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Genetic Diversity of Elite Maize Germplasm for Resistant to SCMV

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Abstract Forty-six elite maize (*Zea mays* L.) inbred lines were evaluated for reaction to sugarcane mosaic virus under field conditions with artificial inoculation in 1999 and 2000. The results showed that 8 lines (K22, CN962, P138, Qi318, Zhongzi01, Jinhuang96B, Qi319, and Pa405) were highly resistant to SCMV, and 7 (Han21, Zhongzi03, Han23, Nongda178, Huobai, K12, and Huangzao4) were resistant. The germplasm basis for resistance to SCMV in 46 inbreds was investigated by SSR markers. The average number of alleles per SSR locus was 3.43 with a range from 2 to 10. The UPGMA cluster analysis showed that the 46 inbreds could be classified into 7 distinct groups with several subgroups, which were generally consistent to their known pedigree information and breeder's experiences. Fifteen resistant inbreds were found in 4 groups (A, B, E, G), which could be used in germplasm improvement for SCMV resistance based on heterotic pattern, respectively. Group E and subgroup GII were identified as two important sources of resistance, which can be employed to synthesize the composites for SCMV resistance. The study provided useful information for germplasm improvement for resistance to SCMV.

Key words *Zea mays* L.; SSR marker; Heterotic group; Sugarcane mosaic virus; Resistance evaluation

玉米抗甘蔗花叶病毒资源的遗传多样性研究*

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摘 要 利用人工接毒方法对 46 份我国主要玉米自交系进行了两年抗甘蔗花叶病毒鉴定, 筛选出高抗系 8 份(K22、CN962、P138、齐 318、中自 01、金黄 96B、齐 319、Pa405), 抗病系 7 份(旱 21、中自 03、旱 23、农大 178、获白、K12、黄早四)。用 SSR 标记研究了 46 份自交系的遗传多样性。49 对引物共检测出 168 个等位基因变异, 每对引物检测等位基因 2～10 个, 平均为 3.43 个。UPGMA 聚类分析表明, 供试自交系可分为 7 群, 划群结果与系谱和育种家经验基本相符。15 份抗病毒自交系分散于 4 群(A、B、E、G), 依据杂种优势原理, 可用于改良同一群内感病系的抗性; 其中群 E 和亚群 GII 被鉴定为抗病毒种质, 可用于组建抗病群体。本文的研究结果为抗甘蔗花叶病毒玉米种质改良提供了重要信息。

关键词 玉米; SSR 标记; 杂种优势群; 甘蔗花叶病毒; 抗性鉴定

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Mosaic disease, caused by sugarcane mosaic virus (SCMV), is one of the most important diseases of maize in China, which causes 10%～15% yield losses annually. It was reported as a sever

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disease of maize in 1960s, and became more serious in 1980s and 1990s. Up to date, the disease has been spread in several provinces[1]. SCMV is naturally transmitted by aphids in a non-persistent manner. It becomes especially severe when maize is planted in continuous cropping system. According to the taxonomy of Shukla *et al.* (1989)[2], maize dwarf mosaic virus (MDMV) strain B was reidentified as SCMV. SCMV is more prevalent than MDMV in China and Europe, and MDMV is predominantly distributed in southern parts of USA[3,4]. It is impossible to control SCMV directly with chemicals. Likewise, the attempt to control aphids is not effective because of the non-persistent mode of virus transmission. Development of resistant hybrids is the effective strategy to control SCMV.

Studies on the resistance to MDMV and SCMV have been conducted with U. S. and European maize germplasm[4,5]. Evaluation for resistance to SCMV in Chinese maize germplasm has been done, but these evaluations were mainly conducted with local varieties and released hybrids[6]. However, little is known on genetic diversity of maize germplasm for resistance to SCMV. The information can greatly facilitate germplasm improvement and breeding activities for resistance to SCMV.

Genetic relationship can be estimated by analysis of pedigree, heterosis, morphological traits, isozymes or using molecular markers. Since 1980s, the genetic base of Chinese maize germplasm has been investigated by examining percentage of parental lines in their hybrids utilized in different times, and heterotic grouping has been clustered by pedigree and geographic derivation[7,8]. DNA-based markers provide a powerful tool in the assessment of the genetic relationships among the breeding materials. Simple sequence repeats (SSRs) have proven to be highly polymorphic and useful as genetic markers in many plant species including maize[9~12]. SSRs represent the approach for the identification and pedigree validation of maize genotypes compared to other PCR-based methods. The patterns of genetic divergence by SSRs were consistent with their known pedigree[12,13]. The objectives of the study were (1) to evaluate 46 elite inbred lines for reaction to SCMV under field conditions with artificial inoculation; (2) to analyze the genetic diversity by SSRs and to assign them to heterotic groups, and (3) to provide a strategy of germplasm improvement for resistance to SCMV.

1 Materials and Methods

1.1 Plant materials

Forty-six inbred lines, which are currently used in Chinese maize breeding program were included in the study (Table 1). The inbred lines were maintained by selfing from seed of individual ears.

Table 1 Evaluation of 46 inbred lines for resistance to SCMV (1999 and 2000, Tangshan)

No.	Line	1999		2000		Average over two years	
		DI%	RE	DI%	RE	DI%	RE
1	K22	0.0	HR	0.0	HR	0.0	HR
2	CN962	0.0	HR	0.0	HR	0.0	HR
3	P138	0.0	HR	0.0	HR	0.0	HR
4	Qi318	0.0	HR	3.2	HR	1.6	HR
5	Zhongzi01	7.8	HR	0.0	HR	3.9	HR
6	Jinhuang 96B	0.0	HR	8.9	HR	4.5	HR
7	Qi319	6.9	HR	2.4	HR	4.7	HR
8	Pa405	15.0	R	4.6	HR	9.8	HR
9	Han21	22.6	R	14.3	R	18.5	R
10	Zhongzi03	0.0	HR	31.7	MR	16.6	R
11	Han23	17.6	R	16.4	R	17.0	R
12	X178	13.6	R	21.2	R	17.4	R
13	Huobai	27.5	R	15.0	R	21.3	R
14	K12	23.8	R	23.1	R	23.5	R
15	Huangzao 4	15.5	R	32.4	MR	23.9	R
16	Hai9-21	31.8	MR	34.3	MR	33.1	MR
17	CA339	34.5	MR	32.2	MR	33.4	MR
18	CA156	34.4	MR	31.5	MR	32.9	MR
19	Ye478	46.4	S	42.9	S	45.2	S
20	Qi205	46.4	S	46.8	S	46.6	S
21	Zhongzi451	44.2	S	56.3	S	50.2	S
22	Dhuang212	60.4	S	43.6	S	52.0	S
23	H21	57.8	S	49.3	S	53.6	S
24	Wenhuang	53.8	S	62.4	S	58.1	S
25	Dan340	40.2	S	77.8	S	59.0	S
26	Zong31	67.1	S	54.7	S	60.9	S
27	CA181	58.8	S	65.2	S	62.0	S
28	5213	67.1	S	60.0	S	63.5	S
29	Zi330	78.3	S	50.0	S	64.2	S
30	Shen5003	71.9	S	56.9	S	64.4	S

Table 1 (Continued)

No.	Line	1999		2000		Average over two years	
		DI%	RE	DI%	RE	DI%	RE
31	Huang C (HC)	82.4	S	50.5	S	66.5	S
32	Tie7922	82.4	S	56.5	S	69.4	S
33	B73	69.0	S	72.9	S	70.9	S
34	CA091	95.6	S	50.0	S	72.8	S
35	Ji53	100.0	S	50.0	S	75.0	S
36	48-2	81.2	S	72.2	S	76.7	S
37	B77	72.1	S	83.3	S	77.7	S
38	U8112	81.4	S	75.7	S	78.6	S
39	Mo17	98.0	S	65.0	S	81.5	S
40	Danhuang 02	85.9	S	80.0	S	83.0	S
41	Ye515	90.9	S	75.6	S	83.3	S
42	374	100.0	S	66.7	S	83.4	S
43	Ye107	87.5	S	85.4	S	86.5	S
44	444	87.5	S	89.1	S	88.3	S
45	7884	89.4	S	91.8	S	90.6	S
46	E28	100.0	S	96.2	S	98.1	S

RE: Resistance evaluation

1.2 Field tests and artificial inoculation

Field tests were performed at Tangshan Institute of Agricultural Sciences, Hebei province in 1999 and 2000. The inbreds were evaluated for reaction to SCMV as single-row plot in a complefe block design with two replicates. The plot was 5m long with 0.76m spacing between rows. Plots were over-planted and thinned to 18 plants in each row.

Virus inocula were prepared from infected seedlings. Leaves with mosaic symptom were homogenated in 0.01 mol/L phosphate buffer (pH 7.0) in 1∶10 dilution. Plants at the 4- to 5- leaf stage were rubbed with the inoculum containing carborundum twice within a week interval. Plants were evaluated for virus symptoms at weekly intervals, beginning at 7-10 days after the initial inoculation.

1.3 SSR primer selection

All 150 SSR primer pairs selected from the *phi*

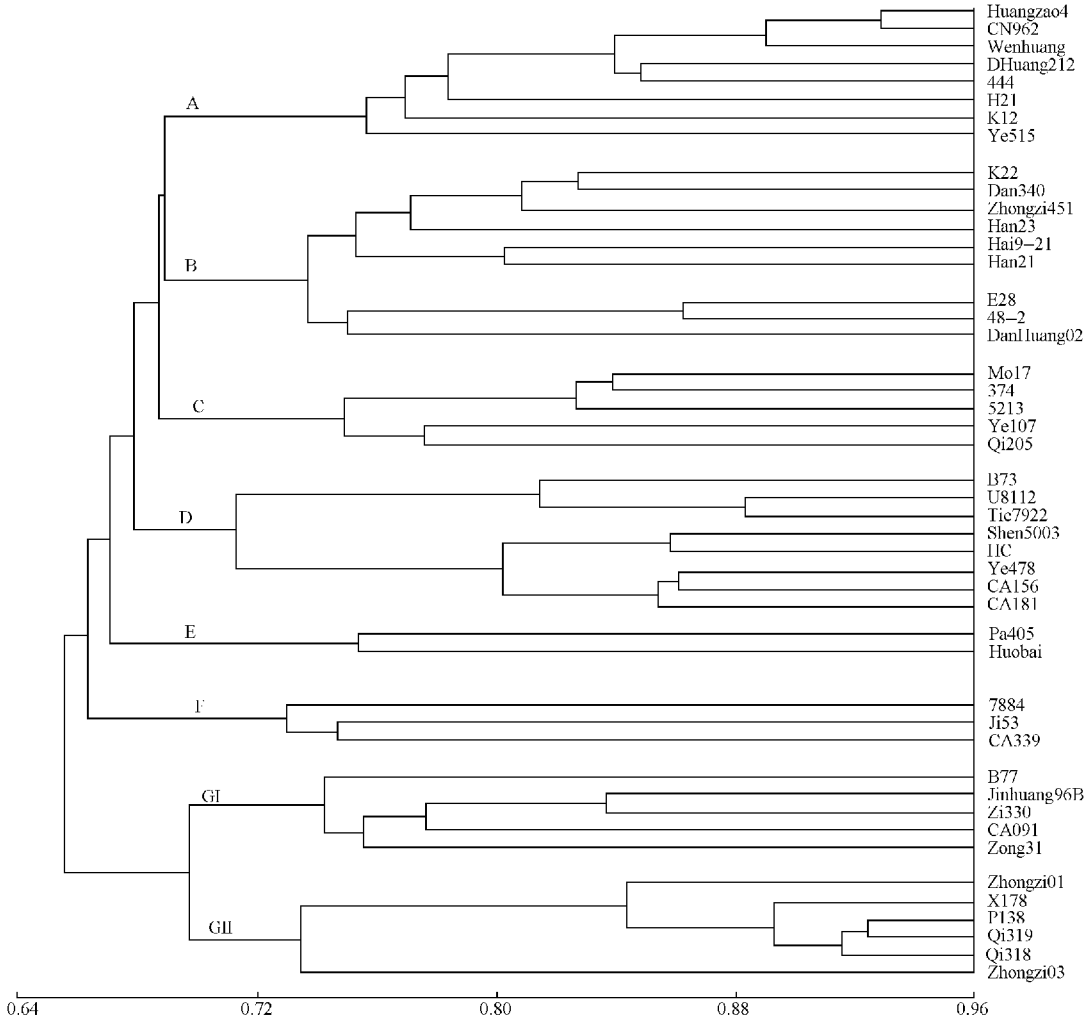

Fig. 1 Dendrogram of 46 maize inbred lines based on SSR markers

set were assayed using the sample of 15 typical inbred lines. A final set of 49 primer pairs given stable amplification profile was chosen for further analysis. Detail information regarding each of the 49 primers was presented in Table 2.

1.4 Amplification and detection conditions

Genomic DNA was extracted from a bulk of ten plant leaves of each line using a modified CTAB procedure[14]. The PCR reactions were performed using a PTC-200 Thermal Cycler (MJ Research, Watertown, MA). The amplification program was one cycle of initial denaturation at 94℃ for 1 min; 35 cycles of 94℃ for 1 min, 60℃ for 2min, and 72℃ for 2 min; and one cycle of a final extension at 72℃ for 5 min. The amplified products were kept at 4℃ until electrophoresis.

PCR reaction volume was 20μL containing 10mmol/L Tris-HCl, 50mmol/L KCl, 0.001% Gelatin, 2.5mmol/L $MgCl_2$, 0.16mmol/L each of 4dNTP, 10% glycerol, 0.3μmol/L SSR primer, 1Unit *Taq* DNA enzyme, and 50ng DNA template. The reaction mix was overlaid with 28μL of mineral oil. After amplification, 3-4μL 5XSGB were added to each tube. The amplification products were separated by electrophoresis in a Model 16cm ×20cm ×0.1cm ATTO AE-6220 vertical gel system using 1×TBE on a 12% un-denatured polyacrylamide gel with 28 lanes. ΦX174/*Hae*III used as DNA ladder marker was loaded into lane 1 per comb. The gels ran at 250 consistent voltages for 2.5 or 3h. After electrophoresis, the gel was silver-stained by the procedure that rinsing by 10% Acetic Acid for 30 min; quick rinsing by water for three times; staining by 0.1% Silver Nitrate for 30 min; rinsing briefly with water; developing by 2.5% Na_2CO_3, and stopping by rinsing gels briefly with 3% Na_2-EDTA (or 10% Acetic Acid), respectively. The gel was carefully slided onto a UV transillluminator and photographed by Fotodyne MP-4 camera with 20 cm ×26 cm hood and Type 665 polariod film.

1.5 Data analysis

The rating system was taken on a scale of 0 (symptomless) to 3 (severe stunting with few ear formed), and disease index (DI) (%) was used to distinguish resistant or susceptible genotypes, which represented 0-10 high resistant (HR); 10.1-30 resistant (R); 30.1-40 moderate resistant (R), and >40 susceptible (S). $DI = \Sigma(\text{No. of infected plants} \times \text{rated scale}) \times 100/(\text{Total plants} \times \text{maximum scale})$

Fragments amplified by SSR primers were scored as present (1), absent (0) or missing (9). Genetic similarity (*GS*) was estimated from the allele data using a simple matching coefficient such that $GS = m/(m + n)$, where m = the number of matches and n = the number of mismatches[15]. Cluster analysis of 46 maize inbred lines was performed based on the matrix of *GS* using Unweight Pair Group Method Using Arithmetic Averages (UPGMA) clustering algorithm. The *GS* matrix and cluster were performed with NTSYS-pc version 1.8 software[16].

2 Results

2.1 Resistance of 46 maize inbred lines to SCMV

Most of elite inbred lines used currently in maize breeding showed high susceptibility to SCMV (Table 1), such as Mo17, Shen5003, Ye107, Zi330, Ye478, Dan340, Tie7922, U8112, *etc*. Of the 46 inbred lines tested, 8 (K22, CN962, P138, Qi318, Zhongzi01, Jinhuang96B, Qi319, and Pa405) were rated as highly resistant, 7 (Han21, Zhongzi03, Han23, X178, Huobai, K12, and Huangzao4) as resistant, 3 (Hai9-21, CA339, and CA156) as moderately resistant, and others as susceptible. The reaction of 46 inbreds to SCMV were generally consistent over two years.

2.2 Characterization of SSR markers

A total of 49 SSR primers from *phi* set were used to assay genetic variation among 46 inbred lines, and produced 168 alleles (Table 2). The number of alleles per SSR locus varied from 2 to 10, with an average of 3.43. The size of alleles ranged from 63bp to 336bp. Most of 49 primers detected only one allele per inbred line, while several primers amplified two bands in some lines.

2.3 Genetic similarities and cluster analysis of 46 inbred lines

GS values (Data unshown) among 46 lines caculated based on 168 alleles ranged from 0.562 between Wenhuang vs Zong31 up to 0.928 between Huangzao4 vs CN962.

The UPGMA algorithm clustered 46 inbred lines into several distinct groups based on 168 alleles, designated A to G (Fig. 1), respectively. Cluster A contains Huangzao 4 and its derivatives, which is named as Sipintou group. Cluster B contains the lines from Luda Red Cob group, such as Dan340 and E28. Cluster C is of Lancaster group, such as Mo17. B73 and several inbreds from PN germplasm are found in Cluster D. Cluster E consists of Pa405 and Huobai, and cluster F contains 7884, Ji53 and CA 339. Cluster G consists of two subgroups GI and GII. GI contains five lines, i.e. B77, Jinhuang 96B, Zi330, CA 091 and Zong31. GII contains six lines that all derived from PN germplasm. Basically heterotic grouping of 46 inbred lines by SSR markers was in agreement with their known pedigrees and breeder's experience. One discrepancy was that K22 and Dan340 were clustered into Cluster B. K22 was a conversion of Ye478. Such incongruity can be explained by several factors[17].

Table 2 Chromosome loci of 49 SSR primers, number of alleles and size range detected among 46 lines

Code	SSR locus	Bin no.	No. alleles	Size range (bp)	Code	SSR locus	Bin no.	No. alleles	Size range (bp)
1	phi056	1.01	4	84- 93	26	phi126	6.00	10	134- 194
2	phi097	1.01	2	97- 100	27	phi077	6.01	8	124- 144
3	phi037	1.08	3	130- 158	28	phi078	6.05	2	122- 126
4	phi011	1.09	2	110- 122	29	phi081	6.05	3	160- 169
5	phi055	1.09	4	103- 115	30	phi070	6.07	2	73- 83
6	phi064	1.11	10	69- 113	31	phi123	6.07	2	143- 146
7	phi120	1.11	5	64- 88	32	phi057	7.01	3	145- 151
8	phi083	2.04	3	126- 134	33	phi034	7.02	4	120- 141
9	phi090	2.08	2	141- 151	34	phi114	7.02	5	135- 167
10	phi127	2.08	4	112- 128	35	phi116	7.06	4	151- 173
11	phi029	3.04	3	146- 164	36	phi119	8.02	3	162- 170
12	phi053	3.05	5	170- 194	37	phi115	8.03	3	93- 113
13	phi073	3.05	3	90- 99	38	phi014	8.04	2	157- 163
14	phi046	3.09	3	140- 152	39	phi121	8.04	2	99- 102
15	phi072	4.00	4	142- 162	40	phi015	8.08- 8.09	4	82- 102
16	phi074	4.04	3	89- 95	41	phi080	8.09	5	140- 165
17	phi096	4.04	2	102- 112	42	phi028	9.01	3	63- 78
18	phi086	4.08	2	70- 73	43	phi017	9.02	3	101- 107
19	phi092	4.08	2	120- 128	44	phi022	9.02	3	124- 148
20	phi019	4.11	4	93- 102	45	phi061	9.03	2	80- 88
21	phi113	5.03- 5.04	4	120- 336	46	phi065	9.03	3	132- 152
22	phi048	5.07	3	157- 169	47	phi059	10.02	2	146- 156
23	phi058	5.07	2	148- 151	48	phi050	10.03	3	80- 88
24	phi085	5.07	3	70- 90	49	phi062	10.04	2	161- 164
25	phi128	5.07	3	100- 110					

Cluster analysis showed that there were at least four groups among 46 inbred lines, which have been found in Chinese maize germplasm[18], i.e. Singpingtou (A), Luda Red Cob (B), Lancaster (C), and PA (D). The genetic distance (GD) between Sipingtou and Luda Red Cob groups is closer, and both had similar GD with groups Lancaster and PA, respectively. It is subgroup GII that could be mentioned as it contains six inbred lines that all derived from PN germplasm. GII probably can be named as group PB compared with PA in which the inbred lines also derived from PN germplasm years ago. GD between PA and PB was remarkable based on SSR markers. It seems that PB is a distinct group that clearly differentiates from PA and other groups. Accurate genetic relationship and heterosis among PB, PA, Lancaster and two domestic groups (Sipingtou, Luda Red

Cob) would be investigated using a large number of inbred lines and more SSR markers

3 Discussion

Under field conditions with artificial inoculation, 8 and 7 inbred lines show high resistance and resistance to SCMV, respectively. However, 28 elite inbred lines used currently in maize breeding are high susceptibility, indicating that germplasm basis for resistance to SCMV in maize breeding program is narrow. Based on genetic analysis, 46 inbred lines are clustered into 7 distinct groups Group A contains 2 resistant lines, Huangzao 4 and CN962, and Group B contains 3 resistant lines, K22, Han 23 and Han21. Group E and subgroup GII contains 2 and 6 resistant lines, respectively. Subgroup GI contains 1 resistant line, Jinghuang 96B.

Results of the study are useful for germplasm improvement for resistance to SCMV based on heterotic pattern. Huangzao 4 and CN962 both show a significant resistance to SCMV, which could be used to improve Wenhuang, Dhuang212, 444, H21 and Ye515. K22, Han23 and Han21 showing resistance to SCMV are in Group B, so that these lines could be utilized to improve Dan340, Zhongzi451, E28, 48-2 and Danhuang02. Group E and Group GII, which contain a large number of resistant germplasm, possess a high potential utilization in improvement of resistance to SCMV. However, no resistant inbred lines can be found in Groups C, D and F.

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