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ORIGINAL ARTICLE

Fine mapping and distribution analysis of hybrid necrosis genes *Ne1* and *Ne2* in wheat in China

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Abstract

Key message **Flanking markers useful for identifying hybrid necrosis alleles were identified by fine mapping *Ne1* and *Ne2* and the distribution of the two necrosis genes was investigated in Chinese elite wheat varieties.**

Abstract Hybrid necrosis of wheat is caused by the interaction of two dominant complementary genes *Ne1* and *Ne2* present separately in normal parents and is regarded as a barrier to gene transfer in wheat breeding. However, the necrosis alleles still occur at a high frequency in modern wheat varieties. In this study, we constructed two high-density genetic maps of *Ne1* and *Ne2* in winter wheat. In these cultivars, *Ne1* was found to be located in a span interval of 0.50 centimorgan (cM) on chromosome 5BL delimited by markers *Nwu_5B_4137* and *Nwu_5B_5114*, while *Ne2* co-segregated with markers *Lseq102* and *TC67744* on 2BS. Statistical analysis confirmed that the dosage effect of *Ne1* and *Ne2* also existed in moderate and severe hybrid necrosis systems, and the symptoms of necrosis can also be affected by the genetic background. Furthermore, we clarified the discrete distribution and proportion of the *Ne1* and *Ne2* in the 10 China's agro-ecological production zones. We concluded that 26.2% and 33.2% of the 1364 cultivars (lines) were genotyped with *Ne1Ne1ne2ne2* and *ne1ne1Ne2Ne2*, respectively and introduced modern cultivars should directly affect the frequencies of necrosis genes in modern Chinese cultivars (lines), especially that of *Ne2*. Taking investigations in spring wheat together, we proposed that hybrid necrosis alleles could positively affect breeding owing to their linked excellent genes such as *Lr13*. Additionally, based on the pedigrees and hybridization tests, we speculated that the *Ne1* and *Ne2* in winter wheat may directly originate from wild emmer and introduced cultivars or hexaploid triticale, respectively.

Introduction

The hybrid offspring of some normal parents can exhibit a phenotype of gradually premature senescence or death. This phenomenon is known as hybrid necrosis and coincides with the Bateson–Dobzhansky–Muller (BDM) model of postzygotic hybrid incompatibility (Orr 1996). To date, several hybrid necrosis systems classified according to the extent of necrosis have been identified in plants (Bomblies and Weigel 2007). The severest type of hybrid necrosis results in death at the seedling stage resembling a form of reproductive isolation, indicating it plays a vital role in speciation in nature (Bomblies and Weigel 2007; Chen et al. 2014; Hermsen 1963a). In terms of breeding practice, hybrid necrosis does limit the use of certain combinations of parents from diverse germplasm pools, therefore, this postzygotic incompatability was considered to be a barrier to crop improvement (Bizimungu et al. 1998; Hermsen 1963a). In common wheat (*Triticum aestivum* L.), the inheritance of

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hybrid necrosis is caused by the interaction of two dominant complementary genes *Ne1* and *Ne2* located on chromosome arms 5BL and 2BS, respectively (Nishikawa et al. 1974). The necrosis can start at any point during development from the 1–2-leaf stage onwards depending on the nature of crosses (Hermsen 1963a). When leaf tissues reach a certain physiologic maturity stage, the necrosis program is activated, then leaves begin gradual yellowing, wilting and necrosis from the tip progressively to the base of the lowest leaf to the flag leaf (Caldwell and Compton 1943). Finally, hybrid necrosis results in dwarfism of wheat plants, reduced growth rate, poor fertility/infertility or even death before the heading period (Caldwell and Compton 1943). Therefore, fine mapping of *Ne1* and *Ne2* is of great significance for guiding the selection of parents in wheat breeding and cloning the necrosis genes.

The first genetic map of *Ne1* and *Ne2* was generated by Chu (2006) in spring wheat using simple sequence repeat (SSR) markers to screen two backcross populations consisting of 100 and 94 individuals, respectively. The genetic distances from *Ne1* and *Ne2* to their nearest SSR markers, *Xbarc74* and *Xbarc55*, were 2.0 centimorgan (cM) and 3.2 cM, respectively (Chu et al. 2006). Recently, two high-density maps of *Ne1* have been published (Li et al. 2021; Si et al. 2021a), in which *Ne1* was delimited into an approximate 4 Mb interval; however, high-throughput molecular markers for screening the necrosis gene have not yet been developed. Although *Ne2* has been cloned (Hewitt et al. 2021; Si et al. 2021b; Yan et al. 2021), the mechanism of hybrid necrosis is still unclear and the application of gene-specific selective markers for *Ne2* in breeding is limited. Therefore, to develop efficient molecular markers and clone these two genes will contribute to optimizing the breeding program and understanding the mechanism of hybrid necrosis.

Surveys have shown that *Ne1* and *Ne2* are widely distributed among different wheat species, subspecies and cultivars throughout the world (Hermsen 1963b; Oetmann and Zeller 1989; Pukhalskiy et al. 2000, 2008a, 2018; Zeven 1965). The distribution of *Ne1* and *Ne2* could be established according to the global lists of *Ne* carriers published from 1963 to 1981(Hermsen 1963b; Zeven 1965, 1967, 1968, 1969, 1971, 1973, 1976, 1981). Although these surveys involved tests of 5,541 wheat cultivars (lines) worldwide, only 154 Chinese wheat cultivars were included. As a result of international cooperation, the frequencies of *Ne1* and *Ne2* have changed in wheat populations in different regions, although the differences in their distribution in different countries remain (Bomblies and Weigel 2007; Pukhalskiy et al. 1998, 2000, 2008b; Vikas et al. 2013). China is the top wheat-producing country worldwide (https://www.fao.org/faostat/zh/#data/QCL/visualize), therefore, systematic analysis of the distribution and proportion of necrosis genes in wheat cultivars is important to elucidate the mechanisms by which these *Ne1* and *Ne2* affect wheat plant breeding in China and even the world.

Based on the distinct variation of necrosis in different F_1-generations (*Ne1ne1Ne2ne2*), Hermsen distinguished hybrid necrosis into nine grades (0–8) and three levels (grades 6–8 indicate severe necrosis, 3–6 moderate necrosis and 0–3 weak necrosis). These variations in wheat were found to be determined by the occurrence of three *Ne1* alleles(*Ne1w*, *Ne1m* and *Ne1s*) and five *Ne2* alleles (*Ne2w*, *Ne2wm*, *Ne2m*, *Ne2ms* and *Ne2s*) (*w* = weak, wm = mid-weak, *m* = moderate, ms = mid-strong, *s* = strong) (Hermsen 1963a). Moreover, he demonstrated that the degree in weak necrosis system (grades 0–3, normal seed) is affected by the number of dominant *Ne* alleles (gene dosage) (Hermsen 1963a). Recently, by genotyping and phenotypic identification of the F_2 population of Pan555/Zheng891, Li confirmed that incomplete dominance at the two *Ne* loci controlled the timing and severity of necrosis in moderate and severe hybrid necrosis systems (Li et al. 2021). However, the dose–effect of hybrid necrosis genes in hybrids needs to be fully confirmed by other different evidences.

During wheat breeding to improve the resistance of high-yield cultivars using a powdery mildew resistance gene (*PmAS846*) donor N9134 (Xue et al. 2012), we observed several necrotic F_1 combinations by crossing normal green wheat lines. Considering the importance of wheat breeding and the gap in our knowledge of the mechanism of hybrid necrosis in wheat, we further investigated the hybrid necrosis locus in winter wheat line N9134 and compared it with that in spring wheat. In this study, we revealed the dose–effect of *Ne1* and *Ne2* on the intensity of hybrid necrosis. We then constructed two detailed genetic maps of *Ne1* and *Ne2* in winter wheat mainly using kompetitive allele-specific PCR (KASP) markers and allele-specific PCR (AS-PCR) markers. Furthermore, we clarified the discrete distribution, proportion and genotype frequencies of the two *Ne* genes in China's 10 agro-ecological production zones (north China winter wheat region, Yellow and Huai River valley winter wheat region, middle and lower Yangtze River valley winter wheat region, south-western winter wheat region, south China winter wheat region, north-eastern spring wheat region, northern spring wheat region, northwestern spring wheat region, Qinghai–Tibet spring–winter wheat region, Xinjiang winter–spring wheat region). Finally, we traced the origin of *Ne1* and *Ne2* in the two backcross mapping populations analyzed in this study.

Materials and methods

Plant materials

The common wheat line N9134 was selected from the hybrid progeny of 5B nullisomic (5BN) of Abbondanza/

wild emmer accession AS846 (*Triticum dicoccoides*, WE As846, obtained from Sichuan Agricultural University) (Fig. S1). N9134 and N0439 (derived from N9134/Xiaoyan 22) are both excellent winter wheat lines developed at Northwest A&F University. Zhoumai 22 (ZH22) and Xinong 509 (XN509) are main commercial cultivars in the Yellow and Huai River Valleys winter wheat cultivation region of China. The F_1 generations of reciprocal crosses (e.g., N9134/ZH22 and ZH22/N9134, etc.), the corresponding F_2 populations and BC_1F_1 populations were used to find, verify, and investigate the hybrid necrosis in our wheat cultivars (Table S1). The two BC_1F_1 populations were also employed for fine mapping of *Ne1* and *Ne2*. Specifically, 1,143 BC_1F_1 plants, derived from the backcrosses of ZH22 (*Ne2* carrier)// [(N9134/ZH22) or (ZH22/N9134)], was used to map the *Ne1* allele in necrotic (*Ne1ne1Ne2_*) and normal (*ne1ne1Ne2_*) plants. The other BC_1F_1 population consisted of 1,006 individuals and derived from N9134 (*Ne1* carrier) was used to map the *Ne2* allele.

The two cultivars (lines), an Australian spring wheat cultivar Spica carrying *Ne1s* (Zhang et al. 2016), the CIMMYT synthetic hexaploid wheat (SHW) line TA4152-60 carrying *Ne1* (Chu et al. 2006), were used for allelism tests with *Ne1* in N9134 (hereafter, *Ne1* in N9134 is designated *Ne1-nw* for convenience).

The seeds of other cultivars (lines) used for genotyping the two necrosis genes (Table S2) were obtained from the Chromosome Engineering Laboratory, Northwest A&F University or the germplasm pool of "Precise identification and innovative utilization of wheat germplasm resources" (2016YFD0100102-6). In which, 3034 wheat materials, including 2781 modern Chinese cultivars (MCC), 66 Chinese landraces (CL) and 187 introduced modern cultivars (IMC), were identified for agronomic traits and resistance to stripe rust and aphid, so as to screen excellent germplasm resources. Then the excellent germplasm resources will be developed and utilized to cultivate better wheat varieties.

Allelism tests

To test the relationship between *Ne1* in N9134, Spica and TA4152-60, N9134 was first crossed with these two *Ne1*-allele carriers to obtain the F_1 gametes, which were further crossed with ZH22 to form two F_1 test cross (TF_1) populations (i.e., ZH22//(N9134/Spica or Spica/N9134) and ZH22//(N9134/TA4152-60 or TA4152-60/N9134). Each TF_1 population was consisted of more than 140 progeny plants.

Investigation of the plant phenotypes and statistical analysis

In the construction of hereditary analysis populations, all crosses, backcrosses and self-crosses were bagged. Seeds were planted at a density of one spike per row in the experimental field of Northwest A&F University, Yangling, Shaanxi, China. In previous years, the seeds were generally planted at the beginning of October, and each was then numbered in early December when the plants reached approximately 15 cm in height. The first investigation of the phenotype at the seedling stage was conducted in the early stage of winter tillering. The second investigation was conducted around the vernal equinox in following year when wheat plants were at the early jointing stage. The third investigation was carried out when plants were at the heading stage in mid-April. The investigations were conducted by direct observation of the obviously contrasting phenotypes of the necrotic and normal plants, as well as the differences from the normal parents. After summarizing the survey data, Chi-squared (χ^2) tests were conducted to determine the fitness of segregation ratios to theoretical Mendelian ratios.

Sampling and DNA extraction

Following the second investigation, the second leaf of plants was sampled and numbered at the jointing stage. After being freeze-dried, the samples (approximately 0.5 g of each fresh leaf in a 2.0 mL centrifuge tube) were ground using a medium throughput ball mill (TL2010, DHS Life Science & Technology Co., Ltd, Beijing, China). Genomic DNA (gDNA) was extracted using the cetyltrimethyl ammonium bromide (CTAB) method (Saghai-Maroof et al. 1984) with some improvements to the extraction buffer due to the use of relative old leaf tissue in this study. The specific composition of the modified gDNA extraction buffer is as follows: 0.8 mol/L sodium chloride (NaCl), 0.14 mol/L D-Sorbital, 0.22 mol/L trismetyl aminomethane (Tris, pH 8.0), 22 mmol/L ethylene diamine tetraacetic acid (EDTA, pH 8.0), 0.8% CTAB, 0.8% Sarcosin, 3% polyvinyl pyrrolidone-K30 (PVP-K30) and 2% 2-mercaptoethanol, to reduce the content of secondary metabolites such as pigment and phenols.

Screening and development of molecular markers

In this study, we used some previously reported flanking molecular markers of *Ne1* (Chu et al. 2006) and *Ne2* (closely related to *Lr13* and *LrLC10*) (Chu et al. 2006; Qiu et al. 2020; Zhang et al. 2016). The other SSR markers were obtained from the GrainGenes Database (https://wheat.pw.usda.gov/GG3/). Specific-Locus Amplified Fragment Sequencing (SLAF-Seq) (Sun et al. 2013) was applied for typing and screening of single nucleotide polymorphism (SNP) sites between the three pools (Table S3). A total of 96,862 polymorphic SLAF tags were obtained, including 14,885 for 2B and 11,622 for 5B, in the SLAF-seq experiment, which was constructed by enzyme digestion of HaeIII

with a sequencing depth of 14.31x. 5,796 and 5,979 SNP sites of the *Ne1* and *Ne2* alleles were obtained respectively according to the SLAF-Seq data from the positive (a) and negative (b or c) pools (Table S3). The upstream and downstream 500-bp flanking sequence of those SNP sites were obtained from the Chinese spring reference genome sequence (International Wheat Genome Sequencing Consortium, IWGSC RefSeq v1.0). The SNP sites were then converted into AS-PCR or KASP markers. The SLAF-Seq was conducted by Biomarker Technology Co., Ltd (Beijing, China). The primers were designed using PolyMarker (http://www.polymarker.tgac.ac.uk) and synthesized by Beijing AuGCT DNA-SYN Biotechnology Co. Ltd. (Beijing, China). The markers used in this study are listed in Table S4. The SSR, AS-PCR and InDel molecular marker assays were performed according to previously reported protocols (Xue et al. 2012). KASP assays were performed according to the manufacturer's protocol (LGC Genomics; http://www.lgcgroup.com).

The polymorphic markers were screened using bulked segregant analysis (BSA). DNA samples from the two parents, the heterozygous F_1, positive pool (20 necrotic BC_1F_1 plants) and the negative pool (20 normal BC_1F_1 plants) were screened separately for *Ne1* and *Ne2*. The selected markers were further independently tested in a small number of individuals (23 necrotic plants and 22 normal plants) from the corresponding BC_1F_1 population, then the polymorphic markers were selected for fine mapping of the two necrosis genes in all the individuals of the two BC_1F_1 populations, respectively.

Construction of the genetic linkage maps

Linkage analysis was performed using QTL IciMapping V4.2 (http://www.isbreeding.net/software/) with grouping based on a threshold logarithm of the odds (LOD) score of 3.0, ordering by the algorithm of nnTwoOpt algorithm (nearest neighbor was used for tour construction, and two-opt was used for tour improvement) and rippling according to the sum of adjacent recombination frequencies (SARF) (Meng et al. 2015). Genetic distances were calculated using the Kosambi mapping function (Kosambi 1943).

Hybrid necrosis pedigree analysis and genotype detection of wheat cultivars

N9134 and ZH22 are regarded as the fundamental carriers of *Ne1* and *Ne2,* respectively in winter wheat. The pedigrees of these cultivars was traced using hybrid tests to determine the presence of hybrid necrosis genes in their parents and ancestors (Fig. S1). Hybrid tests were also applied to classify the genotype of the wheat cultivars by carrying out crosses with *Ne1* or *Ne2* carriers. Independent genotype detection was conducted using the developed closely linked KASP markers (*Nwu_5B_4137* and *Nwu_5B_5114* for the *Ne1* locus; *Nwu_2B_4204* and *Nwu_2B_4249* for the *Ne2* locus).

Trypan blue stain for necrotic leaves

In order to distinguish the extent of cell necrosis in the leaves of BC_1F_1 necrotic plants derived from different female parents, we performed trypan blue staining. The top three leaves of wheat at jointing stage were boiled in trypan blue staining solution (10 mL of lactic acid, 10 mL of glycerol, 10 mL of liquid phenol, and 10 mL of distilled H_2O, 10 mg of trypan blue) for 1 min (Rate et al. 1999). Then these tissues were cleared in saturated chloral hydrate solution, and stored in water.

Fluorescence in situ hybridization (FISH)

To identify the differences in the karyotype of the cultivars carrying the *Ne1* alleles, FISH was performed according to the protocol described by Tang et al. (2014) and using the oligonucleotide probes Oligo-pTa535 (Tamra-5', red) and Oligo-pSc119.2 (6-FAM-5′, green), which were synthesized by Shanghai Invitrogen Biotechnology Co. Ltd. (Shanghai, China). Fluorescent signals were scanned and photographed with an Olympus BX53 microscope equipped with a Photometrics SenSys CCD DP80 camera (Wang et al. 2016).

Results

Genetic analysis of hybrid necrosis

Test crosses of WL711, Manitou and Pan555 (*Ne2*-carriers), as well as Spica and TA4152-60 (*Ne1*-carriers) (Table S1) showed that N9134 (or N0439) is a carrier of *Ne1* allele, while ZH22 (or Z8425B) carries *Ne2* allele and XN509 carries neither. Then, through systematic genetic analysis (Table S1) of winter wheat cultivars (lines) we confirmed that the hybrid necrosis in the winter wheat investigated in this study was also controlled by two complementary dominant alleles, and its genetic pattern was the same as that reported in spring wheat (Chu et al. 2006; Qiu et al. 2020; Zhang et al. 2016). The specific results are summarized as follows. All the F_1-generation plants derived from crosses of N9134 (or N0439)/ZH22 (or Z8425B) developed necrosis consistently while the F_2 populations segregated at the ratio of 9:7(necrotic: normal) at the seedling stage. Each of the BC_1F_1 populations showed a segregation ratio of 1:1 for necrosis compared with the normal phenotype and all plants in the F_1 generations of XN509/ZH22 and XN509/N0439 exhibited the normal phenotype. And the populations from XN509//N0439/ZH22 and XN509/N0439//ZH22

crosses showed the segregation ratios of 1:3 and 1:1, respectively, for necrosis compared with the normal phenotype. The hybrid necrosis traits of plants at different growth stages and cultivation conditions are shown in Fig. 1.

The dosage effect still exists in the moderate and severe hybrid necrosis system

The F_1 plants derived from N9134 and ZH22 exhibited moderate or severe necrosis (Fig. 4c) according to the necrosis grade criteria defined by Hermsen (1963a). Through genetic analysis, we found one line had no living plants and only three lines exhibited necrosis of all plants among the 140 $F_{2:3}$ lines derived from randomly selected necrotic F_2 plants (Table 1, Table S5). This means that there were no more than four double dominant homozygous lines. Even the ratio of double dominant homozygous lines to heterozygous lines was 4:136, it was not fit to, 1:8 ($p = 7.83E^{-04}$), the theoretical Mendelian ratios without dosage effect. By analyzing the mortality rates of BC_1F_1 and F_2 populations from seedling stage to heading stage, we found that the proportion of theoretical *Ne1Ne1Ne2Ne2* genotype was highly correlated with the abnormal mortality of F_2 population (Table S6). Additionally, based on this observation combined with the theoretical ($F_2 = 9{:}7$, $F_3 = 25{:}11$) and practical separation ($F_2 = 8{:}7$, $F_3 = 16{:}11$) ratios of the F_2 and F_3 populations derived from necrotic plants (Table 1, Table S1), we concluded that most plants with genotype *Ne1Ne1Ne2Ne2* die before heading stage and are unable to produce offspring. That is, regardless of the necrosis grades, the *Ne1ne1Ne2ne2*-plants (double heterozygous, dosage 2) should be the weakest generally and also coincide with the corresponding F_1-plants in terms of the necrotic intensity, while the plants with genotype *Ne1Ne1Ne2Ne2* (double dominant homozygous, dosage 4) should always have the strongest degree of necrosis, and most will die before heading stage in the moderate and severe hybrid necrosis system.

High-density genetic maps of the *Ne1* and *Ne2* in winter wheat

Based on the linked markers of *Ne1* (Chu et al. 2006) and *Ne2* (*Lr13* and *LrLC10*) (Chu et al. 2006; Qiu et al. 2020; Zhang et al. 2016) reported in spring wheat, we constructed two genetic maps of these genes in winter wheat using 269 and 264 BC_1F_1 plants, respectively (Fig. S2). Two high-density genetic linkage maps of these genes were then constructed mainly using the developed KASP markers and AS-PCR markers in the two BC_1F_1 populations consisting of 1,006 and 1,143 plants, respectively (Fig. 2). The detailed genetic map of the *Ne2* locus contained 25 molecular markers. Both the InDel marker *Lseq102* (co-segregated with *LrLC10*) and SSR marker *TC67744* co-segregated with *Ne2*. These markers were located at approximately 156.59 and 157.76 million bases (Mb), respectively, on chromosome arm 2BS in Chinese spring RefSeq v1.0. Furthermore, *Ne2* was located between the two flanking tightly linked InDel markers, Lseq54 and Lseq22, with a genetic interval of 0.18 cM and a physical distance of about 4.45 Mb (Fig. 2a, b). The SNP markers *Nwu_5B_4137* and *Nwu_5B_5114* were identified as the two closest linked markers on either side of the *Ne1* locus, with the genetic interval of 0.50 cM (Fig. 2c). The two markers were found to be located at approximately 383.40 Mb and 388.01 Mb on chromosome arm 5BL in Chinese spring RefSeq v1.0, with the physical interval 4.61 Mb (Fig. 2d).

Distribution, proportion and genotype frequencies of *Ne1* and *Ne2* are discrete in China's wheat regions

Based on the planting area and yield of China's 10 agro-ecological production zones (CHINA STATISTICAL YEARBOOK 2019, http://www.stats.gov.cn/tjsj/ndsj/2019/indexch.htm), we chose wheat cultivars (lines) for investigation according to the proportion grown in each area from the "Precise identification and innovative utilization of wheat germplasm resources" program germplasm nursery. The selected materials (consisting of 1,178 MCC, 65 CL and 121 IMC) were genotyped using molecular marker and/or hybrid test. Among them, 99 were detected by the both two methods, and 98 out of the 99 had the consistent genotyping for *Ne1* or *Ne2* (Table S2). In accordance with this, the two groups of genotype frequencies obtained separately using the two methods were also showed a highly positive correlation ($r = 0.919$, Fig. 3a). After de-redundancy, 26.2% of the cultivars (lines) were genotyped as *Ne1Ne1ne2ne2*, while 33.2% were genotyped as *ne1ne1Ne2Ne2* (Fig. 3b). These results in Fig. 3c demonstrated discrete differences in the distribution and proportion of *Ne* genes from different wheat region, with highly variable correlation coefficients among the regions (Fig. 3d). Typically, *Ne1Ne1ne2ne2* was significantly dominant in the region IV, whereas *ne1ne1Ne2Ne2* was significantly dominant in the IMC population. Cultivars (lines) in the region III had the highest frequency of *ne1ne1ne2ne2* (Fig. 3e).

Similarly, according to the CL, IMC and MCC classification (Fig. 3f), we found that the CL population had the highest frequency of both the *ne1ne1ne2ne2* and *Ne1Ne1ne2ne2* genotypes, while the IMC population showed the relatively lowest frequency of *ne1ne1ne2ne2*, but with the highest frequency of *ne1ne1Ne2Ne2*. Intriguingly, the *Ne2* gene frequency in the MCC population was 2.3 times higher than that in the CL population due to the introduction of the IMC into wheat breeding program of China. Moreover, the proportions of the three genotypes distributed in the MCC population were highly correlated with the average of those

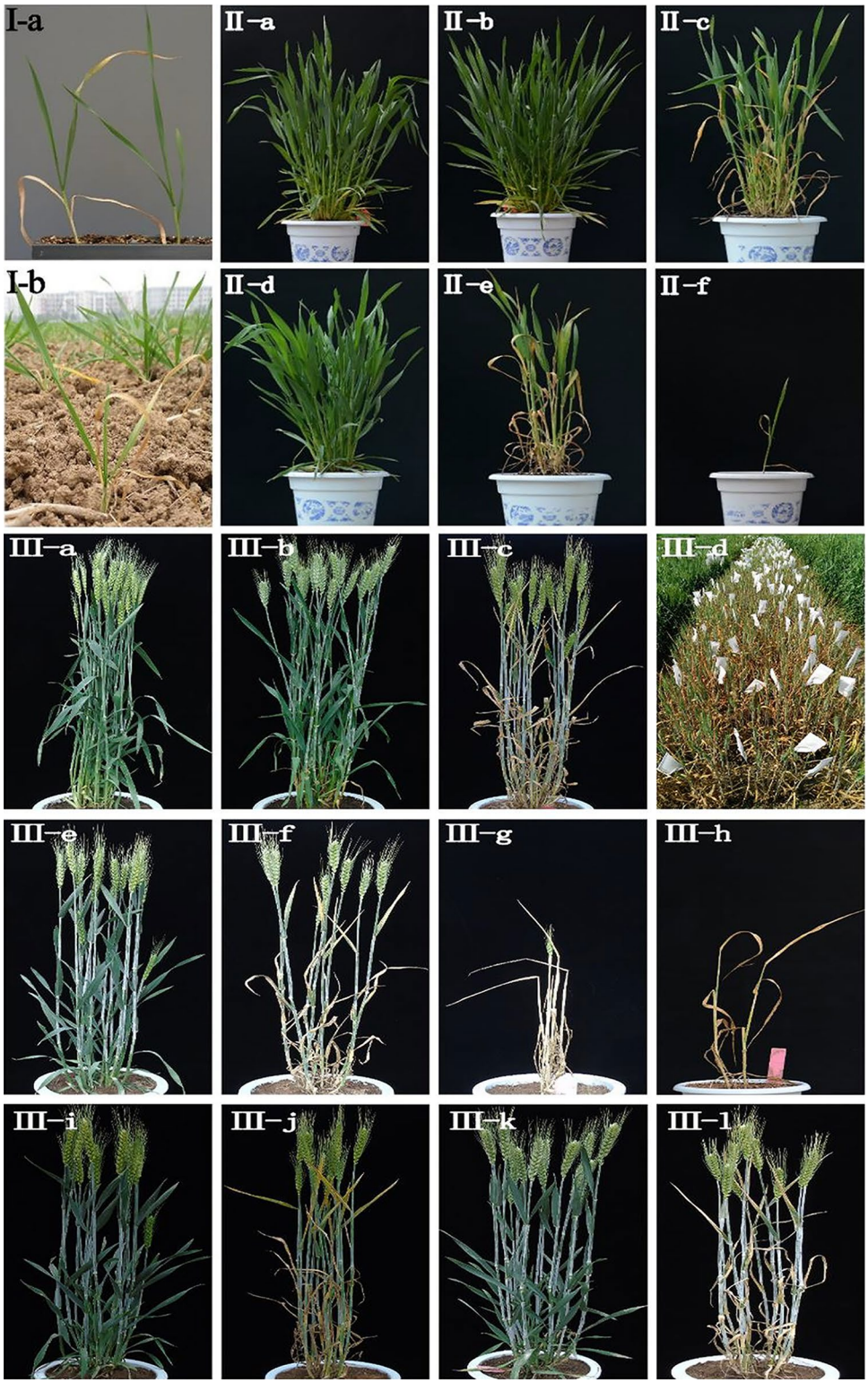


Fig. 1 Hybrid-necrosis traits of plants derived from N9134 and Zhoumai 22 (ZH22) at different growth stages. F_2 seedling with normal and necrotic phenotypes were grown under greenhouse (I–**a**) and in field (I-**b**) conditions. Images of jointing stage seedlings grown under field conditions: N9134 (II–**a**), ZH22 (II–**b**), F_1 (II–**c**), and F_2 with normal (II–**d**) and necrotic (II–**e**, **f**) phenotypes. Images of filling plants grown under field conditions: N9134 (III–**a**), ZH22 (III–**b**), F_1 (III–**c**, **d**), F_2 with normal (III–**e**) and necrotic (III–**f**, **g**, **h**) phenotypes, BC_1F_1 with normal (III–**i**) and necrotic (III–**j**) phenotypes derived from N9134//N9134/ZH22, and BC_1F_1 with normal (III–**k**) and necrotic (III–**l**) phenotypes derived from ZH22//N9134/ZH22. Plants in images marked II and III were photographed after transferring into pots

in the CL and the IMC ($r = 0.971$; Fig. 3g). These findings indicated that the genotype frequencies of *Ne* genes in the MCC are formed by the interaction of the CL (contributing *Ne1* allele) and the IMC (contributing *Ne2* allele).

Ne1-nw is inherited from WE As846, while *Ne2* in ZH22 is inherited from the IMC or hexaploid triticale

To clarify the origins of *Ne1-nw* and *Ne2* in ZH22, we traced the pedigree of these genes separately. The pedigree

Table 1 Genetic analysis of dosage effect associated with moderate and severe necrosis

Years	Female parent	Male parent	Genetic population	Necrotic plants	Normal plants	Seeds	Investigation condition	Theoretical mendelian ratios (Necrotic: Normal)	*p* value	Theoretical Mendelian ratios with dosage effect (Necrotic: Normal)	*p* value
2017–18	N0439	ZH22	F_2	110	71	264	Seedling, field	9:7	0.220	8:7	0.045*
2012–13	ZH22	N0439	F_2	137	132	380	Jointing, field	9:7	0.079	8:7	0.429
2015–16	ZH22	N0439	F_2	128	132	420	Heading, field	9:7	0.023*	8:7	0.185
2013–14	ZH22	N0439	F_3	241	172	800[a]	Heading, field	25:11	$9.93E^{-07}$**	16:11	0.708
2018–19[b]	N9134 (ZH22)	ZH22(N9134)	F_3 ($F_{2:3}$)	986	717	2637	Heading, field	25:11	$4.44E^{-25}$**	16:11	0.253
2018–19[b]	N9134 (ZH22)	ZH22(N9134)	$F_{2:3}$	3[c]	136[c]	140[c]	Heading, field	1:8	$7.83E^{-04}$**	0:8	–

*$P < 0.05$; **$P < 0.001$

[a]The seeds were randomly selected from the mixed seeds of the F_2 necrotic plants

[b]The same population, investigated as individual plants and lines. The details of the $F_{2:3}$ population are shown in Table S5

[c]The number of the $F_{2:3}$ lines having necrotic plants only; The number of $F_{2:3}$ lines containing both necrotic and normal plants; The number of spikes selected from necrotic F_2 plants. *P*-values obtained by chi-squared analysis of the ratios of the genetic population and theoretical Mendelian ratios

of N9134 indicated that *Ne1* is inherited from WE As846 (Fig. S1a). We found that chromosome 5B of N9134 can only be inherited from WE As846, which was consistent with a previous study of *PmAS846* (Xue et al. 2012). In addition, when ZH22 was crossed with WE As846, the F_1 plants showed necrosis similar to the F_1-progeny of ZH22/N9134, while the offspring of ZH22/Abbondanza were normal. This result also confirmed that *Ne1-nw* is inherited from WE As846. However, based on these results, we could only infer that the *Ne2* in ZH22 is inherited from one of the five ancestral parents, four introduced modern cultivars or one hexaploid triticale. The F_1-plants obtained by crossing N9134 and Z8425B showed hybrid necrosis, while those of the other three crosses between N9134 and Zhoumai 12, Yumai 49, and Zhoumai 9 did not; therefore, we proposed that *Ne2* in ZH22 was inherited from Z8425B. However, since Z8425B is derived from five ancestral cultivars (2 Italian cultivars (lines) named St2422/464 (Zhengyin 4) and St1472/506 (Zhengyin 1), 1 Russian named Прелгорная 2 (Erythrospermum-315H160/Wumang 1), 1 Mexican cultivar named Nainari60, and a hexaploid triticale line Guangmai 74) (Fig. S1b), the exact donor of *Ne2* remains to be verified.

Genetic background causes different hybrid necrosis phenotypes

In our study, we observed differences in necrosis phenotypes between the two BC_1F_1 populations only with different female parents (Fig. 4). Specifically, the necrosis processes in the BC_1F_1 plants with female parent N9134 involved yellowing in the leaves from the tip to base, and the whole leaf remained fresh and moist for a short period, followed by gradually drying from the tip. These observations indicated that this type of necrosis may occur independently through two independent processes that result in yellowing and dryness (Fig. 4a). However, the processes in the BC_1F_1 plants from backcross of ZH22 involved gradually yellowing and drying of the leaves almost simultaneously from the tip to base. These observations indicated that this type of necrosis occurs through only one process (Fig. 4b). This phenomenon was also being observed in F_1 offspring of different female parents (Fig. 4c). In BC_1F_1 plants with female parent ZH22, trypan blue staining revealed a large number of necrotic and dried-out tissues that could not be stained because the DNA in these cells had degraded and trypan blue could not bind to (Fig. S3b, S3d). However, the yellow region of BC_1F_1 leaves from N9134 could be dyed light blue, indicating that cells in these tissues were normal or in the early stage of necrosis (Fig. S3a, S3c). Since the dosage and environmental conditions were identical in these two models, we proposed that N9134 and ZH22 may confer differential sensitivities to the activated senescence process, thus representing an example

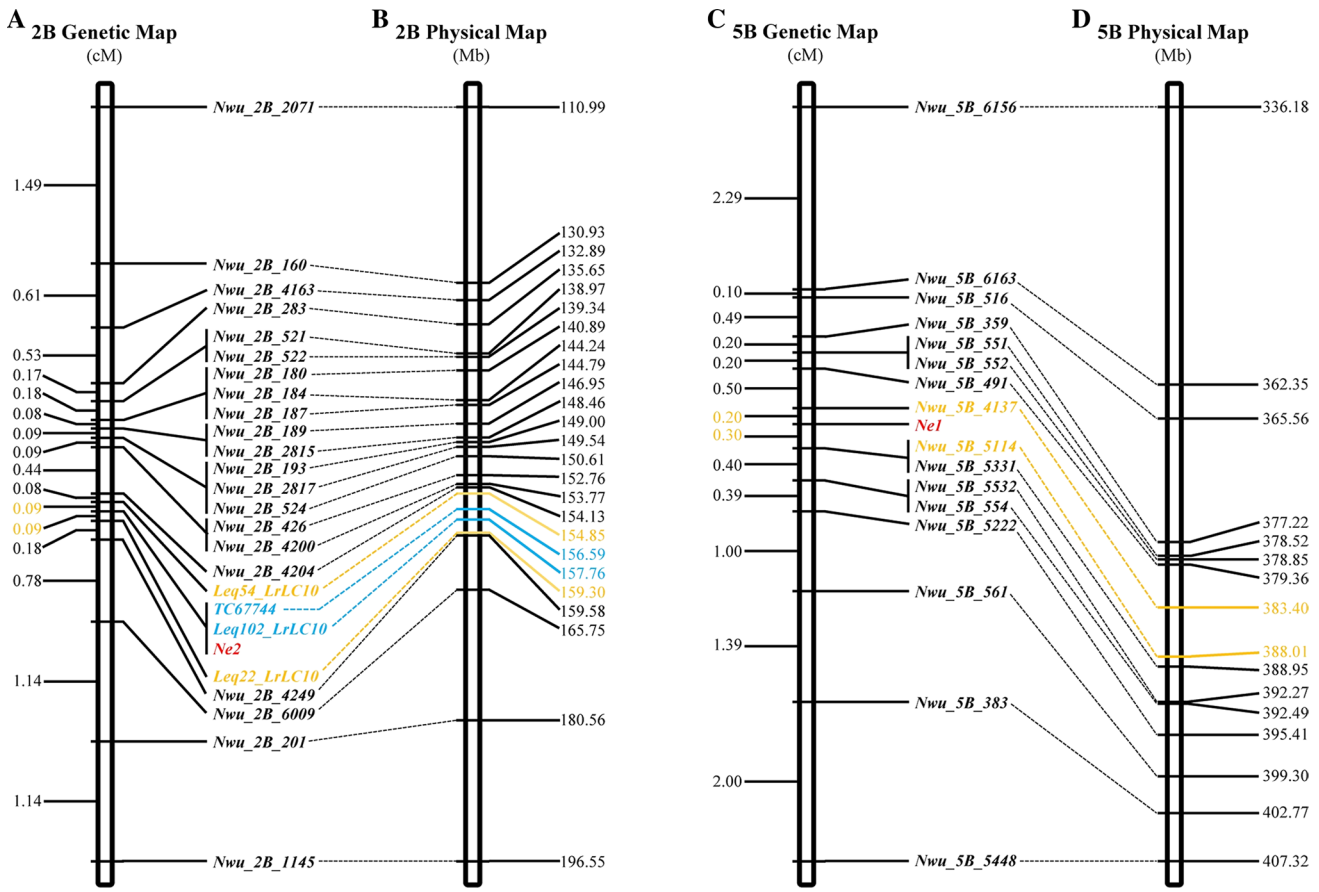


Fig. 2 Genetic linkage maps of *Ne1* and *Ne2* with high-density molecular markers in winter wheat. **a** Genetic linkage map of *Ne2* in ZH22; markers are indicated on the right and genetic distances are indicated in centimorgan (cM) on the left. **b** The corresponding physical location of the polymorphic linkage markers on 2BS of Chinese Spring RefSeq v1.0; the physical distances are indicated as million bases (Mb) on the right. **c** and **d** shows the same information for *Ne1* on 5BL (N9134)

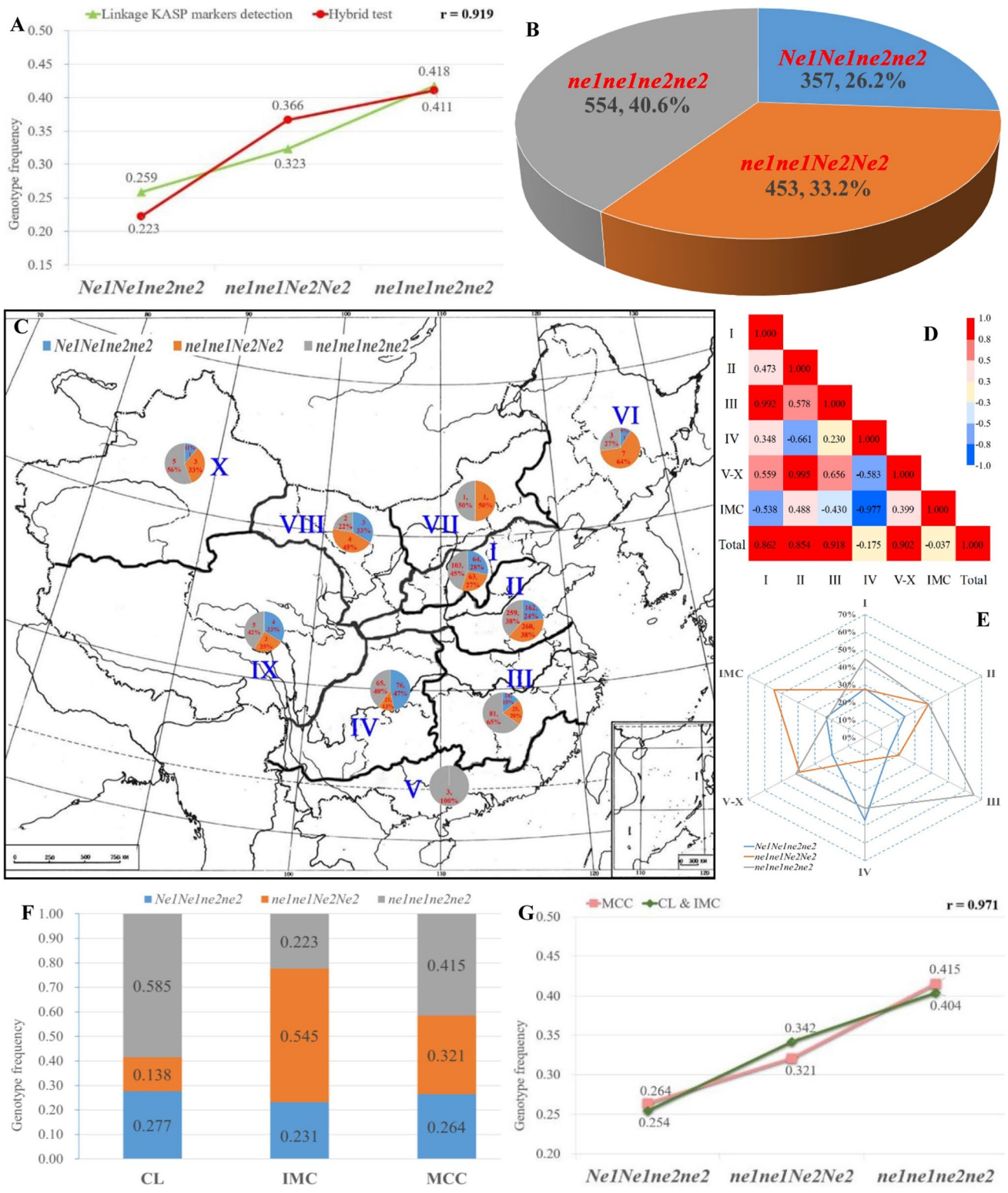
of the effect of genetic background on the manifestation of hybrid necrosis.

Discussion

Ne1-nw is implicated as a novel hybrid necrosis allele

To date, three *Ne1* alleles (*Ne1w*, *Ne1m* and *Ne1s*) have been reported in wheat (Hermsen 1963a). The existence of other alleles is suggested by the differences between the multiple alleles of *Ne1*, not only at the phenotypic level identified in allelism tests, but also the linked molecular markers (Chu et al. 2006; Huang et al. 2020; Qiu et al. 2020; Zhang et al. 2016). The allelism tests (Table S7) performed in this study indicate that the loci of *Ne1s* in Spica and *Ne1* in TA4152-60 are distinct from *Ne1-nw*. Additionally, karyotype analysis revealed that the genetic backgrounds of these genes are very different (Fig. S4). Since Spica and TA4152-60 are spring wheat cultivars (lines), it can be inferred that *Ne1-nw* has a different origins from the other two *Ne1* alleles.

Fig. 3 Distribution, proportion and genotype frequencies of hybrid necrosis genes in China. **a** The genotype frequencies obtained from linkage KASP markers detection and hybrid tests showed a strong positive correlation. **b** Hybrid necrosis genes frequencies for all the 1,364 wheat cultivars (lines). **c** Distribution and genotype frequencies of hybrid necrosis genes in each of China's 10 agro-ecological production zones shown for the 1,246 Chinese wheat cultivars (lines) [I, north China winter wheat region (230); II, Yellow and Huai River valley winter wheat region (681); III, middle and lower Yangtze River valley winter wheat region (124); IV, south-western winter wheat region (162); V, south China winter wheat region (3); VI, north-eastern spring wheat region (11); VII, northern spring wheat region (2); VIII, north-western spring wheat region (9); IX, Qinghai–Tibet spring–winter wheat region (12); X, Xinjiang winter–spring wheat region (9).]. **d** The different wheat regions were with discrepant correlation coefficients and **e** comparison for the frequencies of hybrid necrosis genes. **f** The frequencies of hybrid necrosis genes and **g** their correlation coefficients analyzed according to the classification of Chinese landraces (CL), introduced modern cultivars (IMC) and modern Chinese cultivars (lines; MCC)



Due to the difference in the *Ne1* allele frequencies between tetraploid (emmer, durum and timopheevi) and the hexaploid wheat (Hermsen 1963a; Mori and Tsunewaki 1992; Tsunewaki 1970; Zeven 1969), it is generally accepted that the multiple *Ne1* alleles differentiated genetically before domestication. In this study, we demonstrated that *Ne1-nw* was derived directly from WE (tetraploid wheat). Comparison of the *Ne1* locus interval in Chinese Spring (IWGSC RefSeq v1.0) with that in WE (Zavitan WEWSeq v1.0), revealed a fragment (residing an initiation factor 4F

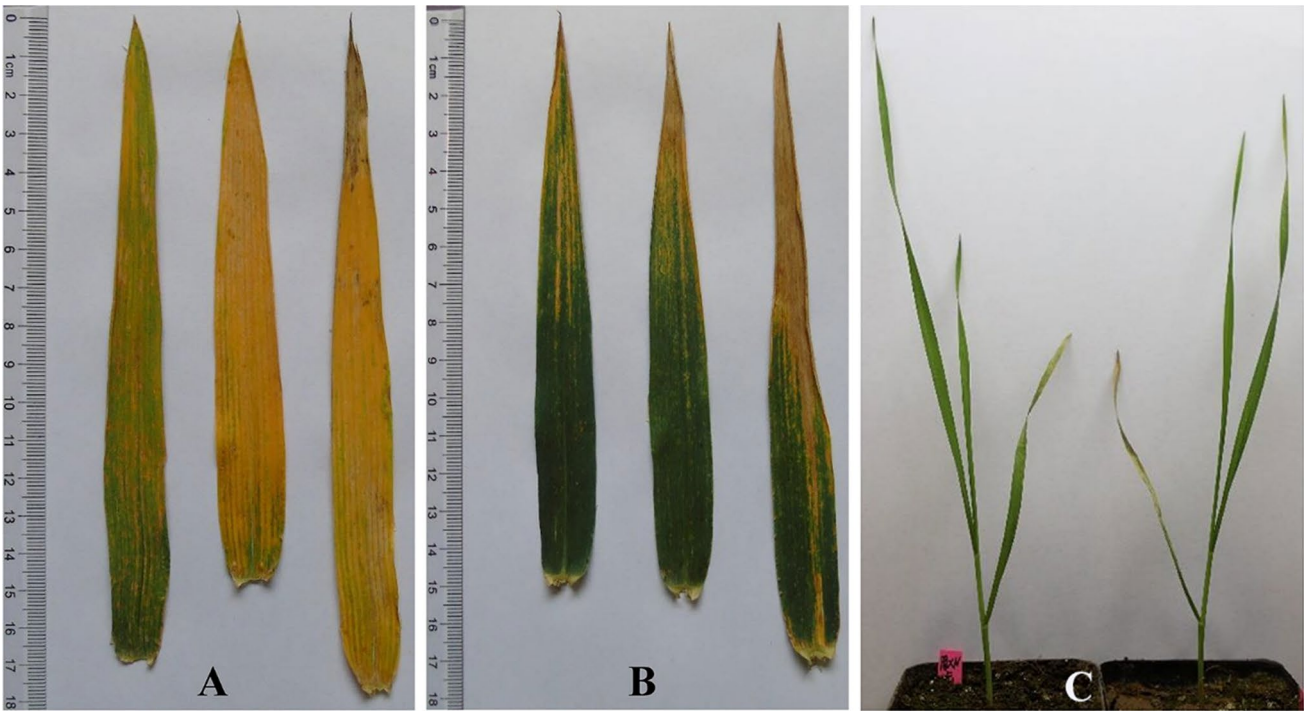


Fig. 4 Necrotic leaves of BC_1F_1 and F_1 plants with different female parents. **a** Flag leaves of the cross of N9134//(ZH22/N9134 or N9134/ZH22). **b** Flag leaves of the cross of ZH2//(ZH22/N9134 or N9134/ZH22). **c** Necrotic phenotypes of seedlings of F_1-generations from N9134/ZH22 (left) and ZH22/N9134 (right)

subunit-encoding gene, an alpha/beta-hydrolases superfamily protein-encoding gene and three *HtrA* genes) as a tandem duplication in Chinese Spring. It is not surprising that the structure and function of these repeat genes changed over the course of evolution. We further speculate that the tandem repeat genes in the *Ne1* loci perform parallel or redundant functions. Therefore, this indicates that these *Ne1* alleles are different, thus implicating *Ne1-nw* derived from WE as a novel hybrid necrosis gene.

Ne1 may be related to serine protease HtrA

We mapped *Ne1* to a physical interval from 383.40 to 388.01 Mb on chromosome 5B of Chinese spring RefSeq v1.0, which was consistent with the interval mapped by Li and Si (Li et al. 2021; Si et al. 2021a). The *Ne1* intersection region of the three genetic maps contains 14 candidate genes (2 encoding initiation factor 4F subunit, 2 alpha/beta-hydrolases superfamily protein, 1 late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein and 9 serine protease HtrA). The *Ne1* allele in ZN17 was associated with a 2.89 Mb deletion on chromosome arm 5BL containing four high-confidence genes (1 alpha/beta-hydrolases superfamily protein-encoding gene and 3 *HtrA* genes) (Si et al. 2021a). Additionally, all the three *HtrA* genes in the deletion were significantly differentially expressed between the positive and negative pools according to our BSR-seq data (unpublished). HtrA proteases share common mechanisms of activation with classic serine proteases such as trypsin, chymotrypsin and elastase (Clausen et al. 2011). It was reported that HtrA proteins participate in defense against stresses causing aberrations in protein structure, and disturbances in their function may induce carcinogenesis or arthritic and neurodegenerative disorders (Zurawa et al. 2007). Chen (2014) demonstrated that the interaction of *Hwi1* (comprises 2 LRR-RLK genes) and *Hwi2* (encodes a putative subtilisin-like protease with serine protease activity) can activate the autoimmune response in the basal nodes of hybrids in rice. Recently, *Ne2* has been cloned and confirmed to be a typical CC-NBS-LRR-type R gene (Hewitt et al. 2021; Si et al.2021b; Yan et al. 2021). Therefore, we speculate that *Ne1* is a serine protease and the enzyme or its substrate is specifically recognized by *Ne2*, leading to the constitutive activation of the disease resistance response. This hypothesis remains to be tested in further investigation of isolated *Ne1*.

The IMC should directly affect the frequencies of necrosis genes in MCC, especially *Ne2*

In the nine reports from 1963 to 1981, 1,467 of the 5,541 cultivars (26.5%) reported globally carried *Ne1* and 1,189 (21.5%) were *Ne2* carriers all over the world, while 60 carried *Ne1* (39.0%) and 15 carried *Ne2* (9.7%) among the 154 Chinese wheat cultivars (lines) (Hermsen 1963b; Zeven 1965, 1967, 1968, 1969, 1971, 1973, 1976, 1981). In contrast, among the 1,178 MCC used in this study, 311 cultivars (26.4%) carried the *Ne1Ne1ne2ne2* genotype and 378 cultivars (32.1%) carried the *ne1ne1Ne2Ne2* genotype (Table S2, Fig. 3f). Strikingly, in comparison with these previous reports, the frequency of the *Ne1* carriers in China showed a decrease of 12.6% (from 39.0 to 26.4%), while the frequency of *Ne2* carriers rose considerably from 9.7 to 32.1%. These trends are consistent with those of previous reports (Bomblies and Weigel 2007). These findings imply that these two *Ne*-genes were unconsciously selected by breeders, and *Ne2* has greater selective advantages than *Ne1*, probably due to the leaf rust resistance conferred by *Ne2* (*Lr13*) (Hewitt et al. 2021; Si et al.2021b; Yan et al. 2021) or other tightly linked loci conferring advantageous traits. This inference is also supported by the fact that many modern cultivars derived from Z8425B, a backbone parent which was widely used in wheat breeding in China (Fig. S1b). Moreover, considering the variation of the frequency between the CL, IMC and MCC (Fig. 3f), we speculate that the increase in the *Ne2* allele in China is caused by the decisive effect of IMC. This indicates the important contribution of the IMC to wheat breeding in China, as well as the robust constitution of the MCC genome (Chen et al. 2019; Hao et al. 2020).

Hybrid necrosis alleles could also positively affect wheat breeding

Hybrid necrosis is generally considered to be a barrier to gene flow at the inter- or intra-specific levels (Presgraves 2010; Rieseberg and Willis 2007; Zhou et al. 2020) and is usually regarded as a negative influence on breeding (Hermsen 1963a; Vikas et al. 2013) that must be avoided. Nevertheless, necrosis occurs only when both *Ne1* and *Ne2* are present in the same plant (Hermsen 1963a), which alleviates the necessity for elimination of both alleles simultaneously. In fact, both the 'negative effectors' *Ne1* and *Ne2* are linked with advantageous genes (Bomblies and Weigel 2007; Xue et al. 2012; Zeven 1981; Zhang et al. 2016), that is, both could positively affect breeding when they occur independently in an individual. Therefore, if breeders reserve F_1 plants showing hybrid necrosis, the high-quality plants might be separated in F_2 generation, increasing the chance of obtaining progeny with superior comprehensive traits. Taken together, the information presented here represents a possible foundation from which the traditional cognition of hybrid necrosis can be adjusted to allow wheat breeders to avoid missing potential elite offspring of necrotic plants. In addition, the fact that *Ne1* and *Ne2* are still widely distributed all over the world instead of being eliminated (Bomblies and Weigel 2007; Vikas et al. 2013) could also support this view. Meanwhile it also hints that the diversity of the excellent germplasm resources should be not sufficient enough for wheat breeding (Hao et al. 2020; Zhou et al. 2020).

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Author contribution statements WJ, HZ, MZ and SL designed the research. MZ, SL, YZW and SW performed the research. MZ, SL, CC, CW and YJW contributed to the development and investigation of materials. MZ, SL and YZW contributed by collecting the samples for DNA extraction. MZ, SL and YZW developed the molecular markers and constructed the genetic maps. S.W. conducted fluorescence in situ hybridization. MZ, SL and HZ analyzed the data and contributed to writing the article.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Bizimungu B, Collin J, Comeau A, St-Pierre CA (1998) Hybrid necrosis as a barrier to gene transfer in hexaploid winter wheat x triticale crosses. Can J Plant Sci 78:239–244

Bomblies K, Weigel D (2007) Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. Nat Rev Genet 8:382–393

Caldwell RM, Compton LE (1943) Complementary lethal genes in wheat: causing a progressive lethal necrosis of seedlings. J Hered 34:67–70

Chen C, Chen H, Lin YS, Shen JB, Shan JX, Qi P, Shi M, Zhu MZ, Huang XH, Feng Q, Han B, Jiang L, Gao JP, Lin HX (2014) A two-locus interaction causes interspecific hybrid weakness in rice. Nat Commun 5:3357

Chen H, Jiao C, Wang Y, Wang Y, Tian C, Yu H, Wang J, Wang X, Lu F, Fu X, Xue Y, Jiang W, Ling H, Lu H, Jiao Y (2019) Comparative population genomics of bread wheat (*Triticum aestivum*) reveals its cultivation and breeding history in China. bioRxiv:519587

Chu CG, 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

Clausen T, Kaiser M, Huber R, Ehrmann M (2011) HTRA proteases: regulated proteolysis in protein quality control. Nat Rev Mol Cell Biol 12(3):152–162

Hao C, Jiao C, Hou J, Li T, Liu H, Wang Y, Zheng J, Liu H, Bi Z, Xu F, Zhao J, Ma L, Wang Y, Majeed U, Liu X, Appels R, Maccaferri M, Tuberosa R, Lu H, Zhang X (2020) Resequencing of 145 landmark cultivars reveals asymmetric sub-genome selection and strong founder genotype effects on wheat breeding in China. Mol Plant 13(12):1733–1751

Hermsen JT (1963a) Hybrid necrosis as a problem for the wheat breeder. Euphytica 12:1–16

Hermsen JT (1963b) Sources and distribution of the complementary genes for hybrid necrosis in wheat. Euphytica 12:147–160

Hewitt T, Zhang J, Huang L, Upadhyaya N, Li J, Park R, Hoxha S, McIntosh R, Lagudah E, Zhang P (2021) Wheat leaf rust resistance gene Lr13 is a specific Ne2 allele for hybrid necrosis. Mol Plant 14(7):1025–1028

Huang S, Liu S, Zhang Y, Xie Y, Wang X, Jiao H, Wu S, Zeng Q, Wang Q, Singh RP, Bhavani S, Kang Z, Wang C, Han J, Wu J (2020) Genome-wide wheat 55K SNP-based mapping of stripe rust resistance loci in wheat cultivar Shaannong 33 and their alleles frequencies in current Chinese wheat cultivars and breeding lines. Plant Dis 105(4):1048–1056

Kosambi DD (1943) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Li N, Tan Q, Ding J, Pan X, Ma Z (2021) Fine mapping of Ne1, the hybrid necrosis gene complementary to Ne2 in common wheat (*Triticum aestivum* L). Theor Appl Genet. https://doi.org/10.1007/s00122-021-03860-9

Meng L, Li HH, Zhang LY, Wang JK (2015) QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. Crop J 3:269–283

Mori N, Tsunewaki K (1992) Distribution of the necrosis and chlorosis genes in two wild tetraploid wheats, *Triticum dicoccoides* and *T. araraticum*. Jpn J Genet 67:371–380

Nishikawa K, Mori T, Takami N, Furuta Y (1974) Mapping of progressive necrosis genes, *Ne1* and *Ne2* of common wheat by the telocentric method. Jpn J Breed 24:277–281

Oetmann A, Zeller FJ (1989) Distribution and origin of hybrid necrosis genes in German winter wheat (*Triticum aestivum* L.) cultivars. Plant Breed 103:207–211

Orr HA (1996) Dobzhansky, Bateson, and the genetics of speciation. Genetics 144:1331–1335

Presgraves DC (2010) The molecular evolutionary basis of species formation. Nat Rev Genet 11:175–180

Pukhalskiy VA, Bilinskaya EN (1998) Identification of hybrid necrosis genes in winter varieties of common wheat *Triticum aestivum* L. Genetika 34:1639–1647

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

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

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

Pukhalskiy VA, Udachin RA, Bilinskaya EN (2008b) Hybrid necrosis genes in aboriginal wheats of Middle Asia in the light of the problem of the primary centers of biodiversity of the *Triticum* L. genus. Euphytica 165:533–543

Qiu L, Wang H, Li Y, Wang W, Liu Y, Mu J, Geng M, Guo W, Hu Z, Ma J, Sun Q, Xie C (2020) Fine mapping of the wheat leaf rust resistance gene *LrLC10* (*Lr13*) and validation of its co-segregation markers. Front Plant Sci 11:470

Rate DN, Cuenca JV, Bowman GR, Guttman DS, Greenberg JT (1999) The gain-of-function Arabidopsis acd6 mutant reveals novel regulation and function of the salicylic acid signaling pathway in controlling cell death, defenses, and cell growth. Plant Cell 11:1695–1708

Rieseberg LH, Willis JH (2007) Plant speciation. Science 317:910–914

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Si Y, Zheng S, Niu J, Tian S, Shi X, He Y, Li Y, Ling HQ (2021) Fine mapping of hybrid necrosis gene Ne1 in common wheat (*Triticum aestivum* L.). Theor Appl Genet 134(8):2603–2611

Si Y, Zheng S, Niu J, Tian S, Gu M, Lu Q, He Y, Zhang J, Shi X, Li Y, Ling HQ (2021b) *Ne2*, a typical CC-NBS-LRR-type gene, is responsible for hybrid necrosis in wheat. New Phytol. https://doi.org/10.1111/nph.17575

Sun X, Liu D, Zhang X, Li W, Liu H, Hong W, Jiang C, Guan N, Ma C, Zeng H, Xu C, Song J, Huang L, Wang C, Shi J, Wang R, Zheng X, Lu C, Wang X, Zheng H (2013) SLAF-seq: an efficient method of large-scale *de novo* SNP discovery and genotyping using high-throughput sequencing. PloS one 8:e58700

Tang Z, Yang Z, Fu S (2014) Oligonucleotides replacing the roles of repetitive sequences pAs1, pSc119.2, pTa-535, pTa71, CCS1, and pAWRC.1 for FISH analysis. J Appl Genet 55:313–318

Tsunewaki K (1970) Necrosis and chlorosis genes in common wheat and its ancestral species. Seiken Ziho 22:67–75

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

Wang Y, Quan W, Peng N, Wang C, Yang X, Liu X, Zhang H, Chen C, Ji W (2016) Molecular cytogenetic identification of a wheat–*Aegilops geniculata* Roth $7M^g$ disomic addition line with powdery mildew resistance. Mol Breed 36:1–10

Xue F, Ji W, Wang C, Zhang H, Yang B (2012) High-density mapping and marker development for the powdery mildew resistance gene *PmAS846* derived from wild emmer wheat (*Triticum turgidum* var. *dicoccoides*). Theor Appl Genet 124:1549–1560

Yan X, Li M, Zhang P, Yin G, Zhang H, Gebrewahid TW, Zhang J, Dong L, Liu D, Liu Z, Li Z (2021) High-temperature wheat leaf rust resistance gene Lr13 exhibits pleiotropic effects on hybrid necrosis. Mol Plant 14(7):1029–1032

Zeven A (1965) First supplementary list of genotypes of hybrid necrosis of wheat varieties. Euphytica 14:239–243

Zeven A (1967) Second supplementary list of genotypes of hybrid necrosis of wheat varieties. Euphytica 16:18–22

Zeven A (1968) Third supplementary list of wheat varieties classified according to their genotype for hybrid necrosis. Euphytica 17:46–53

Zeven A (1969) Fourth supplementary list of wheat varieties classified according to their genotype for hybrid necrosis. Euphytica 18:43–57

Zeven A (1971) Fifth supplementary list of wheat varieties classified according to their genotype for hybrid necrosis and geographical distribution of *Ne*-genes. Euphytica 20:239–254

Zeven A (1973) Sixth supplementary list of wheat varieties classified according to their genotype for hybrid necrosis and geographical distribution of *Ne*-genes. Euphytica 22:618–632

Zeven A (1976) Seventh supplementary list of wheat varieties classified according to their genotype for hybrid necrosis and geographical distribution of *Ne*-genes. Euphytica 25:255–276

Zeven A (1981) Eighth supplementary list of wheat varieties classified according to their genotype for hybrid necrosis. Euphytica 30:521–539

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

Zhou Y, Zhao X, Li Y, Xu J, Bi A, Kang L, Xu D, Chen H, Wang Y, Wang YG, Liu S, Jiao C, Lu H, Wang J, Yin C, Jiao Y, Lu F (2020) *Triticum* population sequencing provides insights into wheat adaptation. Nat Genet 52:1412–1422

Zurawa-Janicka D, Narkiewicz J, Lipińska B (2007) Characterization of the HtrA family of proteins. Postepy Biochem 53(1):27–36