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Pyramiding Resistance Genes to Northern Leaf Blight and Head Smut in Maize

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ABSTRACT

Northern leaf blight (NLB) and head smut are two important diseases of maize (*Zea mays*) in China. The use of resistant cultivars is the most effective, economical and environmentally friendly means to cope with these diseases. For combining alleles for resistance to both NLB and head smut, parental inbred Ent17 with NLB resistance and parental inbred Ent12 with head smut resistance were crossed. The resistance screening for F_2, F_3 generations of the cross Ent17×Ent12 were conducted based on the phenotypic values and marker assisted-selection. Two pyramided lines carrying *Ht1*, *Ht2* and head smut resistance *QTL*, three lines carrying *Ht1* and head smut resistance *QTL* were found. The result revealed that lines carrying *Ht1*, *Ht2* and head smut *QTL* had resistance level and yield over donor Ent17, Ent12, lines carrying *Ht1* and *Ht2*, and lines carrying *Ht1* and head smut *QTL*, suggesting that marker assisted-selection strategy can be used effectively to select high yielding and resistance level in breeding materials in maize. © 2012 Friends Science Publishers

Key Words: Maize; Northern corn leaf blight; Head smut; Pyramiding; MAS

INTRODUCTION

Northern leaf blight (NLB), caused by *Helminthosporium turcicum* Pass., and head smut (HS), caused by *Sphacelotheca reilana* (Kühn) Clint, are two important diseases of maize (*Zea mays*) in China. The use of resistant cultivars is the most effective, economical, and environmentally friendly means to control epidemics of NLB and HS, and pyramiding of resistant genes/quantitative trait loci (QTLs) against both NLB and HS into elite cultivars would be a promising way to improve maize resistance against these diseases.

Marker assisted-breeding has been used to effectively integrate major genes or QTLs with large effect into single genotypes (Huang *et al*., 1997; Hittalmani *et al*., 2000; Castro *et al*., 2003a b; Richardson *et al*., 2006). The completion of maize genome sequence have made it possible to identify and map precisely a number of genes through linked DNA markers and improve desired traits by refined molecular breeding strategies (www.maizesequence.org/). There were many reports about molecular mapping on NLB resistance genes *Ht1* (Bentolila *et al*., 1991; Van *et al*., 2001), *Ht2* (Zaitlin *et al*., 1992; Van *et al*., 2001; Yin *et al*., 2003), *Ht3* (Van *et al*., 2001), *HtN* (Simcox *et al*., 1993; Van *et al*., 2001) and *QTL* (Dingerdissen *et al*., 1996) and HS resistance *QTL* (Lu *et al*., 1999; Lübberstedt *et al*., 1999; Chen *et al*., 2008). Marker assisted-selection can be used for pyramiding these resistance genes and developing broad-spectrum resistance to NLB and HS.

According to the previously reported linked-markers to resistance genes *Ht1*, *Ht2*, *Ht3, HtN1* and *QTLs* to NLB and head smut resistance *QTLs*, tightly linked SSR markers falling in or nearby the reported markers were chosen from the public database of maize genome (http://www.maizeGDB.org) to monitor the presence or absence of these genes in breeding populations. The objectives of this study were to pyramid the resistance genes to both NLB and head smut with the help of marker assisted-selection, to determine resistance levels of pyramiding resistance alleles.

MATERIALS AND METHODS

Plant materials and crosses: Parental inbred Ent17 with NLB resistance and parental inbred Ent12 with head smut resistance were provided by International Maize and Wheat Improvement Center (CYMMIT), parental inbred Liao3162

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susceptible to both NLB and head smut were provided by Maize institute, Liaoning Academy of Agricultural Sciences, Shenyang, Liaoning Province, China. For combining alleles for resistance to both NLB and head smut, Ent17 was crossed with Ent12. The resistance screening for F_2, F_3 generations of the cross Ent17×Ent12 were conducted based on the phenotypic values and marker assisted-selection. The plants of F_2 and lines of F_3 with disease severity greater than or within two standard deviations of the mean susceptible control Liao3162 were discarded.

Disease assessments: Field experiments were conducted during the crop seasons of 2008 and 2009. In 2008 crop season, the parents, 5 F_1 and 190 F_2 seeds were space-planted about 8 cm apart for facilitating note-taking of individual plants and F_3 generation was produced in the field in Shenyang, Liaoning Province, China. In 2009 spring, the two parents and 162 F_3 resistance lines of Ent17×Ent12, with about 30 seeds from each line and completely randomized three replications, were planted in a 4m row with 20 cm apart between rows in two nurseries in Shenyang. Mixed isolates of *H. turcicum* races collected from diseased leaves in the field sections were used for NLB resistance assessment. The isolates were grown on lactose-casein hydrolysate agar. The cultures and medium were mixed with water in a Waring blender. Colony growth development was monitored by measuring colony diameter at 2 day intervals for 7 days or longer. The cultures prepared for inoculations were grown for 7 to 9 days and then harvested. The resulting suspension was filtered through two layers of cheese cloth, and calibrated 20 mL of the inoculum containing 2×10^3 conidia/mL was sprayed using an approximately 8-L garden sprayer onto all the plants at three to five-leaf stage. At flowering stage around two months after the inoculations, severity of NLB was rated as a 0~9 scale, based on the percentage of plants showing NLB infection, in which 1=0~3% diseased leaf area, highly resistant, 3=6~10% diseased leaf area, resistant, 5=11~30% diseased leaf area, moderately resistant, 7=31~70% diseased leaf area, moderately susceptible, and 9=over 70% diseased leaf area, highly susceptible. The sori containing teliospores of *S. reilana* were collected from the field in the previous growing season and mixed with soil at a ratio of 1:1,000 before plantation. The mixture of soil and teliospores were used to cover maize kernels when sowing seeds to conduct artificial inoculation. Individual plants at maturity stage were scored for the presence/absence of sorus in either ear or tassels as an indicator for susceptibility/resistance, and the mean percent disease incidence using averaged three replicate data was subjected for further analysis, where 0~5% highly resistant, 5~20% resistant, 20~50% susceptible and over 50% highly susceptible.

At maturity stage, averaged plant height data were collected for F_3 lines. After harvest, the averaged ear length, ear diameter and kernel number per ear in F_3 lines were scored.

Primer selection and PCR amplification: According to previously reported linked-markers to resistance genes *Ht1*, *Ht2*, *Ht3, HtN1* and *QTLs* to NLB and resistance *QTLs* to head smut, tightly linked SSR markers falling in or nearby the reported markers were chosen from the public database of maize genome (http://www.maizeGDB.org) and synthesized by Baoshengwu Inc., Dalian, China. If the resistance gene fell in two linked marker interval, all SSR markers inside the interval from the public database of maize genome were selected, if the resistance gene was outside of the two linked marker interval, SSR markers were selected down to *10 cM* in the *vicinity* of the closest linked marker to the resistance genes, and if there was only one marker linked to the resistance genes, SSR markers from the public database of maize genome were selected within the upper and lower 10 cM of the linked marker. Total 10 pairs of SSR markers were chosen to conduct the present study (Table I).

Genomic DNA was extracted from each plant for F_1 and F_2, and more than 5 plants of two-leaf stage seedlings for each of the parents, susceptible control Liao 3162 and F_3 lines using the cetyltrimethyl ammonium bromide (CTAB) method (Saghai-Maroof *et al*., 1984). PCR amplifications were performed in a GeneAmp® PCR System 9700 Thermo-cycler and cycling profile were based on the protocol of Senior *et al.* (1993) with slight modifications. A 10 μL reaction mixture consisting of 25 ng of template DNA, 1.0 μL 10X PCR buffer (Boshengwu, Dalian, China), 0.6 unit of Taq DNA polymerase, 0.2 mM each of dCTP, dGTP, dTTP, and dATP and 0.2 μL of each primer (40 μmol/L) synthesized by Boshengwu, Dalian, China. After 5 min of denaturation at 94°C, amplifications were programmed for 30 consecutive cycles, each consisting of 45s at 94°C, 45s at either 55-65°C (depending on the individual SSR primer pairs), 1 min at 72°C and followed by a 10 min extension step at 72°C. After amplification, 6 μL of formamide loading buffer [98% formamide, 10 mM EDTA (pH 8.0), 0.5% (W/V) xylene cyanol and 0.5% (W/V) bromophenol blue] was added to the PCR products. After 4 min denaturation at 94°C, 7 μL of the PCR product and loading buffer mixture for each sample was loaded in a 8% polyacrylamide gel. After electrophoresis, the gel was silver-stained according to the recommendation of the manufacturer.

Statistical analysis: F_3 field data were used for the statistical analysis. Statistic software SPSS13.0 was employed to conduct analysis of variance (ANOVA). Genotypic variation (df=16) was partitioned into four parts according to Richardson *et al.* (2006): Parents (df=1), resistance lines (df=4), parents versus controls (df=2), and lines within QTLs (df=9). The pooled error term was used as the denominator for *F*-test.

RESULTS

The results for disease assessment in the field during 2008 showed that Ent17 was resistant to NLB (severity 5%)

Table I: Sequences of SSR primers used to identify resistance genes *Ht1, Ht2, Ht3, HtN1* and head smut resistance QTLs

SSR primers	Sequences		Chromosome	Taget gene/QTL
bnlg198	GTTTGGTCTTGCTGAAAAATAAAA	GCTGGAGGCCTACATTATTATCTC	2.07	*Ht1*
bnlg1335	GAAGGTTGCTCTTCCACTGG	TGGTTTGTGCAAGTGTCACC	2.07	*Ht1*
umc2210	AGCGGGTCGATCTTTCTCTTAGTT	GATGCACCATTTCAGTGAGCGAT	8.06	*Ht2*
umc1665	CAATCAGGAGCCAGGGAGATG	CTTAAACTTGTCGAGACGGTCCTG	8.06	*Ht2*
umc1029	AACACCTGCTGGATATGGATCACT	GGAAGAAAAATGTCGACCTGCTC	7.04	*Ht3*
bnlg1666	GCTGGTAGCTTTCAGATGGC	TGTCCCTCCTCCAGTTTCAC	7.04	*Ht3*
bnlg240	AAGAACAGAAGGCATTGATACATAA	TGCAGGTGTATGGGCAGCTA	8.06	*HtN1*
umc1728	AGTACTTTCAGGCAGGGACCTTCT	AACGCACTTCTTGTAGCTGTAGGG	8.06	*HtN1*
bnlg1016	CCGACTGACTCGAGCTAACC	CCGTAACTTCCAAGAACCGA	1.04	QTL for heat smut
umc1849	TCCTTGTTGAAGATTTTATTTCTGCT	GGCTTTAAGTGATGCTCAAACGTA	1.04	QTL for heat smut

Table II: The selected pyramided-resistance lines through phenotyping and marker assisted-selection

Line No. and parent	Carrying NLB resistance gene/head smut resistance QTL	Plant height (cm)	Ear length (cm)	Ear Diameter (cm)	Kernel number per ear	Severity of NLB (%)	Severity of head smut (%)
Line 7	*QTL*	285	14.41	4.62	455.20	3.76	0
Line 13	*QTL*	318	14.20	4.64	452.40	3.67	0
Line 28	*QTL*	292	14.58	4.55	458.33	3.53	0
Line 30	*QTL*	300	14.20	4.58	448.93	3.79	1
Line 2	*Ht2*	292	13.80	4.72	445.90	5.39	10
Line 18	*Ht2*	304	13.73	4.68	454.80	5.94	10
Line 19	*Ht2*	312	14.02	4.82	448.50	5.90	6
Line 4	*Ht1* + *Ht2*	292	14.10	4.86	462.00	5.68	6
Line 9	*Ht1* + *Ht2*	285	14.00	4.83	464.33	5.50	8
Line 3	*Ht1* + *QTL*	318	14.63	4.37	463.28	3.98	0
Line 5	*Ht1*+ *QTL*	292	14.57	4.41	462.16	3.86	0
Line 32	*Ht1* + *QTL*	300	14.68	4.56	460.39	3.89	0
Line 16	*Ht1*+*Ht2*+ *QTL*	326	15.03	4.36	471.33	4.22	0
Line 31	*Ht1*+*Ht2*+ *QTL*	313	15.24	4.31	478.67	4.20	0
Ent17	*Ht1* and *Ht2*	283	14.03	4.70	465.67	5.92	15
Ent12	*QTL*	252	14.62	4.58	456.30	0	0
Liao 3162	check	266	13.47	4.73	437.30	8.78	56.1

Note: QTL in the table refers to QTL with resistance to head smut

Table III: The results of variance analysis for agronomic, yield traits and resistance level

Contrast	F value					
	Plant height (cm)	Ear length (cm)	Ear Diameter (cm)	Kernel number per ear	Severity of NLB (%)	Severity of head smut (%)
Ht1 + *Ht2* vs. Ent17	0.701	0.383	5.208*	2.824	4.141	10.280
*QTL*vs Ent12	54.000	3.160	0.274	1.662	15.851	1.222
Ent17 vs Ent12	1.422	23.216**	1.537	1677.653**	1682.227**	--
Ht1 + *Ht2*+*QTL* vs Ent17	2.686	4.787	5.476*	16.706**	67.590**	75.000**
Ht1 + *Ht2*+*QTL* vs Ent12	342.659**	12.766**	5.784**	182.978**	790.092**	—
Ht1 + *Ht2*+*QTL* vs *Ht2*	12.314*	13.412*	5.356*	55.155**	97.126*	63.000**
Ht1 + *Ht2*+*QTL* vs *QTL*	13.742*	14.735*	8.414**	19.268*	13.126**	1.450
Ht1 + *Ht2*+*QTL* vs *Ht1*+ *QTL*	6.754*	10.943*	2.551	6.619*	5.197*	—
Ht1 + *Ht2*+*QTL* vs *Ht1*+ *Ht2*	16.589*	42.781**	20.849*	39.759*	75.846**	40.800**
Ht1 +*QTL* vs *Ht2*	7.664	10.405**	2.911**	4.794**	288.746**	74.000**
Ht1 +*QTL* vs *QTL*	16.582	3.566**	1.272**	2.379**	7.764**	—
Ht1 +*QTL* vs *Ht1*+*Ht2*	18.175**	13.134**	5.370**	0.238*	253.786**	45.600**
Ht1+*Ht2* vs *Ht2*	7.252**	1.131	1.332	11.376**	10.516**	27.802**
Ht1+*Ht2* vs *QTL*	14.543**	3.462*	6.735**	4.657*	225.952**	45.720**

Note: QTL in the table refers to QTL with resistance to head smut

*Denotes Note: F value is significant at $P < 0.05$. **Denotes that F value is significant at $P < 0.01$

and susceptible to head smut (severity 45%), Ent12 was resistant to head smut (severity 10%) and susceptible to NLB (severity 75%). Susceptible check Liao3162 was susceptible to both NLB and head smut. F_1 plants of cross Ent17×Ent12 were resistant to NLB and head smut. Among 190 F_2 plants, 162 plants were resistant to NLB, or head smut, or both of them and 28 plants were susceptible to both NLB and head smut. In 2009 spring, 162 F_3 resistant lines were assessed again in the field together with marker assisted-selection. Among the 162 F_3 lines, lines 3, 5 and 32

Table IV: The contrast result for parents of resistant gene and QTL of pyramided lines

Source of variation	DF	F value					
		Plant height (cm)	Spike length (cm)	Ear Diameter (cm)	Kernel number per ear	Severity of NLB (%)	Severity of head smut (%)
Replications	2	0.202	0.250	1.584	0.923	0084.	0.022
Genotypes	16	10.749**	14.901**	6.269**	14.666**	531.980**	5857.841**
Parents	1	1.422	23.216**	1.537	1677.653**	1682.227**	—
Pyramided lines	4	5.142**	37.170**	19.022**	28.844**	255.348**	9.250**
Parents vs check	2	0.538	8.267*	0.600	2342.197**	1830.341**	31758.242*
Lines within pyramided lined							
QTL	3	17.846**	2.300	0.519	1.597	3.733	0.250
Ht2	2	4.825	0.761	0.795	2.503	17.307**	—
Ht1 + Ht2	1	2.774	0.527	0.303	1.512	2.859	—
Ht1+ QTL	2	7.493*	0.453	0.652	0.150	2.140	—
Ht1 + Ht2 + QTL	1	2.473	2.375	2.777	25.631**	0.18	—
error	34	36.883	19.7059	4.06061	21.16276	3.73769	

Note: QTL in the table refers to QTL with resistance to head smut

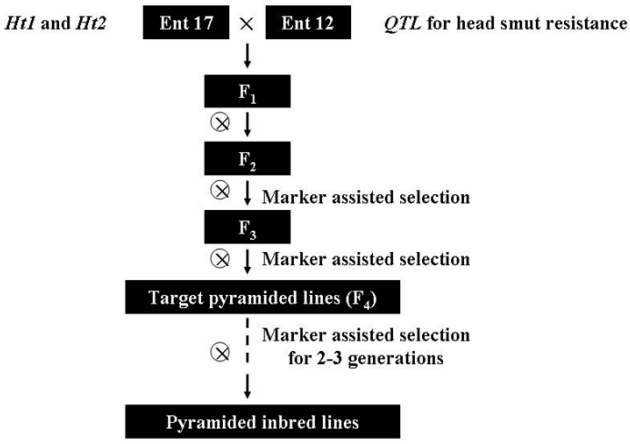
*Denotes that F value is significant at $P < 0.05$. **Denotes that F value is significant at $P < 0.01$

contained band patterns of SSR markers linked to *Ht1* and head smut resistance *QTL*, lines 16 and 31 contained band patterns of SSR markers linked to *Ht1*, *Ht2* and head smut resistance *QTL* (Table II). The other 157 F_3 resistant lines only had band patterns of SSR markers linked to *Ht1,* or *Ht2,* or head smut resistant *QTL,* or none of them. In order to compare the agronomic traits and disease severity with above-mentioned 5 pyramided lines, three highly resistant lines (line 78, 81 & 110) only with band patterns of SSR markers linked to *Ht1*, three highly resistant lines (line 2, 18 & 19) only with band patterns of SSR markers linked to *Ht2*, and four highly resistant lines (line 7, 13, 28 & 30) only with band patterns of SSR markers linked to head smut resistance QTL were selected as checks for statistical analysis (Table II). In the 162 F_3 resistant lines, no lines with band patterns of SSR markers linked to *Ht3* and *HtN1*. Meanwhile, the parent Ent17 and Ent12 were also not found to have the band patterns of SSR markers linked to *Ht3* and *HtN1*, suggesting that parent Ent 17 and Ent 12 did not carry *Ht3* and *HtN1*. Furthermore, there were not *Ht3* and *HtN1* resistant genes among the 162 F_3 resistant lines.

ANOVA revealed significant differences between pyramided lines for the resistance level, agronomic and yield traits (Tables III & IV) Ent17 with *Ht1* and *Ht2*, Ent12 with head smut resistance *QTL* were significantly different from susceptible check Liao3162 for all components. There were significant differences between Ent17 and Ent12 for the resistance level and yield traits.

Comparing pyramided lines carrying *Ht1* and *Ht2* with resistance donor Ent17, lines carrying head smut resistance QTL alleles with resistance donor Ent12, no significant differences were found for all the components. Pyramided lines with *Ht1*, *Ht2* and *QTL* showed significant differences from Ent17 for yield traits and NLB resistance level, from Ent12, lines with *Ht2* and lines with *Ht1* and *Ht2* for all the components. By comparing lines carrying *Ht1* and head smut resistance *QTL* with lines carrying *Ht1*, lines carrying *Ht2*, lines carrying head smut resistance *QTL*, and lines carrying *Ht1* and *Ht2*, it was revealed significant differences for the yield traits and resistance level. Lines carrying *Ht1* and *Ht2* were significantly different from lines carrying *Ht2* for the agronomic traits and resistance level, from lines carrying head smut resistance *QTL* for all the components.

Fig. 1: The diagrammatic representation of pyramiding breeding for resistance to both NCLB and head smut



DISCUSSION

Conventional breeders select genotypes indirectly through phenotype traits. Experienced breeders can accurately judge phenotype traits, overcome the influence of environmental factors on phenotype traits, and implement intentional selection during self-cross breeding. However, selection from early generations, like F_2 or F_3 generations, often leads to the wrong choice due to the subjectivity and influence of environmental factors on phenotypic traits, which the selected plants or lines have no target traits or required genetic background, especially for head smut breeding because of its quantitative traits. With the help of molecular markers tightly linked to resistance genes,

marker-assisted breeding will help the breeders to make selection at early stage, provide opportunities for breeders to pyramid different resistance genes and develop high-yielding, multi-resistant maize inbred lines.

There are many reports about the use of marker-assisted technology leading to the release of varieties in different crop species. With the help of marker-assisted selection and genetic transformation, an elite Indica rice line IR50 was obtained by pyramiding blast resistance gene Piz5 and bacterial blight resistance gene Xa21 (Narayanan *et al*., 2002).

New soybean lines pyramided genes Rsv1, Rsv3, and Rsv4 for SMV resistance using microsatellite markers have been successfully developed (Shi *et al*., 2009). In order to avoid the selection of new virus strains and to create more durable resistances, pyramiding of resistance genes has been effectively used as a promising strategy (Werner *et al*., 2005).

The present study is an early stage for pyramiding breeding (Fig. 1). F_1 was just for producing F_2, F_3 and more advanced generations. The phenotype resistance assessment combined with marker assisted-selection started from F_2. Due to non-repeatable F_2 data, data from F_3 generation was employed to preliminarily explain pyramiding resistance genes/QTLs to both NLB and head smut using statistical program SPSS. Two pyramided lines carrying *Ht1*, *Ht2* and head smut resistance *QTL*, three lines carrying *Ht1* and head smut resistance *QTL* were found. Comparing with conventional phenotypic selection, marker assisted-selection had exact choice for required genetic background and pyramided inbred lines could be created after 4-5 generations proceeding selection from F_2 (Fig. 1). The ANOVA analysis revealed that lines carrying *Ht1*, *Ht2* and head smut *QTL* had resistance level and yield over parental lines Ent17 and Ent12, lines carrying *Ht1* and *Ht2*, and lines carrying *Ht1* and head smut *QTL*, suggesting that marker assisted-selection strategy can be used effectively to select high yield and high resistance level breeding materials in maize.

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REFERENCES

Bentolila, S., C. Cuitton, N. Bouvet, A. Sailland, S. Nykaze and G. Freysainet, 1991. Identification of RFLP marker tightly linked to the *Ht1* gene in maize. *Theor. Appl. Genet.*, 82: 393–398

Castro, A.J., F. Capettini, A.E. Corey, T, Filichkina, P.M. Hayes, A. Kleinhofs, D. Kudrna, K. Richardson, S. Sandoval-Islas, C. Rossi and H. Vivar, 2003a. Mapping and pyramiding of qualitative and quantitative resistance to stripe rust in barley. *Theor. Appl. Genet.*, 107: 922–930

Castro, A.J., X.M. Chen, P.M. Hayes and M. Johnston, 2003b. Pyramiding Quantitative Trait Locus (QTL) Alleles Determining Resistance to Barley Stripe Rust: Effects on Resistance at the Seedling Stage. *Crop Sci.*, 43: 651–659

Chen, Y.S., Q. Chao, G.Q. Tan, J. Zhao, M.J. Zhang, Q. Ji and M.L. Xu, 2008. Identification and fine-mapping of a major QTL conferring resistance against head smut in maize. *Theor. Appl. Genet.*, 117: 1241–1252

Dingerdissen, A.L., H.H. Geiger, M. Lee, A. Schechert and H.G. Welz, 1996. Interval mapping of genes for quantitative resistance of maize to setosphaeria turcica, cause of northern leaf blight, in a tropical environment. *Mol. Breed.*, 2: 143–156

Hittalmani, S., A. Parco, T.V. Mew and Z.N. Huang, 2000. Fine mapping and DNA marker-Asisted pyramiding of the three major genes for blast resistance in rice. *Theor. Appl. Genet.*, 100: 1121–1128

Huang, N., E.R. Angeles, J. Domingo, G. Magpantay, S. Singh, G. Zhang, N. Kumaravadivel, J. Bennett and G.S. Khush, 1997. Pyramiding of bacterial blight resistance genes in rice: maker-assisted selection using RFLP and PCR. *Theor. Appl. Genet.*, 95: 313–320

Lu, X.W. and J.L. Brewbaker, 1999. Molecular mapping of *QTLs* conferring resistance to *Sphacelotheca reiliana* (Kühn) Clint. *Maize Genet. Cooper. Newslett.*, 73: 361–369

Lübberstedt, T.G., X. Liu, A.E. Melchinger and X.C. Xia, 1999. *QTL* mapping of resistance to *Sporisorium reiliana* in maize. *Theor. Appl. Genet.*, 99: 593–598

Narayanan, N.N., N. Baisakh, C.M. Vera Cruz, S.S. Gnanamanickam, K. Datta and S.K. Datta, 2002. Molecular breeding for the development of Blast and Bacterial Blight resistance in Rice cv. IR50. *Crop Sci.*, 42: 2072–2079

Richardson, K.L., M.I. Vales, J.G. Kling, C.C. Mundt and P.M. Hayes, 2006. Pyramiding and dissecting disease resistance QTL to barley stripe rust. *Theor. Appl. Genet.*, 113: 485–495

Saghai-Maroof, M.A., K. Soliman, R.A. Jorgensen and R.W. Allard, 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci. USA*, 81: 8014–8018

Shi, Y., H. Kui, X. Guo, Z. Gu, Y. Wang and A. Wang, 2009. Genetic linkage map of the pearl oyster, *Pinctada martensii* (Dunker). *Aquacult. Res.*, 41: 35–44

Simcox, K.D., M. McMullen and R. Louie, 1993. Mapping the *HtN1* resistance gene to the long arm of chromosome 8. *Maize Genet. Cooper. Newslett.*, 67: 118–119

Van, S.D., C.A. Lambert and A. Lehmensick, 2001. Scar markers for the *Ht1*, *Ht2*, *Ht3* and HtN1 resistance genes in maize. *Maize Genet. Conf. Abst.*, 43: 134

Werner, K., W. Friedt and F. Ordon, 2005. Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2). *Mol. Breed.*, 16: 45–55

Yin, X.Y., Q.H. Wang, J.L. Yang, D.M. Jin, F. Wang, B. Wang and J.R. Zhang, 2003. Fine mapping of the *Ht2* (*Helminthosporium turcicum resistance* 2) gene in maize. *Chinese Sci. Bull.*, 48: 165–169

Zaitlin, D., S. DeMars and M. Gupta, 1992. Linkage of a second gene for NCLB resistance to molecular markers in maize. *Maize Genet. Cooper. Newslett.*, 66: 69–70

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