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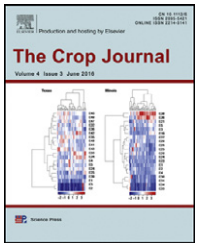


# Metabolite variation in hybrid corn grain from a large-scale multisite study

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ABSTRACT

Metabolite composition is strongly affected by genotype, environment, and interactions between genotype and environment, although the extent of variation caused by these factors may depend upon the type of metabolite. To characterize the complexity of genotype, environment, and their interaction in hybrid seeds, 50 genetically diverse non-genetically modified (GM) maize hybrids were grown in six geographically diverse locations in North America. Polar metabolites from 553 harvested corn grain samples were isolated and analyzed by gas chromatography–mass spectrometry and 45 metabolites detected in all samples were used to generate a data matrix for statistical analysis. There was moderate variation among biological replicates and across genotypes and test sites. The genotype effects were detected by univariate and Hierarchical clustering analyses (HCA) when environmental effects were excluded. Overall, environment exerted larger effects than genotype, and polar metabolite accumulation showed a geographic effect. We conclude that it is possible to increase seed polar metabolite content in hybrid corn by selection of appropriate inbred lines and growing regions.



## 1. Introduction

Corn is one of the most important cereal crops worldwide for food, feed, and energy. Starch, protein, oil, and fiber represent the major nutritional components and economic value in corn grain [1], and are the main targets of plant breeding and biotechnology [2–6]. In contrast, polar metabolites in corn grain are of low abundance (~5%) relative to the cumulative biomass of major seed components [1]. However, seed metabolite content is closely associated with food and feed quality, and increasing attention is being paid to foods that provide health benefits beyond basic nutrition (functional foods). Two different strategies are widely applied to improve the nutritional value of corn grain. One is to increase the

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content of beneficial metabolites: breeding for high vitamin A and high lysine corn are two noteworthy examples [7–9]. A second strategy is to reduce anti-nutritional metabolite content, for example, developing lower-phytic-acid (PA) corn [10–15].

A common feature of these two strategies is targeting a single metabolite or a few metabolites at a time for corn breeding. Generally, both are successfully implemented for seed trait improvements of greatest importance for industrial or nutritional value. However, this approach does not take into account the complexity of metabolic networks. Alteration in the amount of one particular metabolite could potentially affect other metabolites, with some changes desirable and others not. This tradeoff was demonstrated by the recent detailed characterization of a low-PA mutant: lower PA and higher free phosphorus (P) in the PA mutant improved grain food quality, but a decrease in gamma tocopherol and increase in Hg, As, and Al content compromised its nutritional value [16,17].

In recent years the genetic basis of seed metabolite profiles has been investigated, and several metabolite QTLs have been identified [18,19]. These studies also revealed that seed metabolite content is sensitive to environmental fluctuations, so that the heritability of metabolite traits is usually low. In addition, improved seed metabolite traits were often accompanied by yield penalties in several crop species [20,21]. An exception was recently discovered in hybrid plants, in which certain metabolite contents can be enhanced by mechanisms that do not incur a yield penalty [22]. These findings showed that it is practical to improve some seed metabolite traits without yield loss.

The metabolite inheritance pattern in hybrid seeds still remains largely unexplored. However, it is clear that genotype, environment, and the interactions between these two factors play different roles in shaping the seed metabolite profile and contents. Yang et al. [23] generated 30 genetically related corn hybrids by crossing six female inbred lines (from a common Stiff-Stalk progenitor) with five different male inbred lines (from Non-Stiff Stalk), and grew them in two geographically similar locations in Illinois. Parallel metabolic and transcriptional profiling revealed marked variation even among genetically related corn hybrids. This study also suggested that hybrid seed metabolite content is a multigene trait and that the genetic interactions among these genes remains poorly understood. Reynolds et al. [24] performed composition analysis of corn grain from seven hybrids grown at four test sites and found both genotype and environment to be determinants of seed biochemical composition. Harrigan et al. [18] reported that corn hybrid (genotype) influenced the number and type of grain metabolites in response to water deficit. Metabolic profiling of corn hybrids derived from 48 inbreds crossed to two different testers showed that metabolite pool size was highly dependent on genotype; certain metabolite classes showed a tester effect, while others showed either non-interacting or interacting tester and location effects [25,26]. These studies suggested that manipulation of seed metabolite content can be achieved by selection of appropriate tester lines for hybrid production. Röhlig et al. [27] showed that growing season was the most prominent factor influencing metabolite variation when four cultivars were grown at one location for three consecutive seasons. Furthermore, by metabolite profiling of one cultivar grown for three years at four locations, the authors found that natural variation in corn grain metabolite pools was the result of interplay between location, season, and genotype. Different chemical classes could show differences in a genotype- or environment-dependent manner. Recently, Cong et al. [28] reported that crude protein, manganese, β-carotene, and all amino acids except lysine in maize grain were more affected by environment than by genotype. In contrast, most proximates and fibers, all fatty acids, lysine, and most minerals, vitamins, and secondary metabolites in maize grain were affected by genotype more than by environment. A strong interaction between genotype and environment was seen for some analytes.

In this study, 50 genetically diverse non-GM maize hybrids were grown at six locations representing three different climate zones in North America. Forty-five polar metabolites in corn grain were then quantified and different statistical methods were compared for metabolite signature extraction. This study provides additional insight about metabolite variability in hybrid corn seed and its implications for nutritional improvement.

## 2. Materials and methods

### 2.1. Plant materials

Fifty non-GM maize hybrid varieties (or genotypes) from DuPont-Pioneer HiBred were grown in six locations (Texas, Kansas, Illinois, Nebraska, Minnesota, and Ontario). At each site 20 varieties were selected based on their maturity zone (Table S1). Each test site was divided into three blocks and the selected 20 varieties were grown in each block in a randomized manner. Grain samples ($F_2$ seeds) were collected at the $R_6$ stage from all varieties in all blocks. In each block, five hand-pollinated ears were collected from the same variety and the shelled ears were pooled as one sample. One or two samples were collected per block per variety, so that three or six samples per variety were collected from each test site [29–30].

### 2.2. Polar metabolite extraction and derivatization

Metabolites were extracted from lyophilized ground powder of grain samples. Samples of dry weight 5.5–6.5 mg were weighed and transferred into 2 mL microfuge tubes and 0.75 mL of chloroform was added. Samples were incubated at 55 °C with rotation for 30 min, and then 0.75 mL of deionized water (containing 5 μg $mL^{-1}$ ribitol internal standard) was added and incubated for an additional 30 min. Samples were then centrifuged at 1500×$g$ for 15 min to allow phase separation. Of the upper aqueous phase, 660 μL was transferred into a 2 mL glass autosampler vial and evaporated to dryness in CentriVap Console (Labconco, USA). Test samples from the same site were divided into batches (Table S2). One reference sample was included with each batch and analyzed three times during the batch run to monitor instrumental variation. The grain reference samples were obtained by pooling and mixing thoroughly powdered grain from all Illinois varieties, and metabolites were extracted as described above.

The dried extracts were dissolved in 120 μL of 20 mg $mL^{-1}$ methoxyamine hydrochloride in pyridine and incubated at 37 °C