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The disease-resistant study of maize chromosome segment introgress lines to common smut

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ABSTRACT

The maize chromosome segment introgress lines play an importment role to minimize the losses caused by common smut. Ustilago maydis was injected into 8 maize chromosome segment introgress lines(CSILs). 8 CSILs have different chromosome segments of donor maize HB522 respectively. There are 4 kinds of varieties in 8 CSILs expressing highly resistance to common smut. Chr3-7's grain yield is highest among other seven CSILs and two parents. chr3-7 is good SSILs for resistance to common smut study and resistance breeding.

KEYWORDS

Maize; Common smut; Ustilago maydis; Disease-resistant; CSILs.

INTRODUCTION

The maize smut is caused by Ustilago maydis infecting. Ustilago maydis infects seedling, leaf, stem nodes, axillary bud, ear, tassel and immature meristem of root, that gene ratetumour of vary in size to lead to reduction of output. Smut is an important plant disease of maize producing area in the world, the incidence of smut is universal. Usually, reduction of output is under 2% and seriously, reduction of output runs up to upper 15% [1-4]. In 2000, the maize smut ranged in north central of Bair in Left Banner in China and disease attacking area run up to 1.3million ha, the average disease incidence in field is 20%, the serious block is 100% (5333 ha). Smut occur in spring maize area and summer maize area of northeast, northwest, north China, East China [5-7]. With the increasing of maize continuous cropping years, disease incidence presents ascendant tendency too. For clearing up pathogens, preventing maize from disease by chemistry, enhancing planting management, we have got some achievements, but the cost was too high to bear. Thus, the selection, cultivation, appraisal and utilize of resisting breed are effect method to control disease[10]. In China, researchers have launched lots of works in current planting breed, emphasis popularizing breed and appraisal breed of country and excavated some resisting breed to popularize and plant [11-13], as well as exploited some disease-resist antout standing inbred lines to popularize actually. The genetic foundation research of maize smut has an important significance to exploit new species of smut-resistance and selecting and planting of disease-resistant species. Pathologists and botanists distinguish disease-resistant character between quality character and quantity character. Among them, quality resistance is decided by monogene, therefore, it is easy to research while quantity resistance is controlled by oligogene and polygenes. Quantity character is affected by environment factor easily and it is so difficult to discover the main effect gene site quantity, gene interaction, interaction mechanism of gene and environment. In maize, majority disease-resistant character is quantity character[14]. Smut-resistance is controlled by oligogene [15], researcher take it as quantity character to study. T. Lubberstedt etc (1998) structured 4 independent Fn line groups by 4 backbone inbred lines to locate QTL of maize smut. They found 19 different QTL distributing 10 chromosomes, among them, 5 of QTL locate at third chromosome, one locate at 3.05bin, two locate at 3.06 bin, two locate at 3.08 bin [18]. Jun-qiang Ding etc（2008）used recombination inbred lines and 246 SSR sign having polymorphism to locate smut QTL, and found 6 QTL distributing at 3, 5, 8 chromosome. Among them, they found 3 QTL on 3 chromosomes, relatively more QTL on third chromosomes [19]. Chromosomes segment introgress line (CSILs)is QTL appraisal, fine mapping, map-based cloning, interaction analysis and breed improvement ideal material [20]. In our study, we took backbone inbred lines(Z3) as receptor while HB as donor to structure CSILs as material, carried out disease-resistant appraisal of smut, disease-resistant gene fine mapping of smut, marker-aided breeding of molecular markers and germplasm resources innovation.

MATERIALS AND METHODOLOGY

We used Z3 of backbone inbred lines as receptor while HB522 as donor to structure CSILs, 8 Z3 was provided by Central China Agricultural University. In April 20 of 2008, we arranged 56 materials above, sowed them in Hebei North University farm, single line, 60cm line spacing, 30cm row spacing. We preinstalled a handling group, a contrast group, planted them respectively, fertilized the yards 40kg/acre by diammonium phosphate, exerted an additional fertilizationin in jointing stage and macrostoma stage respectively, total fertilization valum was 25kg/acre. We selected 220 sign locating at third chromosome and having difference between donor and receptor to carry out a whole gene scanning to each chromosome segment Genetic background. We selected 18 SSR locating at third chromosome to evaluated onator chromosome segment, which being replaced, in each CSILs. Sahai-Maroof etc (1994) used improvement CTAB to extract maize blade DNA. The reaction system of SSR analysis is 10 × buffer 2 μL, template DNA 6 μL, glycerol2 μL, 5U•μL-1 Taq enzyme 0.15 μL, 2.5 mmol•L-1 dNTP 0.7 μL, 5 μmol•L-1primer 1.2 μL, made up to 20 μL by DNase-free ddH2O. We dipped the PCR product into 6% polyacrylamide gel to carry out an EMSA. We used a1 and a2 strains of Ustilago maydis provided by Central China Agricultural University to carry on our experiment. Refer to Jun-qiang Ding (2008) [18] method, we prepared Ustilago maydis spore soliquoid. In the jointing stage, we dripped the spore soliquoid 2ml (concentration is 10^8) upon spear leaf. 4 weeks after inoculation, disease bract appeared on nutrition organ and reproduction organ of maize. We investigated the plant numbers attacked by smut of 56 introgress line, counted the disease incidence, examined the disease attacking situation of roots, stems, leaves, tassel, ear to define disease attacking class. In maize mature stage, we reaped treatment group and control group, further counted the disease attacking numbers of treatment group ear, accomplished threshing of treatment group and control group and counted yield of area.

(1) Disease incidence(%)=Disease attacking plants numbers/ Total investigation plants numbers
(2) Disease attacking class: measured by disease index
(3) Disease index(%)=$\sum(a\delta/NK)\times100$

a:disease attacking plant numbers, δ: disease attacking class, N: total counting planting numbers, K: the highest disease attacking class. We divided disease class refer to АиЮРку (1999) method.

Refer to Грисенко, Дудка etc (1980), we defined plant disease-resistant class according to plant disease index[6].

RESULT AND DISSCUSS

TABLE 1: Assessment standard of maize resistance to *Ustilago maydis* diease

Disease Incidence	assessment standard
>30%	HS
15.1%~30%	S
10.1%~15%	MR
2.1%~10%	R
<2%	HR

4 weeks after inoculating Ustilago maydis into 8 CSILs and Z3 parent, symptom express stable. The ear position of Z3 receptor parent is attacked by smut seriously. The top of 86% plant ear appear 4cm×3cm or 3cm×2cm or more small area tumors, the number is from1to 12. HB522 donor parent and CSILs chr3-7 was both not attacked by disease. For the CSILs, 64 % and 40% plant ear position of chr3-8 and chr3-2 appear tumour respectively, the biggest area of tumour is 10cm×10cm. The ear position of chr3-3, chr3-4 and chr3-5 all appear tumour the biggest area is 4cm×4cm, tumour number is about 5, less than chr3-8 and chr3-2. chr3-1and chr3-6 is attacked slightly by disease relatively. The top of ear appear 4cm×2cm and 3cm×2cm or more small tumour, the number is from 1 to 20. There are huge differences between disease attacking class of 8 CSILs, donor parent and receptor parent.

For smut-resistant, the difference of 8 CSILs, Z3 receptor parent, HB22 donor parent is very significant. The variation range of plant disease incidence is 0-86%, among them, HB522 and chr3-7 disease incidence is 0, the disease incidence of Z3 is the highest. The variation range of disease class is 0-6, among them, Z3 is the highest while HB522 and chr3-7 is the lowest. The variation range of disease index is 0-85.71, among them, Z3 is the highest while HB522 and chr3-7 is the lowest. For 8 CSILs, HB522 and chr3-7 express highly resistance, chr3-1 express middle resistance, chr3-6 express susceptible, chr3-2, chr3-3, chr3-4, chr3-5 express highly susceptible. Z3 express highly susceptible, HB 522 express highly resistance and chr3-7 is good at disease-resistant.

TABLE 2: CSILs resistantance to *Ustilago maydis*

CSILs	Disease Incidence	Disease class	disease index	Resistance assessment
z3(CK)	0.86	6.00	85.71	HS
HB522(CK)	0.00	0.00	0.00	HR
chr3-1	0.11	2.00	3.70	MR
chr3-2	0.40	4.00	26.67	HS
chr3-3	0.40	2.00	13.33	HS
chr3-4	0.50	2.00	16.67	HS
chr3-5	0.43	2.00	14.29	HS
chr3-6	0.29	2.00	9.52	S
chr3-7	0.00	0.00	0.00	HR
chr3-8	0.64	3.00	31.82	HS

In maize mature stage, we reaped the treatment group and control group, completed threshing, counted maize yield. Except chr3-7, other 7 CSILs, Z3 treatment group and control group, the maize yield declined 115.00g-1041.53g, the descender extent is 20.72-48.60%. The descender extent of chr3-8 line is the biggest, the second one is chr3-2 line, the yield declined 878.00g, the descender extent is 47.08%. For yield, HB522 donor parent and highly resistance chr3-7 has not significant difference contrasting control group. chr3-7 line yield exceed two parents and other lines, the highest yield is 2193.75g.

Analyzing the genetic structure of 8 CSILs by means of 18 SSR sign of the third chromosome, we found, each ILs contain single or multiple chromosome segment of donor parent. Except the bnlg1447 sign position of chr3-4 and chr3-5, other lines all have donor segment. Different IL lines have different donor segment number, chr3-5 lines has 3 donor segments, chr3-4 and chr3-2 both have 2 donor segments and other 5 lines all have only one donor segment. Detecting the receptor genetic background of 8 CSILs by means of other 182 SSR sign excepting 18 SSR above, they all recovered and turned into receptor. The chr3-7 lines was replaced by donor chromosome segment only at the phi046 and nearby position, it enhanced ability of smut-resistance. For 8 CSILs have different genetic structure, ability of smut-resistance has significant difference either.

TABLE 3: Yield of CSILs inoculated *Ustilago maydis*

CSILs	Yield(treatment)/g	Yield/(CK)	Descender extent/g
z3(Receptor)	867.00	1212.5	345.50(28.49%)
HB522(donor)	816.45	851.55	35.10(4.12%)
chr3-1	440.00	555	115.00(20.72%)
chr3-2	987.00	1865	878.00(47.08%)
chr3-3	657.50	1305	647.50(49.62%)
chr3-4	990.00	1485	495.00(33.33%)
chr3-5	1151.25	1410	258.75(18.35%)
chr3-6	957.86	1550	592.14(38.20%)
chr3-7	2211.43	2193.75	-17.68(-0.81%)
chr3-8	1101.67	2143.2	1041.53(48.60%)

TABLE 4: Hereditary constitution of CSILs

CSILs	Locus of chromosome segment of donor		
chr3-1	phi049		
chr3-2	bnlg1325	(nc030)	
chr3-3	bnlg1647		
chr3-4	umc2369	bnlg1447	
chr3-5	bnlg1447	umc1012	phi036
chr3-6	umc1644		
chr3-7	phi046		
chr3-8	umc1010		

CSILs are an important material usually used in genetics and breeding study. Using backbone inbred line Z3 as receptor while HB522 as donor to structure CSILs, we carried out a SSR molecule mark analysis on prospect and background of 8 CSILs, they are all third CSILs, 5 lines are single segment introgress lines, among them, 3 lines contain 2-3 donor chromosome segments. Screening different CSILs of 8 genetic structures, we hope to get a new breeding material, which has extreme disease-resistant ability. In maize growth period, by means of artificial inoculating Ustilago maydis, we can get a stable phenotype to appraise heredity [19]. So far, in China, the spore injection method is a smut-resistant appraisal inoculation one in area. In our study, in jointing stage, we inoculated Ustilago maydis by spear leaf injection method, sent Ustilago maydis into plant through maize tender tissues organ. In experiment area, the attacking of smut is very even. HB522 is highly resistance donor parent and the spike of Z3 receptor parent is high susceptivity for smut, there are huge different among them. 8 CSILs of two parents above express smut-resistant variation. The donor segments position of 8 CSILs are different. Chr3-7 line is the CSILs, whose segments nearby Z3 at phi046 site is replaced by HB522 segments, expressing highly smut-resistance. The donor chromosome segments contained in Chr3-7 change the Z3 phenotype, the yield exceed it's parents and become a material having resistance phenotype. Lots of researchers have begin to study the resistance of smut, it was regarded as quantity and character. 8 CSILs presents 4 phenotype, they are highly resistance (chr3-7), moderate resistance (chr3-1), sensitivity (chr3-6), high susceptivity (chr3-2, chr3-3, chr3-4, chr3-5). Andrew M, et al (2007) structured A188 and CMV3 recombination inbred group as material to carry out the QTL mapping study and found the smut-resistant QTL existing nearby 3.05 and 3.08bin[25]. Using 22 backbone inbred lines, Jun-qiang Ding etc structured recombination inbred group as material to carry out an experiment, found the smut-resistant QTL existing nearby 3.01bin, 3.08bin, 3.09bin[18], furthermore, it had significant additive genetic effect[19]. chr3-7 is the single segment nearby phi046(3.07bin), the phenotype different with Z3 parent is that, inserting of donor chromosome segment may knockout or insert synergistic gene having smut-resistant to the site original gene. We hope to discover the essential reason further.

CONCLUTION

(1)HB522 and chr3-7 express highly resistance, chr3-1 express middle resistance, chr3-6 express susceptible, chr3-2, chr3-3, chr3-4, chr3-5 express highly susceptible, Z3 express highly susceptible, HB522 express highly resistance and chr3-7 is good at disease-resistant.

(2) 8 CSILs of two parents express smut-resistant variation.

(3) Inserting of donor chromosome segment may knockout or insert synergistic gene having smut-resistant to the site original gene.

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