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Development of SCAR Marker Related to Summer Stress Tolerance in Tall Fescue (*Festuca arundinacea*)

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Abstract

Summer stress tolerance (SST) is one of the most important breeding objectives in tall fescue (*Festuca arundinacea*), an important perennial cool-season grass. However, breeding for better SST is generally complicated by the many environmental factors involved during the growing season. Utilizing the bulked segregant analysis (BSA), we were able to identify one marker related to SST from 100 inter-simple sequence repeat (ISSR) markers and 800 random amplified polymorphic DNA (RAPD) markers, and successfully developed a dominant sequence characterized amplified region (SCAR) marker T_SC856 from the UBC856 sequence. Furthermore, the SCAR marker was tested in different clones of new populations, which were identified under complex summer stress (high temperature and humidity, Pythium blight, and brown patch), and it exhibited relatively high consistency (77%) with the phenotype. We believe that with more markers obtained in the future, better efficiency is likely to be achieved in breeding for improved SST in tall fescue and possibly other species as well. Further studies that analyze the factors relating to the SCAR marker are needed.

Keywords: *Festuca arundinacea*, summer stress tolerance, SCAR marker, marker-assisted selection

Introduction

Tall fescue (*Festuca arundinacea*) is a common perennial cool-season grass (Poaceae) spread throughout the temperate regions of the world. It is widely used in parks, home lawns, athletic fields, golf courses and soil conservation sites. The optimum temperature for the growth of tall fescus is 16 °C～24 °C (Emmons, 1994). When grown and managed in temperate and transition climatic zones, quality of tall fescue decreases often suffers greatly during summer months as evidenced by stunt growth, severe disease and insect pressure, and dead patches. The comprehensive resistance exhibited by tall fescue against multiple summer abiotic and biotic stresses is referred as summer stress tolerance (SST). Tall fescue genotypes with better SST have the potential to maintain better overall quality through summer time, especially in the transition zone. Therefore the improvement of tall fescue SST resistance is an important target in tall fescue breeding efforts.

Many previous studies evaluating summer performances of cool-season grasses haven focused on the impact of a single specific stress, such as heat (Du *et al*., 2011; He and Huang, 2007; Kim *et al*., 2010; Lefsrud *et al*., 2010; Xu and Huang, 2008; Xu *et al*., 2006; Xu *et al*., 2010; Xu *et al*., 2011), drought (Abraham *et al*., 2004; Merewitz *et al*., 2010), brown patch (*Rhizoctonia* ssp.) (Bonos *et al*., 2006; Dong *et al*., 2008; Watkins and Meyer, 2004), and Pythium blight (*Pythium* ssp.) (Allen *et al*., 2005), etc. Among these commonly studies stresses, heat and drought are the two major ones limiting the summer growth and quality of cool-season turfgrasses. And reports have been made on the combined the effects of both heat and drought, as well as their impacts on the summer health of cool-season grass species (Abraham *et al*., 2008; Su *et al*., 2007; Wang and Huang, 2004). In a study done in New Jersey, USA, Bonos and Murphy (1999) evaluated growth and performance of Kentucky bluegrass (KBG, *Poa pratensis*) genotypes under summer stresses (heat and drought) and identified genotypes that were relatively more tolerant to these stresses. The growth of KBG is also reported to be limited by drought and heat stress during summer in North-western China (Liu *et al*., 2008). However, there are limited reports on the impacts of combined stress from heat, high humidity, diseases and insects, as commonly encountered in field condition during summer months.

Commercial tall fescue is allohexaploid ($2n=6x=42$) with a large genome ($5.27\text{-}5.83\times10^9$ bp) (Seal, 1983). Due to a cross-pollinated habit, each seed or plant of the grass is genetically unique. Therefore, intraspecific genetic variation in tall fescue is commonly observed for individual selection breeding (i.e., recurrent selection and ecotype breeding). However, due to the heterogeneity of soil physiochemical characteristics and the variation in spreading rate of fungal pathogen in field, the selection efficiency of conventional breeding programs targeting at summer stress tolerance is

relatively low.

Along with the development of molecular technology, transgenic tall fescue lines have been reported with improved stress resistance. Wu *et al*. (2006) obtained transgenic tall fescue plants with enhanced salt and osmosis tolerances, containing *CBF1* gene from *Arabidopsis thaliana* via *Agrobacterium tumefaciens* - mediated transformation. Dong *et al*. (2007) obtained transgenic plants exhibiting resistance to two major fungal diseases greay leaf spot (*Magnaporthe grisea*) and brown patch (*Rhizoctonia solani*). Meanwhile, molecular marker techniques were also applied in tall fescue researches, such as linkage maps construction, genetic diversity analysis, cultivar identification, and so on. The first tall fescue linkage map was reported by Xu *et al*. (1995), generated from the F2 population of HD28-56 and 'Kentucky-31'. It consisted of 108 restriction fragment length polymorphisms (RFLPs) and covered 1274 cM on 19 linkage groups with a mean marker interval of 17.9 cM. Ten years later, an amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) based genetic linkage map of tall fescue was constructed by Saha *et al*. (2005), who combined the female (HD28-56) map and the male (R43-64) map into an integrated map, which covered 1841 cM on 17 LGs with an average marker interval of 2.0 cM. Furthermore, molecular mapping of locating quantitative trait loci (QTLs) related to forage digestibility in tall fescue was also reported by Saha *et al*. (2009). Base on RFLP data, the results of genetic diversity showed that variation within tall fescue cultivars was high (Xu *et al*., 1994). Analyzed by SSR, random amplified polymorphic DNA (RAPD), and inter-simple sequence repeat (ISSR) markers, Mester *et al*. (1999) classified 30 somaclones into four distinct groups. Using 461 AFLPs from six primer combination, Mian *et al*. (2002) clustered 16 tall fescue plants in groups. In addition, identification of 12 commercial varieties of tall fescue was successful via RFLPs (Busti *et al*., 2004), in which seven specific bands were distinguished.

As an effective breeding method, marker-assisted selection (MAS) has been applied in many species successfully. It is especially useful for some complex traits. Unfortunately, there are no reports on MAS of SST in tall fescue. Considering the hereditary characteristics of tall fescue, this study was designed to identify molecular markers (ISSR and RAPD) that are linked to SST trait via pseudo-bulked segregant analysis (BSA) method, using tall fescue clones with varying SST. Also, a sequence characterized amplified region (SCAR) marker developed from polymorphic random molecular marker was tested in other clones. Our purpose of this study is to seek and offer sequence specific markers that could be valuable in assisting breeding for summer stress tolerance in tall fescue, and hopefully render future breeding efforts of this kind more simplicity and reliability.

Materials and methods

Plant materials

Selection population: In the autumn of 2007, 130 tall fescue clone lines (genotypes) from different varieties and lines (Tab. 1) were planted in Funing County, Jiangsu Province, China. Each clone line included twenty-two plants (4-5 tillers per plant). Funing County is located at 33'N latitude and 119'E longitude, and it has four distinct seasons featuring high temperature and plenty of rainfall in summer. Soil type of this area is sandy loam. Till the end of the experiment in 2009, we only fertilized twice (late autumn: 88 kg N·ha^{-1}; early spring: 34 kg N·ha^{-1}). No pesticide was used for insects and diseases control and weeds were hand-picked twice a year in late autumn and early June.

Tab. 1: Identification of summer stress tolerance in different tall fescue germplasms

Clones	Cultivar[a]	Summer Stress Tolerance[b]	Clones	Cultivar[a]	Summer Stress Tolerance[b]
TF01	Southeast	T	TF34	Triple A	S
TF02	Paraso	T	TF35	Plantation	S
TF03	SHED	T	TF36	Plantation	S
TF04	Plantation	T	TF37	SND×98-19	S
TF05	Triple A	T	TF38	SND×98-19	S
TF06	Plantation	T	ST01	SHED	T
TF07	Plantation	T	ST02	SHED	T
TF08	Plantation	T	ST03	SHED	T
TF09	Plantation	T	ST04	SHED	T
TF10	Triple A	T	ST05	SHED	T
TF11	Plantation	T	ST06	SHED	T
TF12	Triple A	S	ST07	SHED	T
TF13	Plantation	S	ST08	SHED	T
TF14	Triple A	S	ST09	SHED	T
TF15	Plantation	S	ST10	SHED	T
TF16	Plantation	S	ST11	SHED	T
TF17	SND×98-19	S	ST12	SHED	T
TF18	SND×98-19	S	WT01	SHED	S
TF19	SND×98-19	S	WT02	SHED	S
TF20	SND×98-19	S	WT03	SHED	S
TF21	SND×98-19	S	WT04	SHED	S
TF22	SND×98-19	S	WT05	SHED	S
TF23	98-19×SND	S	WT06	SHED	S
TF24	98-19×SND	S	WT07	SHED	S
TF25	98-19×SND	S	WT08	SHED	S
TF26	98-19×SND	S	WT09	SHED	S
TF27	SND	S	WT10	SHED	S
TF28	98-19	S	WT11	SHED	S
TF31	Plantation	S	WT12	SHED	S
TF32	Triple A	S	WT13	SHED	S
TF33	Triple A	S			

[a]SHED is Shanghai Evergreen Dwarf Tall Fescue (registered by "Shanghai Crop Cultivars Examination and Approval Committee" under the code of 'Hu Nong Pin Ren Turf (2005) No. 1'). SND is Shangnong Dwarf Tall Fescue. SND and 98-19 are breeding lines from Grass Research Group of Shanghai Jiaotong University (He et al., 2001). SND×98-19 is the hybrid of SND and 98-19, maybe including the SND inbred seeds. 98-19×SND is the hybrid of 98-19 and SND, maybe including the 98-19 inbred seeds.

[b]T, Summer stress tolerant; S, Summer stress sensitive.

Testing population: In 2005, plants of tall fescue variety 'Shanghai Evergreen Dwarf' were grown in Sanjiagang area, Shanghai, China. The seeding rate was 20 $g \cdot m^{-2}$. Clone lines were constructed from fifty plants (including summer stress tolerant and sensitive plants) selected under summer stress (mainly insect and disease pressure), and transplanted to research farm at School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China. Subsequently, twelve clones performed well under rainy and high temperature conditions and thirteen other clones performed poorly under same conditions were selected in autumn of 2008 and 2009. All these plants were used as testing population.

Shanghai is located at 31'N latitude and 121'E longitude. The climate here is similar to Funing County, with an average temperature of 27.9 °C in July (recorded highest temperature of 40.7 °C) and an annual precipitation of 1087 mm (He *et al*., 1997). The Soil type at testing location is loam. Management program was similar to that of selection population.

Identification of summer stress tolerance: We identified SST from clone lines of selection population and clones of testing population. The identification was operated after the full duration of summer stress and before recovery started to occur. Based on the status of plants (survival rate, green leaf index, degree of leaf curling, disease incidence, and so on), three breeders marked each clone by visual inspection independently. Scoring is based on a scale of 1 to 9, with 9 being the best growing state (He *et al*., 2001). Summer stress tolerant and summer stress sensitive clone lines (Tab. 1) were selected, respectively, for future work. Some of the summer stress sensitive clones lost green shoots due to summer stress, but they were able to recover from crown in the next year.

DNA extraction and pseudo-BSA: The same amounts of leaves were collected from each plant per clone line, and genomic DNA mixture of each clone lines was extracted with the CTAB method (Clark 1998). Extracted DNA samples were visualized after electrophoresis on 0.8% agarose gels in 1×TAE. DNA concentration and purity was measured with a UV spectrophotometer. The DNA was adjusted to a final concentration of 20 $ng \cdot \mu L^{-1}$ with TE buffer (pH 8.0) and stored at -20 °C until use.

Equal amounts of DNA from eleven summer stress tolerant clone lines and eleven summer stress sensitive clone lines were pooled to construct two DNA bulks for BSA (Michelmore *et al*., 1991), summer stress tolerant pool (STP) and summer stress sensitive pool (SSP). Obviously, the polymorphism between these two DNA pools was affected by multiple factors rather than only one. To indicate the difference with the traditional BSA approach, we refer our approach as pseudo-BSA.

RAPD and ISSR analyses: A total of 100 ISSR primers and 800 RAPD primers were used to screen polymorphisms in the two bulks. The primers that were able to amplify polymorphic bands between the two pools were tested in the 22 individual clone lines that made up the pools for further check of the polymorphism. RAPDs and ISSRs were all according to sequences of NAPS Unit standard primers (University of British Columbia, Canada). All the primers were synthesized by Sangon Biological Engineering Technology and Service Co. Ltd, Shanghai.

PCR reactions for ISSR and RAPD markers were carried out in 10-μl mixture [20 ng genomic DNA, 10 pmol primer, 100 $\mu mol \cdot L^{-1}$ dNTPs, 1×Taq Buffer, 1.5$mmol \cdot L^{-1}$ $MgCl_2$, and 0.5 unit of Taq DNA polymerase (TaKaRa, Talien, China)]. The reaction conditions were as follows: 94 °C for 3min, followed by 35 cycles at 94 °C for 10s, an annealing temperature depending different primers for 40s, and 72 °C for 45s and a final extension at 72 °C for 6 min. The amplification products were separated on 1.5% agarose gels, and then soaked in a 1.0 $\mu g \cdot mL^{-1}$ of ethidium bromide water solution for 10 min. Then, the gels were illuminated by UV light, and photographed with a Tanon3500 imaging system (Tanon Science & Technology Co., Ltd., Shanghai, China).

Cloning and sequencing of the ISSR fragment: Sequence-related amplified polymorphism fragment was retrieved with the Gel Extraction Kit (DV805A, TaKaRa, Talien), and the quality of fragment extracted was checked by 0.8% agarose gels. The target band was cloned into the PMD18-T vector (D101A, TaKaRa, Talien). Positive colonies bearing DNA of the expected size were sequenced with ABI 3700 Sequencer (Sangon, China).

Conversion of ISSR marker to SCAR (sequence characterized amplified region) marker: According to the sequence of the fragment, SCAR primers were then designed using the Primer Premier5.0. The SCAR primers were tested in two pools and 22 clones. The condition of the SCAR amplification was as follows: 94 °C for 3 min, followed by 29 cycles at 94 °C for 10s, 63 °C for 40s, and 72 °C for 30s and a final extension at 72 °C for 6 min. We obtained one polymorphic SCAR marker, named as T_SC856 (Forward primer sequence: 5'ACACACACACACACACCAATTG3'; Reverse primer sequence:5'ACACACACACACACACTAC CTC3').

Results

Identification of summer stress tolerant clones in selection population

Throughout the whole summer season, the 130 clone lines of tall fescue planted exhibited very distinct summer hardiness. Some clones (i.e., TF18) almost lost all above ground tissues (Fig. 1a), while some other clones (i.e., TF02) were still growing well, with abundant green and dense leaves (Fig. 1b). Obviously, the former type was summer stress sensitive clones, and the latter was summer stress tolerant ones. Careful examination of the dead plants revealed large populations of millipedes (*Spirobolus bungii*) nearby.

Finally, eleven summer stress tolerant clone lines (TF01-TF11) and twenty-five summer stress sensitive clone lines (TF12-TF28, TF31-TF38) were selected from the selection population (Tab. 1). All the summer stress tolerant ones and 11 of the summer stress sensitive ones (TF12-TF22) were selected randomly to construct the DNA bulks.

Identification of markers related to the summer stress tolerance trait

Of the 100 ISSR primers tested, 13 primers were polymorphic between the two bulks (Fig. 2). Unfortunately, none of the 800 RAPD primers showed polymorphism. From the successful reaction system, about 5.5 marker bands were amplified clearly per primer. Upon further testing with 22 clone lines DNA that made up the pools, only one ISSR marker (UBC856) was able to generate the same polymorphic bands in the clone lines and two bulks (Fig. 3a). It was a dominant marker appearing brightly in SSP, and the size of the band was about 1300bp.

Fig. 1. Identification of tall fescue clone lines in Funing County, Jiangsu, China. Summer stress sensitive clone line TF18 (a) and summer stress tolerant clone line TF02 (b)

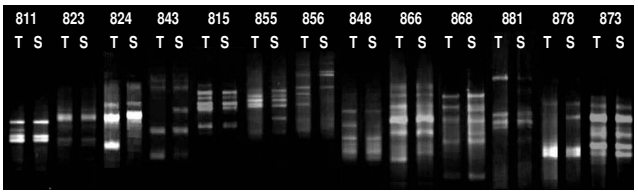

Fig. 2. Polymorphic bands amplified by 13 ISSR primers in two bulks. T, summer stress tolerant pool; S, summer stress sensitive pool. Number indicates ISSR primer names (i.e. 811 means UBC811)

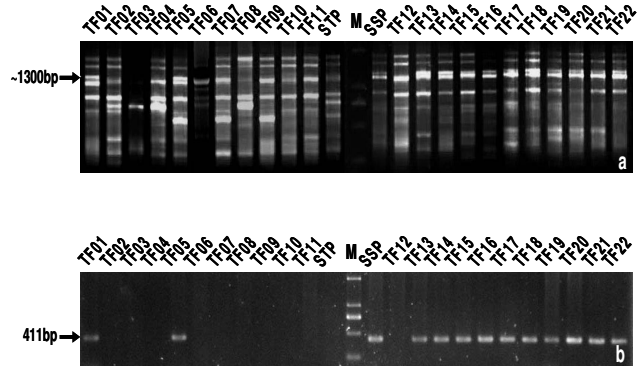

Fig. 3. PCR amplification of the UBC856 (a) and T_SC856 (b) in two bulks and 22 clone lines that comprising the two bulks. STP, summer stress tolerant pool; SSP, summer stress sensitive pool

Conversion of ISSR marker to SCAR marker

The ISSR marker related to summer stress tolerance were converted into SCAR marker (T_SC856). The polymorphic product was 411bp, appeared brightly in SSP and almost none in STP (Fig. 3b). Among the 22 individuals of the two pools, the polymorphic bands amplified by the SCAR marker were identical to those of its corresponding ISSR marker. In the future, this anchor marker could be used for large-scale screens for summer stress tolerance of MAS.

Anchor marker verified in the testing population

Summer stress tolerant clones and summer stress sensitive clones were selected from "Shanghai Evergreen Dwarf" testing population. Combined with the clone lines (TF23-TF28, TF31-TF38) identified in selection population (not include in the bulk construction), they were all tested by the SCAR maker. According to the comparison between polymorphism of SCAR amplification and phenotype of SST in the field, 4 of 13 WT clones (WT3, WT5, WT6, and WT11) were not consistent (Fig. 4b); 2 of 12 ST clones (ST08 and ST12) were not consistent (Fig. 4a). Besides, 3 of 14 clone lines (TF31, TF35, and TF36) were inconsistent (Fig. 4c). Taken together, accuracy of the molecular marker selection reached 77%.

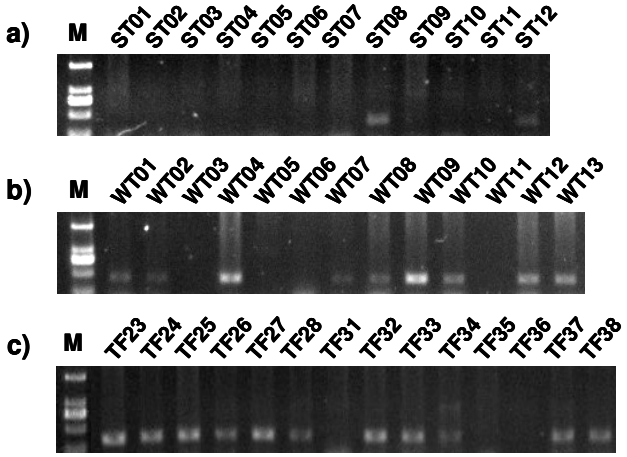

Fig. 4. Amplification of the SCAR marker T_SC856 in summer stress tolerant clones (ST) (a) and summer stress sensitive clones (WT) (b) from SHED testing population, and several summer stress sensitive clones from the selection population (c)

Discussion

It generally takes turfgrass breeders more than ten years to breed a new variety, from single plant selection and cloning to quality evaluation via field tests. Through efforts in conventional phenotypic selection, we have indeed obtained some summer stress tolerant varieties and lines of tall fescue in Shanghai, China (He *et al.*, 2001; He *et al.*, 2002). However, released varieties would be usually replaced by more recent ones after five years or so.

Combining phenotyping with the molecular marker polymorphism selection could certainly accelerate the breeding process, especially for some complex traits. In order to do so, abundant molecular markers closely linked to target traits have to be available to breeders first. Several applications of turfgrass SCAR markers have been reported. For example, SCAR markers created by RAPD bands were used to differentiate some species of bentgrass, and identify progenies derived from hybridization among bentgrass species (Scheef *et al*., 2003).

Identification and detection of *Phoma sclerotioides* was operated by sequence-characterized DNA markers in the brown root rot of alfalfa (Larsen *et al*., 2002). Humaid *et al*. (2004) detected the genetic variation and *Fusarium* resistance in seven tufgrass genotypes with RAPD and SCAR markers. Our work is the first report of the development of SCAR marker relating to summer stress tolerance trait. And it showed the relatively high accuracy of the molecular marker selection in different clones. It is undoubtedly a worthwhile effort using MAS for summer stress tolerance in tall fescue and could be potentially extended to other species.

With continued trend in global warming, grass breeders have been paying more attention to SST in tall fescue. Of course, this is not an easy breeding target to work with. SST in turfgrass species is a very practical trait, but is also very complex indeed. Each involving factor (i.e. heat, disease, insect, drought, water-logging tolerance, etc.) in SST is a complex trait itself controlled by multiple genes. For example, several QTLs related to these factors have been located in diploid ryegrass, which is a main research object in grasses. Pfender *et al*. (2011) detected three stem rust resistance QTLs by SSR, sequence-tagged site (STS), and restriction-site associated DNA (RAD). The most prominent QTL accounted for more than 30% of the phenotypic variance. Pearson *et al*. (2011) identified 37 QTLs for morphological traits influencing water logging tolerance. Certainly, heat tolerance is one of the most important SST components, and some studies on heat tolerance genes have been reported. In C3 *Agrostis* grass species, Xu *et al*. (2007) identified and characterized an expansin gene *AsEXP1* associated with heat tolerance. Kim *et al*. (2010) overexpressed an *Arabidopsis* 2-Cys Prx in transgenic tall fescue plants to enhance heat tolerance.

Factors involved with the combined summer stress could offer different levels of impact depending on the specific climate of a given region or a specific year. In a wet years or regions with ample summer precipitation, summer stress would be dominated by high temperature, high humidity, and brown patch/Pythium blight promoted by these environmental condition. In a dry years and regions with less scarce rainfall, the main stress would be heat and drought.

In this study, the polymorphism of SCAR markers corresponds well with the phenotypic identification in the field, between Funing County and Shanghai. As a result of the environmental factors analysis of the two locations in these years, they were both characterized by high temperature, ample rainfall, and Pythium blight promoting in summer. Later, we identified the polymorphism of the SCAR in another tall fescue population in 2011. However, the result was not ideal (data not shown). The analysis of 2011 found lower temperature, less rainfall, and better field management than those in 2008 and 2009. Under the summer stress of 2011, there were only some plants wilting in the afternoon, and of course, resulted in no brown shoots. It was determined that there were no significant correlations ($p > 0.05$) between T_SC856 and wilting trait. Instead, the SCAR marker maybe linked to heat, high humidity or Pythium blight tolerance. Yet, it is hard to single out which factor relates to the SCAR marker developed indeed. Further studies that specifically look at heat, high humidity tolerance, etc., under carefully controlled environment are needed to verify the factor.

Although this study reported a SST related SCAR marker with an accuracy closed to 80%, it is impractical to base MAS for SST on this one marker alone. Phenotype, affected by many uncertain factors, is unlikely to express consistently if plant materials were selected using one molecular marker, even though they are linked tightly. As a result, if locations and years change, MAS for SST based on one molecular maker is likely to fail. Currently, we are testing the SCAR marker in more clones from different locations and years. Next, many more types of molecular markers related to SST trait need to be developed. A combination use of two or more molecular markers for summer stress tolerance selection could be more accurate and stable. Development of multiple markers linked to SST will certainly be good news for MAS breeding in tall fescue and other species as well.

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References

Abraham EM, Huang BR, Bonos SA, Meyer WA (2004). Evaluation of drought resistance for Texas bluegrass, Kentucky bluegrass, and their hybrids. Crop Sci 44:1746-1753.

Abraham EM, Meyer WA, Bonos SA, Huang BR (2008). Differential responses of hybrid Bluegrass and Kentucky bluegrass to drought and heat stress. HortScience 43:2191-2195.

Allen TW, Han DY, Bowen KL (2005). Effect of environmental characteristics on *Pythium* and *Mesocriconema* spp. in golf course greens in Alabama. Can J Microbiol 51:287-293.

Bonos SA, Murphy JA (1999). Growth responses and performance of Kentucky bluegrass under summer stress. Crop Sci 39:770-774.

Bonos SA, Clarke BB, Meyer WA (2006). Breeding for disease resistance in the major cool-season turfgrasses. Annu Rev Phytopathol 44:213-234.

Busti A, Caceres ME, Calderini O, Arcioni S, Pupilli F (2004). RFLP markers for cultivar identification in tall fescue (*Festuca arundinacea* Schreb.). Genet Resour Crop Evolution 51:443-448.

Clark MS (1998). Plant Molecular Biology – A Laboratory Manual. Springer-Verlag, Heidelberg.

Dong S, Tredway LP, Shew HD, Wang GL, Sivamani E, Qu R (2007). Resistance of transgenic tall fescue to two major fungal diseases. Plant Sci 173:501-509.

Dong S, Shew HD, Tredway LP, Lu J, Sivamani E, Miller ES, Qu R (2008). Expression of the bacteriophage T4 lysozyme gene in tall fescue confers resistance to gray leaf spot and brown patch diseases. Transgenic Res 17:47-57.

Du H, Wang Z, Yu W, Liu Y, Huang B (2011). Differential metabolic responses of perennial grass *Cynodon transvaalensis*×*Cynodon dactylon* (C_4) and *Poa pratensis* (C_3) to heat stress. Physiol Plant 141:251-264.

Emmons RD (1994). Turfgrass Science and Management. 2nd ed. New York: Delmar Publishers TM 62～81.

He YL, Hu XH, Zhang AM, Xu R, Qi J (1997). A study on field experiment of introduced varieties of cool season turf grass. J Shanghai Agr Coll. 15:276-282 (in Chinese with English summary).

He YL, Hu XH, Tang J (2001). Breeding, comparison and extension of new lines of cool-season turf-type tall fescue-"98-8" and "Shangnong Dwarf Tall Fescue". Pratac Sci 18:60-64 (in Chinese with English summary).

He YL, Hu XH, Chen W, Xu H (2002). Breeding of new lines of turf-type tall fescue. Grassl China 24:33-39 (in Chinese with English summary).

He Y, Huang B (2007). Protein changes during heat stress in three Kentucky bluegrass cultivars differing in heat tolerance. Crop Sci 47:2513-2520.

Humaid AA, Motawei MI, Abdalla MY, Mana F (2004). Detection of genetic variation and Fusarium resistance in turfgrass genotypes using PCR-based markers (ISSR and SCAR). J Food Agric Environ 2:225-229.

Kim KH, Alam I, Lee KW, Sharmin SA, Kwak SS, Lee SY, Lee BH (2010). Enhanced tolerance of transgenic tall fescue plants overexpressing 2-Cys peroxiredoxin against methyl viologen and heat stresses. Biotechnol Lett 32:571-576.

Larsen RC, Hollingsworth CR, Vandemark GJ, Gritsenko MA, Gray FA (2002). A rapid method using PCR-based SCAR markers for detection and identification of *Phoma sclerotioides*: the case of brown root rot disease of alfalfa. Plant Dis 86:928-932.

Lefsrud MG, Sorochan JC, Kopsell DA, McElroy JS (2010). Pigment concentrations among heat-tolerant turfgrasses. HortScience 45:650-653.

Liu JR, Xie XR, Du JX, Sun JX, Bai XM (2008). Effects of simultaneous drought and heat stress on Kentucky bluegrass. Sci Hort-Amsterdam 115:190-195.

Merewitz E, Meyer W, Bonos S, Huang BR (2010). Drought stress responses and recovery of Texas x Kentucky hybrids and Kentucky bluegrass genotypes in temperate climate conditions. Agron J 102:258-268.

Mester Z, Murenyetz L, Gyulai G, Janowszky J, Hangyel TM, Bottka S, Heszky L (1999). Somaclonal variation of tall fescue (*Festuca arundinacea* L.) analyzed by RAPD-, ISSR-, and SSR-PCR. In Vitro Cell Dev Biol-Plant 35:175.

Mian MAR, Hopkins AA, Zwonitzer JC (2002). Determination of genetic diversity in tall fescue with AFLP markers. Crop Sci 42:944-950.

Michelmore RW, Paran I, Kesseli RV (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828-9832.

Pearson A, Coqan NO, Baillie RC, Hand ML, Bandaranayake CK, Erb S, Wang J, Kearney GA, Gendall AR, Smith KF, Forster JW (2011). Identification of QTLs for morphological traits influencing waterlogging tolerance in perennial ryegrass (*Lolim perenne* L.). Theor Appl Genet 122:609-622.

Pfender WF, Saha MC, Johnson EA, Slabaugh MB (2011). Mapping with RAD (restriction-site associated DNA) markers to rapidly identify QTL for stem rust resistance in *Lilium perenne*. Theor Appl Genet 122:1467-1480.

Saha MC, Mian R, Zwonitzer JC, Chekhovskiy K, Hopkins AA (2005). An SSR- and AFLP-based genetic linkage map of tall fescue (*Festuca arundinacea* Schreb.). Theor Appl Genet 110:323-336.

Saha M, Kirigwi F, Chekhovskiy K, Black J, Hopkins A (2009). Molecular Mapping of QTLs Associated with Important Forage Traits in Tall Fescue. In: Yamada T and G Spangenberg (eds). Molecular Breeding of Forage and Turf 251-258. Springer, New York.

Scheef EA, Casler MD, Jung G (2003). Development of species-

specific SCAR markers in bentgrass. Crop Sci 43:345-349.

Seal AG (1983). DNA variation in *Festuca*. Heredity 50:225-236.

Su KM, Bremer DJ, Keeley SJ, Fry JD (2007). Effects of high temperature and drought on a hybrid bluegrass compared with Kentucky bluegrass and tall fescue. Crop Sci 47:2152-2161.

Wang ZL, Huang BR (2004). Physiological recovery of Kentucky bluegrass from simultaneous drought and heat stress. Crop Sci 44:1729-1736.

Watkins E, Meyer WA (2004). Morphological characterization of turf-type tall fescue genotypes. HortScience 39:615-619.

Wu GT, Chen JQ, Hu ZH, Lang CX, Chen XJ, Wang FL, Jin W, Xia YW (2006). Production of transgenic tall fescue plants with enhanced stress tolerances by *Agrobacterium tumefaciens*-mediated transformation. Sci Agric Sin 5:330-338.

Xu C, Huang B (2008). Root proteomic responses to heat stress in two *Agrostis* grass species contrasting in heat tolerance. J Exp Bot 59:4183-4194.

Xu JC, Tian J, Belanger FC, Huang B (2007). Identification and characterization of an expansin gene *AsEXP1* associated with heat tolerance in C3 *Agrostis* grass species. J Exp Bot 58:3789-3796.

Xu S, Li JL, Zhang XQ, Wei H, Cui LJ (2006). Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool-season turfgrass species under heat stress. Environ Exp Bot 56:274-285.

Xu WW, Sleper DA, Krause GF (1994). Genetic diversity of tall fescue germplasm based on RFLPs. Crop Sci 34:246-252.

Xu WW, Sleper DA, Chao S (1995). Genome mapping of polyploid tall fescue (*Festuca arundinacea* Schreb.) with RFLP markers. Theor Appl Genet 91:947-955.

Xu Y, Gianfagna T, Huang B (2010). Proteomic changes associated with expression of a gene (ipt) controlling cytokinin synthesis for improving heat tolerance in a perennial grass species. J Exp Bot 61:3273-3289.

Xu Y, Zhan C, Huang B (2011). Heat Shock Proteins in Association with Heat Tolerance in Grasses. Int J Proteomics doi:10.1155/2011/529648.