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Genetic Mapping and Characterization of Lethal Necrotic Mutants in Rice (*Oryza sativa* L.)

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Abstract

The spread of tissue necrosis leads to plant death. We isolated 18 lethal necrotic mutants induced in rice (*Oryza sativa* L.) by gamma-irradiation. The necrotic lethality among the 18 mutants was controlled by single recessive genes designated *necrotic lethality*1 (*nec*1) to *nec*1*8*. These mutants display pale-green leaves from the third-leaf stage and leaf-tip necrosis, which spreads to the whole plant, killing it. Genetic mapping and histochemical analysis of the lethal necrotic mutants were conducted. At least four independent loci on chromosomes 2 and 4 controlled necrotic lethality. Therefore, the genetic causes of lethal necrosis vary among mutant stocks. Histochemical analysis at 12 days after sowing showed that H_2O_2 accumulated in the necrotic parts of leaves, and that cell death occurred throughout the leaf. Mutants for early necrotic lethality (<24 days to lethality) were characterized by the rapid spread of H_2O_2 accumulation throughout the third leaf. Mutants for late necrotic lethality (>35 days to lethality) were characterized by the incomplete spread of H_2O_2 accumulation within the third leaf.

Keywords

Rice, Mutant, Necrotic Lethality, Linkage Mapping, Reactive Oxygen Species

1. Introduction

Necrosis of plant tissues usually shows as brown or black discoloration. Stresses such as fungal infections can cause necrosis in any tissues [1]. Necrosis is common in relation to nutrient supply; for example, deficiencies in potassium can cause severe necrotic lesions in leaves [2] [3] [4] [5] [6]. In addition, hybrid necrosis, caused by deleterious epistatic interactions, results from spontaneous activation of plant defenses associated with leaf necrosis [7] [8] [9]. Necrotic lesion formation has been reported in several mutants. The maize *lethal leaf spot* 1

(*lls*1) shows enhanced resistance to fungal infections due to necrotic lesion formation [10]. The Arabidopsis *botrytis-susceptible* 1 (*bos*1) mutant develops necrosis to resist infection by *Dickeya dadantii* [11]. Several lesion mimic mutants (LMMs) of rice show broad-spectrum resistance to blast and bacterial blight [12]. Excessive phosphate accumulates in the shoots of the *phosphate-accumulator* 2 (*pho*2) mutant of Arabidopsis, which develops necrotic symptoms [13] [14]. The rice *leaf tip necrosis* 1 (*ltn*1) mutant lacks a protein containing a ubiquitin-conjugating domain, which regulates phosphate accumulation, and develops leaf tip necrosis [15]. The phenotypes in most lesion mimic and necrotic mutants are often influenced by environmental factors [16]. Light and temperature affect the severity of mutant phenotypes in rice *zebra necrosis* [17] and *faded green leaf* [18] mutants and maize *lls*1 mutants [10] [19]. Under short-day conditions, a barley lesion-mimic mutant, "1661", fails to reach maturity and does not produce seeds [20]. These mutants can survive to maturity. In addition, mutants in which necrosis spreads to the whole plant have been reported in maize: at least eight recessive mutants (*nec*1 - *nec*7, *nec-t*; [21] [22] showed leaf discoloration, necrotic leaf tips, spots, or transverse bands before death. However, rice mutants showing necrotic lethality have not yet been isolated. In plants, reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide and hydroxyl radicals are constantly produced as metabolic byproducts in chloroplasts, mitochondria, and peroxisomes [23] [24]. Under stress, ROS production exceeds the cells' scavenging capacity, and the ROS radicals induce necrotic lesions and alter the expression of certain genes in many signaling pathways, eventually leading to accelerated cell death [23] [24]. In maize, Arabidopsis, and rice [25] [26] [27] [28], the accumulation of ROS in particular, H_2O_2 and the presence of dead cells are studied as biochemical markers constitutively expressed in LMMs [29]. They are commonly monitored by staining using histochemical dyes: 3',3'-diaminobenzidine (DAB) and trypan blue, respectively [29] [30]. Although H_2O_2 is known to cause cell death leading to necrotic lesions in many plants [31], little is known about ROS-induced necrotic lethality. In this study, we isolated 18 gamma-irradiated rice mutants with necrotic lethality at the seedling stage. We analyzed them to map the genes responsible for their necrotic lethality and histochemically characterized them for H_2O_2 accumulation and cell death.

2. Materials and Methods

2.1. Plant Materials

Two rice cultivars; a *japonica* cultivar, "Taichung 65" (T65) and *indica* cultivar "IR24" were gamma irradiated as described by Yamagata *et al.* [32]. The spikelets of these cultivars were exposed to 100 - 150 Gy dosage just before anthesis using the Co^{60} γ-ray irradiation facility of Institute for Irradiation and Analysis of Quantum Radiation, Kyushu University. Self-pollinated M_1 plants were grown and M_2 seeds were harvested and used to generate M_3 plants. Self-pollination and screening of these mutant stocks were conducted and the resulting mutant

stocks were maintained in the Plant Breeding Laboratory, Kyushu University. These mutants were designed as LEM (lethal mutants) (Table 1). Seeds of the mutant lines were sown on 25 May 2016 on sieved soil containing 2.63 $g \cdot m^{-2}$ of NPK, and the seedlings were grown in a greenhouse under natural conditions at Kyushu University Farm, Fukuoka, Japan (33˚36'55"N). For phenotypic characterization, ten plants were observed per mutant line. Surviving *nec18* mutant seedlings were transplanted into the paddy field and observed until 15 August 2016. For histochemical analysis, plants were grown in a phytotron maintained at 25˚C under natural sunlight in 2016 and 2017 at Kyushu University. For genetic mapping of the mutants, we crossed the wild-type segregants of twelve T65-derived mutant lines, LEM1 (*nec*1), LEM2 (*nec*18), LEM9 (*nec*4), LEM11 (*nec*16), LEM16 (*nec*5), LEM17 (*nec*6), LEM18 (*nec*8), LEM20 (*nec*14), LEM25 (*nec*15), LEM29 (*nec*9), LEM36 (*nec*10), and LEM37 (*nec*11) with *japonica* cultivar "Hinohikari", and crossed normal segregants of two IR24_derived mutant lines, LEM41 (*nec*17) and LEM43 (*nec*13) with T65. The resulting F_2 populations in which necrotic lethality segregated were used to map necrosis genes.

Table 1. Identification of genes responsible for necrotic lethality in the 18 mutant lines.

Gene	Mutant line	Background	DTL	Segregation for necrotic lethality			*P*[a]	BSA	H_2O_2 accumulation[b]
				Wild type	Necrotic lethality	Total			
*nec*1	LEM1	T65	16	68	21	89	0.76	2L	Entire
*nec*2	LEM4	T65	16	87	19	106	0.09	-	-
*nec*3	LEM5	T65	16	88	20	108	0.12	-	-
*nec*4	LEM9	T65	16	82	23	105	0.46	2S and 2L	Entire
*nec*5	LEM16	T65	16	83	29	112	0.83	2S and 2L	Entire
*nec*6	LEM17	T65	16	87	20	107	0.13	2S and 2L	Entire or Tip
*nec*7	LEM18	T65	16	80	21	101	0.33	2L	Entire
*nec*8	LEM24	T65	19	71	23	94	0.91	-	-
*nec*9	LEM29	T65	19	87	20	107	0.13	2L	Entire
*nec*10	LEM36	T65	19	154	49	203	0.78	2L	Entire
*nec*11	LEM37	T65	19	100	27	127	0.33	2L	Entire or Tip
*nec*12	LEM28	T65	21	74	22	96	0.64	-	-
*nec*13	LEM43	IR24	21	75	36	111	0.07	2L	Entire
*nec*14	LEM20	T65	24	63	14	77	0.17	4	Entire
*nec*15	LEM25	T65	35	80	20	100	0.25	4	Tip
*nec*16	LEM11	T65	35	86	22	108	0.27	4	Tip
*nec*17	LEM41	IR24	35	83	18	101	0.10	2S	Transverse band
*nec*18	LEM2	T65	82	83	22	105	0.34	4	Tip

[a]Probability in χ^2 test of a 3:1 ratio. [b]Observations on the third leaves of mutant at 12 days after sowing.

2.2. Development of Indel Markers

To find polymorphic indel sites in temperate *japonica*, we conducted *de novo* whole-genome sequencing of T65 by next-generation sequencing on a Roche/454 GS-FLX Titanium sequencer (Roche, Basel, Switzerland) and *de novo* assembly in GS *De Novo* Assembler software (Roche). The assemblies were used for BLAST similarity searches of Nipponbare pseudo-molecule build 4.0 (BLAST). Polymerase chain reaction (PCR) primers for polymorphic sites with >5-bp insertion/deletion variation were designed in Primer 3 software [33] (**Supplementary Table S1**, **Supplementary Table S2**).

2.3. Genotyping

Fresh leaves of F_2 seedlings and their parents were collected and ground in 1× TE buffer in a Multi-Beads Shocker (Yasui Kikai, Osaka, Japan), and their total genomic DNA was extracted using a modified potassium acetate method [34]. The obtained DNA was amplified by PCR with simple sequence repeat (SSR) markers [35] [36] [37] [38] [39] and indel markers (**Supplementary Tables S1**, **Supplementary Table S2**). PCR was performed in 15-μL mixtures containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 200 μM each dNTP, 0.2 μM each primer, 0.75 units of *Taq* polymerase (Takara, Otsu, Japan), and approximately 5 ng of template DNA in a GeneAmp PCR system 9700 (Applied Biosystems). PCR conditions were 95˚C for 5 min, followed by 35 cycles of 95˚C for 30 s, 55˚C for 30 s, and 72˚C for 30 s. PCR products were separated in 4% agarose gels (Agarose HT, Amresco Inc., Solon, OH, USA) in 0.5× TBE buffer at 250 V.

2.4. Gene Mapping

To determine the loci responsible for necrotic lethality, we used bulked segregant analysis (BSA) [40] to detect markers tightly linked to causal genes using 43 SSR and 38 indel markers polymorphic between T65 and Hinohikari, and 84 SSR and 3 indel markers polymorphic between IR24 and T65. Bulks of wild-type and mutant plants were each composed of 10 individuals from the F_2 generation. Markers showing polymorphism between the two bulks were selected and used for linkage analysis of the F_2 populations based on calculation of recombination value by the maximum likelihood method [41]. The estimated recombination values were converted to map distances by Kosambi's mapping function [42] to construct linkage maps.

2.5. ROS Accumulation

To observe excessive accumulation of ROS in necrotic mutants, we used DAB staining to assess H_2O_2 accumulation [43]. Because necrosis was observed in third leaf of all the mutants at 12 days after sowing (DAS), third leaves of necrotic lethal mutants and wild-type plants at 12 DAS were immersed in 1 mg mL^{-1} DAB in distilled water and incubated for 8 h at 25˚C to allow DAB uptake, with three replicates. After staining, chlorophyll was completely removed by in-

cubation in 96% ethanol at 80˚C for 1 h. The leaf samples were preserved in 70% ethanol and later photographed.

2.6. Detection of Cell Death

Trypan blue staining was used to assess cell death as a result of necrosis [12]. In brief, fresh leaves (third leaf) were submerged in a solution of 2.5 mg·mL^{-1} trypan blue, 25% (w/v) lactic acid, 23% (v/v) water-saturated phenol, and 25% (v/v) glycerol in distilled water. The samples were heated in boiling water for 2 min, placed in chloral hydrate solution (25 g in 10 mL of distilled water) for destaining, and preserved in 70% glycerol.

3. Results

3.1. Identification of Mutants for Necrotic Lethality

Necrotic lethality of mutant seedlings was observed in the self-pollinated progeny of the heterozygous plants in all 18 mutant lines. Wild-type and lethal necrotic seedlings segregated in a 3:1 ratio, indicating that the lethal necrosis was controlled by single recessive genes, designated *necrotic lethality*1 (*nec*1) to *nec*18 (**Table 1**).

3.2. Phenotypic Characterization of Necrotic Mutants

Before the third-leaf stage at 6 DAS, the phenotypes of the 18 lethal necrotic mutants were similar to those of the wild-type plants except for the *nec*1*7* mutant, which had yellow-green leaves by 4 DAS (**Supplementary Table S3**). At the third-leaf stage, at 7 DAS, the second and third leaves of the *nec*1 mutant were pale green (**Figure 1(a)**, and leaf-tip necrosis began in the second leaf. The necrosis spread to the entire leaf blade of the second and third leaves at 12 DAS (**Figure 1(b)**, **Supplementary Table S3**), and the plant died at 16 DAS (**Supplementary Table S3**), during the forth-leaf stage (**Figure 1(c)**).

Similar pale-green discoloration and expansion of the necrosis to the entire plant were observed in all mutants except *nec*17. Different days to lethality (DTL) were recorded: 16 DTL in *nec*2, *nec*3, *nec*4, *nec*5, *nec*6, and *nec*7, 19 DTL in *nec*8, *nec*9, *nec*10, and *nec*11, 21 DTL in *nec*12 and *nec*13, 24 DTL in *nec*14, and 35 DTL in *nec*15, *nec*16, and *nec*17 (**Table 1**, **Supplementary Table S3**). *nec*18 seedlings transplanted into the paddy field at 19 DAS died at 82 DAS (**Figure 1(d)**; **Supplementary Table S3**). In contrast to the other lines, the *nec*17 plants became yellow-green as early as 4 DAS and developed transverse necrotic bands at the second- to third-leaf stage (**Figure 1(e)**, **Figure 1(f)**; **Supplementary Table S3**). The necrosis in *nec*17 gradually spread to the whole plant, and plants died at 35 DAS.

3.3. Bulked Segregant Analysis

BSA of 14 mutant lines revealed that in the F_2 population between *nec*1 and Hinohikari, wild-type and mutant plants segregated in a 69:27 ratio, fitting a 3:1

Figure 1. Three representative mutants for necrotic lethality. (a)-(c) Early necrotic lethal mutant, *nec*1. (a) *nec*1 (right) shows pale-green discoloration in second and third leaves at 7 days after sowing (DAS) while wild-type plant (left) possesses second and third leaves with green color; (b) Leaf-tip necrosis in *nec*1 (right) spread to entire leaf blade of second leaf at 12 DAS; (c) Complete death of whole plant of *nec*1 (right) occurs at 16 - 19 DAS. (d) *nec*18 survived in paddy field until 82 DAS; (e)-(f) Transverse bands of necrosis in *nec*17. (a)-(c), (e) Left and right plants were wild types and mutants, respectively.

ratio (Table 2). At indel markers QSTS96 and QSTS109, on the long arm of chromosome 2 (2L), the *nec*1 bulks showed an intensive band derived from T65, but the wild-type bulks showed heterozygous-like bands derived from T65 and Hinohikari alleles (Supplementary Figure Sl). BSA of the other 13 mutants suggested candidate regions on the short and long arms of chromosome 2 (2S and 2L) and on the long arm of chromosome 4 (Table 1; Supplementary Table S4).

3.4. Validation by Linkage Mapping

Among the 14 necrotic lethal mutants in which responsible regions were identified, we conducted linkage analysis for *nec*1, *nec*13, *nec*16, *nec*17, and *nec*18. *nec*1 was located between QSTS103 and QSTS109 on chromosome 2L (Figure 2(b); Supplementary Table S6). *nec*13 was located in the same region, between QSTS96 and QSTS109 (Figure 2(c); Supplementary Table S7). *nec*17 was located between RM3703 and RM7082 on chromosome 2S (Figure 2(a); Supplementary Table S5). *nec*16 and *nec*18 were located on different regions of chromosome 4L: *nec16* tightly linked to RM3785 and RM7051 (Figure 2(d); Supplementary Table S8), and *nec18* tightly linked to QSTS141 and QSTS142 (Figure 2(e); Supplementary Table S9).

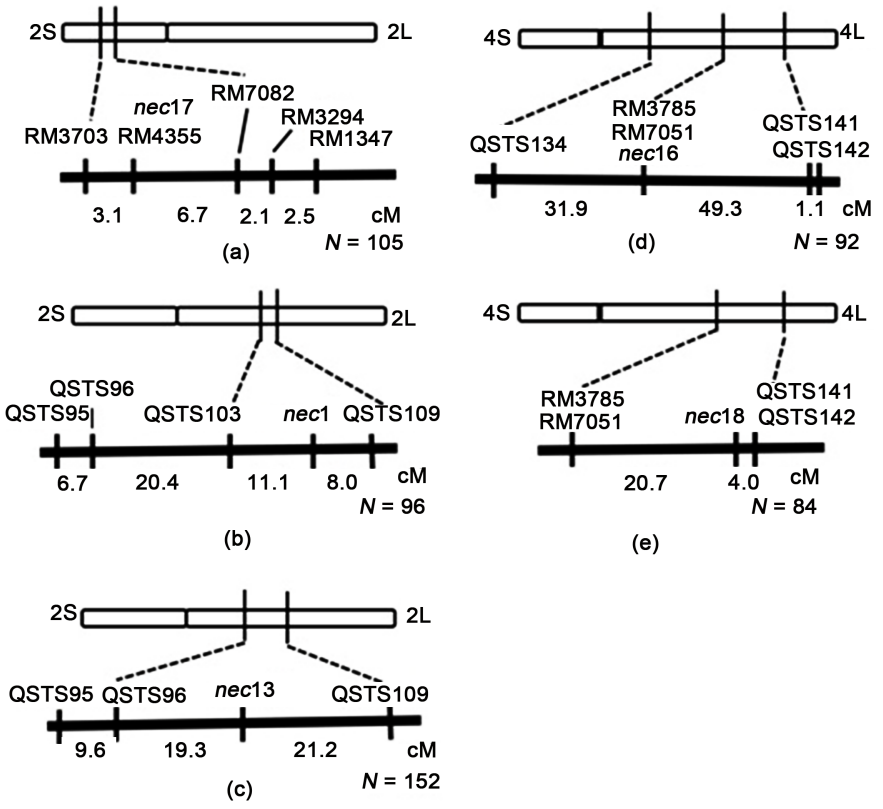

Figure 2. Linkage maps of genes for necrotic lethality in rice. (a) *nec*17; (b) *nec*1; (c) *nec*13; (d) *nec*16; (e) *nec*18.

Table 2. Segregation of wild-type and mutant plants in F_2 populations.

Cross combination		Gene	F_1 phenotype	Segregation for necrotic lethality			
Female	Male			Wild type	Necrotic lethality	Total	P^a
Hinohikari	LEM1	*nec*1	wild type	69	27	96	0.48
Taichung 65	LEM43	*nec*13	wild type	110	42	152	0.45
Hinohikari	LEM11	*nec*16	wild type	76	20	96	0.35
Taichung 65	LEM41	*nec*17	wild type	78	34	112	0.19
Hinohikari	LEM2	*nec*18	wild type	69	27	96	0.48

a. Probability in χ^2 test of a 3:1 ratio.

3.5. Allelism between *nec*1 and *nec*13

*nec*1 and *nec*1*3* were located in the same region on chromosome 2 L (Figure 3(b), Figure 3(c)). We evaluated allelism in F_1 progeny of crosses between *nec*1 and *nec*13. If *nec*1 and *nec*13 are located at different loci, F_1 plants derived from a cross between *nec*1 heterozygotes (+/*nec*1) and *nec*13 heterozygotes (+/*nec*13) would show only wild-type segregants. However, necrotic segregants were observed in F_1 derived from the cross between the heterozygous plants in three replicates, indicating that *nec*1 and *nec*1*3* are located at the same locus (Table 3).

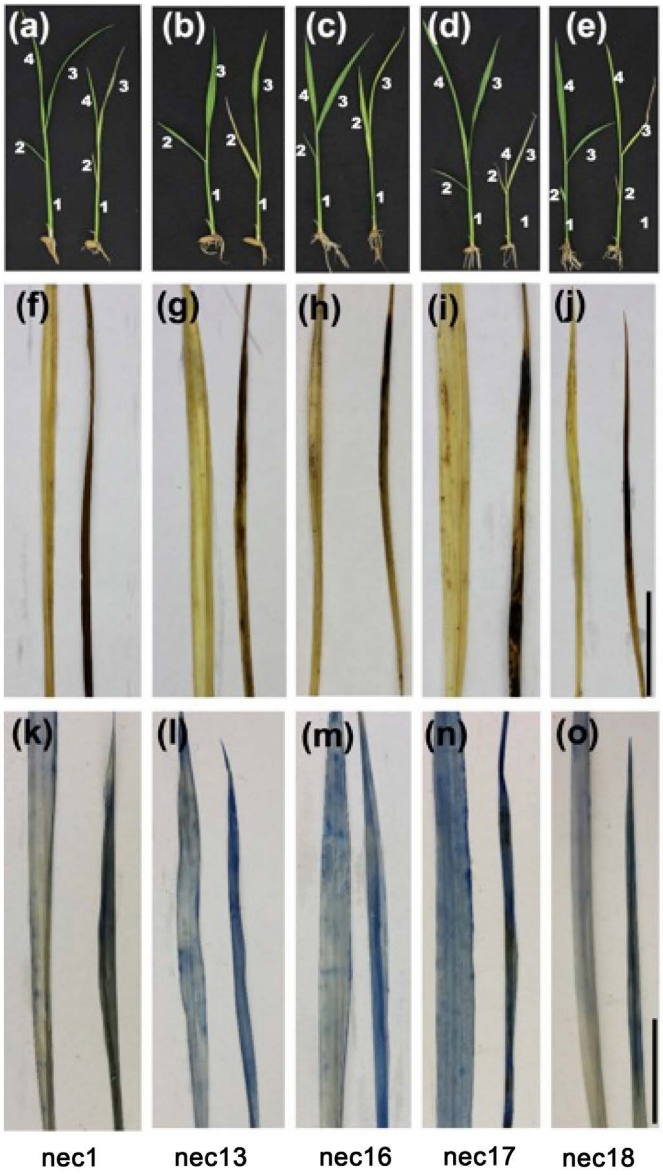

Figure 3. ROS accumulation and cell death in the third-leaves of necrotic mutants at 12 DAS. (a)-(e) Morphological features of wild type (left) and necrotic mutants (right); (f)-(o) Histochemical staining in the third leaf blade of wild type (left) and mutant (right) seedlings at 12 DAS by (f)-(j) DAB staining of H_2O_2 and (k)-(o) trypan blue staining for cell death. Bar = 5 cm. Left and right plants were wild types and mutants, respectively.

Table 3. Segregation in F_1 population derived from *nec*1 × *nec*13.

Cross combination[a]				Segregation for necrotic lethality in F_1		
Female		×	Male	Wild type	Necrotic lethality	Total
LEM43-4	(+/*nec*13)		LEM1-5 (+/*nec*1)	34	6	40
LEM43-8	(+/*nec*13)		LEM1-7 (+/*nec*1)	16	19	35
LEM43-12	(+/*nec*13)		LEM1-12 (+/*nec*1)	20	20	40

a. Genotypes are represented in parentheses.

3.6. Histochemical Characterization of Lethal Necrosis

Of the eighteen mutants, fourteen were initially characterized for their H_2O_2 accumulation of the third leaves at 12 DAS (**Supplementary Figure S2**). Five lethal necrotic mutants showed phenotypic differences in patterns of H_2O_2 accumulation and cell death at 12 DAS: *nec*1 and *nec*13 showed early necrotic lethality (<24 DTL; **Figure 3(a)**, **Figure 3(b)**), and *nec*16, *nec*17, and *nec*18 showed late necrotic lethality (>35 DTL; **Figures 3(c)-(e)**, **Supplementary Figure S2**; **Table 1**). Intense brown staining indicated H_2O_2 accumulation throughout the third leaves of *nec*1 (**Figure 3(f)**) and *nec*13 (**Figure 3(g)**), and in the other early necrotic lethal mutants (*nec*4, *nec*5, *nec*7, *nec*9, *nec*10, and *nec*14: **Table 1**; **Supplementary Figure S2**), as well as in the tips of the third leaves of *nec*16 (**Figure 3(h)**), *nec*18 (**Figure 3(j)**), and *nec*15 (**Table 1**; **Supplementary Figure S2**). The staining pattern of the third leaves in *nec*6 and *nec*11 were not clearly classified into the entire or leaf-tip types. The third leaves of *nec*17 showed a transverse banding pattern of brown staining (**Figure 3(i)**). Trypan blue staining revealed deep blue throughout the leaf blades of *nec*1, *nec*13, *nec*16, *nec*17, and *nec*18 (**Figures 3(k)-(o)**), but not in the wild-type plants. Taken together, these results suggest that excessive accumulation of H_2O_2 caused necrotic symptoms in the mutants.

4. Discussion

We identified 18 mutants characterized by necrotic seedling lethality. The necrotic lethality was controlled by single recessive genes which we designated *nec*1 to *nec*18 (**Table 1**). The necrosis began with leaf discoloration and necrosis formation at the leaf tip, or with transverse banding segments, during the third-leaf stage, and plants died during the fourth- to fifth-leaf stages, between 16 and 82 DAS (**Figure 1**; **Supplementary Table S3**). The initiation of necrosis at the third-leaf stage, the spread of necrosis from the leaf tip to the entire leaf, and early necrotic lethality were similar to those of lethal necrotic mutants in maize *nec*4, *nec*5, *nec*7, and *olive-necrotic*-8147 [21] [44] [45] [46].

A number of rice mutants with disease-like lesions were morphologically categorized into spotted leaf mutants (*spl*: [47] [48] [49], lesion mimic mutants (*lmm*: [27] [50], cell death and resistance mutants (*cdr*: [51] and zebra necrosis mutants (*zn*: [17] [52]. DAB staining indicated that ROS accumulation was limited to the necrotic lesions. In our study, the lethal necrotic mutants showed necrosis of the whole leaves and eventually plant death. The mutants with early necrotic lethality (*nec*1, *nec4* - *nec7*, *nec9* - *nec*11, *nec*13, and *nec*14) had rapid spread of H_2O_2 accumulation throughout the leaf at 12 DAS (**Table 1**; **Supplementary Figure S2**). Those with late necrotic lethality (*nec*15, *nec*16, *nec*18), in contrast, had H_2O_2 accumulation only at the leaf tip at 12 DAS.

Genetic mapping by BSA and linkage analysis revealed at least four independent loci that control necrotic lethality in 14 of the mutants (**Table 1**). The mutants with causal gene on chromosome 2L (*nec*1 and *nec*13) showed early lethal-

ity within 21 DAS, with excessive H_2O_2 accumulation throughout their leaves (**Figure 2**, **Figure 3** and **Supplementary Table S3**). These phenotypes have also been reported in a necrotic mutant of maize, *nec-t* [22]. *nec-t* has abnormal chloroplast development and lacks photosynthetic ability. The mutants with causal genes on chromosome 4 (*nec*16 and *nec*18) had late necrotic lethality (>35 DTL) and excessive ROS production progressing from their leaf tips (**Figure 2**, **Figure 3**, **Supplementary Figure S2**, **Supplementary Table S3**). Additionally, *nec*17 on chromosome 2S, induced late necrotic lethality (35 DTL) with distinct transverse bands (**Figure 3**). Rice *ZEBRA-NECROSIS* (*ZN*) encodes a thylakoid-bound protein involved in the photoprotection of developing chloroplasts during early leaf development, and the *zn* mutant shows leaves with transverse necrotic bands due to accumulation of light-induced ROS [17]. The above-mentioned mutants would be important resources for investigating mechanisms such as stress tolerance, photoprotection of chloroplasts, and nutrient deficiency in plants.

5. Conclusion

We identified 18 lethal necrotic mutants induced in rice by gamma-irradiation. The necrotic lethality was found to be controlled by single recessive genes designated *necrotic lethality*1 (*nec*1) to *nec*18. Among them, five mutants were precisely mapped on the rice chromosomes: *nec*17 on chromosome 2S, *nec*1 (*nec*13) on chromosome 2L, and *nec*16 and *nec*18 on different regions of chromosome 4L. Thus at least four independent loci control necrotic lethality in rice. The histochemical analysis of these mutants at 12 DAS showed that H_2O_2 accumulated in the necrotic parts of leaves, and that cell death occurred throughout the leaf. Mutants for early necrotic lethality (*nec*1 and *nec*13) were characterized by the rapid spread of H_2O_2 accumulation throughout the third leaf. Mutants for late necrotic lethality (*nec*16, *nec*17 and *nec*18) were characterized by the incomplete spread of H_2O_2 accumulation within the third leaf.

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Supplementary

Table S1. List of markers for linkage analysis of mutants induced from Taichung 65.

Chr.	Position[a]	Marker name	Marker type	Forward primer sequence	Reverse primer sequence	Reference
1	548,641	RM3148	SSR	GCTTTGGTATTTGCAGGTTCACG	CTATTGCTCGAACACTTTGCTTCTCC	McCouch *et al.* (2002)
1	2,575,209	QSTS60	Indel	AAAGCTCTGAGTGGCTTTGC	TTGGTTTGTCGATATGTTCAGC	This study
1	4,635,844	QSTS76	Indel	GCGAAAACACAATGCAAAAAG	GTGTGGGGGAAGCAAGAG	This study
1	23,968,523	RM5	SSR	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG	Panaud *et al.* (1996)
1	26,035,809	RM3475	SSR	GTCGGTTTGCCTAGTTGAGC	TTCCTCGGTGTATGGGTCTC	McCouch *et al.* (2002)
1	30,718,595	QSTS82	Indel	TAACAACGGGGGCCTAGATG	GCCGGTGGTGAAGACGAC	This study
1	40,246,974	RM3520	SSR	GAGGCTATATGCTCATGCTC	AAACCTGCAAATGCACAG	McCouch *et al.* (2002)
2	1,878,328	RM7562	SSR	AGACATGCCAATGTGATGGC	TCGGTAGTATGGGGCTTGTC	McCouch *et al.* (2002)
2	4,414,049	RM3865	SSR	AACCATGGACAGTTGAACAC	CTCCGACAAGAACTTCCTC	McCouch *et al.* (2002)
2	5,477,492	RM6378	SSR	CTGATCATCTCATGCCTCCTACG	TCCATCTCCCAATATGACCAACC	McCouch *et al.* (2002)
2	5,852,749	QSTS88	Indel	GCCATGGAAAAGAAAAGCTG	TGATCGATGGATAGCCACAC	This study
2	7,112,707	QSTS89	Indel	TTTTGCACGGTTTTGTATGG	AACCGATGTCTGCATCCAAG	This study
2	9,984,944	QSTS90	Indel	GAGGAGGAGGAACGAAGAGG	GTTCGCTCCTGACCTTCG	This study
2	9,998,681	QSTS91	Indel	GAGGAGGAGGAACGAAGAGG	GTTCGCTCCTGACCTTCG	This study
2	10,186,627	RM3501	SSR	CTACAATGATTCCATGCCTGTCC	TCCGGCTCAAGCTACAGTTAAGG	McCouch *et al.* (2002)
2	17,297,690	QSTS95	Indel	TGAGGACAAGCATGTGAAGG	CAATGGACGCTAGTGGAACC	This study
2	18,744,542	QSTS96	Indel	GCTCGTGTTCGTGCATCTATC	GCAACAACGAGAACGAGAAC	This study
2	20,751,269	QSTS103	Indel	TTCGTTTCGAGTAACAAAGCAC	GTGTTAGGGAAGCACATTCG	This study
2	24,254,973	QSTS105	Indel	AATATTGTAAACAAAAACTCAAAATCC	TTCTTCTCTCTCCGCCTTCC	This study
2	24,518,130	QSTS109	Indel	AATATAGTTTGGTCAAAAGAACAAAAG	CGGATAATCTTGATGAGTGAACC	This study
2	24,531,197	QSTS110	Indel	TGTTGGTGGTCAGTTCAACTTC	TCCCGCCTTCCATTTATTAC	This study
2	24,656,209	QSTS111	Indel	CGGAGGCAGAGTAAGAATCG	CAATCCCGATTGTCGGATAC	This study
2	32,812,348	QSTS114	Indel	GAAAATTTGCATTCACTCCTACC	TTGAAAAACACGGGAATTTTATAG	This study
2	33,019,362	RM6733	SSR	TCCATGTGCACAATCCAATTCC	GTGACGGCATGAGAGTGTTTGTAGG	McCouch *et al.* (2002)
2	33,254,219	QSTS115	Indel	CGCAGTGGCTATAACCCAAG	CCGCATTAGAATCCTTCTCG	This study
2	34,676,703	RM213	SSR	ATCTGTTTGCAGGGGACAAG	AGGTCTAGACGATGTCGTGA	Chen *et al.* (1997)
2	35,424,303	RM2265	SSR	AACTGACCGTATATTAGCCA	TGACCGCCTCTATTATATTG	McCouch *et al.* (2002)

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2	35,450,160	RM3850	SSR	AAGTTGAGAATGAGGGACAA	TTCGGAAGTGAAAAGGTAAT	McCouch *et al.* (2002)
2	35,729,140	RM3274	SSR	GCTGCTGCTGCTACAGTTTG	GCGAGCTCCAGCATTTAAAC	McCouch *et al.* (2002)
2	35,160,161	RM208	SSR	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC	Chen *et al.* (1997)
3	8,170,550	RM5639	SSR	GGAAGAACAGAGTTGCTCGG	GTGCCATTTATTTCCGTCCC	McCouch *et al.* (2002)
3	1,437,206	RM3372	SSR	GAGCGACCAAAGAATCCAAG	CCACGGGGAGCTGATGAAG	McCouch *et al.* (2002)
3	5,971,990	RM3467	SSR	ATAATGGCAGGGTTGTCTCG	CTCGGTGAGCCTCCTACAAC	McCouch *et al.* (2002)
3	16,285,889	QSTS125	Indel	TTGAATCAATCACACATCTTATTCAG	TCCATATGGCCCAACATAAAC	This study
3	28,479,745	RM1350	SSR	CGCCCTAGTAGATAGGTAATTG	AAATCAGCAAGAAAGCTCTG	McCouch *et al.* (2002)
4	24,222,895	RM3785	SSR	ACCTTTTCTTGGCTTGAGGG	GCTTTTGCTACTTTTGGGGG	McCouch *et al.* (2002)
4	24,277,170	RM7051	SSR	CTCGATGAGCTTGGCGTC	TTCAGTGTTCATCGCCTCTG	McCouch *et al.* (2002)
4	5,275,427	QSTS132	Indel	TTTCGATGCTGTCAATCTACG	TCGATCCATCCACTTCCTTC	This study
4	13,164,721	QSTS134	Indel	CTTGTATTGCGTGCAGGATG	TTGGCTTCAATGCATACACC	This study
4	31,466,041	QSTS141	Indel	CCATGCATCCTCCTGTTCC	CTGTTGATGGGTCCCAATTC	This study
4	31,485,856	QSTS142	Indel	AGCTTTTTGGGTGATGTTGG	GGATGCTCTTTCTCCACCAC	This study
5	9,320,757	RM6836	SSR	TTGTTGTATACCTCATCGAC	AGGGTAAGACGTTTAACTTG	McCouch *et al.* (2002)
5	82,104	RM3529	SSR	CGCGCCACCTCGATATATAC	GCTCAGGTTAACCAAGGTGG	McCouch *et al.* (2002)
5	4,311,860	QSTS145	Indel	GAGACGGTCCCAACAAAGAC	CGTTTTAAACCATCCTTAGACG	This study
5	17,292,400	QSTS152	Indel	GAAACTCGGCCAATTGTAAC	TGACATGGCTGTCTTCAACC	This study
6	3,168,368	RM204	SSR	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC	Chen *et al.* (1997)
6	10,151,590	QSTS157	Indel	TGCTGTCATGCCAACTTACC	AAATGGCAGCATCTTCAAGC	This study
6	23,054,814	QSTS171	Indel	TGAAAACCGTGTCACTGTCC	GGGGCTCTAATCACCTCCAG	This study
6	25,612,879	RM6395	SSR	GGCTTCGGCTTCTGAACTAGC	CGACTAAGCAGCAGTAACAATCTCG	McCouch *et al.* (2002)
6	28,563,631	RM3307	SSR	CAGTGCTCTCGAACATGGAG	CTGCATTGTAAACGGTCGAG	McCouch *et al.* (2002)
7	1,186,748	RM6697	SSR	GCAAGATCCAGTCGATTTGG	ATAACATGAGCATCTCCCCG	McCouch *et al.* (2002)
7	23,596,003	RM5847	SSR	TGAGATGAGAGATAGACTCC	AACAGATGAAGGCTATTTTA	McCouch *et al.* (2002)
7	4,692,609	RM6872	SSR	CACCACGATATCCACCTCTAGC	CCTAGGATGAACACTGATGATGG	McCouch *et al.* (2002)

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7	11,713,561	QSTS176	Indel	GTGGCAGCTTGAGAGCATTC	TTTAATCCAAGCTTAGGCAACC	This study
7	13,552,392	QSTS180	Indel	GGAGCTCGTACAGAAAAATCG	CCGGAGGTATATTCCTTGTTTG	This study
7	17,831,145	QSTS182	Indel	GGCAATGGAACTTTTTGATTTG	ATGCACTTTCTGTCTCAGTTTG	This study
7	21,243,983	QSTS191	Indel	CAGTTTGGCACTGGCCTATC	GCATTTCACTCAAGCAATGG	This study
7	28,894,378	RM1306	SSR	TGCCAATTACCTTCCCGTAC	TGCTCCGTATTGCTGCTATG	McCouch *et al.* (2002)
8	20,645,571	RM223	SSR	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG	Chen *et al.* (1997)
8	26,294,879	QSTS215	Indel	TCCCTTAACTTGTCAACAAATCC	GCCACGTCAGCTAAAACCTC	This study
8	27,898,042	RM3155	SSR	GAAAAGGACAGGGGAAAAGC	GATCGTTCGTGTTCGTGTTG	McCouch *et al.* (2002)
9	5,048,789	QSTS222	Indel	CAACCAGCGAAACATACTGC	ACGGGAGGAAGATGACCAC	This study
9	11,045,262	QSTS227	Indel	TTTTTGCTTCTCACCCATTC	TTGCATGAACAGAGGTTTGC	This study
9	12,583,087	QSTS228	Indel	ATATCATTCCGGTGCCTACC	GTGCGCATGTGTGTGTACTG	This study
9	14,644,334	QSTS229	Indel	AGTGGATGGATGGATGGATG	CGTCGTCTCTTCCGTTTTTC	This study
9	20,246,472	RM3808	SSR	CGTTAGCGAAACGAACAGTG	CAGTGGCTCGGTAATCGC	McCouch *et al.* (2002)
9	17,054,142	RM3600	SSR	TGCCCACACATGATGAGC	AACGGGCAAGAGATCTTCTG	McCouch *et al.* (2002)
10	2,522,494	QSTS237	Indel	GACCAGAGACTGCCCCATC	TCACTTCAAGTCTGTGGTTTCAG	This study
10	22,133,218	QSTS248	Indel	TGCAATTGACTACTACTCCAAGG	TCAAACAAACAATCGTGAAGC	This study
10	17,490,576	RM6704	SSR	AATCGAATCTGGATATCTTG	CTTCTACCTAGCTACCGAGA	McCouch *et al.* (2002)
10	15,903,727	RM5304	SSR	CATCTTGAATCCTCCTTCGACTCC	GGCAGCGATAGCAGGAAGAGG	McCouch *et al.* (2002)
11	794,611	QSTS250	Indel	CGTTGGATAATTAATGGAGATCG	CACAAGCACCAAACCCAAC	This study
11	4,484,051	QSTS255	Indel	CATTGCTACGAGGCACAATC	CCGCGCACGTAGGTATATG	This study
11	7,007,889	QSTS263	Indel	ATAAAATCCGGCGAGACAAG	GAAGCGAGTGCTCCTAGCTC	This study
11	21,626,773	RM206	SSR	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	Chen *et al.* (1997)
11	28,173,902	RM144	SSR	CATGTTGTGCTTGTCCTACTGC	AGCTAGAGGAGATCAGATGGTAGTGC	Temnykh *et al.* (2000)
12	905,092	RM1080	SSR	AGAGCCCTCGTAAGCCAAAG	GGTCGTGAATCTCCTCCAAG	McCouch *et al.* (2002)
12	4,919,671	RM3455	SSR	TGAATCCACACTCGCAGATC	GCCAGTCCACGATTGGTC	McCouch *et al.* (2002)
12	25,036,187	RM3739	SSR	AGTTGCGCAGCTAATCGATC	AAGATCCAACGGGTTCTGTG	McCouch *et al.* (2002)
12	8,828,165	RM101	SSR	GTGAATGGTCAAGTGACTTAGGTGGC	ACACAACATGTTCCCTCCCATGC	Temnykh *et al.* (2000)
12	19,156,079	RM1246	SSR	AGCTCGATCCCCTAGCTCTC	TTGGAGAAGGTCACCTGCC	McCouch *et al.* (2002)
12	25,067,717	RM5715	SSR	GCAGAAGAGAGAAATGAAAG	CTTGTCTACGTAGCATGACA	McCouch *et al.* (2002)

[a]Position on rice pseudo-molecule build 4.0.

Table S2. List of markers for linkage analysis of the mutants induced from IR24.

Chr.	Position[a]	Marker name	Marker type	Forward primer sequence	Reverse primer sequence	Reference
1	6,273,454	RM8111	SSR	AGGTAACTAAGCTAGGTGTT	TAGGTACAGTAATACCAAGC	McCouch *et al.* (2002)
1	8,915,941	RM8083	SSR	GATGTGCAAATTATCA TGTGTTTTGTGAGG	ATAGTAGGCCCACA CCTGACAGGTTGTA	McCouch *et al.* (2002)
1	9,938,600	RM8046	SSR	AGTACGATTTCTGT CAGCGTTGCTTAGT	GGATGAAAGTTGATGG ATGATCTACTTGTT	McCouch *et al.* (2002)
1	10,703,670	RM23	SSR	CATTGGAGTGGAGGCTGG	GTCAGGCTTCTGCCATTCTC	Chen *et al.* (1997)
1	18,973,690	RM24	SSR	GAAGTGTGATCACTGTAACC	TACAGTGGACGGCGAAGTCG	Chen *et al.* (1997)
1	23,968,523	RM5	SSR	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG	Panaud *et al.* (1996)
1	30,732,007	RM128	SSR	AGCTTGGGTGATTTCTTGGAAGCG	ACGACGAGGAGTCGCCGTGCAG	Temnykh *et al.* (2000)
1	38,002,959	RM6333	SSR	AGAGAAGACACGGTGGATGG	CAAACTCCTCATTTCGCTCC	McCouch *et al.* (2002)
1	40,161,142	RM104	SSR	GGAAGAGGAGAGAAAGATGTGTGTCG	TCAACAGACACACCGCCACCGC	Temnykh *et al.* (2000)
1	42,919,515	RM8136	SSR	ATGTAAGCTAGGTAGAGCTG	GCGTACGTACGTAAGTAATA	McCouch *et al.* (2002)
2	346,475	RM6800	SSR	CAAGCCTACATGGCCTAGACTCC	ATCCATGATCCATCATCCATGC	McCouch *et al.* (2002)
2	1,083,895	RM154	SSR	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC	Temnykh *et al.* (2000)
2	1,660,838	RM7033	SSR	AGAATAACTCCAGCCCACACTGG	GCGGTGATTTCTGATGACATTCC	McCouch *et al.* (2002)
2	3,863,789	RM3703	SSR	GAGAGAGAGGGAAGGGAAGG	GCTCCCCGACATTTAAACTG	McCouch *et al.* (2002)
2	4,264,267	RM4355	SSR	GGGATGAGAGTAGAAGGCA	TATATGGCAAGCCTAGCG	McCouch *et al.* (2002)
2	5,100,932	RM7082	SSR	TCTCCAACAGCAGCGAGG	GACCCGGCCTTCTACCTAAC	McCouch *et al.* (2002)
2	5,204,941	RM3294	SSR	TTACACACACTACGGACGCG	CCTGGTGGTACCTCTCTTAATC	McCouch *et al.* (2002)
2	5,314,413	RM1347	SSR	AACAAATTAAACTGCCAAG	GTCTTATCATCAGAACTGGA	McCouch *et al.* (2002)
2	6,732,637	RM5897	SSR	GGCATCTTCCCCTCTCTCTC	CCAACCCAAACCAGTCTACC	McCouch *et al.* (2002)
2	7,541,911	RM3505	SSR	GATGAGGTGGGACGACGAC	TCTTCACAGTGACGAAACCG	McCouch *et al.* (2002)
2	8,759,533	RM71	SSR	CTAGAGGCGAAAACGAGATG	GGGTGGGCGAGGTAATAATG	Temnykh *et al.* (2000)

Continued

2	8,980,509	RM5699	SSR	ATCGTTTCGCATATGTTT	ATCGGTAAAAGATGAGCC	McCouch *et al.* (2002)
2	10,186,627	RM3501	SSR	TCCTAGTGCATCAGCACAGC	GTCCGTTTCAGCAAGCAAAC	McCouch *et al.* (2002)
2	15,999,806	RM5812	SSR	CGCTGACATCTTGCCCTC	GTAGGACCCACGTGTCATCC	McCouch *et al.* (2002)
2	17,297,690	QSTS95	Indel	TGAGGACAAGCATGTGAAGG	CAATGGACGCTAGTGGAACC	This study
2	18,744,542	QSTS96	Indel	GCTCGTGTTCGTGCATCTATC	GCAACAACGAGAACGAGAAC	This study
2	17,946,057	RM6611	SSR	CACACACGCACGGTTAGATC	CTCCTCACCTCTTCCCCTTC	McCouch *et al.* (2002)
2	18,169,378	RM6844	SSR	CAGAGCAGGAACAGATGCTG	GTCCAAGAAAGGCACGAGAG	McCouch *et al.* (2002)
2	19,361,410	RM341	SSR	CAAGAAACCTCAATCCGAGC	CTCCTCCCGATCCCAATC	Temnykh *et al.* (2000)
2	20,996,870	RM6023	SSR	AAGGAAGCAGCGATGTGAAG	GAGCTAGAGATCACCTGGCG	McCouch *et al.* (2002)
2	24,518,130	QSTS109	Indel	AATATAGTTTGGTC AAAAGAACAAAAG	CGGATAATCTTGATGAGTGAACC	This study
2	26,260,867	RM3730	SSR	TGCGAGTATCTTCAAGGCAG	ATTGAGGGGGCTAATCATCC	McCouch *et al.* (2002)
2	27,172,434	RM5470	SSR	CATGGATTGTCTGGGCCTAG	AAGACATACCCTGAGTGTGGG	McCouch *et al.* (2002)
2	28,291,970	RM5631	SSR	CGTCCAAGAAATATTGCAGT	GTGAGACAGAATCCTTACGC	McCouch *et al.* (2002)
2	29,604,207	RM6	SSR	GTCCCCTCCACCCAATTC	TCGTCTACTGTTGGCTGCAC	Panaud *et al.* (1996)
2	29,655,949	RM318	SSR	GTACGGAAAACATGGTAGGAAG	TCGAGGGAAGGATCTGGTC	Temnykh *et al.* (2000)
2	34,709,116	RM3789	SSR	TTCCCGAATTAAGCAGATATA	CTGTAGACCATTGACTGGTG	McCouch *et al.* (2002)
3	8,170,550	RM5639	SSR	GGAAGAACAGAGTTGCTCGG	GTGCCATTTATTTCCGTCCC	McCouch *et al.* (2002)
3	12,901,320	RM5551	SSR	GACTAGTCCGGCCGTACATG	AGTTTCTTGTGTGACGCGTG	McCouch *et al.* (2002)
3	22,203,310	RM6832	SSR	GTTGTAAATGCCTGAGTGC	AAAGAGCTAAACCGCTAGG	McCouch *et al.* (2002)
3	26,547,224	RM347	SSR	CACCTCAAACTTTTAACCGCAC	TCCGGCAAGGGATACGGCGG	Temnykh *et al.* (2000)
3	31,185,979	RM448	SSR	TCTGATCTTGATGCAGGCAC	TCTCCCGATTTGGACAGATC	Temnykh *et al.* (2001)
3	33,589,206	RM7000	SSR	CCCTTCTTTTCAACTGAATA	TTGTAACAATGAACTCGTTC	McCouch *et al.* (2002)
4	19,913,395	RM6679	SSR	TTTAGGCCGTAAGAGCGAAC	GAATTTGAGTAGCTGGCTCC	McCouch *et al.* (2002)

Continued

4	28,733,259	RM303	SSR	GCATGGCCAAATATTAAAGG	GGTTGGAAATAGAAGTTCGGT	Temnykh *et al.* (2000)
4	32,869,409	RM348	SSR	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC	Temnykh *et al.* (2000)
5	456,843	RM3796	SSR	ATTAGCCTTTAATTCCACTG	ATACAAACAAACAGCTTGTG	McCouch *et al.* (2002)
5	2,084,750	RM4710	SSR	AACTGGTTACAAAGACATGG	TCATCTACATATGGGGACAC	McCouch *et al.* (2002)
5	8,799,525	RM6082	SSR	AACCCTAGAATCGGCGCTG	CACCGATGACAACGAGGAC	McCouch *et al.* (2002)
5	19,107,688	RM163	SSR	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT	McCouch *et al.* (2002)
5	22,817,739	RM3870	SSR	TACATCTCCGGCGTTTACAC	CCAAGGTTGAAACAGGAAGC	McCouch *et al.* (2002)
5	26,712,707	RM2357	SSR	CCTCCGTTTCACAATGTAAC	CTGATGCTACCAGAATCCTC	McCouch *et al.* (2002)
5	29,161,099	RM6346	SSR	ACTTTGATCGATCAGCCACC	AGGTGGTGGAGATGAAGCAG	McCouch *et al.* (2002)
6	3,168,380	RM8125	SSR	CTCGTACCATCGGCTGT	AACTTACTGTGACT GACTTGGTCA	McCouch *et al.* (2002)
6	5,108,560	RM111	SSR	CACAACCTTTGAGCACCGGGTC	ACGCCTGCAGCTTGATCACCGG	Temnykh *et al.* (2000)
6	9,320,757	RM6836	SSR	TTGTTGTATACCTCATCGAC	AGGGTAAGACGTTTAACTTG	McCouch *et al.* (2002)
6	19,152,497	RM3	SSR	ACACTGTAGCGGCCACTG	CCTCCACTGCTCCACATCTT	Panaud *et al.* (1996)
6	28,216,527	RM340	SSR	GGTAAATGGACAATCCTATGGC	GACAAATATAAGGGCAGTGTGC	Temnykh *et al.* (2000)
6	30,349,942	RM345	SSR	ATTGGTAGCTCAATGCAAGC	GTGCAACAACCCCACATG	Temnykh *et al.* (2000)
7	2,711,818	RM427	SSR	TCACTAGCTCTGCCCTGACC	TGATGAGAGTTGGTTGCGAG	Temnykh *et al.* (2001)
7	3,174,334	RM5711	SSR	GTCCATGCATCCATCTCTAG	ACGGAAGGAATACGTCTGTA	McCouch *et al.* (2002)
7	5,762,184	RM6728	SSR	GGGTATGTGTCGCTATTTTA	GAAATCTGGAATTTTCCCTA	McCouch *et al.* (2002)
7	17,132,877	RM3795	SSR	CATTTGCATGGAGAGGATAG	TCATCTTCATTTCATTTCACC	McCouch *et al.* (2002)
7	22,135,427	RM10	SSR	TTGTCAAGAGGAGGCATCG	CAGAATGGGAAATGGGTCC	Panaud *et al.* (1996)
7	25,599,925	RM18	SSR	TTCCCTCTCATGAGCTCCAT	GAGTGCCTGGCGCTGTAC	Panaud *et al.* (1996)
7	28,871,445	RM1362	SSR	AAACAGGCCCTTAGTGCATG	CTACCATGGCGGCTTATGTC	McCouch *et al.* (2002)

Continued

8	12,288,044	RM331	SSR	GAACCAGAGGACAAAAATGC	CATCATACATTTGCAGCCAG	Temnykh *et al.* (2000)
8	19,002,002	RM4815	SSR	AGTAAATTTCACAAAACTTC	GTGATACAATGCATTAAATA	McCouch *et al.* (2002)
8	20,971,647	RM5887	SSR	CAATGATGGTGGTGAAAATC	GCTCATCTAGAAATCACCGA	McCouch *et al.* (2002)
8	26,140,941	RM5493	SSR	GCAGGACACAGTCACACAGG	AGATTCTTTCACCGGTGACG	McCouch *et al.* (2002)
8	27,834,839	RM3496	SSR	CGCTGAAAATACTGAATTGA	AGATGCATTTATTCCGAAAG	McCouch *et al.* (2002)
9	7,259,847	RM5526	SSR	TCAGCCTGGCCTCTCTTATC	ATGATCCTCCACCCACTAGC	McCouch *et al.* (2002)
9	11,694,729	RM3769	SSR	TGCATGCTTCGTTCAGCTAG	GTCTCCGAGCTCCTCAGGTC	McCouch *et al.* (2002)
9	14,313,180	RM5657	SSR	TATGTGCATTTGTAAGGTGA	GCTTTAGATTATTGAGCGAG	McCouch *et al.* (2002)
9	16,818,078	RM7175	SSR	ACAGTAAACGTGGTGCCTCC	AGAAGTAGCCTCGAGGACCC	McCouch *et al.* (2002)
9	21,906,494	RM7306	SSR	TCGATCCAACGCTAGCTACC	CGGAATGGGGAGGAGATC	McCouch *et al.* (2002)
10	17,490,576	RM6704	SSR	AATCGAATCTGGATATCTTG	CTTCTACCTAGCTACCGAGA	McCouch *et al.* (2002)
10	21,122,837	RM3451	SSR	CGGCGAGATAACAATTCTCC	GCGTGATGATATGGTATCGG	McCouch *et al.* (2002)
10	21,982,081	RM496	SSR	GACATGCGAACAACGACATC	GCTGCGGCGCTGTTATAC	Temnykh *et al.* (2001)
11	2,327,413	RM7557	SSR	GTGTACTGCCATGAAAGGCC	GAAGTGCCTTTGCAGGAGAG	McCouch *et al.* (2002)
11	8,908,333	RM202	SSR	CAGATTGGAGATGAAGTCCTCC	CCAGCAAGCATGTCAATGTA	Chen *et al.* (1997)
11	23,829,201	RM7277	SSR	GCTGAACGTTTCAATATGTA	GTTTGTAGGGAGTTTAATGG	McCouch *et al.* (2002)
11	26,668,576	RM6688	SSR	GTGCCGTTTAATACGTAGAC	AAGGAAACTTTTTTTTGCTC	McCouch *et al.* (2002)
12	2,181,948	RM7315	SSR	CACAAAGGCGTGTGGGTTAG	GAGTCACGGGATGTTGCC	McCouch *et al.* (2002)
12	7,566,745	RM2529	SSR	CATTAAAATCAGTGGGACTG	AGGCATTTCCTGATATGATC	McCouch *et al.* (2002)
12	19,156,079	RM1246	SSR	AGCTCGATCCCCTAGCTCTC	TTGGAGAAGGTCACCTGCC	McCouch *et al.* (2002)
12	21,282,401	RM1986	SSR	TAACGGAGGGAGTAGTTTTC	GAACCTACATATCGAGAGCA	McCouch *et al.* (2002)
12	27,420,423	RM2197	SSR	ACTGAGAACTTTAATCATCG	GAACAACTTTGAAGAGAAAC	McCouch *et al.* (2002)

[a]Position on rice pseudo-molecule build 4.0.

Table S3. Phenotypic characterization of necrotic mutants in rice (2016).

Mutant line	Organ	Days after sowing (DAS)[a]																											
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	32	35	82
LEM 1 *nec1*	I[st] leaf	G	G	G																								
	2[nd] leaf	G	G	G	PG	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	Dead	Dead														
	3[rd] leaf	G	G	G	PG	PG	PG	PG/D	PG/D	PG/D	PG/D	PG/D	YG/D	Dead														
	4[th] leaf							G	G	PG	PG	PG	PG	Dead														
LEM4 *nec2*	I[st] leaf	G	G	G																								
	2[nd] leaf	G	G	G	G	PG	PG	PG/D	PG/D	PG/D	PG/D	PG/D	Dead	Dead														
	3[rd] leaf	G	G	G	G	PG	PG	PG	PG	PG	PG	PG	PG/D	Dead														
	4[th] leaf							G	G	PG	PG	PG	PG	Dead														
LEM5 *nec3*	I[st] leaf	G	G	G																								
	2[nd] leaf	G	G	G	G	G	PG/D	PG/D	PG/D	D	D	D	Dead	Dead														
	3[rd] leaf	G	G	G	G	G	PG	PG	PG	PG/D	PG/D	PG/D	PG/D	Dead														
	4[th] leaf							G	G	G	G	G	PG	Dead														
LEM9 *nec4*	I[st] leaf	G	G	G																								
	2[nd] leaf	G	G	G	G	PG	PG	PG	PG	PG	PG	PG	Dead	Dead														
	3[rd] leaf	G	G	G	G	PG	PG	PG	PG	PG	PG	PG	Dead	Dead														
	4[th] leaf									G	G	G	YG	Dead														
LEM16 *nec5*	I[st] leaf	G	G	G																								
	2[nd] leaf	G	G	G	G	G	PG/D	YG/D	YG/D	YG/D	YG/D	YG/D	Dead	Dead														
	3[rd] leaf	G	G	G	G	G	PG	YG	YG	YG/D	YG/D	YG/D	YG/D	Dead														
	4[th] leaf							YG	YG	YG	YG	YG	YG	Dead														
LEM17 *nec6*	I[st] leaf	G	G	G																								
	2[nd] leaf	G	G	G	G	G	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	Dead	Dead														
	3[rd] leaf	G	G	G	G	G	PG	PG	PG	PG/D	PG/D	PG/D	PG/D	Dead														
	4[th] leaf							G	G	G	G	G	PG	Dead														
LEM18 *nec7*	I[st] leaf	G	G	G																								
	2[nd] leaf	G	G	G	G	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	Dead	Dead														
	3[rd] leaf	G	G	G	G	YG	YG	YG	YG	YG	YG	YG	YG/D	Dead														
	4[th] leaf							G	G	G	G	G	YG	Dead														
LEM24 *nec8*	I[st] leaf	G	G	G																								
	2[nd] leaf	G	G	G	G	PG	PG	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	Dead	Dead	Dead	Dead											
	3[rd] leaf	G	G	G	G	PG	PG	YG	YG	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	Dead	Dead											
	4[th] leaf							YG	YG	YG	YG	YG	YG	YG	YG	YG	Dead											
LEM29 *nec9*	I[st] leaf	G	G	G																								
	2[nd] leaf	G	G	G	G	PG	PG	PG/D	PG/D	PG/D	PG/D	PG/D	Dead	Dead	Dead	Dead	Dead											
	3[rd] leaf	G	G	G	G	PG	PG	PG	PG	PG	PG	PG	YG/D	YG/D	YG/D	Dead	Dead											
	4[th] leaf							G	G	G	G	G	PG	PG	PG	PG	Dead											
LEM36 *nec10*	I[st] leaf	G	G	G																								
	2[nd] leaf	G	G	G	G	G	PG	PG	PG	PG	PG	PG	YG/D	Dead	Dead	Dead	Dead											
	3[rd] leaf	G	G	G	G	G	PG	PG	PG	PG	PG	PG	YG/D	YG/D	YG/D	Dead	Dead											
	4[th] leaf									G	G	G	PG	PG	PG	PG	Dead											

[a]G, PG, YG, D and Dead represent green, pale-green, yellow green, drying and dead, respectively.

Mutant line	Organ	Days after sowing[a]																											
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	32	35	82
LEM37 *nec*11[b]	1st leaf	G	G	G																								
	2nd leaf	G	G	G	G	YG	YG	YG	YG	YG/D	YG/D	YG/D	Dead	Dead	Dead	Dead	Dead											
	3rd leaf	G	G	G	G	YG	YG	YG	YG	YG/D	YG/D	YG/D	Y/ D	Y/ D	Y/ D	Y/ D	Dead											
	4th leaf							YG	YG	YG	YG	YG	YG	Y	Y	Y	Dead											
LEM28 *nec*12	1st leaf	G	G	G																								
	2nd leaf	G	G	G	G	PG	PG	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	Dead	Dead	Dead	Dead	Dead	Dead									
	3rd leaf	G	G	G	G	PG	PG	PG	PG	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	Dead	Dead	Dead	Dead									
	4th leaf							G	G	PG	PG	PG	YG	YG	YG	YG	YG	YG	Dead									
LEM43 *nec*13	1st leaf	G	G	G																								
	2nd leaf	G	G	G	PG	PG/D	PG/D	YG/D	YG/D	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead									
	3rd leaf	G	G	G	PG	PG	PG	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	Dead									
	4th leaf												G	PG	PG	PG	PG	PG	Dead									
LEM20 *nec*14	1st leaf	G	G	G																								
	2nd leaf	G	G	G	G	PG	PG	PG/D	PG/D	PG/D	PG/D	PG/D	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead						
	3rd leaf	G	G	G	G	PG	PG	PG	PG	PG	PG	PG	PG/D	PG/D	PG/Dry	PG/Dry	Dead	Dead	Dead	Dead	Dead	Dead						
	4th leaf							G	G	G	G	G	PG	PG	PG	PG	PG	PG	PG	PG	PG	Dead						
LEM25 *nec*15	1st leaf	G	G	G																								
	2nd leaf	G	G	G	G	PG	PG	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	Dead	Dead	
	3rd leaf	G	G	G	G	PG	PG	PG	PG	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	Dead	
	4th leaf							G	G	PG	PG	PG	PG	PG	PG	PG	PG	PG	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	Dead	
	5th leaf																					G	G	G	PG	PG	Dead	
LEM11 *nec*16	1st leaf	G	G	G																								
	2nd leaf	G	G	G	PG	PG	YG	YG	YG	YG	YG	YG	YG/D	YG/D	YG/D	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
	3rd leaf	G	G	G	PG	PG	YG	YG	YG	YG	YG	YG	YG	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	Dead
	4th leaf							G	G	YG	YG	YG	YG	YG	YG	YG	YG	YG	YG	YG	YG	YG	YG	YG	YG	YG/D	Dead	
	5th leaf																											
LEM41 *nec*17[c]	1st leaf	YG	YG	YG/TB																								
	2nd leaf	YG	YG	YG/TB	YG/TB	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	
	3rd leaf	YG	YG	YG/TB	YG/TB	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	
	4th leaf							YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	YG/TB/D	Dead	Dead	
	5th leaf																		G	G	G	G	G	G	G	YG/TB/D	Dead	
LEM2 *nec*18	1st leaf	G	G	G	G																							
	2nd leaf	G	G	G	G	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	PG/D	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
	3rd leaf	G	G	G	G	PG	PG	PG/D	PG/D	PG/D	PG/D	PG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	YG/D	Dead
	4th leaf							G	G	G	G	G	YG	YG	YG	YG	YG	YG	YG	YG	YG	YG	YG	YG	YG/D	YG/D	YG/D	Dead
	5th leaf																					G	G	G	G	G	G	Dead

[a]G, PG, YG, D and Dead represent green, pale-green, yellow green, drying and dead, respectively; [b]Yellow-green leaves turned completely yellow before drying in *nec*11; [c]Transverse necrotic bands appeared since 6DAS in *nec*17.

Table S4. Inferred markers linked to causal genes by bulked segregant analysis.

Gene	Original cultivar	Chromosomal locations	Markers positively suggested[a]
*nec*1	Taichung 65	2L	QSTS91, QSTS95, QSTS96, QSTS103, QSTS109
*nec*4	Taichung 65	2L and 2S	QSTS89, QSTS91, QSTS95, QSTS96, QSTS103, QSTS109, RM3850,
*nec*5	Taichung 65	2L and 2S	RM7562, RM3865, QSTS88, QSTS89, QSTS91, RM3850
*nec*6	Taichung 65	2L and 2S	QSTS88, QSTS89
*nec*7	Taichung 65	2L	QSTS89, QSTS91, QSTS95, QSTS96, QSTS103
*nec*9	Taichung 65	2L	QSTS91, QSTS95, QSTS96, QSTS103, QSTS109, QSTS114
*nec*10	Taichung 65	2L	QSTS91, QSTS95, QSTS96, QSTS103, QSTS109
*nec*11	Taichung 65	2L	QSTS91, QSTS95, QSTS96, QSTS103, QSTS109
*nec*13	IR24	2L	QSTS95, QSTS96, QSTS109
*nec*14	Taichung 65	4L	RM3785, RM7051, QSTS141, QSTS142
*nec*15	Taichung 65	4L	RM3785, RM7051, QSTS141, QSTS142
*nec*16	Taichung 65	4L	QSTS134, RM3785, RM7051, QSTS141, QSTS142
*nec*17	IR24	2S	RM3703, RM4355
*nec*18	Taichung 65	4L	QSTS134, QSTS141, QSTS142

[a]Marker positions are indicated in Supplementary **Table 1** and **Table 2** for the mutants induced from Taichung 65 and IR24, respectively.

Table S5. Linkage analysis of *nec*17 and DNA markers on chromosome 2.

Gene pair[a]		Number of plants										Recombination value (%)	Map distance (cM)
A(a)	B(b)	AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb	Total		
*nec*17	RM3703	26	44	1	_	_	_	1	0	26	98	3.1	3.1
*nec*17	RM4355	17	57	0	_	_	_	0	0	29	103	0.0	0.0
*nec*17	RM7082	20	50	2	_	_	_	0	5	23	100	6.7	6.7
*nec*17	RM3294	21	53	1	_	_	_	0	6	20	101	6.9	6.9
*nec*17	RM1347	18	54	3	_	_	_	0	7	22	104	9.2	9.3
RM3703	RM4355	16	8	1	1	43	0	0	1	26	96	6.5	6.5
RM3703	RM7082	16	8	1	4	36	2	0	6	20	93	12.6	12.9
RM3703	RM3294	17	9	1	4	38	1	0	7	17	94	13.1	13.5
RM3703	RM1347	14	11	1	4	37	3	0	8	19	97	15.7	16.3
RM4355	RM7082	15	1	0	3	49	2	0	5	23	98	5.8	5.8
RM4355	RM3294	16	1	0	3	52	1	0	6	20	99	5.8	5.8
RM4355	RM1347	14	2	0	3	51	3	0	7	22	102	7.7	7.8
RM7082	RM3294	20	0	0	0	53	1	0	3	19	96	2.1	2.1
RM7082	RM1347	17	2	0	0	54	1	0	2	23	99	2.6	2.6
RM3294	RM1347	18	2	0	0	57	2	0	1	20	100	2.5	2.5

[a]Capital and lower-case letters represent the Taichung 65 alleles and IR24 alleles, respectively.

Table S6. Linkage analysis of *nec1* and DNA markers on chromosome 2.

Gene pair[a]		Number of plants										Recombination value (%)	Map distance (cM)
A(a)	B(b)	AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb	Total		
*nec*1	QSTS95	26	33	10	_	_	_	5	7	14	95	30.1	34.8
*nec*1	QSTS96	27	33	8	_	_	_	4	10	12	94	29.7	34.2
*nec*1	QSTS103	20	44	5	_	_	_	0	6	21	96	11.1	11.3
*nec*1	QSTS109	18	47	3	_	_	_	0	5	22	95	8.0	8.0
*nec*1	QSTS110	18	47	4	_	_	_	1	4	22	96	10.0	10.1
QSTS95	QSTS96	28	1	1	1	36	3	0	5	18	93	6.6	6.7
QSTS95	QSTS103	17	11	3	7	30	3	0	8	16	95	20.4	21.6
QSTS95	QSTS109	11	14	5	8	25	7	0	12	12	94	31.9	37.8
QSTS95	QSTS110	11	13	7	8	25	7	1	12	11	95	35.6	44.7
QSTS96	QSTS103	18	11	2	10	32	1	0	7	13	94	19.4	20.5
QSTS96	QSTS109	12	14	4	10	28	5	0	10	10	93	29.9	34.5
QSTS96	QSTS110	12	13	6	10	28	5	1	10	9	94	33.8	41.1
QSTS103	QSTS109	13	6	1	6	38	5	0	8	18	95	15.5	16.0
QSTS103	QSTS110	13	6	1	8	37	5	1	8	17	96	17.9	18.7
QSTS109	QSTS110	18	0	0	1	50	1	0	1	24	95	1.6	1.6

[a]Capital and lower-case letters represent the Hinohikari and Taichung 65 alleles, respectively.

Table S7. Linkage analysis of *nec*13 and DNA markers on chromosome 2.

Gene pair[a]		Number of plants										Recombination value (%)	Map distance (cM)
A(a)	B(b)	AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb	Total		
*nec*13	QSTS95	31	60	19	_	_	_	2	10	29	151	21.6	23.2
*nec*13	QSTS96	30	66	13	_	_	_	1	13	28	151	18.4	19.3
*nec*13	QSTS109	33	61	14	_	_	_	5	6	31	150	20.0	21.2
QSTS95	QSTS96	26	5	2	5	63	2	0	11	36	150	9.5	9.6
QSTS95	QSTS109	19	12	2	15	36	17	4	19	25	149	28.5	32.3
QSTS96	QSTS109	21	8	2	13	44	20	4	14	23	149	25.6	28.3

[a]Capital and lower-case letters represent the Taichung 65 alleles and IR24 alleles, respectively.

Table S8. Linkage analysis of *nec*16 and DNA markers on chromosome 4.

Gene pair[a]		Number of plants										Recombination value (%)	Map distance (cM)
A(a)	B(b)	AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb	Total		
*nec*16	QSTS132	28	27	16	_	_	_	2	8	9	90	34.2	41.8
*nec*16	QSTS134	28	27	11	_	_	_	1	8	9	84	28.2	31.9
*nec*16	RM3785	21	50	0	_	_	_	0	0	19	90	0.0	0.0
*nec*16	RM7051	21	52	0	_	_	_	0	0	19	92	0.0	0.0
*nec*16	QSTS141	18	37	17	_	_	_	3	6	9	90	37.8	49.3
*nec*16	QSTS142	19	38	16	_	_	_	3	7	9	92	36.9	47.3
QSTS132	QSTS134	26	4	0	1	27	2	2	4	16	82	9.5	9.6

Continued

QSTS132	RM3785	9	18	2	6	21	8	6	9	9	88	39.5	53.7
QSTS132	RM7051	9	19	2	6	21	8	6	10	9	90	39.9	54.7
QSTS132	QSTS141	7	14	8	7	13	14	7	14	4	88	53.2	
QSTS132	QSTS142	7	15	8	8	14	13	7	14	4	90	53.2	
QSTS134	RM3785	9	18	1	6	20	8	2	9	9	82	33.6	40.8
QSTS134	RM7051	9	19	1	6	21	8	2	9	9	84	33.7	40.9
QSTS134	QSTS141	9	13	7	7	12	14	3	14	3	82	48.3	101.1
QSTS134	QSTS142	9	13	7	7	14	14	3	14	3	84	48.2	100.2
RM3785	RM7051	21	0	0	0	50	0	0	0	19	90	0.0	0.0
RM3785	QSTS141	12	7	2	6	28	15	3	6	9	88	29.9	34.5
RM3785	QSTS142	12	7	2	7	29	14	3	7	9	90	30.0	34.7
RM7051	QSTS141	12	7	2	6	30	15	3	6	9	90	29.4	33.7
RM7051	QSTS142	12	7	2	7	31	14	3	7	9	92	29.5	33.8
QSTS141	QSTS142	21	0	0	0	43	0	1	0	25	90	1.1	1.1

[a]Capital and lower-case letters represent the Hinohikari and Taichung 65 alleles, respectively.

Table S9. Linkage analysis of *nec*18 and DNA markers on chromosome 4.

Gene pair[a]		Number of plants										Recombination value (%)	Map distance (cM)
A(a)	B(b)	AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb	Total		
*nec*18	QSTS132	16	33	11	_	_	_	5	10	7	82	43.0	64.6
*nec*18	QSTS134	11	30	18	_	_	_	4	10	7	80	48.8	109.7
*nec*18	RM3785	22	36	1	_	_	_	0	12	9	80	18.0	18.8
*nec*18	RM7051	23	37	1	_	_	_	0	14	9	84	19.6	20.7
*nec*18	QSTS141	30	30	0	_	_	_	0	3	20	83	4.0	4.0
*nec*18	QSTS142	30	31	0	_	_	_	0	3	19	83	4.0	4.0
QSTS132	QSTS134	10	6	3	5	28	9	0	4	13	78	21.8	23.4
QSTS132	RM3785	7	10	2	15	22	5	0	15	2	78	40.2	55.4
QSTS132	RM7051	8	11	2	15	23	5	0	16	2	82	39.5	53.5
QSTS132	QSTS141	8	9	4	16	16	10	5	8	5	81	45.9	79.0
QSTS132	QSTS142	8	9	4	16	17	9	5	8	5	81	45.8	78.0
QSTS134	RM3785	9	4	1	9	23	7	4	17	2	76	40.5	56.4
QSTS134	RM7051	9	5	1	10	23	7	4	19	2	80	41.5	59.4
QSTS134	QSTS141	4	8	3	15	14	10	10	10	5	79	53.9	
QSTS134	QSTS142	4	8	3	15	15	9	10	10	5	79	54.1	
RM3785	RM7051	22	0	0	0	48	0	0	0	10	80	0.0	0.0
RM3785	QSTS141	14	7	0	14	25	9	0	1	9	79	21.9	23.5
RM3785	QSTS142	14	8	0	14	25	9	0	1	8	79	22.8	24.6
RM7051	QSTS141	15	7	0	15	25	11	0	1	9	83	22.9	24.8
RM7051	QSTS142	15	8	0	15	25	11	0	1	8	83	23.7	25.8
QSTS141	QSTS142	30	0	0	0	33	0	0	0	19	82	0.0	0.0

[a]Capital and lower-case letters represent the Hinohikari and Taichung 65 alleles, respectively.

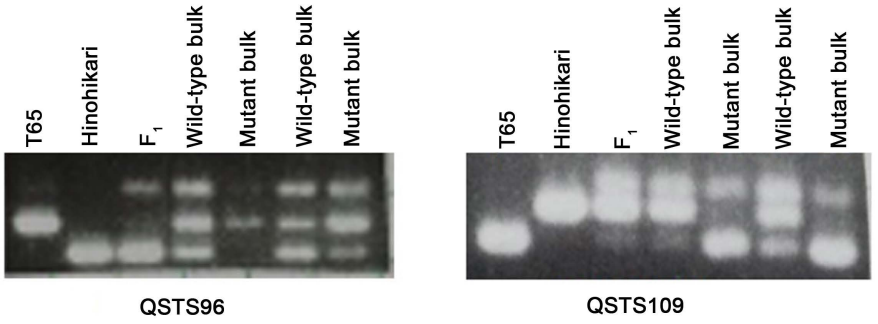

Figure S1. Detection of DNA markers linked to *nec1* locus on chromosome 2 by bulked segregant analysis.

Figure S2. Characterization of mutants for necrotic lethality in rice by DAB staining.