**Evaluation of the Potential Usefulness of exPVP Maize Germplasm in Varietal Development for Organic Systems Using Participatory Variety Testing and Machine Learning Models**

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# **ABSTRACT**

The rapid increase in demand for organic agricultural products has transformed the organic industry from a niche industry into a well-developed alternative to conventional farming systems. However, the current domestic organic food supply does not satisfy the increasing demand. As part of the solution, new crop varieties are needed that perform well in organic systems. The objective of this study was to use a participatory research approach to characterize the performance of maize hybrids developed by conventional and organic breeding programs under real-world organic management practices. A set of twelve cultivars developed by four breeding programs with varying breeding objectives were evaluated in organic on-farm trials. For this purpose, we created a Variety Testing Network (VTN) collaborating with eight farmers in Illinois and four farmers in Indiana. In each year, seven experimental hybrids from the set were selected and tested together with the commercial check by the VTN. Each farmer planted the hybrids in a four-row 30-meter strip plot using an unreplicated, completely randomized design. The hybrids were evaluated for grain yield, grain moisture, test weight, kernel weight, plant height, ear height, and protein, starch, and oil content in the grain. The hybrids differed significantly for all agronomic performance traits across farms, which varied for their crop rotation lengths, cover crop, planting densities, weed management, and nitrogen sources. The grain yield across farms ranged from 1.6 t ha-1 to 14.8 t ha-1 with an average of 9.1 t ha-1 for the three years of the study. The mean protein, starch, and oil content across farms was 8.0%, 69.6% and 4.0%, respectively. The UIUC hybrids derived from exPVP sources had higher grain yield and starch content than those developed from organic inbreds, whereas organic hybrids were distinguished by higher protein contents. Reliability estimates showed that UIUC hybrids had more 50% probability to outperform the commercial check variable across environments while organic experimental hybrids had a lower reliability below 30%. Farmers agronomic management practices such as cover crop types, nitrogen source, planting density and crop rotation length significantly affected the performance of hybrids hence causing a significant genotype by environment interaction. Due to the high yield response of the UIUC hybrids under on-farm conditions, the study shows that the Illinois Elite Maize Association Mapping Panel derived from intercrossing exPVP inbreds can be a potential useful for breeding corn hybrids for organic farming systems in the U.S Corn Belt. However, these experimental hybrids lacked adequate tolerance to high weed pressure and performed poorly in farms with low nitrogen levels. Therefore, the identification and integration of new sources of genetic diversity for these traits would be beneficial for better adaptation to organic systems.

# **INTRODUCTION**

The demand for organic agricultural products has rapidly increased in the last decades due to the increasing consumer awareness of the impacts of conventional production systems on food quality, animal welfare, environment, and human health (Oroian et al., 2017). The organic agricultural industry has transformed from a mere niche industry into a well-developed alternative to conventional systems and a secure source for non-GMO and chemical-free food with the high nutritional value produced using environmentally friendly methods (Annunziata and Pascale, 2009). According to the Organic Trade Association (OTA) 2021 organic industry survey, U.S. organic sales reached a record high of 62 billion dollars in 2020, reflecting a 12 percent growth rate and more than twice the 2019 rate of 5 percent (OTA, 2021). In addition, there is a growing expansion in the certified organic acreage and the number of certified organic farms since more farmers are transitioning from conventional to organic operations. The 2019 organic survey by the U.S. Department of Agriculture's (USDA) National Agricultural Statistics Service (NASS) reported a 17 percent increase in certified organic farms from 14200 in 2016 to over 16500 farms. In addition, the total certified organic acreage increased by 9 percent since 2016 to over 5.5 million acres in 2019. The leading U.S. states with the highest number of certified organic farms in 2019 included California, Wisconsin, and New York, with over 3000, 1360, and 1320 farms, respectively (NSAC, 2020).

Despite the perpetual increase in certified organic farms and the steady expansion of the U.S. organic acreage, the current domestic organic food supply does not satisfy the increasing product demand (Brock et al., 2019). Organic agricultural systems rely on natural ecosystems as the sustainable alternative sources for nutrient supply and pest control instead of synthetic chemical inputs used in conventional systems. However, modern cultivars developed using traditional breeding methods require intensive management and inputs to outcompete weeds, pests, and diseases (Andersen et al., 2015). The Organic Foods Production Act (OFPA), enacted under Title 21 of the 1990 Farm Bill to regulate the organic production standards (SARE, 2003), prohibits the use of chemical inputs such as pesticides and synthetic fertilizers (Gold, 2007 and Osman et al., 2016). Consequently, the productivity of modern cultivars under organic systems is often lower compared to their performance under conventional systems, hence creating a yield gap between the two systems (Andersen et al., 2015; de Ponti et al., 2012; Delbridge and King, 2014). Various studies and survey data from the USDA-NASS shows that the average organic maize yield is 35 percent below an average conventional yield (Kniss et al., 2016; Langemeier and O'Donnell, 2020). The yield gap between conventional and organic systems is due to the lack of crop varieties that are well adapted to the extreme biotic, abiotic stresses like nutrient deficiency, weed pressure, and pest competition prevalent in organic systems (Kniss et al., 2016). Over 95 percent of crop varieties commercially available to organic growers were bred under conventional high-input conditions. They, therefore, lack the resilience to produce high yields when grown under complex and diverse organic conditions with low soil nutrients and high weed and pest pressure (Bueren et al., 2011 and Chozin et al., 2017). In addition, certain traits such as nutritional value, gametophytic incompatibility, fertilizer-uptake and use efficiency, weed pressure and insect resistance that are of high preference to organic growers are not highly prioritized in conventional germplasm selection and breeding programs (Goldstein et al., 2019).

The selection for conventional crop varieties is usually conducted on research stations under high input favorable conditions, hence obtaining high heritabilities and distinct variation between genotypes (Dawson et al., 2008). Varieties selected under such conditions are bred for farmers that use similar management practices and inputs to those used in research stations (Desclaux, 2005). However, the complexity and heterogeneity of organic environments, management practices, and farmers' interests hinder the breeding and selection of crop varieties with a wide range of performance potential (Dawson et al., 2008). Varieties selected under high input homogenous environments perform lower under complex heterogeneous conditions with reduced genetic variance due to the associated genotype-by-environment interaction (Bänziger and Cooper, 2001). Due to the reduced genetic variance, the probability of obtaining significant performance differences among the test varieties under complex and diverse conditions to identify superior genotypes is low (Dawson et al., 2008).

Consequently, high-performing crops varieties under conventional systems do not necessarily perform well under complex and heterogeneous organic conditions. Therefore, there is an inevitable need to establish decentralized breeding programs that utilize direct selection under target environments instead of selection under favorable homogeneous on-station conditions (Bänziger and Cooper, 2001). The establishment of separate decentralized programs with a unique focus on improving and developing cultivars well adapted to organic conditions and addressing organic growers' traits of interest will facilitate the organic industry to maximize its full production potential (Murphy et al., 2007). Participatory plant breeding (PPB) is one of the used approaches in breeding for marginal heterogeneous environments. The PPB approach is an on-farm breeding method that aims at establishing close collaborations between farmers and breeders to develop crop varieties that are well-adapted to the farmer's specific regions and agronomic management practices (Zystro et al., 2012). The involvement of farmers in the breeding and selection processes promotes genetic diversity, empowers farmers, and enables the development of crop varieties that meet farmers' preferences and are well-adapted to their unique growing conditions and management practices (Thro and Spillane, 2000).

Participatory research varies depending on the degree of involvement and collaboration between the farmer and the breeder. These collaboration levels include conventional, where farmers contract land to the research, consultative where the farmer is consulted for input throughout the project, collaborative where both the farmer and breeder jointly make decisions, and collegial where the farmer has the total control over the project (Ashby, 2009 and Zystro et al., 2012). The study in this paper utilized the participatory variety testing (PVT) approach, which is a consultative form of participatory research. While PPB involves the active participation of farmers in the breeding and selection process of genotypes from segregating material, PVT only involves the farmers in the on-farm testing of finished or near-finished varieties from a breeding program. The tested products under PVT may include varieties in advanced testing stages, released cultivars or advanced lines or populations in inbreeding and outcrossing crops, respectively. The three primary phases of PVT include identifying farmers' needs and traits of interest, selecting the suitable varieties with the corresponding traits from the program germplasm pool, and the on-farm experimentation through farmer testing networks (Joshi and Witcombe, 1998). However, the identification and selection of suitable varieties for the PVT on-farm testing rely on the availability of adequate genetic diversity to attain significant genetic progress in the program (Al Bari and Horsley, 2014). The expired Plant Variety Protection (exPVP) maize germplasm is a publicly available source of genetically diverse plant materials for public and private research. The germplasm contains a set of elite inbred parents that have been used in the development of numerous commercial hybrids in the United States since the 1980s (White et al., 2020).

The U.S. Plant Variety Protection Act, established in 1970, grants breeders in public and private institutions exclusive rights and intellectual property protection to produce and commercialize novel plant varieties for 20 years (Alston and Venner, 2002). After the expiration PVP certificate, the PVP materials become publicly accessible through the North Central Regional Plant Introduction Station in Ames, Iowa. Over 460 exPVP inbred maize lines are currently available for public breeders and researchers to use, and 750 additional lines will be added by 2028 (White et al., 2020). Incorporating the exPVP germplasm into organic breeding programs could be a crucial step to explore the potential of these inbred lines in new hybrid combinations with desirable traits (Al Bari and Horsley, 2014). A study by Huffman et al. (2018) to determine under organic conditions the combining ability of maize inbred parents from different breeding programs revealed that hybrids with an exPVP parent performed better than hybrids derived from inbred lines developed by the study cooperators.

Machine Learning Models

Recent progress in machine learning has led to significant developments in data analytics within the agricultural sector, particularly in predicting crop yields. The increased adoption of machine learning models and algorithms is attributed to technological advancement, allowing cost-effective and high throughput phenotyping expanding the availability of large agricultural datasets.

Phenotyping and data collection in agriculture is shifting from the traditional destructive low throughput methods to non-destructive, rapid, high-throughput protocols ranging from the use of ground-based imaging, aerial phenotyping to remote sensing platforms that can capture different developmental biotic and abiotic stresses different crops or

Several HTP platforms exist and are presently employed to phenotype diferent biotic and abiotic stress-associated traits in various crops (Table 1)

During the last decade, there has been rapid adoption of ground and aerial platforms with multiple sensors for phenotyping various biotic and abiotic stresses throughout the developmental stages of the crop plant. High throughput phenotyping (HTP) involves the application of these tools to phenotype the plants and can vary from ground-based imaging to aerial phenotyping to remote sensing.

with destructive, low throughput phenotyping protocols/ methods being substituted by non-invasive high-throughput methods

The expanding availability of agricultural data, encompassing remote sensing, sensor information, and historical crop yield data, has spurred the adoption of machine learning to transform intricate datasets into actionable insights for refining farming practices and boosting crop productivity. Machine learning models have proven effective in pinpointing genetic variations, comprehending the influence of weather conditions, and identifying optimal management strategies in agriculture. Various models, including linear regression, decision trees, and deep learning algorithms, have been employed for crop yield forecasting, with deep learning algorithms exhibiting notable advancements in prediction accuracy owing to improvements in computational capabilities and increased access to extensive datasets. Through the application of advanced machine learning techniques to analyze extensive data from commercial sweet corn fields, coupled with historical weather and genotype information, a more profound understanding of the intricate interactions and variables influencing maize production can be attained. This facilitates data-driven decision-making to optimize crop performance and enhance overall yields.

This study demonstrates that the conventional exPVP germplasm might contain inbred maize lines with undiscovered potential crucial for developing superior hybrids with desirable agronomic traits for organic systems. Our study aimed to use a participatory research approach to characterize the performance of maize hybrids developed by conventional and organic breeding programs under real-world organic management practices. The specific objectives of the study were to (1) characterize the agronomic performance of experimental maize hybrids from organic and conventional breeding programs using a Participatory Testing Network, (2) determine the effect of agronomic management heterogeneity across farms on hybrid performance (3) use machine-learning models to predict the reliability of experimental hybrid performance across different organic fields.

# **MATERIALS AND METHODS**

## **Germplasm Sources**

A core set of cultivars from four different breeding programs with varying breeding objectives was evaluated in on-farm trials. The Mandaamin Institute, an organic breeding program focused on developing maize varieties with enhanced protein quality, soil-nutrient uptake, and efficiency, developed four hybrids (ORG1, ORG2, ORG4, and ORG5). The selection process was conducted under organic conditions to develop the parental inbred lines used in these hybrid crosses. Montgomery Consulting, a conventional breeding program focusing on native insect resistance, native herbicide resistance, seed quality, and grain chemical composition, developed three hybrids (KEV1, KEV2 and KEV3). The parental lines of these hybrids were selected and developed under conventional conditions. Five hybrids (UIUC1, UIUC2, UIUC3, UIUC4, and UIUC7) were derived from ExPVP materials and developed at the University of Illinois. The ExPVP inbred lines were selected based on their high yield response in hybrid combination and their food-grade qualities. A certified organic commercial hybrid (59R5) developed by Great Harvest Organics was used at each farmer trial as a check. Each year, seven experimental hybrids and the check were selected from the core set and tested by all farmers in the variety-testing network. A description of the characteristics of each hybrid is provided in the supplemental Table 13.

## **Strip Trial Locations**

A participatory variety testing approach was used to test different hybrids within a maize variety-testing network (VTN) of farmers in Illinois and Indiana. Throughout the three years, the VTN comprised eight farmers in Illinois and four farmers in Indiana. In 2018, the on-farm strip trials were conducted with six farmers in Illinois and one farmer in Indiana. In 2019, the strip trials were conducted at three farmer locations in Illinois and three farmers in Indiana. In 2020, the hybrids were tested with four farmers in Illinois and three farmers in Indiana. Supplemental Table 14 provides a detailed summary of the location, coordinates, and elevation of the participating farms each year.

## **Experimental Design and Management**

Eight experimental hybrids were planted in each location using a completely randomized design (CRD) with no replications. Each farm location was considered an individual replication for the corresponding year of testing. Experimental hybrids were planted in four-row 30.5 meters long strip plots. The planting density and the row spacing varied from farmer to farmer and ranged from 69,000 plants ha−1 to 87,500 plants ha−1 and 0.76 m to 1 m, respectively. The seed for all the experimental hybrids was produced in our organic nursery at the University of Illinois and was not treated before planting. Although management practices such as weed control and manure sources varied from farmer to farmer, all farmers used methods and inputs that followed the organic standards and regulations established by the USDA's National Organic Program (NOP) under the OFPA Act. All farm management and field history information was collected from each farmer. Throughout the three years of the study, the hybrids were tested under four cover crop type levels (grasses, legumes, mixed and bare fallow), six planting densities (69000, 74000, 79000, 81500, 84000, and 87500 plants per hectare), four crop rotation lengths (2, 3, 4 and 5 years), six organic manure sources (duck, chicken, turkey, cattle, cover crops, and none) and three weed pressure levels (low, moderate, and high). Rotation systems with less three years were classified as intensive, while more complex and diverse rotations above three years were categorized as extensive. Planting density from 69000 to 74000 plants ha−1 were classified as low, 79000 to 81500 as moderate and 84000 to 87500 as high. Supplemental Table 15 provides a detailed summary of the different management practices per farm in each year.

## **Phenotypic Data Collection**

The hybrids were evaluated for grain yield (YLD), plant height (PHT), ear height (EHT), stem diameter (SD), test weight (TWT), and grain quality such as protein (PROT), oil (OIL) and starch content (STR). The trait data were collected during the post-flowering farm visits. PHT and EHT were recorded as the mean of five average plants and measured according to the genome to field standard operating procedure (G2F Initiative, 2018). Plant height was recorded in centimeters and was measured as the distance from the soil line to the ligule of the flag leaf. Similarly, ear height was scored in centimeters and measured from the soil line to the primary ear node or the ear leaf. A digital caliper was used to measure the small and large stem diameters (SDS and SDL) above the second node. At the R6 stage, we visited all participating VTN farms to harvest ears from each strip plot. Two subsamples of ears in an area of 0.0004 ha (1000th of an acre) per subsample were manually harvested. Subsequently the ears were dried and shelled using an Agriculex SCS-2 Single Corn Sheller. Grain yield was calculated in metric tones per hectare at 15.5% grain moisture. Grain quality and chemical composition traits such as protein, starch, and oil content were analyzed using a Inframatic-9500 Near Infrared (NIR) spectrometer in 2018 and 2019 and a Perten DA 7200 in 2020. Each strip sample was divided into two subsamples containing 100 kernels. Each subsample was subjected to the grain analyzer and average values for each measured parameter were obtained from the two sub-sample readings.

## **Reliability Estimation**

Reliability was used calculated as the probability of a given experimental hybrid to outperform the commercial check variety over abroad range of environments. The reliability of each experimental hybrid was estimated using the formula below as described by Eskridge and Mumm (1992).

P (Z > - /)

where Z is a standard normal variable, and is the difference between the yield response of the ith experimental hybrid and the commercial check at a given farmer’s field. Therefore, and is the mean and standard deviation of , respectively, across all environments. The reliability (P) for the ith experimental hybrid was estimated as the probability of the obtained Z-score value using the NORM.S. DIST () function in Excel program.

In addition, a nonparametric approach was used to estimate reliability as the ratio of the number of the environments where the experimental hybrid outperformed the check hybrid to the total number of environments where the hybrid was tested, as described by Eskridge and Mumm (1992). Reliability values were interpreted as a measure of riskiness of a given experimental hybrid where a cultivar with a reliability of near 0.5 is riskier since it outperforms the check in only 50% of the environments while a cultivar with reliability above 0.8 is less risky since it outperforms the check in more than 80% of the environments.

## **Statistical Analysis**

The statistical analysis was conducted using R software version 4.0.5 (R Core Team, 2021) in the RStudio environment (RStudio Team, 2016). A principal component analysis (PCA) was performed using the prcomp () function in R to identify patterns of hybrid performance across the different variables such as management practices and farmer location. The ggbiplot () function in R was used to construct a biplot to visualize such patterns in the dataset and presented in figure 1. The analysis was conducted in two stages. The first stage was a simple model to estimate the genotype and farmer effects on the performance of the experimental hybrids across the different on-farm locations. The second stage analysis was conducted with a complex linear mixed model to explore the effect of the heterogeneity of the farmers management practices on the performance of the hybrid.

### **First stage analysis**

In the first stage of the two-stage analysis, a combined three-year analysis of variance was conducted to estimate the genotype and location effect on hybrid performance. Since hybrids were unreplicated in each location, sub-samples collected in each strip were treated as biological replication to obtain enough degrees of freedom to estimate the error variance. Means for the measured morphological traits, standard errors, minimum and maximum values were calculated. To estimate the main effects and interactions between effects, a linear mixed-effect model below was generated using the lmer () function in the lme4 package in RStudio.

Where:

yijkl = the response for the ith genotype grown by the jth farmer in the kth year

µ = overall mean

= fixed effect of the ith genotype

= fixed effect of the jth farmer

= random effect of the kth year

= fixed interaction terms between the ith genotype and the jth farmer

= sum of random interaction terms of the genotype, farmer, and year.

= random error term

Consequently, an analysis of variance was conducted using the anova () function. Two-way interaction plots between farmers and hybrids were constructed using the interaction.plot () function. A post-hoc test for multiple comparisons of treatment means (year, farmer, and hybrid) was conducted using LSD.test () function using alpha of 0.05 and adjusted p-values were obtained using a Bonferroni correction.

### **Second stage analysis**

To evaluate the impact of management on the performance of the experimental hybrids, a forward stepwise regression was conducted. Using the lmer () function in the lme4 package in RStudio, multiple linear mixed models were generated by using by regressing the hybrid performance on the different management practices. The Akaike's Information Criteria (AIC) was applied to select the best-fit model with the lowest AIC values using the 'AICcmodavg' Package. The final model with the maximum likelihood and therefore used for further statistical analysis for the strip trials with a coefficient of determination of (R2=0.62) is shown below:

Where:

yijkl = response for the ith genotype in Jth year of testing within the kth manure source, lth cover crop type, mth weed pressure rate, nth rotation length and oth planting density.

µ = overall mean

Gi = Fixed effect of the ith genotype (i=1, 2, 3…13)

= Random effect of the jth year of experimentation (i=1, 2,3)

Mk = fixed effect of the kth organic manure source (j=1, 2, 3…7)

Cl = fixed effect of the lth cover crop type (k=1, 2, 3, 4)

Wm = fixed effect of the mth weed pressure rate (l=1, 2, 3)

Rn = fixed effect of the nth rotational length (m=1, 2)

Do = fixed effect of the oth planting density (n=1, 2, 3)

Eijklmno = Random error term

An analysis of variance was conducted using the anova () function in R studio. To check the assumptions of normality and homoscedasticity, the Shapiro-Wilk's test and the Breusch-Pagan Test for Homoscedasticity were conducted. A post-hoc test for multiple comparisons of treatment means was conducted using LSD.test () function using alpha of 0.05 to identify significant differences between trait means.

## **Machine Learning Models**

We applied different machine-learning models to conduct a logistic regression and predict the probability of tested experimental hybrids to outperform the check hybrid across the different tested farms. In this study, check varieties were selected based on the predominantly grown commercial organic hybrids in the regions where farmers in the testing network were located. For this reason, different check varieties were utilized across the testing network. Farms in Illinois and Indiana shared a common commercial check (59R5) from Great Harvest Organics while farms in Wisconsin tested 2 different checks (FOS8507 and FOS8500) from Foundation Organic Seed. In addition, farmers in Illinois and Indiana tested a similar set of experimental hybrids while farmers in Wisconsin tested a different set of experimental organic hybrids, except one (ORG4) that was common across all farms and years. Therefore, to conduct a combined prediction analysis for the experimental hybrid reliability, we used the common hybrid (ORG4) across all fields as the check variety.

# **RESULTS**

**Principal component analysis**

Result of the principal component analysis are presented in a biplot chart showed in **Figure 1**. Hybrid performance was clustered based on breeding programs they were developed and the breeding objectives of the program. The UIUC conventional experimental hybrids and the commercial check were grouped together for their high grain yield, plant height, ear height, test weight and starch content, while the organic hybrids were clustered for their high protein and oil contents. No specific patterns were identified for hybrid performance under farms with management practices.

**Reliability of the experimental hybrids**

Reliability estimates for all experimental hybrids were calculated using the normal distribution method and a non-parametric approach and denoted as and , respectively using 59R5 commercial hybrid as the check variety. Reliability estimates are presented in **Table 1**. Reliability estimates for both non-parametric and normal distribution approach were very similar across all experimental hybrids. The highest reliability was 0.56 while the lowest was 0.03. Three conventional experimental hybrids (UIUC2, UIUC3 and UIUC4) had reliabilities above 0.5. Individual year analysis showed that these three experimental hybrids had reliabilities of 0.64, 0.65 and 0.61, respectively, in 2019 as shown in supplemental Table 12. Two conventional UIUC experimental hybrids (UIUC1 and UIUC7) showed reliabilities below 0.5. However, these estimates were higher than most of the organic experimental hybrids. The reliability estimates for all organic experimental hybrids were lower than 0.5. The organic experimental hybrid (ORG4) had the highest reliability of 0.31. The conventional experimental hybrids from the Montgomery breeding program also had low reliabilities below 0.3.

## **Mean performance of the experimental hybrids in Stage one analysis**

Trait means from the first stage analysis and the analysis of variance results are presented in **Table 2.** The grain yield across all farms and years ranged from 1.6 t ha-1 to 14.8 t ha-1 with a mean of 9.1 t ha-1. Hybrid plant height across all locations ranged from 105 cm to 330 cm with a mean of 232, while ear heigh ranged from 45 cm to 177 cm with a mean of 108 cm. The large stalk diameter ranged from 29 mm to 14 cm while the small stem diameter ranged from 12 mm to 26 mm. The analysis of variance in **Table 1.** showed that the farmers had a significant effect on grain yield and both stalk diameters but no significant effect on plant height and ear height. Results also show a highly significant effect of the genotype across all measured morphological traits. There was also a significant genotype by environmental effect showed due to the observed significant hybrid by farmer interaction. Interaction plots between farms and hybrids are presented in Figures 2 and 3. Trait means, and treatment mean separations for morphological traits across years are presented in **Table 3.** Mean grain yield in 2020 was significantly higher 2019, and 2018. Mean grain yield in 2019 was also significantly higher than 2018. Mean plant height was significantly higher in 2020 while no significant difference was observed in 2019 and 2018. Ear height was significantly higher in 2020 and significantly lower in 2019. No significant differences were observed for mean stalk diameters in 2018 and 2019.

Mean hybrid performance for each farmer was calculated and mean comparisons between farmers were conducted and presented in **Table 4.** Farmers in 2018 were compared separately from farmers in 2019-2020 since two different sets of experimental hybrids were tested in these two growing periods. In 2018, there was a significant difference in the means of all measured morphological trait across farmers. Farmer B obtained the highest mean yield of 11.2 t ha-1 while farmer E had the lowest mean grain yield of 5.5 t ha-1. Farmer B also obtained the highest plant height, ear height, large and small stalk diameters. The combined analysis for farmers in 2019 and 2020 showed that there was a significant difference in the means of the measured morphological traits across all participating farmers. Farmer L obtained the highest mean grain yield of 11.4 t ha-1 while farmer H obtained the lowest yield of 7.8 t ha-1.

The analysis of variance between experimental hybrid means using the first stage model and mean separations for all measured traits are presented in **Table 5**. Results showed significant differences between hybrid trait means across the two growing periods. In 2018, the check variety obtained the highest mean grain yield of 10 t ha-1 ORG2 showed the lowest yield of 6.8 t ha-1. There was a significant difference between the performance of the check and the rest of the experimental hybrids. There was no significant mean yield difference between three organic hybrids (ORG1, ORG2 and ORG5). ORG4 yielded significantly higher than rest of the organic hybrids. There was no significant yield difference between the three Montgomery experimental hybrids. A combined analysis for 2019 and 2020 showed a significant difference between trait means across the experimental hybrids. Conventional UIUC hybrids yielded significantly higher than the organic hybrids except UIUC7. There was no significant yield difference between the check and the UIUC hybrids, while significant yielded differences were observed between the check and all organic hybrids. There was no significant yield difference between the organic hybrids.

## **Phenotypic correlation coefficient**

Estimates of the phenotypic correlation for all measured traits are shown in **Table 6** **and 7.** Since the grain quality traits were measured with different grain analysis equipment in the 3 years (Inframatic-9500 in 2018 and 2019 and Perten DA 7200 NIR in 2020), a combined correlation analysis was conducted for 2018-2019 and a separate analysis for 2020 results. In both analyses, grain yield showed a significant positive phenotypic correlation with plant height (r= 0.55\*\*\* and 51\*\*\*), ear height (r= 0.29\*\* and 0.44\*\*\*), test weight (r= 0.31\*\* and 0.59\*\*) and 300 kernel weight (r= 0.32\*\*\* and 0.46\*\*\*). We also observed a positive correlation between grain yield with small stem diameter (r= 0.39\*\*\*), large stem diameter (r= 0.44\*\*\*) for 2018 and 2019 data. This relationship was not evaluated for 2020 due to the absence of stem diameter data for this particular year of study. Plant height and test weight were the most yield attributing traits, followed by 300 kernel weight, large stem diameter, ear height, small stem diameter, test weight, and moisture content. Although both analyses' correlation trends between morphological traits were similar, we observed different correlations between morphological and grain quality traits as the grain analyzing equipment changed. From the 2018 and 2019 grain quality data obtained from the Inframatic-9500 grain analyzer, grain yield showed a non-significant positive correlation with protein content (r= -0.19), a non-significant negative correlation with oil content (r= -0.18) and almost no correlation with starch content (r= 0.03). However, with the 2020 grain quality data obtained from the Perten DA 7200 NIR grain analyzer, grain yield showed a negative correlation with protein content (r= -0.19), a significant negative correlation with oil content (r= -0.55\*\*\*) and a significant positive correlation with starch content (r = 0.59\*\*\*). In both analyses, starch content showed a significant positive correlation with test weight (r= 0.55\*\*\* and 0.64\*\*\*) and a significant negative correlation with both protein content (r= -0.73\*\*\* and -0.61\*\*\*) and oil content (r= -0.86\*\*\* and -0.79\*\*\*). The starch content from the Inframatic-9500 grain analyzer showed a negative correlation between 300-kernel weight and starch content (r= -0.18). In contrast, a positive correlation was observed when the starch data from the Perten DA 7200 NIR grain analyzer (r = 0.15) was used. Oil content showed a significant negative correlation with kernel weight (-0.34\*\*), test weight (0.74\*\*\*) and plant height (0.32\*) in the 2020 data analysis.

## **Analysis of variance across agronomic management treatment groups**

The combined analysis results showing the mean squares of the morphological and grain quality characteristics for the experimental hybrids are presented in Table 8. The variety effect was highly significant (p<0.01) across all measured traits. The year effect was moderately significant (p<0.05) for grain yield and highly significant (p<0.01) across the rest of the measured morpho-physiological and grain quality traits. Manure source showed a highly significant effect (p<0.01) on all the measured traits but oil content, where no significant effect was observed. Weed pressured showed a highly significant effect (p<0.01) on plant height, ear height, grain yield and test weight, a moderately significant effect (p<0.05) on moisture and no significant effect on the measured grain quality trait. Cover crop type showed a highly significant effect on plant height, ear height, moisture content, kernel weight, a moderately significant effect on grain yield, protein and oil content. However, no significant effect was observed on test weight and starch content. The effect of rotation length was highly significant (p<0.01) on kernel weight and protein content, moderately significant on grain yield, moisture content and starch content, and not significantly on the rest of the traits. Planting density significantly affected plant height, ear height, moisture content, and test weight and was moderately significant on grain yield, protein, and starch content. No significant plant density effect was observed on kernel weight and oil content.

## **Performance characterization of hybrids for using the complex model**

### **Morpho-physiological traits**

Morpho-physiological traits mean values for each hybrid and statistical mean comparisons are presented in Table 9. Similar to the simpler model in the first stage analysis, the analysis with the complex model showed that the grain yield for all conventional experimental hybrids (UIUC1, UIUC2, UIUC3 and UIUC4) was significantly higher than all hybrids bred under an organic breeding program except UIUC7. No significant difference was observed between the grain yield of the four best performing conventional experimental hybrids and the commercial check. Grain yield for the conventional experimental hybrids from Montgomery Consulting (KEV1, KEV2 and KEV3) was not significantly different from the organic hybrids. UIUC3 recorded the highest mean grain yield of 10.5 t ha-1, while ORG2 recorded the lowest mean yield of 6.8 t ha-1. In general, conventional experimental hybrids from the UIUC program and the commercial check recorded the highest plant height compared to hybrids from the other breeding programs. UIUC3, UIUC4 and the check were significantly taller than the rest of the studied hybrids. The highest (114.4 cm) and lowest (94.5 cm) ear heights were recorded by UIUC4 and KEV3, respectively. UIUC2 had the largest mean stem diameter (23.0 mm), while ORG5 had the lowest mean stem diameter value (20.0 mm). The test weight of the experimental hybrids from conventional breeding programs and the commercial check was significantly higher than that of hybrids from the organic breeding program.

### **Grain quality characteristics**

The mean values for the measured grain quality traits and statistical comparisons and separations between hybrids are presented in Table 10. The kernel weight of experimental hybrids from conventional breeding programs and the commercial check was significantly higher than that of all organic hybrids except for ORG1. UIUC4 recorded the highest mean kernel weight of 0.28 g, while both ORG2 and ORG4 had the lowest weight of 0.24. There was no significant difference in kernel weight between the UIUC conventional experimental hybrids and the commercial check. Although organic hybrids obtained lower grain yield, they generally had a higher grain protein content than conventional hybrids and the commercial check. ORG2 recorded the highest mean protein content of 9.9%, while the commercial check had the lowest of 7.5%. Although most hybrids from conventional breeding programs showed low protein content, some hybrids from this set (KEV2, KEV3 and UIUC7) recorded a higher protein content that was relatively comparable to that of the organic hybrids. The Montgomery conventional hybrids and the commercial check recorded the highest starch content values, which were significantly higher than all UIUC conventional hybrids and all organic hybrids except ORG5. Mean starch content values for ORG1 and ORG4 were significantly lower than the rest of the studied hybrids. KEV1 had the highest starch content of 71.8 %, significantly different from the rest of the studied hybrids. In general, conventional hybrids had a higher starch content than organic hybrids. In addition, mean oil content values were significantly higher in organic and Montgomery hybrids than those of the UIUC hybrids and the commercial check. ORG4 recorded the highest oil content of 5.0 %, significantly higher than the rest of the organic hybrids, all conventional hybrids, and the commercial check. The commercial check recorded the lowest oil content of 3.3%.

## **Effect of agronomic management practices on hybrid performance**

We observed a significant genotype by management (G x E) effect where different agronomic practices influenced the experimental hybrids' performance, as shown in Figure **2 and 3**. Experimental hybrids performed differently across farms depending on the management practices conducted by the farmer. For example, the organic hybrid ORG4 was the least performer in 2018 under farmer E but was one of the best three performers under farmer B as shown in Figure 2. Similarly in 2019 and 2020, the check variety was one of the least performer under farmer H, but obtained the highest yield under farmers G, F and L as shown in Figure 3. The effect of results of how different management practices affected the performance of the experimental hybrids are shown in Table 10 and Figure 4. Farms that used grasses as a cover crop in the previous season before the hybrid strip plots were planted yielded significantly higher than farms that used legumes, mixed cover crops and farms bare fallow (no cover crops) as shown in Figure 4B. No significant difference in hybrid performance was observed for farms that used legumes, mixed cover crops and farms with bare fallow. There was a significant difference between grain yield performances under the different planting densities as shown in Figure 4C. The highest grain yield (9.4 t ha-1) was obtained under moderate (79000-81500 plants ha−1) density, which was 5.3% and 10% higher than the yield under low (69000-74000 plants ha−1) and high (84000-87500 plants ha−1) plant density, respectively. In addition, there was a significant difference in the performance of the hybrids across the different rotation systems as presented in Figure 4D. Farms with short-term rotation length (≤3 years) obtained higher yields than farms with long-term rotation systems (> 3 years). The level and efficiency of weed management also significantly influenced hybrid performance as presented in Figure 4E. Farms with low weed pressure yielded significantly higher (10 t ha-1) than farms with moderate (8.4 t ha-1) and high (7.3 t ha-1) weed pressure. Lastly, the source of the manure used in the strip plots significantly influenced the performance of the hybrids as shown in Figure 4F. Farms that used either poultry or livestock manure sources yielded significantly higher than farms than farmers that did not use any manure or used cover crops as the only nutrient source. No significant yield difference was observed between poultry and livestock manure sources.

# **DISCUSSIONS**

Results of the reliability estimates for both non-parametric and normal distribution approach were very similar across all experimental hybrids. Eskridge and Mumm (1992) reported similar positive correlation between the two methods of estimating reliability. The highest reliability was 0.56 while the lowest was 0.03. Three conventional experimental hybrids (UIUC2, UIUC3 and UIUC4) had reliability above 0.5. This means that these experimental hybrids had more than 50% chance of outperforming the check across the tested environments. In 2019, the reliability estimates were for these three hybrids were above 0.6. The reliability estimates for all organic experimental hybrids were lower than 0.5. The conventional experimental hybrids from the Montgomery breeding program also had low reliabilities below 0.3. Reliability is a measure of riskiness of a cultivar relative to the check cultivar. A higher reliability shows a high probability of the experimental cultivar to outperform the check while hybrids with low probability are riskier to grow compared to the check (Eskridge et al., 1993) and (Nuland and Eskridge, n.d.). The results from this study show that experimental hybrids from UIUC are more reliable compared to the organic hybrids and conventional hybrids from Montgomery.

The mean grain yield obtained across all farmers participating in the variety-testing network was 8.96 t ha-1. Using the FINBIN data (a public farm financial database) from 2015 to 2019, Langemeier and O’Donnell (2020) reported that the average national corn grain yield across all certified organic growers during this period was 8.43 t ha-1. Therefore, the grain yield performance across the VTN was 0.5 t ha-1 above the national organic corn yield average. Average plant and ear height across all hybrids was 232 cm and 108 cm, respectively. After evaluating hybrid varieties' performance under organic systems, Lorenzana and Bernardo (2008) also reported similar average plant and ear heights of 250 cm and 103 cm, respectively. The mean protein, starch and oil content across farmers was 8.01%, 69.61% and 3.99%, respectively. According to Zhang et al. (2008), the protein, starch and oil composition in typical maize grains ranges from 8-10%, 70-75%, and 4-5%, respectively, thus agreeing with our results.

## **Phenotypic correlation**

Since two pieces of equipment with different calibrations were used for grain composition analysis (Inframatic-9500 for 2018 and 2019 strip trial samples and Perten DA 7200 NIR for 2020 samples), a combined correlation analysis was conducted for 2018-2019 and a separate analysis for 2020 phenotypic data. The results obtained from both analyses revealed that grain yield had a significant positive correlation with plant height, ear height, test weight, kernel weight and stem diameter. Ogunniyan and Olakojo (2014) reported similar results of a significant positive phenotypic correlation of grain yield with plant height (r= 0.55) and ear height (r= 0.45). Bocanski et al. (2009) and Bartaula et al. (2019) also found a significant correlation of plant height, ear height and test weight with grain yield. The significant correlation of these traits agronomic traits with grain yield implies that the simultaneous selection for these traits could potentially result in the indirect selection for higher grain yield of the experimental hybrids (Bartaula et al., 2019).

Correlations between morphological and grain composition traits changed with the grain analyzer equipment used in the grain analysis. In 2018 and 2019 (Inframatic-9500), grain yield showed a non-significant correlation with protein, oil, and starch. Conversely, in 2020 (Perten DA 7200 NIR), grain yield showed a negative correlation with protein content, a significant negative correlation with oil content and a significant positive correlation with starch content. Mahesh et al. (2013) reported a significant negative phenotypic correlation between grain yield and oil content (r= -0.23), grain yield and protein content (r = -0.15) and a significant positive correlation between yield and starch (r= 57), thus agreeing with our 2020 correlation results. Starch content was significantly correlated to test weight but inversely correlated to protein and oil content. Similar relationships between starch with oil and protein content were reported by Guo et al. (2013) and Mahesh et al. (2013). Oil content also showed a significant positive correlation with protein in 2018-2019 and a non-significant positive correlation in 2020. Zhang et al. (2008) reported a similar significant positive relationship between oil and grain protein (r=0.49).

The analysis with the Inframatic-9500 data showed a negative correlation between kernel weight and starch content, while the data from Perten DA 7200 NIR showed a positive correlation between the two traits. Ryu (2010) reported a positive correlation between starch and kernel weight (r= 0.33\*) and a negative correlation between protein and kernel weight (r = -0.48\*\*). A positive correlation between starch and kernel weight was expected since the kernel endosperm primarily comprises starch, which accounts for over 65 – 75% of the dry kernel weight (Ryu, 2010 and Liu et al., 2016). Therefore, heavier kernels contain more starch than lighter ones. In addition, a negative correlation was observed between oil content and kernel weight and between oil content and grain yield. Similar results were reported by Dudley et al. (1974) from the Illinois long-term selection experiment where the selection of high oil for nine cycles showed a negative linear relationship with kernel weight and yield. The correlation trends from Perten DA 7200 NIR data were more comparable to the expected correlation values of the studied grain composition traits than the Inframatic-9500. This might be attributable to the difference in calibration between the two machines used for grain quality analysis across years.

## **Analysis of variance across management treatment groups**

The variety mean squares were highly significant across all measured traits. This is attributed to the presence of genetic variability for the studied traits in the germplasm used for the study. The significant phenotypic variability across the experimental hybrids paves the way for trait improvement through simple selection (Bello et al., 2012). The observed significant genetic variability is attributed to the different sources from which the experimental hybrids were obtained and the breeding programs' objectives. Four hybrids were acquired from the Mandaamin institute (organic), three hybrids from Montgomery Consulting (conventional), five hybrids from the University of Illinois (conventional) and one commercial check developed by Great Harvest Organics (certified organic). The Montgomery hybrids were developed under conventional management systems with the breeding objective of enhancing grain nutrients, increasing disease and native insect resilience, increasing drought tolerance and nutrient-use efficiency. The Mandaamin hybrids were derived from South American native varieties and developed under organic management with the breeding objective of enhancing protein quality and nutrient-use efficiency (Goldstein, 2021). The experimental hybrids from the University of Illinois were derived from the inbred lines that are part of the exPVP germplasm. The principal component analysis emphasizes the variation in the response of the tested hybrids and shows evidence of performance patterns based on these breeding programs.

The study results showed that the UIUC experimental hybrids were significantly taller with higher grain yield, heavier kernels with higher test weight and starch content than the organic hybrids. The grain yield of four of the five UIUC experimental hybrids (UIUC1, UIUC2, UIUC3 and UIUC4) was significantly higher than the other breeding programs. No significant difference was observed between the check and the UIUC hybrids for grain yield, ear height, stem diameter, test weight, and protein content. The check hybrid had a higher starch content than all UIUC hybrids. Our results agree with Huffman et al. (2018), who compared the performance of hybrids developed through crossing inbred lines from different cooperators, including exPVP and commercial lines. This study showed that hybrids with either exPVP or commercial line as a parent yielded significantly higher than hybrids developed exclusively with cooperators’ inbreds. Lorenzana and Bernardo (2008) also reported similar results after evaluating the performance of test-crosses of exPVP derived recombinant under conventional and organic production systems. The results showed that some test-crosses had high yields under organic and conventional systems, indicating that high-performing hybrids can be identified and developed through screening past conventional germplasm under organic management conditions. In contrast, organic hybrids were characterized by their higher protein and oil content, lower test weight, lighter kernels with low starch content. These results were expected since these hybrids were acquired from a breeding program that selects for higher protein content and nitrogen-use efficiency (Goldstein, 2021). Since oil content is positively correlated with grain protein and negatively correlated with starch as previously reported by Zhang et al. (2008), Guo et al. (2013) and Mahesh et al. (2013), the selection for high protein in the organic hybrids indirectly increased the oil content and reduced the grain starch. The Illinois long-term selection experiment results also show a trade-off between oil content and grain yield. The selection for high oil content indirectly reduced the studied population's grain yield (Dudley et al. 1974). Therefore, the low yields in the organic hybrids can be an indirect result of selecting for high protein and indirect selection for high oil.

We also observed a significant year effect on all the measured morpho-physiological and grain quality traits. The significant year effect on all studied traits indicates the influence of the environment on the expression of these traits since the environmental conditions, particularly the weather, varied from year to year. In 2018, most farmers experienced prolonged heavy rains during the mid-summer (Kerschner, 2018), which impeded the timely weeding of the strip plots. In addition, participating farmers in the testing network reported prolonged heavy rainfalls during the 2019 late spring season that caused late planting (Good, 2019 and Ford, 2020) and a prolonged dry summer that caused drought stress in most fields (Grant, 2019). Even though participating farmers in 2020 experienced more conducive growing conditions throughout the season than the previous two years, some farmers were affected by the destructive derecho that swept across the Midwestern Corn Belt in early August. However, none of the participating farmers in the variety-testing network reported major damages from the derecho. The above-mentioned weather hindrances explain the significant differences in the mean grain yield performance between years as presented in Figure 4A. The highest mean grain yield of 10 t ha-1 was obtained in 2020, which was 10 and 20 percent higher than the mean yield obtained in 2019 and 2018, respectively. The study results also agree with Schnitkey et al. (2020), who reported that the 2020 corn yields across the Midwest states were above state yield trends estimated using 40 years of historical yield data. Our results also agree with the 2020 USDA Crop Report that showed that the 2020 national maize yields were 4 percent higher than the 2019 production level (Barrett, 2021). However, the 2018 national average maize yields were 5 percent higher than the 2019 yields (USDA, 2020a) and the 2019 state yields were 14 percent lower than 2018 state yields (USDA, 2020b). These results contradict with the results obtained in this study since we obtained a 10 percent yield increase from 2018 to 2019. This difference can also be attributed to the difference in the hybrids used in the study in these 2 years. In 2018, we only tested the organic hybrids from the Mandaamin institute and the conventionally developed hybrids from Montgomery consulting, while additional hybrids from the UIUC breeding program were added to the testing set in 2019 and 2020 and the Montgomery hybrids were excluded in the last two years of testing. Based on the results presented in Table 10, UIUC hybrids were better performing compared to the Montgomery set tested in 2018.

## **Effect of farmers management practices on hybrid performance**

The analysis of variance showed that farmers’ management practices as manure sources, cover crop types, weed pressure management, rotation length, and planting density significantly influenced the experimental hybrids' response, as shown in Table 11 and Figure 4. The effect of each management practice on hybrid performance is elaborated in detail below.

### **Organic nitrogen sources**

The manure used by the farmers as a nitrogen source showed a significant effect on all the measured traits except for oil content, where no significant effect was observed. Results presented in Figure 4F show a significant difference between farmers that used supplemental manure sources and farmers that did not apply manure or only relied on cover crops as a source of organic nitrogen. No significant difference in hybrid performance was observed when poultry (chicken, duck or turkey liter) or cattle manure was used as a source of organic nitrogen. Khaliq et al. (2004) reported similar results where the mean grain yield of two maize hybrids evaluated under poultry and cattle manure did not show a statistically significant difference, while similar hybrids under the control treatment without organic manure application obtained the lowest grain yield and harvest index. Ogidi and Payebo (2021) also evaluated the effect of cattle and chicken manure on kernel yield and reported a significant difference in yield response. Although chicken manure obtained a higher grain yield than cattle manure, both treatments yielded significantly higher than the control treatment with no manure application. These results are similar to our findings where farmers who did not apply manure or only used cover crops yielded significantly lower than farmers who used cattle or poultry manure. This study provides evidence that the organic manure application and cover crops enhanced the growth and performance of the experimental maize hybrid. Manure application also influenced grain chemical composition and quality, as presented in Table 11. Farmers who applied either cattle or poultry manure obtained significantly higher grain protein content than farmers who exclusively used cover crops or used bare fallow with no manure application. Protein content for farmers using cover crops was not significantly different from farmers with no fertility source. Kramberger et al. (2009) reported similar results where maize grain nitrogen content was not significantly different under cover crops and control treatment (bare fallow). However, the crop response to the different cover crop types (legumes, grasses or mixed) may vary, as explained in the cover crop section below.

### **Cover crop types**

In this study, cover crop type significantly affected plant height, ear height, moisture content, kernel weight, grain yield, protein and oil content. Results showed that farmers who used grass cover crops prior to the strip plots obtained a higher yield than legumes, mixed cover crops and bare fallow, as presented in Figure 4B. These results contradict our expectations and disagree with previous findings reported from similar studies. Marcillo (2018) conducted a meta-analysis of 65 studies that evaluated the effect of winter crop cover on maize yield in the Unites States and Canada from 1965 to 2015. The results showed that grass cover crop species neither decreased nor increased maize yields, while legume cover crops increased by over 30 percent. Caporali et al. (2004) reported that maize grain yield following legume (hairy vetch and clover) cover crops was 22 and 11 percent higher than yield following grass (rye grass) cover crops and bare fallow control, respectively. Piva et al. (2021) also conducted a similar experiment in Brazil to compare the effect of legume (vetch) and grass (rye grass and white oats) cover crop species on maize grain yield. Their results show that the legume cover crop increased the stem diameter, grain yield and yield components (number of grains per row and ear length) compared to the grass cover crop species. In addition, there was no significant yield difference between the grass cover crops and the bare fallow control treatment Piva et al. (2021). Dube et al. (2013) reported that the maize grain yield and grain protein content was significantly higher under a legume cover crop (grazing vetch) than both grass (oats) and the weedy bare fallow treatments.

The results observed in this study are attributed to the additional management practices conducted in the fields with grass cover crops. In addition to the low weed pressure, the use of optimum planting density and the addition of poultry manure as a nitrogen source, the farmer applied fish bone, formulated molasses, micronized soft rock phosphate and sodium borate in the strip plots, which added additional soil nutrients like calcium, phosphorus and boron. Fish bones contain 60-70% of minerals, mainly phosphorus and calcium (Ahuja et al., 2020), which are essential for plant growth. Micronized soft rocks phosphate and sodium borate are organically approved soluble fertilizers applied as foliar solutions to supplement phosphorus and boron content in the soil (Greer and Adam, 2005). Adequate soil phosphorus availability improves seed germination, root growth and development and enables the translocation of other minerals into the plant, hence improving yield (Hajabbasi and Schumacher, 1994). Barry and Miller (1989) evaluated the effect of phosphorus nutrition during the maize seedling state on the final grain yield and reported treatments with higher phosphorus concentration resulted in greater kernel number, increasing grain yield compared to treatments with lower P concentration. Therefore, a combination of management practices like weed management, use of recommended planting density, and application of organic nitrogen and supplementation of additional nutrients like phosphorus and boron might explain the high grain yield obtained from plots with grass cover crops compared to plots with either legume or mixed cover crops. However, since only one farmer in the network used these soluble foliar supplements, a detailed study with more farmers is necessary to provide more statistical evidence to support this observation.

### **Weed pressure management**

Weed pressure significantly affected plant height, ear height, grain yield, test weight, and moisture but had no significant effect on the grain chemical composition components. As expected, the grain yield reduced as the rate of weed competition increased. The highest grain yield (10 t ha-1) was obtained at low weed pressure, 16% and 27% higher than moderate and high weed pressure, respectively, as shown in Figure 4E. Plant height and ear height followed a similar trend where the tallest plants were observed under low weed pressure and the shortest under high weed pressure, as presented in Table 11. Similar results were reported by Adeniyan et al. (2015) and Fuksa et al. (2004), where plant height and grain yield were highest under weed-controlled treatments and lowest under weedy plots. Landau et al. (2021) used machine-learning techniques to examine the effect of weeds and weed control of maize yield loss utilizing a database of 205 multi-location trials conducted at the University of Illinois for 27 years since 1992. They reported similar results showing that inadequate weed control was the primary driver for an average yield loss of over 50 percent. High weed pressure is the major agronomic challenge in organic agriculture since it reduces the crop yield potential through imposing a competition for nutrients, light and water and can lead to 100 percent crop loss if uncontrolled (Teasdale and Cavigelli, 2010, McErlich and Boydston, 2013, and Chauhan, 2020).

### **Planting density**

Planting density significantly affected plant height, ear height, moisture content, test weight, grain yield, protein and starch content. Moderate plant density (79000-81500 plants ha−1) was the optimum density that obtained the highest grain yield of 9.4 t ha-1, which was 5.3 % and 10% higher than low (69000-74000 plants ha−1) and high (84000-87500 plants ha−1) plant densities, respectively, as presented in Figure 4C. In addition, average kernel weight, protein, and oil content were higher at low and moderate densities than high densities as shown in Table 11. Coulter et al. (2010) reported that even though the general plant population for organic maize growers in the Midwest is between 69000 to 79000 plants ha−1, organic producers might increase grain yield by planting at higher rates above 79000 plants ha−1. This is evident from this study results that farmers that planted at rates higher than the recommended rates obtained higher yields. However, an optimum rate reached (79000-81500 plants ha−1), and grain yield started to diminish as farmers excessively deviated beyond this level. Yan et al. (2017), Yu et al. (2019), and Zhang et al. (2021) conducted similar studies to evaluate the effect of increasing planting density on maize grain yield. All studies reported similar results showing that grain yield increases with increasing plant density until an optimum rate is reached. Zhang et al. (2021) reported that although increasing the planting density significantly increased the leaf area index and the intercepted photosynthetically active radiation, it reduced the plant photosynthetic capacity, stomatal conductance and leaf chlorophyll content hence diminishing crop productivity and yield stability due to reduced resource use-efficiency. Yan et al. (2017) also reported that high plant density reduced the post-Silking nitrogen accumulation, leaf nitrogen concentration and net photosynthesis, which resulted in low grain yield and nitrogen use efficiency. Since the nitrogen content is an indicator of the protein content (Duvnjak et al., 2021), the reduction in nitrogen accumulation under high plant density reported by Yan et al. (2017) explains the low protein content we obtained at high densities compared to the protein content at lower densities as presented in Table 11. The results from our study and previously reported studies indicate that high plant densities cause crowding stress, reducing the plants’ nutrient use efficiency, and resulting in low crop productivity.

### **Rotation length**

Rotation length significantly affected kernel weight and protein content, grain yield, moisture content, and starch content. The study results presented in Figure 4D showed that grain yield was 11 percent higher under intensive (short-term) rotation systems than under extensive (long-term) systems. These results contradict previous studies by Stanger and Lauer (2008), Bowles et al. (2020) and Wagner et al. (2021). Previous studies suggest that extended and more diverse crop rotation systems improved soil health and nitrogen availability and reduced pest, disease, and weed pressure, enhancing crop productivity in the long run (Bowles et al., 2020). Wagner et al. (2021) studied the effect of crop rotation complexity from continuous cropping to 2-year rotations and 4-year rotations on maize grain yield. Results showed that grain yields increased by 29% and 48% under 2-year and 4-year rotations, respectively, compared to continuous mono-cropping systems. In addition, increasing crop rotation complexity and length significantly increased maize grain yields, soil organic carbon, soil nitrogen, soil organic matter, and improved water retention, resulting in stable yields and more resilience to adverse conditions (Wagner et al., 2021). Stanger and Lauer (2008) also studied the effect of six different crop rotation sequences varying from continuous cropping (corn on corn) to 5-years rotations on corn yield response. Results showed an increasing yield trend as the crop rotation complexity increased from continuous corn system to 5-year rotations system. In our study, the lower yields obtained under complex rotation systems might be attributed to the influence of other management practices like weed management and manure sources. Only 33 percent of the farmers that used complex extended rotation systems had low weed pressure in their fields, while 67 percent had moderate and high weed pressure. In addition, 50 percent of these farmers also used either cover crop exclusively as their fertility source or did not use any other complementary sources before planting the strip trial plots. As discussed earlier, organic manure application in addition to cover crops enhanced performance of the hybrids as compared to systems with no addition fertility sources. Wagner et al. (2021) also reported that diverse rotation systems with fertilizer supplements obtained higher corn yields than when no fertilizer was applied to these systems. However, our results show that grain protein and starch were 4.0% and 2.3% higher under complex cropping systems than intensive systems as presented in Table 11. The high protein content implies that these systems had more nitrogen availability than intensive systems. Therefore, the lower yields obtained under complex systems was mostly due to poor weed management and not due to insufficient of soil nitrogen. Poor weed management is the major driver of crop yield loss due to the competition for nutrients, light and water (Teasdale and Cavigelli, 2010, McErlich and Boydston, 2013, and Chauhan, 2020).

# **CONCLUSIONS**

The study demonstrated that the Illinois Elite Maize Association Mapping Panel developed at the University of Illinois, derived from the ExPVP germplasm, is useful to start a breeding program focusing on developing hybrid varieties for organic farming systems in the U.S Corn Belt. The experimental hybrids developed from this germplasm exhibited significantly higher grain yields than the organically bred varieties across the different farm locations and organic management practices. Reliability estimations showed that the UIUC experimental hybrids had more than a 50% probability to outperform the check since their performance was comparable and sometimes higher than the check variety. However, the performance of the experimental hybrids was greatly influenced by the breeding programs that developed them and their corresponding breeding objectives. Organic hybrids were selected for higher N-use efficiency and protein content for animal feed, while conventional hybrids were selected for higher grain yield and starch content. These objectives were reflected in the results of this study since organic hybrids obtained lower yields, higher protein and oil content. The conventional hybrids were characterized for their higher yields and starch content. In addition, results showed that the experimental hybrids lacked adequate tolerance to high weed pressure and performance under low nitrogen levels, as revealed by the lower yields obtained from farms with high weed rates and farms with no organic manure applications. Therefore, the identification and integration of new sources of genetic diversity for these traits would be beneficial for better adaptation to organic systems.

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