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Genetic Gain from Phenotypic and Genomic Selection for Quantitative Resistance to Stem Rust of Wheat

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Abstract

Stem rust of wheat (*Triticum aestivum* L.) caused by *Puccinia graminis* f. sp. *tritici* Eriks. and E. Henn. is a globally important disease that can cause severe yield loss. Breeding for quantitative stem rust resistance (QSRR) is important for developing cultivars with durable resistance. Genomic selection (GS) could increase rates of genetic gain for quantitative traits, but few experiments comparing GS and phenotypic selection (PS) have been conducted. Our objectives were to (i) compare realized gain from GS based on markers only with that of PS for QSRR in spring wheat using equal selection intensities; (ii) determine if gains agree with theoretical expectations; and (iii) compare the impact of GS and PS on inbreeding, genetic variance, and correlated response for pseudo-black chaff (PBC), a correlated trait. Over 2 yr, two cycles of GS were performed in parallel with one cycle of PS, with each method replicated twice. For GS, markers were generated using genotyping-by-sequencing, the prediction model was initially trained using historical data, and the model was updated before the second GS cycle. Overall, GS and PS led to a 31 ± 11 and 42 ± 12% increase in QSRR and a 138 ± 22 and 180 ± 70% increase in PBC, respectively. Genetic gains were not significant but were in agreement with expectations. Per year, gains from GS and PS were equal, but GS led to significantly lower genetic variance. This shows that while GS and PS can lead to equal rates of short-term gains, GS can reduce genetic variance more rapidly. Further work to develop efficient GS implementation strategies in spring wheat is warranted.

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STEM RUST OF WHEAT caused by the fungal pathogen *P. graminis* is a globally widespread and highly damaging disease capable of causing severe yield losses in susceptible cultivars (Park, 2007). In 1998, a new race group, Ug99, then capable of infecting over 80% of the world's wheat germplasm (Singh et al., 2008), was discovered in Uganda. Ug99 has since migrated as far north as Iran, and has evolved to overcome an even larger set of major-effect resistance genes, increasing the susceptibility of commercially grown cultivars to about 90% (Jin et al., 2008, 2009). The emergence and continued evolution of Ug99 has prompted efforts to rapidly develop

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Abbreviations: BLUP, best linear unbiased prediction; C_0, cycle zero population; C_1, cycle one population; C_2, cycle two population; G-BLUP, genomic best linear unbiased prediction; GBS, genotyping-by-sequencing; GS, genomic selection; PBC, pseudo-black chaff; PS, phenotypic selection; QSRR, quantitative stem rust resistance; TP, training population.

Ug99-resistant cultivars adapted to vulnerable regions. The deployment of QSRR, also referred to as slow-rusting adult plant resistance, is advocated because it is generally more durable (Parlevliet, 2002).

Pseudo-black chaff, black discoloration on the glumes and stems, is associated with QSRR. At least four loci are involved in PBC expression including *Sr2* (Hare and McIntosh, 1979; Bariana et al., 2001; Yu et al., 2011; Singh et al., 2013), which is associated with both PBC and durable QSRR. Moreover, the *Sr2*-linked PBC expression can be modified in either direction due to modifiers at other loci (Bhowal and Narkhende, 1981). Although PBC can be a useful morphological marker for QSRR, it is an undesirable trait because in farmers' fields' it can give the appearance of the black chaff disease produced the bacterium *Xanthomonas translucens* f. sp. *undulosa* (E. F. Sm., L.R. Jones & Reddy) Hagb. (Hagborg, 1942). Breeding programs often select plants with low PBC expression.

Genomic selection may be an appropriate marker-assisted breeding strategy for QSRR. With GS, reviewed by Heffner et al. (2009) and Lorenz et al. (2011), a statistical model trained with phenotypic and genotypic data from a relevant population is used to predict, based on markers only, the breeding values of new selection candidates that have been genotyped. This enables selection to occur before phenotyping, potentially leading to greater genetic gain per unit time.

Selection experiments are useful for comparing breeding methods, assessing responses to selection, identifying what unselected traits may exhibit correlated selection responses and measuring changes in inbreeding and genetic variance. Few GS experiments have been conducted. Massman et al. (2013) achieved 14 to 50% greater gain from GS compared with marker-assisted recurrent selection. Asoro et al. (2013) found that selection based on markers and phenotype was significantly more effective than selection based on pedigree and phenotype.

Very few realized genetic-gain experiments have been done comparing GS based on markers only with PS in crop plants (Combs and Bernardo, 2013), and none have been reported in wheat. Comparing GS with PS is important because in many crops, such as wheat, PS is the most commonly used breeding method for quantitative traits. The objectives of this study were to (i) compare realized genetic gain per unit time from GS based on markers only with that of PS for QSRR in spring wheat, (ii) determine if realized gains are consistent with expected gains based on selection theory, and (iii) compare GS with PS in terms of impact on inbreeding and genetic variance and correlated response for PBC.

Materials and Methods

Genetic Material

A historical population, used for initial GS model training, consisted of 374 lines selected from the CIMMYT international stem rust screening nurseries. Major resistance genes effective against stem rust race TTKST were confirmed absent in 365 of the individuals based on seedling tests conducted at the USDA–ARS Cereal Disease Laboratory, St. Paul, Minnesota (Rutkoski et al., 2014).

The cycle-zero population, C_0, was founded from 14 individuals selected from the historical population based on their agronomic performance, complementarity for different traits and absence of major resistance genes effective against TTKST. Quantitative stem rust resistance levels among the founders ranged from moderately resistant to moderately susceptible. To generate C_0, the founders were intermated for two generations by hand pollination. Matings were designed to mimic a large panmictic population. For the first round of intermating, a partial-diallel crossing scheme (Kempthorne and Curnow, 1961), with each parent involved in seven cross combinations, was used to generate 49 F_1 progenies. The F_1 progenies were confirmed based on simple-sequence repeat genotyping. In the next round of intermating, F_1 progenies were intercrossed so that each participated in at least one cross and crosses between F_1 progenies with common parents were avoided. Eighty-four double cross F_1 progenies resulted and were selfed to increase seed, resulting in double cross F_2 progenies. Five hundred four double cross F_2 individuals sampled from each of the 84 families became C_0. To measure the variance in selection response, the 504 individuals were split into two replicate populations of size 252.

Breeding Schemes

Each replicate of C_0 underwent one cycle of PS and two cycles of GS (Fig. 1). For the first GS cycle, C_0 individuals were genotyped, and their breeding values were predicted using the 374 historical lines for model training. The best five C_0 individuals were selected and intermated based on their S_1 progeny using a half-diallel crossing scheme. At least six S_1 progenies were used for each selected individual and two to three successful crosses were made per combination. One generation of self-pollination was performed after intermating for seed increase to create the GS cycle-one populations (C_1) of size 272 and 285 for replicate one and two, respectively. For the second GS cycle, the 504 C_0 individuals, genotyped and phenotyped for two seasons, were added to the model training population (TP) because the initial TP was not closely related to C_1. Cycle-one population individuals were genotyped, their breeding values predicted, and the best five C_1 individuals were selected. Intermating and seed increase was performed as before to create the GS cycle-two populations (C_2). For PS, C_0 individuals were selected using a pedigree best linear unbiased prediction (BLUP) model that included individuals' own phenotypic records from two seasons. Pedigrees traced back to the founders of C_0. The best five C_0 individuals were selected. Intermating and seed increase was performed as before to create the PS C_1 populations. Full details about the genotyping, phenotyping, and statistical modeling procedures are described in the subsequent sections.

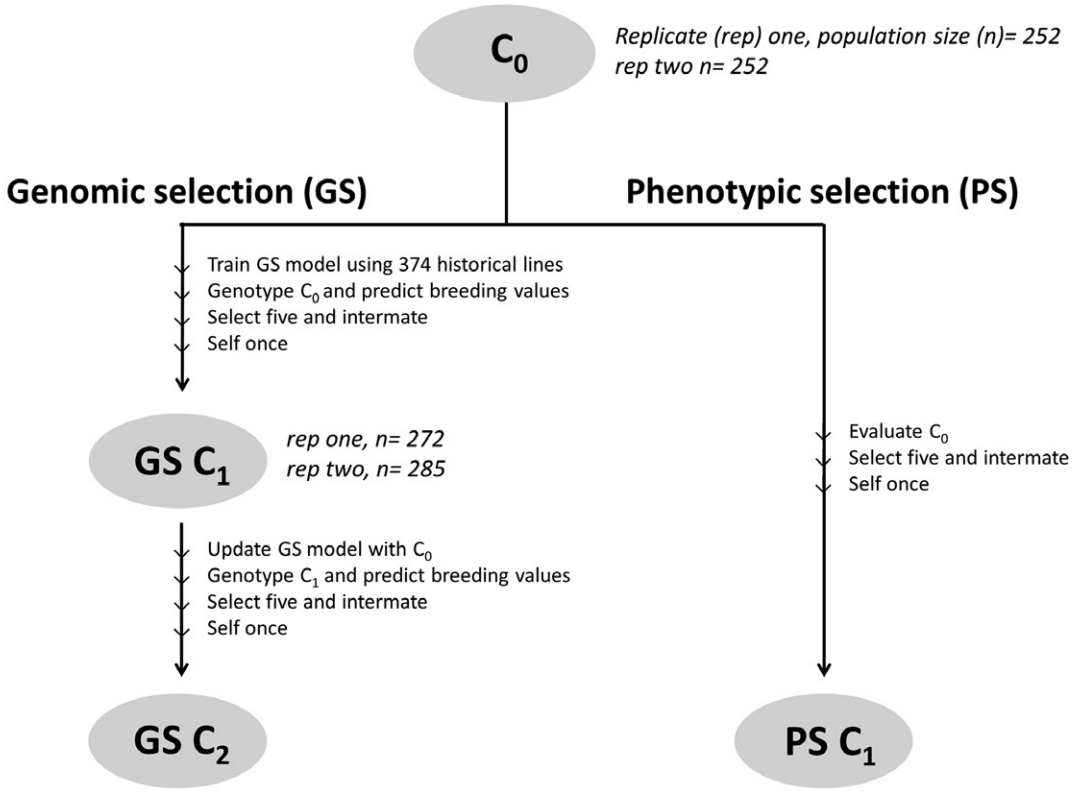

Figure 1. Genomic selection (GS) and phenotypic selection (PS) schemes. C_0 is the cycle zero population; PS C_1 is the population resulting from one cycle of PS; and GS C_1 and GS C_2 are the populations resulting from one and two cycles of GS, respectively.

Genotypic Data

Genotypic data was generated and processed in two batches: the first was before the first cycle of selection and included the historical and C_0 populations, and the second was before the second cycle of selection and included the GS C_1 population. All genotypic data were generated using genotyping-by-sequencing (Elshire et al., 2011) according to the protocol described by Poland and Endelman et al. (2012). Due to the heterozygosity in C_0 and C_1, polymorphisms were called in C_0 and C_1 based on the polymorphic markers that were discovered in the historical population. A total of 27,434 markers were scored. Due to relatively low read depth, a large proportion of heterozygous loci were likely called as either parental allele at random. Marker genotypes were recoded as –1, 0, or 1; homozygotes for the minor allele were coded as –1, heterozygotes were coded as 0, and homozygotes for the major allele were coded as 1. For the first batch of genotypic data, markers with more than 80% missing data were removed, resulting in 20,882 markers (Supplementary Dataset S1). For the second batch, markers with pairwise $r^2 < 1$ were selected and markers with ≥80% missing data were removed, resulting in 18,653 markers (Supplementary Dataset S2). Mean imputation was used to handle missing data (Rutkoski et al., 2013). In C_0 and C_1, mean imputation was performed within full-sib families.

Phenotypic Data

Genomic Selection Model Training and Phenotypic Selection

The model training population for the first cycle of GS (the historical population of 374 lines) was evaluated for QSRR at the international Ug99 stem rust screening nurseries at the Kenya Agricultural Research Institute, Njoro, Kenya, and the Ethiopian Institute of Agricultural Research, Debre Zeit, Ethiopia, between 2005 and 2011. There were 16 total environments with each individual appearing in about four environments (Supplementary Dataset S3). Planting and inoculation details were as described by Rutkoski et al. (2014). For PS and GS model updating before GS C_2, all individuals from C_0 with adequate seed were evaluated in the Njoro 2012 off-season, June through October, and the Njoro 2012 main season, December through April, using an augmented lattice square design (Federer, 2002). Checks consisted of 60 individuals from historical and selection candidate populations with abundant seed. Planting and inoculation procedures were consistent with previous seasons. Individuals from C_0 were evaluated based on their S_1 or S_2 progeny.

Realized and Expected Gain Estimation

The zero-cycle, one-cycle, and two-cycle populations were evaluated based on their S_1 or S_2 progenies in Njoro and Debre Zeit during the 2014 off-season, January through May, to estimate cycle means, genetic variances,

Table 1. Mean stem rust severity, mean pseudo-black chaff (PBC), mean level of inbreeding, and genetic variance for each population and for each selection method.

Population†		Number of individuals evaluated	Mean stem rust severity	Mean PBC	Mean level of inbreeding	Genetic variance
C_0	Rep. 1	240	39.14	0.56	0.5	79.92
	Rep. 2	241	37.81	0.53	0.5	74.42
	Mean	241	38.48 ± 0.66	0.54 ± 0.02	0.5	77.17 ± 2.75
PS C_1	Rep. 1	94	18.11	1.96	0.57	81.67
	Rep. 2	241	26.42	1.11	0.53	102.3
	Mean	168	22.26 ± 4.15	1.54 ± 0.43	0.55 ± 0.02	91.98 ± 10.31
GS C_1	Rep. 1	258	35.03	1.14	0.57	70.44
	Rep. 2	267	33.67	0.67	0.59	76.13
	Mean	263	34.35 ± 0.68	0.91 ± 0.23	0.58 ± 0.01	73.29 ± 2.85
GS C_2	Rep. 1	288	31.3	1.21	0.69	33.33
	Rep. 2	280	22.31	1.37	0.73	51.14
	Mean	284	26.8 ± 4.5	1.29 ± 0.08	0.71 ± 0.02	42.23 ± 8.91

† C_0, cycle zero; PS C_1, phenotypic selection cycle one; GS C_1, genomic selection cycle one; GS C_2, genomic selection cycle two; Rep., replicate.

and genetic correlations (Supplementary Dataset S6). Experimental design, planting, and inoculation procedures were the same as those used for C_0 evaluation described above, except there were 64 checks. Urediniospores of races TTKST and TTKSK (Ug99 lineage) of stem rust were used for inoculating field trials at Njoro and Debre Zeit, respectively. Quantitative stem rust resistance was evaluated in both locations, and PBC on the glumes was scored on a zero-to-five scale at Njoro where conditions were favorable for PBC expression. The number of individuals evaluated per population to estimate realized and expected gains is shown in Table 1. Entry mean heritabilities within and across environments were calculated according to Hallauer et al. (2010).

Statistical Models for Selection

Genomic Selection Cycle One

Genomic selection model training was done in two stages. In the first stage, genetic values of the historical individuals were estimated. The R (R Development Core Team, 2010) package lme4 (Bates and Maechler, 2010) was used to fit the mixed model:

$$y_{ij} = \mu + \beta_i + g_j + \varepsilon_{ij}$$

where y_{ij} is the phenotype, μ is the mean, β_i (i = 1,...16) is fixed environment effect, g_j (j = 1,…374) is a random genotype effect with $g_j \sim N(0, \sigma^2_g)$, where σ^2_g is the genetic variance, and ε_{ij} is the residual with $\varepsilon_{ij} \sim N(0, \sigma^2_\varepsilon)$ where σ^2_ε is the residual variance.

In the second stage, genetic values g_j (j = 1,…374) of the historical individuals were used in Bayesian ridge regression (Pérez et al., 2010), based on the following model:

$$g_j = \sum_{k=1}^{20,882} x_{jk} m_k$$

where x_{jk} (k = 1,…20,882) are the marker genotypes and m_k are the marker effects. Bayesian ridge regression assumes Gaussian distributed marker effects with common marker variances and is the Bayesian equivalent to ridge regression BLUP. Predicted breeding values of the C_0 individuals were estimated as $\sum_{k=1}^{20,882} x_{jk}\hat{m}_k$.

Phenotypic Selection Cycle One, and Genomic Selection Cycle Two

To estimate genetic values for PS C_1 and to train the GS model before GS C_2, we estimated historical and C_0 genetic values using ASReml-R (Gilmour et al., 2009) to fit the following mixed model:

$$y_{ijk} = \mu + \beta_i + g_j + r_k + \varepsilon_{ijk}$$

where y_{ijk} is the phenotype, μ is the mean, β_i (i = 1,2) is fixed environment effect, g_j (j = 1,…504) is a random genotype effect with $g_j \sim N(0, \mathbf{A}\sigma^2_g)$ where $\mathbf{A}$ is a relationship matrix calculated using pedigrees that traced back to the C_0 founders (Supplementary Dataset S5) and σ^2_g is the genetic variance, r_k (k = 1,2) is random block effect for blocks only within the Njoro main season 2012 environment with $r_k \sim N(0, \sigma^2_r)$ where σ^2_r is the block variance, and ε_{ijk} is the residual. A two-dimensional, first-order autoregressive (AR1 × AR1) variance model was used to model the plot errors in the row and column direction (Gilmour et al., 1997) within the Njoro off-season 2012 environment. The solutions for g_j were used as the estimates of genetic values.

The R package rrBLUP (Endelman, 2011) was used to implement the genomic BLUP (G-BLUP) model

$$\mathbf{g} = \mathbf{Zu} + \varepsilon$$

where $\mathbf{g}$ is a vector of the genetic values of the historical and C_0 individuals, $\mathbf{u}$ is a vector of genomic estimated breeding values with $\mathbf{u} \sim N(0, \mathbf{G}\sigma^2_u)$ where σ^2_u is the genetic variance, $\mathbf{G} = \mathbf{MM}'$ is a marker-based relationship matrix containing the historical, C_0 and C_1 individuals, where $\mathbf{M}$ is the a matrix numerically coded marker genotypes, and ε is as vector of residuals $\varepsilon \sim N(0, \sigma^2_\varepsilon)$ where σ^2_ε is the residual variance. Breeding value predictions from G-BLUP with $\mathbf{G} = \mathbf{MM}'$ are equivalent to those from ridge regression BLUP (Hayes et al., 2009).

Realized Gain Calculations

Stem Rust Quantitative Resistance

Adjusted populations means for each replicate of C_0, GS C_1, PS C_1, and GS C_2 were calculated using the mixed model:

$$y_{ijkl} = \mu + \beta_i + g_j + r_k + p_l + \varepsilon_{ijkl}$$

where, y_{ijkl} is the phenotype, μ is the mean, β_i (i = 1,2) is a fixed environment effect, g_j (j = 1,…1950) is a random genotype effect with $g_j \sim N(0, \sigma_g^2)$, where σ_g^2 is the genetic variance, r_k (k = 1,2) is random effect for blocks in the Njoro environment where $r_k \sim N(0,\sigma_r^2)$ and where σ_r^2 is the block variance, p_l (l = 1,…8) is a fixed population effect, and ε_{ijkl} is the residual. An AR1 × AR1 variance model was used to model the variance structure of the plot errors in the row and column direction within the Njoro environment. The overall mean of each population was calculated as $p_l + \mu$. For summary data, the mixed model described above, except excluding the population effect was fitted to calculate genetic values for the individuals. To calculate genetic values within environment, the environment effect was also removed, and the model was fit separately for Njoro and Debre Zeit.

Realized gains were calculated by subtracting the C_1 and C_2 population means by their respective C_0 population means. Percentage gain was calculated as realized gain divided by the C_0 population mean. To express gain from selection in terms of stem rust resistance, percentage gain and realized gain values were multiplied by –1. Paired two-tailed *t*-tests were used to test differences in gain per cycle and per unit time between GS and PS and differences between observed and expected gains. One-sided, one-sample *t*-test was used to test if genetic gains were significant.

Correlated Response for Pseudo-black Chaff

To calculate correlated response for PBC, adjusted population means were estimated using the mixed model:

$$y_{ijk} = \mu + g_i + r_j + p_k + \varepsilon_{ijk}$$

where y_{ijk} is the phenotype, μ, g_i,(i = 1,…1876), r_j, (j = 1,2), and p_k (k = 1,…8) are as described in the previous model, and ε_{ijk} is the residual with $\varepsilon_{ijk} \sim N(0,\sigma_\varepsilon^2)$ where σ_ε^2 is the residual variance. Population mean estimates were used to calculate realized gain and percentage realized gain. Paired two-tailed *t*-tests were used to test for differences between GS and PS gains per cycle and per unit time. One-sided, one-sample *t*-tests were used to test if genetic gains were significant.

Expected Gain from Selection Calculations

General Formulas

Expected gain (G) in QSRR from PS was calculated based on the general formula for gain from selection on both sexes:

$$G = 2ck\sigma_A^2 / \sigma_y$$

(Hallauer et al., 2010) where c is the covariance (2 × the coefficient of kinship) between the selection units and the individuals in the improved population, $k = S/\sigma_y$ where S is the difference between mean of the selected individuals and the population mean, σ_A^2 is the additive genetic variance, and σ_y is the phenotypic standard deviation on an entry mean basis. In all cases c was equal to 0.75 because the selected parents were inbred to a coefficient F = 0.5 during population development. For details on how to calculate coefficients of kinship for estimation of c, see chapter five of Falconer and Mackay (1996).

Expected gain in QSRR from GS and expected gain in PBC from PS and GS was calculated based on the general formula for gain from correlated trait selection:

$$G_Y = 2ckh_X h_Y r_{A_{XY}} \sigma_A$$

(Hallauer et al., 2010), where Y is the trait of interest, X is the trait directly under selection, h_Y is the selection accuracy of trait Y estimated as $\sigma_{AY} / \sigma_{yY}$ where σ_{AY} and σ_{yY} is the additive genetic and phenotypic standard deviation of trait Y respectively, and $r_{A_{XY}}$ is the genetic correlation between trait X and Y. In the case of GS, $h_X = 1$ and $r_{A_{XY}}$ is the GS accuracy.

Estimation of Parameters

Genomic selection accuracy was measured as the Pearson's correlation (r) between genetic values estimated phenotypically, and the genomic estimated breeding values used as the criteria for selection, divided by the phenotypic selection accuracy, h_Y (Falconer and Mackay, 1996). The genetic correlation between PBC and QSRR was estimated for each population using variances and covariances from bivariate models fit using PBC and QSRR phenotypic data. In all cases, parameters from the specific population under selection were used to estimate expected gain.

To estimate genetic variances for QSRR, nongenetic effects were first removed by fitting the mixed model:

$$y_{ij} = \mu + \beta_i + r_j + \varepsilon_{ij}$$

where, y_{ij} is the phenotype, μ is the mean, β_i (i = 1,2) is a fixed environment effect, r_j (j = 1,2) is random effect for blocks in the Njoro environment where $r_j \sim N(0,\sigma_r^2)$ where σ_r^2 is the block variance, ε_{ij} is the residual, and plot errors in the row and column direction within the Njoro environment are modeled using an AR1 × AR1 variance model. The adjusted phenotypic values y' (the

residuals from the previous model) were then used to fit population-specific models, $y_i' = \mu + g_i + \varepsilon_i$, where μ is the mean, g_i ($i = 1,\ldots n$) is a random genotype effect with $g_i \sim N(0, \sigma_g^2)$, n is the number of entries and σ_g^2 is the genetic variance, and ε_i is the residual with $\varepsilon_i \sim N\left(0,\sigma_\varepsilon^2\right)$ where σ_ε^2 is the residual variance. Estimates of genetic variance $\hat{\sigma}_g^2$ were used to approximate the additive genetic variance, $\hat{\sigma}_A^2$, and σ_y was estimated as $\hat{\sigma}_g + \frac{\hat{\sigma}_\varepsilon}{e}$ (Hallauer et al., 2010), where $e = 2$ is the number of environments.

To estimate genetic variance for PBC, nongenetic effects were removed by fitting the mixed model:

$$y_i = \mu + r_i + \varepsilon_i$$

where y_i is the phenotype, μ is the mean, r_i ($i = 1,2$) is random effect for blocks with $r_i \sim N(0,\sigma_r^2)$ where σ_r^2 is the block variance, and ε_i is the residual with $\varepsilon_i \sim N\left(0,\sigma_\varepsilon^2\right)$. Adjusted phenotypic values y' were used to fit population specific models as described above for QSRR. Estimates of genetic variance $\hat{\sigma}_g^2$ were used to approximate $\hat{\sigma}_A^2$ and σ_y was estimated as $\hat{\sigma}_g + \hat{\sigma}_\varepsilon$.

The difference between mean of the selected individuals and the population mean (S) of GS for QSRR was estimated using the mixed model:

$$y_{ijklm} = \mu + \beta_i + g_j + r_k + p_l + S_{(l)m} + \varepsilon_{ijklm}$$

where y_{ijklm} is the phenotype, μ is the mean, β_i ($i = 1,2$) is a fixed environment effect, g_j ($j = 1,\ldots 1950$) is a random genotype effect with $g_j \sim N(0,\sigma_g^2)$ where σ_g^2 is the genetic variance, r_k ($k = 1,2$) is random effect for blocks in the Njoro environment where $r_k \sim N(0,\sigma_r^2)$ where σ_r^2 is the block variance, p_l ($l = 1,\ldots 8$) is a fixed population effect, $S_{(l)m}$ ($m = 1,2$) is a factor nested within population indicating selected vs. not selected by GS, ε_{ijklm} is the residual, and an AR1 × AR1 variance model was used to model the variance structure of the plot errors in the row and column direction within the Njoro environment. The solutions for $S_{(l)m}$ ($m = 1,2$) were used as estimates of S for GS. Likewise, S of PS for QSRR was estimated using the same mixed model but $s_{(l)m}$ was a factor indicating selected vs. not selected by PS. To calculate S of GS and PS for PBC we fit the same models as described above except the environment effect was removed, there were 1876 genotypes, and the AR1 × AR1 variance model was not used.

Estimation of Mean Level of Inbreeding

For each set of individuals selected at each cycle of selection, the mean level of inbreeding resulting from one generation of random mating and one generation of selfing was calculated based on pedigrees using the R package pedigreemm (Vazquez et al., 2010), which uses the algorithm described in the appendix of Sargolzaei and Iwaisaki (2005) to calculate inbreeding coefficients.

The expected level of inbreeding due to drift alone was calculated using 10,000 simulations of the breeding scheme used in this study with selections made at random. Briefly, in each simulation, five individuals were randomly selected from C_0, and pedigrees from all possible combinations of crosses were recorded. Mimicking the actual population development procedure, from each cross, pedigrees were generated for S_1 families of size 27, simulating the C_1 population. This was repeated using C_1 as the initial population, generating C_2. All pedigrees generated were combined and inbreeding coefficients calculated. From each simulation the mean levels of inbreeding for C_1 and C_2 were saved and the overall means for C_1 and C_2 were computed. To determine if observed inbreeding was significantly different from expected, observed inbreeding levels were compared with the expected inbreeding distribution.

Results

Realized Gain Trial

Disease pressures were high in both Njoro, Kenya, and Debre Zeit, Ethiopia, during the 2014 off-season, and heritability within Njoro and Debre Zeit was 0.79 and 0.62, respectively. Entry mean heritability across environments was 0.78. Estimates of genetic values were consistent across environments (Figure S1) with a correlation of 0.67. Population mean by environment interaction was observed, however there was only one instance of a crossover interaction. Adjusted population means for QSRR and PBC are summarized in Table 1.

Gain from Selection for Quantitative Stem Rust Resistance (QSRR)

Mean GS accuracy in C_0 and C_1 was 0.37 and 0.69 ± 0.11, respectively. Mean PS accuracy, h, in C_0 was 0.84 ± 0.01. Percentage total gain from two generations of GS and one generation of PS, was 31 ± 11 and 42 ± 12%, respectively. Total gains were not significant for GS ($p = 0.1$) and PS ($p = 0.09$). Differences between realized and expected gains (Supplementary Table S1; Fig. 2A, B) were also not significant for GS cycle one ($p = 0.47$) and two ($p = 0.82$), and PS ($p = 0.92$). In our two selection schemes, one cycle of PS required the same amount of time as two cycles of GS (Figure S2). On a per-unit time basis, realized gain from GS and PS was not significantly different, $p = 0.69$.

Mean Level of Inbreeding and Genetic Variance

Inbreeding increased by 0.05 ± 0.02, 0.08 ± 0.01, and 0.21 ± 0.02 relative to C_0 after one cycle of PS, one cycle of GS, and two cycles of GS, respectively (Supplementary Table S1; Fig. 3A). Due to drift alone, inbreeding levels were expected to increase by 0.04 ± 0.01 and 0.11 ± 0.02 relative to C_0 after one and two cycles of selection, respectively. Observed inbreeding was significantly greater ($p < 0.025$) than expected values under drift for all selection populations except for replicate two of PS C_1 ($p = 0.15$). Inbreeding levels between GS and PS were not

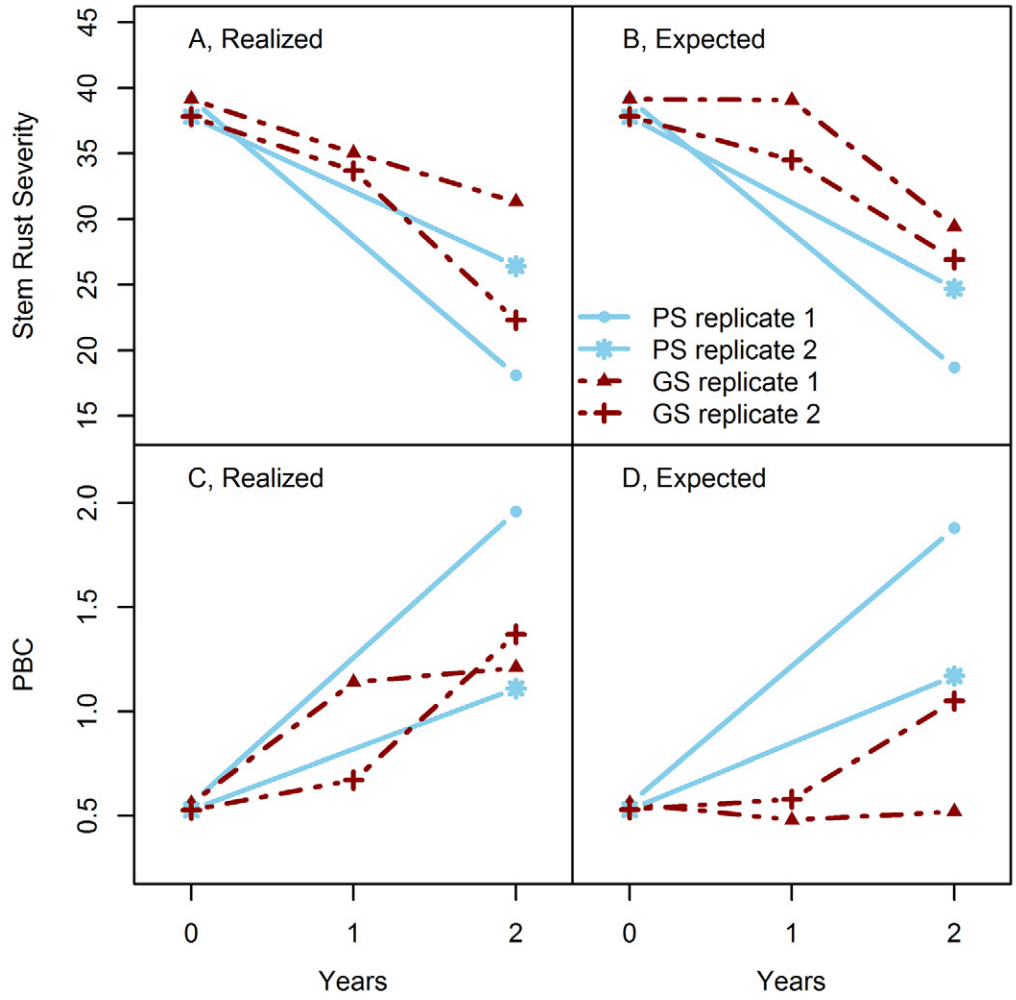

Figure 2. Realized and expected gains in quantitative stem rust resistance (QSRR) and pseudo-black chaff (PBC) per year due to phenotypic selection (PS) and genomic selection (GS) for QSRR. Lower stem rust severity corresponds to greater QSRR. (A) Realized gains in QSRR; (B) expected gains in QSRR; (C) realized gains in PBC; (D) expected gains in PBC. GS, red, triangles, plus signs, and dashed lines; PS, blue, circles, asterisks, and solid lines. The *x* axis indicates the year. One GS cycle requires 1 yr and one PS cycle requires 2 yr.

significantly different per cycle ($p = 0.49$) or per unit time ($p = 0.24$). Mean levels of inbreeding for each population are summarized in Table 1. Realized change in genetic variance from one cycle of PS and GS were not significantly different ($p = 0.15$; Supplementary Table S1; Fig. 3B). However, two selection cycles were completed for GS, leading to a significant reduction in genetic variance ($p = 0.02$) on a per-unit time basis compared with PS.

Correlated Response in Pseudo-black Chaff

The phenotypic selection accuracy, *h*, of PBC was 0.88 $\pm$ 0.01 in C_0 and 0.77 $\pm$ 0.07 in C_1 from GS. Genetic correlation between PBC and QSRR in C_0 was 0.66 $\pm$ 0.07. Mean GS accuracy in cycle one and two was 0.13 $\pm$ 0.07 and 0.36 $\pm$ 0.14, respectively. Total percentage gain from GS and PS was 138 $\pm$ 22 and 180 $\pm$ 70%, respectively. Gains in PBC from GS and PS (Supplementary Table S1; Fig. 2C) were not significantly different per cycle ($p = 0.18$) and per unit time ($p = 0.71$). Overall genetic gain was significant for GS ($p = 0.04$) and not significant for PS ($p = 0.12$). Realized and expected gains (Supplementary Table S1; Fig. 2C, D) were in agreement with the exception of GS cycle one ($p = 0.01$).

Discussion

Effectiveness of Selection

For both GS and PS, percentage gain per cycle was high, suggesting that recurrent selection is a highly effective breeding strategy for QSRR. This is in agreement with other studies of recurrent selection for quantitative rust resistance in cereals that have also reported high percentage gain per cycle, ranging from 6 to 28% (Ceballos et al., 1991; Abedon and Tracy, 1998; Díaz-Lago et al., 2002; Long et al., 2006).

The second cycle of GS was more effective than the first because the prediction model was updated with C_0 data before the second GS cycle. This lead to a 1.86$\times$ increase in accuracy. Without model updating, GS accuracy in the second cycle would have dropped to -0.02 $\pm$ 0.31. Overall gain per unit time from GS would have likely improved had we performed the first cycle of selection solely based on phenotype and begun GS in the

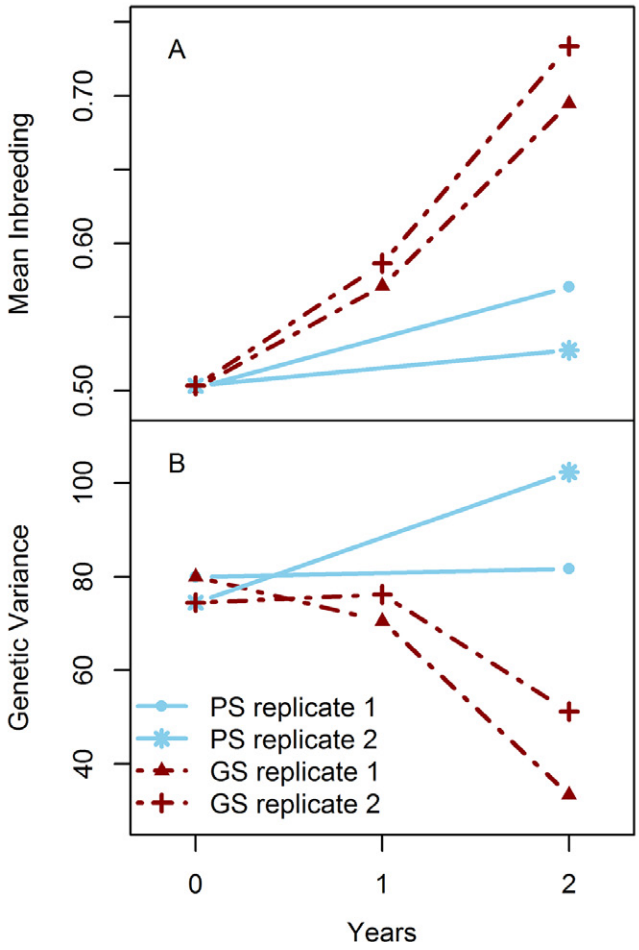

Figure 3. Change in realized mean inbreeding and genetic variance per year of genomic selection (GS) and phenotypic selection (PS). (A) Inbreeding; (B) genetic variance; GS, red, triangles, plus signs, and dashed lines; PS, blue, circles, asterisks, and solid lines. The *x* axis indicates the year. One GS cycle requires 1 yr and one PS cycle requires 2 yr.

second cycle of selection when C_0 data was available for model training. This would have increased cycle one duration 2× and cycle one accuracy 2.28×.

Expected and Realized Gain

In general, estimates of realized gains were in agreement with expected gains based on theory, with GS cycle one for PBC being the only exception. The large standard errors about the mean gain per unit time from GS and PS contributed to the lack of significant differences between selection methods and between observed and expected gains. Expected variation in selection response depends on the rate of inbreeding (Hill, 1977), which is $1/2N$ assuming a noninbred base population. Variance in selection response could have been decreased by increasing *N*, using optimum contribution selection (Meuwissen, 1997; Grundy et al., 1998) to control the rate of inbreeding, or by optimizing selection to maximize response while constraining its variance (Meuwissen and Woolliams, 1994).

Impact of Selection on Genetic Variance

Genomic selection lead to a significantly greater loss in genetic variance per unit time compared with PS, largely because GS enabled two cycles of selection for every one cycle of PS. However, GS may also have led to a more rapid loss of genetic variance because of the way GS affects allele frequencies. Genomic selection based on markers only cannot act on alleles that are either not represented in the training population or not in linkage disequilibrium with markers. Thus, under GS, the frequencies of such alleles are affected by drift alone. On the other hand, PS can act on favorable low frequency alleles thus driving these alleles more rapidly to intermediate frequencies, leading them to generate more variance (Casellas and Varona, 2011). In the case of alleles that are already at intermediate frequencies, represented in the training population and in LD with markers, GS leads to more rapid allele fixation compared with PS (Jannink, 2010), leading these alleles to generate less variance. Over the course of this study, PS may have brought low frequency alleles to intermediate frequencies, leading to increased genetic variance from these loci, in turn leading to the overall increase in genetic variance. In contrast, GS may have brought moderate effect resistance alleles, such as *Sr2*, from intermediate frequency to near fixation therefore reducing the genetic variance generated from these loci, leading to an overall decrease in genetic variance.

An increased rate of inbreeding from GS compared with PS is another factor that can lead to a greater loss of genetic variance from GS. Although compared with PS, GS did not lead to significantly higher inbreeding values per cycle or per unit time; inbreeding levels were numerically higher and may have contributed to the greater loss in genetic variance due to GS. Over longer timescales, stochastic simulation studies have indicated that selection based on markers only may lead to an increased rate of inbreeding due to the reduction in the breeding cycle duration and due to the loss in accuracy of the Mendelian sampling term compared with PS where phenotypic information on the selection candidates is available (Jannink, 2010; de Roos et al., 2011). Deterministic simulations based on selection index theory have indicated that GS can lead to an equal or lower inbreeding rate compared with PS, largely due to better estimation of the Mendelian sampling term (Dekkers, 2007; Daetwyler et al., 2007). However, these studies assumed that the GS prediction accuracy was high (0.8 and 0.85) and that the variance of GS prediction was split evenly between within- and between-family effects. In practice, GS accuracy may be largely due to prediction of between-family effects, especially when GS accuracy is low (Jannink, 2010). Long- or medium-term selection experiments will be needed to better understand how the implementation of GS will impact inbreeding rates in breeding programs. Likewise, optimization of mating strategies in GS will be a critical area of theoretical and applied research to minimize inbreeding.

Correlated Response in Pseudo-Black Chaff

The significant correlated response for PBC that we observed is expected because at least two of the loci affecting both QSRR and PBC are known to be linked or pleiotropic (Hare and McIntosh, 1979; Bariana et al., 2001; Yu et al., 2011; Singh et al., 2013). Percentage gain in PBC was substantially higher than percentage gain in QSRR. This may be because the correlation between the two traits is due to either linkage or pleiotropy at relatively few loci or because the loci affecting PBC are not strictly additive. To efficiently produce germplasm with high QSRR and low PBC, selection should be based on an index including both traits. If PBC expression is modified by few loci, marker-assisted selection against alleles that increase PBC could be effective. This is especially important because expression of PBC is strongly affected by environment.

Conclusions

This study showed that on a per-unit time basis and using equal selection intensities, GS could perform as well as PS for improvement of QSRR in wheat; however, GS can also result in significantly less genetic variance over the same time largely because it reduces in the breeding cycle duration. For short-term selection programs, rapid loss in genetic variance may not be an issue; however, if long-term GS programs also experience faster losses of genetic variance this could reduce rates of genetic gain. Longer-term GS experiments should be conducted to better characterize the effect of GS on genetic variance and to assess rates of long-term gain. In addition, optimum contribution selection (Meuwissen, 1997; Grundy et al., 1998) and weighting low-frequency favorable alleles (Jannink, 2010) should be tested to help reduce the reduction in genetic variance due to inbreeding and the fixation of loci.

The GS scheme used in this experiment may be considered less favorable than PS from a practical standpoint because it was more costly, used more genetic variance, and did not lead to an increase in the rate of genetic gain. This outcome is specific to our particular breeding schemes and objectives. More theoretical and applied research is needed to develop effective and cost-effective GS breeding strategies for spring wheat, and, ultimately, individual breeding programs will need to begin testing GS to develop the best breeding strategy for their specific resources and objectives.

Supplemental Information

Supplementary Dataset S1 = Batch1GBS.txt

Supplementary Dataset S2 = Batch2GBS.txt

Supplementary Dataset S3 = TrainingPhenoData1.csv

Supplementary Dataset S4 = TrainingPhenoData2.csv

Supplementary Dataset S5 = Pedigree.csv

Supplementary Dataset S6 = Trial2014.csv

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