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ORIGINAL PAPER

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Molecular mapping of hybrid necrosis genes *Ne1* and *Ne2* in hexaploid wheat using microsatellite markers

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Abstract Hybrid necrosis is the gradual premature death of leaves or plants in certain F_1 hybrids of wheat (*Triticum aestivum* L.), and it is caused by the interaction of two dominant complementary genes *Ne1* and *Ne2* located on chromosome arms 5BL and 2BS, respectively. To date, molecular markers linked to these genes have not been identified and linkage relationships of the two genes with other important genes in wheat have not been established. We observed that the F_1 hybrids from the crosses between the bread wheat variety 'Alsen' and four synthetic hexaploid wheat (SHW) lines (TA4152-19, TA4152-37, TA4152-44, and TA4152-60) developed at the International Maize and Wheat Improvement Center (CIMMYT) exhibited hybrid necrosis. This study was conducted to determine the genotypes of TA4152-60 and Alsen at the *Ne1* and *Ne2* loci, and to map the genes using microsatellite markers in backcross populations. Genetic analysis indicated that Alsen has the genotype *ne1ne1Ne2Ne2* whereas the SHW lines have *Ne1Ne1ne2ne2*. The microsatellite marker *Xbarc74* was linked to *Ne1* at a genetic distance of 2.0 cM on chromosome arm 5BL, and *Xbarc55* was 3.2 cM from *Ne2* on 2BS. Comparison of the genetic maps with the chromosome deletion-based physical maps indicated that *Ne1* lies in the proximal half of 5BL, whereas *Ne2* is in the distal half of 2BS. Genetic linkage analysis showed that *Ne1* was about 35 cM proximal to *Tsn1*, a locus conferring sensitivity to the host selective toxin Ptr ToxA produced by the tan spot fungus. The closely linked microsatellite markers identified in this study can be used to genotype parental lines for *Ne1* and *Ne2* or to eliminate the two hybrid necrosis genes using marker-assisted selection.

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Introduction

Hybrid necrosis, characterized by necrosis of leaf and sheath tissues on hybrid plants, has been frequently observed in F_1 hybrids between genotypes of common wheat (*Triticum aestivum* L., $2n=6x=42$, AABBDD genomes) and between common wheat and tetraploid wheat (*T. turgidium* L., $2n=4x=28$, AABB) (Tsunewaki 1992). Hybrid necrosis is usually lethal or semilethal, resulting in gradual death or loss of productivity (Tomar et al. 1991; Tomar and Singh 1998). Therefore, hybrid necrosis is a serious barrier either for combining desirable traits from different genotypes of common wheat or for transferring genes from related species to commercial cultivars (Bizimungu et al. 1998; Tomar et al. 1991). In addition, hybrid necrosis can complicate the genetic analysis of particular traits because it may prohibit the development of desirable mapping populations.

Hybrid necrosis is controlled by the complementary dominant genes *Ne1* and *Ne2*, which are located on chromosome arms 5BL and 2BS, respectively (Tsunewaki 1960; Zeven 1972; Nishikawa et al. 1974). The *Ne1* and *Ne2* genes are both widely distributed among different subspecies, varieties, and commercial cultivars of common wheat (Tsunewaki 1970, 1992). Pukhalskiy et al. (2000) investigated the geographical distribution of hybrid necrosis genes in bread wheat. They found that noncarrier genotypes of the *Ne1* and *Ne2* genes are predominant in most continents. However, 7.5 and 55.1% of total genotypes in North America carry the *Ne1* and *Ne2*, respectively. On the contrary, 47.9 and 6.7% of the genotypes in Africa have *Ne1* and *Ne2*,

respectively (Pukhalskiy et al. 2000). Singh et al. (2000) reported that about 70% of the recently developed Indian and Mexican spring wheat cultivars carry *Ne2*. Although these studies showed that breeding has caused changes in necrotic genotype frequencies in wheat populations from different regions, the wide distribution of the *Ne1* and *Ne2* genes in bread wheat throughout the world poses a problem for wheat breeding (Singh et al. 2000).

Previous studies on hybrid necrosis involved classical genetic analysis and surveys on the distributions of the *Ne1* and *Ne2* genes (Pfeffer and Zeller 1987; Oetmann and Zeller 1989; Singh et al. 1989; Gupta and Gupta 1993; Pukhalskiy and Bilinskaya 1998; Pukhalskiy et al. 2000). The current approach to test if a genotype carries a necrosis gene relies on hybridizations with testers (*Ne1Ne1ne2ne2, ne1ne1Ne2Ne2*). Molecular markers tightly linked to the two hybrid necrosis genes will be very useful for wheat breeders to genotype their base germplasm and parental lines as well as to eliminate the dominant alleles from commercial cultivars using marker-assisted selection. However, the *Ne1* and *Ne2* genes have not previously been mapped using molecular markers and thus the precise linkage relationships between *Ne* genes and other agronomically important genes have not been established.

In addition to *Ne1*, chromosome 5BL harbors *Tsn1*, a gene that confers sensitivity to the host selective toxin Ptr ToxA produced by the tan spot pathogen [*Pyrenophora tritici-repentis* (Died.) Drechs.] (Faris et al. 1996). The Ptr ToxA causes severe necrosis on the leaves of wheat genotypes carrying *Tsn1*, but the toxin has no effect on the genotypes lacking *Tsn1* (Ciuffetti and Tuori 1999; Anderson et al. 1999). Since the genetic relationship between *Tsn1* and *Ne1* has not been investigated, it remains unknown if hybrid necrosis and toxin-induced necrosis have a common or related genetic basis.

During the process of transferring desirable genes from synthetic hexaploid wheat (SHW) (×*Aegilotriticum* spp.) into commercial cultivars of hard red spring wheat (HRSW), we observed that the F_1 hybrids between the HRSW variety 'Alsen' and four elite CIMMYT SHW lines (TA4152-19, TA4152-37, TA4152-44, and TA4152-60) developed hybrid necrosis. Alsen is currently a leading HRSW variety planted in North Dakota and has been grown on 20–40% of the spring wheat acreage each year in North Dakota since it was released in 2000 (North Dakota Agricultural Statistics Service 2004). Genotyping Alsen and the elite CIMMYT SHW lines for the hybrid necrosis genes would be useful for wheat breeders to use them in future breeding schemes. Thus, the hybrid necrosis observed in these hybrids provided an excellent tool for mapping *Ne1* and *Ne2* using molecular markers. The four elite CIMMYT SHW lines are known to be insensitive to Ptr ToxA (Xu et al. 2004), whereas Alsen carries *Tsn1*. Thus, Alsen and the four SHW lines are useful for determining the linkage relationship of *Ne1* and *Tsn1* on chromosome 5BL. Therefore, this study was conducted to determine the genotypes of Alsen and the SHW lines at the *Ne1* and *Ne2* loci, to map the two hybrid necrosis genes using microsatellite (SSR) markers, and to clarify if a common genetic basis underlying the hybrid necrosis and toxin-induced necrosis exists by determining the linkage relationship between *Tsn1* and *Ne1* on chromosome 5BL.

Materials and methods

Plant materials

The HRSW variety Alsen and four SHW lines developed at CIMMYT were used in this study. Alsen was released by the North Dakota Agricultural Experiment Station in 2000 and it is a derivative of scab-resistant spring wheat germplasm ND2710 (PI 633976) (Frohberg et al. 2004). ND2710 has a pedigree of ND2603 ('Sumai 3'/'Wheaton')/'Grandin' (Frohberg et al. 2004). The original seed of Alsen was provided by Dr. Mohammad Mergum, Department of Plant Sciences, North Dakota State University, Fargo, ND, USA. Four elite CIMMYT SHW lines including TA4152-19 [Pedigree: Dverd 2/*Aegilops tauschii* (221)], TA4152-37 [68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (878)], TA4152-44 [68.111/RGB-U//WARD/3/*Ae. taucshii* (629)], and TA4152-60 [Scoop 1/*Ae. tauschii* (358)] were previously identified to be resistant to tan spot and Stagonospora nodorum blotch [casual agent: *Phaeosphaeria nodorum* (E.Mull.) Hedjar.] (Xu et al. 2004). The seed of the SHW lines used in this study was provided by Dr. Bikram S. Gill, the Wheat Genetics Resource Center (WGRC), Kansas State University, Manhattan, KS, USA.

Alsen was used as the male parent in crosses with the four SHW lines to transfer disease resistance. All F_1 hybrids showed hybrid necrosis, suggesting that Alsen carries one of the two dominant complementary genes (*Ne1* or *Ne2*) and the four SHW lines carry the other of the two complementary genes. To determine the genotypes of Alsen and the SHW lines at the *Ne1* and *Ne2* loci and to map the two hybrid necrosis genes using microsatellite (SSR) markers, we used the F_1 plants derived from the cross between Alsen and TA4152-60 to develop segregating populations. F_2 plants were used to identify the hybrid necrosis gene carried by Alsen and to evaluate the genetic linkage relationship between *Ne1* and *Tsn1*. In comparison with the F_2 population, backcross populations can generate more genotypic information for linkage analysis since only one of the two necrosis genes actually segregated, which made it easier to genotype each plant. Therefore, we developed two backcross populations consisting of 100 and 94 individuals derived from crossing the F_1 plants to Alsen (TA4152-60/2*Alsen) and TA4152-60 (TA4152-60/Alsen//TA4152-60), respectively, which were used for molecular mapping of *Ne1* and *Ne2* with microsatellite markers.

To classify the necrotic phenotype, the F_2 and BC_1 plants were grown in 6-in. clay pots and super-cell cones (Stuewe and Sons, Inc., Corvallis, OR, USA), respectively, with Sunshine SB100 Bedding Bark Mix (Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada) fertilized with Osmocote Plus 15-19-12 (Scotts Sierra Horticultural Product Company, Marysville, OH, USA) in a greenhouse at 22–25°C under supplemental sodium halide lights with a 16-h photoperiod. At the three-leaf stage, plants with complete necrotic death of the first leaf were scored as positive for hybrid necrosis and those with all normal leaves were scored as non-necrotic (Fig. 1).

Ptr ToxA infiltration

Ptr ToxA was purified from *P. tritici-repentis* race 2 (isolate 86–124) as described by Zhang et al. (1997) and provided by S.W. Meinhardt, Department of Chemistry, North Dakota State University, Fargo, ND, USA. Toxin infiltration was performed according to Xu et al. (2004). When the secondary leaf was fully expanded, approximately 25 µl Ptr ToxA (10 µg ml^{-1}) was used to infiltrate the first leaf at the two-leaf stage, but for plants showing necrosis the second leaf was used. Leaves were evaluated 4 days after infiltration and scored as insensitive (−) or sensitive (+).

DNA isolation and microsatellite marker analysis

Genomic DNA was isolated from young leaves according to Faris et al. (2000). Wheat microsatellite markers located on 2B and 5B were selected based on previously published maps (Röder et al. 1998; Somers et al. 2004; Sourdille et al. 2004; Song et al. 2005). The selected primers were synthesized according to the sequences queried from GrainGenes (http://www.wheat.pw.usda.gov/). In addition, many plants expressing necrosis in backcross populations were not amenable to Ptr ToxA infiltrations because they had only one normal leaf, which was used for DNA extraction. Therefore, the microsatellite marker *Xfcp1*, which is linked to *Tsn1* at 0.3 cM (Lu et al. 2006), was used to estimate the genetic distance between *Tsn1* and *Ne1*.

PCR reaction mixtures contained 200 nM of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 1 unit Taq polymerase (QIAGEN Sciences, Inc., Germantown, MD, USA), and 100–200 ng of template DNA. The amplifications were performed in a GeneAmp 9700 (Applied Biosystem, Foster City, CA, USA) thermal cycler. After 4 min at 94°C, 45 cycles were performed with 1 min at 94°C, 1 min at 50, 55, or 60°C (depending on the annealing temperatures of primers), 2 min at 72°C, and a final extension step of 10 min at 72°C.

The PCR products were mixed with 6 µl loading buffer (40% sucrose, 0.2% each of bromophenol blue and xylene cyanol). Electrophoresis was carried out on 10% polyacrylamide gels (0.4 mm thick) in 1× TBE (90 mM Tris-borate, 2 mM EDTA) at 60 W for 2 h. Gels were stained using 0.001% SYBR Green II (Sigma-Aldrich, St. Louis, MO, USA) for 30 min and scanned using a Typhoon 9410 variable mode imager (GE Healthcare, Waukesha, WI, USA). Linkage analysis was performed using MAPMAKER (Version 3.0b) (Lander et al. 1987) with a minimum LOD threshold of 3.0 and the Kosambi mapping function (Kosambi 1944).

Results

Genetic analysis of the F_2 population

Alsen is sensitive to Ptr ToxA while the SHW line TA4152-60 is insensitive to the toxin, indicating that Alsen carries *Tsn1*. *Tsn1* and *Ne1* are both located on chromosome arm 5BL (Faris et al. 1996; Nishikawa et al. 1974). Therefore, the phenotypic segregation of sensitivity to Ptr ToxA and autonecrosis in the F_2 population can be used to determine the genotypes of TA4152-60 and Alsen at the *Ne1* and *Ne2* loci. Based on

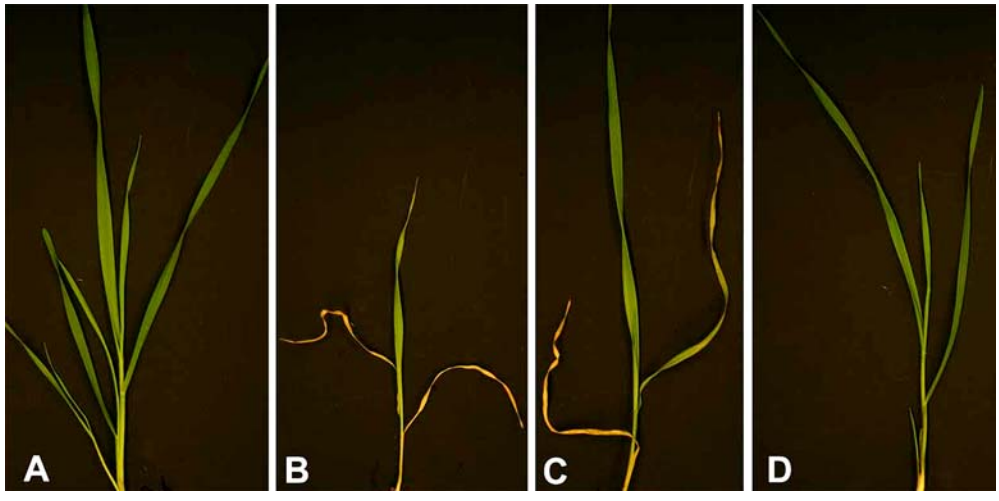

Fig. 1 Photographs of the seedling plants of hard red spring wheat cultivar 'Alsen' (**a**), F_2's with hybrid necrosis (**b** and **c**), and synthetic hexaploid wheat line TA4152-60 (**d**)

the reactions to Ptr ToxA and hybrid necrosis, four types of F_2 plants derived from TA4152-60/Alsen were observed: (1) sensitive to Ptr ToxA and showing hybrid necrosis (S&Hn), (2) sensitive to Ptr ToxA and with no hybrid necrosis (S&N), (3) insensitive to Ptr ToxA and showing hybrid necrosis (I&Hn), and (4) insensitive to Ptr ToxA and with no hybrid necrosis (I&N) (Table 1).

Hypothetically, if there was no linkage between *Ne1* and *Tsn1*, the expected segregation ratio in the F_2 population would approximate to 27 S&Hn:21 S&N:9 I&Hn:7 I&N [calculated from (3 S:1 I) × (9 Hn:7 N)] (Table 1). Because *Ne1* and *Ne2* are located on different chromosomes, this ratio would not be influenced by which necrosis gene is carried by Alsen. If *Ne1* and *Tsn1* were linked and Alsen carries *Ne1*, the expected segregation ratio in the F_2 would be 9 S&Hn:3 S&N:0 I&Hn:4 I&N (Fig. 2). On the other hand, if *Tsn1* was linked to *Ne1* and Alsen carries *Ne2*, the expected segregation ratio in the F_2 would be 6 S&Hn:6 S&N:3 I&Hn:1 I&N (Fig. 2). Therefore, if the observed segregation ratio falls between 27:21:9:7 and 9:3:0:4, it would suggest that Alsen carries *Ne1*, otherwise, Alsen would carry *Ne2* if the observed ratio falls between 27:21:9:7 and 6:6:3:1.

In this study, the observed segregation ratio of 165 F_2 plants was 67 S&Hn:61 S&N:21 I&Hn:16 I&N. The χ^2 test showed that the observed segregation was significantly different from the ratio of 9:3:0:4 ($P < 0.0001$), but it was not significantly different from the ratios of 27:21:9:7 ($P = 0.6$–0.7) and 6:6:3:1 ($P = 0.05$–0.10), suggesting that Alsen has the genotype *ne1ne1Ne2Ne2* whereas the SHW line TA 4152-60 has *Ne1Ne1ne2ne2* (Table 1). Because the observed ratio fits the ratio of 27:21:9:7 better than 6:6:3:1, it is suggested that *Ne1* is at a considerable distance from *Tsn1*.

Molecular mapping *Ne1* and *Ne2*

Results from F_2 analysis indicated that Alsen has the genotype *ne1ne1Ne2Ne2* whereas the TA4152-60 has *Ne1Ne1ne2ne2*. Therefore, the two populations derived from the backcrosses TA4152-60/2*Alsen and TA4152-60/Alsen//TA4152-60 were used to map *Ne1* and *Ne2* genes, respectively. The χ^2 test showed that segregation of the hybrid necrosis phenotype fits the expected ratio of 1:1 ($P = 0.4$–0.5), indicating that both were suitable mapping populations (Table 2).

A total of 64 microsatellite markers selected from previously published maps of chromosome 2B were surveyed for polymorphism between TA4152-60 and Alsen. Of these, only six detected polymorphic fragments linked to the *Ne2* gene in the population derived from the backcross TA4152-60/Alsen//TA4152-60 (Fig. 3a). *Xbarc55* was the marker most closely linked to *Ne2* at a genetic distance of 3.2 cM.

Comparison of the physical map of chromosome 2B (Sourdille et al. 2004) with our 2B genetic map indicated that our genetic map accounted for most of the physical size of the chromosome (Fig. 3a). The marker *Xgwm257*, which is the most distal short arm marker on our chromosome 2B map, is located in the distal 16% of 2BS (bin 2BS3-0.84-1.00) on the physical map. On the long arm, *Xwmc175* is the most distal on our map and it is located in the distal 50% of the arm (bin 2BL4-0.50-0.89). *Ne2* is flanked by markers *Xgwm148* and *Xbarc55*, which are both located in deletion bin 2BS1-0.53-0.75. This indicates that *Ne2* is located in the same bin, and shows that even though *Ne2* is physically far from the centromere, it is genetically close due to the commonly observed phenomenon of suppressed recombination in proximal regions.

A total of 40 microsatellite markers selected from previously published maps of chromosome 5B were surveyed for polymorphism between TA4152-60 and Alsen. Of these, five microsatellite primer sets detected polymorphic fragments and were subsequently mapped in the TA4152-60/2*Alsen population (Fig. 3b). *Xbarc74* was the marker most closely linked to *Ne1* at a genetic distance of 2.0 cM. The genetic distance between *Ne1* and *Xfcp1*, which is known to be 0.30 cM proximal to *Tsn1* (Lu et al. 2006), was 35.4 cM, suggesting that *Ne1* is about 35 cM from *Tsn1*. Sourdille et al. (2004) and Somers et al. (2004) indicated that *Xbarc216* is located in the centromeric region of chromosome 5B. The genetic distance between *Ne1* and *Xbarc216* is 8.3 cM, suggesting that *Ne1* is located on 5BL at a genetic distance of about 9 cM from the centromere.

Comparison of the deletion-based physical map presented by Sourdille et al. (2004) with the genetic map of 5B developed in this research indicated that *Ne1* is physically located in the proximal 55% of the long arm (Fig. 3b). Because *Xbarc216* lies physically close to the centromere and *Xbarc74* lies within bin 5BL6-0.29-0.55, we are unable to determine if *Ne1* exists in bin 5BL6-0.29-0.55 or bin C-5BL6-0.29. Nevertheless, *Ne1* lies in a proximal region of the chromosome arm, which is known to possess fewer genes than the distal regions and have a lower frequency of recombination (Akhunov et al. 2003).

Table 1 The segregation and χ^2 test for sensitivity to Ptr ToxA and hybrid necrosis in an F_2 population derived from the cross of TA4152–60/Alsen

Segregation ratio	χ^2 value	Probability
Observed ratio[a] S&Hn:S&N:I&Hn:I&N = 67:61:21:16		
Expected ratio		
If no linkage between Ne1 and Tsn1 S&Hn:S&N:I&Hn:I&N = 27:21:9:7	1.41	0.6–0.7
If Ne1 closely linked to Tsn1 and Alsen carries Ne1 S&Hn:S&N:I&Hn:I&N = 9:3:0:4	∞	< 0.0001
If Ne1 closely linked to Tsn1 and Alsen carries Ne2 S&Hn:S&N:I&Hn:I&N = 6:6:3:1	6.77	0.05–0.1

[a] *S* sensitive to Ptr ToxA, *I* insensitive to Ptr ToxA, *Hn* hybrid necrosis, *N* no hybrid necrosis

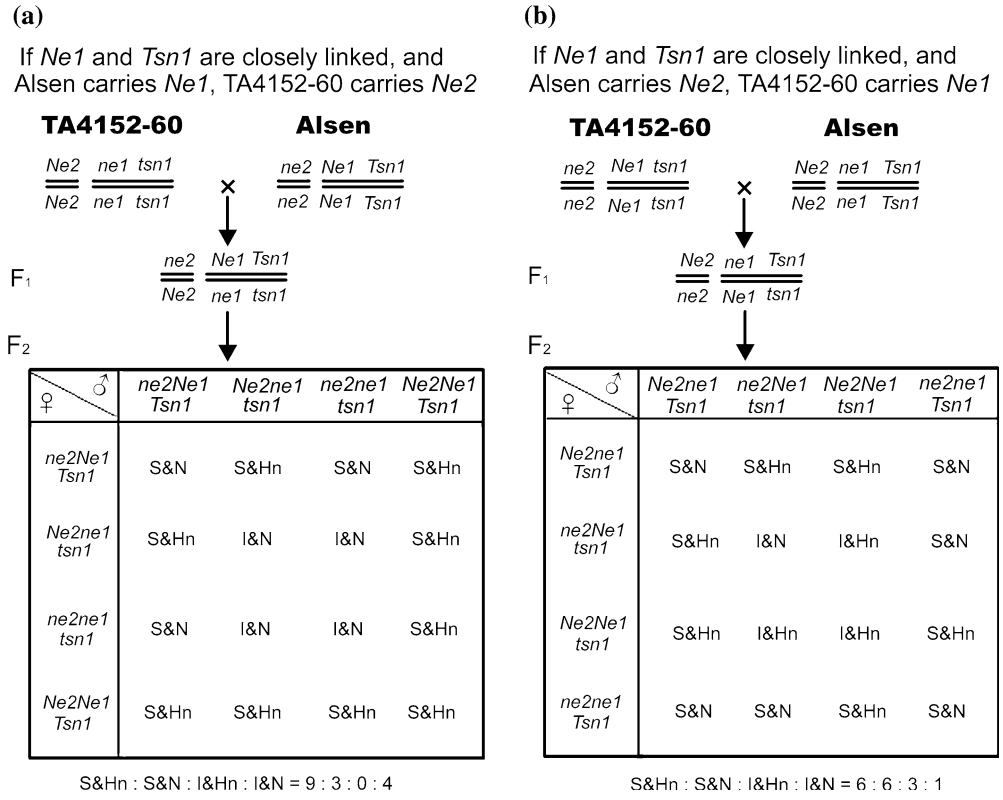

Fig. 2 Expected segregation ratios in the F_2 population derived from the cross TA4152-60/Alsen. *S* sensitive to Ptr ToxA, *I* insensitive to Ptr ToxA, *Hn* hybrid necrosis, *N* no hybrid necrosis

Discussion

Hybrid necrosis in wheat was first described in the 1940s (Caldwell and Compton 1943). Subsequently, a series of classical studies revealed that this phenomenon is genetically controlled by two complementary dominant genes *Ne1* and *Ne2* located on chromosome arms 5BL and 2BS, respectively (Tsunewaki 1960; Hermsen 1966; Zeven 1972; Nishikawa et al. 1974). The widespread occurrence of the two genes among elite lines of common wheat ecotypes throughout the world poses an obstacle in certain breeding efforts and genetic studies. Knowledge of the allelic state of major commercial cultivars and elite germplasm at the *Ne1* and *Ne2* loci would provide a guide for breeders and geneticists regarding parental selections in development of new cultivars/germplasms and mapping populations.

Table 2 The segregation and χ^2 test for hybrid necrosis in two backcross populations

Population	Observed segregation (necrosis:non-necrosis)	χ^2 1:1 ratio	
		Value	Probability
TA4152-60/2*Alsen	54:46	0.64	0.4–0.5
TA4152-60/Alsen//TA4152-60	51:43	0.68	0.4–0.5

The genetic analysis presented in this study showed that the HRSW variety Alsen and an elite CIMMYT SHW line TA4152-60 have the genotypes *ne1ne1Ne2Ne2* and *Ne1Ne1ne2ne2*, respectively. Because the F_1 hybrids of Alsen with the other three elite CIMMYT SHW lines TA4152-19, TA4152-37, and TA4152-44 exhibited hybrid necrosis, the three SHW lines should also have the genotype *Ne1Ne1ne2ne2*. Several studies demonstrated that the *Ne1* and *Ne2* genes are both present among different genotypes of hexaploid common wheat but only *Ne1* is commonly found in tetraploid wheat species (Hermsen 1963b; Tsunewaki 1970, 1992; Tomar et al. 1991). Because SHW lines are produced from hybrids between tetraploids and *Ae. tauschii*, necrosis genes found in SHW lines are usually *Ne1* derived from the tetraploid wheat parent. Therefore, Alsen or other *Ne2* carriers should not be used as parents in developing mapping populations for genetic studies of desirable traits in the SHW lines.

A number of early studies showed that although hybrid necrosis always occurred when the two dominant complementary genes *Ne1* and *Ne2* were brought together either in homozygous or heterozygous condition in a hybrid, large variation in the degree of necrosis was observed in different crosses (Hermsen 1963a, b, 1966; Singh et al. 1992). Hermsen (1963a) used a 0–9 scale to rate the degree of necrosis and classified the necrosis into three levels: (1) weak (0–3)—hybrids produce normal seeds, (2) moderate (3–6)—hybrids produce premature

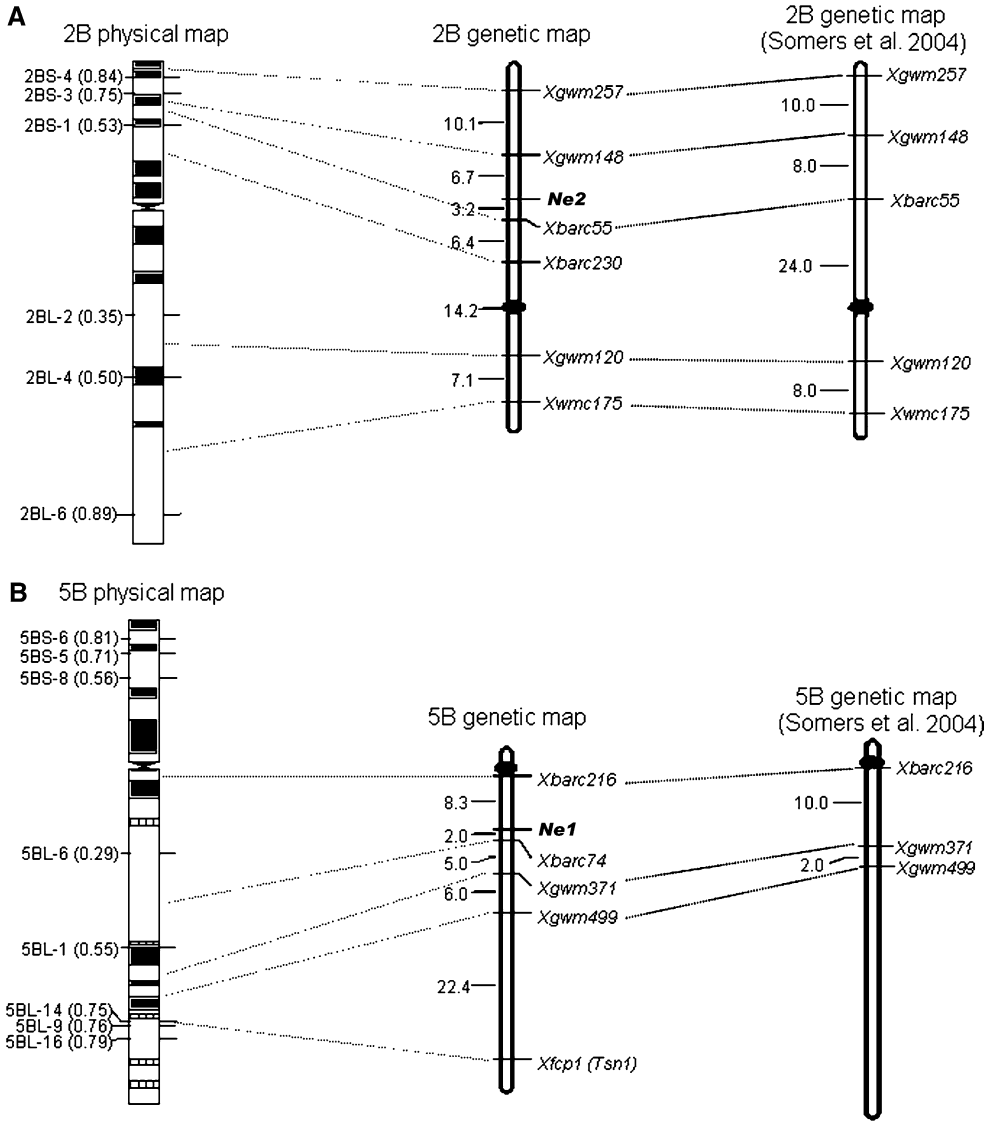

Fig. 3 Physical and genetic maps of chromosomes 2B (**a**) and 5B (**b**), which possess hybrid necrosis genes *Ne2* and *Ne1*, respectively. Physical maps including the deletion line designations and fraction breakpoints are indicated on the *left*. *Dark regions* represent C-bands. Genetic linkage maps of 2B and 5B with mapped *Ne2* and *Ne1* genes from this study were constructed using the populations derived from the backcrosses TA4152-60/Alsen//TA4152-60 and TA4152-60/2*Alsen, respectively, they are indicated in the *middle* with centiMorgan (cM) distances between markers indicated along the *left side* and molecular markers along the *right*. Physical locations of markers on the genetic maps are indicated by *dotted lines* extending from the marker to the appropriate deletion bin as presented in Sourdille et al. (2004). The genetic linkage maps of 2B and 5B developed by Somers et al. (2004) with markers in common with those used in this research are shown on the *right*

seeds, and (3) severe or strong (6–9)—hybrids produce no seed. The degree of necrosis in F_1 hybrids largely depends on different combinations of the three alleles *w* (weak), *m* (moderate), and *s* (strong) of *Ne1* and *Ne2* genes (Hermsen 1963a; Singh et al. 1992). So far, three *Ne1* alleles ($Ne1^w$, $Ne1^m$, and $Ne1^s$) and five *Ne2* alleles ($Ne2^w$, $Ne2^{mw}$, $Ne2^m$, $Ne2^{ms}$, and $Ne2^s$) have been differentiated (Hermsen 1963b; Zeven 1972). We observed that the necrosis in the F_1 hybrids of Alsen with the four elite CIMMYT SHW lines started at the tip of the first leaf when the second leaf became fully expanded. Then the necrosis gradually progressed to younger leaf tissue but new leaves were always normal as described by Hermsen (1966). After the flowering stage, the necrosis gradually progressed to the whole plant and caused leaves and sheaths to senesce prior to plant maturity. The plants produced premature or shriveled seed. This observation indicated that the hybrids of Alsen with the four elite CIMMYT SHW lines had moderate necrosis.

Expressivity of hybrid necrosis is affected by the dosage of *Ne* alleles, genetic background, and environment (Hermsen 1963b; Zeven 1972). We observed that although the F_1 hybrids from cross of TA4152-60/Alsen developed moderate necrosis, some F_2 plants could not survive due to severe necrosis at the seedling stage. The severe necrosis of some F_2 plants was probably caused

by increased dosage of two dominant genes *Ne1* and *Ne2* due to homozygosity. Therefore, the populations derived from the F_1 hybrids with moderate hybrid necrosis might not be suitable for genetic analysis due to loss of some genotypes. However, because the plants with moderate hybrid necrosis could produce viable seed, moderate hybrid necrosis should not be a serious obstacle for transferring desirable genes from a donor parent to a recurrent parent using backcross methods.

The locations of the *Ne1* and *Ne2* loci on genetic linkage maps were previously investigated using classical genetic analysis in a few studies. Using telocentric analysis, Nishikawa et al. (1974) mapped *Ne1* at 10.5 ± 2.0 cM from the centromere on 5BL and *Ne2* at 9.4 ± 1.5 cM from the centromere on 2BS. Mirua et al. (1992) established a gene order of breakpoint—*Ne1–Vg1–Ibf-B1* and mapped the *Ne1* 6 cM from the centromere of 5BL using the morphological marker *Vg* (winter variegation), the isozyme marker *ibf-B1*, and 5B/7B translocation breakpoints. They observed that ear emergence and plant height were associated with both *Ne1* and *Vg* (Mirua et al. 1992). The gene *Ne2* was linked to the dwarf gene *D2* (Hermsen 1967). Singh (1993) reported that the gene *Lr13* for adult-plant resistance to leaf rust was tightly linked with the $Ne2^m$ allele. All wheat lines carrying *Lr13* have $Ne2^m$ (Wamishe and Milus 2004). Since Alsen was found to have *Lr13* (Oelke and Kolmer 2005), it should have $Ne2^m$. This is consistent with the fact that hybrids between Alsen and the four elite CIMMYT SHW lines had moderate necrosis.

Availability of a large number of molecular markers and high density of linkage maps in wheat provide useful tools for more precisely mapping the necrosis genes. Using microsatellites we identified multiple markers linked to *Ne1* on 5BL and *Ne2* on 2BS. The closely linked microsatellite makers *Xbarc74* and *Xbarc55* identified in this study might be useful for evaluating whether the parents are carriers for hybrid necrosis genes before a cross is made. Furthermore, elimination of the hybrid necrosis alleles would be helpful in gene transfer via hybridization, and it can be accomplished using a backcross method coupled with marker-assisted selection. The molecular mapping results of our work revealed the genetic linkage relationship of *Ne1* with the well-documented locus *Tsn1*. The genetic distance between *Ne1* and *Tsn1* suggests that hybrid necrosis and toxin-induced necrosis are controlled by different genetic systems.

The study of mechanisms underlying hybrid necrosis is an interesting research topic. Khanna-Chopra et al. (1998) reported that the occurrence of hybrid necrosis was related to increased levels of superoxide anions. High superoxide content was associated with increased lipid peroxidation and membrane damage (Dalal and Khanna-Chopra 1999). Dalal and Khanna-Chopra (2001) observed that hybrid plants had increased peroxidase and superoxide dismutase activity, but had decreased catalase activity as compared to their parents during the progression of necrosis (Dalal and Khanna-Chopra 2001). They concluded that hybrid necrosis in wheat leaves is associated with oxidative stress without a well-coordinated antioxidant defense system (Dalal and Khanna-Chopra 2001). Details regarding the molecular basis and mechanisms associated with hybrid necrosis in wheat are still not well understood. The molecular mapping work conducted in this study revealed the locations of the two hybrid necrosis genes in the wheat genome, and thus provides a possibility to further uncover the structure, function, and products of the two necrosis genes.

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