Marker-Assisted Breeding in Maize for Cold Regions of Japan

Hiroyuki ENOKI

CONTENTS

次へ進む

農業・食品産業技術総合研究機構 北海道農業研究センター

Marker-Assisted Breeding in Maize for Cold Regions of Japan

Hiroyuki ENOKI

I. INTRODUCTION

Maize (*Zea mays* L.) is a grass and belongs to the large and important family, Grameneae. It is one of the three major cereals together with rice and wheat, and its cultivation area is about 140 million ha in the world. The most prominent differences between maize and other major grasses are photosynthesis and reproductive system, which are C_4 photosynthesis and hermaphrodite. This photosynthesis system contributes to the highest productivity in grasses and the morphological characteristics make maize outbreeding species. Maize is utilized in several ways as a food for humans (including starch, oil, bourbon whiskey and other products), biodegradable plastic and biomass fuel (ethanol). However, maize is primarily used as a feed grain for livestock.

Maize is mainly cultivated as a harvested grain used for livestock feed, and its production has supported the animal industry. Maize is also utilized as whole plant silage for ruminant feed. The whole plant silage of maize surpasses other forage crops in the average yield of dry matter and digestible nutrients per hectare. The silage maize is particularly suitable for cropping in cold regions, because it can be harvested 14 to 21 days before grain maturity. In Japan, almost all maize is cultivated for the whole plant silage. Its cultivation area was 84,400 ha in 2006. About 40 % of the maize cultivation area (35,900 ha) is distributed in Hokkaido.

Hybrid maize has been predominant in the recent maize cultivation. The utilization of hybrids contributes to achieving superior productivity, uniformity and adaptability. SHULL (1908, 1909, 1910) suggested the pure-line (inbred line) method of maize breeding, and described an outline of the breeding methods of hybrid maize. JONES (1918) suggested use of the double cross hybrid, which is a cross between two single crosses. The double cross formula made the hybrid maize practical. According as the improvement of seed productivity of inbred lines, single-cross hybrids arose in early 1960s and have recently become the primary type of maize hybrids (HALLAUER 1990).

The breeding of hybrid maize is based on the heterosis expressed in crosses between inbred lines. The degree of heterosis in crosses is varied depending on the difference in genetic structure between parents, and greater heterosis is obtained when distantly related groups of inbred lines are crossed. The combination of inbred lines or breeding materials which express greater heterosis than others is called a "heterotic pattern", and each group is called a "heterotic group". The establishment of heterotic groups based on the heterotic pattern is indispensable for the hybrid breeding program because the degree of heterosis of inter-group hybrids is generally superior to intra-group hybrids. In the U.S.A. Corn Belt, there are two major dent heterotic groups, the Lancaster Sure Crop (LSC) and Reid Yellow Dent (RYD). The hybrids obtained by crossing the LSC with the RYD inbred lines are highly productive, and almost all U.S.A. Corn Belt hybrids are based on use of the LSC by the RYD heterotic pattern (SMITH 1988). In Europe, a common heterotic pattern is the U.S.A. dent inbred lines crossed with European flint inbred lines.

In the hybrid breeding, information on the genetic diversity and relationship among the breeding materials is needed to choose source materials for new inbred lines, assign inbred lines to heterotic groups, and

choose testers for trials of hybrid combinations. The diversity among and within the groups of breeding materials also determines the future prospects of success in the breeding programs (MELCHINGER 1999). The genetic diversity among the inbred lines has usually been assessed based on morphological data such as the endosperm type, the pedigree record of inbred lines, and the amount of heterosis expressed in the crosses. However, these descriptors present several limitations. The morphological characteristics often do not reliably portray their genetic relationships. The pedigree records of inbred lines are affected by selection and gene drift during inbreeding (MESSMER et al. 1993). Testcross designs with several testers are extremely expensive and time-consuming.

In Japan, the maize breeding program is based on use of the heterotic pattern between the U.S.A. dent and flint inbred lines. The source materials of the flint inbred lines are the Caribbean flint in intermediate and warm regions, and the Northern flint and European flint in the cold regions. The local varieties belonging to the Northern flint were introduced from the North America in the 1870s (INOUE 1984). The Northern flint inbred lines developed from them are superior in low-temperature germination as well as low-temperature growth (MONMA and OKABE 1985). Flowering time of the Northern and European flint inbred lines are generally earlier than the dent inbred lines. The heterotic pattern between the dent and Northern flint or European flint combines the high productivity of the U.S.A. dent and the cold tolerance and earliness of the flint inbred lines into a hybrid. The breeding system has been used for developing several hybrids which are well adapted to the cold regions of Japan (KANEKO et al. 1975; KUSHIBIKI et al. 1979; HASEGAWA 1984; MIURA et al. 1989, 1995; KOINUMA et al. 2004)

The assessment of genetic diversity among the inbred lines bred in Hokkaido compared with representative inbred lines, such as the European flint inbred lines, the Corn Belt dent inbred lines and the Canadian inbred lines, is considered to be useful for the evaluation of the local breeding materials, and the exploitation and introduction of other germplasm. The Northern flint germplasm is one of the origins of the Corn Belt dent (GOODMAN and BROWN 1988) and European flint germplasm (FREI 2000). The assessment of the genetic relationship between the Northern flint and other germplasm will provide useful clues for evaluating the role that the Northern flint has played in the establishment of the heterotic groups. The introduction of new elite germplasm adaptable to cool conditions is necessary to exploit a higher level of heterosis and the progress of the breeding in the cold regions of Japan. However, it is difficult to introduce elite inbred lines from the U.S.A. or Europe, because most of them are proprietary. Therefore, the maize breeders rely on the introduction of two breeding materials, the one is commercial hybrids bred in the U.S.A. and Europe, and the other is elite inbred lines bred in the intermediate and warm regions of Japan. Regarding the use of commercial hybrids, it is difficult to assign the inbred lines developed from the European hybrids to either the dent or flint group (INOUE 1984), because the European hybrids are usually crosses between the dent and European flint inbred lines. Therefore, the inbred lines developed from them are mixtures of the dent and flint germplasm. Since assignments from testcross data are extremely expensive and time-consuming, a more convenient assignment method should be developed to promote use of the inbred lines developed from the European hybrids.

Some of the dent inbred lines bred in the intermediate and warm regions are superior in productivity and lodging tolerant, and are considered to be favorable resources for improving the productivity of early silage maize hybrids. Early flowering is a key component in the yield of maize in the cold regions (FREI 2000). However, early-maturing elite dent inbred lines are scarce, because the dent inbred lines are commonly late-maturing in the cold regions of Japan. Improvement of the flowering time of the inbred lines bred in the intermediate and warm regions is usually performed by crossing them with early inbred

lines. However, these methods are time-consuming as well as laborious.

Plant breeders will need to develop and apply new technologies at a faster pace to improve breeding materials more effectively. DNA marker technology provides scientists with a powerful approach for identifying and mapping quantitative trait loci (QTL) (STUBER et al. 1999). In breeding programs, DNA marker technology is useful for marker-assisted selection (MAS) as a substitute for or to assist in phenotypic selection (COLLARD et al. 2005), the analysis of genetic diversity among the breeding materials (MELCHINGER 1999), and the discrimination of varieties for the protection of breeders' rights (TERZI et al. 2005).

In the hybrid breeding, molecular marker analysis provides information on the genetic diversity among the breeding materials. This information is useful for obtaining a clearer description of existing heterotic groups and identification of new heterotic groups in a systematic manner (MELCHINGER 1999). STUBER and GOODMAN (1983) reported that isozyme variation could provide an accurate estimate of the genetic distance among the inbred lines. Restriction fragment length polymorphism (RFLP) markers have been used to assess the genetic diversity and assign the inbred lines to the groups of the U.S.A. dent germplasm (LEE et al. 1989; MELCHINGER et al. 1990, 1991), European germplasm (MESSMER et al. 1992a, 1992b, BOPPENMAIER et al. 1992), and U.S.A. dent and European germplasm (DUBREUIL et al. 1996). PINTO et al. (2003) have reported that the assignment from the RFLP markers was very similar to those from estimates of the specific combining ability estimated from testcross hybrids.

Regarding the use of marker technology in breeding systems, the cost, labor, and time required for molecular marker analysis are major constraints (MELCHINGER 1999; STUBER et al. 1999; COLLARD et al. 2005). Obviously, the cost of analysis depends mainly on the choice of marker types and the number of markers required for sufficient accuracy. Simple sequence repeats (SSR) analysis presents the potential advantages of reliability, reproducibility, discrimination, standardization and cost-effectiveness over RFLP analysis (SMITH et al. 1997). SSR loci provide a high level of polymorphism in maize (SENIOR and HEUN 1993). SENIOR et al. (1998) have reported that SSR analysis using high quality agarose gels can conveniently assess the genetic diversity of maize inbred lines. REIF et al. (2003) have also reported that SSR markers are a valuable complementation to field trials for identifying heterotic groups.

BARBOSA-NETO et al. (1997) have pointed out that marker loci should be chosen uniformly over the entire genome in the genetic diversity studies to avoid biases due to sampling, and the precision of genetic similarity (GS) estimates increased as the number of marker loci increased. However, it is preferable to determine the minimum number of markers required for the assignment with sufficient accuracy in breeding programs. SMITH et al. (2000) reported that, when the genetic distance were estimated from selected loci showing the allele frequency of 0.4 or greater in Stiff Stalk Synthetic (BSSS) and non-BSSS group, the estimates were significantly correlated with F_1 grain yield and the degree of heterosis for grain yield. The number of SSR loci required for the assignment will be reduced with sufficient accuracy by selection of the loci considering differences in the allele frequency. So far, no assignment from selected loci with differences in allele frequency between maize heterotic groups has been reported.

However, MAS is a potential tool for improving the flowering time of the late maturity dent inbred lines. The Northern flint populations, one of the heterotic groups in the cold regions of Japan, are potential donors of earliness. In Europe, the introduction of the Northern flint populations has played a key role in the adaptation of maize (REBOURG et al. 2003; SOENGAS et al. 2003). If QTLs for the early flowering of the Northern flint can be identified, MAS using markers linked to the QTLs will accelerate the transfer of

this trait to maize germplasm bred in the intermediate and warm regions in the breeding programs. It will be an efficient method to introduce the earliness of the Northern flint germplasm to the late maturing dent inbred lines across the heterotic pattern. DNA markers have been efficiently used to detect and characterize QTLs for flowering time and/or plant stature in the dent inbred lines (BEAVIS et al. 1994; BERKE and ROCHEFORD 1995; VELDBOOM and LEE 1996; AUSTIN and LEE 1996), the dent and European flint inbred lines (REBAI et al. 1997), and tropical inbred lines (RIBAUT et al. 1996). In the Northern flint, near-isogenic lines (NILs) created by crossing the dent inbred lines with Gaspe type lines (a Northern flint open-pollinated population) were used to identify and characterize the QTLs related to the early flowering (KOESTER et al. 1993; VLADUTU et al. 1999). However, there have been no reports on QTL analysis of the early flowering of elite inbred lines of the Northern flint.

Moreover, the elucidation of the mechanism of the early flowering of the Northern flint will help us to understand the maize adaptation to the cold regions and will help improve the flowering time of the dent inbred lines bred in the intermediate and warm regions by MAS. Candidate gene approach has been applied in plant genetics for the characterization and cloning of QTLs (PFLIEGER et al. 2001). In maize, polymorphisms in specific markers for *vp1* and *kn1* are associated with anther culture response in Chi-square tests (WASSOM et al. 2000), and an 18-bp indel in the *ccoaomt2* first exon was associated with cell wall digestibility variation (GUILLET-CLAUDE et al. 2005). In terms of flowering time, polymorphisms in the potential candidate gene *d8* are associated with differences in the flowering time in association tests (THORNSBERRY et al. 2001).

The present study was carried out to develop marker-assisted breeding to overcome above mentioned limitations and accelerate the breeding of excellent maize varieties adapted to the cold regions of Japan. The objectives of the three studies are to 1) assess the genetic diversity among the inbred lines adapted to the cold regions of Japan from SSR analysis of the 60 loci distributed uniformly throughout maize genome, 2) establish an assignment method of the inbred lines developed from the European hybrids from the mean GS estimates derived from a smaller number of SSR loci, which were chosen based on the differences in the mean allele frequency between the dent and flint groups, and 3) identify QTLs for the early flowering of an elite Northern flint inbred line toward establishment of MAS for the early flowering.

次へ進む　目次に戻る

II. SSR Analysis of Genetic Diversity among Maize Inbred Lines Adapted to Cold Regions of Japan

1. INTRODUCTION

Information on the genetic diversity and relationship among the breeding materials is indispensable for the development of new maize inbred lines, the assignment of maize inbred lines to the heterotic groups, and the choice of testers for trials of hybrid combinations in the maize breeding programs. In addition, a comparison of the genetic diversity among representative inbred lines of the U.S.A. [LSC and Iowa Stiff Stalk Synthetic (BSSS; an improved synthetic population representative RYD)], European (European flint), Canadian and local inbred lines are useful for the evaluation of the local breeding materials, and the exploitation and introduction of other germplasm. The utilization of molecular markers that directly evaluate the genetic differences between the inbred lines has been attempted to assess the genetic diversity among the maize inbred lines (MELCHINGER 1999). SENIOR and HEUN (1993) have reported that SSR loci provide a high level of polymorphism in maize. SSR analysis presents the potential advantages of reliability, reproducibility, discrimination, standardization and cost-effectiveness over RFLP analysis (SMITH et al. 1997).

The objectives of the present study were to (1) evaluate the discrimination ability of the SSR analysis of the 60 loci distributed uniformly throughout the maize genome, (2) assess the genetic diversity among the inbred lines adapted to the cold regions of Japan and representative inbred lines, and (3) assign the inbred lines developed from the hybrids to the heterotic groups.

次へ進む　前に戻る　目次に戻る

II. SSR Analysis of Genetic Diversity among Maize Inbred Lines Adapted to Cold Regions of Japan

2. MATERIALS AND METHODS

Plant material

Fifty one maize inbred lines were chosen to represent maize diversity among the breeding materials adapted to the cold regions of Japan, and 14 maize inbred lines introduced from the U.S.A., Canada and Europe were used for comparison (Table 1). Twenty of the 65 inbred lines were developed from the flint germplasm and 26 from the dent germplasm. Additionally 19 inbred lines which were developed from the European hybrids by crossing the early maturing dent with European flint were subsequently designated as "miscellaneous". Among all pairs of the inbred lines, those without common parentage were designated as "unrelated". Genomic DNA was isolated from a bulk of five seedlings from each inbred line using a modified CTAB procedure (SAGHAI-MAROOF et al. 1984).

SSR marker selection

Based on their chromosome loci, we chose 100 SSR primers from Maize Genetics and Genomics Database (MaizeGDB: http://www.maizegdb.org/) and assayed their preliminary discriminatory power using a sample of 16 inbred lines. Primers were excluded from the present study if they did not show different band sizes or consistently failed to amplify in the 16 inbred lines. A final set of 60 SSR primers was selected for further analysis (Table 2). Among this set of SSR loci, 31 (51%) were di-repeats, 7 (12%) tri-repeats, 8 (13%) tetra-repeats, 3 (5%) penta-repeats, 1 (2%) a hexa-repeat and 10 (17%) were unknown. Each chromosome had at least five SSR loci, and the mean map distance between neighboring SSR loci was approximately 21.1 cM.

Amplification and detection conditions

The reactions were carried out in a DNA Thermal Cycler (PERKIN-ELMER, NORWALK, GA., USA). The reaction consisted of a denaturation step of 1 min at 96 ℃, followed by a touchdown procedure as described by MELLERSCH and SAMPSON (1993). This procedure began with 1 min at 96 ℃, 1 min at 65 ℃, and 2 min at 72 ℃. The annealing temperature was then reduced at each cycle by 0.5℃ until a final annealing temperature of 55 ℃ was reached. The last cycle was repeated 20 times and was terminated at 72 ℃ for 2 min. Then, the reaction was finished with a continuous cycle at 4 ℃. The 10-µl reaction mix consisted of 20 nM of each primer, 1 unit of Taq DNA polymerase (PROMEGA, CO., INC.), 200 µM each dNTP, 1× reaction buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 100 µg mL^{-1} gelatin: pH 8.3), and 30 ng of template DNA. Reactions were stopped with 10 µl of loading-dye (50% deionized formamide, 40 % glycerol, 20 mM EDTA, 0.6 mg mL^{-1} bromphenol blue). After the reaction, 20 µl of the reaction mixture was heated at 96 ℃ for 2 min, placed on ice, then loaded onto a 10 % polyacrylamide denaturing gel (16 cm × 16 cm) containing 8 M urea. After the run, gels were soaked for 15 min in

distilled water to reduce the concentration of urea in the gel so that denatured DNA would anneal and thus be able to be stained with ethidium bromide (EtBr).

Statistical analysis

Estimates of genetic similarity (GS) were calculated for all possible pairs of the inbred lines according to the following equation (DICE 1945; NEI and LI 1979):

$$GS(i,j)=2N(i,j)/[N(i)+N(j)],$$

where GS(i,j) is the GS estimate between inbred lines i and j, N(i,j) is the total number of bands common to i and j, and N(i) and N(j) is the number of bands for the inbred lines i and j, respectively. The standard error [SE(i,j)] of GS(i,j) was estimated by

$$SE(i,j) = \{GS(i,j)[1-GS(i,j)]/[N(i)+N(j)]^{0.5}\}$$

(DUBREUIL et al. 1996). This estimator is strictly equivalent to the jackknife estimator used in published studies (e.g. MELCHINGER et al. 1991; MESSMER et al. 1992b) and overestimates the actual variances when loci have been chosen to optimize the genome coverage (DUBREUIL et al. 1996). Thus, in the present study, estimated SE values had to be considered as an upper limit for the actual values. Average linkage (UPGMA) cluster analysis and principal coordinate analysis (PCOA) were performed with the matrix of GS estimates using appropriate procedures of the program NTSYS-pc (ROHLF 1989). The mean GS estimates of the inter- and intra-groups were calculated among all possible pairs of the inbred lines belonging to each group. In order to estimate the mean genetic similarity of unrelated pairs of the inbred lines (GS_{MUR}) with the highest precision, GS_{MUR} was calculated from all GS estimates of 520 unrelated pairs among 26 dent inbred lines and 20 flint inbred lines. The miscellaneous inbred lines were assigned to the groups from the difference between the differences between the values of GS_{MUR} and GS for unrelated pairs between the miscellaneous inbred lines, the significance of the differences between the values of GS_{MUR} and GS for unrelated pairs between the miscellaneous inbred lines and dent or flint inbred lines being determined by t-tests at 0.05 level. The MALECOT coancestry coefficient (*f*) (MALECOT 1948) was calculated for all pairs of the inbred lines with known pedigrees. The *f* value for the unrelated pairs of the inbred lines was set to zero. For a given *f* value of two lines, the expected genetic similarity (GS_{EXP}) was calculated by

$GS_{EXP} = f + (1 - f)\, GS_{MUR}$, (MESSMER et al. 1993). The polymorphic-index content (PIC) for each SSR locus was determined as described by SMITH et al. (1997). PIC is a measure of the allele diversity at a locus and is equal to

$PIC = 1 - \sum h_k^2$, where h_k is the frequency of the kth allele. When calculated in this manner, PIC is synonymous with the term "gene diversity" as described by WEIR (1996).

次へ進む 前に戻る 目次に戻る

II. SSR Analysis of Genetic Diversity among Maize Inbred Lines Adapted to Cold Regions of Japan

3. RESULTS AND DISCUSSION

Allele diversity at the 60 SSR loci

The sixty SSR primers produced 433 alleles among the 65 maize inbred lines, and the number of alleles at the SSR loci ranged from 2 to 17, with the mean allele being 7.3, and the PIC values for the SSR loci ranged from 0.41 to 0.91, with the mean PIC being 0.69 (Table 2). The mean PIC value in the present study was higher than 0.62 determined by SMITH et al. (1997) and 0.59 determined by SENIOR et al. (1998). The higher PIC value probably resulted from excluding the SSR primers with low discriminatory power by the preliminary test. Di-repeat SSR loci gave a higher mean allele (9.3) and mean PIC (0.74), and tri-, tetra- and penta-repeat SSR loci gave lower mean alleles (3.4-5.0) and mean PIC (0.56-0.59) (Table 3). The highest mean PIC value of the di-repeat SSR loci is consistent with the results of SMITH et al. (1997) and SENIOR et al. (1998). SMITH et al. (1997) have reported that di-repeat SSR loci abound with alleles, but some di-repeat SSR loci tend to produce additional stutter bands. In the present study, because of exclusion by the preliminary test for the SSR primers, stutter bands did not appear.

Accuracy of the genetic similarity calculated from 60 SSR data

The f and GS estimates for 56 pairs among the inbred lines with a pedigree record ranged from 0.063 to 0.750 and from 0.224 to 0.826, when the unrelated pairs of the inbred lines were excluded. The correlation between the GS estimates and *f*, and the GS_{EXP} for them was significant ($r = 0.70$ and $r = 0.70$, $P < 0.01$ respectively; Fig. 1). This significant correlation agrees with the results of MESSMER et al. (1993), DUBREUIL et al. (1996) and SMITH et al. (1997). However, deviations between the GS estimates and GS_{EXP} averaged 0.10. The deviations increased especially with increasing *f*, and the mean GS estimate for $f = 0.5$ pairs (0.548) was lower than the GS_{EXP} (0.637) and ranged widely from 0.311 to 0.826. The differences between the GS estimates and coancestry coefficient are considered to result from the effects of selection and gene drift.

The SE of individual GS estimates had a mean of 0.06 and ranged from 0.04 to 0.06. BARBOSA-NETO et al. (1997) have reported that marker loci should be chosen uniformly over an entire genome in genetic diversity studies, avoiding biases due to sampling, and the precision of GS estimates increases as the number of marker loci increases. However, given the high cost and intensive labor of DNA-marker assays, it is necessary to choose the minimum number of markers required for a given level of precision in GS estimates. We chose the 60 SSR primers uniformly over the maize genome from the MaizeGDB, with the mean SE of the GS estimates in the present study being almost equal to the mean SE (0.06) in DUBREUIL et al. (1996). When the genome was uniformly covered with marker loci, an estimate of SE overestimates the actual variance of the GS estimates (DUBREUIL et al. 1996), making the precision of the present study sufficient for estimating GS among maize inbred lines. Therefore, the SSR analysis of the 60 loci provided

sufficient accuracy for the GS estimates, and this analysis system appears to be effective for the assessment of the genetic diversity among the maize inbred lines.

Analysis of the mean genetic similarity

The GS_{MUR} estimate of 520 unrelated pairs among the 26 dent inbred lines and 20 flint inbred lines was 0.271. The GS_{MUR} was used as criterion for the genetic diversity among and between each group. Both the mean GS estimate (0.405) for unrelated pairs among the dent inbred lines bred in Hokkaido and the mean GS estimate (0.424) for unrelated pairs among the Northern flint inbred lines bred in Hokkaido were higher than the mean GS (0.289) estimates for unrelated pairs among the representative dent inbred lines (Table 4). Each value was also greater than GS_{MUR}. Therefore, the dent and Northern flint inbred lines bred in Hokkaido were considered to have a narrower genetic diversity than the representative dent inbred lines, respectively. However, the mean GS estimate between the dent and the Northern flint inbred lines bred in Hokkaido (0.273) is almost equal to GS_{MUR}, and the dent inbred lines bred in Hokkaido were distantly related to the Northern flint inbred lines bred in Hokkaido. This result supports the observation of heterosis in crosses of the dent inbred lines bred in Hokkaido with the Northern flint inbred lines bred in Hokkaido.

The Canadian flint inbred line CO12 had the greatest mean GS estimate (0.452), and the European flint inbred line F283 had the second greatest (0.381), with the Northern flint inbred lines bred in Hokkaido (Table 5). This result agrees with the origin of the local varieties in the cold regions of Japan, which were introduced from the North America. However, another European flint inbred line F2 had a smaller mean GS estimate (0.298) with the Northern flint inbred lines bred in Hokkaido. European flint inbred lines were selected from the European open-pollinated populations, which presumably traces back to tropical flints from the West Indies and Caribbean islands (WALLACE and BROWN 1956). Therefore, their progenitors seem to be different from the Northern flint. However, the result suggests that the Northern flint was one of their progenitors. Two LSC inbred lines, Mo17 and CM37, had the greatest mean GS estimate (0.341 and 0.323, respectively) with the Northern flint inbred lines bred in Hokkaido among the representative dent inbred lines, and the mean GS estimates were higher than GS_{MUR}. The Corn Belt dents were developed from mixing the Southern dents with the Northern flints (GOODMAN and BROWN 1988). These inbred lines are considered to have received more genes from the Northern flint than other dent inbred lines.

Two BSSS inbred lines, B73 and A679, had the greatest mean GS estimate (0.348 and 0.342, respectively) with the dent inbred lines bred in Hokkaido among the representative dent inbred lines. C103-related inbred lines (C103 and Mo17) had a comparatively greater mean GS estimate (0.314) with the dent inbred lines bred in Hokkaido than GS_{MUR}. However, Oh43-related inbred lines (Oh43 and A619) and the Canadian dent inbred lines (CM37 and CMV3) had low mean GS estimates (0.256 and 0.253, respectively) with the dent inbred lines bred in Hokkaido. The dent inbred lines bred in Hokkaido were developed from the U.S.A. and European hybrids. From isozyme and chromatographic data, the U.S.A. varieties appear to be heavily dependent on the usage of B73, A632, Oh43 and Mo17, or their close derivatives (SMITH 1988). And, in the LSC inbred lines, C103-related inbred lines are not comparatively similar to Oh43-related inbred lines in RFLP and SSR analysis (MUMM and DUDLEY 1994; SENIOR et al. 1998). The GS estimates among the dent inbred lines bred in Hokkaido and the representative dent inbred lines indicate that the dent inbred lines bred in Hokkaido are comparatively similar to the BSSS and C103-

related inbred lines, and are not similar to the Oh43-related and Canadian dent inbred lines. The U.S.A. hybrids used as source of the dent inbred lines bred in Hokkaido are considered to have originated from the BSSS inbred lines crossed with the C103-related inbred lines.

Nineteen miscellaneous inbred lines were grouped from the relative magnitude of the mean GS estimates with the dent and flint inbred lines (Table 6). Six miscellaneous inbred lines (Ho34, Ho36, Ho65, Ho66, Ho73 and To113) had significantly greater mean GS estimates with the flint inbred lines than GS_{MUR}, and are considered to be similar to the flint inbred lines. Three miscellaneous inbred lines (Ho59, Ho63 and To132) had significantly greater mean GS estimates with the dent inbred lines than GS_{MUR}, and are considered to be similar to the dent inbred lines. Four miscellaneous inbred lines (Ho64, Ho67, Ho81 and To133) had significantly greater mean GS estimates with the dent and flint inbred lines than GS_{MUR}, and are considered to be intermediate with the dent and flint inbred lines. However, six miscellaneous inbred lines (Ho3, Ho4, Ho37, Ho42, Ho49 and Ho50) did not have significantly greater GS estimates with the dent and flint inbred lines than GS_{MUR}, and are considered not to be similar to the dent and flint inbred lines.

Cluster analysis

Cluster analysis classified the 65 inbred lines into four main clusters (Fig. 2). The first main cluster consisted of the entire flint inbred lines and four miscellaneous inbred lines. This cluster was subdivided into two subclusters. All Northern flint inbred lines bred in Hokkaido were classified into the subcluster with CO12 and F283. Two miscellaneous inbred lines (Ho34 and Ho81) were included in this subcluster. The F2 were classified into the other subcluster with two miscellaneous inbred lines (Ho65 and To113). The second main cluster consisted of B73, A679, the entire dent inbred lines bred in Hokkaido and eight miscellaneous inbred lines. Therefore, the dent inbred lines bred in Hokkaido appear to be more similar to the BSSS inbred lines than the C103-related inbred lines. In the cold regions of Japan, some cool-weather damages occur (MONMA and OKABE 1985), and the specific genotype suitable for the cold regions was prior-selected; as a result the genetic diversity of these dent inbred lines is considered to closely incline toward one side. The cluster was subdivided into three subclusters with one subcluster consisting of the dent inbred lines bred in Hokkaido and three miscellaneous inbred lines (Ho59, Ho63 and Ho64), and the other consisting of five miscellaneous inbred lines (Ho66, Ho67, Ho73, To132 and To133). B73 and A679 were on the edge of this main cluster. The third main cluster consisted of representative LSC inbred lines (Oh43, A619, Mo17 and C103) and INRA258-related miscellaneous inbred lines (Ho3, Ho4, Ho36, Ho37, Ho42, Ho49 and Ho50). The fourth main cluster consisted of five representative dent inbred lines (CM37, CMV3, CO158, H99 and W79A).

Principal coordinate analysis

PCOA revealed similar groupings of the inbred lines (Fig. 3). The first and second principal coordinates, termed PC1 and PC2, explain 9.0% and 6.2% of the total variation in the SSR data. The representative dent inbred lines were clearly separated from the representative flint inbred lines with respect to PC1, but were widely spread with respect to PC2. The dent inbred lines were loosely divided into two groups, with the one consisting of B73, A679 and the dent inbred lines bred in Hokkaido, and the other consisting of LSC inbred lines, W79A and CO158. Among the flint inbred lines, CO12 and the Northern flint inbred lines bred in Hokkaido were firmly grouped. F2 and F283 were slightly separated from this group.

Among the 19 miscellaneous inbred lines, INRA 258-related miscellaneous inbred lines were grouped and were independent of the dent and flint inbred lines, except for Oh43 and A619. The genotype of INRA 258 consisted of Minnesota 13 (M13) and European flint, and Oh43 is partially related to M13 (GERDES and TRACY 1993). Thus, the INRA 258-related miscellaneous inbred lines were more similar to the Oh43-related inbred lines than the European flint inbred lines. In addition, the INRA 258-related miscellaneous inbred lines had a lower mean GS estimate with the dent inbred lines bred in Hokkaido and Northern flint inbred lines bred in Hokkaido, and they are considered to be valuable for breeding materials in the cold regions of Japan.

The miscellaneous inbred lines, except for the INRA258-related miscellaneous inbred lines, were spread between the dent and flint inbred lines with respect to PC1. Among them, Ho34, Ho59, Ho63 and To132 were clearly assigned to the dent or flint groups, and this result agrees with the relative magnitude of mean GS estimates and the results of the cluster analysis. The empirical knowledge of heterosis supports this result. On the other hand, the assignment of other miscellaneous inbred lines was different according to the mode of analysis. From the PCOA and relative magnitude of mean GS estimates, they were classified into the intermediate or flint group. However, from the cluster analysis, Ho66, Ho67 and Ho73 were classified into the dent group. The empirical knowledge of heterosis agrees with the results of the PCOA and relative magnitude of mean GS estimates. PCOA is suitable for faithful portrayals of the relationships between larger groups of inbred lines, and cluster analysis is reliable for depicting close relationships between the inbred lines (MELCHINGER 1999). Though the assignment of the miscellaneous inbred lines is important in the breeding programs, the results may be changed according to the analysis, so we should assign them with these results and the empirical knowledge of heterosis. The clear assignment of the inbred lines developed from the hybrids is difficult based on their pedigree record. However, by comparison with the representative inbred lines, the genetic diversity and relationships among the inbred lines were evaluated. These results provide a useful criterion for the exploitation and introduction of the breeding materials for hybrids which adapt to the cold regions of Japan.

次へ進む　前に戻る　目次に戻る

III. Selection of SSR Sets in Assignment to Dent and Flint Groups of Maize Inbred Lines Derived from European Hybrids

1. INTRODUCTION

The European hybrids are usually obtained by crossing the early-maturing dent and European flint lines. Therefore, since the inbred lines developed from the European hybrids are mixtures of the dent and flint germplasm, it is difficult to assign them to either dent or flint groups from the morphological data such as the endosperm types and/or pedigree records (INOUE et al. 1984). In the previous chapter, SSR analysis of the 60 loci distributed uniformly throughout the maize genome was effective for the assessment of the genetic diversity among the inbred lines, and was sufficient to assign them to the flint or dent groups. However, it is preferable to determine the minimum number of markers required for the assignment with sufficient accuracy in the breeding programs. SMITH et al. (2000) reported that, when the genetic distance was estimated from selected loci showing the allele frequency of 0.4 or greater in BSSS and non-BSSS inbred lines, the estimates were significantly correlated with F_1 grain yield and the degree of heterosis for grain yield. Taking differences in allele frequency for selection of SSR loci into consideration, the number of SSR loci for the assignment will be reduced with sufficient accuracy.

The objective of the present study was to assign the inbred lines developed from the European hybrids, whose genetic backgrounds were unknown, to the dent or flint groups from the mean GS estimated from a smaller number of SSR loci compared with the original 60 SSR loci. The loci were chosen from the differences in mean allele frequency in dent and flint groups. Moreover, to evaluate the accuracy of the assignment method from each subset of SSR loci, we investigated the testcross performance of several inbred lines developed from the European hybrids that were not consistently assigned from the subsets of SSR loci.

次へ進む 前に戻る 目次に戻る

III. Selection of SSR Sets in Assignment to Dent and Flint Groups of Maize Inbred Lines Derived from European Hybrids

2. MATERIALS AND METHODS

Plant materials

Seventy-seven maize inbred lines were chosen to represent maize inbred lines adapted to the cold regions of Japan, and 11 maize inbred lines (A509, A619, A679, B73, C103, CO12, CO158, F2, F283, Oh43 and W79A) introduced from the U. S. A., Canada and Europe were used for comparison. Thirty-five of the above 88 inbred lines were developed from the dent germplasm and 21 from the flint germplasm including the Northern flint and European flint. The remaining thirty-two inbred lines were developed from the European hybrids whose pedigree records were unknown and were designated as "miscellaneous". Total DNA was isolated from a bulk of five seedlings from each inbred line using the modified CTAB procedure (SAGHAI-MAROOF et al. 1984).

Selection of SSR loci

The sixty SSR primer pairs, which were shown in chapter II, were used. Amplification and detection methods are described also in chapter II. At least five SSR loci were located on each chromosome, and the mean map distance between adjacent SSR loci was approximately 21.1 cM. Details of the SSR loci and their map locations were given in chapter II and in the MaizeGDB.

Subsets of the 60 SSR loci were chosen from differences in allele frequency above 0.4 (Set 1) and 0.5 (Set 2) in 35 dent and 21 flint inbred lines, respectively (See Table 7). SMITH et al. (2000) showed that the genetic distance estimates between the BSSS and non-BSSS inbred lines, from selected loci with differences in allele frequency of 0.4 or greater, were significantly correlated with F_1 grain yield and the degree of heterosis for grain yield. SENIOR et al. (1998) showed that many loci with differences in allele frequency of 0.5 or greater in the BSSS and LSC groups were located in regions where QTLs for yield heterosis in a B73 × Mo17 cross were detected. B73 and Mo17 are representative inbred lines of the BSSS and LSC, respectively.

Statistical analysis

Estimates of GS for all possible pairs of the inbred lines were calculated from the polymorphism data of each subset of SSR loci according to the following equation (DICE 1945, NEI and LI 1979) using the NYSYS-pc program (ROHLF 1989):

$$GS(i,j)=2N(i,j)/[N(i)+N(j)],$$

where GS(i,j) is the GS estimate between inbred lines i and j; N(i,j) is the total number of bands common to both lines; and N(i) and N(j) are the numbers of bands specific to line i and line j, respectively. The mean GS estimates of the 32 miscellaneous inbred lines with dent group (GS-D) and flint group (GS-F) were calculated from the polymorphism data of each subset of SSR loci. Then, these miscellaneous inbred

lines were assigned to the groups from the difference between the values of GS-D and GS-F, the significance of the differences between the values of GS-D and GS-F being determined by t-tests. If the GS-D value was significantly higher than the GS-F value, the miscellaneous inbred line was assigned to the flint group, and vice versa. If there was no significant difference between the GS-D and GS-F values, it was assigned to the intermediate type. The accuracy of the assignment from each subset of SSR loci was evaluated by comparison with the results of the assignment from the original full set of SSR loci.

Testcross designs

Four miscellaneous inbred lines (Ho83, Ho86, To119 and To132), which were differently assigned from the two subsets of SSR loci used, were crossed with 3 dent and 3 flint inbred lines, which were representative testers in the cold regions of Japan, respectively. Twenty-four F_1 crosses were planted at the National Agricultural Research Center for Hokkaido Region (NARCH), Sapporo, Hokkaido, Japan on May 16 in 2003. The experimental design was from randomized blocks with two replications. Plant density was 6.84 plants / m^2, 34 plants were 0.195m apart in 3.5m rows spaced at 0.75m. Ten plants per plot were harvested by cutting at 5cm height from the ground level on September 26, when most of the F_1 crosses had reached the mid-dent stage. The means for the dry matter yield and days to silking of the crosses between each miscellaneous inbred line and testers were calculated and were used for the evaluation of heterosis. If the means for the dry matter yield of the crosses with the dent testers were significantly higher than those with the flint testers (p=0.05), the miscellaneous inbred line was assigned to the dent group, and vice versa. If there was no significant difference in the mean values for the dry matter yield between the crosses with the dent and flint testers (p=0.05), the mean values for the days to silking were used for preliminary assignment.

次へ進む 前に戻る 目次に戻る

III. Selection of SSR Sets in Assignment to Dent and Flint Groups of Maize Inbred Lines Derived from European Hybrids

3. RESULTS

Selection of SSR loci

The full set of 60 SSR loci carried 463 alleles among the 88 maize inbred lines and the numbers of alleles at each of the SSR loci ranged from 2 to 17, with a mean value of 7.7. The mean GS estimate among the 35 dent inbred lines was 0.36, with a variance of 0.011. The mean GS estimate among the 21 flint inbred lines was 0.43, with a variance of 0.009. The mean GS estimate between the 35 dent inbred lines and the 21 flint inbred lines was 0.25, with a variance of 0.002.

Two subsets of SSR loci, Set 1 and Set 2, were chosen from the differences in allele frequency in the dent and flint groups, respectively (Table 7). The numbers of SSR loci in Set 1 and Set 2 were 25 and 14, respectively. The SSR loci of Set 1 and Set 2 carried a total number of 176 and 99 alleles among the 88 inbred lines, respectively. These values accounted for 38% and 21% of the allele number of the full set of SSR loci, respectively. For the 1st, 6th and 9th chromosomes, the percentages of the SSR loci belonging to Set 1 and Set 2 ranged from 67% to 86% and from 60% to 67%, respectively. In contrast, there was no SSR locus belonging to Set 1 for the 3rd and 4th chromosomes.

Assignments from each set of SSR loci

The thirty-two miscellaneous inbred lines were subjected to the assignment to the dent or flint groups from the mean GS estimates with each group using the two subsets and the full set of SSR loci (Table 8). From the original full set of SSR loci, 4 miscellaneous inbred lines were assigned to the dent group, 12 to the flint group, and the other 16 to the intermediate type. In the assignment from Set 1 SSR loci, 27 of them were assigned consistently from the results from the full set of SSR loci. The assignment of the residual 5 inbred lines (Ho75, Ho85, To97, To112 and To119) depended on the SSR loci used. In the analysis of the full set of SSR loci for these 5 inbred lines, the values of GS-D were not significantly different from the respective GS-F, and the inbred lines were assigned to the intermediate type. In the analysis of Set 1 SSR loci, the GS-F values of 4 of these 5 inbred lines (Ho75, Ho85, To97 and To119) were significantly higher than the respective GS-D, while the GS-D value of To112 was significantly higher than the GS-F. Therefore, Ho75, Ho85, To97 and To119 were assigned to the flint group, and To112 was assigned to the dent group.

The assignment of 25 inbred lines from Set 2 SSR loci gave the same results as those from the full set of SSR loci, whereas the assignment of the residual 7 inbred lines (Ho62, Ho75, Ho85, To97, To112, To131 and To132) from Set 2 SSR loci was different. In the analysis from the full set of SSR loci, the GS-D values of 6 inbred lines (Ho62, Ho75, Ho85, To97, To112 and To131) were not significantly different from the respective GS-F, and the inbred lines were assigned to the intermediate type. In the analysis of Set 2 SSR loci, the GS-F values of these inbred lines were significantly different from the respective GS-D. The GS-F values of 4 of these 6 inbred lines (Ho75, Ho85, To97 and To131) were significantly higher

than the respective GS-D, while the GS-D values of Ho62 and To112 were significantly higher than the respective GS-F. Therefore, Ho75, Ho85, To97 and To131 were assigned to the flint group, and Ho62 and To112 were assigned to the dent group. However, the assignment of To132 from Set 2 SSR loci gave opposite results to those from the full set of SSR loci; To132, which was assigned to the flint group from Set 2 SSR loci, was assigned to the dent group from the full set of SSR loci.

Testcross designs

The means for the dry matter yield and days to silking of the crosses of the 4 miscellaneous inbred lines (Ho83, Ho86, To119 and To132) with the dent and flint testers are shown in Table 9. Assignments for these inbred lines from the two subsets of SSR loci were inconsistent. In Ho83 and Ho86, there was no significant difference between the GS-D and GS-F values from either set of SSR loci. In To119 and To132, the assignments differed depending on the set of SSR loci used.

The means for the dry matter yield and days to silking ranged from 157 to 177 kg/a, and from 78.7 to 82.3 days, respectively. There were no significant differences between the means of the crosses with the dent and flint testers, either for the dry matter yield or days to silking. The means for the dry matter yield of the crosses of To119 with the dent testers were significantly higher those that with the flint testers (p=0.05). The means for the dry matter yield of the crosses of the other inbred lines with the dent testers were not significantly different from those with the flint testers. The means for the days to silking of the cross of Ho86 with the dent testers were not significantly different from those with the flint testers. However, the means for the days to silking of the crosses of Ho83 and To132 with the flint testers were significantly earlier than those with the dent testers (p=0.05).

次へ進む　前に戻る　目次に戻る

III. Selection of SSR Sets in Assignment to Dent and Flint Groups of Maize Inbred Lines Derived from European Hybrids

4. DISCUSSION

Accuracy of the assignments from each set of SSR loci

BARBOSA-NETO et al. (1997) have reported that the precision of GS estimates from molecular markers decreased as the number of marker loci decreased. However, in the present study, the results of the assignment of the 32 miscellaneous inbred lines using reduced numbers of SSR loci mostly agreed with the results from the original full set of SSR loci.

The use of testcross data is effective for estimating the relative combining abilities of inbred lines (HALLAUER et al. 1988). Comparison of the assignments from the mean GS estimates and the results from the testcrosses showed that the assignments of Ho83 and Ho86 from the SSR loci corresponded to the testcross data. The inbred lines were assigned to the intermediate type by SSR analysis, and the means for dry matter yield were not significantly different between those of their crosses with the dent and flint testers. The assignments of To119 and To132 differed depending on a set of SSR loci used. To119 was assigned to the intermediate type from the full set and Set 2 SSR loci, while to the flint group from Set 1 SSR loci. The crosses of To119 with the dent testers showed a higher dry matter yield than those with the flint testers, indicating that the assignment from Set 1 SSR loci corresponded to the testcross data. To132 was assigned to the dent group from the full set and Set 1 SSR loci, and to the flint group from Set 2 SSR loci. For To132, the earlier silking date of the crosses with the flint testers indicated that To132 was more likely to belong to the dent group, although the dry matter yield was not significantly different between the crosses of To132 with the dent and flint testers. Therefore, the results of the assignments from the full set and Set 1 SSR loci were more similar to the testcross data than Set 2 SSR loci. The results of the testcrosses indicated that the assignment from Set 1 SSR loci was considered to be more accurate than Set 2 SSR loci. The results also showed that the assignment from Set 1 SSR loci was as accurate as the full set of SSR loci. The results of the assignments from Set 2 SSR loci were more different from the full set of SSR loci than Set 1 SSR loci. The lower accuracy of the assignment from Set 2 SSR loci may be attributed to the omission of several genome regions for the assignment of the dent and flint groups.

The chromosome position of selected SSR loci

The SSR loci belonging to Set 1 and Set 2 were mostly located on the 1st, 6th and 9th chromosomes and not on the 3rd or 4th chromosomes. SENIOR et al. (1998) reported that SSR loci with significant differences in allele frequency in the BSSS and LSC groups were located on the 3rd, 5th, 6th, 8th, 9th and 10th chromosomes. In the present study, many SSR loci on the 6th and 9th chromosomes displayed differences in allele frequency above 0.4 in dent and flint groups adapted to the cold regions of Japan. This indicates that the SSR loci on the 6th and 9th chromosomes may show a large difference in allele frequency when other maize groups were tested. On the other hand, in many SSR loci on the 1st chromosome, the difference in allele frequency in the dent and flint groups was above 0.4 in the present

study, while SENIOR et al. (1998) reported the absence of SSR locus with a difference in allele frequency in the BSSS and LSC groups on the 1st chromosome. Conversely, the 3rd chromosome carried many SSR loci with a difference in allele frequency in the BSSS and LSC groups (SENIOR et al. 1998), while there was no SSR locus with a difference in allele frequency in the dent and flint groups on the same chromosome in the present study. These results indicate that the 1st chromosome may be a characteristic region for the assignment of the inbred lines adapted to the cold regions of Japan, while the 3rd chromosome is the characteristic region for the assignment of the BSSS and LSC groups. Further investigations for other maize groups may provide useful information on the characteristic regions for the assignment in maize.

We evaluated the utility of smaller sets of SSR loci chosen from the 60 loci in assigning the miscellaneous inbred lines to the dent or flint groups. Importantly, the assignment from the 25 key SSR loci (Set 1) was as accurate as the full set SSR loci distributed uniformly throughout the maize genome. This means that only 41.7% of the full set SSR loci need to be examined. It is expected that we can perform more efficient testcross designs in the breeding programs by selecting the crosses from Set 1 SSR loci in advance. Moreover, information about mean GS estimates by Set 1 SSR loci will be a useful index for selecting parent of materials for new dent or flint inbred lines. SENIOR et al. (1998) reported that many of the loci with significant allele frequency in BSSS and LSC groups were located in regions where QTLs had been detected for yield heterosis in a B73 × Mo17 cross. In the present study, the region with significant difference in allele frequency in the dent and flint groups could not be assigned to the region concerning heterosis for grain yield in the crosses between the dent and flint groups because there is no report of QTL analysis for it. In the future, QTL analysis for yield traits of dent × flint crosses would provide useful information in elucidating the mechanisms of heterosis in the crosses between the dent and flint groups.

次へ進む 前に戻る 目次に戻る

IV. Mapping of Quantitative Trait Loci Associated with Early Flowering of a Northern Flint Maize Inbred Line

1. INTRODUCTION

Early flowering has become a key component in the yield of maize in the cold regions (FREI 2000). The introduction of the Northern flint populations has played a key role in the adaptation of maize to the cold regions of Japan in chapter II and to most European regions (REBOURG et al. 2003; SOENGAS et al. 2003). The inbred lines developed from the local varieties of the Northern flint in Japan are early-maturing and superior in low-temperature germination as well as low-temperature growth (MONMA and OKABE 1985). However, early-maturing elite dent inbred lines are scarce, because the dent inbred lines are commonly developed from temperate maize germplasm, and tend to be medium- to late-maturing in the cold regions of Japan. This situation is limited to use the hybrids between the dent and Northern flint inbred lines. To overcome this limitation, an efficient method to introduce favorable alleles across the heterotic pattern is desirable to improve the flowering time of the dent inbred lines using the Northern flint germplasm. Elucidation of the mechanism of the early flowering of the Northern flint will help us to understand the maize adaptation to the cold regions and improve the flowering time of the temperate germplasm by MAS. Every major gene or qualitative gene can be involved in the quantitative type of trait variation and may behave as a QTL (ROBERTSON 1985). Recently, the candidate gene approach has been applied in plant genetics for the characterization and cloning of QTLs (PFLIEGER et al. 2001).

The objectives of the present study were to identify the QTLs for the early flowering of an elite Northern flint inbred line and investigate the relationship between the QTLs and the candidate genes related to flowering time.

次へ進む　前に戻る　目次に戻る

IV. Mapping of Quantitative Trait Loci Associated with Early Flowering of a Northern Flint Maize Inbred Line

2. MATERIALS AND METHODS

Plant material

To85, a donor parent of the "early flowering" genes, is a Northern flint inbred line developed in Hokkaido (N43, E141). Mi29, the recurrent parent, is a late-flowering dent inbred line developed in Kyushu (N31, E131). One hundred fifty $F_{2:3}$ lines derived from the cross of Mi29 and To85 were used for QTL analysis in the present study. The plant materials were developed at NARCH, Sapporo (N43, E141), Hokkaido. The cross was made in 2001, and unselected F_2 plants were self-pollinated in 2002 to produce 150 $F_{2:3}$ lines.

Trait evaluation

The parents, the F_1 line, and the 150 $F_{2:3}$ lines were planted at NARCH on 16 May in 2003. The experimental design involved randomized blocks with two replications, and the plant density was 68,376 plants/ha; 17 plants were 0.195 m apart in each 3.5-m long row spaced at 0.75-m intervals. The fertilizer application and cultivation regimes were consistent with optimum maize production for this region.

Flowering dates, plant height (PH), and ear height (EH) were measured on a plot basis. Flowering dates were recorded in terms of accumulated growing degree days in degrees Celsius (CROSS and ZUBER 1972). The number of accumulated growing degree days (GDD) in ℃ to the pollen shedding date (POL) was calculated from the date of planting to the date by which 50% of the plants had shed pollen. The GDD to silking date (SLK) was measured from the date of planting to the date by which 50% of the plants had silks that had emerged from the primary ear shoot. GDD were calculated using the following formula:

GDD = [(maximum ℃ + minimum ℃)/2] - 10 ℃, where 10 ℃ was used for the minimum temperature and 30 ℃ was used for the maximum temperature when the actual temperatures exceeded these limits. After all of the plants had completed their pollen shed, we measured the mean PH and EH for each plot from 5 competitive plants. PH and EH were measured from the ground level to the node of the tassel and the node attaching the primary ear, respectively.

Molecular markers

Genomic DNAs were isolated from the seedlings of the 150 F_2 plants to produce the F_3 lines. The 110 SSR primers used in the present study were from the MaizeGDB. The methods of isolating genomic DNA and amplifying and detecting SSR markers were described in chapter II. We chose four candidate genes (*an1*, *d8*, *vp1*, and *ck2α*) related to flowering traits from the previous reports (BENSEN et al. 1995; THORNSBERRY et al. 2001; SUZUKI et al. 2001; TAKAHASHI et al. 2001). The primer sites for the determination of the parents sequences of the candidate genes were determined from published cDNA sequences (GenBank accession (gb) MZEAN1A, AF413120, ZMVP1MCW, and Y11526, respectively). An insertion and deletion (InDel) marker used for the detection of the parents' genotype of *d8* was

obtained from THORNSBERRY et al. (2001). Two InDel markers and one cleaved amplified polymorphic sequence (CAPS) marker used for the detection of the parents' genotype of *an1*, *vp1*, and *ck2α* were made from the partial sequence data of the respective genes. The PCR reactions for the InDel and CAPS markers were carried out in a DNA Thermal Cycler (BIO-RPOL LABORATORIES, HERCULES, California). The 10-µl reaction mixture consisted of 20 nM of each primer, 1 unit of Taq DNA polymerase (PROMEGA, CO., INC.), 200 µM of each dNTP, 1 x reaction buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 100 µg mL^{-1} gelatin: pH 8.3), and 30 ng of template DNA. The reaction began with an initial denaturation step of 1 min at 96 ℃, then a cycle of 1 min at 96 ℃, 1 min at 54 ℃, and 2 min at 72 ℃ was repeated 30 times. The reactions were then finished with a continuous cycle at 4 ℃ and were stopped with 2 µl of loading-dye (40 % glycerol, 20 mM EDTA, and 0.6 mg mL^{-1} bromphenol blue). PCR products of partial fragments of *d8* and *an1* were loaded onto 12 % polyacrylamide non-denaturing gels. After the run, the reaction products were stained with EtBr to make them visible. PCR products of partial fragments of *vp1* were loaded onto 1.5 % agarose gels. After the run, these gels were stained with EtBr. After the PCR procedure, 1 unit of *AfaI* and 1 µl of buffer (100 mM Tris-HCL pH 7.5, 100 mM $MgCl_2$, 10 mM DTT) were added to the PCR products of a CAPS marker of *ck2α*, and a 20-µl final volume was reached by adding distilled deionized water. The reaction mixture was then incubated at 37 ℃ for 2 h. Digested PCR products were loaded onto 1.5 % agarose gels. After the run, the reaction products were stained with EtBr to make them visible.

Linkage analysis

Chi-square analyses were performed on each SSR marker to detect deviations from the expected Mendelian segregation of a 1:2:1 ratio or a 3:1 ratio. Linkage analysis was performed using Mapmaker/EXP 3.0 with the Kosambi function (LANDER et al. 1987). QTL analysis was performed using composite interval mapping with the QTL Cartographer version 2.0 computer program (WANG et al. 2001-2004). A significant threshold was determined using 1,000 permutations, and an LOD threshold of 3.6 was established. Additionally, the total percentage of variation accounting for all of the significant QTLs was determined for each trait in a multiple model.

The genetic effects and percentages of phenotypic variation attributable to individual putative QTLs were estimated at the peaks of significant regions. Because $F_{2:3}$ progeny were used for trait evaluation, the estimates of dominant effects are expected to be reduced by half from heterozygous F_2 plants. In this instance, estimates of dominant effects were doubled in accordance with established procedures (MATHER and JINKS 1971). Average levels of dominant were calculated as the ratio d/a, where "a" was the additive effect and "d" was the dominant effect estimated for the $F_{2:3}$ population. Gene action was determined based on the average level of dominant by using the criteria of STUBER et al. (1987): Additive (A) = 0-0.20; partial dominant (PD) = 0.21-0.80; dominant (D) = 0.81-1.20; and over dominant (OD) > 1.20.

次へ進む 前に戻る 目次に戻る

IV. Mapping of Quantitative Trait Loci Associated with Early Flowering of a Northern Flint Maize Inbred Line

3. RESULTS

Variation of flowering time and plant stature in F_3 lines

The parental checks (To85 and Mi29) were significantly different for all traits, with Mi29 having the greater values. The mean temperature of GDD per day for a pollen shedding date between To85 and Mi29 was 9.6 ℃. The differences in SLK and POL between Mi29 and To85 were 184.1 ℃ and 193.0 ℃, respectively (Table 10). SLK and POL for the $F_{2:3}$ lines showed fitted normal distributions, and all $F_{2:3}$ lines were distributed between the parents (Fig. 4). The coefficients of variation for SLK and POL in the $F_{2:3}$ lines were 2.5% and 1.8%, respectively. The mean values of SLK and POL in all $F_{2:3}$ lines and for F_1 were earlier than the mid-parental values of those traits. The differences in PH and EH between To85 and Mi29 were 22.6 and 35.6 cm, respectively (Table 10). PH and EH for the $F_{2:3}$ lines showed fitted normal distributions (Fig. 4). The coefficients of variation for PH and EH in the $F_{2:3}$ lines were 3.7% and 7.4%, respectively. The ranges of PH and EH in the $F_{2:3}$ lines were broader than the parents. The value of PH in F_1 was higher than the mid-parental values, but the mean of the $F_{2:3}$ lines was smaller than the mid-parental values. The means of EH in the $F_{2:3}$ lines and F_1 were higher than the mid-parental values. A high correlation between SLK and POL was found (r_p = 0.82**) (Table 11). Significant correlations were found between SLK and PH, POL and PH, POL and EH, and PH and EH, but those correlations were lower than that between SLK and POL.

Linkage map

The 110 SSR markers, 1 CAPS marker, and 3 InDel markers produced the linkage map and were included in ten linkage groups (Fig. 5). The total length of the map was 1287 cM, with a mean density of 12.4 cM. The length of the longest gap between two loci, located on chromosome 4, was 26.5 cM. The map was largely in agreement with the maize consensus genetic map (POLACCO and COE 2002). Only one highly significant distortion (P < 0.01) from expected segregation ratios was identified at locus *bnlg1371* on chr.6. The loci of the candidate genes *an1*, *d8*, and *ck2α* were located on chr.1 and *vp1* was located on chr.3, respectively.

QTL analysis

The three QTLs for SLK were identified on chr.1, chr.8, and chr.9, and accounted for 48.6% of the total phenotypic variation (Table 12 and Fig. 5). The four QTLs for POL were identified on chr.1, chr.3, chr.8, and chr.9, and accounted for 63.7% of the total phenotypic variation. The amount of phenotypic variation explained by each QTL ranged from 4.9% to 36.0%. To85 alleles at these loci contributed to decreases in SLK and/or POL. The additive effect of these alleles ranged from -11.1 ℃ to -27.7 ℃ for SLK and from -

10.0 ℃ to -18.5 ℃ for POL. None of the gene actions of the QTLs for SLK and/or POL showed over dominant. The QTL on chr.8 for SLK explained 36% of the total phenotypic variation. The additive effect of the To85 allele at this locus was the largest among the three QTLs for SLK and contributed to decreasing SLK by 27.7 ℃, which was equivalent to about 3 days in Sapporo. The position and gene action of the QTL on chr.8 for POL were in accordance with the position and gene action for SLK, and the gene action of To85 alleles at this locus was partially dominant. The QTLs for SLK and POL were identified at closely linked loci on chr.9. Those QTLs explained 4.9% and 8.1% of the total phenotypic variation of SLK and POL, respectively, and they were the smallest among the QTLs detected in the present study. On the other hand, three QTLs on chr.1, and chr.3 were independently identified for SLK or POL, respectively. The additive effects of the To85 alleles at these loci contributed to decreased the values of SLK or POL from 11.1 ℃ to 18.5 ℃, which were equivalent to about 1 or 2 days in Sapporo. The 1-LOD support interval of the QTL on chr.1 for SLK ranged from 129.5 cM to 157.7 cM, which did not include the locus of *d8* (158.8 cM: LOD = 0.2), but included the locus of an1 (140.7 cM). All QTLs for SLK and POL were completely separated from the loci of *vp1* and *ck2α*.

The two QTLs for PH were identified on chr.3 and chr.4, and accounted for 42.7% of the total phenotypic variation. The four QTLs for EH were identified on chr.1, chr.3, and chr.9, and accounted for 52.5% of the total phenotypic variation. The amount of phenotypic variation explained by each QTL ranged from 6.2% to 23.0%. To85 alleles of QTLs on chr.4 for PH and on chr.1 and chr.9 for EH contributed to increased the values of PH and EH, respectively. One of the six QTLs for PH and EH was detected in the common region on chr.3. Their gene action showed over dominant, and the Mi29 allele showed positive effects at these loci. The QTL on chr.3 for PH was detected at the linked locus of the QTL for POL. However, the peaks of these QTLs were clearly separated by over 20 cM.

次へ進む 前に戻る 目次に戻る

IV. Mapping of Quantitative Trait Loci Associated with Early Flowering of a Northern Flint Maize Inbred Line

4. DISCUSSION

The relationships between QTLs and correlated traits

A total of 13 QTLs were detected comprising 6 loci for PH and EH in the present study. The total amounts of phenotypic variation of the QTLs detected in the present study, ranging from 42.7% for PH to 63.7% for SLK, were comparable to those in the previous reports (BERKE and ROCHEFORD 1995; VELDBOOM and LEE 1996; AUSTIN and LEE 1996; RIBAUT et al. 1996). Of these loci defining the QTLs regions, five were associated with the QTLs for two or more traits. Correlated traits often have common QTLs that are significantly associated with each trait (PATERSON et al. 1991; ABLER et al. 1991; BEAVIS et al. 1994; VELDBOOM and LEE 1996). In the present study, SLK was highly correlated with POL (r_p = 0.82), and two QTLs for SLK and POL were detected in common regions. The directions of the additive effect for the QTLs coincided. These QTLs were probably the main contributors to the high correlation between SLK and POL.

Flowering time tends to be positively correlated with PH (i.e., taller plants flower later), and the improvement of flowering time is often attended by a decrease of PH. This is not desirable, because PH is one of the components related to the yield of silage maize. In the previous reports, the correlation between flowering time (SLK and POL) and PH varied, ranging from -0.05 to 0.66 (BEAVIS et al. 1994; VELDBOOM and LEE 1996). In the present study, moderate correlations were found between SLK and PH and between POL and PH (r_p = 0.31, and 0.36, respectively). Only one QTL on chr.3 for POL was detected at the linked locus of the QTL for PH with a negative effect from the same parent, To85, and these QTLs may have contributed to the moderate correlation between POL and PH. However, the peaks of these QTLs were clearly separated, and separation of these QTLs would not be difficult using the markers linking them. In addition, the peaks of all QTLs for SLK were separated from those of all QTLs for PH. These results indicated that selection for early flowering and tall plants was possible in this population, because the major QTLs for the flowering traits contributed little to PH. In other combinations of traits, high correlations had been found between PH and EH in the previous studies (r_p = 0.82; VELDBOOM and LEE 1996 and r_p = 0.90; BEAVIS et al. 1994). BEAVIS et al. (1994) detected four of six QTLs for PH and EH in common regions. VELDBOOM and LEE (1996) reported that only two of seven QTLs for PH and EH were detected in common regions, but these two QTLs had large effects. In the present study, the QTLs on chr.3 for PH and EH with the positive effects from Mi29 were detected in the common region. The effect of these QTLs was not large (8.0% and 8.1% of the phenotypic variation for PH and EH, respectively), suggesting that it caused a moderate correlation between PH and EH (r_p = 0.35) in the present study.

QTL for flowering time in maize

An important consideration for QTL analysis is whether the locations and effects of the QTLs detected in one population are observed in other populations. This information provides more genetic and functional clues to define the characteristic of the QTLs. The region on chr.1, which was associated with the QTL for SLK in the present study, had been associated with QTLs for SLK in other populations (VELDBOOM and LEE 1996; AUSTIN and LEE 1996; RIBAUT et al. 1996) and in testcross progeny of $F_{6:8}$ and $F_{2:3}$ lines (AUSTIN et al. 2001). In addition to chr.1, several other regions with the QTLs in the present study had been associated with flowering time in other populations. The region on chr.1, which was associated with the QTL for POL in the present study, had been associated with QTLs for POL in another population (BEAVIS et al. 1994). The region on chr.9 associated with QTL for POL and SLK was previously reported (VELDBOOM and LEE 1996; RIBAUT et al. 1996; BERKE and ROCHEFORD 1995). These results indicate that these QTLs will frequently contribute to flowering time in different environments and populations.

The QTL on chr.8 was identified as having the largest effect for SLK in the present study but has not been reported in other populations such as the dent and European flint. In populations of the Gaspe type Northern flint, QTLs for the flowering time have been detected on chr.8 (KOESTER et al. 1993; VLADUTU et al. 1999), and were associated with PH. It is difficult to conclude whether the QTL on chr.8 in the present study was the same as the QTLs detected in the populations of the Gaspe type Northern flint, because it is difficult to accurately compare the effects of the QTLs detected in several studies due to the interaction between genetic and environmental effects. However, the QTL on chr.8 was not associated with PH in the present study. The present result raises the possibility that the QTL on chr.8 is not the same as the QTLs on chr.8 that were detected in the populations of the Gaspe type Northern flint.

The candidate genes for flowering time

The information regarding the candidate genes for the QTLs will provide efficient molecular markers, since recombinations between markers and the QTLs would be absent from these genes (PFLIEGER et al. 2001). The relationship of the InDel and CAPS markers of the genes related to the flowering time was detected in the linkage analysis. The region on 1L was associated with the QTL for SLK and contained the locus of the InDel markers of *an1*. The *an1* mutant has been cloned and characterized by BENSEN et al. (1995). The mutant has a phenotype of an andromonoecious dwarf that responds to gibberellin and has a delayed flowering time. The *id1*, a mutation that causes a delay of flowering time (SINGLETON 1946), is also located on chr.1 near *an1* (Polacco and Coe 2002). The region on chr.8 near *bnlg1067* was strongly associated with the flowering time in the present study. The *epc1*, a mutation that reduces the duration of the juvenile vegetative phase and causes early flowering, was located on chr.8 near *bnlg1067* (0.4cM) (VEGA et al. 2002). These observations support the relationship proposed by ROBERTSON (1985), in which alleles with quantitative and qualitative effects reside at the same locus and provide the genetic information to the candidate genes for the QTL on chr.1 and chr.8, respectively.

The *d8* mutant has a phenotype of an andromonoecious dwarf but does not respond to gibberellin (PHINNEY 1956). THORNSBERRY et al. (2001) reported that the *d8* polymorphisms are associated with differences in flowering time among 92 maize inbred lines, and the 6-bp deletion just downstream from the SH-2-like domain had a large estimated effect of reducing the flowering time by 7-11 days. However, the locus of *d8* was not significantly associated with SLK and POL in the present study. This result was surprising, because the polymorphism of the InDel marker of *d8* (a 6-bp deletion just downstream from the SH-2-like domain; THORNSBERRY et al. 2001) was detected in the present study. Recently, CHARDON et

al. (2004) reported that the locus of *d8* was not associated with the flowering time in the meta-analysis. ANDERSEN et al. (2005) also reported that the *d8* polymorphisms were not significantly associated with the flowering time when population structure was considered in the association test in elite European inbred lines. We could not conclude whether the *d8* polymorphism was associated with the flowering time in maize or not, because QTL mapping provides less precise genetic information than do mutants with qualitative effects, and the environment in the present study was different from the previous studies. In order to perform a more precise examination, we are developing NILs for each locus.

In the present study, we detected the QTLs related to the early flowering in the Northern flint inbred line. All QTLs for the flowering time in the present study are expected to improve the flowering time of the dent inbred lines bred in the intermediate and warm regions, because they have little effect on plant stature. In the near future, we will characterize the QTLs for MAS in the breeding programs and investigate the relationship between the QTLs and the candidate genes in detail using NILs.

次へ進む 前に戻る 目次に戻る

V. GENERAL DISCUSSION

Information on the genetic diversity and relationship among the breeding materials is indispensable for the maize breeding programs. The maize breeders usually strive to keep and expand the genetic diversity among the breeding materials. Especially in the cold regions, the genetic diversity is usually restricted by the cool climate, which shortens the maize growing period. The maize breeders have been attempting to introduce new elite germplasm, the hybrids and inbred lines bred in the intermediate and warm regions, to improve the breeding materials in the cold regions. A more convenient method than conventional breeding methods for introducing these materials is required. The objective of the present study was to develop marker-assisted breeding, assessing and expanding the genetic diversity using DNA markers, in order to improve the maize breeding materials in the cold regions of Japan.

The object of chapter II is the selection of SSR loci for the assessment of the genetic diversity and relationship among the breeding materials adapted to the cold regions of Japan. Recent advances in the maize genome project have allowed the breeders to easily use SSR loci from the 1,748 SSR map in the MaizeGDB (SHAROPOVA et al. 2002) for selection procedures. The 60 SSR loci were selected based on the chromosome position and discrimination ability by the preliminary test. The set of 60 SSR loci was useful for estimating the GS among the inbred lines, and assessing the genetic diversity and relationship among the breeding materials with sufficient accuracy. The results in chapter II suggested that the set of 60 SSR loci will be useful for genome-wide diversity analysis and various views in the maize breeding program.

This analysis will be efficient for monitoring the genetic diversity among the breeding materials. In the previous reports, there were moderate associations between GS and mid-parent heterosis for yield traits (SMITH et al. 1990, 2000; MELCHINGER et al. 1992; DHILLON et al. 1993; MARSAN et al. 1998; BARBOSA et al. 2003). To maintain a high level of heterosis, the breeding materials are requested to keep a high level of the genetic diversity. The monitoring of the genetic diversity will help the breeders understand the sequential changes of the genetic diversity in the breeding program, and be alerted against the radical reduction of the genetic diversity.

In addition, this analysis will be useful for assessing the genetic relationship between the breeding materials and representative inbred lines. The Northern flint is one of the origins of the European flint germplasm (FREI 2000). However, the contribution of the Northern flint to establishment of the European flint germplasm has been unknown. The present result of the analysis revealed that the Northern flint germplasm was possibly related to the progenitors of the European flint inbred line F283. This result was surprising, because the Northern flint germplasm has recently had very limited use in the European breeding programs (FREI 2000). However, after the present study, REBOURG et al (2003) and SOENGAS et al. (2003) also reported that the introduction of the Northern flints into the Europe made a major contribution to the germplasm of traditional varieties in most European regions. These results indicated the contribution of the Northern flint germplasm for expansion of the maize cultivation area in the cold regions.

In the present study, another European flint inbred line, F2, was found to be dissimilar to the Northern flint

inbred lines bred in Hokkaido. Their progenitors seem to be independent of the Northern flint. This result suggested that introduction of the European flint germplasm will lead to expansion of the genetic diversity among the flint breeding materials in Hokkaido. The maize breeders have used the European hybrids to introduce the European flint germplasm into the breeding materials. However, the inbred lines developed from the European hybrids (miscellaneous inbred lines) are a mixture of the dent and flint germplasm, and are difficult to assign. A convenient assignment method for the miscellaneous inbred lines was needed to introduce the European flint germplasm systematically.

The result of the analysis based on the 60 SSR loci also revealed that the dent inbred lines bred in Hokkaido were much more similar to the BSSS inbred lines than the LSC inbred lines. This result was unexpected, because some of them were developed from the U. S. hybrids, and the most widely used hybrids in the U. S. are crosses between the RYD and LSC inbred lines (HALLAUER 1990; GERDES and TRACY 1993). These results indicated that the utilization of the LSC inbred lines will be one of the most effective approaches to expanding the genetic background of the dent breeding materials in Hokkaido.

The set of 60 SSR loci will be applicable to profiling of the inbred lines. The maize profile will describe the detailed genetic relationship among the inbred lines. The profile will help the breeders to choose the testers in the testcross trials. Recently, the inbred lines bred in Hokkaido have been provided to private seed companies as the parent lines of maize hybrids. These private seed companies have requested detailed assignment results of the inbred lines. The analysis based on the 60 SSR loci can portray the genetic relationship and provide detailed assignment results of the inbred lines. The disclosure of the profile of the inbred lines will promote the utilization of them by the private seed companies.

The analysis based on the 60 SSR loci suggested that the introduction of other germplasm is effective for improving the breeding materials in the cold regions of Japan. The object of chapter III and chapter IV is the development of convenient methods to introduce additional germplasm, as well as the European hybrids and dent inbred lines bred in the intermediate and warm regions using DNA markers.

The object of chapter III is the selection of SSR sets from the 60 SSR loci (full set) in the assignment of the miscellaneous inbred lines to the dent or flint groups. Predicting hybrid performance has always been a primary concern in the hybrid breeding programs. The assignment of the inbred lines is useful for avoiding the intra-group hybrids in which the degree of heterosis expressed is usually poor. The mean GS of the miscellaneous inbred lines with the dent and flint inbred lines was a reliable criterion for the assignment of them (MESSMER et al. 1992a). Furthermore, the GS estimates from selected loci with differences in allele frequency in heterotic groups were significantly correlated with the degree of heterosis for grain yield (SMITH et al. 2000). In the present study, the 25 key SSR markers (Set 1) were selected based on the differences in allele frequency in the dent and flint groups. The mean GS with the dent and flint inbred lines estimated from Set 1 SSR loci will be useful for assigning the miscellaneous inbred lines with sufficient accuracy. Set 2 SSR loci composed of 14 markers was also selected in the present study. The accuracy of the assignment from Set 2 SSR loci was low in comparison with those of Set 1 SSR loci, but it was adequate for the preliminary assignment.

Each of the three SSR sets (full, Set 1 and Set 2) will be applicable to a different process of the breeding program. Set 2 SSR loci will be suitable for the preliminary assignment for developing lines. The assignment from Set 1 SSR loci will help the breeders choose the testers for the miscellaneous inbred lines, when the breeders design testcross trials. After the testcross trials, selected elite inbred lines will be analyzed by the full set of the SSR loci in order to profile them and monitor the genetic diversity. These SSR sets will be useful for the systematic introduction of the European germplasm.

The object of chapter IV is the identification of the QTLs for the early flowering of the elite Northern flint inbred line, To85, and the relationship between the QTLs and the candidate genes related to the flowering time. A total of 7 QTLs for silking date and pollen shedding date were detected at 5 loci in the present study. These QTLs for the flowering time will be useful for the improvement of the flowering time of the dent inbred lines bred in the intermediate and warm regions, because they contributed little to the plant height, which directly affects the yield of silage maize. The QTL on chr.8 was particularly identified as having the largest additive genetic effect for the silking date (about 3 days at Sapporo) in the present study and previous reports (BERKE and ROCHEFORD 1995; VELDBOOM and LEE 1996; AUSTIN and LEE 1996; RIBAUT et al. 1996). These results suggested that the QTL on chr.8 played a key role in the adaptation of the Northern flint to the cold regions, and will take a central role in the improvement of the flowering time of the dent inbred lines in Hokkaido.

Early flowering is a key component in the yield of maize in the cold regions (FREI 2000). Improvement of the flowering time of the dent inbred lines bred in the intermediate and warm regions is usually performed by selfing after direct crossing or by back-crossing with the early inbred lines, including the Northern flint inbred lines. However, an unexpectedly large donor region was often retained on NIL in the back-crossing strategy (VLADUTU et al. 1999). This is not desirable in the hybrid breeding programs, because the degree of heterosis is affected by the genetic similarity between the heterotic groups (MELCHINGER 1999). The QTLs detected in the present study will lead to MAS for the flowering time. It will be an effective tool for introducing favorable alleles for the early flowering into the dent inbred lines from the Northern flint inbred lines across the heterotic pattern.

The identification of the QTLs is important in the elucidation of the mechanism of quantitative traits. The candidate gene approach has been applied in the plant genetics for the characterization and identification of QTLs (PFLIEGER et al. 2001). The information from the rice genome studies is useful for the study of *Gramineae* including maize, because comparative maps have been constructed between rice and maize (AHN and TANKSLEY 1993), and the Comparative Map Viewer (CMap tool) allows a user to view and compare maps between and among the species in GRAMENE (http://www.gramene.org/). In rice, genes concerning flowering time, *Hd1*, *Hd3a*, *Hd6*, *Se5* and *Ehd1*, have been identified (YANO et al. 2000; YAMAMOTO et al. 2000; TAKAHASHI et al 2001; IZAWA et al. 2000; DOI et al. 2004). The rice candidate genes have been located onto the maize genome using a synteny conservation approach based on comparative mapping between the maize genetic map and japonica rice physical map (CHARDON et al. 2004). This information is useful for choosing the candidate genes of the QTLs for the flowering time in maize.

In the present study, in order to screen the candidate gene for the early flowering of the Northern flint, the DNA markers for the candidate genes including the rice gene were used for linkage analysis. The *an1* and *id1* were chosen for the candidate genes for the QTL on chr.1, and the*epc1* for the QTL on chr.8. However, no rice candidate genes were located near the QTLs in the present study. Almost all identified rice genes for the flowering time contribute to the photoperiodic control. However, the maize temperate cultivars were virtually photoperiod insensitive (BONHOMME et al. 1994). For this reason, the rice candidate genes for the flowering time were not associated with the QTLs in the present study.

The alternative strategy of the candidate gene approach for identifying the QTLs is a map-based cloning strategy. It has been thought that the application of map-based cloning to the maize genome studies is difficult because the maize genome is abundant in highly repetitive regions (SANMIGUEL et al. 1996). Recently, using the rice genome sequence, a major QTL controlling the differences in fruitcase structure

between maize and teosinte, *tga1*, was identified by a map-based cloning strategy (W*ANG* et al. 2005). S*ALVI* et al. (2002) have also attempted to identify the gene for *Vgt1* which is the QTL for the flowering time. Furthermore, the maize genome sequencing in the Maize Genome Project will be completed in 1-2 years (MARTIENSSEN et al. 2004). The maize genome sequence will be a powerful resource for the map-based cloning strategy as in rice and Arabidopsis, and will make the map-based cloning strategy for the QTLs in maize laboratories possible in the near future.

In the cold regions of Japan, the maintenance and expansion of the genetic diversity among the breeding materials are important in the breeding programs. In the present study, the marker-assisted breeding for the assessment and expansion of the genetic diversity among the breeding materials in the cold regions of Japan was developed. The SSR marker set selected will be useful for monitoring the genetic diversity, profiling the inbred lines, and systematically introducing of other elite germplasm such as the European hybrids. The QTLs detected in the present study will be useful for improving the flowering time of the dent inbred lines bred in the intermediate and warm regions, in order to introduce them into the breeding materials in the cold regions of Japan. These tools for marker-assisted breeding will solve the restriction of the genetic diversity by the cool climate conditions, and lead the breeders to future success in the maize hybrid breeding for the cold regions of Japan.

次へ進む　前に戻る　目次に戻る

Marker-Assisted Breeding in Maize for Cold Regions of Japan

Hiroyuki ENOKI

SUMMARY

In the cold regions of Japan, the maintenance and expansion of the genetic diversity among the breeding materials are important in the maize (*Zea mays* L.) hybrid breeding programs. The present study was carried out to develop marker-assisted breeding to accelerate the breeding of excellent maize varieties adapted to the cold regions of Japan. The objectives of the three studies are to 1) assess the genetic diversity among the inbred lines adapted to the cold regions of Japan from SSR analysis of the 60 loci distributed uniformly throughout maize genome, 2) establish an assignment method of the inbred lines developed from the European hybrids from the mean GS estimates derived from a smaller number of SSR loci, which were chosen based on the differences in the mean allele frequency between the dent and flint groups, and 3) identify QTLs for the early flowering of an elite Northern flint inbred line toward establishment of MAS for the early flowering.

Information on the genetic diversity and relationships among the breeding materials is necessary for the hybrid maize breeding programs. Simple-sequence repeats (SSR) analysis of the 60 loci distributed uniformly throughout the maize genome was carried out for 65 inbred lines adapted to the cold regions of Japan in order to assess the genetic diversity among the inbred lines and assign them to the heterotic groups. The mean value (0.69) of polymorphic-index content (PIC) for the SSR loci provided sufficient discrimination ability for the assessment of the genetic diversity among the inbred lines. The correlation between the genetic similarity (GS) estimates and coancestry coefficient was significant (r=0.70). The average-linkage (UPGMA) cluster analysis and principal-coordinate analysis (PCOA) for a matrix of the GS estimates showed that the Northern flint inbred lines bred in Hokkaido were similar to the Canadian Northern flint inbred line CO12 and the European flint inbred line F283, and the dent inbred lines bred in Hokkaido were similar to the BSSS inbred lines such as B73. These associations correspond to the known pedigree records of these inbred lines. The results indicate that the SSR analysis is effective for the assessment of the genetic diversity among the maize inbred lines and the assignment of the inbred lines to the heterotic groups.

The maize breeders commonly assign the inbred lines to the groups in order to maintain the high level of hybrid vigor obtainable from the crosses. The results in chapter II indicated that the GS estimated from the 60 SSR loci was effective for the assignment of the maize inbred lines derived from the European hybrids, which contain mixtures of the dent and flint germplasm, to the dent or flint groups adapted to the cold regions of Japan. We evaluated a simplified assignment method using a smaller number of SSR loci. Two subsets were chosen from the full set of 60 SSR loci distributed uniformly throughout the maize genome. Set 1 composed of 25 loci and Set 2 composed of 14 loci were chosen based on the difference in allele frequency (0.4 for Set 1 and 0.5 for Set 2) in the dent and flint groups. The SSR loci of Set 1 and Set 2 carried a total of 176 and 99 alleles among 88 inbred lines, respectively. The numbers of alleles for Set 1 and Set 2 were 38% and 21% of the number of alleles for the full set of SSR loci, respectively. The assignments from mean GS estimates using Set 1 SSR loci almost all corresponded to the full set of SSR loci. Furthermore, the assignments of several inbred lines from Set 1 SSR loci were ascertained by the testcross data. The results indicated that the assignment using Set 1 SSR loci with a similar accuracy to the

full set of the SSR loci is an efficient method in the maize breeding programs.

Flowering time is a trait of interest to the maize breeders because of its importance in the selection of appropriate parents of hybrids. We detected quantitative trait loci (QTLs) associated with the early flowering of the Northern flint inbred line, To85, using 150 $F_{2:3}$ lines derived from a cross of To85 and a late dent inbred line, Mi29. We used 110 SSR, 1 cleaved amplified polymorphic sequence (CAPS), and 3 insertion and deletion (InDel) markers for 4 candidate genes (*an1*, *d8*, *ck2α*, and *vp1*) related to the flowering time in maize in order to construct a framework linkage map. The $F_{2:3}$ population exhibited a wide range of variation in growing degree days (GDD), silking (SLK), and pollen shedding (POL). Using composite interval mapping with an LOD threshold of 3.6, seven QTLs associated with SLK and/or POL were detected on chr.1, chr.3, chr.8, and chr.9. The region on chr.1 associated with the QTL for SLK contained the locus of *an1*. The peak of the QTL on chr.8 with the largest effect for SLK was near *bnlg1067*, which was near the locus of *epc1*. These QTLs did not greatly affect the plant height and/or ear height. These results provide information that allows us to choose the candidate genes and improve the flowering time of the maize inbred lines bred in the intermediate and warm regions through marker-assisted selection.

In the present study, the marker-assisted breeding method for the assessment and expansion of the genetic diversity among the breeding materials in the cold regions of Japan was developed. This will be useful for monitoring the genetic diversity, profiling the inbred lines and systematically introducing other elite germplasm such as the European hybrids and dent inbred lines bred in the intermediate and warm regions. These tools for the marker-assisted breeding will solve the restriction of the genetic diversity due to the cool climate conditions, and lead the breeders to the future success in the maize hybrid breeding under the cold regions of Japan.

次へ進む　前に戻る　目次に戻る

REFERENCES

□□□ ABLER, B. S. B., EDWARDS, M. D. and STUBER, C. W. (1991) :Isoenzymatic identification of quantitative trait loci in crosses of elite maize inbreds. Crop Sci., 31, 267-274.

□□□ AHN, S. and TANKSLEY, S. D. (1993) :Comparative linkage maps of the rice and maize genomes. Proc. Natl. Acad. Sci. U. S. A., 90, 7980-7984.

□□□ ANDERSEN, J. R. SCHRAG, T. MELCHINGER, A. E. ZEIN, I. and LUBBERSTEDT, T. (2005): Validation of dwarf8 polymorphisms associated with flowering time in elite European inbred lines of maize (*Zea mays* L.). Theor. Appl. Genet., 111, 206-217.

□□□ AUSTIN, D. F. and LEE, M. (1996): Genetic resolution and verification of quantitative trait loci for flowering and plant height with recombinant inbred lines of maize. Genome, 39, 957-968.

□□□ AUSTIN, D. F., LEE, M. and VELDBOOM, L. R. (2001): Genetic mapping in maize with hybrid progeny across testers and generations: plant height and flowering. Theor. Appl. Genet., 102, 163-176.

□□□ BARBOSA, A. M. M., GERALDI, I. O. BENCHIMOL, L. L. GARCIA, A. A. F. SOUZA, C. L. and SOUZA, A. P. (2003): Relationship of intra- and interpopulation tropical maize single cross hybrid performance and genetic distances computed from AFLP and SSR markers. Euphytica, 130, 87-99.

□□□ BARBOSA-NETO, J. F. HERNANDEZ, C. M. O'DONOUGHUE, L. S. and SORRELLS, M. E. (1997): Precision of genetic relationship estimates based on molecular markers. Euphytica, 98, 59-67.

□□□ BEAVIS, W. D. SMITH, O. S. GRANT, D. and FINCHER, R. (1994): Identification of quantitative trait loci using a small sample of topcrossed and F_4 progeny from maize. Crop Sci., 34, 882-896.

□□□ BENSEN, R. J. JOHAL, G. S. CRANE, V. C. TOSSBERG, J. T. SCHNABLE, P. S. MEELEY, R. B. and BRIGGS, S. P. (1995): Cloning and characterization of the maize *an1* gene. Plant Cell, 7, 75-84.

□□□□ BERKE, T. G. and ROCHEFORD, T. R. (1995): Quantitative trait loci for flowering, plant and ear height, and kernel traits in maize. Crop Sci., 35, 1542-1549.

□□□□ BONHOMME, R. DERIEUX, M. and EDMEADES, G. O. (1994): Flowering of diverse maize cultivars in relation to temperature and photoperiod in multilocation field trials. Crop Sci., 34, 156-164.

□□□□ BOPPENMAIER, J. MELCHINGER, A. E. BRUNKLAUS-JUNG, E. GEIGER, H. H. and HERRMANN, R. G. (1992): Genetic diversity for RFLPs in European maize inbreds: I. Relation to performance of flint × dent crosses for forage traits. Crop Sci., 32, 895-902.

□□□□ CHARDON, F. VIRLON, B. MOREAU, L. FALQUE, M. JOETS, J. DECOUSSET, L. MURIGNEUX, A. and CHARCOSSET, A. (2004): Genetic architecture of flowering time in maize as inferred from quantitative trait loci meta-analysis and synteny conservation with the rice genome. Genetics, 168, 2169-2185.

□□□□ COLLARD, B. C. Y. JAHUFER, M. Z. Z. BROUWER, J. B. and PANG, E. C. K. (2005): An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica, 142, 169-196.

□□□□ CROSS, H. Z. and ZUBER, M. S. (1972): Prediction of flowering dates in maize based on different

methods of estimating thermal units. Agron. J., 64, 351-355.

□□□□ DHILLON, B. BOPPENMAIER, S. J. POLLMER, W. G. HERRMANN, R. G. and MELCHINGER, A. E. (1993): Relationship of restriction-fragment-length-polymorphisms among European maize inbreds with ear dry-matter yield of their hybrids. Maydica, 38, 245-248.

□□□□ DICE, L.R. (1945): Measures of the amount of ecological association between species. Ecology, 26, 297-302.

□□□□ DOI, K., IZAWA, T. FUSE, T. YAMANOUCHI, U. KUBO, T. SHIMATANI, Z. YANO, M. and YOSHIMURA, A. (2004): *Ehd1*, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of *Hd1*. Genes Dev., 18, 926-936.

□□□□ DUBREUIL, P. DUFOUR, P. KREJCI, E. CAUSSE, M. VIENNE, D. GALLAIS, A. and CHARCOSSET, A. (1996): Organization of RFLP diversity among inbred lines of maize representing the most significant heterotic groups. Crop Sci., 36, 790-799.

□□□□ FREI, O. M. (2000): Changes in yield physiology of corn as a result of breeding in northern Europe. Maydica, 45, 173-183.

□□□□ GERDES, J. T. and TRACY, W. F. (1993): Pedigree diversity within the lancaster surecrop heterotic group of maize. Crop Sci., 33, 334-337.

□□□□ GOODMAN, M. M. and BROWN, W. L. (1988): Races of corn. In: Sprague, G. F. and Dudley, J. W. (eds) Corn and Corn Improvement, 3rd ed. Madison, Wisconsin, pp 33-80.

□□□□ GUILLET-CLAUDE, C. BIROLLEAU-TOUCHARD, C. MANICACCI, D. FOURMANN, M. BARRAUD, S. CARRET, V. MARTINANT, J. P. and BARRIERE, Y. (2005): Genetic diversity associated with variation in silage corn digestibility for three *O-methyltransferase* genes involved in lignin biosynthesis. Theor. Appl. Genet., 110, 126-135.

□□□□ HALLAUER, A. R. (1990): Methods used in developing maize inbreds. Maydica, 35, 1-16.

□□□□ HALLAUER, A. R., RUSSELL, W. A. and LAMKEY, K. R. (1988): Corn breeding. In: SPRAGUE, G. F. and DUDLEY, J. W. (eds) Corn and Corn Improvement, 3rd ed. Madison, Wisconsin, pp. 463-564.

□□□□ HASEGAWA, H. (1984): "Daiheigen", maize Norin-Ko No. 23. New Technology, 21, 50-54.

□□□□ INOUE, Y. (1984): Specific combining ability between six different types of maize (*Zea mays* L.) obtained from a diallel set of eleven open-pollomated. Japan. J. Breed., 34, 17-28.

□□□□ IZAWA, T., OIKAWA, T. TOKUTOMI, S. OKUNO, K. and SHIMAMOTO, K. (2000): Phytochromes confer the photoperiodic control of flowering in rice (a short-day plant). Plant J., 22, 391-399.

□□□□ JONES, D. F. (1918): The effects of inbreeding and crossbreeding upon development. Conn. Agric. Exp. Stn. Bull., 207, 5-100.

□□□□ KANEKO, K., HASEGAWA, H. and INOUE, Y. (1975): New cultivar of silage corn, "Hokuyu". Res. Bull. Hokkaido Natl. Agric. Exp. Stn., 110, 97-111.

□□□□ KOINUMA, K., MIURA, Y. SATO, H. ENOKI, H. and TAKAMIYA, Y. (2004): Breeding of a silage maize cultivar, "Ohzora", with high yielding ability and lodging resistance. Res. Bull. Natl. Agric. Res. Cent. Hokkaido Reg., 180, 1-17.

□□□□ KOESTER, R. P., SISCO, P. H. and STUBER, C. W. (1993): Identification of quantitative trait loci controlling days to flowering and plant height in two near isogenic lines of maize. Crop Sci., 33, 1209-1216.

□□□□ KUSHIBIKI, H., NAKANO, H. and KUWAHATA, S.(1979): "Wase-homare" a new silage corn cultivar. Bull. Hokkaido Pref. Agric. Exp. Stn., 41, 91-103.

□□□□ LANDER, E. S., GREEN, P. ABRAHAMSON, J. BARLOW, A. DALY, M. J. LINCOLN, S. E. and NEWBURG, L. (1987): MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics, 1, 174-181.

□□□□ LEE, M., GODSHALK, E. B. LAMKEY, K. R. and WOODMAN, W. W. (1989): Association of restriction fragment length polymorphisms among maize inbreds with agronomic performance of their crosses. Crop Sci., 29, 1067-1071.

□□□□ MALECOT, G. (1948) : Les mathematiques de l'heredite. Masson & Cie, Paris.

□□□□ MARSAN, P. A., CASTIGLIONI, P. FUSARI, F. KUIPER, M. and MOTTO, M. (1998): Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers. Theor. Appl. Genet., 96, 219-227.

□□□□ MARTIENSSEN, R. A., RABINOWICZ, P. D. O'SHAUGHNESSY, A. and MCCOMBIE, W. R. (2004): Sequencing the maize genome. Curr. Opin. Plant Biol., 7, 102-107.

□□□□ MATHER, K. and JINKS, J. L. (1971): Biometrical Genetics. Chapman and Hall, London.

□□□□ MELCHINGER, A. E. (1999): Genetic diversity and heterosis. In: Coors J.G. and Pandey, S. (eds) The Genetics and Exploitation of Heterosis in Crops. Madison, Wisconsin, pp 99-118.

□□□□ MELCHINGER, A. E., LEE, M. LAMKEY, K. R. HALLAUER, A. R. and WOODMAN, W. L. (1990): Genetic diversity for restriction-fragment-length-polymorphisms and heterosis for 2 diallel sets of maize inbreds. Theor. Appl. Genet., 80, 488-496.

□□□□ MELCHINGER, A. E., MESSMER, M. M. LEE, M. WOODMAN, W. L. and LAMKEY, K. R. (1991): Diversity and relationships among united-states maize inbreds revealed by restriction-fragment-length-polymorphisms. Crop Sci., 31, 669-678.

□□□□ MELCHINGER, A. E., BOPPENMAIER, J. DHILLON, B. S. POLLMER, W. G. and HERRMANN, R. G. (1992): Genetic diversity for RFLPs in European maize inbreds: II. relation to performance of hybrids within versus between heterotic groups for forage traits. Theor. Appl. Genet., 84, 672-681.

□□□□ MELLERSCH, C. and SASMPSON, J. (1993): Simplifying detection of microsatellite length polymorphisms. Bio. Techniques, 15, 582-584.

□□□□ MESSMER, M. M., MELCHINGER, A. E. BOPPENMAIER, J. BRUNKLAUS-JUNG, E. and HERRMANN, R. G. (1992a): Relationships among early European maize inbreds: I. Genetic diversity among flint and dent lines revealed by RFLPs. Crop Sci., 32, 1301-1309.

□□□□ MESSMER, M. M., MELCHINGER, A. E. BOPPENMAIER, HERRMANN, J. R. and BRUNKLAUS-JUNG, E. (1992b): RFLP analyses of early-maturing European maize germplasm: I. Genetic diversity among flint and dent inbreds. Theor. Appl. Genet., 83, 1003-1012.

□□□□ MESSMER, M. M., MELCHINGER, A. E. HERRMANN, R. and BOPPENMAIER, J. (1993): Relationships among early European maize inbreds: II. Comparison of pedigree and RFLP data. Crop Sci., 33, 944-950.

□□□□ MIURA, Y., HASEGAWA, H. MONMA, E. OKABE, T. and INOUE, Y. (1989): Breeding of a new cultivar of silage corn "Kitayutaka" and its agronomic characteristics. Res. Bull. Hokkaido Natl. Agric. Exp. Stn., 151, 15-26.

□□□□ MIURA, Y., HASEGAWA, H. SATO, H. MONNA, E. OKABE, T. and INOUE, Y. (1995): Breeding of "Kita-asahi" silage corn and its characteristics. Res. Bull. Hokkaido Natl. Agric. Exp. Stn., 161, 33-44.

□□□□ MONMA, E. and OKABE, T. (1985): Genetic variation in germination rates and seedling growth at

low temperatures in inbred lines of corn (*Zea mays* L.). Res. Bull. North Japan Natl. Agrhc. Exp. Stn., 143, 137-148.

□□□□ MUMM, R. H. and DUDLEY, J. W. (1994): A classification of 148 U.S. maize inbreds: I. cluster analysis based on RFLPs. Crop Sci., 34, 842-851.

□□□□ NEI, M. and LI, W. H. (1979): Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA, 76, 5269-5273.

□□□□ PATERSON, A. H., DAMON, S. HEWITT, J. D. ZAMIR, D. RABINOWITCH, H. D. LINCOLN, S. E. LANDE, E. S. and TANKSLEY, S. L. K. (1991): Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. Genetics, 127, 181-197.

□□□□ PFLIEGER, S., FEBVRE, V. and CAUSSE, M. (2001): The candidate gene approach in plant genetics: a review. Mol. Breed., 7, 275-291.

□□□□ PHINNEY, B. O. (1956): Growth response of single-gene dwarf mutants in maize to gibberellic acid. Proc. Natl. Acad. Sci. USA, 42, 185-189.

□□□□ PINTO, R. D. C., SOUZA JR., C. L. CARLINI-GARCIA, L. A. GARCIA, A. A. F. and SOUZA, A. P. (2003): Comparison between molecular markers and diallel crosses in the assignment of maize lines to heterotic groups. Maydica, 48, 63-73.

□□□□ POLACCO, M. L. and COE Jr., E. (2002): IBM neighbors: a consensus genetic map. Maize GDB (http://www.maizegdb.org/).

□□□□ REBAI, A, BLANCHARD, P. PERRET, D. and VINCOURT, P. (1997): Mapping quantitative trait loci controlling silking date in a diallel cross among four lines of maize. Theor. Appl. Genet., 95, 451-459.

□□□□ REBOURG, C., CHASTANET, M. GOUESNARD, B. WELCKER, C. DUBREUIL, P. and CHARCOSSET, A. (2003): Maize introduction into Europe: the history reviewed in the light of molecular data. Theor. Appl. Genet., 106, 895-903.

□□□□ REIF, J. C., MELCHINGER, A. E. XIA, X. C. WARBURTON, M. L. HOISINGTON, D. A. VASAL, S. K. BECK, D. BOHN, M. and FRISCH, M. (2003): Use of SSRs for establishing heterotic groups in subtropical maize. Theor. Appl. Genet., 107, 947-957.

□□□□ RIBAUT, J. M., HOISINGTON, D. A. DEUTSCH, J. A. JIANG, C. and GONZALEZDELEON, D. (1996): Identification of quantitative trait loci under drought conditions in tropical maize. 2. Flowering parameters and the anthesis-silking interval. Theor. Appl. Genet., 92, 905-914.

□□□□ ROBERTSON, D. S. (1985): A possible technique for isolating genic DNA for quantitative traits in plants. J. Theor. Biol., 117, 1-10.

□□□□ ROMERO-SEVERSON, J. (1998) : Maize microsatellite-RFLP consensus map. Maize DB (http://www.agron.missouri.edu).

□□□□ ROHLF, F. J. (1989): NTSYS-pc Numerical taxonomy and multivariate analysis system, version 2.00. New York Exeter. Publ., N. Y.

□□□□ SAGHAI-MAROOF, M. A., SOLIMAN, K. M. JORGENSON, R. and ALLARD, R. W. (1984): Ribosomal DNA spacer length polymorphisms in barley: mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. USA, 81, 8014-8018.

□□□□ SALVI, S., TUBEROSA, R. CHIAPPARINO, E. MACCAFERRI, M. VEILLET, S. BEUNINGEN, L. ISAAC, P. EDWARDS, K. and PHILLIPS, R. L. (2002): Toward positional cloning of Vgt1, a QTL controlling the transition from the vegetative to the reproductive phase in maize. Plant Mol. Biol., 48, 601-13.

□□□□ SANMIGUEL, P., TIKHONOV, A. JIN, Y. K. MOTCHOULSKAIA, N. ZAKHAROV, D. MELAKE-BERHAN, A. SPRINGER, P. S. EDWARDS, K. J. LEE, M. AVRAMOVA, Z. and BENNETZEN, J. L. (1996): Nested retrotransposons in the intergenic regions of the maize genome. Science, 274, 765-768.

□□□□ SENIOR, M. L. and HEUN, M. (1993): Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. Genome, 36, 884-889.

□□□□ SENIOR, M. L., MUROHY, M. M. GOODMAN, M. M. and STUBER, C. W. (1998): Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. Crop Sci., 38, 1088-1098.

□□□□ SHAROPOVA, N., MCMULLEN, M. D. SCHULTZ, L. SCHROEDER, S. SANCHEZ-VILLEDA, H. GARDINER, J. BERGSTROM, D. HOUCHINS, K. MELIA-HANCOCK, S. MUSKET, T. DURU, N. POLACCO, M. EDWARDS, K. RUFF, T. REGISTER, J. C. BROUWER, C. THOMPSON, R. VELASCO, R. CHIN, E. LEE, M. WOODMAN-CLIKEMAN, W. LONG, M. LISCUM, J. E. CONE, K. DAVIS, G. and COE, J. E. H. (2002): Development and mapping of SSR markers for maize. Plant Mol. Biol., 48, 463-481.

□□□□ SHULL, G. H. (1908): The composition of a field of maize. Am. Breeders' Assoc. Rep., 4, 296-301.

□□□□ SHULL, G. H. (1909): A pure-line method of corn breeding. Am. Breeders' Assoc. Rep., 5, 51-59.

□□□□ SHULL, G. H. (1910): Hybridaization methods in corn breeding. Am. Breeders' Mag., 1, 98-107.

□□□□ SINGLETON, W. R. (1946): Inheritance of indeterminate growth in maize. J. Hered., 37, 61-64.

□□□□ SMITH, J. S. C. (1988): Diversity of united states hybrid maize germplasm: isozymic and chromatographic evidence. Crop Sci., 28, 63-69.

□□□□ SMITH, J. S. C. CHIN, E. C. SHU, L. H. SMITH, O. S. WALL, S. J. SENIOR, M. L. MITCHELL, S. E. KRESOVITCH, S. and ZIEGLE, J. (1997): An evaluation of the utility of SSR loci as molecular markers in maize (Zea mays L.): comparisons with data from RFLPs and pedigree. Theor. Appl. Genet., 95, 163-173.

□□□□ SMITH, O. S. SMITH, J. S. C. BOWEN, S. L. TENBORG, R. A. and WALL, S. J. (1990): Similarities among a group of elite maize inbreds as measured by pedigree, F_1 grain-yield, grain-yield, heterosis, and RFLPs. Theor. Appl. Genet., 80, 833-840.

□□□□ SMITH, O.S. SULLIVAN, H. HOBART, B. and WALL, S. J. (2000): Evaluation of a divergent set of SSR markers to predict F_1 grain yield performance and grain yield heterosis in maize. Maydica, 45, 235-241.

□□□□ SOENGAS, P. ORDAS, B. MALVAR, R. A. REVILLA, P. and ORDAS, A. (2003): Heterotic patterns among flint maize populations. Crop Sci., 43, 844-849.

□□□□ STUBER, C. W. and GOODMAN, M. M. (1983): Allozyme genotypes for popular and histrically important inbred lines of corn, Zea mays L. USDA Agr. Res. Results, Southern. Ser., no. 16.

□□□□ STUBER, C. W. EDWARDS, M. D. and WENDEL, J. F. (1987): Molecular marker-facilitated investigations of quantitative trait loci in maize: II. factors influencing yield and its component traits. Crop Sci., 27, 639-648.

□□□□ STUBER, C. W. POLACCO, M. and SENIOR, M. L. (1999): Synergy of empirical breeding, marker-assisted selection, and genomics to increase crop yield potential. Crop Sci., 39, 1571-1583.

□□□□ SUZUKI, M. KAO, C. Y. COCCIOLONE, S. and MCCARTY, D. R. (2001): Maize *vp1* complements Arabidopsis *abi3* and confers a novel ABA/auxin interaction in roots. Plant J., 28, 409-418.

□□□□ TAKAHASHI, Y. SHOMURA, A. SASAKI, T. and YANO, M. (2001): Hd6, a rice quantitative trait locus

involved in photoperiod sensitivity, encodes the α subunit of protein kinase *CK2*. Proc. Natl. Acad. Sci. USA, 98, 7922-7927.

□□□□ TERZI, V. MORCIA, C. GORRINI, A. STANCA, A. M. SHEWRY, P. R. and FACCIOLI, P. (2005): DNA-based methods for identification and quantification of small grain cereal mixtures and fingerprinting of varieties. J. Cereal Sci., 41, 213-220.

□□□□ THORNSBERRY, J. M. GOODMAN, M. M. DOEBLEY, J. KRESOVICH, S. NIELSEN, D. and BUCKLER, E. S. (2001): Dwarf8 polymorphisms associate with variation in flowering time. Nature Genetics, 28, 286-289.

□□□□ VEGA, S. H. SAUER, M. ORKWISZEWSKI, J. A. J. and POETHIG, R. S. (2002): The early phase change gene in maize. Plant Cell, 14, 133-147.

□□□□ VELDBOOM, L. R. and LEE, M. (1996): Genetic mapping of quantitative trait loci in maize in stress and nonstress environments: II. plant height and flowering. Crop Sci., 36, 1320-1327.

□□□□ VLADUTU, C. MCLAUGHLIN, J. and PHILLIPS, R. L. (1999): Fine mapping and characterization of linked quantitative trait loci involved in the transition of the maize apical meristem from vegetative to generative structures. Genetics, 153, 993-1007.

□□□□ WALLACE, H. A. and BROWN, W. L. (1956): Corn and its early father. Michigan State Univ. Press.

□□□□ WANG, H. NUSSBAUM-WAGLER, T. LI, B. ZHAO, Q. VIGOUROUX, Y. FALLER, M. BOMBLIES, K. LUKENS, L. and DOEBLEY, J. F. (2005): The origin of the naked grains of maize. Nature, 436, 714-719.

□□□□ WANG, S. C. J. BASTEN, Z and ZENG, B. (2001-2004): Windows QTL Cartographer 2.0. Department of Statistics, North Carolina State University, Raleigh, NC. (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm).

□□□□ WASSOM, J. J. MEI, C. ROCHEFORD, J. M. and WIDHOLM, J. M. (2000): Allelic variation in candidate genes associated with QTL for the maize anther culture response. Maydica, 45, 267-276.

□□□□ WEIR, B. S. (1996): Genetic data analysis II. 2nd ed. Sinauer Associates Inc Sunderland MA.

□□□□ YAMAMOTO, T. LIN, H. X. SASAKI, T. and YANO, M. (2000): Identification of heading date quantitative trait locus Hd6 and characterization of its epistatic interactions with Hd2 in rice using advanced backcross progeny. Genetics, 154, 885-891.

□□□□ YANO, M. KATAYOSE, Y. ASHIKARI, M. YAMANOUCHI, U. MONNA, L. FUSE, T. BABA, T. YAMAMOTO, K. UMEHARA, Y. NAGAMURA, Y. and SASAKI, T. (2000): Hd1, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS*. Plant Cell, 12, 2473-2484.

次へ進む 前に戻る 目次に戻る

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to Professor Dr. YOSHIO SANO, Hokkaido University, and Dr. KEIICHI KOINUMA, National Agricultural Research Center for Hokkaido Region, for their kind guidance, reading the manuscript and making a number of helpful suggestions. I wish to thank Cooperator Dr. KAZUTOSHI OKUNO, National Institute of Agrobiological Sciences, for his kind guidance and reading the entire text in its original form. Also, I wish to thank Professor Dr. KEISUKE KITAMURA and Dr. YUJI KISHIMA, Hokakido University, for reading the entire text in its original form.
Further, my thanks are due to Mr. YASUO MIURA, Mr. HISASHI SATO, The Ministry of Agriculture, Forestry and Fisheries, and Mr. KAZUYOSHI MIKI, Nagano Chushin Agricultural Experiment Station, for their encouragement and helpful supports. Thanks are also due to Mr. TAKAO SATO, Mr. TOMOFUMI SHIINA, Mr. TAKURO NAKAMURA, Mrs. KIMIKO NAKAKO, Mrs. MINAKO SATO, Mrs. SHIZUKO KIRIKI and Mrs. RYOUKO TAKADA, National Agricultural Research Center for Hokkaido Region, for their helpful supports.

Finally, I would like to thank all the member of Plant Breeding laboratory, Corn Breeding laboratory and Grassland Operation Section in National Agricultural Research Center for Hokkaido Region for their helpful suggestions and supports.

次へ進む 前に戻る 目次に戻る

Marker-Assisted Breeding in Maize for Cold Regions of Japan

Hiroyuki ENOKI

和文要約

雑種強勢を利用した飼料用トウモロコシ育種において、育種材料内の遺伝的多様性に関する情報は、育種母材の選択、自殖系統のヘテロティックグループへの系列分けおよび組合せ能力検定試験のテスター選択に不可欠である。さらに、育成自殖系統と代表的な自殖系統との近縁関係に関する情報は、育成自殖系統内の遺伝的多様性の評価および新たな育種材料の導入の検討に利用できる。また、外国や温暖地域で育成された育種材料の導入は、寒地での育種材料の遺伝的多様性の拡大および改良に有効な方法である。しかし、ヨーロッパF_1品種由来自殖系統は、デント種およびフリント種の遺伝子が混在するため、ヘテロティックグループへの系列分けが難しい。また、寒地での育種材料として温暖地域育成デント種の導入には、開花期の改良が必要であるものの、デント種には優れた早生系統が極めて少ない。そのため、フリント種から早生性を導入することが望まれている。

本研究では、DNAマーカーによる近縁度解析により、寒地適応型トウモロコシ自殖系統の近縁関係を解明するとともに、新たな優良育種材料を効率的に導入するため、DNAマーカーによるヨーロッパF_1品種由来自殖系統の効率的系列分け法および温暖地域育成デント種自殖系統の開花期の効率的改良法を開発することを目的とした。

１） SSRマーカーによる北海道育成寒地適応型トウモロコシ自殖系統間の近縁度解析

寒地適応型トウモロコシ自殖系統間の近縁関係を明らかにするため、染色体上に偏り無く分布する60個のSSR（Simple Sequence Repeat）マーカーを選定した。これらのSSRマーカーは、遺伝的多様性の指標であるPolymorphic-index contentの平均値が0.69と、これまでの報告より高い値を示した。また、このSSRマーカーセットにより推定した自殖系統間の近縁度と系譜に基づき算出した近交係数との間に有意な相関関係を示した（r =0.70**）。以上の結果から、このSSRマーカーセットは自殖系統間の近縁関係の解析に利用できると考えられた。自殖系統間の近縁関係を明らかにするため、推定した近縁度を用い、樹形図（UPGMA）、Principal-coordinate analysisおよび平均近縁度による解析を行った。いずれの解析方法でも、北海道で育成されたデント種自殖系統とフリント種自殖系統は明確に分類された。さらに、代表的な自殖系統との近縁関係では、北海道育成北方型フリント種およびデント種自殖系統は、カナダ育成北方型フリント種CO12およびBSSS系列デント種とそれぞれ近縁であった。しかし、北海道育成の両系列の遺伝的多様性は、代表的なデント種自殖系統より低かった。また、北海道育成自殖系統は、ヨーロッパフリント種自殖系統F2やLSC系列デント種およびカナダ育成デント種と比較的遠縁であった。したがって、これらの系列に属する育種材料の導入は、北海道の育種材料の遺伝的多様性の拡大および改良に有効であると考えられた。ヨーロッパF_1品種由来自殖系統の近縁度解析の結果は、これまでの育種経験と一致していたことから、このSSRマーカーセットは、これらの自殖系統の系列分けに有効な方法であると結論された。

２） ヨーロッパF 品種由来自殖系統の系列分けに有効なSSRマーカーセットの選定

本研究に供試した60個のSSRマーカーによる近縁度解析は、１）の結果からヨーロッパＦ$_1$品種由来自殖系統　（混在型自殖系統）　の系列分けに有効であることが示された。本実験では、より簡便な系列分け法を開発するため、両系列間での多型の出現頻度の差　（40%および50%）　に基づき、２つのSSRマーカーセット（セット１およびセット２）を選定した。セット１およびセット２のSSRマーカー数は、それぞれ25個および14個であった。これらのセットの多型数も60個のSSRマーカーに比べ、38%および21%と大幅に少なく、60個のSSRマーカーより簡便な解析が可能だと考えられた。これらのSSRマーカーセットによる系列分けの精度を評価するため、混在型32自殖系統における系列分けの結果を比較検討した。その結果、セット１による系列分けは、60個のSSRマーカーに基づく結果にほぼ一致した。セット２による系列分けは、60個のSSRマーカーに基づく結果とは若干異なる様相を示した。セット１の系列分けでは、混在型4自殖系統による組合せ能力検定試験の結果ともほぼ一致していたことから、SSRマーカーセット１による系列分けは、育種的利用の観点から十分な精度を有していると考えられた。

３）北方型フリント種の早生性に関するQTL解析

フリント種自殖系統の早生性を、効率的にデント種自殖系統に導入するため、早生性に関する選抜マーカーの開発を目的として実験を行った。本実験では、北方型フリント種の早生系統To85とデント種の晩生系統Mi29の交雑後代Ｆ$_{2:3}$150系統を供試した。MaizeGDBに登録されている110個のSSRマーカー、イネの開花期関連遺伝子の相同遺伝子である*ck2α*の遺伝子内変異を識別するCAPSマーカーおよびトウモロコシで開花期への影響が示唆されている*an1* ,*d8* および*vp1* の遺伝子内変異をそれぞれ識別するInDelマーカーを用い、全長1287cM、平均遺伝距離12.4cMの連鎖地図を作成した。QTL解析は、LOD値の閾値を3.6に設定し、Composite interval mappingにより絹糸抽出期、雄穂開花期、稈長および着雌穂高について行った。その結果、開花期に関する合計７つQTLを、第１染色体、第３染色体、第８染色体および第９染色体に検出した。*an1*　遺伝子および*epc*　遺伝子の連鎖マーカーbnlg1067の近傍にそれぞれ開花期に関するQTLを検出した。本実験で検出した開花期に関するQTLについては、いずれも稈長および着雌穂高への大きな影響が認められなかった。以上の結果から、本実験で検出した開花期に関するQTLは、温暖地域育成デント種自殖系統の開花期の改良に利用できるものと考えられた。また、これらの情報は、早生化に関わるQTLの候補遺伝子を選択する際にも有効であると考えられた。

本研究では、DNAマーカーを利用して寒地適応型トウモロコシ自殖系統内の遺伝的多様性の解明および拡大に有効な方法を開発した。これらの方法は、遺伝的多様性の経時的調査、自殖系統のプロファイリングおよび優良な育種材料　（ヨーロッパＦ$_1$品種および温暖地域育成デント種自殖系統）　の導入に利用できる。これらのDNAマーカーを利用した選抜技術の利用は、寒地適応型飼料用トウモロコシ育種材料の遺伝的多様性の拡大および優良遺伝子の集積を促進し、寒地適応型優良Ｆ$_1$品種育成に大きく寄与することが期待される。

前に戻る　目次に戻る

Table 1. Derivation and group of 65 inbred lines used in SSR analysis

Inbred line	Derivation	Group[1)]
Flint inbred lines bred in the cold regions of Japan (n=17)		
Ho11	Mutu	N. flint
Ho20	N206×N28	N. flint
Ho22	Mutu	N. flint
N19	Sakashita	N. flint
N21	Sakashita	N. flint
N85	Iwanai zairai A	N. flint
N138	Kiwase	N. flint
N146	Iwanai zairai B	N. flint
To15	Kiwase×(Yamamotoshu, Sakashita)	N. flint
To38	Kiwase×(Yamamotoshu, Sakashita)	N. flint
To39	Kiwase×(Yamamotoshu, Sakashita)	N. flint
To77	To15×To31	N. flint
To79	To15×To31	N. flint
To82	To15×To36	N. flint
To85	To15×To31	N. flint
To88	To9×To15	N. flint
To90	To15×To36	N. flint
Dent inbred lines bred in the cold regions of Japan (n=15)		
Ho32	Pioneer 3747	Dent
Ho40	Pioneer 3790	Dent
Ho43	Pioneer 3732	Dent
Ho45	Pioneer 3747	Dent
Ho47	Pioneer 3747	Dent
Ho52	Pioneer 3732	Dent
Ho54	Pioneer 3790	Dent
Ho57	Pioneer 3389	Dent
Ho60	Pioneer 3790	Dent
Ho68	DK403	Dent
Ho72	4332	Dent
Ho77	Pioneer 3897	Dent
Ho78	X9232	Dent
Ho79	N4545	Dent
To92	Pioneer 3747	Dent
Representative flint inbred lines (n=3)		
CO12	unknown, Canada-	N. flint
F2	Lacaune population	E. flint
F283	F227×F228 (50%F7)	E. flint
Representative dent inbred lines (n=11)		
A619	(A171×Oh43)×Oh43	LSC (Oh43)
A679	B73×A662	BSSS
B73	Iowa SSS C5 Sel.	BSSS
C103	Lancaster Surecrop	LSC (C103)
CM37	KE3	LSC
CMV3	A21×W185	LSC
CO158	Dekalb 46	Dent
H99	Illinois Syn. 60C	LSC
Mo17	CI187.2×C103	LSC (C103)
Oh43	Oh40B×W8	LSC (Oh43)
W79A	Minn. 13	M13
Miscellaneous origin (n=19)		
Ho3	INRA258	
Ho4	INRA258	
Ho34	(N85×Ho11)×Oh545	
Ho36	(Ho4×Ho3)×N204	
Ho37	(Ho4×Ho3)×W452	
Ho42	(Ho4×Ho3)×N204	
Ho49	N85×Ho4	
Ho50	N85×Ho4	
Ho59	PH1407	
Ho63	EXP736	
Ho64	EXP736	
Ho65	SH6061	
Ho66	SH7329	
Ho67	SH7329	
Ho73	Astrid	
Ho81	LG2080	
To113	DEA	
To132	LG2266	
To133	LG2266	

[1)] N. flint, E. flint and dent stand for Northern flint, European flint, and inbred line developed from dent hybrid, respectively.

Table 2. Allele numbers and PIC values for SSR loci found in 65 maize inbred lines

SSR locus	No. chr.[1)]	cM[1)]	Repeat class[1)]	No. alleles	PIC value
phi056	1	6.1	3	4	0.69
bnlg1007	1	39.7	2	11	0.86
phi001	1	60.7	2	14	0.90
bnlg1273	1	85.1	2	8	0.75
bnlg1564	1	111.2	2	9	0.84
bnlg1597	1	135	2	2	0.42
phi120	1	150.5	3	4	0.64
bnlg1017	2	25.7	2	10	0.84
bnlg469	2	48.9	-	5	0.67
phi083	2	80.9	4	6	0.66
nc003	2	89.3	2	12	0.82
phi127	2	106.1	4	4	0.62
bnlg1520	2	130	2	9	0.72
umc1057[2)]	3	-	3	2	0.42
bnlg1523[2)]	3	27	2	8	0.48
phi036	3	38.8	2	7	0.52
phi053	3	51.4	4	4	0.63
bnlg197	3	71.9	-	12	0.89
phi046	3	82.2	4	2	0.41
phi047	3	103.7	3	3	0.62
phi072	4	14.4	4	4	0.47
phi021	4	39.1	2	12	0.76
bnlg252	4	68.3	-	5	0.58
bnlg2291	4	77.4	2	6	0.67
bnlg1444	4	98	2	17	0.91
bnlg1565	4	124.7	2	6	0.75
phi024	5	21.6	3	5	0.67
phi008	5	50.2	3	3	0.40
dupssr10	5	71.7	2	15	0.84
bnlg1237	5	95.3	2	5	0.41
bnlg1695	5	122.7	2	12	0.88
bnlg389	5	144.5	-	6	0.62
phi126	6	4.9	2	7	0.81
bnlg249	6	17	-	11	0.79
bnlg1371	6	22.8	2	10	0.76
nc010	6	42.2	4	4	0.61
phi070	6	91.3	5	4	0.54
phi123	6	102.5	4	3	0.50
bnlg2132	7	13.1	2	7	0.59
umc1066	7	35.5	6	5	0.49
bnlg657	7	52.7	-	9	0.79
bnlg434	7	60.5	-	7	0.75
dupssr13	7	87.4	2	8	0.67
phi116	7	113.8	-	4	0.71
bnlg2235	8	26	2	11	0.84
bnlg125	8	35.9	2	4	0.66
bnlg162	8	55	-	6	0.76
bnlg1152	8	66.7	2	10	0.80
bnlg1823	8	85.7	2	9	0.77
phi080	8	112.1	5	5	0.67
phi028	9	19.4	3	3	0.49
bnlg1401	9	33.3	2	9	0.75
phi065	9	45.6	5	4	0.50
bnlg1270	9	69.8	2	6	0.76
bnlg1525	9	88.1	2	11	0.79
phi041	10	0	4	5	0.78
bnlg1451	10	30.3	2	12	0.85
bnlg210	10	46.9	-	7	0.57
bnlg1518	10	55.1	2	10	0.83
bnlg1250	10	67.3	2	10	0.83

[1)] Loci and repeat class were referred from MaizeGDB and the SSR Consensus 1998 (Romero-Severson 1998).

[2)] umc1057 and bnlg1523 assigned to bin 3.02 and bin 3.03 were referred from MaizeGDB.

Table 3. Summarized information score statistics in repeat class

Repeat class	Mean No. alleles	Mean PIC value	PIC standard error
2	9.3	0.74	0.02
3	3.4	0.56	0.05
4	4.0	0.59	0.04
5	4.0	0.57	0.05
6	5.0	0.49	-
3～6	3.9	0.57	0.03

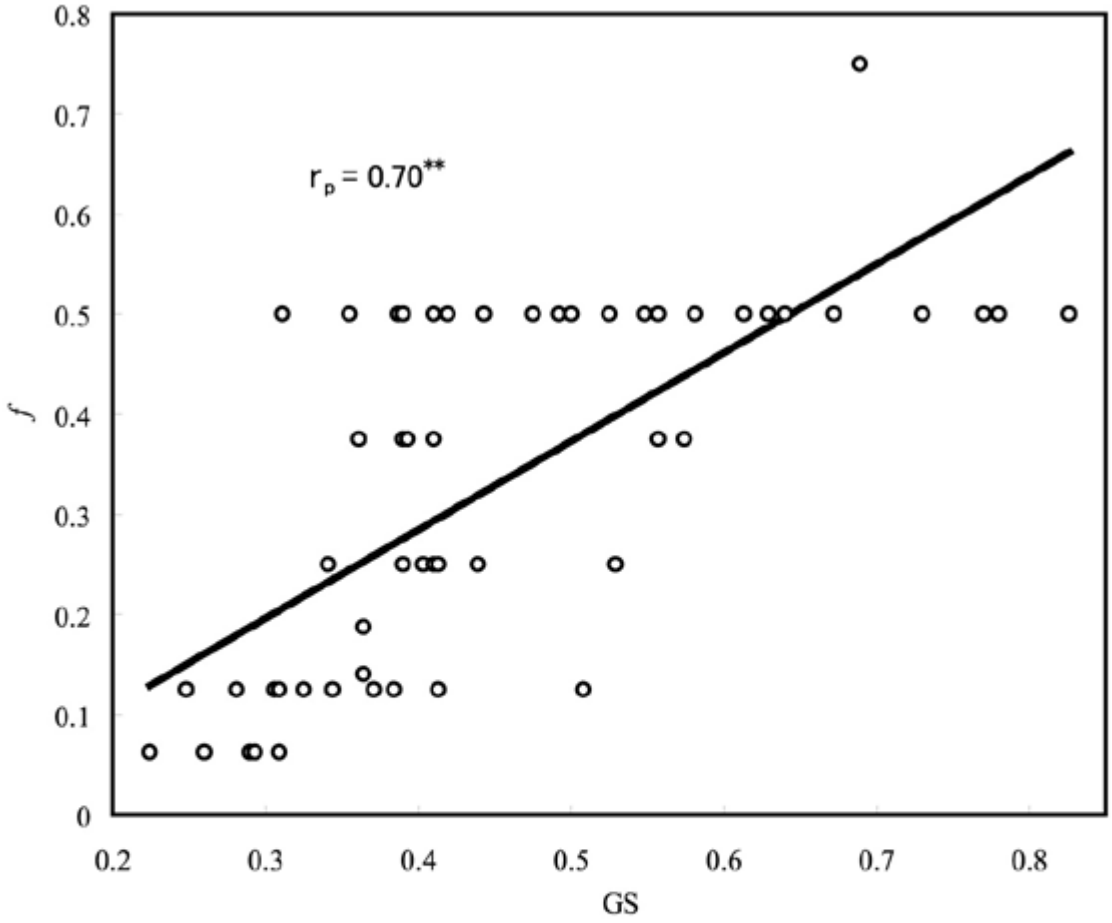

Fig. 1. Correlation between Malecot ancestory coefficient (f) and genetic similarity (GS) calculated from 60 SSR data[1)]

[1)] The f and the GS estimates for 56 pairs among inbred lines with a pedigree record ranged from 0.063 to 0.750 and from 0.224 to 0.826, when the unrelated pairs of inbred lines were excluded.

Table 4. Mean genetic similarities (GS) within and among groups calculated from the 60 SSR data[1)]

Group	SSR-based mean GS with		
	Representative dent inbred lines (n=11)	Dent inbred lines bred in Hokkaido (n=15)	Northern flint inbred lines bred in Hokkaido (n=17)
Representative dent inbred lines	0.289	0.295	0.284
Dent inbred lines bred in Hokkaido		0.405	0.273
Northern flint inbred lines bred in Hokkaido			0.424

[1)] The mean genetic similarity estimates of 520 unrelated pairs among the 26 dent and 20 flint inbred lines (GS_{MUR}) was 0.271.

Table 5. Mean genetic similarities (GS) between the representative inbred lines and the inbred lines bred in Hokkaido calculated from the 60 SSR data[1)]

Representative inbred line	Group	SSR-based mean GS with	
		Dent inbred lines bred in Hokkaido (n=15)	Northern flint inbred lines bred in Hokkaido (n=17)
C103	LSC (C103)	0.321	0.266
Mo17	LSC (C103)	0.307	0.341
Oh43	LSC (Oh43)	0.283	0.289
A619	LSC (Oh43)	0.228	0.242
CM37	LSC (Canada)	0.256	0.323
CMV3	LSC (Canada)	0.249	0.279
H99	LSC	0.313	0.305
W79A	M13	0.291	0.272
B73	BSSS	0.348	0.257
A679	BSSS	0.342	0.294
CO158	dent	0.311	0.251
F2	European flint	0.232	0.298
F283	European flint	0.195	0.381
CO12	Northern flint	0.262	0.452

[1)] The mean genetic similarity estimates of 520 unrelated pairs among the 26 dent and 20 flint inbred lines (GS_{MUR}) was 0.271.

Table 6. Mean, minimum, and maximum of genetic similarities (GS) for unrelated pairs between the miscellaneous inbred lines and the dent or flint inbred lines calculated from 60 SSR data[1,2)]

		SSR-based GS with					
		Dent inbred lines (n=26)			Flint inbred lines (n=20)		
Inbred line	Derivation	Mean	Min	Max	Mean	Min	Max
Ho3	INRA258	0.257	0.131	0.361	0.247	0.163	0.361
Ho4	INRA258	0.227	0.131	0.311	0.238	0.179	0.325
Ho34	(N85×Ho11)×Oh545	0.257	0.182	0.364	0.383	0.217	0.529
Ho36	(Ho4×Ho3)×N204	0.284	0.197	0.377	0.343	0.295	0.407
Ho37	(Ho4×Ho3)×W452	0.280	0.180	0.377	0.240	0.197	0.295
Ho42	(Ho4×Ho3)×N204	0.239	0.163	0.341	0.261	0.179	0.339
Ho49	N85×Ho4	0.269	0.161	0.368	0.262	0.161	0.387
Ho50	N85×Ho4	0.298	0.180	0.423	0.271	0.195	0.358
Ho59	PH1407	0.351	0.228	0.488	0.285	0.210	0.348
Ho63	EXP736	0.333	0.210	0.455	0.280	0.211	0.371
Ho64	EXP736	0.356	0.222	0.496	0.329	0.270	0.416
Ho65	SH6061	0.298	0.149	0.430	0.304	0.180	0.430
Ho66	SH7329	0.296	0.198	0.430	0.354	0.262	0.430
Ho67	SH7329	0.303	0.195	0.410	0.360	0.309	0.426
Ho73	Astrid	0.283	0.148	0.393	0.333	0.246	0.426
Ho81	LG2080	0.312	0.224	0.406	0.377	0.301	0.621
To113	DEA	0.294	0.213	0.397	0.329	0.231	0.628
To132	LG2266	0.312	0.228	0.439	0.280	0.230	0.359
To133	LG2266	0.332	0.240	0.468	0.306	0.226	0.372

[1)] Standard error of estimated GS ranged from 0.04 and 0.06

[2)] The mean genetic similarity estimates of 520 unrelated pairs among 26 dent and 20 flint inbred lines (GS_{MUR}) was 0.271.

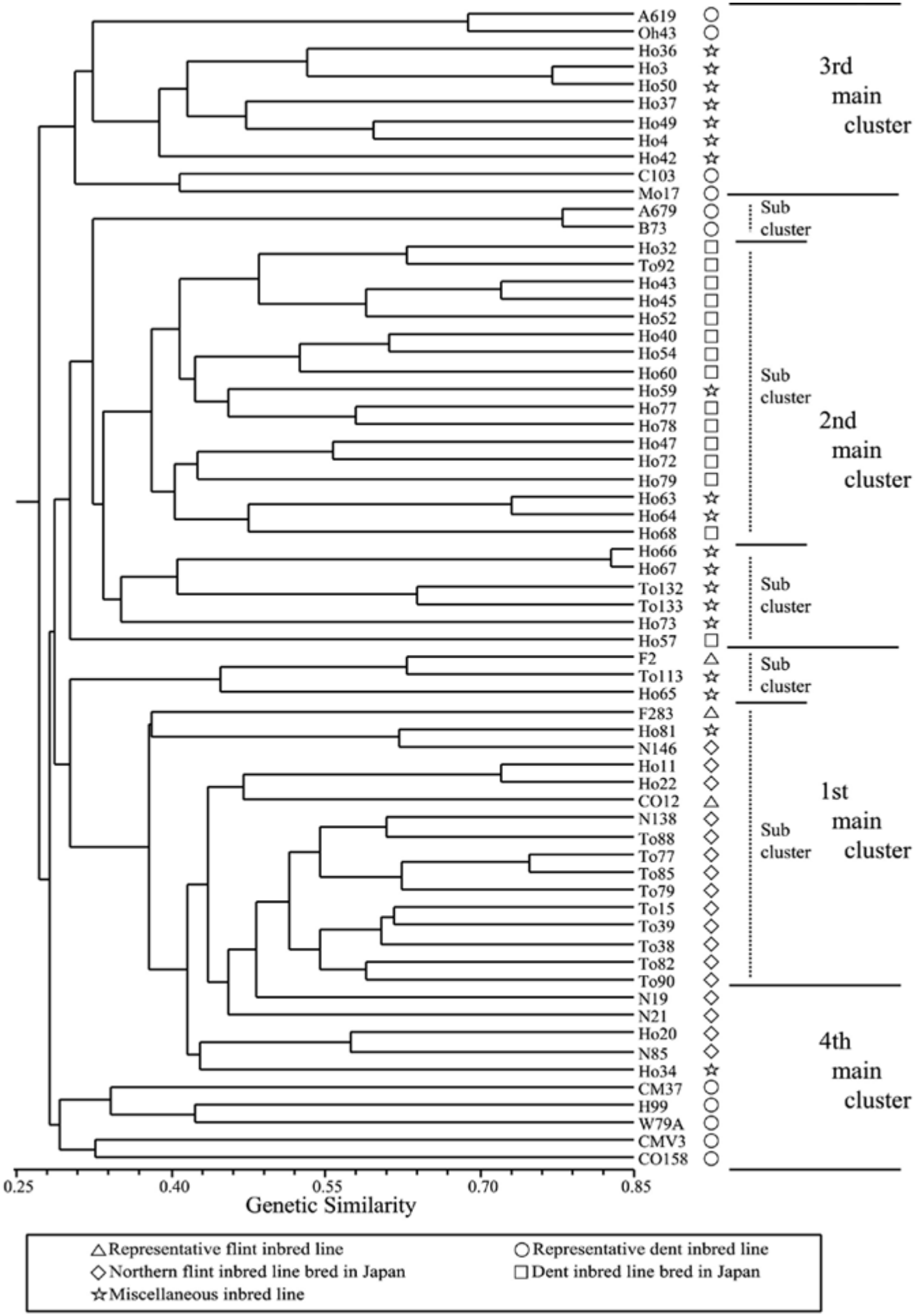

Fig. 2. Dendrogram constructed with a Unweighted Paired Group Method Using Arithmetic Average (UPGMA) clustering algorithm from the pairwise matrix of genetic similarities (GS) among the 65 maize inbreds

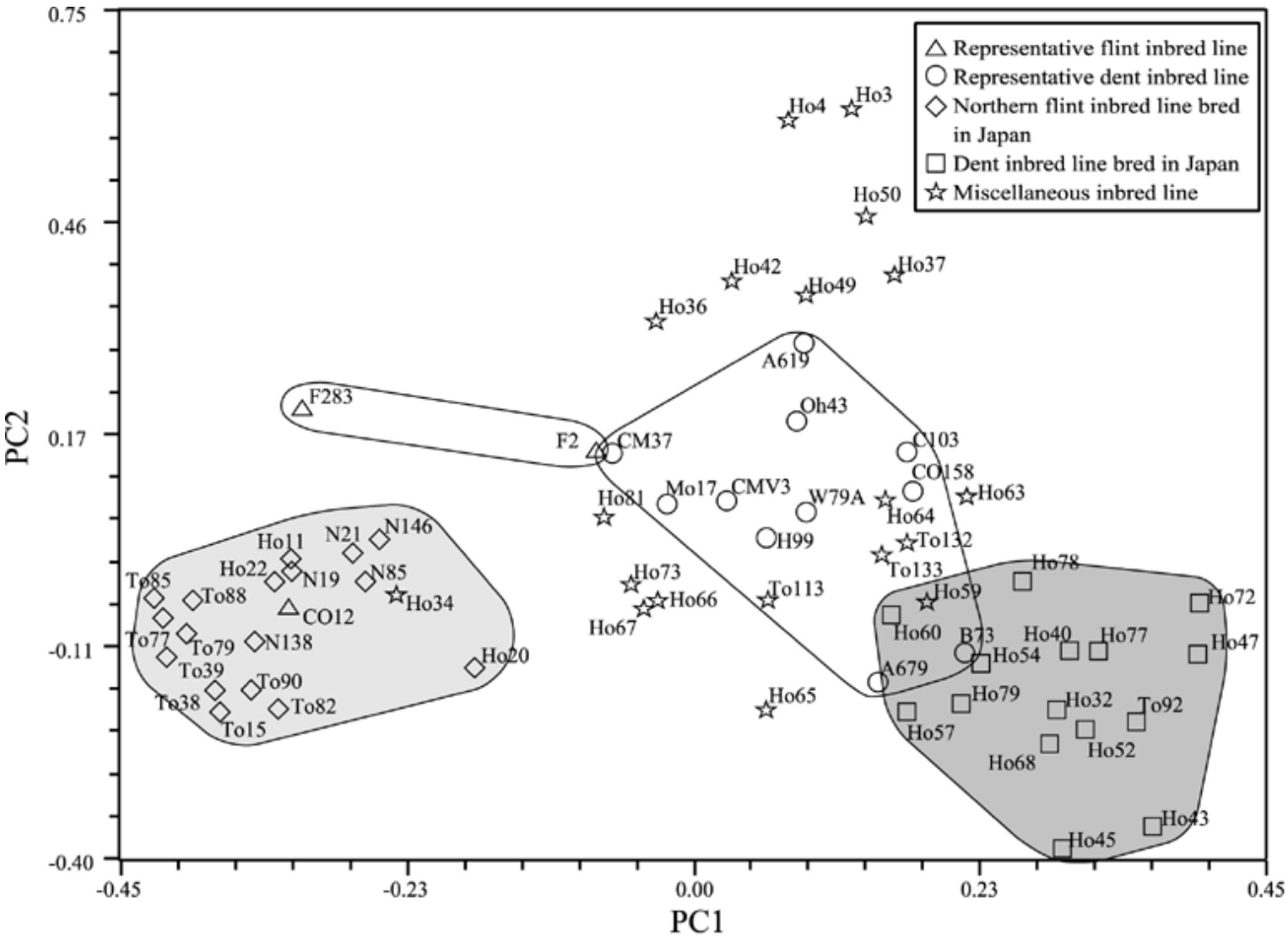

Fig. 3. Associations among the 65 maize inbred lines revealed by principal coordinate analysis (PCOA) performed on genetic similarities (GS) calculated from the 60 SSR data

Table 7. SSR loci and alleles with frequency difference above 0.40 (Set 1) and 0.50 (Set 2) between dent and flint groups[1)]

Locus[2)]	Chr.	cM[3)]	Type[4)]	Frequency		Locus[2)]	Chr.	cM[3)]	Type[4)]	Frequency	
				Dent	Flint					Dent	Flint
phi056*	1	6.1	D	0.54	0.00	nc010*	6	42.2	D	0.74	0.00
			F	0.09	0.90				F	0.20	0.81
bnlg1007	1	39.7	F	0.00	0.43	phi123	6	102.5	D	0.57	0.14
bnlg1273*	1	85.1	F	0.09	0.81				F	0.40	0.86
bnlg1564	1	111.2	D	0.49	0.00	umc1066*	7	35.5	D	0.46	0.00
			F	0.09	0.57				F	0.34	0.90
bnlg1597*	1	135.0	D	0.66	0.00	phi116*	7	113.8	F	0.14	0.71
			F	0.34	1.00	bnlg1152	8	66.7	D	0.49	0.00
phi120	1	150.5	D	0.43	0.00				F	0.03	0.52
bnlg1017	2	25.7	D	0.46	0.05	phi080	8	112.1	F	0.17	0.62
phi083	2	80.9	D	0.57	0.14	phi028	9	19.4	F	0.03	0.48
bnlg1520*	2	130.0	F	0.23	0.76	bnlg1401*	9	33.3	D	0.69	0.00
phi024*	5	21.6	D	0.69	0.10	phi065	9	45.6	F	0.40	0.81
bnlg389*	5	144.5	D	0.46	0.05	bnlg1525*	9	88.1	F	0.06	0.71
			F	0.29	0.90	bnlg1518*	10	55.1	F	0.06	0.67
bnlg249*	6	17.0	D	0.63	0.05	bnlg1250*	10	67.3	D	0.51	0.05
			F	0.00	0.62				F	0.06	0.71
bnlg1371	6	22.8	F	0.14	0.57						

[1)] All the SSR loci listed were included in Set 1; * indicates SSR loci of Set 2.

[2)] Loci were designated based on MAIZE GDB and the SSR Consensus 1998 (Romero-Severson 1998).

[3)] Values indicate the centimorgan from the short arm edge.

[4)] D and F stand for the dent and the flint alleles with frequency differences above 0.40, respectively.

Table 8. Mean genetic similarities (GS) between the miscellaneous inbred lines and the dent (GS-D) or flint (GS-F) inbred lines estimated by using the original fullset, Set 1, and Set 2 of SSR loci

Inbred line	Full set of SSR loci				Set 1 SSR loci				Set 2 SSR loci			
	GS-D (n=35)	GS-F (n=21)	t-test[1)]	Assign-ment[2)]	GS-D (n=35)	GS-F (n=21)	t-test[1)]	Assign-ment[2)]	GS-D (n=35)	GS-F (n=21)	t-test[1)]	Assign-ment[2)]
Ho34	0.25	0.37	***	F	0.17	0.39	***	F	0.19	0.48	***	F
Ho59	0.35	0.27	***	D	0.32	0.23	***	D	0.31	0.22	***	D
Ho62	0.32	0.30		I	0.25	0.29		I	0.30	0.22	**	D
Ho63	0.33	0.27	***	D	0.35	0.21	***	D	0.33	0.16	***	D
Ho64	0.34	0.32		I	0.31	0.29		I	0.27	0.28		I
Ho65	0.29	0.29		I	0.24	0.27		I	0.27	0.29		I
Ho66	0.28	0.34	***	F	0.26	0.41	***	F	0.27	0.53	***	F
Ho67	0.28	0.34	***	F	0.27	0.39	***	F	0.28	0.46	***	F
Ho73	0.29	0.32		I	0.29	0.32		I	0.29	0.33		I
Ho75	0.30	0.33		I	0.26	0.33	***	F	0.27	0.37	***	F
Ho76	0.30	0.36	***	F	0.29	0.38	***	F	0.21	0.42	***	F
Ho81	0.30	0.36	***	F	0.25	0.37	***	F	0.22	0.37	***	F
Ho82	0.32	0.31		I	0.29	0.28		I	0.33	0.27		I
Ho83	0.29	0.27		I	0.27	0.25		I	0.29	0.24		I
Ho84	0.28	0.33	**	F	0.22	0.33	***	F	0.18	0.34	***	F
Ho85	0.25	0.29		I	0.28	0.39	***	F	0.28	0.43	***	F
Ho86	0.29	0.28		I	0.27	0.22		I	0.26	0.24		I
To48	0.25	0.47	***	F	0.17	0.56	***	F	0.16	0.70	***	F
To97	0.30	0.32		I	0.23	0.32	***	F	0.25	0.33	**	F
To105	0.20	0.44	***	F	0.13	0.57	***	F	0.11	0.60	***	F
To106	0.23	0.30	***	F	0.19	0.38	***	F	0.19	0.48	***	F
To112	0.31	0.28		I	0.33	0.25	**	D	0.35	0.24	**	D
To113	0.29	0.31		I	0.26	0.28		I	0.31	0.28		I
To115	0.26	0.25		I	0.23	0.25		I	0.30	0.24		I
To117	0.25	0.26		I	0.23	0.27		I	0.25	0.24		I
To119	0.22	0.25		I	0.22	0.29	**	F	0.27	0.27		I
To125	0.27	0.37	***	F	0.21	0.40	***	F	0.23	0.47	***	F
To127	0.24	0.42	***	F	0.20	0.52	***	F	0.15	0.71	***	F
To128	0.25	0.39	***	F	0.21	0.48	***	F	0.15	0.67	***	F
To131	0.33	0.29		I	0.29	0.28		I	0.23	0.32	***	F
To132	0.32	0.27	**	D	0.29	0.24	**	D	0.21	0.29	***	F
To133	0.34	0.29	**	D	0.32	0.27	**	D	0.34	0.27	**	D

[1)] *** and ** stand for the significance of difference between the values of GS-D and GS-F at the 0.001 and 0.01 levels, respectively.

[2)] D, F, and I indicate dent, flint, and intermediate type.

Table 9. Dry matter yield and days to silking in the crosses of the 4 miscellaneous inbred lines with 3 dent and 3 flint tester inbred lines[1,2)]

	Tester group	Ho83	Ho86	To119	To132	Mean[3)]
Dry matter yield (kg/a)	Dent	157	166	177 >*	169	167
	Flint	159	170	157	161	162
Days to silking (d)	Dent	80.7 >*	82.3	80.3	81.7 >*	81.3
	Flint	78.7	82.0	81.3	80.0	80.5

[1)] Least significant difference (p =0.05) and coefficient of variance are 15 kg/a and 4.5% for dry matter yield, and 1.3 days and 0.8% for days to silking, respectively. * indicates significance at the 0.05 level.

[2)] Each value is the mean of 3 crosses of the miscellaneous inbred lines with dent or flint tester inbred lines.

[3)] Means of the crosses of the dent and flint testers with the 4 miscellaneous inbred lines.

Table 10. Means and ranges for flowering time and plant stature of parents, F_1, and $F_{2:3}$ lines from the cross of Mi29 and To85

	Trait[1)]			
	SLK	POL	PH	EH
	GDD °C	GDD °C	cm	cm
Means				
To85	446.3	442.3	170.5	48.3
Mi29	630.4	635.3	193.1	83.9
F_1	485.4	485.4	191.1	78.0
$F_{2:3}$ lines	527.9	504.9	172.9	61.3
Range				
$F_{2:3}$ lines	458.2-606.2	458.2-567.0	136.5-201.1	37.0-84.8
C.V.[2)]	2.5%	1.9%	3.7%	7.4%

[1)] SLK, POL, PH, and EH stand for the growing degree days to silking, growing degree days to anthesis, plant height, and ear height, respectively.

[2)] C.V. stands for coefficients of variance.

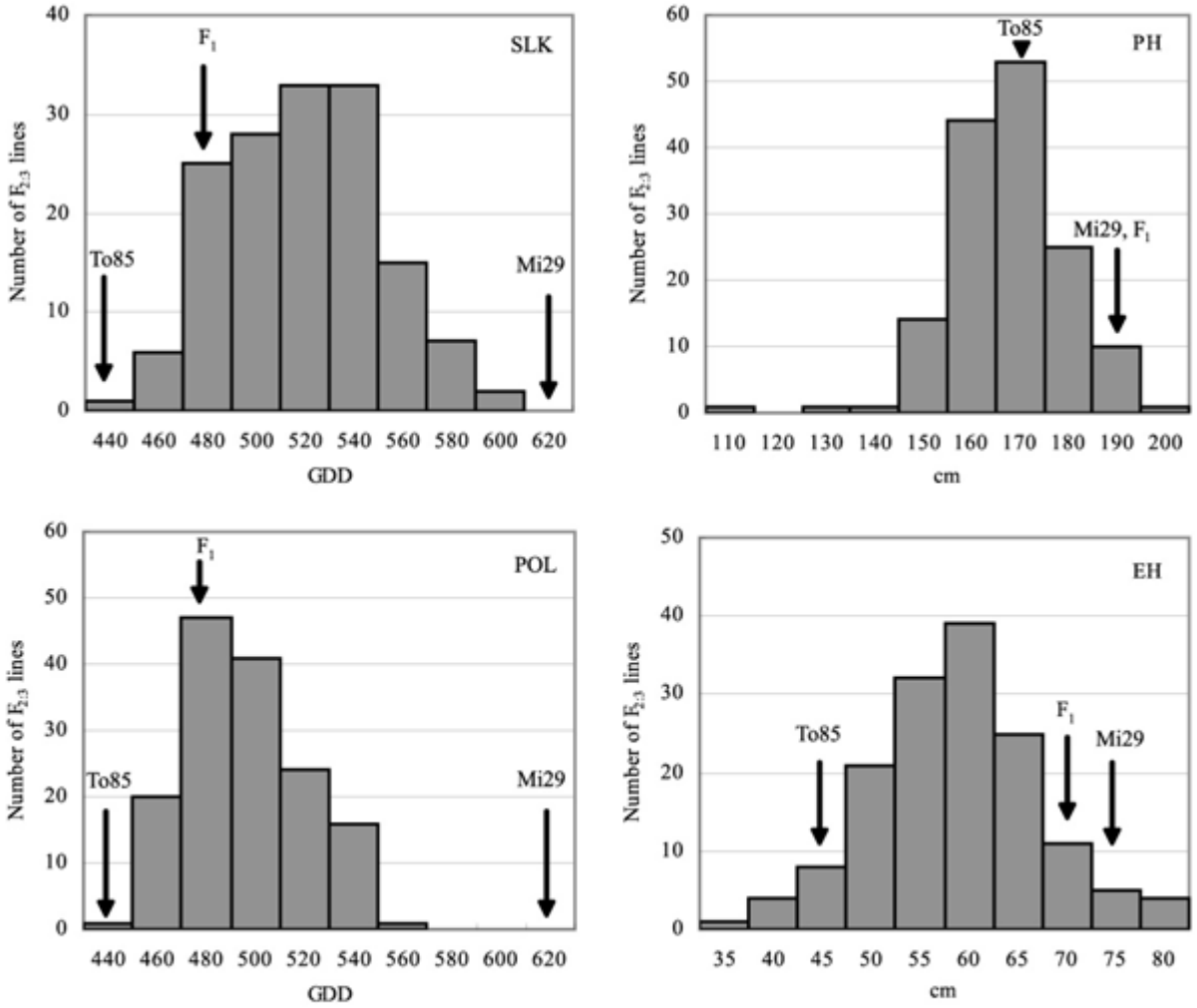

Fig. 4. Distribution of SLK, POL, PH, and EH in segregation of the $F_{2:3}$ lines from the cross of Mi29 and To85 [1)]

1) SLK, POL, PH, and EH stand for the growing degree days to silking, growing degree days to pollen shedding plant height, and ear height, respectively.

Table 11. Correlations among flowering time and plant stature in 150 $F_{2:3}$ lines[1,2)]

	POL	PH	EH
SLK	0.82**	0.31**	0.14
POL		0.36**	0.41**
PH			0.35**

[1)] SLK, POL, PH, and EH stand for the growing degree days to silking, growing degree days to anthesis, plant height, and ear height, respectively.

[2)] * and ** show significance at the 0.05 and 0.01 levels, respectively.

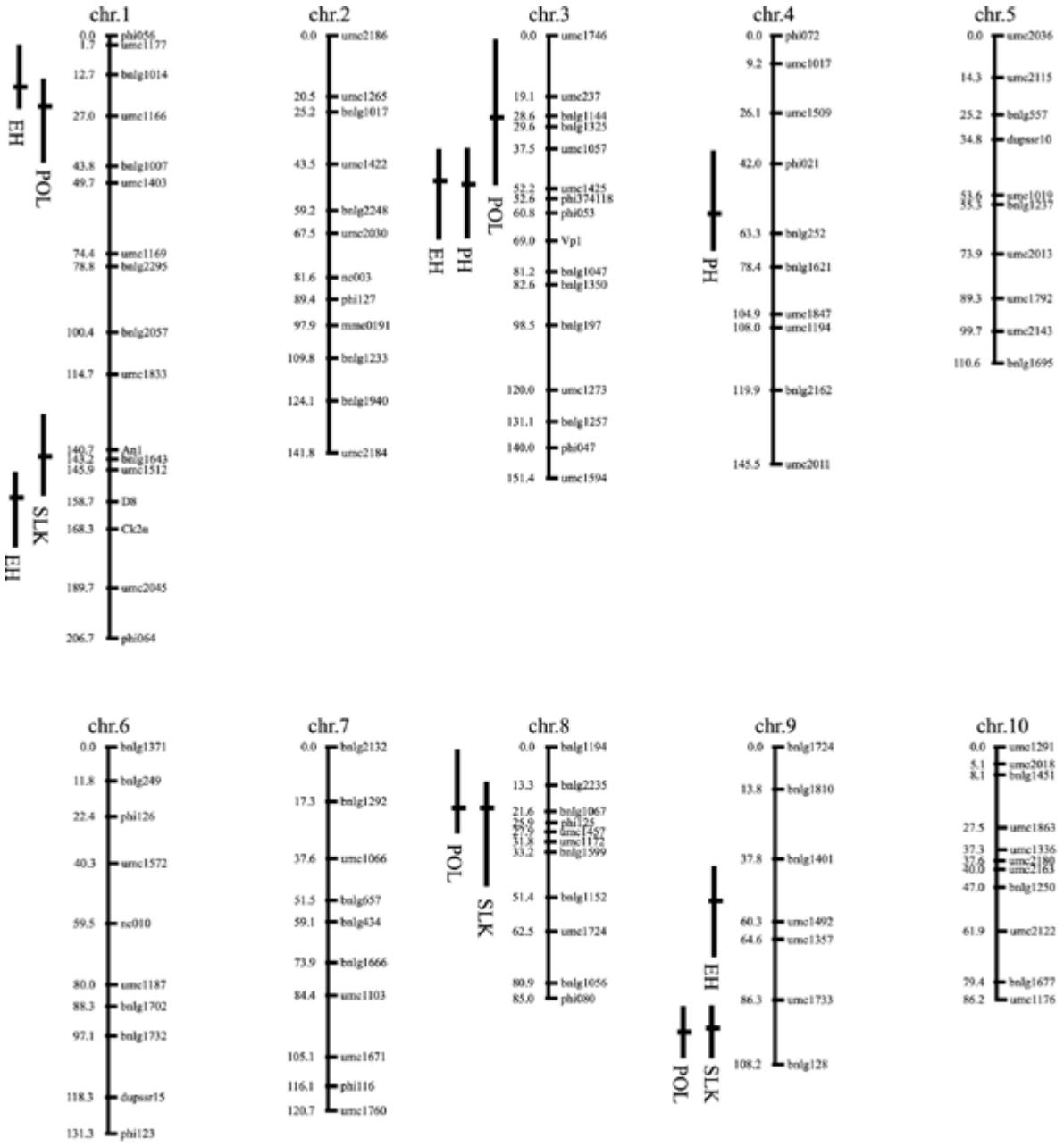

Fig. 5 Genetic linkage map for Mi29xTo85 $F_{2:3}$ lines and chromosome position of QTLs for SLK, POL, PH, and EH
Positions of DNA marker loci are given in cM to the right of the linkage groups relative to the first locus (position 0.0) in each chromosome. QTL positions for the $F_{2:3}$ lines are indicated on the left of the linkage groups. One-LOD support intervals (LOD > 3.6) are indicated by vertical bars, with the position of the maximum LOD peak indicated by horizontal bars. SLK, POL, PH, and EH stand for the growing degree days to silking, growing degree days to pollen shedding, plant height, and ear height, respectively.

Table 12. Genomic locations, percentages of phenotypic variation, and genetic effects of QTLs for flowering time and plant stature detected for 150 $F_{2:3}$ lines[1)]

Chr.	QTL position (cM)	Nearest locus	LOD score	Genetic effect[2)]			Gene action[3)]	Direction[4)]	Phenotypic variance[5)](%)
				a	d	d/a			
SLK				GDD					
1	143.2	bnlg1643	5.3	-11.1	6.1	-0.55	PD	Mi29	7.0
8	19.3	bnlg1067	12.4	-27.7	-20.4	0.74	PD	Mi29	36.0
9	98.3	bnlg128	4.8	-12.3	-1.3	0.11	A	Mi29	4.9
								total	48.6
POL				GDD					
1	24.7	umc1166	11.7	-18.5	-1.9	0.10	A	Mi29	27.9
3	28.6	bnlg1144	9.3	-14.9	3.1	-0.21	PD	Mi29	18.9
8	19.3	bnlg1067	8.3	-15.1	-10.2	0.68	PD	Mi29	18.9
9	100.3	bnlg128	4.1	-10.0	-9.7	0.97	D	Mi29	8.1
								total	63.7
PH				cm					
3	51.5	umc1425	9.7	-4.2	8.1	-1.93	OD	Mi29	8.0
4	58.0	bnlg252	9.7	7.1	3.5	0.49	PD	To85	23.0
								total	42.7
EH				cm					
1	18.7	bnlg1014	4.3	-3.1	2.5	-0.80	D	Mi29	6.2
1	157.9	D8	8.4	5.2	4.2	0.81	D	To85	18.8
3	49.5	umc1425	7.2	-3.4	4.1	-1.20	OD	Mi29	8.1
9	53.8	umc1492	5.9	4.6	2.3	0.51	PD	To85	14.9
								total	52.5

[1)] SLK, POL, PH, and EH stand for the growing degree days to silking, anthesis, and plant stature, respectively.

[2)] Additive effects are associated with the allele from To85. A negative value means that the To85 allele decreases the value of the trait.

[3)] Gene action is determined from the ratio d/a. A, PD, D and OD stand for additive, partial dominant, dominant, and over dominant, respectively.

[4)] Direction of response is the parent whose additive value of a marker allele decreased the value of the trait

[5)] Totals are the percentage of phenotypic variation accounted for in the multiple QTL model.

Project

Community

B73 Assembly Tools
Genome Browser
Locus Lookup
Go!
Locus Pair Lookup
Downloads
more...

Other Tools
BLAST
Bin Viewer
1 | 2 | 3 | 4 | 5
6 | 7 | 8 | 9 | 10
B
Pathways
POPcorn

Data Centers
Genetic
Maps
Loci
QTL
Stocks
Cytogenetics
Variations/Alleles
Genomic
Markers/Probes
Sequences
Functional
Gene Products
Images
Mutant Phenotypes
Colleagues
People/Organizations

References

MaizeGDB is a community-oriented, long-term, federally funded informatics service to researchers focused on the crop plant and model organism *Zea mays*.

Maize Genome Sequencing

Line	Genome Browsing	Other Links
B73	MaizeGDB Genome Browser	Status
	maizesequence.org	Information
		Publication
Mo17	phytozome	Publication
Palomero	Palomero Toluqueño	Publication

For more information, please visit our sequence resource page.

Be on Top of Recent Advances!

Project Oversight

MaizeGDB is guided by members of the community of maize geneticists through feedback sent to us through the website, and by guidance from the MaizeGDB Working Group. MaizeGDB's areas of concentration for 2007 - 2012 are described on this page.

Funding Sources

USDA ars NSF

This page last updated 11:25 am, Mar 29, 2011.

Outreach
MaizeGDB Tutorials

What's New

March 29: Need somewhere to put your short reads since GenBank closed the SRA? Try EMBL-EBI's ENA!

March 23: Reconstructed chromosomes from the maize tetraploidy event from J. Schnable and M. Freeling (Reference) available via the Genome Browser.

March 22: Looking to align many sequences to B73 RefGen_v2 and deploy tracks in the MaizeGDB Genome Browser? Try ZeAlign!

March 5: The 2011 Maize Meeting Program is now available on the Maize Meeting page or directly here.

See older items...

Upcoming

Anticipated in July: The release of B73_RefGen_v3 along with its respective annotations.

Database

Last update:
April 5, 2011
Next update:
May 3, 2011

Maize Genetics Cooperation Newsletter

Staging site for Online Issues

Search

Google™ Custom Search

Maize Newsletter Issue 35 (1961)
Maize Newsletter Issue 34 (1960)
Maize Newsletter Issue 33 (1959)
...
Maize Newsletter Issue 25 (1942)
Maize Newsletter Issue 24 (1942)
Maize Newsletter Issue 23 (1942)
Maize Newsletter Issue 22 (1942)
Maize Newsletter Issue 21 (1942)
Maize Newsletter Issue 20 (1942)
Maize Newsletter Issue 19 (1942)
Maize Newsletter Issue 18 (1942)
Maize Newsletter Issue 17 (1942)
Maize Newsletter Issue 16 (1942)
Maize Newsletter Issue 15 (1941)
Maize Newsletter Issue 14 (1940)
Maize Newsletter Issue 13 (1939)
Maize Newsletter Issue 12 (1938)
Maize Newsletter Issue 11 (1937)
Maize Newsletter Issue 10 (1936)
Maize Newsletter Issue 09 (1935)
Maize Newsletter Issue 08 (1934)
Maize Newsletter Issue 07 (1934)
Maize Newsletter Issue 06 (1934)
Maize Newsletter Issue 05 (1934)
Maize Newsletter Issue 04 (1933)
Maize Newsletter Issue 03 (1933)
Maize Newsletter Issue 02 (1932)
Maize Newsletter Issue 01 (1932)

Instructions for Submission of notes for the Maize Genetics Cooperation. Newsletter.

Please report any errors or non-working links to Mary Schaeffer

This project is supported by both an endowment at the Univeristy of Missouri that was set up by Ed Coe with contributions from Maize Cooperators and the USDA-ARS, through support to MaizeGDB. MaizeGDB hosts the archival site.

Please Note: Notes submitted to the Maize Genetics Cooperation Newsletter may be cited only with consent of the authors.

Last Modified: Feb 2009

Windows QTL Cartographer V2.5_009

Release Date: 18 - MAR - 2011

Shengchu Wang, Christopher J. Basten and Zhao-Bang Zeng
Program in Statistical Genetics, North Carolina State University

Windows QTL Cartographer maps quantitative trait loci (QTL) in cross populations from inbred lines. WinQTLCart includes a powerful graphic tool for presenting and summarizing mapping results and can import and export data in a variety of formats.

WinQTLCart incorporates the modules found in its command-line sibling, QTL Cartographer, and provides a graphical interface to QTL Cartographer's features.

What's new

1. Add new function: Import – CSV format that can use to create mcd source data file. Sample file "NSimuB1-01-OnePop.csv" is in folder NCSU\WinQTLCart2.5\Examples.
2. Add function of score statistics procedures in MIM analysis.
3. Updated the User's Manual (4/16/2010).
4. Fixed the IM, CIM permutation error.
5. Fixed errors in result graph display.
6. Add new information in MIM summary.
7. Fixed problem in Multiple Trait IM-CIM permutation.
8. Fixed problem of Category trait MIM new model.
9. Improved single marker analysis.
10. More Improvement of MIM summary.
11. Improved MIM graph display.
12. Correct error on MIM for RI cross
13. Correct error on graph display.

Download

Updated version of WinQTLCart v2.5.009 released at MAR 18, 2011
Click the link below to begin downloading.

WinQTLSetup25009

Old Version

WinQTLSetup25008 (01/25/2011) WinQTLSetup25007 (01/08/2011)

WinQTLSetup25006 (11/06/2010) WinQTLSetup25005 (09/10/2010)
WinQTLSetup25004 (08/03/2010) WinQTLSetup25003 (07/24/2010)
WinQTLSetup25002 (07/17/2010) WinQTLSetup25001 (07/13/2010)
WinQTLSetup25000 (06/26/2010) WQTLSetup2.50

Please let us know who you are

Please send us an email with your name and company name, and any comments you may have about WinQTLCart. Knowing who our users are is important to us for the continual development of the software. One email is enough, rather than one for each upgrade you download. We appreciate hearing from you!

Citation

Wang S., C. J. Basten, and Z.-B. Zeng (2011). Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)