Plant Science 235 (2015) 37–45

Contents lists available at ScienceDirect

Plant Science

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

Candidate gene association mapping for winter survival and spring regrowth in perennial ryegrass

Xiaoqing Yu[a], Paula M. Pijut[b], Stephen Byrne[c], Torben Asp[c], Guihua Bai[d], Yiwei Jiang[e,*]

[a] Department of Agronomy, Iowa State University, Ames, IA 50011, USA
[b] USDA-Forest Service, Northern Research Station, Hardwood Tree Improvement and Regeneration Center, West Lafayette, IN 47907, USA
[c] Department of Molecular Biology and Genetics, Faculty of Science and Technology, Research Centre Flakkebjerg, Aarhus University, Forsøgsvej 1, Slagelse 4200, Denmark
[d] USDA-ARS Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506, USA
[e] Department of Agronomy, Purdue University, West Lafayette, IN 47907-2054, USA

ARTICLE INFO

Article history:
Received 2 December 2014
Received in revised form 12 February 2015
Accepted 4 March 2015
Available online 11 March 2015

Keywords:
Association mapping
Candidate gene
Lolium perenne
Winter survival
Regrowth

ABSTRACT

Perennial ryegrass (*Lolium perenne* L.) is a widely cultivated cool-season grass species because of its high quality for forage and turf. Susceptibility to freezing damage limits its further use in temperate zones. The objective of this study was to identify candidate genes significantly associated with winter survival and spring regrowth in a global collection of 192 perennial ryegrass accessions. Significant differences in winter survival (WS), percentage of canopy green cover (CGC), chlorophyll index (Chl), and normalized difference vegetation index (NDVI) were found among accessions. After controlling population structure, *LpLEA3* encoding a late embryogenesis abundant group 3 protein and *LpCAT* encoding a catalase were associated with CGC and Chl, while *LpMnSOD* encoding a magnesium superoxide dismutase and *LpChl Cu-ZnSOD* encoding a chlorophyll copper–zinc superoxide dismutase were associated with NDVI or Chl. Significant association was also discovered between C-repeat binding factor *LpCBF1b* and WS. Three sequence variations identified in *LpCAT*, *LpMnSOD*, and *LpChl Cu-ZnSOD* were synonymous substitutions, whereas one pair of adjacent single nucleotide polymorphisms (SNPs) in *LpLEA3* and one SNP in *LpCBF1b* resulted in amino acid change. The results demonstrated that allelic variation in *LpLEA3* and *LpCBF1b* was closely related to winter survival and spring regrowth in perennial ryegrass.

1. Introduction

Perennial ryegrass (*Lolium perenne* L.) ($2n = 2x = 14$) is a cool-season grass species from the family *Poaceae* with a self-incompatible and outcrossing nature [1]. Native to Europe, Asia, and Northern Africa, it is one of the most important perennial grasses worldwide [2]. Widely cultivated in the temperate regions, perennial ryegrass has the highest forage quality of all cool-season grasses for feeding dairy cattle and sheep and is also a primary turf species with rapid growth and establishment [3]. Growing perennial ryegrass also benefits ecosystems by improving carbon sequestration, and soil formation, protection, and nutrient cycling [4]. However, perennial ryegrass used in cultivation is susceptible to extreme cold temperatures because of its relatively poor winter hardiness, which limits expanding the use of this grass in temperate zones. Genetic mechanisms of winter hardiness in perennial grasses are not well understood, owing to the quantitative trait of winter hardiness as well as the complex genetic nature and genomes of most popular perennial grasses. Exploration of genetic mechanisms underlying winter survival and spring regrowth is valuable for breeding programs aimed at improving winter hardiness of perennial grasses.

Winter hardiness is a complex trait, which is controlled by multiple genes involved in different pathways or physiological processes. The up-regulation of cold-regulated and dehydration-responsive ice-recrystallization-inhibition genes, and down-regulation of photosynthesis and respiration-related genes were found in cold-acclimatized perennial ryegrass, but not in non-acclimatized plants, suggesting a role of these genes in freezing tolerance [5]. Candidate genes involved in water movement across the membrane, cellular dehydration, and antioxidant metabolism may also facilitate winter hardiness of the plants. For example, cold stress induced a gradual increase in root osmotic hydraulic conductivity of rice (*Oryza sativa* L.), which accompanied a coordinated up-regulation of root aquaporin gene expression especially *OsPIP2;5* under low root temperature, indicating that root water

* Corresponding author. Tel.: +1 765 494 0651; fax: +1 765 496 2926.
E-mail address: yjiang@purdue.edu (Y. Jiang).

http://dx.doi.org/10.1016/j.plantsci.2015.03.003
0168-9452/

uptake function during the cold acclimation process was possibly regulated through aquaporins [6]. When a gene encoding late embryogenesis abundant (LEA) protein in barley (*Hordeum vulgare* L.) (HVA1) was introduced into mulberry (*Morus alba*), the mulberry plants showed increased cold tolerance [7]. A novel LEA protein in wheat (WCI16) was induced during cold acclimation and contributed to freezing tolerance in winter wheat (*Triticum aestivum* L.) [8]. Transcriptome profiling analysis revealed that genes encoding antioxidant enzyme such as catalase, peroxidase, and Cu–Zn superoxide dismutase was induced by cold stress [9,10]. At the protein level, the freezing-tolerant cultivar of strawberry (*Fragari ananassa*) [11] and zoysiagrass (*Zoysia japonica*) [12] had more stress-responsive proteins including those antioxidant and detoxification enzymes than the freezing-sensitive cultivars. Induction of cold-regulated proteins such as LEA, antifreeze proteins, and detoxification enzymes was common across some plant species [5,13], suggesting a protective role of these proteins in freezing tolerance.

C-repeat-binding factor (CBF)-dehydration-responsive element-binding factor (DREB) is an important pathway that regulates cold acclimation. *CBF* genes appeared to be ubiquitous in plant species [14–17]. It was estimated that up to 20% of cold-induced transcriptional changes were involved in *CBF1-3* in *Arabidopsis thaliana* [18]. A large cold responsive *CBF3* subfamily was identified in purple false brome (*Brachypodium distachyon*), while *CBF4* homologs were absent from the genome [19]. In perennial ryegrass, Tamura and Yamada [14] found 10 putatively distinct *CBF* genes that were similar to either the *HvCBF3* or *HvCBF4* subgroups in barley, and some of these genes were responsive to cold treatment. Overexpression of *CBF* genes from *Arabidopsis*, rice, wheat, and perennial ryegrass induced strong expression of stress-responsive genes in transgenic *Arabidopsis* plants, resulting in increased tolerance to freezing stresses [20–22]. Similarly, when two genes from winter wheat (*TaCBF14* and *TaCBF15*) were introduced into spring barley, transgenic plants were able to survive freezing temperatures several degrees lower than that which proved lethal for the wild-type spring barley [17]. In addition, the deletion of nine *CBF* genes in tetraploid wheat was associated with significant reductions in survival after exposure to freezing temperatures [23]. The results indicated a regulatory function of *CBF* in freezing tolerance.

The study of natural variation of winter hardiness holds great potential to dissect the genetic network controlling freezing tolerance [16,24–26]. Analysis of gene and trait association in a natural population allows identification of SNPs associated with freezing tolerance and winter survival traits. For example, through candidate-gene association analysis, two SNPs in *ScCBF15* and one in *ScCBF12* were significantly associated with frost tolerance in 201 genotypes from five Eastern and Middle European winter rye populations [16]. One major quantitative trait locus (QTL) in *LpCBFIIIc* was associated with freezing tolerance in 109 perennial ryegrass plants with the majority of the plants possessing the superior allele [25]. Through genome-wide association analysis, *FR-H1* and *FR-H2* controlling low temperature tolerance QTL were identified, which explained 25% of the phenotypic variation of winter hardiness in barley [24]. In *Arabidopsis*, natural variation of *CBF* genes was a major cause of divergence in freezing tolerance in four populations [27]. McKhann et al. [15] demonstrated that the Versailles core collection of 48 *Arabidopsis* accessions varied largely in freezing tolerance, polymorphism in the *CBF* genes as well as expressions of *CBF* and cold-regulated gene (*COR*), but *CBF* or *COR* gene expression was not closely correlated with freezing tolerance. The results suggested that a complexity of mechanisms underlying natural variation of freezing tolerance, and the *CBF* genes alone, cannot explain all differences in phenotype.

Although a large number of genes have been identified in the process of cold acclimation and freezing tolerance, little is known about whether allelic diversities of candidate genes contribute to winter survival and spring regrowth in perennial grass species. Given the fact that functional genes involved in dehydration, antioxidant, water movement across membranes, as well as regulatory genes such as *CBF* may influence winter survival of the plants, thus this study was designed to identify associations between candidate genes and winter survival related traits in a global collection of perennial ryegrass accessions. Perennial ryegrass is a diploid grass with more genetic and genomic information available for this species [28–33] than for any other major economically important perennial forage and turf grass species. The knowledge gained from this study will benefit the genetic improvement of winter hardiness in perennial ryegrass, and will also be valuable for investigation of other major cool-season perennial grass species with more complex genomes.

2. Materials and methods

2.1. Plant materials

The experiment was conducted at the Pinney Purdue Agriculture Center (PPAC) (Wanatah, IN, USA; 41°26′ N and 86°54′ W) and Turfgrass Research and Diagnostic Center (TRDC) at Purdue University (West Lafayette, IN, USA; 40°25′ N and 86°54′ W). A global collection of 192 accessions were used in the study, representing a wide range of ecotype diversity (Fig. 1, Supplemental Table S1). The panel included 72 wild, 66 cultivated, and 54 accessions with uncertain pedigree according to germplasm bank classification [32]. All the accessions were confirmed as diploid by flow cytometry [34]. Each accession was vegetatively propagated multiple times by tillers in a greenhouse to maintain genetic uniformity. The grasses were planted in the field at PPAC and TRDC in August 2008. Each location had three replications for each accession with the same genotype across locations. Soil type was sandy loam in PPAC and silt loam in TRDC. The maintenance of plants in the field during the study followed the routine practices for perennial ryegrass. The air temperatures of the two locations were shown in Supplemental Fig. S1.

2.2. Phenotypic traits

Phenotypic traits were recorded in two locations and years as well as different dates within a particular year. Specifically, winter survival (WS) was rated visually on April 25 at PPAC and April 27 at TRDC in 2009 using a 1–4 scale in which 1, 2, 3, and 4 represented completely dead plants without recovery, low, moderate, or high recovery of green tissues, respectively (Fig. 2). This observation served as a preliminary assessment of freezing tolerance and winter survival for the mapping population. The surviving plants were well established the second year after planting, and measurements focused only on traits related to spring regrowth. Percentage of canopy green cover (CGC), chlorophyll index (Chl), and normalized difference vegetation index (NDVI) parameters were recorded as indicators of spring regrowth on March 12, April 5, and April 23, 2011 at TRDC and April 12 and May 24, 2011 at PPAC. Canopy green coverage was rated visually as percentage of grass recovered after winter with zero for no green tissue coverage and 100% for complete green tissue coverage. A FieldScout CM 1000 Chlorophyll Meter (Spectrum Technologies, Inc., Plainfield, IL, USA) was used to measure Chl by setting wavelengths at 700 nm and 840 nm to estimate the quantity of chlorophyll in the leaves. This “point-and-shoot” technology instantly measures reflectance at these two wavelengths at the canopy level, and provides fast and reliable measurements of chlorophyll. Measurement distance between the meter and grass canopy was around 100 cm.