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AN EVALUATION OF THE UTILITY OF SSR LOCI AS MOLECULAR MARKERS IN MAIZE (*ZEA MAYS* L.): COMPARISONS WITH DATA FROM RFLPS AND PEDIGREE

Document prepared by Smith, J.S.C et al. and presented on behalf of ASSINSEL

AN EVALUATION OF THE UTILITY OF SSR LOCI AS MOLECULAR MARKERS IN MAIZE (*Zea mays* L.): COMPARISONS WITH DATA FROM RFLPS AND PEDIGREE

J.S.C. Smith, E.C.L. Chin, H. Shu, O.S. Smith, S.J. Wall[1], M.L. Senior[2], S.E. Mitchell[3], S. Kresovich[3] and J. Ziegle[4]

SUMMARY

The utility of 131 Simple Sequence Repeat (SSR) loci to characterize and identify maize inbred lines, validate pedigree and show associations among inbred lines was evaluated using a set of 58 inbred lines and four hybrids. Thirteen sets of inbred parent-progeny triplet pedigrees together with four hybrids and their parental lines were used to evaluate levels of non-Mendelian scoring. Results were compared to those obtained using 80 RFLP probes. Over all inbred triplets, 2.2% of SSRs and 3.6% of RFLPs were scored in a non-Mendelian fashion. The PIC values ranged from 0.06 to 0.91 for SSRs and from 0.10 to 0.84 for RFLPs. Mean values for PIC (a measure of discrimination ability) for SSRs and RFLPs were similar, approximately 0.62. However, PIC values for nine SSRs exceeded the maximum PIC for RFLPs. Di-repeats gave the highest mean PIC scores for SSRs but this class of repeats can result in "stutter" bands that complicate accurate genotyping. Associations among inbreds were similar for SSR and RFLP data, closely approximating expectations from known pedigrees. SSR technology presents advantages of reliability, repeatability, discrimination, genetic interpretation, standardization and cost effectiveness over RFLPs and most other PCR amplification methods. These advantages promote the use of SSRs for the identification and pedigree validation of maize genotypes.

Key Words: Simple Sequence Repeat, Microsatellite, SSR, Maize, Variety Identification

INTRODUCTION

Microsatellites, or Simple Sequence Repeats (SSRs) are short nucleotide sequences, usually from 2-3 bases(b) in length that are repeated in tandem arrays. Polymorphisms are revealed because of differences in the numbers of tandem repeats that lie between sequences that are otherwise conserved for each locus. Microsatellite loci have proven to be highly polymorphic and useful as genetic markers in humans (Weber and May, 1989), in other animals including the timber rattlesnake (Villarreal et al., 1996), the cat family (Menotti-Raymond and O'Brien, 1995), grizzly bear (Craighead et al., 1995), Koala (Houlden et al., 1996), cattle (Glowatzki-Mullis et al., 1995; Usha et al., 1995), fungi (Sastry et al., 1995) and plant species including Arabidopsis (Depeiges et al., 1995), bur oak (Dow et al., 1995), seashore paspalum (Liu et al., 1995), rapeseed (Kresovich et al., 1995; Charters et al., 1996), soybean (Akkaya et al., 1992; 1995; Rongwen et

[1] Pioneer Hi-Bred International, Inc., Research Technology Services, Research and Product Development, 7300 NW 62nd Avenue - P.O. Box 1004, Johnston, IA 50131-1004
[2] North Carolina State University, Department of Crop Science, Raleigh, NC 27695
[3] USDA-ARS, Plant Genetic Resources Conservation Unit, 1109 Experiment Street, Griffin, GA 30223-1197
[4] Perkin Elmer - Applied Biosystems Division, 850 Lincoln Center Drive, Foster City, CA 94404

al., 1995), sugar beet (Mörchen et al., 1996) sweet potato (Jarret and Bowen, 1994) and wheat (Plaschke et al., 1995; Roder et al., 1995).

Senior and Heun (1993) provided preliminary evidence that SSRs are present in the genome of maize (*Zea mays* L.) and that they could be useful for genetic analyses. The usefulness of an additional 131 SSRs as genetic markers and discriminators of maize germplasm is reported in this study.

The objectives of this paper are to:

1) compare parent-progeny (triplet) SSR profiles for inbred and inbred-hybrid triplets;
2) compare parent-progeny (triplet) RFLP profiles for inbred triplets;
3) compare the triplet data for SSRs and for RFLPs;
4) present the polymorphic index content (PIC) for each SSR;
5) present the polymorphic index content for each RFLP locus;
6) compare the PIC values for SSRs and for RFLPs;
7) present the associations among inbreds on the basis of SSR data;
8) present the associations among inbreds on the basis of RFLP data;
9) present the associations among inbreds on the basis of pedigree data; and
10) present correlations of pairwise genetic distances between inbreds for the SSR, RFLP and pedigree data.

We discuss the potential of SSRs to provide a set of molecular markers that can a) uniquely identify inbred lines of maize and b) show associations among those inbred lines in relation to pedigrees and heterotic groups.

METHODS

DNA was extracted from 58 maize inbred lines (Table 1) and from four maize hybrids (Pioneer® hybrids 3183, 3377, 3732, and 3747). The 58 inbreds encompass a broad range of genetic diversity for Corn Belt materials, including pairs of lines that span pedigree relationships from unrelated to highly related. Among these inbred lines were 13 sets of triplets (a progeny line and both its parents) that provided opportunities for tests of inheritance and/or reliable band scoring. In addition, four hybrids were also profiled, providing additional opportunities to check scoring and inheritance of polymorphisms. A proprietary DNA extraction method for which patent protection is being sought was used. However, the CTAB procedure (Saghai-Maroof et al., 1984) also provides DNA suitable for amplification by these SSRs and provides equivalent results. SSR loci were individually amplified using DNA of each inbred and hybrid using protocols described by Chin et al., (1996), except that fluorescent labeled primers were used. Samples containing 0.5 µl of the PCR products, 0.5µl GENESCAN 500 internal lane standard labeled with N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMARA) (Perkin Elmer - Applied Biosystems) and 50% formamide were heated at 92°C for 2 min, placed on ice, then loaded on 6% denaturing acrylamide gels (24 cm well-to-read format). DNA samples were electrophoresed (29 watts) for 7 hrs on an ABI Model 373A automative DNA sequencer/fragment analyzer equipped with GENESCAN 672 software v. 1.2 (Perkin Elmer - Applied Biosystems). DNA

fragments were sized automatically using the "local Southern" sizing algorithm. PCR products from individual samples were assigned to specific alleles at each locus based on "binning" of a range of sizes (± 0.5 bp) as determined by ABI GeneScan™ and GENOTYPER™ software using the "local Southern" algorithm (Elder and Southern, 1987). Primer pairs for 200 potentially useful SSR loci were identified from sequence data that were published in Genbank, from di-repeat libraries made by Ben Burr (Brookhaven National Laboratory) and from additional sequence data. An initial screen of nine inbred lines was used to evaluate utility (Chin et al., 1996). Sequence data for primers to amplify these SSRs are available *via* the electronic maize database (Maize DB), (Polacco, 1996). Attempts were made to profile all of the 58 inbred lines and four hybrids with these SSRs. It was possible to obtain profiles for all of the inbreds and hybrids included in this survey for one hundred and thirty-one SSRs (Table 2). Genomic locations for most SSRs are provided according to the nomenclature used in Coe et al. (1995). Among this set of SSRs, 59 (45%) were di-repeats, 36 (27%) were tri-repeats, 21 (16%) were tetra-repeats, 7 (5%) were penta-repeats, 5 (4%) were hexa-repeats, 2 (2%) were septa-repeats, and 1 (1%) was an octa-repeat.

RFLP data were obtained by Linkage Genetics (Salt Lake City , Utah) using DNA extraction and other protocols described by Helentjaris et al., (1985). Eighty single-locus probes that collectively sampled every chromosome arm were used.

Polymorphic Index Content (PIC) values were calculated using the algorithm:

$$PIC = 1 - \sum_{i=1}^{n} f_i^2$$

where f_i^2 is the frequency of the i^{th} allele.

PIC provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles. PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles in equal frequencies). For example, a marker locus that reveals five alleles, but where one allele is found in very high frequency (e.g. freq. = 0.9), has overall less discriminatory capability than a locus that also has five alleles but in which those alleles are found in more equal frequencies.

Genetic distances between pairs of inbred lines from SSR and RFLP data were calculated from comparisons of the band scores using a modified Nei's distance (Nei and Li, 1979). Pedigree distances between pairs of inbreds were calculated from 1-Malecot's Coefficient of relatedness (Kempthorne, 1969; Delannay et al., 1983). Associations among inbreds from SSR, RFLP and pedigree data were revealed using average linkage cluster analysis.

RESULTS

SSRs that failed to amplify against the majority of inbreds or which gave amplified products that could not be clearly resolved were re-amplified and electrophoresed a second time. If results were still poor, then primers were redesigned (designated with '-2' following the SSR locus

name) for further evaluation. If amplified products still failed to yield clearly scorable profiles for less than 95% of the inbred lines, then those SSRs were discarded from this study. This exercise resulted in scorable data being obtained for the 58 inbreds and four hybrids from 131 SSRs (Table 2). Primers with different sequences for loci already published (Coe, 1996) may result in amplification products with different molecular weights from those that are obtained using the initial primer sequences.

Thirteen parent-progeny triplets were available for examinations of inheritance and scoring accuracy. For SSRs, non-Mendelian scores (where an amplified product was scored in a progeny inbred that had not been scored in one or both parental inbreds) ranged from 0 to 7 of the SSRs (0 - 5.3% of SSRs) per triplet. The mean was 2.85 incidences of non-Mendelian scoring (2.2% of all SSRs) per triplet. For RFLPs the range of non-Mendelian scores was from 0 to 7 RFLPs per triplet (0- 8.8% of RFLPs per triplet). The mean for RFLPs was 2.85 (3.6% of RFLPs) incidences of non-Mendelian scoring per triplet.

PIC values for SSRs are presented in Tables 3a and 3b. PIC values for SSRs ranged from 0.06 to 0.91; the mean PIC for SSRs was 0.62. SSRs that had the highest PIC values were di-repeats; representing 17% of all di-repeats that were used in profiling the 58 inbred lines. Di-repeats were the predominant class of SSRs for the highest PIC values of 0.91 down to a PIC value of 0.62. For PIC values from 0.62 down to 0.09, tri-repeats were the predominant class of repeats, except for the ranges of 0.58-0.53 and 0.46-0.35 where tri- and tetra-repeats were equally represented. PIC values for RFLPs (not presented) ranged from 0.10 to 0.84; the mean PIC value for RFLPs was 0.63. The mean PIC values for SSRs according to source of DNA was 0.57 (from RFLPs), 0.61 (from cDNA) and 0.74 (from genomic DNA, di-repeat class only).

Associations among inbreds on the basis of pedigree, RFLP and SSR data are presented in Figures 1, 2 and 3, respectively. Associations of inbreds on the basis of pedigree (Figure 1) were similar to that which could be expected on the basis of either marker method (Figures 2 and 3). Very similar associations of inbreds were revealed from analyses of the RFLP and the SSR data (Figures 2 and 3). The correlations of pairwise distances among all pairs of inbred lines for SSRs compared to RFLPs was $r = 0.85$. The correlation for pairwise distances between all pairs of inbreds for RFLPs compared to pedigree was $r = 0.80$; the correlation for SSRs with pedigree data was $r = 0.81$.

DISCUSSION

A. Non-Mendelian scoring and repeatabilty of scoring

Non-Mendelian scoring does not necessarily indicate non-Mendelian inheritance. First, bands can be mis-scored and given the wrong allelic designation. The discriminative ability of gel separation technology, effective use of molecular weight marker ladders, use of internal genomic standard check inbreds or hybrids, visual checking of scoring and manual data entry are factors that all determine capabilities to score bands accurately and repeatedly. Artifactual "stutter" bands, that are especially prone to occur from di-repeat SSRs, can also cause incorrect genetic scoring of bands, although Pertin et al. (1995) describe how 'stutter' bands can aid in automated

genotyping. Second, there may have been residual heterozygosity remaining within an inbred at the time it was originally used to make the parental cross for subsequent progeny development by successive self-pollination and selection. Subsequent self-pollinations of the parent stock would then reduce or eliminate that heterozygosity so that later sources of the parental line (which would then be the representative sources of that line for profiling) would not carry all of the alleles that were still in heterozygous condition when the line was used as a parent in a breeding cross. Third, the progeny line could have been contaminated by outsourced pollen due to poor pollen control during its development (i.e., the pedigree could be incorrect). Fourth, a parental stock could have changed genetically after the time it was used to make the parental cross from which the progeny line was subsequently derived, either by mutation, contamination by an outsource of pollen, or by physical mixing of seed from another genotype. High mutation rates have been reported for microsatellites (Levinson and Gutman, 1987; Jeffreys et al., 1988; Kelly et al., 1991; Wierdl et al., 1996).

Incidences of non-Mendelian scoring were identified in this study whenever 1) the progeny line was scored with a band that was not scored in at least one of the parental lines; and 2) whenever the parental lines were scored as both having the same band, but that band was then not scored as being present in the progeny. Therefore, we did not consider instances as non-Mendelian scoring where a parental line was heterozygous but the progeny did not receive both bands; those were more likely to represent occurrences of residual heterozygosity in the parent and, therefore, did not indicate any problems in either inheritance or in band scoring. Nevertheless, incidences of non-Mendelian scoring, including instances of true non-Mendelian inheritance plus contributions of residual heterozygosity, outcrossing, and mis-scoring of bands, were very low for SSRs; lower than for RFLPs. Therefore, this study provides no evidence that previously reported hypervariability of SSRs (Levinson and Gutman, 1987; Jeffreys et al., 1988; Kelly et al., 1991; Wierdl et al., 1996) will cause them to yield data that will be unreliable in characterizing maize inbred lines and hybrids, at least in respect of contemporary and parental germplasm. A more thorough investigation of mutation rates will be necessary before SSR data can be used to provide reliable measures of phylogeny among germplasms that are unrelated or very distantly related by pedigree (Nauta and Weissing, 1996).

The incidences of non-Mendelian scoring among parent-progeny inbred lines were lower for this set of SSRs (2.2% of SSRs) than for RFLPs (3.6% of RFLPs). The incidence of non-Mendelian scoring for inbred parent-hybrid triplets was also low (2.3% of SSRs). The level of non-Mendelian scoring for SSRs can be improved. For example, the omission of eight SSRs that were involved in non-Mendelian scores for two or more of the triplets would then result in 1.3% of SSRs being associated with problematic scoring.

SSR technology can be more reliable and repeatable than RFLP technology because the methodology that is available to separate amplified bands, to determine molecular weights, and to translate those molecular weights into discrete alleles is very precise and accurate (Schwengel et al., 1994; Mitchell et al., 1996). SSRs can be amplified under high stringency conditions, thereby reducing the chances that non-allelic bands will be amplified. SSRs can be separated on acrylamide sequencing gels in contrast to the less discriminative agarose gels that are used for RFLPs. SSRs can be co-electrophoresed with comprehensive molecular weight standard ladders in each sample lane, whereas RFLP data are scored with the aid of comprehensive genomic

ladders in flanking lanes and/or one or two co-migrating molecular weight standards. Finally, available technology facilitates optical scoring of SSRs as an integral component of the electrophoretic procedure thereby promoting the use of procedures that can further eliminate human error.

B. Discrimination ability.

Mean PIC values for the SSRs and RFLPs used in this study were essentially identical. However, the maximum PIC value for SSR loci was 0.91 and the PIC values of nine (7%) SSR loci exceeded the maximum PIC value of 0.84 that was shown by RFLP probes. Consequently, a subset of these SSR loci will have a higher mean PIC value than would an equivalent number of RFLP loci.

Di-repeats gave the highest PIC values. However, di-repeats can present scoring problems because of a tendency to produce additional "stutter" bands. Most of the 69 SSRs that were not carried forward into this profiling set of 131 SSRs were di-repeats that presented this and other problems. However, within this set of 131 SSRs, incidences of non-Mendelian scoring, which would have been inflated by mis-scoring of stutter bands as alleles, were usually not apparent for SSRs that were di-repeats. An exception was bngl 619 that was scored in a non-Mendelian manner for four of 13 triplets.

The increase in PIC value shown by these SSRs over single copy RFLPs for maize is much less than that shown by SSRs compared to single copy RFLPs in soybean or wheat. That is because this class of RFLPs reveal many more polymorphisms among elite Corn Belt Dent and Flint germplasm of *Zea mays*, than is the case for *Glycine max*, *Triticum aestivum* and *Triticum durum* (Plaschke et al., 1995; Röder et al., 1995; Rongwen et al., 1995; Smith, 1995). Consequently, SSRs do not provide the same degree of increase in discrimination power in maize as has been reported for soybean, wheat, or tomato. SSRs can, nonetheless, provide a useful increase in discriminatory power over RFLPs in maize.

C. Associations among inbred lines

Both SSR and RFLP data provide associations of inbred lines that largely concur with expectations based upon pedigree data. There is a major split between Stiff Stalk and non-Stiff Stalk pedigreed inbreds with subdivisions that further break out very largely according to pedigree background (compare Figure 1 with Figures 2 and 3).

Both SSR and RFLP data show lines that are the most closely related by pedigree to be those that are also closely related on the basis of marker information. For example, the following pairs of lines are closely related by pedigree and molecular data provide confirmation: B64, PHWK9, PHMM9-PHV94,PH165-PHG29 and PHJ76-PHK29. However, there are some differences in associations among inbreds according to whether SSR or RFLP data are used. For example, SSR data indicate that the Iodent lines PH165, PHK42 and PHG29 are more closely associated than are the Stiff Stalk lines B64 and PHWK9; RFLP data reveal the opposite.

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CONCLUSIONS

SSRs exceed the capabilities of RFLPs with regard to the characterization and unique identification of maize germplasm. SSRs can be more reliably and repeatedly scored than RFLPs, they can provide greater power of discrimination than RFLPs and they can reveal associations among inbred lines that are reflective of pedigree. Further increases in efficiency can be effected through the simultaneous amplification, gel separation and scoring of more individual SSR loci. These technical developments are underway (Mitchell et al., 1996) and should result in the provision of maize SSR profiling technology that will be faster and more cost effective than RFLP technology.

SSR profiling has the potential to be standardized across laboratories to an extent that exceeds what has been possible using RFLPs or most amplification methods that utilize arbitrary primers. SSRs require only that primer sequence data, together with amplification, gel running and scoring procedures be shared among laboratories. The needs to physically share vectors containing probes and to then harvest them from bacteria or to amplify them *via* the PCR process that are prerequisites for RFLP technology are done away with by the use of SSRs. The challenges to maintain standard wash conditions in order to achieve highly repeatable DNA-membrane binding for standardization of profiling by RFLPs are eliminated and replaced by more robust PCR annealing and amplification conditions that can readily be practiced by different laboratories.

In contrast, there are greater prospects for achieving standardization among laboratories with the use of SSRs. The availability of a useful publicly or commercially provided set of SSRs can preclude the need for additional, independent and significant investments that would be needed to develop further sets of SSRs, at least as regards the need to characterize the identities and pedigrees of inbred line and hybrids. Additionally, commercial equipment that provides band separation, molecular weight determination, allele designations of bands, and databasing already is in widespread use. This, or equivalent equipment, when combined with standard extraction and amplification conditions, can provide standardization of the whole SSR profiling process for a specific crop species. Even without total standardization of the process, however, SSR technology still offers substantial advantages over RFLPs in terms of higher repeatabilities and discrimination power.

SSRs, as RFLPs, reveal the co-dominantly inherited multi-allelic products of loci that can be readily mapped. Therefore, SSR technology present distinct advantages over most other PCR amplification methods, at least with respect to the identification of specific genotypes because SSR profiles are highly discriminatory and the banding profiles can be interpreted genetically without the need to repeatedly map amplified bands to marker loci in different populations. Commercial products are already available that are instrumental in helping to provide for the highly discriminative and reliable separation of polymorphisms, their scoring and databasing. As a result, it should be anticipated that SSR profiling will replace RFLPs as the method of choice in the identification of maize inbred lines and hybrids for a multitude of applications.

Table 1 List and pedigree background of inbred lines used in the present SSR and RFLP profiling study.

Inbred	Pedigree Background*
A632	BSSS[1]C0 (94%), Minnesota 13[2] (6%)
B73	BSSS[1] (100%)
Mo17	Lancaster Sure Crop[2] (50%), Krug[2] (50%)
PH165	Iodent[2] (59%), Long Ear[2] (20%), Minnesota 13[2] (11%), Troyer Reid[2] (5%)
B64	BSSS[1]C0 (87.5%), Maiz Amargo[2] (12.5%)
PH595	Midland Yellow Dent[2] (25%), Southern U.S. Landrace Synthetic (19%), Funks G4949 (12.5%), Illinois Long Ear[2] (12.5%), Illinois Two Ear (12.5%)
PH642	BSSS[1]C0 (87.5%), Iodent[2] (9%).
PH814	Lancaster Low Breakage (25%), Southern U.S. Landrace Synthetic (19%), Osterland Yellow Dent[2] (16%), Funks G4949 (13%), Midland Yellow Dent[2] (6%), Tuson B[3] (6%), Brookings 86[4] (5%)
PH848	Minnesota 13[2] (12.5%), Osterland Yellow Dent[2] (12.5%), SRS303[5] (12.5%), Iodent[2] (12%), Reid Yellow Dent[2] (12%), Lancaster Sure Crop[2] (6%), Longfellow Flint[2] (6%), MHW[6] (6%)
PHB09	BSSS[1]C0 (62.5%), Minnesota 13[2] (25%)
PHB46	BSSS[1]C0 (50%), Alberta Flint[2] (25%), Osterland Yellow Dent[2] (25%)
PHB47	BSSS[1]C0 (87.5%), Brookings 86[4] (12.5%)
PHB76	SmithTC[7] (25%), Midland Yellow Dent[2] (12.5%), NW Dent[8] (12.5%), Southern U.S. Landrace Synthetic (9%), Minnesota 13[2] (8%), Funks G4949 (6%), Illinois Long Ear (6%), Illinois Two Ear[2] (6%), Osterland Yellow Dent[2] (6%)
PHB89	Coker 616 (25%), Lancaster Sure Crop[2] (12.5%), Midland Yellow Dent[2] (12.5%), Southern U.S. Landrace Synthetic (9%), Minnesota 13[2] (8%), Funks G4949 (6%), Funks Yellow Dent[2] (6%), Illinois Long Ear[2] (6%), Illinois Two Ear (6%)
PHBE2	Iodent[2] (18%), Southern U.S. Landrace Synthetic (9%), Minnesota 13[2] (9%), Osterland Yellow Dent[2] (6%), Midland Yellow Dent[2] (6%), Long Ear (6%), Funks G4949 (6%), Lancaster Low Breakage (5%)
PHBG4	Iodent[2] (27%), Minnesota 13[2] (11%), Long Ear (9%), Coker 616

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(8%), Midland Yellow Dent[2] (6%), Lancaster Sure Crop[2] (6%), Southern U.S. Landrace Synthetic (6%)

PHG12 BSSS[1]C0 (37.5%), Lancaster Low Breakage (25%), M3204[9] (25%)

PHG29 Iodent[2] (59%), Long Ear (20%), Minnesota 13[2] (13%), Troyer Reid[2] (5%)

PHG31 Iodent[2] (44%), Long Ear (15%), Minnesota 13[2] (11%), Midland Yellow Dent[2] (6%), Southern U.S. Landrace Synthetic (5%)

PHG35 Iodent[2] (29%), Midland Yellow Dent[2] (13%), Minnesota 13[2] (11%), Southern U.S. Landrace Synthetic (9%), Long Ear (9%), Funks G4949 (6%), Illinois Long Ear (6%), Illinois Two Ear (6%)

PHG39 BSSS[1]C0 (69%), Maiz Amargo[2] (25%)

PHG42 Iodent[2] (30%), Lancaster Low Breakage (10%), Southern U.S. Landrace Synthetic (9%), Osterland Yellow Dent[2] (9%), Minnesota 13[2] (7%), Funks G4949 (6%)

PHG45 Iodent[2] (59%), Long Ear (20%), Minnesota 13[2] (13%), Troyer Reid[2] (5%)

PHG50 Iodent[2] (35%), Long Ear (12%), Minnesota 13[2] (12%), Osterland Yellow Dent[2] (7%), SRS 303[5] (6%), Reid[2] (6%)

PHG53 BSSS[1]C0 (91%), Maiz Amargo[2] (6%)

PHG55 PROCOMP[10] (50%), Minnesota 13[2] (6%), Osterland Yellow Dent[2] (6%), SRS 303[5] (6%), Iodent[2] (6%), Reid[2] (6%)

PHG69 BSSS[1] (50%), BSSS[1]C0 (25%), Alberta Flint (13%), Osterland Yellow Dent[2] (13%)

PHG71 BSSS[1]C0 (47%), Iodent[2] (30%), Long Ear (10%), Minnesota 13[2] (9%)

PHG74 BSSS[1]C0 (89%), Minnesota 13[2] (5%)

PHG80 Dockendorf 101[11] (50%), BSSS[1]C0 (38%)

PHG81 BSSS[1] (50%), Iodent[2] (30%), Long Ear (10%),Minnesota 13[2] (6%)

PHG83 Iodent[2] (30%), Lancaster Low Breakage (13%), Long Ear (10%), Southern U.S. Landrace Synthetic (9%), Osterland Yellow Dent[2] (9%), Minnesota 13[2] (7%), Funks G 4949 (6%)

PHG84 Midland Yellow Dent[2] (13%), Southern U.S. Landrace Synthetic (9%), Minnesota 13[2] (8%), Funks G4949 (6%), Illinois Low Ear (6%), Illinois Two Ear (6%), Osterland Yellow Dent[2] (6%), SRS 303[5] (6%), Iodent[2] (6%), Reid[2] (6%)

PHG86 BSSS[1] (50%), BSSS[1]C0 (44%), Maiz Amargo[2] (6%)

PHJ76 BSSS[1] (50%), BSSS[1]C0 (38%)

PHK29 BSSS[1]C0 (63%), BSSS[1] (25%), Brookings 86[4] (6%)

PHK42 Iodent[2] (59%), Long Ear (20%), Minnesota 13[2] (13%), Troyer Reid[2] (5%)

PHMK0 BSSS[1]C0 (38%), Southern U.S. Landrace Synthetic (21%), BSSS[1] (13%), Dockendorf 101[11] (13%)

PHMM9 BSSS[1]C0 (53%), Dockendorf 101[11] (25%), Maiz Amargo[2] (13%)

PHN46 Southern U.S. Landrace Synthetic (12%), Iodent[2] (10%), Lancaster Low Breakage (9%), Osterland Yellow Dent[2] (9%), Funks G4949 (8%), Minnesota 13[2] (6%), Midland Yellow Dent[2] (6%)

PHN65 BSSS[1] (50%), Minnesota 13[2] (6%), Osterland Yellow Dent[2] (6%), SRS 303[5] (6%), Iodent[2] (6%), Reid[2] (6%)

PHP38 BSSS[1]C0 (66%), Maiz Amargo[2] (13%), BSSS[1] (13%)

PHP85 BSSS[1]C0 (48%), BSSS[1] (38%), Maiz Amargo[2] (6%)

PHPE5 Iodent[2] (22%), Southern U.S. Landrace Synthetic (9%), Midland Yellow Dent[2] (9%), Minnesota 13[2] (8%), Long Ear (8%), Coker 616 (6%), Funks G4949 (6%), Illinois Long Ear (5%), Illinois Two Ear (5%)

PHR03 Iodent[2] (25%), Minnesota 13[2] (11%), Long Ear (8%), Southern U.S. Landrace Synthetic (6%), Midland Yellow Dent[2] (6%), Lancaster Sure Crop[2] (6%)

PHR63 Iodent[2] (29%), Coker 616 (13%), Minnesota 13[2] (10%), Long Ear (10%), Lancaster Sure Crop[2] (6%), Midland Yellow Dent[2] (6%), Southern U.S. Landrace Synthetic (5%)

PHR92 BSSS[1]C0 (69%), Maiz Amargo[2] (25%)

PHT11 BSSS[1]C0 (47%), BSSS[1] (25%), Maiz Amargo[2] (13%), Alberta Flint (6%), Osterland Yellow Dent[2] (6%)

PHT55 BSSS[1]C0 (69%), Maiz Amargo[2] (25%)

PHV25 Iodent[2] (30%), Midland Yellow Dent[2] (13%), Long Ear (10%), Southern U.S. Landrace Synthetic (9%), Minnesota 13[2] (7%), Funks G4949 (6%), Illinois Long Ear (6%), Illinois Two ear (6%)

PHV35 BSSS[1] (50%), BSSS[1]C0 (34%), Maiz Amargo[2] (13%)

PHV78 Iodent[2] (15%), Southern U.S. Landrace Synthetic (14%), Midland Yellow Dent[2] (13%), Funks G4949 (9%), Illinois Long Ear (6%), Illinois Two Ear (6%), Lancaster Low Breakage (6%), Long Ear (5%), Minnesota 13[2] (5%), Tuson B[3] (5%)

PHV94 BSSS[1]C0 (53%), Dockendorf 101[11] (25%), Maiz Amargo[2] (13%)

PHW52 BSSS[1] (50%), BSSS[1]C0 (34%), Maiz Amargo[2] (13%)

PHW53 Iodent[2] (21%), Osterland Yellow Dent[2] (11%), Minnesota 13[2]

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(10%), Long Ear (7%), Lancaster Low Breakage (6%), SRS 303[5] (6%), Reid[2] (6%), Southern U.S. Landrace Synthetic (5%)

PHWK9 Maiz Amargo[2] (50%), BSSS[1]C0 (50%)

PHZ38 BSSS[1] (50%), BSSS[1]C0 (41%)

PHZ51 Osterland Yellow Dent[2] (14%), Lancaster Low Breakage (13%), Southern U.S. Landrace Synthetic (9%), Minnesota 13[2] (8%), Funks G4949 (6%), SRS 303[5] (6%), Iodent[2] (6%), Reid[2] (6%)

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*Contributions of 5% or greater by pedigree are provided.
[1]Iowa Stiff Stalk Synthetic
[2]pen-pollinated variety
[3]Derived from Tuson, an open-pollinated variety from the West Indies
[4]Population derived from Minnesota 13 open-pollinated variety
[5]Stiff Root and Stalk or Stalk Rot Synthetic selection from Krug
[6]Dawes open-pollinated variety from Nebraska most likely from Reid obtained from Mount Haleb, Wisconsin
[7] Smith top-cross derived from HAT0 fling synthetic
[8] Northwest Dent, open-pollinated variety once grown in northwest and north central U.S.
[9] Synthetic from Mississippi
[10] Composite of Southern U.S. prolific germplasm and Corn Belt lines made by W.L.,Brown in the 1960's; known as "BS11" at Iowa State University
[11] Hybrid once sold by Dockendorf

Table 2. SSR markers, their map locations (where known) and primer sequences (5'⌫3'); forward primers listed first, followed by the reverse primers. Genomic locations in parentheses remain to be validated.

SSR LOCUS	GENOMIC LOCATION	PRIMER SEQUENCE	
phi056	1.01	ACGCCCAGATCTGTTCCTTCTC	ATGGCGGCAGGCCGATTGTT
phi097	1.01	TGCTTCACATTCAGTCACCGTCAG	CCACGACAGATGATTACCGACC
bngl439	1.03	TTGACATCGCCATCTTGGTGACCA	TCTTAATGCGATCGTACGAAGTTGTGGAA
phi001	1.04	TGACGGACGTGGATCGCTTCAC	AGCAGGCAGCAGGTCAGCAGCG
bngl615	1.07	CTTCCCTCTCCCCATCTCCTTTCCAA	GCAACCTGTCCATTCTCACCAGAGGATT
bngl100	1.08	TGCACGCACGGGCACTGAAC	TAAGACATCTATGGCCACCGGAG
phi002	1.08	CATGCAATCAATAACGATGGCGAGT	TTAGCGTAACCCTTCTCCAGTCAGC
phi037	1.08	CCCAGCTCCTGTTGTCGGCTCAGAC	TCCAGATCCGCCGCACCTCACGTCA
phi038	1.08	TCAGACTCCGCCCAGCAATCATCTG	AGCCTAGTGCTTATCTTGAAGGCTT
phi039	1.08	ACCGTGTCTAATGTGTCCATACGG	CGTTAGGAGCTGGCTAGTCTCA
phi011	1.10	GAGCTTCAGCAAGAGCATCCAG	CAACGCGATCGATGTGAGCACA
phi055	1.10	GAGATCGTGTGCCCGCACC	TTCCTCCTGCTCCTCAGACGA
phi094	1.10	AAAGAGGAGGAACGCGAAGGAC	TCACATCCTGGCGGTCACCA
bngl504	1.11	CGGCAGCTCCAGCACCGGCAT	AGTGTCCACATACCGCCACACACGTTT
phi064	1.11	CCGAATTGAAATAGCTGCGAGAACCT	ACAATGAACGGTGGTTATCAACACGC
phi120	1.11	GACTCTCACGGCGAGGTATGA	TGATGTCCCAGCTCTGAACTGAC
bngl339	1S	CCAACCGTATCAGCATCAGC	GCAGAGCTCTCATCGTCTTCTT
phi098	2.02	GAGATCACCGGCTAGTTAGAGGA	GTATGGTTGGGTACCCGTCTTTCTA
bngl108	2.04	GCACTCACGCGCACAGGTCA	CGCCTGCCAAGGTACATCAC
phi083	2.04	CAAACATCAGCCAGAGACAAGGAC	ATTCATCGACGCGTCACAGTCTACT
nc003	2.06	ACCCTTGCCTTTACTGAAACAACAGG	GCACACCGTGTGGCTGGTTC
phi127-2	2.07	ATATGCATTGCCTGGAACTGGAAGGA	AATTCAAACACGCCTCCCGAGTGT
phi090	2.085	CTACCTATCCAAGCGATGGGGA	CGTGCAAATAATTCCCCGTGGGA
bngl371-2	2L	ATCTAATCGCAACGCGAAGCAGAGA	TATCGACCGTAGCTCCGACTGT
phi099	3.02	TACAAAAATCAGGACTGCGAAAAACCCAA	GTCGGTGTGTGATCCTTCCAC
bngl602	3.04	CCCGATAGCCAAGCTCTCGCCAA	AGCTCGTGGACCGAACAAGCCCA
nc030	3.04	CCCCTTGTCTTTCTTCCTCC	CGATTAGATTGGGGTGCG
phi029	3.04	TTGTCTTTCTTCCTCCACAAGCAGCGAA	ATTTCCAGTTGCCACCGACGAAGAACTT
phi053	3.05	CTGCCTCTCAGATTCAGAGATTGAC	AACCCAACGTACTCCGGCAG
phi073	3.05	TTACTCCTATCCACTGCGGCCTGGAC	GCGGCATCCCGTACAGCTTCAGA
phi046	3.08	GATCTTGCCCGGAACTCTGAC	ATCTCGCGAACGTGTGCAGATTC
phi047	3.09	GGAGATGCTCGCACTGTTCTC	CTCCACCCTCTTTGACATGGTATG
phi072	4.01	ACCGTGCATGATTAATTTCTCCAGCCTT	GACAGCGCGCAAATGGATTGAACT
phi021	4.02	TTCCATTCTCGTGTTCTTGGAGTGGTCCA	CTTGATCACCTTTCCTGCTGTCGCCA
phi026	4.04	TAATTCCTCGCTCCCGGATTCAGC	GTGCATGAGGGAGCAGCAGGTAGTG
phi074	4.04	CCCAATTGCAACAACAATCCTTGGCA	GTGGCTCAGTGATGGCAGAAACT
phi079	4.04	TGGTGCTCGTTGCCAAATCTACGA	GCAGTGGTGGTTTCGAACAGACAA
bngl252	4.05	CGTTCTCCGTACAGCACAGACCAACGT	CTCAGATGAACTCCTCAGCAGCTGTAGCCT
phi096	4.05	CAACAATGTCGTCGTCGCTCTATC	GACGACCGTTGAAACTGGTGCTTT
phi066	4.08	CCATCCTTGAGGTGGTGTGAC	GAAGGAGCAGTAGCACTTGGTG
phi086	4.08	TACGTCGACGAGATCACTGGTC	CCACCATGATGCACCCACACT
phi092	4.08	GTGGGGGAGCCTACTACAGG	GACGAGGCCATCATCACGGT
phi093	4.08	AGTGCGTCAGCTTCATCGCCTACAAG	AGGCCATGCATGCTTGCAACAATGGATACA
bngl589	4.10	GGGTCGTTTAGGGAGGCACCTTTGGT	GCGACAGACAGACAGACAAGCGCATTGT
phi006	4.10	AGGCGGCGTGCTGAACACCT	CGCTTCATCTCCCGTGACAATG
phi019	4.10	TCCGCCTTTGTACCAATACAAGCCA	ATCCATCTTCAGGTAGCAGGGGT
phi076	4.10	TTCTTCCGCGGCTTCAATTTGACC	GCATCAGGACCCGCAGAGTC
phi024	5.00	ACTGTTCCACCAAACCAAGCCGAGA	AGTAGGGGTTGGGGATCTCCTCC
bngl143	5.01	GCACTGCCGGAGTGCCTTCT	ATGCCGTGATCTGTGACATCTAACC

bngl105	5.02	GACCGCCCGGGACTGTAAGT	AGGAAAGAAGGTGACGCGCTTTTC
bngl219	5.02	TGTTCCTGTACGGAGGCACTTCAA	TTCCAAGGTAATCCTCGCCTCAG
bngl557-2	5.02	TTCCTCCAAGGTCGCGTTTCAC	AGGAAAGGGATGGGAAGAACCGAA
phi113	5.02	GCTCCAGGTCGGAGATGTGA	CACAACACATCCAGTGACCAGAGT
phi008	5.03	CGGCTACGGAGGCGGTG	GATGGGCCCACACATCAGTC
bngl603-2	5.04	AGCTGGCCCCTGTGAATGGT	GCAACGTCCCTGGTTAGTTGAG
bngl653	5.04	CGCATTGCCATGGATGAAGAACTGG	GCAAGCGCCTCACAAGGTATGCACA
phi069	5.04	AGACACCGCCGTGGTCGTC	AGTCCGGCTCCACCTCCTTC
bngl278	5.06	GTGGGCGACTAACGCAATCTC	ATGCATCAACGTAACTCCCTCTCGT
bngl609	5.06	GCTCGTTCTCGCCAGTGTGCCG	GGCCCGAGCCATCTCTGCTGC
phi085	5.06	CGAGACCACCATCATCTGGAAG	TTTGCAATCGCTTCGGGGACC
phi087	5.06	GAGAGGAGGTGTTGTTTGACACAC	ACAACCGGACAAGTCAGCAGATTG
phi101	5.06	TGTTCGCCGTCTAGCCTGGATT	TCATCAGCAACGACGACTACTCC
phi058	5.07	AGGTGCTGGACACAGACTTCAAC	ACTGAGATCCAGGCTCCTCTTC
phi128-2	5.07	TTGCYCGGTATGAAGAAAATAGTCTTTCC	ATCTTGCAACTAGACTGAGGCAACCA
bngl150-2	5S	AGTAGAAAGAAAAACCCCCTCCCC	AAATCTGGGATCTCTGCCAATGGC
bngl238	6.00	CTTATTGCTTTCGTCATACACACACATTCAT	GAGCATGAGCTTGCATATTTCTTGTGG
phi036	6.00	CCGTGGAGAGACGTTTGACGT	TCCATCACCACTCAGAATGTCAGTGA
phi075	6.00	GGAGGAGCTCACCGGCGCATAA	AAAGGTTACTGGACAAATATGCGTAACTCAACATTGGA
phi077	6.01	GAGAAGAGGATCAGGTTCGTTCCA	CGCGTTGTACATCTTGCCTGCTT
phi126-2	6.01	TCCTGCTTATTGCTTTCGTCAT	GAGCTTGCATATTTCTTGTGGACA
bngl107	6.02	AGCAATGCATTATCTTTTGGGACAAACCCA	CAACAACAAGTGGCTGGCTAGGGTGAA
bngl391-2	6.02	GATAGAACCAGATATCACAGCATCAGAAG	ACGCAGCTCTCCTTCGTTTGTTC
bngl480	6.03	GACATTTCCAATGGCGGCTTTCC	TCTAGTTATTCCAAGCCCTGGGC
phi031	6.03	GCAACAGGTTACATGAGCTGACGA	CCAGCGTGCTGTTCCAGTAGTT
phi129	6.03	GTCGCCATACAAGCAGAAGTCCA	TCCAGGATGGGTGTCTCATAAAACTC
nc013	6.04	AATGGTTTTGAGGATGCAGCGTGG	CCCCGTGATTCCCTTCAACTTTC
phi102	6.05	TGAATCTAAACATAACTTATGTCTAGGTACATAGCAAA	CCTCGGATTCCGGATTGTAAGTCA
phi070	6.06	GCTGAGCGATCAGTTCATCCAG	CCATGGCAGGGTCTCTCAAG
phi123-2	6.06	GGAGACGAGGTGCTACTTCTTCAA	TGTGGCTGAGGCTAGGAATCTC
phi025	6.07	GCAACATCCTGGAGAGCCACTACAAGG	ACAGCCTGTTTTCCTGGACAGTGAACTC
phi078	6.07	CAGCACCAGACTACATGACGTGTAA	GGGCCGCGAGTGATGTGAGT
phi081	6.07	AAGGAACTGGTGAGAGGGTCCTT	AGCCCGATGCTCGCCATCTC
bngl161	6S	GCTTTCGTCATACACACACATTCA	ATGGAGCATGAGCTTGCATATTT
phi057	7.01	CTCATCAGTGCCGTCGTCCAT	CAGTCGCAAGAAACCGTTGCC
phi112	7.01	TGCCCTGCAGGTTCACATTGAGT	AGGAGTACGCTTGGATGCTCTTC
phi034	7.02	TAGCGACAGGATGGCCTCTTCT	GGGGAGCACGCCTTCGTTCT
phi114	7.02	CCGAGACCGTCAAGACCATCAA	AGCTCCAAACGATTCTGAACTCGC
phi091	7.03	ATCTTGCTTCCATAAGATGCACTGCTCT	CTCAGCTTCGGTTCCTACACAGT
phi082	7.05	CACAGCACAGGCAGTTCG	CGCGGCAAAAGATCTTGAACACCT
phi051	7.06	GGCGAAAGCGAACGACAACAATCTT	CGACATCGTCAGATTATATTGCAGACCA
phi116	7.06	GCATACGGCCATGGATGGGA	TCCCTGCCGGGACTCCTG
phi049	7.07	GTNTGGCCATACCGTACTGCTTCT	TCCAGTTCTTCCGAAACGAAAGGG
bngl669	8.03	GCACGCACCAGCAGTCGGCAGT	CGGCCTAGTGGGCATGGAGCCT
phi115	8.03	CTAGTGGGCGAACAACTGGTAAG	AAAGAGACCGTGTCAGGATTGCC
phi119	8.03	GGGCTCCAGTTTTCAGTCATTGG	ATCTTTCGTGCGGAGGAATGGTCA
phi125	8.03	ACCGCCGGTGCGAGTTGAAG	CTTGGGATTGCCCTCATCCAC
phi121	8.04	AGGAAAATGGAGCCGGTGAACCA	TTGGTCTGGACCAAGCACATACAC
phi014	8.05	AGATGACCAGGGCCGTCAACGAC	CCAGCTTCACCAGCTTGCTCTTCGTG
phi060	8.05	ACATGCAGAAGCTTGGCATCAAGG	GCTGAGCGATCAGTTCATCCAG
bngl162	8.06	ACTAGCAGCAGTAAAACCTAATAAAGGGA	CAAGTAGCTAGCATGCATTTGCAGTGT
bngl666	8.06	AAAAGGCAAGTAGCTAGCATGCATTTGCAG	GGCTCACGTCCGTATCCAAACCAACA
phi015	8.08	GCAACGTACCGTACCTTTCCGA	ACGCTGCATTCAATTACCGGGAAG
phi080	8.08	CACCCGATGCAACTTGCGTAGA	TCGTCACGTTCCACGACATCAC
phi067-2	9.01	CTGCAAAGGTAAGCACTAGGATGCT	CATCATTGATCCGGGTGTCGCTTT

phi068	9.01	GTACACACGCTCCGACGATTAC	TCTTCTCCACCAGAGCCTTGTAAG
phi017	9.02	CGTTGGCGACCAGGGTGCGTTGGAT	TGCAACAGCCATTCGATCATCAAAC
phi028	9.02	TCTCGCTGTCCTTCGATTAGTACGG	AATGCAGGCGATGGTTCTCCGGCCT
phi033	9.02	ATCGAAATGCAGGCGATGGTTCTC	ATCGAGATGTTCTACGCCCTGAAGT
phi043	9.02	AGCTGTACCGCTACATTTGCGATACCAA	TCACAGTCAGGCCGAACGCTTCGTAG
phi044	9.02	TTATTGGTCCCTCTCCCGTCCCAGA	AGCATACCCCAATGGTCAACAGGGA
bngl127	9.03	CATGTATACGAGAAGCACCCTAT	ATCGTAACTCAGCGGTTTGTG
bngl244	9.03	GATGCTACTACTGGTCTAGTCCAGA	CTCCTCCACTCATCAGCCTTGA
bngl430	9.03	CTTATCGAGCATCTTCCTTCTCTCC	TCCGGTGATGCTCCAGCGAC
phi022	9.03	TGCGCACCAGCGACTGACC	GCGGGCGACGCTTCCAAAC
phi027	9.03	CACAGCACGTTGCGGATTTCTCT	GCGTACGTACGACGAAGACAC
phi061	9.03	GACGTAAGCCTAGCTCTGCCAT	AAACAAGAACGGCGGTGCTGATTC
phi065	9.03	AGGGACAAATACGTGGAGACACAG	CGATCTGCACAAAGTGGAGTAGTC
phi016	9.04	TTCCATCATTGATCCGGGTGTCG	AAGGAGCAACATCCCATCCAGGAA
phi032	9.04	CTCCAGCAAGTGATGCGTGAC	GACACCCGGATCAATGATGGAAC
phi042	9.04	ATGTGGCCATCATTCAATGCTGTAGAC	ACACATGCAGGTGCAGCCAGA
phi040	9.05	GGGATATATGTCCCCCACAATCGT	GGCCCTAAGCGAAAATCTATGCTGA
bngl128	9.07	CACCTGGAGGGACCCATTCC	AGGACCACAGGATCCATCATCCT
bngl619	9.07	ACCCATCCCACTTTCCACCTCCTCCT	GCTTTCAGCGAATACTGAATAACGCGGA
phi118-2	10.00	ATCGGATCGGCTGCCGTCAAA	AGACACGACGGTGTGTCCATC
phi041	10.00	TTGGCTCCCAGCGCCGCAAA	GATCCAGAGCGATTTGACGGCA
phi052	10.02	CAGAATGGGACGACAAGGTCATC	GGGACACTTCTAGCAGGATCTGTTT
phi059	10.02	AAGCTAATTAAGGCCGGTCATCCC	TCCGTGTACTCGGCGGACTC
phi063	10.02	GGCGGCGGTGCTGGTAG	CAGCTAGCCGCTAGATATACGCT
bngl275	10.03	AGAAAAGAGAGTGTGCAATTGTGATAGAG	AATGGGTGCCTCGCACCAAG
bngl640	10.03	TGCGGATCCAACACGGACTGTCC	GCAGGCTCTCCGCCCACACCTC
phi050	10.03	TAACATGCCAGACACATACGGACAG	ATGGCTCTAGCGAAGCGTAGAG
phi054	10.04	AGAAAAGAGAGTGTGCAATTGTGATAGAG	AATGGGTGCCTCGCACCAAG
phi062	10.04	CCAACCCGCTAGGCTACTTCAA	ATGCCATGCGTTCGCTCTGTATC
phi071	10.04	GGAGTTCATCAGCTACCCCATCT	TTCTGCTTGTTGATCTGCACCCAC
phi084	10.04	AGAAGGAATCCGATCCATCCAAGC	CACCCGTACTTGAGGAAAACCC
bngl236	10.06	CGCTTTGCAGTACCAGTACACAC	GACGACAACTGCAGAGTACCAGA
bngl594	10.06	CGAGCGCTTTGCGAGTACCAGTACACA	CTGCGTGCGTCCAGCCTCCACT
6754/5		AAAAGGCCGTCAGAGCAGAACTGA	GTGACCGTGCCGTTGTATCACAA
7662/3		ATGCTTCCCTCGCAGCAGATTTCA	GTCGCCTTTTTCTACTAGCTGGTAG
7676/7		TGCTGCGCGCTCCACCAC	AGCTGCAGCTCGTCAATCAGG
7680/1		CTGGGCCACCAGCTTTGACC	CCGATGTGGGGTACGACCTC
7684/5		GCGGGCGACGCTTCCAAAC	CGCGCCCAAAACGCTTTCCC
7768/9		CCATGCCCATGGATGTTATTGCC	GCGGACCATGCATCCATCAAAC
10168/9		GAATTGGGAACCAGACCACCCAA	ATTTCCATGGACCATGCCTCGTG
bngl149		CATCCTCCAAAAGCACTACGT	CAGCTGTCCGACACTTATTCTGTA
bngl468		AGGGTGTACAGGTCCAAGTCCAA	AATGTGGGTCGTCAGCCATCAG
phi111		ATCTCGCGAACGTGTGCAGATTCT	TCGATCTTTCCCGGAACTCTGAC
bngl182	(1.03)	AGACCATATTCCAGGCTTTACAG	ACAACTAGCAGCAGCACAAGG
bngl421	(1.05)	ACAACTAGCAGCAGCACAAGG	GGGGCAAGGACTTGTCGGT
bngl125-2	(2.03)	AAGCAGAGGCTGCTCTCACTGA	AAATCAATGGCAAGGGACCTCGTAG
bngl381-2	(2.03)	TGGCGGCCGCTCTAGTAACT	AGGGTTTCCATGGGCAGGTGT
bngl166	(2.04)	GCCAACGTTTCCAGCCTGA	CTCCGTTTGCCCGAGTCC
bngl420	(2.04)	CTTGCGCTCTCCTCCCCTT	GGCCAGCTCACTGCTCACT
bngl198-2	(2.07)	CTGAAAAATAAAATCATGGTTTGTGCAAGTGTCA	ATGCACTGTGCACTGGCATTCACA
bngl197	(3.07)	GCGAGAAGAAAGCGAGCAGA	CGCCAAGAAGAAACACATCACA
bngl490	(4.05)	GCCCTAGCTTGCTAATTAACTAACA	ACTGTAAGGGCAGTGGACCTATA
bngl667	(4.05)	CGTGGATGTAAGGGGGCGCGCT	GGCCGCTGCTCAACACAGGCAG
bngl118-2	(5.08)	GCCTTCCAGCCGCAACCCT	CACTGCATGCAAAGGCAACCAAC
bngl389-2	(5.08)	CGTCGGCCAACAGGGTATC	CTCGCACGCGGTCTTCTTC

bngl386	(5.09)	CACCCTCCCTTTGCAGGTA	TGGTTTATCAGATAACGATTCAGC
bngl249	(6.01)	CCGGTCGCAGTTAGTAGATGAT	TCGGCGTTGATTTCGTCAGTA
bngl176	(6.04)	AGTTCACGTCCAGCTGAATGACAG	CGCGCATCGCATGCTTATCCTA
bngl147-2	(7.00)	TATGACCTTCTTTGGACGCTGACAC	ATTTGTTGTGCTAGCTTCGCCCAAG
bngl657	(7.02)	TCTGAGGATGCCCAATCATGCGC	CGTTTCCGTTCGTCACCAGCTCG
bngl434	(7.03)	GTGCAAAGGGGAGAGAGGAA	TCGCCGTTCTTCGCCTTAG
bngl155	(7.04)	ACCGAGTAGCCGAGACACG	AGAGTCCTGGAGCCACATGAG
bngl240	(8.07)	AAGAACAGAAGGCATTGATACATAA	TGCAGGTGTATGGGCAGCTA

Table 3a. Polymorphic Index Content (PIC), number of bands and repeat class for SSR markers.

PIC	Marker	Number of bands	Repeat Class
0.91	bngl619	11	2
	zct155	12	2
0.89	zca381	9	2
0.87	bngl176	10	2
	phi001	10	2
0.86	bngl105	8	2
0.85	bngl127	8	2
	bngl609	7	2
	phi026	9	2
0.84	bngl238	8	2
	phi015F	7	4
	phi119	5	2
0.83	zct161	8	2
	zct166	8	2
0.82	nc003	8	2
	phi064	7	4
	zca391_2	7	2
0.81	bngl128	7	2
	phi054	8	2
0.80	phi043	6	2
0.79	zag557	5	2
0.78	bngl615	7	2
	phi034	4	3
	phi079	3	5
0.77	zag249	6	2
	zca468	4	2
0.76	bngl182	5	2
	phi042	4	4
	phi127	4	4
	zct197	6	2
0.75	phi049	7	3
	phi083	4	4
	phi085	3	5
0.74	bngl421	6	2
0.74	bngl602	4	2
	phi037	5	2
	phi120	5	3

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PIC	Marker	Number of bands	Repeat Class
	zct118_2	5	3
0.73	bngl108	5	2
	zct434	6	2
0.72	phi021	4	2
	phi113	3	4
	zca150_2	3	2
0.71	bngl504	3	2
	phi056	5	3
	phi061	3	8
	phi073	3	3
	zag240	4	2
	zca147_2	3	2
0.70	bngl278	4	2
	phi075	3	2
	zct420_2	7	2
0.69	bngl653	3	2
	nc013	5	2
	phi047	4	3
	phi093	3	4
0.68	phi070a	4	5
0.67	_76623	2	2
	phi029	3	2
	phi116	5	7
0.66	bngl439	4	2
	phi036	4	2
	zct339	7	2
0.65	_67623	4	4
	bngl666	7	2
0.64	phi068	6	2
0.63	bngl1430	4	2
	bngl589	5	2
	phi078	2	4
0.62	_7676	2	3
	phi008	4	3
0.62	phi011	4	3
	phi025	2	2
	phi101	3	3
0.61	bngl162	4	2
	phi027	3	5
	phi046	3	4

PIC	Marker	Number of bands	Repeat Class
	phi057	4	3
0.60	bngl657	5	2
	phi006	4	3
	phi031	4	4
	phi041	5	4
	phi091	3	5
	phi099	5	2
0.59	phi017	3	3
	phi019	2	3
	phi128_2	4	3
0.58	phi032	2	4
	phi076	3	6
	phi121	2	3
	phi123_2	2	4
0.57	phi112	5	2
0.56	phi058	2	3
	phi129	5	4
0.55	phi024	2	3
0.54	bngl252	4	2
	phi069	4	3
	phi115	2	6
0.53	phi038	2	2
	phi050	3	4
	phi074	2	3
0.52	phi016	3	3
	phi066	2	3
	phi081	3	6
	phi084	2	3
0.51	phi071	2	3
	phi072	5	4
0.48	_76801	4	3
	phi062	2	3
0.47	phi118_2	4	3
0.46	phi102	2	2
	zag389	3	2
0.45	phi022	2	4
	phi055	3	3
	phi059	2	3
0.44	_76845	2	4
0.43	phi051	5	7

PIC	Marker	Number of bands	Repeat Class
0.41	_101689	2	4
0.36	phi097	2	3
0.35	phi096	2	5
0.34	phi040	3	3
0.31	phi094	2	3
0.28	phi090	2	5
0.26	phi028	3	3
	phi060	3	6
0.24	phi082	4	2
0.23	phi033	4	3
0.20	phi014	2	3
0.18	phi052	3	6
0.09	phi098	3	2
0.06	phi044	2	4

Table 3b. Polymorphic Index Content (PIC) for SSR markers used in the present study; SSRs are sorted by class of repeat unit.

Class of Repeat Unit	Marker	Number of Bands	PIC
2	bngl619	11	0.91
	zct155	12	0.91
	zca381	9	0.89
	bngl176	10	0.87
	phi001	10	0.87
	bngl105	8	0.86
	bngl127	8	0.85
	bngl609	7	0.85
	phi026	9	0.85
	bngl238	8	0.84
	phi119	5	0.84
	zct161	8	0.83
	zct166	8	0.83
	nc003	8	0.82
	zca391_2	7	0.82
	bngl128	7	0.81
	phi054	8	0.81
	phi043	6	0.80
	zag557	5	0.79
	bngl615	7	0.78
	zag249	6	0.77
	zca468	4	0.77

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Class of Repeat Unit	Marker	Number of Bands	PIC
	bngl182	5	0.76
	zct197	6	0.76
	bngl421	6	0.74
	bngl602	4	0.74
	phi037	5	0.74
	bngl108	5	0.73
	zct434	6	0.73
	phi021	4	0.72
	zca150_2	3	0.72
	bngl504	3	0.71
	zag240	4	0.71
	zca147_2	3	0.71
	bngl278	4	0.70
	phi075	3	0.70
	zct420_2	7	0.70
	bngl653	3	0.69
	nc013	5	0.69
	_76623	2	0.67
	phi029	3	0.67
	bngl439	4	0.66
	phi036	4	0.66
	zct339	7	0.66
	bngl666	7	0.65
	phi068	6	0.64
	bngl1430	4	0.63
	bngl589	5	0.63
	phi025	2	0.62
	bngl162	4	0.61
2	bngl657	5	0.60
	phi099	5	0.60
	phi112	5	0.57
	bngl252	4	0.54
	phi038	2	0.53
	phi102	2	0.46
	zag389	3	0.46
	phi082	4	0.24
	phi098	3	0.09
3	phi034	4	0.78
	phi049	7	0.75
	phi120	5	0.74
	zct118_2	5	0.74
	phi056	5	0.71
	phi073	3	0.71
	phi047	4	0.69

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Class of Repeat Unit	Marker	Number of Bands	PIC
	_7676	2	0.62
	phi008	4	0.62
	phi011	4	0.62
	phi101	3	0.62
	phi057	4	0.61
	phi006	4	0.60
	phi017	3	0.59
	phi019	2	0.59
	phi128_2	4	0.59
	phi121	2	0.58
	phi058	2	0.56
	phi024	2	0.55
	phi069	4	0.54
	phi074	2	0.53
	phi016	3	0.52
	phi066	2	0.52
	phi084	2	0.52
	phi071	2	0.51
	_76801	4	0.48
	phi062	2	0.48
	phi118_2	4	0.47
	phi055	3	0.45
	phi059	2	0.45
	phi097	2	0.36
	phi040	3	0.34
	phi094	2	0.31
	phi028	3	0.26
	phi033	4	0.23
	phi014	2	0.20
4	phi015F	7	0.84
	phi064	7	0.82
	phi042	4	0.76
	phi127	4	0.76
	phi083	4	0.75
	phi113	3	0.72
	phi093	3	0.69
	_67623	4	0.65
	phi078	2	0.63
	phi046	3	0.61
	phi031	4	0.60
	phi041	5	0.60
	phi032	2	0.58
	phi123_2	2	0.58
	phi129	5	0.56

Class of Repeat Unit	Marker	Number of Bands	PIC
	phi050	3	0.53
	phi072	5	0.51
	phi022	2	0.45
	_76845	2	0.44
	_101689	2	0.41
	phi044	2	0.06
5	phi079	3	0.78
	phi085	3	0.75
	phi070a	4	0.68
	phi027	3	0.61
	phi091	3	0.60
	phi096	2	0.35
	phi090	2	0.28
6	phi076	3	0.58
	phi115	2	0.54
	phi081	3	0.52
	phi060	3	0.26
	phi052	3	0.18
7	phi116	5	0.67
	phi051	5	0.43
8	phi061	3	0.71

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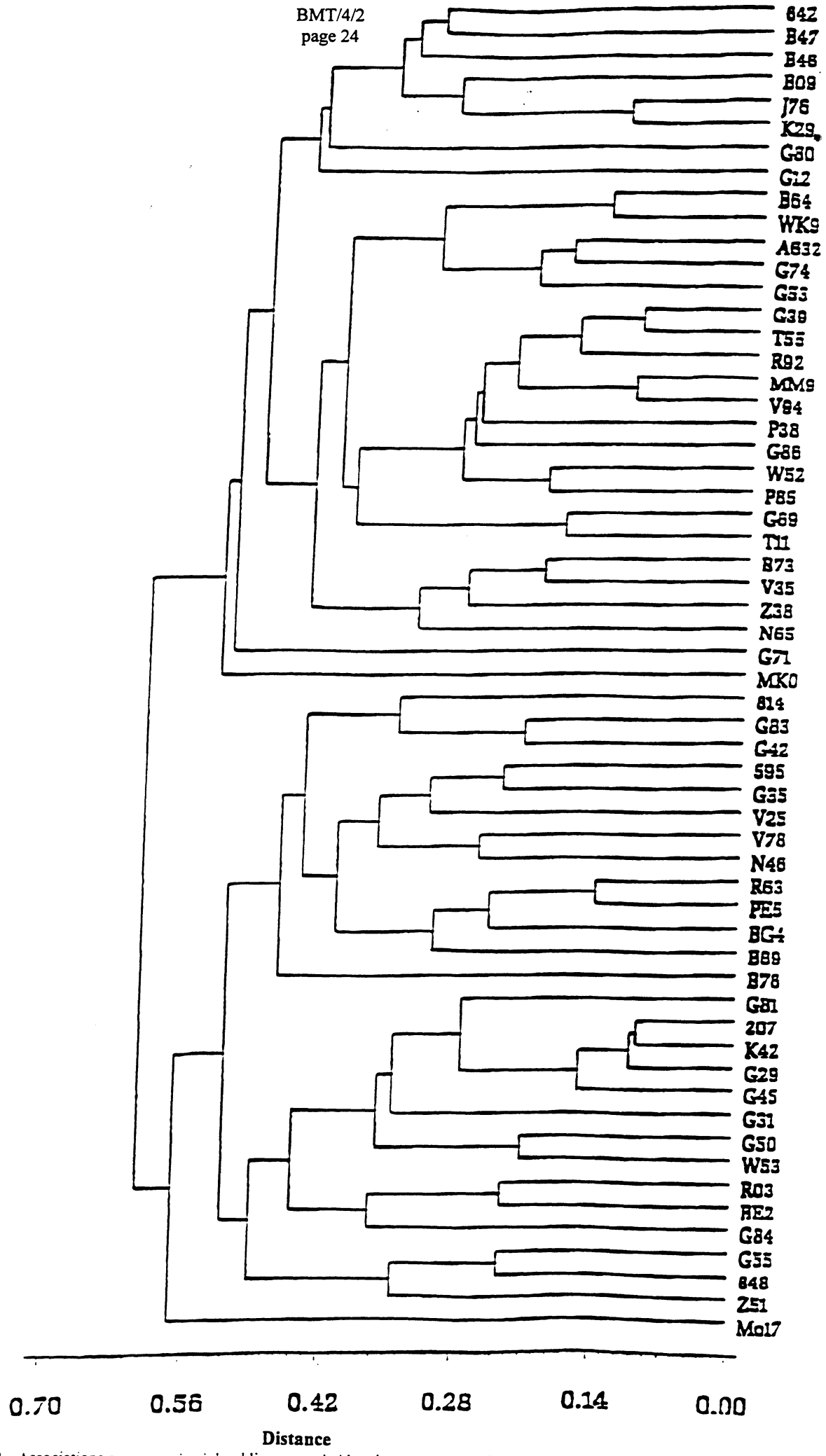

Figure 1. Associations among maize inbred lines revealed by cluster analysis of pedigree distance data.

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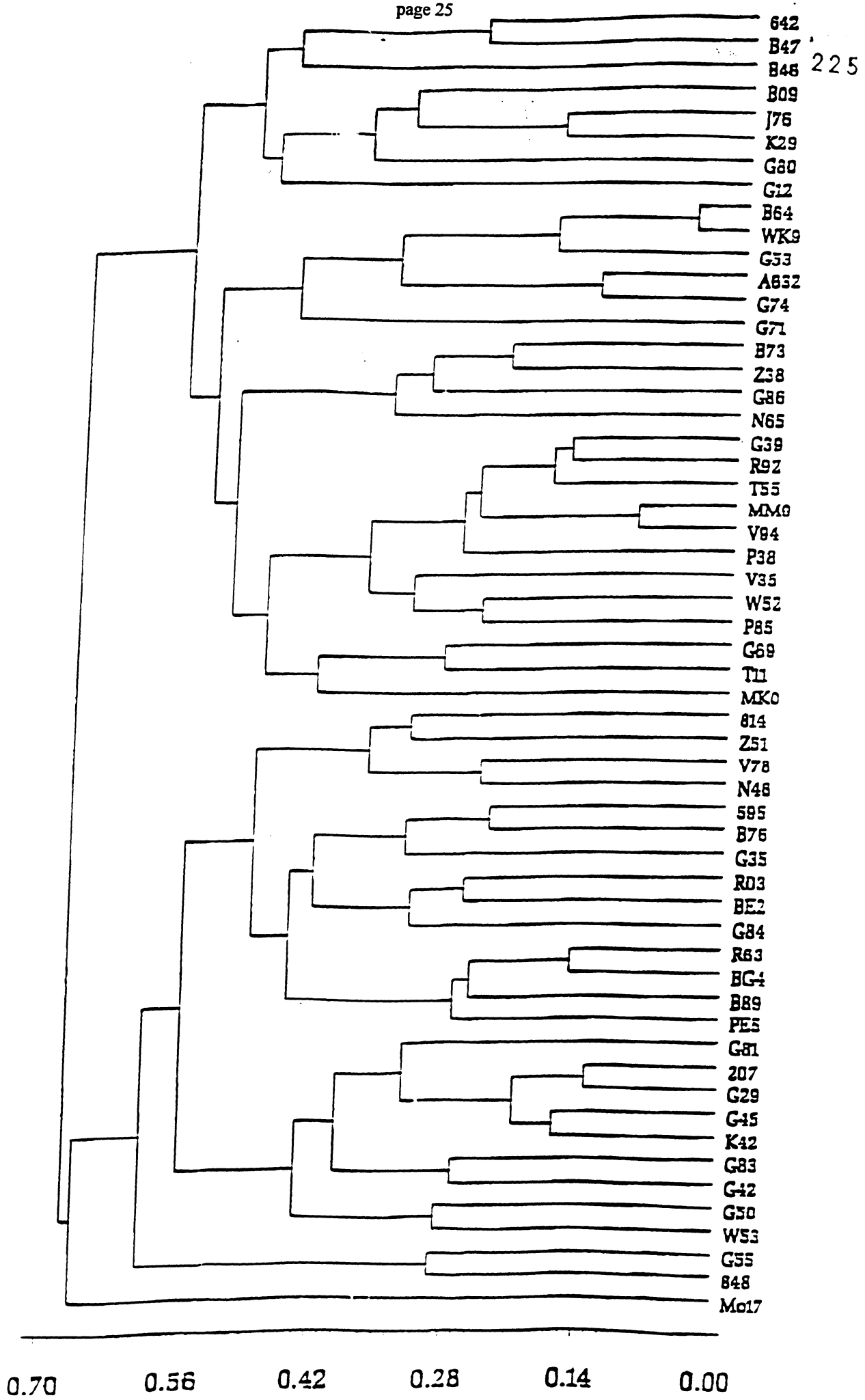

Figure 2. Associations among maize inbred lines revealed by cluster analysis of RFLP distance data.

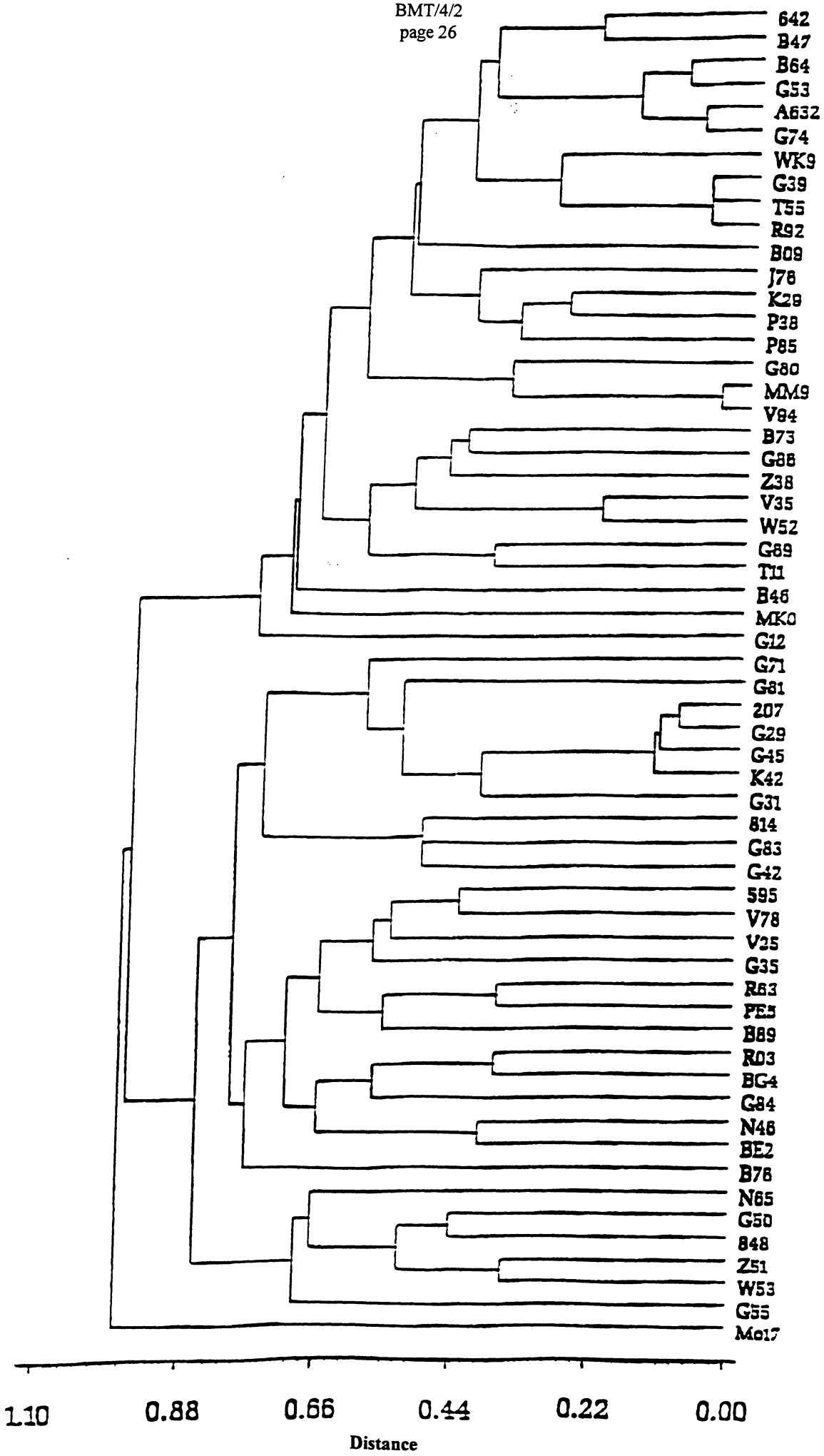

Figure 3. Associations among maize inbred lines revealed by cluster analysis of SSR distance data.

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