Hindawi
International Journal of Agronomy
Volume 2022, Article ID 9953200, 14 pages
https://doi.org/10.1155/2022/9953200

Research Article

Estimation of Breeding Parameters from Phenotypic Data of F4:5 RIL in Ethiopian Malt Barley (*Hordeum distichum* L.) Breeding Population

Endeshaw Tadesse,[1,2] **Peer Wilde**,[3] **Kassahun Tesfaye**,[4] **Yohannes Fekadu**,[5] **Tesfahun Alemu**,[6] **and Rebeka Gebretsadik**[7]

[1]*Addis Ababa University, Department of Microbial Cellular and Molecular Biology, P.O. Box 1176, Addis Ababa, Ethiopia*
[2]*Oromia Agricultural Research Institute(IQQO), Sinana Agricultural Research Center (SARC), P.O. Box 208, Bale Robe, Ethiopia*
[3]*KWS LOCHOW GMBH, Ferdinand-Von-Lochow-Str.5 29303, Bergen, Germany*
[4]*Institute of Biotechnology, Addis Ababa University, P.O. Box 32853, Addis Ababa, Ethiopia*
[5]*Ethiopian Institute of Agricultural Research, P.O. Box 2003, Addis Ababa, Ethiopia*
[6]*Ethiopian Institute of Agricultural Research (EIAR), Holeta Agricultural Research Center (HARC), P.O. Box 2003, Holeta, Addis Ababa, Ethiopia*
[7]*Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ), Supporting Sustainable Agricultural Productivity (SSAP), P.O. Box 100009, Addis Ababa, Ethiopia*

Correspondence should be addressed to Endeshaw Tadesse; etadde12@gmail.com

Received 1 July 2022; Revised 17 August 2022; Accepted 20 August 2022; Published 3 October 2022

Academic Editor: Mehdi Rahimi

The critical stage for any breeder is the selection of crossing parents to drive improved inbred for subsequent breeding cycles. In our study, we estimate breeding parameters such as mid-parent value (MPV), variances among and within crosses, the heritability of relevant traits and their correlations, the usefulness of crosses, and regression of cross means on MPV. 900 F4:5 Recombinant inbred lines (RILs) derived from 30 crosses were tested together with their parental lines in a modified split-plot p-rep design at two locations. The analysis revealed significant genetic variation among parents, crosses, and RIL for almost all traits. Heritability for parents ranged from 49.50% (malt extract) to 93.60% (plant height) and heritability for crosses ranged from 29.52% (grain protein concentration) to 87.0% (days to maturity), whereas heritability for RIL was the lowest with 27.40% for beta-glucan and the highest to 73.60% for thousand kernel weight, respectively. Significant ($P < 0.01$) genotypic correlations with high impact for practical breeding were found between malting traits. Accordingly, the genotypic correlation ranged from −0.73 to 0.78 whereas the phenotypic correlation ranged from −0.60 to 0.65, respectively. Significant ($P < 0.01$) regression of cross-mean on MPV where R^2 ranges from 0.27 to 0.70 and is higher than 0.5 for most of the traits demonstrates that cross means can accurately be predicted from MPV and selection among crosses at an early stage is highly effective. Based on the usefulness criterion, 16 superior crosses were identified compared to the planet as the actual leading malt variety. Starting from a simple additive genetic model with random mating, we discuss deviations from the initial model and their impact on the actual estimates implying how to design a state-of-the-art cereal breeding program.

1. Introduction

Plant breeding programs are based on the effective maintaining and reuse/shuffling of genetic variation aimed at generating new and improved combinations of alleles and assembling them in a single superior genetic background[1, 2]. Genetic variation can be obtained from germplasm sources such as landraces or exotic lines from foreign countries that could be directly used to exploit the inherent genetic variance. Alternatively, elite lines are combined in

crosses to form a base population from which new and improved lines are developed ("second cycle breeding") [3–6]. Hence, the genetic variance can be structured into variance between crosses and within crosses. The variance between crosses is the variation observed between crosses generated from different parental lines [6]. This should be distinguished from the variance within crosses which is the variation between recombinant inbred lines (RILs) generated from a given cross through subsequent selfing.

Thus, breeding can be regarded as a two-step selection process [6]. The first step is the selection between crosses and the corresponding selection gain depends on the genetic variance among cross means (σ_c^2). The second step is the selection among RIL within crosses and the corresponding selection gain depends on segregation variance (σ_{gij}^2) among lines within a given cross of parent i and parent j which has been selected in the first step. Therefore, information about σ_c^2 and σ_{gij}^2 is important to the breeder to optimize the allocation of resources with regard to the number of crosses exploited and the number of RIL evaluated per individual cross [7]. For example, if σ_c^2 is large and σ_{gij}^2 is small, the breeder will invest more into crosses and less into the number of lines per cross. Also, the choice of crosses decides on which genes will be (re)combined to obtain superior progenies [8–10].

In cross formation, parental selections play a major role which in turn depends on heritability which is an important parameter in practical plant breeding, because it determines the genetic gain. Breeders evaluate cultivars of interests across multiple locations and several years which are known as multienvironmental trials (METs) [11].

Heritability can be estimated in different ways. For instance, the standard heritability estimate as the ratio of σ_g^2 to σ_p^2 assumes that the trial design is completely balanced, genotype effects are independent, and variance and covariance are homogeneous [12–14]. However, when considering segregating populations, the RILs of a given cross share the same parental lines and RILs from two crosses might be related to each other as half-sibs because one of the parental lines is identical. In the case of testing the entries in different environments, the respective variances and covariances are not necessarily homogeneous. Under such circumstances, heritability should be based on BLUP (Best Linear Unbiased Predictor). Hence, [11] suggested fitting the genetic term as a random effect (BLUP) and explained heritability as a function of the ratio: mean-variance of a difference of two BLUPs for the genotypic effect divided by twice the genotypic variance.

Correlations between traits can be caused by phenotypic, genotypic, and environmental effects. Phenotypic correlation (rp) occurs when phenotypic values of the two traits are correlated due to genetic and/or nongenetic effects. Genetic correlation (rg) can arise from pleiotropy caused by the same genes influencing two different traits and/or linkage disequilibrium between genes controlling different characters [15, 16]. Environmental correlation is the correlation of environmental effects [15]. For example, drought might affect plant height and thousand-grain weight in the same direction. Trait correlations are important phenomena in crop improvement because enhancement in one trait may have a negative impact on another trait which is also important [17, 18].

Regression of cross-mean (CM) on mid-parent (MPV) values is relevant for practical breeding because MPV could be used as a predictor of the offspring or CM for the traits of interest. In our experimental context, the offspring mean is identical to the mean of all RIL derived from a cross between the parents P_1 and P_2. The association between MPV and offspring can be explained [15] through the regression of offspring on mid-parent values ($bOMPV$):

$$\text{bOMPV} = \frac{\text{cov}_{\text{OMPV}}}{\sigma^2_{\text{MPV}}}, \tag{1}$$

where COV_{OMPV} is the covariance of the offspring with the mid-parent and σ^2_{MP} is the variance of the phenotypic means of P1 and P2. For the denominator, we can derive assuming that the two parents have the same variance:

$$\sigma^2{}_{MP} = \frac{1}{2}\left(\sigma^2 P1 + \sigma^2 P2\right). \tag{2}$$

Under the assumptions of an idealized population, the variance of the phenotypic means of the individual parents can be dissected into

$$\begin{aligned} \sigma_P^2 &= \sigma_G^2 + \sigma_E^2, \\ \sigma_G^2 &= \sigma_A^2 + \sigma_I^2. \end{aligned} \tag{3}$$

σ_G^2 is the variance of genetic effects, σ_A^2 is the variance of additive effects, σ_I^2 is the variance of epistatic effects, and σ_E^2 is the variance of environmental (e.g., genotype × environment-interaction) effects.

The potential of a cross for a given quantitative trait can be quantified by the usefulness criterion [19]. U is determined by the cross-mean (μ) and the genetic gain ($ih\sigma$) from exploiting the segregation variance within the cross: $U = \mu + ih\sigma$, where σ is the genetic standard deviation within the cross. i (α) is the selection intensity depending on the selection rate. $\alpha(h)$ is the selection accuracy as defined above.

In summary, our objectives were to (i) estimate and compare mid-parent value and cross-mean, (ii) estimate and compare the variance between means of crosses (σ_c^2) and segregation variance of recombinant inbred line within crosses (σ_g^2), (iii) estimate the heritability of the traits under selection and correlation among them, and (iv) estimate the usefulness of crosses.

2. Materials and Methods

2.1. Germplasm. Thirty (30) crosses derived from 17 parents were conducted at Holeta Agricultural Research Center in the year 2018 (Table 1), and 30 RIL per cross had been developed in an SSD procedure in the following growing seasons. RILs used for testing descend from F4 single plants and were tested in F5-generation (F4:5-L). Thus, RIL populations comprising 900 entries were used in this study. The 17 parental lines and 13 well-known malt barley varieties were included as checks. The parental lines used in the crossing program were organized from released malt barley varieties and elite germplasm

Table 1: List of the 17 parental lines, origin, and their agronomic profile.

SN	Parents	Origin	Description
1	Burton	USA, UM	Resistant to pests (such as aphids and others)
2	M135	USA, UM	
3	MN Brite	USA, UM	
4	G 13–64	Europe	Malt quality and high Yield, Late heading, short height, but susceptible to leaf disease
5	Planet	Europe	
6	IBON 14/15–144	ICARDA	Stem stiffness, drought tolerance, and well adapted to the Ethiopian environment
7	IBON 13/2	ICARDA	
8	IBON 13/33	ICARDA	
9	ICARDA GP-67	ICARDA	
10	ICARDA GP-75	ICARDA	
11	IBON 13/14–129	ICARDA	
12	MBHIBYT-23	ICARDA	
13	MBHIBYT-22	ICARDA	
14	IBON 13/14–128	ICARDA	
15	IBON 13/14–41	ICARDA	
16	HB1963	Ethiopia	Resistance to biotic and a biotic stress and well adapted
17	Bekoji-1 x Grace	Ethiopia	

Source: HARC, 2020, UM = University of Minnesota.

selected from national variety trials based on their good line per se performance. The parental selection and combination were based on criteria such as high yield, adaptiveness to the Ethiopian environment, and good malt quality. The origin of the parents used for crosses includes Europe, the USA, ICARDA, and Ethiopia. The European sources are known for their malt quality and high yield under favorable farming conditions but also for late heading date, short plant height, and susceptibility against leaf diseases. USA sources excel for their resistance to insects such as aphids, ICARDA lines are good sources for stem stiffness and drought tolerance and are well adapted to the Ethiopian environment whereas the Ethiopian sources are generally innate with, e.g., good adaptation to low soil pH and resistance to other biotic and abiotic stress.

2.2. Experimental Design and Field Management. The experiment was conducted in 2020 at two Agricultural Research Stations Holeta (altitude 2390 m.a.s.l with an average annual rainfall of 1100 mm and Max and Min temperature of 22.2 and 6.13°C) and Bekoji (substation of Kulumsa Agricultural Research Center) (altitude of 2780 m.a.s.l. with an annual mean rainfall of 1049.6 mm and Max and Min temperature is 19.6 and 8.3°C), respectively. A modified split-plot *p*-rep design was used in which crosses were allocated to main plots and RILs of a given cross were randomized as subplots within the main plots. The main plots were nested within blocks. The total entry number was 900 (30 crosses by 30 RIL each), from which 450 entries were replicated at Holeta and the remaining were replicated at Bekoji. In addition, the parents of the crosses and check varieties were tested in 5 replicates per site. Thus, the main plot comprised 50 subplots with 15 RIL replicated twice, 15 unreplicated RIL, and 5 parents/checks). Entries were grown in two-rowed observation plots with plot lengths of 1 m, 0.2 m spacing between rows, and 1 m path which made a net plot area of 0.4 m^2.

2.3. Agronomic Data. Data were recorded on 8 quantitative characters of each at both locations using tablets equipped with field scorer 4 android Katmandoo applications, developed and supported by the Department of Agriculture and Fisheries in Queensland. The following data were recorded as indicated in Table 2.

2.4. Data Analysis. Data were subjected to statistical analysis to test for outliers, homogeneity of variance, and normality of residuals using R software, version 3.4.2 (R core Team, 2017). Estimation of variance components among parents (checks), crosses, and RIL within crosses was computed based on mixed model procedures using asreml-r (Commercial Package based on R software). The basic model assumption was checked with a graphical overview and outliers were replaced by NA (NA stands for not available in R syntax). The group which contains checks were considered fixed whereas the genotype which contains parents, crosses, and RILs with crosses was considered a random factor.

2.4.1. Estimation of Variance Components. A spatial model was fitted to produce adjusted genotype means for each environment [20, 21]. After spatial adjustment, a combined analysis was conducted across the environment [22, 23]. The linear mixed model for the combined analysis contains different dummy groups to predict the adjusted mean precisely [24]. The model was defined as

$$Y = \mu + \text{group} + \text{pg} + \text{loc} + \text{cross} + \text{cross: loc} + \text{Entry} + \text{Entry: loc} + \text{row: loc} + \text{col: mainp: loc} + e. \quad (4)$$

Attaching the dummies to (group {groupRILs, groupCHK}), parental group (pg) (pgParent, pgechk) where pgParent is parents that are used in the cross, and pgechk is nonparents that are included as additional checks. In addition, for each treatment factor (cross and entry), dummies have been attached as shown in the following model, and the

respective design factors for rows, columns, and main plots were considered per location.

$$Y = \mu + \text{group} + \text{pg} + \text{loc} + \text{cross: pgParent} + \text{cross: pgParent: loc} + \text{Entry: pgechk} + \text{Entry: pgechk: loc} + \text{Entry: groupCHK: pgParent} + \text{Entry: groupRIL} + \text{Entry: groupCHK: pgParent: loc} + \text{Entry: groupRIL: loc} + \text{row: loc} + \text{col: mainp: loc} + e, \quad (5)$$

where Y is the total observation for the trait of interest, μ is the overall mean of RIL, group is to identify checks, and RIL is the only fixed factor, and crosses, checks, and RILs within crosses are assigned as genotypes in the model. Dummies were included to estimate the effect of one factor by switching off other effects. Accordingly, for pgParent, pgechk, groupCHK, and groupRIL dummies were attached to estimate the effect of parents, echek, checks, and RIL by switching off one factor over the other. echk identifies extra checks besides the 17 parents actually used in crossing, CHK represents the total checks used in the experiment (i.e., including both parents and nonparents), RIL is recombinant inbred lines derived from the crosses, and loc assigns locations. An example of other interactions with environments (Entry.loc) is defined as the interaction between location and set of genotype groups, i.e., crosses, checks, RILs within crosses. row.loc and (mp.col.loc) are rows, columns, and main plots effects, respectively, nested within locations, and e is the random error for the model.

2.4.2. Estimate of Heritability (Broad Sense). Heritability was estimated based on Cullis et al. [25]. Specific allocation for checks crosses and RIL was taken into account when estimating their respective heritability.

$$H^2\text{Cullis} = 1 - \frac{\text{average standard error from BLUP}}{2 * \sigma_g^2}, \quad (6)$$

where σ_g^2 is genotype variance from a model construct.

2.4.3. Theory Assumptions. We assume that RIL represents a line population developed under a single seed descent (SSD) procedure:

(i) Forces driving changes of allele frequencies such as selection, migration, drift, and mutation [15] are assumed to be negligible

(ii) Linkage equilibrium [15] between QTL controlling a trait is given

(iii) Epistasis is absent or of minor importance

With these assumptions given a simple additive genetic model can be used as suggested by [6, 25].

2.4.4. Parameters Estimation for Phenotypic Data. Best Linear Unbiased Predictions (BLUPs) were computed for means of parents, crosses, and RILs within crosses and estimated as random effects with distribution μ~MVN (0, $\sum \sigma_\mu^2$) being $\sum \sigma_\mu^2$ a relationship matrix among the level of the random effect. The genetic variance of mid-parents was estimated from the genetic variance of the female (σ_f^2) and male (σ_m^2) parental lines (σ_p^2) $\sigma_p^2 = (\sigma_m^2 + \sigma_f^2)/2$. The genetic variance among crosses σ_c^2 is the variance component accounting for variation between the 30 crosses and is estimated as the variance component assuming crosses as random [6]. The average segregation variance σ_g^2 is the variation of RILs within crosses. Under the assumptions specified above, a quantitative genetic interpretation of the breeding parameters is given in Table 3.

3. Results

3.1. Mean of Parents. The MPV (Table 4) of all 30 crosses represents the parental per se performance. Accordingly, Burton with the planet was found to be good in their mean performance in terms of DH (76.6 days), TKW (46.2 g), ME (80.7%), GPC (11.1%), and FR (70%), respectively. Similarly, the planet with MBHIBYT-22 also indicated good performance in terms of DH (80.8 days), PH (89.5 cm), ME (81.4%), GPC (11.1%), and FR (70%), respectively (Table 4). These indicate that the parents involved in the crossing program were relatively early maturing, with good malt quality traits with optimum grain weight. Further, some potential parental lines (e.g., planet or HB1963) have been used by Ethiopian farmers for malt barley production. These lines were taken as reference lines and supposed to be surpassed by the RIL derived from their crosses. We used the variety planet (European origin) in 13 of the crosses to transfer the malting quality and high yield potential under favorable agronomic conditions. Thus, the mid-parents were split into a group with and without a planet as a crossing partner. Hence, considering the difference between the two groups, we had observed −0.8. −0.7, 0.7, and 0.7 for DH, DM, PH, and TKW, respectively. This implied that the variety planet contributed to earliness, tallness, and better TKW. Similarly, the desired difference was observed for FR (1.1) and BG (−15) which revealed that the planet has positive effects on malt quality in terms of ME, FR, and BG.

Table 2: List of agronomic and quality parameters collected.

SN	Variables	Code	Nature of Variables	Collection method
1	Heading date	DH	Quantitative	Number of days from date of sowing till about 75% of plants in the plot was flowering
2	Maturity date	DM	Quantitative	Number of days from sowing to the date when 75% of the peduncle turned to yellow straw color and when no green color remained on glumes and peduncles of the tagged plant
3	Plant height	PH	Quantitative	The actual measurement of plant height from ground level to the tip of the spike excluding the awns and recorded as the average of five randomly selected plants in each plot
4	Thousand kernel weight	TKW	Quantitative	Weights were measured by taking the mass of carefully counted a thousand kernels on sensitive electronic balance (±0.1 g) after kernels were adjusted to 12.5% moisture content
5	Grain protein concentration	GPC	Quantitative	Total protein content in the grain, expressed as a percentage. estimated using the NIRS machine
6	Malt extract	ME	Quantitative	Solid material extracted from finely ground malt, expressed as a percentage. estimated using the NIRS machine
7	Friability	FR	Quantitative	Indicator of endosperm modification after malting by a simple milling test in a friabilimeter, expressed as a percentage. estimated using the NIRS machine
8	Beta-glucan	BG	Quantitative	a principal constituent of the barley endosperm cell wall, expressed as mg/estimated using the NIRS machine

The presence of planet variety resulted in the difference observed between the two groups.

3.2. Mean and Ranges of RIL. The mean of the RIL population (Table 5) estimated overall RIL within and across crosses reflects the general performance of the progeny produced from the parental lines. RIL performance ranged from 74.64 to 88.76 days for DH (mean 81.20 days), 126.50 to 139.60 days for DM (mean 133.10 days), 75.09 to 102.50 cm for PH (mean 91.51 cm), 79.4 to 81.39% for ME (mean of 80.68%), and 10.52 to 12.13% for GPC (mean11.21%). Minimum and maximum values and the respective standard deviation (SD) reveal a considerable variation that can be exploited by selection. For all traits, SD is higher than SE of means indicating that phenotypic variation is influenced by genetic variation.

3.3. Variance among Parents, Crosses, and Lines within Crosses. Analysis of variance (Table 6) revealed significant ($P < 0.05$) genetic variation among parents for DH, PH, and GPC, while other traits showed nonsignificant differences among parents. Significant genetic variances among crosses were observed for DH, DM, TKW, ME, FR, and GPC but not for PH, and BG. Genetic variance among RIL within crosses was highly significant ($P < 0.001$) almost for all traits except for ME and GPC. For two traits (DM, ME), the ratio of genetic variances σ_c^2: $\sigma_p^2/2$ was estimated to be larger than one. In contrast, the ratio for PH was found to be significantly smaller than one. Significant ($P < 0.05$) genotype × location interaction variances were consistently observed for parents, crosses, and RIL for DM, PH, TKW, FR, and BG which indicates that genotypes differ in response to the two environments.

3.4. Genetic Variance within Crosses (σ_g^2). The average genetic variance of RIL within crosses was estimated in a combined analysis as shown in Table 6. Besides, for each of the 30 crosses, the variance of RIL was estimated separately (Table 7) hypothesizing that the genetic variance (σ_g^2) and/or their genotype × location interaction variance are different from cross to cross. Except for ME, FR, and BG relevant differences between the crosses with regard to σ_g^2 were observed. The range of σ_g^2 was very high for DH (2.56–10.48), DM (1.25–7.7), PH (3.0–29.7), TKW (1.92–36.28), FR (3.12–23.6), and BG (114.2–455.7), and for these traits, the respective SD exceeds SE (Table 7). It is noteworthy that there is a difference between the estimates shown in Table 6 to the genetic variance of RIL explained in Table 7. The genetic variance of RILs shown in Table 6 is generated from BLUE, whereas the genetic variance shown in Table 7 is generated from BLUP predicted mean values. These BLUP predicted mean values are actually penalized by the shrinkage effect, which leads to much smaller estimates in Table 6 compared to Table 7.

3.5. Broad Sense Heritability (H^2) of Traits among Parents, Crosses, and RIL. Heritability across both environments (Figure 1) showed moderate to high estimates with a range of 27.4 to 87.4% depending on the source of genetic variation (parents, crosses, and RIL) and trait (Figure 1). For the parents, H^2 ranges from 49.5% for ME to 93.6% for PH, for the crosses H^2 ranges from 29.5% for GPC to 87.0% for DM, whereas for the RIL H^2 extends from 27.4% for BG to 73.6 for TKW, respectively. Compared to parents and crosses, H^2 for RIL are smaller for most traits. This is in line with the genetic and the interaction location variance components shown in Table 5. Concerning the average standard error going into the H^2 formula, it has to be taken into account that testing intensity for parents is higher compared to RIL.

Table 3: Parameter estimation and their quantitative genetic expectation assuming an additive model.

Parameters	Genetic expectations	References
Generation means		
Mid-parent value	$\mathrm{MPV}_{ij} = (m_i + m_j)/2$	[6]
Cross-mean	$C_{ij} = (m_i + m_j)/2$	
Genetic variance		
Among parents	$\sigma_p^2 = (\sigma_m^2 + \sigma_f^2)/2 = \sigma_A^2$	
Between means of crosses	$\sigma_c^2 = \sigma_A^2$	
Among lines within crosses	$\sigma_g^2 = \sigma_A^2$	
Heritability	$H^2 Cullis = 1 -$ average standard error from BLUP$/\sigma_g^2$	Cullis et al. 2006
Correlation		
Phenotypic	$(\sigma_p^2/2) = \sigma_c^2 = \sigma_g^2 = \sigma_A^2.$	[15]
Genotypic	$r_g = (\mathrm{COV}(g_1, g_2))/(\sqrt{\mathrm{Var}(g_1)}\sqrt{\mathrm{Var}(g_2)})$	
Regression of cross mean on MPV	$c_{ij} = m + b_{ij}(MP_{ij}) + e_{ij}$	[6]
Usefulness of crosses	$U_{ij} = C_{ij} + \Delta G_{ij} = C_{ij} + ih\sigma_{gij}$	Schnell & Utz, 1975

m, m_i, and m_j = mean of the RIL population, parent i and j, resp.; MPV_{ij} = mid-parent value of parent i and j; C_{ij} = predicted cross-mean; σ_A^2 = variance of additive effects; σ_c^2, σ_g^2 = genotype variance among crosses and among lines within crosses, resp.; r_p = phenotypic correlation, COV (trait 1, trait 2) = the covariance between the trait values, the denominator is the product of the square root of the phenotypic variance of trait 1 and trait 2; r_g = genetic correlation, COV ($g1$, $g2$) = covariance of the genetic effects g_1 and g_2 for trait 1 and trait 2, the denominator is the product of the square root of genetic variance of trait 1 and trait 2; b_{ij} = the slope of regression; ε_{ij} = error term; U_{ij} is the usefulness of the cross C_{ij},, R_{ij} = genetic gain, which is a result of segregation variance, heritability (h^2) within the cross, and the selection intensity (i).

Table 4: BLUP predicted mid-parent values across two environments.

P1	P2	Cross		DH	DM	PH	TKW	ME	GPC	FR	BG
Planet	MBHIBYT-23	Cr	1	82.0	133.7	90.9	48.3	80.6	11.1	68.0	338.9
IBON 13/2	G13–64	Cr	10	80.7	131.4	92.9	48.2	80.4	11.2	66.7	376.7
Burton	ICARDA GP-67	Cr	11	80.4	131.7	92.0	46.0	80.6	11.1	68.2	373.3
G13-64	MBHIBYT-22	Cr	12	81.6	134.0	87.6	45.6	81.1	11.1	69.9	352.1
Planet	IBON 14/15–144	Cr	13	81.1	132.9	91.1	47.8	81.1	11.1	68.7	342.0
Planet	IBON 13/14–128	Cr	14	81.0	133.3	89.8	46.7	80.9	11.2	67.5	352.5
Planet	IBON 14/15–129	Cr	15	80.9	134.5	91.2	47.3	81.4	11.1	68.4	337.9
Planet	IBON 13/14–41	Cr	16	82.7	136.2	93.3	49.3	80.7	11.2	66.5	338.6
Planet	MBHIBYT-22	Cr	17	80.8	133.0	89.5	46.7	81.4	11.1	70.0	348.2
M 135	G13–64	Cr	18	81.2	134.1	91.5	47.1	80.6	11.2	67.0	360.3
Planet	ICARDA GP–75	Cr	19	80.4	132.8	90.8	47.6	80.9	11.2	67.1	341.1
MN Brite	IBON 13/14–128	Cr	2	79.6	131.5	88.7	45.2	80.7	11.2	67.5	398.3
Burton	G13–64	Cr	20	79.7	130.2	90.7	46.5	80.6	11.2	67.4	373.5
G13-64	ICARDA GP-75	Cr	21	81.1	133.8	89.0	46.4	80.6	11.2	67.0	345.0
IBON 13/2	ICARDA GP-67	Cr	22	81.3	132.9	94.2	47.7	80.4	11.1	67.6	376.6
Burton	Planet	Cr	23	79.6	130.5	90.3	46.2	80.7	11.1	69.5	369.8
Burton	IBON 13/14–128	Cr	24	80.1	131.3	89.4	46.0	80.6	11.2	67.3	382.3
IBON 13/33	G13–64	Cr	25	81.1	130.2	86.5	43.2	80.8	11.2	67.8	314.0
M 135	Planet	Cr	26	81.1	134.4	91.1	46.8	80.8	11.1	69.1	356.6
IBON 13/2	Planet	Cr	27	80.6	131.7	92.5	47.9	80.6	11.1	68.8	373.1
M 135	IBON 13/14–128	Cr	28	81.6	135.2	90.2	46.6	80.6	11.2	66.9	369.1
(Bekoji-1 xGrace)	Planet	Cr	29	81.3	132.4	92.5	47.2	81.0	11.1	69.7	359.0
HB 1963	IBON 14/15–144	Cr	3	81.8	134.0	89.2	46.6	80.8	11.1	68.7	345.9
Planet	ICARDA GP–67	Cr	30	81.3	133.7	92.4	46.7	81.0	11.1	68.4	343.5
G13-64	IBON 13/14–128	Cr	4	81.7	134.3	87.9	45.6	80.6	11.2	67.5	356.4
IBON 14/15-144	IBON 13/14–128	Cr	5	81.2	131.5	89.4	43.6	80.6	11.2	67.4	388.9
IBON 14/15-144	ICARDA GP-67	Cr	6	81.5	132.0	92.0	43.6	80.6	11.1	68.3	379.9
MN Brite	Planet	Cr	7	79.2	130.8	89.6	45.4	80.9	11.1	69.7	385.8
HB 1963	IBON 13/14–128	Cr	8	82.0	133.1	88.9	46.1	80.4	11.2	66.9	387.1
ICARDA GP-67	HB 1963	Cr	9	85.8	138.0	93.3	48.9	81.3	11.2	68.6	348.4
Mean				81.1	133.0	90.6	46.6	80.8	11.1	68.1	360.5
LSD				0.45	0.67	0.69	0.53	0.10	0.02	0.39	7.31
Mean planet crosses ($N = 9$)*				80.5	132.6	90.8	46.9	80.9	11.1	68.7	357.0
Mean Nonplanet crosses ($N = 13$)*				81.3	133.3	90.1	46.2	80.7	11.2	67.6	372.0
Δ(Nonplanet vs. planet crosses*))				−0.8	−0.7	0.7	0.7	0.2	0.0	1.1	−15.0

*crosses had been selected to balance the gametic contributions of parental lines in the two groups. P1, P2 = Parent 1 and Parent 2, DH = days to heading, DM = days to maturity, PH = plant height, TKW = thousand kernel weight, ME = malt extract, FR = friability, GPC = gain protein concentration, BG = beta-glucan.

Table 5: Mean, minimum and maximum values estimated from 30 F4:5 RIL per cross based on 30 crosses tested across two environments.

	DH	DM	PH	TKW	ME	FR	GPC	BG
Min	74.64	126.50	75.09	35.16	79.76	52.40	10.52	317.40
Max	88.76	139.60	102.50	58.16	81.39	76.17	12.13	430.00
Mean	81.20	133.10	91.51	46.88	80.68	66.65	11.21	363.40
SD	2.47	1.99	3.49	2.74	0.24	0.23	2.96	15.55
SE	0.08	0.07	0.12	0.09	0.01	0.01	0.10	0.52
LSD	0.163	0.143	0.25	0.184	0.02	0.02	0.204	1.06

DH = days to heading, DM = days to maturity, PH = plant height, TKW = thousand kernel weight, ME = malt extract, FR = friability, GPC = gain protein concentration, BG = beta-glucan, SD = standard deviation, SE = standard error of RIL means.

Table 6: Variance components for the genetic and genotype × location effects of parents, crosses, and RIL within crosses estimated from the combined analysis over locations.

Source of variation	DF	DH	DM	PH	TKW	ME	FR	GPC	BG
Parents (σ_p^2)	16	27.01*	13.64	240.65*	15.70	0.68	55.99	0.67*	7725.53
Parent: Loc	16	2.60	14.21*	22.92*	4.79*	1.24	29.63*	0.15	4401.44*
Crosses (σ_c^2)	29	7.90*	20.36*	15.13	10.87*	0.82*	25.88*	0.07*	3398.14
Cross: Loc	29	0.99	2.66*	55.70*	3.11*	0.30*	14.94*	0.25	6762.4*
RIL (σ_g^2)	870	11.14***	9.15***	30.66***	14.13***	0.34*	27.17**	0.196	1811.44*
RIL: Loc	870	3.55**	8.29***	39.28***	2.82**	0.27*	42.69***	0.11*	13984.4***
Ratio σ_c^2: $\sigma_p^2/2$		**0.60**	**2.99***	**0.13***	**1.39**	**2.41****	**0.92**	**0.22**	**0.89**
Ratio σ_c^2: σ_g^2		**0.71**	**2.23***	**0.50***	**0.77**	**2.41****	**0.95**	**0.37**	**1.88***

*, **, ***significance at $\alpha = 0.05$, 0.01, 0.001, respectively, DF = degree of freedom, DH = days to heading, DM = days to maturity, PH = plant height, TKW = thousand kernel weight, ME = malt extract, FR = friability, GPC = gain protein concentration, BG = beta-glucan.

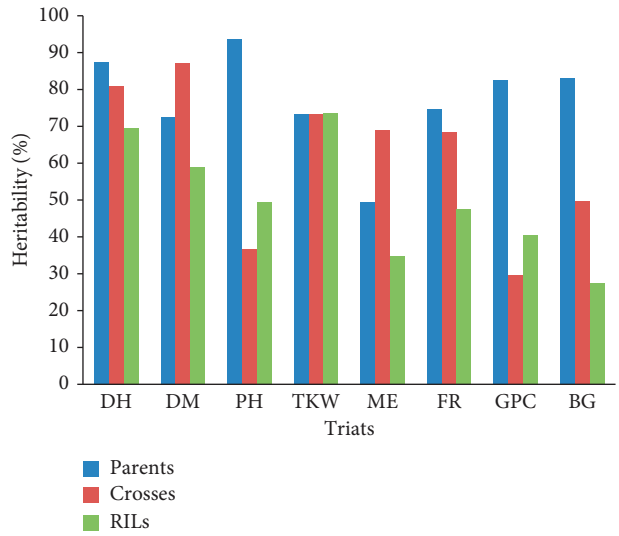

Figure 1: Heritability of traits for parents, crosses, and RILs across. DH=days to heading, DM=days to maturity, PH=plant height, TKW=thousand kernel weight, ME=malt extract, GPC=gain protein concentration, FR=friability, BG=beta-glucan, Hol=Holeta, Bek=Bekoji.

3.6. Phenotypic and Genotypic Correlation between Traits. The phenotypic and genotypic trait correlation values obtained were low to moderate with absolute values ranging between 0.00 and 0.78 (Table 8). Accordingly, positive and significant ($P < 0.01$) phenotypic and genotypic correlations were found between ME and FR ($r = 0.58$ and 0.60) and DH and DM ($r = 0.65$ and 0.78) whereas the negative and significant phenotypic and genotypic correlation was between FR and GPC ($r = -0.60$ and -0.73), respectively (Table 8). The positive significant correlation observed between ME versus FR and DH versus DM reveals that selection for one trait will lead to automatic selection for the other, leading to more rapid progress in selection for both traits, whereas in the case of negative correlation, simultaneous improvement of the traits is difficult. Overall, correlations among malting quality traits were higher than the correlations among agronomic traits.

Table 7: Mean minimum and maximum genetic variances (σ_g^2) among RILs within crosses estimated from 900F4:5 based on the 30 malt barley crosses for traits studied.

Parameter	Traits							
	DH	DM	PH	TKW	ME	FR	GPC	BG
Mean	6.3	4.1	12.6	7.73	0.06	9.04	0.05	249.3
Minimum	2.56	1.25	3.08	1.92	0.03	3.12	0.02	114.2
Maximum	10.48	7.7	29.7	36.28	0.13	23.6	0.13	455.7
SE of σ^2g'	0.45	0.29	0.97	1.35	0.004	0.79	0.01	16.79
SD	2.44	1.59	5.31	7.62	0.02	4.39	0.03	90.54

:SD>SE σ^2g' = average genetic variance among RILs within crosses estimated from mixed model

DH days to heading, DM days to maturity, PH plant height, TKW thousand kernel weight, ME malt extract, FR friability, GPC gain protein concentration, BG beta-glucan, SD standard deviation, SE standard error.

Table 8: Correlation of traits studied (genotypic correlation above diagonal).

	DH	DM	PH	TKW	ME	FR	GPC
DH	*	0.78	0.01	0.02	0.01	-0.11	0.07
DM	0.65	*	0.19	0.13	-0.14	-0.15	0.12
PH	-0.08	0.06	*	0.35	0.01	0.02	0.07
TKW	0.02	0.17	0.35	*	-0.19	-0.29	0.39
ME	0.00	-0.02	-0.03	-0.09	*	0.60	-0.48
FR	-0.05	-0.10	-0.08	-0.27	0.58	*	-0.73
GPC	0.06	0.01	0.17	0.40	-0.35	-0.60	*

Deep green desired direction and strong relation, light green desired direction and moderate correlation, very light green desired direction and weak correlation, deep red undesired direction and moderate correlation, light red undesired direction and weak correlation, correlations values above 0.2 and below -0.2 are significant ($P < 0.01$).

Table 9: Association of Mid-Parent Values (MPV) to Cross Means (CM).

Parameter	Trait							
	DH	DM	PH	TKW	ME	FR	GPC	BG
Regression coefficient	1.51***	1.60***	0.84***	1.61***	2.07***	2.17**	2.64**	1.35***
SE	0.27	0.30	0.15	0.20	0.32	0.62	0.76	0.26
R^2	0.52	0.50	0.52	0.70	0.58	0.28	0.27	0.47
H^2 crosses	0.81	0.87	0.37	0.73	0.69	0.68	0.42	0.49
H^2 parents	0.87	0.72	0.93	0.73	0.49	0.74	0.81	0.83

*, **, ***indicates significance at P = 0.05, 0.01, and 0.001, resp. DH = days to heading, DM = days to maturity, PH = plant height, TKW = thousand kernel weight, ME = malt extract, GPC = gain protein concentration, FR = friability, BG = beta-glucan.

3.7. Regression of Cross-Mean (CM) on Mid-Parent Value (MPV). Regression coefficients of the cross-mean on mid-parent values studied were highly significantly different from zero for all traits (Table 9). The portion of the cross-mean variance attributed to the regression coefficient is quantified by the coefficient of determination (R^2). R^2 ranges from 0.27 to 0.70 and is higher than 0.5 for most of the traits. This indicates that the MPV was an accurate predictor of the CM performance. Regression and determination coefficients increase if the phenotypic covariance between MPV and CMs in the nominator is large and vice versa they decrease if phenotypic variances in the denominator are large. H^2 for MPV and CM indicates how much phenotypic variance is inflated compared to their genotypic variances.

3.8. Usefulness of Crosses. Since the interest of the breeding program is typically both increasing the mean value of the breeding population and identifying superior RIL, crosses can be ranked based on the usefulness criterion (Schnell and Utz, 1975). To illustrate a practical application of the usefulness parameter (Table 10), the 30 crosses of the experiment were evaluated for their potential to deliver a RIL that can outperform the well-known malting variety planet for five important field and malt quality traits (PH, TKW, ME, FR, and BG). U has been calculated as $U_{ij} = C_{ij} + ih\sigma$, and as selection intensity $U(i = 1)$ was chosen. This selection intensity corresponds to a selection rate of α~0.3. The best 8 crosses surpass the planet with their selected fraction $U(i = 1)$ for all 5 traits. Thus, assuming sufficient RILs/crosses are available, there is a good chance to select one "all-rounder"

Table 10: Usefulness of crosses ($U_i = 1$).

Cross		Traits					No of traits with fraction $U_{(i=1)}$ > Planet	
Parent 1	Parent 2	PH	TKW	ME	FR	BG		
Planet	MBHIBYT-22	92.51	48.75	82.48	74	398.76	5	Superior
ICARDA GP-67	HB 1963	94.59	52.01	81.75	69.92	366.92	5	
Planet	ICARDA GP-67	96.15	46.79	81.7	70.15	379.76	5	
(Bekoji-1 xGrace)	Planet	96.47	50.52	81.22	70.6	383.94	5	
G13-64	MBHIBYT-22	92.57	47.44	81.22	72.49	360.53	5	
MN Brite	Planet	93.21	48.83	80.95	73.56	387.75	5	
IBON 13/2	G13-64	95.66	52.01	80.85	69.62	426.82	5	
Planet	ICARDA GP-75	94.84	51.59	80.74	66.4	375.29	5	
Planet	IBON 14/15-144	92.49	50.8	82.1	72.95	331.16	4	
Planet	IBON 14/15-129	94.8	49.39	82.04	70.75	325.37	4	
Burton	ICARDA GP-67	92.14	47.47	81.59	73.5	357.02	4	
Planet	IBON 13/14-128	91.94	47.96	81.51	72.71	316.08	4	
Burton	Planet	93.9	46.95	81.1	72.58	326.38	4	
HB 1963	IBON 13/14-128	91.32	48	80.54	67.26	409.86	4	
IBON 13/2	Planet	92.52	48.28	80.28	66.72	365.73	4	
HB 1963	IBON 14/15-144	93.61	48.99	80.23	69.92	386.2	4	
M 135	G13-64	98.07	54.16	81.05	65.32	337.05	3	Inferior
MN Brite	IBON 13/14-128	90.05	44.04	80.87	70.57	433.47	3	
IBON 14/15-144	ICARDA GP-67	94.46	44.44	80.539	74.71	384.9	3	
IBON 14/15-144	IBON 13/14-128	92.06	43.64	80.43	68.47	433.06	3	
Burton	IBON 13/14-128	95.28	50.45	80.34	62.72	453.6	3	
M 135	IBON 13/14-128	93.8	51.18	79.9	62.6	429.05	3	
Planet	MBHIBYT-23	92.96	50.68	79.74	68.7	354.34	3	
IBON 13/2	ICARDA GP-67	98.13	55.88	79.72	60.92	385.92	3	
Burton	G13-64	93.42	50.55	79.6	61.23	405.84	3	
M 135	Planet	90.76	46.04	81.28	69.96	349.81	2	
IBON 13/33	G13-64	89.06	42.74	81.23	70.25	306.89	2	
G13-64	IBON 13/14-128	90.82	46.35	80.9	74.55	349.32	2	
Planet	IBON 13/14-41	97.02	57.01	80.34	66.06	344.92	2	
G13-64	ICARDA GP-75	94.25	50.26	80.24	63.07	350.39	2	
Mean		93.6	49.1	80.9	69.1	373.9	5	
Planet		91	46.6	80.7	66.2	359	0	

RIL out of these crosses, which has a good performance for all 5 traits green color in Table 10. The second-best group of 8 crosses fits the U criterion for 4 traits (blue color). The third and fourth groups (9 and 5 crosses, respectively) are considered inferior because for more traits compromises have to be accepted. If the breeder could predict the most attractive crosses at the beginning of the breeding cycle, he would invest in higher numbers of RIL of these crosses and discard the low-performing crosses to save breeding capacity.

4. Discussion

4.1. Comparison of Generation Means. Means of 30 mid-parents values (μMPV) and their 900 derived RIL (μRIL) were estimated across Holeta and Bekoji (Table 11). Differences Δ (μMPV–μRIL) across all crosses were small in general and nonsignificant in a t-test. On average, RIL was found to be slightly later and taller and provided with a higher TKW. With regard to malt quality traits, RIL deviated slightly in the undesired direction. Three driving forces for deviations between generation means shall be discussed in the following.

(i) Differences between generation means could be due to shortcomings in the accuracy of our experimental data. Compared to Bekoji, the accuracy of Holeta was lower. Alternative designs to the p-rep design chosen might lead to higher accuracies and are discussed below.

(ii) Selection and also drift effects during line development from F2- to F4- generation are potential reasons for a difference between generation means.

Table 11: Comparison of generation means of mid-parent values (μMPV) and derived recombinant inbred lines (μRIL) estimated across two environments.

Generation means	DH	DM	PH	TKW	ME	GPC	FR	BG
Mid-Parents								
All crosses ($N = 30$)	81.1	133.0	90.6	46.6	80.8	11.1	68.1	360.5
Planet crosses ($N = 9$)	80.5	132.6	90.8	46.9	80.9	11.1	68.7	357.0
Nonplanet crosses ($N = 13$)	81.3	133.3	90.1	46.2	80.7	11.2	67.6	372.0
RIL								
All crosses ($N = 30$)	81.2	133.1	91.5	46.9	80.7	11.2	66.7	363.0
Planet crosses ($N = 9$)	79.8	132.0	91.0	46.4	81.1	11.2	68.2	347.2
Nonplanet crosses ($N = 13$)	81.9	134.2	91.3	46.6	80.5	11.2	65.8	381.9
Δ(mMP-mRIL)								
All crosses ($N = 30$)	−0.1	−0.2	−0.9	−0.3	0.1	−0.1	1.4	−2.5
Planet crosses ($N = 9$)	0.7	0.6	−0.2	0.5	−0.2	−0.1	0.5	9.7
Nonplanet crosses ($N = 13$)	−0.5	−0.9	−1.2	−0.4	0.2	−0.1	1.8	−9.9

In these generations, entries are grown as single plants which could efficiently be selected on traits such as DH, PH, or TKW. Because not more than 30 RIL/cross had been sampled, drift effects cannot be excluded.

(iii) Based on the genetic architecture of the traits, epistasis could be an explanation for the difference between the two generations. The absence of significant differences between the generations' means would suggest the following: (1) epistatic effects are of minor importance in the germplasm investigated and/or (2) positive and negative epistatic effects have canceled each other. In line with this, [6] reported that epistatic effect was the main reason for the observed difference between generations' mean. The fact that deviations between parents and RIL were larger and differing in sign when considering the planet and nonplanet crosses separately speaks in favor of the second "canceling" hypothesis. In any case, epistasis can be considered a potential source of bias and breeders should try to limit its impact. For example, in the future, when assessing mid-parent values, breeders could estimate the breeding value instead of the genotypic value of the putative parental lines and use them to predict cross-means. In line with this, many studies showed the involvement of epistasis in generation mean on barley [25], durum wheat [28], or bread wheat [29–32].

(iv) Finally, all three driving forces might be confounded in the phenotypic data and might have canceled each other to a certain extent.

4.2. Variance among Mid-Parents, Crosses, and Lines within Crosses. In the previous sections of this study, a simple genetical model has been proposed. In short, this model assumes that all parental lines (i) originate from one random mating population in linkage equilibrium and (ii) are mated randomly to form crosses. (iii) Parental lines are assumed to be homozygous. (iv) From these crosses, homozygous RILs are derived by an SSD process in the absence of forces driving changes in allele frequency. (v) As a mode of inheritance, additive gene action and absence of epistasis have been postulated. With this model, we expect the genetical variances of parents, crosses, and RILs:

$$\frac{\sigma_p^2}{2} = \sigma_c^2 = \sigma_g^2 = \sigma_A^2. \quad (7)$$

The following deviations of the breeding population studied in the real experiment from the idealized population described above will be addressed. In particular, the impacts of these deviations on genetic variances are discussed.

(i) 17 parental lines have been used to produce crosses. As shown in Table 1, lines have different geographical origins such as the USA, Europe, Ethiopia, and ICARDA. Diversity analysis based on KASP markers (Marker data not shown) groups the lines into 3 (sub)populations. Thus, the assumption that parental lines originate from one population is not met in our experiment. If populations differ in their allele frequencies and means, crosses between them will lead to more heterozygous F1 plants. Variance within crosses derived from these F1 genotypes will be increased compared to the variance among crosses [6] and accordingly shrink the ratio σ_c^2: σ_g^2.

(iii) Another model assumption is that parental lines are randomly intercrossed. With 17 parental lines $(17 \times 16)/2 = 136$ crosses are feasible. Out of these potential 136 crosses, only 30 crosses had been investigated for our experiment. This small section led to variation in the gametic contributions of the parental lines. For example, the planet had been involved in 13 crosses, whereas Bekoji × Grace contributed only to one cross. Further, the small sample of crosses could have led to assortative or disassortative mating [15], which means that lines with a similar or dissimilar genotype for a given trait have been crossed more often than those that would occur by chance. Assortative mating increases the variance between crosses and decreases the variance within crosses. The opposite holds true for

disassortative mating. Breeders often follow these nonrandom matings by "best × best" and "parents complementary" crossing designs, respectively. Which of these forces leading to deviation from random mating predominated in our case has to be inspected for each trait separately.

(iii) Parents were assumed to be homozygous. Adopting the homogeneity threshold of 95%, we know this assumption does not hold true for 6 out of the 17 parents (marker data not shown). The more heterogeneous lines taken for crosses are, the more the variance within crosses increases, and correspondingly, the variance among crosses decreases.

(iv) RILs used in our experiment are lines derived from individual F4-SP and tested in F5-generation. F4-SP is expected to be homozygous for 87.5% of the loci having been heterozygous in the ancestral F1 genotype. The remaining 12.5% of the loci are still heterozygous and do not contribute to segregation variance between RILs within a given cross. In case fully homozygous RILs were produced, e.g., by double haploid culture, 100% of the loci would have been homozygous and a higher segregation variance is expected. As a consequence, the RIL generation chosen for our experiment will contribute to inflating the ratio σ_c^2: σ_g^2. In the SSD procedure leading to the final RILs, no change of allele frequency due to drift, selection, migration, and mutation has been assumed. In practical breeding, drift could occur due to bottlenecks in the preceding generations. Selection on traits such as earliness, plant height, or TKW is straightforward to have happened. (Im)migration of foreign germplasm into the progeny of a cross can be caused by, e.g., technical mixture or wrong pedigree assignment.

(v) From analyzing the generation means in the previous chapter, we should be careful to regard epistatic effects and their respective variance as nonexistent. This conclusion is in line with findings in the literature [6] for wheat and [11] for barley.

Recurring to the balance sheet of the genetical variances estimated in our experiment, the following conclusions can be drawn. The parameter $\sigma_p^2/2$ subdivides the variance among individual parental lines by 2 and is meant as an estimator of the variance between mid-parents. Taking the arguments summarized under (ii), this estimator is rather imperfect and should be replaced by the variance among mid-parents used in our experiment. Ratio σ_c^2: σ_g^2 was assumed to be equal to one. Taking the arguments summarized under (i)-(iv), we see severe violations of the assumptions made in the initial model, and therefore, we cannot anymore expect these two variances to be equivalent. For DH, PH, TKW, FR, and GPC, we observed a tendency for σ_c^2 to be smaller than σ_g^2. The opposite tendency was found for DM, ME, and BG. In particular, arguments under (ii) are of importance here. In contrast to the companion study of [6], in our case, mating of parental lines is highly unbalanced and cannot be regarded as random.

The breeder might use outstanding RILs from this experiment for intercrossing and for starting a new breeding cycle. The resulting population will be much closer to the assumptions defined above than the actual breeding population and deliver variance estimates which are more representative of a situation close to classical second cycle breeding. Nevertheless, the actual estimates are a highly valuable starting point to improve breeding methodology. Irrespective of their actual ratio, the two variances, σ_c^2 and σ_g^2, proved to be significantly deviating from zero for almost all traits. Accordingly, the breeder can exploit both of them for selection. A similar result has been reported on wheat by [6] with large size of σ_g^2 among RIL within crosses.

4.3. Genetic Variance within Crosses (σ_g^2). Combining parents from different genetic backgrounds, origins, and performances, a high variation of the estimates for the genetic variance of RILs within crosses is expected. The results obtained in this study confirmed this expectation for most traits. σ_g^2 estimated varied from cross to cross for DH, DM, PH, TKW, and GPC. In contrast, this had not been observed for the quality traits ME, FR, and BG. As an indicator for the variation of cross-specific σ_g^2, the standard deviation of the estimates in comparison to their mean standard error was taken. The standard error depends on the number of RILs per cross and the testing intensity in terms of testing sites and replications. With only 30 RILs/cross and only two locations, σ_g^2 can only very roughly be estimated for an individual cross. Therefore, for breeding methodological studies, the mean σ_g^2 found in our experiment should be taken as a starting point and as a rule-of-thumb figure. In line with this, [6] reaches a similar conclusion for most traits studied in their winter wheat experiment.

4.4. Broad Sense Heritability of Traits. In line with our result, [33] reported a wide heritability value of the same magnitude in some quantitative traits in barley. Similarly, [34] conducted an extensive review on heritability, and [33] in their study on barley genotype under irrigated conditions concluded that for most agronomic traits, heritability values are generally high (>80%). Heritability of malt quality traits ranged from moderate to high value as reported by [35].

In our study, H^2 of parental lines was higher compared to the estimates for crosses and RIL. A genetic explanation for this finding comes from the model (Table 11) expecting the variance of homozygous parental lines to be twice as large compared to the variance among crosses. Another reason for a higher parental heritability is attributed to experimental design. For the standard scenario, phenotypic variance as a denominator in the heritability formula has been defined as $\sigma_p^2 = \sigma_g^2 + \sigma_{gl}^2/n_l + \sigma_{gy}^2/n_y + \sigma_{gly}^2/(n_l n_y) + \sigma_e^2/(n_l n_y n_r)$ with σ_g^2 variance of the genetic effects, σ_{gl}^2 variance of the genotype × location interaction effects, σ_{gy}^2 variance of the genotype × year interaction effects, σ_{gly}^2 variance of the genotype × location × year interaction effects, σ_e^2 variance of

the error effects on a single plot basis, and n_l, n_y, and n_r as the number of locations, years, and replications, respectively. Parents were five times replicated at each location of this experiment. Therefore, the effect of the experimental error on phenotypic variance could be reduced considerably.

In our study, RILs were tested across two locations in a *p*-rep design that practiced replicating half of the RILs at each location. So far, comprehensive studies are lacking on how this design increases as compared to a design with a single replication per location such as augmented RCBD. Heritability could be classified as low $H^2 \leq 0.2$, moderate $0.2 \leq H^2 \leq 0.50$, and high $H^2 \geq 0.5$, and most of the heritability obtained in this study falls into the moderate and high classes. Taking into account the fact that genetic gain is proportional to the square root of heritability (h) the h-values are even higher.

Further, because overall selection intensity has to be distributed to all traits under selection, for the individual trait, only a negative selection with discarding the low performers can be realized. With the h-values achieved in our experiment, the risk is low to falsely discarding an entry that in reality is high performing. Nevertheless, when asking the question of how heritability can be increased, two driving forces can be mentioned besides increasing genetic variances: (i) testing intensity can be enhanced by involving more years, locations, and replications, and (ii) employing trial analytics reduces masking of the genotypic effects. In our case, testing intensity hardly can be increased, because available seeds will suffice for not more than 3 plots. Generally, enhancing testing intensity is costly and will suffer from diminishing returns. In contrast, employing trial designs and analytics with "phenotypic spatial correction, location data quality evaluations, and the generation of breeding values" [36] is hypothesized to be highly effective in terms of optimized use of limited phenotyping capacity.

4.5. Phenotypic and Genotypic Correlation between Traits. The strong negative phenotypic and genotypic correlation between GPC and FR ($r = -0.60, -0.73$) is in line with [12, 37], who suggested that the breeder should take into account this negative relation while conducting selection. Similarly, [38] reported a strong negative correlation between FR and GPC. From F2- to F4-generation, breeders advance their cross progenies as single plants grown in Belg- (F2- and F4-SP) and Meher- (F3-SP). Single plant performance can be used to select field traits such as DH, PH, and TKW. Single plant selection on these traits is known to be cheap and effective when conducted by skilled breeders. As shown in Table 10, the genetic correlation of these traits to malt traits is generally low except the correlation TKW-GPC ($r_g = 0.39$). Apparently, a stringent selection on these traits will hardly affect malt quality, in particular, if only a negative selection for TKW by discarding entries with a very low TKW is practiced. If successfully preselected in the way described in the F4:5 generation, the breeder can focus on selecting the RIL populations on malt traits and can realize higher selection intensity for these traits.

When it comes to the selection of malt traits, the breeder should consider $r_g = 0.60$ for ME-FR which is in the direction desired for malt quality improvement. Other significant correlations for ME-GPC ($r_g = -0.48$) and FR-GPC ($r_g = -0.73$) are also in the desired direction if GPC does not fall below the minimum value required for malting. The best way to exploit these trait correlations is to combine all single traits in an index targeting to improve malt quality as a complex trait.

4.6. Regression of Cross-Mean (CM) on Mid-Parent Value (MPV). MPV could be used as a predictor of CM for the traits of interest. For all traits under study, regression coefficients were found to be highly significant deviating from zero (Table 8). The corresponding coefficients of determination (R^2) indicate how much of the cross-mean variance is explained by the regression coefficient. They were estimated to be higher than 0.5 for all field traits and ME. Lower R^2-values were observed for FR, GPC, and BG. Usually, phenotypic data from parental lines are known from the preceding breeding cycle. In case of the breeding scheme currently applied by the Ethiopian barley team, field and quality data are available for two years (PON and PVT) and for grain yield only for one year (PVT). In an additional year, parental lines are tested in a Parental Performance Test (PPT). Aggregating all data from three years, entries have been tested for field and quality traits in $2 + 2+4 = 8$ environments from PON, PVT, and PPT, respectively. Analogously, yield data are available in $0 + 2+4 = 6$ environments from PON, PVT, and PPT, respectively. Further, in the future, there will be pedigree and phenomic (from NIRS spectra, see [39]), and estimated breeding values are available. By employing all data, very accurate MPV estimates should be used in the future.

In our experiment, 17 parental lines were involved. Derived MPV estimates are a powerful tool for the plant breeder as can be demonstrated by the following consideration. In our case, means of $(17 \times 16)/2 = 136$ crosses or $17 \times 16 = 272$ backcrosses to both parents can be predicted. To predict cross-means and select among crosses is highly advantageous if the variance between crosses is high as has been found in our experiment (Table 4).

4.7. Usefulness of Crosses. Applying the usefulness criterion for the current study, out of 30 crosses, 16 crosses were classified as superior (Table 10). Their $U_{(i=1)}$ surpassed the performance of the best-registered variety "planet" for 4–5 important traits of malt barley. As discussed before, an accurate estimation of cross-specific σ_g^2 is extremely demanding from an experimental point of view. The prediction of this parameter and its integration into the usefulness criterion had been an unsolved problem until the advent of genomic prediction [40, 41]. As a resource for developing and phenotyping candidate entries often is the main limiting factor in breeding programs, focusing on the most promising cross will be the way to obtain outstanding or transgressive segregants in the subsequent generation [6, 40, 42–44].

Crosses can be ranked/selected based on usefulness (U) criteria as the interest in breeding is typically both increasing the mean value of the population and identifying superior

RIL. Hence, in this study, the known malting variety planet was used as a threshold to select RILs that can outperform the planet using $U_{(i=1)}$. Accordingly, about 16 crosses (8 crosses outperforming the planet at least by 5 economic traits and 8 crosses at least by 4 traits) were selected for further evaluation. If this could be predicted at an early stage of the breeding cycle, the breeder would have a good chance to discard nonuseful and low-performing crosses and save breeding capacity. Applying the usefulness criterion leads to a reduced number of lines contributing to the next breeding cycle and a higher variance of the gametic contribution of the remaining parental lines. In the long run, this could lead to the loss of genetic diversity and genetic gain. To counterbalance this trend, the breeder should follow the recommendation of [41] and generate more RIL in crosses with a lower mean but regarded as indispensable for maintaining genetic diversity. Thus, segregation variance can be exploited more extensively and the chances for RIL meeting the required performance are enhanced.

5. Conclusions

Genetic variation in a breeding population is the basis for crop improvement and is required to achieve genetic gains in a breeding program. This variation can be exploited through the use of genetic different sources such as landrace, exotic lines, or elite breeding lines in the actual breeding program. This genetic variation could be structured into between crosses and among lines within crosses. The breeder can use the estimates of these variances to optimize his/her breeding resources. Hence, irrespective of their actual ratio, the two variances, σ_c^2 and σ_g^2, in our study proved to be significantly deviating from zero for almost all traits. Accordingly, the breeder can exploit both of them for selection. Further, cross selection based on mid-parent value and usefulness were found parameters to be exploited in practical breeding.

Data Availability

Data are available on request from the authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors thank the GIZ-SSAP, the Oromia Institute of Agricultural Research (IQQO) for financing, and the Ethiopian Institute of Agricultural Research (EIAR) for hosting the experiment.

References

[1] J. A. d. C. Andrade, "Genetic variability and breeding potential of Flintisa composite of maize in two levels of technology," *Crop Breeding and Applied Biotechnology*, vol. 19, no. 2, pp. 145–152, 2019.

[2] L. Brbaklić, D. Trkulja, S. Mikić et al., "Genetic diversity and population structure of Serbian barley (*Hordeum vulgare* L.) collection during a 40-year long breeding period," *Agronomy*, vol. 11, no. 1, p. 118, 2021.

[3] R. Bernardo, "Reinventing quantitative genetics for plant breeding: something old, something new, something borrowed, something BLUE," *Heredity*, vol. 125, no. 6, pp. 375–385, 2020.

[4] R. Bernardo, "Upgrading a maize breeding program via two-cycle genomewide selection: same cost, same or less time, and larger gains," *Crop Science*, vol. 61, no. 4, 2021.

[5] J. Shrimali, A. S. Shekhawat, and S. Kumari, "Genetic variation and heritability studies for yield and yield components in barley genotypes under normal and limited moisture conditions," *Journal of Pharmacognosy and Phytochemistry*, vol. 6, no. 4, pp. 233–235, 2017.

[6] H. F. Utz, M. Bohn, and A. E. Melchinger, "Predicting progeny means and variances of winter wheat crosses from phenotypic values of their Parents," *Crop Science*, vol. 41, pp. 1470–1478, 2001.

[7] H. Y. Hung, C. Browne, K. Guill et al., "The relationship between parental genetic or phenotypic divergence and progeny variation in the maize nested association mapping population," *Heredity*, vol. 108, no. 5, pp. 490–499, 2012.

[8] F. Addisu and T. Shumet, "Variability, heritability and genetic advance for some yield and yield related traits in barley (*Hordeum vulgare* L.) landraces in Ethiopia," *International Journal of Plant Breeding and Genetics*, vol. 9, no. 2, pp. 68–76, 2015.

[9] N. Akgun, "Selcuk journal of agriculture and food sciences genetic variability and correlation studies in yield and yield related characters of barley (*Hordeum vulgare* L.) genotypes," *Selcuk Journal Agriculture and Food Science*, vol. 30, no. 2, pp. 88–95, 2016.

[10] N. H. Ghimire and P. M. Mahat, "Genetic variability and correlation coefficients of major traits in cold tolerance rice (*Oriza sativa* L.) under mountain environment of Nepal," *International Journal of Applied Sciences and Biotechnology*, vol. 7, no. 4, pp. 445–452, 2019.

[11] H. Oakey, A. Verbyla, W. Pitchford, B. Cullis, and H. Kuchel, "Joint modeling of additive and non-additive genetic line effects in single field trials," *Theoretical and Applied Genetics*, vol. 113, no. 5, pp. 809–819, 2006.

[12] F. Laidig, H. P. Piepho, D. Rentel, T. Drobek, U. Meyer, and A. Huesken, "Breeding progress, variation, and correlation of grain and quality traits in winter rye hybrid and population varieties and national on-farm progress in germany over 26 years," *Theoretical and Applied Genetics*, vol. 130, no. 5, pp. 981–998, 2017.

[13] H. P. Piepho and J. Möhring, "Computing heritability and selection response from unbalanced plant breeding trials," *Genetics*, vol. 177, no. 3, pp. 1881–1888, 2007.

[14] P. Schmidt, J. Hartung, J. Rath, and H. P. Piepho, "Estimating broad-sense heritability with unbalanced data from agricultural cultivar trials," *Crop Science*, vol. 59, no. 2, pp. 525–536, 2019.

[15] D. S. Falconer and T. F. C. Mackay, "Introduction to quantitative genetics (fourth edition)," in *Trends in Genetics* Vol. 12, Longman Pub Group, Harlow, UK, 1996.

[16] A. Hailu and S. Alamerew, "Correlation and path coefficient analysis of yield and yield associated traits in barley (*Hordeum vulgare* L.) germplasm," *Advances in Crop Science and Technology*, vol. 4, no. 2, 2016.

[17] J. A. Al-Tabbal and A. H Al-Fraihat, "Genetic variation, heritability, phenotypic and genotypic correlation studies for

yield and yield components in promising barley genotypes," *Journal of Agricultural Science*, vol. 4, no. 3, pp. 193–210, 2011.

[18] M. Iqbal, K. Khan, and J. Bakht, "Genotypic and phenotypic associations among physiological traits in subtropical maize," *Sarhad Journal of Agricultural*, vol. 25, no. 4, p. 5, 2009.

[19] F. W. Schnell and H. F. Utz, "F1-leistung und elternwahl in der zü chtung von selbstbefruchtern," *Berichtü ber die Arbeitstagung der Vereinigung österreichischer Pflanzenzüchter, BAL Gumpenstein, Gumpenstein, Austria*, pp. 243–249, 1975.

[20] B. Cullis, B. Gogel, A. Verbyla, and R. Thompson, "Spatial analysis of multi-environment early generation variety trials," *Biometrics*, vol. 54, no. 1, p. 1, 1998.

[21] J. Möhring and H. P. Piepho, "Comparison of weighting in two-stage analysis of plant breeding trials," *Crop Science*, vol. 49, no. 6, pp. 1977–1988, 2009.

[22] B. Cullis, B. Gogel, A. Verbyla, and R. Thompson, "Spatial analysis of multi-environment early generation variety trials author(s): brian Cullis, bev gogel, arūnas verbyla and robin thompson," *International Biometric Society Stable*, vol. 54, no. 1, pp. 1–18, 1998.

[23] A. Smith, B. Cullis, and A. Gilmour, "Applications: the analysis of crop variety evaluation data in Australia," *Australian & New Zealand Journal of Statistics*, vol. 43, no. 2, pp. 129–145, 2001.

[24] H. P. Piepho, E. R. Williams, and L. V. Madden, "The use of two-way linear mixed models in multitreatment meta-analysis," *Biometrics*, vol. 68, no. 4, pp. 1269–1277, 2012.

[25] A. J. Wright and C. C. Cockerham, "Selection with partial selfing. II. Family selection 1," *Crop Science*, vol. 26, no. 2, pp. 261–268, 1986.

[26] B. R. Cullis, A. B. Smith, and N. E. Coombes, "On the design of early generation variety trials with correlated data," *Journal of Agricultural, Biological, and Environmental Statistics*, vol. 11, no. 4, pp. 381–393, 2006.

[27] A. H. Madakemohekar, L. C. Prasad, and R. Prasad, "Generation mean analysis in barley (*Hordeum vulgare* L.) under drought stress condition," *Plant Archives*, vol. 18, no. 1, pp. 917–922, 2018.

[28] M. Salmi, A. Benmahammed, L. Benderradji et al., "Generation means analysis of physiological and agronomical targeted traits in durum wheat (Triticum durum Desf.) cross," *Revista Facultad Nacional de Agronomia Medellin*, vol. 72, no. 3, pp. 8971–8981, 2019.

[29] H. Abbasi Holasou, F. Rahmati, F. Rahmani, M. Imani, and Z. Talebzadeh, "Elucidate genetic diversity and population structure of bread wheat (*Triticum aestivum* L.) cultivars using IRAP and REMAP markers," *Journal of Crop Science and Biotechnology*, vol. 22, no. 2, pp. 139–151, 2019.

[30] T. Javanmard, F. S. Saleh-Abadi, and M. R. Bihamta, "Estimation of some genetic parameters through generation mean analysis in melon," *Indian Journal of Animal Research*, vol. 52, no. 6, pp. 619–624, 2018.

[31] J. Ojaghi and E. Akhundova, "Genetic analysis for yield and its components in doubled haploid wheat," *African Journal of Agricultural Research*, vol. 5, no. 4, pp. 306–315, 2010.

[32] A. A. Said, "Generation mean analysis in wheat (*Triticum aestivum* L.) under drought stress conditions," *Annals of Agricultural Science*, vol. 59, no. 2, pp. 177–184, 2014.

[33] R. M. Sayd, R. F. Amabile, F. G. Faleiro, A. P. L. Montalvão, and M. C. Coelho, "Agronomic characterization of high-yielding irrigated barley accessions in the Cerrado," *Pesquisa Agropecuaria Brasileira*, vol. 52, no. 2, pp. 84–94, 2017.

[34] R. F. Amabile, F. G. Faleiro, F. Capettini, W. Q. Ribeiro Júnior, J. R. Peixoto, and B. C. de Almeida, "Variabilidade genética de acessos elite de cevada para sistemas irrigados no cerrado com base em marcadores RAPD," *Bioscience Journal*, vol. 30, no. 4, pp. 1118–1126, 2014.

[35] M. Bhatta, L. Gutierrez, L. Cammarota et al., "Multi-trait genomic prediction model increased the predictive ability for agronomic and malting quality traits in barley (*Hordeum vulgare* L.)," *G3 Genes|Genomes|Genetics*, vol. 10, no. 3, pp. 1113–1124, 2020.

[36] J. N. Cobb, R. U. Juma, P. S. Biswas et al., "Enhancing the rate of genetic gain in public-sector plant breeding programs: lessons from the breeder's equation," *Theoretical and Applied Genetics*, vol. 132, no. 3, pp. 627–645, 2019.

[37] I. E. Matthies, M. Malosetti, M. S. Röder, and F. Van Eeuwijk, "Genome-wide association mapping for kernel and malting quality traits using historical European barley records," *PLoS One*, vol. 9, no. 11, Article ID e110046, 2014.

[38] R. Huerta-Zurita, J. Barr, R. D. Horsley, and P. B. Schwarz, "Predicting malt fermentability in malting barley breeding lines," *Journal of the American Society of Brewing Chemists*, vol. 78, no. 1, pp. 50–62, 2020.

[39] R. Rincent, J. P. Charpentier, P. Faivre-Rampant et al., "Phenomic selection is a low-cost and high-throughput method based on indirect predictions: proof of concept on wheat and poplar," *G3 Genes|Genomes|Genetics*, vol. 8, no. 12, pp. 3961–3972, 2018.

[40] C. Lehermeier, S. Teyssèdre, and C. C. Schön, "Genetic gain increases by applying the usefulness criterion with improved variance prediction in selection of crosses," *Genetics*, vol. 207, no. 4, pp. 1651–1661, 2017.

[41] T. Osthushenrich, M. Frisch, C. Zenke-Philippi et al., "Prediction of means and variances of crosses with genome-wide marker effects in barley," *Frontiers of Plant Science*, vol. 9, p. 1899, 2018.

[42] A. Allier, C. Lehermeier, A. Charcosset, L. Moreau, and S. Teyssèdre, "Improving short and long term genetic gain by accounting for within family variance in optimal cross selection," 2019, https://www.biorxiv.org/content/10.1101/634303v1.full.

[43] A. Allier, L. Moreau, A. Charcosset, S. Teyssèdre, and C. Lehermeier, "Usefulness criterion and post-selection parental contributions in multi-parental crosses: application to polygenic trait introgression," *G3 Genes|Genomes|Genetics*, vol. 9, no. 5, pp. 1469–1479, 2019.

[44] P. Bijma, Y. C. J. Wientjes, and M. P. L. Calus, "Breeding top genotypes and accelerating response to recurrent selection by selecting parents with greater gametic variance," *Genetics*, vol. 214, no. 1, pp. 91–107, 2020.