaches in dissecting complex traits and assessing gene effects. Genetic fingerprinting of *Hordeum spontaneum* has revealed genetic marker associations with eco-geographic factors and experimentally imposed stresses (Forster *et al.*, 2000). Examples of QTL associated with valuable traits are increasing in wide range of crops including rice, wheat, maize, sorghum, barley, forage and turf grasses.

Useful information on the genetic basis of abiotic stress tolerance has been obtained by moving genes between plants of the same or closely related species. Gene introgression achieved by conventional cross pollination means that created a range of genetic variation available to understand and manipulate genetic adaptation to environmental change is greatly enhanced. In barley, greater variation to abiotic stress exists in primitive landraces and gene pools in related wild species (Forster *et al.*, 2000), which are easily hybridized with cultivated barley to provide extended sources of variation. Drought and cold tolerance has been improved in hybridization within the *Lolium / Festuca* species complex (Humphreys *et al.*, 1998). White clover plants have been produced with varying resource allocation in stolons and rhizomes, which also affects tolerance to stresses such as cold and drought (Marshall *et al.*, 2001). Advances in understanding the effectiveness of stress responses are also being made using transgenic plant analysis (Hasegawa *et al.*, 2000). However, it is also true that drought/salinity tolerant transgenic crop plants are yet away from the reach of farmers.

Stress Responses and Physiology

Tall fescue (*F. arundinacea*) $2n=6x=42$, a cool season perennial grass species is the most important forage species. Tall fescue is better adapted to avoid drought than other cool-season grasses such as other perennial ryegrasses partially due to bigger

root size (length or mass) and spatial distribution. A link was found between drought tolerance of tall fescue and enhanced deeper root growth under water limiting conditions (Huang and Fry, 1998; Huang and Gao, 2000). This findings has been used in drought tolerance breeding programmes by selecting low shoot-to-root ratios in turf type tall fescue populations (Bonos *et al.*, 2004). Obligatory summer dormancy (defined as plant dormancy in response to increased day length and probably high temperature) has been found in some cool-season perennial grasses (Ofir and Kigel, 1999). Obligatory summer-dormant tall fescue had better drought tolerance (Malinowski, *et al.*, 2005). The mechanism of obligatory summer-dormancy remained to be understood at the physiological, biochemical and molecular levels.

Alfalfa (*Medicago sativa* L., $2n=4x=32$) is grown extensively throughout temperate and tropical regions for green fodder, hay, silage and pasture. Alfalfa combines high biomass productivity, optimal nutritional profiles and adequate persistence, thus making it ideal for dairy cattle and other livestock (Brummer, 2004). As a perennial forage crop, alfalfa is a fairly hardy species and has a relatively high level of drought tolerance compared with many other legume forages (Barnes and Sheaffer, 1995). The greater drought tolerance of alfalfa is partially due to deeper roots and the ability to extract more available water in the root zone (Hall, 2001). Alfalfa becomes dormant during periods of cold or severe drought and may last for 1 to 2 years until the temperature or moisture available to resume growth (Barnes and Sheaffer, 1995). Screening for salt responsive proteins in two contrasting alfalfa cultivars using a comparative proteome approach revealed two novel proteins NAD synthetase and biotin carboxylase-3, as salt-responsive. These results provide new insight of salt stress tolerance in alfalfa (Rahman *et al.*, 2015). Effects of rhizobial strains on the amino acid composition in alfalfa under salt stress indicated that proline, glutamine, arginine, GABA and histidine substantially accumulated in salt stresses nodules, suggesting an enhanced production of amino acid associated with osmoregulation, N storage or energy metabolism to counteract salt stress (Bernard *et al.*, 2016).

The mechanism controlling winter hardiness in alfalfa (Lucerne) have been investigated with biochemical and molecular approaches. The differences in the level of freezing tolerance between non-hardy and hardy alfalfa cultivars was found to be related to the capacity of the plants to accumulate raffinose and stachyose in their roots and crowns other

than the capacity to accumulate sucrose (Castonguay *et al.*, 1995) earlier than non-dormant plants. During drought or salt stress, plants induce processes that regulate osmotic adjustment to maintain sufficient cell turgor partially through accumulation of compatible solutes comprised of mainly nontoxic low molecule chemicals *viz.*, sucrose, fructose sugar alcohols, proline and glutamic acid in shoots and roots. Accumulation of proline upon dehydration due to water deficit, high salinity and low temperature has been reported in bacteria, algae and higher plants and the causal relationship between increased proline accumulation and plant tolerance of hyper-osmotic stresses has been demonstrated (Hare *et al.*, 1999). Proline content in alfalfa leaves and roots increased dramatically when plants were subjected to drought (Goicoechea, *et al.*, 1998). Two genes controlling the transcriptional regulation of key proline cycle enzymes in alfalfa have been identified and cloned (Miller *et al.*, 2005).

White clover (*Trifolium repens* L. $2n=4x=32$) is an allotetraploid forage legume species widely distributed in the world due to its wide range of climatic adaptation (Pederson, 1995). But it is less tolerant to drought compared with other perennial temperate forage legumes because of its shallow root system and inability to effectively control transpiration (Annicchiarico and Piano, 2004). The major feature of white clover is its soloniferous habit. It spreads by growth of stolons with adventitious roots developing at the nodes. The persistence, under water stress, is largely dependent on the ability of vegetative stolons to survive variable periods of drought (Williams, 1987). So the development of a strong network of stolons is a prerequisite and stolon characters have a major focus of breeding efforts in this species (Sanderson *et al.*, 2003). Biochemical studies indicated that when white clover was stressed with water deficit, the *de novo* amino acid synthesis including proline was increased in both leaves and roots (Lee *et al.*, 2005). The phenomenon may serve as adaptive response during first few days in drought stress, as the transient increase of amino acid concentration was followed by decrease of protein synthesis that make the plants grow slower.

Cowpea (*Vigna unguiculata* L. Walp.) which is grown in varied environments from tropical to arid/semi-arid regions, enhanced drought and heat tolerance would be desirable. Enhanced drought tolerance in cowpea is accompanied with (i) better water-use efficiency and tolerance to water-deficiency and extreme heat conditions, (ii) better recovery of plants

after drought is removed *i.e.*, on re-watering. Two types of drought tolerance mechanisms observed. Type 1 : Lines stopped growth, conserved moisture in all the plant tissues and stayed alive for over two weeks and gradually entire plant dried together. Type 2 : Lines continued slow growth, mobilizes moisture from lower leaves to growing tips and remain live for longer time while lower leaves die one by one. Better regeneration after re-watering.

Both types of drought tolerance are dominant traits controlled by a single dominant gene Rds1 and Rds2 respectively. Test of allelism indicated that Type 1 is dominant over type 2 (when together) and the F₂ population between two types segregated in the ratio 3:1 (Type1:Type 2) plants. Breeding efforts to combine deep root systems with drought tolerance to enhance plants ability to absorb moisture from receding water after the rains ceases. Several drought tolerant lines have been identified *viz.*, TVu 11986, TVu 11979, IT93K – 451-1 (Type 1), while Dan Ila, IT89KD – 288 – 40, IT97K – 1025 – 18, IT 99K – 687, IT 99 K – 695 (Type 2) (Singh and Matusi, 2002).

The beach cowpea (*Vigna marina* ssp. *oblonga*) growing on sandy beaches in subtropical and tropical regions closest of the sea has potential to be a gene source for breeding salt tolerant cultivars. Chankaew *et al.*, (2014) for the first time reported the mapping of QTL for salt tolerance in *Vigna marina*, and multiple internal mapping consistently identified one major QTL which can explain 50% of phenotypic variance. The flanking marker may facilitate transfer of salt tolerance from this sub species into related *Vigna* crops.

Improvement of Stress Tolerance by Intergeneric Hybridization

Wide hybridization with relative species followed by chromosome and/or chromosome fragment introgression has been considered an efficient way to transfer, salt and other stress tolerance gene(s) to the target species to widen the gene pool. Intergeneric hybrids between *Lolium* (Ryegrass) and *Festuca* (Fescue) species have received much attention by forage breeders. Ryegrasses are considered the ideal grasses due to their rapid establishment, ability to withstand heavy grazing, good palatability and high nutritious value (Humphreys *et al.*, 2003). However, their growth is restricted only to some European countries, some regions in Australia, New Zealand and Southeast US because they are not sufficiently robust to meet many of the environmental challenges in less

favorite agricultural areas (Thomas *et al.*, 2003). Among the genetically closed relatives of ryegrasses are *Festuca* species that show better adaptation to abiotic and biotic stresses. Most of the species in this genus are more persistent due partially to their better developed root system (Ervin and Koski, 1998; Humphreys *et al.*, 1998). The close taxonomic relationship between the *Lolium* and *Festuca* species makes it possible to hybridize between them and transfer genes through recombination of homoeologous chromosomes. This is the major reason why a man-made species *Festulolium* has been used in many grass breeding programmes worldwide (Casler *et al.*, 2001; and Yamada *et al.*, 2005). In hybrids between *Lolium* spp. and *F. arundinacea* and its progenitors, intergeneric chromosome pairing and recombination is very frequent, but chromosomes of *Lolium* and *Festuca* can be distinguished by genomic in-situ hybridization (GISH). This has allowed a novel approach to breeding for stress tolerance, termed introgression mapping, whereby a range of different *festuca* genes has been introduced into *Lolium*, thereby “dissecting” complex QTL. GISH can determine the location of alien *Festuca* gene and genetic marker ascribed to targeted sequence (Humphreys *et al.*, 1998). Gene for drought resistance transferred from *F. arundinacea* were mapped onto chromosome 2 in two introgression lines of *Lolium multiflorum* (Humphreys and Pasakinskiene, 1996).

Drought tolerant ryegrass Tall fescue is a valuable gene source for drought tolerance. In back crossing programme involving *Lolium multiflorum* (the recurrent parent) and *F. arundinacea*, the diploid *L. multiflorum* phenotype was rapidly recovered with inclusion of a small number of genes from the fescue parent. In field drought trials, it was found that 3 % of the derivatives of these backcross populations were more drought resistant than *L. multiflorum* parental populations and as drought resistant as *F. arundinacea*. After polycrossing of selected drought resistant *Lolium*-like plants followed by one cycle of selection, the mean drought resistance of most progeny lines was significantly improved, in some cases to near that of *F. arundinacea* (Humphreys and Thomas, 1993). Tetraploid genotypes of perennial ryegrass demonstrated higher tolerance to cold and drought stress conditions, better spring growth and regrowth after cuts and higher dry matter yield in an evaluation of 128 diploid and 25 tetraploid ryegrass populations in short periods of lower than -10°C temperature with slim snow cover (Kemesyte *et al.*, 2017). Association analysis of single nucleotide polymorphism in Lp IRI1

gene with freezing tolerance traits in perennial ryegrass indicate that allelic variation in the LpIRI1 plays an important role in cell membrane integrity of perennial ryegrass during freezing and can be exploited for developing more freezing tolerant cultivars (Aleliunas *et al.*, 2015 and Dabkeviciene *et al.*, 2017).

White clover is a highly heterozygous out-crossing species with considerable variations available for improvement of many traits, but this is not the case for some desirable attributes including drought tolerance. Direct selection for drought tolerance has been carried out in the field but success has been limited (Abberton and Marshall, 2005). In white clover drought tolerance improvement programme, introgression has also been used as a route to transfer the morphological or physiological traits from its related wild species that show more drought tolerance or have better persistence. Hybrids of white clover (*T. repense* L.) and related species Kura clover (*T. ambiguum* M. Bieb) and ball clover (*T. nigrescens* L. Viv.) have been developed to introgress key traits such as drought tolerance and grazing tolerance into white clover gene pool. Caucasian or Kura clover is very persistent species with good drought tolerance due partially to its rhizomous habit (Meredith, *et al.*, 1995). A range of backcross hybrids using white clover as recurrent parent have been generated (Abberton *et al.*, 1998). In the third generation of backcross, individual plants that were white clover but with more rhizomes as well as stolons were obtained and their drought tolerance were superior to the white clover parent (Abberton *et al.*, 1998; Marshall, *et al.*, 2001).

Improvement of Stress Tolerance by Genetic Transformation

Genetic improvement of forages by conventional plant breeding is slow because of the fact that most forage species are self-incompatible, which limits inbreeding to concentrate desired genes for use in rapid development of new cultivars. Genetic transformation allows the direct introduction of desirable genes, thus offers new opportunities for molecular breeding of forages. Like many other crops, drought tolerance is an important target for improvement in alfalfa. As cuticular waxes play a pivotal role in limiting transpirational water loss across the plant surface, it is expected that genetic engineering of plant waxes may eventually increase environmental stress tolerance in crops of agronomic importance (Vogg *et al.*, 2004).

Medicago truncatula is an omnimediterranean forage legume species and is closely related to world's most important forage legume alfalfa, *Medicago sativa* (May, 2004). Two genes designated as WXP_1 and WXP_2 cloned from *Medicago truncatula* are novel transcription factor genes, which activate wax production in the acyl-reduction pathway. Over expression of WXP_1 and WXP_2 under the control of the CaMV35S promoter led to significant increase in cuticular wax loading on leaves of transgenic alfalfa. The increase in wax production was mainly contributed by the increase in C-30 primary alcohol. The electron microscopy scanning revealed that the density of wax crystalline structures on both adaxial and abaxial surfaces of mature leaves was higher in transgenic than in control plants. Transgenic leaves showed reduced water loss and chlorophyll leaching. Transgenic alfalfa plants with increased cuticular waxes showed enhanced drought tolerance demonstrated by delayed wilting after watering was ceased and quicker and better recovery when the dehydrated plants were re-watered (Zhang *et al.*, 2005).

A common problem in irrigated agriculture is the gradual buildup of salts in the root zone; which can be detrimental to sustained crop production. Salt stress significantly limits productivity of alfalfa via its adverse effect on growth and symbiotic nitrogen-fixation capacity. Recent progress has been made in the identification and characterization of the mechanisms that allow plants to tolerate high salt concentration. Identification of different sodium transporters *e.g.*, plasma membrane Na^+/H^+ antiporters allows the engineering of crop plants with improved salt tolerance (Apse and Blumwald, 2002). The antiporters are prevalent membrane proteins present in Bacteria, yeasts, animals and plants. The vacuolar Na^+/H^+ antiporter catalyzes the exchange of Na^+ and H^+ across the plasma membrane contributing to the regulation of internal pH, cell volume and sodium concentration. The vacuolar Na^+/H^+ antiporter can pump Na^+ from cytoplasm into vacuole, to maintain a higher K^+/Na^+ ratio in cytoplasm than in vacuoles, protecting cell from sodium toxicity.

Recently a vacuolar Na^+/H^+ antiporter gene cloned from rice was over expressed in perennial ryegrass by agro bacterium mediated transformation. The transgenic ryegrass plants had dramatically improved salt tolerance under 100-350 m mol/L NaCl treatment. The leaves of transgenic plants accumulated higher concentration of Na^+ , K^+ and proline than those of the control plants (Wu, *et al.*, 2005). Transcription

factors (TF) play classical roles in regulating various abiotic stress responses. The current developments in understanding TFs, with particular emphasis on their function in orchestrating plant abiotic stress responses have been discussed (Chen *et al.*, 2018 and Khan *et al.*, 2018).

Drought Tolerance via Symbiosos with Endophyte

Tall fescue (*Festuca arundinacea*) plants, the most widely planted forage grass, are commonly symbiotically infected with the endophytic fungus *Neotyphodium coenophialum* (Bouton and Easton, 2005). The relationship between the endophytic fungus and plant is generally considered mutualistic because endophyte significantly improves host plant tolerance to drought along with increased persistence and vigour and in turn plant provides the symbiont with nutrients, protection and reliable and efficient dissemination (Schardl *et al.*, 2004). Endophyte-infected grasses are better adapted than non-infected grasses to abiotic stresses *i.e.*, drought and marginal soil conditions due to direct changes affecting water status in shoots and indirect changes in root morphology and function (Malinowski and Belesky, 2000).

These adaptations may arise from a chemical signaling system in the symbiotum. Apparently, drought signals sensed by roots can be received by endophyte and induce a range of responses in host plants. Less is known about the chemistry of these signals. The possible mechanism of drought tolerance includes improved water uptake from the soil as of extensive root system - decreased root diameter and increased root hair length (Malinowski and Belesky, 2000), better control of transpiration by rapid stomata closure (Elmi and West, 1995), better water storage in tiller base by reduced leaf conductance (Elbersen and West, 1996) and enhanced drought tolerance by inducing rapid accumulation of compatible solutes like glucose, fructose, sugar alcohols proline and glutamic acid in shoots and roots. The fungal metabolites like mannitol and loline alkaloids also significantly increased with the water deficit (Bush *et al.*, 1997 and Nagabhyru *et al.*, 2013).

Animals feeding on endophyte infected (E^+) tall fescue cultivars suffer from fescue toxicosis which causes poor weight gain and reproduction problems (Sleper and West, 1996). Ergot alkaloids, especially ergovaline derived from the endophyte association are considered to be responsible for most animal problems (Lyons *et al.*, 1986). However, introducing endophyte-free tall fescue varieties has not been very successful

because of their poor persistence once exposed to abiotic stresses. A very useful approach is to isolate naturally occurring, non-ergot-producing strains and re-infecting elite varieties. One such novel endophyte strain AR542 has been selected in New Zealand, which was used to re-infect tall fescue varieties Jsup and Georgia 5 in the United States (Bouton *et al.*, 2002). More novel endophyte isolations have been characterized and are being used for re-infection of tall fescue and perennial ryegrass cultivars and breeding lines. Another promising approach to deal with this problem is genetic manipulation of *Neotyphodium* spp. endophyte to eliminate the toxin from the symbiosis (Panaccione *et al.*, 2001).

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