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ARTICLE

Association mapping of germinability and seedling vigor in sorghum under controlled low-temperature conditions

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Abstract: Sorghum is one of the world's most important food, feed, and fiber crops as well as a potential feedstock for lignocellulosic bioenergy. Early-season planting extends sorghum's growing season and increases yield in temperate regions. However, sorghum's sensitivity to low soil temperatures adversely impacts seed germination. In this study, we evaluated the 242 accessions of the ICRISAT sorghum mini core collection for seed germination and seedling vigor at 12 °C as a measure of cold tolerance. Genome-wide association analysis was performed with approximately 162 177 single nucleotide polymorphism markers. Only one marker locus (Locus 7-2) was significantly associated with low-temperature germination and none with vigor. The linkage of Locus 7-2 to low-temperature germination was supported by four lines of evidence: strong association in three independent experiments, co-localization with previously mapped cold tolerance quantitative trait loci (QTL) in sorghum, a candidate gene that increases cold tolerance and germination rate when its wheat homolog is overexpressed in tobacco, and its syntenic region in rice co-localized with two cold tolerance QTL in rice. This locus may be useful in developing tools for molecular breeding of sorghums with improved low-temperature germinability.

Key words: sorghum, association mapping, SNP, early-season cold tolerance.

Résumé : Le sorgho est l'une des plus importantes cultures au monde, autant pour sa production de fibres que d'aliments pour les humains et les animaux, ainsi que pour son potentiel en tant que matière première pour la production de bioénergie lignocellulosique. Un semis hâtif permet de rallonger la saison de croissance et d'augmenter les rendements dans les régions tempérées. Cependant, la sensibilité du sorgho aux basses températures du sol a des impacts négatifs sur la germination des graines. Dans cette étude, les auteurs ont évalué 242 accessions de la collection nucléaire minimale (« mini core collection ») de l'ICRISAT pour leur germination et la vigueur des plantules à 12 °C afin d'en mesurer la tolérance au froid. Une analyse d'association pangénomique a été réalisée au moyen d'environ 162 177 marqueurs mononucléotidiques (SNP). Seul un marqueur (Locus 7-2) a montré une association significative avec la germination à basse température et aucun à la vigueur des plantules. L'association entre le Locus 7-2 et la germination à basse température était supportée par quatre évidences : une forte association au sein de trois expériences indépendantes, une colocalisation avec des locus de caractères quantitatifs (QTL) identifiés antérieurement chez le sorgho, la présence d'un gène candidat qui accroît la tolérance au froid et le taux de germination lorsque l'orthologue de ce gène présent chez le blé est surexprimé chez le tabac, et le fait que la région correspondante chez le riz contient deux QTL pour la tolérance au froid. Ce locus pourrait s'avérer utile en vue du développement de sorghos dotés d'une meilleure germination à basse température à l'aide d'outils de sélection moléculaires. [Traduit par la Rédaction]

Mots-clés : sorgho, cartographie par association pangénomique, SNP, basses températures en début de saison.

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Introduction

The response of plants to suboptimal temperatures is in part related to the center of origin of a given plant species. Plants with centers of origin in temperate regions such as wheat, barley, and Arabidopsis are tolerant to chilling but not to freezing, while those originated from tropical and subtropical regions such as sorghum, maize, and rice are more sensitive to chilling stress; tolerance cannot be induced by cold acclimation (Zhu et al. 2007). Due in part to its drought tolerance and low-input requirements (Saballos 2008), sorghum (*Sorghum bicolor* L. Moench) is an important food, feed, and fiber crop as well as a top candidate for the production of lignocellulosic bioenergy. In the United States, sorghum is grown in the Great Plains from Texas to South Dakota, which spans several plant hardiness zones with changes in latitude (Daly et al. 2012). To increase grain yield, early planting has been adopted to extend sorghum's growing season in the higher latitude temperate regions; however, problems arise when seeds fail to germinate (poor stand density) or exhibit poor seedling vigor due to sensitivity to early-season cold. Poor cold tolerance in sorghum reduces early-season growth, lowers biomass and grain yield, and limits this crop to more semi-tropical regions across the globe (Burow et al. 2011; Knoll et al. 2008; Peacock and Heinrich 1982; Saballos 2008).

While originated in the northeastern quadrant of tropical Africa, sorghum was carried by early humans to diverse ecosystems in Africa and Asia, and these regions included temperate regions (e.g., northeastern China) where the crop experienced chilling stress. Thus, worldwide germplasm collections are likely to harbor natural variation to chilling tolerance that will be useful for crop improvement (Upadhyaya et al. 2009). Genetic variation in early-season cold tolerance is measured in sorghum by evaluating low-temperature germination in the laboratory, field emergence, and (or) seedling vigor during early- and late-season field plantings. At a low temperature of 15 °C, both tolerant and sensitive sorghum varieties can achieve full or close to full germination given sufficient time. Franks et al. (2006) demonstrated that germination near 15 °C was most effective at differentiating tolerant from sensitive sorghum varieties. Although germination at low temperatures in a growth chamber may be correlated with that observed in early-season field planting, the correlation is neither high nor consistent. For example, Knoll et al. (2008) did not find a correlation, while other reports indicated moderate (R = 0.44) to high (R = 0.66) correlations between low-temperature germination in growth chambers and early-season field conditions (Tiryaki and Andrews 2001; Yu et al. 2004). It should be noted that both Tiryaki and Andrews (2001) and Yu et al. (2004) used 15 °C for low-temperature germination, while Knoll et al. (2008) used 13 °C. Lower correlation (0.32–0.37) was reported by Burow et al. (2011) when 12 °C was used in the cold germination test. Such discrepancy may be caused by variability in field conditions (Knoll et al. 2008), and it was suggested to use a temperature-controlled growth chamber for testing germinability as an indicator for cold tolerance but field emergence at early-season planting as the main screening method (Burow et al. 2011).

Several loci underlying early-season cold tolerance in sorghum have been mapped. Knoll et al. (2008) identified one quantitative trait locus (QTL) for low-temperature germination on chromosome 3 (SBI-03) and one QTL on SBI-01 for both early- and late-season seedling emergence and seedling vigor; one QTL for both early and late emergence on SBI-02 and one QTL for early-season seedling vigor on SBI-04. Burow et al. (2011) detected one QTL for both low- and optimal-temperature germinability on SBI-02, one QTL for field emergence on SBI-09, and two QTL on SBI-01 that aligned with a QTL associated with late field emergence identified by Knoll et al. (2008). Bekele et al. (2014) mapped two low-temperature germination QTL for 2009 (on SBI-01 around 51.2 cM and on SBI-03 around 56 cM) and one for 2010 (on SBI-06 around 6 cM). Using 194 genotypes for association mapping with DArT markers, Fiedler et al. (2012) identified five QTL with final emergence under cold conditions. No association mapping with a large number of single nucleotide polymorphism (SNP) markers has been conducted so far to identify genes related to cold tolerance in sorghum.

To gain further insight into the genetics of early-season cold tolerance in sorghum, we conducted an association study with 162 177 SNP markers to map QTL for low-temperature germinability and vigor in the 242 landraces of the ICRISAT sorghum mini core collection (Upadhyaya et al. 2009) under controlled conditions at 12 °C.

Materials and methods

Germinability assay and seedling vigor evaluation

The plant materials consisted of 242 sorghum germplasm accessions from the ICRISAT mini core collection (Upadhyaya et al. 2009). Seeds from the 242 accessions were harvested from ICRISAT field plot in the 2010 and 2012 postrainy seasons and stored at 4 °C and 20%–30% relative humidity. Three independent and replicated experiments were conducted for both germination (G) and vigor (V): Exp I using the 2010 seeds and Exp II and Exp III using 2012 seeds harvested from at least 5–10 plants for each accession. Exp I and Exp II were within six months of seed harvest and Exp III was conducted one year after the harvest.

For germination assays (germinability) in Exp I and Exp II, 25 seeds of each accession were arranged on standard germination papers in each of the two replicate (Whatman®181 Grade) in Petri dishes (9 cm diameter) and incubated at 12 °C for 10 days. In Exp III, 20–25 seeds for each of the four replicates of each accession were placed between 400T standard paper towels from

SeedBuro™ (Des Plaines, Ill., USA) in plastic trays. Distilled water was added to the trays and allowed to wick up to the level of the seeds which were then incubated in a seed germinator at 12 °C, a temperature also used for evaluation of early-season cold tolerance by Burow et al. (2011). After 10 days in each of the three experiments, the percentage of germinated seeds was recorded.

Seedling vigor was visually scored in Exp I and Exp II in one to three grades, 1 (long radicle - high vigor), 2 (medium radicle length - moderate vigor), and 3 (short radicle - poor vigor). In Exp III, radicle length was used to measure seedling vigor as radicle length has been traditionally employed as a component of seedling vigor index (Abdul-Baki and Anderson 1973). Radicle length was manually measured in millimetres (mm) in four replicates and measurements from 20 germinating seeds were averaged for each replicate, excluding non-germinating, deformed, or off-type seedlings. Exps I and II had two replicates each and Exp III contained four replicates. Replicates in each experiment were averaged for association analysis.

For heritability estimates, data were analyzed using residual (or restricted) maximum likelihood (REML) in GenStat software (VSN International 2013). Variance due to genotypes (δ^2_g), genotype (g) × environment (e) (δ^2_{ge}), and error (δ^2_e) were estimated. Heritability in broad sense (H^2) was estimated using δ^2_g/δ^2_p; where δ^2_p is total phenotypic variance which was estimated as $\delta^2_p = \delta^2_g + \delta^2_{ge}/ne + (\delta^2_e)/(ne \times nr)$, where ne equals the number of environment or season in this case and nr equals the number of replications.

Association mapping

SNP markers used in this study were generated by genotyping-by-sequencing approach (Elshire et al. 2011). These were detailed in Morris et al. (2013) (265 k dataset; those starting with "S") and Wang et al. (2013) (15 k dataset; those starting with "Chr"). SNPs were named with the "S" or "Chr" prefix followed by chromosome number and position in base pair (bp). The 281 441 SNPs were filtered so that minor allele frequency was at least 5%. Approximately 162 177 SNPs remained after filtering and were used for association analysis in this study. To minimize the possibility of false positive associations, the data was analyzed using the mixed linear model (MLM; Yu et al. 2005) as implemented in TASSEL 5.0 (Bradbury et al. 2007). A previous study in the same mini core collection (Upadhyaya et al. 2013) have shown that MLM with kinship index (K model) produces similar results as MLM with K and population structure indices (QK model) or MLM with K and principal component indices (PK model). Therefore, only the K model was used in this study and the kinship index was generated in TASSEL with SNP markers developed by Wang et al. (2013). The significance of associations between marker and trait was based on $p < 0.0001$, as used in other association studies (Agrama and Yan 2009; Courtois et al. 2013; Famoso et al. 2011), with multiple markers reproducible in at least two experiments. Accessions that carried the same allele in an associated marker were averaged to estimate allelic effect on germination according to Jia et al. (2012).

Co-localization with previously mapped QTL

Simple sequence repeat (SSR) markers previously mapped to early-season cold tolerance QTL in sorghum were localized to the genome based on sequence information provided in Map Viewer at NCBI (http://www.ncbi.nlm.nih.gov/mapview/). If the position of an SSR was not given in Map Viewer or provided in Ramu et al. (2010) or Mace and Jordan (2011), the primer sequences used to amplify the given SSR were queried against the sorghum genome database (Sorghum bicolor v. 3.1; http://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Sbicolor_er; Paterson et al. 2009) for physical localization. Maps of chromosomes based on the physical distances in mega base pairs (Mb) were drawn in Microsoft Excel.

Alignment with rice genome

The sorghum genomic regions containing markers associated with low-temperature germinability and vigor were aligned with their syntenic regions in rice. To find these regions in rice, genes within 50 kb (Wang et al. 2013) on each side of the marker using Sorghum bicolor v. 3.1 were used to search against rice protein sequence in Phytozome 10.3 (Oryza sativa v. 7.0, http://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Osativa) to identify the syntenic region in rice. Identified rice regions were then aligned with mapped QTLs underlying cold tolerance in rice (Andaya and Mackill 2003; Yang et al. 2013) based on location of rice genetic markers.

Results

Phenotypic analysis

As expected, there was tremendous variation in germinability in all three experiments (Fig. 1), although broad-sense heritability ranged from 0.816 to 0.983. Fourteen accessions failed to germinate in Exp III but all germinated in Exp I and Exp II. Least variation was observed in germinability in Exp II (Fig. 1) which had 77 accessions fully germinated, compared to 12 in Exp I and 0 in Exp III. Germinability in Exp I and Exp II was positively correlated with a Pearson's coefficient (R) of 0.54; as was the correlation in seedling vigor between Exp I and Exp II with a Pearson's coefficient of 0.61 (Table 1). Correlations of germinability in Exp I and Exp II with those in Exp III were both 0.27. The low correlation was probably due to longer-stored seeds used in Exp III. Seedling vigor among Exp I, Exp II, and Exp III was highly correlated. Seedling vigor was correlated with germinability ($R = 0.74$) in Exp III and such correlations were 0.54 and 0.32 in Exp I and Exp II, respectively. These suggest that high germinability under the cold condition is correlated with

Fig. 1. Boxplot showing phenotype distribution in (A) germination and (B) vigor and frequency distribution of low-temperature germinability of the sorghum mini core accessions in (C) Exp I, (D) Exp II, and (E) Exp III. Box edges represent the upper and lower quantile with median value shown as line inside the box. Whiskers represent 1.5 times the quantile of the data. Individuals falling outside the range of the whiskers are shown as asterisk. In Exp III, vigor was measured by radicle length in millimetres (mm). In Exp I and Exp II, vigor was visually rated with 1 as most and 3 as least vigorous. [Colour figure available online.]

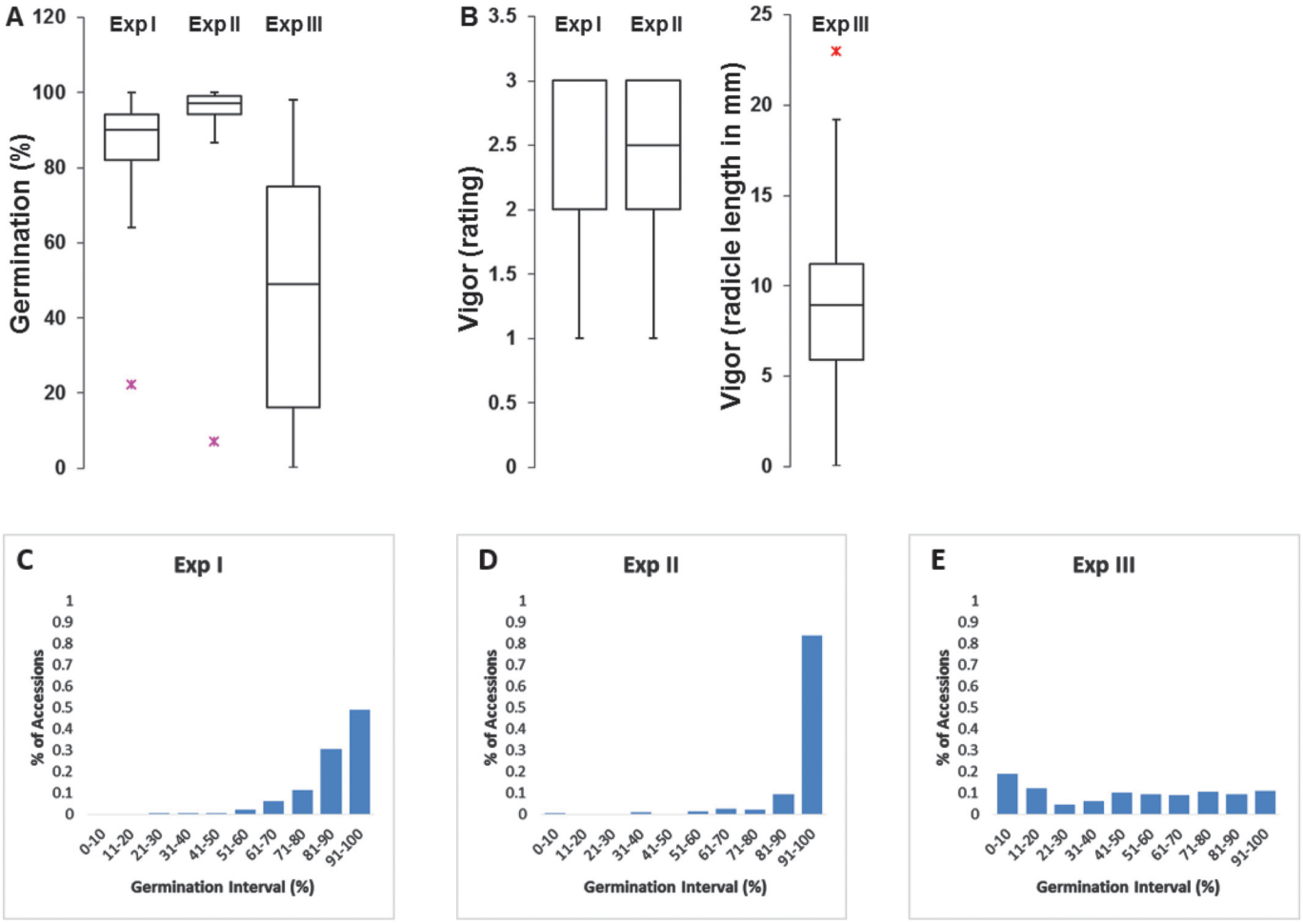


Table 1. Pearson's correlation among experiments (I–III) and between germination (G) and seedling vigor (V).

	II G	I V	II V	III G	III V
I G	0.54	0.54	0.39	0.27	0.37
II G	—	0.29	0.32	0.27	0.37
I V	—	—	0.61	0.40	0.49
II V	—	—	—	0.43	0.60
III G	—	—	—	—	0.74

Note: All significant at $p = 0.00001$.

seedling vigor, i.e., plants with strong germinability are more likely to have a strong vigor under cold conditions. Six accessions were consistently ranked among the top 10% based on vigor in all three experiments: IS 1212 (China), IS 14779 and IS 15170 (Cameron), IS 22986 (Sudan), and IS 7305 and IS 7310 (Nigeria). Based on germination, five accessions were consistently ranked among the top 10% in all three experiments: IS 602 (USA), IS 1233 (China), IS 7305 (Nigeria), IS 10302 (Thailand), and IS 20956 (Indonesia). IS 24503 (South Africa), IS 29714, and IS 29772 (both from Zimbabwe) were consistently ranked among the bottom 10% in germination in all three experiments. IS 7305 from Nigeria was among the top 10% based on both germinability and vigor. These accessions may be useful for future breeding studies.

Association mapping

Using the defined association criteria described in Material and Methods (association of multiple markers with $p < 0.0001$ in more than one environment/experiment), we identified only one locus named 7-2 that met the stringent criteria. Locus 7-2 was associated with low-temperature germinability (Table 2; Fig. 2). Table 2 lists three SNP markers, S7_56998511, S7_56991738, and S7_56991483 that were associated with low-temperature germinability. Although association p values for S7_56991738 and S7_56991483 were above the threshold of 0.0001 in some cases, the pattern of association was observed in all three experiments, with p values of 0.000025 and 0.00094, respectively, for averaged data (Table 2).

Table 2. SNP markers associated with sorghum seed germinability at 12°C.

Linked marker	S7_56998511	S7_56991738	S7_56991483
Missing data rate (%)*	12.1	32.1	52.9
Exp I			
p	0.00793	0.02353	**0.000013**
R^2	0.043	0.052	0.149
Allele/Effect†	A/+4.0	T/+6.5	G/+18.4
Exp II			
p	0.06571	0.00017	0.12065
R^2	0.026	0.128	0.024
Allele/Effect	A/+5.3	T/+8.1	G/+19.0
Exp III			
p	**0.000048**	0.00154	0.11807
R^2	0.097	0.088	0.022
Allele/Effect	A/+20.2	T/+23.7	G/+26.0
Average‡			
p	**0.0000061**	**0.000025**	0.00094
R^2	0.114	0.162	0.104
Allele/Effect	A/+12.6	T/+14.9	G/+22.2

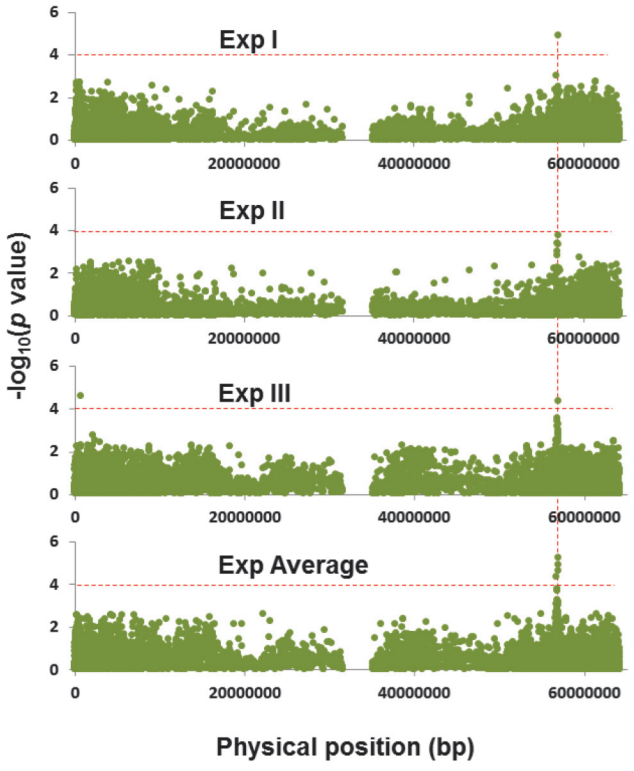
Note: *p* values < 0.0001 are in bold. Allelic effects (Allele/Effect) were calculated as described in Materials and Methods.

*Missing data rate was the ratio of number of accessions without genotyping information to the total number of 242 accessions.

†Unit for Allele Effect is percentage in germination.

‡These were the association mapping results from the average of Exp I, II, and III.

Fig. 2. Chromosome Manhattan plots of Locus 7-2 linked to sorghum seed germinability at 12 °C in all three experiments (Exp I, Exp II, Exp III, and Exp Average). Horizontal dashed line indicates the threshold *p* value. [Colour figure available online.]



In addition to lowest *p* value, S7_56998511 also had lowest missing data rate, while S7_56991483 had the highest missing data rate (Table 2). For the 113 accessions genotyped by S7_56991483, the G allele of the SNP locus confers increased germination rate compared to the T allele (Table 2). Similarly, in S7_56991738 and S7_56998511, one allele increased germination rate over the other across experiments (Table 2). It is possible that these three markers be used in predicting low-temperature germinability upon further improvement.

Co-localization with previously mapped QTLs and candidate gene

As a way to validate the above associations, we co-localized the SNP locus with early-season cold tolerance QTL mapped previously in sorghum. Locus 7-2 was probably covered by the field early-emergence QTL flanked by Xtxp278 and Xtxp295 (Burow et al. 2011). Additionally, Locus 7-2 was next to three overlapping emergence QTL close to sPb-5796 (Fiedler et al. 2012) (Fig. 3A).

To find candidate genes potentially responsible for low-temperature germinability, we used the annotated genome database Sorghum bicolor v. 3.1. For the three markers linked to low-temperature germinability, S7_56998511 is located at the end and S7_56991483 and S7_56991738 in the promoter region of Sobic.007G140900, coding for a CBL-interacting protein kinase in sorghum. Its rice homolog, LOC_Os08g34240 (CBL-interacting protein kinase 6 or *OsCIPK06*), shares 82% identity and 89% similarity with Sobic.007G140900 protein. LOC_Os08g34240 is located on *Os*-08 between 21466751 and 21468619 bp (Fig. 3B).

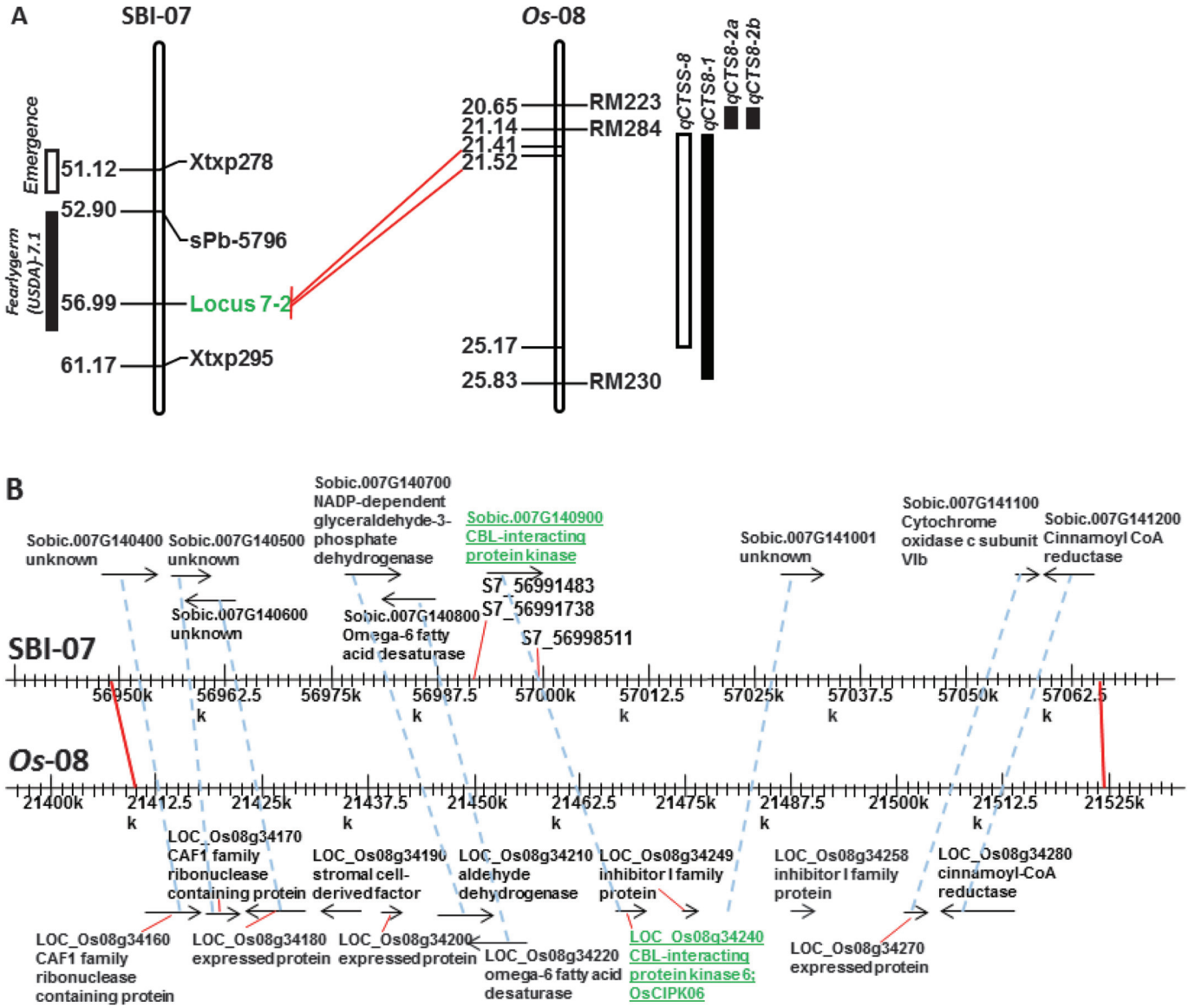
Rice genomic region syntenic to Locus 7-2

To identify the rice genomic region syntenic with Locus 7-2, nine sorghum genes annotated in Phytozome 10.3 (Sorghum bicolor 3.1; http://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Sbicolor_er) in the 100 kb range (50 kb on each side) of the locus were used: Sobic.007G140400, Sobic.007G140500, Sobic.007G140600, Sobic.007G140700, Sobic.007G140800, Sobic.007G140900, Sobic.007G141001, Sobic.007G141100, and Sobic.007G141200. Except Sobic.007G141001, all others have homologs in rice (Fig. 3B). Search of GenBank rice protein database showed that the eight sorghum genes are syntenic with an 111-kb region on rice chromosome 8 (*Os*-8) from 21 410 964 to 21 521 976 bp (Fig. 3B). This region in rice is covered by three QTL for seedling cold tolerance mapped in two independent studies using different populations (Fig. 3A). The rice homolog of Sobic.007G140900, LOC_Os08g34240, is also located in the region (Fig. 3B). This further validates our mapping of Locus 7-2 with low-temperature germinability in sorghum.

Discussion

Association mapping can be a powerful tool in identifying genes linked to a phenotype. To minimize false

Fig. 3. (A) Left: Co-localization of Locus 7-2 with previously mapped QTLs. Closed and open bars to the left of SBI-07 indicate QTLs mapped by Burow et al. (2011) and Fiedler et al. (2012), respectively. Linked SSR markers from previous QTL studies and SNP loci identified in the current study are shown to right of the chromosome. Physical positions of markers in mega base pairs (Mb) are shown to the left of the chromosome. Right: Syntenic region on rice chromosome 8 (*Os*-8) with three seedling cold tolerance QTL by Andaya and Mackill (2003) and one by Yang et al. (2013). (B) Gene location in the syntenic regions of SBI-07 (top) and *Os*-08 (bottom). The sorghum candidate gene for low-temperature germinability (Sobic.007G140900) and its rice homolog (LOC_Os08g34240) are underlined and, like other homologs, connected by dashed lines. [Colour figure available online.]



positive associations, we examined association of multiple markers in a locus as in other studies (Atwell et al. 2010; Morris et al. 2013). Furthermore, an association had to be observed in more than one experiment/environment. However, we did use relaxed p values as it has been shown that stringent requirement for p values such as Bonferroni correction may miss potential target genes producing false negative associations (Famoso et al. 2011; Zhao et al. 2011). Also in selecting linkage disequilibrium block size when identifying rice syntenic region, we chose genes 50 kb on each side of the locus. This is because linkage disequilibrium block size in the sorghum genome ranges from 10–30 kb (Hamblin et al. 2005; Wang et al. 2013) to 100 kb (Bouchet et al. 2012).

Although it has been shown previously that not all cold tolerance QTL can be mapped in all experiments (Burow et al. 2011; Bekele et al. 2014), Locus 7-2 was found linked to low-temperature germinability in all three independent experiments in this study (Table 2; Fig. 2). To further validate the mapping of Locus 7-2, we co-localized the locus with two previously mapped cold tolerance QTL in sorghum and identified the rice genomic

region syntenic with the locus (Fig. 3). The rice region has been mapped in at least two (possibly three) seedling cold tolerance QTL in two studies using populations generated from different parental varieties. Using a recombinant inbred populations from a cross of M-202 and IR50, Andaya and Mackill (2003) mapped a seedling cold tolerance QTL (*qCTS8-1*) between SSR markers RM284 (located at 21.14 Mb) and RM 230 (located at 25.83 Mb) on rice chromosome 8 (Fig. 3). They also mapped two overlapping QTL between RM223 (located at 20.65 Mb) and RM284: *qCTS8-2a* for cold-induced wilting tolerance and *qCTS8-2b* for cold-induced necrosis tolerance. Yang et al. (2013) used F_3 from a cross of Nipponbare and LPBG to delineate a seedling cold tolerance QTL (*qCTSS-8*) between 21.14 and 25.17 Mb on rice chromosome 8. *qCTS8-1* and *qCTSS-8* almost overlap perfectly, with the syntenic region covered by the overlapping QTL (Fig. 3A). In addition, Locus 7-2 spans the candidate gene, Sobic.007G140900, which is most homologous to rice LOC_Os08g34240 (*OsCIPK06*) (Fig. 3B). The wheat homolog of this gene, *TaCIPK14*, confers cold tolerance and increased germination rate in transgenic tobacco overexpressing *TaCIPK14* (Deng et al. 2013). These further support our conclusion that Locus 7-2 is associated with low-temperature germinability in sorghum.

Previous studies have investigated the relationship among the component traits for early-season cold tolerance in sorghum. First, low- and optimal-temperature germination were highly correlated (R = 0.883 in Knoll et al. 2008, 0.74 in Burow et al. 2011, and 0.756–0.893 in Bekele et al. 2014), indicating that a variety that germinates well at low temperature also likely germinates well at optimal temperature. Second, under field conditions, a strong correlation was also observed between early- and late-season emergence (R = 0.739) (Knoll et al. 2008). Although early emergence reflects a variety's tolerance to early-season cold, a variety that germinates well during early planting when temperature is low also germinates well during late planting when temperature is warmer. These suggest that germinability of a variety can be used as a measure of tolerance to early-season cold. QTL mapping also supports this correlation between germination at low and optimal temperatures. Bekele et al. (2014) mapped both low- and optimal-temperature germination QTL on the top of SBI-02 and QTL for low- and optimal-temperature emergence on SBI-06 around 20–30 cM. Two overlapping low- and optimal-temperature germination QTL were also mapped on SBI-03 around UBC171 (Knoll et al. 2008) and on SBI-02 around 99–102 cM (Burow et al. 2011).

We also identified IS 7305, a Caudatum landrace from Nigeria, that showed increased germinability and vigor under low-temperature condition. In field evaluations at ICRISAT, Patancheru, India, in the 2010–2011 and 2011–2012 postrainy seasons and two environments (irrigated and water stressed), IS 7305 showed higher number of basal tillers (2.17), flowered early (74 days), and had higher panicle length (18.6 cm) as compared to the check, IS 33844 (77 days to flowering, basal tiller 1.50, panicle length 15.0 cm), a sorghum cultivar widely grown during rabi (postrainy) season in India. IS 7305 is resistant to pathotype 6 of Downey mildew (*Peronosclerospora sorghi*) (Radwan et al. 2011) in the USA and moderately resistant to anthracnose (Sharma et al. 2012) with a rating of 3.6 based on two seasons data (2009 and 2010) at ICRISAT, Patancheru, India, on a 1–9 scale where 1 to resistant and 9 is highly susceptible. IS 7305 has high seed iron (37.3 mg/kg seed) content than control IS 33844 (28.9 mg/kg seed) (Upadhyaya et al. 2016). This accession may be useful in future breeding programs.

In conclusion, we have mapped one locus associated with low-temperature germinability and none with seedling vigor in sorghum. This locus was in close proximity of previously mapped QTLs on SBI-07 and was significantly associated with low-temperature germinability in all three independent experiments. The mapping of this locus was supported by four lines of evidence: strong associations in all three experiments, co-localization with previously mapped cold tolerance QTL in sorghum, the locus spans a gene that has been previously show to confer cold tolerance and germination rate, and its rice syntenic region co-localizing with three cold tolerance QTL in rice. One of the linked markers, S7_56998511, has the potential to be used in marker-assisted breeding upon further testing of more accessions. Future work will involve evaluating additional growth parameters such as speed of germination at more than one low temperature and in the field to confirm associations reported in this study. This is important because results from this and similar studies may facilitate the development of molecular markers for breeding sorghums tolerant to early-season cold.

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