Fine Mapping of Hybrid Necrosis Gene *Ne1* in Common Wheat (*Triticum Aestivum* L.)

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Abstract

Hybrid necrosis is characterized as progressive chlorosis and necrosis of plant leaves, tillers or whole plants premature death in certain wheat hybrids, which is caused by the combination of two complementary loci *Ne1* and *Ne2* located on chromosome arms 5BL and 2BS, respectively. Hybrid necrosis is a serious barrier for combining desirable traits from various genotypes of wheat and the full utilization of heterosis in wheat. In this study, we fine mapped *Ne1* on chromosome 5BL, and finally delimited *Ne1* to a 4.06-Mb region with large heterogeneous inbred families (HIFs) from the RILs of 'Zhengnong 17' × 'Yangbaimai'. Further characterization of the near isogenic lines derived from HIFs confirmed that *Ne1* was tightly linked with a 2.89-Mb fragment that is deleted in Zhengnong 17. Additionally, a diagnostic InDel marker *5B-InDel385* for *Ne1* was developed and was used to assess the existence of *Ne1* in a diverse panel of 501 wheat accessions. Among them, 122 (61%) out of 200 landraces showed the presence of *Ne1* gene, whereas only 79 (26%) out of 301 modern cultivars carried the *Ne1* gene. The drastic decrease of *Ne1* frequency in modern cultivars indicates that the *Ne1* has been subjected to heavily selection pressure. This study provides a good foundation for the marker-assisted selection, gene cloning and functional studies of *Ne1* in wheat.

Key Message

Hybrid necrosis gene *Ne1* was delimited into an approximately 4.06-Mb region on chromosome 5BL using heterogeneous inbred families, and a diagnostic marker for *Ne1* was developed.

Introduction

Hybrid necrosis is characterized as premature senescence or death of leaves, tillers and even the whole plants in certain hybrids of wheat (Caldwell and Compton 1943; Hermesen 1963a). In some cases, hybrid necrosis is lethal or semilethal, resulting in gradual death or loss of productivity (Chu et al. 2006; Takumi et al. 2013; Tsunewaki 1960). Although weak hybrid necrosis has no obvious phenotypic effects, it caused segregation distortion in progenies from the intraspecific crosses (Takumi et al. 2013). Thus, hybrid necrosis was a barrier to gene transfer in elite lines and commercial cultivars and affected the full utilization of heterosis (Bizimungu et al. 1998; Galaiev 2016; Tomar et al. 1991).

Hybrid necrosis is controlled by the interaction of two complementary dominant genes *Ne1* and *Ne2*, located in chromosome arms 5BL and 2BS, respectively (Chu et al. 2006; Kandel et al. 2017; Nishikawa et al. 1974; Pukhalskiy et al. 2018; Zeven 1972). Both *Ne1* and *Ne2* genes are widely distributed among tetraploid and hexaploid wheat (Takumi et al. 2013; Tsunewaki 1992; Vikas et al. 2013). Based on the strength of necrotic symptoms, three dominant alleles (*Ne1^w*, *Ne1^m*, and *Ne1^s*) were identified on the *Ne1* locus, and five dominant alleles (*Ne2^w*, *Ne2^{wm}*, *Ne2^m*, *Ne2^{ms}*, and *Ne2^s*) were found at the *Ne2* locus (Hermesen 1960; Hermesen 1963a; Pukhalskiy et al. 2019). When a wheat genotype carries only *Ne1* (*Ne1Ne1ne2ne2*) or *Ne2* (*ne1ne1Ne2Ne2*), it grows normally. When *Ne1* and *Ne2* recombined together in a genotype (*Ne1Ne1Ne2Ne2*), hybrid necrosis appears, and the degree of necrosis is determined by the

combination of different alleles at the *Ne1* and *Ne2* loci (Hermesen 1963a). However, the molecular mechanisms of wheat hybrid necrosis are still largely unknown.

Since widespread occurrence of *Ne1* and *Ne2* in wheat elite lines and cultivars, it is important to access their allele types to avoid the occurrence of necrotic F₁ and to ensure successful hybridization (Chu et al. 2006; Galaiev 2016). Before molecular markers are developed, researchers relied on hybridizations with fixed testers carrying *ne1ne1Ne2^sNe2^s* or *Ne1^sNe1^sne2ne2* to test if a genotype carries a necrosis gene (Hermesen 1963a). This approach is time-consuming, laborious, and low accuracy because the symptom of hybrid necrosis is affected by genetic background and environmental factors (Hermesen 1963a). Chu et al. (2006) mapped the hybrid necrosis genes *Ne1* and *Ne2* using SSR markers and demonstrated that markers *Xbarc74-5B* and *Xbarc55-2B* were closely linked to *Ne1* and *Ne2* at genetic distances of 2.0 cM and 3.2 cM, respectively. Due to the genetic distances between the markers and *Ne* genes and the lack of coordination of alleles of markers with alleles of *Ne* genes, these two markers were only adopted by several studies in assessing the *Ne* genes in wheat cultivars (Galaiev 2016; Kandel et al. 2017). Therefore, it is necessary to develop a more convenient and efficient diagnostic marker for assessing the *Ne* genes in wheat germplasm.

Although hybrid necrosis has been reported in the 1940's (Caldwell and Compton 1943), the *Ne* genes are still not fine mapped and isolated. In this study, we developed a recombinant inbred line (RIL) population segregating at the *Ne1* locus, and identified its HIFs. With them, we precisely mapped *Ne1* in a region of ~ 4.06 Mb on chromosome 5BL, and developed an InDel marker co-segregated with *Ne1*. Further, we analyzed the distribution of *Ne1* in bread wheat varieties with the InDel marker.

Materials And Methods

Plant materials

A RIL (F₈) population containing 188 lines was developed using single seed descent from a F₂ population derived from the cross of 'Zhengnong 17' (ZN17, *ne1ne1Ne2Ne2*) with 'Yangbaimai' (YBM, *Ne1Ne1ne2ne2*), two Chinese hard white winter wheat cultivars. The two parents exhibited normal growth, but some RILs (24/188) from the population showed different levels of hybrid necrosis. This RIL population was used for mapping *Ne1* and *Ne2* genes. Two independent heterogeneous inbred families (HIF1 and HIF2) segregated for leaf necrosis from the RILs were identified and verified with markers linked to *Ne1*. Heterozygous F₉ individuals in HIF1 and HIF2 were identified using a *Ne1*-specific marker and self-pollinated to produce near-isogenic line (NIL) population for fine mapping of *Ne1*. Homozygous F₉ individuals with *Ne1^{YBM}* or *ne1^{ZN17}* from HIF1 were selected as NILs (NIL-*Ne1^{YBM}* and NIL-*ne1^{ZN17}*) for agronomic trait evaluation.

An additional HIF derived from the RIL (F₈) population of Zhengzhou 6903 × Yumai 14 was developed for validating the deletion of 2.89-Mb segment. In total, 501 wheat varieties (200 landraces and 301 cultivars) were used for studying the *Ne1* distribution in wheat germplasm (Supplementary Table S1).

Seven cultivars (carriers of known alleles of the gene *Ne1* or *Ne2/Lr13*) were applied to check the accuracy of the new diagnostic marker(s) (Supplementary Table S1).

Phenotypic evaluation and statistical analysis

The RIL population was evaluated at two different environmental conditions in the wheat growth season 2016–2017: one was in the experimental station in Beijing (40° 16' N, 116° 24' E) and the other one was in the experimental station of Zhaoxian county, Hebei province (37° 50' N, 114° 49' E). The HIFs used for fine mapping of *Ne1* were planted in the experimental station of Zhaoxian county, Hebei province, during the wheat growth season 2017–2018. All the seeds for the experiments were sown in single row plots (one meter long with 11 plants per row, 25 cm between rows, and 10 cm between plants). Student's *t* test was used to test for statistical significance (*, $P < 0.05$; **, $P < 0.01$).

Investigation of necrosis was taken at the grain filling stage. Ten representative plants were selected from each NIL-*Ne1*^{YBM} and NIL-*ne1*^{ZN17} lines, and their agronomic traits were assessed at maturity, including tiller number, plant height, thousand-kernel weight, grain length and grain width. The traits of grain size were measured with a camera-assisted phenotyping system (Hangzhou Wanshen Detection Technology Co., Ltd, Hangzhou, China).

Association analysis for Ne genes

Genomic DNA was extracted from young leaves by the CTAB method (Chatterjee et al. 2002). The two parents and 188 RILs of ZN17/YBM population were genotyped with the wheat660K SNP array (https://wheat.pw.usda.gov/ggpages/topics/Wheat660_SNP_array_developed_by_CAAS.pdf). The association analysis between polymorphic SNPs and necrosis scores was conducted with the function of GLM (Generalized Linear Model) implemented in the software TASSEL Version 5.0 (<https://tassel.bitbucket.io/>). The threshold of *P*-value was set to 3.42×10^{-7} to select associated SNPs based on the Bonferroni adjustment of independent SNPs. Genome coordinates presented in this study are based on Chinese Spring RefSeq v1.0 assembly and gene annotations are based on the updated RefSeq v1.1 annotation (IWGSC 2018).

Molecular marker development

For fine-mapping *Ne1*, SSR markers were designed on chromosome 5BL from ~ 290–400 Mb using BatchPrimer3 (<http://batchprimer3.bioinformatics.ucdavis.edu/>) based on the Chinese Spring RefSeq v1.0 assembly (IWGSC 2018), and dCAPS markers were developed by dCAPS Finder 2.0 (<http://helix.wustl.edu/dcaps/dcaps.html>). A 10-μl PCR system contained 2 μl DNA template (~ 80 ng), 2 μl H₂O, 1 μl mixed primers, and 5 μl 2×Taq PCR StarMix. PCR reaction was done as follows: 5 min at 94 °C; 7 cycles of 30 s at 94 °C, 30 s at 63 – 56 °C (dropping 1 °C per cycle), 30 s at 72 °C; 25 cycles of 30 s at 94 °C, 30 s at 56 °C, 30 s at 72 °C; 3 min at 72 °C. Then, the PCR products were separated on 5% agarose or 12% non-denaturing PAGE gels. The primers used in this study are listed in Supplementary Table S2.

Results

Ne1 induced the necrotic phenotype and yield decrease

In the RIL population of ZN17/YBM, 24 out of 188 RILs showed different degrees of necrosis (Supplementary Fig. S1). Most of the necrotic lines exhibited necrosis at the leaf tips during the seeding stage, preceded gradually to the middle and bottom of the leaves at jointing and flowering stage, and dried at the late grain filling stage (Supplementary Fig. S1a-c), while the two parents ZN17 and YBM grew normally. At the grain filling stage, six lines showed severe necrosis that resulted in premature death of the leaves (Supplementary Fig. S1d) and shriveled grains; three lines exhibited weak necrosis at only flag leaf tips (Supplementary Fig. S1e); two lines had yellow leaves (Supplementary Fig. S1f).

The necrotic phenotype in the NIL-*Ne1*^{YBM} firstly appeared on the lower leaves at the tillering stage (Fig. 1a) and became more evident during the heading period in the field. A more detailed observation indicated that the leaf necrosis initiated from the bottom leaves and progressed gradually to the upper leaves, and advanced from the tip to base of the leaf (Fig. 1b). Necrosis formation in NIL-*Ne1*^{YBM} leaves appeared to follow a developmental pattern, in which the severity of necrosis is correlated positively with the age of leaves. At the grain filling stages, only the flag leaf or upper leaves remained partially green, and the rest of leaves were necrotic or dried in the NIL-*Ne1*^{YBM}, while at the same stage the leaves of NIL-*ne1*^{ZN17} remained green and healthy (Fig. 1c). In addition, the NIL-*Ne1*^{YBM} displayed smaller leaves and fewer tillers, with a shorter plant height (Fig. 1a, c) compared to those of the NIL-*ne1*^{ZN17}.

Yield-related agronomic traits of the NIL-*Ne1*^{YBM} and NIL-*ne1*^{ZN17} lines were evaluated after harvest. Compared to the normal NIL-*ne1*^{ZN17}, the NIL-*Ne1*^{YBM} showed fewer tillers (Fig. 2a), shorter plant height (Fig. 2b), smaller (Fig. 2c) and thinner grain size (Fig. 2d). The thousand-kernel weight of the NIL-*Ne1*^{YBM} (34.62 g) was 33.13% smaller than that of the NIL-*ne1*^{ZN17} (51.77 g; Fig. 2e).

Genetic mapping of *Ne1*

The two parents and 188 RILs of ZN17/YBM population were genotyped with the wheat660K SNP array. After removing the SNPs with > 5 percentage data missing values and multiple mapping sites on RefSeq v1.0 (Keeble-Gagnere et al. 2018), 146,351 high-quality polymorphic SNPs were obtained. The associations of SNPs and the necrotic phenotype were conducted with the function of GLM implemented using software TASSEL Version 5.0. There were 1,844 SNPs significantly associated with the phenotype at the threshold of *P*-value (3.42×10^{-7}). Among these SNPs, 916 (49.67%) and 870 (47.18%) SNPs were mapped on chromosome 2BS and 5BL, respectively, and the rest 58 SNPs (3.15%) were distributed on 2A, 3A, 5A, 2D, 4D and 5D chromosome (Fig. 3a). These consisted with previous reports that hybrid necrosis was controlled by *Ne1* on 5B and *Ne2* on 2B in common wheat (Chu et al. 2006; Nishikawa et al. 1974; Zeven 1972). The significant SNPs on 5BL were in ~ 297 Mb to 399 Mb and those on 2BS located in ~ 110 Mb to 180 Mb (Fig. 3b). These results suggested that *Ne1* and *Ne2* were located to the regions of ~ 297 Mb – 399 Mb on 5BL and ~ 110 Mb – 180 Mb on 2BS, respectively.

Genetic analysis and fine mapping of *Ne1* with HIFs

To test the genetics of the *Ne1* locus on 5B, 252 individuals were randomly selected from HIFs-5B population (HIF₂), which segregated at the *Ne1* locus. Among them, 53 individuals showed severe necrosis, 134 plants were characterized as moderate necrosis, and 65 plants displayed a normal phenotype at the grain filling stage (Supplementary Fig. S2). The segregation ratio of severe necrosis: moderate necrosis: normal fitted the expected ratio of 1:2:1 ($\chi^2 = 2.16$, $P < 0.05$). These results suggested that the necrotic phenotype of HIFs-5B population was controlled by a semi-dominant nucleus gene.

Based on the IWGSC RefSeq v1.0 and the flanking sequence of the associated SNPs, 50 molecular markers were developed in the *Ne1* region (~ 297 Mb – 399 Mb) on chromosome 5BL. Among them, four markers were found to be polymorphic between ZN17 and YBM. The four polymorphic markers were applied to assess the genotype of 682 randomly selected individuals from HIFs-5B population. With these four markers, *Ne1* was delimited into the region flanked by markers *5B-378* and *5B-388*. Then, the rest 3402 individuals of HIFs-5B population were analyzed with these two markers for screening additional recombinants. A total of 23 recombinants were identified between the markers *5B-378* and *5B-388*. Subsequently, we developed new polymorphic molecular markers (InDel and SNP markers) and genotyped the recombinant plants to further narrow the candidate interval of *Ne1*. Eventually, *Ne1* was mapped to a 4.06-Mb physical interval delimited by markers *5B-383* and *SN-2142* (Fig. 3c, d).

The *ne1* allele is associated with a 2.89-Mb fragment deletion on chromosome 5BL in ZN17

In order to clone *Ne1* gene, we designed 57 SSR markers in the range of ~ 383 Mb to 387 Mb between the markers *5B-383* and *SN-2142* on 5BL based on IWGSC RefSeq v1.0, and found most SSR markers were of presence/absence between ZN17 and YBM, but not a product size differences. We speculate that this phenomenon should be caused by missing a large segment in this interval in ZN17. In order to confirm this speculation, we re-sequenced ZN17 and YBM for 10X sequencing depths. Sequence reads were aligned to the reference genome of Chinese Spring (RefSeq v1.0), and SNPs and InDels located in the region between the markers *5B-383* and *SN-2142* were called by the HaplotypeCaller module (Chai et al. 2018). We found continuously missing sequences from ~ 383,441,497 to 386,325,646 (RefSeq v1.0) in ZN17, but not in YBM (Supplementary Table S3). This suggested that there is an approximate 2.89-Mb segment deletion between the markers *5B-383* and *SN-2142* in ZN17. To verify the missing segment in ZN17, we designed primers to amplify six high confident genes located on the missing segment of chromosome 5BL in the NILs and the two parents ZN17 and YBM (Supplementary Table S2). All six genes were detected in YBM and NIL-*Ne1*^{YBM}, but not in ZN17 and NIL-*ne1*^{ZN17}. These results further confirmed the deletion of the *Ne1* gene candidate region in ZN17. Therefore, it is not possible to clone *Ne1* with the population developed from the cross between ZN17 and YBM.

Development, validation and application of the diagnostic marker for *Ne1*

To confirm that the missing of the 2.89-Mb fragment on 5BL is associated with *Ne1* gene, we screened a new HIF (HIF₃) from a RIL (F₈ generation) population from the cross between ‘Zhengzhou 6903’ and

'Yumai 14'. The HIF3, as HIF1 and HIF2, segregated in necrotic phenotype in the field, and showed the same PCR product pattern as in HIF2 with the molecular marker *5B-378*. We also found that all six genes located in the 2.89-Mb fragment could be identified in Yumai 14 (carrying *Ne1*), but not in Zhengzhou 6903 (carrying *ne1*). This indicated that the large fragment deletion in *ne1* varieties might not be rare. Therefore, we picked *5B-InDel385* as the diagnosis marker for *Ne1* gene among the six markers. *5B-InDel385* divided wheat into two major haplotypes (H1 and H2) based on the presence/absence of the 2.89-Mb fragment on 5BL. The H1 haplotype was the necrotic-*Ne1* allele carrying the 2.89-Mb fragment; oppositely, H2 haplotype possesses the normal-*ne1* allele with the 2.89-Mb fragment deletion.

To test the efficiency of the diagnostic marker *5B-InDel385*, 1034 individuals of HIF3-5B population (derived from HIF3) were genotyped and analyzed. As expected, all normal plants were H2 haplotype and all necrotic individuals were H1 haplotype. Collectively, this data revealed that *Ne1* was co-segregated with the marker *5B-InDel385* on chromosome 5BL in wheat.

The *5B-InDel385* was also validated in some cultivars - carriers of known alleles of the gene *Ne1* or *Ne2/Lr13*: including Kubanka (*T. durum*, *Ne1^s*), Chinese Spring (*Ne1^w*, *ne2*), Vakka (*Ne2^w*), Blackhull (*Ne2^s*), Sonalika (*Ne2^m*), Manitou (*Lr13*), and Frontana (*Lr13*). Notably, all the above-mentioned varieties grow normally. The marker *5B-InDel385* assay showed that all of the cultivars carrying *Ne2/Lr13* belonged to H2 type containing *ne1* allele. The other two cultivars, Kubanka and Chinese Spring known to possess *Ne1* allele were of the H1 type. Based on these results, we concluded that the marker *5B-InDel385* can accurately distinguish *Ne1* from *ne1*.

Using the diagnostic marker, we firstly characterized 259 wheat accessions (29 landraces and 230 cultivars) from China. As shown in Fig. 4a, the *Ne1* allele was frequent in landraces (62%). Conversely, the frequency of *Ne1* decreased sharply in the modern cultivated wheat varieties (32%) in China (Fig. 4b). We studied the geographical distribution of *Ne1* in China and found the frequency of *Ne1* was highest in Henan (41.51%), followed by Shaanxi (41.38%), Shanxi (33.33%), Beijing (31.25%), Hebei (28.57%), Jiangsu (29.41%), and Shandong (5.13%; Table 1). Furthermore, 242 common wheat landraces/cultivars from diverse origin were detected with *5B-InDel385*. The results showed that *Ne1* and *ne1* were widely distributed throughout the world. The *Ne1* allele was present in 104 of 171 landraces (61%) from USDA collections (from South, West and Central Asia; Fig. 4c). On the contrary, in a subset of 71 common wheat cultivars from USA, the *Ne1* allele was just observed in 7% of the accessions (Fig. 4d). In total, 122 landraces (61%) showed the presence of *Ne1* (Fig. 4e), whereas only 79 modern cultivars (26%) carried *Ne1* (Fig. 4f). This suggested that *Ne1* allele was subjected to a high selected pressure in wheat breeding.

Table 1
Distribution of *Ne1* in wheat cultivars from China

Agroecological region	NO. of accessions	<i>5B-InDel385</i>			
		H1 (<i>Ne1</i>)		H2 (<i>ne1</i>)	
		NO. of accessions	Percentage of the allele	NO. of accessions	Percentage of the allele
Henan	53	22	41.51%	31	58.49%
Shaanxi	29	12	41.38%	17	58.62%
Shanxi	15	5	33.33%	10	66.67%
Beijing	16	5	31.25%	11	68.75%
Hebei	42	12	28.57%	30	71.43%
Jiangsu	18	5	27.78%	13	72.22%
Shandong	39	2	5.13%	37	94.87%
Sichuan ^a	11	8	-	3	-
Anhui ^a	3	2	-	1	-
Zhejiang ^a	2	1	-	1	-
Yunnan ^a	2	0	-	2	-
Total	230	74	32.17%	156	67.83%
^a Wheat accessions from these regions were not included for the <i>Ne1</i> frequency analysis because of limited entries in this study.					

Putative *Ne1* candidate genes

To identify the candidate genes for *Ne1*, we analyzed the predicted genes on the 5BL from 383.03 Mb to 387.10 Mb of the Chinese Spring RefSeq v.1.0 sequence. Based on the RefSeq v1.1 annotation, 54 genes were identified in this region. There are 28 genes in the 2.89-Mb missing fragment, 6 genes are in the region between the marker *5B-383* and the deletion region, and 20 genes between the marker *SN-2142* and the deletion region (Supplementary Table S4). The re-sequencing of ZN17 and YBM showed that the major difference in the *Ne1* candidate region between the two parents was the 2.89-Mb deletion and there was no amino acid difference in the rest 26 genes outside the deletion bin though there were 385 SNPs/InDels in the candidate interval (Supplementary Table S5).

To predict the *Ne1* gene, we analyzed the expression profiles of the genes in the candidate region of Chinese Spring using the wheat expVIP expression platform (<http://www.wheat-expression.com/>). It is known that the necrotic phenotype is caused by the interaction of *Ne1* and *Ne2*, and starts from

seedlings, and 'Chinese Spring' carries the *Ne1^w* allele (Hermsen 1963a; Zhang et al. 2016). Therefore, we hypothesize that *Ne1* should express in leaves and shoots at its whole growth period. We found that 18 of 54 candidate genes expressed (above two transcripts per million) in at least three RNA-seq samples of Chinese Spring (leaves/shoots and roots, n = 40) at different developmental stages (Supplementary Table S6). Among them, 9 of 18 genes are high-confidence genes, including two auxin-responsive *SAUR* protein, two serine protease HtrA-like, two alpha/beta-Hydrolases superfamily protein, one serine protease *HTRA3*, one initiation factor 4F subunit (*DUF1350*), one RING/U-box superfamily protein, one trypsin-like serine protease (Supplementary Table S7). We speculated that one of the 9 genes might be the *Ne1* gene.

Discussion

Hybrid necrotic phenotype is influenced not only by gene dosage effect, but also by genetic background

Wheat hybrid necrosis phenotypically manifests itself as premature gradual perishing of leaves, sheaths or entire plants (Caldwell and Compton 1943; Hermsen 1963a), which reduces the crop yield. Hybrid necrosis is induced by the interaction of two complementary dominant genes *Ne1* and *Ne2* (Hermsen 1963a), located on chromosomes 5B and 2B, respectively (Chu et al. 2006; Nishikawa et al. 1974). Previously, *Ne1* was considered as a dominant gene based on the segregation ratio of necrotic phenotype in populations derived from the cross between two parents carrying *Ne1Ne1ne2ne2* and *ne1ne1Ne2Ne2* (Bizimungu et al. 1998; Hermsen 1963a; Kandel et al. 2017). The *Ne1* had the dosage effect and carrier of *Ne1Ne1Ne2Ne2* showed heavier necrotic symptoms, compared to *Ne1ne1Ne2Ne2* (Hermsen 1963b). In this study, we found heterozygous individuals at the *Ne1* locus derived from HIF1 and HIF2s were significantly different from those of homozygous plants (Supplementary Fig. S2), showing moderate intensity of necrosis, suggesting that the necrotic phenotype caused by the *Ne1* locus has a dosage effect and *Ne1* is a semi-dominant gene. The various necrosis levels observed in the 24 lines of ZN17 × YBM RIL population suggested that genetic background played an important role in hybrid necrosis (Supplementary Fig. S1).

The candidate gene for *Ne1* was likely to be located in the 2.89-Mb DNA fragment

Previously, the microsatellite marker *Xbarc74-5B* was developed for *Ne1* with a genetic distance of 2.0 cM between the marker and *Ne1*, and targeted the genome region from 402,787,119 bp to 402,787,293 bp on 5B (Chinese Spring RefSeq v.1.0) (Chu et al. 2006). In this study, we further delimited the *Ne1* locus to an interval with a physical distance of 4.06-Mb, from 383.03 to 387.10 Mb (RefSeq v.1.0), and found that *Ne1* was co-segregated with a 2.89-Mb DNA fragment in the interval. The comparison of this region in multiple wheat genomes reveal this 2.89-Mb DNA fragment deletion is not rare (Supplementary Fig. S3) (Walkowiak et al. 2020). Thus, based on the 2.89-Mb DNA deletion, we developed the diagnostic marker *5B-InDel385*, which co-segregates with *Ne1* in large HIF-5B populations (4084 plants in total). Because *5B-InDel385* is an InDel marker, it distinguished *Ne1* from *ne1* among wheat germplasms, but not the three alleles of *Ne1*. As expected, although Kubanka (*T. durum*, *Ne1^s*) and Chinese Spring (*Ne1^w*, *ne2*) carry different *Ne1* alleles (Zhang et al. 2016), both were classified as H1 haplotype (*Ne1*) according to

5B-InDel385. Thus, we can efficiently select germplasms with *ne1* instead of *Ne1* to design crosses to avoid hybrid necrosis in wheat breeding with *5B-InDel385*.

In this study, we mapped *Ne1* to 383.03 Mb – 387.10 Mb on 5BL (Chinese Spring RefSeq v.1.0). There are 54 predicted genes in this region, including 36 low confidence genes and 18 high confidence genes (IWGSC 2018). The re-sequencing of ZN17 and YBM revealed that 28 out of 54 genes located in 2.89-Mb deletion region in ZN17 and the other 385 SNPs/InDels did not lead to amino acid change between the both parents. Thus, it was likely that the expression differences of candidate genes leads to the necrotic phenotype, as the proteomic analysis of hybrid necrosis with F₁₄ NIL revealed that *Ne1* possibly encodes protein(s) (Pan et al. 2017). We examine the expression profiles of 54 genes in the 4.06 Mb genomic region of Chinese Spring and found that 9 high-confidence genes were detected in leaves/shoots or roots samples (Supplementary Table S6, 7). Thus, we hypothesized that these 9 genes were the most likely candidates for *Ne1*, especially *TraesCS5B02G212800*, *TraesCS5B02G212900*, *TraesCS5B02G213100* and *TraesCS5B02G213200*, which located on the 2.89-Mb missing fragment. However, based on the available data, we cannot determine which gene is *Ne1*. Therefore, to determine the *Ne1* gene, the quickest way might be knocking out the 9 most likely candidate genes separately in the background of *Ne2* and checking the disappearance of necrotic phenotype as a large segregation population could not break the linkage among these genes in current study.

The frequency of *Ne1* gene was impacted by selection pressure

In this study, 501 landraces/cultivars from diverse origin were analyzed with the diagnostic marker *5B-InDel385*. The frequency of *Ne1* carrier was higher in landraces, both from China and the USDA–ARS National Small Grains Collection (from Central, West and South Asia; Fig. 4a, c). In contrast, the allele frequency of *Ne1* was significantly lower (32%) in modern cultivated wheat varieties in China (Fig. 4b), and only 7% in cultivars from the United States (Fig. 4d). This is consistent with the finding obtained by Pukhalskiy et al. (2000), wherein 9.1% wheat cultivars of North and South-Central USA were *Ne1* carriers. Therefore, breeding related changes of *Ne1* frequency in common wheat is nonrandom and the *Ne1* gene was subject to heavy selection pressure in breeding programs (Pukhalskiy et al. 2008; Pukhalskiy et al. 2000).

Since hybrid necrosis is the result of interactions between *Ne1* and *Ne2*, the frequency of *Ne1* can be influenced by the presence of *Ne2* in wheat breeding programs. It is well known that the *Ne2* gene is tightly linked to the *Lr13* gene (a widely distributed brown rust resistance gene in wheat) located on chromosome 2BS, and genetic and mutational analyses indicate that they are the same gene (McIntosh et al. 1995; Zhang et al. 2016). The closely linked between *Ne2^m* and *Lr13* led to a sharply increase in the presence of *Ne2^m* in wheat in the United States, as *Lr13* may initially be highly favored in the selection of rust-resistant wheat (Pukhalskiy et al. 2000; Zhang et al. 2016). So, the continuous use of *Lr13/Ne2^m* may explain the significantly decrease frequency of *Ne1* among the USA cultivars to avoid hybrid necrosis.

Conclusion

Using large HIFs and new molecular markers, hybrid necrosis gene *Ne1* was mapped to a 4.06-Mb region (383.03 Mb – 387.10 Mb) on chromosome 5BL, and was co-segregated with the InDel marker *5B-InDel385*. The significantly difference of *Ne1* frequencies between landraces and modern cultivars indicated that *Ne1* was subjected to strongly selected pressure in wheat breeding.

Declarations

Funding

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Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

Availability of data and material

All data generated or analysed during this study are included in this article and its supplementary information files.

Code availability

Not applicable.

Authors' Contributions

HL and SZ conceived the project; SZ and YS developed the RIL and HIFs populations; YS carried out experiments and analyzed the data; ST and JN assisted in marker development and collected data for the RIL population; XS, SZ, YH and YS analyzed the data of re-sequencing; YL provided several cultivars-carriers of known alleles of gene *Ne1* or *Ne2/Lr13* and helped check the wheat accessions information; YS wrote the manuscript; HL and SZ revised the manuscript.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

References

1. Bizimungu B, Collin J, Comeau A, St-Pierre CA (1998) Hybrid necrosis as a barrier to gene transfer in hexaploid winter wheat x triticales crosses. *Can J Plant Sci* 78:239–244
2. Caldwell RM, Compton LE (1943) Complementary lethal genes in wheat causing a progressive lethal necrosis of seedlings. *J Hered* 34:67–70
3. Chai L, Chen Z, Bian R, Zhai H, Cheng X, Peng H, Yao Y, Hu Z, Xin M, Guo W, Sun Q, Zhao A, Ni Z (2018) Dissection of two quantitative trait loci with pleiotropic effects on plant height and spike length linked in coupling phase on the short arm of chromosome 2D of common wheat (*Triticum aestivum* L.). *Theor Appl Genet* 131:2621–2637
4. Chatterjee A, Moulik SP, Majhi R, Sanyal SK (2002) Studies on surfactant-biopolymer interaction. I. Microcalorimetric investigation on the interaction of cetyltrimethylammonium bromide (CTAB) and sodium dodecylsulfate (SDS) with gelatin (Gn), lysozyme (Lz) and deoxyribonucleic acid (DNA). *Biophys Chem* 98:313–327
5. Chu C-G, Faris JD, Friesen TL, Xu SS (2006) Molecular mapping of hybrid necrosis genes *Ne1* and *Ne2* in hexaploid wheat using microsatellite markers. *Theor Appl Genet* 112:1374–1381
6. Galaiev O (2016) Identification and distribution of alleles of hybrid necrosis gene *Ne2* in soft wheat cultivars (*Triticum aestivum* L.). *Agric Sci Pract* 3:22–27
7. Hermesen JGTh (1963a) The genetic basis of hybrid necrosis in wheat. *Genetica* 33:245–287
8. Hermesen JGTh (1963b) Hybrid necrosis as a problem for wheat breeder. *Euphytica* 12:1–16
9. IWGSC (2018) Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science* 361:eaar7191
10. Kandel DR, Glover KD, Berzonsky WA, Gonzalez-Hernandez JL, Ali S, Chao S, Bhusal SJ (2017) Flanking SSR markers for alleles involved in the necrosis of hybrids between hexaploid bread wheat and synthetic hexaploid wheat. *J Crop Improv* 31:879–892
11. Keeble-Gagnere G, Rigault P, Tibbits J, Pasam R, Hayden M, Forrest K, Frenkel Z, Korol A, Huang BE, Cavanagh C, Taylor J, Abrouk M, Sharpe A, Konkin D, Sourdille P, Darrier B, Choulet F, Bernard A, Rochfort S, Dimech A, Watson-Haigh N, Baumann U, Eckermann P, Fleury D, Juhasz A, Boisvert S, Nolin M-A, Dolezel J, Simkova H, Toegelova H, Safar J, Luo M-C, Camara F, Pfeifer M, Isdale D, Nystrom-Persson J, Koo D-H, Tinning M, Cui D, Ru Z, Appels R, Iwgc (2018) Optical and physical mapping with local finishing enables megabase-scale resolution of agronomically important regions in the wheat genome. *Genome Biol* 19:112
12. McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts. An atlas of resistance genes. CSIRO Publishing, Melbourne
13. Nishikawa K, Mori T, Takami N, Furuta Y (1974) Mapping of progressive necrosis gene *Ne1* and *Ne2* of common wheat by the telocentric method. *Jpn J Breed* 24:277–281

14. Pan SR, Pan XL, Pan QY, Shi YH, Zhang L, Fan Y, Xue YR (2017) *Ne2* encodes protein(s) and the altered RuBisCO could be the proteomics leader of hybrid necrosis in wheat (*Triticum aestivum* L.). *J Genet* 96:261–271
15. Pukhalskiy VA, Bilinskaya EN, Kudryavtsev AM (2018) Analysis of hybrid necrosis genes in winter common wheat (*Triticum aestivum* L.) cultivars. *Russ J Genet* 54:1487–1492
16. Pukhalskiy VA, Bilinskaya EN, Martynov SP, Dobrotvorskaya TV, Obolenkova GA (2008) New data on the distribution of hybrid necrosis genes in winter bread wheat (*Triticum aestivum* L.) cultivars. *Russ J Genet* 44:171–179
17. Pukhalskiy VA, Martynov SP, Dobrotvorskaya TV (2000) Analysis of geographical and breeding-related distribution of hybrid necrosis genes in bread wheat (*Triticum aestivum* L.). *Euphytica* 114:233–240
18. Pukhalskiy VA, Zuev EV, Bilinskaya LN, Kudryavtsev AM (2019) Distribution of hybrid necrosis alleles in genotypes of aboriginal common wheat cultivars from Afghanistan, Palestine, and Transjordan. *Russ J Genet* 55:955–961
19. Takumi S, Motomura Y, Iwata JCM, Kobayashi F (2013) Segregation distortion caused by weak hybrid necrosis in recombinant inbred lines of common wheat. *Genetica* 141:463–470
20. Tomar SMS, Kochumadhavan M, Nambisan PNN (1991) Hybrid weakness in *Triticum dicoccum* Schubl. *Wheat Inf Serv* 72:9–11
21. Tsunewaki K (1960) Monosomic and conventional gene analysis in common wheat. III. Lethality. *Japan J Genet* 35:71–75
22. Tsunewaki K (1992) Aneuploid analysis of hybrid necrosis and hybrid chlorosis in tetraploid wheats using the D-genome chromosome substitution lines of durum wheat. *Genome* 35:594–601
23. Vikas VK, Tomar SMS, Sivasamy M, Kumar J, Jayaprakash P, Kumar A, Peter J, Nisha R, Punniakotti E (2013) Hybrid necrosis in wheat: evolutionary significance or potential barrier for gene flow? *Euphytica* 194:261–275
24. Walkowiak S, Gao L, Monat C, Haberer G, Kassa MT, Brinton J, Ramirez-Gonzalez RH, Kolodziej MC, Delorean E, Thambugala D, Klymiuk V, Byrns B, Gundlach H, Bandi V, Siri JN, Nilsen K, Aquino C, Himmelbach A, Copetti D, Ban T, Venturini L, Bevan M, Clavijo B, Koo D-H, Ens J, Wiebe K, N'Diaye A, Fritz AK, Gutwin C, Fiebig A, Fosker C, Fu BX, Accinelli GG, Gardner KA, Fradgley N, Gutierrez-Gonzalez J, Halstead-Nussloch G, Hatakeyama M, Koh CS, Deek J, Costamagna AC, Fobert P, Heavens D, Kanamori H, Kawaura K, Kobayashi F, Krasileva K, Kuo T, McKenzie N, Murata K, Nabeka Y, Paape T, Padmarasu S, Percival-Alwyn L, Kagale S, Scholz U, Sese J, Juliana P, Singh R, Shimizu-Inatsugi R, Swarbreck D, Cockram J, Budak H, Tameshige T, Tanaka T, Tsuji H, Wright J, Wu J, Steuernagel B, Small I, Cloutier S, Keeble-Gagnère G, Muehlbauer G, Tibbets J, Nasuda S, Melonek J, Hucl PJ, Sharpe AG, Clark M, Legg E, Bharti A, Langridge P, Hall A, Uauy C, Mascher M, Krattinger SG, Handa H, Shimizu KK, Distelfeld A, Chalmers K, Keller B, Mayer KFX, Poland J, Stein N, McCartney CA, Spannagl M, Wicker T, Pozniak CJ (2020) Multiple wheat genomes reveal global variation in modern breeding. *Nature* 588:277–283

25. Zeven AC (1972) Determination of the chromosome and its arm carrying the *Ne1*-locus of *Triticum aestivum* L., Chinese Spring and the *Ne1*-expressivity. *Wheat Inf Serv* 33–34:4–6
26. Zhang P, Hiebert CW, McIntosh RA, McCallum BD, Thomas JB, Hoxha S, Singh D, Bansal U (2016) The relationship of leaf rust resistance gene *Lr13* and hybrid necrosis gene *Ne2m* on wheat chromosome 2BS. *Theor Appl Genet* 129:485–493

Supplementary Information

Fig. S1-S3

Fig. S1 Different types of necrosis of representative recombinant inbred lines.

Fig. S2 Comparison of necrosis levels (a) and grain characters (b) of individuals with different *Ne1* alleles derived from HIF2. Phenotype was observed at the grain filling stage. Bar = 10cm. Values represent the means and SD of five replicates. Student's *t* test was used for statistical analysis.

Fig. S3 Collinearity analysis of the 2.89-Mb DNA fragment deletion region on chromosome 5BL. Genomic DNA sequences of 'Norin 61', 'CDC Landmark', PI190962, 'Zhengnong 17', 'Yangbaimai' and 'Chinese Spring'. The genes marked in red are located on the 2.89-Mb deletion fragment in the candidate region of *Ne1*.

Tables S1-S8

Table S1 Alleles of *Ne1* in wheat accessions detected by the diagnostic marker *5B-InDel385*

Table S2 List of the markers used in this study

Table S3 The 2.89-Mb deletion in the candidate region of *Ne1* in ZN17

Table S4 Annotation of the candidate genes of *Ne1*

Table S5 Variant comparison of the candidate genes of *Ne1*

Table S6 Expression pattern of 'Chinese Spring' genes in the fine-mapped interval of *Ne1* on chromosome 5BL with expVIP expression platform (<http://www.wheat-expression.com/>).

Table S7 Most likely candidate genes for *Ne1*

Figures

Figure 1

Comparison of necrosis levels of individuals with different *Ne1* alleles derived from HIF1. (a) The phenotypes of NIL-*Ne1*^{YBM} and NIL-*ne1*^{ZN17} at the tillering stage. (b) Morphology of the leaves at the jointing stage. (c) Phenotype of whole plant at the grain filling stage. Bar = 10 cm.

Figure 2

Yield-related trait comparisons of the NIL-Ne1YBM and NIL-ne1ZN17 for tiller number (a), plant height (b), grain length (c), grain width (d) and thousand kernel weight (e). Student's t test was used to generate P values. **, P<0.01. Error bars indicate the standard deviation.

Figure 3

Fine mapping of Ne1 on chromosome 5B. The distribution of significant associated SNPs among whole genome (a) and on chromosome 5B (b) detected by the GLM method. (c) Physical distance between molecular markers and numbers left in each region indicate the number of recombinants. Red block indicated fragment deletion in ZN17. (d) Graphical illustrations of the recombinant genotypes and phenotypes in the Ne1 interval.

Figure 4

Frequency of alleles Ne1 (haplotype H1) and ne1 (-2.89 Mb, haplotype H2) in wheat landraces and cultivars collected around the world. (a) and (b) Landraces and modern cultivars collected from China. (c) Landraces from USDA collection (from South, West and Central Asia). (d) Modern cultivars from USA. (e) and (f) all the landraces and cultivars accessions used to estimate allele frequencies (summarized in Supplementary Table S1).

Supplementary Files

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