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The Analysis of the Genotype Segregation at Ssr Loci among Double-Cross F_1 Population Resistant to Northern Corn Leaf Blight and Head Smut in Maize

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Abstract: With the help of previously reported linked-markers to resistance genes *Ht1*, *Ht2*, *Ht3, HtN1* and *QTLs* to northern corn leaf blight (NCLB) and resistance *QTLs* to head smut, total 94 tightly linked SSR markers falling in or nearby the reported markers were chosen from the public database of maize genome to conduct this study. Ten (10) SSR markers showing polymorphisms among 3 parents (one resistant to *Exserohilum turcicum*, one resistant to head smut and one susceptible to both of them) were used to detect 32 double-cross F_1 plants. The analysis of the genotypes segregation at the SSR loci showed parental preference toward Ent12 with resistance to NCLB. In addition to 4 kinds of band patterns of normal genotype, the double-cross F_1 population also produced abnormal genotype bands same as Parents Ent17, Ent12 or three parental hybrid bands, respectively. These results are valuable reference for the pyramiding multi-resistance genes to both *Exserohilum turcicum* and head smut and the application of double-cross F_1 plants in maize breeding.

Key words: Maize · Double-Cross F_1 Population · SSR Marker · Genotype Segregation

INTRODUCTION

Heterosis utilization is one of the important means of plant breeding. Maize is the first crop of heterosis utilization in China. In early 70s, maize hybrids, first double-cross, then single-cross, have been cultivated in Chinese maize production. Over the past 10 years, biotechnology is widely used in crop breeding and developed into an emerging discipline in breeding. The molecular-assisted selection makes it possible to conduct study on multi-gene pyramiding breeding techniques and theories, to gradually establish a maize molecular breeding system connecting conventional breeding, which might provide new technical means for maize breeding, to speed up the process of maize breeding.

There were many reports about molecular mapping on NCLB resistance genes *Ht1*[1,2], *Ht2* [2,3,4], *HtN* [2,5] and *QTL* [6] and head smut resistance QTL [7,8]. With the rapid development in plant genomics, there emerged a large number of molecular markers, genetic mapping, genomic sequence and gene expression data. In the era of information explosion, it is urgent to combine biological sciences and computer science, which leads to the emergence of bioinformatics. Breeders can combine different crops, different experiments, different mapping populations to construct comparative QTL mapping, establish relationship between QTL with the DNA sequences, which facilitate the effective use of different crop genomics information and development of new molecular markers. At present, there are over 2000 pairs of simple sequence repeats (SSRs) markers published in the public database of maize genome, which are important resources for molecular-assisted selection.

According to the previously reported linked-markers to resistance genes *Ht1*, *Ht2*, *Ht3, HtN1* and *QTLs* to NCLB 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 to genotype double-cross F_1 population of (Liao-3162×Ent17)×(Liao-3162×Ent12). The objective of this study was to track multi-resistance genes in

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the double-cross F_1 population, to provide valuable reference in the application of double-cross F_1 plants in maize breeding.

MATERIALS AND METHODS

Plant Materials: Parental inbred Ent17 with NCLB resistance and parental inbred Ent12 with head smut resistance were from International Maize and Wheat Improvement (CYMMT), parental inbred Liao-3162 susceptible to both corn leaf blight resistance and head smut was provided by Maize institute, Liaoning Academy of Agricultural Sciences.

The Construction of Double-Cross F_1 Population: In the 2007 spring, inbred lines Ent17, Ent12 and Liao-3162 were planted in the experiment field in Maize Institue, Liaoning Academy of Agricultural Sciences, Shenyang, China. The crosses Liao-3162×Ent17 and Liao-3162×Ent12 were made in the silking period, seeds of two single-crosses F_1 were harvested in October and were planted in experiment field in Hainan, China in the November of the same year 2007. The double-cross F_1 (Liao-3162×Ent17) × (Liao-3162×Ent12) was made in silking period. In spring 2008, the three parents and double-cross F_1 population were again planted in Maize Institute, Liaoning Academy of Agricultural Sciences, Shenyang, China. At the 6th-leaf stage, about 5 kilogram leaves from each of 5 plants for each parent and single plant for double-cross F_1 population were collected for DNA extraction according to Reif *et al*. [9].

SSR Primer Selection: According to the previously reported linked-markers to resistance genes *Ht1*, *Ht2*, *Ht3, HtN1* and *QTLs* to NCLB 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 (http://www.maizeGDB.org), were selected, if the resistance gene was outside of the two linked marker interval, SSR markers, from the public database of maize genome (http://www.maizeGDB.org), were selected down to 10 cM in the vicinity of closet linked marker to the resistance gene, except all SSR markers inside the interval and if there was only one marker linked to the resistance gene, SSR markers, from the public database of maize genome (http://www.maizeGDB.org), were selected within the upper and lower 10cM of the linked marker.

PCR Amplification: PCR reactions were performed in a GeneAmp® PCR System 9700 thermo-cycler. A 10µl 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 (Boshengwu, Dalian, China), 0.2 mM each of dCTP, dGTP, dTTP and dATP (Boshengwu, Dalian, China) 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 45, 50, 55, or 60°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, about 7µl of the PCR product and loading buffer mixture for each sample was loaded for electrophoresis in an 8% polyacrylamide gel. After electrophoresis, the gel was silver-stained according to the recommendation of the manufacturer.

Statistical Analysis of SSR Genotyping Segregation of Double-Cross F_1 Population: The genotype band pattern from Ent17 was recorded as A, from Ent12 as B, from Liao-3162 as C; the hybrid genotype band pattern from both Ent17 and Liao-3162 were recorded as AC, from both Ent12 and Liao-3162 as BC□ from Ent17 and Ent12 as AB and from Ent17, Ent12 and Liao3162 as ABC; missing was recorded as '□'. The "chi test" procedure in the data analysis of Microsoft Office Excel 2003 was used to calculate *P* values.

RESULTS

SSR Polymorphism among the Parental Inbred of Double-Cross F_1: A total of 94 SSR markers, from the public database of maize genome (http://www.maizeGDB.org), were selected based on the previously reported mapping of resistance genes to NCLB and head smut, of which 15, 27, 4 and 9 SSR fell in linked marker interval, or near to the closely linked marker to resistance genes*Ht1*, *Ht2*, *HtN1* or *QTLs*, respectively. There were 10, 8, 6, 6 and 7 SSR primers fell in confidence interval of resistance *QTLs* to head smut on chromosomes 1, 2, 3, 4 and 9. Of the 94 selected SSR markers, 10 SSR markers were polymorphic among 3 parental inbred (Table 1), accounting for 10.5□.

Table 1: The polymorphic SSR markers among the three parents

Chromosome location	No. of detected markers	No. of polymorphic SSR markers among three parents	The proportion of polymorphism (%)
1	10	0	—
2	23	6	26
3	6	0	—
4	9	0	—
7	4	0	—
8	36	4	11
9	6	0	—

Table 2: Genotype segregation of double-cross F_1 population at 10 pairs of SSR marker loci

				Observed No. of normal genotypes					Observed No. of abnormal genotypes		
SSR primers	Plant No. of Ent17 genotype□A□	Plant No. of Ent12 genotype□B□	Plant No. of Liao 3162 genotype□C□	A	AC	BC	AB	χ^2 analysis for 1:1:1:1 (P Value)	B	C	ABC
Umc1637a	17	13	12	6	4	6	5	0.913630	0	0	2
Umc2005	18	13	14	4	6	5	5	0.940242	0	0	3
Umc1947	22	8	15	3	13	2	6	0.006324	0	0	0
Dupssr24	18	3	13	8	10	3	0	0.007548	0	0	0
Umc1864	23	12	12	7	7	0	9	0.043392	3	5	0
Umc1665	20	15	5	10	0	5	10	0.011726	0	0	0
Umc2210	19	11	3	7	2	1	10	0.012858	0	0	0
Bnlg1152	23	12	0	11	0	0	12	3.87E-05	0	0	0
Bnlg1662	25	13	12	12	3	3	4	0.015715	0	0	6
Bnlg1940	26	7	9	19	4	0	3	2.67E-07	4	5	0
Total	211	107	95	87	49	25	64		7	10	11

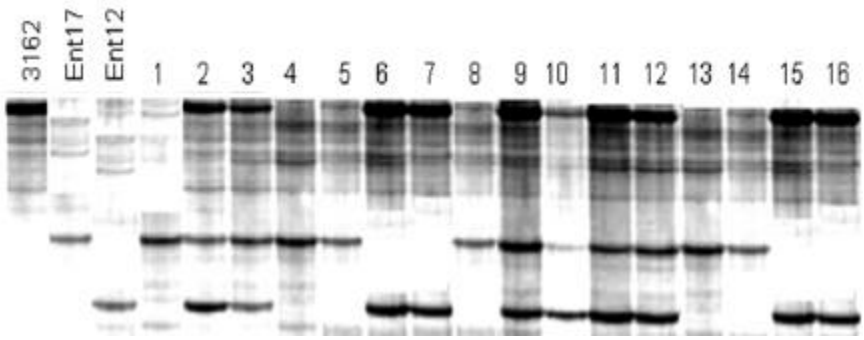

Fig. 1: The genotype segregation among some plants of double-cross F1 population at SSR marker *bnlg1662* loci. 1□16 refer to some plants of double-cross F1 population

None of the SSR markers showed polymorphism among 3 parental inbred on chromosomes 1, 3, 4, 7 and 9. There were 6 and 4 SSR markers on chromosomes 2 and 8, respectively, suggesting that the resistance genes in our study were mainly located on chromosomes 2 and 8.

The Genotype Composition of Double-Cross F_1 Population: 32 plants of double-cross F_1 were genotyped using 10 polymorphic SSR markers among three parental inbred and 405 polymorphic bands were detected, of which 211 bands were the same as A of Ent17 band pattern, 121 bands same as B of Ent12 ban pattern, 73 bands same as C of Liao3162, with the ratio 3:1.5:1, suggesting that the segregation of SSR loci in the double-cross F_1 population was skewed to parental inbred Ent17 with NCLB resistance. There were only 2 of the 10 polymorphic SSR markers *umc1637a* and *umc2005* showing theoretical ratio 1:1:1:1 of genotype segregation, the remaining 8 SSR markers were segregated skewedly, accounting for 80% (Table 2). Theoretically, this double-cross F_1 population only had 4 genotyping band patterns (Fig. 1), which were same as A from Ent17,

AC from Ent17 and Liao3162, BC from Ent12 and Liao3162 and AB from Ent17 and Ent12. In fact, when SSR primers umc1637a, umc2005, umc1864, bnlg1662 and bnlg1940 were used to genotype the double-cross F_1 population, band patterns B from Ent12, C from Liao3162 and ABC from Ent17, Ent12 and Liao-3162 were also detected.

DISCUSSION

At present, Most currently cultivated maize cultivars in China have a narrow genetic basis for resistance, which can not meet the requirements of maize production. In recent years, crop genome research at home and abroad led to the establishment of molecular marker-assisted selection system. The combination of molecular marker-assisted selection with conventional breeding technique might select targeted genes accurately and quickly, thersefore shorten the life span of breeding.

In the present study, a total of 94 SSR markers, from the public database of maize genome (http://www.maizeGDB.org), were selected based on the previously reported mapping of resistance genes to NCLB and head smut, of which 10 polymorphic SSR markers among three parental inbred were located chromosomes 2 and 8. The reason might be due to the parental inbred selected for this study carrying resistance genes on chromosomes 2 and 8. In addition, the results of this study showed that SSR loci segregation of double-cross F_1 population was skewed to Ent17 with NCLB resistance, probably due to gene deletion, mutation and micro-rearrangements during meiosis. Of the 32 plants of double-cross F_1, many plants had both NCLB resistance gene *Ht2* (located on chromosome 2) and head smut resistance *QTL* (located on chromosome 2), consistent with theoretically low exchange probability of different alleles located on the same chromosome during meiosis, most gamete genotypes from the same parental genotypes and few recombinant genotypes. In contrast, 2 or multi-pairs of alleles, located on different chromosomes, independently segregated during meiosis, freely combined among the different alleles and allocated to different gametes.

In our study, there were segregation loci of only SSR markers *umc1637a* and *umc2005* consistent with theoretical genotype segregation ratio (1:1:1:1) in double-cross F_1 population. Skewed separation, showing performance of biological evolution, is a very common phenomenon in nature, speculated that the reason may be caused by linked molecular marker segregation distortion, different environments, the presence of transposons, marker locus missing data in experiment, or man-made selection errors. In addition to theoretical band patterns same as A from Ent17, AC from Ent17 and Liao-3162, BC from Ent12 and Liao3162 and AB from Ent17 and Ent12, we also found band patterns B from Ent12, C from Liao-3162 and ABC from Ent17, Ent12 and Liao-3162 while using SSR primers umc1637a, umc2005, umc1864, bnlg1662 and bnlg1940 to genotype the double-cross F_1 population. These anomalous genotypes might be due to parental inbred Ent17 with NCLB and head smut resistance genes located on the same chromosome 2, which led to very little chance to exchange during meiosis, the emergence of the same genotypes as parental inbred in double-cross F_1 population. In addition, unequal fragment exchange at anomalous separation loci during the process of chromosome pairing also contributed to the occurrence of anomalous genotypes.

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