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Single nucleotide polymorphisms in *TaER* genes and their association with carbon isotope discrimination in wheat genotypes under drought

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Abstract

Candidate gene association studies implicate the detection of contributing single nucleotide polymorphism (SNP) for the target traits and have been recommended as a promising technique to anatomize the complex characters in plants. The *ERECTA* gene in plants controls different physiological functions. In this study, we identified SNPs in 1.1 kb partial sequences of *TaER-1* and *TaER-2* of wheat (*Triticum aestivum* L.). Thirty-nine SNPs were identified in the coding regions of *TaER-1* gene in 33 wheat genotypes, of which 20 SNPs caused non-synonymous mutations while 19 SNPs produced synonymous mutations; 31 SNPs were located in the coding regions of *TaER-2* gene in 26 genotypes, of which 18 SNPs caused non-synonymous mutations and 13 SNPs caused synonymous mutations. In addition, 32 SNPs in *TaER-1* and 9 SNPs in *TaER-2* were also identified in the non-coding regions. Moreover, the significant genetic associations of SNPs of *TaER-1* and *TaER-2* genes with carbon isotope discrimination, stomatal conductance, photosynthetic rate, transpiration rate, intrinsic water use efficiency (iWUE), leaf length, leaf width, stomatal density, epidermal cell density, and stomatal index were noted in wheat genotypes. This study confirms the importance of *TaER-1* and *TaER-2* genes which could improve iWUE of wheat by regulating leaf gas exchange and leaf structural traits. These identified SNPs may play a critical role in molecular breeding by means of marker-assisted selection.

Additional key words: association analysis, leaf anatomy, photosynthetic rate, stomatal conductance, stomatal density, transpiration rate, *Triticum aestivum*.

Introduction

Bread wheat (*Triticum aestivum* L.) is often cultivated in arid to semi-arid zones. With changing climate, water scarcity has turn into alarming constraints for wheat (Farooq *et al.* 2015). Different cultivation techniques have been found for enhancing the water use efficiency (WUE) of crops under rain-fed and restricted irrigation environments, but WUE is also reliant on the plant itself (Farooq *et al.* 2014, 2015, Hussain *et al.* 2016). Carbon isotope discrimination (CID) is a stable and delicate indicator negatively associated to plant water use efficiency (WUE; Farquhar and Richards 1984). Consequently, CID is suggested as an alternative

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Abbreviations: abx - abaxial, adx - adaxial; CID - carbon isotope discrimination; E - transpiration rate; ED - epidermal cell density; *ER - ERECTA gene*; g_s - stomatal conductance to water vapour; iWUE - intrinsic water use efficiency; LL - leaf length; LRR-RLK - leucine-rich repeat receptor-like kinase; LW - leaf width; MAS - marker-assisted selection; P_N - photosynthetic rate; QTN - quantitative trait nucleotide; SD - stomatal density; SI - stomatal index; SNP - single nucleotide polymorphism; WUE - water use efficiency.
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to WUE for use in the breeding strategies for C_3 crops including wheat (Kumar and Singh 2009).

The *ERECTA* gene (*ER*) is the main contributor to CID in *Arabidopsis*, and improves WUE under both adequate and inadequate water environments (Masle *et al.* 2005). The *ER* gene translates a leucine-rich repeat receptor-like kinase (LRR-RLK) to regulate organ progression and flower growth in *Arabidopsis* by regulation cell expansion and multiplication (Shpak *et al.* 2003). Transgenic plants of *Arabidopsis*, due to over-expression of this gene, modify WUE and drought resistance through changes in mesophyll cell proliferation, epidermal cell expansion, stomatal density and cell to cell contact. In wheat, two distinctive homologues of *TaER* genes (*TaER1* and *TaER2*) have recently been isolated, having a similar intron/exon structure, and they encode a putative LRR-RLK (Huang *et al.* 2013).

It is challenging to work on the innate complexity of phenotypic characteristics through customary linkage based methods, because these are laborious, costly, and time consuming (Mayles *et al.* 2009). In contrast, candidate gene association investigations implicate the discovery of causative polymorphisms for superior characters. Therefore, it is a significant technique for cloning based on genetic mapping and marker-assisted selection (MAS) which are revealed as extremely stable markers and associated with specific plant trait (Andersen and Luebberstedt 2003, Kim *et al.* 2005).

Association genetics is an un-controlled experimentation in nature, which may produce greater mapping resolution than the linkage mapping (Neale and Savolainen 2004). Investigations related to association studies have been extensively used in crops (Rashwan 2011) including wheat to locate the chromosome regions controlling qualitative traits (Breseghello and Sorrells 2006) or resistance to glume blotch (Tommasini *et al.* 2007) and Russian wheat aphid (Peng *et al.* 2009). However, association mapping has not been used in wheat under water stress because phenotyping of drought tolerance in a natural population is very difficult.

The identification of SNPs through genetics based association analysis may yield the discovery of causative quantitative trait nucleotides (QTNs), which can be utilized further for gene-assisted selection by synthesizing the genetic markers to select the superior planting materials depending upon their DNA sequences. This could be an important study to increase the production potential of wheat crop by optimizing the physiological and agronomic parameters under drought. This study was conducted to discover the SNPs in the copies of wheat *TaER* genes and to detect the genetic association between discovered SNPs and the leaf structure and water use related physiological traits in wheat genotypes.

Materials and methods

Plants and experimental conditions: A set of 49 wheat genotypes, collected from major winter wheat (*Triticum aestivum* L.) growing regions of China, was used in this experiment (Table 1 Suppl.). The collection consisted of 46 released cultivars and one landrace being extensively cultivated in China and also used in different breeding projects. Two Australian genotypes were also used based on published CID (Rebetzke *et al.* 2006). The selected genotypes represent a diverse range of CID values, but did not differ greatly in phenology. The origin and planting region of the planting material is given in Table 1 Suppl. These plants have already been used in our previous experiments (Yasir *et al.* 2013).

This experiment was conducted at the Institute of Water Saving Agriculture in Arid Regions of China, Northwest A & F University, Yangling (34°17.7′ N, 108°4.05′ E; 526 m a.s.l.), Shaanxi, China, during the growing seasons of 2011 - 2012. With the intention to simulate the water stressed conditions and to nullify the influence of erratic rainfall, the experiment was laid out under rainout shelter and irrigation. The experimental soil was "Loutu" having the bulk density of 1.4 g cm^{-3}, organic matter content of 0.86 %, and pH 7.7. All the genotypes were planted on 20 October in the two cropping seasons in two-row plots (100-cm rows with 6.7-cm spacing between plants and 25-cm between rows), with a plant density of 60 plants m^{-2}. Irrigation was applied in all treatments after sowing, to ensure homogeneous germination. Three restricted irrigations, each of 40 mm, were given by a water-meter at the tillering, stem elongation, and booting stages.

Measurement of phenotypic traits: For CID determination, 10 g of grains from individual genotype was taken and ground to make a very fine powder. The $^{13}C/^{12}C$ were analyzed using an isotopic ratio mass spectrometer (*Delta V*, *Thermo Fisher Scientific*, Bremen, Germany) interfaced with an element analyzer (*Flash EA1112 HT*) in the Laboratory of Stable Isotopes, Chinese Academy of Forestry Sciences (Beijing, China). Results were expressed as $\delta^{13}C$ (‰) = $[(R_{sample}/R_{reference}) - 1] \times 1000$, where R being $^{13}C/^{12}C$ ratio. A secondary standard (potato starch) calibrated alongside *Pee Dee Belemnite* carbonate was used for comparison. The CID value was calculated by the formula given by Farquhar *et al.* (1989).

Photosynthetic rate (P_N), stomatal conductance to water vapour (g_s), and transpiration rate (E) were recorded from sun-exposed flag leaf of the main tillers during grain filling stages using a portable photosynthesis

system (*Li-Cor 6400*; Lincoln, NE, USA). The intrinsic water use efficiency (iWUE) was calculated as P_N/g_s.

At grain filling stages, average leaf length (LL) and leaf width (LW) of flag leaves were measured from 10 plants selected randomly from each genotype. At this stage stomatal density (SD) and epidermal cell density (ED) were also calculated on both sides of the flag leaves by impression method (Wang and Clarke 1993). The stomatal index was calculated using the following equation: $I = [S/(S+E)] \times 100$, where I is the stomatal index, S and E are the stomatal density and epidermal cell density, respectively.

DNA extraction, amplification, and the partial *TaER* sequence: Genomic DNA was extracted from young leaves of all 49 genotypes using a cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980). Thereafter, to confirm the quality and concentration of genomic DNA, gel electrophoresis and *Gene Quant Pro* spectrophotometer (*Amersham Biosciences*, Piscataway, USA) were used.

Allohexaploid wheat consists of three similar genomes, and therefore, most of the genes are present as three similar sequences of homologous copies. Based on the conserved regions of the wheat cDNA sequence of *TaER-1* and *TaER-2* (Huang *et al*. 2013), one pair of primers Tr1-F (5'-GCAATACTCGGCATTGCTCT-3') and Tr1-R (5'-ATGCCAAAGTCCGTAAGGTG-3') were designed using *Premier Primer 5* software (http:// www.premierbiosoft.com) and synthesized by *Sunny Biological Technology Company* (Shanghai, China). PCR amplification was performed on the genomic DNA of 49 wheat genotypes under cycling conditions of 94 °C for 5 min, 40 cycles at 94 °C for 45 s, 56 °C for 30 s, and 72 °C for 70 s, followed by a final extension at 72 °C for 5 min using a *Gradient Thermal Cycler* (*Eppendorf*, Hamburg, Germany).

The PCR products were passed through agrose gel (1 %), stained with ethidium bromide and visualized under *BioRad* (Hercules, USA) imaging system. The target segments were gel purified by gel extraction kit (*Biomiga*, San Diego, CA, USA), and then ligated to pMD-18T vector (*TaKaRa*, Dalian, China) and transformed into *Escherichia coli strain* DH5α. The clones selected were confirmed and sequenced by *Shanghai Sunny Biotech*, Shanghai, China. At least six clones from each genotype were sequenced.

Sequence analysis and SNP discovery: Sequences obtained for each genotype were assembled, and then all the sequences from each genotype were aligned with the sequence of *Aegilops tauschii* **(**DD genome) and *Aegilops speltoides* (BB genome) separately using *BioEdit v 7.0.4.1* (Hall 1999). The genotypes sequences having 98 - 99 % similarity with DD sequence were separated and grouped into *TaER-1*, while the sequences having 98 - 99 % similarity with BB genome sequences were separated and grouped into *TaER-2*.

The intron locations were identified by aligning the genomic sequences of *TaER-1* and *TaER-2* to their corresponding cDNA sequences using *BioEdit* software. Sequences were also confirmed for their similarity by using *BLASTn* (http://blast.ncbi.nlm.nih.gov/). Sequences corresponding to *TaER-1* and *TaER-2* were aligned separately to discover SNPs using CLC Free Workbench 4 software (*CLC Bio*, Aarhus, Denmark). Finally, all the sequences were translated into amino acids and were aligned to detect the synonymous and non-synonymous mutations among 33 genotypes in *TaER-1* and 26 genotypes in *TaER-2*.

Association between the SNPs was discovered and the values of leaf structural and leaf physiological traits were calculated with the general linear model (GLM), which executes association analysis by a least square fixed effects model with the traits analysis by association, evolution, and linkage (*TASSEL*, *v.2.1*).

Results

Partial DNA sequences of *TaER-1* (1 105 bp) and *TaER-2* (1 108 bp) were cloned and sequenced from 33 and 26 wheat genotypes, respectively. The partial sequence of *TaER-1* showed high similarity with *Triticum aestivum* at chromosome 7D and 7B (100 and 93 %, respectively), *Hordeum vulgare* (100 %), *Brachypodium distachyon* (94 %), *Sorghum bicolor* (87 %), *Zea mays* (87 %), and *Oryza sativa* (89 %) (Table 2 Suppl.). The partial sequence of *TaER-2* was found to be similar when compared with *Triticum aestivum* at chromosome 7D and 7B (95 and 93 %, respectively), *Hordeum vulgare* (97 %), *Brachypodium distachyon* (93 %), *Sorghum bicolor* (86 %), and *Oryza sativa* (90 %) (Table 3 Suppl). Afterward, sequences of *TaER-1* and *TaER-2* were aligned with the *ER1* (JQ599260.2) and *ER2* (JQ599261.2) whole length cDNA sequence of *Triticum aestivum*, respectively, to find out the intronic and exonic regions. From the alignment, it was obtained that both the *TaER-1* and *TaER-2* sequences fall in the three exonic and two intronic regions.

Partial DNA sequences of *TaER-1* gene from 33 genotypes were aligned together by using *CLC* sequence viewer software (*CLC Bio*) to discover the SNPs. Seventy-one SNPs were observed in total, of which 39 SNPs were found in coding regions (Table 4 Suppl.) while 32 SNPs in non-coding regions (Table 5 Suppl.). Among the 71 SNPs detected, 58 were caused by

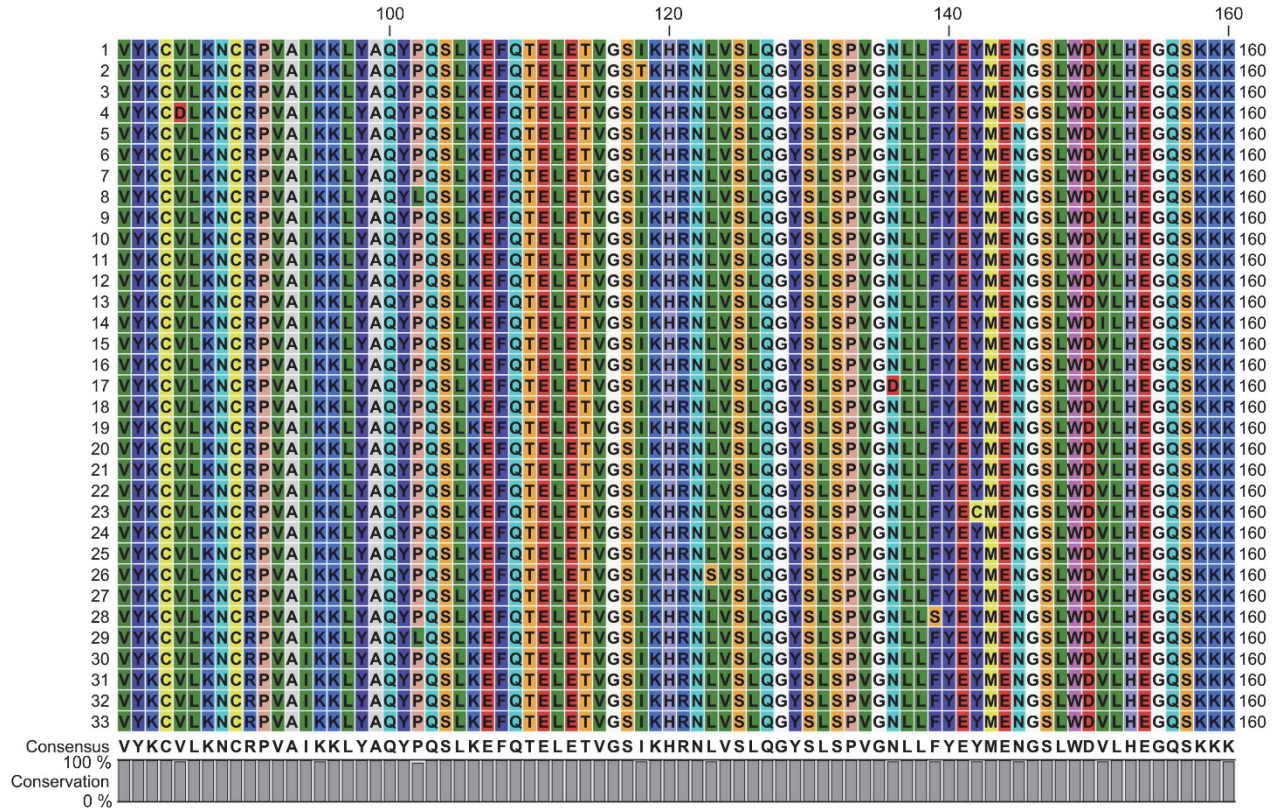

Fig. 1. Comparison of amino acid sequences (a segment of 81 - 160 amino acids) of partial *TaER-1* gene from 33 wheat genotypes. *Vertical axis* shows the amino acid sequences from 1 to 33 genotypes, while *horizontal axis* shows the non-synonymous mutation by a different colour within each column. Names of genotypes are given in Table 8 suppl. The complete sequences of amino acids showing non-synonymous mutations are given in Fig. 1. Suppl.

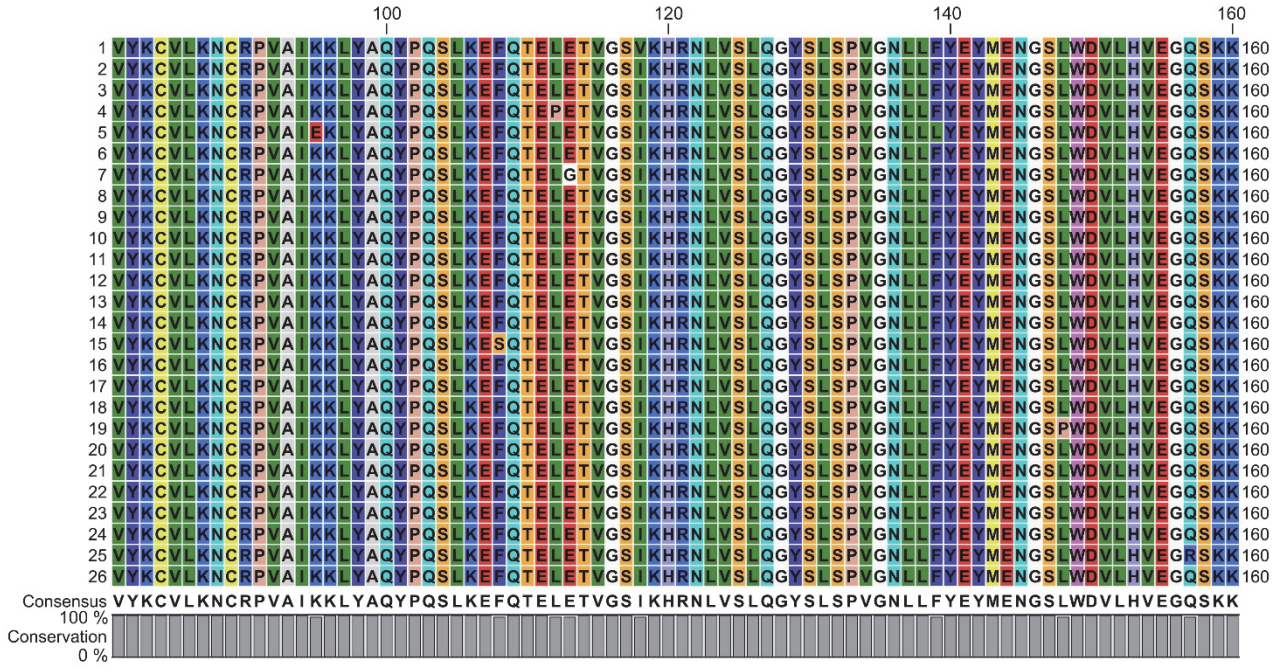

Fig. 2. Comparison of amino acid sequences (a segment of 81 - 160 amino acids) of partial *TaER-2* gene from 26 wheat genotypes. *Vertical axis* shows the amino acid sequences from 1 to 26 genotypes, while horizontal axis shows the non-synonymous mutation by a different colour within each column. Names of genotypes are given in Table 9 Suppl. The complete sequences of amino acids showing non-synonymous mutations are given in Fig. 2. Suppl.

the transition mutation whereas the other 13 were caused by transversion mutation. For the sequence alignment of *TaER-2* gene from the 26 genotypes, a total of 40 SNPs were identified, where 31 SNPs were detected in the exonic region (Table 6 Suppl.), and 9 SNPs in the intronic region (Table 7 Suppl.). For synonymous and non-synonymous mutation study, sequences from exonic regions were translated and aligned among 33 genotypes for *TaER-1* and 26 geno-types for *TaER-2*, respectively using *CLC* sequence viewer software. Fig. 1 shows a segment of 81 - 160 amino acid sequences cropped from full length sequences of *TaER-1* from the 33 genotypes (Fig. 2 Suppl.). The alignment of these sequences identified 20 non-synonymous (Fig. 1 Suppl.) and 19 synonymous mutations in *TaER-1* sequence. Similarly, Fig. 2 displaying a cropped segment of 81 - 160 amino acid sequences from the full length sequences of *TaER-2* from 26 genotypes (Fig 2 Suppl.). Their alignment resulted in 18 non-synonymous (Fig. 2 Suppl.) and 13 synonymous mutations within the open reading frame of *TaER-2*.

Genetic association study between SNPs in *TaER-1* and phenotypic variations on 33 wheat genotypes (Table 1) revealed that there were 18 SNPs significantly associated with CID, gas exchange parameters (P_N, g_s, E, and iWUE), and leaf structural traits (LL, LW, SD, ED,

Table 1. Association analysis of morpho-physiological traits with SNPs in partial *TaER-1* gene in wheat based on 33 sequenced samples (* - $P < 0.05$; ** - $P < 0.01$; the percentage given in the parentheses is the contribution of locus to the trait).

Exon/intron	SNP position	Translation	Amino acid	Traits associated with SNP
Exon 24	57	GCT/GCC	Ala	SIabx* (12 %)
	76	CCT/TCT	Pro-Ser	SIabx* (15 %)
	90	AAA/AAC	Lys-Asn	g_s* (12.8 %)
	94	ATC/GTC	Ile-Val	P_N* (12.7 %), SIadx* (17.4 %), E* (19 %)
Exon 25	371	GCC/GTC	Ala-Val	SDabx* (14 %)
	397	AGG/GGG	Arg-Gly	EDadx** (20.4 %), SIabx* (17.3 %)
	417	AGT/AGC	Ser	SIabx* (15 %)
	433	GGG/AGG	Gly-Arg	P_N* (19 %), E** (28.3 %)
	467	GTT/GAT	Val-Asp	EDabx* (11.9 %), EDadx* (15.2 %), LL* (13.3 %), SDadx* (14.5 %)
	518	CCG/CTG	Pro-Lue	g_s* (17.8 %), iWUE* (13.3 %)
	566	ATC/ACC	Ile-Thr	SIabx* (15.0 %)
	591	CTT/CTC	Leu	CID* (13.5 %)
	619	AAT/GAT	Asn-Asp	iWUE*** (32.9 %)
	629	TTC/TCC	Phe-Ser	EDadx** (20.4 %), SIabx* (17.3 %)
	638	TAC/TGC	Val-Cys	LL** (20.0 %)
	647	AAT/AGT	Asn-Ser	EDabx* (11.9 %), EDadx* (15.2 %), LL* (13.3%), SDadx* (14.5%)
	664	GTT/ATT	Val-Ile	CID* (13.5 %)
Exon 26	943	AAA/GAA	Lys-Glu	LW** (20.1 %), SDadx* (12.2 %)

Table 2. Association analysis of morpho-physiological traits with SNPs in partial *TaER-2* gene in wheat based on 26 sequenced samples (* - $P < 0.05$; ** - $P < 0.01$; the percentage given in the parentheses is the contribution of locus to the trait).

Exon/intron	SNP position	Translation	Amino acid	Traits associated with SNP
Exon 25	340	TCC/CCC	Ser-Pro	CID* (19.3 %)
	412	AGA/GGA	Arg-Gly	SIabx** (25.7 %), SIadx* (23.9 %)
	453	AGC/GGC	Gln	iWUE* (16.6 %)
	467	AAT/AGT	Asn-Ser	E** (25 %)
	553	TTG/CTG	Leu	EDadx* (17.5 %), SDadx* (15.3 %)
	569	GTA/GCA	Val-Ala	SIadx* (16.6 %)
	570	GTA/GTG	Val-Ala	E** (25 %)
	593	GTC/GCC	Val-Ala	CID* (19.3 %)
	599	AAG/AGG	Lys-Arg	CID* (19.3 %)
	611	TAT/TGT	Tyr-Cys	EDadx* (17.5 %), LW* (22.3 %), SDabx* (23.4 %), SDadx* (21.3%)
Exon 26	995	AAG/AGG	Lys-Arg	P_N* (23.3 %), g_s* (18 %)
	1006	ACC/GCC	Thr-Ala	E** (25 %)

and SI). The SNP in exon 25 at position 397 was correlated (20.4 %; software *TASSEL*) with ED on adaxial surface of the leaf (EDadx) and conversion of amino acid arginine to glycine [*i.e*., change in nucleotide sequence (AGG/GGG) which produced change in amino acid sequence]. Similarly, SNP at position 433 was highly correlated (28.3 %) with E and with the amino acid conversion from glycine to arginine, and SNP at position 619 was highly correlated (32.9%) with iWUE and with the amino acid change from asparagine to aspartic acid. The SNP at position 629 was highly correlated (20.4 %) with EDadx and with the amino acid change from phenylalanine to serine, while the SNP at 638 correlated (20 %) with LL by altering the amino acid valine to cysteine. The SNP in exon 26 at position 943 was also highly correlated with LW by inducing the change in amino acid lysine to glutamic acid (Table 1).

There were also some SNPs which were associated with multiple traits. The SNP in exon 24 at position 94 was simultaneously associated with P_N, SI on adaxial surface, and E, and with the change from isoleucine to valine. Two SNPs in exon 25 at position 467 and 647 were associated with four traits (EDabx, EDadx, LL, and SDadx) and altered amino acids from valine to aspartic acid and from asparagine to serine, respectively. Two SNPs in exon 25 at position 591 and 664 were also associated with carbon isotope discrimination (CID), but the magnitude of the association was not very strong (13.5 %). Other SNPs were found significant but they exhibited weaker correlation with different phenotypic traits given in the Table 1. The association between SNPs and above mentioned traits indicates that these SNPs can produce any change in these parameters.

Genetic association study of SNPs in *TaER-2* with phenotypic variations on 26 wheat genotypes (Table 2) revealed that there were 12 SNPs associated with CID, gas exchange parameters (A, g_s, E, and iWUE), and leaf structural traits (LL, LW, SD, ED, and SI). There were four SNPs, which showed highly significant correlation with different traits. In exon 25, the SNP at position 412 was highly correlated (25.7 %) with SI on adaxial surface and with the amino acid change from arginine to glycine. Furthermore, two SNPs, in the same exon 25, at positions 467 and 570 were also highly correlated (25 %) with E and with the amino acid conversion from asparagine to serine and valine to alanine, respectively. Similarly, the SNP in the exon 26 at position 1006 was highly correlated with E (25 %) and with altered threonine to alanine.

The SNP in exon 25 at position 412 was simultaneously associated with SI on abaxial and adaxial surfaces. Two SNPs, one in exon 25 at position 553 and second in exon 26 at position 995 were simultaneously associated with two traits. The SNP in exon 25 at position 611 was associated with four traits (EDadx, LW, SDabx, and SDadx) and with the amino acid change from tyrosine to cysteine. Moreover, three SNPs in exon 25 at positions 340, 593, and 599 were also associated with CID, but the magnitude of the association was not very strong (19.3 %). Other SNPs were also significant but they also exhibited weaker correlation with different phenotypic traits given in the Table 2.

Discussion

The SNP frequency in wheat has been estimated as one in every 86 bp sequence length to one in every 540 bp sequence length (Somers *et al*. 2003). In our study, one SNP was identified at each 16 bp (1 106 bp sequence of *TaER-1* gene) to 28 bp (1 108 bp of *TaER-2* gene). The comparison of obtained SNP frequency in these genes with other studies has not been done because the full length of *TaER-1* and *TaER-2* genes have been discovered recently (Huang *et al.* 2013). When we talk about SNP detection methods, it is found that more SNPs can be discovered by direct sequencing rather than other methods and the number of SNPs can be different in different genomic areas. Analysis showed that SNP frequency varied along the partial sequences of both genes amplified in these experiments. Across the partial length of both *TaER-1 TaER-2* sequences, the higher nucleotide variation occurred in the coding regions than in the non-coding regions. Our results are in contrast to those of Ching *et al.* (2002), Nasu *et al.* (2002), and Hamblin *et al.* (2004), who found higher frequency of SNPs in the non-coding regions than in the coding regions. The possible cause for obtaining more number of SNPs within the coding region might be the structure of this gene. It can only be confirmed and validated when the complete sequences of *TaER-1* and *TaER-2* genes are identified. The resulting protein, produced with the different combinations of amino acids, can only be changed due to non-synonymous mutations rather than synonymous mutations. Therefore, non-synonymous SNPs causing variation in protein sequence can influence structural, functional, and biochemical attributes of the enzymes produced in the plant system (Bromberg and Rost 2007). It may eventually bring about variation in phenotypic parameters of the plants, particularly important for the adaptation to drought. The *ERECTA* gene encodes a kind of protein which takes part in the normal growth and development of plant epidermis (Torii *et al.* 1996; Shpak *et al.* 2003, 2004). The variation in leaf physiological and structural parameters and their associations with SNPs discovered in *TaER-1* and *TaER-2* confirmed the findings of Masle *et al.* (2005) that *ERECTA* gene has the tendency to maximize the capacity

of electron transport and Rubisco carboxylation, and finally, affects the photosynthetic capacity in *Arabidopsis*. Moreover, Casson and Gray (2008) found that ER, ERL1, and ERL2 influence transpiration efficiency in *Arabidopsis* by affecting the stomatal related traits.

Although the association was found between *TaER-1*/*TaER-2* and different leaf characters, the possibility of errors due to the structure of population cannot be neglected, mainly when a sample of small size is used (Yu and Buckler 2006). It is therefore necessary for association analysis to use a set of random markers based on SNPs in order to test population structure in future experiments. As we used partial sequences of TaER-1 and TaER-2 for detecting the SNPs and associating them with the traits, definitely, some genetic associations might have not been discovered. In future, candidate genes with full length sequences consisting of intron, exon, promoter, 5'-end, 3'-end un-translated regions are recommended for the detection of SNPs that can provide the locus to the candidate gene (Zhu *et al.* 2008, Lau *et al.* 2009).

Conclusions

The present study revealed a strong association between SNPs in both *TaER-1* and *TaER-2* genes with different leaf physiological (P_N, E, and iWUE) and structural (SD, ED, SI, LL, and LW) parameters. The results of this study confirm the importance of *ERECTA* gene which could improve WUE by regulating stomatal patterning of the plants. This indicates that SNPs in *TaER-1* and *TaER-2* genes might modify stomatal patterning and cause variation in the phenotypic traits in wheat. These SNPs, if validated, have the potential to be used in marker assisted selection for the early selection and prediction of wheat plant characteristics.

References

Andersen, J.R., Lubberstedt, T.: Functional markers in plants. - Trends Plant Sci. **8**: 554-560, 2003.

Breseghello, F., Sorrells, M.E.: Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. - Genetics **172**: 1165–1177, 2006.

Bromberg, Y., Rost, B.: SNP: predict effect of non-synonymous polymorphisms on function. - Nucl. Acids. Res. **35**: 3823-3835, 2007.

Casson, S., Gray, J.E.: Influence of environmental factors on stomatal development. - New Phytol. **178**: 9-23, 2008.

Ching, A., Cladwell, K.S., Jung, M., Dolan, M., Smith, O.S., Tingey, S., Morgante, M., Rafalski, A.J.: SNP frequency, haplotype structure and linkage disequlibrium in elite maize inbred lines. - BMC Genet. **3**: 19-33, 2002.

Farooq, M., Hussain, M., Siddique, K.H.M.: Drought stress in wheat during flowering and grain filling periods. - Crit. Rev. Plant Sci. **33**: 331-349, 2014.

Farooq, S., Shahid, M., Khan, M.B., Hussain, M., Farooq, M.: Improving the productivity of bread wheat by good management practices under terminal drought. - J. Agron.Crop Sci. **201**: 173-188, 2015.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T.: Carbon isotope discrimination and photosynthesis. - Annu. Rev. Plant Physiol. Plant mol. Biol. **40**: 503-537, 1989.

Farquhar, G.D., Richards, R.A.: Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. - Aust. J. Plant Physiol. **11**: 539-552, 1984.

Hall, T.: BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. - Nucl. Acids Symp. Ser. **41**: 95-98, 1999.

Hamblin, M.T., Mitchell, S.E., White, G.M., Gallego, J., Kukatla, R., Wing, R.A., Paterson, A.H, Kresovich, S.: Comparative population genetics of the panicoid grasses: sequence polymorphism, linkage disequilibrium and selection in a diverse sample of *Sorghum bicolor*. - Genetics. **167**: 471-483, 2004.

Huang, L., Yasir, T.A., Phillips, A.L., Hu, Y.G.: Isolation and characterization of *ERECTA* genes and their expression patterns in common wheat (*Triticum aestivum* L.). - Aust. J. Crop Sci. **7**: 381-390, 2013.

Hussain, M., Farooq, S., Jabran, K., Ijaz, M., Sattar, A., Hassan, W.: Wheat sown with narrow spacing results in higher yield and water use efficiency under deficit supplemental irrigation at the vegetative and reproductive stage. - Agronomy **6**: 22, 2016.

Kim, M.Y., Van, K., Lestari, P., Moon, J.K., Lee, S.H.: SNP identification and SNAP marker development for a GmNARK gene controlling supernodulation in soybean. - Theor. appl. Genet. **110**: 1003-1010, 2005.

Kumar, S., Sing, B.: Effect of water stress on carbon isotope discrimination and Rubisco activity in bread and durum wheat genotypes. - Physiol. mol. Biol. Plants **15**: 281-286, 2009.

Lau, E.T., Ho, W.S., Julaihi, A.: Molecular cloning of *cellulose synthase* gene, *SpCesA1* from developing xylem of *Shorea parvifolia* spp. *parvifolia*. - Biotechnology **8**: 416-424, 2009.

Masle, J., Gilmore, S.R., Farguhar, G.D.: The *ERECTA* gene regulates plant transpiration efficiency in *Arabidopsis*. - Nature **436**: 866-870, 2005.

Mayles, S., Peiffer, J., Brown, P.J., Eroz, E.S., Zhang, Z., Costich, D.E., Buckler, E.S.: Association mapping: critical considerations shift from genotyping to experimental design. - Plant Cell **21**: 2194-2202, 2009.

Murray, M.G., Thompson, W.F.: Rapid isolation of high molecular weight plant DNA. - Nucl. Acids Res. **8**: 4321-4325, 1980.

Nasu, S., Suzuki, J., Ohta, R., Hasegawa, K., Yui, R., Kitazawa, N., Monna, L., Minobe, Y.: Search for and analysis of single nucleotide polymorphisms (SNPs) in rice (*Oryza*

sativa, Oryza rufipogon) and establishment of SNP markers. - DNA Res. **9**: 163-171, 2002.

Neale, D.B., Savolainen, O.: Association genetics of complex traits in conifers. - Trends Plant Sci. **9**: 325-330, 2004.

Peng, J.H., Bai, Y., Haley, S.D., Lapitan, N.L.V.: Microsatellite-based molecular diversity of bread wheat germplasm and association mapping of wheat resistance to the Russian wheat aphid. - Genetica **135**: 95-122, 2009.

Rashwan, A.M.A.: Study of genotype and phenotypic correlation for some agro-agronomic traits in okra (*Abelmoschus esculents* (L.) Moench). - Asian J. Crop Sci. **3**: 483-486, 2011.

Rebetzke, G.J., Richard, R.A., Condon, A.G., Farquhar, G.D.: Inheritance of carbon isotope discrimination in bread wheat (*Triticum aestivum* L.). - Euphytica. **150**: 97-106, 2006.

Shpak, E.D., Berthiaume, C.T., Hill, E.J., Torii, K.U.: Synergistic interaction of three ERECTA-family receptor-like kinases controls *Arabidopsis* organ growth and flower development by promoting cell proliferation. - Development **131**: 1491-1501, 2004.

Shpak, E.D., Lakeman, M.B., Torii, K.U.: Dominant-negative receptor uncovers redundancy in the *Arabidopsis* ERECTA leucine-rich repeat receptor-like kinase signaling pathway that regulates organ shape. - Plant Cell **15**: 1095-1110, 2003.

Somers, D.J., Kirkpatrick, R., Moniwa, M., Walsh, A.: Mining single-nucleotide polymorphisms from hexaploid wheat ESTs. - Genome **49**: 431-437, 2003.

Tommasini, L., Schnurbusch, T., Fossati, D., Mascher, F., Keller, B.: Association mapping of *Stagonospora nodorum* blotch resistance in modern European winter wheat varieties. - Theor. appl. Genet. **115**: 697-708, 2007.

Torii, K.U., Mitsukawa, N., Oosumi, T., Matsuura, Y., Yokoyama, R., Whittier, R.F., et al.: The *Arabidopsis ERECTA* gene encodes a putative receptor protein kinase with extracellular leucine-rich repeats. - Plant Cell **8**: 735-746, 1996.

Wang, H., Clarke, J.M.: Genotypic, intraplant, and environmental variation in stomatal frequency and size in wheat. - Can. J. Plant Sci. **73**: 671-678, 1993.

Yasir, T.A., Min, D.H., Chen, X.J., Condon, A.G., Hu, Y.G.: The association of carbon isotope discrimination (Δ) with gas exchange parameters and yield traits in Chinese bread wheat cultivars under two water regimes. - Agr. Water Manage. **119**: 111-120, 2013.

Yu, J., Buckler, E.S.: Genetic association mapping and genome organization of maize. - Curr. Opin. Biotechnol. **17**: 155-160, 2006.

Zhu, C., Gore, M., Buckler, E.S., Yu, J.: Status and prospect of association mapping in plants. - Genome **1**: 5-20, 2008.