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Phenotypic Structures and Breeding Value of Open-Pollinated Corn Varietal Hybrids

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ABSTRACT

The growing interest in using open-pollinated varieties (OPVs) and varietal hybrids (OPVhs) of corn (*Zea mays* L.) reflects the value of large plasticity levels available in their plant, ear, and kernel traits. We estimated variation, broad sense heritability, general and specific combining ability, and pairwise phenotypic distances based on 35 traits measured on 46 OPVhs grown under four environments. Average grain yield across environments was inversely related to its variance; we identified OPVhs with large grain yield and small variance along with the traits contributing to this large and stable grain yield. Estimates of heritability ranged from 0.35 to 0.85, with decreasing average values for kernel, ear, and plant traits. Effects of general combining ability and its interaction with the environment for most traits were more important than specific combining ability effects. Largest grain yield and specific combining ability effects resulted from crosses among OPVs of different source germplasm origin. Pairwise phenotypic distances among OPVhs indicate the presence of "functional" OPVh groups based on multiple traits and their interaction with the environment.

Keywords: combining ability, open-pollinated corn, secondary traits, yield stability, *Zea mays*

INTRODUCTION

Though modern corn hybrids are mainly F1 crosses made between inbreds, it was recognized already in the late 1800's that some degree of heterosis for yield is possible in F1 crosses between open-pollinated varieties (Richey 1922; Sprague and Eberhart 1977). In some cases, open pollinated varieties (OPVs) and varietal hybrids (OPVhs) of corn (*Zea mays* L.) are considered (Prasanna *et al*. 2001; Vaz Patto *et al*. 2008) to have more stable yield and broad adaptation as compared with hybrid corn (HYB). Some organic and low-input farmers, in particular, prefer OPVs and OPVhs over HYBs due perhaps to their perceived drought tolerance and superior quality (i.e., protein and oil content) as opposed to known and documented superiority to other hybrids, and nutritious value as livestock feed (Lauer *et al*. 2001; Scott *et al*. 2008), and human food (Kamara *et al*. 2004; Ortiz *et al*. 2008). In addition, OPVhs seed is less expensive to produce than the commercially-available HYBs. Farmers often resort to selecting and planting saved corn seed from their previous harvest (Kutka and Smith 2007), thus enhancing genetic diversity on the farm (Vaz Patto *et al*. 2008).

OPVs of the Corn Belt Dent were developed in the early 19th century by the hybridization of two highly differentiated races, the Northern Flints and the Southern Dents, followed by mass selection (Ho *et al*. 2005). However, the original genetic base for OPVs (~800 varieties) was reduced over time and many of these varieties are already extinct. Potentially, a genetic bottleneck of U.S. corn occurred when widely adapted and popular OPVs were selected for early inbred development (Ho *et al*. 2005). The evolution of OPVs of the 1930s to single-cross HYBs of the 1990s was associated with an increase in rate of hybrid improvement and in area of adaptation, and with a decrease in genetic diversity within and among commercial corn genotypes (Tollenaar and Wu 1999). Pedigrees of modern hybrids probably contain a disproportionate amount of Reid Yellow Dent ancestry (Tracy and Chandler 1996). OPVs yielded 15% less than the first HYBs available in the 1920s (Duvick 1999). More recently, however, average grain yield (GY) estimates of OPVs at different localities in the Northern Corn Belt of the U.S. (e.g., Wisconsin, Iowa, Minnesota, and North Dakota) ranged from 5.0 to 9.3 Mg ha^{-1}, which is 18-20% less than the best HYBs (e.g., Carena 2005). The development of corn tolerant to drought (Monneveux *et al*. 2006) and low inputs (Kamara *et al*. 2003b) and the development of corn with superior protein quality (Reeves and Cassaday 2002) were among the most important achievements of corn breeding research during the last 50 years. New quality protein corn synthetics, which are superior OPVs formed from inbred lines, feature special characteristics such as low and uniform ear placement, resistance to root lodging (Carena 2005), and much higher levels of essential amino acids and protein than those contained in HYBs (Scott *et al*. 2008).

Genetic vulnerability to abiotic and biotic stresses of current corn germplasm is a strong indicator of its limited diversity and may limit future gains from selection. OPVs, as well as landraces and synthetic populations, may be useful in improving corn yield in the Northern Corn Belt (Taller and Bernardo 2004). Hence, we witnessed an increased interest in commercializing outstanding populations and/or population hybrids in certain agricultural sectors of the U.S., Europe and Africa. This interest is demonstrated by increased research efforts on OPVs, OPVhs and landrace corn under organic and other low-input farming in North America (Carena 2005; Kutka and Smith 2007), Europe (Vaz Patto *et al*. 2008), Africa (Kamara *et al*. 2004), Latin America (Ortiz *et al*. 2008), and China (Song 1999). Farmers and public plant breeders continue to improve OPVs (Song 1999); however, unless new high-yielding hybrid populations are made available to farmers, it is unlikely that

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OPVs or OPVhs with economically competitive GY would be developed in the near future (Carena 2005). Nevertheless, and due to low heritability of GY under low-input conditions, Monneveux *et al.* (2008) suggested using secondary traits as a viable alternative to improving yield (*per se*) of OPVs. These traits are easy to observe and measure, genetically variable and highly heritable, and stable over the measurement period.

Heterosis, as a predictor of single-cross hybrid value, could increase the efficiency of hybrid breeding programs; however, the environment can differentially affect the performance of inbreds and hybrids, thus altering the observed heterosis. A heterotic group (HG) consists of a set of cultivars with common ancestry that combines well with cultivars in another heterotic group. A heterotic pattern refers to a specific beneficial combination between two heterotic groups. Tracy and Chandler (2006) stated that "Corn-Belt Dent heterotic patterns were created by breeders, and are *not* the result of historical and geographic contingencies." However, it is a perennial finding that crosses between cultivars of different types often show heterosis even if there has been no conscious effort to breed for heterosis (Sprague and Eberhart 1977). We would argue that making better use of OPV's in hybrid combinations entails identifying these novel, unplanned heterotic patterns.

Combining ability to detect good combiners for grain yield, in addition to phenotypic and developmental traits, is the ultimate factor determining future usefulness of OPVs to develop hybrids (Rasmussen and Hallauer 2006). The magnitude of additive gene action, as estimated by general combining ability (GCA), is a measure of the average performance of a line in hybrid combinations; whereas, that of non-additive gene action, as estimated by specific combining ability (SCA), is a measure of the deviation of crosses on the basis of average performance of the lines involved (Baker 1978). The objectives of this study on 46 OPVhs grouped into eight heterotic groups on the basis of their female parents, and evaluated under four environments (i.e., location-years) were (1) to estimate their broad-sense heritability, general (GCA) and specific (SCA) combining ability for GY and 34 secondary phenotypic traits, (2) to elucidate the multivariate relationships between GY and each of 34 plant, ear, and kernel secondary traits (*senso* Monneveux *et al.* 2008), and (3) to identify high-yielding OPVhs within female HGs, and the associated secondary traits having a large impact on GY and its stability across environments.

MATERIALS AND METHODS

Genetic material

Forty-six OPVhs derived from crosses between corn HGs were used in field experiments in Morris, MN (45° 41′ N, 95° 48′ W, elevation 370 m; 2004 and 2005), Brookings, SD (44° 20′ N, 96° 47′ W, elevation 490 m; 2004), and in Colfax, WI (45° 00′ N, 91° 43′ W, elevation 300 m; 2004). The hybridization scheme indicates that there were 13 HGs, 11 and 10 of which were used as female and male parents, respectively (**Table 1**). Differences between female, but not male, HGs, using multivariate analysis of variance, were found (Jaradat 2010) to be highly significant for all traits under study; therefore, we used female HGs as a classificatory categorical source of variation in subsequent statistical analyses.

Full description and pedigrees of HGs used in this study are available from the USDA's Plant Introduction Germplasm site (www.ars-grin.gov). The following is a brief description of the genetic material for the purpose of this study. Leaming is an old open pollinated variety that has its own heterotic group and was improved by Troyer (2000). Lancaster, Northwestern Dent, and Minnesota 13 are versions of old open pollinated varieties (Troyer 2000) that were improved and made available to the USDA program by A. F. Troyer, Dekalb, Illinois; Lancaster and Minnesota 13 are non-stiff stalk OPVs (Troyer 2000). Nokomis Gold is a population developed by the Michael Fields Agricultural Institute out of native varieties and Corn Belt Dents; it appears to be mainly non-stiff stalk in its combining ability. TEPR-EC6 is an open pollinated variety derived from Pioneer Prolific Composite (Troyer 2000) and it is a non-stiff stalk. BS21(R)C7 is cycle 7 of a synthetic variety which originated from a cross between BS5 and BS20 (Hallauer *et al.* 2000); its genetic background includes about 54% Reid Yellow Dent, 18% Minnesota 13, 13% European Flint, and 15% from sources of unknown origin. BS22(R)C7 is cycle 7 of a synthetic population developed by inter-mating 16 early-maturing inbred lines (Hallauer *et al.* 2000); its genetic background includes 45% Reid Yellow Dent, 13% Lancaster Sure Crop, 9% Minnesota 13, and 33% from sources of unknown origin. BS33(S)C5 is a synthetic derived from Leaming by USDA and Iowa State University breeding programs (Anon. 2008). BSSS(R)C15 is cycle 15 of the Iowa stiff stalk synthetic that produced many excellent inbreds in the 'stiff stalk' heterotic group (Hallauer *et al.* 1983). Ten of the 16 original lines used to create this synthetic had Reid Yellow Dent ancestry and it is generally regarded as being a major contributor of that genetic background (Troyer 2004). BS13(S)C10 is a strain of BSSS developed after 7 cycles of half-sib and 10 cycles of S2 recurrent selection. BSCB1(R)C15 is a strain of Iowa Corn Borer Synthetic (BSCB1) developed after 15 cycles of reciprocal half-sib recurrent selection with BSSS as a tester (Hallauer *et al.* 1983). HPAL C#1 was developed at the University of Nebraska by crossing a number of commercial inbreds in the High Plains Region and, in hybrids, it combines well with the stiff stalk HG. For the purpose of this study, we considered HPALC #1, Lancaster, Leaming, Minnesota 13, Nokomis Gold, Northwestern Dent, and TEPR-EC6 as unique heterotic groups. The Iowa BS-OPVs generally have a large proportion of Reid Yellow Dent in their ancestry; therefore, they were treated as one HG in this study.

Table 1 Crossing scheme between 13 OPVs used to produce 46 OPVhs evaluated under four environments in the upper Midwest (MN, SD and WI) of the US.

Female (horizontal)/ male (vertical) parents	**BSCB1(R)C15**	**BS22(R)C7**	**BS33(S)C5**	**BSSS(R)C15**	**HPAL C#1**	**Lancaster**	**Leaming**	**Minnesota 13**	**Nokomis Gold**	**Northwestern Dent**	**TEPR-EC6**	**Total**
BSCB1(R)C15	0			X								1
BS13(S)C10	X			X								2
BS21(R)C7		X	X		X	X	X	X	X	X	X	9
BS22(R)C7		0	X		X	X	X	X	X	X	X	8
BS33(S)C5			0		X	X	X	X	X	X	X	7
HPAL C#1					0	X	X	X	X	X	X	6
Lancaster						0			X	X	X	3
Leaming						X	0		X	X	X	4
Minnesota 13						X	X	0	X	X	X	5
TEPR-EC6									X		0	1
Total	1	1	2	2	3	6	5	4	8	7	7	46

Experimental layout

All field experiments were laid out in a randomized complete block design with three replicates in Morris (2004-2005), MN, and two replicates in each of Brookings, SD (2004), and Colfax, WI (2004). Each plot consisted of four rows 6.25 m long and 0.7 m between rows and 0.20 m among plants within rows. Management practices (i.e., seedbed preparation, seeding rate, sowing date, fertilizer rates and time of application, and weed control) were performed according to local standards for each location. The inner two rows in each plot were trimmed to a length of 5.25 m before combine harvesting, and GY was adjusted to 155 g kg^{-1} of moisture.

Plant sampling and measurements

At physiological maturity, five random single plants from the two inner rows in each OPVh, replicate and location, with a total of 50 plants per OPVh, were cut at the soil surface and photographed with ear(s) attached for further phenotypic analyses, then cob(s) were digitally photographed after being detached and their husks were removed. Morphological quantitative traits were measured on skeletal images, and fractal dimensions for plants and tassels were developed using the box count as applied by ImageJ software (Rasband 2004). Images of each plant and tassel were covered by a sequence of grids made of squares decreasing in size, then a fractal dimension (*Do*) was calculated based on the squares intersected by the image. Stalk lodging at the plot level was recorded under typical lodging-conducive conditions in Brookings, SD, on a scale of 1 (0% lodged plants) to 5 (100% lodged plants). Root damage caused by the Western rootworm larva (*Diabrotica virgifera virgifera* LeConte) was scored on roots of three plants $plot^{-1}$ taken at random from each plot and replicate and expressed as scores ranging from 1 (no damage) to 6 (severe damage) (Hills and Peters 1971).

Statistical analyses

Grain yield (Mg ha^{-1} and g $plant^{-1}$) was adjusted to 155 g kg^{-1} grain moisture at harvest for each location-year combination (i.e., environment). In order to satisfy assumptions of uni- and multi-variate analyses of variance, all variables were subjected to the Levene test of homogeneity of variances and to the Shapiro-Wilk W test for normality, then the appropriate data transformation was carried out (Zar 1996). Transformed data was back-transformed for reporting. Mean square error variances of each individual analysis by environment were found to be homogeneous based on Bartlett's χ^2 test conducted before statistical analysis was carried out across environments. The variance components for OPVhs within a HG pooled across all HGs [$\sigma^2_{OPVh(HG)}$], environments [σ^2_E] and OPVhs within HGs x environment interaction [$\sigma^2_{OPVh(HG)*E}$] were estimated using the restricted maximum-likelihood method (Payne *et al*. 2006). The phenotypic variance of an OPVh mean was estimated as $[\sigma^2_P]=[\sigma^2_{OPVh(HG)}]+[\sigma^2_{OPVh(HG)*E}]/e+[\sigma^2_{Error}]/er$; where e is the number of environments and r is the number of replicates in each of the e environments. Broad sense heritability (H^2) was estimated as the ratio between $\sigma^2_{OPVh(HG)}$ as an estimator of genetic variance (σ^2_g) and σ^2_P (Falconer 1981). In the diallel analyses, OPVhs were considered fixed effects, and replicates and environments (E) were considered random effects. To test for phenotypic similarity (or dissimilarity) between these OPVhs and to recover much of the expected structure of the HGs, the unweighted paired-group method with arithmetic average (UPGMA) clustering procedure was employed to construct a dendrogram of pairwise phenotypic distances (PPD) between OPVhs using all plant, ear and kernel traits in addition to grain yield in all four environments.

The magnitude of additive and non-additive gene actions as estimated by GCA and SCA, respectively, were quantified to determine the future usefulness of OPVs for hybrid development. Significance of OPVhs, GCA and SCA mean squares were estimated with *F*-tests, using their interaction with the environment as an error term. The significance of GCAxE and SCAxE were determined using the corresponding interaction with E as error terms. GCA effects of the parents and SCA effects of the crosses, as well as their mean squares across environments, were estimated following Griffing's Method IV diallel analysis (Griffing 1956). Since parental inbreds (i.e., OPVs) *per se* were not included in the diallel analyses, mean trait (e.g., GY) of an OPV in hybrids was used as inbred values for GCA (based on female and male parent performance), SCA and mid-parent heterosis calculations (Bhatnagar *et al*. 2004). Variances due to GCA and SCA in the diallel analyses were used to estimate the ratio of additive genetic variance to total variance according to Baker (1978) as $[2\sigma^2 GCA/(2\sigma^2 GCA+\sigma^2 SCA)]$; where $\sigma^2 GCA$ and $2\sigma^2 SCA$ are variance components due to GCA and SCA, respectively. Mid-parent heterosis was calculated as the percent deviation of OPVh's GY from the average of its female and male parent. Finally, two groups of OPVhs were identified on the basis of deviation of their mean GY and its standard deviation (SD) across environments from the overall mean GY and SD, and scatter plots were constructed for comparison and to identify high and stable yielding OPVhs across environments. Relevant modules in STATISTICA 9.1 (StatSoft Inc. 2010) and GenStat 10.1 (Smith *et al*. 2005; Payne *et al*. 2006) were used in performing the statistical analyses, unless otherwise indicated.

RESULTS

Sources of variation and heritability of GY and secondary traits

Variance components analysis for individual traits and the level of significance for all four sources of variation (i.e., E, HG, OPVhs(HG) and OPVhs(HG)xE; **Table 2**) indicated that most traits displayed significant differences due to variation among HGs; whereas the least number of traits displayed significant differences due to variation among environments (E) or due to OPVhs (HG)xE. The traits that displayed significant differences due to E ($p<0.05$; kernel moisture content at harvest) also displayed significant differences due to OPVhs(HG)xE; whereas the traits that displayed marginal ($p<0.09$; stalk thickness, kernels per plant, and ear weight), or no significant differences due to E (most traits, except GY per plant, and plant dry weight) showed no significant differences due to OPVhs(HG)xE (**Table 2**). In addition, we observed that the few traits with significant differences due to OPVhs(HG)xE tended to have below-average H^2 estimates (e.g., plant dry weight, apical sterility, and kernel moisture content at harvest).

Most broad sense heritability estimates were either small ($H^2<0.50$; ear leaf angle, and apical sterility) or moderate ($0.50>H^2<0.65$); whereas two of the ear traits (ear weight, and ear harvest index) and one kernel trait (test weight) had H^2 values ≥ 0.80. On average, H^2 increased from 0.53 for plant traits, to 0.62 for ear traits, and to 0.69 for kernel traits. Genotypic correlations of GY per plant and the plant, ear and kernel traits were generally stronger than phenotypic ones. Strong and positive genotypic ($r=0.27$ to 0.89; $p<0.05$) and phenotypic ($r=0.22$ to 83; $p<0.05$) correlations were found between most traits and GY across environments. However, a number of traits (e.g., ear height, tassel length, tassel peduncle length, stem lodging, root damage, apical sterility, and kernel moisture content at harvest) displayed strong negative genotypic and phenotypic correlations with GY per plant (**Table 2**). Apical sterility and 1000-kernel weight expressed significant non-linear, negative and positive correlations with GY, respectively.

Phenotypic distances between OPVhs

Pairwise phenotypic distances (PPDs) between all OPVhs based on plant, ear and kernel traits, in addition to GY per plant and per hectare, using the UPGMA clustering procedure are presented in **Fig. 1**. Apart from four BS-OPVhs and two HPAL-OPVhs, the UPGMA clustering procedure separated the stiff stalk and the non-stiff stalk groups from each other at about 75 PPDs (**Fig. 1**). The dendrogram (**Fig. 1**) suggests that the between-HGs variability was larger than the within-HGs variability based on plant, ear and kernel

Table 2 Significant variance components ($p<0.05$, otherwise non-significant, ns), among and within eight female heterotic groups (HGs), whole model adjusted R^2, and correlation with grain yield per plant (GY) of 34 plant, ear and kernel traits measured on 46 open pollinated varietal hybrids (OPVhs) averaged over four environments.

No	Trait	Significant % variance (p<0.05)		Adjusted R^2	Heritability	Correlation with GY	
	Plant architecture	**HGs**	**OPVh(HG)**	**Model vs. error**	H^2	**Genotypic**	**Phenotypic**
1	Grain yield/plant, g	73	11	0.78	0.61	1.00	1.00
2	Plant dry weight, g	74	14	0.85	0.56	0.76	0.83
3	Plant height, cm	69	10	0.87	0.63	0.35	0.28
4	Ear height, cm	68	30	0.74	0.54	-0.56	-0.43
5	Ear:plant height, ratio	22	5 (ns)	0.89	0.52	-0.45	-0.39
6	Ears per plant	25	10 (ns)	0.72	0.47	-0.63	-0.47
7	Stalk thickness, mm	75	18	0.67	0.62	0.52	0.36
8	Tassel length, cm	79	8 (ns)	0.85	0.64	-0.55	-0.56
9	Tassel peduncle length, cm	80	10 (ns)	0.73	0.62	-0.32	-0.26
10	Tassel branching length, cm	82	4 (ns)	0.81	0.60	0.33	0.25
11	Tassel primary branches, cm	51	19 (ns)	0.85	0.59	0.52	0.34
12	Tassel 2nd branches, cm	20 ns	11 (ns)	0.83	0.51	0.42	0.40
13	Number of leaves per plant	70	5 (ns)	0.87	0.55	0.73	0.68
14	Leaves above ear per plant	80	2 (ns)	0.78	0.52	0.55	0.48
15	Ear leaf angle	18 ns	22	0.68	0.35	0.63	0.62
16	Ear leaf length, cm	28 ns	69	0.78	0.63	0.33	0.36
17	Ear leaf width, cm	75	4 (ns)	0.87	0.51	0.32	0.24
18	Plant fractal dimension, *Do*	71	0.0 (ns)	0.68	0.59	0.73	0.66
19	Tassel *Do*	76	0.0 (ns)	0.86	0.65	0.63	0.52
20	Stem lodging †	65	10 (ns)	0.73		-0.73	-0.56
21	Root damage †	52	15	0.60		-0.66	-0.38
	Ear traits						
22	Ear length, cm	79	2 (ns)	0.74	0.60	0.75	0.60
23	Ear peduncle length, cm	43	27	0.61	0.55	0.25	0.27
24	Ear diameter, cm	77	0.0 (ns)	0.72	0.52	0.78	0.67
25	Kernel rows per ear	31	2 (ns)	0.85	0.62	0.65	0.50
26	Apical sterility, cm	34	53	0.79	0.45	-0.35	-0.25
27	Kernels per ear	84	5 (ns)	0.81	0.64	0.65	0.48
28	Kernels per plant	79	11 (ns)	0.73	0.52	0.79	0.65
29	Ear weight, g	83	12	0.83	0.80	0.69	0.52
30	Ear harvest index, %	64	30	0.92	0.85	0.89	0.82
	Kernel traits						
31	Test weight , kg l^{-1}	73	21	0.83	0.82	0.62	0.56
32	1000 kernel weight, g	82	12	0.96	0.64	0.54	0.37
33	Kernel moisture at harvest (%)	85	7 (ns)	0.73	0.54	-0.65	-0.53
34	Kernel length, mm	88	0.0 (ns)	0.88	0.74	0.35	0.33
35	Kernel width, mm	46	4 (ns)	0.78	0.69	0.27	0.22

†; in Brookings, SD only.

traits in addition to GY. The UPGMA procedure, based on PPDs, clustered those OPVhs having a consistent response across environments together; therefore, the groups of OPVhs within each cluster are expected to have a small OPVhs(HG)xE interaction for those traits.

OPVhs having Northwestern Dent in their pedigree were the only ones to cluster together at a PPD <25 whereas the remaining OPVhs formed more or less heterogeneous groups at different PPDs. Based on the PPDs, four "functional" OPVh groups, each of which can be divided into two sub-groups, were delineated at PPD ≤25. The first and second groups are dominated by Nokomis Gold and TEPR-EC6 as female HG parents, the third is exclusively a Northwestern Dent group whereas the fourth is more complex than the first three and was dominated by Minnesota 13, Lancaster and Leaming as female HG parents. The remaining cluster, composed of four BS-OPVhs, and two HPAL-OPVhs, was totally separated from the first four (PPD=100).

General and specific combining ability for GY

Level of significance derived from the combined analyses of variance for grain yield and 34 plant, ear and kernel traits measured on 46 OPVhs averaged across four environments are presented in **Table 3**. The analysis of variance across environments indicated that environmental effects were significant for GY and for about 50% of plant architectural traits, and for most ear and kernel traits considered as yield components. There were significant ($p<0.05$) effects due to OPVhs for the majority of plant traits, and for all ear and kernel traits. Environmental effects were not estimated for stem lodging and root damage because these were scored in one location. GCA effects were significant ($p<0.05$) for all traits, except for ear peduncle length, kernel length, and kernel width whereas SCA effects were not significant for four plant architectural traits, and for most ear and kernel traits. The two-way interactions between sources of variation were mostly significant for OPVhxE, and GCAxE, but not for SCAxE. Grain yield per plant, tassel second branching length, plant fractal dimension, apical sterility, and kernels per plant were significantly impacted by all main sources of variation and their interaction; whereas plant dry weight, tassel fractal dimension, and test weight exhibited similar responses, except for the non-significant effect of SCAxE. Two groups of traits (plant height, ear height and ear/plant ratio; and leaves above ear, and leaf ear angle) exhibited similar significant responses to all factors, except E and SCAxE.

The ratio of additive to total genetic variance ranged from 42 for apical sterility to 88% for ear harvest index. Almost 50% of all traits had ratios >75% whereas only three traits had ratios <50%. Ear height, ear length, ear weight, test weight, 1000-kernel weight, in addition to ear harvest index, had ratios >80% whereas ratios for the remaining traits ranged from 60 to 75%. All GCA estimates for GY based on female parents (i.e., GCA_f) were significant ($p<0.05$) whereas almost 50% of those based on male parents (GCA_m) were not (**Table 4**). GCA_f effects ranged

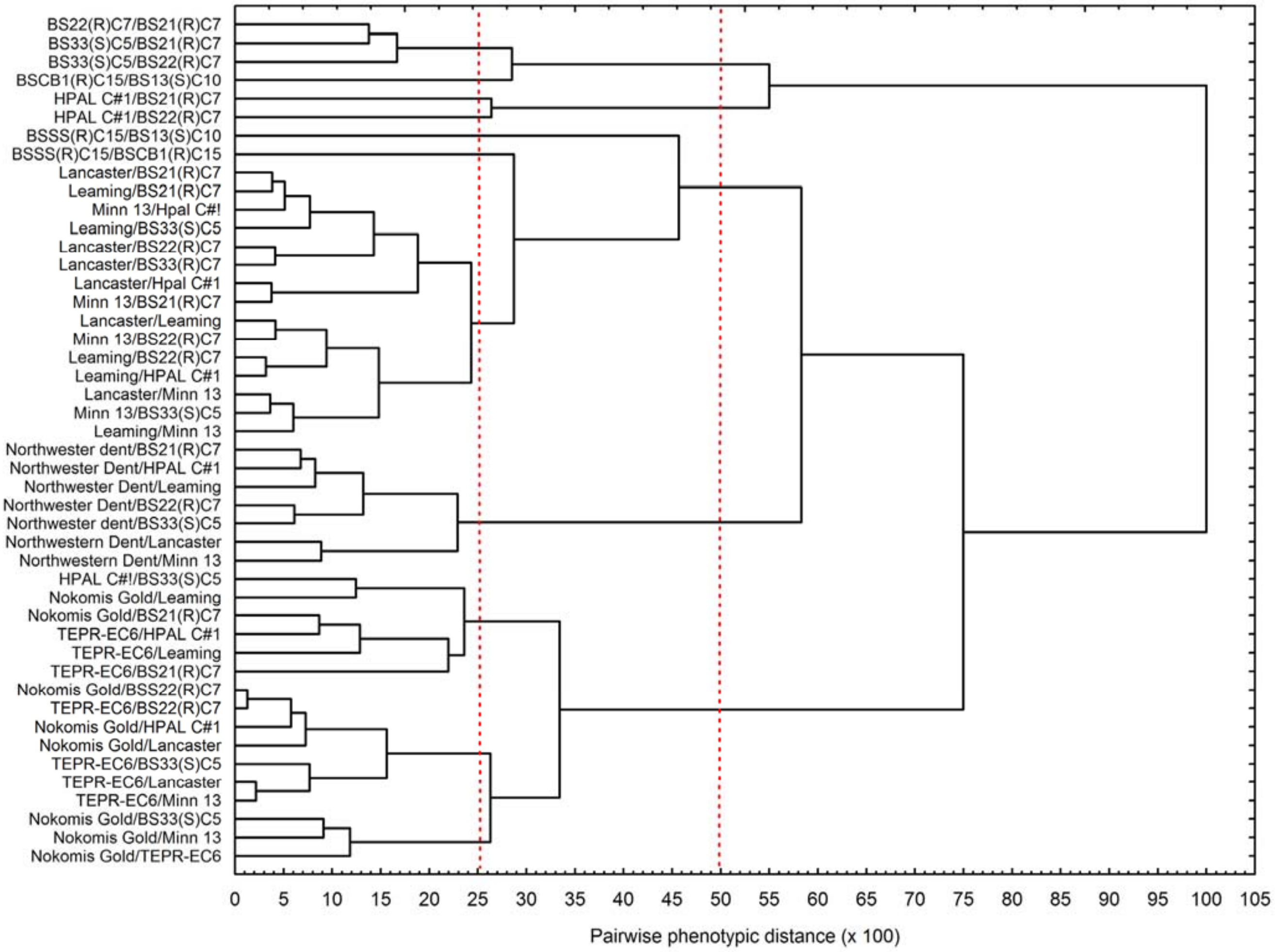

Fig. 1 Pairwise phenotypic distances between 46 open pollinated corn varietal hybrids in eight female heterotic groups and based on UPGMA clustering procedure using 35 plant, ear and kernel traits and GY in four environments.

from -0.71 for Northwestern Dent to 0.61 Mg ha^{-1} for BS33(S)C5; however, GCA$_m$ effects were smaller in magnitude and ranged from -0.37 for BSS33(S)C5 to 0.39 Mg ha^{-1} for BS21(R)C7. The SCA effects exhibited a wide range of values; 12 and 13 of the OPVhs had significant ($p<0.05$) positive and negative SCA effects, respectively whereas those of the remaining 21 OPVhs were not significant. The top five performing crosses with high positive SCA effects were TEPR-EC6/BS21(R)C7, BSSS(R)C15/BS13(S)C10, HPALC#1/BS21(R)C7, Nokomis Gold/BS21(R)C7, and Minnesota 13/BS21(R)C7. Heterosis for GY (%) was positive, exhibited a wide range (from 3 to 44%), and varied among HGs. Northwestern Dent as a female parent showed the smallest heterosis, followed by Lancaster and Leaming whereas HPAL C#1 and TEPR-EC6 consistently exhibited large values.

Stability of GY across environments

A scatter plot and relationships between grain yield and its standard deviation in two distinct groups of OPVhs are presented in **Fig. 2**. The overall average GY (4.75 Mg ha^{-1}) was associated with a large level of variation expressed as standard deviation (S.D., 1.25 Mg ha^{-1}) or as coefficient of variation (C.V.=28%). Two distinct groups of OPVhs were identified on the basis of the deviation of their mean GY and its S.D. from the overall average GY (4.75 Mg ha^{-1}) and standard deviation (S.D.=1.25 Mg ha^{-1}). The first group (**Fig. 2A**), composed of 18 mostly non-stiff stalk OPVhs with average GY >4.5 Mg ha^{-1} and S.D. <1.05 Mg ha^{-1}, was more compact than the second group (**Fig. 2B**) which was composed of 28 more dispersed, mostly stiff stalk OPVhs, the majority of which (24 OPVhs) have GY <4.5 Mg ha^{-1} and S.D. >1.2 Mg ha^{-1}. Moreover, GY of high-yielding OPVhs tended to be less variable than GY of low-yielding OPVhs in both groups; GY and its S.D. were significantly and negatively correlated in the first ($r=-0.92$; $p<0.001$) and second groups ($r=-0.68$; $p<0.01$), respectively. The first group of OPVhs was composed of BS-OPVhs (5.7 Mg ha^{-1}; 25% C.V.), HPAL-OPVhs (6.05 Mg ha^{-1}; 14% C.V.), Nokomis Gold-OPVhs (4.9 Mg ha^{-1}; 20% C.V.), and TEPR-OPVhs (5.1 Mg ha^{-1}; 18% C.V.). The second group was composed of Lancaster-OPVhs (4.4 Mg ha^{-1}; 32% C.V.), Leaming-OPVhs (4.3 Mg ha^{-1}; 35% C.V.), Minnesota 13-OPVhs (4.4 Mg ha^{-1}; 32% C.V.), and Northwestern Dent-OPVhs (3.3 Mgha^{-1}; 48% C.V.). Three of the Nokomis Gold-OPVhs, and three of the BS-OPVhs, in addition to BSCB1(R)C15/BS13(S)C10, had below and above average GY, respectively, as compared to the remaining OPVhs.

DISCUSSION

The limited diversity of current corn germplasm can lead to genetic vulnerability to abiotic and biotic stresses and it may limit future gains from selection; therefore, OPVs, in addition to landraces and synthetic populations, may be useful in improving corn in the Northern Corn Belt (Taller and Bernardo 2004), and elsewhere in developed (Tollenaar and Lee 2002) and developing countries (Kamara *et al*. 2003a). This germplasm pool of OPVhs proved to harbor large levels of "functional" variation for most plant, ear and kernel traits, a prerequisite initially sought for breeding OPVs (Vasal *et al*. 1999), the diversity of which was maintained during the last ~ 50 years at levels much larger than those of HYBs (Ho *et al*. 2005). Only a few corn breeders or farmers, especially in the US, today have experience with the forma-

Table 3 Level of significance derived from the combined analyses of variance for grain yield and 34 plant, ear and kernel traits measured on 46 open pollinated varietal hybrids (OPVhs) averaged across four environments.

No	Trait	Source of variation							Additive/total genetic variance
	Plant architecture	**E**	**OPVhs**	**GCA**	**SCA**	**OPVhxE**	**GCAxE**	**SCAxE**	**Percent**
1	Grain yield/plant, g	**†	**	*	**	*	*	*	62
2	Plant dry weight, g	*	*	**	ns	*	*	*	71
3	Plant height, cm	ns	*	*	*	*	*	ns	60
4	Ear height, cm	ns	*	**	**	*	*	ns	83
5	Ear:plant height, ratio	ns	*	*	*	*	*	ns	48
6	Ears per plant	*	*	*	*	*	*	*	75
7	Stalk thickness, mm	ns	ns	*	ns	ns	ns	ns	62
8	Tassel length, cm	*	*	*	*	*	ns	ns	71
9	Tassel peduncle length	ns	*	*	ns	ns	ns	ns	62
10	Tassel branching length	ns	*	*	*	*	*	ns	74
11	Tassel primary branches	*	ns	**	*	**	**	**	72
12	Tassel 2nd branches, cm	*	**	*	*	*	**	*	79
13	Number of leaves/plant	*	*	*	ns	*	*	ns	65
14	Leaves above ear/ plant	ns	*	*	*	*	*	ns	62
15	Ear leaf angle	ns	*	*	*	*	*	*	72
16	Ear leaf length, cm	ns	*	*	*	*	ns	ns	52
17	Ear leaf width, cm	ns	ns	*	*	*	ns	*	48
18	Plant fractal, *Do*	*	*	*	*	*	*	*	75
19	Tassel *Do*	*	*	**	**	*	**	ns	78
20	Stem lodging §	0	*	*	*	0	0	0	67
21	Root damage §	0	*	*	*	0	0	0	69
	Ear traits								
22	Ear length, cm	*	*	*	ns	*	**	*	82
23	Ear peduncle length, cm	ns	*	ns	ns	*	ns	ns	65
24	Ear diameter, cm	ns	*	*	ns	*	**	*	79
25	Kernel rows per ear	ns	*	*	ns	*	*	*	77
26	Apical sterility, cm	*	*	*	*	*	*	*	42
27	Kernels per ear	*	*	**	ns	*	**	**	76
28	Kernels per plant	*	*	**	*	*	*	*	78
29	Ear weight, g	*	*	**	ns	*	*	*	86
30	Ear harvest index, %	*	*	**	ns	*	*	ns	88
	Kernel traits								
31	Test weight , kg l^{-1}	*	*	**	**	*	*	ns	82
32	1000 kernel weight, g	*	*	**	ns	*	*	ns	83
33	Kernel moisture (%)	**	*	**	ns	*	*	ns	75
34	Kernel length, mm	ns	*	ns	*	*	*	ns	59
35	Kernel width, mm	ns	**	ns	*	*	*	ns	54

†; * and **, significant at the 5 and 1% level of probability, respectively; ns; Not significant.
§; in Brookings, SD only.

tion of synthetics or composites for release as high-yielding OPVs (Kutka and Smith 2007). Therefore, this and other germplasm pools of potentially high-yielding OPVs and OPVhs are useful to broaden the genetic base of OPVs available to farmers (Soengas *et al.* 2003) and for the production of high quality human food (Prasanna *et al.* 2001) and for livestock feed (Lauer *et al.* 2001; Scott *et al.* 2008).

Sources of trait variation and heritability

A genetic bottleneck of U.S. corn may have occurred when widely adapted and popular OPVs were selected for early inbred development (Ho *et al.* 2005); therefore, the reduced level of genetic variation for "*secondary*" traits (*senso* Monneveux *et al.* 2008) due to intense selection in corn breeding programs is a concern. The majority of traits, especially those with small, or no-interaction with the environment in this germplasm pool of OPVhs, can be used as components of a selection index (Ortiz *et al.* 2008). As a strong indicator of phenotypic variation (Smith *et al.* 2005; Monneveux *et al.* 2008), there were more statistically significant quantitative differences in phenotypic traits between HGs as compared to differences among OPVhs(HG), and the variance component due to HGs was more important than the variance components due to OPVhs(HG). On the other hand, the variance component due to OPVhs(HG) x E was negligible for most traits as was the case for populations within a corn landrace (Ortiz *et al.* 2008). Broad sense heritability estimates for the majority of traits in these OPVhs are exceptionally large in view of environmental effects and when compared to H^2 estimates in comparable germplasm pools which were bred to contain considerable diversity (Monneveux *et al.* 2005). These OPVhs, due to large phenotypic diversity of many traits, can be selected for further adaptation under specific environmental conditions and management practices (Warburton *et al.* 2008).

General and specific combining ability

The large number of OPVs (Baker 1979) and level of genetic diversity (Jaradat 2010) in 35 plant, ear and kernel traits used in this study allowed for reliable estimation and interpretation of the diallel analyses. The relatively larger additive gene effects than non-additive gene effects for GY and for most traits under study is an indication of the type of gene action in the OPVs (Baker 1978). As suggested by many studies (e.g., Betran *et al.* 2003; Rasmussen and Hallauer 2006), OPVs and populations with the longest [e.g., BS33(S)C5, BS22(R)C7] or different selection history of cultivation (e.g., HPAL C#1, TEPR-EC6) had significantly positive GCA effects and grain yield across environments.

Trait expression in corn changes with change in the environment, and the means and variances of GY and secondary traits are affected by the environment as indicated by the level of significance derived from the combined analyses of variance (**Table 3**), and as suggested by Mon-

Table 4 Estimates and level of significance of general (GCA) and specific (SCA) combining ability, and mid-parent heterosis for grain yield in 46 corn OPVhs (mean GY of an OPV in hybrids was used as inbred values for calculations; Bhatnagar *et al.* 2004).

No	Cross (Female/Male)	Variable			
		GCA_f	GCA_m	SCA	Heterosis (%)
1	BS22(R)C7/BS21(R)C7	0.59*†	0.39*	-0.23	41
2	BS33(S)C5/BS21(R)C7	0.61**		0.27*	37
3	BS33(S)C5/BS22(R)C7		-0.20	-0.26*	44
4	BSCB1(R)C15/BS13(S)C10	0.30*	0.35*	0.18	31
5	BSSS(R)C15/BS13(S)C10	-0.49*		0.48*	22
6	BSSS(R)C15/BSCB1(R)C15		-0.34*	-0.47*	34
7	HPAL C #1/BS21(R)C7	0.54*		0.48*	37
8	HPAL C #1/BS22(R)C7			0.20	40
9	HPAL C #1/BS33(S)C5		-0.37*	-0.67**	41
10	Lancaster/BS21(R)C7	-0.53*		0.35*	20
11	Lancaster/BS22(R)C7			0.24	26
12	Lancaster/BS33(S)C5			-0.21	31
13	Lancaster/HPAL C #1		0.23*	0.31*	34
14	Lancaster/Leaming			-0.27*	31
15	Lancaster/Minn 13			-0.42*	34
16	Leaming/BS21(R)C7	-0.59*		0.30*	19
17	Leaming/BS22(R)C7			0.22	25
18	Leaming/BS33(S)C5			0.10	31
19	Leaming/HPAL C #1			-0.23	31
20	Leaming/Minn 13			-0.39*	34
21	Minn 13/BS21(R)C7	-0.63**		0.44*	22
22	Minn 13/BS22(R)C7			-0.26*	25
23	Minn 13/BS33(S)C5			-0.37*	29
24	Minn 13/HPAL C #1			0.21	34
25	Nokomis Gold/BS21(R)C7	0.37*		0.46*	27
26	Nokomis Gold/BS22(R)C7			0.18	30
27	Nokomis Gold/BS33(S)C5			-0.43*	34
28	Nokomis Gold/HPAL C #1			0.09	37
29	Nokomis Gold/Lancaster		-0.22	0.19	38
30	Nokomis Gold/Leaming		0.27*	0.21	39
31	Nokomis Gold/Minn 13		-0.28*	-0.45*	39
32	Nokomis Gold/TEPR-EC6		0.17	-0.24	31
33	Northwestern Dent/BS21(R)C7	-0.71**		0.37*	3
34	Northwestern Dent/BS22(R)C7			-0.24	9
35	Northwestern Dent/BS33(S)C5			-0.27*	15
36	Northwestern Dent/HPAL C#1			0.15	18
37	Northwestern Dent/Lancaster			-0.16	18
38	Northwestern Dent/Leaming			0.35*	19
39	Northwestern Dent/Minn 13			-0.21	21
40	TEPR-EC6/BS21(R)C7	0.54*		0.51*	31
41	TEPR-EC6/BS22(R)C7			-0.22	33
42	TEPR-EC6/BS33(S)C5			-0.30*	38
43	TEPR-EC6/HPAL C #1			0.25*	41
44	TEPR-EC6/Lancaster			-0.17	40
45	TEPR-EC6/Leaming			0.22	40
46	TEPR-EC6/Minn 13			-0.30*	42

†; *, ** Significantly different from zero at the 5 and 1% level of probability, respectively.

neveux *et al.* (2008). The value of secondary traits in breeding (e.g., for adaptation) has been demonstrated by examining the genetic correlation with GY (**Table 2**; Monneveux *et al.* 2008). Significant GCAxE and SCAxE (**Table 3**) means that the magnitude of differences among the GCA and SCA effect of OPVs were different across environments (Melani and Carena 2005). Significant GCAxE for most traits indicates that GCA effects associated with parents were not consistent over locations whereas the non-significant SCA effects for some ear and kernel traits may suggest a small role of dominance gene action, although heritability estimates are reasonably large for a few of these traits (Rasmussen and Hallauer 2006).

Modeling GY

The short growing season, especially of the MN environment in this study, may not allow OPVh plants to develop the maximum number of leaves necessary for the optimum use of the limited growing period (Barata and Carena 2006; D'Andrea *et al.* 2008). Plant and tassel fractal dimensions (*Do*) were positively correlated with GY. Total number of leaves and leaves above ear per plant, but not ear leaf dimensions, contributed positively to GY (**Table 2**). Tassel *Do* reflects the size, dry weight, and branching of the tassel, all of which were reported (Uribelarrea *et al.* 2002; Monneveux *et al.* 2005) to correlate significantly with yield components and with GY. Tassel size is heritable (Monneveux *et al.* 2005) and a small tassel with many secondary branches (Uribelarrea *et al.* 2002) positively correlates with GY. Reduction in tassel size, when combined with increased branching, leads to pollen being shed as tassel differentiates and may lead to a larger number of kernels per ear and reduced apical sterility.

Grain yield in corn was reported to be more strongly associated with kernel number/ear than with number of ears/plant or with kernels/plant (Monneveux *et al.* 2005); however, in this study, ear length and kernel rows per ear were positively associated with GY. Large ears, especially at anthesis (Monneveux *et al.* 2005), constitute strong sinks that would result in a larger number of kernels/ear or per unit area if number of kernels/plant and 1000-kernel weight

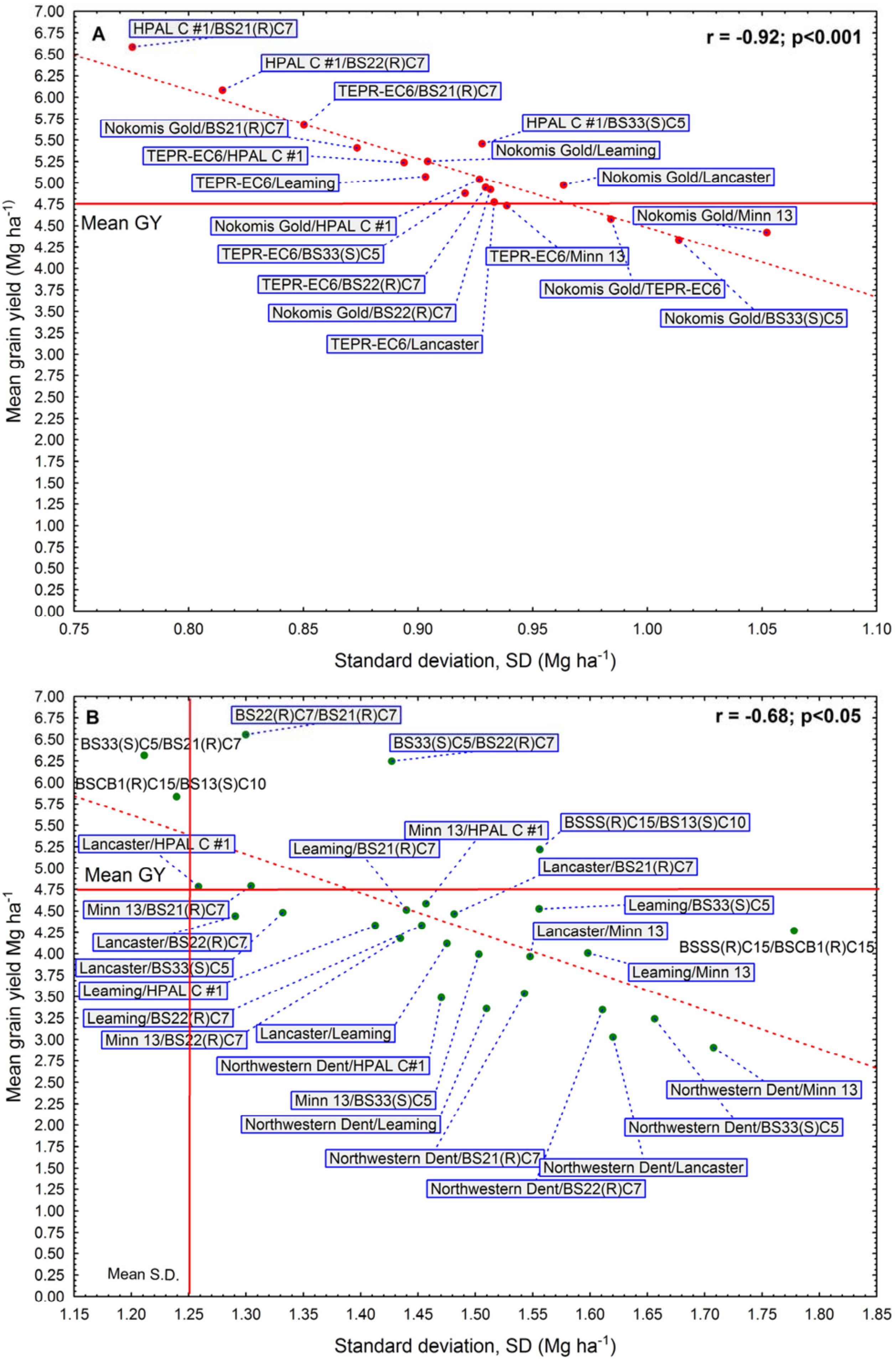

Fig. 2(A-B) Scatter plot and relationships between GY and its standard deviation in two distinct groups of open pollinated corn varietal hybrids in eight female heterotic groups and grown under four environments.

do not exhibit a strong negative relationship. This trade-off can be minimized by selection for increased post-silking biomass production, longer leaf area duration and increased post-silking light interception (D'Andrea *et al.* 2008). Large levels of variation were found in this study for number of kernels/ear and number of ears/plant; however, depending on source-sink relationships, larger number of ears/plant as a typical OPV and OPVh trait, may become a better alternative sink than larger average size of individual ears; the later may lead to increased competition between neighboring grains and to increased abortion if the source became limiting (Monneveux *et al.* 2008). Kernel moisture at harvest was one of a few traits significantly influenced by the environment. All OPVhs had kernel moisture content >15.5% at harvest and there were significant differences among OPVhs(HGs), mainly due to interaction with the environment (**Table 2**). Kernel moisture at harvest is considered as important as GY for the northern Corn Belt (Barata and Carena 2006). Fast dry down at maturity (Soengas *et al.* 2003) or low kernel moisture content at harvest (Barata and Carena 2006) can stabilize yield by partially evading drought hazards, and will help reduce potential drying cost.

Phenotypic pairwise distances among OPVhs

The pairwise phenotypic distances constructed by the UPGMA (**Fig. 1**) shows that a few OPVhs are considerably differentiated from the others, even within the HG [e.g., HPAL C#1/BS33(S)C5, TEPR EC6/Leaming and BSSS(R)C15/BSCB1(R)C15]. The variation in a similar set of phenotypic traits (Pinheiro de Carvalho *et al.* 2008) was instrumental in correctly (88.4%) classifying 43 open pollinated corn populations whereas population differentiation of corn landraces for quantitative traits was caused by changes of covariation and correlation among quantitative traits, both of which may respond to selection and management practices (Pressoir and Perthaud 2004). The dendrogram in **Fig. 1** appears to provide valuable information about the relatedness of different OPVhs. HPAL C#1 is derived from modern hybrids, therefore, we expect it to cluster with the BS-OPVhs (with the exception of BS33), as they contain a considerable amount of Reid Yellow Dent in their ancestry. In principle, BS33(S)C5 should have been separated out and analyzed together with Leaming. However, the current data analysis strongly suggested that its grouping with other BS-OPVhs was correct since hybrids made with BS33(S)C5 as a female parent behaved more like other BS-OPVhs than Leaming. Hybrids that had Lancaster, Leaming, and Minnesota 13 as female parents clustered closer together than they did with those having Nokomis Gold or TEPR-EC6 as female parents. Nokomis Gold and TEPR-EC6 have little Reid Yellow Dent in their ancestry, and showed the greatest distance from BS-OPVhs and HPAL C#1-OPVhs. OPVhs in the former and latter groups generally showed average and above-average GY, respectively, with relatively small variances (**Fig. 2**) and may be useful to develop future varietal hybrids if they respond to selection for GY in reciprocal combinations.

Stability of GY across environments

A strong relationship between yield stability and phenotypic variability was demonstrated in this study by a number of OPVhs having small yield variation associated with above-average GY across four environments (**Fig. 2A**). Broad adaptation and yield stability, both expressed as small yield variation across environments and small OPVhs(HG)xE interactions, and considered as preferred traits for OPVhs (Song 1999), were experimentally confirmed for a large number of OPVhs. A few high-yielding OPVhs, with reasonably stable GY across environments, can be identified in **Fig. 2B**. OPVs showed impressive yield stability under typical smallholder farmers' conditions in Ethiopia (Reeves and Cassaday 2002), sub-Saharan Africa (Kamara *et al.* 2004), and China (Song 1999) and demonstrated a high level of uniformity in field trials as well as high grain quality (Carena 2005). Improved OPVs may be less costly to grow, and more profitable than HYBs under certain environmental and management conditions and have the added advantage of yield buffering capacity as a result of plasticity, especially under less favorable growing conditions.

CONCLUSIONS

There is some renewed interest in open pollinated varieties and open pollinated varietal hybrids of corn, especially among organic and low-input farmers, due to their stable yield and broad adaptation as compared with hybrid corn. In addition, open pollinated varietal hybrids may have better drought tolerance, prolificacy, superior quality, and nutritious value as livestock feed, and human food. We estimated broad-sense heritability of 35 plant, ear, and kernel traits in 46 open pollinated varietal hybrids grown under four environments. We identified varietal hybrids with a large GY potential associated with small variability across environments, along with the phenotypic traits contributing to this large and stable yield. The information on the variability available in these varietal hybrids and their breeding potential is of value for corn breeders in pursuing breeding and selection objectives for organic and low-input farming and for farmers to stabilize yield through increased diversity on the farm.

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