Crop Science

ORIGINAL ARTICLE

Crop Breeding & Genetics

Canopy coverage phenotyping and field spatial variability adjustment as an efficient selection tool in soybean breeding

Diana M. Escamilla[1] | **Meng Huang**[2] | **Leah McHale**[3] | **Dechun Wang**[4] | **Brian Diers**[5] | **Alencar Xavier**[1,6] | **Katy Martin Rainey**[1]

[1]Department of Agronomy, Purdue University, West Lafayette, Indiana, USA

[2]College of Pharmacy, The University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas, USA

[3]Department of Horticulture and Crop Science, The Ohio State University, Columbus, Ohio, USA

[4]Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, Michigan, USA

[5]Department of Crop Sciences, University of Illinois, Urbana, Illinois, USA

[6]Department of Biostatistics, Corteva Agriscience, Johnston, Iowa, USA

Correspondence

Katy Martin Rainey, Department of Agronomy, Purdue University, 915 West State Street, West Lafayette, IN 47907, USA. Email: krainey@purdue.edu

Assigned to Associate Editor Vincent Pantalone.

Funding information

North Central Soybean Research Program

Abstract

Accurate estimation of grain yield potential in soybean [*Glycine max* (L.) Merr.] progeny rows (PRs) by measuring yield itself is challenging due to the small number of seeds available. To obtain more precise estimates of soybean yield potential and control nongenetic sources of variability, soybean breeders in the United States use secondary traits, visual selection (VIS), adjustments for field spatial variation, pedigree (PED) information, and unmanned aerial systems–based plant phenotypes; however, there are limited comparisons among the different PR testing procedures. We conducted a selection experiment in 2018 PR populations developed for yield and diversity from four soybean breeding programs. Then, we compared the performance of the lines selected using 13 selection categories in 2019 preliminary yield trials (PYTs). The sources of information used across categories included spatially adjusted (SP) traits, PED information, the canopy of the plant measured by aerial and ground digital images, reproductive length (RL), and grain yield (YLD). SP trait covariates and canopy data were the information sources most highly associated with lower yield ranks and higher yield performance of PYT. The most effective secondary trait was average canopy coverage (ACC) measured by high-throughput phenotyping (HTP) platforms. Our selection experiment shows that ACC used as a secondary trait in combination with SP trait covariates effectively selects high-yielding lines from non-replicated experiments. Based on the scenarios considered in this study, it may be possible to increase the gain from selection by phenotyping secondary traits using HTP and implementing spatial variation adjustments in PR trials, which could help enhance crop productivity.

Abbreviations: ACC, average canopy coverage; BLUP, best linear unbiased predictor; CAM, camera; CC, canopy coverage; HTP, high-throughput phenotyping; LM, linear model; MG, maturity group; MLM, mixed linear model; PED, pedigree; PR, progeny row; PYT, preliminary yield trial; RGB, red–green–blue; RL, reproductive length; SP, spatially adjusted; UAS, unmanned aerial system; VIS, visual selection; Yield, adjust yield means; YLD, grain yield.

1 | INTRODUCTION

Increasing yield is a continuous objective in soybean [*Glycine max* (L.) Merr.] breeding programs to ensure food security for the ever-growing world populations (Razzaq et al., 2021; Xiong et al., 2022). In the face of climate change, improving crop productivity and breeding efficiency is a difficult task (Xiong et al., 2022). Breeders measure the progress and efficiency of their programs through the increase in performance achieved annually, known as genetic gain, which can be defined as the increment in average genetic value of a population over cycles of selection (Rutkoski, 2019; Xu et al., 2017). In the context of phenotypic selection, the expected genetic gain from a single selection cycle, also known as the breeder's equation, is described as:

$$\Delta G = ih\sigma_A/t \qquad (1)$$

where ΔG is the change in yield from the parental generation to the progeny of selected parents in a single breeding cycle, h is the square root of the narrow-sense heritability, i is the intensity of selection, σ_A is the additive genetic standard deviation, and t is the breeding cycle time (Falconer et al., 1996; Meuwissen, 2001; Rutkoski, 2019; Xu et al., 2017). In soybeans, higher genetic gains are needed to meet the growing demand of seeds for food, feed, oil, and a variety of industrial applications (Xu et al., 2017), with a projected increase in soybean consumption of 17% by 2029 (OECD and Food and Agriculture Organization of the United Nations, 2020).

A crop's yield potential is the maximum yield obtained under ideal conditions without stress. In soybeans, yield potential ranges from 7,500 to 11,000 kg/ha (Van Roekel et al., 2015). While soybean yields in the United States for 2021 averaged 3,600 kg/ha, ranging from 2,000 to 4,000 kg/ha in different states (Soy Stats, 2022), which reveals an expansive room for improvement in yield. Soybean's current genetic gain for yield across maturity groups (MGs) of 29 kg/ha per year (Rincker et al., 2014) is lower than the genetic gain (77 kg/ha per year) observed in maize (*Zea mays* L.; Duvick et al., 2003). The slower increases in soybean yield through breeding could result from constraints in genetic diversity, heritability estimations, selection intensity, and breeding cycle time (Xu et al., 2017). Based on the breeder's equation, breeders could improve the rate of soybean genetic gains through several strategies—first, the reduction of the breeding cycle time through rapid generation advances, marker-assisted selection, optimized breeding schemes, and genomic selection (Cobb et al., 2019; Sinha et al., 2021; Xu et al., 2017); second, unlocking and creation of new genetic variation through mutation, gene mapping and discovery, and transgenes and genome editing, combined with appropriate diversity management (Michel et al., 2022; D. Müller et al., 2018; Sinha et al., 2021; Xu et al., 2017);

Core Ideas

- Average canopy coverage and spatial field adjustments effectively selected high-yielding lines from progeny rows.
- Reproductive length, visual selection, and pedigree were the least informative sources for selection.
- Selection for high-yielding lines was more efficient when canopy coverage was measured via aerial imagery.

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third, accurate trait heritability estimation through more precise phenotyping, increased number of replications and tested environments, characterization and measurement of environmental factors, the use of standardized agronomic practices and appropriate statistical methods (Sinha et al., 2021; Xu et al., 2017); finally, increasing selection intensity through higher population sizes and the use of new technologies to reduce the cost of testing (Sinha et al., 2021).

Integrating multiple disciplines and using the latest technologies for genotyping, envirotyping, and phenotyping can increase genetic gains and crop productivity to accelerate the development of climate-resilient crops (Xiong et al., 2022). Next-generation sequencing technologies enable us to obtain information on the genetic makeup of plant germplasms at a low cost that, in addition to genome editing, could play a crucial role in discovering and creating new genetic variations (He et al., 2014; Varshney et al., 2014; Xu et al., 2017). At the same time, high-throughput phenotyping (HTP) platforms allow the measurement of many morphological and physiological traits over time across large populations (He et al., 2014; Houle et al., 2010; Xu et al., 2017). These high-throughput measurements are more repeatable and precise than manual measurements, thus improving heritability estimations (Andrade-Sanchez et al., 2014; Duddu et al., 2019; Lane & Murray, 2021). In addition, the systematic characterization and measurement of environmental factors, known as envirotyping, could help control environmental errors and improve the precision of phenotypic measurements (Xu, 2016; Xu et al., 2017). Thus, phenotyping, genotyping, and envirotyping strategies could help enhance genetic gain in combination with modern breeding pipelines and acceptable agronomic practices.

In soybeans, a typical breeding program starts with segregating populations, and once they are near homozygosity (F_4–F_6), individual plants are selected to grow out progeny rows (PRs; Orf et al., 2016). Due to the limited seed produced by individual plants, PR trials are commonly unreplicated single rows grown in small plots evaluated for yield, visually assessed, and scored for other traits (Orf et al., 2016; Sun

et al., 2015). PR trials are the first selection stage in breeding populations and observed yield values are initial indicators of yield potential for the F_{4-}, F_{5-}, or F_6-derived lines (Sun, 2014). Rather than estimating yield precisely in unreplicated PR trials, the objective is to select soybean lines with the highest yield potential (Martin et al., 2006). The new lines can be ranked using several methods, including raw yield values, the best linear unbiased estimation (BLUE) of genotypes calculated in a mixed model by defining genotypes as fixed effects, and the best linear unbiased prediction (BLUP) of genotypes calculated by defining genotypes as random effects (Martin et al., 2006; Molenaar et al., 2018; Piepho et al., 2008). BLUP estimation based on the mixed linear model (MLM) methodology has been used in plant breeding to estimate the random genotype effects in single unreplicated and multiple environment trials (Federer, 1998; Piepho et al., 2008; Santos et al., 2002). If using marker- or pedigree (PED)-based MLM, breeding values can be estimated (Piepho et al., 2008). The single-trait BLUP is the standard method for selecting lines with the highest genetic merit in soybean breeding programs (Martin et al., 2006; Piepho et al., 2008; Volpato et al., 2019). Usually, 8%–10% of the lines in the PR stage advance for later evaluations in replicated yield trials. Higher genetic merit is associated with a higher ability to produce superior progeny (Piepho et al., 2008). Therefore, accurate estimation of phenotypes, genotypic values, or breeding values to select candidate lines is crucial to the success of breeding programs. However, limitations at the PR stage led to inaccurate measurements of yield and, therefore, inaccurate BLUP estimations (Moreira et al., 2019), which constrain the rate of genetic gain for soybean yield.

Genomic selection approaches have been successfully applied in different scenarios and are becoming a routinely applied tool to assist in the selection of complex traits (Gapare et al., 2018; Perez-de-Castro et al., 2012; Velazco et al., 2019; Zhang et al., 2016; Zhu et al., 2021); thus, it can potentially replace the PR yield testing. It is important to note that genomic selection has also its limitation in the cost of genotyping, especially when thousands of new selection candidates are generated each generation in breeding programs (Zhu et al., 2021). In addition, PR testing is still a valuable tool for screening and validation.

Indirect selection for yield through secondary traits that can be measured more precisely is a common practice among breeders to improve PR selection effectiveness. HTP platforms such as unmanned aerial systems (UASs) enable the measurement of traits like average canopy coverage (ACC), hyperspectral canopy reflectance, and normalized difference vegetation index (NDVI), used to characterize biomass accumulation dynamics. These traits can improve data quality for yield selection, especially in unreplicated trials where yield estimations are challenging (Montesinos-López et al., 2017; Moreira et al., 2019; van Eeuwijk et al., 2019; Xavier

et al., 2017). For example, ACC measured in the early soybean season showed high heritability ($h^2 = 0.7$) and a strong genetic correlation with yield (0.87), enabling indirect selection (Xavier et al., 2017). In addition, another study reported that yield prediction models incorporating ACC at the PR stage successfully selected high-yielding soybean lines (Moreira et al., 2019). Furthermore, growth period traits such as maturity (R8) and reproductive length (RL) also have a positive correlation with yield (Xavier et al., 2017). The higher yield observed in later maturity cultivars is a confounding factor when measuring yield potential (Moreira et al., 2019), and R8 is regularly used as a covariate to avoid erroneous estimates. In the case of RL, there is limited information on its use for indirect selection in soybean breeding pipelines.

Nongenetic effects, such as field spatial variation caused by soil heterogeneity, management practices, pest pressure variation, and experimental error, are the principal limitations of early generation selection in self-pollinated crops (Bernardo, 2003; Sun et al., 2015). Field spatial variation can be measured as the trait correlation among plots, which can affect the yield ranks and impact the accuracy of selection (Gilmour et al., 1997; Sun et al., 2015); however, appropriate spatial adjustment of the phenotypic data can help to remove these field spatial variation trends and increase selection accuracy (Lado et al., 2013). Strategies to eliminate field spatial variation from phenotypic data include modeling variance–covariance matrixes, row–columns design, and moving means (Cullis et al., 1998; Lado et al., 2013; Leiser et al., 2012; B. U. Müller et al., 2010; Peiris et al., 2008). Yield prediction models accounting for spatial field variation decreased the error variance and increased the accuracy of yield selection in early generations (Sun et al., 2015). At the same time, yield prediction models that use PED information exploit genetic correlation among relatives and allow better differentiation of genetic and environmental variance leading to more accurate breeding value estimates (Piepho et al., 2008). Thus, modeling yield performance based on spatially adjusted (SP) phenotypic data and PED in combination with good experimental designs could improve the accuracy of yield prediction models and trait heritability, increasing genetic gains.

The selection of plant rows at the PR stage is crucial in soybean breeding programs. Through the years, soybean breeders have identified nongenetic sources of variability and imprecise phenotypes as constraints in the estimation accuracy of the genetic values or breeding values used in PR selections (Bernardo, 2003; Sun et al., 2015). To obtain more precise phenotypes and control nongenetic sources of variability at the PR stage, soybean breeders use yield-related traits for indirect selection, visual selection (VIS), SP trait covariates , PED information, and UAS-based plant phenotypes (Lado et al., 2013; Montesinos-López et al., 2017; Moreira et al., 2019; Xavier et al., 2017). These new UAS-based plant phenotypes are used as secondary traits or covariates to improve

yield estimations (Moreira et al., 2019; Xavier et al., 2017). A selection experiment compared direct selection for yield with indirect selection using ACC and ACC as a covariate in the PR stage of soybean breeding and identified that ACC used as a covariate was an effective method for selecting high-yielding lines (Moreira et al., 2019). However, there are limited comparisons among the different PR testing procedures used by public breeding programs in the north-central region of the United States and their efficacy when used in soybean breeding pipelines. Selection experiments help compare breeding methods by measuring their realized gain. Thus, we aimed to conduct a selection experiment in populations developed for yield and diversity that compares the yield performance of lines selected using 13 selection categories that combine different sources of information. The sources of information include VIS, SP traits, PED information, grain yield (YLD) itself, and secondary traits such as canopy coverage (CC) and RL.

2 | MATERIALS AND METHODS

2.1 | Description of breeding populations

This research was a collaborative effort among public soybean breeding programs in the north-central region of the United States (Table 1). For the selection experiment, we used 2018 PR populations from MGs II to IV developed within the soybean breeding program pipelines of the University of Illinois (IL), Purdue University (IN), Michigan State University (MI), and The Ohio State University (OH). In this study, the PR populations correspond to typical public soybean breeding germplasm, which is genetically more diverse and encompasses more diverse breeding objectives than commercial breeding. Each breeding program was responsible for the general management and experimental design of a typical PR test. In 2018, breeders grew their PR populations under modified augmented designs with replicated maturity checks, including IA1022 (MG 1.8), IA2102 (MG 2.5), IA3048 (MG 3.5), LD06-7620 (MG 4), and MN1410 (MG 1.5). The breeder at the University of Illinois split its PR populations into two blocks, and we referred to them as Illinois set A (ILA) and Illinois set B (ILB). PR population's size ranged from 677 to 4,933 plots of unique experimental lines, grown in single-row plots that were between 1.2- and 3.2-m long and had 0.76-m row spacing. A total of 183 parents were used across breeding programs to develop the PR populations, with some parents overlapping between breeding programs (Figure S1).

Breeders advanced selected lines at the PR stage to the 2019 preliminary yield trials (PYTs). PYTs were organized by maturity with lines randomly assigned to environments, standard checks, and an experimental design to adjust for field variation. The experimental units correspond to two row plots, 3.2 m-long with 0.76 m of row spacing and no borders. Due to field limitations, field variation, and logistics within each breeding program, breeders used different experimental designs for their populations. For example, the breeder at Purdue University evaluated their 1,143 advanced lines in 174 incomplete blocks at two locations, each block of nine lines with a minimum of one and a maximum of three checks per incomplete block. For PYT at OH, the breeder grouped their 884 advanced lines based on maturity. From these lines, 721 lines were evaluated at two locations with two replications, and 163 were evaluated at one location with two replications. For PYT at MI, they evaluated 118 advanced lines at three locations, with two replications in one location and no replication in the others. Lastly, the breeder at the University of Illinois evaluated two sets of 533 (ILA) and 560 (ILB) advanced lines at one location in two replications, with the lines grouped by maturity into 10 and 14 trials, respectively. In this project, there were nine participating breeding programs; however, in five breeding programs only a small percentage of their advanced PRs were grown in 2019 PYT, making comparisons between selection categories impossible, and therefore they were excluded from the analysis.

2.2 | Phenotypic data

The phenotypic data used by the breeders to make 2018 PR selections included YLD (kg/ha), R8, RL, and CC (Table 1). YLD was converted to kg/ha and plots were adjusted to 13% seed moisture using harvest time seed moisture. The RL was the difference between days to maturity (R8) and days to flowering (R1). R1 was the number of days until 50% of the plants in a plot had one open flower on the main stem, and R8 was the number of days from August 31 until 95% of pods reached maturity (Fehr & Caviness, 1971). The performance of the selected lines in the 2019 PYT was measured based on the yield data.

Breeding programs at IN and MI collected CC data in PR populations using aerial imagery, while the breeding program at IL collected CC using ground-based imagery. To gather the imagery data from PR populations, breeders agreed to collect the images following the same protocol. Images were collected on days with good weather conditions, at noon (~10 a.m. to 2 p.m.), without intense wind and clouds. A fixed-wing Precision Hawk-III UAS equipped with a 14-megapixel RGB Nikon 1-J3 digital camera (CAM) was used to take aerial images in IN, with similar platforms used in PR populations in MI. UASs were flighted at 50 m, yielding a spatial resolution of 1.50 cm/pixel with 70%–80% lateral and forward overlap. Ground-based red–green–blue (RGB) pictures (640 × 480 pixels) were taken at the center of each plot from ~ 1.5 m above the ground, at a 45° angle from the horizon, using a

TABLE 1 Selection categories used to select experimental lines from 2018 progeny rows in four public soybean breeding programs.

Breeding program	Breeding program ID	Number of selection candidates	Selection categories	Selection intensity (%)
Purdue University	IN	4903	YLD|SPYLD + SPACC	8
Purdue University	IN	4903	YLD|SPYLD + PED	7
Purdue University	IN	4903	ACC|SPACC + PED	8
Purdue University	IN	4903	Random	8
The Ohio State University	OH	4939	VIS	5.5
The Ohio State University	OH	4939	RL|SPRL	8
The Ohio State University	OH	4939	RL|SPRL + PED	8
The Ohio State University	OH	4939	Random	7
Michigan State University	MI	677	ACC|SPACC	4
Michigan State University	MI	677	ACC|PED	7
Michigan State University	MI	677	ACC|SPACC + PED	6
Michigan State University	MI	677	Random	7
University of Illinois	ILA	1625	VIS	11
University of Illinois	ILA	1625	YLD|CC	8
University of Illinois	ILA	1625	CC|CAM	8
University of Illinois	ILA	1625	YLD	8
University of Illinois	ILA	1625	Random	8
University of Illinois	ILB	3261	YLD	8
University of Illinois	ILB	3261	YLD|CC	8
University of Illinois	ILB	3261	CC	8
University of Illinois	ILB	3261	Random	2

Abbreviations: ACC, average canopy coverage; Cam, camera; CC, canopy coverage; ILA, Illinois set A; ILB, Illinois set B; IN, Indiana; MI, Michigan; OH, Ohio; PED, pedigree; RL, reproductive length; SPACC, spatially adjusted average canopy coverage; SPRL, spatially adjusted reproductive length; SPYLD, spatially adjusted yield; VIS, visual selection; YLD, yield.

tripod and pocket CAMs. Ground-based imagery was collected 37 days after planting (DAP), and aerial images across different sampling dates of early-season canopy development. Four sampling dates were used to collect aerial images from the PR populations in IN, at 39, 45, 50, and 64 DAP, and two sampling dates at 17 and 47 DAP for PR populations in MI.

CC was measured using the percentage of plot image pixels classified as canopy pixels. Hearst (2019) described the multilayer mosaic methodology used for aerial image analysis, plot extraction, and classification. Plot extraction from orthorectified RGB images results in several plot images from different perspectives, and the number of plot images from the same date varies among plots (Moreira et al., 2019). The Excess Green Index (ExG) and Otsu thresholding were used to segment individual plot images and separate the canopy vegetation from the background. As described previously by Moreira et al. (2019), the median CC values were calculated from replicated plot images for each sampling date; then, ACC for each plot was calculated by averaging the median CC among sampling dates. Ground-based plot images for CC estimations were classified using the same Excess Green Index (ExG) and Otsu thresholding methods as the UAS plot clips (Hearst, 2019). Because ground-based canopy

percentages were derived from individual plot pictures, we referred to them as "CC" values. In contrast, canopy data from aerial images obtained from multiple clips per plot are called "ACC."

2.3 | PR selection categories and statistical models

Within the existing pipeline of each breeding program, PR selections were performed as usual; however, breeders also participated in the selection experiment to compare the agronomic performance of lines selected by breeders using their usual selection methods to lines selected through prediction of yield performance using new sources of data and information. The selection experiment was conducted in all states with an equal target selection intensity across breeding programs; however, some experimental units were not grown in the PYT because there was not enough seed to be grown in the replicated experiments, and there were slight deviations from the target selection intensity in some categories of the breeding programs (Table 1, Figure S2). In addition, breeders provided block-range-row information of each line for spatial

correction of field variation in the phenotypes and parental information to enhance selection accuracy among families.

A total of 13 selection categories were evaluated across breeding programs and each breeding program used at least three different categories, including random selection as a control. Selection categories used by each breeding program are listed in Table 1. The breeding program of University of Illinois had two sets (A and B) of PR populations, and the breeder applied different selection categories to each one of them. The 13 selection categories include: (1) VIS; (2) line's performance for YLD; (3) line's performance for ground-based CC; (4) random selection category (R); (5) an LM to predict corrected YLD values using CC as independent variable (YLD|CC); (6) an LM to predict corrected CC estimation with the CAM as independent variable (CC|CAM); (7) an LM to predict corrected ACC values using SPACC as independent variable (ACC|SPACC); (8) an LM to predict corrected RL values using SPRL as independent variable (RL|SPRL); (9) an LM to predict corrected YLD values using SPACC and SPYLD as independent variables (YLD|SPYLD + SPACC); (10) a PED-based MLM to estimate the line's breeding values for YLD using SPYLD values as fixed effect (YLD|SPYLD + PED); (11) a PED-based MLM to estimate the line's breeding values for ACC using SPACC values as fixed effect (ACC|SPACC + PED); (12) a PED-based MLM to estimate the line's breeding values for RL using SPRL values as fixed effect (RL|SPRL + PED); and (13) a PED-based MLM to estimate the line's breeding values for ACC with no fixed effects (ACC|PED). We fit the LMs using ordinary least squares (OLS) regression in R software, and the models can be described as:

$$Y_i = \beta_0 + \beta_1 x_{i,1} + \beta_2 x_{i,2} + e_i \tag{2}$$

where Y_i is the vector of observations (dependent variable); β_0, β_1, and β_2 are the regression coefficients; $x_{i,1}$ and $x_{i,2}$ are the vector of observations of the independent variables; and e_i is the vector of residuals $e_i \sim N(0, \sigma_e^2)$ in the models with one independent variable. For the model with two independent variables, $e_i \sim MVN(0, \sigma_e^2)$, where σ_e^2 is the residual variance. The new adjusted phenotypes associated to each PR was calculated as the residual effect $= \mathbf{y_i} - \mathbf{\hat{y}_i}$; then, the residuals were used as selection criteria in the respective selection categories. We ran PED-based MLM to estimate variance components and the best linear unbiased predictors (BLUPs) in REMLF90 (Misztal et al., 2014). The univariate MLM can be described as:

$$Y_i = \mu + x_i + g_i + e_i \tag{3}$$

where Y_i is the phenotype, μ is the mean, x_i is the SP covariate (i.e., SPACC, SPYLD, or SPRL) fitted as a fixed effect, g_i ($i = 1,\ldots$ number of genotypes) captures the random geno-type effect with $g_i \sim N(0, \mathbf{A}\sigma_a^2)$, where $\mathbf{A}$ is the relationship matrix calculated using PEDs tracked back to PR founders and σ_a^2 is the additive genetic variance, and e_i is the residual term distributed as $e_i \sim N(0, \sigma_e^2)$, where σ_e^2 is the residual variance. We obtained the SP covariates by using the moving average of neighbor plots described by Lado et al. (2013) and estimated through functions NNscr/NNcov of R package NAM (Xavier et al., 2015). Finally, we fitted a random model without fixed effects that can be described as:

$$Y_i = \mu + g_i + e_i \tag{4}$$

where Y_i, g_i, and e_i were previously described in Equation (3). The solutions of g_i correspond to the BLUPs. From variances components, we estimated narrow-sense heritability (h^2) as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2} \tag{5}$$

where σ_a^2 and σ_e^2 were described previously. We calculated Pearson correlation coefficients between traits measured in each breading program. We obtained adjusted values for phenotypes calculated as $\mu + g_i$ to express them in units. For selections, we ranked the PRs in 2018 according to each selection category and selected approximately 8% of progenies for breeding program (Table 1). Some categories had deviations from the targeted selection intensity (8%) due to field and seed limitations and logistics. Selected lines overlapped among categories (Figure S2); thus, the total selected PRs by breeding program were 1,143 for IN, 884 for OH, 119 for MI, 533 for ILA, and 560 for ILB.

2.4 | Evaluation of PYT

We performed analysis of variance (ANOVA) on YLD (kg/ha) for each breeding program to determine the level of the significance of genotype differences, location, replications, and blocks; the ANOVA was performed using the "*lm*" function implemented in R. We estimated the genetic values as the BLUP for each line within each breeding program to evaluate the PYT line's performance. We obtained the BLUPs by a MLM analysis using restricted maximum likelihood implemented in the R package 'lme4' (Bates et al., 2015). For IN, the model can be described as:

$$Y_{ijk} = \mu + f(x) + g_i + l_j + b_k + e_{ijk} \tag{6}$$

where Y_{ijk} is the phenotype (YLD, R8), μ is the intercept, $f(x)$ is the spatial covariate based on a moving-average of neighbor plots as described by Lado et al. (2013) and estimated through functions NNscr/NNcov of R package NAM

(Xavier et al., 2015), g_i ($i = 1, \ldots$ number of genotypes) captures the genotype effect with $g_i \sim N(0, \sigma_g^2)$, where σ_g^2 is the genetic variance, l_j ($j = 1, \ldots$ number of location) is the location effect with $l_j \sim N(0, \sigma_l^2)$, where σ_l^2 is the location variance, b_k ($k = 1, \ldots$ number of incomplete blocks) is the incomplete blocks effect with $b_k \sim N(0, \sigma_b^2)$, where σ_b^2 is the block variance, and e_{ijk} is the residual term distributed as $e_{ijk} \sim N(0, \sigma_e^2)$, where σ_e^2 is the residual variance. Maturity is a confounding factor influencing yield (Moreira et al., 2019); thus, we fitted an additional model for YLD using R8 as a covariate in Equation (6) to avoid misinterpretation of the results. For PYT in OH, the model can be described as:

$$Y_{ijkl} = \mu + g_i + l_j + t_k + b_l + e_{ijkl} \tag{7}$$

where Y_{ijkl} is the phenotype (YLD, R8); μ, g_i, e_{ijkl}, and l_j were described previously for model in Equation (6); t_k ($k = 1, \ldots$ number of trials) is the effect of the trials where lines were grouped based on maturity with $t_k \sim N(0, \sigma_t^2)$, where σ_t^2 is the trials variance; and b_l ($l = 1, \ldots$ number of blocks) is the effect of blocks with $b_l \sim N(0, \sigma_b^2)$, where σ_b^2 is the block variance. We fitted an additional model for YLD including R8 as a covariate in Equation (7). For PYT in MI, the model can be described as:

$$Y_{ijk} = \mu + g_i + l_j + b_k + e_{ijk} \tag{8}$$

where Y_{ijk} is the phenotype (YLD, R8); μ, g_i, e_{ijk}, and l_j were described previously for the model in Equation (6); and b_k ($k = 1, \ldots$ number of blocks) is the effect of blocks with $b_k \sim N(0, \sigma_b^2)$, where σ_b^2 is the block variance. We fitted an additional model for YLD including R8 as a covariate in Equation (8). For PYT in ILA and ILB, the model can be described as:

$$Y_{ijk} = \mu + g_i + t_j + b_k + e_{ijk} \tag{9}$$

where Y_{ijk} is the phenotype (YLD, R8); μ, g_i, and e_{ijk} were described previously in Equation (6); t_j is the effect of the trials where lines were grouped based on maturity ($j = 1, \ldots$ number of replications) with $t_j \sim N(0, \sigma_t^2)$, where σ_t^2 is the replication variance; and b_k ($k = 1, \ldots$ number of blocks) is the effect of blocks with $b_k \sim N(0, \sigma_b^2)$, where σ_b^2 is the block variance. We fitted an additional model for YLD including R8 as a covariate in Equation (9). We adjusted the BLUPs from all models as $u + g_i$ to express the phenotypes with units. As described by Moreira et al. (2019), the selection method that increases the population means the most between generations would be the preferred method. So, we used two-sample t-tests to compare adjusted yield means (Yield) and adjusted yield means for maturity (Yield|R8) among selection categories within each breeding program. Other variables

we measured from PYT include yield-ranks (ranks) and R8.

2.5 | Yield ranks and sources of information

We created a combined dataset for Yield, Yield|R8, yield ranks, and R8 from 2019 PYT across breeding programs including dummy variables for the different sources of information used in 2018 PR selections. We grouped the sources of information into five categories: (1) SP that include all spatially adjusted traits used across categories; (2) the canopy of the plant (Canopy) that include CC measured by aerial and ground digital images; (3) the breeder's visual selection (VIS); (4) grain yield (YLD); and (5) RL. Lines selected by any given source will contain a 1 in the respective source column, and several sources could select the same line. To estimate the relationship between the five sources of information and the response variables Yield, Yield| R8, Ranks, and R8, we performed a multiple regression analysis using the 'lm' function implemented in R software, and the model can be generally expressed as Equation (2). Where y is the vector of observations of the response variable (Yield, Yield|R8, Ranks, and R8), $\boldsymbol{\beta}$ (slope) is the vector of unknown parameters to estimate, $\mathbf{X}$ is the incidence matrix linking the sources of information as independent variables to the observations, and $\mathbf{e}$ is the vector of residuals with $\mathbf{e} \sim MVN(0, \sigma_e^2 \mathbf{I})$, where σ_e^2 is the residual variance and $\mathbf{I}$ is an identity matrix.

3 | RESULTS

3.1 | Phenotypes of 2018 PR

Table 2 shows the narrow-sense heritabilities and phenotypic Pearson's correlation of trait values for lines phenotyped in each breeding program. R8 had the highest narrow-sense heritability, followed by RL, YLD, ACC, and CC. The narrow-sense heritability values range from 0.12 to 0.61, with some variation between breeding programs. YLD had higher narrow-sense heritability than ACC at IN, and lower than CC at ILA and ILB. The lower heritability in IL could be a result of the shorter row plots compared to the other collaborators. R8 had higher narrow-sense heritability than YLD at IN and OH. RL had lower narrow-sense heritability than YLD at OH. All secondary traits showed positive correlations with YLD. The correlations of CC and YLD were 0.21 in IN and 0.38 in ILA and ILB. R8 had positive correlations with YLD in IN (0.11) and MI (0.48). RL correlation with YLD was 0.48 in OH. PYT across breeding programs showed significant differences among entries, locations, replications, and blocks; except in PYT at IN and ILB where entries did not show significant differences (Table S1).

TABLE 2 Narrow-sense heritability (diagonal/bold) and Pearson correlation coefficient (above diagonal) for traits phenotyped in 2018 progeny rows in each public breeding program.

Breeding program ID		*r*		
		YLD	ACC	R8
IN	YLD	**0.38**	0.21	0.11
IN	ACC	–	**0.27**	0.24
IN	R8	–	–	**0.47**
		YLD	RL	R8
OH	YLD	**0.48**	0.43	0.47
OH	RL	–	**0.46**	0.79
OH	R8	–	–	**0.61**
		ACC	–	–
MI	ACC	0.12	–	–
		YLD	CC	
IL	YLD	**0.15**	0.38	–
IL[a]	CC	–	**0.24**	–

Abbreviations: ACC, average canopy coverage; CC, canopy coverage; h^2, narrow-sense heritability; IL, University of Illinois Urbana Champaign; IN, Purdue University; MI, Michigan State University; OH, The Ohio State University; r, Pearson's coefficients; RL, reproductive length; YLD, grain yield.

[a]We combined Illinois set A (ILA) and Illinois set B (ILB) datasets to estimated variance components and h^2 for University of Illinois.

3.2 | Selection category performance in PYT 2019

To examine the selection categories' performance, we compared the adjusted mean Yield and aYield|R8 between selected classes (Figure 1). Results were similar when comparing categories for Yield and Yield|R8 across populations. For PYT at IN, Yield and Yield|R8 were slightly higher for lines selected by ACC|SPACC + PED than for lines selected from the other categories, with only significant differences for Yield between ACC|SPACC + PED and random selection. For PYT at OH, there were no significant differences between categories, which include VIS, RL|SPRL, and RL|SPRL + PED. For PYT at MI, we observed higher Yield performances for lines selected by ACC|SPACC + PED followed by ACC|PED category, with no visible difference between lines selected by ACC|SPACC and at random; however, there were only significant differences between categories ACC|SPACC + PED and random. Selection categories YLD and YLD|CC had the highest yield performance in PYT of ILA, with significant differences in yield compared to the other classes, which include CC|CAM, VIS, and random selection. For PYT of ILB, there were no significant differences in yield performance among classes, but YLD, YLD|CC, and CC categories had slightly higher yields than lines selected at random. Figure S3 shows the distribution of adjusted R8 values and mean comparisons between selected

classes. Lines selected by RL|SPRL and RL|SPRL + PED exhibited later maturity than lines selected by VIS in PYT at OH; while lines selected by using yYLD showed later maturity than lines selected by VIS and CC|CAM in PYT at ILA. Figures S4 and S5 show the number of lines selected per selection category and the distribution of the adjusted yield values and mean comparisons between selected classes for the breeding programs that were not included in this report.

Table 3 shows each breeding program's 10 top-ranked lines in PYT 2019. For IN, the categories that selected the highest number of the 10 top-ranked lines were ACC|SPACC + PED (six lines), followed by YLD|SPYLD + SPACC (four lines). There was no predominant category for OH and the different categories equally selected the 10 top-ranked lines. ACC|SPACC + PED was the most effective category in selecting top-ranked lines in MI (eight lines). YLD|CC, YLD, and VIS categories equally selected high-yielding lines for ILA while YLD|CC selected the majority of 10 top-ranked lines in ILB (seven lines). The correlations between yield performance of selected lines in 2018 PR and 2019 PYT were 0.68 for IN, 0.58 for OH, 0.73 for ILA, and 0.54 for ILB.

3.3 | Yield ranks versus sources of information

Figure 2 shows the significance and the regression coefficients obtained by modeling the relationship between the response variables of 2019 PYT (yield ranks, Yield, Yield|R8, and R8) and the sources of information used for 2018 PR selections. Table S2 has a complete summary of the multiple linear regressions by response variables. Positive coefficients indicate that using any given source of information for PR selections led to increases in the response variable, while negative coefficients will indicate the opposite. Yield rank had a significant relationship with all sources of information. As breeders look for lower yield ranks, efficient sources of information for PR selections will be those with the negative and highest coefficients. SP had the highest negative coefficient, followed by canopy, YLD, and VIS. In contrast, RL and PED had positive coefficients with yield ranks. Yield and Yield|R8 had a significant relationship with the different sources of information except for VIS and PED. The most efficient sources of information for selecting high-yielding lines were SP, followed by canopy and YLD. In contrast, RL was the least efficient with a negative coefficient for Yield and Yield|R8. R8 was only significantly associated with YLD, VIS, and RL. As YLD and RL had positive coefficients for R8, using them for PR selections could indirectly select for later maturity soybeans. At the same time, VIS had a negative coefficient with R8 which indicates that PR selections by VIS could indirectly select for earlier maturity.
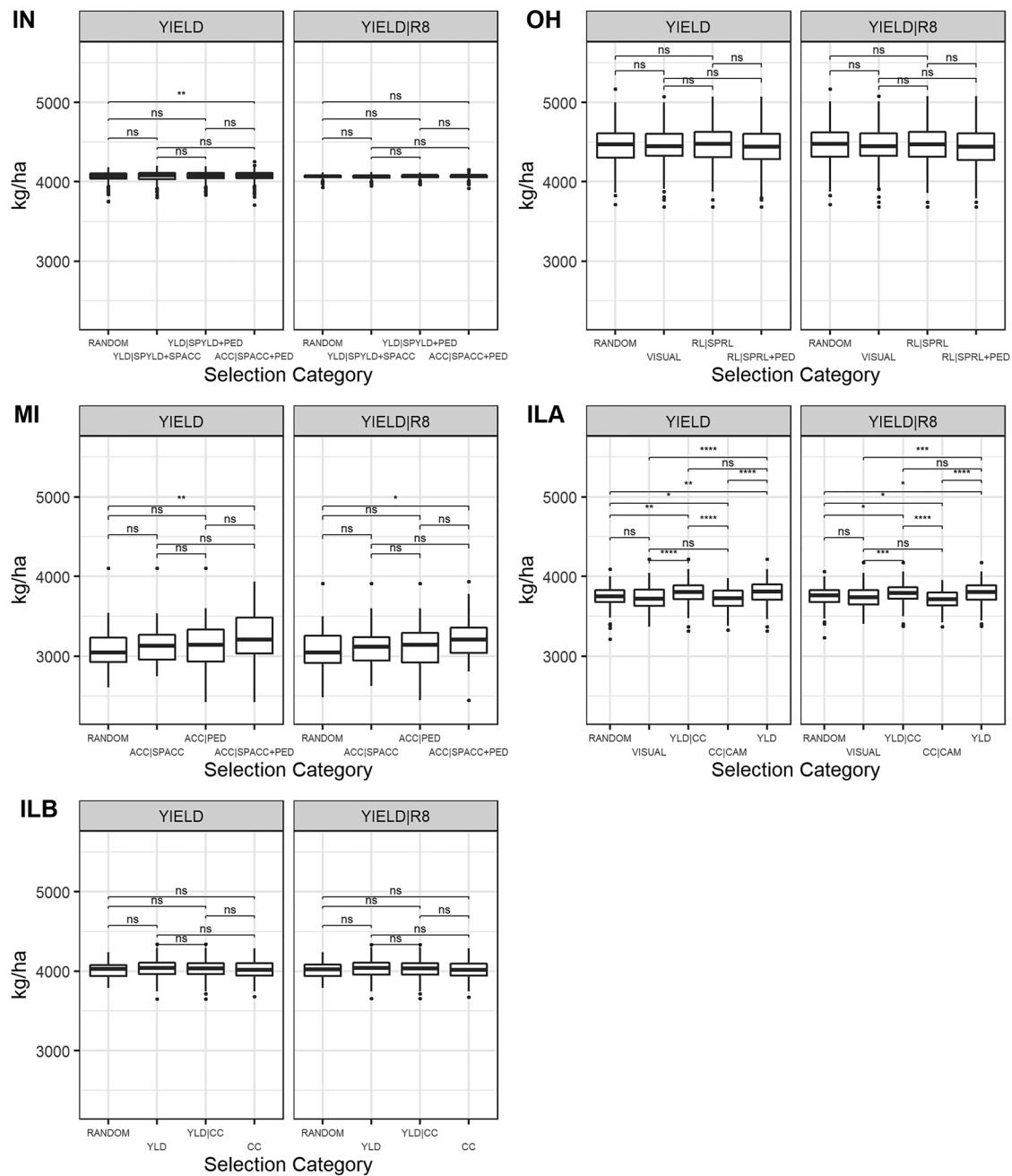
FIGURE 1 Boxplots of adjusted yield (Yield; kg/ha) and adjusted yield given R8 (Yield|R8) distribution for lines selected by each selection categories for 2019 preliminary yield trials (PYTs) in Indiana (IN), Ohio (OH), Michigan (MI), Illinois A (ILA), and Illinois B (ILB). The line crossing the box plots represents the median for each class. ACC, average canopy coverage; CAM, camera; CC, canopy coverage; PED, pedigree; RL, reproductive length; SPACC, spatially adjusted average canopy coverage; SPRL, spatially adjusted reproductive length; SPYLD, spatially adjusted grain yield. [ns] not significant, $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

4 | DISCUSSION

Narrow-sense heritabilities reported in this study (Table 2) were low and similar to narrow-sense heritabilities reported for soybean PR populations by Moreira et al. (2019). Heritability estimation accuracy is limited by how well characterized the environmental factors are and the precision of the measurements of the phenotypes (Xu et al., 2017); therefore,

as suggested by Moreira et al. (2019), low heritabilities are expected in PR populations since they are grown in unreplicated single row plot trials which challenge the estimation of the genetic parameters of the lines.

The effectiveness of a secondary trait as a predictor of yield potential depends on the strength of its phenotypic and, most importantly, genetic correlations with yield (Xavier et al., 2017). CC and ACC had positive correlations with

TABLE 3 Progeny row selection categories that selected the ten top-ranked lines in 2019 preliminary yield trials (PYTs) for each breeding program.

Rank	PYT IN	PYT OH	PYT MI	PYT ILA	PYT ILB
1	YLD|SPYLD + SPACC	Random	ACC|SPACC ACC|PED	VIS YLD|CC YLD	YLD YLD|CC
2	YLD|SPYLD + SPACC	VIS RL|SPRL RL|SPRL + PED	ACC|SPACC + PED	YLD|CC YLD	YLD YLD|CC
3	ACC|SPACC + PED	RL|SPRL	ACC|SPACC + PED	YLD|CC YLD	YLD|CC
4	ACC|SPACC + PED	VIS RL|SPRL RL|SPRL + PED	ACC|SPACC + PED	VIS YLD	CC
5	ACC|SPACC + PED	RL|SPRL	ACC|PED ACC|SPACC + PED	VIS	YLD|CC
6	YLD|SPYLD + SPACC	VIS RL|SPRL	ACC|SPACC + PED	VIS	YLD|CC
7	YLD|SPYLD + SPACC YLD|SPYLD + PED ACC|SPACC + PED	VIS RL|SPRL RL|SPRL + PED	ACC|PED	VIS	CC
8	ACC|SPACC + PED	Random	ACC|PED ACC|SPACC + PED	YLD| CC YLD	YLD YLD|CC
9	ACC|SPACC + PED	Random	ACC|PED ACC|SPACC + PED	Random	YLD
10	Random	Random	ACC|PED ACC|SPACC + PED	VIS	YLD YLD|CC

Abbreviations: ACC, average canopy coverage; CC, canopy coverage; ILA, Illinois set A; ILB, Illinois set B; IN, Indiana; MI, Michigan; OH, Ohio; PED, pedigree; RL, reproductive length; SPACC, spatially adjusted average canopy coverage; SPRL, spatially adjusted reproductive length; SPYLD, spatially adjusted yield; YLD, yield; VIS, visual selection.

YLD, but their strength was lower than previously reported (Table 2; Jarquin et al., 2018; Moreira et al., 2019; Xavier et al., 2017). A low phenotypic correlation may result from a strong genotype by environment interaction, with genetic and environmental correlations acting in opposite directions (Falconer et al., 1996; Moreira et al., 2019; Xavier et al., 2017); thus, two traits could have a low phenotypic correlation but still have a strong correlation at the genetic and environmental levels (Falconer et al., 1996; Moreira et al., 2019). Phenotypic correlations of YLD with RL and R8 were positive, as described in other studies, with some differences in the strength of the correlations due to specific characteristics of the populations and the environments (Cheng et al., 2011; Xavier et al., 2017; M. Xu et al., 2013).

In soybean breeding, it is a common practice to adjust YLD for maturity to control for its confounding effect on yield estimations (Moreira et al., 2019). We observed positive correlations of R8 with ACC, and YLD in 2018 PR (Table 2), as described by Xavier et al. (2017); thus, selection for ACC and YLD could lead to indirect increases in maturity; and including ACC and R8 in the yield prediction models may be necessary. In 2019 PYT, we observed significant differences in R8 between lines selected from the different categories in OH, ILA, and ILB. Lines selected by YLD, CC, and RL exhibited later maturity (Figure S3); thus, indirect selection for maturity is more likely to occur when the methods used for selection include RL and YLD data without adjustment for

maturity. To avoid possible confounding effects of maturity, we evaluated the performance of selection categories by comparing them for Yield and yield\|R8, but there were no major differences between them. In addition, there were some deviations from the target selection intensity in some categories; however, losing experimental units is not uncommon in field experiments, and it is even more likely in the PR stage, given the limited number of seeds and the small plot sizes. Different selection intensities could lead to erroneous comparisons among categories; however, in this selection experiment, the deviations from the target selection intensity were in a few categories and minor (Table 1).

Indirect selection for yield based on ACC\|SPACC + PED resulted in better yield performance than ACC prediction models fitting PED and SP information separately (Figure 1, MI); however, there were not significant differences among the three categories, ACC\|SPACC + PED was the most effective in selecting top-ranked lines (Table 3). Our findings align with previous reports where adjusting phenotypes for field spatial variation and using PED information enhanced genetic selection for soybean seed composition and yield (Bernardeli et al., 2021; da Silva et al., 2021; Lado et al., 2013; Muir, 2007). da Silva et al. (2021) and our findings demonstrated that modeling additional sources of information, such as PED and adjusting for field spatial variation could lead to a more accurate estimation of genetic values in non-replicated experiments.
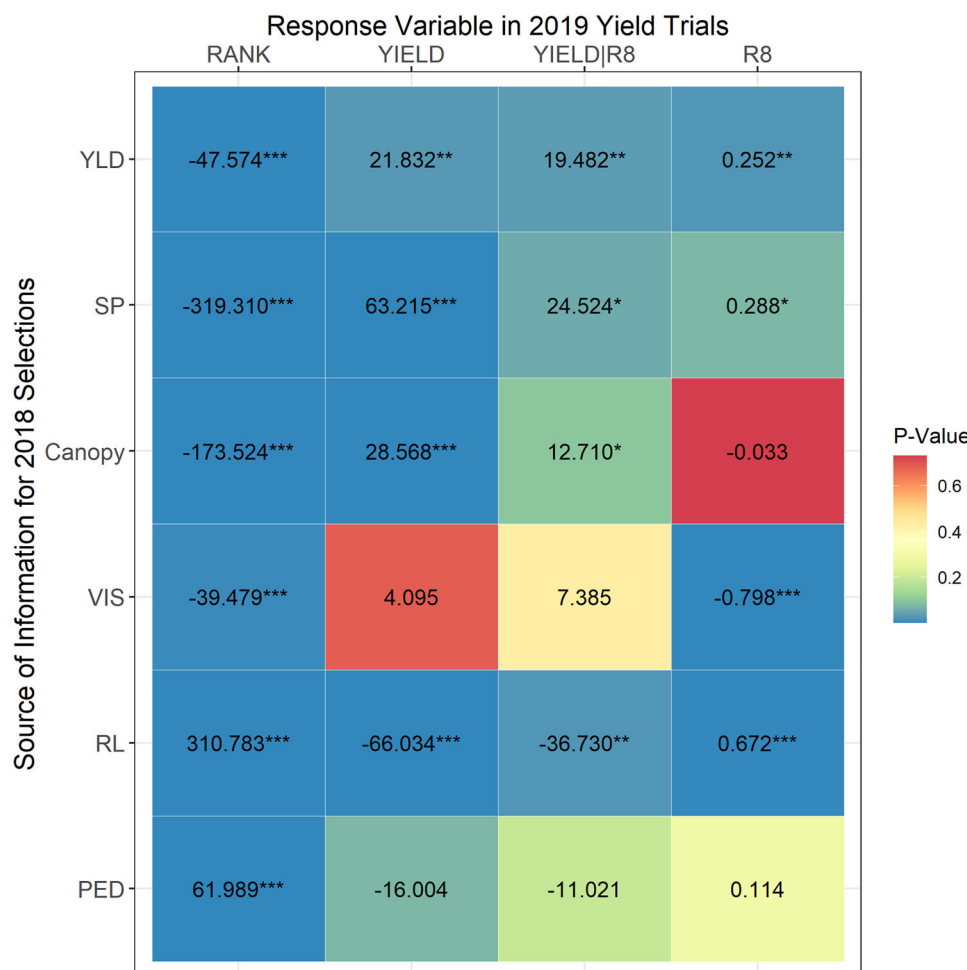
FIGURE 2 Coefficients of regression models of yield ranks (RANK), adjusted yield means (kg/ha; YIELD), and adjusted yield means for maturity (kg/ha; YIELD|R8) across preliminary yield trials (PYTs) in 2019 by the sources of information used for progeny row (PR) selections in 2018. The table is color coded for the *p*-values with *$p < 0.1$; **$p < 0.05$; ***$p < 0.01$. Canopy, canopy coverage; PED, pedigree; RL, reproductive length; SP, spatially adjusted traits; VIS, visual selection; YLD, grain yield.

Successful indirect selection for yield occurs if the yield gains are more significant than direct selection. Indirect selection for yield using ACC|SPACC + PED was not significantly different from the direct selection using YLD|SPYLD + PED and the direct selection category YLD|SPYLD + SPACC using ACC data as covariate (Figure 1, IN); however, ACC|SPACC + PED was more effective than the other categories in selecting the highest yielding lines (Table 3). Moreira et al. (2019) did not find significant differences between direct and indirect selection for yield in PR using ACC either; still, several authors have recognized that CC can be advantageous in early soybean selections when obtaining accurate yield data is challenging and other phenotypic data are not available, under unexpected climatic events that partially lose plots, and as a covariate to help improve the selection model's performance (Jarquin et al., 2018; Moreira et al., 2019; Xavier et al., 2017). In addition, larger test sizes could be possible under indirect selection.

ACC|SPACC + PED was the most effective category for selecting high-yielding lines in IN and MI (Table 3). ACC was measured from 39 to 64 DAP with four sampling dates in IN and at 17 and 47 DAP in MI. Schmitz et al. (2021) showed green CC measured using normalized difference vegetative index (NDVI) at R5 as a single observation effectively predicted yield. At the same time, Jarquin et al. (2018) indicated no differences in the predictive ability of genomic prediction models, including canopy data collected during the early stages of the growing season and collecting the canopy information throughout the whole growing season. Our results and previous findings by Schmitz and Kandel (2021) and Jarquin et al. (2018) suggest that ACC data collected early or later in the season with two or more sampling dates are equally successful in selecting high-yielding lines.

Predicting yield from PR data of RL was ineffective in selecting lines with higher genetic potential (Figure 1, OH, Table 3). Smith and Nelson (1987) previously reported that early generation selection for RL was an unreliable method

for identifying high-yielding lines. Besides this study, to our knowledge, there are no other reports of RL as a trait for PR testing. We did not measure ACC and RL in the same PR populations and there is no way to know if RL was more or less correlated to YLD than ACC; however, previous studies reported lower heritabilities and lower correlations of RL with YLD than ACC (Smith & Nelson, 1987; Xavier et al., 2017; Xavier et al., 2017), which could explain the lower performance of PR selection categories using RL for indirect yield selection compared to others. In addition, RL positively correlates to YLD and could still be used as a covariate to help improve the yield selection model's performance.

Selection categories using YLD and adjusted mean YLD given CC (YLD|CC) outperformed selections made using only CC in ILA and ILB (Figure 1, ILA, ILB). It suggests ACC could be more effective than CC for indirect yield selection. Single-point CC estimates from ILA and ILB were made via ground-based plot images, while ACC from IN and MI was measured using aerial images across several sampling dates. Bai and Purcell (2019) reported that aerial images taken vertically above the top of the field might better assess the soybean canopy; thus, leading to better estimates of PR's genetic potential. It is important to note that there were no field spatial variability adjustments for the traits in the PR selection categories of ILA and ILB. As mentioned before, accounting for spatial variation within a location can led to better estimates of the genetic values of the lines; thus, several sampling dates, PED, and spatial field variation adjustments in the prediction models are probably other factors contributing to the higher efficacy of selection categories using ACC information. When selecting the best phenotyping strategy, it is also essential to consider that ground point measurements are more time consuming and unsuitable for large populations, which is not much of a problem for aerial HTP methods. In contrast, aerial phenotyping is highly dependent on weather conditions, and training is required (Bai & Purcell, 2019). The contrasting results of the efficacy of PR selections using ACC or CC could also result from the specific environmental influences on the trait phenotypes.

SP traits (and canopy data were the more effective sources of information on selecting high-yielding lines with lower yield ranks and smaller or no effect on maturity (Figure 2). The advantages of using CC data and spatial variation adjustments as sources of information for soybean selection have been demonstrated in previous studies (da Silva et al., 2021; Jarquin et al., 2018; Moreira et al., 2019; Xavier et al., 2017). On the contrary, VIS, RL, and PED were not reliable sources of information to select high-yielding lines. VIS has not been proven effective in modifying several traits (Ordás et al., 2012), but it is more convenient than harvesting plots for yield measurements; however, manual data collection (VIS)

is time consuming, error prone (Cobb et al., 2019), and may not be precise enough to increase genetic gains in soybean PR selections. It is noteworthy that VIS for PR selections was an effective strategy in one breeding program, suggesting some breeders may have developed through their years of experience a higher ability to assess PR populations visually. It would be interesting in the future to explore the application of computer vision, machine learning, and deep learning algorithms to classify PR based on the breeder's visual criteria. On the other hand, PEDs represent the expected average relationships describing the potential transmission of genes, but they do not capture mendelian segregation; thus, the joint use of PED and genomic information could provide better estimates of genetic similarities between genotypes and could be used to optimize prediction of complex traits (Velazco et al., 2019). Finally, as described previously, RL had been reported as an unreliable method for PR selections (Smith & Nelson, 1987); RL is also highly correlated to maturity and will likely lead to the indirect selection of lines with later maturity. RL, VIS, and PED information may still be helpful when combined with other sources of information such as ACC, YLD, and genomic information.

In this study, we demonstrated that UAS-based ACC used as a secondary trait in combination with SPACC is an **e**fficient selection strategy for PR. Adjusting for field spatial variation is a common practice for the analysis of plant breeding (da Silva et al., 2021); while HTP is an emerging solution to increase selection intensity and improve phenotyping accuracy, which could enable greater gain for selection (Cobb et al., 2019). Our results are similar to those Moreira et al. (2019) obtained. The differences between our study and the one performed by Moreira et al. (2019) were that we studied a more comprehensive set of selection methods used by soybean breeders and compared several sources of information such as PED, SP traits, YLD, CC, and RL. In addition, Moreira et al. (2019) conducted the selection experiment in IN, while we conducted the selection experiment in IN, IL, MI, and OH. Based on the scenarios considered in this study, results indicate that it may be possible to increase the effectiveness of PR selections by phenotyping secondary traits using HTP and implementing spatial variation adjustments in PR selection trials. Data captured by HTP platforms could also be used as part of PED or genomic prediction schemes (Cobb et al., 2019), and breeders must carefully evaluate the value of these alternatives in their breeding pipelines. Further studies should (1) assess the benefits of using SP traits and ACC in multi-environmental trials, (2) evaluate other scenarios with different combinations of the sources of information evaluated in this study and additional ones, and (3) evaluate the long-term genetic gain on yield when using ACC for PR selections.

5 | CONCLUSION

Integrating new HTP technologies into public plant breeding programs could help enhance selection accuracy by increasing broad sense heritability. However, with the abundance of new technologies and new traits available, breeders need to carefully evaluate the impact of these new traits and technologies on selection accuracy. We evaluated different sources of information and combinations of them as selection criteria for PR selections in public breeding programs and compared their performance in PYT of advanced lines. The sources of information included PED, SP traits, YLD, aerial (ACC) and ground measured CC, and RL. In this study, we demonstrated that UAS-based ACC used as a secondary trait in combination with SPACC is an efficient selection strategy for PR. Alternatively, VIS can also be an effective selection method, but it is subject to the expertise of the breeder performing it. Contrarily, RL and PED information were the least effective sources for selecting PR with high genetic potential; however, they could still be valuable information sources when used as covariates. ACC, measured using aerial images through several sample dates, was a more effective selection method than single-time CC, measured via ground-based plot images. Further research to evaluate canopy measurements in multi-environment trials using different technologies and sampling dates is necessary to ensure the maximum attainable genetic gains when using ACC for soybean selection.

AUTHOR CONTRIBUTIONS

Diana M. Escamilla: Conceptualization; formal analysis; investigation; methodology; visualization; writing—original draft. **Meng Huang**: Formal analysis; writing—review and editing. **Leah Mchale**:Data curation; writing—review and editing. **Dechun Wang**: Data curation; writing—review and editing. **Brian Diers**: Data curation; writing—review and editing. **Alencar Xavier**: Conceptualization; methodology. **Katy Martin Rainey**: Conceptualization; methodology; project administration; writing—review and editing.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Derived data supporting the findings of this study are available from the corresponding author (Katy Martin Rainey) on request.

ORCID

Diana M. Escamilla https://orcid.org/0000-0001-9293-9581
Leah McHale https://orcid.org/0000-0003-1028-2315
Brian Diers https://orcid.org/0000-0003-3584-5495
Alencar Xavier https://orcid.org/0000-0001-5034-9954
Katy Martin Rainey https://orcid.org/0000-0001-8541-5851

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SUPPORTING INFORMATION

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