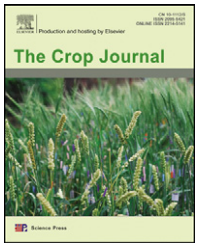
THE CROP JOURNAL 3 (2015) 37–45

HOSTED BY

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect



Mapping the glaucousness suppressor *Iw1* from wild emmer wheat "PI 481521"

Zongchang Xu[a], *Cuiling Yuan*[a], *Jirui Wang*[b], *Daolin Fu*[a,*], *Jiajie Wu*[a,*]

[a]*State Key Laboratory of Crop Biology, Shandong Key Laboratory of Crop Biology, Shandong Agricultural University, Tai'an 271018, China*
[b]*Triticeae Research Institute, Sichuan Agricultural University, Chengdu 611130, China*

ARTICLE INFO

Article history:
Received 9 June 2014
Received in revised form
9 September 2014
Accepted 28 September 2014
Available online 13 December 2014

Keywords:
Waxiness
Triticum turgidum ssp. *durum*
T. turgidum ssp. *dicoccoides*
Wheat 90K iSelect array

ABSTRACT

Many species of Triticeae display a glaucous phenotype. In wheat, glaucousness/waxiness on spikes, leaves and shoots is controlled by wax production genes (*W* loci) and epistatic inhibitors (*Iw* loci). In this study, a suppressor of glaucousness from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) accession "PI 481521" was investigated in a pair of durum (*T. turgidum* ssp. *durum* cv. "Langdon", LDN)—wild emmer wheat chromosome substitution lines, LDN and "$LDN_{DIC521\text{-}2B}$". Genetic analysis revealed that the non-glaucous phenotype of $LDN_{DIC521\text{-}2B}$ was controlled by the dominant glaucous suppressor *Iw1* on the short arm of chromosome 2B. In total, 371 2B-specific marker differences were identified between LDN and $LDN_{DIC521\text{-}2B}$. The location of the *Iw1* gene was mapped using an F_2 population that stemmed from LDN and $LDN_{DIC521\text{-}2B}$, generating a partial linkage map that included 19 simple sequence repeats (SSR) and ten gene-based markers. On the current map, the *Iw1* gene was located within the *Xgwm614*–BE498111 interval, and cosegregated with BQ788707, CD893659, CD927782, CD938589, and *Xbarc35*. Mapping of *Iw1* in $LDN_{DIC521\text{-}2B}$, a publically accessible and widely distributed line, will provide valuable information for marker-assisted selection of the agronomically important trait of glaucousness.

1. Introduction

Epicuticular wax (EW) is an important surface structure on plants. In general, EW may affect water relations, protect plants from radiation, provide a physical barrier for toxic substances, enhance canopy reflectance, and increase grain yield [1–3]. Epicuticular wax also plays important roles in plant defense against bacterial and fungal pathogens, and impacts plant–insect interactions [2,4].

Plant EW contains a variety of long chain-length hydrocarbons, such as alcohols, aldehydes and alkanes, each of which also contains various homologues [5]. When compounds accumulate in the wax layer, especially those compounds enriched in one single homologue, they form ordered microcrystalline structures,

Abbreviations: CAPS, cleaved amplified polymorphic sequence; CSL, chromosome substitution line; dCAPS, derived cleaved amplified polymorphic sequence; EST, expressed sequence tag; EW, epicuticular wax; InDel, insertion–deletion; NILs, near-isogenic lines; NT, nulli-tetrasomic; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

* *Corresponding authors*. Tel.: +86 538 8242213; fax: +86 538 8242226.
E-mail addresses: dlfu@sdau.edu.cn (D. Fu), jiajiewu@sdau.edu.cn (J. Wu).
Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.

http://dx.doi.org/10.1016/j.cj.2014.09.004

which cause light-scattering effects and glaucousness [6]. Therefore, plant EW can be grouped into non-glaucous and glaucous epicuticular waxes [7]. Glaucous EW is associated with high concentrations of β-diketones, C_{29} and C_{31} hydrocarbons, primary alcohols, triterpene ketones, and esters within the EW hydrocarbon matrix [7–9]. In xeric or semiarid plants, the glaucous EW improve water status under drought stress conditions [10].

In the Triticeae, many species have evolved with both glaucous and non-glaucous phenotypes, such as *Aegilops tauschii* [11], *Hordeum vulgare* [12,13], tetraploid wheat [14] and polyploid wheat [15,16]. In wheat, major compounds such as β-diketones and hydroxy-β-diketones cause glaucousness [17,18]. Glaucous phenotypes in wheat (*Triticum aestivum* L.) are mainly controlled by two wax production loci (*W1* and *W2*) and closely associated inhibitor genes *Iw1* and *Iw2*, that are epistatic to *W1* and *W2* [19]. Wax composition was recently studied in six near-isogenic lines (NILs) varying in different *W* and *Iw* combinations in the genetic background of common wheat "S-615" [18]. NILs *W1W2iw1iw2*, *W1w2iw1iw2*, and *w1W2iw1iw2* are glaucous, whereas *w1w2iw1iw2*, *W1W2Iw1iw2*, and *W1W2iw1Iw2* are non-glaucous. In general, the glaucous NILs are similar in wax load and wax composition, and β-diketones account for ca. 60% of the total wax. However, the levels of β-diketones are dramatically reduced in the non-glaucous NILs, accounting for 8% of total wax in *w1w2iw1iw2* and becoming undetectable in *W1W2Iw1iw2* and *W1W2iw1Iw2* [18].

The wax production genes as well as the inhibitors were mapped on wheat chromosome arms 2BS and 2DS [19]. The *Iw1* locus is ca. 2 cM distal to the *W1* locus, whereas the *Iw2* locus is ca. 131 cM distal to *W2*. However, *Iw1* and *Iw2* are likely orthologs in wheat homologous group 2 [19,20]. In wheat, two *Iw* loci, potential equivalents of *Iw1* and *Iw2*, were mapped on the distal ends of the short arms of chromosome 2B and 2D [21,22]. More recently, Adamski et al. [23] mapped the *Iw1* gene within a sub-cM interval containing a single colinear gene in *Brachypodium* and rice (*Oryza sativa* L.). It was shown that *Iw1* inhibits formation of β- and hydroxy-β-diketones in wheat EW on peduncles and flag leaf tissues.

In tetraploid wheat, several sets of chromosome substitution lines have been developed in durum cultivar "Langdon" (LDN, *T. turgidum* ssp. *durum*) [24–26]. In the Langdon background, the substitution line $LDN_{DIC521\text{-}2B}$ carries a pair of 2B chromosomes from wild emmer wheat PI 481521 (*T. turgidum* ssp. *dicoccoides*, DIC) [26]. Langdon has a glaucous phenotype, and $LDN_{DIC521\text{-}2B}$ is non-glaucous. In this study, we report the mapping of the *Iw1* locus using an F_2 population developed from Langdon and $LDN_{DIC521\text{-}2B}$.

2. Materials and methods

2.1. Plant materials

This study was conducted on tetraploid wheat Langdon and $LDN_{DIC521\text{-}2B}$ (*T. turgidum* L., $2n = 4x = 28$, AABB). Langdon was released by the North Dakota Agricultural Experiment Station in 1956 [27]. $LDN_{DIC521\text{-}2B}$ is a substitution line, in which the chromosomes 2B pair of Langdon is replaced by the homologous pair from the wild emmer accession "PI 481521" [26]. Reciprocal crosses were made between Langdon and $LDN_{DIC521\text{-}2B}$, and F_1, F_2, and F_3 plants were used to analyze the visual EW phenotypes. The parental lines for the study were obtained from USDA-ARS, Fargo, ND, USA.

2.2. Evaluation of glaucous and non-glaucous phenotypes

The visual EW phenotypes on flag-leaf sheaths, peduncles, and glumes were evaluated at the plant booting and heading stages. Glaucous EW is visible waxiness that contributes to the bluish color of organs; the transparent non-glaucous EW allows a natural reflection of green light from the investigated tissue surfaces.

2.3. Polymerase chain reaction

Genomic DNA of leaf tissues was extracted from plants at the jointing stage using the Sarkosyl method [28]. PCR amplifications were performed in 20 μL mixes containing 1× PCR buffer (1.5 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} each of dCTP, dGTP, dTTP, and dATP; Promega, Madison, USA), 0.4 μmol L^{-1} of both forward and reverse primers, 100 ng DNA template, 0.4 U of *Taq* DNA polymerase (Promega), and ddH_2O. Amplifications were conducted in an ABI 9700 Thermal Cycler (Life Technologies, Grand Island, NY, USA). Amplification cycles included an initial denaturation (94 °C for 5 min); 40 cycles of denaturation (94 °C for 30 s), annealing (58 °C for 30 s) and extension (72 °C for 30 s); and a final extension (72 °C for 10 min). PCR products were separated on 6% PAGE gels and examined under UV light.

2.4. Development of gene-based markers on wheat chromosome 2B

Gene-based markers provide informative data for genetic mapping and comparative genomics. To develop this type of marker, we utilized DNA polymorphisms in the gene region, which normally corresponds to expressed sequence tags (EST) and transcriptome-derived single nucleotide polymorphisms (SNP). To differentiate the 2B chromosomes between durum and wild emmer, we genotyped Langdon and $LDN_{DIC521\text{-}2B}$ using the wheat 90K iSelect SNP array, in which all SNP probes were generated from data mining of genomic sequences and wheat transcriptomes [29]. PCR markers were developed from selected SNPs showing polymorphism between Langdon and $LDN_{DIC521\text{-}2B}$. In addition, BE444541 and BE498396 were chosen to develop 2B-specific markers; these are two wheat ESTs belonging to the distal 6S deletion bin in the group 2 consensus map [30]. Three closely linked EST markers recently developed for the *Iw1* gene [23] were also integrated into current mapping effort.

2.5. Construction of a genetic linkage map

Data for the Langdon/$LDN_{DIC521\text{-}2B}$ F_2 population was used to construct a chromosome 2B linkage map. In addition to 10 gene-associated markers, 40 simple sequence repeats (SSR) were chosen from those previously mapped to chromosome 2B [31–33]. The resulting 19 polymorphic SSR markers were used to map the chromosome 2B inhibitor of wax production.