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Plant Growth and Morphophysiological Modifications in Perennial Ryegrass under Environmental Stress

Fuchun Xie, Rahul Datta and Dong Qin

Abstract

Perennial ryegrass (*Lolium perenne* L.) is a popular and important cool-season turfgrass used in parks, landscapes, sports fields, and golf courses, and it has significant ecological, environmental, and economic values. It is also widely used as forage and pasture grass for animals around the world. However, the growth of perennial ryegrass is often affected by various abiotic stresses, which cause declines in turf quality and forage production. Among abiotic stresses, drought, salinity, temperature, and heavy metal are the most detrimental factors for perennial ryegrass growth in different regions, which result in growth inhibition, cell structure damage, and metabolic dysfunction. Many researches have revealed a lot useful information for understanding the mechanism of tolerance to adverse stresses at morphophysiological level. In this chapter, we will give a systematic literature review about morphological and physiological changes of perennial ryegrass in response to main stress factors and provide detail aspects of improving perennial ryegrass resistance based on research progress. Understanding morphophysiological response in perennial ryegrass under stress will contribute to improving further insights on fundamental mechanisms of perennial ryegrass stress tolerance and providing valuable information for breeding resistance cultivars of perennial ryegrass.

Keywords: perennial ryegrass, morphology, physiology, abiotic stress, stress resistance

1. Introduction

Urban green areas have important various functions contributing to the quality of human health. Well-kept lawns enhance the esthetic value of the entire city and are involved in phytoremediation, leading to an improvement in the quality of the air and soil [1–4]. Perennial ryegrass (*Lolium perenne* L.) is an important and widespread perennial cool-season grass cultivated in temperate climates, originating in Europe, temperate Asia, and North Africa [5]. Perennial ryegrass is commonly used in home lawns, sport fields, and parks with rapid growth and establishing rate, and other elements for ecosystem service due to its massive root system, superior regeneration,

and tillering ability. It is also widely used as nutritive forage and pasture grass for animals around the world [6–8]. Moreover, numerous perennial ryegrass genotypes and hybrids are now released by commercial utilities [9, 10].

In fields, the growth and development process of plants needs to counteract various environmental stresses such as salinity, drought, cold, heat, and heavy metal [11–13]. Harsh environmental conditions may result in growth inhibition, cell structure damage, and metabolic dysfunction [14–20]. Moreover, stresses will further be intensified for the potential impact of climate change in future. Thus, maintaining proper growth of turfgrass with minimal inputs under abiotic stress conditions is a great challenge for turfgrass industry. This challenge could be addressed through improving the stress tolerance of turfgrass [14, 21]. Understanding morphological and physiological mechanisms of turfgrass adaptation to various abiotic stresses is a key step for the development of stress-tolerant ability and cost-effective and efficient management practices [13]. Morphophysiological mechanisms of turfgrass in abiotic stresses tolerance involve phenotypic changes, multiple physiological and biochemical response, and complex metabolic processes, such as water and nutrient relations, carbohydrate metabolism, protein metabolism, hormone metabolism, as well as antioxidant defenses [22, 23]. Current studies on morphophysiological mechanism controlling turfgrass adaptations to various growth conditions have provided important information for production of abiotic stress-tolerant germplasms and the further understanding of regulation mechanism of turfgrass response to abiotic stresses [13, 24, 25]. However, the mechanisms of the adaptive responses are integrated but are not necessarily the same [14]; thus, studies on how perennial ryegrass adapts to stress conditions will become more important with the increasing pressure of utilizing both ecological and economical strategies in the turf management. Furthermore, insights into mechanisms of stress resistance in perennial ryegrass will aid in identifying important characteristics for selecting the criteria of improving stress tolerance and will ultimately lead to better selection of new cultivars adapted to adverse environments. This chapter, therefore, focuses on an extensive overview of the current understanding of changes in physiology and growth/development of perennial ryegrass under various abiotic stresses. In addition, strategies for improving the stress tolerance of perennial ryegrass are also presented. This review can contribute to the better understanding of the mechanisms of perennial ryegrass response to environmental stresses and can provide valuable information for improving resistance characteristics of perennial ryegrass by breeding. Moreover, enhancing our understanding of physiological effects of abiotic stresses can provide guidelines for the practical management strategies of the maintenance of high-quality turf under limited resource availability.

2. Abiotic stresses

Abiotic stresses are major environmental conditions that reduce plant growth, productivity, and quality. Plants have evolved mechanisms to perceive these environmental challenges, transmit the stress signals within cells as well as between cells and tissues, and make appropriate adjustments in their growth and development for survive and reproduce [26–29]. The morphological and physiological changes of perennial ryegrass under abiotic stress will be discussed in this chapter.

2.1 Responses of drought stress in perennial ryegrass

Growth and development processes are inhibited when plant is exposed to drought stress [30–36]. Morphological adjustments, such as biomass allocation and leave

changes, have been proposed as the key mechanisms used by turfgrass to enhance survival under drought [37]. There is a series of morphology changes in perennial ryegrass under drought stress. Drought stress reduced the turf quality (TQ), number of live tillers and dry-matter yield [38–40]. Moreover, drought significantly enhanced root to shoot ratio (R/S) in perennial ryegrass to an less extents, depending on the intensity, the reason may be that perennial ryegrass in drought stress develop a large R/S to maintain water and nutrient uptake [39]. The leaves of perennial ryegrass under drought stress were also dramatically different from that of nonstressed perennial ryegrass, for example, under drought stress, the diurnal variation in the rate of leaf extension was smaller but the leaves tended to grow faster at night compared to normal irrigation controls; however, water stress ultimately reduced the rate of leaf extension and leaf area in perennial ryegrass [40]. Furthermore, the leaves' epidermis of perennial ryegrass under drought reduced the stomatal size and increased the numbers per unit leaf area. Drought also resulted deeper ridging on leaf ad-axial surface, smaller epidermal cells and bigger ridge angle [40]. Under drought stress, leave stomata of perennial ryegrass began to close to reduce their evapotranspiration rate (ET), at leaf water potentials below—13 bars [40].

Drought stress causes significant physiological changes, including photosynthesis, osmotic adjustment substances, proteins, and antioxidant metabolism, in perennial ryegrass. For instance, the content of leaf total nitrogen and leaf relative water content (RWC) were tested to decrease, on the contrary, antioxidant activity including ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione S-transferase (GST), amino acids such as aspartic acid, threonine, serine, glutamic acid, abscisic acid (ABA) concentration, and proline content increased under drought stress [41]. Photosynthesis is the primary process controlling plant growth and adaption to drought stress [42]. The canopy photosynthesis of perennial ryegrass at saturating light intensity was reduced by about half in the stressed field swards and by more than 80% in the stressed simulated swards [38]. Drought stress inhibits photosynthesis, which may be the result of low CO₂ availability caused by stomatal closure and/or the inhibition of photochemical reactions and carbon assimilation metabolism [43]. In addition to the photosynthesis, starch is also considered as a buffer for imbalance between acquisition by photosynthesis and C-sink activities such as growth and respiration resulted from drought, also stress due to the excessive use of inorganic fertilizer [44]. However, use of green manure also has risk of xenobiotic contamination [45–47], and the soluble sugars, including sucrose, fructose and glucose, are involved in multiple physiological functions such as respiration, turgor maintenance, signaling and defense. Under drought conditions, starch of perennial ryegrass significantly decreased in shoots, but did not change in roots, which indicated that perennial ryegrass in drought condition preferentially allocates carbon not only to root growth, but also to root storage, while soluble sugars were enhanced in both shoots and roots. Accumulation of soluble sugars has been widely reported for plants upon water stress as a means to provide osmotic protection [39], which suggested that increasing of soluble sugars was benefit to plants to maintain growth and active metabolic activities under water deficit.

It is generally accepted that there is a noticeable genotypic variation in perennial ryegrass for drought stress responses. The research showed that one self-pollinating genotype “S10” showed higher RWC, shoot dry weight (SDW), proline, ABA, nitrogen and amino acid contents, and antioxidant enzymes activities in comparison with two commercial genotypes of “Vigor” and “Speedy” [41]. Proteins involved in carbon and energy metabolism, photosynthesis, tricarboxylic acid cycle (TCA) cycle, redox, and transport categories were upregulated in the two commercial genotypes of “Vigo” and “Speedy,” while the protein profile of the “S10” changed

slightly under drought stress, and the reason may be that self-pollination in the genetic background of the “S10” genotype may have a lower variation in response to drought stress conditions [41]. Additionally, other research indicated that tetraploid perennial ryegrass exhibited a greater biomass under severe drought, whereas diploids had a greater biomass under the current rainfall [48, 49]. Moreover, tetraploid perennial ryegrass populations were able to develop more shoot and root dry matter than diploid populations in following the application of drought stress [50].

The above researches showed that drought stress caused significantly physiological and morphological changes in perennial ryegrass (**Table 1**) [55, 56]. Thus, the growth of perennial ryegrass is severely restricted by soil water deficits [57]. Increasing drought tolerance of perennial ryegrass via strategies is importance for both water conservation and maintaining growth in water limiting environments. For example, the grass-*Epichloë* endophytic improved water utilization and drought tolerance in perennial ryegrass [58]. Moreover, arbuscular mycorrhizal fungi (AMF) + *Epichloë* treatments increased phosphorus (P) uptake, net photosynthetic rate (P_n), root activity, soluble sugar concentration, peroxidase (POD) activity, and decreased malonyldialdehyde (MDA) concentration in perennial ryegrass under drought stress, the reason may be that Plant-AMF-*Epichloë* symbiosis alleviated the damage caused by drought stress by promoting P uptake, photosynthesis, and the accumulation of osmoregulatory substances [59]. Additionally, application of plant growth regulators (PGRs) have been reported to be a promising way of reducing drought stress impacts [60]. The study manifested that trinexapac ethyl (TE) treatment increased chlorophyll content, proline content, the RWC, soluble sugar content, antioxidant enzymes activities, decreased MDA and hydrogen peroxide (H_2O_2) contents in perennial ryegrass under drought stress, while Paclobutrazol (PAC)- and ABA-treated perennial ryegrasses were all effective in mitigating physiological damages resulting from drought stress [52]. Furthermore, overexpression of some drought-related genes has been shown to effectively improve drought tolerance of plants [61]. According to Patel et al. [53], overexpression of *LpHUB1* gene conferred drought tolerance in perennial ryegrass.

2.2 Responses of temperature stress in perennial ryegrass

Perennial ryegrass can grow throughout the year, and the major constraint on growth is temperature [51, 62]. Perennial ryegrass has an optimal growth temperature of about 20°C, and it is sensitive to high (30–40°C) and low (–20 to 0°C) temperatures [63, 64]. Common perennial ryegrass germinates quickly and can be used as a temporary ground cover while the slower growing bluegrass plants take

Morphological responses	Physiological responses	Strategies
<ul style="list-style-type: none">• Decreased turf quality• Enhanced R/S• Decreased leaf area• Reduce the number of live tillers• Had smaller stomata and epidermal cells• Had bigger ridge angle• Controlled stomatal opening [38–40]	<ul style="list-style-type: none">• Decline biomass• Decline photosynthetic rate• Increased osmotic adjustment substances• Increased antioxidant activity• Increased amino acid content [39, 41, 51]	<ul style="list-style-type: none">• Application of plant growth regulators (PGRs)• Selected drought resistance cultivars from different cultivars• Using endophytes• Using transgenic technology [52–54]

Table 1.
Morphophysiological response of perennial ryegrass under drought stress.

hold in cool temperate region. In warm climates, it is used as an overseed to maintain winter green in the lawn after the warm season grasses go dormant. However, populations of perennial ryegrass will not survive the summer heat. Severe heat stress (40/35°C day/night) caused significant physiological damages, including declining in TQ, RWC, CAT activity, and enhancing in electrolyte leakage (EL) of leaves and MDA content, in perennial ryegrass [65]. Moreover, heat stress decreased plant height (HT), leaf fresh weight (LFW) and leaf fresh dry (LFD), and increased cytokinin and auxin at 35/30°C (day/night) of temperature [66]. Moreover, low temperature is one of the main factors that limit the persistence of perennial ryegrass-dominated grasslands in northern regions. Cold stress decreased TQ, regrowth, dry weight, and tiller density in perennial ryegrass when the winters were mild with short (2–6 weeks) periods of lower than –10°C temperatures and no permanent snow cover [67]. Furthermore, cold stress decreased RWC and increase EL in leaves and roots when perennial ryegrass was exposed to –15 or –25°C [68]. To resolve these problems and maintain high visual quality of perennial ryegrass through the year, it is important to found new cultivars to adapt to temperature stress. Variations of heat- or cold- resistance were also found among different perennial ryegrass cultivars. Thus, selection cold- or heat-tolerant cultivars during perennial ryegrass genotypes can be an effective method for temperature tolerance improvement in perennial ryegrass. The research tested the heat tolerance of 58 cultivars collected from seed companies and research centers in U.S.A., New Zealand, and Europe, the result showed that distinct heat tolerance was found among the cultivars at all the temperature regimes, and the least and most tolerant cultivars were “JPR005” and “JPR178,” respectively [69]. The other research indicated that changes of morphology and physiology were different for heat-tolerant accession “PI265351” and sensitive accession “PI225825” [66]. Similarly, the heat-tolerant populations of perennial grass showed significantly lower degree damage in efficiency of photosystem II and cell membrane stability than the sensitive ones at different levels of stress [70]. Additionally, the study showed that 21 accessions sampled from a larger set of 300 accessions with known winter hardiness, the result showed that the degree of semi-lethal temperature in 21 ryegrass varieties varies from –10.31 to –13.95°C, with 3 accessions possessing significantly greater freezing tolerance than the most freeze-tolerant check “NK200” [71]. Moreover, tetraploid genotypes of perennial ryegrass demonstrated higher tolerance to cold stress conditions, better spring growth, and regrowth after cuts, and higher dry matter yield compared to diploid genotypes [67].

The studies indicate that temperature stress caused the morphological and physiological damage in plant, and the response of genotypes to temperature stress was different [72–80]. Therefore, founding some strategies which could improve cold- or heat-tolerant of perennial ryegrass is important. It was also reported that 24-epibrassinolide promoted carbohydrates accumulation in crowns of perennial ryegrass during cold acclimation by regulation of gene expression and enzyme activities, and which resulted in increased frost tolerance [81]. Moreover, drought preconditioning increased in crown fructans, proline, and total soluble protein content for “Buccaneer” and “Sunkissed” during cold acclimation, which suggested a synergistic effect between drought exposure and low temperature, and drought preconditioning resulted in an improvement in freezing tolerance of perennial ryegrass [82]. Additionally, previous studies have shown that the enzyme activity level and gene expression of antioxidants are associated with cold and heat tolerance in a cool-season perennial grass species [83, 84]. For instance, *LpHOX21* was positively associated with heat tolerance of perennial ryegrass [85]. Similarly, P450 gene (*LpCYP72A161*) showed remarkable upregulation in perennial ryegrass under heat and cold treatment. Therefore, transferring key genes into perennial ryegrass will be beneficial to improve heat or freezing tolerance [86].

2.3 Responses of salt stress in perennial ryegrass

Salinity stress has become a more significant problem in turfgrass management in many areas [13]. Responses of plants to salinity stress occur mainly through two distinct phases over time: osmotic-changing and ion specific phases [87–89]. Like other turfgrasses, salt stress caused morphology, physiology, molecular changes in growth and development of perennial ryegrass, such as TQ LFW, LED, and RWC of perennial ryegrass decreasing after exposure to salinity [89, 90]. The alterations of morphological characteristics of turfgrass under salt stress are derived from the changes of physiological traits such as cell membrane stability [14]. It was reported that MDA content and EL enhanced by NaCl concentration in perennial ryegrass [54]. Simultaneously, superoxide radical (O_2^-), H_2O_2 , and singlet oxygen (O_2) concentration increased observably in perennial ryegrass after salt stress treatment [54, 91]. To scavenge reactive oxygen species (ROS), salt-stressed leaves of perennial ryegrass exhibited greater activities of SOD, APX, and CAT at the initial stage of salt stress, but lower levels of enzyme with the extension of salt stress [89]. Salt stress also negatively affected on the total chlorophyll (Chl), Chl a and Chl b, in perennial ryegrass [89], which showed that salt stress induced Chl decomposition in leaves. Moreover, a further research of PSII changes in perennial ryegrass discovered that quantum yields, efficiencies, and energy fluxes were impacted after salt stress treatment [92, 93]. Additionally, a vast amount of Na^+ accumulated in plants could induce ionic imbalance in the cells. It was reported that Na^+ concentration accumulated rapidly and other ion concentrations including K^+ , Ca^{2+} and Mg^{2+} were decreased in response to salt stress in perennial ryegrass [89].

Salt stress causes dramatically changes in morphology and physiology of perennial ryegrass as showed above and summaries in **Figure 1**. However, these responses varied greatly among different genotypes. The research compared the salt tolerance in 10 accessions of perennial ryegrass, and determined that “PI275660”

Figure 1. Morphophysiological response and strategies for salt stress in perennial ryegrass.

and “BrightStar” showed the best tolerance to salt stress, while “PI231595” and “PI251141” were the most sensitive accessions [5]. The other research reported that the effect on parameters of photosynthetic efficiency in perennial ryegrass “Roadrunner” was less than that in “Nira” under salt stress condition [6]. Moreover, the highest salt tolerance accessions were from the European group, wild accessions and exhibited more variation in functional traits and salt tolerance than commercial cultivars [90]. Some other strategies can also improve the salt-tolerance in perennial ryegrass. Salt-tolerant transgenic perennial ryegrass could be obtained by *Agrobacterium tumefaciens*-mediated transformation of the vacuolar Na^+/H^+ antiporter gene [94]. Additionally, exogenous cytokinin applications alleviated salt-induced leaf senescence in perennial ryegrass [8]. Furthermore, salt tolerance of perennial ryegrass can increase by a novel bacterium strain from the rhizosphere of a desert shrub *Haloxylon ammodendron* induced [95].

2.4 Responses of heavy metals stress in perennial ryegrass

The continuing industrialization has led to extensive environmental problems worldwide [96–98]. Heavy metals produced from industry are released to soil. Thus, high accumulation of heavy metal in soil can induce environmental stress on plants [14]. Research on the response of perennial ryegrass to heavy metal stress has also progressed in recent years. It has been proved that heavy metals can induce damage and affect metabolic processes in perennial ryegrass [98–100]. For example, perennial ryegrass had characters in yield reduction and visible symptoms of phytotoxicity under cadmium (Cd) and zinc (Zn) stress [98]. Moreover, the cellular membrane system was damaged because of elevated MDA and EL contents when perennial ryegrass was exposed to salt condition [101]. According to studies, a dramatic inhibition of root and shoot growth was detected in perennial ryegrass after heavy metals treatment [101–103]. Moreover, the composition of the leaves of perennial ryegrass, including apparently opposite effects on the calcium (Ca), potassium (K) and P levels, was changed under the aluminum (Al) stress [104]. Additionally, ROS bursts occurred in perennial ryegrass under heavy metal stress conditions. For instance, H_2O_2 and $\text{O}_2^{\cdot-}$ were significantly accumulated in perennial ryegrass under Cd stress [105]. Hence, the protection mechanisms in perennial ryegrass such as the antioxidant system were triggered under heavy stress, resulting in the increase of SOD, CAT, and POD activities and their corresponding genes [106]. Moreover, content of fructan, sugar, and starch showed an increasing trend in perennial ryegrass after heavy metal stress [98]. However, certain concentrations of heavy metal were beneficial for the growth of perennial ryegrass [107]. Heavy metal stresses not only induce physiological damage, but also inhibit germination and growth of perennial ryegrass [108].

To improve the heavy metal stress tolerance of perennial ryegrass, several investigations were conducted in recent years. It was reported that signal messengers such as nitric oxide (NO) and glycine betaine (GB) play crucial roles in alleviating Cd and Cu-induced damages in perennial ryegrass [109, 110]. Moreover, the exogenous P was testified to improve the Cd tolerance of perennial ryegrass, the reason may be that exogenous P facilitates chelation-mediated Cd detoxification processes [105]. Similarly, a high dose of P amendment alleviated Mn-toxicity in Mn-sensitive genotype in perennial ryegrass [102]. Furthermore, the addition of biochar to a contaminated mine soil improved the nutrient status of this mine soil and contributed to a better establishment of perennial ryegrass [100]. Additionally, AMF enhance both absorption and stabilization of Cd by perennial ryegrass in a Cd-contaminated acidic soil [96], and ethylene diamine tetra acetate (EDTA) enhanced phytoremediation of heavy metals from municipal waste compost and sludge soil by perennial ryegrass [99, 111, 112].

3. Conclusions and future research perspectives

Significant progress has been made in the understanding of morphological and physiological mechanisms associated with perennial ryegrass tolerance to drought, salinity, temperature, and heavy metal stresses. Harsh stress conditions inhibit the growth and development and decrease TQ, root length, and dry weight in perennial ryegrass. Moreover, physiological response to abiotic stress in perennial ryegrass displays changes of the cell membrane, photosystem, metabolites, and antioxidant system. The contents of MDA and EL are increased, while Chl content and photosynthesis are decreased under stress conditions. To regulate the osmotic potential of the cell after stress treatment, some metabolites such as proline, soluble sugars, and proteins accumulate. Meanwhile, antioxidant enzymes' activities increase in perennial ryegrass for scavenging ROS. Perennial ryegrass has protective responses against unfavorable conditions, but there is a threshold to these physiological changes. To understand the response to abiotic stress and resistance attributes in perennial ryegrass will be beneficial to breeding in future.

For improving the stress tolerance of perennial ryegrass, some practical strategies are exploited currently, such as application of phytohormones, endophytes, and chemical compounds. Further research on increasing perennial ryegrass stress tolerance should pay more attention to transgenic technology to identify effective genes for modifying stress-tolerance ability.

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