Fine Mapping of Two Interacting Loci for Transmission Ratio Distortion in Rice (Oryza sativa L.)

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Transmission ratio distortion (TRD) denotes the observed allelic or genotypic frequency deviation from the expected Mendelian segregation ratios in the offspring of a heterozygote. TRD can severely hamper gene flow between and within rice species. Here, we report the fine mapping and characterization of two loci (TRD4.1 and TRD4.2) for TRD using large F₂ segregating populations, which are derived from rice chromosome segment substitution lines, each containing a particular genomic segment introduced from the japonica cultivar Nipponbare (NIP) into the indica cultivar Zhenshan (ZS97). The two loci exhibited a preferential transmission of ZS97 alleles in the derived progeny. Reciprocal crossing experiments using near-isogenic lines harboring three different alleles at TRD4.1 suggest that the gene causes male gametic selection. Moreover, the transmission bias of TRD4.2 was diminished in heterozygotes when they carried homozygous TRD4.1ZS97. This indicates an epistatic interaction between these two loci. TRD4.2 was mapped into a 35-kb region encompassing one candidate gene that is specifically expressed in the reproductive organs in rice. These findings broaden the understanding of the genetic mechanisms of TRD and offer an approach to overcome the barrier of gene flow between the subspecies in rice, thus facilitating rice improvement by introgression breeding.

Keywords: rice, reproductive isolation, transmission ratio distortion, allele frequency, gametic selection, epistatic interaction

INTRODUCTION

Reproductive isolation is regarded as a driving force in the process of evolution in various species (Ouyang and Zhang, 2013; Baack et al., 2015). The development of reproductive isolation relies on the accumulation of genic incompatibilities, which can lead to non-Mendelian inheritance of alleles and genotypes in the offspring of hybrids (Fishman et al., 2008; Leppälä et al., 2013). Transmission ratio distortion (TRD) is a naturally occurring phenomenon in which one allele is preferentially transmitted to the progeny than the opposite allele in hybrids between species (Jenczewski et al., 1997; Casellas et al., 2012). If one allele at a locus can reduce gametic or zygotic fitness, then the genomic regions linked to it will cause distorted allele or genotype frequencies in heterozygotes (Vogl and Xu, 2000; Xu et al., 2013). TRD is frequently observed and characterized in
intraspecific or interspecific segregating populations from various plant species, such as Arabidopsis (Leppälä et al., 2013; Seymour et al., 2019), cotton (Chandnani et al., 2017; Dai et al., 2017), wheat (Kumar et al., 2007), and rice (Koide et al., 2008a,b, 2012; Li et al., 2017, 2019). In particular, TRD is one of the primary origins of reproductive isolation or speciation and can severely hamper the exchange of genes among rice subspecies. Understanding the mechanisms responsible for TRD is important for using agriculturally interesting alleles from rice germplasm.

Transmission ratio distortion occurs before or after fertilization due to various reasons, such as meiotic drive, gametic competition, inbreeding depression, and hybrid incompatibilities (Huang et al., 2013; Fishman and McIntosh, 2019). It can be caused either by incompatible allelic interaction at a single-locus (Bauer et al., 2007; Corbett-Detig et al., 2013) or by two or multi-loci interaction, in which one gene effect is dependent on the presence/absence of other genes (Giesbers et al., 2019). With regard to rice, numerous loci/regions have been identified for TRD (Li et al., 2017, 2019; Zhang et al., 2020a). A few of them have been cloned and characterized in rice. They revealed that a given locus usually consists of multiple tightly linked genes and it is easily affected by the complex genetic background. For example, several well-known killer–protector and toxin–antidote segregation distortion systems that consist of multiple tightly linked genes have been identified. A killer–protector system contains three closely linked genes at the S5 locus that regulate both hybrid fertility and segregation distortion (Yang et al., 2012). The toxin–antidote system of qHMS7 contains two tightly linked genes (ORF2 and ORF3), of which ORF2 encodes a toxic genetic element that can kill the pollen without the protection of ORF3, leading to segregation distortion in heterozygotes (Tu et al., 2018). The S1 locus, constituted of three genes SIA4, SITPR, and SIA6, also demonstrates a killer–protector system that can eliminate the gametes carrying the Asian allele (S1-s), resulting in a preferential transmission of the African rice S1 allele to the progeny (Xie et al., 2017, 2019). TRD might occur due to two or multi-loci interactions, such as S27/S28, DPL1/DPL2, and S25/S24 regions (Mizuta et al., 2010; Yamagata et al., 2010; Kubo et al., 2011; Nguyen et al., 2017). Despite these well-investigated examples, understanding the genetic and molecular mechanisms of TRD is still incomplete. Therefore, it is critical to identify more loci and candidate genes for dissecting the genetic and molecular basis of such a complex phenomenon.

Our previous studies, using backcross inbred lines (BIL) derived from an intersubspecific cross of the japonica cultivar Nipponbare (NIP, as the donor) and indica Zhenshan97 (ZS97, as the recurrent parent), using a backcross scheme with a marker-assisted selection approach (Sun et al., 2015; Zhang et al., 2020b). A CSSL that harbors two loci/regions of interest was selected to cross with ZS97 to develop an F1 hybrid. Deviation of the allele and genotype frequencies from Mendelian expectations at a target locus was assessed in segregating the progeny of the hybrid. Furthermore, from the CSSL-derived progenies, several independent near-isogenic lines (NILs), each containing only a single introduced heterozygous segment covering either TRD4.1 or TRD4.2 in the otherwise uniform background of ZS97, were developed. Six independent segregating populations were derived from relevant NILs to validate TRD loci. To delimit TRD4.1 and TRD4.2, two large segregated populations were generated to select recombinant individuals per locus. The recombinants with heterozygous target regions were self-crossed to generate progeny for phenotyping TRD. In addition, the reported BIL population from the cross of NIP and 9,311, which were genotyped by the genotyping-by-sequencing method (Yuan et al., 2019), was also used for the analysis of epistatic interaction of particular TRD loci.

For reciprocal cross to test gametic selection, a line (named as NZ) carrying TRD4.1 at a heterozygous state was first obtained by crossing ZS97 with a NIL that harbors the NIP alleles at TRD4.1. Then, two reciprocal populations were generated by crossing NZ as male or female parent with a developed NIL (named as MM) that contains introduced Minghui 63 (MH) alleles at TRD4.1 (Chen et al., 2018). Both NZ and MM have the same genetic background as ZS97.

Uniformly germinated seeds were planted on 96-well plates with the bottoms removed (Li et al., 2015), and the plates were placed in a growth chamber (Dongnan, Ningbo, China) or a greenhouse at 30°C under 16-h light/8-h dark conditions. The 7-day seedlings were used for genotype analysis. Some lines and segregating populations were grown in the experimental field of Huazhong Agricultural University (HAU) in Wuhan (30.48N, 114.2E), China. Each line was planted in a row with 10 individual spacings of 16.7 cm × 26.6 cm for genotyping. The field management was managed according to the local standard practices.

DNA Extraction and Genotype Analysis
Genomic DNA was extracted from young seedling leaves, as described previously (Zhang et al., 2020b). The genome-wide genotyping of CSSLs was conducted using the RICE 6K array (Sun et al., 2015; Chen et al., 2018). For the genotyping of
segregating populations, a number of polymorphic markers, including simple sequence repeat and insertion/deletion markers, were designed (Supplementary Table 1) and used for PCR reaction following the described procedure (Panaud et al., 1996).

**Transmission Ratio Distortion Analysis**

The allele and genotype frequencies were assayed by several polymorphic markers in a segregated population. The statistical Chi-square test ($\chi^2$) was performed using chisq.test function of R to determine whether the observed frequencies are distorted from Mendelian segregation ratios in any segregated population. A genomic region with two or more distorted consecutive markers was considered as one with TRD. Also, the TRD analysis in two subsets of the BIL population was performed using a single-marker analysis model in QTL IciMapping (v4.1), as described previously (Zhang et al., 2020a). A significance level of LOD > 5.0 was set as the threshold to declare the presence of a putative TRD effect in a given bin/marker.

**Gametic and Zygotic Selection**

To determine the cause of TRD (e.g., gametic or zygotic selection) at a given locus, the allele and genotype (NIP and ZS97) frequencies were assayed to test if they fit the Mendelian ratios, respectively. The comparative patterns of the allele and genotype frequencies may infer gametic or zygotic selection in multiple F$_2$ populations. If the allele frequency does fit the theoretical ratio of 1:1, and the genotype frequency is biased to 1: 2: 1, the TRD may be raised by zygotic factors. In this regard, an additional test is also performed to determine whether the observed heterozygote frequency deviated from the theoretical genotype frequency (0.5) in the F$_2$ populations (Fishman and McIntosh, 2019). If the allele frequency is distorted from 1:1, and the genotype frequency fits the ratio of 1: 2: 1, the TRD may result from gametic factors. If there is a distortion of both allele and genotype frequencies from the Mendelian ratios, then the TRD is due to both gametic and zygotic factors.

To track male- and female-specific transmission patterns for a given region using a reciprocal crossing test, diagnostic markers that were polymorphic among the parental lines (NIP, ZS97, and MH) were used for genotyping in the reciprocal cross progeny. The pattern of unequal frequencies through gametic selection is illustrated in Figure 1. If the F$_1$ (ZN) is the male parent (or conversely the female), distortion of genotype (NM and ZM) frequencies in the progeny indicates the gametic selection at the F$_1$-heterozygous locus.

**Pollen Fertility Observation**

Pollen fertility was examined with the I$_2$-KI staining method using mixed pollen grains of more than eight florets from the panicles of each plant, as previously described (Li et al., 2017). A microscope field with at least 200 pollen grains was observed. Pollen fertility was scored as the percentage of filled and stained grains in total grains (Kubo et al., 2011; Li et al., 2017).

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1https://www.r-project.org/
segregation of the two markers (M3 and M4) on chromosome 4 was observed in both allele and genotype frequencies (Table 1 and Supplementary Figure 1). Furthermore, eight additional consecutive markers (M5 to M12) distributed in the two TRD regions were all distorted significantly from the expected Mendelian ratios in the allele and genotype frequencies. The distortion of these markers was all biased toward ZS97 (Table 1 and Supplementary Table 2), suggesting that the ZS97 alleles at these two regions/loci (named TRD4.1 and TRD4.2) were transmitted to the progeny at a higher frequency than the NIP alleles.

### Validation of Transmission Ratio Distortion 4.1 and Transmission Ratio Distortion 4.2

To validate the effect of TRD4.1, two lines that carried heterozygous TRD4.1 along with TRD4.2 homozygous ZS97 or NIP were obtained and self-crossed to produce corresponding segregating populations. As a cluster of molecular markers showing TRD suggests that the chromosomal region may have one or more genes causing TRD, the representative marker (M7) was used to analyze the TRD effect at TRD4.1. A non-Mendelian segregation of TRD4.1 was observed in these two populations (Figure 2 and Table 2). Moreover, the ZS97 alleles at TRD4.1 were preferentially transmitted to the progeny of heterozygotes.

To check the effect of TRD4.2, two lines that harbored heterozygous TRD4.2 and homozygous at TRD4.1 either with ZS97 or NIP alleles were self-pollinated to produce segregating populations (Figure 2 and Table 2). Consistently, TRD4.2 assayed by the representative marker M12 exhibited a significant TRD effect with the preferential transmission of the ZS97 alleles in the progeny of heterozygotes harboring homozygous TRD4.2.

An additional segregated population (n = 902) was also generated from the heterozygotes at both TRD4.1 and TRD4.2 (Figure 2). Frequencies of the nine genotypes assayed by two markers (M7 and M12) linked with TRD4.1 and TRD4.2 did not fit the Mendelian segregation ratio ($\chi^2 = 185.5; P < 2.2 \times 10^{-16}$), given the two loci are linked (Supplementary Table 2). In particular, the number of genotype homozygous TRD4.1$^{ZS97}$TRD4.2$^{ZS97}$ was more than that of TRD4.1$^{NIP}$TRD4.2$^{NIP}$, TRD4.1$^{ZS97}$TRD4.2$^{NIP}$, and TRD4.1$^{NIP}$TRD4.2$^{ZS97}$ in the population. Collective data confirmed that TRD arose from both TRD4.1 and TRD4.2 and a preferential transmission of the ZS97 gametes at the two loci.

### Detection of Other Transmission Ratio Distortion Loci Conditions on Transmission Ratio Distortion 4.1

To determine whether TRD4.1 affects other genomic regions on TRD, the BIL population that was previously developed from the cross of NIP and 9,311 (Zhang et al., 2020b) was divided into two subpopulations according to the TRD4.1 genotype: SubN (n = 75) in which all lines had TRD4.1$^{NIP}$ and Subj (n = 325) that had TRD4.1$^{9311}/9311$ assayed by 10 consecutive bin-markers (from 13.47 to 15.87 Mb) at the TRD4.1 region (Supplementary Table 3). Four and ten regions were detected in SubN and Subj, respectively. Among them, three TRD regions (TR1.3, TR8.2, and TR12.1) were common in both subpopulations. The other seven regions were identified only in Subj. In particular, the TRD4.2 region was detected only in SubN but not in Subj (Supplementary Table 3). These results revealed the effect of TRD4.1 on the detection of other TRD loci in the BIL population.

### Gametic Selection Leading to Transmission Ratio Distortion

To investigate whether TRD4.1 and TRD4.2 are involved in the gametic or zygotic factors, the population segregated at both TRD4.1 and TRD4.2 was analyzed. The results showed that the frequencies of heterozygous genotypes at either TRD4.1 or TRD4.2 were approximately 0.5. The observed frequencies of heterozygotes at either TRD4.1 or TRD4.2 exhibited no significant derivation from the expected

### Table 1 | Identification of TRD4.1 and TRD4.2 in the CSSL-derived segregating populations.

| Loci | Marker | Position (Mb) | Genotype frequency | P value | Allele frequency | Toward$^a$ |
|------|--------|---------------|--------------------|---------|-----------------|-----------|
|      |        |               | P1 | H | P2 | $\chi^2$ (df = 2) | P-value | ZS97 | NIP | $\chi^2$ (df = 1) | P-value |
| TRD4.1 | M5  | 6.46 | 71 | 95 | 20 | 28.05 | 8.1E-07 | 0.64 | 0.36 | 27.97 | 1.2E-07 | ZS97 |
|       | M6  | 8.63 | 79 | 89 | 22 | 34.96 | 2.6E-08 | 0.65 | 0.35 | 34.20 | 5.0E-09 |
|       | M3  | 11.65 | 73 | 95 | 22 | 27.37 | 1.1E-06 | 0.63 | 0.37 | 27.38 | 1.7E-07 |
|       | M7  | 14.06 | 77 | 94 | 20 | 30.90 | 1.9E-07 | 0.64 | 0.36 | 30.86 | 2.8E-08 |
| TRD4.2 | M4  | 19.95 | 67 | 93 | 25 | 19.07 | 7.2E-05 | 0.61 | 0.39 | 19.07 | 1.3E-05 | ZS97 |
|       | M9  | 20.09 | 74 | 94 | 23 | 27.30 | 1.2E-06 | 0.63 | 0.37 | 27.24 | 1.8E-07 |
|       | M10 | 20.17 | 67 | 98 | 18 | 27.16 | 1.3E-06 | 0.63 | 0.37 | 26.24 | 3.0E-07 |
|       | M11 | 20.20 | 70 | 85 | 26 | 22.10 | 1.6E-05 | 0.62 | 0.38 | 21.39 | 3.7E-06 |
|       | M12 | 21.63 | 76 | 88 | 27 | 26.32 | 1.9E-06 | 0.63 | 0.37 | 25.14 | 5.3E-07 |

$^a$ The allele is preferentially transmitted in heterozygotes. P1, P2, and H indicate homozygous ZS97, Nipponbare (NIP), and heterozygous genotypes, respectively.
frequencies (Supplementary Table 2). This non-distortion of heterozygote frequency may indicate a gametophytic barrier. In addition, normal pollen fertility (greater than 95%) of the nine genotypes revealed that TRD was not caused by pollen fertility (Supplementary Table 4). These results confirm that the gametic factors are involved in TRD4.1 and TRD4.2.

To investigate whether the TRD4.1 effect is caused by female or male gametic factors, a reciprocal test cross was made between NILs with a heterozygous (ZN) segment and a MM segment that contains homozygous Minghui 63 alleles at the locus (Figure 1). Segregation of the locus was assessed in a F1 progeny from the crosses. The progenies of the reciprocal crosses (NZ × MM and MM × ZN) were examined for TRD effects. The polymorphic markers M13 and M14 that were tightly linked with TRD4.1 showed normal-Mendelian segregation (no TRD) by the Chi-square test against the expected ratio of 1:1 (NM:ZM = 283:292; \( \chi^2 = 0.70 \)) in the cross of ZN × MM (\( n = 575 \)), in which ZN was used as the female parent. The results suggest that NIP and ZS97 alleles were equally transmitted to the progeny. However, significant distortion of genotype frequencies (NM:ZM = 196:343; \( \chi^2 = 40.09, P < 2.5 \times 10^{-10} \)) was observed in the progeny of the cross of MM × NZ (\( n = 539 \)), in which ZN was used as the male parent (Figure 1). In addition, normal pollen fertility (greater than 95%) of ZN was observed. These results indicate that the ZS97 allele was preferentially transmitted to the progeny over the NIP allele. Therefore, the distortion of genotype frequencies at TRD4.1 in the progeny involved male gamete selection.

**Fine Mapping of Transmission Ratio Distortion 4.1 and Transmission Ratio Distortion 4.2**

To narrow the region of TRD4.1, a mapping population comprising of approximately 6,500 individuals was developed from one plant that harbored only one heterozygous TRD4.1 region and one homozygous TRD4.2\textsuperscript{NIP} within the ZS97 background. Initially, five recombinant individuals (R1 to R5) were obtained using several markers linked with TRD4.1. Then, the recombinants were self-crossed to generate five independent segregating populations for further genotyping (Figure 3). Based on frequencies of the genotypes in each recombinant-derived population using representative markers (M7 or M8), the distorted region covering TRD4.1 was determined in the corresponding recombinant. TRD4.1 was preliminarily mapped to an approximately 590-kb region between markers M7 and M8. For fine mapping of TRD4.1, eight recombinant individuals (R6 to R13) within the interval of M7-M8 were selected and generated eight independent recombinant-derived populations. TRD analysis based on frequency genotypes in recombinant-derived populations delimited TRD4.1 into a 100-kb region between markers M19 and M8. Based on the same approach, fine-mapping of TRD4.2 was conducted on a segregated population (composed of approximately 5,800 individuals) derived from a plant that carried only one heterozygous TRD4.2 region and one homozygous TRD4.1\textsuperscript{NIP} segment in the ZS97 background. Seventeen recombinant individuals in the TRD4.2 region flanked by markers M23 and M12 were obtained (Figures 4A,B). Based on TRD analysis of genotype frequencies in each of the 17 recombinant-derived populations and genotyping with additional markers in the target region, TRD4.2 was

**Table 2** Validation of TRD4.1 and TRD4.2 in four segregating populations.

| Parental genotype | Representative Marker | Observed genotype frequency | Observed allele frequency | Toward |
|-------------------|-----------------------|-----------------------------|--------------------------|-------|
|                   |                       | ZZ  | H   | NN  | Sum  | \( \chi^2 \) (df = 2) | P-value | ZS97 | NIP | \( \chi^2 \) (df = 1) | P-value |       |
| TRD4.1\textsuperscript{m}/TRD4.2\textsuperscript{ZS} | M7                  | 128 | 178 | 63  | 369  | 23.4                       | 8.6E-06 | 0.59 | 0.41 | 22.9                    | 1.7E-06 | ZS97  |
| TRD4.1\textsuperscript{n}/TRD4.2\textsuperscript{NIP} | M7                  | 122 | 174 | 52  | 348  | 28.2                       | 7.7E-07 | 0.60 | 0.40 | 28.2                    | 1.1E-07 | ZS97  |
| TRD4.1\textsuperscript{NIP}/TRD4.2\textsuperscript{ZS} | M12                 | 136 | 181 | 39  | 356  | 53.0                       | 3.2E-12 | 0.64 | 0.36 | 52.9                    | 3.6E-13 | ZS97  |
| TRD4.1\textsuperscript{NIP}/TRD4.2\textsuperscript{NIP} | M12                 | 81  | 184 | 78  | 343  | 1.9                        | 3.9E-01 | 0.50 | 0.50 | 0.1                     | 8.2E-01 | Equal |

\( a,b \) Genotype and allele frequencies assayed by representative markers tightly linked with TRD4.1 or TRD4.2, respectively. ZZ, NN, and H indicate the homozygous ZS97 (ZS), Niponbare (NIP), and heterozygous genotype (het), respectively. \( c \) Toward means preferential allele transmission from heterozygotes. "Equal" indicates that both alleles are transmitted equally to the progeny.
narrowed down to an approximately 34.1-kb region. This region contains nine predicted genes based on the reference genome \(^1\) (Figure 4C). Of them, LOC_Os04g33150 was the only one expressed gene, after removing those annotated as unknown, transposons/retrotransposons, or hypothetical proteins. It was specifically and highly expressed in the pre-emergence inflorescence, 5-d seed, and 25-d endosperm. \(^3\) This gene encodes a desiccation-related protein. Sequence differences that existed in the coding region between NIP and ZS97 may cause two amino acid changes. \(^4\) Therefore, LOC_Os04g33150 is the most likely candidate gene for TRD4.2.

**DISCUSSION**

The present study identified two linked loci TRD4.1 and TRD4.2, and their epistatic interaction caused transmission ratio distortion in several CSSL-derived segregating populations, in which only a single or two NIP segments were targeted within the ZS97 background in rice. Both loci showed a preferential transmission of the ZS97 alleles to the progeny in heterozygotes. This severe TRD with a bias toward the indica allele is consistent with previous results in the NIP/9311 BIL population and other subspecific crosses (Wang et al., 2009; Reflinur et al., 2014; Zhang et al., 2020a).

One of the findings is that the TRD effect on TRD4.2 was dependent on TRD4.1 (Figure 1). TRD4.2 showed severe TRD in the CSSL-derived populations in which the progeny harbored homozygous homzygous TRD4.1\(^{NIPI}\), but normal segregation in the progeny when it carried with homozygous TRD4.1\(^{ZS97}\) (Table 2). Furthermore, TRD4.2 was detected only in SubN that had a fixed NIP genotype at the TRD4.1 region in the NIP/9311 BIL population (Supplementary Table 3). However, TRD4.1 displayed several allelic transmission biases no matter whether TRD4.2 was homozygous or heterozygous NIP alleles (Figure 2). In addition, TRD4.1 from the NIP decreased the incidence of TRD (63%) in a subset (SubN) of the BIL population. These results suggest that epistatic interaction plays an important determinant in the segregation patterns of TRD loci. This is the possible reason that some loci like TRD4.2 have largely gone unnoticed in any previous study on rice.

Another finding in this study is that TRD4.1 is possible only through male gametic selection among the progeny. TRD is a selection mechanism that could be caused by gametic and zygotic factors. In the present studies, using a series of CSSL- and NIL-derived populations, we found that the allele frequencies at TRD4.1 and TRD4.2 in the populations were significantly skewed toward ZS97 (Tables 1, 2); however, the heterozygous genotypes showed fertile pollen and normal segregation in the progeny (Supplementary Table 4). These results indicate that gametic

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\(^1\)http://rice.plantbiology.msu.edu/
\(^2\)http://rice.plantbiology.msu.edu/expression.shtml
\(^3\)http://rice.hzau.edu.cn/rice_rs3/

**FIGURE 3** Fine-mapping of TRD4.1. R1 to R13 represents the recombinant individuals between M15 and M8. Their derived segregating populations were used to validate TRD of TRD4.1. (A) TRD analysis of five (R1 to R5) recombinant-derived segregating populations delimited TRD4.1 into a 590-kb interval. The polymorphic marker of M8 was used to investigate the genotypes of R1 to R5-derived populations. (B) Finely mapping of TRD4.1 to a 100-kb region between M8 and M19 using eight (R6 to R13) recombinant-derived populations. The marker M8 was used to investigate the genotypes of R6- and R7-derived populations. The marker M7 was used to investigate the genotypes of R8 to R13-derived populations. ZS97, H, and NIP denote ZS97, heterozygous, and NIP genotypes at TRD4.1, respectively. **"** Denotes significant distortion of the allele and genotype frequencies by chi-square test at \(P < 0.01\). NS, no significance.
selection was involved in TRD4.1 and TRD4.2. Notably, we used a three-allele reciprocal crossing test to investigate whether there was a female or male gametic selection in TRD4.1 and found that the severe transmission bias of TRD4.1 was due to the male gametic selection of the NIP alleles. It is notable that TRD4.1 was mapped in the common region where SD4.1 was reported to affect segregation distortion and spikelet fertility in both inter- and intra-specific populations (Wan et al., 1996; Li et al., 2017; Zhang et al., 2020a). We further delimited TRD4.1 into a 100-kb interval and TRD4.2 into a 34.1-kb region with one candidate gene (Figures 3, 4), which would facilitate cloning the genes underlying the gametic factors for TRD. Further characterization and functional analysis of TRD4.1 will be required to better understand the male gametic selection through gamete killer, gamete competition, and/or differential fertilization success.

**CONCLUSION**

Two TRD regions (TRD4.1 and TRD4.2) on chromosome 4 were identified and validated using CSSL-derived secondary populations. A significant digenic interaction between TRD4.1 and TRD4.2 affected TRD. Of them, TRD4.2-mediated TRD was dependent on the presence of TRD4.1 alleles, but TRD4.1 was not affected by TRD4.2. Moreover, TRD4.1 and TRD4.2 were delimited to approximately 100-kb and 34.1-kb intervals, respectively. Furthermore, we found that TRD4.1 is male gametic in action with the preferential transmission of the indica ZS97 allele to the progeny. These findings would be helpful for cloning candidate genes and characterizing the molecular mechanisms underlying TRD.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

**AUTHOR CONTRIBUTIONS**

SY designed and conceived the research. CZ, JW, XX, DW, and WS developed the populations. CZ, JW, XZ, XX, and ZY conducted the experiments. CZ and JW analyzed the data. CZ, JW, and SY wrote the manuscript. All authors read and approved the final manuscript.
FUNDING

This research was funded by grants from the National Natural Science Foundation of China (31971864), the Hubei Special Major Projects for Technological Innovation (2020ABA016), and the Earmarked Fund for China Agricultural Research System (CARS-01-06).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.866276/full#supplementary-material

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Supplementary Figure 1 | Primarily mapping TRD regions on chromosome 4 using CSSL-derived populations. (A) Graphical genotype of CSSL91 showing four introduced Nipponbare segments encompassing TRD4.1 and TRD4.2 in the ZS97 background. (B) The allele and genotype frequencies at target regions (markers) showing non-Mendelian segregation by Chi-square test. ZZ, ZN, and NN represent ZS97, heterocytoge, and NIP genotype, respectively.

Supplementary Table 1 | Primers used in this study.

Supplementary Table 2 | Nine genotypic frequencies at the loci TRD4.1 and TRD4.2 in a segregated population.

Supplementary Table 3 | The genomic regions detected for transmission ratio distortion in two subpopulations fixed TRD4.1 Nip or TRD4.19311.

Supplementary Table 4 | Pollen fertility percentage of nine genotypes at TRD4.1 and TRD4.2.
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