ADDENDUM

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Improved abiotic stress tolerance of bermudagrass by exogenous small molecules

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Abbreviations: ABA, abscisic acid; NO, nitrogen oxide; ROS, reactive oxygen species; TCA, tricarboxylicacid; GO, gene ontology; RCAR, regulatory component of ABA receptors; PYR/PYL, pyrabactin resistance/PYR like; SnRK2, SNF1-related protein kinases 2; NCED, 9-cis-epoxycarotenoid dioxygenase; JA, jasmonate; JAZ, jasmonate ZIM-domain.

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As a widely used warm-season turfgrass in landscapes and golf courses, bermudagrass encounters multiple abiotic stresses during the growth and development. Physiology analysis indicated that abiotic stresses induced the accumulation of ROS and decline of photosynthesis, resulting in increased cell damage and inhibited growth. Proteomic and metabolomic approaches showed that antioxidant enzymes and osmoprotectant contents (sugar, sucrose, dehydrin, proline) were extensively changed under abiotic stress conditions. Exogenous application of small molecules, such as ABA, NO, $CaCl_2$, H_2S, polyamine and melatonin, could effectively alleviate damages caused by multiple abiotic stresses, including drought, salt, heat and cold. Based on high through-put RNA seq analysis, genes involved in ROS, transcription factors, hormones, and carbohydrate metabolisms were largely enriched. The data indicated that small molecules induced the accumulation of osmoprotectants and antioxidants, kept cell membrane integrity, increased photosynthesis and kept ion homeostasis, which protected bermudagrass from damages caused by abiotic stresses.

Bermudagrass (*Cynodon dactylon*) is a warm-season turfgrass and widely used on home lawns, golf courses and sport fields. Bermudagrass is adapted to cultivation in a wide range of climate condition. Analysis of natural variations of drought stress tolerance revealed that different bermudagrass cultivars exhibited varied tolerance to drought stress.[1-3] Exogenous application of small molecules could effectively alleviate damages caused by multiple abiotic stresses, including drought, salt and cold.[4-8] Exogenous ABA treatment improved drought stress tolerance in 'TifEagle' cultivar through induction of H_2O_2 and NO, which, in turn, enhanced antioxidant enzyme activities.[4] Our studies indicated that exogenous $CaCl_2$, polyamines, H_2S, and NO treatments were effective to induce bermudagrass tolerance to drought, salt, and cold stresses.[5,6,8]

Abiotic stress tolerance is a complex trait, therefore plants develop different approaches to cope with adverse environmental conditions. About half century ago, Barnett and Naylor found that water stress induced a 10- to 100-fold accumulation of free proline and a 2- to fold6-accumulation of free asparagines.[9] Further studies showed that abiotic stress treatments changed antioxidant enzyme activities, ROS contents, osmoprotectant contents (sugar, sucrose, dehydrin, proline), and photosynthesis.[1-2,5-6,10-14]

Recently, proteomics and metabolomics approaches have been successfully used to detect omic level changes during bermudagrass stress responses. Under water-deficit condition, 32 proteins had increases in the abundance and 22 proteins exhibited decreases in the abundance, which were mainly involved in metabolism, energy, cell growth/division, protein synthesis and stress defense.[15] In previous study, we found that chilling treatment modulated the abundance changes of 28 proteins and in total 51 proteins were regulated by $CaCl_2$ treatment. These proteins were enriched in redox, tricarboxylicacid cycle, glycolysis, photosynthesis, oxidative pentose phosphate pathway, and amino acid metabolisms.[8] Additionally, we identified 39 proteins with significantly changed

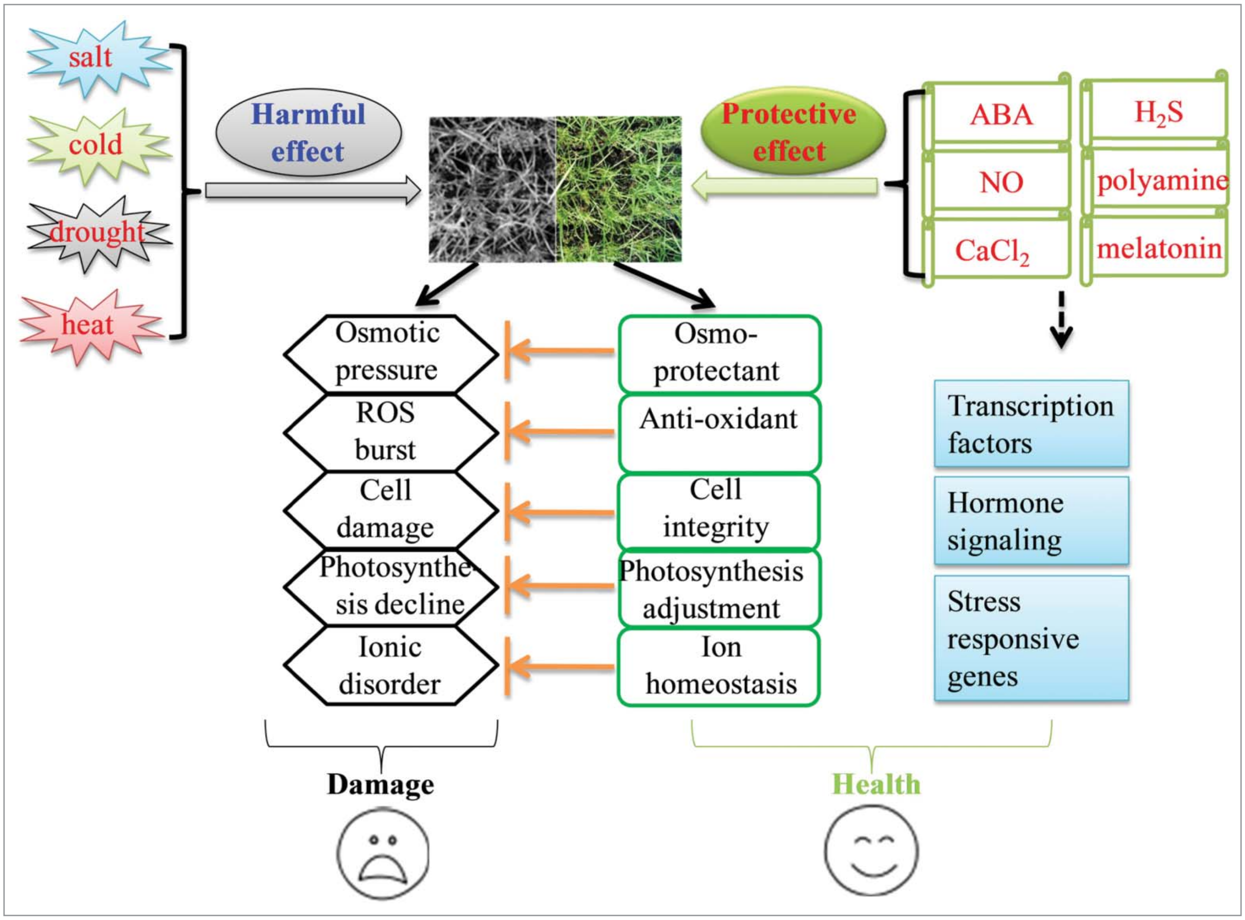

Figure 1. A possible model showing mechanisms involved small molecules induced bermudagrass stress resistance.

abundance after drought stress treatment in leaves and stems of Yukon and Tifgreen cultivars.[3] Among them, proteins involved in photosynthesis, glycolysis, N-metabolism, TCA and ROS pathways were enriched. Pretreatment of exogenous polyamine conferred increased salt and drought tolerances in bermudagrass. Comparative proteomic analysis revealed that 36 commonly regulated proteins by at least 2 types of polyamines in bermudagrass. Among them, proteins involved in ROS, electron transport and energy pathways were largely enriched.[5] Metabolomics analyses confirmed that amino acids, organic acids, sugars, and sugar alcohols were significantly changed after stress treatments.[8,16] All these results indicated that abiotic stresses or exogenous small molecule treatments extensively regulated down-stream protein/enzyme and metabolite biosynthesis.

To further detect up-stream changes in bermudagrass upon stress treatment, macroarray analysis was performed and 189 drought responsive candidate genes were identified, of which 120 were up-regulated and 69 were down-regulated. Functional annotation analysis suggested that upregulated genes were mainly involved in proline biosynthesis, signal transduction pathways, protein repair systems, and removal of toxins, while downregulated genes were mostly related to basic plant metabolism such as photosynthesis and glycolysis.[17]

More recently, high throughput RNA seq analysis showed that exogenous melatonin treatment conferred improved salt, drought, and cold stress tolerances in bermudagrass through modulation of 3933 genes (2361 up-regulated and 1572 down-regulated).[7] Melatonin (*N*-acetyl-5-methoxytryptamine) is a well-known animal hormone which is also found to play important roles in plant development and abiotic stress responses.[18-19] Exogenous melatonin treatment alleviated ROS burst and cell damage induced by abiotic stress in bermudagrass. This was consistent with transcriptomic data which showed that redox-related genes were enriched.[7] Moreover, pathway and GO term enrichment analyses indicated that 8 pathways were over-represented after melatonin pre-treatment, including nitrogen metabolism, major carbohydrate metabolism, TCA/org transformation, transport, hormone metabolism, metal handling, redox, and secondary metabolism. Interestingly, several key genes involved in ABA (*RCAR/PYR/PYL*, *SnRK2*, and *NCED3*) and JA (JAZs) signaling were significantly changed after melatonin pre-treatment, which might be contributed to increased abiotic stress tolerance in bermudagrass.[7]

Conclusions

Based on these observations, a possible model that depicting bermudagrass abiotic stress responses was proposed (Fig. 1). Abiotic stress treatments severely inhibited plant growth and development through osmotic pressure, ROS burst, cell damage, photosynthesis decline, and ionic disorder. Exogenous application of small molecules, including ABA, NO, $CaCl_2$, H_2S, polyamine and melatonin, induced the accumulation of osmoprotectants and antioxidants, kept cell membrane integrity, increased photosynthesis and kept ion homeostasis. These treatments might also induce the expressions of transcription factor and hormone signaling related genes to activate down-stream stress responsive pathways, resulting in stress resistance in bermudagrass.

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