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Lipid- and calcium-signaling regulation of *HsfA2c*-mediated heat tolerance in tall fescue

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ABSTRACT

Heat stress transcription factor A2c (HsfA2c) is known for its positive roles in conferring heat tolerance. The objectives of this study were to examine the effectiveness of selected signaling molecules for improving heat tolerance of a cool-season grass species, tall fescue (*Festuca arundinacea* Schreb.) and to determine the signal transduction pathways regulated by those molecules through the activation of HsfA2c and heat shock proteins (HSP). Foliar application of phosphatidic acid (PA), calcium chloride ($CaCl_2$), hydrogen peroxide (H_2O_2), salicylic acid (SA) and trans-zeatin riboside (t-ZR) all activated expression of *HsfA2c* and several HSP genes, and enhanced tall fescue tolerance to prolonged periods of heat stress (38/33 °C, day/night temperature). Exogenous application of all the molecules increased photosynthetic activities and membrane stability of tall fescue exposed to heat stress, with PA (25 μM) and $CaCl_2$ (10 μM) having more pronounced effects than other treatments. PA- and Ca^{2+}-mediated heat tolerance was associated with the regulation of *HsfA2c* and small HSPs (*Hsp18*) during short-term heat stress and with large HSPs (*Hsp70* and *Hsp90*) during prolonged heat stress, involving genes in PA-synthesis and catabolism, and the downstream genes of the Ca^{2+} signaling pathways. These results suggest the transcriptional regulation of *HsfA2c*-mediated heat tolerance involving lipid and calcium signaling pathways in tall fescue.

1. Introduction

Heat stress is a major detrimental abiotic stress limiting the growth of cool-season plant species during summer months. As sessile organisms, plants have evolved signaling pathways to rapidly detect changes in ambient temperature, which activate transcription factors that induce changes in downstream genes and proteins and regulate various metabolic processes that enable plants to function and survive under heat stress (Mittler et al., 2012). Plant responses to heat stress are very complex and are comprised of many signaling pathways, where many signal molecules involved in each pathway play important roles in high temperature responses (Brestic et al., 2014).

The major signaling molecules that accumulate in response to heat stress include those in metabolic pathways, such as reactive oxygen species (ROS) and hormones, as well as membrane-sensing pathways, such as phospholipids and Ca^{2+} (Brestic et al., 2014). Hydrogen peroxide (H_2O_2) is one of the key elements in the ROS signaling pathway and was found to induce plant heat tolerance through exogenous application (Banzet et al., 1998; Desikan et al., 2001; Larkindale and Huang, 2005). Cytokinins (CK) are among the most well-known hormones that play positive roles in improving abiotic stress tolerances, including heat tolerance (Brestic et al., 2014). Increasing endogenous CK content by foliar application of compounds containing CK, such as seaweed extract-based CK and trans-zeatin riboside (t-ZR), alleviated heat-induced damages in cool-season turfgrass (Zhang and Ervin, 2008). Salicylic acid (SA) has been known to be a stress defense activator (Raskin et al., 1987), and foliar treatment of plants with SA significantly improved plant heat tolerance (Dat et al., 1998; He et al., 2005; Larkindale and Huang, 2005). Plasma membranes are sensitive to heat stress, and changes in membrane fluidity in response to increasing temperature serve pivotal roles in activating stress

Abbreviations: ABI, abscisic acid insensitive; CaM, calmodulin; CDPK, calcium dependent protein kinase; EGTA, ethylene glycol tetraacetic acid; DGK, diacylglycerol kinase; MBF, multiprotein bridging factor; MPK, mitogen-activated protein kinase; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; PDK, phosphoinositide-dependent kinase; PLC, Phospholipase C; PLD, Phospholipase D; RBOHD, respiratory burst oxidase homolog-D; SA, salicylic acid; t-ZR, trans-zeatin riboside; TGD, trigalactosyldiacylglycerol.

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signaling (Saidi et al., 2009; Sangwan et al., 2002). In response to heat stress, an inward calcium flux, which is most likely activated by a temperature-induced increase in fluidity of the plasma membrane, has been considered to be the primary pathway in heat signal transduction (Mittler et al., 2012; Saidi et al., 2009). Application of Ca^{2+} can enhance plant intrinsic heat tolerance and maintain antioxidant activities by decreasing membrane lipid peroxidation (Gong et al., 1997; Jiang and Huang, 2001). Heat-induced changes in membrane fluidity also trigger lipid signaling, resulting in the accumulation of various lipid-signaling molecules, including phosphatidic acid (PA) (Mishkind et al., 2009; Munnik, 2001), which targets phosphoinositide-dependent kinase 1 (PDK1), abscisic acid insensitive 1 (ABI1), Phosphoenolpyruvate carboxylase (PEPC) and calcium-dependent protein kinase (CDPK) in response to various biotic and abiotic stress factors in plants (Testerink and Munnik, 2005). Despite the known positive physiological effects of H_2O_2, CK, SA, Ca^{2+} and PA on heat tolerance, the relative effectiveness of those molecules are not well documented, particularly for heat-sensitive cool-season plant species. Additionally, the molecular factors regulated by the major signaling molecules that may lead to improved heat tolerance are not well understood.

Heat signal transduction pathways are composed of cascades of kinases and some transcription factors (Mittler et al., 2012). As the terminal components of the signal transduction chain, heat stress transcription factors (HSFs) are key factors that receive heat stress signals from upstream pathways and trigger the transcription cascades of downstream heat responsive genes (Mittler et al., 2012). It was reported that calcium-activated calmodulin (Ca^{2+}-CaM) was directly involved in the heat stress signal transduction pathway in wheat (*Triticum aestivum* L.) and up-regulated heat shock proteins (HSPs) under heat stress (Liu et al., 2003). HSPs of various molecular sizes (i.e. HSP18, HSP70, HSP90 and HSP101) are known to be positive regulators of heat tolerance in various plant species (Zhou and Abaraha, 2007). Moreover, pretreatment of whole cell extract of maize (*Zea mays* L.) seedlings with Ca^{2+} increased in vitro DNA binding of HSFs at room temperature, whereas the Ca^{2+} chelator ethylene glycol tetraacetic acid (EGTA) abolished HSF binding when activated by heating (Li et al., 2004). H_2O_2 is also known to be involved in the early phase of heat stress and is required for effective expression of HSPs and HSFs in *Arabidopsis* and rice (*Oryza sativa* L.) (Volkov et al., 2006; Wang et al., 2009). Other signal molecules involved in early perception and transduction of heat stress signals for activating HSF-mediated heat stress response are not well documented.

The plant HSF family is large with a multitude of complex structures, classifications, and functions; however, among all the members of this family, A2 group members of HSF (HsfA2s) are shown to be crucial factors in heat stress response (Scharf et al., 2012). In our previous study, we identified the positive roles of heat stress transcription factor A2c (*HsfA2c*) in conferring heat tolerance in tall fescue (*Festuca arundinacea* Schreb.) (Wang et al., 2016). We hypothesized that PA, Ca^{2+}, H_2O_2, SA, and CK may improve heat tolerance in tall fescue involving the regulation of *HsfA2c* and related HSP genes. The objectives of this study were to examine the effectiveness of these selected signal molecules for improving heat tolerance of cool-season grass species and to determine the signal transduction pathways regulated by those compounds through activating *HsfA2c* and related *HSPs*.

2. Materials and methods

2.1. Plant materials and growth conditions

Seeds of tall fescue (cv. 'Kentucky 31′) were sown in plastic pots (10 cm diameter and 20 cm depth) filled with a mixture of soil and sand (2:1, v/v) and were established in a greenhouse controlled at 25/20 °C (day/night temperature) for a month. During the establishment period, plants were irrigated every two days, fertilized weekly with half strength of Hoagland's nutrient solution (Hoagland and Arnon, 1950), and trimmed every three days to keep the canopy height at 8 cm. Plants were transferred to environmental growth chambers (Chagrin Falls, Ohio, USA) controlled at 22/17 °C, 60% relative humidity, a 14 h photoperiod, and 650 μmol $m^{-2}\,s^{-1}$ photosynthetic active radiation at the canopy level for one week to allow plants to acclimate to growth chamber conditions prior to chemical and heat treatments. And the turfgrass routine managements were same with that in the greenhouse.

2.2. Chemical application and heat treatment

To determine whether or not chemicals could mitigate heat stress in tall fescue, plants were foliar sprayed with a solution every time containing PA, $CaCl_2$, H_2O_2, SA, t-ZR, PA + $CaCl_2$, or PA + EGTA, respectively, or with water as the control. The concentrations and biological functions of each treatment are listed in Table 1. Chemicals were foliar sprayed three times every two days prior to heat treatment and once every 7 d during heat treatment. At each application, 50 mL solution was sprayed onto leaves in each pot. Each exogenous treatment had six pots with approximately 50 plants in each pot.

For heat treatment, plants were exposed to 38/33 °C for 35 days. The control plants were grown at 22/17 °C. Each temperature treatment was repeated in three growth chambers, and plants were distributed randomly in growth chamber every 3 d to minimize any variations possibly caused by differing environmental effects of growth chambers. The treatments were arranged as a split-plot design with temperature as main plots and chemical treatments as sub-plots.

2.3. Physiological measurements

Turf quality is widely used as an evaluation of overall plant growth status based on color, density, and uniformity of grass canopy (Beard, 1973). Turf quality was visually rated for plants of each pot on a scale of 1–9, with 9 indicating the best in all quality components, 6 being the minimally acceptable rating, and 1 indicating completely brown, dead turf.

For photosynthetic indicators, leaf net photosynthetic rate was determined using a LI-6400 portable photosynthesis system (LI-

Table 1
Information for the chemicals applied in tall fescue.

Chemicals Abbreviation	Chemicals Full Name	Sigma SKU	Final Concentration	Biological pathway
PA	phosphatidic acid	P9511	25 μM	Lipid pathway
$CaCl_2$	calcium chloride	C5670	10 mM	Calcium pathway
H_2O_2	hydrogen peroxide	H1009	5 mM	ROS pathway
SA	salicylic acid	S5922	10 μM	Hormone pathway
t-ZR	trans-zeatin riboside	Z0375	1 μM	Hormone pathway
EGTA	ethylene glycol tetraacetic acid	E3889	5 mM	Calcium pathway