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A Review on Kentucky Bluegrass Responses and Tolerance to Drought Stress

Jian Cui, Saud Shah, Shah Fahad and Yajun Chen

Abstract

Kentucky bluegrass (*Poa pratensis* L.) is an excellent cool-season turfgrass and is extensively used in urban green space, parks and sports fields worldwide, but it is sensitive to drought stress. Drought reduces turf quality of Kentucky bluegrass by influences on the shoot density, texture, uniformity, color, growth habit and recuperative capacity. It has been a challenge for breeding water saving cultivars and enhances water use efficiency in Kentucky bluegrass. Many studies have revealed the mechanisms of drought stress tolerance in Kentucky bluegrass via multiple approaches. The morphological and physiological attributes as well as molecular information were discovered for better understanding and improving its drought tolerance. In this chapter, we will draw a systematic literature review about Kentucky bluegrass in response to drought stress and provide future perspectives of Kentucky bluegrass drought resistance research.

Keywords: Kentucky bluegrass, drought stress, tolerance, mechanism

1. Introduction

Drought is one of the major environmental factors that affect plant growth and survival worldwide. Turfgrass as an important part of the green ecosystem in the urban area which provides many kinds of environmental functions such as beautify and green the city, cooling warm weather, and soil stabilization. As urban rapid expansion nowadays, the percentage of land converted into turfgrass has been increasing. Turfgrasses are generally classified into cool- and warm- season groups based on their adaptation to specific ranges in temperature and precipitation, which are mainly governed by latitude and altitude [1, 2]. Kentucky bluegrass (*Poa pratensis* L.) is an excellent cool-season turfgrass and is extensively used in public parks, golf courses and residential lawns in temperate and cold temperate zones and cool plateau areas. This is a highly variable, rhizomatous species. Many improved cultivars of this species have been developed and used in landscaping areas in the world. A wide range of diverse cultivars and accessions of Kentucky bluegrass have been characterized based on pedigree, common turf performance, and morphological characteristics and were grouped into different genotypes such as Common, Compact, Compact-America, Julia, Mid-Atlantic and Midnight types [3]. Although these germplasm possess prominent ornamental value, however, Kentucky bluegrass extremely limiting used by water scarcity in practice, especially in semiarid, arid regions as well as the areas with the increase demand on water for

agricultural, residential and industrial use [4–6]. It has been a challenge to select water saving cultivars and reduce water input in turf management [7]. Drought reduces the turf quality of Kentucky bluegrass by influences on the shoot density, texture, uniformity, color, growth habit and recuperative capacity [8, 9], in response to drought stress, different genotypes of Kentucky bluegrass performed various adaptive mechanisms and strategies in respective to their morphology, physiology and molecular bases. Understanding these mechanisms of Kentucky bluegrass tolerance to drought stress is a key step for improving drought resistant cultivars and reduces water input in management. Here, we summarize research progress in drought stress of Kentucky bluegrass for providing an overview of the field to readers and also for providing guidelines for practical management strategies under limited water availability.

2. Responses to drought stress in Kentucky bluegrass

Kentucky bluegrass, like other agronomic, horticultural, and landscape vegetation, requires water for growth and provides esthetics functional benefits for environments. Main causes of water deficiency may result from low rainfall, inadequate irrigation, as well as summer heat, which could greatly limit growth, and turf quality of Kentucky bluegrass. Like other plants, the ability of Kentucky bluegrass to maintain growth and survive under drought stress is broadly considered as drought resistance. Three major strategies of plant drought resistance are considered as escape, avoidance, and tolerance [2, 10] and are illustrated in **Figure 1**. Drought resistance traits vary genetically by exhibiting survival strategies among Kentucky bluegrass cultivars under water limited conditions [11]. However, these strategies are not mutually exclusive, and Kentucky bluegrass may utilize more than one when facing to water shortage.

2.1 Turf performance and morphological responses

Turf quality (TQ) refers to two aspects including visual quality and functional quality [1]. Turfgrass density, texture, uniformity, color, growth habit and smoothness are the most visible factors influence turf appearance quality, while functional quality such as playability in a particular sport turf is determined not only by some of the visual determinants, but also by other characteristics as well, including rigidity, resiliency, verdure, rooting and recuperative capacity. The drought resistance characteristics of specific species in morphology, growth patterns largely determine the turf quality under drought conditions [12, 13].

Morphological traits on Kentucky bluegrass often change with soil moisture [14], and plants often exhibit a severe decline in TQ and may become dormant during extended drought conditions [15]. Although Kentucky bluegrass can escape by going into dormancy under severe drought conditions especially during summer periods [16]. People still desire to sustain a green surface during drought periods for esthetics, sports, and other eco-functions. Therefore, drought escape is only considered a viable alternative for Kentucky bluegrass in those areas where irrigation is not available and survival of the turfgrass following drought is the primary objective [17].

Water use of the turfgrass canopy is influenced by water loss via shoot transpiration and soil evaporation, and by water uptake from the soil through the root system. The differences among Kentucky bluegrass cultivars in shoot and root characteristics such as leaf orientation and canopy configuration, tiller or shoot density, rooting depth, and root density are associated with water-use rate [18].

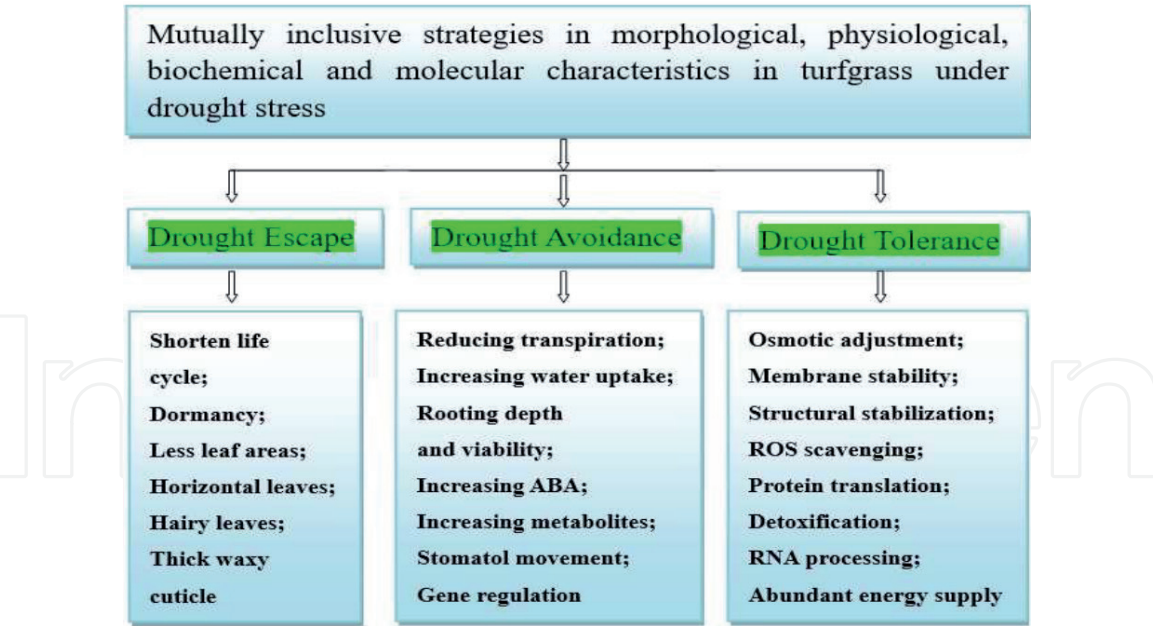


Figure 1.
Drought resistance strategies of turfgrass under drought conditions (illustrated by Jian Cui).

Lower ET rates of grass species were generally characterized with comparatively a high shoot density and relatively horizontal leaf orientation; and also with a low leaf area, including a slow vertical leaf extension rate and a narrow leaf texture [19]. Shoot vertical extension rate was positively correlated with water-use rate for Kentucky bluegrass with upright growth cultivars [20]. Based on a random spaced plant of 61 Kentucky bluegrass cultivars under untrimmed conditions, the morphological properties and comparative water use rate were strongly correlations by use discriminant analysis [21]. Low-water use cultivars had 13% more horizontal leaf orientation, 6% narrower leaf texture, 13% more lateral shoots per plant, 12% slower vertical leaf extension rate, 2% more leaves per shoot, and 7% shorter leaf blades and sheaths than the high-water use cultivar [21]. Low soil water content resulted significantly different in shoot-to-root ratio, vertical growth and survival rate among 11 Kentucky bluegrass cultivars, and more influence on tillering rate than other morphological indicators [11]. The components of leaf epidermis including stomatal apparatus, silica cells and cuticle are very important to the drought resistance of turfgrass canopy. Chen et al. found that leaf epidermis characteristics were associated with drought resistance among Kentucky bluegrass cultivars [22]. The opening or closing status of stomatal apparatus, the silica cell size and density, the thickness of the wax layer on leaf surface were related to the drought resistance and varied among Kentucky bluegrass cultivars (**Figure 2**).

Generally, an extensive deep root system of plants is important for efficient water uptake from the soil. Deep rooting enables plant to avoid water stress by taking up water from deeper in the soil profile when the surface soil is dry. The increased drought resistance of Kentucky bluegrass cultivars was correlation to the increased water uptake activity at the 15 to 30 cm soil depth [23]. Deeper root system in Kentucky bluegrass can avoid drought stress more consistently than total root mass [24]. However, other research found that not all turfgrasses with extensive root system are necessarily high water use. Hence, improvements in drought tolerance in Kentucky bluegrass may not necessarily occur by selecting germplasm with deep rooting or high root to shoot ratios due to no correlation between deep rooting and the ability to withstand long periods of water deficit [18]. Study on Kentucky bluegrass with the relation of Carbon-13 discrimination and water use efficiency (WUE) showed that turfgrass performance under drought may be

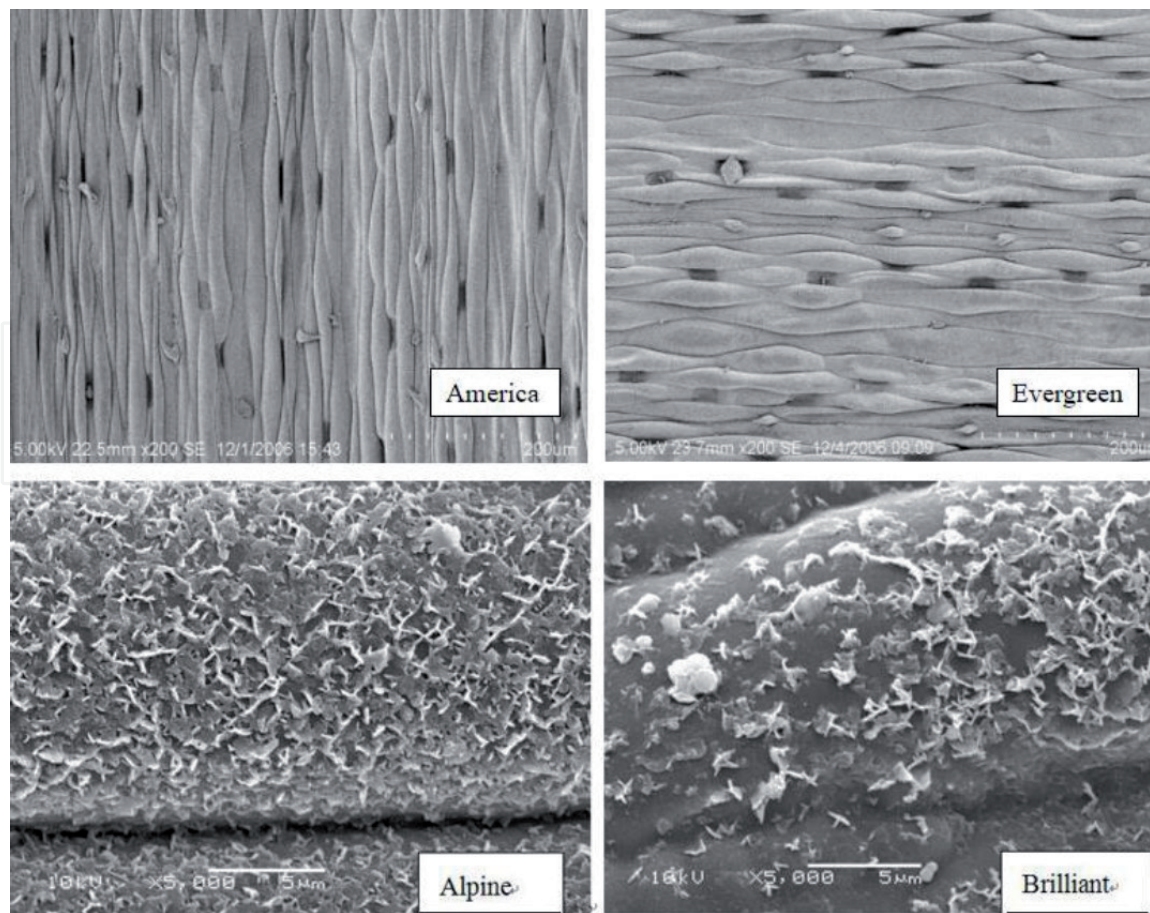


Figure 2.
Leaf epidermis characteristics among Kentucky bluegrass cultivars (photos provided by Yajun Chen).

improved for low Carbon-13 discrimination, which has been shown to be negatively correlated with WUE. Low Carbon-13 discrimination values were associated with less wilt and leaf firing, suggesting that Carbon-13 discrimination may be a useful selection criterion for superior performance under limiting soil moisture [25]. Kentucky bluegrass is a typically rhizomatous perennial, and ramets distributions in the grass clonal system connected by rhizomes largely rely on water status in soil. Drought stress severely influenced all agronomical, anatomical attributes of Kentucky bluegrass especially the seeking water behavior of rhizomes in both homogenous and heterogeneous environments [26]. Understanding these morphological characteristics respond to drought may assist to selecting low-water use Kentucky bluegrass cultivar in future for better adaptation water scarcity environment and saving water in turf management [27].

2.2 Bio-physiological and molecular in responses to drought

Severe drought can cause Kentucky bluegrass to go into dormancy and lose its greenness, which is a survival strategy as we described above, but we do not expect Kentucky bluegrass to adopt escape drought strategy to lose its landscape value during water deficiency. However, three drought resistance strategies including escape, avoidance and tolerance may be used simultaneously in different drought settings for specific turfgrass, and so does Kentucky bluegrass [2]. Drought-avoiding traits on Kentucky bluegrass can exhibit many different morphological characteristics and water absorption ability in response to drought, including previous reports as discussed before on leaf orientation and canopy configuration, tiller or shoot density, rooting system, leaf epidermis, lower ET and the like. Drought avoidance and tolerance are critical strategies for maintenance turf greenness and extending

green period, fitness turf visual and functional quality under short or long drought stress. Many desirable drought resistance attributes on Kentucky bluegrass involve in physiological and biochemical metabolic activities as well as molecular regulators can serve as selection criteria for improving drought resistance cultivars using for turfgrass practical managements. For maintenance structural stabilization under drought stress, strategies on physiological responses of Kentucky bluegrass exhibited by increasing cellular osmotic potential and antioxidant enzyme activities, suppressing reactive oxygen species (ROS) and ABA production, etc.

The sucrose, fructose, glucose, starch as well as carbohydrate metabolism enzymes associated with plant tolerance to severe drought stress and post-drought recovery in two Kentucky bluegrass cultivars 'Midnight' and 'Brilliant' [28]. The differential in accumulation of different types of soluble carbohydrates could be related to the genetic variability and biological functions during drought and post-drought recovery in Kentucky bluegrass. These soluble sugars as osmolytes play critical role in maintenance cellular turgor by increasing osmotic potential and energy sources in photosynthetic process, and sucrose also plays protective roles in proteins and membranes from drought damages [29]. Sucrose, proline, as well as inorganic ions are important osmolytes contributing to in cell osmotic adjustment when turfgrass faced to drought stress [30]. In addition, another interesting result was that superior drought resistance for 'Midnight' Kentucky bluegrass could be characterized by the accumulation of sucrose in association with increased activity of sucrose-synthesizing enzymes (sucrose phosphate synthase and sucrose synthase), suggesting that increased sucrose accumulation resulting from the maintenance of active sucrose synthesis could relate to superior turf performance during water loss in Kentucky bluegrass [28]. Two Kentucky bluegrass cultivars contrasting in drought tolerance were evaluated the photosynthetic responses and underlying enzyme activities during drought stress and re-watering [31]. Compared to 'Brilliant', drought-tolerant 'Midnight' maintained significantly higher net photosynthetic rate, higher enzymatic activity and transcript level of ribulose-1,5-bisphosphate carboxylase (Rubisco), higher enzymatic activity of glyceraldehyde phosphate dehydrogenase (GAPDH) during 10-d drought stress and in responses to re-watering, as well as higher Rubisco activation state upon re-watering, suggesting that carboxylation controlled by Rubisco and carbon reduction regulated by GAPDH could be the key metabolic processes imparting genetic variation in photosynthetic responses to drought stress while active Rubisco, GAPDH and Rubisco activase could all be involved in the superior post-drought recovery in Kentucky bluegrass [32]. Drought-tolerance 'midnight' and drought-sensitive 'Brilliant' as drought research model cultivars of Kentucky bluegrass have been detected 88 drought-responsive proteins by gel electrophoresis and mass spectrometry analyses. Many proteins involved in amino acid or energy metabolism were down regulated under drought stress, but most of those proteins had higher abundance in 'Midnight' than in 'Brilliant'. These proteins may serve as drought stress responsive proteins imparting Kentucky bluegrass adaptation to drought [33].

One of the major drought avoidance strategies in plant is the antioxidant physiological response by increasing antioxidant enzymes activity or by developing an effective reactive oxygen species (ROS) scavenging system to suppress ROS production which is a major cause of cellular injuries induced by drought [34]. Researches have revealed that availability of antioxidant enzyme activity and induced gene relative expression, the accumulation of abscisic acid and the level of membrane fatty acid saturation were important metabolic factors contributing to enhance drought resistance in Kentucky bluegrass although the responses in cultivars, organs and growth stages were varied [35–38]. Some studies found that the activity of superoxide dismutase (SOD) increased while that of catalase (CAT) and peroxidase (POD) decreased in leaves of Kentucky bluegrass when only surface soil drying

was simulated [39]. The increased SOD activity in Kentucky bluegrass leaf cannot inhibit formation and accumulation of free radicals, but only delay accumulation of free radicals to a certain extent for alleviate active oxygen damage to cells [40, 41]. Under drought stress, the increased superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) in leaves and roots of Kentucky bluegrass were associated with lipid peroxidation. In addition, the increases in the activities of ascorbate peroxidase (APX), monodehydroascorbate reductase (MHAR), dehydroascorbate reductase (DHAR) in leaves and that of CAT, glutathione reductase (GR), and MHAR in roots, but reduction in the activities of SOD and DHAR in roots [42]. After 6 days of rewatering, 'Midnight' displayed significantly higher activity levels of CAT, POD, and APX compared with 'Brilliant'. The differential responses of the activities of antioxidant enzymes to drought stress and post-drought rewatering between 'Midnight' and 'Brilliant' indicated that antioxidant enzymes including APX, SOD, GR, MR (monodehydroascorbate reductase), and DR (dehydroascorbate reductase) in the AsA-GSH (ascorbate–glutathione) cycle may play important protective roles involved in scavenging oxidant stress induced reactive oxygen species in Kentucky bluegrass for cellular survival of severe water deficit and post-drought recovery [43]. The increased content of unsaturated lipids in Kentucky bluegrass leaves under drought stress was crucial to maintain cell membrane fluidity and reduce ROS production. Leaf dehydration tolerance and postdrought recovery in Kentucky bluegrass was associated with their ability to maintain relative higher proportion and level of unsaturated fatty acids, particularly linolenic acids and linoleic acids [44, 45]. These researches on antioxidant metabolic responses revealed key information for controlling drought tolerance in turfgrass species and would facilitate the development of drought-tolerant germplasm through biotechnology.

So far, the important physiological mechanism of plant adaptation to drought resistance is ABA (Absciscic acid) accumulation, the amount of the primary chemical of ABA can reach up to more than 50-fold in plants under drought conditions [46, 47]. ABA is one of the most drastic changes observed hitherto in the concentration of a plant hormone responding to an environmental stimulus [37]. The functions and the signaling pathways of ABA in plants' responses to drought stresses have been extensively studied, and it is now well accepted that ABA plays important roles in plant including turfgrass adaptation to environmental stresses [5, 48, 49]. The relationship of ABA accumulation and drought resistance in different genotypes of Kentucky bluegrass varied. Leaf ABA content in drought susceptible cultivars increased sharply after 2 days of drought stress to as much as 34-fold the controls, while in drought resistant cultivars, the content ABA in leaves also increased with drought, but to a lesser extent than in drought sensitive cultivars. In addition, the stomatal conductance, photosynthetic rate, leaf water potential and turf quality in drought resistant cultivars performed less severe decline during drought than drought sensitive cultivars, indicating that stomatal conductance was negatively related to ABA accumulation and the ABA concentration during drought could regulate by stomatal behavior of Kentucky bluegrass [31]. The exogenous ABA application on Kentucky bluegrass demonstrated that ABA sprayed on leaves can help maintain higher turf quality and delayed the quality decline during drought stress. ABA treated grass had higher cell membrane stability indicated by less electrolyte leakage of leaves, and higher photochemical efficiency [50]. ABA effects on shoot growth and stomata, it may also facilitate osmotic adjustment and expression of specific proteins [6]. Further study suggesting that ABA accumulation in response to drought stress could be used as a metabolic factor to select for drought tolerance in Kentucky bluegrass [35]. Some studies on Kentucky bluegrass discovered that the interaction of endogenous hormones could contribute to increase drought resistance. Under drought conditions,

the concentrations of ABA, JA (Jasmonate) and BR (Brassinolide) increased significantly compared to well water plants. Drought stressed Kentucky bluegrass had higher leaf ABA, lower leaf trans-zeatin riboside (ZR), isopentenyl adenosine (iPA), and indole-3-acetic acid (IAA), but similar level of leaf gibberellin A4 (GA4) when contrasted to the control, suggested that drought stress-induced injury to Kentucky bluegrass may be associated with hormonal alteration and may have better photosynthetic function and performance [5, 51].

Recently, a great progress on biological omics such as metabolomics, proteomic and transcriptomic have revealed many drought response metabolites, proteins and molecular factors in major crops [2]. However, information on Kentucky bluegrass involved in molecular mechanisms and genes underlying drought resistance is lacking. Limited researchers have identified some drought-responsive genes and gene signaling transduction pathways as described below. Under drought stress and water recovery treatments, the gene expression patterns of antioxidant enzymes of Kentucky bluegrass were differentially or cooperatively involved in the defense mechanisms in the leaves and roots. For the leaves, the expressions of iron SOD (*FeSOD*), cytosolic copper/zinc SOD (*Cu/ZnSOD*), chloroplastic *Cu/ZnSOD*, and *DHAR* were down-regulated by drought stress but recovered to control level after rewatering, while the expressions of *GR* and *MDHAR* were up-regulated and remained that levels after recovery. For the roots, the expressions of cytosolic *Cu/ZnSOD*, manganese SOD (*MnSOD*), cytosolic *APX*, *GR*, and *DHAR* were down-regulated under drought stress but recovered except for *GR* and *DHAR*, while *MDHAR* expression was up-regulated. No differences in *CAT* transcript abundance were noted among the treatments [42]. Transcriptome sequencing on cuticular wax deposition of Kentucky bluegrass have discovered that a number of genes involved in very longchain fatty acids and cuticular wax biosynthesis, transportation and regulation pathways, and these genes presented differentially expressed patterns between the leaf non-elongation zone and the emerged blade zone [52]. There were 9 candidate reference genes expression stability in the leaves and roots of Kentucky bluegrass under different stresses (drought, salt, heat, and cold), and were evaluated using the GeNorm, NormFinder, BestKeeper, and RefFinder bio-tools. Among 9 genes, *ACT* and *SAM* maintained stable expression in drought-treated leaves, and *GAPDH* combined with *ACT* was stable in drought-treated roots. The expression stability of reference genes in Kentucky bluegrass will be particularly useful in the selection of stress-tolerance genes and the identification of the molecular mechanisms conferring stress tolerance in this species [53]. A comparative transcriptomic study also found that many differentially expressed genes were enriched in 'Plant hormone signal Transduction' and 'MAPK signaling pathway-Plant'. Some key up-regulated genes, including *PYL*, *JAZ*, and *BSK*, were involved in hormone signaling transduction of abscisic acid, jasmonic acid, and brassinosteroid and possibly these genes play important roles in coping with drought stress in Kentucky bluegrass under drought stress [5]. These studies suggest importance of molecular functions and related genes for protection and improvement in Kentucky bluegrass tolerance to drought, however, molecular mechanisms underlying drought tolerance in Kentucky bluegrass are largely unknown.

3. Summary and future perspectives

Global climate change and the continuously growing population in the world, result increasingly limited and more costly in water availability. Water conservation in turf management becomes extremely important. Kentucky bluegrass is one the most popular and widely used cool-season turfgrass for amenity, sports and environmental conservation. Turf industry has released many improved Kentucky bluegrass

cultivars in the world since last century. Among these germplasm, a wide range in water consumption variation represented by the daily evapotranspiration rate [20]. Hence, to identify ideal water conserving properties on Kentucky bluegrass in reducing irrigation requirements by enhance drought tolerance has been pursued by scientists. Many researchers have revealed a great deal of results in the understanding of morphological and physiological traits and mechanisms responding to drought resistance with Kentucky bluegrass. In response to drought stress, various adaptation strategies of Kentucky bluegrass were taken based on morphology, physiology and genetics. Narrow leaf texture, leaf orientation and canopy configuration, shoot and root systems are associated with Kentucky bluegrass drought resistance. Moreover, the physiological characteristics with the increased ABA and metabolites to enhance cell water potential and regulating stomatal movement, the protein translation and ROS scavenging to protect membrane stability are vital strategies for Kentucky bluegrass survive and maintain greenness under drought conditions. However, although some drought-responsive genes and gene signaling transduction pathways as well as proteins for Kentucky bluegrass tolerance to drought stress have been identified, our knowledge of the response is still limited information on how whole plants perceive and conductive these signals under long period drought. On Kentucky bluegrass, systemic study on the molecular mechanisms of drought resistance is still lagging. Identification of novel candidate genes, proteins, metabolites, and molecular markers, and integrating transcriptomics, proteomics and metabolomics to explore intercellular communication and drought resistance manipulating signals are crucial for molecular breeding and marker-assisted selection of Kentucky bluegrass with superior drought tolerance in future.

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