Characterization of RsgWBDC053: A New Greenbug Resistance Gene From Wild Barley (Hordeum Vulgare ssp. Spontaneum)

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Abstract

Greenbug (*Schizaphis graminum* Rondani) is a destructive insect pest that not only damages plants, but also serves as a vector for many viruses. Host plant resistance is the preferred strategy for managing greenbug. To date, only two greenbug resistance genes, *Rsg1* and *Rsg2,* have been reported in barley, with only the former being deployed in cultivars. To breed cultivars with effective resistance against various greenbug biotypes, additional resistance genes are urgently needed to sustain barley production. Wild barley accession WBDC053 (PI 681777), originating from the Baluchistan region of Pakistan, was previously found to be resistant to several greenbug biotypes. In this study, a recombinant inbred line (RIL) population derived from Weskan × WBDC053 was evaluated for response to greenbug biotype E and genotyped using genotyping by sequencing (GBS). A set of 3,347 high quality GBS-derived single nucleotide polymorphisms (SNPs) were then used to map a greenbug resistance gene in this wild barley accession. Linkage analysis placed the greenbug resistance gene in WBDC053, temporarily designated *RsgWBDC053*, in a 2.35 Mb interval (0-2,354,645 bp) in the terminal region of the short arm of chromosome 2H. This interval harbors 15 genes with leucine-rich-repeat (LRR) protein domains. An allelism test between WBDC053 carrying *RsgWBDC053* and STARS1501B carrying *Rsg2* indicated that the former is either allelic or closely linked to the latter. GBS-SNPs *2H_1318811* and *2H_1839499* co-segregated with *RsgWBDC053* and were converted to Kompetitive allele specific PCR (KASP) markers, *KASP-Rsg053-1* and *KASP-Rsg0533-2*. The two KASP markers can be used to select for *RsgWBDC053,* but also have the potential to tag *Rsg2* in barley improvement programs.

Key Message

A new gene for greenbug resistance (*RsgWBDC053*) was identified in the terminal region of chromosome 2HS in wild barley (*Hordeum vulgare* ssp. *spontaneum*), and two KASP markers co-segregating with the gene were developed for use in introgressing greenbug resistance in barley breeding programs.

Introduction

Barley (*Hordeum vulgare* L.) is one of the most widely grown crops in the world and is ranked fourth in world production among food crops in 2017 (FAOSTAT, 2017). The crop has a number of important uses, including malt production for beer and spirits, feed for animals and also food for human consumption (Ullrich 2011). Barley is often cultivated in marginal areas with few additional inputs. Thus, it is important to protect the crop from biotic constraints such as insects to secure sustainable and economic production.

Greenbug (*Schizaphis graminum* Rondani) is one of the most important insect pests in US barley production, with large scale outbreaks occurring every 5-10 years in the southern Great Plains (Starks and Burton 1997; Giles et al. 2000, 2003). Greenbug feeding causes chlorosis, necrosis and even death of plants when they are heavily infested. Although outbreaks occur only occasionally, yield losses can be huge in an outbreak year without expensive pesticide applications.

Growing greenbug resistant barley is the preferred approach for insect control because it reduces production costs and eliminates contamination from pesticide applications. Although considerable efforts have been made to identify sources of resistance, only two barley greenbug resistance genes, *Rsg1* and *Rsg2*, have been identified. *Rsg1* was found in barley cultivar 'Post 90', a reselection of barley cultivar 'Post' derived from the cross Harrison x Will with Will as the donor of the resistance gene (Edwards et al. 1985; Mornhinweg et al. 2004). *Rsg2* was first identified in Jao (PI 426756), a spring barley landrace collected from Sindh, Pakistan in 1976. Two breeding lines carrying *Rsg2*, STARS1501B and STARS1502B, were released in the US in 2018 (Mornhinweg et al. 2018). Previous studies indicated that *Rsg2* was non-allelic to *Rsg1* (Webster and Starks 1984), and that the two genes responded differently to greenbug biotypes TX1, WY10MC, WY81, and WY10B (Porter et al. 2007; Armstrong et al. 2016). *Rsg1* was mapped to an 8.4-cM interval in the terminal region of chromosome 3HL using SSR and SNP markers (Azhaguvel et al. 2014) and has been used in barley breeding. However, *Rsg2* has not been used in breeding and its genomic location remains unknown.

Rsg1 and *Rsg2* confer resistance to some economically important greenbug biotypes. However, new virulent greenbug biotypes continuously occur. For example, Armstrong et al. (2016) identified six new greenbug biotypes in Wyoming alone, including WY81, WY10MC, WY10B, WY12 MC, WY86 and WY4. Among these, biotype WY81 is virulent to *Rsg1*, while WY10MC and WY10B are virulent to *Rsg2*. Therefore, new greenbug resistance genes are urgently needed in barley breeding pipelines to manage greenbug.

Wild barley (*Hordeum vulgare ssp. spontaneum*) is the progenitor of cultivated barley and originated in the Fertile Crescent region. Today, its range extends from North Africa in the west, across Central Asia and into Southern Asia. Wild barley possesses abundant genetic variation and is considered an important source of biotic and abiotic stress resistance. To identify new genes for disease resistance in barley breeding, Steffenson et al. (2007) assembled the Wild Barley Diversity Collection (WBDC), comprised of 318 accessions from the Fertile Crescent, Central Asia, North Africa, and the Caucasus regions. The WBDC was screened for reaction to a set of greenbug biotypes by the USDA-ARS, Stillwater, OK, and only a few accessions were found resistant. One of these accessions (WBDC053) exhibited a unique response profile to a suite of greenbug biotypes, with resistance to greenbug biotypes B, C, E, I, TX1, WY4A, WY4B, WY81, WY12MC, and WY86 and susceptibility to F, H, WY10MC, and WY10B (Armstrong et al. 2016). Therefore, WBDC053 may carry new greenbug resistance gene(s). The objectives of this study were to characterize the genetics of greenbug resistance in WBDC053 and develop genomic tools for its introgression into elite barley cultivars.

Materials And Methods

Mapping population

A set of 154 $F_{5:6}$ recombinant inbred lines (RILs) was developed from the cross Weskan × WBDC053 using single seed descent and used in this study. WBDC053 (PI 681777) is a wild barley accession

originally collected in the Baluchistan region of Pakistan. It was derived from a single plant selection and selfed five times at University of Minnesota, St Paul, MN. (Ames et al. 2015). Weskan, a winter barley cultivar with a pedigree of Purdue 6515A2/KY 66-7-63-1294, was released by the Kansas State University Experiment Station in 1990 and is susceptible to greenbug.

Evaluation of RILs for responses to greenbug biotype E

WBDC053, Weskan and all RILs were evaluated for responses to greenbug biotype E at the USDA-ARS Wheat, Peanut, and Other Field Crop Research Unit, Stillwater, OK in 2020. The experiment was conducted in a greenhouse with supplemental light of 14 h per day at 22 ± 5°C. Greenbug biotype E was increased on susceptible barley cultivar Eight-Twelve (PI 537437). A randomized complete block design with two replicates was used. Screening flats were 152 cm long and 104 cm wide with 8 cm-diameter hills spaced 8 cm apart in the formation of 60 hills (6 x 10) per flat. For each RIL, six seeds were planted in each of two consecutive hills in each replicate, and the two parents were planted in each flat as resistant (WBDC053) and susceptible (Weskan) controls. Flats were infested as soon as seedlings emerged by laying heavily infested leaves from culture pots of greenbug biotype E between the rows. Plants were rated approximately two weeks after infestation when the susceptible checks had died. The dead plants were classified as susceptible, and the normal plants were classified as resistant.

Genotype the RIL population using the genotyping-by-sequencing

Leaf tissue was collected from each RIL and the two parents at the two-leaf stage, dried at -80°C in a Freezemobile 35EL Sentry 2.0 Lyophilizer (Sp Scientific, Warminster, PA), and grounded at 1,500 rpm for one min in a *MiniG*® Automated Tissue Homogenizer (Metuchen, NJ, USA). Genomic DNA was extracted using a method described by Dubcovsky et al. (1994) and quantified using the Quant-iT^{TM} PicoGreen dsDNA assay kits (Thermo Fisher Scientific, Waltham, MA, USA). A protocol described by Mascher (2013) was used to construct GBS libraries. In brief, DNA normalized to 20 ng/μL was digested with *PstI* and *MspI*, barcoded and ligated to a common Y-adaptor using T4 DNA ligase. Then, all DNA samples were pooled, purified and PCR-amplified. DNA fragments of 200–300 bp were size-selected for sequencing on an Ion Proton sequencer (Thermo Fisher Scientific, Waltham, MA, USA). SNPs were called using a universal network-enabled analysis kit (UNEAK) and the reference-based TASSEL GBS pipeline (Elshire *et al.*, 2011; Glaubitz *et al.*, 2014). SNP positions were determined by mapping sequence reads to the barley reference genome *Hordeum vulgare* r1 (Mascher et al. 2017), and SNPs with minor allele frequency greater than 20%, heterozygote frequency less than 5% and missing datapoints less than 11 were selected to map the target gene.

Allelism test

The greenbug resistance gene *Rsg2* has not been mapped. Therefore, an F_2 population was developed from the cross STARS1501B × WBDC053 to determine whether the greenbug resistance gene in WBDC053 is allelic to *Rsg2*. The F_2 population was evaluated for response to greenbug biotype E in two replicates, using the protocols described above for the RIL population.

Mapping the greenbug resistance gene in WBDC053

The ICI QTL Mapping V4.1 software program (Meng et al. 2015) was employed to map the greenbug resistance gene in WBDC053 using the Kosambi mapping function to convert recombination frequency into genetic distance (Kosambi 1944). The GBS-SNPs were grouped using a logarithm of the odds (LOD) threshold of 4.5, and ordered in each linkage group using the Recombination Counting and ORDering (RECORD) algorithm. The linkage map was fine-tuned using the sum of adjacent recombination fractions (SARF) at a window size of 7 as rippling criteria. MapChart 2.2 (Voorrips 2002) was used to draw the linkage map.

Development of KASP markers

Primer 3 (v. 4.0) (http://bioinfo.ut.ee/primer3-0.4.0/) was used to design primers. KASP assays were performed in an ABI ViiA 7 real-time PCR system (ThermoFisher Scientific, MA, USA), with 10 µL reaction mixtures containing 5 µL KASP master mix, 12 µM of each allele-specific primer, 30 µM of common primer and 20 ng genomic DNA. The PCR began at 30°C for 1 min and 94°C for 15 min, and then continued for 40 cycles of 94°C for 20 s and 60°C for 1 min, with a final step of 30°C for 1 min. KASP marker data were analyzed using the ABI ViiA 7 software.

Results

Responses of Weskan × WBDC053 RILs to greenbug biotype E

The two parents and their RILs were evaluated for responses to greenbug biotype E. WBDC053 was highly resistant, while Weskan was highly susceptible, resulting in the death of all plants at the end of the experiment. Of the 154 RILs, 84 were resistant and 64 were susceptible, which fit a 1:1 segregation ratio for a single gene (χ^2 = 2.7, df = 1, p > 0.1). The remaining six lines were still segregating, with an overall combined total of 99 resistant and 42 susceptible plants. Therefore, the greenbug resistance gene in WBDC053 is dominant (χ^2 = 1.7, df = 1, p > 0.18 for a 3:1 segregation ratio).

Linkage analysis

A total of 181.7 million high quality reads were generated from sequencing of the RIL population, with each RIL having 422,433-4,871,356 reads and 100.54 Mb-1.16 Gb sequence. These sequences were mapped to the barley reference sequence *Hordeum vulgare r*1, which led to the identification of 69,506 SNPs with a minor allele frequency greater than 0.2. We further selected 3,347 SNPs which have no more than 10 missing datapoints in the RIL population and mapped 466, 521, 506, 238, 617, 402, and 540 GBS-SNPs on chromosomes H1 to H7, respectively. The remaining 57 SNPs were not located to a specific chromosome. On average, each SNP had 2.5 missing datapoints in the RIL population.

Linkage analysis positioned the greenbug resistance gene in WBDC053, designated *RsgWBDC053*, to the distal end (0-2.35 Mb) on the short arm of chromosome 2H. The gene co-segregated with GBS-SNPs

2H_1318811, *2H_1368709* and *2H_1839499*, and was 0.68 cM distal to SNP *2H_2354645* in the map (Fig. 1).

Allelism test

Given that the genomic location of *Rsg2* is still unknown, we performed an allelism test to determine whether *RsgWBDC053*s allelic to *Rsg2* by evaluating an F_2 population derived from STARS1501B × WBDC053. A total of 1,226 F_2 plants, with 596 in replicate 1 and 630 in replicate 2, were evaluated for response to greenbug biotype E. All 1,226 plants exhibited high resistance, indicating *RsgWBDC366* is either allelic or very close to *Rsg2*.

Development of KASP markers

Two of the three GBS-SNPs co-segregating with *RsgWBDC053*, *2H_1318811* and *2H_1839499*, were converted to KASP markers *KASP-Rsg053-1* and *KASP-Rsg053-2*, respectively. *KASP-Rsg053-1* from *2H_1318811* was located at 1,318,811 bp in the Morex reference genome (*Hordeum vulgare* r1). WDBC053 carries the “G” allele, while Weskan carries the “A” allele at this locus. The allele-specific primers locate from 1,318,791 to 1,318,811 bp and the common primer extends from 1,318,878 to 1,318,899 bp in the *Hordeum vulgare* r1 reference genome (Table 1). *KASP-Rsg053-2* was converted from the GBS-SNP *2H_1839499* at 1,839,499 bp in the reference genome, where Weskan and WDBC053 carry the “C” and “T” alleles, respectively. We used the genomic sequence from 1,839,479 to 1,839,499 bp to design the allele-specific primers and 1,839,544 to 1,939,563 bp to design the common primer (Table 1).

KASP-Rsg053-1 and *KASP-Rsg053-2* were used to genotype the RIL population (Fig. 2), and the KASP genotyping results were in complete agreement with the GBS-SNP data, indicating that *KASP-Rsg053-1* and *KASP-Rsg053-2* can be reliably used to tag *Rsg053* in barley breeding. These markers can facilitate the introgression of the *Rsg* gene in WBDC053 into locally adapted barley breeding lines.

Candidate genes in the target region

The *Rsg* gene in WBDC053 resides in the interval of 0 - 2,354,645 bp (SNP *2H_2354645*) in the terminal region of chromosome 2HS. There are 59 annotated genes in this region with 46 genes encoding proteins with known functions in the *Hordeum vulgare* r1 reference sequence (Table 2). Among the 46 genes, 15 are R genes that encode proteins containing leucine-rich-repeat (LRR), including *HORVU2Hr1G000150*, *HORVU2Hr1G000160*, *HORVU2Hr1G000170*, *HORVU2Hr1G000180*, *HORVU2Hr1G000190*, *HORVU2Hr1G000200*, *HORVU2Hr1G000290*, *HORVU2Hr1G000510*, *HORVU2Hr1G000560*, *HORVU2Hr1G000630*, *HORVU2Hr1G000640*, *HORVU2Hr1G000700*, *HORVU2Hr1G000770*, *HORVU2Hr1G000780*, and *HORVU2Hr1G000900*. Given that the LRR-containing proteins are usually associated with innate immune responses in plants, they are putative candidates for the *Rsg* gene in WBDC053. Genes encoding cytochrome P450-related proteins, aldo/keto reductase, protein phosphatase 2, L-3-cyanoalanine synthase/cysteine synthase, transferase, tetrahydroberberine oxidase/THB oxidase,

and hexokinase etc. were also identified in this region (Table 2). High resolution mapping will be required to identify the candidate *Rsg* gene in WBDC053.

Discussion

RsgWBDC053 is a new gene for greenbug resistance

Wild species has been proven to be an important source for greenbug resistance in small grain cereals. In wheat, seven of the eight greenbug resistance genes originated from wild species or wheat relatives, including *Gb5* from *Ae. speltoides*, *Gb2* and *Gb6* from rye, and *Gb3*, *Gb4*, *Gb7,* and *Gb8* from *Ae. tauschii* (Xu et al. 2020). In this study, we identified a greenbug resistance gene in wild barley for the first time. This finding highlights the value of this subspecies for identifying new resistance genes of value for barley (Henningsen et al. 2021).

The *Rsg* gene in WBDC053 was mapped on chromosome 2H in this study, and *Rsg1* was previously mapped to chromosome 3H, demonstrating they are different genes at two unique loci. The chromosome location of *Rsg2* has not been reported to date; however, a previous study indicated that STARS 1051B (the source of *Rsg2*) and WDBC053 were resistant and susceptible to greenbug biotype F, respectively (Armstrong et al. 2015). This indicates that the two genes are indeed different from each other. To further resolve the relationship between the *Rsg* genes in the two resistance sources, we conducted an allelism test, and the results suggested the *Rsg* gene in WBDC053 either lies at a new locus closely linked with *Rsg2* or is an allele at the *Rsg2* locus. Given that a considerable number of R genes reside in the target region, the greenbug resistance gene in WBDC053 will be given the temporary designation of *RsgWBDC053* until high resolution mapping studies are conducted.

Tocho et al. (2013) mapped a quantitative trait locus (QTL), *QGb.unlp-2H*, for reduced foliar area and dry weight by greenbug infestation on chromosome 2H; thus, this genomic region might harbor a QTL for greenbug tolerance. However, Tocho et al. (2013) focused on tolerance and the two parents in that study showed slight difference in chlorosis. *RsgWBDC053* likely not *QGb.unlp-2H* because WDBC053 shows high resistance to greenbug.

RsgWBDC053 was mapped to a 2.35 Mb interval in the terminal region of chromosome 2H. In this interval, 59 annotated genes were identified with 15 genes carrying LRR protein domains. LRR protein domains commonly confer plant disease resistance by recognizing products of avirulent genes in pathogens. Some of these genes are either very close to or between GBS-SNPs markers that co-segregated with greenbug resistance in the RIL population. For example, *HORVU2Hr1G000510* is 1,579 bp distal to *2H_1318811*, and *HORVU2Hr1G000560*, *HORVU2Hr1G000630*, *HORVU2Hr1G000640*, *HORVU2Hr1G000700*, *HORVU2Hr1G000770*, *HORVU2Hr1G000780*, and *HORVU2Hr1G000900* reside between *2H_1318811* and *2H_1839499*. To date, no aphid resistance gene has been cloned and the mechanisms underlying greenbug or any other aphid resistance are still elusive. Given that LRR genes often provide plant innate immune responses, these LRR genes are good candidates for *RsgWBDC053*. Further fine-mapping of *RsgWBDC053* is needed to identify candidate genes and also resolve its

relationship to *Rsg2*. This should be feasible given the availability of residual heterozygosity in the RIL population. Of the 154 F_6 RILs evaluated in this study, six lines were segregating for resistance to greenbug biotype E. The corresponding F_5 lines of these RILs can be advanced for fine mapping *RsgWBDC053*. Since the gene resides in the gene-rich terminal end of chromosome 3H, we expect high recombination frequency in this region, which may lead to the identification of the causal variant. Additionally, WBDC053 and other WBDC accessions are currently being sequenced by the International Wild Barley Sequencing Consortium (https://iwbsc.umn.edu/). This effort will facilitate the cloning of the greenbug resistance gene from this wild barley accession.

Use of *RsgWBDC053* in barley breeding

Since the greenbug resistance gene identified in this study was from wild barley, it is likely that some undesirable genes will be carried along when introgressing it into adapted germplasm. Thus, pre-breeding will be necessary to efficiently transfer *RsgWBDC053* into modern barley cultivars. The KASP markers developed in this study co-segregated with *RsgWBDC053* in the RIL population and can be used for marker-assisted selection in barley breeding. WBDC053 is resistant to greenbug biotypes B, C, E, I, TX1, WY4, WY81, WY12 MC, and WY86 (Armstrong et al. 2016). Given that *Rsg1* is overcome by biotypes TX1 and WY81 (Armstrong et al. 2016), the resistance gene in WBDC053 will be valuable for enhancing greenbug resistance in combination with *Rsg1*. Molecular markers flanking *Rsg1* are available (Azhaguvel et al. 2014). Further conversion of those markers into KASP markers is still necessary to meet the requirements for high throughput screening of breeding materials. Moreover, *Rsg2* has been recently transferred to elite barley lines by recurrent backcrossing of STARS1501B, a selection from PI 426756, to Weskan, leading to the development of STARS1502B. STARS1502B showed similar grain yield, plant height, and test weight in fields as Weskan (Mornhinweg et al. 2018), suggesting that *Rsg2* does not negatively affect yield or quality traits. *Rsg2* is either allelic or very close to *RsgWBDC053*. Thus, the KASP markers developed in this study have the potential to tag *Rsg2* in barley breeding, depending on whether they are polymorphic between the parents of breeding populations.

The only WBDC accessions found resistant to greenbug biotype by Armstrong et al. (2016) were WBDC053 from Pakistan, WBDC157 from Iraq, and WBDC177 and WBDC366 from Turkmenistan. This suggests that greenbug resistance may have originated in wild barley populations of South and Central Asia and Middle East. To identify additional sources of resistance, greater sampling and testing of wild barley accessions from this region should be conducted. In addition to greenbug resistance, WBDC053 possesses a high level of resistance to powdery mildew, an important foliar disease of barley caused by the fungus *Blumeria graminis* f. sp. *hordei*. The resistance spectrum of WBDC053 was also very wide as it exhibited low infection types to 38 of 40 *B. graminis* f. sp. *hordei* pathotypes tested (Ames et al. 2015).

Declarations

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Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The USDA is an equal opportunity provider and employer.

Conflict of interest

On behalf of all authors, the corresponding authors state that there is no conflict of interest.

Author contributions

XX, DM and GB designed experiments; DM, GL, AB and BL conducted genotyping and phenotyping experiments; BJS provided genetic materials; XX drafted the manuscript and all authors revised the manuscript and approved the final version of the manuscript.

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Tables

Table 1. Alleles, allele-specific primers, and Common primers of *KASP-Rsg053-1* and *KASP-Rsg053-2*

Marker	Alleles*	Allele-specific Primer	Common Primer
KASP-Rsg053-1	G/A	CCAGGTCCCTGCAGTTGAAG	ACTACTGGCTGGTGAATTCCG
		CCAGGTCCCTGCAGTTGAAA	
KASP-Rsg053-2	T/C	CTGCTTGGAACCATGACACC	GTTCATGTGCATAGCGCCC
		CTGCTTGGAACCATGACACT	

*: WBDC053/Weskan

Table 2 Genomic locations and function annotation of the candidate genes in the terminal region of chromosome 2H where *RsgWBDC053* was located.

Gene	Genomic location (bp)	Function
HORVU2Hr1G000010	24939..32359	Polycomb group protein
HORVU2Hr1G000050	139300..149044	ALDO/KETO reductase
HORVU2Hr1G000060	182842..185828	Ethylene response sensor-2-related
HORVU2Hr1G000140	303954..304483	ALDO/KETO reductase
HORVU2Hr1G000090	207003..213166	Protein phosphatase 2C (PP2C)
HORVU2Hr1G000150	555110..557071	Leucine-rich repeat containing protein
HORVU2Hr1G000160	574243..576804	Leucine-rich repeat containing protein
HORVU2Hr1G000170	607918..609884	Leucine-rich repeat containing protein
HORVU2Hr1G000180	619057..624665	Leucine-rich repeat containing protein
HORVU2Hr1G000200	645032..647593	Leucine-rich repeat containing protein
HORVU2Hr1G000190	693030..712161	Leucine-rich repeat containing protein
HORVU2Hr1G000250	723583..723698	Bifunctional L-3-cyanoalanine synthase/cysteine synthase/cysteine synthase C1, mitochondrial
HORVU2Hr1G000260	761023..766626	Bifunctional L-3-cyanoalanine synthase/cysteine synthase/cysteine synthase C1, mitochondrial
HORVU2Hr1G000270	764403..765912	Predicted membrane protein
HORVU2Hr1G000280	766431..831248	L-3-cyanoalanine synthase/ cysteine synthase (ATCYSC1)
HORVU2Hr1G000290	783497..792896	Leucine-rich repeat containing protein
HORVU2Hr1G000360	923158..929823	Sequence-specific DNA binding transportation factor
HORVU2Hr1G000380	952979..954487	C2H2-type zinc finger (zf-C2H2_6)
HORVU2Hr1G000390	1021350..1032930	Polycomb protein Suz12
HORVU2Hr1G000410	1146999..1148640	Transferase family (Transferase)
HORVU2Hr1G000420	1183898..1186327	Transferase family (Transferase)
HORVU2Hr1G000430	1196655..1203088	Cytochrome P450 CYP4/CYP19/CYP26 subfamilies
HORVU2Hr1G000480	1235192..1236988	Cytochrome P450 CYP4/CYP19/CYP26 subfamilies
HORVU2Hr1G000490	1265575..1266439	Tetrahydroberberine oxidase / THB oxidas
HORVU2Hr1G000510	1308095..1317214	Leucine-rich repeat containing protein
HORVU2Hr1G000530	1317594..1320213	Tetrahydroberberine oxidase / THB oxidase

HORVU2Hr1G000540	1322179..1323992	Tetrahydroberberine oxidase / THB oxidase
HORVU2Hr1G000550	1326577..1327546	Cannabidiolic acid synthase / CBDA synthase
HORVU2Hr1G000560	1361514..1366863	Leucine-rich repeat containing protein
HORVU2Hr1G000580	1376247..1380663	Translation initiation factor EIF3-related
HORVU2Hr1G000590	1380974..1386210	G-patch domain (G-patch)
HORVU2Hr1G000630	1465139..1466444	Leucine-rich repeat containing protein
HORVU2Hr1G000640	1466498..1468456	Leucine-rich repeat containing protein
HORVU2Hr1G000660	1491544..1493077	Cytochrome P450 CYP4/CYP19/CYP26 subfamilie
HORVU2Hr1G000670	1501961..1503257	Cytochrome P450 81D1-related
HORVU2Hr1G000700	1539568..1543563	Leucine-rich repeat containing protein
HORVU2Hr1G000770	1712040..1719477	Leucine-rich repeat containing protein
HORVU2Hr1G000780	1681391..1683451	Leucine-rich repeat containing protein
HORVU2Hr1G000840	1729315..1730862	Methanol O-anthraniloyltransferase
HORVU2Hr1G000890	1766506..1768035	Cytochrome P450 CYP2 subfamil
HORVU2Hr1G000900	1797984..1801262	Leucine-rich repeat containing protein
HORVU2Hr1G000940	2084734..2097842	Histone-lysine N-methyltransferase, H3 lysine-36 specifi
HORVU2Hr1G000990	2145801..2162290	Hexokinase (HK)
HORVU2Hr1G001070	2245699..2246897	Late embryogenesis abundant protein (LEA_2)
HORVU2Hr1G001110	2335789..2337923	Cytochrome P450 76C1
HORVU2Hr1G001120	2347405..2351445	Terpene synthase, N-terminal domain (Terpene_synth) // Terpene synthase family, metal binding domain (Terpene_synth_C)

Figures

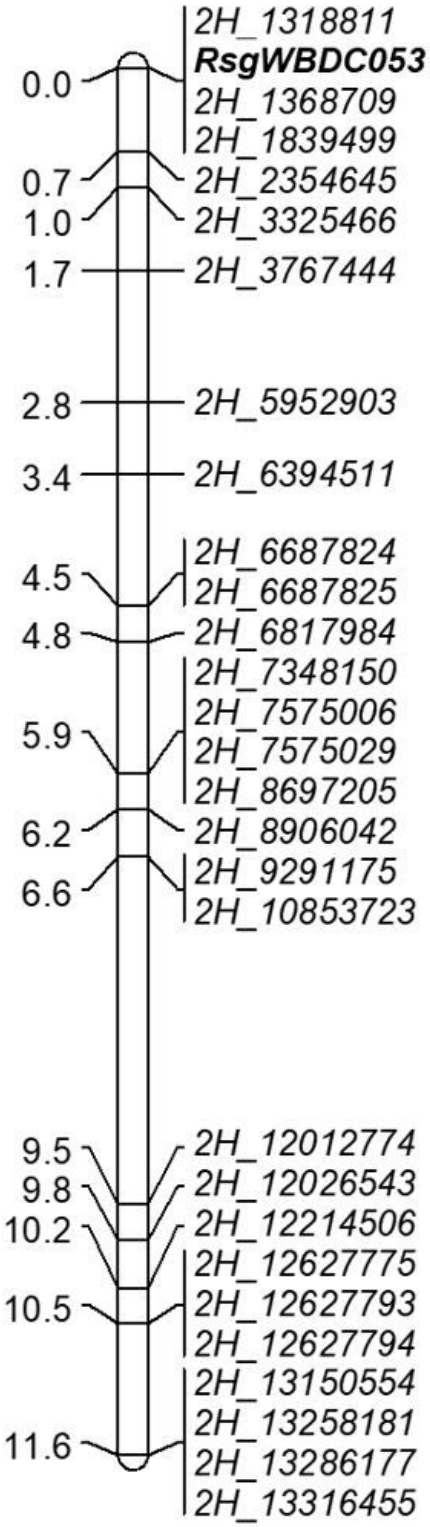

Figure 1

A linkage map containing RsgWBDC053 in the terminal region of chromosome 2H. The GBS-SNPs and genetic distances (in cM) are shown on the right and left sides of the linkage map, respectively. Each SNP was named using a combination of its chromosome and physical location in the Hordeum vulgare r1 reference sequence (in bp). Only a portion of the chromosome 2H linkage map is shown.

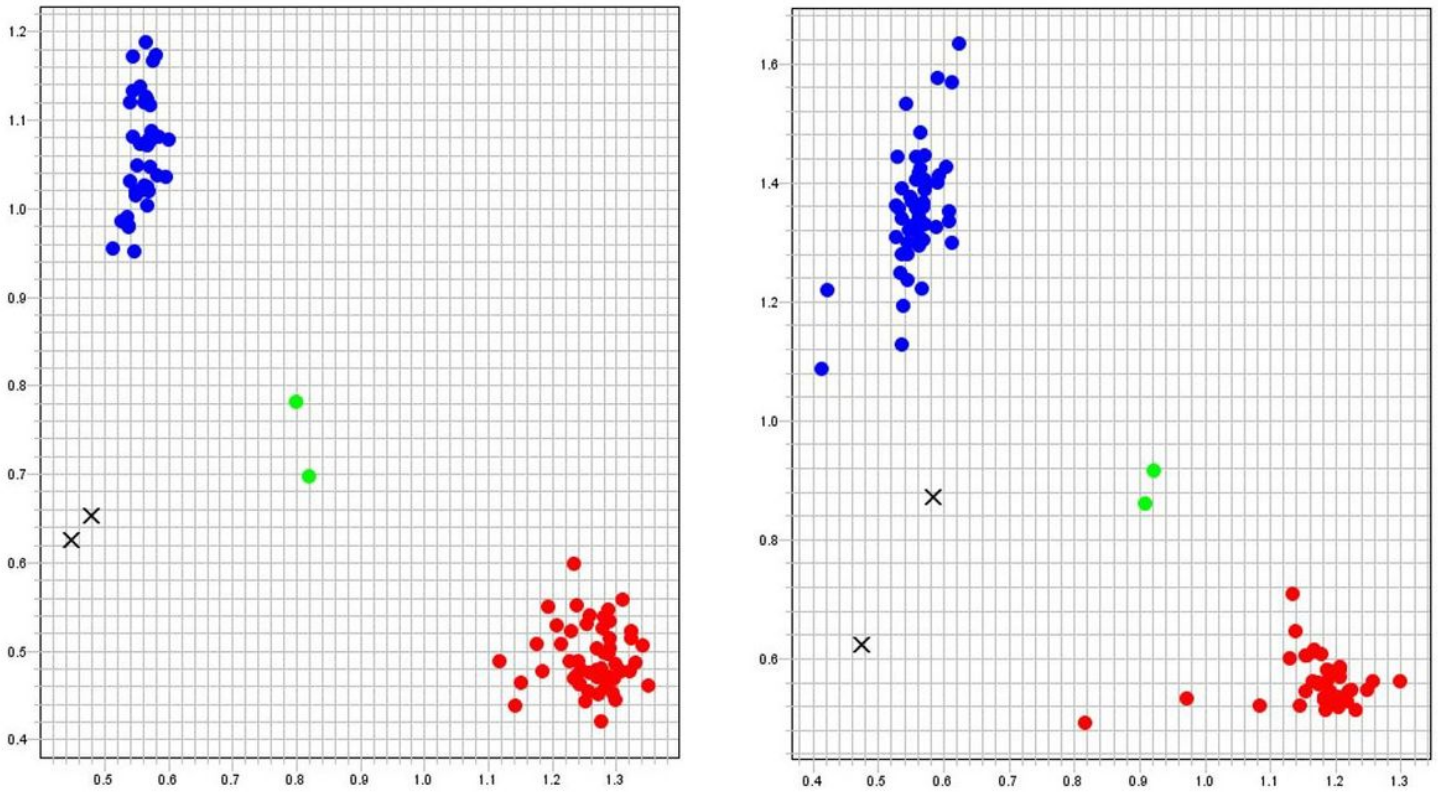

Figure 2

Segregation of the WBDC053 (red dots), Weskan (blue dots) and heterozygous (green dots) alleles at the KASP-Rsg053-1 (left) and KASP-Rsg053-2 (right) loci in a RIL population from WBDC053 × Weskan. The “x” represents negative control.