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Fine mapping of *qhir8* affecting in vivo haploid induction in maize

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Abstract

Key message **The QTL *qhir8* affecting in vivo haploid induction in maize was mapped to a 789 kb region, embryo abortion rate and segregation ratios were analyzed, linkage markers for MAS were developed.**

Abstract The doubled-haploid (DH) technology has become an important tool for line development in modern maize breeding. However, the genetic basis of haploid induction remains elusive. In previous QTL mapping research, *qhir8* besides *qhir1* significantly affected haploid induction rate (HIR). Our objective was to fine map *qhir8* and assess its effect on HIR, segregation distortion (SD) and embryo abortion (EmA). A total of 3989 F_2 plants from the cross of inducers CAUHOI and UH400 were screened for recombinants in the *qhir8* region. F_2 plants and F_3 plants from selfing progenies of 34 recombinant F_2 plants were evaluated for HIR, SD and EmA. In parallel, we developed 31 new markers providing good coverage of the *qhir8* region. We confirmed that *qhir8* has an increasing effect on HIR and EmA, but not on SD. Moreover, we successfully narrowed down the *qhir8* locus to a 789 kb region flanked by markers 4292232 and umc1867.

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C. Liu, W. Li, A. E. Melchinger and S. Chen contributed equally to this work.

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Introduction

The doubled-haploid (DH) technique has become a major tool in maize breeding during the past decades for temperate as well as tropical target regions (Prigge and Melchinger 2012; Prasanna 2012). The main advantages of the method are considerable savings in time for line development, full recovery of the entire genetic variance from the very beginning of a breeding cycle, and easier line maintenance and registration (Schmidt 2003; Melchinger et al. 2005; Seitz 2005). Currently, the DH method in maize relies almost exclusively on in vivo haploid induction. The break through for this method came when inducers were derived from Stock6 that produce maternal haploids at an acceptable rate in different genetic backgrounds and environments (Prigge et al. 2011; Kebede et al. 2011). Most inducers are equipped with morphological markers such as the *R1-nj* gene that allow discrimination of haploid and normal diploid seeds on the basis of coloration of the embryo. Chen and Song (2003) and Melchinger et al. (2013) proposed use of high oil inducers for distinguishing haploid from diploid seed. Successful application of this new method has been demonstrated in several experiments (Melchinger et al. 2014, 2015a, b) and promises to extend the application of in vivo haploid induction to maize germplasm in which the embryo marker cannot be used (Chaikam et al. 2015).

Haploid induction rate (HIR) relates to the proportion of haploid seeds obtained in induction crosses with an inducer. Coe (1959) first reported a stable frequency of haploid kernels ~3.23 % in selfed progenies of Stock6. The use of color genes expressed in the scutellum and aleurone made it more efficiency for haploid identification (Coe and Sarkar 1964). Based on the accurate identification of haploids, they found that the haploid induction rate was subject to both paternal parent and maternal parent (Sarkar and Coe 1966). Lashermes and Beckert (1988) verified that it is a quantitatively inherited trait that can be improved by selection. In a QTL mapping study with RFLP markers, Röber (1999) identified two QTLs, one major QTL located on chromosome 1 and a minor QTL on chromosome 2, which together explained 17.9 % of the phenotypic variance. Barret et al. (2008) identified one major locus controlling in vivo haploid induction in bin 1.04, which is same region as identified by Röber (1999). In a QTL study with four populations all involving inducer UH400 as common parent, Prigge et al. (2012) detected 8 QTLs, with *qhir1* and *qhir8* being two major QTL on chromosome 1 and 9 explaining 66 and 20 % of the genetic variance, respectively. The *qhir1* region in bin 1.04 does not only trigger haploid induction, but also causes gametophytic segregation distortion and embryo abortion (Barret et al. 2008; Prigge et al. 2012; Xu et al. 2013). Meanwhile, *qhir1* was fine-mapped to a 243 kb region in bin 1.04 (Dong et al. 2013), and used for MAS in the development of new inducers (Dong et al. 2014).

For further uncovering the genetic basis of in vivo haploid induction in maize fine-mapping of the major QTL and finally cloning the genes controlling HIR are necessary. In this study, we concentrated on fine mapping of *qhir8,* the second large QTL for HIR. Our goal was to (1) validate and characterize the effect of *qhir8* described previously, (2) pave the way for cloning of the underlying gene(s), and (3) develop markers closely linked to *qhir8* that could be used in marker-assisted selection (MAS) for inducer development.

Materials and methods

Plant materials

Inducer lines CAUHOI and UH400 were used as parents for constructing the mapping population. CAUHOI has HIR of about 2 % (Li et al. 2009), whereas UH400 has a higher HIR of about 8 % (Prigge et al. 2011). UH400 and CAUHOI are both fixed for the *qhir1* region (Prigge et al. 2012), which is regarded as mandatory for haploid induction. Both inducers carry the dominantly expressed marker gene *R1-nj*, which causes purple coloration of both the scutellum and aleurone in normal diploid crossing seeds, but white coloration of embryos in haploid seeds (Nanda and Chase 1966; Neuffer et al. 1997).

The F_1 plants from the cross CAUHOI × UH400 were self-pollinated to develop F_2 populations in 2010 and 2012. In 2011, F_2 populations with 244 and 374 individuals were planted in Beijing and Hainan islands, respectively, to verify the *qhir8* effect on HIR with markers bnlg1272 and phi033. In 2012, 830 F_2 plants were planted in Hainan islands and screened for recombinants in the *qhir8* region with markers bnlg1272 and phi033. All recombinants were self-pollinated to produce F_3 progenies. In 2013 in Beijing and Hainan islands, a total of 17 F_3 populations were employed for fine mapping. Each F_3 plant was genotyped and its HIR was determined by crossing it onto tester ZD958 and growing the testcross seed in the next season. The same year in Beijing, we planted 1287 F_2 individuals for screening for recombinants, however, none of them could be self-pollinated because of serious draught. In Hainan islands, another F_2 population with 618 individuals was planted and screened for recombinants again with markers bnlg1272 and phi033. In 2014, we employed 17 newly screened recombinants in total for fine mapping in Beijing and Hainan islands. Hybrid ZD958 was used as a tester for evaluating the HIR of F_2 individuals or F_3 progenies as described by Dong et al. (2013).

DNA extraction, and marker development

Young leaves were sampled in the field for DNA extraction, which was performed according to the protocol of Murray and Thompson (1980). Development of new markers concentrated on the interval between bnlg1272 and phi033. First, the sequence of the B73 reference genome between markers bnlg1272 and phi033 was downloaded from the MaizeGDB (http://www.maizegdb.org/) for searching for SSRs and single or low-copy sequences by online blast (http://www.maizegdb.org/popcorn-/search/sequence_search/home.php?a=BLAST_UI). Second, these SSRs and low copy sequences were used for the development of SSR markers and InDel markers with PRIMER 3 (http://frodo.wi.mit.edu/primer3/). Third, all markers developed were tested with polyacrylamide gel electrophoresis (PAGE) or agarose gel electrophoresis (AGE) for polymorphism between CAUHOI and UH400. Finally, each polymorphic marker was tested by a linkage group containing more than 45 F_2 recombinants to confirm its position. Markers positioned on chromosome 9 with polymorphism and stable performance were used for subsequent mapping work.

Genotyping

Each DNA sample was electrophoretically analyzed in a 1–3 % agarose gel or a 6 % denaturing polyacrylamide gel with

appropriate markers. First, 3989 individuals in total from F_2 population were screened with markers bnlg1272 and phi033 for recombinants. Then, markers newly developed were used to resolve the crossover point in each recombinant. For each individual in F_3 generation, the two nearest markers flanking the crossover point were used for genotyping. As a result, individuals of each F_3 family were classified into three genotype classes: A for absence of the *qhir8* allele, B for presence of the *qhir8* allele in homozygous state, and H for heterozygosity.

Phenotyping

In the experiment verifying the effect of *qhir8* in the F_2 population in 2011, three plants of hybrid ZD958 were pollinated by each F_2 plant for determining its HIR. For the fine mapping work in F_3 generation, 4–6 ears of ZD958 were pollinated. Normal kernels on the ear of ZD958 were classified into diploid crossing kernels and haploid seeds based on the color of scutellum and aleurone according to Dong et al. (2013). Abnormal kernels without embryo but normal endosperm were classified as embryo abortion (EmA) kernels according to Xu et al. (2013). After classification, all putative haploid seeds were planted in the field to verify their true nature following the protocol of Dong et al. (2013). HIR was calculated according to Dong et al. (2013) as HIR = (number of putative haploids/total number of normal kernels) × (number of true haploids in the field/number of putative haploids) × 100 %. The embryo abortion rate (EmAR) was calculated according to Xu et al. (2013) as EmAR = [number of EmA kernels/(number of normal kernels + number of EmA kernels)] × 100 %.

Results

Effect of *qhir8* in F_2 populations in Beijing and Hainan

A total of 138 and 73 F_2 individuals of different genotype classes were phenotyped in Beijing (2011) and Hainan islands (2011), respectively. The average HIR values of the three genotype classes showed the same trend for Beijing and Hainan (Fig. 1). Wilcoxon rank sum tests indicated that in both environments, HIR of F_2 plants from genotype class B (homozygous for UH400 at *qhir8*) was significantly higher ($P < 0.01$) than HIR of F_2 plants from genotype class A (homozygous for CAUHOI at *qhir8*). The mean HIR of heterozygous F_2 plants (genotype class H) was almost perfect in between the two homozygous classes.

Fine mapping of *qhir8*

In a first step, we concentrated on recombinants in the region from bnlg1272 to phi033. In 2013, we planted in

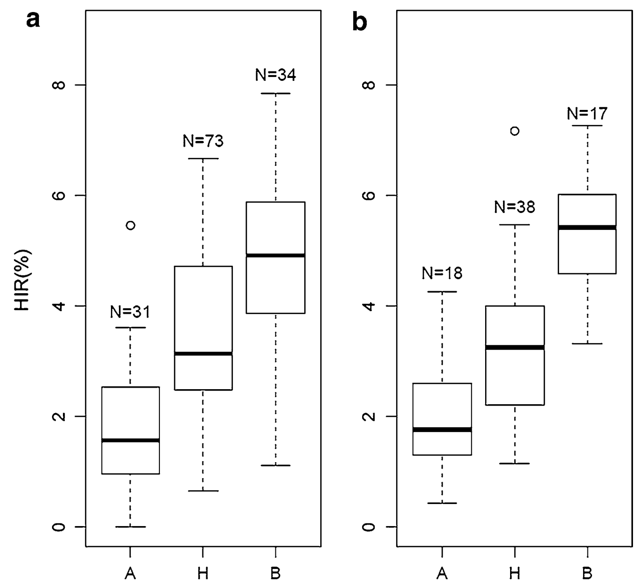
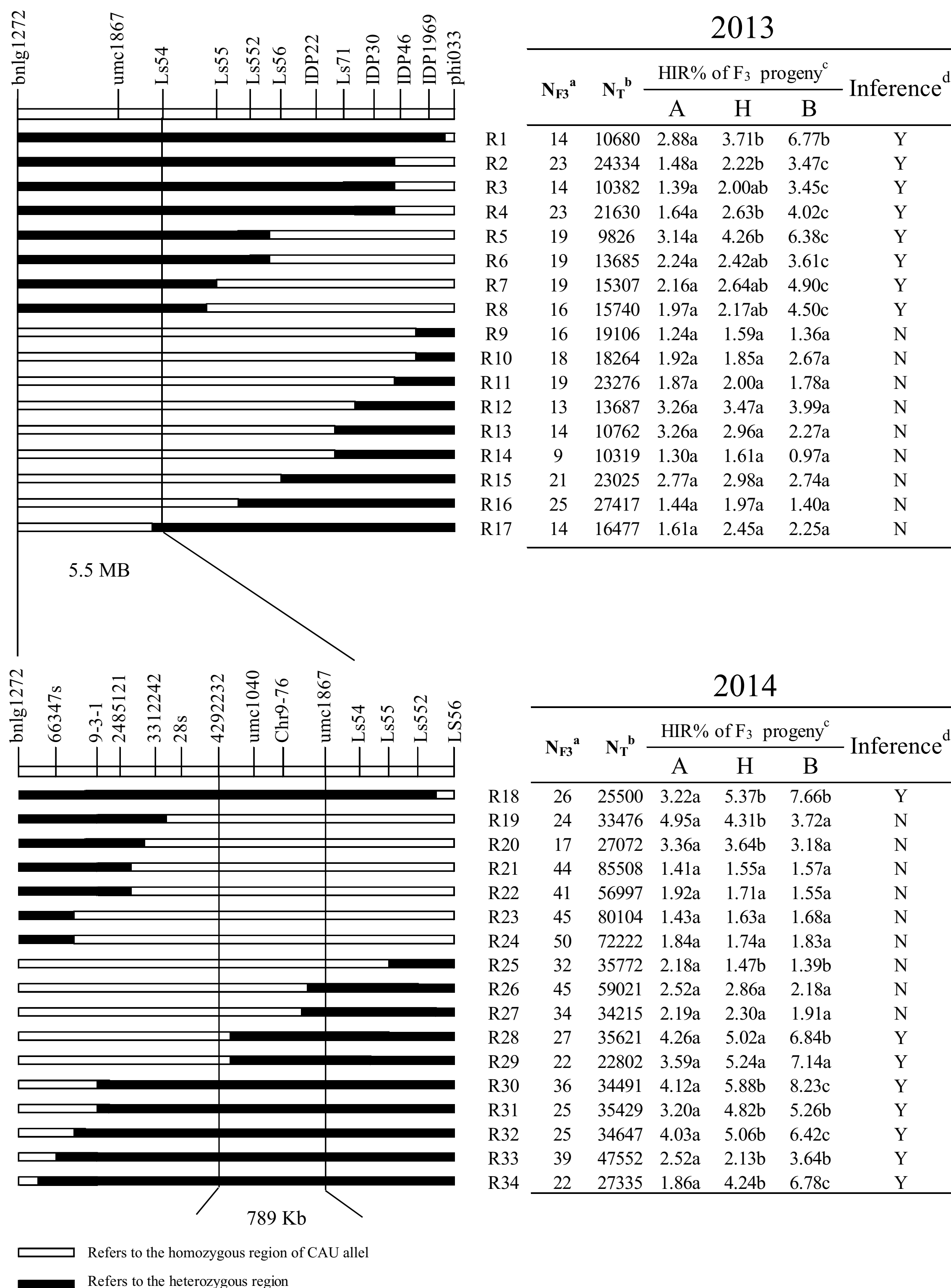


Fig. 1 Effect of the *qhir8* region on HIR in maize was analyzed with an F_2 population derived from cross CAUHOI × UH400. Number (*N*) of F_2 plants classified as *A* homozygous for the CAUHOI haplotype, *B* homozygous for the UH400 haplotype and *H* heterozygous based on genotype of two flanking markers bnlg1272 and phi033 in the *qhir8* region. **a** F_2 plants grown in Beijing in summer 2011, **b** F_2 plants grown in Hainan in winter 2011

Beijing about 30 seeds with good kernel quality from each of 17 F_3 families produced from recombinant F_2 plants. Among the 17 recombinants identified, eight (R1–R8) had crossovers in marker intervals downstream of Ls54 and were heterozygous for the chromosome segment upstream of the putative crossover point (Fig. 2), all of them showed significant differences ($P < 0.05$) for HIR between genotype classes A and B indicating that these recombinants harbor QTL *qhir8* and their location is upstream of the marker Ls55. Conversely, the HIR of F_3 families from the nine recombinants (R9–R17) had crossovers in marker intervals downstream of umc1867 and were heterozygous for the chromosome segment downstream of the putative crossover point with no significant differences ($P < 0.05$) among the three genotype classes (Fig. 2). Altogether, these results indicated that *qhir8* is located upstream of marker Ls54. At the same time, an F_2 population with 618 individuals was planted in Hainan islands and screened for recombinants with markers bnlg1272 and phi033. As a result, 127 recombinants were identified and selfed for fine mapping work next year.

In 2014, the mapping region was further narrowed down from bnlg1272–phi033 to the region bnlg1272–Ls54. To this end, 22 markers were newly developed in the region from bnlg1272 to Ls54 (Supplemental Table. S1) for resolving the newly identified recombinants screened in winter of 2013. F_3 progenies of 17 recombinants (Fig. 2)



2013

	N_{F3}[a]	N_T[b]	HIR% of F_3 progeny[c]			Inference[d]
			A	H	B	
R1	14	10680	2.88a	3.71b	6.77b	Y
R2	23	24334	1.48a	2.22b	3.47c	Y
R3	14	10382	1.39a	2.00ab	3.45c	Y
R4	23	21630	1.64a	2.63b	4.02c	Y
R5	19	9826	3.14a	4.26b	6.38c	Y
R6	19	13685	2.24a	2.42ab	3.61c	Y
R7	19	15307	2.16a	2.64ab	4.90c	Y
R8	16	15740	1.97a	2.17ab	4.50c	Y
R9	16	19106	1.24a	1.59a	1.36a	N
R10	18	18264	1.92a	1.85a	2.67a	N
R11	19	23276	1.87a	2.00a	1.78a	N
R12	13	13687	3.26a	3.47a	3.99a	N
R13	14	10762	3.26a	2.96a	2.27a	N
R14	9	10319	1.30a	1.61a	0.97a	N
R15	21	23025	2.77a	2.98a	2.74a	N
R16	25	27417	1.44a	1.97a	1.40a	N
R17	14	16477	1.61a	2.45a	2.25a	N

2014

	N_{F3}[a]	N_T[b]	HIR% of F_3 progeny[c]			Inference[d]
			A	H	B	
R18	26	25500	3.22a	5.37b	7.66b	Y
R19	24	33476	4.95a	4.31b	3.72a	N
R20	17	27072	3.36a	3.64b	3.18a	N
R21	44	85508	1.41a	1.55a	1.57a	N
R22	41	56997	1.92a	1.71a	1.55a	N
R23	45	80104	1.43a	1.63a	1.68a	N
R24	50	72222	1.84a	1.74a	1.83a	N
R25	32	35772	2.18a	1.47b	1.39b	N
R26	45	59021	2.52a	2.86a	2.18a	N
R27	34	34215	2.19a	2.30a	1.91a	N
R28	27	35621	4.26a	5.02a	6.84b	Y
R29	22	22802	3.59a	5.24a	7.14a	Y
R30	36	34491	4.12a	5.88b	8.23c	Y
R31	25	35429	3.20a	4.82b	5.26b	Y
R32	25	34647	4.03a	5.06b	6.42c	Y
R33	39	47552	2.52a	2.13b	3.64b	Y
R34	22	27335	1.86a	4.24b	6.78c	Y

Fig. 2 Fine mapping of the *qhir8* region. *Left* side the markers mapping to bin 9.01 are shown on *top* of the *bars*. The designations of the F_2 recombinants (R1-R34) in 2013 and 2014 are shown on the right side of each *bar*. *Right* side mean haploid induction rate (HIR %) of F_3 progenies: [a]*N* F_3 number of plants in the F_3 progeny, [b]*N*T total number of kernels from tester ears per F_3 family, [c]HIR of three genotype classes of each family, numbers in a line followed by the same *letter* are not significantly different from each other based on Wilcoxon rank-sum tests at 5 % probability level, [d]significant effect of the segment on haploid induction based on the result of the Wilcoxon rank-sum tests. *A* homozygous for the CAUHOI haplotype, *B* homozygous for the UH400 haplotype and *H* heterozygous, *Y* F_2 recombinant harbors the *qhir8* QTL allele in heterozygous state, *N* F_2 recombinant does not harbor the *qhir8* allele

newly identified with crossovers between bnlg1272 and Ls56 were planted. Recombinant R18 had a similar multi—locus genotype as R6 and corroborated that *qhir8* is located upstream of marker Ls56. Recombinants R19 to R24 with crossover upstream 28 s heterozygous for chromosome segment upstream of the putative crossover point showed in their F_3 progenies no significant differences for HIR among the three genotype classes. However, five recombinants with crossovers downstream 66347s heterozygous for the chromosome segment downstream of the putative crossover point showed significant differences between genotype classes A and B. All together these results suggested that *qhir8* is downstream of marker 3313421. Recombinants R25, R26 and R27 with crossover between chr9–76 and Ls54 and heterozygous for the chromosome segment downstream of the putative crossover point showed no significant differences among three genotype classes, demonstrating that *qhir8* is located upstream of umc1867. The two most important recombinants were R28 and R29, with crossover between 4292232 and umc1040 and heterozygous for the chromosome segment downstream of the putative crossover point, showed significant differences among three genotype classes, corroborating that the causal region was located downstream of marker 4292232. Combining all results, the position of QTL *qhir8* was narrowed down to the region between markers 4292232 and umc1867, with a physical distance of 789 kb according to the B73 reference sequence.

Within the 789 kb intervals, there were 35 genes according to the B73 reference sequence (Supplemental Table. S2), of which, 22 genes were predicted as low confidence genes and transposable elements, and the other 13 genes were protein coding genes. Among the 13 protein coding genes, three genes GRMZM2G079003, GRMZM5G820423 and GRMZM5G878346 had no annotation according to maizeGDB data base (http://www.maizegdb.org). Two genes, GRMZM2G392306 and GRMZM2G113888 encode a NLP transcription factor and TCP transcription factor respectively. Four genes including GRMZM2G465046, GRMZM2G003167, GRMZM2G124276 and GRMZM2G124274 encode putative enzyme related proteins. GRMZM2G435294 encodes a putative Myosin family protein. GRMZM2G094586 encodes PPR_2 repeat. GRMZM2G124288 and GRMZM5G862101 encode putative Dev-Cell death superfamily and a putative ABA induced protein respectively.

Haploid induction was caused by the paternal parent and reciprocal crosses showed that no haploid or EmA kernels occurred (Xu et al. 2013). As a result, the genes affecting male gametophyte development were more likely responsible for haploid induction. The gene GRMZM2G435294 encoding a Myosin putative protein, which may be involved in cytoplasmic streaming, organelle motility, and remodeling in plant growth and development (Jedd and Chua 2002; Prokhnevsky et al. 2008; Bertet et al. 2004), is expressed with high level in the tassel, especially in anthers, and thus might be an important gene affecting male gametophyte development. However, other genes like GRMZM2G392306 and GRMZM2G113888 encoding transcription factors are also important genes, and cannot be excluded. More research is needed to verify the gene(s) underling haploid induction.

Segregation distortion (SD) and embryo abortion rate (EmAR)

None of the F_3 families from all recombinants showed significant distortion ($P < 0.01$) from the expected (1:2:1) Mendelian segregation ratio. Since each one showed no deviation from expected Mendelian segregation ratios, we conclude that *qhir8* has no effect on segregation distortion.

The Wilcoxon rank-sum test indicated a tight association between HIR and EmAR in the experiments in 2013 and 2014 (Table 1). All recombinants supposedly carrying the *qhir8* allele showed in their F_3 families significant higher ($P < 0.05$) EmAR rates for genotype class B than for class A, except for the two recombinants (R1 and R4) that did not reach the significant level ($P < 0.05$). For the majority of (14 of 18) recombinants in which *qhir8* is supposedly absent, no significant differences ($P < 0.05$) were observed among three genotype classes. As the result, we conclude that *qhir8* increases EmAR besides its positive effect on the HIR.

Discussion

Strategies for fine mapping of *qhir8*

Fine mapping of a QTL depends on three key elements: (1) a high marker density in the target region, (2) a sufficient number of recombinants and (3) accurate phenotyping of the selfed progenies of the recombinants (Yang et al. 2012). To meet these requirements, we first, developed 34 markers in the region between bnlg1272 and phi033, including InDels and SSRs, with a marker density of ~320 kb between adjacent markers. Second, a very large F_2 population was constructed with a total of $N = 3989$ plants so as to ascertain enough recombinants. Fortunately, the *qhir8* region located in bin 9.01 displays a high frequency of chromosomal crossover swaps. Among the 3989 F_2 individuals, we found in total 562 recombinants, 34 of which were used for fine mapping. Third, precise phenotype was achieved by crossing each F_3 plant to at least three plants of tester ZD958. Thus, HIR could be determined with high precision on the basis of at least 600 testcross seeds for each F_3 plant.

Table 1 Chi square tests for segregation distortion and Wilcoxon rank-sum tests for embryo abortion rate (EmAR) of different genotype classes among F_3 progeny

ID of F_2 recombinants	No. of F_3 progeny[a]				χ^2 test[b]		EmAR[c]				Inference about q*hir8*[d]
	A	*H*	*B*	Total	*A*:*H*:*B* = 1:2:1	*P* value	*A*	*H*	*B*	*F* value	
R1	4	8	2	14	0.86	0.65	2.25a	2.60a	3.41a	0.41	Y
R2	4	14	5	23	1.17	0.56	1.01a	1.38a	3.62b	5.92E − 08	Y
R3	4	7	3	14	0.14	0.93	0.92a	1.09a	2.59b	1.98E − 03	Y
R4	6	13	4	23	0.74	0.69	1.41a	1.49a	1.95a	0.15	Y
R5	6	8	5	19	0.58	0.75	1.64a	2.28a	4.43b	2.90E − 04	Y
R6	6	7	6	19	1.32	0.52	1.40a	1.33a	3.21b	5.14E − 06	Y
R7	4	10	5	19	0.16	0.92	1.85a	2.14a	3.87b	1.49E − 04	Y
R8	6	5	5	16	2.38	0.30	1.23a	1.78ab	2.21b	0.09	Y
R9	2	8	6	16	2.00	0.37	0.63a	1.26a	0.93a	0.38	N
R10	6	9	3	18	1.00	0.61	1.52a	1.08a	1.72a	0.16	N
R11	5	8	6	19	0.58	0.75	1.64a	0.66b	0.94b	0.01	N
R12	3	8	2	13	0.85	0.66	3.17a	2.58a	3.26a	0.55	N
R13	5	4	5	14	2.57	0.28	2.43a	1.85a	1.95a	0.37	N
R14	4	4	1	9	2.11	0.35	0.94a	1.29a	1.11a	0.42	N
R15	3	10	8	21	2.43	0.30	1.79a	2.03a	1.73a	0.73	N
R16	5	16	4	25	2.04	0.36	1.27a	1.52a	0.73a	0.55	N
R17	2	8	4	14	0.86	0.65	0.75a	1.80b	1.55b	0.08	N
R18	7	12	7	26	0.15	0.93	2.73a	3.60b	4.94b	0.02	Y
R19	8	11	5	24	0.92	0.63	2.52a	2.59a	2.57a	0.99	N
R20	8	5	4	17	4.76	0.09	1.73a	1.81a	1.42a	0.48	N
R21	12	23	9	44	0.50	0.78	1.41a	1.12a	1.28a	0.31	N
R22	9	25	7	41	2.17	0.34	1.61a	1.08b	1.23ab	4.72E − 03	N
R23	13	19	13	45	1.09	0.58	1.40a	0.96b	1.23a	5.90E − 05	N
R24	14	24	12	50	0.24	0.89	1.04a	1.06a	1.05a	0.99	N
R25	8	14	10	32	0.75	0.69	3.33a	1.69b	2.51a	2.27E − 03	N
R26	12	22	11	45	0.07	0.97	1.81a	1.72a	1.47a	0.45	N
R27	3	26	5	34	9.76	0.01	0.10a	0.21a	0.07a	0.68	N
R28	11	7	9	27	6.56	0.04	2.87a	3.75b	4.42b	2.12E − 04	Y
R29	6	7	9	22	3.73	0.16	2.81a	3.17a	5.64b	1.34E − 04	Y
R30	7	22	7	36	1.78	0.41	3.13a	4.95b	5.68b	2.57E − 04	Y
R31	5	12	8	25	0.76	0.68	2.13a	2.95b	4.14c	4.73E − 05	Y
R32	8	10	7	25	1.08	0.58	2.24a	3.62b	3.64b	8.15E − 04	Y
R33	10	14	15	39	4.38	0.11	1.95a	2.68b	3.67c	8.67E − 08	Y
R34	4	14	4	22	1.64	0.44	1.39a	3.09b	4.19b	2.53E − 04	Y

[a] F_3 plants were classified into three genotypes, *A* homozygous for the CAUHOI haplotype, *B* homozygous for UH400 haplotype, *H* heterozygous

[b] χ^2 test for segregation distortion from *A*:*H*:*B* = 1:2:1

[c] Wilcoxon rank-sum tests for EmAR, *A* homozygous for the CAUHOI haplotype, *B* homozygous for UH400 haplotype, *H* heterozygous. Numbers in a line followed by the same letter are not significantly different from each other based on a Wilcoxon rank-sum test at the 5 % probability level

[d] Inference of about segregation of *qhir8* in the F_3 progeny of the F_2 recombinants

Considering that confidence intervals for location of QTL are generally large and associated with uncertainty, we extended the search region beyond markers bnlg1272 and umc1867 delimiting QTL *qhir8* in the study of Prigge et al. (2012). To guarantee that *qhir8* could be fine mapped successfully, we began our fine mapping work by investigating a larger region between markers bnlg1272 and phi033 with a physical distance of 11 Mb, which is 6 Mb larger than the original QTL interval.

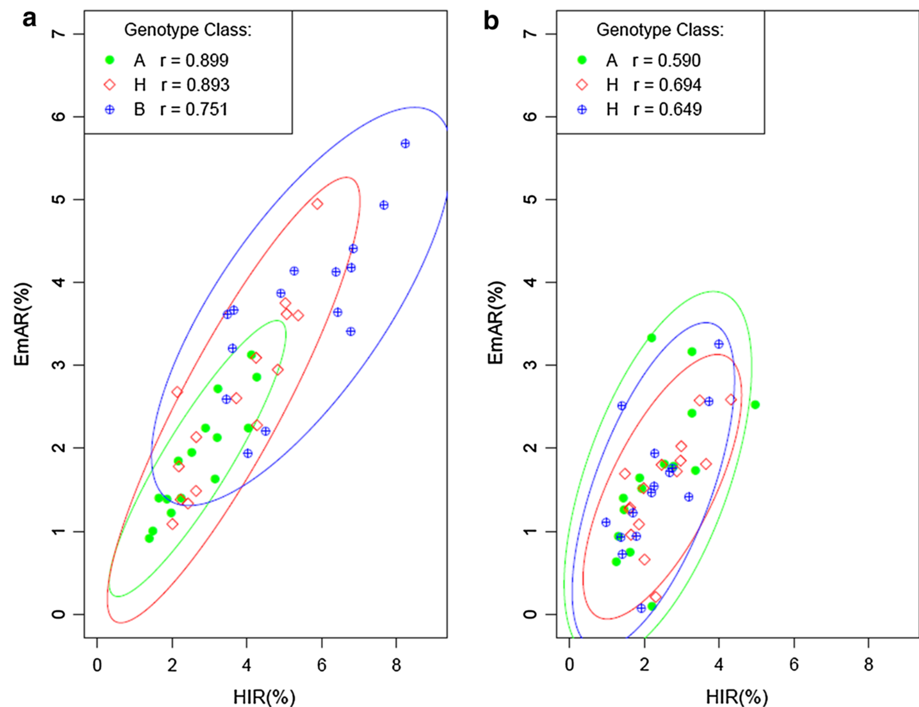


Fig. 3 Correlation between HIR and EmAR, **a** Plot of HIR and EmAR of F_3 families with significant differences among different genotype classes, *A* homozygous for CAUHOI, *B* homozygous for UH400 and *H* heterozygous. **b** Plot of HIR and EmAR of F_3 families with no significant differences among different genotype classes, *A* homozygous for CAUHOI haplotype, *B* homozygous for UH400 haplotype and *H* heterozygous

Effect of *qhir8*

The QTL mapping study of Prigge et al. (2012) indicated that the HIR in cross CAUHOI × UH400 was controlled by two major QTLs (*qhir1* and *qhir8*) and several minor QTLs. The *qhir1* locus, detected by several researchers with different populations (Röber, 1999; Barret et al. 2008), is supposedly mandatory for haploid induction ability (Prigge et al. 2012). However, inducers carrying the *qhir1* locus alone do not have high HIR and can differ substantially for this trait. For example, inducers CAUHOI and UH400 both harbor the *qhir1* allele, but their HIR varies considerably (2 and 8 % respectively) (Prigge et al. 2011; Li et al. 2009). Hence, one can conclude that in addition to *qhir1*, further genes do affect HIR. In our study, both F_2 plants and F_3 plants from recombinants carrying the *qhir8* allele had significantly higher HIR than those genotypes without this allele and recombinants homozygous for the *qhir8* allele had higher HIR than those being heterozygous. This result is in harmony with the hypothesis of Prigge (2012) that *qhir8* acts as enhancer for the function of *qhir1*, and improves the HIR of inducers in the presence of *qhir1*.

QTL *qhir1* was proven to affect not only HIR, but to cause also segregation distortion and embryo abortion (Barret et al. 2008; Xu et al. 2013; Prigge et al. 2012). Xu et al. (2013) speculated that EmA kernels are also haploid or aneuploid, but stop to develop because of shortage of nutrient supply, chromosomal imbalance or other reasons at later development stages (Xu et al. 2013). As a result, the number of kernels heterozygous or homozygous for the *qhir1* allele was less than expected from Mendelian segregation (Xu et al. 2013).

In our study, *qhir8* also increased embryo abortion in addition to HIR, the effect of *qhir8* on EmAR was similar to that on HIR. Thus, there is a strong positive correlation between HIR and EmAR (Fig. 3). However, different from *qhir1*, *qhir8* did not lead to segregation distortion. This result suggests that *qhir8* acts with regard to HIR and EmA in a way different from *qhir1*. On the other hand, Xu et al. (2013) showed that abortion of kernels with *qhir1* lead to the SD effect of *qhir1*. According to our results, *qhir8* could also lead to embryo abortion and we speculate that *qhir8* could increase SD effect of *qhir1,* although it might not cause the SD effect itself. However, further research is warranted to verify this result. We have developed NILs with B73 background harboring each of the two QTLs and both simultaneously and further experiments will be designed for the exploration of the interaction between the two QTLs controlling HIR.

Application for marker-assisted selection (MAS)

Marker-assisted selection (MAS) can improve the efficiency of selection and accelerate the process of breeding (Xu 2010). Markers closely linked to specific QTL are the basis for MAS. Fine mapping of *qhir8* provided closely linked markers for MAS and made it possible screening for presence and selection of the *qhir8* allele in segregating populations.

The *qhir1* locus is considered mandatory for haploid induction (Prigge et al. 2012). However, the HIR of CAUHOI, which carries the *qhir1* but not the *qhir8* allele was on average approximately 2 % in our research based on testers from different genetic backgrounds. Hence, the *qhir1* locus alone cannot meet the standards for an acceptable HIR expected from modern inducers in DH breeding. Our study showed that the *qhir8* locus can more than double the HIR in the presence of *qhir1*. Thus, an elite inducer should harbor at least these two QTLs. Consequently, integrating both *qhir1* and *qhir8* from inducers such as UH400 by MAS into new materials with desirable agronomic properties seems to be a promising way for inducer development in the future. When designing such a MAS breeding program, one must keep in mind that segregation distortion most likely acts against the inducer genotype. Thus, the population size used for MAS should be increased to be prepared against a lower number of desired segregants, as expected under normal Mendelian segregation. Starting with separate selection for *qhir1* and *qhir8* and combining both loci in an advanced generation as suggested by Frisch and Melchinger (2001) could even help to reduce the costs of such a MAS breeding program.

Author contribution statement S. Chen managed the project. S. Chen, C. Liu, designed the experiment. C. Liu, W. Li, Y. Zhong, X. Dong, X. Tian, L. Wang, B. Chen and C. Chen performed experiment. C. Liu, W. Li, H. Hu and A.E. Melchinger performed data analysis. C. Liu, W. Li, A.E. Melchinger and S. Chen wrote the paper.

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Compliance with ethical standards

Conflict of interest We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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