Environmental and Experimental Botany 131 (2016) 101–109

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Osmotic stress- and salt stress-inhibition and gibberellin-mitigation of leaf elongation associated with up-regulation of genes controlling cell expansion

Qian Xu[a,b], Patrick Burgess[b], Jichen Xu[a], William Meyer[b], Bingru Huang[b,*]

[a] National Engineering Laboratory for Tree Breeding, Beijing Forestry University, Beijing, China
[b] Department of Plant Biology and Pathology, Rutgers, The State University of New Jersey, New Brunswick, NJ, United States

ARTICLE INFO

Article history:
Received 16 February 2016
Received in revised form 27 May 2016
Accepted 2 June 2016
Available online 3 June 2016

Keywords:
Grass
Salt stress
Osmotic stress
Leaf elongation
Gene
expression
Gibberellic acid

ABSTRACT

Leaf elongation rates (LER) is sensitive to salt stress and osmotic stress, and gibberellic acid (GA) may mediate stress-inhibition of leaf elongation by affecting genes controlling cell expansion. The objectives of this study were to examine the differential inhibitory effects of osmotic stress and salt stress on LER for fast- and slow-growing genotypes of tall fescue (*Festuca arundinacea*) and to determine whether GA could mitigate stress-induced decline in LER. Plants of fast-growing 'K-31′ and slow-growing 'Bonsai' were grown hydroponically and treated with GA under either osmotic or salt stress. LER of both genotypes were inhibited by osmotic and salt stress (46–86%) due to reduced cell elongation and production rate. 'Bonsai' LER was reduced to a greater extent than that of 'K-31′ due to greater reduction in cell production rate. Exogenous GA application significantly enhanced LER regardless of stress for both genotypes, which was more pronounced in 'Bonsai' than in 'K-31′ caused by greater cell elongation and production rates. Quantitative PCR analysis revealed that four expansin genes (*EXPA4*, *EXPA5*, *EXPA7* and *EXPB7*) and one XET gene (*XET2*) were down-regulated by osmotic stress and *EXPA7* was down-regulated by salt stress in both genotypes. Three expansin genes (*EXPA4, EXPA5* and *EXPA7*) were up-regulated by GA treatment in both genotypes under non-stress conditions, in which *EXPA4* was also up-regulated by GA treatment in both genotypes under osmotic stress. The expression level of *XET1* and *XET3* increased in 'K-31′ and 'Bonsai', respectively, with exogenous GA treatment under non-stress and salt stress. This study demonstrated the roles of GA regulation of genetic variations in leaf differential responses to osmotic and salt stress involving different expansins and XET genes.

1. Introduction

Salinity is a major abiotic factor limiting plant growth and development in areas with limited fresh water supplies and poor-quality water for irrigation. The detrimental effects of salinity can be due to the direct effect of high salt accumulation or the induction of osmotic stress. Cell expansion driving leaf elongation is one of the most sensitive growth traits affected by salt stress or salt-induced osmotic stress (Cramer, 2003). Stress-induced decline in cell and leaf elongation has been reported in various plant species such as tall fescue (*Festuca arundinacea*) during osmotic stress (-2.0 MPa) (Durand et al., 1995) and wheat (*Triticum aestivum*) during salt stress (Hu and Schmidhalter, 2008). The reduction in wheat leaf area by salt stress was attributed to both decreased cell length and number of epidermal cells (Hu and Schmidhalter, 2007). Despite the well-known adverse effects of osmotic and salt stress on leaf expansion, the mechanisms underlying stress-limitation of leaf growth are not well understood.

Cell elongation is governed by factors controlling cell-wall extensibility by embedded cell- wall proteins, such as expansin and xyloglucan endotransglycosylase (XET) (Cosgrove, 1998). It is well known that expansin eases cell wall tension by disrupting the hydrogen bonds linking cellulose microfibrils to matrix polysaccharides or between cross-linked matrix polysaccharides (Cosgrove, 1997). Alternatively, XETs serve important roles in both loosening and strengthening of cell walls through transient matrix cleavage without hydrolysis (Eklöf and Brumer, 2010). Choi et al. (2003) found that over-expressing the expansin gene *OsEXP4*

Abbreviations: GA, gibberellic acid; LER, leaf elongation rates; LM, epidermal cell length; P, cell production rate; PEG, polyethylene glycol 8000; XET, xyloglucan endotransglycosylase.

* Corresponding author.
E-mail address: huang@aesop.rutgers.edu (B. Huang).

http://dx.doi.org/10.1016/j.envexpbot.2016.06.001
0098-8472/

increased coleoptile and mesocotyl length by 31 and 97%, respectively, and anti-sensing the gene decreased lengths by 28 and 43%, respectively, thereby suggesting *OsEXP4* is associated with alterations in cell size and cell wall extensibility. Positive correlations between expansin expression and cell elongation were also demonstrated by over-expressing a soybean (*Glycine max*) expansin gene *GmEXPB2 in Arabidopsis (Arabidopsis thaliana)* (Guo et al., 2011). Palmer and Davies (1996) found that XET activity peaked near the base of the growing zone of maize (*Zea mays*) third leaves and was associated with the maximum relative elemental growth rate in this zone. XET activity, elongation rates, and cell lengths then declined as maize leaves matured. Expression of expansin and XET genes has been positively correlated with improved drought or salt tolerance, such as *CaXTH3* in hot pepper (*Capsicum annuum*) (Cho et al., 2006) and *TaEXPB23 in wheat* (Li et al., 2011). *Members of the expansin and XET gene families serve diverse roles in regulating plant growth and stress tolerance across various plant species* (Cal et al., 2013; Harb et al., 2010; Muller et al., 2007). *For example, TaEXPB23* is responsive to drought or salt stress but not to heat stress in wheat (Han et al., 2012) while *AsEXP1* is responsive to heat stress in *Agrostis stolonifera* (Xu et al., 2007). However, specific expansins and XET genes related to stress-inhibition of leaf growth during water or salt stress and are not well described.

Plant hormones such as gibberellic acid (GA) also serve integral regulatory roles influencing leaf growth rates (Nelissen et al., 2012; Smith et al., 1996). GA is considered to be a primary hormone regulating cell elongation in various plant organs including leaves (Bultynck and Lambers, 2004), internodes (Potter and Fry, 1993), hypocotyls (Cowling and Harberd, 1999), and roots (Ubeda-Tomás et al., 2009). Muhammad et al. (2010) showed that GA_3 applied exogenously at varied concentrations (0.5, 1.0, and 5.0 μM) significantly increased plant height as well as root and shoot biomass of soybean seedlings under salt stress (100 mM NaCl) and it was suggested that GA_3 mitigates the adverse effects of salt stress on root and shoot growth (Hamayun et al., 2010). GA regulates plant growth by means of gene-expression stimulation or repression with subsequent downstream effects on cell elongation and division (Azeez et al., 2010; Lee and Kende, 2002; Smith et al., 1996). For example, induction of *OsEXPA4* gene expression by exogenous GA was positively correlated to rapid elongation of rice internodes (Choi et al., 2003; Lee and Kende, 2002). Despite the positive relationship between GA, cell-wall loosening genes, and cell elongation, little is known as to whether osmotic- and salt-inhibition of leaf growth is mediated by GA-regulated gene expression of cell wall loosening genes. Furthermore, various genes within the expansin and XET gene families may serve different roles in genetic variance, hormonal regulation, and stress responses controlling leaf growth rates. Investigating the variable expression patterns of expansin and XET genes may help to describe the individual roles of specific genes in relation to hormone cues and stress response pathways.

Tall fescue has broad genetic variations in LER with fast and slow-growing (or dwarf-type) genotypes utilized across forage and turfgrass settings (Huang et al., 1998; Huang and Fry, 1998; Volenec and Nelson, 1981). Our preliminary study suggested that 'K-31′ groOS faster than 'Bonsai' due to greater cell elongation and production rates and exogenous GA enhanced LER by stimulating cell elongation and production rates in leaves of both genotypes. The quantitative PCR analysis revealed that four expansin and two XET genes were associated with GA-stimulation of leaf elongation and two expansin genes could contribute to the genotypic variations in LER in tall fescue. It is hypothesized that fast- and slow-growing leaves may exhibit differential responses to osmotic or salt stress as regulated by GA stimulation of expansin and XET gene-expression during either stress. The specific objectives of the current study were to 1) examine differential responses of leaf elongation involving cell elongation and production rates for fast- and slow-growing genotypes of tall fescue exposed to osmotic or salt stress, 2) determine whether GA mitigates the decline in LER induced by osmotic or salt stress, and 3) elucidate on whether differential expression patterns of expansins and XETs contribute to the negative effects of osmotic or salt stress and positive effects of GA on LER.

2. Materials and methods

2.1. Plant material and hydroponic growth conditions

Seedlings of two tall fescue (*Festuca arundinacea*) genotypes, fast-growing 'K-31′ and slow-growing (dwarf-type) 'Bonsai' were established from seeds in plastic bins (54 × 42 × 14 cm) filled with modified Hoagland solution (Hoagland and Arnon, 1950). The nutrient solution contained ammonium sulfate ($(NH4)_2SO_4$, 71.36 mg L^{-1}), potassium nitrate (KNO_3, 27.3 mg L^{-1}), calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, 120.8 mg L^{-1}), potassium phosphate monobasic (KH_2PO_4, 81.65 mg L^{-1}), potassium sulfate (K_2SO_4, 52.28 mg L^{-1}), magnesium sulfate anhydrous($MgSO_4$, 60 mg L^{-1}), EDTA-ferric sodium salt trihydrate (Fe(EDTA)Na, 16.84 mg L^{-1}), boric acid (H_3BO_3, 1.43 mg L^{-1}), manganese chloride ($MnCl_2{\cdot}4H_2O$, 0.91 mg L^{-1}), zinc sulfate ($ZnSO_4$. H_2O, 0.11 mg L^{-1}), cupric sulfate ($CuSO_4$, 0.04 mg L^{-1}), ammonium molybdate ($(NH_4)Mo_7O_{24}{\cdot}4H_2O$, 0.01 mg L^{-1}). The nutrient solution was aerated by air pumps and replaced every 5 d. Nutrient solution pH was adjusted every other day to 5.8 using KOH. Plants were maintained in a walk-in growth chamber (Environmental Growth Chambers, Chagrin Falls, OH) set to 22/17 °C day/night, 60% relative humidity, 16 h photoperiod, and 680 μmol m^{-2} s^{-1} photosynthetically active radiation at the canopy level.

2.2. Osmotic stress, salt stress, and gibberellin treatments

Uniform-sized seedlings were selected at the 3.2 leaf stage according to the Haun Idex (Haun, 1973) when the third leaf was fully expanded for exposure to osmotic and salinity stress. For osmotic stress, seedlings were transferred to modified Hoagland's nutrient solution containing polyethylene glycol (PEG) 8000 with water potential of −0.5 MPa for 2 d, to a −1.0 MPa solution for additional 2 d, and finally to a −1.5 MPa solution for remainder of study. Water potential was continually monitored using a vapour pressure osmometer (Vapro5520, Wescor, Inc. Logan,UT) (Merewitz et al., 2010; Michel, 1983). Non-stress control plants were maintained in identical nutrient solution lacking PEG.

Salt stress was applied in a stepwise manner to avoid salt shock by gradually adding sodium chloride (NaCl) to the nutrient solution with increasing electrical conductivity of 1, 4, and 9 dS m^{-1} for 2 d at each level and finally maintained at 14 dS m^{-1} for remainder of study. The concentration of NaCl was about 6.6 mM, 44 mM, 81 mM, 128 mM at each level respectively. Salt concentration was continually monitored using an electrical conductance meter (Han et al., 2014). Non-stress control plants were maintained in identical nutrient solution lacking NaCl.

For the investigation of gibberellic acid regulation of leaf elongation, seedlings were treated with gibberellin A3 (GA_3) (Sigma-Aldrich, St. Louis, MO). The roots of plants were immersed in the nutrient solution containing 50 μmol L^{-1} GA_3 for 12 h, removed from GA nutrient solution, rinsed with deionized water, and transferred to nutrient solution lacking GA for 3 d, after which time LER was evaluated. A preliminary study evaluated six concentrations (0, 10, 25, 50, 100, and 200 μmol L^{-1}) of GA_3 and 50 μmol L^{-1} GA_3 was found to be the most effective concentration promoting leaf elongation.