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Evaluation of Genetic Heterogeniety among the Corn Landraces Collected from Farmer's Field

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ABSTRACT

This paper describes the variations in eight agronomic traits in three unadpted local landraces and an inbred cultivar of corn. To compare the agronomic traits in field evaluation with molecular marker evaluation, the genotypes of the plant introductions were also checked by 4 microsatellite-SSR loci. The variations of the eight agronomic traits were higher in the local landraces than in the inbred line, which was substantiated by the high genetic variation in the landraces with microsatellite-SSR loci. The level of genetic variation was also different between landraces. Since the genetic evaluation can be easily quantified by the analysis of microsatellite-SSR loci, the threshold level of genetic homogeneity in the population for parental lines in breeding program can be determined and the effort of maintaining the landrace population would be alleviated. As an example in our analysis, the entry from Whachon should not need the same number of selfing generations as the other two landraces to get the level of inbred state, since this line showed lowest intra-genetic variation within the population.

Key words: corn, landraces, genetic heterogeneity, homogeneity, QTL, microsatellite-SSR

INTRODUCTION

Genetic erosion in modern crops has been brought up as a serious problem by many scientists since the limited genetic variabilities have left the current crops very vulnerable to biotic and abiotic stresses. However, the farmer's lines or landraces are still possessing many unselected desirable alleles or genes which can be introgressed to modern crop cultivars. Therefore, thousands of landraces are being introduced into the germplasm or plant breeding institutes around the world each year. Landrace or germplasm accessions are, then, evaluated for their potential to improve current cultivars by per se evaluation in either an area of adaptation or of testcross or combination per se in a multistage evaluation (Abel and Pollak, 1991).

Since corn is an open-pollinated species, the plant introductions can not be utilized directly in plant breeding program until certain level of homogeneity in a population is achieved. To achieve the homogeneity in agronomic performance, the plants are allowed to self-pollinated for several generations. Theoretically, heterozygosity will be reduced as a rate of $(1/2)^n$, where the n is the number of generation. Therefore, the majority of the plants will have homozygotic genotypes in either dominance or recessive in most of the loci after many generations in autogamous or selfed plants. Then, selection and propagation on the basis of individual plant will make the derived population homogeneous in genetic or agronomic performances.

The level of genetic homogeneities in the first entries of the plant introduction will be varied depending on the origin of the plants. Some plant introductions will be highly homozygous in most of the loci, which requires only few generations for selfing to achieve the level of breed true. However, some plant introductions will require many generations of selfing to get the similar

level of homozygosity. To evaluate the genetic homogeneity, a set of morphological and agronomic characters has been utilized conventionally in many plant populations including corn (Pandey et al., 1991; Gouesard et al., 1996). Molecular markers have also been utilized extensively to measure genetic variation with accuracy by eliminating environmental effects on the evaluation (Dudley et al., 1991; Mumm and Dudley 1994; Bernado et al., 1996). Among the many molecular marker systems, microsatellite-SSR analysis has provided the most reliable and informative results in measuring the genetic variation in many plant species (Morgante and Oliveri 1993; Wang et al., 1994; Taramino and Tingey 1996; Powell et al., 1996).

The objective of this study was to develop an efficient and reliable method to evaluate the genetic homogeneities in the corn landraces collected from farmer's field.

MATERIALS AND METHODS

Plant materials and Field experiments

Three unadapted corn landraces were collected from farmer's field from InJae, HwaChon, and BongWha at 1996 and planted at the field laboratory of Hongchon Corn Experiment Station (Doochonmyeon, Hongchongun) at April 26, 1997. As well, one inbred line, *KP12*, was also planted for control. Fifty seeds from each collection and inbred line were planted in the distances of 70cm × 30cm in three rows. Then, the plants were grown as the conventional cultivation method.

Measurements of plant height, ear height, ear length, ear width, and number of ears per plant were done from all the plants after they were fully grown. Days to tasseling and days to silking were the number of days elapsed after sowing for the appearances of tassel and silk, respectively. Grain weight was measured with the randomly chosen 100 grains after the harvested seeds were completely dried.

Microsatellite-SSR analysis

The procedures for plant genomic DNA extraction were followed with protocols of Lee et al.(1994) without modification .

The primers for microsatellite-SSR loci were synthesized according to the published information in elsewhere (Chin et al., 1996). The sequences of the SSR primers are shown in Table 1. The PCR profile was consisted of an initial denaturation of 94℃ for 5 min, followed by 2 cycles of denaturing at 94℃ for 1 min, annealing at 65℃ for 1 min, and extention at 72℃ for 2 min. After the second cycle, the annealing temperature was decreased 1℃ in every second cycle to 55℃. The last cycle was repeated 20 times. When the cycles were completed, the extension cycle was extended for 10 min at 72℃ and the reaction tubes were stored at 4℃. Amplified microsatellite-SSR fragments were, then, separated either in denaturing 6% polyaclyamide or 3% agarose and stained with either silver or ethidium bromide depending on the gel type, respectively.

Statistical Analysis

Analyses of all data were calculated with the all plants in each population. That is, the mean was calculated by dividing the sum of all the measured value with the number of plants in each population. The variance and

Table 1. Sequences of the SSR primers used.

No.	Primer	Chr. no.	Repeat	Sequence
primer 3	ZMGST3	3	AGC/GCT	F* 5' -TTACTCCTATCCACTGCGGCCTGGAC-3' R 5' GCGGCATCCCGTACAGCTTCAGA-3'
primer 6	MZEG3LP	6	AGCTC/ GAGCT	F 5' -GCTGAGCGATCAGTTCATCCAG-3' R 5' -CCATGGCAGGGTCTCTCAAG-3'
primer 7	ZMOPA2	7	AG/CT	F 5' -TGCCCTGCAGGTTCACATTGAGT-3' R 5' -AGGAGTACGCTTGGATGCTCTTC-3'
primer 10	UAZ250	10	AAG/CTT	F 5' -AGAAGGAATCCGATCCATCCAAGC-3' R 5' -CACCCGTACTTGAGGAAAACCC-3'

Note : * F and R mean forward and reverse primer for each SSR locus

standard deviation were calculated with the equations of $\Sigma(Xi-X)/n-1$. Coefficient of variation (C.V.) was the value of $S/M \times 100$. All of the calculations were done with the Microsoft Excel Statistics program.

RESULTS AND DISCUSSION

Variations in agronomic characters

The differences among the populations for the studied traits were significant. The studied characters were plant height, ear height, ear length, ear width, days to tasseling, days to silking, number of ears per plant, and grain weight. Variations in eight agronomic characters within the population of unadapted local landraces were measured and compared to the inbred control (Table 2 and Figure 1). Most of the characters showed normal distribution of the frequency in all of the characters except of the number of ears per plant in the lines studied, which indicate that they are quantitative traits (QTLs). The distribution frequencies revealed that the genetic heterogeneities in the unadapted lines were higher than those of inbred control since the distributions in the unadapted landraces were wider than those of inbred control.

Although we realized that replica experiments to check the variations from environmental effects were necessary, replica experiments were not conducted due

Table 2. Mean (M), standard deviation (S), variance (S2), and coefficient of variation (C.V.) of the eight agronomic traits within the population of an inbred and unadapted local landraces.

			KP12	INJAE	HWACHON	BONGHWA
Plant height	M	cm	141.86	178.78**	205.54**	169.82**
	S		13.11	20.76	15.75	22.14
	S2		172.00	430.99	248.17	490.31
	C.V.	%	9.24	11.6	7.66	13.04
Ear height	M	cm	74.78	128.52**	122.00**	96.28**
	S		10.24	15.25	14.09	18.15
	S2		104.87	232.42	198.47	329.59
	C.V.	%	13.69	11.86	11.40	18.86
Ear length	M	cm	9.33	9.31ns	13.58**	13.64**
	S		1.00	3.17	3.16	2.77
	S2		1.00	10.06	9.98	7.65
	C.V.	%	10.74	34.06	23.27	20.27
Ear width	M	cm	2.43	2.80**	2.70**	2.96**
	S		0.17	0.33	0.39	0.33
	S2		0.03	0.11	0.15	0.11
	C.V.	%	7.13	11.70	14.19	11.20
Days to tasseling	M	day	95.66	94.74*	92.08**	90.36**
	S		1.36	3.90	2.20	1.83
	S2		1.86	15.22	4.85	3.34
	C.V.	%	1.43	4.12	2.39	2.02
Days to silking	M	day	101.42	97.96**	97.88**	96.62**
	S		1.16	3.02	3.45	2.85
	S2		1.35	9.14	11.90	8.12
	C.V.	%	1.15	3.09	3.52	2.95
No. of ear	M	e	1.40	2.44**	1.84**	1.32ns
	S		0.49	0.5	0.58	0.47
	S2		0.24	0.25	0.34	0.22
	C.V.	%	35.35	20.55	31.75	35.70
Grain weight	M	(g/100)	8.13	8.20ns	11.68**	14.06**
	S		0.22	2.80	1.75	2.35
	S2		0.05	7.82	3.05	5.53
	C.V.	%	2.76	34.10	14.95	16.72

Note : *, ** designate significant at 5% and 1%, respectively.

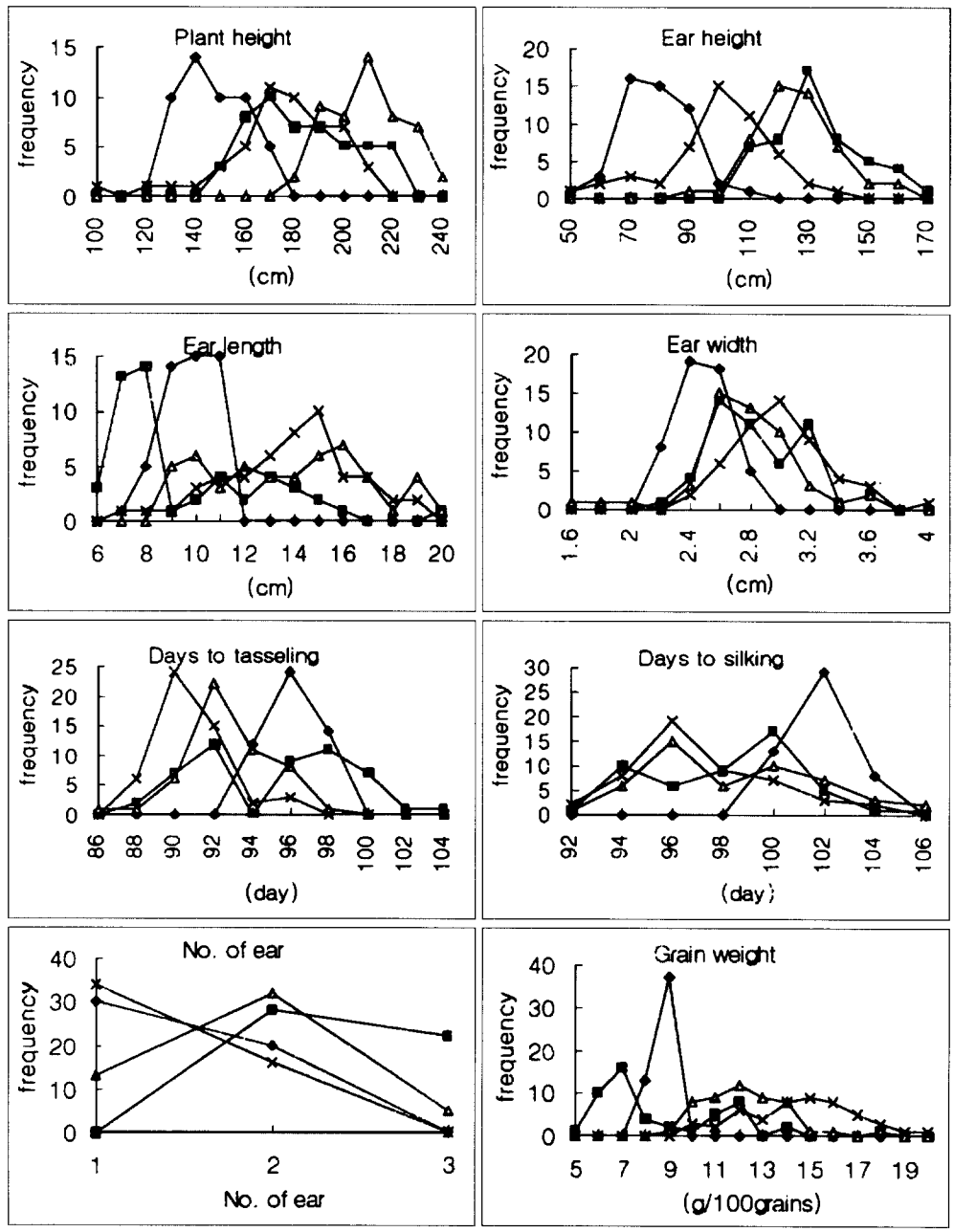

Fig. 1. Distribution frequency of plants showing each agronomic traits in the corn lines analyzed.
—◆— KP12, —■— INJAE, —▲— HWACHON, —×— BONGHWA

to the limit of seeds in each collection. Therefore, we could not rule out the possibility of overestimates of the true value in the variance because the genotype-by-environment component could not be partitioned from the genotypic variance. However, the negation of environmental contribution in other genetical studies conducted in the same experimental field maybe support our data as valid (data not shown). High heritabilities (h^2) were reported in grain yield, ear

length, ear diameter, cob length, grain weight, ear hight by other researchers (Berke and Rocheford 1995; Holthaus and Lamkey 1995; Austin and Lee 1996). Therefore, the interactions with environments would be smaller than the main genetic effects in these traits.

The variances among the unadapted lines in plant height showed higher than the control. However, the line from HwaChon showed lower coeffcient of variation (C.V.) than inbred control since the plant height of this line was significantly taller than the control (significance level at 5%). The same situation was occurred in the ear height since coeffcients of variations among the unadapted lines were lower than those of inbred control, which might be also from the fact that the ear height in the unadapted lines was higher than that of control. The rest of the traits, ear length, ear width, days to tasseling, days to silking, and grain weight, showed that variance and coefficient of variation in the inbred control were lower than those of unadapted landraces. However, variance in the number of ear seemed to be not related with the genetic homogeneity within the population. This could be from the low heritability of this trait. Austin and Lee (1996) demonstrated the trait of ear numbers per plant showed lowest heritability (h^2=0.48) among 8 QTLs for grain yield and yield components.

Variation in microsatellite-SSR analysis

Microsatellites are stretches of tandemly arranged short sequence motifs that individually range from two to six nucleotides (Hamada et al. 1982). The numbers of repeating motifs are highly variable so that they have been used heavily in several genetic analyses for molecular markers (Chin et al. 1996; Senior et al. 1996; Powell et al. 1996). The unadapted and an inbred corn lines were surveyed their genetic homogeneities with 4 sets of microsatellite primers (Table 3). Because each microsatellite-SSR locus could be regarded as independent genetic locus to other microsatellite-SSR loci and each population was consisted of 50 plants, analysis of four microsatellite-SSR loci means that two hundred loci were checked to test the genetic homogeneity in each population. The inbred control line, *KP12*, showed high level of homogeneity in the SSR loci in which only five plants showed different alleles in the set of primer 6. Therefore, the level of homogeneity could be as high as 5/197 (one plant in primer 6 and two plants in primer 7 showed failure in amplification). The *KP12* showed only single types in the loci of primer3, 7 and 10. However, the levels of homogeneity in the unadapted landraces were low as much as 61/199 in InJae, 45/198 in HwaChon, and 59/190 in BongWha. The number of different banding patterns in each line was considerably low in the inbred control. The number of detected alleles were also high in the unadapted lines. While the inbred control had only 5 alleles in four loci, the other lines had 9 alleles in Injae, 10 alleles in HwaChon, 8 alleles in BongWha, respectively. As well, the proportion of plants having microsatellite

Table 3. The number of heterogeneous plants and heteozygotes in each corn line in the SSR loci

	KP12		INJAE		HWACHON		BONGHWA	
	Hetero-geneous	Hetero-zygote	Hetero-geneous	Hetero-zygote	Hetero-geneous	Hetero-zygote	Hetero-geneous	Hetero-zygote
PRIMER 3	0/50 (1)* (1)**	0/50	15/49 (2) (3)	14/49	13/50 (2) (3)	2/50	35/48 (2) (3)	25/48
PRIMER 6	5/49 (2) (3)	1/49	35/50 (2) (3)	30/50	20/49 (3) (4)	1/49	12/50 (2) (3)	4/50
PRIMER 7	0/48 (1) (1)	0/48	6/50 (2) (2)	0/50	9/49 (3) (4)	7/49	10/42 (2) (3)	1/42
PRIMER 10	0/50 (1) (1)	0/50	5/50 (3) (3)	5/50	3/50 (2) (2)	3/50	2/50 (2) (2)	2/50
Total	5/197	1/197	61/199	49/199	45/198	13/198	59/190	32/190

Note : * ( ) : No. of Alleles
** ( ) : No. of banding patterns

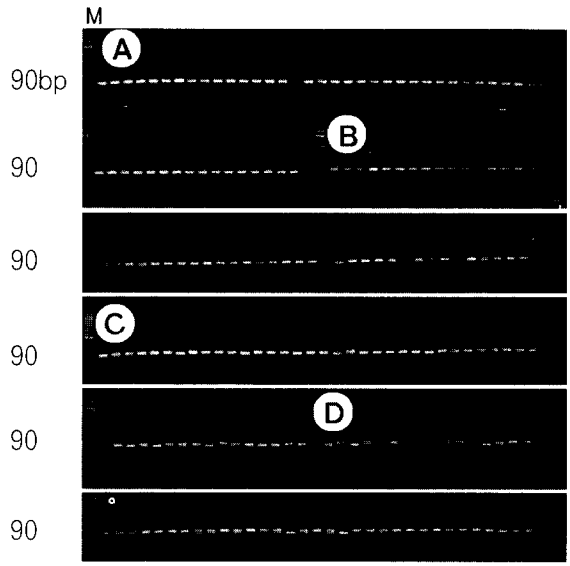

Fig. 2. Microsatellite-SSR alleles in SSR primer 3
Note : A: KP12, B: INJAE, C: HWACHON, D: BONGHWA

loci in heterozygote was also quite high in the unadapted landraces. In the cases of primer 3 in Bongwha and primer 6 in Injae, the heterozyotes were even more common than homozygotes.

When the alleleic frequency in each primer locus was considered separately, a specific allele was predominant across the populations. For example, among the five alleles were detected in the locus of primer 3, only twenty plants in 200 did not carry a predominant allele. Although we did not compare the data with corn lines derived from other sources, the rare alleles in the twenty plants would be the ones derived from either unequal crossing-over or DNA slippage in replication (Wierdl et al. 1997). Inman et al.(1997) demonstrated that there was significantly positive correlation between number of repeats and amount of variation in the microsatellite loci in unadapted natural populations of *Arabidopsis thaliana*. Although we could not determine the length of the repeating units in our case, the number of bases in repeating motif seemed to be related to the amount of variation in our study since the amount variation in the locus of primer 6, which has five base repeating motif [(AGCTC)$_n$], showed the highest amount variation among the four microsatellite loci. Chin et al. (1996) demonstrated that trinucleotide repeat was the most abundant and polymorphic in maize. This would be very important for checking the genetic variation or purity among the plant entries in plant breeding institute. We can minimize the number of PCR reactions to detect variations by selecting the most polymorphic microsatellite-SSR loci.

The amount variation among the unadapted line was significantly higher than the inbred control in the analyses of both agronomical characters and microsatellite-SSR locus. The differences of variation between the unadapted landraces were not determined directly by the lack of appropriate statistical equation, there were differences in variation among the landraces. By the sum of total coefficient varaitions of the eight characters, the landrace entry from HwaChon showed the lowest amount variation among the three plant entries. The number of heterogeneous plants in their microsatellite-SSR loci was also low in the landrace from HwaChon. In order to use as parental lines in breeding programs, the introduced landraces shoud be gone thorough many times selfing to make homozygotes as many loci as possible. However, the proportion of each alleles in the population would not be variable in the subsequent generations by Hardy-Weinberg equilibrium concept, if selection was not practiced in the course of selfing. Therefore, the plants could be selected as a base of ear-to-row after certain number of selfed generations. In other method, the plants would be selected as recurrent selection method. Since the final breeding lines should have certain degree of homozygosity for using parental lines in cross hybridization between lines, the requirement of number of generations in selfing will be variable depending on the lines when they were introduced. For example, the landrace line intriduced from WhaChon needs not be selfed as many generations as others. If we know the level of genetic heterogeneity in the plant introduction and can determine the threshold level of genetic homogeneity in the population for parental lines in breeding program, the effort of maintaining the landrace population would be alleviated. This study showed the standard deviation in eight quantitative traits in an inbred line and three landrace

introductions in corn. Although the number of landraces was not extended, this study would delineate the threshold for inbred in the studied traits. The studied quantitative traits are difficult to assign the genetical contribution to the variation since environmental effects in the quantitative traits are also high in many cases. Therefore, the microsatellite-SSR variations, which can rule out the possible environmental effects in the variation, would complement the defect of agronomic trait analysis.

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