CHEMICAL PRIMING AND PLANT GROWTH REGULATOR EFFECTS ON DROUGHT RESISTANCE CHARACTERISTICS OF CREEPING BENTGRASS

By

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ABSTRACT

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Despite widespread use of plant growth regulators (PGRs) their interaction (PGRs) their interaction with other plant treatments associated with stress tolerance is poorly understood. Greenhouse and growth chamber studies were conducted to determine the effects of seven chemical priming compounds on drought responses under growth chamber and field conditions on creeping bentgrass 'T1' (Agrostis stolonifera). The treatments were: control, trinexapacethyl (TE), salicylic acid (SA), gamma amino butyric acid (GABA), jasmonic acid (JA), TE + SA, TE + GABA, and TE + JA. Leaf relative water content (RWC), soil volumetric water content (SWC), leaf electrolyte leakage (EL), turf quality (TQ), normalized difference vegetation index (NDVI), canopy temperature depression (CTD) and leaf chlorophyll content were measured in plants under well-watered and drought-stressed conditions. Under drought stress in the growth chamber, TE+SA and TE treated plants had a significantly higher TQ and lower EL than the controls. TE+SA, TE, TE+JA, and TE+GABA treated plants had a higher RWC and a lower CTD than the control. In field conditions, plants treated with TE+SA, TE, and TE+JA had a higher SWC, TQ, NDVI, and RWC than the controls. The results from this study suggest that out of the various chemical priming agent treatments tested, TE and TE plus priming agents (TE+SA, TE+JA, and TE+GABA) were most effective in alleviating drought stress of creeping bentgrass through avoidance and tolerance. Treatment with TE alone had the same effect as TE supplemented with SA, GABA or JA.

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То

My mother Bellancile Mukakibibi My Sister Marie Odette Munganyinka David and Victoria Weight Helen K. Swearingen Thank you so much for your support

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BACKGROUND

Water resources are becoming scarce while the demand for water in agriculture, residential and industrial use is increasing worldwide (Fader et al., 2016). Environmental drought occurs when precipitation falls significantly below the long-term average over an extended period. The long-term lack of water translates into the reduction in soil water content and induces many environmental effects such as alteration of plant growth and development (Schild and Dworak, 2013). Drought stress of plants can occur due to the absence of rainfall or irrigation for a period long enough to deplete soil moisture and cause injury to plants. Drought symptoms in plants often are exhibited when water lost through evapotranspiration and cellular water use exceed water uptake and/or availability (Lisar et al., 2012). This review describes the damage caused by drought stress, the response of plants to alleviate the damage, and the exogenous application of priming chemicals to reduce drought stress in plants. Because of variations among C3, C4 and CAM plants in drought response, I focus here on the general understanding of the response, with emphasis on C3-photosynthesis type plants. This type of plant includes creeping bentgrass (*Agrostis stolonifera*), a turf species on which our research was conducted.

Drought stress effects on plant growth and water relations

Drought stress affects plants at all development stages, with consequences that range from morpho- physiological to molecular levels. Drought stress of C3 photosynthetic plants affects many aspects of growth and development. Plant growth consists of cell division, elongation, and differentiation leading to a permanent increase in weight, size, or volume of the whole plant. (Taiz and Zaiger, 2010). Drought stress can cause a reduction in cell elongation and expansion leading to reduced plant height and low leaf area (Nonami, 1998; Bayer et al., 2016; Nelissen et al., 2018). Growth becomes regulated in plants during water stress due in large part to the impairment of photosynthesis, carbohydrate allocation and translocation, and respiratory processes (Hussain et al., 2016).

In C3 photosynthetic plants, stomatal closure causes decreased stomatal conductance and is an early response to drought stress. It is then followed by impairment in Riboluse1,5biphosphate (RuBP) regeneration and adenosine triphosphate (ATP) synthesis leading to the inhibition of subsequent metabolic processes ending in the inability to perform the needed CO₂ photosynthetic assimilation (Flexas and Medrano, 2002). Drought stress also affects the dry matter partitioning and carbohydrate translocation in C3 photosynthetic plants. Some C3 plants may respond to drought stress by allocating a higher proportion of dry matter and soluble sugars to roots and other sinks and decreasing the sugars in leaves (Xu et al., 2015). However, as the stress intensifies this process may be impaired, leading to an inadequate supply of carbohydrates to the sinks. For example, cotton (*Gossypium hirsutum*) plants subjected to drought stress had reduced activity of enzymes involved in sugar metabolism such as sucrose synthase and vacuolar invertase enzymes when compared to the control. The low activity of these enzymes resulted in a low concentration of hexose sugars and a lower fiber elongation of cotton fibers (Tang et al., 2017).

If the stomatal closure is not sufficient to reduce drought incidence and stress to plants, drought stress may cause metabolic effects associated with a loss of cell turgor pressure, impaired enzyme activity, and reduced energy supply (Farooq et al., 2009). Leaf water potential, stomatal resistance, relative water content (RWC), transpiration rate, and canopy temperature are essential physiological parameters in plant–water relationships affected during drought stress (Hossain et al., 2016,). Drought stress reduced RWC and midday water potential of curved lovegrass (*Eragrostis curvula*) (Colom and Vazzana, 2001). Water deficit decreased leaf water potential, osmotic potential, and RWC in sunflower (*Helianthus annuus*) (Tezara et al., 2002). Drought stress affects transpiration, which governs water and nutrient uptake, controls CO₂ gas exchange and reduces fluctuation in leaf temperature. Rice (*Oryza sativa*) plants exposed to drought had reduced transpiration and higher leaf temperature (Siddique et al., 2000).

Drought resistance: escape, avoidance and tolerance mechanisms

Plants possess intrinsic mechanisms to survive or prevent water deficit. They use a range of processes including dehydration escape, avoidance, and tolerance to endure drought periods. Drought escape is a rather narrow classification in which plants exploit rapid phenological development when water is available, followed by dormancy or completion of a life cycle during severe stress (Kramer, 1980). Drought escape strategies are not preferable for aesthetic and functionality of turfgrass species; thus, turfgrass species typically must rely on avoidance or tolerance strategies.

Through drought avoidance mechanisms, plants aim to reduce water use or limit water loss such as by lowering evapotranspiration or transpiration rate and maintaining and increasing water uptake even when the soil is drying (Farooq et al., 2012). Plants can avoid drought in the following ways: by quickly closing their stomata and rolling their leaves to reduce water losses (Boguszewska-Mańkowska et al., 2018). By accumulation of wax on the leaf surface to reduce transpiration water losses in some species like tobacco (*Nicotiana tabacum*) and camelina (*Camelina sativa*) (Lee et al., 2014), or by enhancing water uptake through a well-developed root system via increased rooting depth, rooting density or root/shoot ratio. For example, when tall fescue (*Festuca arundinacea*) and Kentucky bluegrass (*Poa pratensis*) were subjected to increasing levels of drought, tall fescue had more root dry weight, higher turf quality, lower leaf firing and lower canopy depression (Ervin and Koski, 1998). In another experiment, tall fescue genotypes with higher root volume and weight had a higher recuperative ability after exposure to intense drought (Pirnajmedin et al., 2016). Canopy characteristics may also play a role in drought avoidance of plants. Phenotypic characteristics and plasticity in plant biomass, leaf and tiller density affect evapotranspiration and thereby are associated with avoidance (Cuoso et al., 2012).

The categorization of drought resistance mechanisms is not always straightforward and might leave some gray areas. Some mechanisms may fall into multiple categories, and often plants will use several processes at different stages of growth to combat drought. For example, some plants have the flexibility to accelerate or decelerate the conversion from vegetative growth to reproductive growth to avoid complete abortion during severe drought stress (Franks, 2011).

Drought tolerance refers to a plant's ability to deal with cellular water loss, i.e., avoidance traits were not sufficient to prevent cellular water loss. A plant's ability to sustain a certain level of physiological activities under drought stress conditions will indicate how tolerant of drought the plant may be. Tolerance traits are associated with the regulation of many genes and series of metabolic pathways to reduce or repair the resulting stress damage (Mitra, 2001). For instance, the osmotic adjustment is a drought tolerance trait aimed at turgor maintenance (Hossain et al., 2016). Through osmotic adjustment, plants accumulate some organic and inorganic solutes such as alkaloids, sugars, polyols, amino acids, or inorganic ions to reduce osmotic potential in the cell cytoplasm to increase the cell water retention capacity under water deficit (Morgan, 1984). The accumulated solutes are referred to as compatible solutes because even at a higher concentration, they do not compromise the normal functionality of enzymes, membrane or other macromolecules (Kiani et al., 2007). An example of an essential compatible solute is proline; it accumulates in plants under dehydration stress mostly in younger leaves (Pérez-Pérez et al., 2009). Proline has a dichotomous protective ability allowing its hydrophilic side to bind to water molecules and making them accessible to proteins that are attached to its hydrophobic side (Hoekstra et al., 2001).

Another essential tool of drought tolerance used by plants is their antioxidant defense system. The normal metabolism of oxygen that supplies the energy required for plant growth and development and other reactions can generate by-products named reactive oxygen species (ROS). In normal concentrations, ROS are essential in cell signaling and homeostasis. Examples of ROS include H₂O₂, superoxide ($^{\circ}$ O⁻₂) and 1 O₂. (Sharma et al., 2017). Under drought, ROS can accumulate to excessive levels and can lead to cell death through oxidation of essential macromolecules such as through lipid peroxidation (Cruz De Carvalho, 2008). ROS distort the regular structure of membrane proteins and enzymes leading to ion leakage and membrane permeability (Gill and Tuteja, 2010). Plants combat the deleterious effects of ROS by regulating the content and activity of ROS scavengers, which may be enzymatic or non-enzymatic. Enzymatic antioxidants present in plant tissues can include peroxidase, catalase, superoxide dismutase, ascorbate peroxidase and glutathione reductase, while the nonenzymatic actors include reduced glutathione, cysteine and ascorbic acid (Gong et al., 2005; Apel and Hirt, 2004).

Plant growth regulators and drought resistance

Plant growth regulators (PGRs) and plant hormones can significantly influence metabolism, growth, and development of plants. PGRs are the synthetically produced chemicals that have plant hormone activity. Stressful conditions modify the internal content of plant hormones and how they are altered may be dependent on plant species, specific plant tissues, or stage of development. Important plant hormones that are involved in the drought response include auxins, gibberellins (GA), cytokinin (CK), ethylene and abscisic acid (ABA) (Turgeon, 2012, Taiz and Zaiger, 2015). The proportion of growth suppressants increase while growth promoter concentrations may decline under drought conditions (Farooq et al., 2009). GA and CK promote plant growth, while ethylene and ABA have inhibitory effects and are called growth retardants (Taiz and Zeiger, 2015). Drought stress caused a sharp decline in endogenous levels of GA followed by growth inhibition on maize seedlings (Wang et al., 2008).

Hormones that have not been as well studied during drought responses are salicylic acid (SA) and jasmonic acid (JA). In the case of drought tolerance, endogenous SA is said to enhance the early response to environmental water stress and the production of ROS in photosynthetic tissues of *Arabidopsis thaliana* subjected to osmotic stress (Borsani, 2001). Foliar application of SA enhanced a higher RWC, dry mass accumulation, chlorophyll content and a higher activity of antioxidant enzyme; peroxidase (POD) of wheat seedlings under

drought stress (Singh and Usha 2003). JA is said to be a precursor of ABA biosynthesis and accumulate under drought stress (Ollas et al., 2013). Soybean (Glycine *max*) treated with 50 μ M of methyl jasmonate under drought stress had increased activity of superoxide dismutase (SOD), POD, and catalase (CAT) and accumulated more proline. The results of this higher enzymatic antioxidant activity were a higher RWC and a decreased lipid peroxidation (Anjum et al., 2011). The research on the role of JA/SA on drought stress highlight the role of the two through the downstream effects of antioxidant metabolism, more research is needed on their relative interaction or crosstalk with other plant growth hormones under drought stress.

Plant growth regulators can alter endogenous plant hormones that are important to drought responses such as GA. The suppression of GA by PGRs may cause effects on other hormones important in drought responses such as ABA, JA, or SA. This would be similar to how endogenous cross-talk between plant hormones can result in antagonistic or synergistic effects on various phenotypic responses to abiotic stress (Kuppusamy et al., 2009; Depuydt and Hardtke, 2011). The same might happen when PGRs are applied to plants under drought stress.

Priming for drought resistance

Plants have evolved many strategies, such as those mentioned above, to alleviate or overcome the multiple abiotic and biotic stresses that they are faced with. What is less well understood is a plant's stress memory, meaning plants may have a predisposition for enhanced genetic or biochemical modifications leading to a faster and stronger response of tolerance on the subsequent exposure. Plant priming, such as exposure to predisposing stress or chemical treatment, can elicit plant memory-based mechanisms (Bruce et al., 2007; Pastor et al., 2013). An example of abiotic stress predisposing plants for enhanced tolerance to future stress is known as drought preconditioning. For instance, oat-glass plants (*Arrhenatherum elatius*) exposed to double stress (early and late) had higher biomass when compared to the single stressed (only late stress) (Walter et al., 2011). Use of abiotic stresses for preconditioning plants may be difficult to implement; thus, the use of chemical priming may be a viable alternative.

Many chemical priming methods have been investigated to harness a plant's stress memory including the application of inorganic compounds, amino acids, nonprotein amino acids, polyamines, reactive oxygen and nitrogen compounds, antioxidants, organic alcohols, volatile organic compounds, and plant hormones (Merewitz, 2016). For example, application of H_2O_2 and Ca^{2+} increased the RWC, and chlorophyll and led to the normal growth of Rapeseed (Brassica napus) seedlings under drought stress (Khan et al., 2017). Foliar application of SA conferred resistance of Chinese nutmeg tree (Torreya grandis) to moderate drought stress by increasing water content, CO₂ assimilation rate, proline content and promoting the activity of antioxidant enzymes (Shen et al., 2014). The exogenous foliar application of ABA improved the water status and photochemical parameters of two maize (Zea mays) varieties under drought stress (Corrêa de Souza et al., 2013). Foliar spray of GA increases stomatal conductance, net photosynthesis, and transpiration rate of cotton under drought stress (Kumar et al., 2001). Many chemicals have been tried as exogenous priming agents for drought resistance. Their effectiveness varies with species, the degree of stress imposed, and the management conditions. Testing chemical priming methods on turfgrass species is needed to reduce water use and drought-induced losses on high-value turfgrass areas.

How chemical priming and PGR treatments may interact in turfgrass systems are not well studied. Understanding their interaction, whether there is an amplification, nullification, or no effect of the combination is critical to understand for informed turfgrass management decisions. The priming agents and PGRs examined in this thesis are either already highly used in the turf industry or have the potential for use. Therefore, this thesis includes a growth chamber and field experiment aimed to determine the effects of chemical priming with SA, JA, or GABA in combination with or without the plant growth regulator, trinexapac-ethyl.

CHEMICAL PRIMING AND PLANT GROWTH REGULATOR EFFECTS ON DROUGHT RESISTANCE CHARACTERISTICS OF CREEPING BENTGRASS

INTRODUCTION

The application of plant growth regulators (PGRs) is a widespread practice in turfgrass management. PGRs serve many functions including suppression of seed head formation, reducing vertical shoot growth, and improvement of turf quality (Turgeon 2012; Fry and Huang, 2004). Trinexapac-ethyl (TE) is one of the most commonly used PGRs in the turfgrass industry (Fagerness and Yevelton, 2001). TE is a systemic PGR that is applied as a post-emergence foliar spray. TE treatment of plants blocks the conversion of precursor forms of gibberellins (GAs) such as GA20 to GA1 by inhibiting the enzyme 3-ß-hydroxylase (Adams et al., 1992). Because the primary function of GAs in plants is to stimulate cellular elongation, blocking GA biosynthesis in grasses reduces shoot growth which can enhance lateral growth and allow for reduced mowing frequencies (Ervin and Koski, 1998). TE treatment of Kentucky bluegrass (*Poa pratensis*) resulted in significant vertical growth suppression, nearly 50% reduction of the biomass, broader leaves, and higher chlorophyll content when compared to the control (Fan et al., 2009). Whether TE application has additive effects or is synergistic or antagonistic to other plant supplement treatments is not well understood.

In addition to vertical growth suppression, TE may alter plant resistance of some abiotic stresses potentially through both avoidance and tolerance mechanisms. TE treated plants exhibited enhanced drought, heat, and shade tolerance in some turfgrass species (Turgeon, 2012; Jespersen and Huang, 2017). Specifically, for drought stress, creeping bentgrass treated with TE maintained a higher soil volumetric water content (SWC) when compared to the

control after three weeks of imposed drought. TE treated plants had a higher leaf relative water content, sustained growth, and improved quality compared to the control (Mccann and Huang, 2008). Maintenance of higher SWC suggests TE may increase a turfgrass systems potential for drought avoidance. TE application also promoted drought tolerance characteristics, those associated with a plant's metabolism, like the osmotic adjustment. TE-treated plants under drought stress accumulated more soluble sugars and inorganic ions (Ca^{2+} and K^+) leading to a higher turf quality and photochemical efficiency of the leaf when compared to non-treated plants (Bian et al., 2009).

The effects of TE on biotic stresses can be dichotomous. In field studies, application of TE reduced disease symptoms of dollar spot (*Sclerotinia homoeocarpa*) a necrotrophic pathogen of creeping bentgrass (Golembiewski and Danneberger, 1998; Putman and Kaminski, 2011). Creeping bentgrass was more susceptible to brown patch (*Rhizoctonia solani*) disease, another common necrotrophic pathogen, after treatment with TE during warmer season months compared to control field plots (Wong et al., 2009). Management practices and the different parameters in the microenvironment of the grass canopy may contribute to the differences in disease incidence in response to TE application. These microenvironment factors could be humidity, evapotranspiration rates, water use rates, and other factors associated with turfgrass leaf height (Fry and Huang, 2004; Roberts et al., 2016; Elansary and Yessoufou, 2015). However, whether biochemical or metabolic responses to GA repression are a factor in TE-induced abiotic stress tolerance or potential changes in disease susceptibility are not well studied.

Salicylic acid (SA) and jasmonic acid (JA) are essential signaling hormones for abiotic and biotic stress responses in plants. They are intimately linked and interact to control systemic acquired resistance (SAR) and induced systemic resistance (ISR) pathways (Zhang et al., 2004; Durrant and Dong, 2004; Vallad and Goodman, 2004). Repression of GA biosynthesis due to TE treatment should theoretically result in significant hormone crosstalk within plants. GA interaction with SA and JA are involved within the growth-defense tradeoff within plants. SA and GA are antagonistic to each other, with one promoting defense and the other promoting growth (Huot et al., 2014). Krishnan and Merewitz (2015) found that TE application supported a higher level of SA accumulation in Kentucky bluegrass, which follows the growth-defense trade-off model. If growth and GA are suppressed by TE, SA is free to accumulate. For applied purposes in turfgrass management, if TE products are already being applied, applying products, such as costly specialty fertilizers containing SA, maybe a wasteful practice. Alternately, if TE increases susceptibility to some diseases, using combination treatments that may highly promote disease tolerance may be warranted. Thus, determining their singular or combined effects under controlled and under field conditions is needed.

The cross-talk between GA and JA is less clear and is more complex. In some plants, GA and JA interactions seem to work antagonistically whereas in other plants or under different environmental conditions GA can promote JA signaling. In Kentucky bluegrass treated with TE and exposed to drought stress, an increase in JA content was delayed during drought stress compared to plants not treated with TE. At severe levels of relative water content (26%), JA levels were higher in plants treated with TE compared to those not treated with TE (Krishnan and Merewitz, 2015). Thus, it is not clear whether TE interactions with JA during drought

stress could have played any role in stress resistance. Supplementing plants treated with TE with exogenous JA will help elucidate whether plants may benefit from JA during TE treatment and stress conditions.

SA and JA are two hormones that have been shown to contribute to drought stress alleviation. SA increased relative water content, leaf compatible osmolytes accumulation, chlorophyll and seed essential oil content of the fennel plant (Foeniculum vulgare Mill.) grown under drought conditions (Askari and Ehsanzadeh, 2015). Exogenously applied JA improved chlorophyll content, relative water content, dry weight and reduced oxidative stress of three Brassica species (Alam et al., 2014). SA and JA application alleviated the detrimental effects of drought stress in wheat (Triticum aestivum) seedlings by improving the activity of the ascorbate-glutathione (ASA-GSH) enzymatic antioxidant cycle (Kang et al., 2013; Shan et al., 2015). Exogenous application of SA on Kentucky bluegrass (Poa partensis) sods resulted in increases in activity of antioxidant enzymes (SOD, CAT), reduced heat injury, improved turf quality and photochemical efficiency (Ervin et al., 2005). Besides the physiological changes, biochemical changes brought by JA and SA were also reported in turfgrass species. SA application on CBG under drought stress promoted a higher accumulation of amino acids and carbohydrates that alleviated drought stress through an increase in energy metabolism and osmotic adjustment (Li et al., 2016). The metabolic benefits of SA and JA application combined with potential drought avoidance and tolerance characteristics imparted by TE treatment may be beneficial for plants survival under drought stress.

Gamma-aminobutyric acid (GABA) accumulates under drought stress in several plant species. Exogenous application of GABA improved turf quality, relative water content, and other health parameters in several studies on both perennial ryegrass and creeping bentgrass (Krishnan et al., 2013; Li Peng and Huang, 2016). Metabolites like GABA can interact with hormones mentioned above, and function as osmolytes, antioxidants, or reactive oxygen scavengers that help plants to tolerate stress. TE-induced drought tolerance in Kentucky bluegrass was associated with an increase in various proteins and metabolites (Xu and Huang, 2012). In a similar study conducted on tomato(*Solanum lycopersicum*), Pal et al. (2016) found that plants treated with paclobutrazol (a GA inhibitor) in well-watered and drought-stressed plants had accumulated a higher amount of GABA when compared to the untreated. The relationship between TE application and the application of plant metabolites such as GABA in drought stress control is not well understood.

The objective of the study was to investigate the singular and interactive effects of TE with SA, JA, and GABA on creeping bentgrass drought resistance under both controlled and field conditions.

MATERIALS AND METHODS

Growth chamber experiment - plant material and growing conditions. The experiment was conducted in Spring 2016 (March- April) and repeated in fall 2017 (October-November). Sod plugs (10.16 cm diameter) of creeping bentgrass 'T1' were taken in October 2017 from the Hancock Turfgrass Research Center (HTRC) in East Lansing, MI. This field was seeded in fall 2015. Bentgrass sods were cut free of all roots and soil and allowed to establish in 40 cm polyvinylchloride (PVC) tubes filled with a typical Hapludult soil (composition of 56.9% sand, 26.1% silt and 17% clay). After one month of establishment in the greenhouse, plants were transferred to an environmentally controlled growth chamber. Plants were acclimated to the growth chamber conditions for one week before treatment. The grass was trimmed weekly to maintain a turf canopy height at 5-6 cm. Weekly fertilization was given with half-strength Hoagland's Solution until the drought stress treatment began. The conditions of the chamber were maintained at an average day/night temperature of 21°C and 60 % relative humidity and included a light level of 400 μ mol m⁻² s⁻¹ with a 14-hours photoperiod throughout the study.

Growth chamber experiment - treatments. Water treatments included a well-watered and drought stress treatment, with water being completely withheld from drought treated plants. The chemical (PGRs) treatments were; 1. control (no treatment) 2. trinexapac ethyl (TE), 3. salicylic acid (SA), 4. Gamma-aminobutyric acid (GABA), 5. jasmonic acid (JA), 6. TE + SA, 7. TE + GABA, 8. TE + JA. TE was applied twice biweekly at a rate of 1.95 mL L⁻¹ [v/v] (Primo Maxx, Syngenta Corp, Greensboro, NC). SA and JA were applied at a rate of 0.5 mM. This rate was the most common among drought studies conducted by other researchers on cereals (Kang et al., 2013; Marci et al., 2013) and other plant species (Alam et al., 2014; Eraslan

et al. 2007). GABA application rate was based on the previous study conducted in perennial ryegrass (Krishnan et al., 2013). JA, SA, and GABA were sprayed three times a week on similar days. Their first application came two days after the last TE spray. Drought stress was imposed one day after the last JA, SA and GABA application, which meant the first day of drought was on December 09, 2017. Chemicals were applied in the form of a foliar spray, and each pot was sprayed with five mL of a given treatment

Field experiment - plant material and growing conditions. The experiment was performed in Summer 2016 (June-August) and repeated in Summer 2017 (June-August) on individual plots $(1.82 \times 0.91 \text{m})$ of recently sown (2 yrs. old) creeping bentgrass 'T1' at the HTRC. The soil type was a sandy loam soil from the HTRC station. The textural composition of the HTRC soil is 57% sand, 26% silt, and 17% clay (2015 lab test). Plants were mowed three times a week at the height of 3.3 mm with clippings removed and watered three times per week to maintain soil water content at field capacity (30%) before the drought treatment. Fertilization was done once a week with a 28-0-0 liquid fertilizer at the rate of 0.1 lbs. of N per 1000 ft². Preventative fungicide was applied as needed. One month before drought treatments were implemented, a field shelter (10.36 m W x 21.94 m L) was put in place over all field plots evaluated in the study (both watered and drought plots). The static field shelter had a clear plastic canopy that did not significantly affect the light incidence based on light measurements using a light meter (data not shown) (Light Scout Foot-candle meter, Spectrum Technologies Inc., Plainfield IL). The shelter had two wide open short sides, and the long sides were retractable so as not to affect air circulation. Similar static rain-out shelters have been used in previous studies (Karcher et al., 2008). With the shelter in place, all plots were hand watered before drought treatment. The

amount of water used was determined based on evapotranspiration calculations as in the methods of Martin (2011) and Beard (2002). Weather data were collected from the HTRC weather station found within the close vicinity of the field experimental site. Since a rainout shelter was in place, we collected daily maximum and minimum temperatures

Field experiment - treatments. The experiment was performed June through August in 2016, and drought stress imposition started on July 20. For the year 2017, the experiment was conducted in May through August with drought imposition starting on July 15. The chemical treatments were the same as described the growth chamber study. The rates and frequency were also as described above in the growth chamber except for TE which was applied at a rate of 0.125 fl. Oz./1000 ft² (the label rate for putting greens).

Irrigation water was for the well-watered plots was hand supplied with a hose, and the drought stress imposition was done by withholding the water.

Physiological evaluations. The effects of watering treatment on soil water content (SWC) were measured using a soil moisture meter (TDR 100; Spectrum Technologies, Plainfield, IL) in the growth chamber by inserting the 20-cm-long rod vertically in the soil. Field SWC was measured using a hand-held moisture meter (FieldScout TDR 300; Spectrum Technologies, Plainfield, IL) in a 0- to the 7.62-cm-deep soil. Well-watered plants or pots were maintained at approximately 25% soil volumetric water content SWC.

TQ was rated visually based on a scale of 1–9 (9 representing a fully turgid, dense green canopy, and 1 being necrotic plants) by factoring in color, density, and uniformity of the grass (Beard 2002). The normalized difference vegetative index measurement was conducted only

in field conditions using an NDVI turf color meter (FieldScout TCM 500 NDVI Turf Color Meter; Spectrum Technologies, Inc., Plainfield IL)

Canopy temperature depression (CTD) was evaluated to determine leaf temperature using an infra-red thermometer (IR crop temperature meter 2956; Spectrum Technologies, Inc.). CTD was calculated by subtracting ambient temperature from leaf temperature (Barrs & Weatherley 1962). Leaf relative water content (RWC) was determined from 10 mature leaves that were weighed immediately to determine the fresh weight (FW). The leaves were placed in covered Petri dishes filled with water and kept at 4°C overnight to reach full hydration. Leaf samples were blotted dry and weighed immediately to determine the turgid weight (TW). Leaf tissues were dried in an oven at 80°C for 72 hours and weighed to determine the dry weight (DW). RWC was calculated as $(FW - DW) / (TW - DW) \times 100$ (Barrs and Weatherley, 1962).

Leaf chlorophyll content (Chl) was measured in the growth chamber study by taking plant leaf samples and performing extractions in dimethyl sulfoxide and calculated as in the methods of Arnon (1949). The absorbance of extracts was taken with a UV–visible spectrophotometer (GENESYS 10S UV-Vis spectrophotometer, ThermoScientific, Grand Island, NY) at 663 and 645 nm. For the field study, chlorophyll index was measured using a hand-held chlorophyll meter (FieldScout CM 100 chlorophyll meter, Spectrum Technologies Inc., Aurora, IL).

Cell membrane stability of leaves was estimated by measuring electrolyte leakage (EL) ; (Blum and Ebercon, 1981). Approximately ten leaves were taken from each plant then briefly rinsed and submerged in a 15-mL tube containing seven mL of de-ionized water. The samples were then placed on a shaker for 24 h. The conductivity of the immersion water was measured as initial conductivity (C_i). The leaf tissues were then killed by boiling for 20 min and then placed on a shaker for 24 h. The conductivity of the water containing the dead tissues was measured as the maximum conductance (C_{max}). The percentage EL was calculated as (C_i/C_{max}) ×100. EL measurement was conducted in the growth chamber study only.

Experimental design and statistical analysis. Experimental design for the growth chamber study was a split-plot design in a completely randomized design with water as the main plot and chemical treatment as the subplot. Each of the two factors was replicated four times with a total of 64 pots. Experimental design for the field study was a split-plot in a randomized complete block design with two blocks. Water was the main plot and chemical application the subplot; chemicals were applied to individual 1.82×0.91 m plots. Each of the two factors was repeated four times with a total of 64 plots.

The effects of the chemical and drought treatment were analyzed over time by analysis of variance (ANOVA) based on the general linear model procedure of SAS (version 9.4; SAS Institute, Cary, NC). Data were analyzed separately for each year due to differences in sampling dates and weather conditions (for the field study). Fisher's protected least significant difference (LSD) test at 0.05 probability level was used to detect differences between treatment means. The calculated LSD bars were presented in the figures or used to determine the letters presented in the figures and tables where significant chemical or drought effect was observed.

RESULTS

Growth chamber (GC) experiment results

As a significant yearly interaction existed between study years for all the measurements, each year was analyzed separately.

Soil water content (SWC)



Figure 1. A B. Effect of chemical treatment and irrigation on the soil volumetric water content (% SWC) of creeping bentgrass (CBG) in drought and well-watered conditions for 2016 and 2017 growth chamber studies. LSD bars are present on a given date where statistically significant differences were observed between water treatments ($P \le 0.05$). No LSD bar

indicates no significant differences on that date.

In well-watered pots, the average SWC for all days was 21.7 % for 2016 and 19.7% for 2017. In response to water withholding, SWC among pots markedly declined in drought conditions at approximately similar rates. SWC dropped from an average of 22.2% on day (d) one to 1.36 % on 15 d in 2016 and from 20.6 % on d two to 1.07 % on 11 d in 2017 (Fig. 1A and B). There were no significant differences among chemical treatments in SWC either among well-watered plants or among drought-stressed plants in either of the GC studies. Relative water content (RWC)



Figure 2. A B. Relative water content (% RWC) of CBG leaves in response to chemical and water treatments (drought) for 2016 and 2017 growth chamber studies. The horizontal bar represents the chemical treatment average in well-watered condition. LSD bars are present on a given date where statistically significant differences were observed between chemical treatments ($P \le 0.05$). No LSD bar indicates no significant differences on that date.

In well-watered conditions, RWC of all CBG plants did not change significantly throughout the study and averaged 84.7% in 2016 and to 85.4% in 2017 (Fig. 2 A and B). Drought stress caused RWC to decrease over time and significant differences were observed among the chemical treatments on 12 and 15 days in 2016 (Fig. 2A). On day 12, TE+SA and TE (50.1% average) were significantly higher than the control (34.2%), and on 15 d, TE+SA, TE, and TE+JA (average 35.5%) were higher than the control (22%). In 2017 on 8 d, TE+SA treatment (42.3%) had a significantly higher RWC than plants treated with the other chemical applications and the control (26.06% average). On 11 d, TE+SA, TE+JA, TE+GABA, and TE had an average RWC of 26.06% and were significantly higher than the control (14.8%) (Fig. 2B).

Turf quality (TQ)



Figure 3. A B. Effect of chemical treatment on the turf quality rating (1-9 scale; 1= best, 9=best) of creeping bentgrass (CBG) in drought conditions for 2016 and 2017 growth chamber studies. The horizontal bar represents the chemical treatment average in well-watered condition. LSD bars are present on a given date where statistically significant differences were observed between chemical treatments ($P \le 0.05$). No LSD bar indicates no significant differences on that date.

TQ was not significantly different among all plants under well-watered conditions with an average TQ of 8.05 and 8.6 for the 2016 and 2017 studies, respectively (Fig. 3A and B). For the year 2016, under drought stress, TQ declined with statistically significant differences on 12 d and 15 d of the study (Fig. 3A). For example, TE+SA (6.5) and TE (5.5) had a significantly higher turf quality than the control (4) on 12 d. A similar trend was observed on day 15 where TE+SA (4.75) and TE (3.75) were higher than the control (1.75). In 2017, on 8 d and 11 d of the study (Fig.2B), TE (7) and TE+SA (7.5) had a significantly higher turf quality than the control on 8 d (Fig.3B).

Electrolyte leakage (EL)



Figure 4. Electrolyte leakage (% EL) of CBG leaves in response to chemical and water treatments, drought conditions in 2017. The horizontal bar represents the chemical treatment average in well-watered condition. LSD bars are present on a given date where statistically significant differences were observed between chemical treatments ($P \le 0.05$). No LSD bar indicates no significant differences on that date.
In 2016, treatments did not significantly differ in their EL. In 2017, well-watered plants had an average EL of 10.8% and did not change significantly throughout the study. EL in drought-stressed plants increased over time and a significant difference in EL was evident among the priming treatments on day 11. On 11 d, TE+SA had a significantly lower EL (67.2%) than all the other treatments (81.5% average) (Fig. 4).

Canopy temperature depression (CTD)



Figure 5. A B. Canopy temperature depression (CTD) of CBG leaves in response chemical and drought conditions for 2016 and 2017 GC studies. The horizontal bar represents the chemical treatment average in well-watered condition. LSD bars are present on a given date where statistically significant differences were observed between chemical treatments ($P \le 0.05$). No LSD bar indicates no significant differences on that date.

CTDs of the well-watered pots were not different among the chemical treatments in either year and had an average value of -0.68 ° C in 2016 and -5.02 in 2017 (Fig. 5AB). Drought stresstreated plants exhibited significant differences among chemical treatments on 12 d in 2016. On this date, the control (8.075 °C) had a higher CTD than TE+JA, TE+SA and TE+GABA (5.6 °C average) (Fig. 5A). In 2017, CTD increased for drought treated plants over the course of the study and significant differences were observed on 5, 8 and 11 days (Fig. 5B). For example, TE+SA (-2.72 °C) and TE+JA (-2.15°C) were lower than the control on day 5. TE +SA had a significantly lower CTD (-0.9°C) than all other chemical treatments (averaged at 2.15°C) on 8 d, and the similar difference was observed on 11 d (1.3°C versus 3.27°C).

Chlorophyll content

Table 1. Mean chlorophyll content (mg g⁻¹ DW) of plants treated with priming agents used for both drought stressed and well-watered conditions for 2016 growth chamber study. LSD values ($p \le 0.05$) are for comparison between priming chemicals on a given day of measurement.

Duration of treatment (days)							
Priming agent	1 d	7 d	12 d	15 d			
control	22.02	21.12 ab*	17 b	15.45 b			
TE	22.42	23.16 a	20.46 a	18.05 a			
SA	21.58	21.58 ab	17.04 ab	15.86 b			
GABA	22.24	18.87 b	16.26 b	14.49 b			
JA	21.46	22.47 a	17.08 ab	15.91 b			
TE+SA	22.48	22.98 a	20.59 a	18.37 a			
TE+GABA	20.69	22.62 a	20.32 a	18.4 a			
TE+JA	20.74	20.68 ab	17.48 ab	15.53 b			
LSD	NS^{\dagger}	3.3	3.8	1.4			

[†] NS stands for non-significant, *Values followed by the same letter within a column are not significantly different ($p \ge 0.05$) Fisher's LSD test ($\alpha = 0.05$)

In 2016, there was no significant interaction between priming chemicals and irrigation on CBG leaf chlorophyll (Chl) content. However, the single effect of chemicals was noted from days 7 to 15 (Table 1). Chemical priming caused significant changes in Chl in both drought stressed and well-watered conditions on days 7, 12 and 15. For instance, Chl declined from 21.7 mg g^{-1} DW on day 1 to 16.6 on day 15. TE, TE+SA, and TE+GABA had a higher chlorophyll content than the control on days 7 and 15 (Table 1).



Figure 6. Chlorophyll content of CBG leaves in response chemical and drought conditions for the 2017 year. LSD bars are present on a given date where statistically significant differences were observed between chemical treatments ($P \le 0.05$). No LSD bar indicates no significant differences on that date.

Differences in Chl content were noted under drought conditions in 2017 GC study on day 5

and 8. On 5 d the rest of treatments were higher than the control (13.1) on day 5 and the same

trend was observed on day 8 (Fig. 6).

Field study results



Environmental conditions: weather data

Figure 7. A B. Daily maximum and minimum temperature during the experimental period of 2016 and 2017 trial on drought tolerance in creeping bentgrass at HTRC, East Lansing, MI.

The average temperatures showed slight variation between the two years. In 2016, there were more days with a maximum temperature above 25° C than in 2017. In 2016, the average daily maximum was 29.3°C and the daily minimum was 16.8°C In 2017, the max and min were 27°C and 15.1°C, respectively (Fig. 7AB).

Soil water content (SWC)



Figure 8. A B. Effect of chemical priming treatment on the soil volumetric water content (% SWC) of creeping bentgrass (CBG) in drought and well-watered conditions in 2016 and 2017 trials. LSD bars are present on a given date where statistically significant differences were observed between chemical treatments ($P \le 0.05$). No LSD bar indicates no significant differences on that date.

In the well-watered plots, SWC did not significantly differ in both years and had an average of 38.7 % in 2016 and 43.4% in 2017 (Fig. 8 AB). A decline in soil water content (SWC) was observed as a response to drought imposition. In 2016, SWC decreased from an average of 36.8% on 1d to 16.2 % on 14 d (Fig. 8A). Under drought stress, significant differences among the chemicals were recorded on days 7 and 14. TE+SA, TE, TE+JA and JA treatments (average 28.16 %) had a substantially higher SWC than the control (16.5%) on 7 d (Fig. 8A). On 14 d, three treatments (TE+SA, TE, and TE+JA) had an average SWC of 21.9 % and were higher than the controls (11.8%) (Fig.8A). In 2017, SWC declined from 33.1% on 1 d to 13.7% on 17 d (Fig. 8B). Priming chemicals differed significantly in their SWC on days 9 and 13 of drought stress imposition. TE+SA (28.3%) treated plots had a substantially higher SWC than the controls (17.6%) on 9 d (Fig. 8B). On 13 d, the interaction of treatment and irrigation was significant, but no differences among the treatments occurred under drought stress.

Turf Quality (TQ)



Figure 9. A B. Effect of chemical treatment on the turf quality rating (1-9 scale; 1= best, 9=best) of creeping bentgrass (CBG) under drought stress for 2016 and 2017 field trials. The horizontal bar represents the chemical treatment average in well-watered condition. LSD bars are present on a given date where statistically significant differences were observed between chemical treatments ($P \le 0.05$). No LSD bar indicates no significant differences on that date.

In well-watered conditions, plots treated with different priming chemicals had nonsignificantly different TQ with an average of 8.3 in 2016 and 8.4 in 2017 for the duration of the studies (Fig. 9AB). Drought stress caused a decline in Turf quality, and considerable differences were recorded only on 14 d in 2016 (Fig. 9A). On that day control and GABA had a lower TQ rating (3.8 average) than the rest (6.6). For the year 2017, significant differences in TQ were recorded on days 13 and 17 (Fig. 9B). On day 13, control and GABA treatments (2.8 average) had a TQ significantly lower than the rest (5.54 average), the same trend was noted on day 17 (2.6 versus 5.3).

Normalized difference vegetation index (NDVI)



Figure 10. Normalized difference vegetation index (NDVI) rating of CBG plants under drought stress conditions in 2017 field trail (C). The horizontal bar represents the chemical treatment average in well-watered condition. LSD bars are present on a given date where statistically significant differences were observed between chemical treatments ($P \le 0.05$). No LSD bar indicates no significant differences on that date.

Under drought stress, priming chemicals differed in their NDVI rating only in the year 2017. In the well-watered conditions, no significant differences were found among priming agents in either of the years (2016 or 2017). Under drought stress conditions, priming chemical differed in their NDVI on 13 d with control (0.60) GABA (0.61), and SA (0.62) treated plots had a significantly lower NDVI rating than the rest of treatments (0.7 average) (Fig. 10).

Relative water content (RWC)



Figure 11. A B. Relative water content (RWC) of CBG leaves in response to chemical and water treatments (for drought conditions) in 2016 and 2017 field trials. The horizontal bar represents the chemical treatment average in well-watered condition. LSD bars are present on a given date where statistically significant differences were observed between chemical treatments ($P \le 0.05$). No LSD bar indicates no significant differences on that date.

In well-watered conditions, RWC did not vary significantly; the average for all treatments was 91.7 in 2016 and 81.3 %. In 2017 (Fig 10 AB). For the year 2016, differences in RWC were detected among priming agents on day 14 of drought (Fig. 10A). TE+SA (77.3%), TE and TE+JA (75.6 average) were considerably higher than the control (61.1%). For the 2017 year, on day 13 of drought stress differences in RWC were noted among priming agents (Fig. 10B). Four treatments (TE+SA, TE, JA, and TE+JA) averaged an RWC of 56.6% and were considerably higher than the control (47.3%).

Chlorophyll index

Table 2. Mean chlorophyll index of plants treated with priming agents for drought-stressed and well-watered conditions in 2016 and 2017 studies in field conditions under a rainout shelter at East Lansing, MI. LSD values ($p \le 0.05$) are for comparison between priming chemicals on a given day of measurement.

	Chlorophyll index (0.000-999)							
	20)16	-	-	•	2017		
PA	1 d	7 d	14 d	1 d	5d	9 d	13 d	17d
control	326.13a	345.1bc	305.12	278.6	241b	250.0b	234.6 a	232d
	b*	d						
TE	333.25a	367.7ab	332.12	307.1	279.38a	277.1ab	271.8 a	280.6a
	b	с						
SA	326ab	336.5bc	303.37	284.3	254.5ab	257.9ab	236.6 a	243.5bc
		d						d
GABA	311.7b	329.75c	298.25	299.1	266.13a	265.1ab	242.1 a	239.9cd
		d			b			
JA	316.37a	326.13d	301.25	299.0	254.63a	254.3ab	241.0 a	244.1bc
	b				b			d
TE+S	340.75a	361.8ab	307.25	308.6	276.63a	278.6a	270.3 a	276.9ab
А	b	cd						
TE+G	345a	372.25a	321.87	306.6	283.88a	269.0ab	266.4 a	268.5ab
ABA		bc						с
TE+J	340ab	380.88a	330.25	305.8	280.25a	271.8ab	263.8 a	267.9ab
А								с
LSD	31.2	40	ns	ns	30.3	27.6	39	36.3

*Values followed by the same letter within a column are not significantly different (p ≥ 0.05) based on Fisher's LSD test ($\alpha = 0.05$)

In field conditions, there was no significant interaction between priming chemicals and water treatments on the Chl index. However, Chl index was significantly affected by priming agents irrespective of water treatments in both years. For the two years, few significant differences were noted among priming agents regarding their Chl index. GABA and control tended to have lower Chl index when compared to other treatments (Table 2).

DISCUSSION

The effect of drought on SWC was more pronounced in the growth chamber than in field conditions. Over a period of two weeks of drought stress, the SWC of the GC declined in 2017 from 22.2 % to 1.36 %, which corresponds to a 94% decline in SWC. While for two weeks of the field study, SWC declined from 36.8 % to 16.2 % in 2016, which corresponds to a 56 % decline in SWC. These differences are expected due to environmental and experimental differences that occur in field vs. growth chamber studies. Factors that affect evapotranspiration such as temperature, wind disturbances, relative humidity, pot vs. plot size, and other factors can play a major role in the rate of drying during drought studies.

Results from the two years of the growth chamber study show that there were no significant differences among chemical treatments in SWC either among well-watered plants or among drought-stressed plants in either of the GC studies. Contrastingly, in the field study, significant differences in SWC were observed in plots treated with different chemical priming agents and drought stress. TE, TE+SA, TE+JA treated plants showed similar, higher SWC than the controls. Thus, TE treatment may have altered plant canopy water use or water loss characteristics under field conditions. Based on our measurements, it is not clear which of these, water use or water loss, may have been affected by TE and it is not clear why these differences may not have occurred under growth chamber conditions. The field study results suggest the role of TE and TE plus priming agent treatments (TE+JA, TE+SA) are possibly altering drought stress avoidance characteristics in CBG. In another drought stress study, TE has been shown to reduce soil water depletion in CBG by lowering evapotranspiration rates.

Those plants also had a higher SWC and a higher RWC in leaves than the untreated plants in growth chamber conditions (Bian et al., 2009). It is possible that the reduction in cellular elongation and growth caused by GA inhibition by TE could play a role in reducing plant water use or canopy characteristics such as leaf density, which would, in turn, reduce evapotranspiration and thereby maintain higher SWC levels.

Significant differences among priming agents were detected in leaves of CBG for RWC and CTD and NDVI. TE, TE+SA, TE+JA treated plants demonstrated higher leaf RWC than the control in both GC and field studies and a higher NDVI in field conditions. Plants treated with the same treatments (TE, TE+SA, and TE+JA) had a lower CTD than the untreated in the growth chamber study. These results suggest the role of TE and TE based treatments in improving CBG leaf water status and maintenance of leaf surface evaporative cooling under drought stress for the growth chamber study. However, for the field conditions, this could be attributed to the higher SWC associated with TE and TE-related treatments (TE+SA and TE+JA). Other drought stress studies have shown the positive role of TE alone or in combination with other PGRs or osmoregulants on CBG leaf hydration and performance under drought stress. TE+glycine betaine treated CBG plants showed a higher membrane stability, RWC and higher NDVI than plants treated with TE or betaine alone (Burgess and Huang, 2014). Foliar application of TE or indole-3-butyric acid (IBA) alone or in combination improved CBG quality, photosynthesis and stomatal conductance and leaf water relations (Zhang et al., 2017). Additional work to eliminate confounding effects of drought avoidance characteristics altered by TE would be needed to determine if any metabolic effects or tolerance traits are enhanced with priming treatments in plants treated with TE.

Another physiological parameter that was influenced by chemical priming under drought stress is electrolyte leakage. TE+SA treated plants had the lowest EL among all other chemical treatments in the growth chamber experiment, including TE alone. This suggests a possible role of SA in maintaining cell membrane integrity during drought stress. SA alone could contribute to drought stress alleviation by lowering cell membrane damage translated as lower EL, which is similar to results found in another study of CBG (Li et al., 2017) and other species like black cumin (Nigella *sativa*) (Kabiri et al., 2014). TE treated plants supported a higher level of SA accumulation compared to untreated plants under drought stress (Krishnan and Merewitz, 2015). Despite internal upregulation of SA due to TE treatment, it is possible that external SA application could still provide benefits to plants treated with TE under drought stress conditions. GABA, another well-known drought stress priming agent with a potential impact in lowering EL under-drought stress on a number of turfgrass species (Krishnan et al., 2013), was tested here but did not perform well in this study.

Another measured parameter was chlorophyll content. In this study, we found a significant interaction between drought stress and priming chemical treatment only in 2017 GC study. That interaction did show any significant difference among priming agents, but they were all higher than the control. However, a single significant effect of priming agents on chlorophyll was noted in the 2016 GC study and both field studies. TE, TE+SA and TE+GABA treated plants were not significantly different from each other, but all had a higher Chl content than the control in the 2016 GC study. The field studies also showed that TE, TE+SA and TE+GABA and TE+GABA and TE +JA treatments had significantly higher Chl index than the control. This suggests the role of TE in the maintenance of the photosynthesis apparatus in both dry and wet

water treatments in GC. This is in agreement with other studies that documented the role of TE increased for the role of Chl irrespective of water treatment. For example, TE increased Kentucky bluegrass leaf cell density and chlorophyll concentration (Ervin and Koski, 2001). For the field study, it is not clear if this is due to direct effects of TE on Chl or if the higher SWC that was associated with TE and TE-related treatments played a significant role in maintaining plant health and therefore Chl.

In conclusion, it is clear that TE treatment can promote drought stress resistance in CBG. The main goal of the study was to determine whether priming agents in combination with TE provide any benefit or may be unnecessary. SA priming of TE treated plants may have provided a metabolic benefit, which we observed as a lower level of EL. Otherwise, no clear benefits were detected for any of the priming treatments to TE treated plants or due to priming treatment alone. Thus, specialty products or fertilizers containing SA, JA or components that may stimulate an ISR or SAR response may not be needed in plants already treated with TE. However, future research should look at varying rates of priming treatments and other abiotic or biotic stresses to determine whether these priming agents in combination with TE may promote plant health.

APPENDIX

Tables for ANOVA

Table 3. Analysis of variance (AOV) table for SWC of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2016 growth chamber study.

		2016 tr	eatment			
		Days after withh	olding irrigation			
Source of variation	df	Day 1	Day 7	Day 12	Day 15	
rep	3					
Priming agents (PA)	7	NS	NS	NS	NS	
Irrigation (I)	1	NS	*	*	*	
PA×I	7	NS	NS	NS	NS	
Error	45					
Total	63					
*and NS indicate significance at P≤0.05, and not significant at P≥0.05, respectively						

Table 4. Analysis of variance (AOV) table for SWC of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2017 growth chamber study.

		2017 tre	eatment			
		Days after withh	olding irrigation			
Source of variation	df	Day 0	Day 2	Day 5	Day 8	Day 11
Row id (irrig)	3					
Priming agents (PA)	7	NS	NS	NS	NS	NS
Irrigation (I)	1	NS	*	*	*	*
PA×I	7	NS	NS	NS	NS	NS
Error	45					
Total	63					
*and NS indicate significance at P≤0.05, and not significant at P≥0.05, respectively						

Table 5. Analysis of variance (AOV) table for RWC of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2016 growth chamber study.

		2016 tr	eatment		
		Days after withh	olding irrigation		
Source of variation	df	Day 1	Day 7	Day 12	Day 15
rep	3				
Priming agents (PA)	7	NS	NS	*	*
Irrigation (I)	1	NS	*	*	*
PA×I	7	NS	NS	*	*
Error	45				
Total	63				
*and	NS indicate sig	gnificance at P≤0.05,	and not significant at	t P≥0.05, respectively	1

Table 6. Analysis of variance (AOV) table of RWC of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2017 growth chamber study.

		2017 tre	eatment			
		Days after withh	olding irrigation			
Source of variation	df	Day 0	Day 2	Day 5	Day 8	Day 11
rep	3					
Priming agents (PA)	7	NS	NS	NS	NS	*
Irrigation (I)	1	NS	NS	*	*	*
PA×I	7	NS	NS	NS	*	*
Error	45					
Total	63					
*and 1	NS indicate sig	gnificance at P≤0.05, a	and not significant at	P≥0.05, respectively	1	

Table 7. Analysis of variance (AOV) table for TQ of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2016 growth chamber study.

		2016 tr	eatment			
		Days after withh	olding irrigation			
Source of variation	df	Day 1	Day 7	Day 12	Day 15	
rep	3					
Priming agents (PA)	7	NS	NS	*	*	
Irrigation (I)	1	NS	*	*	*	
PA×I	7	NS	NS	*	*	
Error	45					
Total	63					
*and NS indicate significance at P≤0.05, and not significant at P≥0.05, respectively						

Table 8. Analysis of variance (AOV) table of TQ of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2017 growth chamber study.

		2017 tre	eatment			
		Days after withh	olding irrigation			
Source of variation	df	Day 0	Day 2	Day 5	Day 8	Day 11
rep	3					
Priming agents (PA)	7	NS	*	NS	NS	*
Irrigation (I)	1	NS	NS	*	*	*
PA×I	7	NS	NS	NS	*	*
Error	45					
Total	63					
*and 1	NS indicate sig	gnificance at P≤0.05, a	and not significant at	P≥0.05, respectively	/	

Table 9. Analysis of variance (AOV) table for CTD of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2016 growth chamber study.

		2016 tr	eatment		
		Days after withh	olding irrigation		
Source of variation	df	Day 1	Day 7	Day 12	Day 15
rep	3				
Priming agents (PA)	7	NS	NS	NS	NS
Irrigation (I)	1	*	*	*	*
PA×I	7	NS	NS	*	NS
Error	45				
Total	63				
*and	NS indicate sig	nificance at P≤0.05, a	and not significant at	P≥0.05, respectively	/

Table 10. Analysis of variance (AOV) table of CTD of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2017 growth chamber study.

		2017 tr	eatment			
		Days after withh	olding irrigation			
Source of variation	df	Day 0	Day 2	Day 5	Day 8	Day 11
rep	3					
Priming agents (PA)	7	NS	NS	NS	NS	*
Irrigation (I)	1	*	*	*	*	*
PA×I	7	NS	NS	*	*	*
Error	45					
Total	63					
*and 1	NS indicate sig	nificance at P≤0.05, a	and not significant at	P≥0.05, respectively	1	

Table 11. Analysis of variance (AOV) table for Chl. of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2016 growth chamber study.

		2016 tr	eatment		
		Days after with	olding irrigation		
Source of variation	df	Day 1	Day 7	Day 12	Day 15
rep	3				
Priming agents (PA)	7	NS	*	*	*
Irrigation (I)	1	NS	NS	*	*
PA×I	7	NS	NS	NS	NS
Error	45				
Total	63				
*and	NS indicate sig	gnificance at P≤0.05,	and not significant at	t P≥0.05, respectively	/

Table 12. Analysis of variance (AOV) table of chl. of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2017 growth chamber study.

		2017 tre	eatment			
		Days after withh	olding irrigation			
Source of variation	df	Day 0	Day 2	Day 5	Day 8	Day 11
rep	3					
Priming agents (PA)	7	NS	NS	*	*	*
Irrigation (I)	1	*	NS	NS	*	*
PA×I	7	NS	NS	*	*	NS
Error	45					
Total	63					
*and	NS indicate sig	gnificance at P≤0.05, a	and not significant at	P 20.05, respectively	1	

Table 13. Analysis of variance (AOV) table of EL of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2017 growth chamber study.

		2017 tr	eatment							
	Days after withholding irrigation									
Source of variation	df	Day 0	Day 2	Day 5	Day 8	Day 11				
rep	3									
Priming agents (PA)	7	NS	*	NS	NS	*				
Irrigation (I)	1	NS	NS	*	*	*				
PA×I	7	NS	NS	NS	NS	*				
Error	45									
Total	63									
*and 1	NS indicate sig	gnificance at P≤0.05, a	and not significant at	$P \ge 0.05$, respectively	/					

Table 14. Analysis of variance (AOV) table for soil water content of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2016 field study.

Source of variation	df	Day 1	Day 7	Day 14		
rep	3					
Priming agents (PA)	7	NS	*	*		
Irrigation (I)	1	NS	*	*		
PA×I	7	NS	*	*		
Error	45					
Total	63					
*and NS indicate significance at P \leq 0.05, and not significant at P \geq 0.05, respectively						

Table 15. Analysis of variance (AOV) table for soil water content of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2017 field study.

Source of variation	df	Day 1	Day 5	Day 9	Day 13	Day 17
rep	3					
Priming agents (PA)	7	NS	NS	*	NS	NS
Irrigation (I)	1	*	*	*	*	*
PA×I	7	NS	NS	*	*	NS
Error	45					
Total	63					
	*and NS ir	ndicate significance a	at P≤0.05, and not sig	gnificant at P≥0.05, r	respectively	

Source of variation	df	Day 1	Day 7	Day 14			
rep	3						
Priming agents (PA)	7	NS	NS	NS			
Irrigation (I)	1	NS	*	*			
PA×I	7	NS	NS	*			
Error	45						
Total	63						
*and NS indicate significance at P≤0.05, and not significant at P≥0.05, respectively							

Table 16. Analysis of variance (AOV) RWC of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2016 field study.

Table 17. Analysis of variance (AOV) table for RWC of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2017 field study.

Source of variation	df	Day 1	Day 5	Day 9	Day 13	Day 17	
rep	3						
Priming agents (PA)	7	*	NS	NS	*	NS	
Irrigation (I)	1	NS	*	*	*	*	
PA×I	7	NS	NS	NS	*	NS	
Error	45						
Total	63						
*and NS indicate significance at P≤0.05, and not significant at P≥0.05, respectively							

Source of variation	df	Day 1	Day 7	Day 14		
rep	3					
Priming agents (PA)	7	NS	*	*		
Irrigation (I)	1	NS	*	*		
PA×I	7	NS	NS	*		
Error	45					
Total	63					
*and NS indicate significance at P \leq 0.05, and not significant at P \geq 0.05, respectively						

Table 18. Analysis of variance (AOV) of TQ of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2016 field study.

Table 19. Analysis of variance (AOV) table for TQ of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2017 field study.

Source of variation	df	Day 1	Day 5	Day 9	Day 13	Day 17		
rep	3							
Priming agents (PA)	7	NS	NS	*	*	*		
Irrigation (I)	1	NS	NS	*	*	*		
PA×I	7	NS	NS	NS	*	*		
Error	45							
Total	63							
*and NS indicate significance at P≤0.05, and not significant at P≥0.05, respectively								
Total	63 *and NS in	ndicate significance a	at P≤0.05, and not sig	gnificant at P≥0.05, r	espectively			

Source of variation	df	Day 1	Day 7	Day 14	
rep	3				
Priming agents (PA)	7	NS	NS	NS	
Irrigation (I)	1	NS	*	*	
PA×I	7	NS	NS	NS	
Error	45				
Total	63				
*and NS indicate	e significance a	at P ≤ 0.05 , and not sign	gnificant at P≥0.05, r	espectively	

Table 20. Analysis of variance (AOV) of NDVI of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2016 field study.

Table 21. Analysis of variance (AOV) table for NDVI of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2017 field study.

Source of variation	df	Day 1	Day 5	Day 9	Day 13	Day 17	
rep	3						
Priming agents (PA)	7	NS	*	*	*	*	
Irrigation (I)	1	NS	NS	*	*	*	
PA×I	7	NS	NS	NS	*	NS	
Error	45						
Total	63						
*and NS indicate significance at P≤0.05, and not significant at P≥0.05, respectively							

Source of variation	df	Day 1	Day 7	Day 14			
rep	3						
Priming agents (PA)	7	*	*	NS			
Irrigation (I)	1	NS	*	*			
PA×I	7	NS	NS	NS			
Error	45						
Total	63						
*and NS indicate significance at P≤0.05, and not significant at P≥0.05, respectively							

Table 22. Analysis of variance (AOV) of chl. of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2016 field study.

Table 23. Analysis of variance (AOV) table for chl. of CBG, as influenced by priming agents (PA) and irrigation (I) treatments in 2017 field study.

Source of variation	df	Day 1	Day 5	Day 9	Day 13	Day 17		
rep	3							
Priming agents (PA)	7	NS	*	*	*	*		
Irrigation (I)	1	NS	NS	*	*	*		
PA×I	7	NS	NS	NS	NS	NS		
Error	45							
Total	63							
*and NS indicate significance at P≤0.05, and not significant at P≥0.05, respectively								

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