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Classification of North Dakota maize inbred lines into heterotic groups based on molecular and testcross data

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Abstract Establishment of the best combination among heterotic groups, heterotic patterns, is crucial to the development of successful maize (*Zea Mays* L.) hybrids. The use of molecular markers in maize-breeding programs might or might not increase the efficiency of heterosis prediction by classifying diverse inbred lines into heterotic groups. The objectives of present research were to classify elite North Dakota (ND) maize inbred lines into heterotic groups and evaluate the consistency between simple sequence repeat (SSR) grouping and testcross data. Thirteen ND inbred lines representing diverse genetic background were crossed in a diallel mating design in 2000. The crosses and 12 checks were evaluated across four ND environments in 2001 and 2002. In addition, these lines were crossed to commercial inbred testers representing known heterotic groups in 2002. Hybrids between public and private lines were evaluated across three ND environments in 2003. Inbred lines representing Lancaster Sure Crop, Iowa Stiff Stalk Synthetic (BSSS), Minnesota #13, Northwestern Dent, Golden Glow pedigrees and ND inbred lines were screened with 49 SSR markers. Inbred lines ND246, ND278, ND280, ND281, ND282 and ND284 were clustered within the BSSS heterotic group. Inbreds ND277, ND285, ND286, ND290, and ND291 grouped closer to the Lancaster Sure Crop heterotic group. Inbred lines ND257 and ND288 grouped within Minnesota #13. Data from ND278 and ND290 testcrosses showed good combining ability with testers representing more than one heterotic group. Our research shows that groups of genetically similar germplasm could not be identified accurately and reliably with molecular markers even when the available germplasm was diverse contrary what has been suggested. Therefore, extensive field evaluation is recommended to classify unrelated inbred lines of maize.

Keywords Heterotic groups · Inbreds · Maize · Testers · SSR

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Introduction

The development of successful maize (*Zea Mays* L.) hybrids requires establishment of heterotic patterns, defined as the cross between known genotypes that expresses a high level of heterosis (Carena & Hallauer, 2001). The most exploited heterotic pattern is the cross between Iowa Stiff Stalk Synthetic (BSSS) and Lancaster Sure Crop heterotic groups. Crosses among inbred lines that derive from unrelated heterotic groups are known to have better grain yield performance than those crosses among lines belonging to the same group (Moll et al., 1965; Hallauer et al., 1988; Melchinger, 1999).

Molecular markers have shown to be useful classifying unrelated inbred lines into heterotic groups (Smith

et al., 1997; Pejic et al., 1998; Senior et al. 1998; Lu & Bernardo, 2001; Li et al., 2002). Based on this information, the integration of molecular markers in maize-breeding programs can increase their efficiency. However, because of the complexity encountered in multi-trait and multi-stage selection as well as the complexity in the genetics of economically important traits molecular markers still do not have a prominent role in breeding programs (Hallauer, 1999). Therefore, molecular classification of inbred lines into heterotic groups might not be reliable especially for unrelated ones.

Simple sequence repeats (SSR) have been extensively used as genetic markers in eukaryotic genomes (Tautz, 1989). Such markers have a number of advantages when compared to amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), and restriction fragment length polymorphism (RFLP) markers (Pejic et al., 1998; Senior et al., 1998; Gethi et al., 2002).

The intercross of a broad variety of inbred lines developed base populations at North Dakota State University. For each population inbred lines were chosen based on their good combining ability for grain yield performance, stalk and root lodging resistance, and early maturity (Table 1).

The inbred lines utilized in this research were derived from ND base populations and have not been assigned to heterotic groups (Table 2).

The objectives of this study were to classify ND lines into heterotic groups and to evaluate the consistency between SSR grouping and testcross field data.

Table 1 North Dakota maize populations and genotypes used for their development

Populations	Genotypes
NDSA	A90, MS1334, ND376, ND474, ND478, NDB8, SD10, W153R
NDSB	CO303, CV3, ND33, ND405, ND363, MS142, Zapalote Chico
NDSC	A556, CG1, CG5, CO303, MS93, ND474, ND478, ND480, ND481, NDB8, W153R
NDSD	A554, A556, A654, A90, MS141, ND203, ND363, ND364, ND376, ND474, SD5, SDP2, SDP232, SDP236M, SDP254
NDSM	A654, A664, CM105, CM153, ND101, ND245, ND247, ND250, ND363, ND468, ND8RF, PA363, W59E
NDSF	Composite of 65 IL (flint, yellow and white corn)
NDSAB	Derived from 20 full-sib families between NDSA and NDSB
NDSK(FS)C1	Developed after one cycle of reciprocal full-sib selection among full-sib families between NDSA and NDSB
NDSL(FS)C1	Developed after one cycle of reciprocal full-sib selection among full-sib families between NDSA and NDSB
NDSCD	Developed by one cycle of full-sib family selection among 78 full-sib families between NDCD(FS)C1 and NDSD(FS)C1. NDSC(FS)C1 and NDSD(FS)C1 were produced by one cycle of reciprocal full-sib selection from NDSC and NDSD

Material and methods

Phenotypic data

Experiment I

Seed of all entries was multiplied in the 2000 Fargo breeding nursery to produce similar seed quality. A diallel mating design without reciprocals included 13 ND inbred lines as parents. Ten rows, 7 m long and 0.76 m between rows were planted for each hybrid produced. During pollination, all possible pair-row crosses were made. Pair-crosses were harvested and shelled in bulk per cross.

F_1 public hybrids and 12 commercial checks were evaluated in experiments with two replications arranged in a randomized complete block design (RCBD) since the environmental variability among experimental units was not large. Field experiments were conducted in 2001 and 2002 at three ND locations: Fargo (silt clay, Vertic Haplaquoll), Casselton (Beardon silt clay loam, Aeric Caliaquoll), and Oakes (Gardena, coarse-silty soil, mixed Pachic Udic Haploborolls). Experimental units were one-row plots with the same size as the ones used for seed production. The difference, however, was that plant density was greater. Plots were over-planted and thinned to an approximate plant density of 75,000 plants ha^{-1}. Plots were planted and harvested by machines adapted for small experimental plots. Missing data were generated on crosses ND280 $\times$ ND281 and ND286 $\times$ ND288 due to insufficient seed production for both years. Therefore, these two entries were estimated for the diallel analysis.

Analyses of variance were performed for grain yield within environments and data were combined

Table 2 Origin of maize inbred lines used in this study

Inbred line	Pedigree
ND281	NDSAB(MER)C3
ND284	NDSM(M)C1
ND280	(ND245 × ND252) × NDSB(FS)C4
ND291	NDSM(M)C1
ND246	W755 × W771
ND278	NDSL(FS)C3
ND288	NDSB × NDSF
ND282	NDSB(FS)C4
ND285	NDSCD(M)C1
ND286	NDSCD(M)C3
ND257	NDSC(M)C1
ND290	NDSAB(MER)C5
ND277	NDSK(FS)C3

following a mixed model. Genotypes were considered fixed effects and environments random effects. Mean square error variances of each individual analysis were homogenous based on the Bartlett's chi-square test conducted before analysis across environments. *F*-tests were considered significant at $P \leq 0.01$ and $P \leq 0.05$. Genotype by environment interaction mean square was used as the denominator to calculate the *F* value for crosses. Since detectable differences were contributed from parents to offprings (significant differences among crosses), an orthogonal subdivision of the sums of squares for diallel crosses was performed. This allowed the estimation of general combining ability effects (GCA, effects for all crosses that include a common parent) and specific combining ability effects (SCA, effects of each pair of parents for specific crosses) for parents without missing data (Sprague & Tatum, 1941) using Griffing's Method 4 (Griffing, 1956). ND inbred lines included in this study are not an unselected sample. Therefore, Model I (fixed model) was followed and parents were the reference genotypes (genotypes under consideration). On the other hand, this selected set of parents represents an elite group of lines. The Student's test of significance was used to test the null hypothesis that GCA and SCA effect values were equal to zero. Simple linear regression analyses were performed to study the degree of association between the average performance of ND inbred lines and their corresponding GCA values for grain yield. Fisher's protected least significant difference (LSD) was used to compare means at $P \leq 0.05$.

Experiment II

Seed of all entries was multiplied in the Fargo 2003 breeding nursery to produce similar seed quality. A North Carolina Design II (Comstock & Robinson, 1948) was utilized for making testcrosses between ND lines and three commercial testers. These testers represented the major heterotic groups available in the northern Corn Belt: B14, Iodent, and LH82. Two Bt testers developed by Syngenta represented the B14 and Iodent groups. Monsanto provided LH295 representing the LH82 unrelated group. Pair rows, 7 m long and 0.76 m between rows, were planted for each hybrid produced. During pollination, all possible pair-row crosses were made. Pair-crosses were harvested and shelled in bulk per cross.

Private × public as well as commercial F_1 hybrids were planted and harvested following the same procedures of experiment I. Twelve ND inbred lines were crossed to an Iodent Bt tester and a B14 Bt tester from Syngenta, and LH295 from Monsanto. In addition, inbred lines LH176 and LH177 (Monsanto) were crossed to ND278 substituting the cross to LH295, since they have similar background. Inbred line TR4033 (Thurston Genetics), representing the BSSS group was crossed to ND285 in replacement to the B14 Bt tester. Inbred line ND257 was not included in this experiment.

Analyses of variance were performed for grain yield, adjusted to 155 g kg^{-1} grain moisture at harvest and expressed as Mg ha^{-1}, grain moisture at harvest (g kg^{-1}), stalk lodging (%) and flowering (days) using the SAS Lattice procedure for a 9 × 9 partially balance lattice design (SAS, 1989). Efficiency of the lattice relative to a RCBD was calculated with the average of variance (effective error) and the RCBD error. Mean square error variances of each individual analysis were homogenous based on the Bartlett's chi-square test conducted before analysis across environments. Means adjusted by blocks were used when the relative efficiency of lattices was higher than 105% when compared with the RCBD. The effective error mean squares instead of RCBD error mean squares were used as a denominator in the *F*-test when relative efficiency of lattice designs was greater than 105% compared with the RCBD. For the combined analysis, adjusted and unadjusted means were analyzed as a RCBD design, following a mixed model in which genotypes were considered fixed effects and environments random effects. The interaction between genotypes and environments was used as the denominator

to calculated the F value for genotypes. Pooled error was used as denominator to calculate the genotype by environment interaction F value. The genotype by environment mean square was also used to calculate the LSD value for every trait (Bernardo, 2002).

Genotypic data

Plant material

Fresh leaf tissue from 13 ND inbred lines and 27 historically old inbred lines were obtained for genetic analysis (Fulton et al., 1995) (Table 3). Seeds for the 27 historically old inbred lines were kindly provided by the North Central Regional Plant Introduction Station (NCRPIS), part of the United States National Plant Germplasm System.

Seeds were planted in the green house and 5 days after germination 200 mg of the two youngest leaves were collected for DNA extraction. The protocol used for the DNA extraction was originally used for tomato and other herbaceous plants. It yielded in average 628 μg/ml by using fresh young leaves, avoiding an extra step of freezing the leaves before extraction. Inbred line C103 was isolated twice, but did not produce high quantities of DNA. Therefore, this genotype had missing data.

Sixty ng of DNA was used for PCR amplification. Promega Taq polymerase (0.5 U/sample) was used with buffers at 1 × PCR buffer and $MgCl_2$ at 2.5 mM. The PCR amplifications were performed by 30 cycles of 1 min at 94 °C, 1 min at 55–66 °C, and 1 min at 72 °C, ending with 5 min at 72 °C. Reaction products were electrophoresed on 6% polyacrylamide (19 acrylamide: 1 bis-acrylamide) gels and visualized by staining with ethidium bromide (Wang et al., 2003). Photographs were taken from the gels and printed for scoring.

Fifty SSR primers were assayed to represent all 10 maize chromosomes. Primers used in this study were chosen for being present in previous studies and for showing high Polymorphic Index Correlation (PIC) values. Polymorphic Index Correlation (PIC) is the frequency of the ith allele, averaged across loci:

$$\mathrm{PIC} = 1 - \sum {f_i}^2,$$

f_i^2 is the frequency of the ith allele, averaged across loci.

Table 3 Maize inbred lines used for the genetic similarity analysis

No. of lines	Inbred lines	Pedigree
1	A554	[(WD × Wf9) WD(2)]
2	A556	B164–886 × A237
3	A654	A116 × Wf9
4	A90	64 (renamed A48) × 15–28 (renamed A39)
5	B104	BS13(S)C5
6	B14	Iowa Stiff Stalk Synthetic
7	B37	Iowa Stiff Stalk Synthetic
8	B73	Iowa Stiff Stalk Synthetic C5
9	B84	BS13(S2)C0
10	B87	BS22
11	B97	BSCB1(R)C9
12	C103	Lancaster Sure Crop (derived from Noah Hershey)
13	Mo17	C.I.187-2 × C103
14	MS1334	[(Golden Glow × Maize Amargo) × Golden Glow]
15	MS141	MS1334 × [(H × MS206) × (A9 × C105)]
16	MS142	Mich 250
17	MS93	[Oh40B × R53) R53]
18	ND203	Haney's Minnesota 13
19	ND246	W755 × W771
20	ND257	NDSC
21	ND277	NDSK(FS)C3
22	ND278	NDSL(FS)C3
23	ND280	(ND245 × ND252) × NDSB(FS)C4
24	ND281	NDSAB(MER)C3
25	ND282	NDSB(FS)C4
26	ND284	NDSM(M)C1
27	ND285	NDSCD(M)C1
28	ND286	NDSCD(M)C3
29	ND288	NDSB × NDSF
30	ND290	NDSAB(MER)C5
31	ND291	NDSM(M)C1
32	ND33	not known (possibly Manitoba Yellow Flint)
33	ND376	A376 × ND203
34	ND405	ND203 × OH51A
35	ND474	[(WD × Wf9) WD (2)]
36	ND480	Oh51A × ND230
37	NDB8	4 County White yellow recovery
38	OH43	W8 × OH40B
39	SD10	B8 × W56A
40	W153R	[(Ia153 × W8) × Ia 153

The average number of alleles per polymorphic locus was calculated to estimate genetic diversity among inbred lines. Genetic distance estimates between inbreds (i and j) were estimated based on Nei and Li

(1979):

$$GDij = 1 - GSij \text{ where } GSij = 2Nij/(Ni + Nj)$$

Nij is the number of bands (or alleles) found in both i and j. Ni is the total number of bands found in i. Nj is the total number of bands found in j. GDij is equal to one minus the genetic similarity coefficients originally devised by Dice (1945). Jaccard's method (1908) was also tested to calculate genetic distance for repeatability. Using a matrix of genetic similarities, cluster analysis was performed by the Unweighted Pair Group Method using Arithmetic averages (UPGMA) algorithm. The dendogram was constructed with the Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.02i (Exeter Software, Setauket, NY) software package (Rohlf, 1998).

Results and discussion

Experiment I

Grain yield means ranged from 4.60 to 5.50 Mg ha^{-1}. GCA effects ranged from −5.04 to 4.15 (Table 4). Analysis of variance (*F*-test) of the crosses among lines indicated that most of the variation within this set of crosses was due to the average performance of the lines included in the diallel mating design. In eight of 13 instances GCA effects were significant and relatively large when compared to SCA effects. Inbred lines ND246, ND278, ND280, ND282, ND284, ND288, ND290, and ND291 had significant GCA effects. Therefore, the main type of gene action expressed in this unique set of crosses was additive. Base populations for these inbred lines share inbred lines from similar heterotic groups, decreasing SCA effects. SCA sum squares were relatively smaller than the GCA sum squares for grain yield. However, even though non-additive gene effects are on average small, they are important for unique combinations (Hallauer & Miranda, 1988). No linear relationship between average grain yield of crosses, parents and their corresponding GCA values was found (Table 4). The Model I analysis yielded considerable information about the fixed set of parents, most of which were released by ND > AES, that are potential candidates for the production of early-maturing single-cross hybrids.

Table 4 Means of North Dakota maize inbred lines and general combining ability (GCA) effects for grain yield

Inbred line	Yield of crosses†	GCA
	Mg ha^{-1}	
ND246	5.14	−2.12**
ND257	5.48	0.15
ND277	4.85	0.25
ND278	4.83	3.98**
ND280	5.50	−2.91**
ND281	4.88	−1.19
ND282	4.94	4.15**
ND284	4.98	−2.63**
ND285	4.79	−0.33
ND286	5.22	−0.08
ND288	5.45	−5.04**
ND290	4.60	2.80**
ND291	5.18	2.97**
$LSD_{0.05}$	1.30	1.33

*Significant at $P \leq 0.01$

Experiment II

The choice of best parents for a northern maize-breeding program based on this set of lines cannot rely on information obtained from Experiment I only. The average grain yield performance of parents included in a diallel mating design among only ND lines is useful but provides limited information for not only classifying these lines into heterotic groups but also to assess average grain yield performance of parents. The use of commercial testers of known heterotic groups is a common practice to identify the commercial potential of public lines in our ND maize-breeding program. Selected means for the traits analyzed are listed in Table 5.

There were significant differences among crosses for grain yield. Inbred line ND278 had the highest grain yield when crossed to all commercial testers (Iodent Bt tester, LH82, and B14 Bt) and its performance in public × public and public × private hybrid combinations was outstanding when compared to Pioneer hybrids 39R34 and 39H84. This confirms the positive GCA values found for this inbred in experiment I. Inbred line ND290 in crosses with all testers also formed the best hybrids, a result expected based upon its GCA effects. Inbred lines ND282, ND286, and ND291 were parents of the best crosses, but combined well only with lines derived from only one heterotic group. ND282 and

Table 5 Grain yield, grain moisture at harvest, stalk lodging, and flowering of selected entries across environments

Line	Tester	Grain yield (Mg ha^{-1})	Grain moisture (g kg$^{-1)}$	SL (%)	SILK (Days)
ND277	× NP2341Bt	5.17	16.3	12.6	63
ND278	× NP2341Bt	6.38	16.0	8.5	62
ND280	× NP2341Bt	4.93	18.0	20.1	63
ND281	× NP2341Bt	5.81	17.0	11.0	63
ND282	× NP2341Bt	6.32	17.5	4.8	64
ND284	× NP2341Bt	4.40	15.9	16.4	63
ND285	× TR4033	4.71	18.1	38.2	67
ND286	× NP2341Bt	4.88	15.5	6.9	64
ND287	× NP2341Bt	5.03	15.6	18.7	64
ND288	× NP2341Bt	4.26	14.4	1.7	61
ND289	× NP2341Bt	4.16	15.0	10.2	63
ND290	× NP2341Bt	6.55	16.4	0.9	62
ND291	× NP2341Bt	6.37	16.8	13.4	63
ND278	× NP2123Bt	8.00	19.5	3.5	64
ND280	× NP2123Bt	6.60	20.1	27.7	64
ND281	× NP2123Bt	6.03	18.8	24.4	64
ND282	× NP2123Bt	4.55	18.7	20.2	65
ND284	× NP2123Bt	5.24	20.4	29.9	63
ND285	× NP2123Bt	4.63	18.8	22.4	66
ND286	× NP2123Bt	4.77	17.2	15.4	67
ND287	× NP2123Bt	5.41	18.6	13.1	66
ND288	× NP2123Bt	4.38	16.9	7.6	63
ND289	× NP2123Bt	6.89	19.3	4.6	66
ND290	× NP2123Bt	6.36	18.9	5.7	63
ND291	× NP2123Bt	5.28	19.3	10.8	62
ND277	× LH295	4.01	17.7	19.2	67
ND278	× LH177	6.82	18.1	‘8.2	63
ND278	× LH176	6.87	17.6	0.0	65
ND280	× LH295	5.91	19.2	11.4	67
ND281	× LH295	5.95	19.9	18.7	66
ND282	× LH295	6.54	20.4	18.6	67
ND284	× LH295	6.18	19.4	27.0	64
ND285	× LH295	4.56	18.2	25.2	65
ND286	× LH295	6.67	18.6	22.8	67
ND287	× LH177	6.33	18.2	21.8	66
ND288	× LH295	4.41	16.8	6.0	64
ND290	× LH295	6.80	20.1	5.0	65
ND291	× LH295	6.60	21.4	9.3	63
CHECK 1	Syngenta N17–R3	8.28	17.4	10.7	65
CHECK 3	Pioneer 39R34	6.12	15.8	5.2	60
CHECK 2	Pioneer 39H84	6.01	15.8	9.1	62
CHECK 5	N×1870	7.38	17.7	4.4	63
CHECK 4	DK 35–51 RR YG	8.01	17.5	16.9	64
EXP	MEAN	5.50	18.0	15.3	63.7
LSD		1.5	2.5	25.3	2.8
CV		16.4	7.1	59.2	1.7

ND291 showed good GCA effects for grain yield while ND286 did not show significant GCA effects.

Inbred lines are usually developed within narrow and specific heterotic groups with the purpose to exploit heterosis in the final cross. Therefore, they usually combine well when crossed to lines from only one opposite specific heterotic group (Moll et al., 1965; Hallauer et al., 1988; Melchinger, 1999).

However, some lines have demonstrated the flexibility to produce successful crosses with lines representing different heterotic groups. This research confirms that ND278 and ND290, developed from genetically broad-based populations, have good combining ability across heterotic groups. Current modified convergent improvement (Richey, 1931) efforts at NDSU suggest this advantage in flexibility can be incorporated.

Grain moisture at harvest is as important as grain yield for the northern Corn Belt. The challenging very short growing season for North Dakota makes early-maturing maize essential. There were significant differences among crosses for grain moisture at harvest. Grain moisture at harvest ranged from 14.1 to 23.6%. This variability reflects the potential drying costs for the northern corn producer. The best crosses for grain yield that also had low grain moisture at harvest included inbred lines ND278, ND290, ND291, and ND2000 as one of the parents. ND2000 is the latest release of our maize breeding program (Carena & Wanner, 2003) and it was used in hybrid combination as a check. Only two commercial hybrid checks had acceptable grain moisture at harvest: Pioneer 39R34 and Pioneer 39H84 and the earliest tester with highest stalk lodging resistance was the one representing the B14 heterotic group (Table 5). However, Pioneer hybrid checks 39R34 and 39H84 had significantly lower performance than NP2123Bt × ND278. Flowering dates were related with grain moisture at harvest in most cases (Table 5). Significant differences among hybrids were also present for stalk lodging resistance. Hybrid combinations including ND2000, ND278, ND288, and ND290 as parents were the most resistant to stalk lodging across all testers. The averages stalk lodging across testers of these hybrid combinations were below 5.1% while the commercial hybrid check average was 9.3%. It is interesting to note that Monsanto hybrid check DK 35–51 RR YG was above the experiment mean with 16.9% even though it carries the Bt gene.

Genotypic data

Forty-nine primers have been mapped to regions that were dispersed equally throughout the maize genome and produced 184 alleles among the 40 maize inbreds (Table 6). Data from only one pair of primers was discarded, Phi042, due to difficulty in scoring.

To test the number of primers needed to construct an accurate cluster combinations of 20, 40 and 49 primers were performed and compared. Groups formed based on 20 primers did not agree with already known relationships among lines. When 40 and 49 primers were used, grouping was repeatable and in better agreement with previous pedigree information. Based on this information, results were discussed based on 49 SSR primers.

The number of alleles required for a good estimation of genetic similarity depends on the relationship among inbred lines. For intermediately related inbred lines, about 150 alleles are sufficient, according to Tivang et al. (1994) and Pejic et al. (1998). The average number of alleles for this study was 3.8 per locus. This number might be considered low if compared to other studies. For example, Smith et al. (1997) estimated an average of 4.3 alleles per locus while Senior et al. (1998) found 5.2 alleles per locus, Lu and Bernardo (2001) 4.9 alleles per locus, and Liu et al. (2003) 21.7 alleles per locus. However, relatedness of ND inbred lines and the type of marker utilized probably affected the number of alleles per locus found. For instance, Liu et al. (2003) used tropical, subtropical and temperate genetic materials, in addition to U.S. Corn Belt inbred lines. Di-nucleotide markers were not used in this study what might explain the low number of alleles per locus found. Most markers were tetra-nucleotide, representing 71% of the primers. PIC values for the SSR loci ranged from 0.43 to 0.83, with an average of 0.70. The average PIC values for tri, tetra and penta-nucleotides were 0.71, 0.69 and 0.71, respectively. These results agree with Liu et al. (2003), when comparing to the PIC average values of tri-nucleotide or longer-repeat motifs.

Heterotic groups were identified as Northwestern Dent, Golden Glow, BSSS, Wf9, Minnesota #13, Lancaster Sure Crop and Miscellaneous (Fig. 1). The cluster analysis identified five potential heterotic groups for ND inbred lines but no ND lines were grouped within Golden Glow and/or Northwestern Dent. Ironically, Northwestern Dent was one of the most popular land races for North Dakota (Olson et al., 1927).

ND246, ND280, ND281, and ND284 clustered within the BSSS group. ND246 and ND280 are related based on pedigree since ND280 has one ND246 sister line in its background (ND245 and ND252). However, our results disagree with previous research on ND246, an economically important line used to develop LH160, LH161, and LH162. Romero-Severson et al. (2001)

Table 6 Genome location, SSR loci, repeat type, number of alleles produced, PIC values, maximum, minimum, and average values for 40 maize genotypes

Bin no.	SSR locus	Repeat type	No. of alleles	PIC
1.01	phi109275	AGCT	4	0.79
1.04	umc1169	(TTA)4	5	0.83
1.06	umc1122	(CGT)7	4	0.79
1.08	phi002	AACG	2	0.61
1.11	phi064	ATCC	6	0.84
2.01	phi96100	ACCT	5	0.81
2.03	phi109642	ACGG	3	0.67
2.04	phi083	AGCT	6	0.78
2.08	phi127	AGAC	3	0.71
2.10	phi101049	AGAT	5	0.82
3.01	phi104127	ACCG	2	0.50
3.05	phi053	ATAC	5	0.74
3.06	umc2267	(CTTG)5	3	0.69
3.07	umc1399	(CTAG)5	6	0.74
3.08	phi046	ACGC	6	0.73
4.00	phi072	AAAC	3	0.67
4.04	phi096	AGGTG	5	0.58
4.05	phi079	AGATG	4	0.81
4.08	phi092	GCAA	3	0.65
4.08	phi093	AGCT	3	0.68
5.03	phi109188	AAAG	5	0.80
5.04	phi113	GTCT	4	0.74
5.06	phi085	AACGC	4	0.71
5.07	phi128	AAGCG	3	0.72
5.09	umc1153	(TCA)4	5	0.80
6.04	phi031	GTAC	4	0.76
6.04	phi452693	AGCC	4	0.81
6.05	phi078	AAAG	3	0.69
6.05	phi129	ATAC	2	0.51
6.07	phi123	AAAG	3	0.67
7.01	phi057	GCC	4	0.69
7.02	phi034	CCT	5	0.80
7.03	phi114	GCCT	5	0.83
7.04	phi328175	AGGTG	6	0.72
7.05	phi051	AGG	2	0.56
8.03	phi100175	AAGC	3	0.67
8.04	phi014	GGC	2	0.50
8.05	umc1121	AGAT	5	0.77
8.08	phi015	AAAC	5	0.81
8.09	phi233376	CCG	4	0.72
9.01	phi044	CCCT	2	0.49
9.03	phi022	GTGC	3	0.68
9.04	phi032	AAAG	2	0.48
9.05	phi108411	AGCT	2	0.43
10.00	phi041	AGCC	3	0.76
10.02	phi059	ACC	3	0.67
10.02	phi063	TATC	3	0.69
10.02	phi96342	ATCC	2	0.51
10.03	phi050	AAGC	3	0.68
Max.			6	0.84
Min.			2	0.43
Average			3.8	0.70
Total			184	

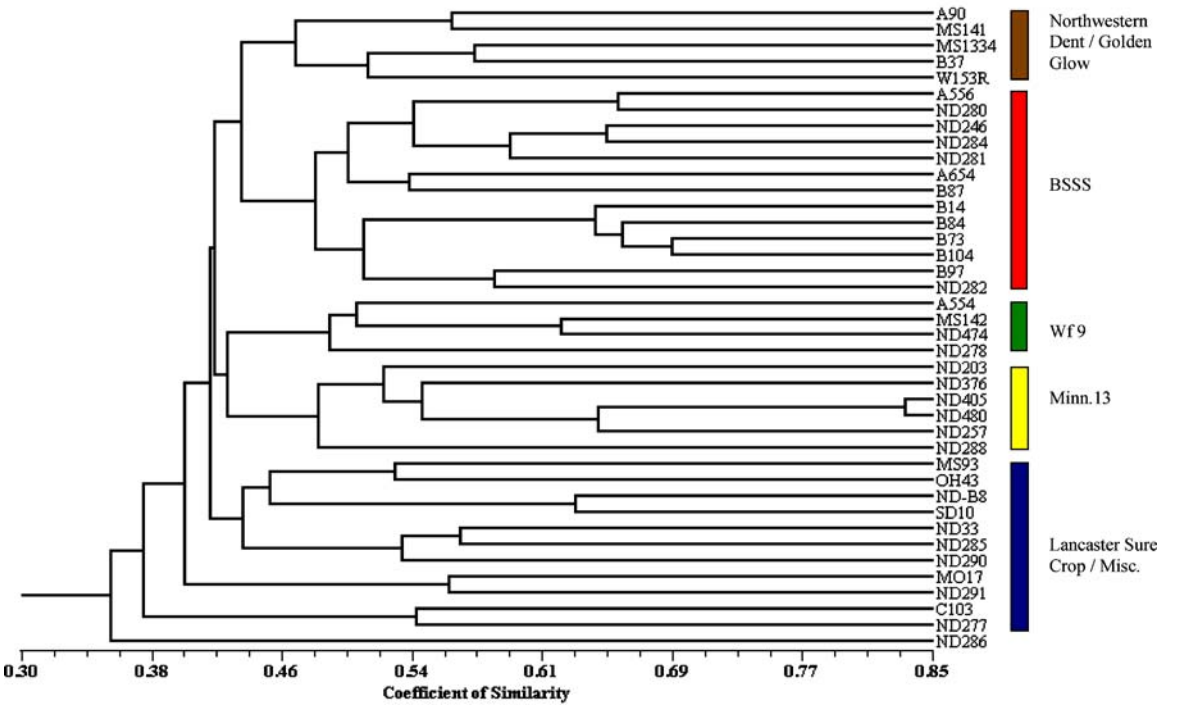

Fig. 1 Associations among 40 maize inbred lines revealed by UPGMA cluster analysis generated by 49 SSR markers

showed that ND246 was clustered between BSSS and Lancaster Sure Crop groups. On the other hand, Liu et al. (2003) found that ND246 clustered within the Lancaster Sure Crop group. Therefore, some caution is needed when interpreting molecular results for this line. Inbred ND278 was closer to A554 and ND474, two inbred lines that have the Wf9 line in their background (Reid Yellow Dent). Wf9 covered 30% of U.S. hectares for a long time, becoming the most popular line of all times (Troyer, 1999). ND257 and ND288 were grouped within Minnesota #13 group and several lines were included within Lancaster sure Crop (ND277, ND285, ND290 and ND291). We did not expect ND lines to be grouped within Lancaster Sure Crop since most of the lines present in the base populations, have BSSS background. This result shows a disagreement between SSR marker and pedigree data. The same can be said about ND286 (Fig. 1).

North Dakota inbred lines may have alleles that when identical in size, may represent alleles that are only identical in state, and may not always be identical by descent (Mumm & Dudley, 1994; Falconer & Mackay, 1996; Senior et al., 1998; and Bernardo et al., 2000). Some ND inbred lines are derived from similar genetic backgrounds, such as ND285 and ND286 (North Dakota State University, 1998). These two inbred lines are derived from population NDSCD, but from cycle 1 and cycle 3, respectively. However, they do not cluster close together. The same was observed for ND284 and ND291. Both lines derived from population NDSM but the first inbred clustered within the BSSS group while the second one clustered within the Lancaster Sure Crop group. Moreover, Yu et al. (2001) found that inbred lines derived from the same open pollinated cultivar did not always show high genetic similarity estimates. In addition, Warburton et al. (2002), utilizing SSR markers for seven CIMMYT populations and 57 inbred lines, found that lines derived from the same population did not cluster together either.

SSR markers were suggested to be very useful to assign inbred lines into known heterotic groups (Melchinger, 1999) especially when distinguishing closely related inbred lines and detecting pedigree relationships that may not be evident by phenotype and/or records. However, the presence of bands with minimum size differences for different sources of the same inbred line can lead to disagreements with pedigree records. This may be due to non-genetic variance, such as DNA extraction, PCR reaction or data scoring (Romero-Severson et al., 2001). Presence of residual

heterozygosity in the original release of the inbred line, divergent selection, random drift, occasional mutation cases, contamination during pollination, human error, genome sampling, and number of markers (Nei, 1987; Tivang et al., 1994) could also explain cases of disagreement with pedigree data.

Conclusions

ND278 and ND282 had the highest GCA effects for grain yield. They were classified within the Wf9 and BSSS groups respectively, both considered Reid Yellow Dent derived groups (Troyer, 2004). Both lines combined well even with testers from the same heterotic group (B14). The effects of sampling, selection (Hallauer et al., 1988), and broad genetic background seem to be sufficient to cause differences in allele frequencies, resulting in heterosis in the cross of inbred lines derived from the same heterotic group. This is in agreement with our current breeding efforts in modified convergent improvement of top public hybrids.

ND inbred lines were also clustered within the Lancaster Sure Crop heterotic group, while their genetic background is mostly represented by Reid Yellow Dent (ND277, ND285, ND286, ND290 and ND291). Among them, ND290 and ND291 had above average GCA effects for grain yield later confirmed with crosses to commercial testers. Therefore, pedigree data did not agree with molecular marker data as in many other cases (Mumm and Dudley, 1994; Bernardo et al., 2000; Gethi et al., 2002; Li et al., 2002; Labate et al., 2003; Liu et al., 2003). As a consequence, field data across environments is essential to classify unrelated inbred lines into heterotic groups.

ND278, ND282, ND290, and ND291 are the best options for breeding programs developing elite products for short growing season areas. The above average combining ability for grain yield and grain moisture at harvest of these lines even across heterotic groups is enough evidence to use them as source of new early-maturing inbred lines. Other ND lines contributed unique characteristics to hybrids such as stalk lodging resistance (e.g. ND288).

There are still large inconsistencies between molecular marker data and field trial data. Our research confirms that extensive multi-environment testing of genotypes should be the priority of maize breeding programs over molecular data collection.

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