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Pollen control and spatial and temporal adjustment in evaluation of kernel composition of maize inbreds

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Abstract

Maize (*Zea mays* L) pollen can have an immediate effect on kernel oil, protein, and starch concentration, and evaluation of kernel composition is often done with self pollination. Procedures for adjusting for spatial or temporal variation have not been studied for kernel composition traits in maize. Our objectives were to (i) characterize the effect of pollen control on protein, oil, and starch concentrations in maize inbreds, (ii) determine the impact of open and self pollination on relative ranking of inbreds, and (iii) determine if spatial or temporal adjustments are useful for kernel composition when plants are open pollinated. Thirty inbreds were evaluated for kernel protein, oil, and starch concentrations in open- and self-pollinated treatments at two planting dates in St. Paul, MN in summer 2011. The inbreds differed significantly for oil, protein, and starch concentrations. However, pollination treatment was not significant for oil, protein, or starch concentration. Simple correlations and rank correlations between open- and self-pollinated treatments were high for oil (0.93), protein (0.83 - 0.85), and starch (0.73 - 0.82) concentrations. Neither spatial nor temporal adjustments improved estimates of open- pollinated kernel composition over unadjusted, open-pollinated means. Overall, our results suggested that when relative performance of inbreds is more important than absolute concentrations, maize inbreds can be evaluated for oil, protein, and starch concentrations without the pollen source confounding differences among entries.

Keywords: maize, pollen control, xenia, kernel composition, spatial adjustment, temporal adjustment

Introduction

When a maize (*Zea mays* L) plant is open pollinated, it produces seed fertilized by pollen from itself and from neighboring maize plants flowering concurrently. The xenia effect, which is the immediate effect of pollen on the developing kernel, has been implicated in kernel oil, protein, and starch concentration (East and Jones, 1920; Curtis et al, 1956; Letchworth and Lambert, 1998), kernel size (Leng, 1949), and kernel development (Pixley and Bjarnason, 1994; Seka and Cross, 1995; Bulant and Gallais, 1998). Double fertilization results in the embryo developing from equal contributions of the maternal and paternal genome, while the endosperm develops from the union of one sperm and two polar nuclei, increasing the influence of the maternal parent on endosperm characteristics (Kiesselbach, 1999).

Multiple studies have agreed that, when investigating oil concentration, entries should be self pollinated as both the maternal parent and pollen parent influence oil concentration (Miller and Brimhall, 1951; Curtis et al, 1956; Letchworth and Lambert, 1998). Large numbers of entries need to be evaluated across multiple environments in inbred development programs or in linkage mapping and association mapping studies to map quantitative trait loci (QTL). Experiments to map QTL for oil and protein concentration in maize have been conducted using only self-pollinated kernel samples (Goldman et al, 1993, 1994; Laurie et al, 2004; Clark et al, 2006; Wassom et al, 2008; Cook et al, 2012).

While previous studies have shown the effect of both male and female parents on kernel composition, most studies have included few entries and have not considered the impact of pollen source on the ranking of entries between open- and self-pollinated treatments (East and Jones, 1920; Curtis et al, 1956; Letchworth and Lambert, 1998). Also, previous studies have utilized germplasm previously selected for either protein or oil concentration instead of germplasm that is more representative of the variation in kernel composition traits in the US Corn Belt (East and Jones, 1920; Miller and Brimhall, 1951; Curtis et al, 1956; Letchworth and Lambert, 1998). Furthermore, procedures for adjusting for spatial or temporal variation have been used for grain yield in maize (Brownie et al, 1993; Moreau et al, 1999) and in wheat (*Triticum aestivum* L; Stroup et al, 1994), but their usefulness for kernel composition traits has not been studied. Therefore, our objectives were to (i) characterize the effect of pollen control on protein, oil, and starch concentrations in maize inbreds, (ii) determine the impact of open and self pollination on relative ranking of inbreds, and (iii) determine if spatial or temporal adjustments are useful for kernel composition when plants are open pollinated.

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Materials and Methods

A collection of 30 maize inbreds were evaluated for kernel protein, oil, and starch concentrations in open- and self-pollinated treatments. The 30 inbreds included 18 publicly developed inbreds and 12 privately developed inbreds with expired US Plant Variety Protection Act certificates. The inbreds used in this study were not chosen based on known kernel composition or known previous selection for kernel composition traits, nor did they contain known major genes modifying kernel traits. The 30 inbreds used were as follows: 4N506, A7, A116, A239, A310, A385, A495, A632, A634, A639, A648, A654, A664, A674, B73, B84, DJ7, DKFAPW, DKMDF-13D, LH82, LH149, NK807, Oh43, Pa91, PHG47, PHG84, PHJ75, PHW17, W64a, and WIL900. Seed for most inbreds was provided by the USDA North Central Regional Plant Introduction Station at Ames, Iowa, USA.

The inbreds were evaluated at two planting dates in St Paul, Minnesota, USA in summer 2011. The 14 day difference between the two planting dates (16 May and 31 May 2011) permitted replication in time, which may influence the availability of pollen from the same plant and the extent of natural self- versus cross-pollination. The experiment for each planting date was conducted in a split-plot design with three replications. The two main-plot treatments were open pollination and self pollination and the subplot treatments were the 30 maize inbreds.

Each inbred was planted in a single row at a plant population density of 86,500 plants ha^{-1}. The plots were 4.72 m long and spaced 0.76 m apart. Border plots were planted around each replication with a balanced bulk of seed from 56 maize inbreds. In the open-pollinated treatment, all plots were allowed to open pollinate. In the self-pollinated treatment, ear shoots were covered prior to silk emergence and 10 - 12 plants were self pollinated. Ears from 6 - 8 plants within a row were hand harvested, bulked, and mechanically shelled. Whole kernel samples were scanned using a Perten DA 7200 near infrared reflectance analyzer (Springfield, Illinois, USA) and commercially available equations were used to predict oil, protein and starch concentrations. Concentrations were converted to a dry matter basis (g kg^{-1}). Analysis of variance (ANOVA) was conducted with pollination treatment and inbred as fixed effects and planting date and replication as random effects using PROC GLM in SAS/STAT software (SAS Institute, 2009). To increase the power of the tests, the error sums of squares for the main plots and subplots were pooled when the corresponding mean squares were not significantly different at P = 0.25 (Carmer et al, 1969). Standard F-tests for combined split-plot experiments were conducted as outlined by McIntosh (1983). Least squares estimates of treatment means were calculated and Fisher's LSD (P = 0.05) was used for mean separation, where applicable.

In a separate field experiment, days to anthesis expressed as growing degree days (GDD) was recorded on the same 30 inbreds as part of a larger experiment grown in an adjacent section of the same field in St Paul in summer 2011. This experiment was conducted in an augmented randomized complete block design with two replications and was planted on the same date (16 May 2011) as the first planting of the kernel composition experiment previously described. Identical crop management practices were applied to both experiments. An ANOVA was conducted and least squares means for inbreds were calculated using PROC GLM in SAS/STAT (SAS Institute, 2009). Least squares means for days to anthesis were used for developing covariates for kernel composition data.

Two methods were used to adjust kernel composition data in the open-pollinated treatment, one based on spatial distribution and the other on temporal distribution. The first method was a nearest neighbor adjustment based on the Papadakis method using two covariates (Wilkinson et al, 1983; Gezan et al, 2010). One covariate was the mean residual from two adjacent rows and the second covariate was the mean residual from two neighboring plots from the adjacent ranges. Border plots were assumed to have the experiment mean for kernel composition traits and were included in the spatial adjustment calculation.

Table 1 - Mean squares (MS) and significance of effects from analyses of variance for oil, protein, and starch concentration for open- and self-pollinated treatments of 30 maize inbreds evaluated at two planting dates in St Paul, Minnesota, USA in summer 2011.

	df	Oil MS	Protein MS	Starch MS
Planting date	1	116	4315*	8591*
Replication/planting date	4	51*	211*	919*
Pollination treatment	1	71	8029	20971
Planting date × pollination treatment	1	27	551*	414
Inbred	29	358*	2761*	3890*
Planting date × inbred	29	9	184*	238*
Pollination treatment × inbred	29	15	232*	395
Planting date × pollination treatment × inbred	29	13	111*	257*
Pooled error	230	9	43	107

* Significant at P = 0.05

Table 2 - Simple and rank correlations of open-pollinated inbred means (unadjusted or after spatial and temporal adjustment) with self-pollinated inbred means for oil, protein, and starch concentrations. All correlations were significantly different from zero ($P < 0.001$).

Adjustment method	Oil		Protein		Starch	
	Simple	Rank	Simple	Rank	Simple	Rank
Unadjusted	0.93	0.93	0.85	0.83	0.82	0.73
Spatial	0.93	0.93	0.85	0.83	0.82	0.75
Temporal	0.91	0.92	0.85	0.84	0.80	0.73

For the temporal adjustment, all plots within each replication that reached anthesis within 100 GDD of a given plot were averaged and used as a single covariate. An analysis of covariance was conducted for each method using the spatial or temporal covariates and least squares means were calculated using PROC GLM in SAS/STAT software (SAS Institute, 2009).

Simple (Pearson) correlations and Spearman rank correlations were calculated between least squares means of inbreds from the open- and self-pollinated treatments and the spatially and temporally adjusted data using PROC CORR in SAS software (SAS Institute, 2009). Fisher z-transformation was used to test the significance of differences among correlations. All comparisons were made in relation to the means of the self-pollinated treatment as these were assumed to be the more accurate estimates of kernel composition for a given inbred.

Results

The inbreds differed significantly in their oil concentration (Table 1), with inbred means ranging from 38 to 59 g kg^{-1} in both the open and self-pollinated treatments (Figure 1). However, effects of pollination treatment and the interaction between pollination treatment and inbred were not significant (Table 1). Rank changes among inbreds between open and self-pollinated treatments for oil concentration were minimal, with simple and rank correlations of 0.93 ($P < 0.001$; Table 2). Simple and rank correlations between adjusted and self-pollinated least squares means were 0.93 for the spatial adjustment and 0.91 - 0.92 for the temporal adjustment ($P < 0.001$; Table 2). No significant difference was found among simple and rank correlations. Temporal adjustments were based on flowering date, and the mean difference between the earliest-flowering inbred (A495) and the latest-flowering inbred (PHG84) was 484 GDD (approximately 18 calendar days). The number of plots used in the each temporal adjustment ranged from 2 to 15.

Inbred, planting date by pollination treatment interaction, planting date by inbred interaction, and pollination treatment by inbred interaction significantly affected protein concentration (Table 1). Protein concentration among inbreds ranged from 93 to 158 g kg^{-1} in the open-pollinated treatment and from 101 to 173 g kg^{-1} in the self-pollinated treatment (Figure 1). The difference between means of the open and self-pollinated treatments for protein concentration was 6 g kg^{-1} for the first planting date and 12 g kg^{-1} for the second planting date. Three inbreds (A7, A310, and LH149) had significantly higher protein concentration in the self-pollinated treatment than in the open-pollinated treatment (Figure 1). The rank correlation for protein concentration between pollination treatments was 0.83 ($P < 0.001$; Table 2). Rank changes among inbreds between open and self-pollinated treatments were minimal with the exception of inbred A310, which moved 20 positions between pollination treatments. Rank correlations between adjusted and self-pollinated least squares means for protein concentration were 0.83 for the spatial adjustment and 0.84 for the temporal adjustment ($P < 0.001$; Table 2). The corresponding simple correlations were 0.85. No significant difference was found among simple and rank correlations.

Inbred, planting date by inbred interaction, and planting date by pollination treatment by inbred interaction significantly affected starch concentration (Table 1), with inbred means ranging from 639 to 736 g kg^{-1} in the open-pollinated treatment and from 637 to 721 g kg^{-1} in the self-pollinated treatment (Figure 1). The rank correlation between pollination treatments for starch concentration was 0.73 ($P < 0.001$; Table 2). Rank changes among inbreds were more erratic for starch concentration than for oil or protein concentration. Rank correlations between adjusted and self-pollinated least squares means for protein concentration were 0.75 for the spatial adjustment and 0.73 for the temporal adjustment ($P < 0.001$; Table 2). The corresponding simple correlations were 0.80 - 0.82. No difference was found among simple and rank correlations.

Discussion

Three primary conclusions were made from this study. First, method of pollination did not influence protein, oil, or starch concentrations. Second, high correlations between open and self-pollination treatments indicated that open-pollinated evaluations would be sufficient when screening a large number of inbreds for oil, protein, and starch concentrations for the purpose of selection. Third, neither spatial nor temporal adjustments improved estimates of open-

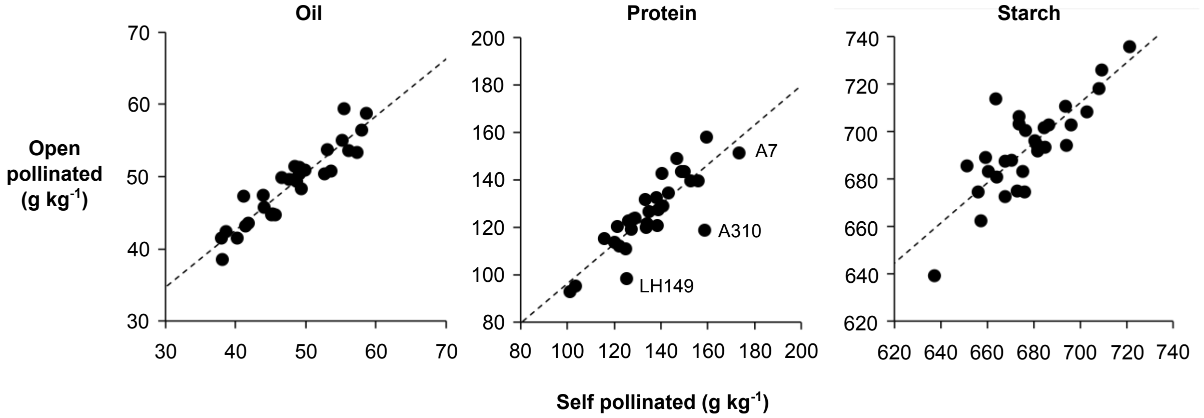

Figure 1 - Means of 30 maize inbreds for kernel composition in open-pollinated and self-pollinated treatments. Inbreds that significantly differed between pollination treatments (P = 0.05) are labeled and the dashed lines represent the simple regression lines.

pollinated kernel composition over unadjusted, open-pollinated means.

When the purpose of the experiment is to determine the actual kernel composition of inbreds (instead of the relative performance of inbreds), open versus self pollination must not have an effect on the mean concentration. Pollination treatment did not significantly affect oil, protein, or starch concentrations among the 30 maize inbreds in this study, and this finding conflicted with previous research. In a study using commercial hybrids and hybrids selected for protein content, mean oil and starch concentrations were significantly greater in the open pollinated treatment, and protein concentration was significantly greater in the self-pollinated treatment (Letchworth and Lambert, 1998). In studies utilizing inbreds and populations derived from the Illinois long-term selection experiment, the pollen parent was found to influence oil and protein concentrations (Miller and Brimhall, 1951; Curtis et al, 1956). For example, oil concentration increased from 42 g kg^{-1} with a low oil pollen parent to 72 g kg^{-1} with a high oil pollen parent (Curtis et al, 1956). The direct comparison of the findings of the present study and those of Miller and Brimhall (1951) and Curtis et al (1956) was confounded by the genetic background of the germplasm studied.

When the purpose of the experiment is to rank and select the best inbreds for kernel composition, pollination treatment by inbred interaction as well as rank changes between pollination treatments must be minimal. Pollination treatment by inbred interaction was significant only for protein concentration. Three inbreds (A7, A310, and LH149) had significant differences between open- and self-pollinated treatments, with all three inbreds having higher protein concentrations in the self-pollinated treatment (Figure 1). However, the simple and rank correlations between open and self-pollinated treatments were high for protein concentration (0.83 - 0.85; Table 2), indicating that selection for protein concentration can be done in open-pollinated experiments. This result supports previous research that found the female parent to have a much greater effect on protein concentration compared to the pollen parent (Letchworth and Lambert, 1998). Similarly, high simple and rank correlations for oil (0.93) and starch (0.73 - 0.82) concentrations between open- and self-pollinated treatments indicated that selection for these traits can be done in open-pollinated experiments (Table 2).

Spatial adjustment by nearest neighbor analysis has been shown to effectively account for plot-to-plot variation for grain yield in maize (Brownie et al, 1993; Moreau et al, 1999) and in wheat (Stroup et al, 1994). In open-pollinated evaluations of kernel composition, pollen from adjacent plots is very likely to land on receptive silks of the plot of interest. Temporal adjustment of kernel composition based on relative days to anthesis was also of interest as only inbreds flowering concurrently can influence kernel composition of a given plot. However, neither spatial adjustment nor temporal adjustment increased the simple or rank correlations between pollination treatments (Table 2). The range of 100 GGD, equivalent to about 4 calendar days during the time of flowering (data not shown), was used in estimating the temporal covariate as it is a reasonable timeframe in which airborne pollen may land on receptive silks in the plot of interest.

In this study, planting date was found to affect protein and starch concentrations but not oil concentration (Table 1). While our experiments were limited to two planting dates at the same location and a previous study (Bulant and Gallais, 1998) indicated that xenia effects were subject to genotype by environment interaction, this previous study also showed that xenia effects were repeatable across environments. The interaction of planting date with inbred was also significant for protein and starch concentrations in the present study (Table 1). These results support previous research showing a significant genotype by environment interaction for protein and starch concentration but not for oil concentration

(Berke and Rocheford, 1995). Our results as well as previous studies indicate the importance of conducting kernel composition experiments in more than one environment.

Overall, our results showed that maize inbreds can be evaluated for oil, protein, and starch concentrations without the pollen source confounding differences among entries when relative performance of inbreds is more critical than absolute concentrations. Spatial and temporal adjustments were not useful for kernel composition. We note that all inbreds in our experiment were of temperate background, and our conclusions may not necessarily apply to other germplasm (e.g., tropical inbreds, landraces, sweet corn, etc.) with different kernel characteristics. Further experiments with larger numbers of temperate inbreds, with other types of germplasm, and with diverse environments would be helpful. Nevertheless, modern genetic methodologies such as genomewide association analysis (Zhu et al, 2008; Yan et al, 2011) and large scale selection experiments in temperate maize require the evaluation of large numbers of entries where absolute kernel composition estimations are not necessary and reducing labor requirements is of interest. Our results suggest that open pollination of the entries would be adequate in such situations.

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