Harnessing genetic diversity in the USDA pea (*Pisum sativum* L.) germplasm collection through genomic prediction

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Abstract

Phenotypic evaluation and efficient utilization of germplasm collections can be time-intensive, laborious, and expensive. However, with the plummeting costs of next-generation sequencing and the addition of genomic selection to the plant breeder's toolbox, we now can more efficiently tap the genetic diversity within large germplasm collections. In this study, we applied and evaluated genomic selection's potential to a set of 482 pea accessions – genotyped with 30,600 SNP markers and phenotyped for seed yield and yield-related components – for enhancing selection of accessions from the USDA Pea Germplasm Collection. Genomic prediction models and several factors affecting predictive ability were evaluated in a series of cross-validation schemes across complex traits. Different genomic prediction models gave similar results, with predictive ability across traits ranging from 0.23 to 0.60, with no model working best across all traits. Increasing the training population size improved the predictive ability of most traits, including seed yield. An increasing trend in predictive ability was also observed with an increasing number of SNPs. Accounting for population structure effects did not significantly boost predictive ability, but we observed a slight improvement in seed yield. By applying the genomic prediction model from this study, we then examined the distribution of nonphenotyped accessions, and the reliability of genomic estimated breeding values (GEBV) of the USDA Pea accessions genotyped but not phenotyped. The distribution of GEBV suggested that none of the nonphenotyped accessions were expected to perform outside the range of the phenotyped accessions. Desirable breeding values with higher reliability can be used to identify and screen favorable germplasm accessions. Expanding the training set and incorporating additional orthogonal information into the genomic prediction framework could enhance prediction accuracy.

Keywords: genomic selection, genomic prediction, reliability criteria, germplasm accessions, pea (*Pisum sativum* L.), next-generation sequencing

Introduction

Pea (*Pisum sativum* L.) is a vitally important pulse crop that provides protein (15.8-32.1%), vitamins, minerals, and fibers. Pea consumption has cardiovascular benefits as it is rich in potassium, folate, and digestible fibers, which promote gut health and prevent certain cancers (Mudryj et al., 2014; Tayeh et al., 2015). Considering the health benefits, the US Department of Agriculture recommends regular pulses consumption, including peas, to promote human health and wellbeing (http://www.choosemyplate.gov/). In 2019, more than 446,000 hectares of edible dry pea were planted with production totaling 1013,600 tonnes in the USA, making it the fourth-largest legume crop (http://www.fao.org) (USDA, 2020). Growing peas also help maintain soil health and productivity by fixing atmospheric nitrogen (Burstin et al., 2015). Recently, pea protein has emerged as a frontrunner and showed the most promise in the growing alternative protein market. The Beyond Meat burger is a perfect example of a pea protein product gaining traction in the growing market. About 20-gram protein in each burger comes from pea (https://www.nasdaq.com/articles/heres-why-nows-thetime-to-buy-beyond-meat-stock-2019-12-05). Another product made from pea, Ripptein, is a non-dairy milk product of pea protein that is gaining tremendous interest as an alternative dairy product (https://www.ripplefoods.com/ripptein/). Additionally, peas are gaining attention in the pet food market as it is grain-free and an excellent source of essential amino acids required by cats and dogs (PetfoodIndustry.com) also serves as animal feed (Facciolongo et al., 2014). As the demand for pea increases, particularly in the growing alternative protein market, genetic diversity expansion is needed to double the current rate of genetic gain in pea (Vandemark et al., 2015).

Germplasm collections serve as an essential source of variation for germplasm enhancement that can sustain long-term genetic gain in breeding programs. The USDA *Pisum* collection, held at the Western Regional Plant Introduction Station at Washington State University, is a good starting point to investigate functional genetic variation. To date, this collection consists of 6,192 accessions plus a Pea Genetic Stocks collection of 712 accessions. From this collection, the USDA core collection comprised of 504 accessions was assembled to represent ~18% of all USDA pea accessions at the time of construction (Simon and Hannan 1995; Coyne et al., 2005). Subsequently, single-seed descent derived homozygous accessions were developed from a subset of the core to form the 'Pea Single Plant'-derived (PSP) collection. The PSP is used to facilitate the collection's genetic analysis (Cheng et al., 2015). The USDA Pea Single Plant Plus Collection (PSPPC) was assembled and included the PSP and Chinese accessions and field, snap and snow peas from US public pea-breeding programs (Holdsworth et al., 2017).

Genomic selection (GS) takes advantage of high-density genomic data and rapidly increases the rate of genetic gain (Meuwissen et al., 2001). As genotyping costs have significantly declined relative to current phenotyping costs, GS has become an attractive option as a selection decision tool to evaluate accessions in extensive germplasm collections. A genomic prediction approach could use only genomic data to predict each accession's breeding value in the collection (Meuwissen et al., 2001; Habier et al., 2007; VanRaden, 2008). The predicted values would significantly increase the value of accessions in germplasm collections by giving breeders a means to identify those favorable accessions meriting their attention from the thousand available accessions in germplasm collection (Longin et al., 2014; Crossa et al., 2016; Jarquin et al., 2016). Several studies used the genomic prediction approach to harness diversity in germplasm collections, including soybean (Jarquin et al., 2016), wheat (Crossa et al., 2016), rice (Spindel et al., 2015), sorghum (Yu et al., 2016), maize (Gorjanc et al., 2016), and potato (Bethke et al., 2019). A pea genomic selection study for drought-prone Italian environment revealed increased selection accuracy of pea lines through genomic prediction (Annicchiarico et al., 2019; Annicchiarico et al., 2020). To the best of our knowledge, no such studies have been performed using the USDA Pea Germplasm Collection, but a relevant study has been made using a diverse pea germplasm set comprised of more than 370 accessions genotyped with a limited number of markers (Burstin et al., 2015).

To date, methods to sample and utilize an extensive genetic resource like germplasm collections remain a challenge. In this study, a genomic prediction approach targeting complex traits, including seed yield and phenology, was evaluated to exploit diversity contained in the USDA Pea Germplasm Collection. No research has been conducted on genomic prediction for the genetic exploration of the USDA Pea Germplasm Collection. Different cross-validation schemes were used to answer essential questions surrounding the efficient implementation of genomic prediction and selection, including determining best prediction models, optimum numbers of markers and population size, and impact of accounting population structure into genomic prediction framework. We then examined the distribution of all nonphenotyped accessions using SNP information in the collection by applying genomic prediction models.

Material and Methods

Plant materials

The Pea Single Plant Plus Collection (Pea PSP) of 292 USDA pea germplasm accessions (Cheng et al., 2015) was used in this study for phenotypic assessment. The USDA Pea Core Collection contains accessions from different parts of the world and represents the entire collection's morphological, geographic, and taxonomic diversity. These accessions were initially acquired from 64 different countries and are conserved at the Western Regional Plant Introduction Station, USDA, Agricultural Research Service (ARS), Pullman, WA (Cheng et al., 2015).

DNA extraction, sequencing, SNP calling

Green leaves were collected from seedlings of each accession grown in the greenhouse with the DNeasy 96 Plant Kit (Qiagen, Valencia, CA, USA). Genomic libraries for the Single Plant Plus Collection were prepped at the University of Minnesota Genomics Center (UMGC) using genotyping-by-sequencing (GBS). Four hundred eighty-two (482) dual-indexed GBS libraries were created using restriction enzyme *Ape*KI (Elshire et al., 2011). A NovaSeq S1 1 x 100 Illumina Sequencing System (Illumina Inc., San Diego, CA, USA) was then used to sequence the GBS libraries. Preprocessing was performed by the UMGC that generated the GBS sequence reads. An initial quality check was performed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Sequencing adapter remnants were clipped from all raw reads. Reads with final length <50 bases were discarded. The high-quality reads were aligned to the reference genome of *Pisum sativum* (Pulse Crop Database https://www.pulsedb.org/, Kreplak et al., 2019) using the Burrow Wheelers Alignment tool (Version .7.17) (Li and Durbin, 2009) with default alignment parameters, and the alignment data was processed with SAMtools (version 1.10) (Li et al., 2009). Sequence variants, including single and multiple nucleotide polymorphisms (SNPs and MNPs, respectively), were identified using FreeBayes (Version 1.3.2) (Garrison and Marth, 2012). The combined read depth of 10 was used across samples for identifying an alternative allele as a variant, with the minimum base quality filters of 20. The putative SNPs from freeBayes were filtered across the entire population to maintain the SNPs for biallelic with minor allele frequency (MAF) < 5%. The putative SNP discovery resulted in biallelic sites of 380,527 SNP markers. The QUAL estimate was used for estimating the Phred-scaled probability. Sites with a QUAL value less than 20 and more than 80% missing values were removed from the marker matrix. The rest markers were further filtered out so that heterozygosity was less than 20%. The filters were applied using VCFtools (version 0.1.16) (Danecek et al., 2011) and in-house Perl scripts.

Missing data were imputed using a *k*-nearest neighbor genotype imputation method (Money et al., 2015) implemented in TASSEL (Bradbury et al., 2007). Single Nucleotide Polymorphism (SNP) data was converted to a numeric format where 1 denotes homozygous for a major allele, -1 denotes homozygous for an alternate allele, and 0 refers to heterozygous loci. Finally, 30,646 clean, curated SNP markers were identified and used for downstream analyses.

Phenotyping

Pea germplasm collections (Pea PSP) were planted following augmented design with standard checks ('Hampton,' 'Arargorn,' 'Columbian,' and ‘1022’) at the USDA Central Ferry Farm in 2016, 2017, and 2018 (planting dates were March 14, March 28, and April 03, respectively). Central Ferry farm is located at Central Ferry, WA at 46°39’5.1’’N; 117°45’45.4” W, and elevation of 198 m. The Central Ferry farm has a Chard silt loam soil (coarse-loamy, mixed, superactive, mesic Calcic Haploxerolls) and was irrigated with subsurface drip irrigation at 10 min d^{-1}. All seeds were treated with fungicides; mefenoxam (13.3 mL a.i. 45 kg-1), fludioxonil (2.4 mL a.i. 45 kg -1), and thiabendazole (82.9 mL a.i.45 kg -1), insecticide; thiamethoxam (14.3 mL a.i. 45 kg -1), and sodium molybdate (16 g 45 kg -1) prior to planting. Thirty seeds were planted per plot; each plot was 152 cm long, having double rows with 30 cm center spacing. The dimensions of each plot were 152 cm x 60 cm. Standard fertilization and cultural practices were used.

The following traits were recorded and are presented in this manuscript. Days to first flowering (DFF) are the number of days from planting to when 10% of the plot's plants start flowering. The number of seeds per pod (NoSeedsPod) is the number of seeds in each pod. Plant height (PH cm) is defined as when all plants in a plot obtained full maturity and were measured in centimeters from the collar region at soil level to the plants' top. Pods per plant (PodsPlant) is the number of recorded pods per plant. Days to maturity (DM) referred to physiological maturity when plots were hand-harvested, mechanically threshed, cleaned with a blower, and weighed. Plot weight (PlotWeight, gm) is the weight of each plot in grams after each harvest. Seed yield (kg ha^{-1}) is the plot weight converted to seed yield in kg per hectare.

Phenotypic data analysis

A mixed linear model was used to extract the best linear unbiased predictors (BLUPs) from this trial for DFF, NoSeedsPod, PH, PodsPlant, DM, and seed yield using the following model:

$$y_{ij} = \mu + G_i + T_j + (T * G)_{ij} + e_{ij} \qquad (1)$$

where y_{ij} is the observed phenotype, μ is the overall mean, G_i is the random genotypic effect, T_j is the random year term, $(T * G)_{ij}$ is the genotype by year interaction, and e_{ij} is the residual error.

The heritability or repeatability for each assessed trait was calculated to evaluate the quality of trait measurements following the equation:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GE}^2/e + \sigma_e^2/er} \qquad (2)$$

where σ_G^2 is the genetic variance, σ_{GE}^2 is variance due to genotype by year interaction, σ_e^2 is the error variance, e is number of environments (number of years), and *r* is the harmonic mean of the replicates (number of relative occurrences of each genotype in a trial). The R package, lme4 (Bates et al., 2015), was used to analyze the data. The trait values derived from BLUPs were used to measure correlation with the ggcorrplot package using ggplot2 (Wickham 2016).

Genomic selection models

The genomic selection models were fitted to a univariate genomic selection model as follows:

$$y_{ij} = X\beta + Zu + \varepsilon \qquad (3)$$

Where *y* is a vector of the observed phenotype, *X* is a fixed effect matrix relating fixed effects of individuals, β is a vector of fixed effect, *Z* is a matrix of random effect, *u* is a random effect vector, and ε is a residual vector.

Seven genomic selection methods were used to predict genomic estimated breeding values in phenotypic forms: ridge regression best linear unbiased prediction approach (RR-BLUP), Gaussian kernel (GAUSS), partial least squares regression model (PLSR), elastic net (ELNET), random forest (RF), BayesCpi, and Reproducing Hilbert Kernel Space (RHKS).

The RR-BLUP approach assumes all markers have an equal contribution to the genetic variance. One of the predominant methods for predicting breeding values is RR-BLUP, comparable to the best linear unbiased predictor (BLUP) used to predict the worth of entries in the context of mixed models (Meuwissen et al., 2001). The RR-BLUP basic frame model is:

$$y = WGu + \varepsilon \qquad (4)$$

where u ~ N (0, Iσ2u) is a marker effect vector, G is the genotype matrix e.g., {aa,Aa,AA} = {–1,0,1} for biallelic single nucleotide polymorphisms (SNPs) under an additive model, and W is the design matrix relating lines to observations (y).

Often, breeders are interested in the total genotypic values rather than genomic estimated breeding values. Therefore, the Gaussian kernel model expands on the basic RR-BLUP to include epistatic effects and non-additive effects with an appropriate kernel function by reproducing kernel Hilbert space (RKHS) (Endelman 2011) to obtain total genotypic values. Both RR-BLUP and Gaussian kernel use the ‘RR-BLUP’ package to run genomic predictions.

Professor Herman Ole Andreas Wold introduced partial least square regression (PLSR) circa 1966 to deal with cases when there are more independent variables (p) than observations (n) (Colombani et al., 2012). PLSR was executed using the ‘pls’ package. In the estimation of regression parameters, PLSR can avoid multicollinearity effects which makes it suited for prediction.

Penalties from Lasso (L1 regularization) and Ridge (L2 regularization) regressions are incorporated into the elastic net (ELNET) model to select highly correlated variables to introduce a grouping effect (Zou and Hastie 2005). The ELNET model is more useful when many predictors (p) are higher than the number of observations (n), such as PLSR. The 'glmnet' package was used to develop an elastic net model (Friedman et al., 2010).

Random forest is a machine-learning algorithm-based genomic selection model that uses an average of multiple decision trees to determine predicted values. This regression model was implemented using the 'randomForest' package (Breiman, 2001).

BayesCpi was used to verify the influence of distinct genetic architectures of different traits on prediction accuracy. The BayesCpi assumes that each marker has a probability π of being included in the model, and this parameter is estimated at each Markov Chain Monte Carlo (MCMC) iteration. The vector of marker effects u is assumed to be a mixture of distributions having the probability π of being null effect and (1-π) of being a realization of a normal distribution, so that $\mathbf{u}_j \mid \pi, \sigma_g^2 \sim N(\mathbf{0}, \sigma_g^2)$ and the vector of residual effects was considered $\mathbf{e} \sim N(\mathbf{0}, \sigma_e^2)$. The marker and residual variances were assumed to follow a chi-square distribution $\sigma_g^2 \sim \chi^2(S_b, \nu_0)$ and $\sigma_e^2 \sim \chi^2(S_b, \nu_0)$, respectively $\nu_0 = 5$, degrees of freedom as prior and S_b shape parameters assuming a heritability of 0.5 (Pérez and de los Campos 2014). The last model used was the Reproducing Hilbert Kernel Space (RHKS). The method is a regression where the estimated parameters are a linear function of the basis provided by the reproducing kernel (RK). In this work, the multi-kernel approach was used by averaging three kernels with distinct bandwidth values chosen according to the rule proposed by de los Campos et al. (2010).

Genomic selection methods RR-BLUP, GAUSS, PLSR, ELNET, RF were carried out using 'GSwGBS' package (Robert Gaynorr 2015) while the Bayesian and RHKS were executed with the BGLR package (de los Campos et al., 2010). The predictive accuracy was estimated using 80% of the observations as a training set and 20 % as a test set. This process was repeated 20 times.

All statistical models were analyzed in the R environment (R Core Team, 2020). We calculated each genomic selection model's predictive ability as the correlation coefficient between predicted genomic estimated breeding values (GEBV) and best linear unbiased predictors (BLUPs) of phenotypes for individual traits. The genomic prediction models also estimated the bootstrap confidence intervals for the predictive accuracy considering 10000 samplings (James et al., 2013).

Determining optimal marker density

The markers were placed into subsets of one thousand (1 K), five thousand (5 K), ten thousand (10 K), fifteen thousand (15 K), twenty thousand (20 K), twenty-five thousand (25 K), thirty thousand (30 K), and all markers together, approximately 31 thousand (~31K) to determine optimal markers for highest prediction accuracy. A 5-fold cross-validation with 20 replicates was used to evaluate predictive ability among subsets of SNPs. The accuracies for each subset of SNPs were averaged across the replicates. All comparisons were made based on the correlation between the observed phenotype and the predicted breeding value. To evaluate the predictive ability of each subset of SNPs, we used the RR-BLUP genomic selection model.

Determining optimal training population size

The impact of training population size on predictive ability was evaluated using a validation set comprising 50 randomly selected lines and training sets of variable sizes. The validation set was formed by randomly sampling 50 lines without replacement. The training population of size n was formed sequentially by adding 25 accessions from the remaining accessions such that its size ranged between 50 to 175. We subset the collection into subgroups of 50, 75, 100, 125, 150, and 175 individuals each. The RR-BLUP model was used to predict specific traits. This procedure was repeated 20 times, and accuracies of each training population size were averaged across iterations. A similar procedure was followed to predict subpopulation 5 using variable training populations 50 to 175 with an increment of 25.

Accounting for population structure in the genomic prediction framework

We explored the confounding effect due to population structure on predictive ability. We investigated subpopulation structure on 482 accessions genotyped with 30,600 SNP markers using the ADMIXTURE clustering-based algorithm (Alexander et al., 2009). ADMIXTURE identifies K genetic clusters, where K is specified by the user, from the provided SNP data. For each individual, the ADMIXTURE method estimates the probability of membership to each cluster. An analysis was performed in multiple runs by inputting successive values of K from 3 to 20. The K-value was determined using ADMIXTURE's CV values. Based on >60% ancestry, each accession was classified into seven subpopulations (K=7). Using ADMIXTURE, we obtained eight subpopulations. Principal component (PC) analysis was also conducted to summarize the genetic structure and variation present in the collection.

To account for the effect of population structure, we included the top 10 PCs or, the Q-matrix from ADMIXTURE into the RR-BLUP model and performed five-fold cross-validation repeated 20 times. Alternatively, we also used the subpopulation (SP) designation as a factor in the RR-BLUP model. Albeit a smaller population size, we also performed a within-subpopulation prediction. As stated above, a subpopulation was defined based on >60% ancestry. Only three significant subpopulations with this cut-off were used: SP5 (N=51), SP7 (N=58), and SP8 (N=41). A leave-one-SP-out was used to predict individuals within the subpopulation with the RR-BLUP model.

Estimating reliability criteria and predicting unknown phenotypes:

The reliability criteria for each of the nonphenotyped lines were calculated using the formula (Hayes et al., 2009; Clark et al., 2012) as follows:

$$r(\mathrm{PEV}) = \sqrt{(1 - (PEV/\sigma_G^2)}$$

where PEV is the prediction error variance, and σ_G^2 is the genetic variance. Nonphenotyped entries were then predicted based on the best-performing model using SNP markers only.

Results

Phenotypic heritability and correlation

Recorded DFF had a wide range of variability from 60 to 84 days with a mean of 71 days. The estimated heritability for DFF was 0.90 (**Table 1**). For the number of seeds per pod, the mean was 5.7 with a heritability estimate of 0.84. The heritability for plant height was 0.81, with an average height of 74 cm. Pods per plant had a heritability estimate of 0.50 with a mean of 18 pods per plant and ranged from 15 to 23 pods per plant. DM had a mean of 104 days with an estimated heritability of 0.51. Seed yield per hectare ranged widely from 1734 to 4463 kg ha^{-1} with a mean yield of 2918 kg ha^{-1} and a heritability value of 0.67. The number of pods per plant was highly and positively correlated with seed yield. Correlation estimation also suggested seed yield was positively correlated with plant height (PH), days to maturity (DM), days to first flowering (DFF) (**Supplementary Figure S1**).

Predictive ability of different genomic selection models

No single model consistently performed best across all traits that we evaluated (**Table 2**), however Bayesian model BayesCpi, Reproducing Kernel Hilbert Space (RKHS), and RR-BLUP, in general, tended to generate better results. Roughly the predictive abilities from different models were similar, although slight observed differences were likely due to variations on genetic architecture and the model's assumptions underlying them. For DFF, the highest predictive ability was obtained from the RR-BLUP and GAUSS (0.60). RR-BLUP, Random Forest (RF), and RKHS models generated the highest predictive ability for pods per plant (0.28). The number of seeds per pod (NoSeedPod) was better predicted by RR-BLUP and Bayes Cpi (0.42). For plant height (PH) highest prediction accuracies were obtained from RF and BayesCpi (0.45). BaysCpi also gave the highest prediction accuracies for DM (0.47). For seed yield, RKHS had slight advantages over other models (0.42). As mentioned above, some differences between the model's accuracy were only marginal and cannot be a criterion for choosing one model (**Table 2**). For example, among the tested models, the highest difference in predictive accuracy, considering NoSeedsPod, had a magnitude of 0.02, a marginal value. The lack of significant differences among genomic prediction methods can be interpreted as either a good approximation to the optimal model by all methods or there may be a need for further research (Yu et al., 2016). Unless indicated otherwise, the rest of our results focused on findings from the RR-BLUP method.

Determining optimal marker density

In general, predictive ability increased with an increasing number of markers (**Figure 1**). The highest reported predictive ability was for the number of seeds per pod (0.30) at 30K markers. Days to first flowering, pods per plant, and plant height obtained the highest predictive ability when all ~31K markers were utilized. We obtained the highest prediction accuracy for seed yield at 15K markers (0.40) than the rest marker densities evaluated.

Determining the optimal number of individuals

Increasing the training population size led to a slight increase in the predictive ability overall for all traits. Across all traits except days to first flowering and plant height, predictive ability reached a maximum with the largest training population size of N=175 (**Figure 2**). A training population comprised of 50 individuals had the lowest predictive ability across all traits. For days to first flowering, and plant height predictive ability did steadily increase up at N= 150, and prediction ability reached the maximum for most traits at highest training population size with N=175. Regardless of population size, predictive ability was consistently higher for days to first flowering, whereas predictive ability was consistently lower for pods per plant (**Figure 2**). However, while predicting subpopulation 5 highest predictive ability was obtained for plant height (**Supplementary Figure S2**).

Accounting for population structure in the genomic prediction model

Population structure explained some portion of the phenotypic variance, ranging from 9-19%, with the highest percentages observed for plant height (19%) and seed yield (17%). Using either ADMIXTURE or PCA to account for the effect due to population structure, we improved the predictive ability. We observed a 6% improvement for days to first flowering and 32% for seed yield compared with models that did not account for population structure.

We also performed within-subpopulation predictions. Presented here are the predictive abilities for subpopulations 5, 7, and 8, as they had at least 40 entries. Subpopulation 8 had the highest predictive ability for days to first flowering (0.68), plant height (0.33), days to maturity (0.43), and seed yield (0.37). The highest predictive abilities for the number of seeds per pod (0.40) and pods per plant (0.12) were obtained from subpopulation 7 (**Table 3**). Notably, predictive ability was generally higher when all subpopulations were run in the model compared to when predictions were made within subpopulations.

Predicting nonphenotyped accessions

The genomic selection model was then used to predict nonphenotyped entries based on their marker information. Based on the distribution of predicted values, none of the predicted phenotypes for nonphenotyped accessions exceeded the top-performing observed phenotypes for seed yield (**Figure 3**). The mean seed yield of predicted entries was 2914 kg/ha, very close to the mean 2918 kg/ha of observed genotypes. The mean of observed and predicted entries were very close for the other five traits (Supplementary Table 1). The predicted phenotypes based on genomic estimated breeding values (GEBV) for number of pods per plant, number of seeds per pod (**Supplementary Figure S3 and S4**), days to first flowering, and days to maturity all fall within the range of observed phenotypes (Similar Figures not added).

Reliability estimation

We obtained reliability criteria across six traits on seed yield and phenology for the 244 nonphenotyped accessions. The average reliability values ranged from 0.30 to 0.35, while the top values ranged from 0.75 to 0.78 for evaluated traits. The higher reliability values were distributed in the top, bottom, and intermediate predicted breeding values (**Supplementary Table S2 to S7**). For seed yield (kg ha^{-1}), the highest reliability was obtained from the bottom 50 genomic estimated breeding values (GEBV) (**Figure 4**). Higher reliability criteria are primarily distributed among the intermediate and top GEBVs for days to first flowering. Predicted intermediate plant height showed the highest reliability, as presented in **Figure 4**.

Discussion

Widely utilized plant genetic resources collections, such as the USDA pea germplasm collection, hold immense potential as diverse genetic resources to help guard against genetic erosion and serve as unique sources of genetic diversity from which we could enhance genetic gain, boost crop production, and help reduce crop losses due to disease, pests, and abiotic stresses (Crossa et al., 2017; Holdsworth et al., 2017; Jarquin et al., 2016; Mascher et al., 2019). As the costs associated with genotyping on a broader and more accurate scale continue to decrease, opportunities increase to utilize these collections in plant breeding. Relying on phenotypic evaluation alone can be costly, rigorous, and time-intensive. However, by incorporating high-density marker coverage and efficient computational algorithms, we can better realize the potential for utilizing these germplasm stocks by reducing the time and cost associated with their evaluation (Yu et al., 2016; Li et al., 2018; Yu et al., 2020). In this study, we evaluated the potential of genotyping-by-sequencing derived markers for genomic prediction. We found that it holds promises for extracting useful diversity from germplasm collections for applied breeding.

In this study, prediction ability values were generally similar among methods, and there was no single model that worked across traits, consistent with results obtained by other authors (Burstin et al., 2015; Spindel et al., 2015; Yu et al., 2016; Azodi et al., 2019). For example, considering only the punctual estimates, RR-BLUP and Gaussian kernel models were the best for DFF, however for PH, DM, and seed yield, the best models were BayesCpi and RF, BayesCpi and RKHS, respectively. In recent work, Azodi et al., (2019) compared 12 models (6 linear and 6 non-linear) considering 3 traits through 6 different plant species, and they did not find any best algorithm for all species and all traits. Newer statistical methods are expected to boost prediction accuracy; however, the biological complexity and unique genetic architecture of traits can be regarded as the root cause for getting zero or slight improvement on prediction accuracy (Yu et al., 2020; Valluru et al., 2019). As data collection accelerates in at different levels of biological organization (Kremling et al., 2019), genomic prediction models will expand and nonparametric models, including machine learning, may play an essential role for boosting prediction accuracy (Azodi et al., 2019; Yu et al., 2020).

A related work in pea has been published but only based on a limited number of markers (Burstin et al., (2015). This work assessed genomic prediction models in a diverse collection of 373 pea accessions with 331SNPs markers and found no single best model across traits, which is consistent with our findings. In this work, the authors reported that traits with higher heritability, such as thousand seed weight and flowering date, were easier to predict, which is expected. We also verified DFF as having the highest heritability and predictive accuracies through all the models. Interestingly, yield components like the number of seeds per pod and pods per plant showed lower predictive accuracy, independent of the model. Consistent with our results, Burstin et al. (2015) also found yield components (seed number per plant) as having lower predictive accuracy and higher standard deviation for prediction. This trait is highly influenced by the environment and showed a lower correlation for prediction coefficients through the years.

We observed an increase in predictive ability for traits as the number of SNPs included in the model increased, but beyond 15K markers, we noted a slight decrease in prediction accuracy for seed yield. Such a decrease in the prediction accuracy could be due to overfitting the model with too many markers resulting in a reduced predictive ability after saturation could be due to the non-genetic effects of the beyond saturated markers (Norman et al., 2018; Hickey et al., 2014). Similarly, the predictive ability increased for all traits except plant height when we increased the model's training population size, suggesting that adding more entries in the study could boost predictive ability. By accounting population structure into genomic prediction framework, we observed an improved prediction accuracy for some traits – seed yield and DFF – but not others. Although the population structure explained 9-19% of the phenotypic variance, we cannot fully and conclusively answer the effect of population structure in prediction accuracy due to smaller population size. In addition, the relatedness among individuals in the training and testing sets needs to be accounted for (Lorenz and Smith, 2015; Rutkoshi et al., 2015; Riedelsheimer et al., 2013).

Previous studies have indicated the importance of considering reliability values when using prediction ability values to select genotypes (Yu et al., 2016). Our study found higher reliability estimates to be spread across all predicted values rather than clustering around one extreme prediction or another. Such findings are advantageous as an extreme predicted value is not always the target for selection. Those accessions with top predicted values and high-reliability estimates would be most well-suited as candidates for a breeding program in selecting for seed yield. However, for a trait such as days to flowering in pea, even low or intermediate predicted values would be suitable candidates when paired with high-reliability values. When predicting nonphenotyped accessions, the means of those predicted entries were close to observed accessions and did not exceed phenotyped germplasm accessions for seed yield. Several accessions in the USDA pea germplasm collection could be readily incorporated into breeding programs for germplasm enhancement by incorporating above-average accessions with high or moderately high-reliability values (Yu et al., 2020).

Conclusions and Research Directions

The research findings demonstrated that the wealth of genetic diversity available in a germplasm collection could be assessed efficiently and quickly using genomic prediction to identify valuable germplasm accessions that can be used for applied breeding efforts With the integration of more orthogonal information into genomic prediction framework (Kremling et al., 2019; Valluru et al., 2019) coupled with the implementation of more complex genomic selection models like a multivariate genomic selection approach (Rutkoski et al., 2015), we can considerably enhance predictive ability. This research framework could greatly contribute to help discover and extract useful diversity targeting high-value quality traits such as protein and mineral concentrations from germplasm collection.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

NBB, CJC, and MAB conceived and designed the manuscript. CJC, DM, and RMcG designed and executed the field and genotyping experiments. YM and PZ performed DNA extraction, constructed the library, and called SNPs. MAB, IV, and SS analyzed data, curated SNPs, and ran genomic selection models. NBB oversaw statistical analyses. MAB, HW, IV, and NBB wrote and edited the overall manuscript. All authors edited, reviewed, and approved the manuscript.

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Table 1. Heritability and summary statistics for seed yield and other agronomic traits

Trait	Mean	Range	SD	CV(%)	H^2
DFF (days)	71	60-84	4.8	6.7	0.90
NoSeedsPod (Nos.)	5.7	4.4-6.9	0.5	8.5	0.84
PH (cm)	74	37.6-108.3	11.5	15.5	0.81
PodsPlant (Nos.)	18	15-23	1.5	8.3	0.50
DM (days)	104	99-112	2.4	2.3	0.51
SeedYield (Kg ha^{-1})	2918	1734-4463	451	15.4	0.67

DFF is days to first flowering; NoSeedsPod is the number of seeds per pod, PH is plant height, PodsPlant is the number of pods per plant, DM is days to physiological maturity, SeedYield is seed yield per hectare, SD is the standard deviation, CV is coefficient of variance, H^2 is heritability in the broad sense.

Table 2. Predictive ability of genomic selection models for seed yield and agronomic traits

Traits	**RR-BLUP**	**GAUSS**	**PLSR**	**ELNET**	**RF**	**BayesCpi**	**RKHS**
DFF (days)	0.60 (0.57-0.63)	0.60 (0.58-0.63)	0.57 (0.53-0.61)	0.57 (0.52-0.61)	0.55 (0.52-0.58)	0.59 (0.55-0.63)	0.54 (0.5-0.58)
NoSeedPod	0.42 (0.37-0.48)	0.41 (0.37-0.47)	0.41 (0.36-0.46)	0.41 (0.35-0.48)	0.40 (0.35-0.45)	0.42 (0.38-0.46)	0.40 (0.34-0.48)
PH (cm)	0.39 (0.33-0.44)	0.38 (0.33-0.44)	0.42 (0.38-0.48)	0.37 (0.31-0.42)	0.45 (0.4-0.5)	0.45 (0.41-0.48)	0.43 (0.39-0.48)
PodsPlant	0.28 (0.22-0.33)	0.26 (02-0.32)	0.25 (0.2-0.31)	0.23 (0.17-0.29)	0.28 (0.22-0.34)	0.23 (0.17-0.29)	0.28 (0.23-0.34)
DM (days)	0.42 (0.36-0.47)	0.41 (0.36-0.47)	0.44 (0.39-0.5)	0.40 (0.34-0.46)	0.41 (0.35-0.46)	0.47 (0.43-0.5)	0.45 (0.4-0.48)
SeedYield (kg ha-1)	0.38 (0.34-0.42)	0.38 (0.34-0.42)	0.31 (0.27-0.36)	0.38 (0.33-0.48)	0.39 (0.35-0.44)	0.35 (0.31-0.39)	0.42 (0.37-0.48)

DFF is days to first flowering, PH is Plant height in cm, DM is days to physiological maturity.

Table 3. Predictive ability within and across subpopulations using RR-BLUP and all markers

Sub pops	DFF	NoSeedPod	PH	PodsPlant	DM	SeedYield
Sub pop 5 (51)	0.27	0.26	0.08	-0.01	0.02	0.18
Sub pop 7 (58)	0.34	0.40	0.22	0.12	-0.01	0.01

Sub pop 8 (41)	0.68	0.35	0.33	0.07	0.43	0.37
SP-	0.50	0.45	0.47	0.25	0.51	0.34
SP+	0.53	0.35	0.42	0.25	0.48	0.45
SP PC10	0.51	0.41	0.44	0.18	0.20	0.43
Var exp (R^2)	0.13	0.09	0.19	0.15	0.15	0.17

DFF is days to first flowering, PH is plant height, DM is days to physiological maturity, SP- does not account for population structure, SP+, refers to the population structure addressed in the model, SP PC10 addresses population structure with 10 PC, Var exp (R^2) refers the variance explained by population structure after fitting a regression model, within parenthesis represent the number of entries in each subpopulation.

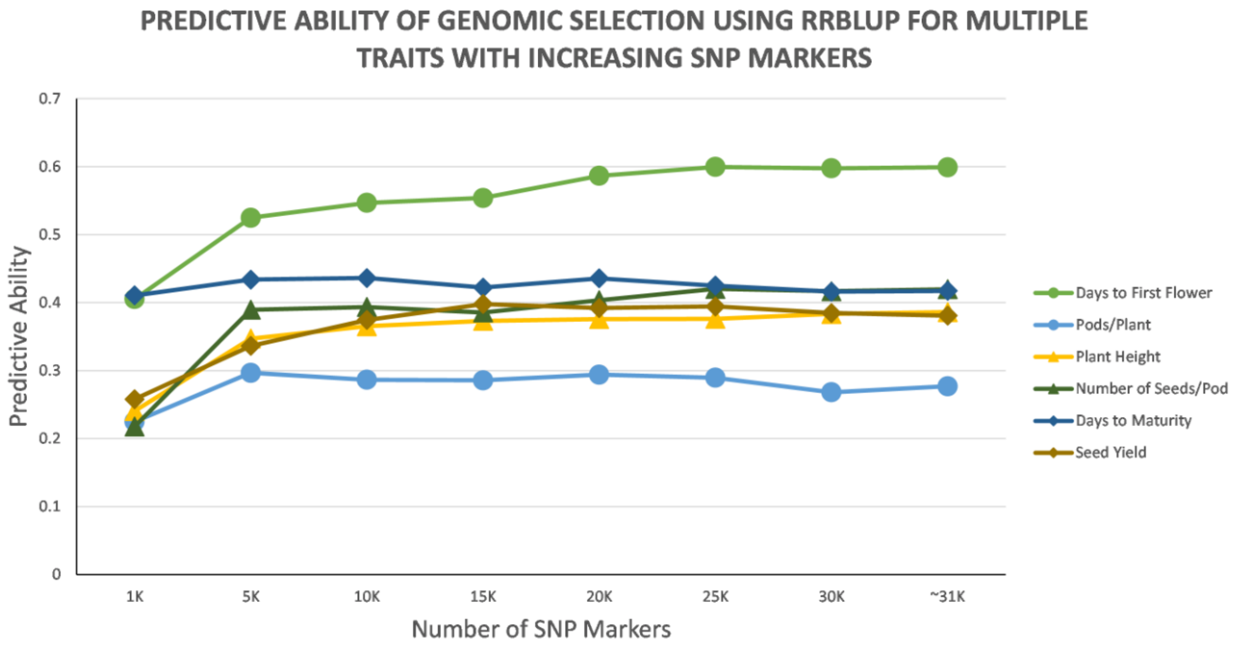


Figure 1. Predictive ability with an increasing number of markers using different models, the x-axis markers are in kilo (K) base pairs, and genomic selection models are within parentheses

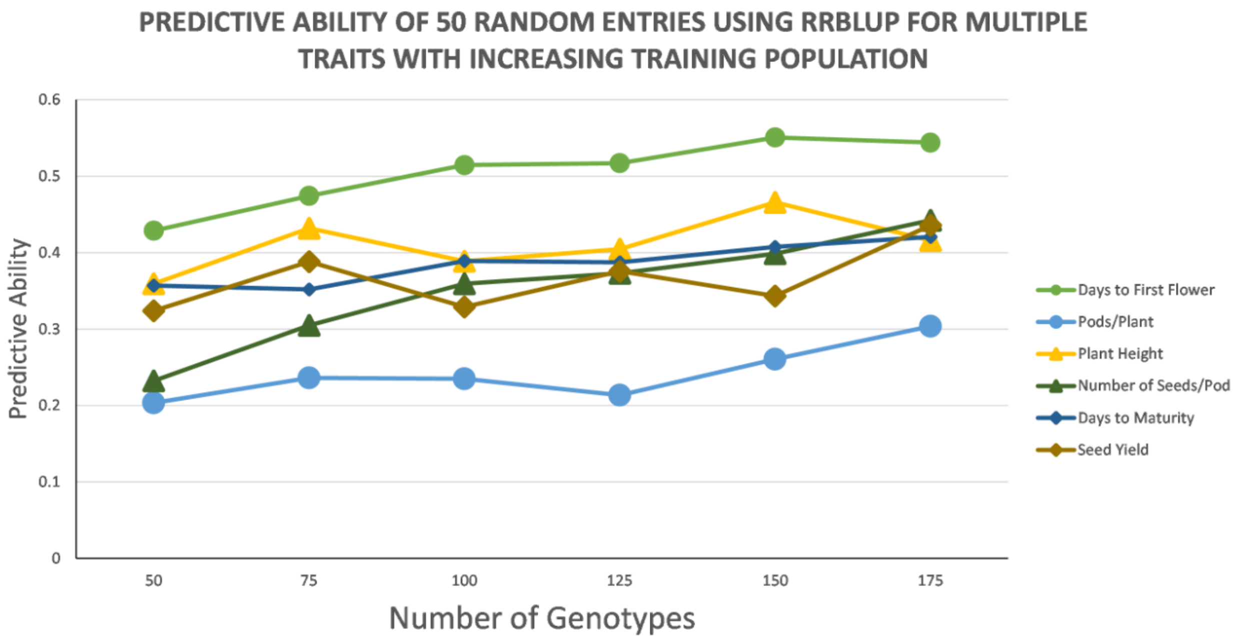


Figure 2. Predictive ability with increasing population size, the x-axis represents the number of populations used in the genomic selection model, and the y-axis is the predictive ability

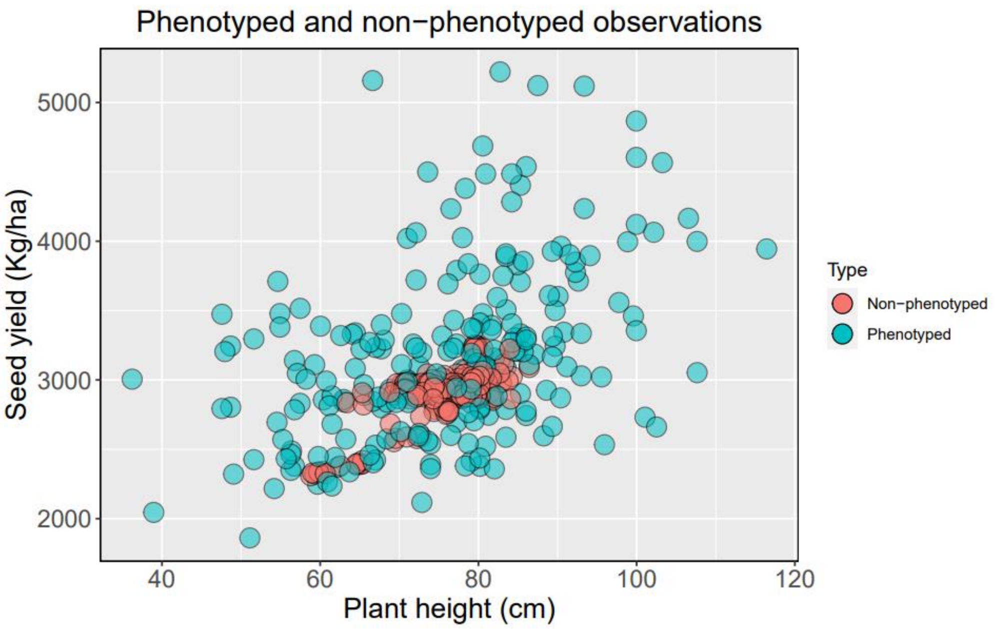
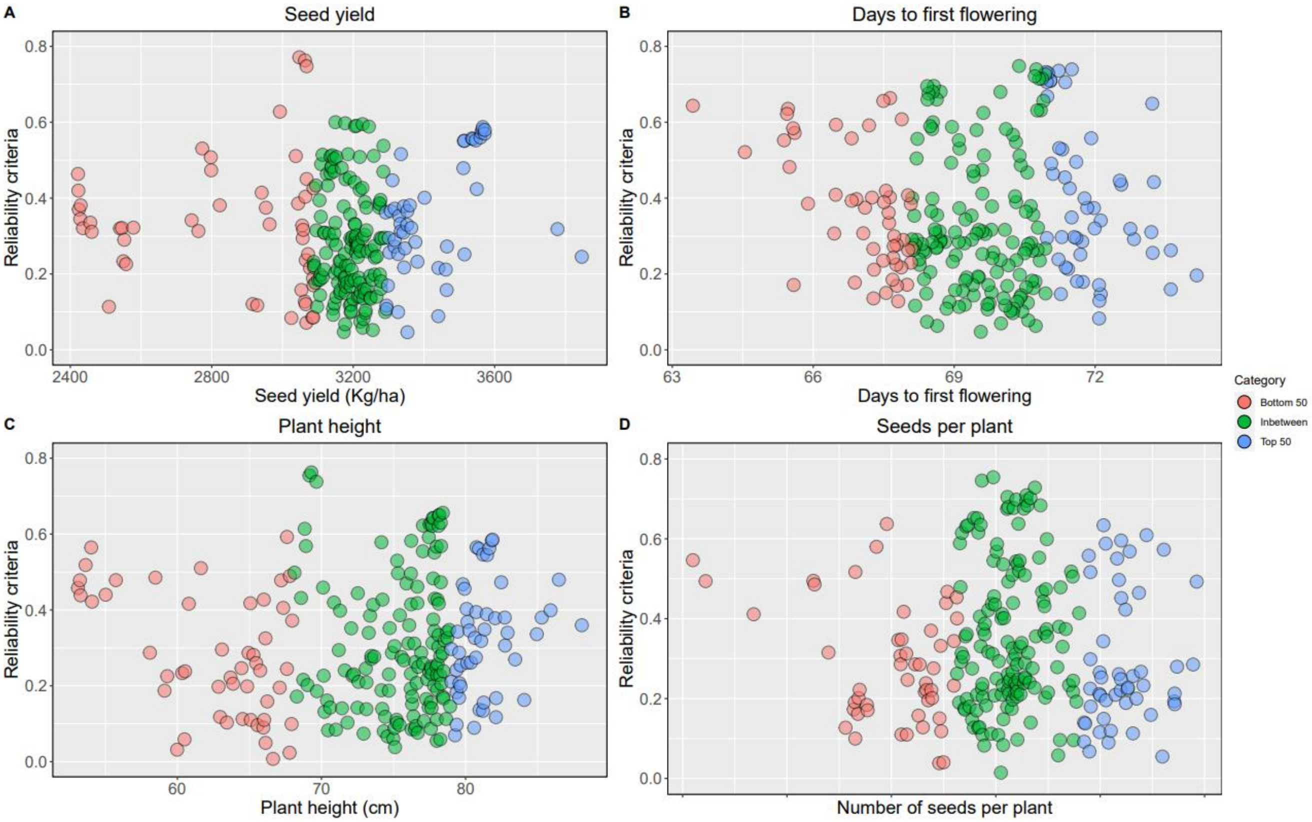


Figure 3. Distribution phenotyped and predicted non-phenotyped accessions of USDA pea germplasm collections for seed yield and plant height



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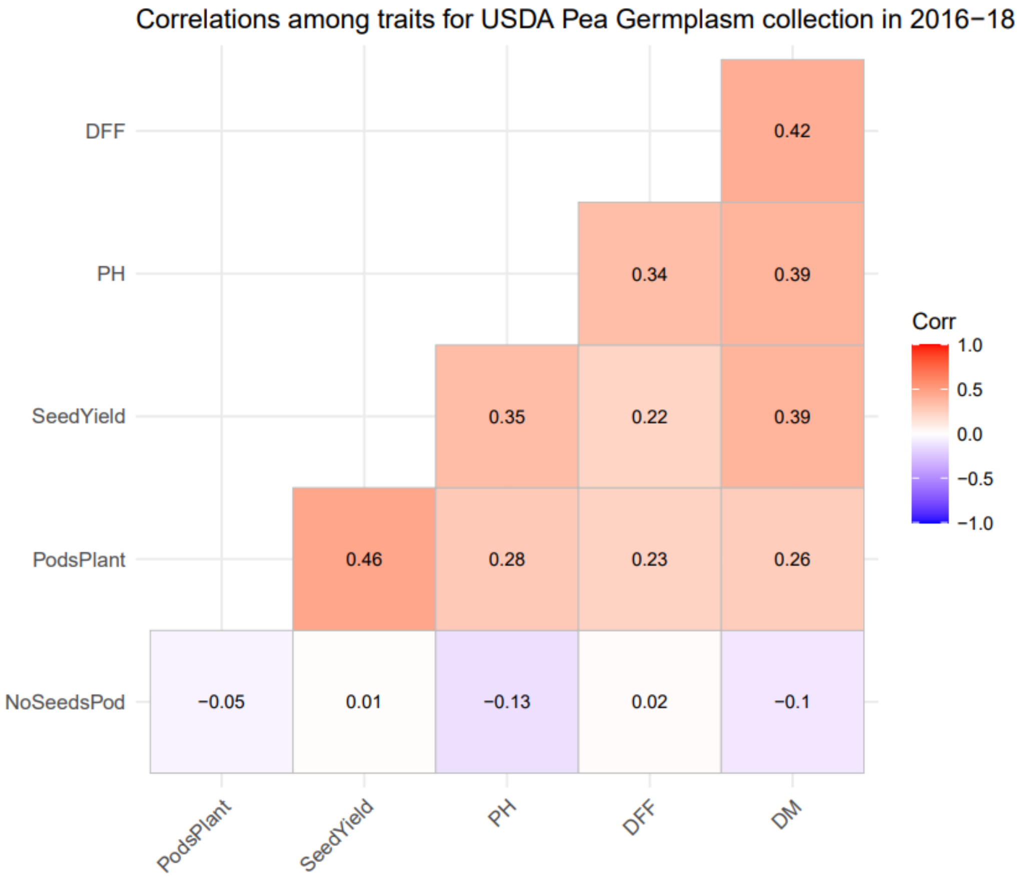
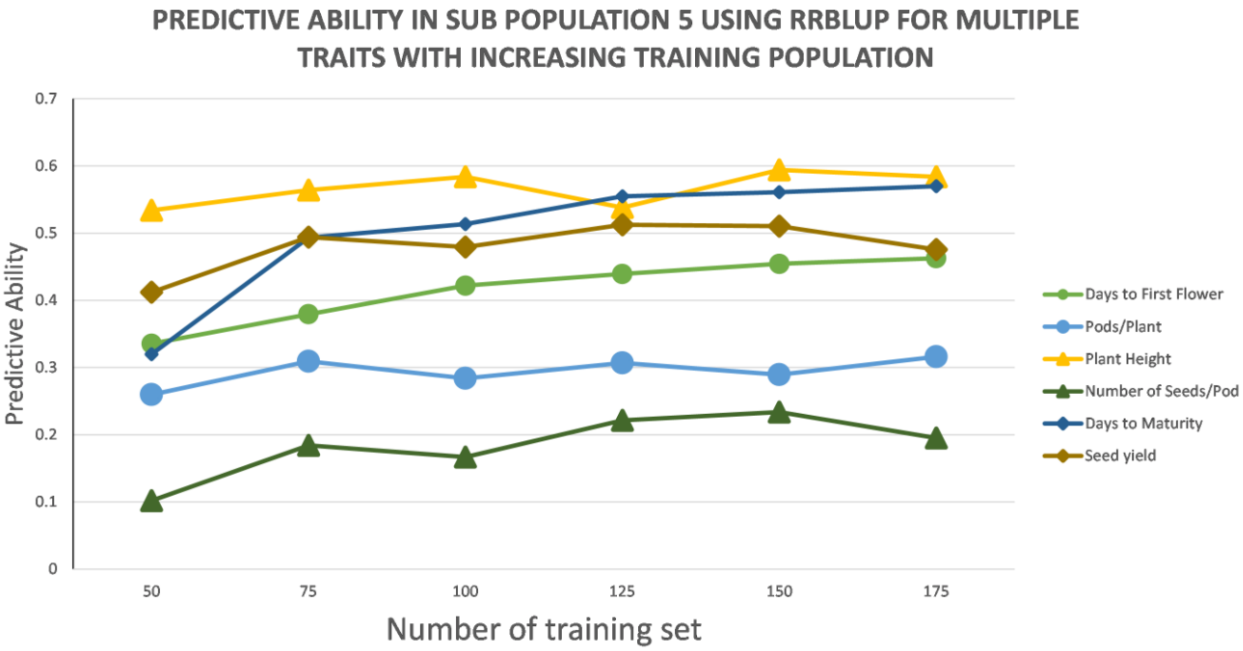
677 Figure 4. Reliability criteria for nonphenotyped lines, the top 50 of the genomic estimated breeding values are blue, and bottom 50 are in red, intermediates are in green. A. reliability estimates for seed

678 yield (Kg/ha), B. days to first flowering, C. plant height, D. seeds per plant



Supplementary Figure S1. Phenotypic correlation among seed yield and agronomic traits evaluated in this study, DFF is days to first flowering, PH is plant height in cm, SeedYield is seed yield in kg ha^{-1}, DM is the days to physiological maturity



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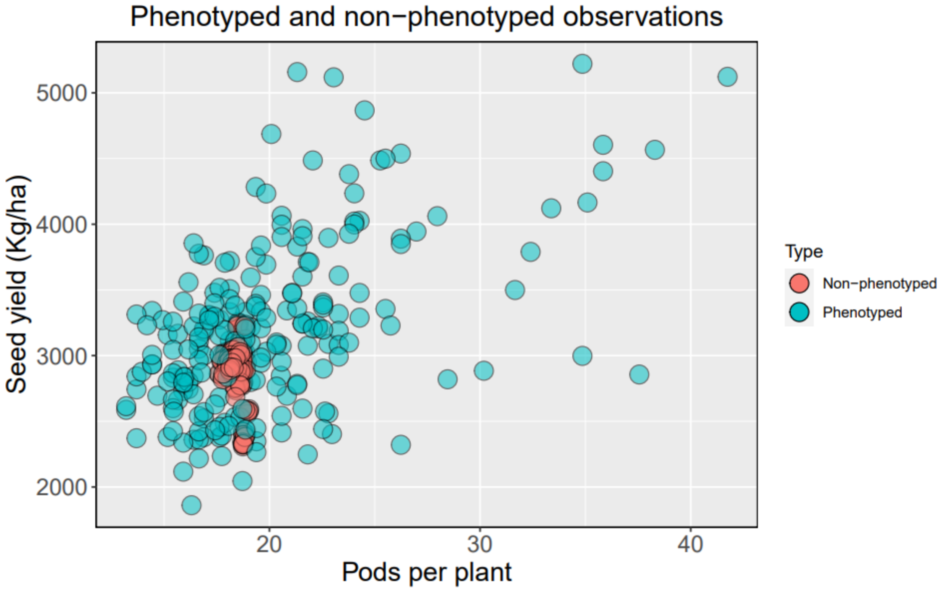
684 Supplementary Figure S2. Predictive ability of subpopulation 5 with increasing training
685 population



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687 Supplementary Figure S3. Distribution of phenotyped and predicted non-phenotyped accessions
688 for seed yield and number of pods per plant in the USDA germplasm collections

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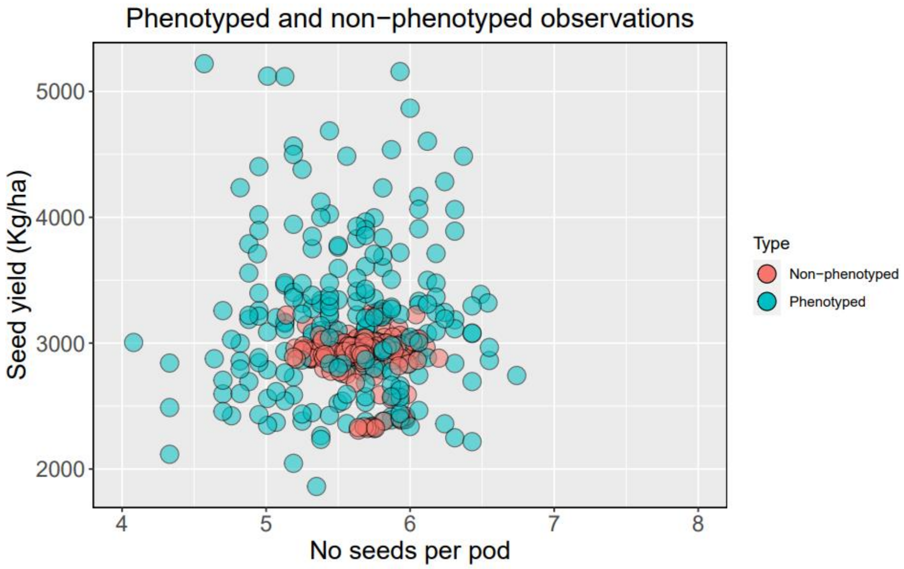

Supplementary Figure S4. Distribution of phenotyped and predicted non-phenotyped accessions for seed yield and number of seeds per pod in the USDA germplasm collections