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PREDICTION OF A CANDIDATE RESISTANCE GENE AND DEVELOPMENT OF THE *LSDCAP8* MARKER RELATED TO HEAD SMUT RESISTANCE IN MAIZE

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Abstract

R proteins, which are encoded by plant disease resistance (*R*) genes, are involved in many plant-pathogen interactions and thereby regulate plant resistance to numerous pathogens. Some R proteins genes contain a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) domain and are therefore known as NBS-LRR proteins. In the present study, a SNP was identified within an exon of the candidate resistance NBS-LRR-containing gene *ZmNL*, which is located on maize chromosome 2. This SNP could distinguish inbred maize lines that are resistant to head smut from those that are susceptible. Therefore, we developed the Derived Cleaved Amplified Polymorphic Sequence marker *LSdCAP8* from this SNP to efficiently distinguish resistant genotypes among 62 maize inbred lines belonging to six heterotic groups. The correlation between the *LSdCAP8* marker genotype and field phenotype was 96.8% and 100% in inbred lines with disease incidences less than 10% and greater than 40%, respectively. This dCAPS marker could be used for highly sensitive selection of germplasm to use for breeding improved resistance to maize head smut.

Key words: Candidate gene; Derived cleaved amplified polymorphic sequence (dCAPS); Single nucleotide polymorphisms (SNP)**;** Head smut; Maize.

Introduction

R genes play important roles in plant-pathogen interactions to regulate resistance to numerous pathogens. Many R proteins encoded by plant disease resistance (*R*) genes contain a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) domain, and are known as NBS-LRR proteins. The NBS domain binds and hydrolyzes ATP to induce conformational changes in proteins, and functions in the formation of the apoptosome and participation in cell defense. There are two subdomains in the NBS domain, including a nucleotide binding (NB) subdomain and ARC subdomains that also appear in the human APAF-1 protein and the *Caenorhabditis elegans* CED-4 protein, in addition to plant R proteins (Yan *et al*., 2005; Shrivastava *et al*., 2011). The *NBS-LRR* gene family is the most common type of R protein and functions in plant resistance to viruses, fungi, bacteria, and nematodes (Hammond-Kosack & Jonathan, 1997; Ellis & Jones, 1998; Meyers *et al*., 1999; Williamson, 1999). To date, genes encoding R proteins have been cloned from crop plants such as grapefruit (Huang *et al*., 2004), maize (Collins *et al*., 1999), rice (Monosi *et al*., 2004; Wang *et al*., 2005), wheat (Wang *et al*., 2009), and from the model plant *Arabidopsis* (Meyers *et al*., 2003).

Bioinformatics provides a useful tool to search for candidate *R* genes underlying QTL for resistance to plant diseases. The putative structures and functions assigned during gene annotations can be compared among genomes and can improve the ability to predict candidate genes (Ouyang *et al*., 2009). At the same time, SNP in candidate genes can be detected during structural analysis of these candidates. Because many SNP genotyping assays require probes with fluorescent labels and apparatus to detect fluorescent signals (Lee *et al*., 2004), converting SNPs into markers that can be amplified by PCR (Polymerase Chain Reaction) is often desirable. The Derived Cleaved Amplified Polymorphic Sequence (dCAPS) method has proven efficient for converting SNP into PCR-based markers (Lee *et al*., 2009; Shahinnia *et al*., 2009; Baloch *et al*., 2016). A sequence containing a site recognized by a restriction enzyme, which also contains the SNP is created in a PCR product using a primer with at least one mismatch to the DNA template. After digestion of the modified PCR product with the corresponding restriction enzyme, SNPs are revealed by changes in the pattern of bands resulting from digestion (Neff *et al*., 1998).

Head smut, which is a fungal disease caused by *Sporisorium reilianum*, causes yield losses of maize as high as 70-80% in growing areas characterized by low temperature or high latitude (Frederiksen, 1977; Bernardo *et al*., 1992; Lu *et al*., 1999). Compared to expensive and environmentally unfriendly means to control the disease such as fungicide treatment of seed, other more efficient, economical, and sustainable approaches include adjustment of sowing dates, appropriate cultivation and management of crops, and breeding for resistance (Lindhout, 2002; Liu *et al*., 2008). Genetic analyses have shown that head smut resistance is quantitative and is under the control of several genes (Ali and Baggett, 1990).

In the present study, we used a microarray including ~14,850 probes to profile gene expression in a smut-resistant near-isogenic line (NIL) and a highly smut-susceptible inbred line after artificial inoculation with *S. reilianum* and thereby analyze the molecular mechanisms of head smut resistance in maize.

We previously used 184 $F_{2:3}$ families from the cross between the smut-resistant inbred 'Mo17' and the smut-susceptible inbred 'Huangzao4' to identify five QTL that influence head smut resistance including one QTL on each of chromosomes 1, 3, and 8; and two QTL on

chromosome 2. Among these, a major QTL located in bin 2.09 explained 43.7% of phenotypic variation in maize resistance to head smut (Li *et al*., 2008). Chen *et al*. (2008) also reported a major QTL in bin 2.09 (*qHSR1*) that controls smut-resistance, which is between the flanking simple sequence repeat (SSR) markers 148152 and STS661 in a mapping population resulting from a cross between the fully resistant inbred 'Ji1037' and the very susceptible 'Huangzao4'. Weng *et al*. (2012) used a combined linkage mapping and association mapping approach to identify three QTL, *qHS1.03*, *qHS2.09*, and *qHS4.03*, that confer resistance to head smut on chromosomes 1, 2, and 4, respectively. The SNP PZE-102187611 linked to the major-effect QTL *qHS2.09* could explain 42.1 % of variation in resistance to *S. reiliana* infection. Although Zuo *et al*. (2015) cloned the *ZmWAK* gene, which encodes a wall-associated kinase, from within *qHSR1*, which confers resistance to head smut in maize, there also could be other resistance genes in this region.

The objectives of the present study were to (i) predict candidate *R* gene(s) in bin 2.09 and develop dCAPS marker(s) based on SNP(s) in candidate *R* genes, and (ii) evaluate the ability of the dCAPS marker(s) to distinguish smut-resistant and susceptible lines among a total of 62 inbreds that represent six maize heterotic groups. The results of the present study will provide useful information and tools for marker-assisted selection in maize breeding for head smut resistance.

Materials and Methods

In the present study, progeny from a cross between susceptible inbred 'Huangzao4' and resistant inbred 'Mo17' were used for QTL mapping of head smut resistance, prediction of *R* genes in bin 2.09, and the development of dCAPS markers. A collection of 62 maize inbred lines were evaluated that included representatives from six maize heterotic groups. These included Lancaster (LAN); PB, which includes lines derived from US hybrid 78599; LRC, which includes lines derived from the Chinese landrace Lv-da-hong-gu; SPT, which includes lines derived from the Chinese landrace Si-ping-tou; PA, which includes lines derived from other modern US hybrids; and finally BSSS, which is derived from the Iowa Stiff Stalk Synthetic population (listed in Table 2). The inbreds 'Mo17', 'Qi319', 'Dan340', 'Huangzao4', 'K10', and 'B73' represent each of the six heterotic groups mentioned above, respectively.

Fgenesh software (www.softberry.com/berry.phtml) was used to analyze sequences from the B73 RefGen_v2 in bin 2.09, and ORF Finder (http://www.ncbi.nlm.nih.gov/projects/gorf/) was used to find putative open reading frames. Then InterProScan (http:// www.ebi.ac.uk/Tools/pfa/iprscan/) was used to predict protein domains in the amino acid sequences predicted to be encoded by the candidate sequences. Finally, candidate sequences were identified by the presence of conserved nucleotide binding site (NBS) and leucine-rich repeat (LRR) domains characteristic of *R* genes. These blast searches were run at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) and MaizeGDB (http://www.maizegdb.org/) databases.

Genomic DNA was extracted from the uppermost two leaves harvested from 20-day-old 'Huangzao4' and 'Mo17' plants following the CTAB procedure (Murray *et al*., 1980; Di *et al*., 2016). Primer sequences were designed using Primer3 software (http://simgene.com/Primer3) using *NBS-LRR* gene sequences obtained from candidate gene prediction. Primer sequences, approximate PCR product sizes, and annealing temperatures used for PCR amplification of *NBS-LRR* gene sequences appear in Table 1. PCR was carried out in 10-μL reactions comprised of 1 U Taq polymerase with 1× PCR buffer with 1.5 mM $MgCl_2$, 0.5 mM of each dNTP, 2.5 μM of each primer, and 50 ng of template DNA. The PCR conditions consisted of initially denaturing for 5 min at 94°C, then 35 cycles of 45 s at 94°C, 45 s at 55-60°C, and 1.5 min at 72°C, with final extension for 10 min at 72°C. After resolving amplification products on 1% agarose gels, bands were excised and DNA fragments were purified from the gel using an Agarose Gel DNA Purification Kit (Tiangen Biotechnology CO, Beijing, China) Fragments were then inserted into the pGEM-T vector (pGEM-T kit, Promega CO., Beijing, China) and sequenced (Sangon Biotech CO., Shanghai, China). DNAMAN 4.0 (Lynnon Biosoft, San Ramon, CA, USA) was used to identify SNP markers by comparing DNA sequences amplified from the smut-resistant and -susceptible lines.

Table 1. Primer sequences, approximate product sizes, and annealing temperatures used for PCR amplification of candidate *NBS-LRR* gene sequences.

Candidate sequence	Primers		*T*m (°C)	Product size (bp)
ZmYC1	F	AGCACATGCACGCTTTCCTC	56	861
	R	AAATGCCCGTACCTGACTAA		
ZmYC2	F	TTTGACTTTAATAGCCACAG	55	1573
	R	CAAGAAGCAGAGCGATGA		
ZmYC3	F	TGCTTGGGAAAATAAACT	56	1071
	R	TTGAGATGCCATGCTGTA		
ZmYC4	F	AAGATCGAGCGACGTAGTTG	60	1044
	R	TAGCACCTCATTCCAACAGA		

Table 2 Primers and restriction enzymes for development of *LSdCAP8* marker.

Primer sequence (5' to 3')[a]		Types[b]	Enzyme	Recognition site	Expected product[c]	SNPs[d]		Sequence of PCR product (5' to 3')
						Location	SNP	
F	CGACAACGCCC GCCATCGCCG	S	*Aci*I	C↓CGC	132/19,113	22	C/G	CGACAACGCCCGCCATCGCCG[C/T]AGTGGCGAGCCCCGCGGCAGC GGGAGGCTTGCCAGCCTCAAGAG GGCATGGTACCTGCTCACCACGC TGCACGCGCGCCACCGCATCGCC ACTGACCTCGGCAACCTCAA
R	TTGAGGTTGCCG AGGTCAGT							

Primer and restriction enzyme combinations were designed using dCAPS Finder 2.0 and Primer 3 software (http://simgene.com/Primer3) to develop dCAPS markers from candidate gene sequences containing SNPs. The dCAPS primers did include necessary mismatches, but did not include the SNPs that were being analyzed. To detect dCAPS markers in this set of maize lines, 25-μl amplification reactions including 50 ng of template maize genomic DNA, 1× PCR buffer, 0.2 mM of each dNTP, 1 U of *Taq* DNA polymerase, plus 15 pmol of each primer (forward and reverse) were carried out. Amplification conditions were adjusted depending on the specific primer sets as follows: initial denaturing for 3 min at 94°C, then 34 cycles each of 30 s at 94°C, 40 s at 67°C, and 40 s at 72°C for, followed by final extension for 10 min at 72°C. Amplified products were then sequenced (Shanghai Sangon Company, Shanghai, China) for confirmation. PCR products were digested using the appropriate restriction enzymes in a 10-μl volume according to the manufacturer's instructions and resolved by electrophoresis on 4.5% PAGE gels to evaluate markers.

A total of 62 maize inbreds were evaluated for resistance to infection with *S. reilianum* in the field in a randomized complete block design including two replications in an area with high smut prevalence in Harbin, Heilongjiang Province, China (45.8° N, 126.5° E) from April through September 2011 and 2012. Each plot consisted of two rows 5 m long with 0.67-m inter-row spacing. Each plot was over-planted, and then plants were thinned to 25 per row. Seeds were artificially inoculated by sowing them mixed with 0.1% *S. reilianum* inoculum mixed with soil. Smut inoculum had been collected during the preceding season and stored under dry, ventilated conditions in cloth bags. Normal maize agronomic practices were followed. At crop maturity, individual maize plants were scored for the presence of *S. reilianum* sori when symptoms of smut had become apparent, and mean smut incidence scores were determined from two replicates per plot (Di *et al*., 2015).

Results and Discussion

Using Fgenesh and ORF-Finder, 1056 sequences including complete ORFs were obtained. Four predicted amino acid sequences containing the conserved nucleotide-binding site (NBS) and leucine-rich repeat (LRR) domains were identified using InterProScan and the candidate sequences were designated ZmYC1, ZmYC2, ZmYC3, and ZmYC4.

DNA sequences corresponding to the candidate ZmYC1, ZmYC2, ZmYC3, and ZmYC4 amino acid sequences were amplified from susceptible 'Huangzao4' and resistant 'Mo17' by PCR. PCR primers designed to amplify the fragments containing the conserved *R* gene domains from these four candidate sequences are shown in Table 1. Purified PCR products that had been cloned into the pGEM-T vector (Promega) were sequenced (Sangon Biotech CO., Shanghai, China). The TA Cloning Kit (TransGen Company, Beijing, China) was used to clone the amplified *ZmYC1*, *ZmYC2*, *ZmYC3*, and *ZmYC4* DNA sequences from both susceptable 'Huangzao4' and resistant 'Mo17' (Fig. 1).

When the candidate *ZmYC* sequences were aligned, a (C→G) mutation at nucleotide position 1044 in the *ZmYC4* DNA sequence was revealed between susceptible 'Huangzao4' and resistant 'Mo17' (Fig. 2), but no SNPs were detected in the *ZmYC1*, *ZmYC2*, or *ZmYC3* sequences.

The *ZmYC4* sequence contains an 894-bp open reading frame (ORF) that encodes a predicted NB-ARC protein of 297 amino acids as determined by InterProScan analysis (Fig. 3).

The 15-kb sequences flanking *ZmYC4* in bin 2.09 in the B73 RefGen_v2 were analyzed using Fgenesh. The full-length 8640-bp sequence containing *ZmYC4* contains two exons and one intron (Fig. 4). The full-length gene was named *ZmNL* (define name) and was deposited in GenBank as GenBank Accession No. KF765443. The lengths of the two exons were 894 bp (from nucleotide position 2314 to 3208 bp) and 2250 bp (from nucleotide position 6014 to 8264 bp), respectively, and the *ZmYC4* sequence was located within the first exon of the *ZmNL* gene.

The SNP within the first exon of the *ZmNL* gene was converted into a dCAPS marker by designing appropriate primers and corresponding restriction enzymes (Table 2). The DNA fragment containing the expected nucleotide mismatch, a SNP, and the sequences of the forward and reverse primers was successfully amplified and sequenced. Figure 5 shows the PAGE profiles of PCR products from 'Huangzao4' and 'Mo17' digested with *Aci*I that we used to identify the dCAPS marker *LSdCAP8*.

A blast using the sequence of the *ZmYC4* PCR product from 'Huangzao4' as a query was performed at the MaizeGDB to identify the *LSdCAP8* marker on maize chromosome 2 (Fig. 6).

The *LSdCAP8* marker was validated by evaluation of head smut resistance in 62 inbred lines representing the LAN, PB, LRC, SPT, PA, and BSSS maize heterotic groups. Mean incidence of head smut in 62 maize inbred lines was evaluated during the 2011 and 2012 growing seasons. The highest incidence of smut was 89.1% in the LRC heterotic group, while the lowest was 0% in the LAN, PB, and BSSS heterotic groups (Table 3).

Table 3. Analysis of predictive power of *LSdCAP8* marker genotype for field phenotype.

Subgroup	Resistance level	Field phenotypic identification of smut resistance	LSdCAP1	
			Marker detection[a]	predictive power
LAN	R	13	13	100.0
	S	3	1	33.3
	HS	0	0	0.0
	Total	16	14	87.5
PB	R	9	9	100.0
	S	5	2	40.0
	HS	1	1	100.0
	Total	15	12	80.0
LRC	R	2	2	100.0
	S	2	0	0.0
	HS	1	1	100.0
	Total	5	3	60.0
SPT	R	0	0	0.0
	S	0	0	0.0
	HS	8	8	100.0
	Total	8	8	100.0
PA	R	3	2	100.0
	S	8	1	12.5
	HS	1	0	0.0
	Total	12	3	25.0
BSSS	R	4	4	100.0
	S	2	0	0.0
	HS	0	0	0.0
	Total	6	4	66.7
All	R	31	30	96.8
	S	20	4	25.0
	HS	11	10	90.9
	Total	62	44	71.0

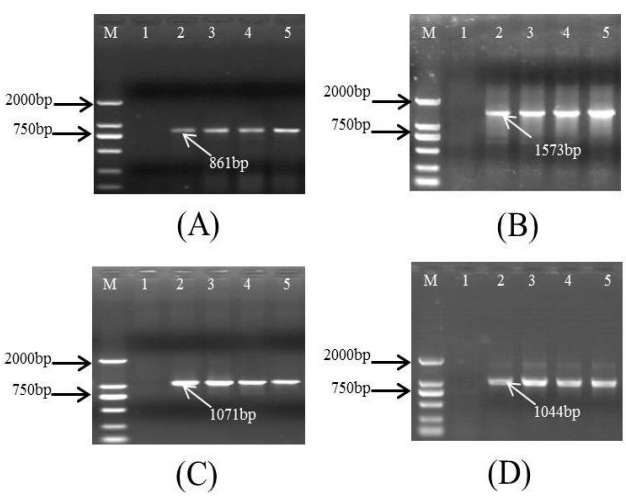


Fig. 1. *ZmYC1*, *ZmYC2*, *ZmYC3*, and *ZmYC4* PCR products of resolved by electrophoresis on 1% agarose gel
A: *ZmYC1*, B: *ZmYC2*, C: *ZmYC3*, D: *ZmYC4*
Note: Lane 1: Control PCR containing no template DNA; Lanes 2-3: PCR products from Mo17; Lanes 4-5: PCR products from Huangzao4; Lane M: Marker 2000 (Company, City, Country).

```
Mo17.txt        GGAGGTGGCCTACGACATCGAAGACTGCGTCGACAACGCC  280
Huangzao4.txt   GGAGGTGGCCTACGACATCGAAGACTGCGTCGACAACGCC  280
Consensus       ggaggtggcctacgacatcgaagactgcgtcgacaacgcc

Mo17.txt        CGCCATCGCCTGAGTGGCGAGCCCCGCGGCAGCGGGAGGC  320
Huangzao4.txt   CGCCATCGCCTCAGTGGCGAGCCCCGCGGCAGCGGGAGGC  320
Consensus       cgccatcgcct agtggcgagccccgcggcagcgggaggc

Mo17.txt        TTGCCAGCCTCAAGAGGGCATGGTACCTGCTCACCACGCT  360
Huangzao4.txt   TTGCCAGCCTCAAGAGGGCATGGTACCTGCTCACCACGCT  360
Consensus       ttgccagcctcaagagggcatggtacctgctcaccacgct
```

Fig. 2. SNP in the *ZmYC4* sequence between susceptible inbred maize line 'Huangzao4' and resistant inbred maize line 'Mo17' SNP (C→G) detected by aligning the *ZmYC4* sequences from 'Huangzao4' and 'Mo17'.

The detection of the *LSdCAP8* marker could predict smut-resistant phenotypes 96.8% of the time in the inbred lines that had disease incidence of less than 10%. When disease incidence was greater than 40% within an inbred line, identification of the allele associated with resistance could predict smut-resistant phenotypes 90.9% of the time.

The *LSdCAP8* marker was not very predictive in lines in which the disease incidence was between 10% and 40%. The predictive ability of the *LSdCAP8* marker varied substantially among these six maize heterotic groups. The *LSdCAP8* marker was most predictive of smut resistance for the SPT subgroup 100%, which might be because this marker was developed from 'Huangzao4', an important member of the SPT subgroup. For the other heterotic groups, relatively good results (87.5%, and 80.0%) were obtained using the *LSdCAP8* marker to predict smut resistance in LAN and PB heterotic groups, respectively. These results indicate that the *LSdCAP8* marker could be used for the identification of disease-sensitive lines.

With the publication of the maize genome sequence, much research is focusing on the discovery of resistance genes and development of functional markers. Several previous studies have reported major QTL in maize bin 2.09 that correspond to the main locus controlling head smut resistance in maize (Li *et al*., 2008; Chen *et al*., 2008; Weng *et al*., 2012). Because *R* genes in plants confer resistance to many pathogens, researchers have cloned more than 70 from several species of plants (Wei *et al*., 2013), the *NBS-LRR* genes, encode predicted proteins that contain a highly conserved nucleotide-binding site (NBS) and leucine-rich repeat (LRR) domain (Dangl *et al*., 2001), it is relatively easy to predict *R* genes in the maize genome using these conserved NBS and LRR domain sequences as queries. Xiao *et al*. (2006) identified 228 resistance gene analogs (RGAs) throughout the maize genome, most of which were based on the sequences of putatively expressed *R* genes. When 153 of these RGAs were mapped to 172 loci identified in the maize genome (Xiao *et al*., 2007), seven of these RGAs were located in the bin 2.09 region, including ZmR-RGA(7), ZmR-RGA(10), ZmMla1(3), ZmR-RGA(11), ZmR-RGA(12), and ZmPbs1(16). The results of that study indicated that *R* genes related to maize head smut resistance could be identified by homology-based methods. In the present study, the sequences of four candidate resistance genes were identified in bin 2.09 were identified by the presence of conserved R protein domains in their predicted amino acid sequences. Further analysis should be performed to confirm whether these candidates are homologous to sequences or parts of *R* genes specifically related to maize head smut resistance. Because the goal for this study was to develop functional markers for molecular marker-assisted selection (MAS), only the *ZmYC4* sequence, which contains a SNP marker distinguishing the susceptible line 'Huangzao4' from the resistant line 'Mo17', was further analyzed. This SNP was located within the first exon of a full-length 8640-bp gene containing NB-ARC and P-loop domains. The NB-ARC domain is one of the most conserved NB-LRR protein domains (Yan *et al*., 2005; Shrivastava *et al*., 2011). The NB domain contains characteristic P-loop, kinase-2, kinase-3a, and hydrophobic GLPL (HD) motifs (Tan and Wu, 2012). Mutations in essential residues in the P-loop that bind nucleotide triphosphates (Saraste *et al*., 1990; Traut, 1994) cause loss-of-function mutations in several NB-LRR proteins (Williams *et al*., 2011). Based on the above results, we deduced that the gene cloned in the present study is an *NBS-LRR* gene in bin 2.09 that co-localizes with the main QTL related to resistance to head smut in maize. This gene was designated *ZmNL* and was deposited in GenBank as Accession No. KF765443.

In future studies, we plan to introduce the *ZmNL* gene into maize and continue its functional analysis. Cloning and characterization of *ZmNL* gene could not only improve understanding of the molecular interactions between maize plants and pathogenic microorganisms, but also provide important information and tools for disease resistance breeding in maize.

Because the major QTL located in bin 2.09 explains 43.7% of the phenotypic variation in resistance to head smut (Li *et al*., 2008), researchers have developed other markers linked closely to this major QTL to further validate it by fine mapping. Chen *et al*. (2008) have developed six markers in this region, including SSR148152, CAPS25082, STS171, SNP661, STS1944, and STSrga840810. A SCAR marker, S130, has also been developed from an AFLP marker from 'Qi319' (Shi *et al*., 2009). Here, we have developed a new dCAPs marker based on a SNP located in the first exon of the candidate *R* gene *ZmNL*. The efficiency of the *LSdCAP8* marker was analyzed in 62 inbred lines representing six maize heterotic groups including LAN, PB, LRC, SPT, PA, and BSSS. The *LSdCAP8* marker, located within an exon of the *R* gene *ZmNL*, could predict smut-resistance phenotypes in the field based on genotype 96.8% and 100% of the time when disease incidence was under 10% or above 40%, respectively. The candidate *R* gene *ZmNL* should be further studied to reveal its function.

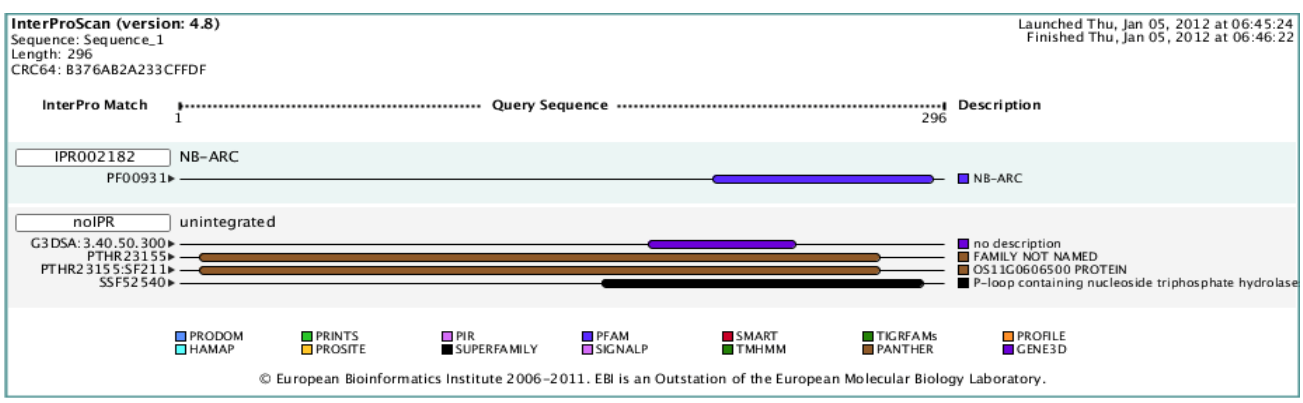
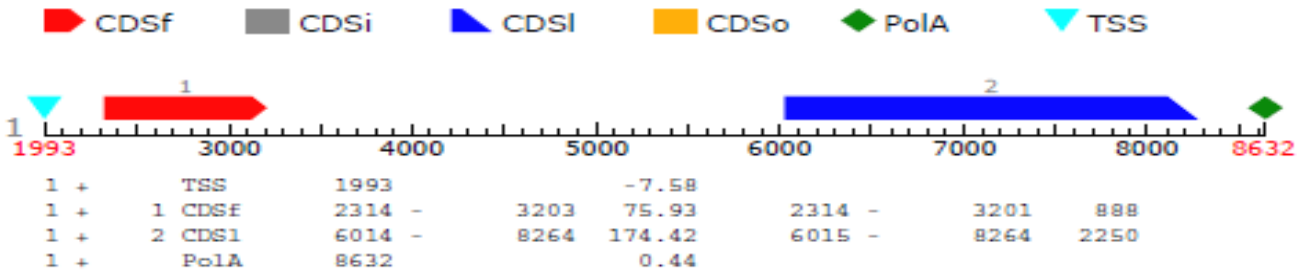


Fig. 3. Conserved NB-LRR domain in the predicted ZmYC4 protein
NB-ARC conserved domain in the predicted ZmYC4 protein identified using InterProScan.

FGENESH 2.6 Prediction of potential genes in Monocot genomic DNA
Seq name: test sequence
Length of sequence: 8640
Number of predicted genes 1: in +chain 1, in -chain 0.
Number of predicted exons 2: in +chain 2, in -chain 0.
Positions of predicted genes and exons: Variant 1 from 1, Score:231.390601



1	+		TSS	1993		-7.58			
1	+	1	CDSf	2314	- 3203	75.93	2314	- 3201	888
1	+	2	CDSl	6014	- 8264	174.42	6015	- 8264	2250
1	+		PolA	8632		0.44			

Fig. 4. Prediction of the *ZmNL* gene structure
TSS indicates the transcription start site, and CDSf and CDSI refer to exon 1 and exon 2, respectively.
The *ZmNL* gene contains exon 1 and exon 2 and one intron predicted using Fgenesh; *ZmYC4* is located within exon 1.

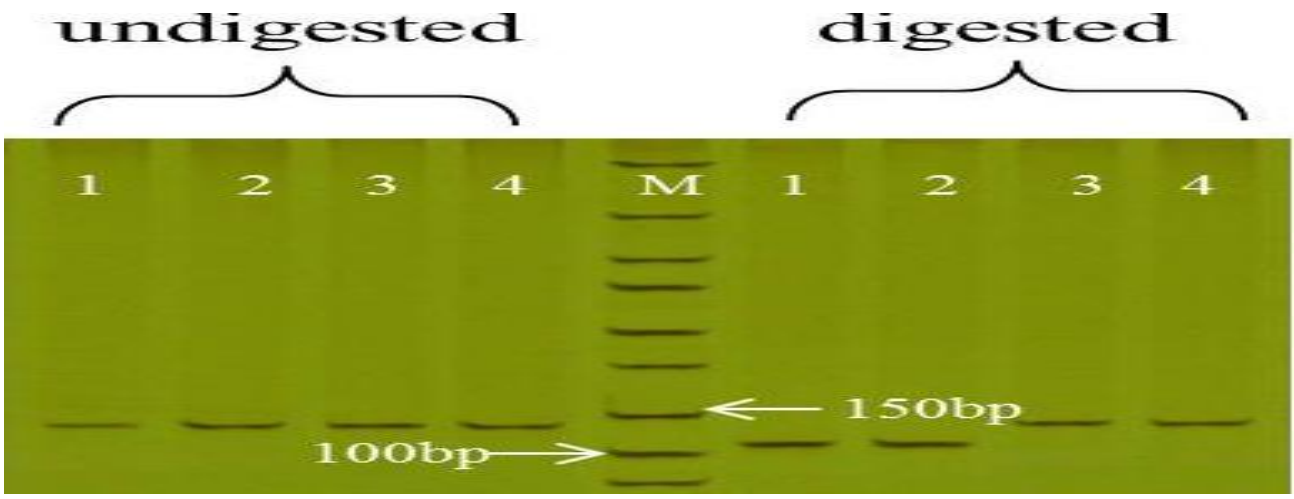


Fig. 5. Electrophoresis of the *LSdCAP8* marker from 'Huangzao4' and 'Mo17'
Lanes 1-4: Digested 115-bp and undigested 140-bp PCR products; M: 50-bp DNA ladder (Tiangen); Lanes 1 and 2: 'Huangzao4'; Lanes 3 and 4 'Mo17' run on polyacrylamide gel.
PCR products amplified from susceptible line 'Huangzao4' could be digested by *Aci*I, and those amplified from resistant line 'Mo17' could not be digested, which indicates that the 'Huangzao4' allele of the *LSdCAP8* marker was associated with susceptibility to head smut in maize.

Visual alignment for chr2

Alignment details for chr2

Alignment details for hit #1 for chr2

```
Score =  126 bits (233.79815042829),  Expect = 9.0397055628929e-60
Identities = 130/132 (0.9848%), Gaps = 0 (0.0000%)
Strand = Plus / Plus


Query  1          CGACAACGCCCGCCATCGCCGCAGTGGCGAGCCCCGCGGCAGCGGGAGGCTTGCCAGCCT  60
                  ||||||||||||||||||| ||||||||||||||||||| ||||||||||||||||||
Sbjct  229020999  CGACAACGCCCGCCATCGCCTCAGTGGCGAGCCCCGCGGCAGTGGGAGGCTTGCCAGCCT  229021058

Query  61         CAAGAGGGCATGGTACCTGCTCACCACGCTGCACGCGCGCCACCGCATCGCCACTGACCT  120
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  229021059  CAAGAGGGCATGGTACCTGCTCACCACGCTGCACGCGCGCCACCGCATCGCCACTGACCT  229021118

Query  121        CGGCAACCTCAA  132
                  ||||||||||||
Sbjct  229021119  CGGCAACCTCAA  229021130
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Fig. 6. Genomic location of *LSdCAP8* marker
The *LSdCAP8* marker was identified by blast on maize chromosome 2 at position 229020999 to 229021130 in B73 RefGen_v2.

Acknowledgments

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