ESTIMATION OF BREEDING PARAMETERS WITHIN AND BETWEEN MALT BARLEY (*HORDEUM VULGARE* L) CROSSES USING PHENOTYPIC TRAITS AND KASP SNP MARKERS

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This is to certify that the thesis prepared by **Mr. Endeshaw Tadesse**, entitled "**Estimation of Breeding Parameters within and Between Malt Barley (*Hordeum distichum* L.) Crosses using Phenotypic Traits and KASP SNP Markers"** submitted in partial fulfillment of the requirement for the Degree of Master of Science in Biology (Applied Genetics) complies the regulation of the University and meets the accepted standards with respect to originality and quality.

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DEDICATION

This piece of work is dedicated to my Mother **Dame Aredo Tola** and my late father **Tadesse Simie Feyisa** who nurtured me with affection and love and has been waiting for my success, but passed away before celebrating the fate of his endeavors. You have successfully made me the person I am becoming. You will always be remembered.

ABSTRACT

The genetic variation in a breeding program is created by crossing genetically divergent parents. The resulting genetic variation between and within crosses is the determining factor for the offspring's performance, which is defined as the level of compliance of the offspring with preset breeding goals. Estimation of breeding parameters plays a key role in crop improvement. The aim of this study was to (i) estimate and compare mid-parent value and the cross mean (ii) estimate and compare the variance between means of crosses (σ^2_c) and segregation variance of recombinant inbred line within crosses (σ^2_g) (iii) estimate correlation among the traits measured and their heritability (iv) estimate and compare Rogers Distances (RD) of parental lines and their correlation to the segregation variance within crosses, and (v) estimate usefulness of crosses. 900, F4:5 recombinant inbred lines randomly derived from 30 crosses were evaluated at two locations in modified split plot p-rep design whereas the parental where genotyped using SNP-KASP markers. The variance component analysis revealed significant ($P < 0.05$) genetic variation among parents, crosses and RIL for almost all traits. Based on generation mean analysis, the means of recombinant inbred line did not deviate significantly from the means of parental lines. The range of σ^2_g for the individual crosses was very high for days to heading, days to maturity, plant height, thousand kernel weight and grain protein concentration and for these traits the respective standard deviation were high. Heritability for parents ranged from 49.50 % (malt extract) - 93.60 % (plant height), heritability for crosses ranged from 29.52 % (grain protein concentration) - 87.0% (days to maturity), whereas heritability for RIL was lowest with 27.40% (beta-glucan) - 73.60% for thousand kernel weight. Significant ($P<0.01$) genotypic correlations with high impact for practical breeding were found between malting traits. Significant ($P<0.01$) regression of cross mean on mid-parent value were obtained for all traits demonstrating that cross means can accurately be predicted from mid parent values and selection among crosses at an early stage of line development is highly effective. Further, based on the usefulness criteria, 16 outperforming crosses were identified that surpass Planet as the actual leading malt variety. Diversity analysis revealed an average RD between lines was 0.46 with a range of 0.32-0.64 and homogeneity within the 17 parental lines varied from 71-100%. The dendrogram grouped the lines into three clusters. Importantly, the high malting European lines were grouped together with some of the ICARDA and Ethiopian lines Correlations of RD to σ^2_g ranged from -0.19 to 0.34 and were not significantly deviating from zero for all traits except DH, hence, RD proved to be not predictive for σ^2_g. In this study the two variances, σ^2_c and σ^2_g, proved to be significantly deviating from zero for almost all traits; hence breeder can exploit both of them for selection. Generally, differences between generation means (Parental lines vs. RIL) were small across all crosses. But when grouping into crosses with and without Planet as a parental line, deviation between parent and RIL were larger possibly due to epistastic effects. Hence breeder should estimate breeding values rather than genetic values of parental lines. The use of mid-parent value and usefulness for cross selection were found to be promising. The national barley breeding program should exploit this result by starting with a large number of initial crosses and reducing them to the most promising crosses. Some heterogeneity was observed within the lines. When originating from technical mixture this should be considered as a wake-up call for the breeder to closely follow maintenance breeding. RD based on KASP markers was not predictive for σ^2_g; hence further research will be required by improving accuracy of σ^2_g estimate and linkage disequilibrium between markers and quantitative trait loci (QTL). Over all, the large diversity revealed among lines indicates the potential that the genotypes have to improve the productivity and quality of the crop and the uses of molecular markers can benefit the breeding program in order to have an effective breeding program by selecting diverse parental genotypes with complementary gene action.

Key Words: Parent, Cross, RIL, Heritability, Usefulness, Genetic Diversity

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BLUE	Best Linear Unbiased Estimator
BLUP	Best Linear Unbiased Prediction
CTAB	Cetyltrimethyl ammonium bromide
COV	Covariance
COP	Coefficient of Parentage
CSA	Central Statistical Agency
CS	Certified Seed
DBARC	Debre Brehan Agricultural Research Center
DH-L	Double Haploid Line
DUS	Distinctness, Uniformity and Stability
EBI	Ethiopian Biodiversity Institute
EGS	Early Generation Seed
EIAR	Ethiopian Institute of Agricultural Research
FAO	Food and Agriculture Organization
G-BLUP	Genomic Best Linear Unbiased Prediction
GEI	Genotype by Environment Interaction
GIZ	Deutsche Gesellschaft für Internationale Zusammenarbeit
GLY	Genotype by Location by Year Interaction
HARC	Holetta Agricultural Research Center
HLW	Hectoliter weight in kilogram
IAR	Institutes of Agricultural Research
IBON	International Barley Observation Nursery
ICARDA	International Center for Research in Arid Dry Land Agriculture
KASP	Kompetitive Allele-Specific PCR
KARC	Kulumsa Agricultural Research Center

Kg ha-1	Kilogram per hectar
MET	Multi-environmental Trial
MVN	Multi-variate Normal distribution
MPV	Mid-Parent Value
MOA	Ministry of Agriculture
NIRS	Near-infrared spectroscopy
NPS	Nitrogen-Phosphate and Sulphur fertilizer
PS	Phenomic Selection
QTL	Quantitative Trait Loci
RD	Rodger's Distance
RIL	Recombinant Inbred line
SARC	Sinana Agricultural Research Center
SSAP	Supporting Sustainable Agricultural Program
SNP	Single Nucleotide Polymorphism
SSD	Single Seed Decent
STS-PCR	Sequence tagged sites Polymerase Chain Reaction
TKW	Thousand kernel weight
UPOV	International Convention for the Protection of New Varieties of Plant
VCU	Value for Cultivation and Use
YPU	Yield Plot Unit

1. INTRODUCTION

Barley (*Hordeum vulgare* L.) is an economically important crop worldwide. Following wheat, rice and maize, barley is the 4th main grain and among the top ten crop plants in the globe (Wegayehu Feleke & Derib Alemu, 2019).The Major producers in the world are Europe, South Africa, Near East, Russia, China, India, Canada, USA, Australia and Ethiopia (Shahbandeh 2020, personal communication). Ethiopia is recognized as a center of diversity, as its barley germplasm have global significance because of improved traits, including disease resistance (Tigist Dejene *et al.*, 2010; Wosen Gebresilassie *et al.*, 2019). The national area coverage, production and productivity of barley have been estimated to be 959,273.4 ha, 2,024,921.7 tons and 2.16 tons/ha, respectively (Endalkachew Aklilu, 2020; Daniel Tadesse & Beyene Derso, 2019; World Bank, 2019).Out of the total land currently under cultivation, barley took 9.8 and 8.3% of the total cultivated land and production of cereals crops, respectively (Zewdie Bishaw and Adamu Molla, 2020).

Barley has diverse agro-ecologies being grown from 1800 to 3400m altitude in different seasons and production systems (Muluken Bentayehu, 2013; Dawit Milkias and Gadisa Muleta, 2021). It makes Ethiopia being the lead producer (2.3%) in Africa (Shahbandeh 2020, personal communication), due to favorable ecologies. For centuries, it has been supplying the basic necessities of life (food, feed, and beverages) for millions of households in the highland areas (Wegayehu Feleke & Derib Alemu, 2019). The majority of barley that farmers grow in Ethiopia is food barley and it is the main ingredient for several staple dishes such as injera, porridge, and bread (Wuletaw Mekuria *et al.*, 2017). One aspect of the unique importance of barley is its industrial use with processing into malt which mainly is used for brewing and distilling (Fang *et al*., 2019). Nevertheless, accurate statistical information on area coverage in the country is scanty. Recently, there is an increasing demand to grow malt barley by farmers, which constitutes 15 -20 percent of the total barley production in relation to the introduction of several new malt factories in the country (Wuletaw Mekuria *et al.*, 2017; Zewdie Bishaw and Adamu Molla, 2020).

Despite its long history of cultivation and wide range of uses, its productivity at the farm level has remained low in Ethiopia due to biotic, abiotic and soil factor (Tura Kusa & Gashaw Guben, 2015). Of these, biotic factors such as disease, weed and insects and abiotic factors such as drought, lack of improved variety, water logging, and marginal managements (such as sub-optimal nutrient management, in appropriate or insufficient use of inputs) are involved (Tura Kaso & Gashaw Guben, 2015; Abiro Tigabie *et al.,* 2017). Thus, to tackle these challenges, a sound crop breeding program that improves resilience, productivity and quality of the crop through the development of well- performing varieties is of prime importance.

In most breeding systems genetic variation is created by crossing genetically divergent parents. In this case, the choice of parents in cross combination is very important. It decides on which part of the initially available genetic variation new cultivars will be based and which gene will be (re) combined by crossing (i.e., first selection step).The resulting genetic variation between and within crosses is the determining factor for the offspring's performance, which is defined as the level of compliance of the offspring with preset breeding goals. Genetic variability can be efficiently exploited by selection depending upon heritability (Bilgin *et al.,* 2010).Trait heritability determines the ratio of phenotypic to genotypic variation (Falconer & Mackay, 1996). Hence, estimating the heritability of these traits will help to improve the breeding methodology thereby the breeders can select the desired traits efficiently (Hossain *et al*., 2021; Schmidt *et al*., 2019).

Furthermore, genotypic and phenotypic correlations of traits are important breeding parameters because selection on one trait may have an impact on other related traits (Akgun, 2016; Saleem *et al*., 2016). It is important to understand the interrelationship between different traits of interest that help in determining which traits to select when improvement of the related complex traits is desired. This trait correlation is happened generally, due to linkage disequilibrium, pleotropic gene action and epistasis effect when we talk of genotypic correlation (Falconer and Mackay, 1996;Azeb Hailu *et al.*, 2016). Correlation coefficients indicate relationships among independent variables and the degree of linear relation between these traits among genetically diverse population for enhanced progress in crop improvement (Heffner *et al.*, 2010). Hence, knowledge

of correlation that exist between the desirable traits can enhance the interpretation of results, dictate breeding methods used and provide the baseline information for planning more efficient breeding program (Negash Geleta *et al*., 2019). Further, the magnitude of trait association in barley is important and their utilization in selection has been reported (Tofiq *et al.*, 2015; Azeb Hailu *et al*., 2016; Laidig *et al.*, 2017; Negash Geleta *et al.*, 2019).

The most challenging steps for breeders are creation of genetic variability (i.e.) how to find the best combination of two (or more) parental genotypes to maximize variance among and within respective breeding population, and consequently, the chance of finding superior progeny in the subsequent generations. As a result, breeder starts with large number of parents to get maximum number of progenies. But to select the superior progenies among the given crosses, much resources and time are wasted. Cross performance depending on both mean of a cross and the variation within a cross could be predicted indirectly based on mid-parent values or phenotypic/genotypic distance estimates calculated from morphological or molecular marker data of parental lines (Utz *et al*., 2001).Specifically, the usefulness of a cross for a given trait is defined as the mean of a defined upper fraction of its progeny lines. It could be predicted as the expected cross mean plus the expected selection gain, which is a function of the selection intensity, square-root of the trait heritability, and the genetic standard deviation of the cross (Utz, 1975). If predictions can be made with sufficient accuracy this approach has the advantage that resources can be saved by focusing on the most promising crosses (Kotzamanidis *et al*., 2008; Utz *et al*., 2001).

In addition to develop high yielding hybrids, significant emphasis should also be paid towards characterization of elite breeding lines and understanding of their genetic diversity and relationships through either morphological traits, biochemical or molecular markers (Sejake *et al.*, 2021). In the last three to four decades, tremendous progress has been made in the development of novel genetic tools such as DNA molecular markers, (i.e., Isozymes, Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), and Inter-Simple Sequence repeats (ISSR), Simple Sequence Repeats (SSR), and Single Nucleotide Polymorphism (SNP)) were developed for various molecular purposes (Berhanu Tadesse *et al.*,

2015; Kassa Semagn *et al.*, 2012;Yang *et al.*, 2017). Markers have contributed extensively to generate the information required in the breeding programs. Currently, molecular markers especially, SNPs is emerged as powerful tools for many genetic applications, including germplasm characterization(genetic diversity, genetic relationship, population structure), quality control(genetic purity, genetic identity, parentage verification), linkage mapping, linkage-based and linkage disequilibrium-based quantitative trait loci (QTL) mapping, marker assisted backcrossing (MABC), marker assisted recurrent selection (MARS) and genomic selection (GS)(Berhanu Tadesse *et al.*, 2017; Kassa Semagn, *et al.*, 2012). SNP data can be obtained using one of the numerous uniplex or multiplex SNPs genotyping platforms that combines a variety of chemistries, detection methods and reaction format. Hence, Kompetitive Allele Specific PCR (KASP), utilized in this study is a uniplex SNP genotyping platform that had been developed into a global benchmark technology. KASP is a homogeneous SNPs genotyping technology initially developed by LGC genomics for in-house genotyping. The effectiveness of KASP in genotyping has been reported by several scholars including (Kassa Semagn, *et al.*, 2012; Khera *et al.*, 2013; Berhanu Tadesse *et al.*, 2017; Sejake *et al.*, 2021).

Therefore, marker based assessment of genetic diversity and similarity (homogeneity) in crossing parent is important for selection as well as to save resource and time. (Prasanna & Hoisington, 2003) indicated that assessment of genetic diversity using molecular markers is helpful to maintain and broaden the genetic base of the elite germplasm; selection of appropriate parental lines and generation of segregating progenies to provide genetic variability for further selection. Many DNA based genetic diversity studies have been conducted on barley using different molecular marker types (AKbulut *et al.*, 2018; Ferreira *et al.*, 2016; Huang *et al.*, 2014; Shtaya *et al.*, 2015). However, their application in the day-to-day activity of the breeding program has been limited in Ethiopia. The above review showed the lack of integrated use of breeding methods and molecular markers in our breeding programs that indicate the importance of developing a project that can provide information on the current genetic diversity of the parental lines and breeding parameters utilized in the Ethiopian barley breeding program. Hence, this study was conducted to achieve the following objectives and supply information to the breeding program of the country.

2. OBJECTIVES OF THE STUDY

2.1. General Objective

The main objective of the study was to estimate the breeding parameters which is important information to improve the efficiency of barley breeding program in Ethiopian

2.2. Specific Objectives

The specific objectives of this study were:-

- To estimate and compare mid-parent value and confirm its predictability for the mean performance of crosses
- To estimate and compare the variance between means of crosses and segregation variance within crosses and give guidance for the breeder on how selection could be practiced
- To determine the correlation and heritability among the measured traits of interest
- To estimate the genetic diversity of parental lines using KASP markers and their correlation to the segregation variance within crosses
- To estimate the usefulness of crosses and identify the best crosses based on criteria

3. LITERATURE REVIEW

3.1. Origin and Distribution of Barley

Barley (*Hordium Vulgare* L) is one of the founder crops of Old World Agriculture. Its domestication is fundamental to understand the origin and early diffusion of agrarian culture (Morrell & Clegg, 2007;Wang *et al.*, 2015). Archaeological remains of barley grains found within the Fertile Crescent (Morrell & Clegg, 2007; Saisho & Purugganan, 2007; Wang *et al.*, 2015), indicate that the crop was domesticated about 8000 B.C. Nevo (1992) and Zohary (1989), explained that *Hordeum spontaneum* and *H. Vulgare* are morphologically similar, with the cultivated form having broader leaves, shorter stem and awns, tough ear rachis, a shorter and thicker spike. The wild progenitor *H. spontaneum* is remain colonizing primary habitats within the Fertile Crescent from Israel and Jordan to south Turkey, Iraqi Kurdistan, and south western Iran (Badr *et al.*, 2000; Morrell & Clegg, 2007). According to Harlan and De wet (1971), the earliest archaeological findings of barley date back to 8000 to 7500 B.C. in Mureybit, Syria. According to the archeological findings, fully domesticated two-rowed barley appeared around 7000 B.C., whereas both six-rowed and naked types appeared by 6000 B.C. The introduction of barley in Ethiopia, where it evolved to a secondary center of diversity is considered to be from the middle East about 5000-6000 years ago (Endashaw Bekele, 1983; Harlan, 1971; Molina-Cano *et al.*, 1987). Since then, the crop has diversified and formed unique morphotypes including the irregular and deficient types (Dawit Milkias and Gadisa Muleta, 2021)

Indeed, the Himalayas, Ethiopia, and Morocco have occasionally been considered centers of barley domestication (Endashaw Bekele, 1983; Molina-Cano *et al.*, 1987).The crop is believed to have a multi-centric origin and it could have been domesticated along a broad area from Morocco to Tibet. However, the evidences from archaeology, genetics and distribution patterns of wild and cultivated barley species available in the Near-East (the Fertile Crescent) point towards a mono-centric origin and domestication of barley in this particular area. Further, Harlan & Zohary (1966) were of the view that “domestication might not have taken place where wild cereals were most abundant”. Thus, cereals like wheat and barley originated in areas adjacent to, instead of in, the regions of greatest abundance of untamed cereals.

This view was endorsed by the studies of Badr *et al*.(2000), who, through molecular analysis, located the probable area of domestication in Israel and Jordan. Similarly, Jones *et al.* (2008), guided by the principle that photoperiod (day length or duration of daily exposure to light) response plays a task in allowing crops to be grown in new environments, based their observations on molecular studies of the photoperiod regulating gene in barley, *Ppd-H1*.They concluded that another area of domestication was situated in Iran, east of the Fertile Crescent. In Ethiopia across different regions, there are known potential areas for their agro ecology suitability and rich biodiversity for barley adaptation and distribution (Dawit Milkias and Gadisa Muleta, 2021). However, the crop is mainly distributed in the highland of the country such as Oromia (mainly Bale and Arsi), some part of Amhara, Tigray and SNNP in the altitude ranging from 1500 to 3500 m, but it is predominantly cultivated between 2000 to 3400 m.a.s.l (Tura Kaso & Gashaw Guben, 2015; Berhane Lakew *et al.*, 1997).

3.2. The Role of Barley in Ethiopian Agriculture

In Ethiopian economy, agriculture contributed more than 31.1% of the total GDP, 85 percent of the labor force and 90% of the export earnings (Zewdie Bishaw and Adamu Molla, 2020; Tarekegn Garomsa, 2016; Dawit Milkias and Gadisa Muleta, 2021). It's the major supplier of raw materials to food processing, beverage and textile industries (Dawit Alemu *et al.*, 2006; ATA, 2014). Agricultural technology is among the most impactful area of modern technology; specifically, play a key role in enhancing agricultural yield, product quality, poverty reduction, and as a whole addressing the national food security program as well as creates spillover effect to the remaining sectors(World Bank, 2019). Nevertheless, the production and productivity in agricultural sector in general and at farm level in specific is low in developing countries such as Ethiopia. This could be due to many factors including biotic and abiotic factors, resulting in economic fluctuation that always suffer from unstable production. Cereal production is the means of livelihood for millions of households in Ethiopia and is the single largest sub-sector within Ethiopia's agriculture, far exceeding all others in terms of its share in rural employment, agricultural land use, calorie intake, and contribution to national income (Dawit Alemu *et al.*, 2006; Rashid, 2010).

Barley plays a significant role in the Ethiopian economy both as food and industrial crop. More than 60% of Ethiopian highlanders depend on barley for food, feeds and other local drink preparations (Tura Kaso & Gashaw Guben, 2015).It has a long history and unique place in Ethiopian agriculture due to many reasons such as, it has history of cultivation that coincided with the start of plow culture, grown both in belg and meher season, hunger breaker/crop that provide relief during food shortage because of its early maturing, used in diversity of recipes and deep rooted in the culture of peoples diet, grow in extreme areas that are marginal to other crops and its straw is an indispensible component of animal feed as well as roof thatching (Tura Kaso & Gashaw Guben, 2015; Berhane Lakew, 2020; Berhane Lakew *et al.*,1997). Recently in Ethiopia, the unique use of barley is as industrial crop which is used for malting and brewing. Malt barley, however, may be a newer addition to Ethiopian agriculture, because it was introduced within the late 1960s to service a booming malting and brewing industries (Zewdie Bishaw and Adamu Molla, 2020). Currently about 15-20% of total land is covered with malt barley productions which cover about 40% of the total malt requirement by breweries whereas 60% of raw material requirement is supplemented from external source (Zewdie Bishaw and Adamu Molla, 2020).

3.3. Genetic Diversity of Barley

Ethiopia is a major center of genetic diversity for many important domesticated crop species including barley due to its different ecological variation and landscape. Barley is one of the most important cereal crops grown as farmers' variety and often as heterogeneous populations over a wide range of climates (Berhanu Bekele *et al.*, 2019; Molina-Cano *et al.*, 1987). Such diversity provides security for the farmers against diseases, pests, drought and other stresses (Melaku Worede, 2000). Genetic diversity is not evenly distributed within the geographical range of the species gene pool. This has been recognized since Vavilov work in the 1920s and has been demonstrated in many studies. Some unique alleles occur in some regions "but not in others. Some of the heterogeneity in the distribution of variation is explained by migration or chance factors such as founder effects and genetic drift (Teseam Tanto *et al.*, 2009).

According to Endashaw Bekele (1985), there are different forces that maintain barley polymorphism in Ethiopia such as balance between mutation and selection, balance between selection and migration, the heterogeneous environment, natural polymorphism, frequency dependent selection, and transient polymorphism among the forces. For the genetic variation of cultivated barley, the wild progenitor (*Hordeum spontaneum*) has significant contributions that are economically important. Some of the traits include earliness, high yield, biomass, resistant genes for abundant genetic variation against physiological stress such as drought and salinity (Muñoz-Amatriaín *et al.,* 2014). The genetic diversity of this crop exists in many environments but, its continuity is particularly observed in marginal areas (mountains) due to the well-marked environmental change over small distance, seasonally and in isolation between the production zones.

Morphological diversity is significantly related to altitude for individual characters like rachilla hair type, spike length, and spike density, spike row number and caryopsis type (Wosene Gebreselassie *et al.,* 2015). Some characters are strongly associated with high altitude and others with low altitude. In high altitude of southeast part of Ethiopia, the spike density, short spike length and six-row type are highly diverse (Alo Aman *et al*., 2020; Fassil Kebebew *et al*., 2001). According to Mulugeta Negassa (l985a), the six-row type is concentrated in Arsi-Bale highlands because of their genes for frost resistance, it also appears that the short spike phenotypic class (<10cm) is frequent at higher altitudes (>2400 m.a.s.l) in Arsi and of this about 12% are dense.

In general, the large genetic diversity of Ethiopian barley landraces could be due to the diversity in soils, climate, altitude and topography, together with geographical isolation for longer periods and this environmental variance play significant role for the diversification through evolutionary process (Tesema Tanto *et al*.,2009; Endashaw Bekele, 1985; Sarkar *et al*., 2014;Tigist Dejene *et al*., 2010). In addition, the relationship between the morphological diversity of barley with biochemical components like hordeins polypeptide, flavonoids are some of the important indications of Ethiopian barley diversity. Based on this Biochemical analysis using hordein polypeptide variation has been applied to the systematic of Ethiopian barley (Sintayehu Debebe *et al*., 2015 & 2019). Ethiopia have more than (>1500) germplasm and recognized as a center of

diversity for barley because of the presence of great genetic diversity and endemic forms. For this reason, the barley germplasm has considerable importance to barley breeding worldwide.

3.4. Barley production in the world and in Ethiopia

In the world, European Union, Russian Federation, Ukraine, Turkey and Canada are the top five largest barley producers where, European Union's produce the largest quantities of barley with an estimated production of nearly 60 million tons followed by Russian federations with a production of about 20 million tons (Shahbandeh, 2021, personal communication). In Africa, Ethiopia, Algeria, Morocco, South Africa and Tunisia were the top five largest barley producers for the year 2020 with estimated production of approximately 2.35 million tones,1.85 million tones,1.35 million tones,0.9 million tones and 0.307 million tons, respectively (Shahbandeh, 2021, personal communication).

Ethiopia accounts nearly 25% of the total production in Africa where barley is the principal cereal crop among highlanders (Berhanu Bekele *et. al*., 2005; Sultan Usman & Adamu Zeleke, 2017). Ethiopia is also recognized as a center of diversity for barley having global significance due to its improved traits, including disease tolerance (Bonman *et al*., 2005). In Ethiopia, both food and malt barley species is cultivated. Approximately, 85% of land allocated for barley in Ethiopia is used for food barley production. On the other hand, nearly 150,000 hectares of land (15 % of total barley land) is used for malt barley production, which is the major input for beer production (Zewdie Bishaw and Adamu Molla, 2021; Danie Tadesse & Beyene Derso, 2019).

Ethiopia is endowed with diverse agro-ecologies suitable for different crops among which barley is the crop grown on areas marginal to others crops. It can be cultivated at altitudes between 1400 and 3400 m, but is primarily grown between altitudes of 2000 and 3200 m. However, the area allocated to barley production in Ethiopia over the past 25 years had been variable (Mulatu Bayeh & Berhane Lakew, 2011). It was estimated to be 0.8 million hectare in the late 1970s, and increased to more than 1 million hectare in the late 1980s. It then declined and remained between 0.8 and 0.9 million hectare till the beginning of the 3rd millennium with productivity, of 1.3 t/ha,

that is about half the world average (Berhanu Bekele, 2006). The ratio of total land under barley to other cereal crops has been decreasing over the past years. This reduction in area in the past could be attributed to a number of factors, among these; most of the area under barley is sown to farmer varieties. These produce poor yields and are within the environment for hundreds of years, and sometimes show significant morphological diversity (Berhane Lakew *et al.,* 1993; Daniel Tadesse & Beyene Derso, 2019). There is low productivity in farmer cultivar compared with bread wheat, the latter having been very recently introduced. Wheat has given significantly higher yields than local barley varieties in the same niche, where barley has been in production for millennia. The other possible reason could be the less contribution of the crop as cash crop in the market before the inception of malt factories in the countries. This in turn forced farmers and other producers to turn their face toward production of cash crops like wheat, Teff and Maize.

3.5. Economic Importance of Barley

In developed countries, barley is employed commercially for animal feed, to manufacture malt, which is primarily utilized in beer production, for seed and to some extent human food consumption. Feed comprises about 70 percent of barley use in developed nations. About 16 percent of barley is used for malting, seed or other industries and 14 percent is used for food (World Bank, 2019). Most of the barley consumed in China and USA was as malting production (more than 70%) while quite 85% of barley consumed in Canada is used as feed. In Ethiopia barley grain is majorly used as, food for human consumption, feed for animals and malt for beer production. Traditionally, barley was used for making local recipes, different types of drinks and other types of food (Mulatu Bayeh & Berhane Lakew, 2011). Approximately, 85% of barley produced in Ethiopia is food, and much of it is consumed at home and retained as seed for up-coming season by farmers with only 15% of the product sold at the local markets.

Specifically, it is utilized in several forms like porridge, bread, soup, roasted grain and for preparing different types of drinks (Mulatu Bayeh & Berhane Lakew, 2011). Its straw is important for animal feed, thatching roofs and bedding. As food, it has some useful merits including medicinal properties (Finocchiaro *et al*., 2012; Grando *et al*., 2005). The grain is rich

in zinc, iron and soluble fibers and has higher content of Vitamins A and E than other cereals. Beta glucan of barley reduces blood cholesterol and low glycemic index is used for Type II diabetic people. Tocols in barley are also helping to reduce serum LDL cholesterol and therefore, the bran flour accelerates gastrointestinal transit time and hence reduces colon cancer. Besides its importance in house consumption, malt is the second economically important and unique use of barley that is used mostly in beer production, but also in hard liquors, malted milk, and flavoring in a variety of foods. Some brewers, distiller grains, and sprouts from malting barley even have desirable protein content for animal diets (Emebiri *et al.*, 2005).

Therefore, with this importance, the expansion of beer industries, the research focus on malt barley and the attention given by the government toward industrialization are main driving force for the expansion of malt barley production both in area coverage and yield potential in Ethiopia. Farmers who operate the barley-based farming system that is in the highlands of Ethiopia have very few alternative crops (Mulatu Bayeh & Berhane Lakew, 2011). This is complicated by the very fact that farmers have only a few cash-generating alternatives, which is critical for their existence. Hence, increasing malt barley production in these areas is extremely important to fill the demand gap as well as to sustain the life of the farming community in the highland. Furthermore, the gap between demand and supply for raw material to feed the existing malt and brewery industries require a great revolution in malt barley production from single small holder farming towards organized production in which business sense is created in farmers.

3.6. Barley production constraints

Ranking the 4th Africa's largest beer producer, Ethiopia has an estimated annual total production capacity of 12.6 million hectoliters of beer which demands 235,000 tons of malt a year (Addisu Bezabih, 2018; Tura Kaso & Gashaw Guben, 2015). The total capacity of the malt factories in the country are accounted for about 52,200tons of malt and covering only between 35-40% of the malt demand and the remaining could be filled through imports, which account for 60-65% of the entire annual requirement (Tura Kaso & Gashaw Guben, 2015).To satisfy the ever-increasing malt demand by the beverage industry, and to make sure dependable and better cash returns to

the farmers, expansion of the malt barley production is extremely important since immense potential areas are available for malt barley production to narrow the gap between national demand and supply.

Despite the past and current malt barley research and production efforts, a huge gap exists between the national malt barley grain demand and supply. This comes from the fact that malt barley production still suffers from traditional farming practices, as well as a number of biotic and abiotic factors posing a challenge to barley production in different parts of Ethiopia. However, their impact varies depending upon the genetic, environmental, management condition and the interactions of these factors. Among the abiotic factors low fertilizer use, improper management practices like crop rotation system, tillage practices (Biruk Gezahegn and Demelash Kefale, 2016; Derebe Terefe *et al*., 2018;Azeb Haile *et al.*, 2012 & 2017), and lack of improved variety (Zewude Bishaw and Adamu Molla, 2020; Daniel Tadesse & Beyene Derso, 2019) has been the major factors imposing yield reduction in barley. The biotic factors include, weeds, disease and insect pests (Bonman *et al.*, 2005; Muluken Goftishu *et al.*, 2009; Tafa Jobie, 2003 & 2004;Teklay Abebe & Muruts Legesse, 2017) were also economically important in barley field causing huge yield loss.

Specifically, plant disease is the major biotic stresses that limit production of cereal crops including barley (Bonman *et al*., 2005). More than 28 pathogenic agent of diseases incur yield and quality loses of barley in Ethiopia had been reported in earlier studies (Bekele Hundie *et al.*, 2004; Taye Bekele *et al.*, 2009; Yitbarek Semeane *et al.*, 2002). Nonetheless, all diseases are not important over all environments and they are specific to certain agro-ecological boundaries based on the environmental factors and host variability. Among several diseases recorded on barley in Ethiopia, the major disease includes net blotch (*Pyrenophora teres*), leaf rust (*Puccinia hodei*) and Scald (*Rhynchosporium secalis*) still incur yield losses as high as 67% depending on the susceptibility of the cultivars and seasons (Amri *et al.*, 2005; Tekley Abebe & Murtus Legesse, 2017). It was reported that net blotch, leaf rust, and in some cases, scald was a widely distributed disease in Bale highlands while spot blotch and scald were most widely distributed disease in Arsi, Shewa and East Wellega regions (BARC, 2000; Chua, 2019; SARC, 1990, 1991).

Moreover, insect pest are other biotic factor and economically important in barley field especially in southeastern Ethiopia. Among economically important insect pest known on barley, shoot fly and Aphids are the common and are significantly threatening barley production in Ethiopia. There are two barley shoot fly species *Delia arambourgi Seguy*(Davidson, 1969) and *D. flavibasis Stein* (Tafa Jobie, 2003) identified in Ethiopia, inflicting considerable yield losses. Both species belongs to the order Diptera and family Anthomyiidae. *Delia flavibasis* has been recently recorded from Ethiopia (Tafa Jobie *et al*. 2004). It is considered as the major pest of barley in Ethiopia and Kenya (Tafa Jobie, 2003). It was confirmed that the losses from barley shoot fly have been estimated to be 40-50 %(SARC, 2005). Unfortunately, malting barley varieties are more susceptible to barley shoot fly (Tafa Jobie, 2003) and almost 100% crop failure can be experienced under severe pest infestation (Muluken Goftishu *et al.*, 2009; Teklay Abebe & Murtus Legesse, 2017; Tafa Jobie, 2003; Tekalign Zeleke *et al.*, 2017).

3.7. Organization of Barley Breeding in Ethiopia

Barley breeding in Ethiopia has a long history and a well-organized research system has been started in 1966 with the establishment of the Holetta Agricultural Research Centre (HARC) of the then Institute of Agricultural Research (IAR), [now the Ethiopian Institute of Agricultural Research (EIAR)] to represent the central highlands of Ethiopia with barley being a major focus in crop research (Mulatu Bayeh & Berhane Lakew, 2011; Berhane Lakew *et al.*, 1997). From 1968 onward, the research system mainly focused on food barley with its share estimated about 90%, and the major activities were targeted toward the improvement of agronomic characters (Mulatu Bayeh & Berhane Lakew, 2011;Shahidur *et al*.,2015). Since then, the breeding program was devoted to improving land races and elite germplasm. Currently, the joint Ethiopian barley breeding is organized mainly under four barley improvement centers (i.e., Sinana Agricultural Research Center (SARC) (National Coordinator)), Holeta Agricultural Research Center (HARC), Kulumsa Agricultural Research Center (KARC), and Debre Brehan Agricultural Research Center (DBARC)), which maintain many other collaborative testing sites. The highland and mid-altitude agro-ecology is covered by SARC and HARC, the low moisture area is represented by

KARC whereas the frost-prone area is considered by DBARC. So far, the program has been released more than 47 food and 24 malt barley varieties for commercial use (MOA, 2020).

3.8. Breeding Scheme Used

Barley breeding in Ethiopia depends on local germplasm for resistance to biotic and abiotic factors and on foreign elite germplasm that contributes grain yield, stiff straw, and grain quality (Berhanu Bekele *et al.*, 2019; Sintayehu Debebe *et al*., 2019; Sana *et al*., 2012). Introduced germplasm especially from International Center for Agricultural Research in the Dry Areas (ICARDA) and other organizations are used in the current Ethiopian barley breeding program (Hernandez *et al*., 2020). The barley breeding program in Ethiopia is following a state-of-the-art breeding approach based on pedigree and Single Seed Descent (SSD) methods (Oeveren, 1993; Collard *et al*., 2017; Sana *et al*., 2012). For further improvement of the program, the national barley breeding team has been jointly working with GIZ-SSAP program providing technical support and strengthening the off-season breeding activities via establishing certain facilities such as an irrigation system at HARC. By using off-season trials, it is possible to substantially shorten the breeding cycle. According to Cobb *et al*.(2019),"cycle time is the easiest to understand, cheapest to manipulate, and but the most powerful parameter for increasing genetic gain". Further, the efficiency of allocation of breeding resources is improved by applying quantitative genetical theory on appropriate dimensioning of the individual breeding steps.

3.9. Germplasm Sources for Barley Breeding Program in Ethiopia

Ethiopia is considered as a primary and secondary gene center for many field crop species including barley that were adapted for centuries to the specific Ethiopian environment and developed wide genetic diversity (Adugna Abdi, 2011). This wide diversity in plant genetic resource provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, which includes farmers preferred traits (high yield, large grain, high biomass etc.), breeder preferred traits (quality, pest and disease resistance, high yielding) (Berhanu Bekele, 2017). Success in any breeding program depends on the available source of genetic variation, knowledge, and understanding of the inheritance of traits of interest.

The Ethiopian Biodiversity Conservation (EBI), gene bank is primarily mandated to the preservation of genetic diversity of crop plants, their wild relatives, and native species important to Ethiopian agriculture. The gene bank serves as a reservoir of potentially useful genes for many purposes, including resistance to diseases, pests, and other environmental stresses, as well as for traits that can improve the yield or quality (IBC, 2007; Wosen Gebreselassie, 2019). Accordingly, more than 15,000 accessions of barley germplasm have been collected and preserved (Adugna Abdi, 2011; Mulatu Bayeh & Berhane Lakew, 2011) in EBI. This number is expected to increase, as there has been regular collection coming to the database year to year (Wosen *et al*., 2019).

The country is also innated with large genetic and morphological diversity of barley. For example, more genetic diversity was reported in Ethiopia than other countries of North Southwest Asia, the middle East, North and Northeast Africa and South Arabia (Pomortsev *et al.,* 2013). Barley germplasm from Ethiopia, especially landraces, has been extensively used in breeding programs throughout the world as sources of novel alleles for disease resistance despite its weaknesses in straw strength and malt quality (Sitayehu Debebe *et al*., 2019). According to Fassil Kebebew *et al*.(2001) and Mulugeta Negassa (1985), many promising lines have been used as donors for disease resistance to commercial varieties in North America and Europe. Furthermore, high genetic diversity was observed in Ethiopian barley for traits such as agronomic and other traits of interest that enrich the gene pool (Ebrahim *et al.,* 2015;Tesema Tanto *et al*., 2009; Berhane Lakew *et al*., 1997;Tigist Shiferaw *et al*., 2020). Report by different scholars revealed that Ethiopian barley landraces have high genetic diversity due to ecological heterogeneity except for spike length. More than 13 studied quantitative traits revealed the variability in a population of 36 barley landraces (Addisu Fikadu & Shumet Tenaw, 2015).

Nevertheless, the introduction of germplasm from ICARDA, Europe and America also played a greater role in the improvement of the Ethiopian barley breeding program (Pomortsev *et al*., 2013; Wosen *et al*., 2019). It has significant contribution in variability for important traits such as malt quality, earliness, short plant height and straw stiffness.

3.10. Traits under selection in the Ethiopian Barley Breeding Program

Apart from yield and resistance against biotic and abiotic stresses, malt barley breeding requires combining QTL (Quantitative Trait Loci) controlling grain quality attributes suited to satisfy the need of malt and brewing industries.

3.10.1. Field and Quality Traits

The first challenge in breeding for multiple traits is to determine and prioritize traits which are most important for the target environment and market (Rana *et al*., 2018a). Several traits of importance can be simultaneously targeted for genetic improvement through field selection (Brasileiro *et al*., 2016; Rana *et al*., 2018a; Sölkner *et al*., 2008). Thus, crop breeding requires development of new genotypes that meet specific agronomic and quality traits (Brasileiro *et al*., 2016; Collard & Mackill, 2008; Sölkner *et al*., 2008). Quality traits for market is also evolving, and needs to be considered if new crop varieties are to easily find their way to consumer or end users (Dawson & Healy, 2017). Hence, field traits provide valuable information for breeders in crop improvement. Last not least, information on field performance is important to farmers when choosing well-adapted crops for a given environment (Summers & Brown, 2013).

The evaluation of agronomic and quality traits in an appropriate environment will help to select suitable varieties and design the breeding program depending on the specific need of the target environment and market (Kabir *et al*., 2015;Rana *et al.*, 2018b). For instance, flowering and maturity date are important adaptive traits describing the main growing period of the variety. Breeders use this trait information to develop early maturing varieties for terminal stress and low moisture areas and other commercial interest (Sayd *et al.*, 2017), when they select crossing parents. On the other hand, shortening the time it takes for a variety to reach maturity gives farmers the opportunity to get their product to the market faster and earn some cash quicker.
It protects their harvest by reducing the chances of insect and disease related damage that comes at later stage and also provides farmers with a buffer against the vagaries of the weather (Reynolds *et al*., 2007).

In addition, plant height is another important trait for farmers in Ethiopia. Accordingly, too long or too short varieties may not get the chance to be selected. Tallness is related to lodging problems whereas shortness is connected with lower biomass yield and can be susceptible to rodents and insects. Likewise, spike length is related to yield potential and is therefore regarded as an important trait when selecting entries as single plants or in observation rows. Similarly, disease resistance traits contributed to yield stability which secures to the economic livelihood of the end-users (Danial *et al*., 2007;Summers & Brown, 2013).

Malting quality is economically very important for farmers, maltsters and other stakeholders in the malt barley value chain (Misganew Ferede & Zena Demissie, 2020;Sebetha *et al*., 2014; Silva *et al*., 2005). It is genetically complex, comprising several different quality sub-traits. These include hot water extract, viscosity, Kolbach index, wort, β-glucan, fermentability, diastatic power and many others (Biadige Kefale & Yadesa Abushu, 2017;Kumar *et al.*, 2013). Among these protein content, malt extract, friability percentage and β-glucan are the major quality traits under selection in the Ethiopian barley breeding program.

These quality traits are assed using Near-Infrared spectroscopic (NIRS) machine. Near-Infrared spectroscopic (NIRS) analysis is a technique that makes use of the naturally existing electromagnetic spectrum (Sileoni *et al*., 2015). The NIR region is the area of the spectrum defined by wavelengths between 700 nm and 2500 nm. It is an accurate and rapid analysis method that is well suited for the quantitative determination of the major constituents of food and malt quality (Yadesa Abeshu & Ashagrie Zewdu, 2020; Richard & Billaut, 2018). This technology is advantageous in that it rapidly provides data for better decision making in malt barley variety development processes (De Sá & Palmer, 2006). Compared to other analysis methods it requires little sample preparation and no chemicals or consumables.

It is a non-destructive, operator friendly, fast (60-90 seconds/sample), reliable and precise technology. The Ethiopian barley breeding program at HARC introduced this technology in collaboration with GIZ-SSAP to enhance the efficiency of the barley breeding program. Regarding the accuracy of the technology, a model calibration study (Utschig *et al*., 2019) was

conducted on four important quality traits such as malt extract, friability, grain protein concentration and β-glucan so far and at least three of them confirmed excellent to good accuracy except β-glucan which shows a low level of accuracy. Accordingly, in the validation set the calibration accuracy achieved for the above-mentioned traits were (R^2 = 0.83, 0.83, 0.95 and 0.65) respectively (Utschig *et al*., 2019).

An innovative application of NIRS technology has been introduced by Rincent *et al*.(2018). In the concept of 'Phenomic selection (PS)' NIRS wavelength data on wheat grain and leaf tissue are used to estimate a kinship matrix and to develop a best linear unbiased predictor ([NIRS-BLUP]). Thus, NIRS data replace the genetic marker information in genomic selection methodology for G-BLUP. The predictors can be used to rank candidate genotypes and to use this ranking for selection e.g., to discard the inferior fraction. PS can be of special interest for breeding programs when genotyping is not available at reasonable costs. The basic idea of this approach, which we call 'PS' is that the absorbance of a sample in the near infrared range is mainly related to its chemical composition, which depends itself on endophenotyphes and genetics. As a result, NIRS are supposed to capture at least part of the genetic variance and therefore, one could use it to make prediction of traits unrelated to the analyzed tissue or in independent environments (Rincent *et al.*, 2018). The process of PS is similar to GS, but instead of reference material and selection candidate being genotyped, they are analyzed by NIRS.

3.10.1.1. Grain Protein Content (GPC)

GPC is one of the most important traits when selecting malting barley varieties. This trait is affected by many factors such as genotypes, cultural practices and growing environments (Derebe Terefe *et al*, 2018; Talab, 2016). Breeders should provide varieties best suitable for growers responsible for the farm management and the final GPC of their harvest (De Sá & Palmer, 2006). A too high protein level (>11.5%) results in lower extract and slows down water uptake during steeping, potentially affecting final malt quality (Kumar *et al.*, 2013; Sainju, 2013; Shrestha & Lindsey, 2019). A very low protein level (< 9.5%), on the other hand, results in a

lack of enzymes necessary to modify the barley kernel and to break down the starch during brewing (Shrestha & Lindsey, 2019).

3.10.1.2. Malt Extract (ME) and Friability Percentage (FR)

ME is the percentage of solid material extracted from finely ground malt and an important quality factor, indicating the amount of beer that can be produced from a given quantity of malt (Yadesa Abeshu & Ahagrie Zewdu, 2020; Dráb *et al.*, 2014). It is perhaps the most important quality trait for maltsters and brewers when selecting or purchasing malt barley. FR on the other hand indicates the degree to which the endosperm has been broken down (modified) during germination. Taking agronomical aspects into account, the level of ME is influenced by the quantity of soil nutrients supplied to the crop during growth and soil moisture. According to Dráb *et al.*(2014) and Fox *et al*.(2003), the amount of extract is influenced by several factors. The first is environment such as growing condition, temperature, fertilizer, and available moisture. The other is determined by several genetic and biochemical components that influence the final level of extract. The influences of these factors are not direct on ME but rather indirectly impacted on protein and starch levels and compositions. Further, high nitrogen rates significantly increased GPC and negatively affected ME and FR in malting barley grain (Meharie Kassie & Kinde Tesfaye, 2019; Kiliç *et al*., 2010; Potterton & McCabe, 2018; Talab, 2016; Wegayehu Feleke & Derib Alemu, 2019).

3.10.1.3. Beta-glucan (BG)

BG is the principal constituent (70%) of the barley endosperm cell wall (Jin *et al*., 2004). This is an essential part of the malting process as the cell walls need to be broken down to liberate the starch granules hiding within the cells of the endosperm. Better malting performance is associated with lower levels of BG content in grains and higher levels of β-glucanase in malted barley (Jamar *et al*., 2011). Thus, brewers rely on well-suited varieties and farmers should have proven this important quality trait for malting and brewing properties (Habschied *et al.*, 2020; Turner *et al*., 2019; DeeKupa, 2015 personal communication).

3.11. DNA markers

Molecular markers are nucleotide sequences and can be investigated through the polymorphism present between the nucleotide sequences of different individuals. An ideal DNA marker should be co-dominant, evenly distributed throughout the genome, selectively neutral, highly reproducible, and having the ability to detect a higher level of polymorphism (Collard *et al.*, 2005; Mondini *et a*l., 2009; Nadeem *et al.*, 2018). Hence, molecular marker technologies are the most advanced and, possibly, the most effective means for understanding the basis of genetic diversity. Among other research activities, recent studies demonstrate how to link polymorphism on the DNA level with the phenotypic expression of relevant agronomic traits or how to predict breeding parameters such as breeding value or segregation variance (Bassi *et al*., 2015; Hill, 2010; Ríos, 2015). Furthermore, the reliability of selection based on the field trials may be further increased by use of DNA marker, whereas crossing based on DNA "fingerprints" is the means for improving the introduction of genetic variation (Ríos, 2015). Potentially, these tools can be helpful for the Ethiopian breeding program to select divergent and complementary parental lines for crosses that result in good progeny performance.

3.12. Factors Determining Genetic Gain

3.12.1. Genetic Variability

Genetic variability in a breeding population is the base for crop improvement and is required to achieve genetic gains in a breeding program. Germplasm sources as described in chapter 3.9 could directly be used to exploit the inherent genetic variance. Autochthonous landraces can be dissected into single plant progenies to test them for their genetic value. Analogously, the Ethiopian barley program regularly grows breeding material or varieties from foreign countries in so-called 'International Barley Observation Nurseries' (IBON) (Tesfahu Alemu *et al*., 2012). In contrast to this and for direct use of genetic variance in advanced breeding cycles, elite lines are combined in crosses to form a base population from which new and improved lines are developed ('second cycle breeding') (Islam Matin *et al*., 2017;Langat *et al.*, 2019;Shrimali *et al.*, 2017).Thus, 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 parents (Utz *et al.*, 2001).This should be distinguished from the variance within crosses which is the variation between Recombinant Inbred Lines (RIL) generated from a given cross through subsequent selfing.

Therefore, breeding can be regarded as a two-step selection process as stated by Utz *et al*.(2001). The first step is the selection between crosses and the corresponding selection gain depends on the genetic variance among cross means (σ^2_c). The second step is selection among RIL within crosses and the corresponding selection gain depends on segregation variance (σ^2_{gij}) among lines within a given cross of parent i and parent j which has been selected in the first step. Thus, information about σ^2_c and σ^2_{gij} is important to the breeder to optimize allocation of resources regarding to the number of crosses exploited and the number of RIL evaluated per individual cross (Hung *et al.*, 2012). For example, if σ^2_c is large and σ^2_{gij} is small, the breeder should invest more into crosses and less into number of lines per cross. The choice of crosses or parent combinations is very important as it decides on which initially available genetic variation new cultivars will be based and which genes will be (re)combined by crossing to obtain superior progenies (Addisu Fekadu & Shumet Tenaw, 2015; Akgun, 2016; Ghimire & Mahat, 2019).

3.12.2. Heritability

Heritability in a broad sense is the proportion of the total genetic variability to the total phenotypic variance (Allard, 1960; Madakemohekar & Prasad, 2015) whereas heritability in a narrow sense is the ratio of additive genetic variance to phenotypic variance (Falconer & Mackay, 1996). Heritability estimates are very important to a breeder since they indicate the possibility and extent to which improvement is possible through selection. Heritability values range from zero where all the variation comes from environment to one where all variation is caused by the genetic composition of the entries. Heritability estimates can be classified into three groups such as low $H^2 \leq 0.2$, moderate $0.2 \leq H^2 \leq 0.50$, high $H^2 \geq 0.5$) (Sight, 2005).

The estimate of heritability (H^2) also indicates the reliability with which the phenotypic value represents the genotypic value and determines the proportion of gain obtained by selection (Akgun, 2016; Falconer & Mackay, 1996; Ghimire & Mahat, 2019).

3.12.3. Selection Intensity

Given heritability is larger than zero, then the smaller the proportion of plants selected, the higher the breeder expects their genetic superiority when compared to the population mean (Rutkoski, 2019; Utz *et al*., 2001). The respective technical terms are selection rate (α) for the portion of plants selected and selection differential (S) to address the difference between the mean of the selected genotypes and the population mean. The selection intensity (i) is a standardized coefficient indicating by how many standard deviations the selection differential will exceed the populations mean. Values for i for a given α can be found in tables or can be derived from or easy to calculate approximation formulae (Utz, 1984). The selection intensity depends very much on the budget of a breeding program allowing for only a small or for a larger number of entries (Cobb *et al.*, 2019). Furthermore, optimizing testing intensity and applying high through-put technologies such as NIRS or molecular marker could be powerful tools in a breeding program to influence and increase selection intensity and hence the genetic gain from selection (Berhanu Bekele *et al*., 2019; Hernández-Bautista *et al.*, 2020; Rincent *et al*., 2018; Ríos, 2015; Yanes-Lane *et al.*, 2020)

3.12.4. Time and Cost Demand

As pointed out by Ceccarelli (2015), time demand for a breeding cycle can be defined as the number of years needed from “cross to cross” (Y1), i.e. from intercrossing parental lines to intercrossing of lines derived from these crosses and identified as superior. This definition refers to all processes needed to increase the frequency of favorable alleles from one parental generation to the next one. In the Ethiopian barley breeding program, Y1= 4 to 5 benefitting from off-season activities in Belg and early intercrossing which then is confirmed or rejected by the subsequent test of parental performance. More common is to define cycle length as the

number of years from “cross to release” (Y2). This includes besides Y1 all years needed to verify in official tests for VCU (Value for Cultivation and Use) and DUS (Distinctiveness, Uniformity, and Stability) as prerequisites for an inscription.

In Ethiopia, it takes 3 Years to conduct the national (NVT1, NVT2) and verification (VVT) trials and to receive an inscription of a variety. Therefore, Y2 equals to 7 or 8 years for the Ethiopian barley breeding program. From a commercial or macro-economic point of view, cycle length could be defined as the time from cross to variety adoption (Y3). This includes beside Y2 all years needed for the seed production chain from Early Generation Seed (EGS) to first Certified Seed (CS) launched to the market. As could be shown by Choferie (personal communication, 2020) in an ambitious and well-organized environment another 3 years are needed for these processes with Y3 summing up to 10 or 11 years. In the past however, Ethiopian seed production institutions need much more time to make Ethiopian farmers benefit from a new variety. Moreover, beside insufficient seed availability, the marginality of the growing environment, the level of farmers’ perception toward technology and their involvement also indirectly influences the breeding efficiency (Bishaw & Turner, 2008) and reduce societal benefit from breeding.

Cost estimation is a key step in a breeding program which is important for proper resource allocation. Relative costs influence e.g., number of crosses, selection pressure at different stage and the number and length of breeding cycles. In the Ethiopian barley breeding program, cost estimation is based on the involvement of different actors at different stage in the processes (i.e, breeder, seed companies and minister of Agriculture).The breeder cost estimation includes all steps from crossing to variety release and early generation seed production (EGS). This corresponds to Y1 and Y2 (as described above). The second pool of costs is managed by the seed companies and includes all items from basic seed to certified seed production and marketing. The final stage is run by the Ministry of Agriculture (MoA) through agricultural extension system and covers the distribution of certified seed and adoption by farmers (Y3).

For the sake of brevity, only Y1 and Y2 cost estimation was looked upon in this thesis. In the Ethiopian barley breeding program, cost is estimated per entries which are equivalent to YPUs (Yield Plot Units) in maize as described by (Heffner *et al.*, 2010). In the US the cost of growing and evaluating a single maize yield trial plot is estimated as 1 YPU = US 20$. However, in case of the Ethiopian barley breeding program 1YPU could be estimated as 200 ETB. The breeding program yearly processes seed production and phenotyping entries based on single plant, observation row and yield plot performance (Table. 1). Variable costs sum up to 626,730 ETB, including Y1 and Y2 cycle, which is approximately equivalent to 3134 YPU.

Since labor cost as the main cost item is low in Ethiopia, the cost for the local barley breeding program is much less than the costs in the US environment as described for maize and winter wheat (Heffner *et al.*, 2010). This is advantageous for Ethiopia because the lower the labor costs the higher is the potential to increase the breeding program size. On the other hand, low costs might suggest a low awareness of the importance of stringent cost management. Vice versa, when labor cost is high there will be a tendency to focus on a good economic management of the breeding program.

Table 1. Summary of rough estimate annual cost for barley breeding program for Y1 and Y2

Cycle	stage	EU*)	N	P	R	no EU*)	Cost (ETB)/EU				total costs(ETB)/stage	derived total YPU
							Research					
							Supply	Labor	NIRS	Total		
Y1	Crossing	crosses	180	1	1	180	30	200	0	230	41400	207
	F1	population	157	1	1	157	30	200	0	230	36110	180.6
	F2	population	78	1	1	78	30	200	0	230	17940	89.7
	F3	population	156	1	1	156	30	200	0	230	35880	179.4
	F4	population	180	1	1	180	30	200	0	230	41400	207
	F4:5	observation	5000	2	1.5	15000	30	40	10	80	400000	2000
	PVT	yield plot	200	4	2	1600	30	100	70	200	40000	200
Y2	NVT I+II	yield plot	50	6	2	600	30	100	70	200	10000	50
	VVT	yield plot	20	6	2	240	30	100	70	200	4000	20
Total											**626730**	**3134**
in % of total		cost type :	Research Supply								29	
			Labor								60	
			NIRS								11	
		cost origin	Seed production								28	
			Phenotyping								72	

*) EU =Experimental units, N, P, and R: number of entries, location and replication, respectively

3.13. Relevant Parameters to Barley Breeding Programs

Many agronomical traits such as grain yield or malt quality are difficult to improve due to low heritability and a quantitative inheritance controlled by several genes. Estimation of breeding parameters helps to characterize the action nature of involved genes as well as to evaluate different selection methods and strategies (Cruz *et al*., 2014). In breeding program, breeders conduct a series of breeding activities with thousands of progenies and followed by intensive evaluation and selection each year until homozygous inbred or lines are maintained (Rincent *et al.*, 2018). Data collected in the course of these activities can be used to estimate breeding parameters with little extra costs besides the work needed for computation.

3.13.1. Means

The breeding potential of base populations depends mainly on two parameters: the mean and the genetic variance (Osthushenrich *et al.,* 2018). The population mean is the average performance of a given population under study across environments. In a breeding experiment the population mean is based on the entries forming the population and their phenotypic values which are the result of genetic and non-genetic or environmental variation. Mathematically expressed:

$$P = G + E$$

Where, P is phenotypic mean, G is the genotypic value and E is Environmental deviation.

As described above (under 3.12.1) in a second cycle breeding program, a population can be dissected into crosses and recombinant inbred lines (RIL) within crosses. Under the assumption of a simple additive gene model i.e. absence of epistasis and linkage equilibrium the cross mean is identical with the mean of the two parental lines. This mean is also called mid-parent value. Due to segregation of the genes and recombination for which the parental lines differ the RIL within a given cross will yield different genotypic values. Under the assumptions above the genotypic values of the RIL trace back to additive effects only. They contribute to their phenotypic means which are actually measured in a plant breeding experiment.

3.13.2. Variance Components

In breeding programs, the amount of variation observed is usually expressed as phenotypic variance. Using again the example given above the phenotypic variance can then be partitioned into a genetic and an environmental variance component (Oakey *et al*., 2006). The variance components are simply the means of the squared values of the genetic or environmental effects. When designing and analyzing plant breeding experiments usually a biometrical model is set up incorporating all relevant effects and defining at the same time whether they are considered to be random or fixed effects(Osthushenrich *et al*., 2018;Piepho *et al.*, 2017). So genetic effects could be partitioned regarding the mode of gene action into additive, dominance or epistatic effects(Oakey *et al.*, 2006). Considering the structuring of the population into crosses and RIL within crosses will allow in estimating the respective effects and their variance components.

Plant breeding experiments are often designed as multi-environmental trials (MET). In this case the interaction between genotypes and environment is considered as an important source of variation(Hill, 2010; Oakey *et al.*, 2006). The evaluation of variance components of important traits reveals information to the breeder which is important when deciding on breeding strategies to be proceeded (Carvalho *et al.,* 2017). When dealing with structured populations, the relative size of genetic variance between crosses compared with the genetic variance of RIL within crosses can be estimated. If the first variance component is larger than the second one the breeder will increase the number of crosses. In case of a constant breeding budget this will lead to a reduction of RIL number per cross. Utz *et al*, (2001) evaluated in wheat 30 crosses from a factorial of 5 high yielding and 6 high quality parental lines and their respective RIL. The authors found the variance between crosses to be equal to those within crosses for most traits. This was expected from quantitative genetic theory in case of predominating additive mode of gene action and with parental lines originating from the same population.

With regard to non-genetic variance components the breeder might be interested to compare genotype x environment interaction (GEI) variance with error or residual variance.

If GEI variance is predominating the breeder will invest into broadening the trial net with additional testing sites or years. Very high error variances could lead the breeder e.g. to search for the underlying causes, to implement row-column adjustments or to increase the number of replications. Laidig *et al*., (2017) compared non-genetic and genetic sources of variation in an extensive study of malt barley traits in Germany. In this case the total variance also included variance due to years and locations.

Table 2. Variance components for important agronomic traits from German Malt Barley Value for cultivation and use (VCU) trials as percentage of total variance (Adopted)

variance component	Trait					
	GY	TGW	HLW	PROT	EXTR	FRIA
G	2	16	10	3	19	18
GY	1	1	1	1	2	3
GL	1	1	1	0	1	1
GYL	3	4	4	2	5	2
E	4	9	7	7	10	13

G, GY, GL, GYL, E: variance component due to genotypic, interaction of genotype with years, locations, years and locations, residual effects, respectively.GY=Grain yield, TGW=Thousand grain weight, HLW=Hectoliter weight, PROT=Protein, EXTR=Extract, FRIA= Friability

The author found that compared to the other traits the genetic variance component for grain yield and protein content was small. Genotype x Location (GL) and Genotype x Year (GY) interaction variances recede into the background compared to the threefold interaction (GLY) and the residual variance (E). A similar analysis could be conducted for the Ethiopian National List trials and then be used to optimize their allocation.

3.13.3. Heritability

In a simple and straightforward way, heritability can be defined as the proportion of phenotypic variation due to variation of the genotypic values (Akgun, 2016; Falconer & Mackay, 1996; Ghimire & Mahat, 2019; Madakemohekar *et al*., 2018). It can be defined in a broad sense as the portion of total genetic variability to the phenotypic variance (Allard, 1960), whereas in a narrow sense it is the ratio of additive genetic variance to the phenotypic variance (Falconer & Mackay,

1996; Piepho *et al*., 2017). Heritability is an important parameter in practical plant breeding, because it determines genetic gain. In plant breeding program breeders evaluate cultivars of interests across multiple locations and several years which are known as multi-environmental trials (MET)(Oakey *et al*., 2006). To quantify and compare the precision in MET breeders might use best linear unbiased estimators (BLUE) for the phenotypic mean of genotype i and then calculate their variance (σ^2_p) as:

$$\sigma^2_p = \sigma^2_g + \sigma^2_{gl}/n_l + \sigma^2_{gy}/n_y + \sigma^2_{gly}/(n_l n_y) + \sigma^2_e/(n_l n_y n_r),$$

With σ^2_g variance of the genetic effects, σ^2_{gl} variance of the genotype x location interaction effects, σ^2_{gy} variance of the genotype x year interaction effects, σ^2_{gly} variance of the genotype x location x year interaction effects, σ^2_e variance of the error effects on a single plot basis and n_l, n_y, n_r as number of locations, years and replications, respectively.

The corresponding heritability estimate ($H^2 = \sigma^2_g / \sigma^2_p$) can easily be calculated by plugging in the estimates for the variance components and the number of locations, years and replications. For instance, applying the H^2 formula to the malt barley variance components estimated for important agronomic traits from German Malt Barley reflects the different genetic architecture of the traits and their response to an increasing number of plots invested into the test. Whereas extensive testing with not more than one plot/entry yields $H^2 > 0.5$ only for TKW and ME, traits such as grain yield need a much higher testing intensity.

Table 3. Heritability's for important agronomic traits from German Malt Barley VCU trials

	Allocation				Trait					
Application	nl	ny	nr	nT	GY	TGW	HLW	PROT	EXTR	FRIA
Extensive	1	1	1	1	0.18	0.52	0.43	0.23	0.51	0.49
PVT	2	1	2	4	0.33	0.74	0.66	0.44	0.72	0.7
Medium	2	2	1	4	0.42	0.79	0.73	0.52	0.78	0.76
NVT	6	2	3	36	0.66	0.93	0.89	0.78	0.91	0.89

nl, ny, nr, nT: number of locations, years, replications, total plots, respectively. *GY=Grain yield, TGW=Thousand grain weight, HLW=Hectoliter weight, PROT=Protein, EXTR=Extract, FRIA= Friability*

The common $H^2 = \sigma^2_g / \sigma^2_P$, as defined above assumes that the trial design is completely balanced, genotype effects are independent, and variance and covariance are homogeneous (Piepho *et al.*, 2017; Piepho & Möhring, 2007; Schmidt *et al.*, 2019). In practical plant breeding however, these assumptions are rarely met. In early generation line testing p-rep (Cullis *et al*., 2006) as an unbalanced trial design is often used with some entries replicated only once and whereas others are tested with two replications. In case of a breeding population structured into crosses and RIL within crosses genotypic effects are correlated. RIL of a given cross share the same parental lines and RIL from two crosses might be related to each other as half sibs because one of the parental lines is identical. In 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, Cullis *et al.*(2006) suggested to fit the genetic term as a random effect. He 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.

3.13.4. Phenotypic and Genotypic Correlations

Correlations between traits occur frequently. Correlations can be caused by phenotypic, genotypic, and environmental effects. Phenotypic correlations (r_p) describe the association between two characters that can be directly observed and measure the extent to which they are linearly related (Al-Tabbal & Al-Fraihat, 2011; Ashebr Baye *et al*., 2020). Phenotypic correlation occurs when phenotypic values of the two traits are correlated due to genetic and/or non- genetic effects. Genetic correlation (r_g) can arise from two sources: the first, called pleiotropy is caused by the same genes influencing two different traits. The second source is linkage disequilibrium between genes controlling different characters(Falconer & Mackay, 1996; Azeb Hailu & Sintayehu Alamerew, 2016). These genes can affect both traits negatively or positively and can cause positive or negative co-variation in two different characters (Iqbal *et al*., 2009). Environmental correlation is the correlation of environmental deviations (Falconer & Mackay, 1996). 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 (Al-Tabbal & Al-Fraihat, 2011; Iqbal *et al*., 2009). Bhatta *et al*.(2020) indicated that grain protein content affects the malting quality of barley due to positive correlation with beta-glucan content, soluble nitrogen, and grain plumpness and negative correlation with malt extract. Further, Sarup, (2020) evaluated in their genomic prediction study genetic correlations between seven malt traits. They concluded that due to unfavorable genetic correlations simultaneous improvement is impossible. Moreover, Huerta-Zurita *et al*.(2020), studied the correlation between BG and viscosity (r=0.57), FR and BG (r = -0.60), FR and Hot water extract (HWE (r = 0.54)), and FR and barley protein (r = - 0.66), respectively.

3.13.5. Regression of Cross Mean (CM) on mid parent Value (MPV)

The regression of cross mean on mid parent (MPV) values is an important parameter in plant breeding because the mid-parent value could be used as a predictor of the cross mean for the traits of interest. As explained in chapter 3.13.1 the cross mean is the mean of all RIL derived from a cross between the parents of a cross (P_1 and P_2). The association between MPV and progeny can be explained through the regression of offspring on mid-parent values that measures the degree of resemblance because of its linkage to causal component of variation (Falconer & Mackay, 1996).

The regression can be dissected into:

$$bOMPV = \frac{covOMPV}{\sigma 2MPV}$$

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 nominator we can derive under the assumptions of an idealized population:

$$COV_{OMP} = ½ (COV_{OP1} + COV_{OP2}) = 1/2VA$$

For the denominator we can derive assuming that the two parents have the same variance:

$$\sigma^2_{MP} = 1/2 (\sigma^2_{P1} + \sigma^2_{P2})$$

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

$\sigma^2_P = \sigma^2_G + \sigma^2_{E,} \sigma^2_G = \sigma^2_A + \sigma^2_I$

with: σ^2_G is the variance of genetic effects, σ^2_A is the variance of additive effects, σ^2_I is the variance of epistatic effects, σ^2_E is the variance of environmental (e.g., genotype x environment-interaction) effects

The additive genetic variance component is considered to be the major genetical component in the variance of phenotypic mean(Andrade, 2019). All variances mentioned above will influence heritability and regression as can be shown in the numerical example of table 19 under chapter 6.6. In the absence of epistasis and environmental variance (table 19, scenario 1), the additive variance is the only source of variation and in this case, heritability regression are high which means that there is a great chance to improve cross mean by selecting on mid-parent value.

However, in case of environmental variation and epistasis, (scenarios 2 to 4) the regression coefficient is reduced to a range 0.90 to 0.34. In this case selecting on MPV will have a lower response on cross means. To reduce the masking effect of epistasis an additive relationship matrix and hence breeding values can be estimated (Oakey *et al*., 2006). In addition, also non-additive genetic effects can be considered. In inbred lines, these non-additive effects reflect epistatic interactions and contribute to their genetic values. For consequence, the breeder will use the mid-parent breeding value as a selection criterion and will thus avoid an undesired bias from epistatic effects. To reduce the masking of environmental variation the breeder will benefit from e. g. appropriate selection of his trial field, good experimental design and analysis or management practice(Oakey *et al.*, 2006; Piepho *et al.*, 2017). The study conducted by Utz *et al*.(2001) on wheat crosses and their progeny found significant variation between mid-parent values and means of crosses. For most traits epistasis was negligible as masking effect.

Accordingly, they found that mid-parent value is a fair predictor of cross mean with correlation values estimated in the range of $0.71 \leq r \leq 0.90$ for many traits studied. Therefore, the breeder can increase the size of the regression by accurate testing of the parents. Oakey *et al*.(2006)

conducted a study in a multi-location trial to model additive and non-additive genetic effects. For most of the individual locations taken for parameter estimation additive variance accounted for more than 90% of the genetic variance and correspondingly epistatic variance was of minor importance. To avoid bias from epistasis the authors recommend using the mean breeding value of the parental lines instead of the phenotypic means as predictor for cross mean.

3.13.6. Usefulness (U) of Crosses

At each breeding cycle, breeders are facing the choice of crosses to generate the genetic variation on which selection will act at the next generation. This could be achieved if the cross obtained from parents with superior breeding values could generate high genetic variance and if transgressive segregants are obtained in the RIL population(Allier *et al.*, 2019). The potential of a cross for a given quantitative trait can be quantified by the usefulness criterion (U, Schnell and Utz, 1975). 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 selection rate α, and h is the selection accuracy indicating the correlation between the phenotypic means and the genotypic effects of the RILs within the cross (Bassi *et al*., 2015; Falconer & Mackay, 1996; Heffner *et al.*, 2010; Meuwissen *et al*., 2013).

When MPV is applied as predictor, CM can be predicted with results from the preceding breeding cycle before producing RILs and without any additional cost (Utz *et al*., 2001). As Utz and Schnell (1979) pointed out the tighter the correlation between mid-parent and cross mean is the more powerful is the selection between crosses and the greater should be the corresponding selection intensity. Whereas mid-parent performance as a predictor of the cross mean can rely on phenotypic data which are rather accurate in many breeding programs, the forecast of the genetic standard deviation within a given cross remained difficult for many years. This changed with the advent of molecular markers of which the effects can be estimated by methods of genomic prediction. Osthushenrich *et al*.(2017) developed an analytical approach, which is highly versatile with regard to mapping functions and mating systems.

The applicability of this approach could be confirmed in a framework which is close to a practical barley breeding program (Osthushenrich *et al*., 2018). The RIL progeny of 14 crosses had been genotyped with 9597 SNP markers and also phenotyped in a field experiment at 5 locations in Germany. As prediction criterion the sum of cross mean + genetic standard deviation within cross was chosen. This criterion was compared with the field performance of the best RIL in a given cross. Prediction accuracy was found sufficient to identify the 50% best crosses. The new tool for prediction of usefulness opens the door for a more efficient use of the limited field-testing capacity. By exploiting crosses with a lower mean but with a large genetic variance also genetic diversity and long-term genetic gain can be maintained.

3.13.7. Genetic Distance within and Between Parental lines based on DNA Markers

Genetic distance measures the degree of genetic difference between species, populations or elite breeding material (Reif *et al*., 2005). DNA-based markers represent genetic differences between individual organisms or species. The level of genetic diversity based on molecular marker between parents has been discussed by Longin *et al*.(2014) as useful information on the genetic diversity for agronomic traits and the similarity of parental lines used in a crossing program. For the latter use, costs for the breeding program can be reduced by e.g. avoiding to make non-useful crosses and to evaluate RIL derived from them in the field.

In the Ethiopian barley breeding program, genotypes are tested in multi-environmental yield trials and intercrossed to form an improved breeding population. Before planning the cross nursery, the breeder should have prior information on the genetic variance within and between candidate genotypes to be used for crossing. The within variance depends on the genetic structure of the candidate genotype. If the single plants (SP) sampled for intercrossing are derived from e.g., a double haploid line or an F3- or F4- SP, this will have an impact on the genetic similarity within the parental genotypes. In case of a DH-SP we expect no heterozygosity at all, accordingly there will be no within-variance in the derived DH-L.

For contrast in F3- or F4-SP most (marker) loci will be homozygous too, but other (marker) loci still will be heterozygous and within-variance will be observed in the derived lines. Even more, in practice, individual SP in the sample could deviate from theoretical expectations due to technical hazards such as mislabeling, technical mixture, or due to a crossing event in the preceding generation. Hence, DNA marker-based analysis could assist in correcting errors and in assessing the genetic similarity or distance within individual barley parental lines.

Analogously, the distance between putative parental genotypes can be assessed by molecular marker(Hung *et al.,* 2012; Longin *et al.,* 2011). For example, lines related to each other due to a common ancestor can be identified. This information can be important because relatedness between crossing partners will reduce the genetic standard deviation within the cross. Before more advanced prediction tools as described above where available, prediction of segregation variance was investigated by using genetic distance between parental lines (Geng *et al*., 2021; Makumbi *et al.,* 2011). Correlation between observed and predicted variance were not always high and the reasons for this are discussed in the literature. For example, Utz *et al.*(2001) postulated that for the prediction of segregation variance marker based genetic distance should restrict on closely linked QTL which are trait specific rather than apply the overall genetic distance as a criterion.

Furthermore, to mention another application field for genetic distances, today varieties with a rather similar phenotype are commercially available. Use of traditional DUS procedures based on morphological differences to protect the right and interest of breeders may be time taking and affected by environmental problems in the testing period (Chen *et al.,* 2016; Okoye *et al*., 2016). Thus, the use of marker as rapid, cost-effective molecular tool for testing DUS criteria could become important in protecting breeders' intellectual property right (Nemera Geleta *et al.,* 2006; Pourabed *et al.,* 2015; Wijesundara *et al.,* 2018;Yang *et al*., 2021). Genetic distance between parental lines can be estimated based on Rodgers distance (Rodgers, 1972), which reflects the portion of alleles that two genotypes have in common and is linearly related to the co-ancestry coefficient under certain assumptions (Reif *et al.,* 2005).

4. MATERIALS AND METHODS

4.1. Description of the study area

The experiment was conducted at two locations, Holeta and Bekoji Agricultural Research Station, in the 2020/21 cropping season. Holeta is located about 30 km west of Addis Ababa, Ethiopia with latitude (09° 04'N), longitude (38° 30'E) and altitude of 2390 m.a.s.l. The maximum and minimum temperature is 22.2 and 6.13 °C. The average annual rainfall is 1100 mm and Vertisol and Nitisol are the two main characteristics of soil type at Holeta with soil pH of 5 (http://www.eair.gov.et). Bekoji, which is the sub-station of Kulumsa Agricultural Research Center, is found in Arsi Zone of the Oromia region and is located about 220 km South-East of Addis Ababa. The latitudinal and longitudinal coordinate of Bekoji is 07°05'N, and 39°30'E with an altitude of 2780 m.a.s. l. and characterized by long-term annual mean rainfall of 1049.6 mm received predominantly during the main growing season from June to October. The maximum and minimum temperature is 19.6 and 8.3°C. Nitosol/Luvisol is the main characteristics of Bekoji with a soil pH of 5. These two locations are well-known EIAR stations selected based on their potential for malt barley productions. Further, they represent major parts of the highland barley growing areas in the country.

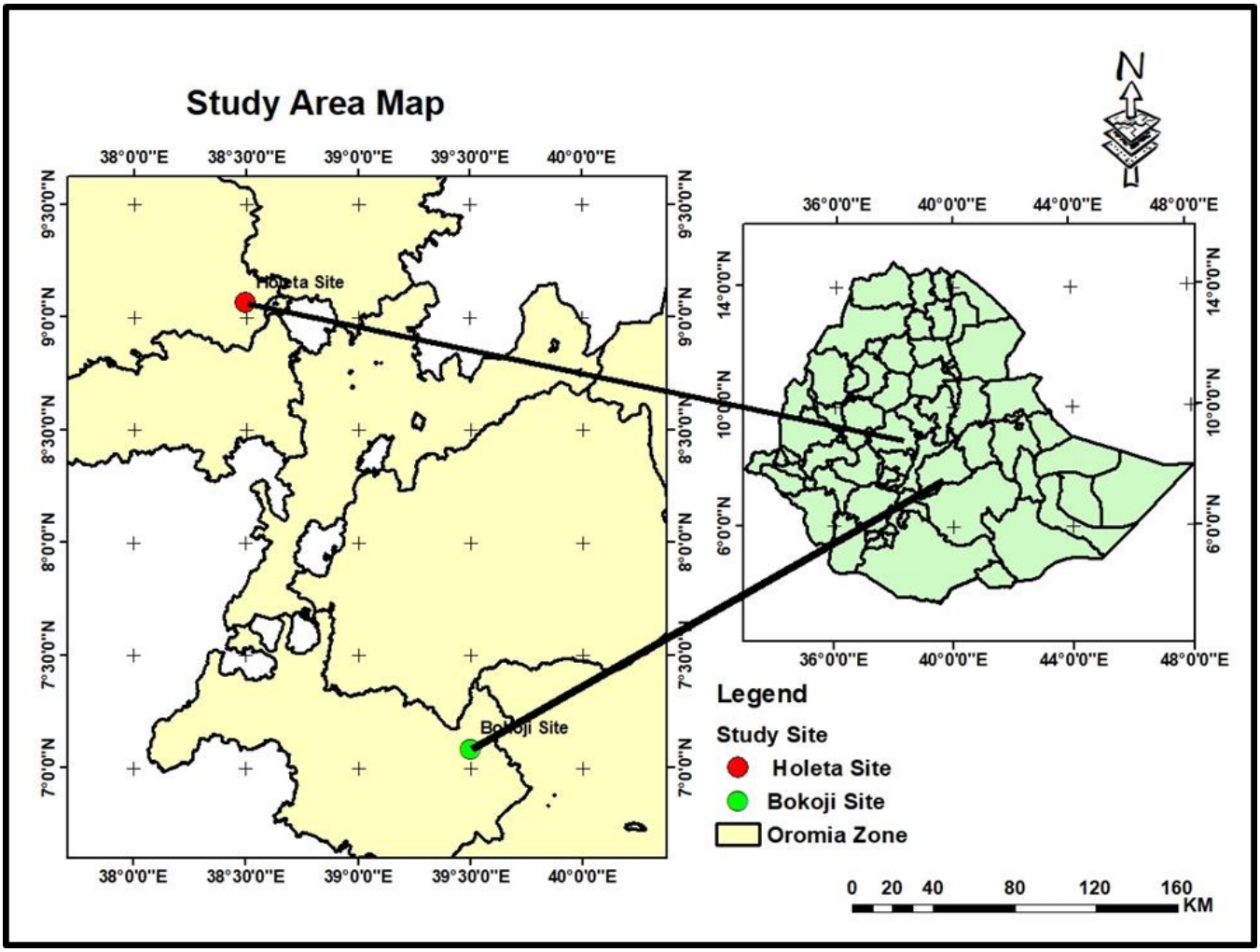

Figure 1. Map of Ethiopia with study area

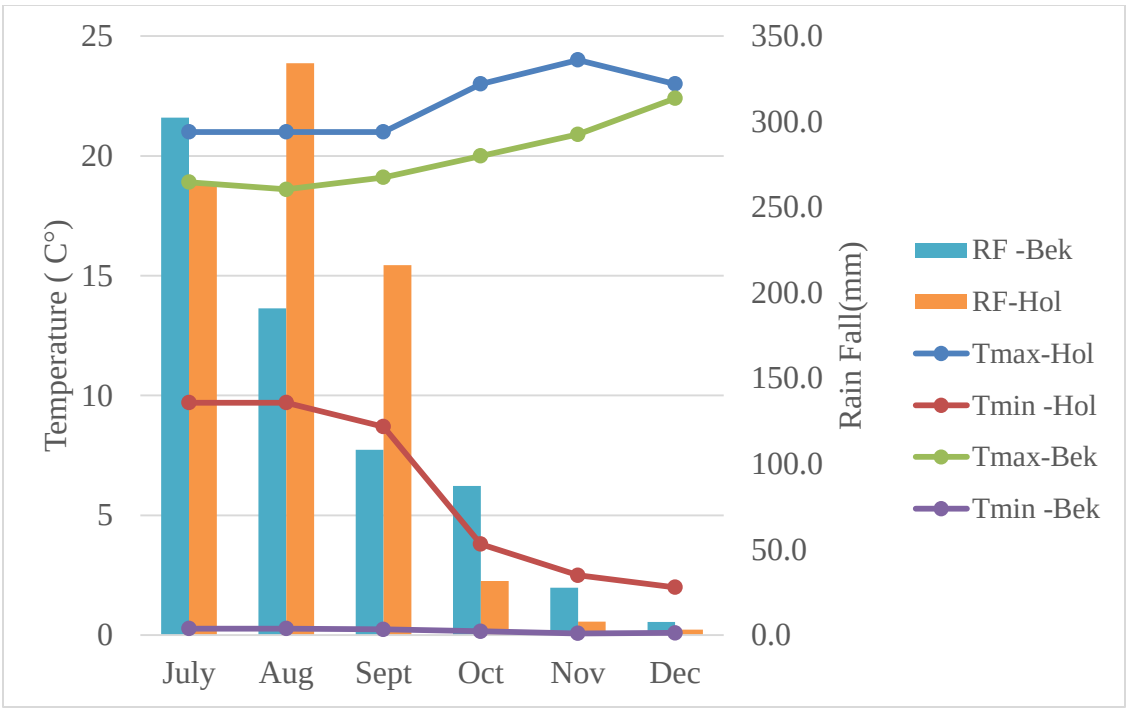

Figure 2. Rain fall (mm), minimum and maximum air temperature(C°) recorded in 2020 main cropping season at Holeta and Bekoji.

4.2. Planting Materials

A total of 30 crosses derived from 17 parents were conducted at Holeta Agricultural Research Center (HARC) in the year 2018 (Table 4 and 5) and 30 RIL per cross had been developed in an SSD procedure in the following growing seasons. A total of 900 RILs descend from F4-SP were tested at F5-generation (F_4:$_5$-L) 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 selected from national variety trials based on their good 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 late maturing, short plant height and susceptibility against leaf diseases.

The USA sources excel for their resistance to insects such as aphids, ICARDA lines are good sources for stem stiffness, drought tolerance and 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. Further, some parents contributed more gametes to crosses than others because of their advantage in certain traits over the others e.g. Planet with good malt quality contributed to 13 crosses. The generation advancement of the lines was conducted both in the long rainy season (Meher) and the short rainy season (Belg) through SSD methods to shorten the breeding cycle. At each generation cross progenies were planted in separate blocks and allowed to inbreed. Only a moderate field selection based on per se F3-SP performance was made. Accordingly, the RIL population used in the experiment can be regarded as a random sample of the cross progenies.

Table 4. 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 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 abiotic stress and well adapted
17	Bekoji-1 x Grace	Ethiopia	

UM= university of Minnesota

Table 5. The 30 Crosses generated from the 17 parental lines described in Table 4

	Planet	Burton	G 13-64	IBON 14/15-144	ICARDA GP-67	M135	MBHIBYT-22	(Bekoji-1 x Grace)	IBON 13/14-128	IBON 13/14-41	ICARDA GP-75	IBON 13/14-129	MBHIBYT-23	No crosses/Parent
Planet		*		*	*	*	*	*	*	*	*	*	*	11
Burton			*		*				*					3
G 13-64						*	*		*		*			4
HB1963				*	*				*					3
IBON 14/15-144					*				*					2
M135									*					1
IBON 13/2	*		*		*									3
IBON 13/33			*											1
MN Brite	*								*					2
No crosses/Parent	2	1	3	2	5	2	2	1	7	1	2	1	1	**30**

*****indicates the number of cross made and used in this study, *(Source HARC, 2020)*

4.3. Experimental Design and Field management

The experiment was arranged in a Modified Split Plot P-rep design. In this design, crosses were allocated to main plots and RILs of a given cross as a subplot where RILs were randomized 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, 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 un-replicated RIL and 5 parents/checks). Per site in total 30 x (15*2 +15 + 5) = 1500 plots were grown for RIL, parents and checks respectively. The plot size was 1 m in length of two rows with 0.2 m spacing between rows which made a net plot area of 0.4 m^2. Plots were separated from each other by one m length path.

The experimental field was prepared following the conventional tillage practice in the experimental area. Seeding was done at the recommended seed rate of 5 g $plot^{-1}$ by hand drilling in the rows uniformly and covered with slight soil manually. The seeding date was 21 June and 19 June 2020 for Holeta and Bekoji, respectively. Fertilizer was applied based on the recommended rate for malt barley production in the area(Girma Chala, 2017). Accordingly, 10.9 kg/ha and 5 kg/ha of Nitrogen-Phosphate and Sulphur fertilizer (NPS) and Urea were used at Holeta excluding the path area whereas 10.85 kg/ha and 4.5 kg/ha of NPS and urea were used at Bekoji. The field was weeded two to three times by hand during the cropping season to control weeds and to reduce weed-crop competition. Moreover, all the necessary field management practices were carried out as per the recommended practices followed by the farming community around the areas to make the testing environment representative for the later applied farming technology. Finally, harvesting was made manually by sickling from both rows at harvest maturity. Threshing was done using a laboratory plot thresher. Finally, the harvested grain at Bekoji was transported to Holeta laboratory for analysis of NIRS quality traits.

4.4. Morpho-physiological Data collected and measured

Data were recorded on eight quantitative characters on each plot and in collaboration with the barley breeding team at Holeta and Bekoji. Data were captured by using a tablet equipped with the field scorer 4 android Katmandoo application developed and supported by the Department of Agriculture and Fisheries in Queensland. This technique is fundamental to modern day plant breeding as it allows an increased scale and efficiency, reduced error and better use of information. A CSV data sheet generated in the computer is imported into the android device or merged using a software program called Kombine. Scored data is later transferred back to the computer through a merging tool as soon as back from the field to reduce the risk of information loss. The following data were collected at field level.

1. **Heading date**: Number of days from date of sowing till about 75% of plants in the plot was flowering.
2. **Maturity Date**: 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:** 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 weigh:** Weights measured by taking the mass of carefully counted thousand kernels on sensitive electronic balance (± 0.1g) after kernels adjusted to 12.5% moisture content.

4.5. Laboratory Quality Data

100 g grains/sample was cleaned and grain moisture was maintained to 12.5% before the quality test. Accordingly, the following traits were measured using non-destructive and robust Infratec^{TM} 1241 Grain Analyzer, Near-Infrared Reflectance Spectroscopy (NIRS), expressed in percentage.

5. **Grain Protein Concentration:** The total protein content in the grain, measured by the NIRs (Henry, 1985) from minimum of 100g grain sample.

6. **Malt Extract:** Percentage of solid material extracted from finely ground malt and measured using NIRs from minimum of 100gm grain sample
7. **Friability:** Indicator of endosperm modification after malting by a simple milling test in a friabilimeter and measured using NIRs from minimum of 100gm grain sample
8. **Beta Glucan:** Similarly, it was estimated from grain sample using NIRs machine with the above mentioned quality parameters.

4.6. Molecular Characterization

A total of 17 parents of the 30 crosses were used for genotyping. For each sample, 5 g seed of parental genotypes were sent to KWS SAAT SE and Co.KGaA breeding Company located at Einbeck, Germany, for genotyping KASP SNP markers. Each parent was sampled with several seeds (23-24 seeds) for homogeneity assessment.

4.6.1. Genomic DNA extraction and genotyping

Genomic DNA extraction was done using modified Ctyle Trimethyl Ammonium Bromide (CTAB) as described elsewhere (Beranu Tadesse *et al*., 2015; Behailu Mulugeta *et al.*, 2021; Kassa Semagn *et al*., 2014). The concentration of DNA was measured using Quant-iT^{TM} PicoGreen®dsDNA assay kit (Invitrogen ^{TM}, KWS, Germany) and normalized to 50 ng/µL for further analysis. The DNA sample was genotyped using KASP genotyping platform (http://www.lgcgroup.com, LGC genomics, Germany). The KASP reaction and components were described as per LGC genomic protocols (http://www.lgcgenomics.com/genotyping/kasp-genotyping-reagents/how-does-kasp-work). The DNA was diluted with distilled water at 1:20 which is optimum for thermal cycling and KASP genotyping prior to using it in downstream applications to secure optimum end result. Two allele-specific oligonucleotide and one common oligonucleotide were designed for each locus to develop the KASP assay. These primers were designed by KBioscience (http://www.lgcgenomics.com) from flanking sequences (50bp each upstream and downstream) around SNP position.

4.6.2. Marker selection and PCR conditioning

Twenty-three markers were selected based their genome coverage, and polymorphic information content(PIC) studies conducted on European malt barley germplasm assuming that this holds true for non-European germplasm (Table 6). Besides, KASP markers are cheaper in comparison to multiplex methods. For screening and characterizing material, they are regarded to be most cost-effective. The KASP assay was carried out in a total reaction volume of 10.4µl with 96-well plate genotyping to combine KASP Master Mix and KASP assay mix. The reaction volume contains 5µl of template DNA, 2.9 Master Mix with volume of 5µl and 0.14µl allele specific forward and reverse common primers. PCR Amplification was carried out in water based thermal cycler (hydro cycler). PCR conditions were programmed for 37 cycles with an initial denaturing step at 94°C for 15 min of one cycle, ten touchdown PCR cycle were programmed for 20's denaturing at 94 °C and annealing from 61 to 55°C by dropping of 0.6°C per cycle for 60sec, the remaining 26 cycles were adjusted to denaturation temperature of 94°C for 20sec and annealing temperature of 55 °C for 60sec.

4.6.3. KASP reaction component and running

KASP genotyping was carried out using 96-well plate formats. Three components, namely the template DNA with the SNP of interest, KASP assay mix containing two different allele specific forward primers with unique tail sequences and one common reverse primer, the KASP master mix containing FAM™ and HEX™ specific FRET (fluorescence resonant energy transfer) cassette for distinguishing varieties plus KASpTaq polymerase, salts, dNTPS and an optimized buffer solution were used. The passive reference dye (ROX) was added to normalize the data by dividing FAM™ and HEX™ according to sample cluster which leads to lighter clustering while viewing using SNP viewer. The fluor labeled part of the FRET cassette is complementary to new tail sequences and binds, releasing the fluor from the quencher to generate a fluorescent signal. Two NTC (No Template Control) were included in genotyping plate to ensure validity/reliability of the genotyping result. Finally, an end point fluorescent read was accomplished to see the genotyping result.

Table 6. Distribution of KASP markers on the seven barley chromosomes

NAME	ALLELE1	ALLELE2	CHROMOSOME	POSITION-pb
GE5194-1118	cyt	gua	chr1H	18807296
GE5381-1950	ade	gua	chr1H	34087634
BOPA1_8743-197	cyt	gua	chr1H	380928570
GE4057-2114	ade	gua	chr1H	555618159
BOPA1_2582-767	ade	gua	chr2H	3326148
GE2052-792	ade	gua	chr2H	196891780
BOPA1_ABC05640-1-1-248	ade	gua	chr2H	702542245
GE13924-403	ade	cyt	chr3H	3981097
GE4593-2007	ade	gua	chr3H	32615385
BOPA1_10248-954	ade	cyt	chr3H	319229422
GE9610-1195	ade	gua	chr3H	654775437
GE2055-947	cyt	gua	chr4H	12270789
GE2274-1226	ade	cyt	chr4H	484881213
GE2878-574	ade	cyt	chr4H	644449770
GE9608-371	ade	gua	chr5H	9861991
GE5004-375	ade	gua	chr5H	229928326
GE6781-1073	ade	gua	chr5H	650559436
GE2795-1707	ade	gua	chr6H	12662354
GE3506-668	ade	gua	chr6H	335738777
GE4126-1180	ade	gua	chr6H	577508169
ABC07611-1-5-315	ade	gua	chr7H	7044961
GE1735-1424	ade	gua	chr7H	110445275
GE4991-1028	ade	gua	chr7H	639792989

4.7. Data Analysis

4.7.1. Phenotypic data analysis

Data were subjected to statistical analysis and model assumptions were checked for homogeneity and normality of residuals. Basic statistical analysis was conducted using open source R software, version 3.6.3. Outliers were replaced by NA (NA stands for not available in R syntax). Estimation of variance components among parents, crosses and RIL within crosses were computed by mixed model procedures using commercial asreml-r (Glimour et al., 1995).

The general mixed model of the experiment was:

$$Y = X\beta + Z\mu + \varepsilon \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Model\ 1$$

Where, Y is the response variable, X and Z are design matrices of the corresponding fixed and random factors respectively. β and μ are the fixed and random factors of the model, respectively, ε is the random error of the model. Thus, variables are customized accordingly to this general mixed model to estimate variance component among parents, crosses, and RIL within crosses.

4.7.1.1. Estimation of Variance Components

Analyses of variance were based on a linear mixed model. A spatial model was fitted to produce adjusted genotype means for each environment (Cullis *et al*., 2016; Mohring and Piepho, 2009). After spatial adjustment, a combined analysis was conducted across environment (Cullis and Gogle, 1998 and Smith *et al*., 2001). The linear mixed model for the combined analysis contains different dummy groups to predict the adjusted mean precisely (Piepho *et al.,* 2012). The model was defined as:

$$Y = \mu + group + pg + loc + cross + cross{:}loc + Entry + Entry{:}loc + row{:}loc + col{:}mainp{:}loc + e$$

Attaching the dummies to (group {groupRILs, groupCHK}), parental group (pg) (pgParent, pgechk) where pgParent=parents that used in the cross, and pgechk=non-parents that included as additional checks. In addition, for each treatment factors (cross and Entry) dummies have been attached as shown in the following model and the respective design factors for rows, columns and main plots was considered per location.

$$Y = \mu + group + pg + loc + cross:pgParent + cross:pgParent:loc + Entry:pgechk + Entry:pgechk:loc + Entry:groupCHK:pgParent + Entry:groupRIL + Entry:groupCHK:pgParent:loc + Entry:groupRIL:loc + row:loc + col:mainp:loc + e --model\ 2$$

Where, Y is the total observation for the trait of interest, μ is the overall mean of RIL, ***group*** to identify checks and RIL as the only fixed factor, ***Crosses***, ***checks,*** and ***RIL within crosses*** are assigned as genotype 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 one factor by switching off the other. e***chk*** identifies extra checks beside the 17 parents actually used in crossing, ***loc*** assigns locations. As an example for other interactions with environments (**Entry.*loc***) is defined as the interaction between location and set of genotype groups i.e. Crosses, checks, RIL within crosses. ***row.loc*** and **(*mp. col. l*oc)** are rows, columns and main plots effects, respectively, nested within locations and ***e*** is the random error for the model.

4.7.1.2. Parameters estimation for phenotypic data

In the following, we assumed that the RIL represents a line population developed through SSD. Further assumptions are:

- Forces driving changes of allele frequencies such as selection, migration, drift, and mutation (Falconer & Mackay, 1996) are assumed to be negligible when developing the RIL in the preceding SSD procedure.
- Linkage equilibrium (Falconer & Mackay, 1996) between QTL controlling a trait is given and epistasis is absent or of minor importance. With these assumptions given a simple additive genetic model can be used as suggested by (Utz *et al.*, 2001;Wright & Cockerham, 1986).

(a). Prediction of Means of Parents, Crosses, and RILs within crosses

Best Linear Unbiased Predictions (BLUP) were computed for means of parents, crosses, and RIL within crosses (model 2) estimated as a random effect with distribution $\mu \sim MVN(0, \sum\sigma^2\mu)$ being $\sum \sigma^2\mu$ a relationship matrix among the level of the random effect.

(b). Mid -parent value (Utz *et al* 2000)

$$\text{mij} = \frac{(\mu f + \mu m)}{2}$$

Where, m_{ij} =mid parent values of homozygous lines that can be derived from the female (f) and the male (m) parent, respectively.

(c). Variance Components

Assuming homozygous parental lines with allele *r* and *s* at loci *k* and *l* as suggested by Utz *et al*., (2001), the genetic variance among parents/checks was estimated from the combined mixed model. The genetic component $\sigma^2_p = (\sigma^2_m + \sigma^2_f)/2$ was estimated from the genetic variance of parental lines from the full model based on ASReml. Variance among parents was estimated from replication effects within the environment and assuming homogeneous plot error for both parents. The genetic variance among crosses σ^2_c is the variance component accounting for variation between the 30 crosses and is estimated as the variance component in the mixed model of F4:5 RILs with environments, crosses and lines within crosses (i.e., crosses, checks, RIL within crosses) assuming crosses as random (Utz *et al*., 2001). The average segregation variance σ^2_g is the variation of RILs within crosses.

Under the assumptions specified above the quantitative-genetic interpretation of variance components was calculated and designed as follow (Utz *et al.*, 2000)

Among mid-parents: $\sigma^2_p = (\sigma^2_m + \sigma^2_f)/2 = \sigma^2_A$

Among crosses: $\sigma^2_c = \sigma^2_A$

Among RIL within Crosses: $\sigma^2_g = \sigma^2_A$

Where, σ^2_A = variance of additive effects.

(d). Heritability (broad sense)

Heritability was estimated based on Cullis *et al., (*2006). Specific allocation for checks crosses and RIL was taken into account when estimating their respective heritability.

$$H2Cullis = 1 - (average\ standard\ error\ from\ BLUP)/(2 * \sigma 2g),$$

Where, σ^2_g = genotype variance from a model construct

(e). Phenotypic and Genotypic Correlation between traits

The genotypic and phenotypic correlation coefficients were computed following Falconer and Mackey (1996) as described below:

$$rg = \frac{COV(g1,g2)}{\sqrt{Var(g1)}\sqrt{Var(g2)}}$$

Where, r_g = genetic correlation, $COV\ (g1,g2)$ = is the covariance of the genetic effects g_1 and g_2 for genotype 1 and genotype 2, the denominator is the product of the square root of genetic variance of trait 1 and trait 2.

$$rp = \frac{COV(g\ 1\ ,g\ 2)}{\sqrt{Var(g1)}\sqrt{Var(g\ 2)}}$$

Where, r_p is the phenotypic correlation. *COV (trait 1, trait 2)* is the covariance between the phenotypic trait values based on model (2). Each of the effects included in the model and not only can the genetic effect contribute to phenotypic covariance. The denominator is the product of the square root of the variance of the phenotypic means for the traits.

(f). Regression of cross means (CM) on mid parent value (MPV).

The regression of the cross means on the mean of their parents (b_{ij}) represents a simple linear regression between two numerical variables, in our case the CM from a model (2) and MPV of the two parents i and j.

$$CM_{ij} = \mu + b_{ij}\,(MPV)_{ij} + \varepsilon_{ij}$$

Where,

CM_{ij} =cross mean of parent i and j, b_{ij} = the slope of regression, MPV_{ij} = mid parent value of parent i and j, ε_{ij} = error term

The coefficient $\boldsymbol{b_{ij}}$ is the slope and shows how many units the CM as the response variable will increase for every one-unit change of the corresponding MPV as the predictor variable. The error term represents deviations coming e.g., from epistasis, cross by environment interaction and mid-parent by environment interaction (Utz *et al*., 2001). The coefficient of determination (R^2) with a range is between 0 and 1 indicates how well the model fits with the data sets.

(g) Usefulness of crosses (U)

The mathematical formula to calculate the usefulness and the selection gain included therein is given by Utz *et al*., (2001).

The subscript i and j stands for parent i and parent j respectively

$$Uij = Cij + Rij = \text{Cij} + \text{ih}\sigma$$

Where, U_{ij} is the usefulness of the cross, C_{ij} is the predicted cross mean; R_{ij} is the genetic gain, which is a result of segregation variance (σ), heritability (h^2) within the cross and the selection intensity (i).

4.7.2. Molecular Data Analysis

Data generated from KASP genotyping were used to compute the homogeneity and genetic distance analysis based on Rodgers distance within and between lines using a Selection Tools Package based on R-language developed by M. Frisch, Justus Liebig University.

h. Genetic Diversity and Similarity Analysis within and between Parental Lines

i. Genetic Diversity

Diversity between parents was estimated by using Rodgers (1972) distance formula described as follow:

$$d_R = \frac{1}{m}\sum_{i=1}^{m}\sqrt{\frac{1}{2}\sum_{j=1}^{ni}(pij - qij)2}$$

Where, p_{ij} and q_{ij} are allele frequencies of the j^{th} allele at i^{th} locus for the two operational taxonomic unit (e.g. the parental lines), n_i represents the number of alleles at the locus, and m refers to the number of loci. d_R is the average Euclidean distance across all loci standardized by $\sqrt{1/2}$ to restrict the values to the interval [0,1] (Reif *et al*., 2005).

ii. Similarity Analysis

The expected heterozygosity (ExHet) within a parental line was estimated as:

$$ExHet = 1 - \sum_{a\in A} fa2$$

Assuming Hardy –Weinberg equilibrium (HWE), the expected heterozygosity ($ExHet$) measures the allelic diversity at a locus. Reciprocally, the expected homozygosity (*ExHom*) within a parental line was estimated as:

$$ExHom = \sum_{a\in A} fa2$$

Where, *A* contains all observed alleles and f_a contains the squared allele frequency of allele *a.* In case allele a is fixed f_a =1, in a given line, ExHom is equal to 1. As a final homogeneity measure, the average *Exhom* estimates across all markers were taken.

5. RESULTS

5.1. Mean of parents

The mid-parent value (MPV) (Table 7) of all 30 crosses represents the parental per se performance. Accordingly, Burton with planet found to be good in their mean performance in terms of DH (79.6 days), TKW (46.2 g), ME (80.7%), GPC (11.1%) and FR (70%), respectively. Similarly, Planet with MBHIBYT-22 also indicated good performance in terms of DH (80.8 days), PH (89.5cm), ME (81.4%), GPC (11.1%) and FR (70%) (Table 7). These indicate that the parents that were involved in the crossing program were relatively early maturing, having 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 line and supposed to be surpassed by the RIL derived from 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. The mid-parents were splitted into a group with and without Planet as a crossing partner. Hence, considering the difference between the two groups we had observed that (-0.8. -0.7, 0.7 and 0.7) for DH, DM, PH and TKW, respectively. This implied that the variety planet contributed for earliness, tallness, and better TKW. In addition it revealed that planet has positive effects on malt quality in terms of ME, FR and BG.

5.2. Mean performance and Ranges of RIL

The mean of the RIL population estimated over all RIL entries within and across crosses reflects the general performance level of the progeny produced from the parental lines (Table 8). Accordingly, RIL performance ranged from 74.64 - 88.76 days for DH (mean 81.20 days), 126.50-139.60 days for DM (mean 133.10 days), 75.09 -102.50 cm for PH (mean 91.51 cm), 79.4-81.39 % for ME (mean of 80.68 %), and 10.52-12.13 % for GPC mean11.21%), respectively. Respective data for field traits showed that performance level can be considered as a typical and representative for the trait expression that the farmers have been experiencing on their farmland.

Table 7. 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
Mean Planet Crosses (N=9)*)				80.5	132.6	90.8	46.9	80.9	11.1	68.7	357.0
Mean NON-Planet Crosses (N=13) *)				81.3	133.3	90.1	46.2	80.7	11.2	67.6	372.0
Δ(Non-Planet 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 gametic contributions of parental lines in the two groups

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

In this study, we obtained higher mean performances of TKW (35.16 -58.16 g) (Table 8) as compared to the values (26.0- 42. 8 g) assessed so far on Ethiopian malt varieties grown at Holeta. In addition, the performances of RIL for malt quality traits such as ME (79.76-81.39 %),

GPC (10.52-12.3 %) and FR (52.40-76.17 %) were observed in our experiment which was by far better than the previous report on similar traits, ME (75.5-80.9 %), GPC (9.6-13.5 %) and FR (31.6-86.5 %). The minimum, maximum value and the respective standard deviation (SD) revealed a considerable variation which can be exploited through selection. For all traits SD is higher than the SE of means.

Table 8. The 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

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

5.3. The estimated Variance among Parents, Crosses, and RIL within Crosses

Analysis of variance revealed significant ($P < 0.05$) variation among the parents for DH (27.01), PH (240.65) and GPC (0.67), whilst other phenotypic traits showed non-significant difference among the parents (Table 9). Genetic variances among crosses proved to be significant for all traits except for PH and BG. Accordingly, higher variance was observed among DH (7.90), DM (20.36), TKW (10.87), ME (0.82), FR (25.88) and GPC (0.07). However, the variance among RIL was the most striking results and found to be highly significant ($P < 0.001$) for all traits except for ME and GPC. For two traits (DM, ME), the ratio of genetic variances Ratio σ^2_c: $\sigma^2 p/2$ deviated to be larger than one. In contrast, the ratio for PH was found to be significantly smaller than one. Significant ($P < 0.05$) genotype x location interaction variances were consistently observed for parents, crosses and RIL for DM, PH, TKW, FR and BG which indicates that the two environments in response to the above traits and informative for future malt barley production.

Table 9. Variance components for the genetic and Genotype x 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^2p)	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^2_c)	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^2_g)	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^2_c: $\sigma^2p/2$		**0.60**	**2.99****	**0.13****	**1.39**	**2.41****	**0.92**	**0.22**	**0.89**
Ratio σ^2_c: σ^2_g		**0.71**	**2.23****	**0.50****	**0.77**	**2.41****	**0.95**	**0.37**	**1.88****

, **, * significance at α = 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*

5.4. Genetic Variance within crosses (σ^2_g)

The average genetic variance of RIL within crosses was estimated in a combined analysis as shown in Table 9. Besides for each of the 30 crosses the variance of RIL was estimated separately (Table 10) hypothesizing that the respective genetic variance (σ^2_g) and/or their genotype x environment interaction variance are different from cross to cross. Except for ME and FR, relevant differences between the crosses with regard σ^2_g were observed. The range of σ^2_g 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), respectively. Similarly, the respective SD value exceeded SE for these traits.

Table 10. The mean, minimum and maximum genetic variances ($\sigma^2 g$) among RILs within crosses estimated from 900F4:5 based on the 30 malt barley crosses based on BLUP mean

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 $\sigma^2_{g,}$	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 $\sigma^2_{g\ '=}$ 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,

5.4. Broad sense heritability (H^2) of Traits among Parents, Crosses, and RIL

Heritability across both environments showed moderate to high estimates with a range of 27.4 – 93.6% depending on the source of genetic variation (parents, crosses and RIL) and traits (Table 11). For the parents, heritability ranges from 49.5 % for ME to 93.6 % for PH, for the cross, heritability ranges from 29.5 % for GPC to 87.0% for DM whereas heritability for RIL extends from 27.4% for BG to 73.6% for TKW, respectively.

Table 11. Heritability of traits from the combined model over location

Heritability	Traits							
	DH	**DM**	**PH**	**TKW**	**ME**	**FR**	**GPC**	**BG**
Parents	87.4	72.5	93.6	73.3	49.5	74.6	82.6	83.00
Crosses	81.0	87.0	36.7	73.4	69.06	68.3	29.52	49.62
RILs	69.4	59.0	49.4	73.6	34.84	47.5	40.48	27.39

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

Heritability was also computed for each location separately (Fig. 3). Accordingly, the heritability of traits at Holeta ranged from (H^2 = 19 % for BG to 93.6 % for PH), (H^2 = 41.4% for GPC to 70.8% for FR), (H^2 = 27.1 % for ME to 77.2 % for PH), whereas heritability of traits at Bekoji ranged from (H^2 = 71.4 % for GPC to 93.4 % for PH), (H^2 = 60.1% for GPC to 92.8 % for DM)

and (H^2 = 46.5% for ME to 89.1% for BG) for parents, crosses and RILs, respectively (Fig.3). This parameter reveals the ability of the respective environment to differentiate parents, crosses and RIL, respectively. This is important in case of seed scarcity to use one location to evaluate and select the respective genotypes or lines based on phenotypic means only. On average Bekoji demonstrated better heritability estimates compared to Holeta. However, assuming a heritability threshold of 20%, both locations provide sufficient heritability estimate between the three groups except parents for BG (H^2 =19%) at Holeta.

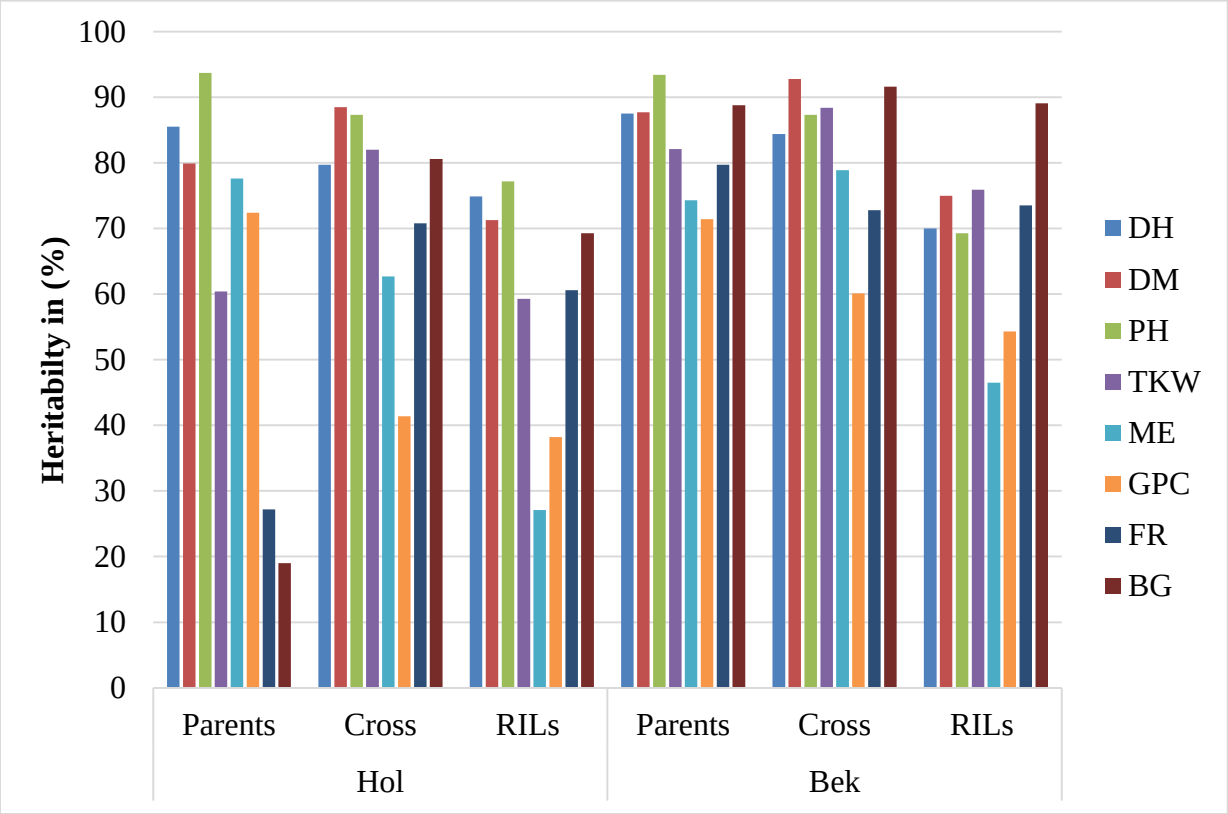

Figure 3. Heritability of traits across the two locations for parents, crosses and RIL

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

5.5. Phenotypic and Genotypic Coefficient of Correlation between Traits

The phenotypic and genotypic trait correlation values obtained in this study was low to moderate with absolute values ranging between -0.73 and 0.78 (Table 12). The correlation coefficient of phenotypic traits was ranged from - 0.08 to 0.65 while the genotypic correlation coefficient ranges from - 0.73 to 0.78. Accordingly, positive and significant ($P<0.01$) phenotypic and genotypic correlations were observed between ME and FR ($r = 0.58$ and 0.60), DH and DM ($r = 0.65$ and 0.78) in the desired direction whereas negative and significant phenotypic and genotypic correlation coefficient between FR and GPC ($r = - 0.60$ and $- 0.73$), respectively (Table 12). The positive and significant correlation observed between ME versus FR and DH versus DM reveals that selection for one trait will leads 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 trait is difficult. Over all, the correlation coefficient among malting quality traits and agronomic traits were weak compared to correlation within.

DH demonstrated highly significant ($P<0.01$) and positive relationship ($r = 0.65$ and 0.78) with DM both at phenotypic and genotypic levels (Table 12). However, it revealed low and positive relationship with TKW ($r = 0.02$), ME ($r = 0.00$ and 0.01) and GPC ($r = 0.06$ and 0.07), both at phenotypic and genotypic level whereas low and negative relationship with FR ($r = -0.05$ and -0.01) for both correlations. Similarly, a weak relationship was observed with PH in opposite direction. Exceptionally, no relationship was observed with ME at the phenotypic level.

On the other hand, PH revealed a positive and moderate correlation in the desired direction with TKW($r = 0.35$, 0.35), whereas weak relationship with DM ($r=0.19$, 0.06), and GPC ($r=0.07$, 0.17) for both genotypic and phenotypic coefficient of correlation. However, poor correlation had been observed with ME ($r = 0.01$) and FR($r = 0.02$) at the genotypic level as well as at phenotypic level ($r= -0.03$, -0.08) in undesired direction. Moreover, TKW was positively and significantly ($P<0.01$) correlated with GPC ($r = 0.39$, 0.40) both at the genetic and phenotypic level whereas negatively and significantly correlated with FR ($r = -0.29$, -0.27), respectively.

Table 12. Correlation of Traits studied (genotypic correlation above diagonal and phenotypic correlation below the 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	*

Correlation between agronomic and malt quality traits. The color represented as 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, very light red = undesired direction and weak correlation. Correlations values above 0.2 and below −0.2 are significant (P < 0.01). 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

Similarly, TKW and ME were found significant only at genotypic level where their relationship was in undesired direction. The correlation between ME and FR (r= 0.58, 0.60) were strong, positive and significant (P<0.01) whereas negative and significant relationship (r = -0.35 and -0.48) with GPC (Table 12).

5.6. Regression of cross mean (CM) on mid parent value (MPV)

Regression coefficients of the cross means on mid parent values estimated showed highly significantly different from zero for all traits (Table 13). 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 (Table 13). 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 numerator is larger and they decrease if their phenotypic variances in the denominator are larger. The respective heritabilities indicate how much phenotypic variance is inflated compared to their genotypic variances.

Table 13. 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*

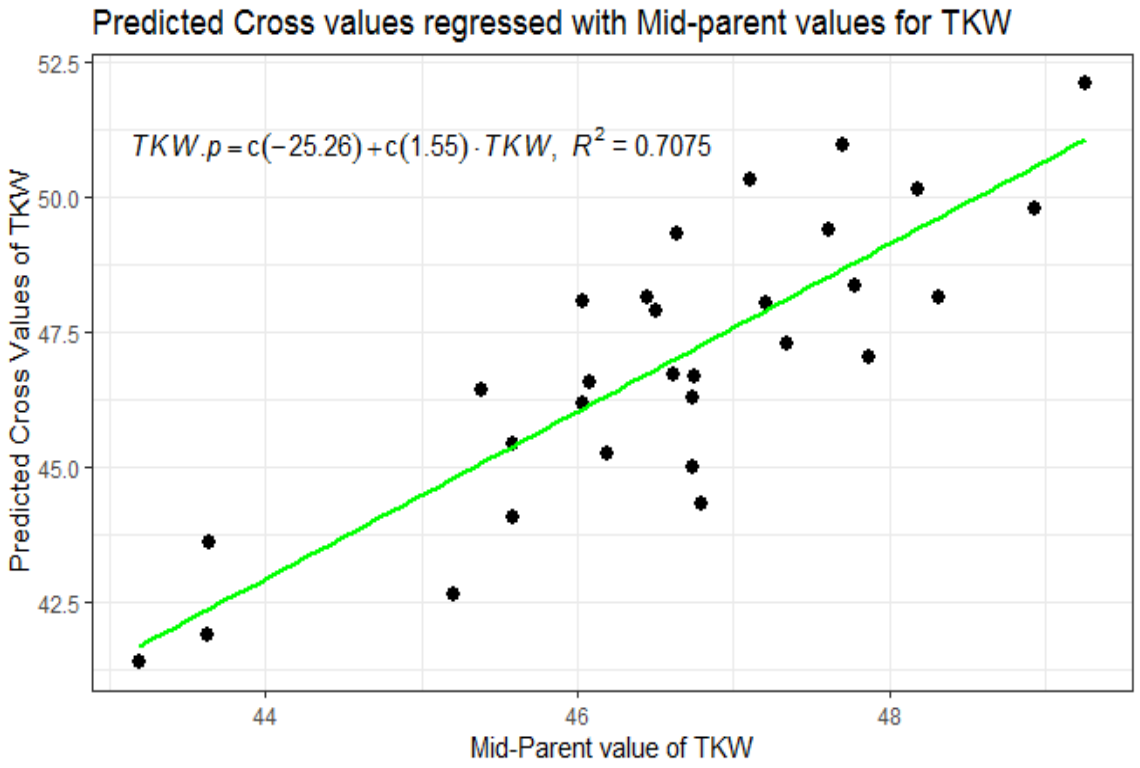

Figure 4a. Regression of cross mean (CM) on mid parent value (MPV) for TKW

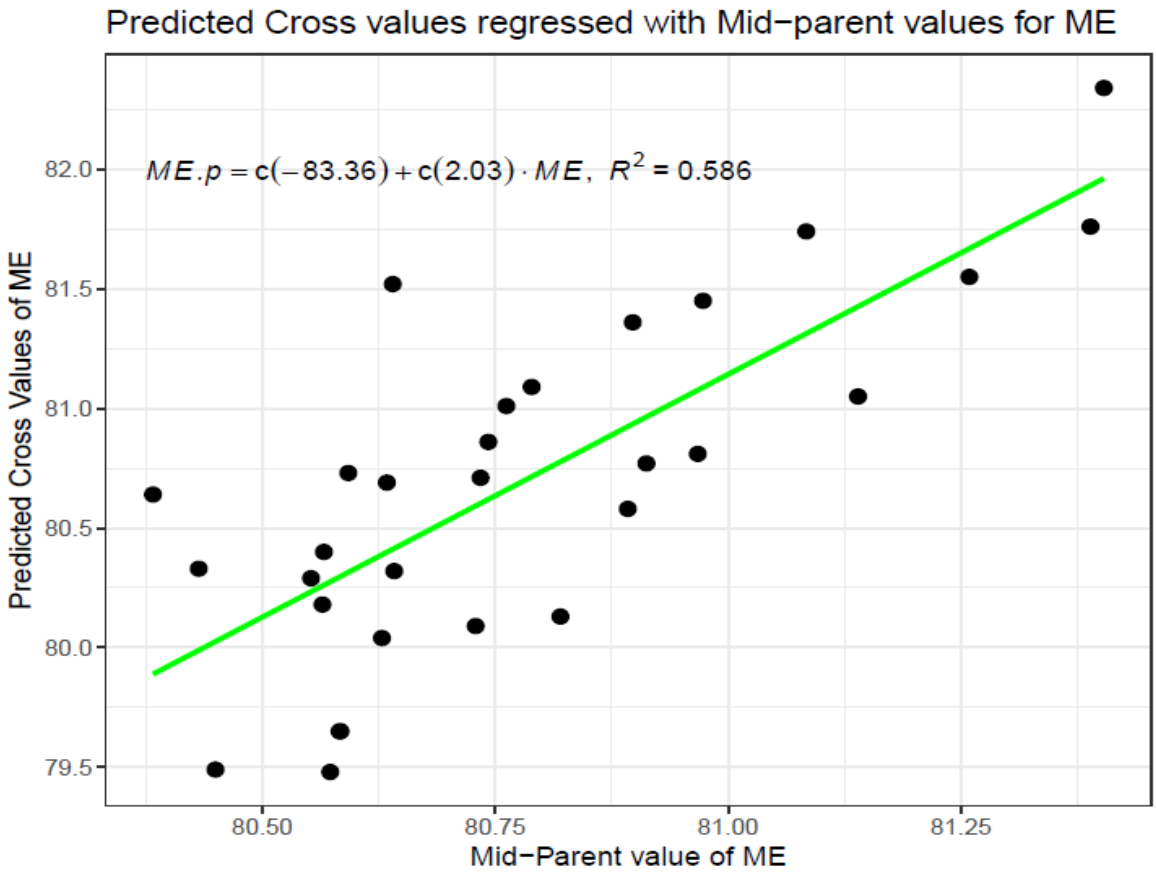

Figure 4b. Regression of cross mean (CM) on mid parent value (MPV) for ME

Concerning individual traits, MPV were accurate predictors for DH, DM, TKW and ME and less accurate for FR, GPC and BG. For this, Fig. 4a and 4b visualizes the distribution of MPV and their respective CM for TKW and ME. Accordingly, we found that about 70% and 58.6% of the variation of cross mean in TKW and ME were explained by the MPV. Hence, Mid-parent value was found to be a perfect predictor for the predicted crosses mean in our trait of interest (TKW and ME).

5.7. Usefulness of Crosses

The interest in breeding program is both increasing the mean value of the breeding population and identifying superior RIL, hence, crosses can be ranked based on the usefulness criterion. To illustrate a practical application of the usefulness parameter (Table 14), 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). The best 8 crosses surpass Planet with their selected fraction $U_{(i=1)}$ for all 5 traits were considered (Table 14). Thus, assuming sufficient RIL/cross are available there is a good chance to select one "all-rounder" RIL out of these crosses, which has a good performance for all 5 traits. Accordingly, five crosses among the best eight were, Planet x MBHIBYT-22 (92.51cm, 48.75 g, 82.48 %, 74 % and 398.76 mgL^{-1}), ICARDA GP-67 x HB 1963 (94.59 cm, 52.01 g, 81.75 %, 69.92 % and 366.92 mgL^{-1}), Planet x ICARDA GP-67 (96.15 cm, 46.79 g, 81.7 %, 70.15 % and 379.76 gmL^{-1}), Bekoji-1 x Grace x Planet (96.47 cm, 50.52 g, 81.22 %, 70.6 % and 383.94 mgL^{-1}), and G13-64 x MBHIBYT-22 (92.57 cm, 47.44 g, 81.22 %, 72.49 % and 360.53 mgL^{-1}) for PH, TKW, ME, FR and BG, respectively(Table 14). These crosses surpassed the reference variety by five economical important traits. Further, among the second-best 8 crosses fits the U criterion for 4 traits (Table 14). The third and fourth groups (9 and 5 crosses, respectively) were 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, it would be easier to invest in higher numbers of RILs of these crosses and discard the low-performing crosses to save breeding capacity.

Table 14. Usefulness of crosses ($U_{i=1}$)

Cross		Traits					No of traits with fraction	
Parent 1	Parent 2	PH	TKW	ME	FR	BG	$U_{(i=1)}$ > Planet	
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 x Grace)	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**	

: $U_{(i=1)}$ > trait expression of Planet

: $U_{(i=1)}$ < trait expression of Planet

U= Usefulness, PH = Plant height, TKW = Thousand kernel weight, ME = Malt extract, FR = Friability, BG = Beta glucan

5.8. Genetic Diversity and Similarity Analysis for within and between Parental Lines

5.8.1. Similarity Analysis

Expected homozygosity for each KASP marker was computed based on the 23-24 grains sampled from each line. The average across markers yields "homogeneity" as the final estimate of genetic similarity between genotypes within the line or purity of the line. The overall average homogeneity across all 24 lines, which included the 17 parental lines of the experiment and seven additional check varieties, was 93.7 (Table 15). The homogeneity within the parental lines varied from 71 – 100 % (Fig.5). Lines released as varieties in Europe are expected to reach a homogeneity value of 100% because of the very strict regulation applied for Distinctness, Uniformity and Stability (DUS) within UPOV-countries. Lines originating directly from breeding programs might be less homogeneous, in the case of the EIAR program they might be equivalent to RIL in F4:5 generation or above. A line may be considered as homogeneous if homogeneity reaches or surpasses the 95% threshold.

Accordingly, line IBON 14/15-144, ICARDA GP-75 and Planet were found to be 100% homogeneous or pure. Concerning the homogeneity threshold, in our case 11 out of 17 (i.e., G13_64 (97%), HB1963 (98%), IBON_14_128 (97%), IBON_14_41 (99%), IBON_13_2 (98%), IBON_14_15_144 (100%), ICARDA GP_75 (100), MNBrite (96%), Planet (100%), Sabini (100%), and Traveler (96%)) of them were among the parental lines that met this criterion while line Bekoji-1 x Grace (71%), contributed most to variation (Table 15). The corresponding average expected heterozygosity, which is the opposite of expected homozygosity, could originate from in-complete inbreeding in-crossing events during line development or technical admixture with other germplasm. Accordingly, Bekoji-1 x Grace which was contributed heterozygosity percentage accounted for 29% of the mixture either due to in-complete inbreeding in breeding events during lines development or technical mix-up during maintenance, cleaning, storage or others.

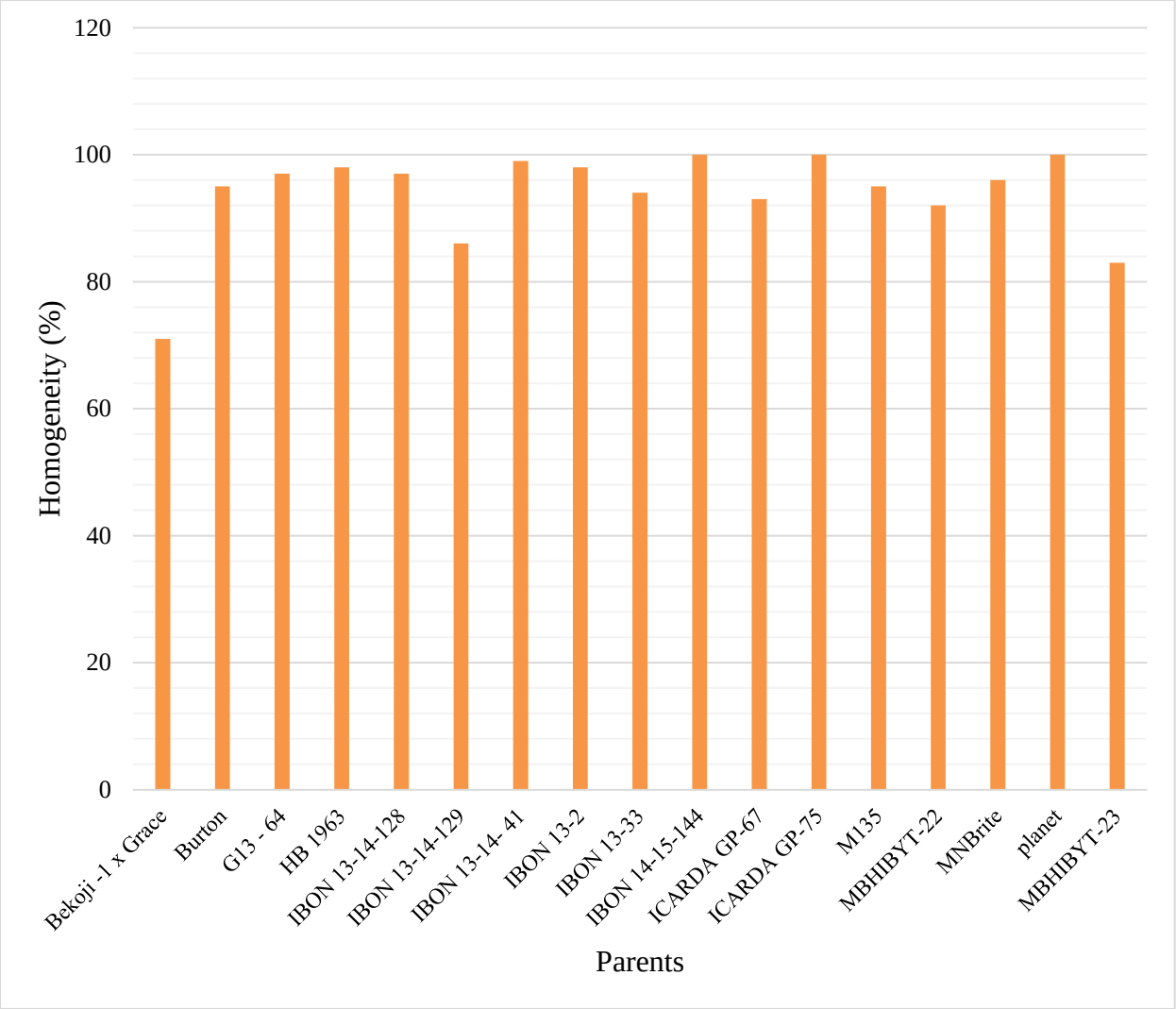

Figure 5. Homogeneity (percent) of parental lines realized in the 30 crosses based on KASP markers

Table 15. Homozygosity of the parental lines across the 24 grain samples for each individual KASP marker

Parents	Homogeneity (%)	missing	GE3506-668	ABC07611-1-5-315	GE4057-2114	GE4126-1180	GE4593-2007	GE4991-1028	GE5004-375	GE5194-1118	GE6781-1073	GE9608-371	GE9610-1195	GE1735-1424	GE13924-403	GE2052-792	GE2055-947	GE2274-1226	GE2795-1707	GE2878-574	BOPA1_2582-767	BOPA1_10248-954	BOPA1_8743-197	BOPA1_ABC 05640-1-1-248
Bekoji-1xGrace	71	0	0.52	1	1	0.5	0.5	0.51	0.51	0.56	1	0.66	0.51	0.56	1	1	0.66	0.5	0.52	1	0.77	0.77	0.81	0.5
Burton	95	0	0.92	1	1	0.92	0.92	0.92	0.92	0.92	1	0.92	0.92	1	0.92	0.92	0.92	0.92	0.92	1	0.92	1	1	1
G13_64	97	0	1	1	0.92	0.92	0.92	1	1	1	1	1	1	1	1	1	0.92	0.92	1	0.92	0.92	0.92	1	0.92
HB 1963	98	0	1	1	1	0.71	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.84	1
IBON_14_128	97	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0.92	0.96	1	1	0.61	1	1	1	0.92
IBON_14_129	86	0	0.77	1	1	0.92	0.77	0.92	1	1	1	0.51	0.77	0.77	0.84	1	1	0.84	0.92	0.77	0.84	0.77	0.84	0.77
IBON_14_41	99	0	1	1	1	1	1	1	1	1	0.96	0.88	1	1	1	1	1	1	1	1	1	1	0.92	0.92
IBON_13-2	98	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.52	1	1	1	1	1	1
IBON_13-33	94	0	1	1	1	1	1	1	1	1	1	0.52	1	1	1	1	1	0.55	1	1	1	1	1	0.52
IBON_14_15_144	100	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ICARDA GP_67	93	0	0.92	1	1	0.92	1	0.92	0.84	1	0.77	1	0.92	0.52	1	1	1	0.77	1	1	0.84	1	1	1
ICARDA GP_75	100	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Irina	100	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M_21 X Karne	89	0	0.65	1	1	0.6	1	1	0.96	1	1	0.7	1	1	NA	0.6	0.54	1	1	1	0.57	1	1	1
M135	95	0	1	0.77	1	1	1	1	1	1	1	1	1	1	1	0.55	1	1	1	1	1	1	1	0.5
MBHIBYT_22	92	0	1	1	1	1	0.92	0.92	1	NA	1	0.92	0.92	1	0.92	0.61	1	0.92	1	0.55	1	1	1	0.58
MNBrite	96	0	1	1	1	1	1	1	1	1	0.51	1	1	0.96	1	1	1	1	1	0.58	1	1	1	1
Planet	100	0	1	1	1	1	1	1	1	1	1	0.96	1	1	1	1	1	1	1	1	1	1	1	1
EH1847	87	0	1	1	1	0.92	0.92	0.92	0.72	0.92	0.85	0.92	0.85	0.85	0.72	0.78	0.92	0.92	0.92	0.85	0.92	0.78	0.5	0.85
Holker	91	0	1	1	1	0.67	1	1	1	1	1	1	1	1	0.67	1	0.96	0.67	0.67	1	0.67	1	0.67	1
IBON_174_03	92	0	1	1	1	0.71	1	1	1	1	0.5	0.92	1	1	1	1	1	1	0.92	0.51	1	1	1	1
MBHIBYT_23	83	0	1	1	1	1	0.5	0.56	0.5	0.96	0.92	0.59	1	0.92	0.5	0.78	0.92	0.67	1	1	0.56	1	1	0.67
Sabini	100	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Traveler	96	0	0.92	1	1	0.92	1	0.92	1	0.92	0.92	1	1	1	0.92	0.92	1	0.92	0.92	1	0.92	1	0.92	0.92

5.8.2. Genetic Diversity Analysis

Genetic distance among parental lines was estimated with Rogers Distance (RD) (Table 16). The analysis revealed an average distance between all parental lines of 0.40 with a range of 0 to 0.64. Lines MBHIBYT_22 and IBON13_14_128 proved to be identical (RD = 0.0), whereas the line combination ICARDAGP_75 and G13_64 exhibited the largest distance among the parents involved (RD = 0.64). Concerning the genetic distance estimate among parents actually combined in the investigated 30 crosses range from 0.32 - 0.64 with an average of 0.46. Looking in to specific crosses, Planet x MNBrite, IBON14_15_144 x ICARDA GP_67, and G13_64 x IBON13_2 were among the parents registered the second largest distance involved in the crossing program (RD=0.55). Furthermore, the third group of parents that exhibited large distance, Planet x ICARDA GP-75, IBON 14/15-128 x Planet, IBON13_2 x Planet, MBHIBYT_22 x Planet, and M135, IBON13_14_128 x MNBrite, G13_64 x MNBrite, IBON13_2 x ICARDA GP_67, IBON13_14_128 x IBON 14_15_144, HB 1963 x IBON 14_15_144, Burton x G13_64, and Burton x IBON13_14_128 were among the list of parents actually realized in the 30 cross having (RD = 0.5) (Table 16).

Moreover, the respective dendrogram visualizes differences among groups of the parental lines and indicated the discriminating power of KASP marker (Fig. 6). It clustered the lines into three major groups with 3 lines in the first group (ICARDAGP_75, HB1963 and MNBrite), 5 lines in the second group (IBON13_33, M135, Burton, IBON13_2, MBHIBYT_23) and the remaining 9 lines in the third group. Lines with USA origin (MNBrite, M135, and Burton) are placed in the first two groups and the two European lines (Planet and G13_64) in the third group. Line Bekoji-1 x Grace is adjacent to these two lines which is plausible with grand-parental line Grace also descending from Europe. The third cluster was the largest consisting of lines from the 3 regions (Ethiopia: Bekoji-1x Grace, Europe: Planet and G13_64 and ICARDA-Morocco: IBON 14_15_144, ICARDA GP 67, IBON 13_14_41, IBON 13_14_129, IBON 13_14_128 and MBHIBYT-22), where the majority of the ICARDA lines were found in this cluster. The similarity of lines originating from different regions indicates that there is some gene flow from region to regions through germplasm exchange.

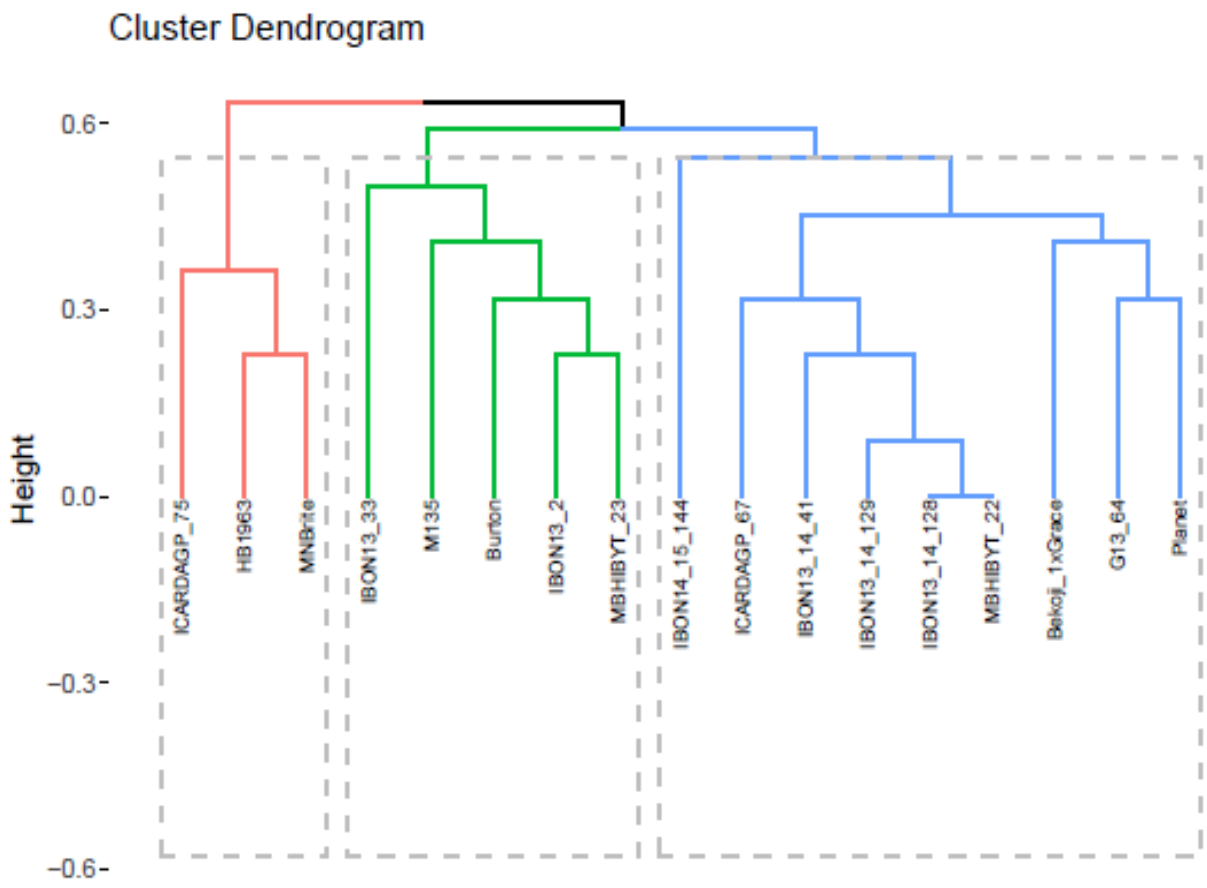

Figure 6. Clustering of parental lines based on RD

In the diversity analysis using 22 markers result revealed that major allele frequency ranged from 0.53 to 0.94(mean 0.73), gene diversity ranged from 0.11 to 0.50(mean 0.36) whereas polymorphic information content (PIC) ranged from 0.10 to 0.37(mean 0.28) (Table 17), respectively.

Table 16. Rogers Distances (RD) between the 17 parental lines, above diagonal parental line combinations realized in the 30 crosses.

	Bekoji_1x Grace	Burton	G13_64	HB1963	IBON13_ 14_128	IBON13_ 14_129	IBON13_ 14_41	IBON13_ 2	IBON13_ 33	IBON14_ 15_144	ICARDA GP_67	ICARDA GP_75	MBHIBY T_22	M135	MN Brite	Planet	MBHIBY T_23
Bekoji_1xGrace																0.36	
Burton	0.45		0.50		0.50						0.36					0.55	
G13_64	0.50	0.50			0.36			0.55	0.36			0.64	0.36		0.50		
HB1963	0.32	0.32	0.27		0.36					0.50	0.32						
IBON13_14_128	0.32	0.50	0.36	0.36						0.50				0.36	0.50	0.50	
IBON13_14_129	0.50	0.50	0.27	0.45	0.09											0.32	
IBON13_14_41	0.36	0.36	0.32	0.32	0.23	0.23										0.36	
IBON13_2	0.50	0.32	0.55	0.45	0.27	0.36	0.32				0.50					0.50	
IBON13_33	0.60	0.50	0.36	0.45	0.45	0.45	0.32	0.45									
IBON14_15_144	0.45	0.55	0.50	0.50	0.50	0.50	0.45	0.32	0.50		0.55					0.45	
ICARDAGP_67	0.45	0.36	0.50	0.32	0.32	0.32	0.27	0.50	0.50	0.55						0.45	
ICARDAGP_75	0.59	0.59	0.64	0.36	0.55	0.64	0.50	0.45	0.55	0.60	0.50					0.50	
MBHIBYT_22	0.32	0.50	0.36	0.36	0.00	0.09	0.23	0.27	0.45	0.50	0.32	0.55				0.50	
M135	0.60	0.50	0.45	0.45	0.36	0.36	0.23	0.27	0.45	0.50	0.50	0.36	0.36			0.50	
MNBrite	0.45	0.36	0.50	0.23	0.50	0.50	0.27	0.50	0.50	0.45	0.36	0.32	0.50	0.50		0.55	
Planet	0.36	0.55	0.32	0.32	0.50	0.32	0.36	0.50	0.50	0.45	0.45	0.50	0.50	0.50	0.55		0.45
MBHIBYT_23	0.36	0.27	0.32	0.32	0.32	0.50	0.36	0.23	0.50	0.36	0.45	0.50	0.32	0.32	0.36	0.45	

RD across all line combinations: Min 0.00
Max 0.64
Mean 0.40

RD across line combinations realized in crosses: Min 0.32
Max 0.64
Mean 0.46

Table 17. Marker position and summary statistics

Marker	Chr	position	Major.Allele. Frequency	Gene Diversity	PIC
GE5194-1118	1H	18807296	0.88	0.21	0.19
BOPA1_8743-197	1H	380928570	0.76	0.36	0.30
GE4057-2114	1H	555618159	0.82	0.29	0.25
BOPA1_2582-767	2H	3326148	0.71	0.42	0.33
GE2052-792	2H	196891780	0.65	0.46	0.35
BOPA1_ABC05640-1-1-248	2H	702542245	0.71	0.42	0.33
GE13924-403	3H	3981097	0.65	0.46	0.35
GE4593-2007	3H	32615385	0.59	0.48	0.37
BOPA1_10248-954	3H	319229422	0.88	0.21	0.19
GE9610-1195	3H	654775437	0.82	0.29	0.25
GE2055-947	4H	12270789	0.71	0.42	0.33
GE2274-1226	4H	484881213	0.53	0.50	0.37
GE2878-574	4H	644449770	0.53	0.50	0.37
GE9608-371	5H	9861991	0.65	0.46	0.35
GE5004-375	5H	229928326	0.82	0.29	0.25
GE6781-1073	5H	650559436	0.59	0.48	0.37
GE2795-1707	6H	12662354	0.82	0.29	0.25
GE3506-668	6H	335738777	0.94	0.11	0.10
GE4126-1180	6H	577508169	0.53	0.50	0.37
ABC07611-1-5-315	7H	7044961	0.88	0.21	0.19
GE1735-1424	7H	110445275	0.71	0.42	0.33
GE4991-1028	7H	639792989	0.59	0.48	0.37
Mean			0.73	0.36	0.28
Min			0.53	0.11	0.10
Max			1.00	0.50	0.37

5.8.3. Correlation of segregation variance (σ^2g) to Rodger's Distance (RD)

Correlations of σ^2_g to RD ranged from 0.34 to -0.19 and were not significantly deviating from zero for all traits except DH (Table 18).

Table 18. Traits correlation with Rodger's Distance among parental lines

Traits	DH	DM	PH	TKW	ME	GPC	FR	BG
r (σ^2g -RD)	0.344	-0.191	-0.075	-0.213	-0.040	0.010	0.034	0.068

DH = days to heading, DM = days to maturity, PH = plant height, TKW = thousand kernel weight, ME = malt extract, FR= friability, GPC= grain protein concentration, BG = beta glucan. Correlations < 0.2 and <-0.2 are non -significantly deviating from zero.

5.9. Time and Cost Demand

Breeding cost is the major factors that influence success in breeding. In here only specific cost related to this experiment is investigated. Currently phenotyping is the main cost factor in the Ethiopian barley breeding program though genotyping might be one of the future tools to be applied. In this study the total cost estimated for F4:5 RIL were 471,016 including genotyping cost and 240,000 without genotyping costs (App. Table 21). This could be converted to YPU which is equivalent to 1155.1 and 1200 YPU with genotyping and without, respectively.

In addition, time demand in breeding as discussed under 3.12.4 and which can be described as number of years from intercrossing parental lines to intercrossing of lines derived from these crosses and identified as superiors an important factor that contribute to success in breeding and in improving the genetic gain from selection. As described earlier Ethiopian barely breeding follows efficient breeding methods ('state - of the - art 'through SSD methods) to shorten breeding cycle as much as possible.

6. DISCUSSION

6.1. Comparison of generation means

Means of 30 mid parents values (μ_{MPV}) and of their 900 derived RIL (μ_{RIL}) were estimated across Holeta and Bekoji (Table 19). Differences between the generation means Δ (μ_{MPV} - μ_{RIL}) across all crosses were small in general and non-significant in a t-test. On average RILs were found to be slightly later, taller and provided a high TKW. With regard to malt quality, traits RIL deviated slightly into the undesired direction. Three driving forces for deviations between generations shall be discussed in the following.

(i) Differences between generation means could be due to shortcomings of the accuracy of our experimental data. Standard errors of the respective means could be taken as parameters to address this point.

(ii) Selection effects during line development from F2- to F4- generation are a potential reason for a difference between generation means. In line development entries are grown as single plants which could efficiently be selected on traits such as DH, PH or TKW, hence selection was imposed for more heritable traits.

(iii) Based on the genetical 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: (1) epistatic effects are of minor importance in the germplasm investigated and/or (2) positive and negative epistatic effects have cancelled each other. In line with this, Utz *et al.*(2001) 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 NON-Planet crosses separately speaks in favor of the second “cancelling” hypothesis. In any case epistasis can be considered as a potential source of bias and breeders should try to limit its impact. For example, in 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.

This result is in agreement with reports from different scholars dealt with the involvement of epistasis in generation mean analysis in barley (Madakemohekar *et al*., 2018), wheat (Holasou *et al.*, 2019; Javanmard *et al*., 2018;Ojaghi & Akhundova, 2010; Salmi *et al*., 2019; Said, 2014).

(iv) Finally, all three driving forces might be confounded in the phenotypic data and might have cancelled each other to a certain extent. The variety Planet has been used in 13 crosses as parent to introgress malt quality and high grain yield under favorable agronomic conditions.

Table 19. Comparison of generation means of Mid-parent values (μ_{MPV}) and derived Recombinant Inbred Lines (μ_{MPV}) 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
NON-Planet 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
NON-Planet Crosses(N=13)	81.9	134.2	91.3	46.6	80.5	11.2	65.8	381.9
$\Delta_{(mMP-mRIL)}$								
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
NON-Planet Crosses(N=13)	-0.5	-0.9	-1.2	-0.4	0.2	-0.1	1.8	-9.9

The Planet-effect can be roughly estimated by comparing 9 crosses ("Planet Crosses"), where Planet has been involved as a crossing partner and 13 other ("NON-Planet Crosses") crosses with other parental lines having been combined. In this subset of 9 +13 crosses as far as possible the other lines had been balanced to contribute roughly equal to both groups. RIL descending from Planet are earlier, have a slightly lower PH and TKW and excel by better malt quality (ME, FR, BG) compared to the RIL from the other group. The differences assessed here can be compared with the annual genetic gain achievable e. g. for ME and FR as two key quality traits. Annual genetic gains of German malt breeding programs are estimated (Laidig *et al.*, 2017) as 0.08 and 0.47 %/year, respectively. Thus, a ME (FR) difference between Planet and NON-Planet RIL of 0.6 (2.4) % is equivalent to 7.5 (5.1) years of intensive breeding work.

On the other hand, results from Ethiopian National Variety trials 2020 (Tesfahun 2021, personal communication) show Planet to have a lower grain yield than varieties better adapted to the Ethiopian target environment with a grain yield level of not more than 2-4 t/ha. So, potentially means of the Planet-crosses for grain yield could also be lower. Data from the preliminary variety tests in Meher season 2021 can be used to confirm or reject this hypothesis. In summary, a conscious and anticipatory parental selection in the early phase of a breeding program is one of the prerequisites for obtaining outstanding and transgressive segregants in the subsequent generations.

6.2. Variance among Mid-Parents, Crosses and Line within Crosses

In the previous chapters 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 RIL are derived by an SSD process in the absence of forces driving changes of allele frequency and (v) As a mode of inheritance, additive gene action and absence of epistasis has been postulated.
With this model we expect for the genetical variances of parents, crosses and RIL:

$$\sigma^2_p/2 = \sigma^2_c = \sigma^2_g = \sigma^2_A$$

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

(i) 17 parental lines have been used to produce crosses. As shown in Table 1 lines have different geographical origins such as USA, Europe, Ethiopia and ICARDA. In addition, diversity analysis based on KASP markers (Fig.6) grouped lines into 3 (sub-) populations. Thus, the assumption, 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 (Utz *et al*., 2001) and accordingly shrink the ratio of σ^2_c: σ^2_g.

(ii) Another model assumption is that parental lines are randomly intercrossed. With 17 parental lines (17 x 16)/2=136 crosses are feasible. Out of these potential 136 crosses, only 30 crosses had actually been investigated for our experiment. This small section led to variation in terms of different gametic contributions of the parental lines. For example, Planet had been involved in 13 crosses, whereas Bekoji x Grace contributed only to one cross. Further, the small sample of crosses could have led to assortative or disassortative mating (Falconer and Mackay, 1996), which means lines with a similar or dissimilar genotype for a given trait have been crossed more often than 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 non-random matings by 'best x best' and 'parents complementary' crossing designs, respectively. However, which of these forces leading to deviation from random mating predominated in our case is impossible to assess.

(iii) Parents were assumed to be homozygous. Adopting the homogeneity threshold of 95% (Kassa Semegn *et al*., 2012), we know this assumption does not hold true for 6 out of the 17 parents. The more heterogenous lines taken for crosses are the more the variance within crosses increases and correspondingly the variance among crosses decreases. In line with this, Utz *et al.*(2001) proved that crossing parents derived from heterogeneous two distinct population of winter wheat resulted in higher variance within crosses compared among crosses.

(iv) RIL 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 homozygous in the ancestral F1 genotype. The remaining 12.5% of the loci are still heterozygous and do not contribute much to segregation variance between RIL within a given cross. In case of fully homozygous RIL were produced e.g., by double haploid culture 100% of the loci would have been homozygous and a higher segregation variance is expected. For consequence, RIL generation chosen for our experiment will contribute to inflating the ratio σ^2_c: σ^2_g.

In the SSD procedure leading to the final RIL changes 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, PH 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. For consequence, the SSD procedure chosen for our experiment will contribute to deflating the ratio σ^2_c: σ^2_g.

(v) From analyzing the generation means in the previous chapter we should be careful to regard epistatic effects and their respective variance as non-existent. This conclusion is in line with findings in the literature (Utz *et al.*, 2001) for wheat and for barley (Oakey *et al.*, 2006).

Recurring to the balance sheet of the genetical variances estimated in our experiment the following conclusions can be drawn.

The parameter $\sigma^2_p/2$ subdivides the variance among individual parental lines by 2 and is meant as an estimator of the variance between mid-parents. Taken the arguments summarized under (ii) this estimator is rather imperfect and should be replaced by the variance among mid-parents actually used in our experiment. Ratio σ^2_c: σ^2_g was assumed to be equal to one. Taken 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, GPC we observed a tendency for σ^2_c to be smaller σ^2_g. 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 (Utz *et al.*, 2001), in our case mating of parental lines is highly unbalanced and cannot be regarded as random. The breeder might use outstanding RIL 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 for a situation close to classical second cycle breeding. Nevertheless, the actual estimates are highly valuable starting points for considerations to

improve breeding methodology. Irrespective of their actual ratio, the two variances, σ^2_c and σ^2_g, proved to be significantly deviating from zero for almost all traits. Accordingly, the breeder can exploit both of them for selection. Similar result has been reported on wheat by Utz *et al.* (2001) with large size of σ^2_g among RIL within crosses. Further, Koide *et al.*(2019) reported higher genetic variance between crosses of Rice on DH. However, the σ^2_g observed in this study was larger enough than the previous study report; hence provide a good chance for breeder to select outstanding RIL within crosses.

6.3. Genetic Variance within crosses (σ^2_g)

Combining parents from different genetic backgrounds, origins and performances, resulted in a higher genetic variance of RIL within crosses. Accordingly, the result obtained in this study confirmed this genetic expectation except for certain traits (Table 10). σ^2_g estimated from individual crosses was considered to vary from the cross to cross for DH, DM, PH, TKW and BG. In contrast this had not been observed for the quality traits ME and GPC. In line with Utz *et al.* (200) found that heterogeneity of σ^2_g was significant heading date, kernel weigh, lodging and plant height.

As an indicator for the variation of cross-specific σ^2_g, the standard deviation of the estimates in comparison to their mean standard error was taken. The standard error depends on the number of RIL per cross and the testing intensity in terms of testing sites and replications. With only 30 RIL/cross and only two locations, σ^2_g can only very roughly be estimated for an individual cross. Therefore, for breeding methodological studies the mean σ^2_g should be taken as a starting point and as a rule-of-thumb-figure.

6.4. Broad sense heritability of Traits

Heritability estimates could provide a valuable indication of expected improvement through selection. Higher variable ranges of heritability estimate were obtained for parents, crosses and RILs within crosses (Table 10 and Fig. 3). Looking across traits the estimates for the parental group heritability ranged from 49.5 (ME) to 93.6 (PH), for the crosses from 29.5 (GPC) to 87.0

(DM) whereas RIL heritability estimates differed from 27.39 (BG) to 73.6 (TKW), respectively. Heritability could be classified as low $H^2 \leq 0.2$, moderate $0.2 \leq H^2 \leq 0.50$, high $H^2 \geq 0.5$) (Sight, 2005). Hence, most of the heritability obtained in this study fall into the moderate and high class. This implied that the possibility for improvement through selection will be high. The higher value of heritability estimates of traits are indictors of greater portion of genetic components in relation to environmental and genotype x environmental interaction components in the total phenotypic variance of a trait (Ebadi *et al.*, 2016; Falconer, 1989). It is one of the most important genetic parameters that a breeder use to assess a quantitative characteristics and to select subject that can obtain genetic gain in barley and other crops(Amabile *et al.*, 2014). In agreement with this result, Addisu Fekadu & Shumet Tenaw (2015), evaluated 36 barley genotype and reported high value of H^2 for the same metric traits. Sayd *et al.*(2017) reported wide heritability value of the same magnitude in some quantitative traits in barley. Similarly, Amabile *et al*.(2014) conducted an extensive review on heritability and Sayd *et al*.(2017) in his study on barley genotype under irrigated condition concluded that for most agronomic traits, heritability values are generally high (>80%). Heritability of malt quality traits were ranged from moderate to high value as reported by Bhatta *et al*.(2020). Looking in to trait specific H^2, for instance ME (H^2=49. 5, 69.6 and 34.48) for parent, cross and RIL, which was lower than the value reported by Sarup, (2020)(H^2=0.66) and (Bhatta *et al*., 2020)(H^2= 0.67) for parent and RIL, but higher for cross.

The heritability of parental lines was higher compared to the estimates for crosses and RIL. A genetic explanation for this finding as derived from the model described in 4.7.1.2 is that the variance of homozygous parental lines is twice as large compared to the variance among crosses. Another reason for a higher parental heritability is attributed to experimental design. Phenotypic variance as denominator in the heritability formula has been defined as $\sigma^2_p = \sigma^2_g + \sigma^2_{gl}/n_l + \sigma^2_{gy}/n_y + \sigma^2_{gly}/ (n_l n_y) + \sigma^2_e/ (n_l n_y n_r$ (assumption of balanced data). Parents were five times replicated at each location in this experiment that contributed for low experimental error and the average experimental error contribution to the phenotypic variance greatly reduced consequently provide high heritability. This option could be realized because there was sufficient seed available from the parental lines. In case of RIL seeds descend from the harvest ware of single plants and hardly more than three plots/RIL can be supplied with seeds. In the case of the crosses

Utz *et al*, (2001) proposed to subdivide the total number of RIL from each cross into sets comprising 11 RIL. These sets were then distributed on four environments (two years times two locations). As can be seen from the formula above this design will increase heritability if σ^2_{gl} and σ^2_{gy} and σ^2_{gly} are important masking variances. The heritability estimate for agronomic traits was found to be higher than that of the quality traits in most cases. This was due to larger genotype by environment interaction variances influencing the quality traits compared to agronomic traits. In line with this, for the parental lines Utz *et al*.(2001) reported only a heritability of 42.2% for GPC, whereas for the same testing intensity heritabilities for DH, PH and TKW were higher and ranged from 78.8 to 97.7%.

In our study, RILs were tested across two locations in a p-rep design that practiced replicating a portion of the RILs in various locations. This provides an opportunity to get a better number of observations for treatment to improve the estimation accuracy. Even the p-rep advantage explained recently, there is no comprehensive study on how the design impacts the heritability estimates as compared to the single replication per location trials such as augmented RCBD. That showed the importance of developing activities to assess the cons and pros of the prep design with other well-established breeders' designs. Nevertheless, when asking the question of how heritability can be increased two driving forces can be mentioned beside increasing genetic variances: (i) testing intensity can be enhanced by involving more years, locations and replications and (ii) employing trial analytics which reduce 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" (Cobb *et al*. 2019) is hypothesized to be highly effective in terms of optimized use of limited phenotyping capacity. No final conclusions can be presented here to which extent the trial analytics employed in our experiment contributed to an increase of heritability compared to employing less advanced approaches.

6.5. Phenotypic and Genotypic Correlation between Traits

As described in previous chapters, phenotypic correlation(rp) occurs when phenotypic values of the two traits are correlated due to their genetic and/or non-genetic effects whereas the genetic correlation (rg) resulted from pleiotropy (is caused by the same genes influencing two different traits) and linkage disequilibrium between genes controlling different characters. Estimate of phenotypic and genotypic correlation coefficients ranged from - 0.073 to 0.78 (Table 12). In specific it revealed that positive and strong phenotypic and genotypic correlations between ME and FR (r=0.58 and r = 0.60) as well as DH and DM (r = 0.65 and 0.78) (Table 12). This strong positive correlation implied that the two traits were genetically correlated, so that indirect selection operating for one trait can be applied for the others too. In addition, the observed significant positive correlation could arise either from strong coupling linkage between the genes or was the result of pleiotropic genes that controlled these traits in the same direction. In agreement with this, Negash Geleta *et al*.(2019) and Laidig *et al*.(2017), reported similar results on barley. Similarly, Ashebr Baye *et al*.(2020) also reported that DH had significant positive correlation with DM in wheat which implied that increasing 75% DH would increase DM.

The strong negative phenotypic and genotypic correlation between GPC and FR (r = - 0.60, - 0.73) is in line with the result of Laidig *et al*.(2017) and Matthies *et al*.(2014), who both suggest that breeder should take in to account this negative relation while conducting selecting. Moreover, Huerta-Zurita *et al*.(2020) reported strong negative correlation between FR and GPC. The negative genetic correlation implied that the traits are correlated but in opposite magnitude. In fact, the negative phenotypic correlation might arise partially from selection for higher FR and ME which are genetically negatively correlated with GPC, and simultaneously for higher yield. Further, the observed negative phenotypic correlation between FR and GPC can be caused partially due to environmental effects such as variation in nitrogen supply that increase GPC. In any case, it implied that indirect selection is not an option for improvement.

The two correlation coefficients slightly differed from each other in most cases. The genotypic correlation coefficient was found to be higher in magnitude than that of phenotypic correlation coefficient values for most of the traits, indicating inherent association of the traits, so that selection for the correlated traits could give a better gain. Thus, the covariance between traits was mainly due to the presence of linkage and the pleiotropic effect of different genes, whereas non-genetic effects did not contribute much to phenotypic covariance. This is in line with the previous reports of Jimera Haile *et al*, (2015); Kefyalew Taye *et al*, (2016), and Endalkachew Aklilu *et al*. (2020) who analogously explained low phenotypic correlation. In addition, other research findings also revealed a lower magnitude of phenotypic compared to genotypic correlation (Sintayehu Debebe *et al*., 2016; Akalu Gebru *et al*, 2018; Ashebr Baye *et al.*, 2020). In general, strong, positive and significant association of traits justify the possibility of correlated response to selection and strong, negative and significant relation of traits prohibit the simultaneous improvement of those traits (Endalkachew Aklilu *et al*., 2020).

6.6. Regression of cross mean (CM) on mid parent value (MPV)

Regression of cross mean on mid parent (MPV) values is an important parameter in plant breeding because the 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 13). 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 for ME. However, lower R^2-values were observed for FR, GPC and BG. This could be due to the fact that, malt quality traits are highly influenced by environmental effect (Laidig *et al*., 2017), hence accurate estimation of MPV and CM for these specific traits might be influenced more as justified in the scenario 3 and 4 in table 19 below. This implied that, in the future breeder can reduce the impact of environmental influence by conducing accurate experiment (such as representative site selection, appropriate replication) with good experimental management.

In the following hypothetical example (Table 19) the dependency of regression and determination coefficients on genetic and non-genetic variances is illustrated. In scenario 1 with pure additive but no epistatic and non-genetic variance regression and R^2 equal to one. In this case CM is perfectly predictable by MP. In scenario 2, we assume that the breeder again conducts a perfect experiment without any non-genetic variance. But in this case, there is also epistatic variance involved. Accordingly, regression and R^2 values drop down. Scenarios 3 and 4 are more realistic for practical plant breeding where some environmental variance is involved. In these cases, R^2 values are reduced to 0.61 and 0.34, respectively. Our result fits in Senario 3 and 4 based on the R^2 estimated on traits of our interest. Practically the breeder cannot influence the mode of inheritance of a trait with epistatic effects that can affect the R^2 on regression. In contrast, the breeder can avoid the influence of the masking environmental effects by designing appropriate experimental design and experiment handling, there by assessing MP and/or CM more or less accurately.

Table 17. Cross Mean (CM)-Mid-parents (MP) Regression and the respective Coefficient of Determination (R^2) as depending on genetic and non-genetic variances

	scenario	Variances					Broad sense Heritability		CM-MP Regression	R^2
		additive (A)	epistatic (I)	non-genetic (E)	Phenotypic Means MP	Phenotypic Means CM	MP	CM		
1	A, but no I + E	100	0	0	50	50	1.00	1.00	1.00	1.00
2	A+I, no E	90	10	0	50	45	1.00	1.00	0.90	0.90
3	A + I + E	90	10	10	60	55	0.83	0.82	0.75	0.61
4	A+I + E'	90	10	30	80	75	0.63	0.60	0.34	0.34
assumptions:		*parents are pure lines*								
		total genetic variance:			*100*					

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 x16)/2=136 crosses or 17 x16= 272 backcrosses to both parents can be predicted. To predict cross means and to select among crosses is highly advantageous if variance between crosses is high as has been found in our experiment (Table 9).

6.7. Usefulness of crosses

Applying this criterion for the current study out of 30 crosses 16 crosses were classified as superior (Table 14). Their $U_{(i=1)}$ surpassed the performance of the best registered variety 'Planet' for 4-5 important traits of malt barley. As discussed in chapter 6.3 an accurate estimation of cross-specific σ^2_g 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 (Lehermeier *et al*., 2017; Osthushenrich *et al*.,2018) .

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 with available resources. In line with this, different scholars were reported its applicability in breeding (Allier *et al*., 2019; Bijma *et al.*, 2020; Lehermeier *et al*., 2017; Utz *et al*., 2001).

On the other hand, among the crosses classified as "inferior" there might be some crosses which were useful to maintain genetic variance or to introgress other valuable traits such as tolerance to soil acidity which are not assessed in this experiment. In this case the usefulness criterion can serve as a guideline for the allocation of resources. By generating more RIL in such crosses segregation variance can be exploited and the chances can be enhanced for RIL reaching the performance level for the 5 traits mentioned and contributing by other valuable traits. Most probably this approach is more efficient than to simply create many small families with the same number of progeny as it is often practiced in breeding programs (Osthushenrich *et al*., 2017).

Furthermore, 17 parental lines contributed their gametes to initial populations with highly unbalanced gametic contributions (Fig. 7). Planet as a well-known malting variety contributed more than other lines due to its high malt quality and agronomic traits and hence, selection based on usefulness criteria increases variance of gametic contribution.

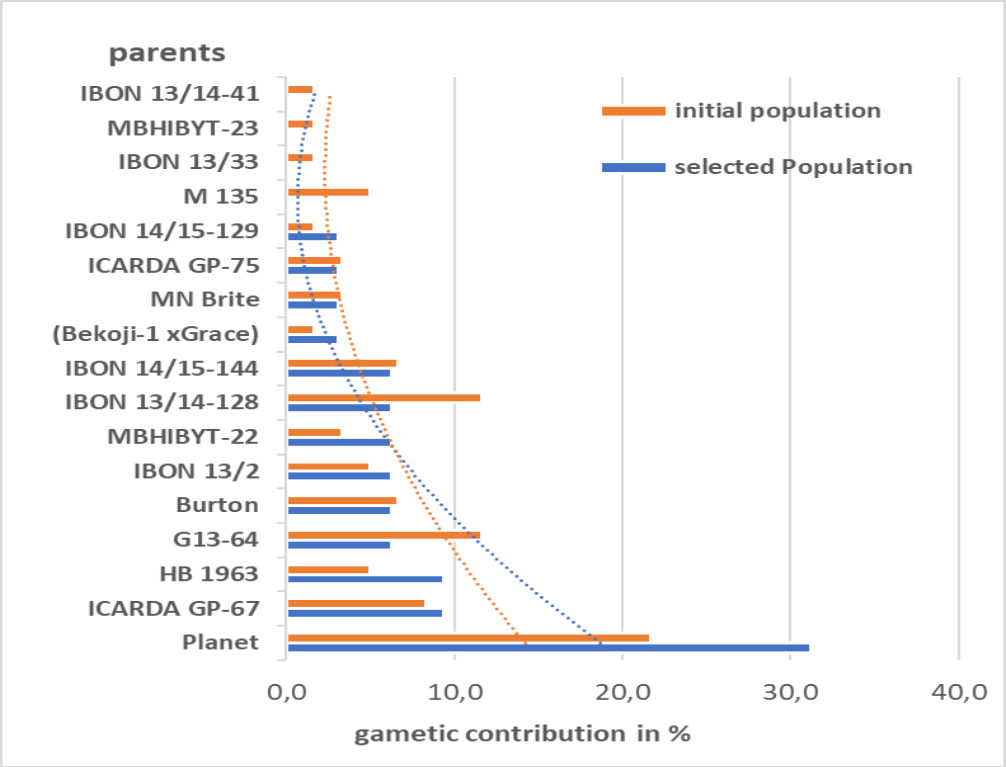

Figure 7. Impact of selection on Usefulness on gametic contribution of parental line

6.8. Homogeneity and Diversity Analysis within and between Parental Lines

6.8.1. Homogeneity Analysis

In our study a marker-based test of the parental lines revealed that homogeneity calculated as the average expected homozygosity across all markers, ranges from 71 (Bekoji-1 x Grace) to 100% (IBON14_15_144, ICARDAGP_75, Planet). The remaining group of lines ranged from 83-98% homogeneity. Heterogeneity, the opposite of homogeneity, within lines is hypothesized to originate from in-complete inbreeding, in-crossing events or technical admixture with another germplasm. In case the last ancestor genotype of a parental line is still heterozygous for a KASP marker we expect p (A) =p (a) =0.5 for the two alleles A and a, correspondingly ExHom= 0.5 in the derived line. Not more than 24 grains per parental line have been genotyped. Due to sampling the observed homogeneity is binomially distributed. It can be shown that in around 94% of the marker analyses observed homogeneity should range between 0.5 and 0.56. The range actually observed could be inflated to some extent by drift effects when developing the final line or when

creating bottlenecks in maintenance breeding. For parental line Bekoji_1xGrace most of the markers analyzed are within this range. So, for this entry incomplete inbreeding is the most likely cause for the low value observed for homogeneity. Hence, this implied that breeder should consider his maintenance breeding or crossing event very carefully. For the rest, no heterozygous individuals had been found in the seed sample of the line. This is expected in a strictly self-pollinating crop such as barley when lines derived from a heterozygous ancestor individual are multiplied over several generations.

In line with this, Chimwemwe *et al.* (2021) reported genetic purity as percent of heterozygous loci among 30 maize inbred lines ranging from 0.0 to 57.6 with an overall mean of 10.43% using 92 SNPs based on KASP genotyping assay. Out of 30 inbred lines they found not more than 18 of them showing 100% genetic homogeneity. According to this author, it indicates the potential discrimination power of KASP markers in purity testing. Similarly, Romdhane *et al.* (2018) used 17SSR marker to test fingerprinting and genetic purity assessment of parents and F1 hybrids in barley. The authors reported that from all tested markers, five of them showed high discrimination power and confirmed the true-to-typeness of hybrid and the parental lines. Moreover, a quality control study conducted on maize inbred lines collected from various sources by Berhanu Tadesse *et al*. (2015) using two marker type showed that homogeneity was varying from 49 to 100% for KASP and 74 to 100% for GBS with overall average homogeneity of 79% and 89% for the two marker respectively.

For the third group in our study, homogeneity estimated between 83-98% can plausibly be explained by admixture of foreign genotypes. Around 2-10 % foreign genotypes (Fig.8) added e.g., by technical mistake or unintended blending could have let to the homogeneity observed in this group. These values have to be considered as a lower limit, because admixture genotypes carrying by chance the same marker allele as the parental line itself cannot be detected. Accordingly, the frequency of undetectable admixture depends on the allele frequency in previous breeding populations about which no conclusive statements can be made here.

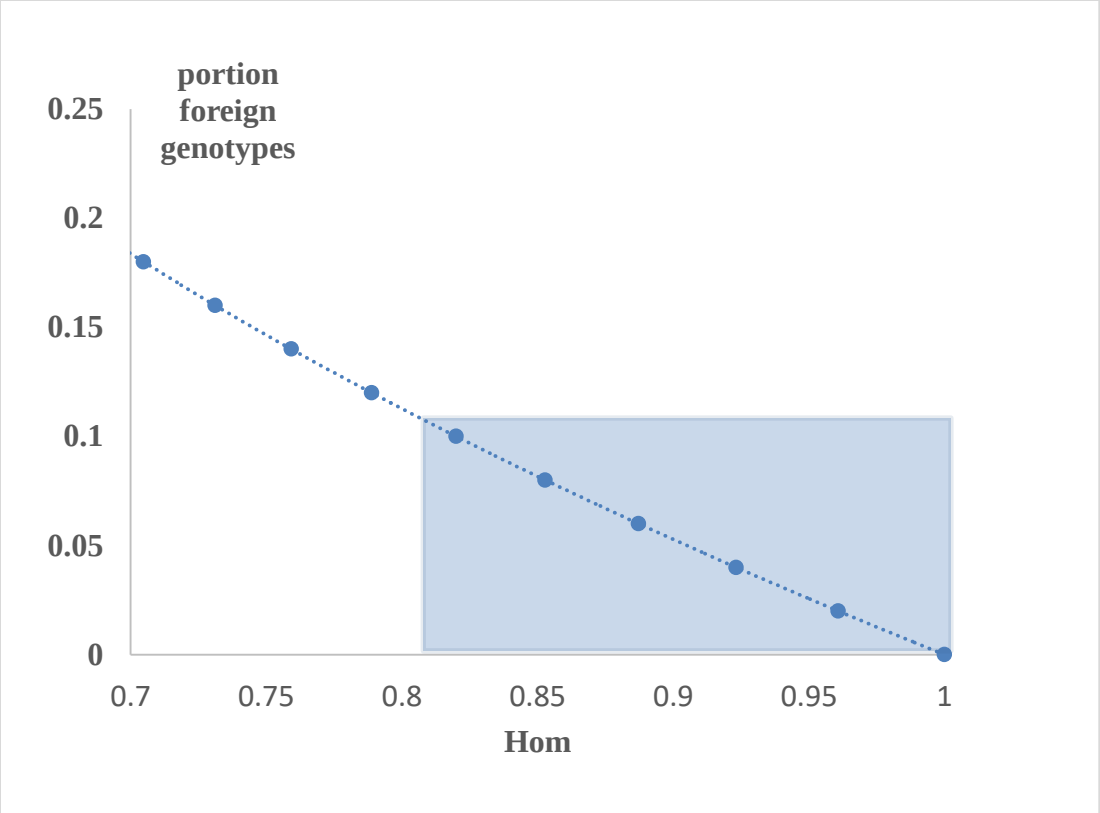

Figure 5. Portion of foreign genotypes explaining the observed average homogeneity in parental line samples

When lines descending from incompletely inbred ancestors are used for crossing with only one plant sampling effects cannot be avoided. If by chance a genotype rather distant from the other line members is chosen for making the crosses covariance between mid-parent value and cross mean will be reduced. In practice breeders often use more than one plant pair to produce a cross due to security reasons and also for having a larger number of F1 seeds available. In this case the sampling effects mentioned above can be regarded as negligible. When admixture genotypes are used for crossing instead of regular line members the covariance between mid-parent and cross mean will drop down dramatically because in this case the RIL population is related to only one of the putative parents but not to the other. Accordingly, the corresponding coefficients for regression and determination will also be reduced. In this case, the breeder forecasting cross mean based on an erroneous mid-parent value will make a rather inaccurate or even a mis-leading prediction.

6.8.2. Diversity Analysis

Genetic diversity in a population can be affected by genetic drift and selection (Condón *et al*., 2008). This can be happened due to increasing in genetic vulnerability, because of the loss some important genes such as disease resistance genes and evolution of plant pathogenic populations hence diminish responses to selection as a consequence of the depletion of genetic diversity. The current study result showed RD between parental lines ranging from 0.32 to 0.64 with an average of 0.46. The dendrogram (Figure 6) clustered the parents into three distinct classes. Importantly, the high malting European lines were grouped with some the ICARDA and Ethiopian lines (Cluster-III with blue color). Similarly, the USA lines were also grouped together with ICARDA lines (cluster -II with green color). Further, the third group contains ICARDA, USA and Ethiopian lines (Cluster -I with red color). Obviously ICARDA lines are related with European, USA and Ethiopian germplasm, which implies that there is gene flow or germplasm exchange between countries or regions. In agreement with this, Sinteyahu Debebe *et al.*(2015), reported higher genetic diversity among barley germplasm from Ethiopia, ICARDA and US in his study of genetic diversity and genome wide association mapping. He reported that the mapping panel were highly structured according to spike row-type, geographical origin and breeding history. On the other hand, Ferreira *et al*.(2016), found higher genetic diversity in Brazilian barley using SSR marker. Similarly, AKbulut *et al*.(2018), also reported higher genetic diversity among 68 Turkish and Azerbaijan barley germplasm using ISSR and RAPD marker.

Another important result of the diversity analysis is that Lines MBHIBYT_22 and IBON13_14_128 were detected to be identical (RD = 0.0). Fortunately, no cross between the seed samples had been made and waste of breeding capacity was avoided. To generalize this outcome a target of a diversity analysis could be to avoid intermating of close relatives. For this purpose, pedigree data could be used alternatively or complementary to RD from anonymous DNA markers. Lines which share more than e.g., two grandparental lines would then not be crossed with each other. A second alternative has been outlined by (Rincent *et al.*, 2018). They used NIRS spectra data to compute relationship matrices between genotypes. Compared to DNA marker analysis, pedigree analysis has the advantage of cost-effectiveness, but the disadvantage

that it cannot capture variation of relationship beyond the classical coefficient of co-ancestry. The NIRS spectra approach is also cost-effective because NIRS data are collected anyhow for other purposes and it should also be able to assess relationship beyond the coefficient of co-ancestry. Further research is needed to compare the three options and depending on results, resources and breeding context the breeder might use one or the other.

6.8.3. Correlation of Segregation Variance (σ^2g) of Traits with Rodger's Distance (RD)

Correlation of segregation variance of traits to RD (Table 17) was found only for DH significant, whereas for all other traits correlations were non-significant. In line with this, (Makumbi *et al*., 2018 and Njeri *et al.*, 2017) found low correlation GD and heterosis in maize inbred lines evaluated across different environments. Similar result was also reported by (Pfunde *et al.*, 2015). Further, a study conducted on maize by Longin *et al.*, (2011) revealed for grain moisture content a low correlation between RD (rp= 0.17) among parents and variance within crosses, whereas grain yield showed moderate correlation of RD (rp =0.55) between the two parameters. Utz *et al*. (2001) obtained similar correlations on wheat for heading date, plant height and kernel weight, but no association for grain yield among parents and within segregation variance.

Often breeders expect high segregation variance for crosses generated from parents with high genetic distance. However, in this study we observed poor correlation between RD and segregation variance. Some outstanding cross was obtained from a cross combination between Planet and MBHIBYT-22 (RD=0.50), and ICARDA GP-67 and HB 1963 (RD = 0.32) (Table 17 and Fig 6). Similarly, Planet when combined with ICARDA GP-67 (RD=0.46) and Bekoji-1 x Grace (RD=0.36), were among the best crosses selected based on usefulness criteria. In this study we found that RD was not strong predictor of segregation variance of trait investigated except DH. In line with this result, Hung *et al.*(2012) reported in maize that genetic distance among parents had no predictive value for progeny variation. Furthermore, Burkhameret *et al.* (1998) reported that the genetic distance computed from coefficient of parentage (COP), STS-PCR primer sets and AFLP primer combination, or combination of them was not a strong predictor of progeny genetic variance or number of transgressive segregates for single traits in

hard red spring wheat, but significant correlation was reported between COP and STS-PCR based genetic distance and overall variance.

To generalize the results from a more theoretical point of view, low correlation of σ^2_g with RD in our study can be due to: (i) linkage disequilibrium between markers and quantitative trait loci (QTL) was low, (ii) the number of markers were limited, (iii) accuracy in estimation of σ^2_g was limited or (iv) heterogeneity of parental lines caused sampling by gametes used for the crosses.

6.9.Time and Cost Demand

Time and cost demand at each breeding cycle influence annual genetic gain. From breeder's equations $G = ir\sigma_g/a$, where i: selection intensity, r: selection accuracy, σ_g: standard deviation of the gain criterion, a: number of years needed for a breeding cycle (Falconer & Mackay, 1996), we understand that genetic gain per cycle increase as the number of years needed for a breeding cycle (denominator) decreases. Hence the time demand in a breeding program when line development duration is long is a major factor to reduce annual gain from selection. In this regard (Heffner *et al.*, 2010) compared gain from using GS and MAS and found that on a per year basis, expected annual gain from GS exceed that of MAS by about three fold for maize and two fold for winter wheat. The authors conclude that if moderate selection accuracies are practiced, GS dramatically improve gain from selection by shortening the breeding cycle. So far Ethiopian barley breeding did not use molecular breeding to shorten cycle length, but could benefit from rapid generation advancement (RGA), by using SSD methods and two seasons/year as recommended by Cobb *et al*. (2019).

In addition, resource demand in barley breeding program is reduced with the use of NIRS to test quality in an early generation. For instance, in this specific study NIRS was used for ME, FR, GPC and BG. These parameters are estimated with time duration of 60 to 90 sec per sample and used to select outperforming RIL in F4:5-L generations and based on their performance in observation rows. Accordingly, only those entries enter the much more costly multi-environmental yield trials (PVT) which have shown to meet minimum quality standards. When consistently applied this means that selection intensity in yield trials can be reserved almost

exclusively to yield improvement. This example shows that assigning selection on a given trait to the appropriate selection unit (observation row vs. yield plot) will increase cost efficiency.

More generally, cost estimation in this study indicates that stringent breeding cost estimation will increase selection efficiency. Since breeding costs influence number of crosses, and selection rates at different stages, strong cost management system is highly advantageous to increase gain from selection. In public breeding program accurate breeding cost estimation is often not practiced, which will have a negative impact on the country's economy. This study cannot provide more than highlight information how public breeding program could practice breeding cost estimation and increase breeding efficiency as well as gain from selection. In Ethiopia, labor cost is cheap and skills of employees is often underestimated compared to developed countries such as USA as described by Heffner *et al. (*2010) for maize and wheat (YPU). The cost of YPU in our case is low (Table 1 and Appendix Table 2) which mean a good opportunity to increase our breeding size.

7. CONCLUSIONS AND RECOMMANDATIONS

7.1. Conclusion and implication to improve malt barley in Ethiopia

Ranking 4th most important crop as food and feeds on the world, barley takes a special place in Ethiopian dishes for centuries as well as important cash-generating crop for millions of small scale households. It has a wide adaptability ranging from 2000-4000 m.a.s.l covering a wide range of agro-ecologies and different growing seasons. The increase in malt barley production in Ethiopia is rather recent and is linked with the booming of the domestic malt and beer industries. Despite its importance, yield performance of barley has remained low in Ethiopia. Hence, alleviating this challenge depends on the effectiveness of the breeding program and its ability to develop outstanding varieties by exploiting the available genetic variation through improvement in breeding parameters.

Generation means

Means of mid-parent values and their derived RIL were estimated across two environments. Differences between generations were small across all crosses. However, deviations between parents and RIL were larger and differing in sign when considering the Planet and NON-Planet crosses separately. This finding speaks in favor of the hypothesis that positive and negative epistatic effects have cancelled each other. In any case epistasis should be considered as a source for deviation from a simple additive genetic model. 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. Discussing epistasis should not distract from the core message arising from the strong similarity of parental and RIL mean; breeders can determine the performance level of the RIL population by intercrossing high-performing parents. In practical breeding therefore RIL with a proven superiority to their parents are preferably to be used as new parents. In turn, untested germplasm with foreign origin should serve as donors for specific traits only.

Genetic Variances

Starting with a simple additive model we expected for the genetical variances of parents, crosses and RIL to be equivalent: $\sigma^2_p/2 = \sigma^2_c = \sigma^2_g$ and to be translatable into additive variance (σ^2_A). A detailed analysis showed that major assumptions made in the model are not met in our experiment. The breeder might use outstanding RIL 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 for a situation close to classical second cycle breeding. Nevertheless, the actual estimates are highly valuable starting points for considerations to improve breeding methodology. Irrespective of their actual ratio, the two variances, σ^2_c and σ^2_g, proved to be significantly deviating from zero for almost all traits. Accordingly, the breeder can exploit both of them for selection.

Correlations among traits

Correlations among traits are important because selection on one trait could have a desirable or undesirable impact on another trait. 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 on-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 12 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 pre-selected in the way described the breeder can focus on selecting the RIL populations on malt traits in F4:5 generation and can realize higher selection intensity for these traits. Concerning selection on malt traits, the breeder should consider rg=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.

Heritabilities

Heritability could be classified as low $H^2 \leq 0.2$, moderate $0.2 \leq H^2 \leq 0.50$, high $H^2 \geq 0.5$) and most of the heritability obtained in this study fall into the moderate and high class. Taking into account 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 often not more than 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 discard an entry which in reality is high performing. Nevertheless, when asking the question of how heritability can be increased two driving forces can be mentioned beside increasing genetic variances: (i) testing intensity can be enhanced by involving more years, locations and replications and (ii) employing trial analytics which reduce 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" (Cobb *et al*. 2019) is hypothesized to be highly effective in terms of optimized use of limited phenotyping capacity. No final conclusions can be presented here to which extent the trial analytics employed in our experiment contributed to an increase of heritability compared to employing less advanced approaches.

Regression CM-MP

Regression of cross mean (CM) on mid-parent value (MPV) was found to be indicative for cross performance prediction. The coefficient of determination (R^2) ranged from 0.27 to 0.70 and was found to be > 0.5 for most traits. This indicates that the mid-parent value was an accurate

predictor of the cross-mean performance. In the discussion chapter we showed regression and determination coefficient depend on how accurately mid-parent values have been estimated.

Usually, phenotypic data from parental lines are known from the preceding breeding cycle. In the case of the breeding scheme applied by the Ethiopian barley team, field and quality data are available from two years (PON and PVT) and for grain yield only data from 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 information (from NIRS spectra, see Rincent *et al*., and (2018)) estimated breeding values will be available. In conclusion by employing all possible data from different sources, breeder will benefit from very accurate MPV estimates in future.

Usefulness of crosses

Crosses can be selected based on usefulness (U) criteria as the interest of breeding is typically both increasing the mean value of 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 Planet using $U_{(i = 1).}$ Accordingly, about 16 crosses (8 crosses outperforming Planet at least by 5 economic traits and 8 crosses at least by 4 traits) were selected for further evaluations. If this could be predicated at an early stage of the breeding cycle, the breeder would have good chance to discard non-useful and low-performing crosses and save breeding capacity. In the discussion (Figure 7) we showed that applying the usefulness criterion leads to a reduced number of lines contributing to the next breeding cycle and to a higher variance of gametic contribution of the remaining parental lines. In the long run this could lead to loss of genetic diversity and genetic gain. To counterbalance this trend the breeder should follow the recommendation of (Osthushenrich *et al.*, 2018) and generate more RIL in crosses with a lower CM 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.

Homogeneity Analysis

In this study similarity and diversity analysis from genetic marker covering 24 samples examined on average 93.7 overall average homogeneities were obtained. However, within parental lines, average homogeneity was ranged from 71 -100%. Assuming that a line could be homogeneous if it surpasses the 95% threshold or 5% deviating as defined by Semegn *et al*. (2012). Hence, only 11 out of 17 parents meet this threshold. So the breeder should work to have a good level of homogeneity within plants in each variety to improve the breeding efficiency and get the target genetic combination from the crossing. Probably, the corresponding average heterogeneity originates from in-complete inbreeding, in-crossing events or technical admixture with other germplasm. Using not fully inbred lines as a parent for intercrossing is supported by the breeder's interest to keep cycle length (Y1, defined as number of years from intercrossing to intercrossing) short. When a line with an outstanding genotypic or breeding value has been identified it should be intercrossed as early as possible and no time should be wasted until complete homozygosity is reached.

If heterogeneity can be traced back to in-crossing events or technical admixture this should be considered as a wake-up call for breeders to adapt technical processes in line development and maintenance breeding. For example, in-crossing or foreign genotypes can be detected with much higher diagnostic power when single plant-progenies instead of single ear-progenies are grown in rows preferably established with a very low seed density. The former progenies comprise much more plants and deviations from line phenotype can be assessed much easier and the respective progenies can be discarded.

Genetic Diversity Analysis

Genetic diversity analysis among the parental lines based on Rogers Distance (RD) showed that the average distance between all parental lines was 0.40 with a range of 0 to 0.64. The cluster dendrogram classified the lines in to three groups (Cluster -I = 3, Cluster-II =5 and Cluster-III = 9). The clustering grouped lines from USA in to the 1^{st} and 2^{nd} group whereas line from Europe

together in to the 3rd group. Acquiring genetic diversity by intercrossing germplasm from foreign countries has the advantage of fast introgressing important QTL as exemplified by Planet as a donor of high malt quality. On the other hand, this approach bears a high risk to lose adaptation to the specific needs of the Ethiopian target environment. For consequence the breeder should intercross preferably lines excelling by a high genetic diversity and with a proven performance in the target environment. Outstanding RIL of the actual experiment will be the parents of choice for building up a high performing and environmentally stable breeding population. In general, the diversity analysis showed that the parental lines used in this crossing were from diverse origins with the majority of them confirmed homogeneous seed sources. The parental genetic diversity analysis using molecular markers can benefit the breeding program in order to have an effective breeding program by selecting diverse parental genotypes with complementary gene action.

Correlation of Segregation Variance (σ²g) of Traits with Rodger's Distance (RD)

In this experiment, correlations among RD and σ^2_g almost for all traits except DH were low and non-significant. This implies that the RD between parental lines is not strong predictor for σ^2g. As long as genomic prediction of segregation variance is not available the breeder is well advised to use the average σ^2_g as estimated in our experiment as a placeholder and as a rule-of-thumb-value. With this approach the breeder could apply the Usefulness criterion to validate the chance of a cross to surpass e.g., the actual leading reference variety for one or several important traits.

Time and Cost Demand

Accurate estimate of time and cost in breeding is found to save resource and increase gain per unit of time. Public plant breeder should create awareness of their breeding costs and use rapid generation advancement (RGA) methods such as SSD in their breeding program to shorten variety release time, hence increase gain from selection.

Overall, genetic variation in a breeding population is the base for crop improvement and is required to achieve genetic gains in a breeding program. This variation can be exploited through the use of germplasm such as land race, exotic lines or elite breeding lines. 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. Further, cross selection based on mid-parent value and usefulness were found parameters to be exploited in practical breeding.

7.2. Recommendations

Based on the result specific to this study, the following recommendations are made for future consideration. In bold letters eventual topics of master thesis are highlighted

- Comparison of generation means (P vs. RIL) and the ratio of genetic variances revealed that for most traits a simple additive genetic model could not be rejected. Nevertheless, when selecting putative parental lines epistasis as a potential bias should be taken into account. Accordingly, breeders should strive to **estimate breeding values** and use them for prediction of cross means.
- In this study sufficient amount of genetic variation was observed among parents, crosses and RIL. Parameters estimated in the experiment can be used to further improve **allocation of breeding resources** such as number of crosses, RIL within crosses and test locations and individual steps in the breeding scheme. Ration of σ^2_c **and** σ^2_g**,** proved to be significantly deviating from zero for almost all traits, hence breeder can **exploit both of them for selection.**
- Correlations among traits suggest investigating opportunities to **select on DH, PH, TKW in Belg and Meher season** based on F2-F4-SP phenotypes. If this can be confirmed selection intensity in F4:5-L generation could mainly be reserved for quality traits.
- Moderate to high heritability was observed in this study. This implied that testing intensity with the use of two locations and a p-rep design generally was appropriate to select on the traits investigated in this study. On the other hand, striking differences of heritabilities achieved in Hol and Bek suggest to improve homogeneity of the test field and to identify chances to increase experimental accuracy. For designing future

experiments, it is recommended to **investigate heritabilities achieved with the actual p-rep design with respective estimates based on a two or three location test with just one replication per location.**

- Mid-parent value (MPV) proved to be useful predictor of cross mean (CM) ranging from ($0.27 < R^2_{(MPV-CM)} < 0.70$) for most traits. In breeding programs MPV can be estimated at early enough with little costs. Hence, it's highly recommended that breeder should use this parameter and improve its breeding efficiency with little cost. Further, **accurate estimation of CM and MPV** is very important as it increases R^2 and helps to employ MPV as a cross prediction parameter to select the best crosses in the future breeding program.
- In our experiment the usefulness parameter for individual crosses detected crosses with a high potential to yield outstanding RIL. **Applying the usefulness parameter for cross selection** means the ratio of initial crosses: exploited crosses should be discussed. In view of the actual breeding capacity the segregation variance of possibly not more than 20-30 crosses can be exploited with sufficient numbers of RIL. Hence, it's promising tool in future.
- Based on KASP markers diversity and similarity of parental lines were analyzed and yielded important lessons learned for breeding steps such as intercrossing or maintenance breeding. Because KASP technology will probably be not available in the next years Ethiopian breeder should search for alternatives. A **critical and innovative revision of e.g., NIRS technology** and of standard operations procedures is highly recommended.
- **Time and cost of breeding cycle** is a powerful parameter that influences the decision making process, hence breeder should seriously consider in breeding program. In individual breeding steps, it should be seriously considered by breeders and its impact on decision making processes, organization of the breeding team and finally the genetic gain should be targeted.

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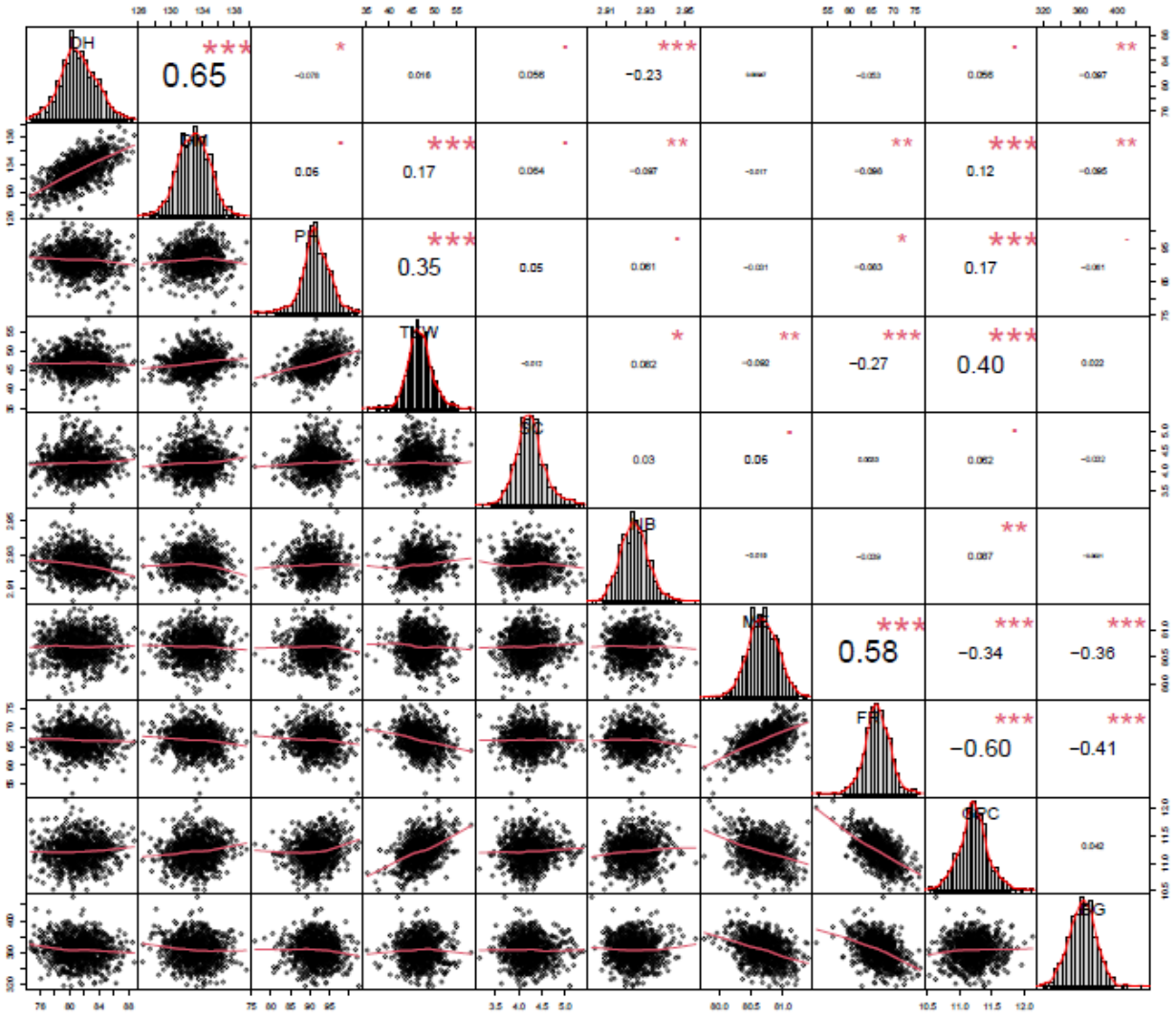
9. APPENDICES

Appendix 1. Trait correlation and their Significance level



Appendix 2. Summary of rough estimate of annual costs for barley breeding program for Y1 and Y2 cycle

cycle	Stage	EU*)	N	P	R	no EU*)	costs (ETB)/EU					total costs (ETB)/stage	derived total YPU
							Research supply	Labor	NIRS	Genotyping	Total		
Y1	crossing	crosses	180	1	1	180	30	200	0		230	41400	207
	F1	population	157	1	1	157	30	200	0		230	36110	180.6
	F2	population	78	1	1	78	30	200	0		230	17940	89.7
	F3	population	156	1	1	156	30	200	0		230	35880	179.4
	F4	population	180	1	1	180	30	200	0		230	41400	207
	F4:5	observation	3000	2	1.5	9000	30	40	10		80	240,000	1200
	F4:5		17	1	1	17	30	40	10	13589.2	13589.2	231016.4	1155.082
	PVT	yield plot	200	4	2	1600	30	100	70		200	40000	200
Y2	NVTI+II	yield plot	50	6	2	600	30	100	70		200	10000	50
	VVT	yield plot	20	6	2	240	30	100	70		200	4000	20
Total												**697746.4**	**3489**
in % of Total		cost type:	Research supply									17	
			Labor									43	
			NIRS									7	
		cost origin	Seed production									25	
			Phenotyping									75	

*) EU= Experimental units N, P, R: number of entries, places and replications, resp.

Appendix 3. Mean of the extra checks of malt barley cultivars for different traits evaluated in two environments

Echecks	**DH**	**DM**	**PH**	**TKW**	**ME**	**FR**	**GPC**	**BG**
Behati	81.4	133.0	92.0	47.0	80.7	66.8	11.2	362.9
EH1847	81.0	132.9	92.0	46.8	80.6	66.4	11.3	361.3
Explorer	81.4	133.4	90.7	46.8	80.8	67.5	11.2	358.4
Fatima	81.4	133.3	91.0	46.7	80.8	67.0	11.2	362.2
Grace	81.4	133.4	90.9	46.9	80.7	66.8	11.2	363.9
HB1533	81.1	133.0	92.1	46.8	80.6	66.3	11.3	361.9
HB1964	81.0	133.1	92.0	47.1	80.7	66.5	11.2	365.4
Henerk	81.4	133.3	90.9	46.8	80.8	67.0	11.2	358.8
Holker	81.3	133.1	92.0	46.8	80.6	65.9	11.2	361.2
IBON-173/4	80.8	133.0	91.6	47.0	80.7	66.1	11.2	370.3
M-21	81.1	133.1	92.0	47.0	80.6	66.2	11.3	363.0
Sabini	80.9	132.9	91.4	46.8	80.7	66.7	11.2	366.4
Traveller	81.4	133.4	91.2	46.9	80.8	66.9	11.2	361.9
Mean	**81.20**	**133.12**	**91.51**	**46.88**	**80.68**	**66.63**	**11.21**	**363.00**
V_p	**0.05**	**0.03**	**0.27**	**0.01**	**0.01**	**0.19**	**0.00**	**9.91**
SD	**0.21**	**0.18**	**0.52**	**0.09**	**0.07**	**0.43**	**0.05**	**3.15**
SE	**0.06**	**0.05**	**0.14**	**0.03**	**0.02**	**0.12**	**0.01**	**0.87**