ARTICLE

Identification of heterotic patterns between expired proprietary, NDSU, and industry short-season maize inbred lines

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Abstract: *Maize (*Zea mays *L.) inbred lines are under restricted use, protected by Patent and Plant Variety Protection (PVP) laws. Research objectives were i) to identify and validate heterotic groups and patterns, and ii) to determine if ex-PVP lines are useful for continued genetic progress in short-season environments. Three groups of crosses were made following North Carolina Mating Design II (NCII) including 12 NDSU, 24 ex-PVP lines, and seven current industry testers. Hybrids were planted in four different experiments at six ND environments following partially balanced lattice experimental designs in 2011 and 2012. Top heterotic patterns were selected based upon grain yield and other agronomic traits. Our research indicates most ex-PVP lines are genetically narrow and may not be immediately useful. Less protection (5-yr vs. 20-yr) might increase usefulness of ex-PVP lines. This change in intellectual property will allow public breeders to develop better versions of industry lines carrying known weaknesses.*

Key words: *Zea mays L., ex-PVP, heterotic groups, SCA.*

INTRODUCTION

Maize breeding for hybrid production is a confidential and highly profitable business. In order to protect this business, The USA decided to protect inbred parents and hybrids by the U.S. Patent/or U.S. Plant Variety Protection Act (PVPA). Expired-PVP (ex-PVP) inbred lines, after being protected for 20 years, are maintained at the North Central Regional Plant Introduction Station (NCRPIS) at Ames, IA (Mikel 2006, Bari and Carena 2014). These lines have become annually available after protection ended. They potentially represent new germplasm sources for many public and private breeding programs for study and use (Nelson et al. 2008). However, many breeders doubt their usefulness due to their original development date, which is over 20 years old.

Maize breeding programs are dependent on the identification and utilization of heterotic groups and heterotic patterns (Melani and Carena 2005). Assigning and validating ex-PVP inbred lines to heterotic groups will be useful to exploit desirable heterotic patterns. Heterotic groups represent groups of germplasm sources that when crossed with each other produce consistently better crosses than when crosses are made within those groups (Hallauer and Carena 2009). Identifying heterotic patterns, which are crosses between known genotypes (from different heterotic groups) expressing a high level of heterosis (Carena and Hallauer 2001, Troyer 2006, Mendes et al. 2015), is key to the development

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of successful maize hybrids (Eyherabide and Hallauer 1991, Barata and Carena 2006, Carena and Wicks III 2006). The North American dent maize germplasm is composed of multiple heterotic groups that when crossed to each other can optimize hybrid performance (Mikel and Dubley 2006). The identification of genotypes in these groups helps exploit suitable heterotic patterns. Dubreuil et al. (1996) emphasized that the accurate assignment of inbred lines to heterotic groups is a prerequisite for efficient utilization of germplasm. Heterotic groups in dent maize have been subdivided into Iowa Stiff Stalk Synthetic (BSSS) and non-BSSS (Lu and Bernardo 2001). A similar grouping consists of Reid Yellow Dent (related to BSSS), Lancaster, Iodent, and miscellaneous heterotic groups (Gethi et al. 2002). Troyer (1999) divided maize into five genetic backgrounds: Reid Yellow Dent (Iodent Reid and BSSS), Minnesota 13 (W153R and SD105), Northwestern Dent (A48, A509, and A78), Lancaster Sure Crop (Mo17 and Oh43), and Leaming (Oh07). The Reid Yellow Dent is the largest group, and has made significant contributions to commercial hybrids.

There are several methods to classify maize inbreds into heterotic groups. Two major classification methods are widely used across the world (Fan et al. 2009). The traditional method uses specific combining ability with line-pedigree information, and/or field hybrid-yield information, to assign maize lines to heterotic groups. A more challenging method is to use different molecular markers to compute genetic similarity (GS) or genetic distance (GD) estimates to assign maize lines to a particular heterotic group, which is not always accurate. Fan et al. (2009) executed a third approach, by using heterotic group's specific and general combining ability (HSGCA) to classify inbreds into heterotic groups. They claimed their way is efficient compared to SSR markers and yield-based specific combining abilities. Menkir et al. (2004) classified inbred lines into heterotic groups by yield-based specific combining ability and molecular marker based approaches. They reported that yield-based combining ability derived heterotic groups did not match with groups established using molecular markers. Melchinger (1999) extensively discussed the potentials of DNA markers in assigning inbreds of unknown genetic origin to established heterotic groups. However, he concluded that if a large number of genotypes are available and proven testers exist, the testcross performances should be the main criteria for classifying materials into heterotic groups. In addition, Barata and Carena (2006) observed large inconsistencies between molecular marker-based classification and field trial based classification (e.g., testcross and diallel data) of a diverse set of inbreds. They concluded that groups of similar germplasm and heterosis properties could not be identified accurately and reliably with molecular markers. Consequently, they recommended extensive field evaluation across environments to classify inbred lines into heterotic groups. Mating designs (Hallauer et al. 2010) can be used to test a large set of progenies, extensively over locations and years to classify inbreds to heterotic groups. Many ex-PVP lines do not have assigned heterotic groups yet; an approximation can be deduced based on PVP documents and their genesis. Moreover, reported heterotic groups may not be stable in different situations. The objectives of the study were i) to identify and validate heterotic groups and patterns of ex-PVP inbreds, industry testers, and NDSU inbred lines, and ii) to determine if ex-PVP lines are useful for continued genetic progress in short-season environments.

MATERIAL AND METHODS

For full details on plant materials, crossing procedures, and field trials see Bari and Carena (2014). Ex-PVP lines were selected because they had the fewest number of silking days and growing degree-days in their public descriptions. Along with current testers and NDSU lines, they represent earliness pools for northern U.S. maize breeding. Ex-PVP materials were requested and obtained from the North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA. Additional information on the the 12 NDSU lines and industry testers utilized can be found in Carena and Wanner (2003, 2009), Carena et al. (2003, 2010), and Carena (2013). Industry testers represent known heterotic groups available in the northern U.S. Corn Belt. T1 is an Iodent line, T2 is B14-derived SS line, T3 is another Iodent line, T4 is a B14/B73 derived SS line, T5 is an LH82 derived non-SS line, T6 is B14-derived SS line, and T7 is a B14/B73 derived SS line.

Twelve NDSU lines were crossed with 12 ex-PVP lines in the 2010 NDSU Fargo summer nursery following the North Carolina Mating Design II (NCII) (Comstock and Robinson 1948). The same 12 NDSU lines were crossed with another set of 12 ex-PVP lines, following a second NCII mating design at the 2010-2011 northern New Zealand winter nursery. All 24 ex-PVP lines (i.e. the lines used in the first and second sets of crosses) were also crossed in the winter with seven current industry inbred testers following a third NCII design. Hybrids were planted in four different partially balance lattice design trials along with five industry hybrids as checks across six North Dakota (ND) environments. Experimental checks represented a wide maturity range for ND (83RM to 100RM). Experimental plots were planted and harvested using machines that had been modified for small experimental plots. Plant density was approximately 80,000 plants ha^{-1}. Fertilization and field management practices were as recommended for ensuring optimum maize production. While harvesting, approximately 500 g seed samples were kept from each plot for grain quality assessment.

Data were recorded on an individual plot basis. Grain weight, grain moisture, and test weight were measured electronically on the combine while harvesting. Grain yield (Mg ha^{-1}) was adjusted to 155 g kg^{-1} grain moisture. Root lodging was measured as percentage of plants in a plot leaning at an angle greater than 30° from vertical while stalk lodging was measured as a percentage of plants in a plot with stalks broken at or below top ear. Lodging notes were taken just before harvest and analyzed as percentages to total stands per plot. Grain protein (g kg^{-1}), grain starch (g kg^{-1}), and grain oil (g kg^{-1}) data were collected with near infrared technology (OmegAnalyzer G, Bruins Instruments).

Plot means of all phenotypes were used for statistical analyses. Analyses of variance were performed for all traits at each location, as well as combined across locations and years using SAS 9.3 software (SAS 2010), for the four experiments. Data were collected and summarized in Excel files and then exported to SAS for analyses. Homogeneity of error variances was tested before combining data across environments. Mean comparisons among genotypes were assessed by Fisher's protected least significant difference (FLSD) at <0.05 level of significance, which has been shown to be an appropriate test for detecting differences (Carmer and Swanson 1971). A combined ANOVA was computed for grain yield (Mg ha^{-1}), grain moisture at harvest (g kg^{-1}), test weight (kg hL^{-1}), root lodging (%), stalk lodging (%), grain protein (g kg^{-1}), grain starch (g kg^{-1}), and grain oil (g kg^{-1}). General and specific combining ability effects were estimated considering year by location combination as environments. Combining abilities were further partitioned into male, female, and interactions of male and female (Scott et al. 2009). SCA effects for yield, along with mean grain yield were utilized to estimate and validate heterotic groups of short season ex-PVP and NDSU lines from the known heterotic groups of testers. Pedigrees of particular inbred lines were also used to determine heterotic groups when inbreds combined well with contrasting heterotic testers.

RESULTS AND DISCUSSION

SCA effects and means for grain yield have been widely used to classify maize heterotic groups (Menkir et al. 2004, Melani and Carena 2005, Fan et al. 2008). Therefore, we used SCA effects and mean grain yield of research trial III to validate heterotic groups of our first set of 12 ex-PVP inbred lines. In this trial we used hybrids between ex-PVP lines and known industry testers. We arranged SCA effects in descending order and selected the lines with top SCA effects (Table 3). Inbreds that were combining well with two contrasting testers (belonging to different heterotic groups) are also presented in Table 1.

Table 1. SCA effects for grain yield and mean grain yield per se, utilized in trial III to determine heterotic groups of first set of 12 ex-PVP maize inbred lines

Hybrids	SCA Effects (Mg ha^{-1})	Yield (Mg ha^{-1})	Testers	Testers HG†	Ex-PVP inbreds	Ex-PVP inbreds HG
T4 x PH207	1.31	9.22	T4	SS	PH207	Iodent?
T4 x Q381	1.20	9.36	T4	SS	Q381	UR?
T1 x Lp5	1.03	8.75	T1	Iodent	Lp5	SS?
T1 x CR1Ht	0.96	9.29	T1	Iodent	CR1Ht	Lancaster
T1 x NK794	0.77	8.75	T1	Iodent	NK794	SS
T1 x LH52	0.71	8.61	T1	Iodent	LH52	Lancaster
T1 x DK78010	0.56	7.68	T1	Iodent	DK78010	SS?
T7 x DK78010	0.47	7.38	T7	SS	DK78010	SS?
T1 x LH54	0.52	8.50	T1	Iodent	LH54	Lancaster
T3 x DKFAPW	0.44	7.17	T3	Iodent	DKFAPW	SS
T2 x DJ7	0.41	6.79	T2	SS	DJ7	SS?
T4 x NK779	0.34	6.16	T4	SS	NK779	Non-SS
T5 x NK807	0.21	6.01	T5	Non-SS	NK807	SS

†known heterotic group (HG) of testers were used to assign the HG of ex-PVP inbred lines; 'SS' refers to Stiff Stalk, 'non-SS' refers non-Stiff Stalk

Heterotic group determination for ex-PVP lines (first set of 12 ex-PVP maize inbred lines)

Mikel (2006) and GRIN have proposed heterotic groups of ex-PVP inbred lines based on the disclosure made by industry. Our study wanted to validate the classification.

SS inbreds

Lp5 combined well with Iodent tester T1, resulting in a grain yield average value of 8.75 Mg ha^{-1} and SCA effect of 1.03 Mg ha^{-1}. Lp5, therefore, seems to be in agreement with the PVP documents as presented in Table 1 (Mikel 2006). DK78010 combined well with both Iodent type tester T1 and SS tester T7. This inbred had above average combining ability with testers of the same and opposite heterotic groups. Even though this line would require more testing efforts, combining well across heterotic groups is a positive and desirable attribute of an inbred. Barata and Carena (2006) also found ND278 and ND282 combined very well across testers. So, the reported heterotic group, SS of DK78010 may not be an accurate classification. Inbred NK807 combined well with non-SS tester T5, therefore,it could belong to the SS group. However, NK807 has Lancaster background so further testing might be needed (Mikel 2006). DKFAPW combined well with the Iodent tester T3. Therefore, the SS background of DKFAPW could be correct. Inbred NK794 combined well with Iodent tester T1 and seems to belong to the SS group. The combining ability of DJ7 was above average with SS tester T2. Therefore, it showed the inbred may not belong to the SS group as published by Mikel (2006). The unrelated proportion of DJ7 could combine well with SS testers.

Non-SS inbreds

Q381 combined very well with SS tester T4 (with a yield of 9.36 Mg ha^{-1} and SCA effect of 1.20 Mg ha^{-1}) (Table 1). The heterotic background of Q381 was proposed as unrelated. In our extensive evaluation, Q381 seems to belong to a non-SS group. PH207 combined well with SS tester T4 (with a yield of 9.22 Mg ha^{-1} and SCA effect of 1.31 Mg ha^{-1}). Our evaluation infers that PH207 has a non-SS background, in addition to Iodent. NK779 combined well with the SS tester T4.NK779 evaluation seems to agree with the non-SS heterotic group assignation, which supports the line's MN13 and unrelated composition (Mikel 2006).

Lancaster inbred

CR1Ht combined very well with Iodent tester T1 (with a yield of 9.29 Mg ha^{-1} and SCA effect of 0.96 Mg ha^{-1}) (Table 1). CR1Ht was reported to have Lancaster and MN13 genetic backgrounds (Table 1), which agrees with our findings. LH52 and LH54 also combined well with Iodent tester, which agrees with past research suggestingthat both lines belongto the Lancaster group.

Heterotic group determination for ex-PVP lines (second set of 12 ex-PVP maize inbred lines)

SS inbreds

PHJ40 combined well with Iodent tester T1 (Table 2). The results for PHJ40 were in agreement with the PVP documented report for this line. Similar results confirmed data for NKS8324, CR14, and LH205, as expected. The combining ability of NKS8324 with Iodent tester T1 was above average. CR14, LH205 (with a yield of 7.62 Mg ha^{-1} and SCA effect of 0.77 Mg ha^{-1}), and RS710 (with a yield of 6.72 Mg ha^{-1}and SCA effect of 0.72 Mg ha^{-1}) also combined well with Iodent tester T1.

Non-SS inbreds

PHK05 and PHK76 combined well with SS tester T4 (Table 2). Based on our results, PHK05 and PHK76 belong to the non-SS group, as expected. Similarly, OQ603, PHP02 (with a yield of 7.47 Mg ha^{-1} and SCA effect of 0.54 Mg ha^{-1}), and L127 combined well with SS testers. PHR25 combined well with SS tester T4, so it could be considered Iodent but further evaluation would be needed to confirm this. PHT77 combined well with SS tester T7. The heterotic classification of PHT77 somewhat agrees with Mikel (2006), who reported that the line belongs to Lancaster and unrelated groups.

Heterotic group determination for NDSU lines

The first set of 12 ex-PVP lines was crossed to 12 NDSU lines and the resulting hybrids were evaluated in trial I.

SS inbreds

ND2004 combined well with non-SS inbred NK779 and SS inbred NK807 (Table 3). The testcross results from Carena and Wanner (2009) infer that ND2004 has a SS heterotic group (Table 2). However, ND2004 has unique combining ability with both heterotic groups based on these trials, which has been useful due to its excellent properties as male and female. ND2002 combined well with the Lp5 line, which was defined as mostly SS. The unrelated proportion of Lp5 might have contributed to combine well with the SS part of the line ND2002 (Table 3). Both ND2002 and ND2004 have been licensed exclusively. ND2003 and ND08-343 combined well with Lancaster line LH54.Testcross data from Carena and Wanner (2009) supports the SS background for ND2003 validating previous information. Also, data confirmed ND08-343 belongs to the SS heterotic group. ND2006 had an above average combining ability with SS and unrelated inbred Lp5. However, ND2006 was reported to have an SS heterotic background so more testing is needed. ND2010 combined well with the Lancaster inbred PH207 and SS inbred NK807. Further testing is, therefore, needed to confirm heterotic grouping of ND2010. ND2007 combined well with Iodent inbred PH207. ND2007 could carry an SS background based on the heterotic combinations tested and its pedigree.

Table 2. SCA effects for yield and mean grain yield per se, utilized in trial IV to determine heterotic groups of second set of 12 ex-PVP maize inbred lines

Hybrids	SCA effects (Mg ha^{-1})	Yield (Mg ha^{-1})	Testers	Testers HG[†]	Ex-PVP	Ex-PVP inbreds HG
T1 x LH205	0.77*	7.62	T1	Iodent	LH205	SS
T1 x RS710	0.72	6.72	T1	Iodent	RS710	SS
T6 x PHP02	0.54	7.47	T6	SS	PHP02	Non-SS
T1 x CR14	0.39	6.53	T1	Iodent	CR14	SS
T7 x OQ603	0.35	6.75	T7	SS	OQ603	Non-SS
T6xPHK76	0.35	6.64	T6	SS	PHK76	Non-SS
T1 x NKS8324	0.33	7.01	T1	Iodent	NKS8324	SS
T4 x L127	0.33	6.88	T4	SS	L127	Non-SS
T4 x PHR25	0.31	6.43	T4	SS	PHR25	Iodent?
T7 x PHT77	0.24	7.08	T7	SS	PHT77	Lancaster
T1 x PHJ40	0.12	6.58	T1	Iodent	PHJ40	SS
T4 x PHK05	0.17	5.57	T4	SS	PHK05	Non-SS

* Significance at $P<0.05$; [†]testers’ heterotic group (HG) were used to derive ex-PVP inbreds’ HG; ‘SS’ refers Stiff Stalk, ‘non-SS’ refers non-Stiff Stalk

Table 3. SCA effects for yield and mean grain yield per se, utilized in trial I to determine heterotic groups of NDSU maize lines

Hybrids	SCA effects (Mg ha^{-1})	Yield (Mg ha^{-1})	Ex-PVP Inbreds	Ex-PVP’s HG[†]	NDSU Inbreds	NDSU’s HG
ND2005 x NK779	0.75*	5.42	NK779	Non-SS	ND2005	SS/Non-SS?
ND2004 x NK779	0.52	5.45	NK779	Non-SS	ND2004	SS?
ND2004 x NK807	0.39	5.46	NK807	SS	ND2004	SS?
Lp5 x ND2002	0.46	6.34	Lp5	SS & UR	ND2002	SS?
DJ7 x ND291	0.45	5.54	DJ7	SS & UR	ND291	SS/non-SS
ND2003 x LH54	0.41	5.54	LH54	Lancaster	ND2003	SS
ND2007 x PH207	0.39	5.20	PH207	Iodent	ND2007	SS
ND2011 x DJ7	0.37	5.25	DJ7	SS & UR	ND2011	Non-SS
ND08-343 x LH54	0.32	5.06	LH54	Lancaster	ND08-343	Non-SS
ND2010 x PH207	0.31	5.78	PH207	Iodent	ND2010	SS?
ND2010 x NK807	0.32	5.45	NK807	SS	ND2010	SS?
ND2006 x Lp5	0.27	4.92	Lp5	SS & UR	ND2006	SS?
DK78010 x ND2001	0.25	4.90	DK78010	SS	ND2001	SS/non-SS
ND2000 x Q381	0.17	4.96	Q381	Non-SS	ND2000	SS
ND2000 x 794	0.16	4.68	NK794	SS	ND2000	SS

* Significance at $P<0.05$; [†]Ex-PVP inbreds’ heterotic groups (HG) were used to derive NDSU inbreds’ HG; ‘SS’ refers Stiff Stalk, ‘non-SS’ refers non-Stiff Stalk;

Non-SS inbreds

The highest SCA effect was observed in hybrids between ND2005 and NK779 (with a yield of 5.42 Mg ha^{-1} and SCA effect of 0.75* Mg ha^{-1}) (Table 3). The heterotic group of ND2005 was presented as non-SS (Bari and Carena 2014). However, ND2005 combined well with testers from two heterotic groups (Carena and Wanner 2009). Barata and Carena (2006) reported that certain inbred lines, especially if genetically diverse, could have the advantage to combine well across heterotic groups. ND2011 combined well with DJ7 confirming to belong to a non-SS heterotic group. We observed good combining ability between ND2001 and DK78010, leaving room to an alternative heterotic group. ND2001 was derived from MN13 (Carena et al. 2010).

SS/non-SS lines

Inbred ND2000 has been largely used in the short-season market and has shown to have unique capabilities to combine with both SS and non-SS inbred lines (Table 3). However, Carena and Wanner (2003) reported, through molecular marker analysis and yield data, that it belonged to the SS group. Further investigation is needed. ND291 showed good combining ability with DJ7.ND291 showed a broad heterotic base of SS/non-SS groups, which is in agreement with the previous molecular marker analysis and yield trial data (Carena et al. 2003).

Heterotic pattern detection

The most used heterotic pattern utilized in the U.S. is the one represented by BSSS x Lancaster, normally used with genetically narrow germplasm. Table 4 shows two heterotic patterns for ex-PVP and current industry lines. The SS x non-SS pattern was represented by hybrid T4 x Q381. The Iodent x Lancaster heterotic pattern was represented in the hybrids T1 x CR1Ht and T4 x PH207. The three hybrids had significantly ($P \leq 0.05$) higher yield than the average of industry checks while similar agronomic characteristics. In addition, these hybrids also had significantly higher ($P \leq 0.05$) protein and oil content, but statistically lower starch than the top check. Hybrids were comparable to the top check. Table 5 showed a few unique combinations. The top yielding hybrid was T1 x LH205, representing the Iodent x SS heterotic pattern. It had similar agronomic properties to the top check, but showed statistically higher grain moisture at harvest and more

Table 4. Heterotic patterns of selected maize hybrids between current industry testers and first set of ex-PVP inbred lines, from the combined analysis across six environments, trial III

Hybrids	**Yield** (Mg ha^{-1})	**MSTR**[†] (g kg $^{-1}$)	**TWT**[‡] (kg hL $^{-1}$)	**PRL**[§] (%)	**PSL**[¶] (%)	**Protein** (g kg $^{-1}$)	**Oil** (g kg $^{-1}$)	**Starch** (g kg $^{-1}$)	**Heterotic Pattern**[#]
T4 x Q381	9.36	151	68.39	1.32	1.41	113	41.67	694	SS x non-SS
T1 x CR1Ht	9.29	150	66.28	1.18	4.18	114	44.01	684	Iodent x Lan
T4 x PH207	9.22	142	69.81	1.85	1.74	112	41.73	695	SS x non-SS
T1 x Lp5	8.75	173	65.55	6.64	5.19	110	41.80	694	Iodent x SS
T1 x NK794	8.75	167	66.83	0.00	3.36	112	41.31	693	Iodent x SS
T6 x Q381	8.72	146	73.44	4.39	0.28	113	39.63	695	SS x non-SS
T1 x LH52	8.61	151	67.94	3.03	1.72	114	42.95	691	Iodent x Lan
T1 x LH54	8.50	169	65.13	0.00	0.81	116	41.78	690	Iodent x Lan
T6 x CR1Ht	8.49	150	73.19	0.71	0.18	116	42.35	690	SS x Lan
T7 x Q381	7.95	156	70.70	1.47	3.19	107	37.86	702	SS x non-SS
T3 x LH52	7.87	138	69.78	0.73	2.28	120	42.93	686	Iodent x Lan
T1 x DJ7	7.80	185	65.45	1.85	3.58	112	43.17	692	Iodent x Lan
T6 x DKFAPW	7.70	143	71.28	0.67	1.95	113	39.68	694	SS x SS?
T1 x DK78010	7.68	151	65.98	1.85	3.49	111	40.51	694	Iodent x SS
Pioneer 38N88[††]	9.41	144	70.86	0.33	0.33	103	39.58	704	
Mean of Checks[‡‡]	8.05	147	70.09	0.75	2.80	101	39.58	706	
Exp. Mean[§§]	6.78	153	69.92	3.18	3.78	111	41.58	694	
LSD (0.05)	1.15	7	1.39	5.04	4.25	5	1.24	5	
CV (%)	21.15	5	2.47	197.11	140.08	5	3.69	1	

[†] Grain moisture at harvest,[‡] test weight,[§] percent root lodging,[¶] percent stalk lodging,[#]'Lan' refers to Lancaster, 'SS' refers to Stiff stalk
[††] best performing check of the trial, [‡‡] mean of five industry checks, [§§]Experimental mean of 72 entries; hybrids were selected based on an index combining higher yield, lower grain moisture, higher test weight, lower per cent root and stalk lodging, and higher grain quality traits.

***Table 5*.** Heterotic patterns of selected maize hybrids between industry testers and second set of ex-PVP inbreds, from combined analysis across six environments, trial IV

Hybrids	Yield (Mg ha^{-1})	MSTR[†] (g kg $^{-1}$)	TWT[‡] (kg hL $^{-1}$)	PRL[§] (%)	PSL[¶] (%)	Protein (g kg $^{-1}$)	Oil (g kg $^{-1}$)	**Starch** (g kg $^{-1}$)	**Heterotic Pattern[#]**
T1 x LH205	7.62	198	66.97	2.24	1.51	111	45.11	690	Iodent x SS
T7 x PHP02	7.55	189	70.56	0.07	1.10	102	38.04	707	SS x non-SS
T6 x PHP02	7.47	179	69.87	4.57	1.95	106	38.75	704	SS x non-SS
T7 x PHT77	7.08	185	68.45	0.30	1.45	106	36.63	705	SS x Lan
T1 x S8324	7.01	187	67.56	1.13	2.11	111	47.33	689	Iodent x SS
T4 x PHP02	6.93	182	66.82	1.42	3.23	108	41.20	699	SS x non-SS
T1 x PHT77	6.90	178	67.52	0.01	5.51	104	39.80	703	Iodent x Lan
T4 x L127	6.88	182	69.41	0.43	8.11	107	44.16	697	SS x non-SS
T7 x OQ603	6.75	195	69.45	0.63	0.74	103	38.85	702	SS x non-SS
T1 x RS710	6.72	158	69.68	0.55	4.34	105	44.75	697	Iodent x SS
T6 xPHK76	6.64	190	72.79	1.41	2.77	107	39.13	704	Non-SS x SS
T1 x PHJ40	6.58	157	71.96	1.61	3.94	105	42.57	700	Iodent x SS
T4 x PHR25	6.43	158	69.07	2.19	0.02	108	41.88	698	SS x Iodent
T6 x L127	6.24	184	73.15	0.41	1.54	113	41.33	695	SS x non-SS
T6 x PHR25	6.16	154	71.64	0.12	2.09	110	39.01	699	SS x Iodent
Pioneer 38N88[††]	7.22	164	68.96	1.38	1.82	103	41.97	703	
Checks mean[‡‡]	6.49	165	69.79	0.50	3.89	104	40.77	703	
Exp. Mean[§§]	5.93	174	69.91	2.36	4.74	109	42.00	697	
LSD (0.05)	1.24	11	1.72	3.85	6.54	4	1.44	4	
CV (%)	25.73	8	3.05	201.30	170.25	5	4.25	0.73	

[†] Grain moisture at harvest,[‡] test weight,[§] percent root lodging,[¶] percent stalk lodging,[#]'Lan' refers to Lancaster, 'SS' refers to Stiff stalk [††] best performing check of the trial, [‡‡] mean of five checks, [§§] Experimental mean of 64 entries; hybrids were selected based on an index combining higher yield, lower grain moisture, higher test weight, lower per cent root and stalk lodging, and higher grain quality traits.

grain protein and oil content. Hybrid T7 x PHP02 represented the typical SS x non-SS heterotic pattern with higher test weight, stalk lodging resistance, and starch content than the average of checks.

Most NDSU lines are genetically broad-based and have unique capabilities to combine well with a wide range of inbred lines, which is reflected in the more diverse heterotic patterns observed. The universal combination of SS x non-SS/ Lancaster is prevalent in trial II. However, in trial I, we found inbred lines with combining ability across heterotic groups. This is often an advantage to develop outstanding hybrids with lines serving as male and/or female parents and further testing is encouraged. Hybrids ND2002 x CR1Ht and ND2002 x PHP02 are recommended as they showed statistically similar grain yield and agronomic traits when compared with the best performing check.

Short- season maize lines were categorized into SS, non-SS, Lancaster, and Iodent heterotic groups. Some of them belonged to both SS and non-SS because same inbreds showed above average combining ability with testers derived from different backgrounds. Heterotic groups are conceptual (Hallauer and Carena 2009) and often are not confined to a particular group. A specific line from one heterotic group may fall into another heterotic group based on performance in a particular hybrid combination. Pedigree information is still a good reference in order to classify inbred lines into heterotic groups and patterns, and these have demonstrated to be very useful to exploit. They represent a cost efficient way to group inbred parents to identify top and unique hybrid combinations. We found the SS x non-SS heterotic pattern to be frequent in our trials representing short-season ex-PVP and current industry genetic materials, in agreement with Mikel and Dubley (2006). Further sampling for larger genetic diversity (Carena et al. 2009, Carena 2011, Sharma and Carena 2012) is needed to develop alternative and productive heterotic patterns. Integration of exotic germplasm improvement with inbred line development has produced NDSU inbred lines associated with different heterotic groups. Ex-PVP lines do not seem to represent a useful source of genetic variation for continued genetic progress in short-season environments. Less protection will encourage the use ex-PVP lines as a complement to genetically broad-based maize. The future relies on the next generation of maize short-season products with increase diversity (Carena 2013).

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