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Chlorophyll-Deficient Gene and Morphological Variations in Korean Populations of Maize (*Zea mays*)

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We studied cultivated and naturalized Korean maize populations to determine the extent to which the chlorophyll-deficient mutation and the phenotypic variations of two morphological characters (i.e., red coleoptiles and epicotyls, and the number of the first root hairs) are maintained. The frequency of the chlorophyll-deficient mutant gene (2.73% on average) was highly variable. Frequencies of red coleoptiles and epicotyls also were higher than expected from a mutation-selection balance. The average number of hairy phenotypes within populations was 1.8, ranging from 0.0 to 4.0. Naturalized populations were closely related to with cultivated communities. Most striking, however, was the more significant difference among populations than within populations with regard to both the frequency of chlorophyll-deficient mutant genes and the phenotypic variations of our two morphological characters. On a per-gene basis, the majority of the phenotypic variation (mean of 73.3%) resided among populations.

Keywords: chlorophyll-deficient mutant, maize, phenotypic variations, *Zea mays*

Zea mays L. (Graminae) is an important food source for meeting the current and future needs of the world. Also known as maize or corn, this tropical American native is the only cereal to be domesticated in the Americas (Chrispeels and Sadava, 1994). Maize has been cultivated in developing countries, including approximately 50 million ha in the tropics, mainly at low elevations (Chrispeels and Sadava, 1994). As this crop has moved out of its center of origin, it has given rise to a number of varieties with different properties (Moore et al., 1995) and enormous genetic variation. Because the potential yield for maize is larger than for wheat or rice, it should assume a proportionally larger and more important role in world food production.

From both agricultural and evolutionary points of view, wild relatives of cultivated plant species offer an important system for investigating the gene pool available for crop improvement (Doebley, 1989; Escalante et al., 1994). They also provide information about the progress of domestication (Doebley, 1989). Thus, it is very important to study established populations of naturalized maize in terms of crop evolution.

Marker genes are valuable tools in genetic and breeding research (Wehner et al., 1998). Most detrimental genes are maintained at only low frequencies in natural populations (0.001 to 0.005), primarily because of the mutation-selection balance (Crumpacker, 1967; Ohnishi, 1982). In this study, we investigated a case of balanced polymorphism involving a recessive lethal gene. Our objectives were to 1) determine the occurrence and distribution of the chlorophyll-deficient mutant gene in naturalized populations of diploid maize in Korea; 2) estimate the extent to which the genes for red coleoptile and epicotyl (*rc*), and for first root hairy (*fh*) are maintained in cultivated and naturalized populations; and 3) describe how genetic variation is distributed within and among populations of this species.

MATERIALS AND METHODS

This study comprised 11 populations each of naturalized and cultivated Korean maize (Table 1). One hundred randomly selected seeds from each population were sown into soil in wooden boxes. The potting medium consisted of mainly peat and vermiculite, with smaller amounts of bark ash and sand. When the plants reached the foliar-leaf stage, they were transplanted to pots (four per pot), and grown outdoors until the bolting stage. The chlorophyll-deficient phenomenon and any morphological variations were monitored at both the cotyledon and the two -leaf stages.

The degree of polymorphism was quantified using Shannon's index of phenotypic diversity (Bowman et al., 1971):

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$$Ho = -\Sigma p_i \log p_i,$$

where p_i is the frequency of a particular phenotype i (King and Schaal, 1989), and H_O can be calculated and compared for different populations (Paul et al., 1997). Let

$$H_{POP} = 1/n\ \Sigma\ H_O$$

be the average diversity over n different populations and let

$$H_{SP} = -\Sigma p \log p$$

be the diversity calculated from the phenotypic frequencies p in all the populations considered together (Paul et al., 1997). Then the proportion of diversity present within populations, H_{POP}/H_{SP} can be compared with that between populations, $(H_{SP} - H_{POP})/ H_{SP}$

Homogeneity of variance among populations was tested by Bartlett's statistics. The G_{ST} coefficient corresponded to the relative amount of differentiation among populations (Nei, 1977). Furthermore, gene flow between the pairs of populations was calculated from G_{ST} values by $Nm = 1/4(1/G_{ST} - 1)$ (Wright, 1965). In addition, χ^2-statistics were used to detect significant differences in allele frequencies among populations for each locus (Workman and Niswander, 1970).

We tested the null hypothesis that the cultivated lines (populations) and the naturalized lines (populations) of morphological characters are equal (two-tailed paired-sample t test). [Data for both 1-2W and 7-2S were excluded in this analysis because we ultimately failed to collect adequate samples of those populations.]

Nei's genetic distance was calculated for each pairwise combination of populations (Nei, 1972). The correlation between geographical and genetic distance was tested using a modified Mautel's test, as given by Smouse et al. (1986). We also constructed a phylogenetic tree by the neighbor joining (NJ) method of Saitou and Nei (1987), using the NEIGHBOR program in PHYLIP version 3.57 (Felsenstein, 1993).

Table 1. Study codes of populations and localities.

Code	Localities
Cultivated population	
1-1S	Anei-myen, Hamyang-gun
4-1S	Uogyang-myen, Kechang-gun
5-1S	Meongseok-myen, Chinju-city
8-1S	Changma-myen, Changreng-gun
9-1S	Chilbuk-myen, Haman-gun
10-1S	Dong-myen, Changwon-city
11-1S	Habuk-myen, Yangsan-gun
12-1S	Ungchon-myen, Ulsan-city
14-1S	Kijang-up, Pusan-city
15-1S	Baedun-myen, Goseong-gun
16-1S	Dosan-myen, Tongreng-gun
Naturalized population	
4-2W	Gajo-myen, Kechang-gun
5-2W	Changjae-dong, Chinju-city
7-2W	Burim-myen, Uireng-gun
8-2W	Youngsan-myen, Changreng-gun
9-2W	Kaya-myen, Haman-gun
10-2W	Buk-myen, Changwon-city
11-2W	Sangbuk-myen, Yangsan-gun
12-2W	Unyang-myen, Ulsan-city
14-2W	Cangan-up, Yangsan-gun
15-2W	Hoaha-myen, Goseong-gun
16-2W	Sanyang-myen, Tongreng-gun

RESULTS

A total of 1976 Korean maize plants were sampled, or more than 78 individuals per population. Among these, 70 (3.5%) showed chlorophyll-deficient phenotypes (Table 2). The frequency of this mutation was highly variable, averaging 2.73%. Although many populations were monomorphic at this locus, those of

Table 2. Gene frequencies of the chlorophyll-deficient mutant and heterozygosity among 22 maize populations in Korea.

Pop.	Total	+/+	+/*ch*	*ch*/*ch*	χ^2	% of *ch*	H_{EP}
1-1S	92	75	11	6	9.72***	12.5	0.206
4-1S	95	95	0	0	0	0	0
5-1S	89	89	0	0	0	0	0
8-1S	93	93	0	0	0	0	0
9-1S	91	91	0	0	0	0	0
10-1S	97	97	0	0	0	0	0
11-1S	87	87	0	0	0	0	0
12-1S	93	66	10	17	12.93***	23.7	0.375
14-1S	88	88	0	0	0	0	0
15-1S	84	84	0	0	0	0	0
16-1S	78	78	0	0	0	0	0
4-2W	97	97	0	0	0	0	0
5-2W	87	87	0	0	0	0	0
7-2W	77	77	0	0	0	0	0
8-2W	95	95	0	0	0	0	0
9-2W	94	94	0	0	0	0	0
10-2W	90	90	0	0	0	0	0
11-2W	85	85	0	0	0	0	0
12-2W	89	63	11	15	11.22***	23	0.358
14-2W	92	92	0	0	0	0	0
15-2W	88	88	0	0	0	0	0
16-2W	95	95	0	0	0	0	0
Total	1976	1906	32	38	513.1***	2.73	0.053

*, $P<0.05$; ***, $P<0.001$.

1-1S, 12-1S, and 12-2W had very high genotype frequencies (12.5%, 23.7%, and 23.0%, respectively). Heterozygosity for each population is shown in Table 2. Although population 12-1S had the highest variation among those three polymorphic populations, the heterogeneity of variance over the three was not significantly different ($0.995 < p < 0.999$, Bartlett's test). The G_{ST} value was 0.21, which is higher than the averages estimated from allozyme data for other outcrossing plant species.

The frequencies of the three genotypes expressing the red coleoptile gene (*rc*) in each population are presented in Table 3. A chi-square test revealed that all the observed values of these genotypes in each population were significant according to the Hardy-Weinberg ratio. The frequencies for *rc* were relatively high in the cultivated populations, with a maximum of 95.0% in 14-1S. Although values for cultivated populations were higher than for naturalized populations, the heterogeneity of variance over all the populations was not significantly different ($0.995 < p < 0.999$, paired *t* test). The frequency of *rc* was higher than that of chlorophyll deficiency, while its G_{ST} value (0.109) was about half that for the latter.

The number of first root hairs was not linked to the traits of the chlorophyll-deficient or the normally green coleoptile gene. The number of hairy phenotypes averaged 1.8 within populations, ranging from 0.0 to 4.0 (Table 4). The frequency of *rc* generally was low, averaging 5.6%. Populations of 11-1S and 11-2W had few first root hairs at this locus, but those of 5-1S and 5-2W had many. On a per-locus basis, the proportion of total genetic variation due to differences among populations (G_{ST}) was 0.482, indicating that half of the total variation was within populations.

Table 3. Morphological variation of green or red coleoptiles and epicotyls, and expected heterozygosity among 22 maize populations in Korea.

Pop.	Total	+/+	+/*rc*	*rc*/*rc*	χ^2	% of *rc*	H_{EP}
1-1S	30	30	0	0	0.00	0.0	0.00
4-1S	30	0	18	12	30.64	70.0	0.42
5-1S	30	3	10	17	31.89	73.3	0.39
8-1S	30	0	21	9	24.13	65.0	0.46
9-1S	30	26	4	0	0.16	6.7	0.12
10-1S	30	30	0	0	0.00	0.0	0.00
11-1S	30	0	22	8	27.47	63.3	0.46
12-1S	30	17	13	0	2.69	21.7	0.34
14-1S	30	0	3	27	488.90	95.0	0.10
15-1S	30	3	20	7	13.55	56.7	0.49
16-1S	30	0	30	0	37.50	50.0	0.50
4-2W	30	15	8	7	3.32	36.7	0.46
5-2W	30	24	6	0	0.40	10.0	0.18
7-2W	30	30	0	0	0.00	0.0	0.00
8-2W	30	4	2	24	98.45	83.3	0.28
9-2W	30	30	0	0	0.00	0.0	0.00
10-2W	30	30	0	0	0.00	0.0	0.00
11-2W	30	6	15	9	6.19	55.0	0.50
12-2W	30	24	6	0	0.40	10.0	0.18
14-2W	30	30	0	0	0.00	0.0	0.00
15-2W	30	6	23	1	13.63	41.7	0.49
16-2W	30	0	30	0	37.50	50.0	0.50
Total	660	308	231	121	23.09	35.8	0.46

The phenotypic frequencies for the number of first root hairs (*fh*) were calculated and used to estimate phenotypic diversity (H_O) within populations (Fig. 3 and Table 5). Although Korean populations are small, isolated, and patchily distributed, they maintain high levels of diversity; our mean H_O was 1.552. Shannon's index of phenotypic diversity for *rc* was used to partition the diversity into within- and among-population components. The proportion of diversity within populations, H_{POP}/H_{SP} indicated that only 4.8% of the total diversity was among populations. Thus, the majority of the phenotypic variation (95.2%) was within populations (Table 5).

In studying the frequencies of the chlorophyll-deficient mutant gene and the phenotypic variations of our two morphological characters, we found that one

Table 4. Morphological variation of the first root hairs and expected heterozygosity among 22 maize populations in Korea.

		No. of first root hairs							
Pop.	Total	0	1	2	3	4	5	Mean	H_{EP}
1-1S	30	3	4	3	17	3	0	2.3	0.63
4-1S	30	3	9	14	4	0	0	1.3	0.66
5-1S	30	0	0	0	7	23	0	3.8	0.36
8-1S	30	5	14	10	0	0	1	0.8	0.64
9-1S	30	8	5	4	4	9	0	1.9	0.78
10-1S	30	4	6	0	17	3	0	2.1	0.61
11-1S	30	28	2	0	0	0	0	0.0	0.12
12-1S	30	3	5	7	6	9	0	2.3	0.78
14-1S	30	3	13	12	2	0	0	1.0	0.64
15-1S	30	1	22	6	1	0	0	0.5	0.42
16-1S	30	3	5	9	6	7	0	2.1	0.78
4-2W	30	3	13	6	8	0	0	1.2	0.69
5-2W	30	0	3	3	5	19	0	3.2	0.55
7-2W	30	15	7	8	0	0	0	0.5	0.62
8-2W	30	0	0	0	6	19	5	4.0	0.53
9-2W	30	0	3	5	4	8	10	3.5	0.76
10-2W	30	11	5	4	10	0	0	1.3	0.71
11-2W	30	29	1	0	0	0	0	0.0	0.06
12-2W	30	4	6	5	12	3	0	1.9	0.74
14-2W	30	0	0	1	20	9	0	3.3	0.46
15-2W	30	3	13	5	3	6	0	1.4	0.72
16-2W	30	13	7	4	4	2	0	0.9	0.72
Total	660	139	143	106	136	120	16	1.8	0.59

of the most striking results was that differences were more significant among rather than within Korean maize populations. On a per-gene basis, the proportion of total phenotypic variation was due to differences within populations (Table 6). The proportion of diversity, G_{ST}, indicated that only 26.7% of the total phenotypic diversity was within populations. Thus, the majority of the phenotypic variation (73.3%) resided among populations (Table 6).

Genetic identity values among pairs of populations ranged from 0.860 to 0.991 (data not shown). In a phylogenetic tree based on the three morphological characters, the positions of the populations did not completely match their corresponding geographical positions (data not shown). Likewise, the correlation between genetic distance and geographic distance in Korea was relatively low ($r = 0.37$, $p<0.05$), which indicates that geographically close populations are not necessarily genetically similar. Therefore, the remainder, or about 86% ($1 - r^2$), of the variation was caused by unknown factors.

Table 5. Phenotypic diversity of the first root hairs and expected heterozygosity among 22 maize populations in Korea.

Pop.	*Ho*	*Hpop*
1-1S	1.281	0.058
4-1S	1.216	0.055
5-1S	0.543	0.025
8-1S	1.134	0.052
9-1S	1.550	0.070
10-1S	1.143	0.052
11-1S	0.245	0.011
12-1S	1.552	0.071
14-1S	1.140	0.052
15-1S	0.776	0.035
16-1S	1.552	0.071
4-2W	1.267	0.058
5-2W	1.048	0.048
7-2W	1.039	0.047
8-2W	0.910	0.041
9-2W	0.851	0.039
10-2W	1.301	0.059
11-2W	0.146	0.007
12-2W	1.486	0.068
14-2W	0.745	0.034
15-2W	0.776	0.035
16-2W	1.420	0.065

Table 6. Partitioning of the phenotypic diversity into within- and among-populations of maize.

Gene	H_{SP}	H_{POP}	H_{POP} / H_{SP}	$(H_{SP} - H_{POP})/H_{SP}$
ch	0.178	0.037	0.211	0.790
rc	0.178	0.019	0.109	0.891
fh	2.181	1.051	0.482	0.518
Mean	0.846	0.368	0.267	0.733

DISCUSSION

Balanced polymorphisms of chlorophyll-deficient mutants have been reported in *Dactylis glomerata* (Apirion and Zohary, 1961), *D. glomerata* ssp. *woronowii* (Curran, 1963), *Fagopyrum esculentum* (Ohnishi, 1982), and *Cucumis melo* (Ray and McCreight, 1996). Likewise, variable flower colors (white, yellow, orange, burnt orange, and amber) have been reported for groundnut (*Arachis hypogaea* L.) (Smartt, 1960; Dwivedi et al., 1996), while several yellow genes have been identified as controlling flesh color in watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] (Henderson, 1991; Henderson et al., 1998). Except for these chlorophyll-deficient and flower color polymorphisms, direct estimates of the frequency of detrimental mutants are still scarce for plant populations (Wehner et al., 1998). Sprague and Schuller (1961) and Ohnishi (1982) have concluded that the frequency of detrimental mutants

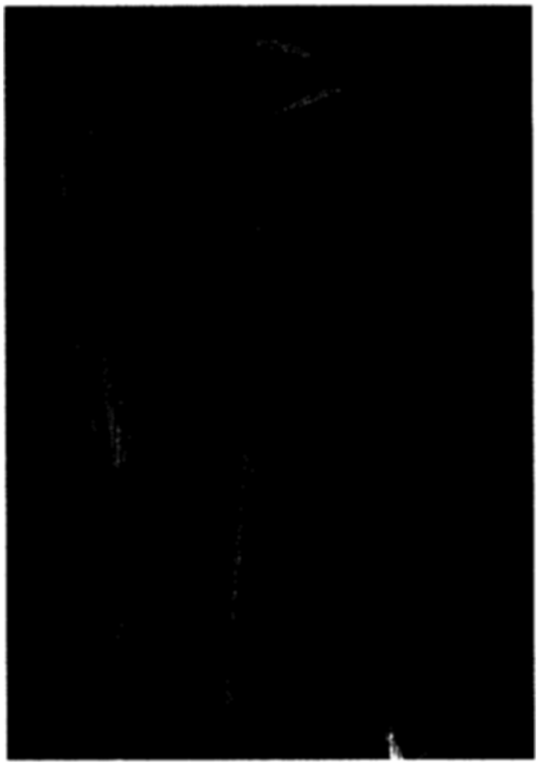

Figure 1. The chlorophyll-deficient mutant of maize populations in Korea. Left, albino; Middle, partial albino; Right, normal leaves.

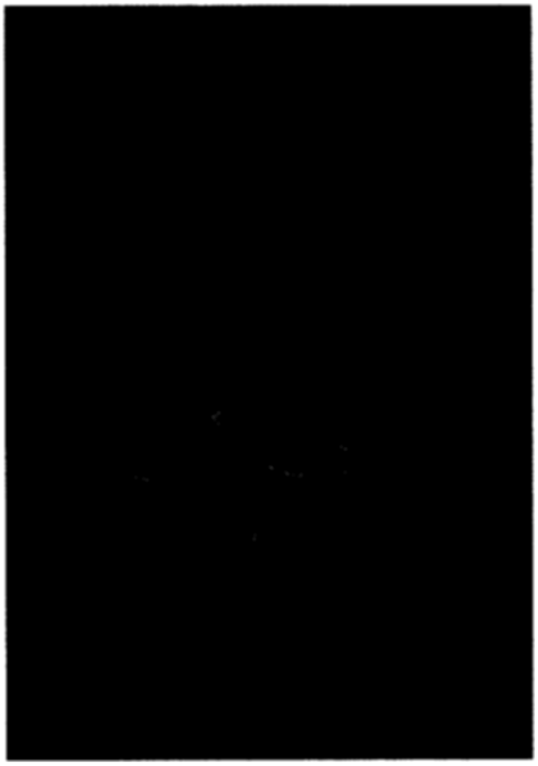

Figure 2. Morphological variation of green or red coleoptiles and epicotyl of maize populations in Korea. Left, green; Right, red coleoptile.

Figure 3. Morphological variation of the number of first root hairs. Left, one hair; Middle, two hairs; Right, four hairs.

at each locus is low, while cases of balanced polymorphism for detrimental mutants also are probably quite rare (Ohnishi, 1999).

Our frequencies of chlorophyll-deficient mutants are similar to those expected from a mutation-selection balance. The amounts and kinds of genetic variation in a population may be affected by a number of factors (Huh, 1999). For example, the amount of variation is always increased by mutation, but always decreased by genetic drift. Other factors, such as selection, migration, and the mating system, may either increase or reduce variation depending on the particular situation. Combining two or more of these factors can generate virtually any amount or pattern of variation. However, these supposed results of balancing selection may be a vast simplification of the actual forces that determine the amount of genetic variation in any particular situation (Crow and Kimura, 1970).

If the frequency of the detrimental alleles is maintained via a mutation-selection balance, the equilibrium frequency at each locus (p) is given by the following formulae (Crow and Kimura, 1970):

$$p = n/hs \text{ or } p = \sqrt{u/s},$$

where u is the mutation rate, s is the selection coefficient of recessive homozygotes, and h is the dominance of heterozygotes. Mutation rates for maize seed and seedling lethality have been estimated to range from 0.38×10^{-6} to 171.6×10^{-6} (Sprague and Schuller, 1961). Therefore, the mutation rate of their detrimental alleles should be between 10^{-6} and 10^{-5}. As calculated before, the gene frequencies at locus were 0.0007 for the chlorophyll-deficient mutants, a value that lies within the range of our mutation-selection hypothesis. From this we conclude that the detrimental chlorophyll-deficient gene is probably maintained by mutation-selection balance in the Korean maize populations.

Conspicuous differences existed among allele frequencies in populations, especially for Ulsan (12-1S and 12-2W), whose frequency of chlorophyll-deficient mutants was the highest of all the populations, most being monomorphic at this locus. The frequency of the chlorophyll-deficient mutant in both Ulsan populations (cultivated and naturalized) was twice that of the Hamyang population (1-1S).

Jenkins (1924) reported a range of 0.0 to 35.5% for chlorophyll-deficient types in fast selfed-generation progenies derived from 16 open-pollinated varieties. Hayes and Brewbaker (1924) also reported frequencies of chlorophyll-deficient types ranging from 10.0 to 39.4% in six varieties. Maize is primarily a cross-pollinating species, a feature that has contributed to its broad morphological variability and geographical adaptability.

The frequencies of red coleoptiles and epicotyls were higher than those expected from a mutation-selection balance, being polymorphic in 16 of our populations (72.7%) (Table 3). The mean frequency of *rc* in all samples lay outside the range of our mutation-selection hypothesis. Thus, we conclude that this character of coleoptile and epicotyl polymorphism is not maintained by the mutation-selection balance in Korean maize.

The number of first root hairs also varied greatly between populations, from a low of 0.0 for 11-1S to a high of 4.0 for 8-2W. Thus, we consider the morphological character of the number of first root hairs to be a key trait for distinguishing maize populations.

We conclude from this study that naturalized Korean maize populations are closely related to their cultivated populations. The average number of individuals exchanged between populations per generation was low for maize (*Nm* = 0.69). Although drift may lead to genetic differentiation among populations, this present distribution of naturalized maize suggests that the level of gene flow in established populations (based on allozyme analysis and amplified fragment length polymorphism; AFLP) is caused primarily by pollen and seed dispersal (unpublished data). Nevertheless, the locally uneven distribution of three detrimental genes among our populations indicates that selection has acted differently on these three characters.

One of the most striking results from this study was the more significant difference among rather than within populations. Even when the outcrossed and wind-pollinated mating systems of maize are taken into account, the mean identity value of 0.915 among 22 populations is lower than expected for a cogeneric species (Hamrick and Godt, 1989). This low value is especially surprising when one considers the narrow geographic area over which the Korean maize collections were made.

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