**Effects of phosphate fertilizer and lime on selected soil chemical properties, yield and grain quality of malt barley (*Hordeum vulgare* L.**) **on acid soils in Southeast Ethiopia**

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### ABSTRACT

*Soil acidity and associated low availability of soil phosphorus are major constraints that limit the productivity and quality of malt barley in the highlands of Ethiopia. A field experiment was conducted at Bekoji and Kofele to determine the effects of lime and phosphorus fertilizer on agronomic and economic performance, the grain quality of malt barley, and selected soil chemical properties. Factorial combinations of four phosphorus fertilizer rates (0, 10, 20 and 30 kg P ha-1) and three lime rates (0, 1.5 and 3 ton ha-1) were laid out in randomized complete block design with three replications. The results revealed significant effects of lime and location interaction on plant height, number of tillers per plant, spike length, grain yield biomass yield, moisture content, thousand grain weight and grain plumpness of malt barley. The effect of the lime interaction by location was significant on the sieve test, thousand grain weight, exchangeable acidity, and CEC. The maximum grain yield (5tha-1) and dry biomass (12.8tha-1) were obtained from application of 30 kgha-1 P and 3tha-1 of lime. Barley yield, plant height, spike length, thousands of grain weight, exchangeable acidity, CEC, and thousands of grain weights were significantly affected by the interaction effects of lime by location. All quality parameters were significantly affected due to the main effect of location. The main effect of P and lime affected plumpness and thousand grain weights. The phosphate applied from zero to 30 kg ha-1 has significantly increased grain yield from 2.85 to 4.38 ton ha-1. Similarly, lime applied at 1.5 and 3 tonha-1 increased soil pH and yield of malt barley yield, suggesting that it has reclaimed soil acidity. Combined applications of 20 kg P ha-1 and 3ton lime ha-1 were found to be economically feasible with the highest net benefit (56340 birr ha-1) and marginal rate of return (316.8%).*

**Keywords:** Grain protein, grain yield, hectoliter weight, soil acidity

1. **INTRODUCTION**

Barley (*Hordeum vulgare* L.) is one of the main food and industrial crops produced in Ethiopia. Barley is the fifth most important cereal as a staple food and an economically important crop in Ethiopia, behind *teff* (*Eragrostis teff*), maize (*Zea mays*), sorghum (*Sorghum bicolor)* and wheat (*Triticum aestivum L.).* (CSA, 2020) in terms of total annual production and annual cultivation area in the country and third after wheat and tef in the southeastern highlands of Ethiopia (CSA, 2020).

There is an increasing demand for malt barley from the growing brewing industries in Ethiopia. However, the country is still unable to produce enough malt barley grains to satisfy the demand of brewers. Currently, only 50% of the demand for malt barley is met domestically (Addisu, 2018; (Agegnehu *et al.*, 2014) despite the huge potential the country has for sufficient malt barley production to satisfy the demands of local brewers and export abroad. The total estimated demand for malt barley in 2015 was approximately 72,000 tons, of which 35% were supplied from local barley farms and the remaining 65% (63,526 tons of malt) imported at a cost of $38 million (ICARDA, 2016). To satisfy the ever-increasing demand for raw materials by the beverage industry and ensure reliable and higher cash returns to farmers, the expansion of malt barley production is very important, as there are immense potential areas available for malt barley production to meet national demand (ICARDA, 2016).

The low productivity and poor quality of malt barley are the main reasons for the apparent shortage of malt grains in the country. The national average productivity of malt barley in the 2018/19 cropping season was estimated at 2.01 ton ha-1 (CSA, 2020) which is far below the world average productivity of 2.4 tons ha-1 (FAOSTAT, 2016 and USDA, 2017).

Soil acidity, P fixation in acid soils, lack of improved varieties and insufficient use of improved crop management technologies, diseases, insect pests, weeds and poor soil fertility are some of the challenges that explain the low barley productivity in Ethiopia (Derebe *et al*., 2018). Poor soil fertility, especially phosphorus deficiency, is one of the main challenges in barley production. Most cultivated land in Ethiopian highlands is characterized by heavy rains and is prone to soil acidity due to the removal of an ample amount of exchangeable cations through leaching, crop mining, and runoff compared to grazing and forest land (IFPRI, 2010, Temesgen, 2014). For example, Tadesse *et al*. (2018) reported that the malt barley yield increased by 70.74% over the untreated control treatment due to the application of P at 40 kg ha-1 in central Ethiopia. Similarly, Temesgen *et al*. (2016) by additions of 10, 20 and 30 kg P ha−1 obtained barley grain yield increase of approximately 29, 55 and 66%, respectively, compared to the control (without P) in central Ethiopia.

Soil acidity is another critical challenge to barley productivity and production in the Ethiopian highlands. Currently, more than 40% of arable land in Ethiopian soils is estimated to be affected by soil acidity and is expanding both in scope and intensity (Abebe, 2007; Woubshet *et al.*, 2017). In strongly acid soils, especially soils with pH values less than 5.5, P is fixed by Fe and Al, which become unavailable to growing crops (Taye *et al*., 1996). Barley is one of the most sensitive crops to soil acidity (Abebe, 2007; Woubshet, 2017). Its growth and productivity are severely affected when grown in soils with a pH lower than 6.3. Currently, most of Ethiopia's highlands are severely affected by soil acidity (pH < 5.5) causing up to 50% loss of barley grain yield (Wassie and Shiferaw, 2009).

Several agricultural practices have been recommended to overcome the problem of infertility of tropical acid soils around the world. Among them, the most common and widely used method is liming, which is defined as the application of ground calcium and/or magnesium carbonates, hydroxides and oxides aimed at increasing the soil pH, modifying its physical, chemical and biological properties (Temesgen *et al.* 2016). The application of lime to acid soils will neutralize acidity and increase soil pH to the desired levels, thus avoiding direct toxicity of roots of crops or plants, increasing the availability of nutrients, particularly P, and stimulating and increasing microbial activity. Therefore, P deficiencies and Al3+ toxicities often occur simultaneously in many acid soils and are believed to be responsible for poor crop yields in acid soils. However, since fixed P would be released for plant uptake after liming, the amount of additional P added must be determined experimentally (Waigwa *et al*., 2003).

Therefore, Al3+ toxicity and P deficiency are the two main factors that limit barley production in acid soils and are partly responsible for seasonal malt barley shortages in the highlands of Ethiopia. Therefore, liming and P fertilization appear to be the most important operations required to increase malt barley productivity in the highlands of Ethiopia. Therefore, it is important to determine and recommend the optimal lime and P rates that should be incorporated into specific acid soils to raise their pH to the required level and improve the grain yield and grain quality of malt barley.

1. **MATERIALS AND METHODS**
	1. **Description of the Study Areas**

The experiment was carried out during the 2017/18 cropping season at the Bekoji and Kofele research substations of the Kulumsa Agricultural Research Center in southeastern Ethiopia. Geographically, the Bekoji and Kofele sub-stations are located at 7° 32' 24'' N latitude and 39o 13' 15'' E longitude; and 7o18'43" N latitude and 39o13'21"E longitude, respectively, at an attitude of 2780 and 2360 meters above sea level (Berhane *et al.,* 1996). The average monthly mean minimum and maximum temperatures in Bekoji during the growing season were 15 and 20 ° C, respectively, with a total annual precipitation of 1026 mm, while Kofele had monthly mean minimum and maximum temperatures of 6.7 and 22.7 ° C, respectively, with a total annual precipitation of 1212 mm.

The Bekoji soil was classified as a mixture of *nitisols* and *vertisols, while* that of Kofele was classified as *eutric nitosols* (Berhane *et al.,* 1996). Pre-plant soil samples were analyzed from both locations, and the results along with the corresponding ratings and references are summarized in **Table 1**. Consequently, the soils of both locations are in the strongly acidic category, low in their available phosphorus, in soil organic carbon, and in total nitrogen content with medium CEC.

#### Table 1.Some of the physicochemical properties of experimental soils before sowing

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Bekoji |  | Kofele | References |
| Soil properties | Value  | Rating  |  | Value  | Rating  |  |
| Textural composition (%) |  | - |  |  |  |  |
| Sand | 10.0 | - |  | 16.0 |  |  |
| Silt | 53.0 | - |  | 50.0 |  |  |
| Clay | 37.0 | - |  | 34.0 |  |  |
| Textural class | SL\* | - |  | SL\*  |  |  |
| pH(1:2.5H2O) | 5.17 |  SA† |  | 4.98 | SA† | Tekalign *et al* (1991) |
| Organic carbon (%) | 1.30 | Low |  | 1.52 | Low | Havlin *et al*. (1999) |
| Total N (%) | 0.08 | low |  | 0.11 | low | Tekalign *et al.* (1991) |
| Available P (mg kg-1) | 6.70 | Low |  | 7.10 | Low | Tekalign *et al.* (1991) |
| CEC (cmol(+)/kg) | 20.15 | Medium |  | 18.05 | Medium | Landon (1991) |

*SA = strongly acidic; SL\* = silt loam*

* 1. **Determination of Lime Requirements (LR) of Experimental Soils**

According to the report by Taye *et al*. (2002), the soil liming requirement has been adopted for Ethiopian soils based on the analysis results of exchangeable acid and effective CEC to be equivalent in the following formula. LR = Where LR = lime requirement in kg ha-1, Exch. A is the exchangeable acidity in Cmol (+) kg-1, BD is bulk density in kg m-3.

 The quantity of exchangeable acidity used in these calculations of lime requirements of the soils of the study sites is shown in **Table 1**. Consequently, the lime requirements of both sites were found to be similar which 3.0 t ha-1.

* 1. **Treatments and Experimental Design**

A factorial experiment consisting of four rates of P fertilizer (0, 10, 20 and 30 kg P ha-1) and three rate of lime (0.0, 1.5 and 3 tha-1) was laid out in randomized complete block design with three replications. Triple superphosphate (20% P) and high quality limestone (98% CaCO3, 99.5% <250 µm in diameter) were used as a source of P and liming material, respectively. Gross plot size of 3m × 4 m (12 m2) with net plot size of 2.5m×3.5m (8.75m2) was used in this experiment with a spacing of 0.5 m between the plots and 1 m between the blocks.

* 1. **Experimental Procedure and Field Management**

At both locations, the total area of land required for the experiment was marked and ploughed by oxen as required frequencies. The final preparation of the land for lime incorporation and malt barley seed sowing was then carried out manually with a hand rake. Following the preparation of the terrain, the experimental field was laid out, and the plots for each treatment were marked according to the design. Then lime was incorporated into the plots according to treatment at a depth of 20 cm, one month before the sown malt barley to give enough time for the lime to react and neutralize the acids in the soils at both locations.

The malt barley variety, *Bekoji-1,* which was released for production around the study areas, was seeded by hand drilling in each plot in rows 20 cm apart at a seed rate of 75 kg ha-1. The entire dose of phosphorus fertilizer was applied at sowing according to the treatment. Half of the recommended dose of nitrogen fertilizer (23 kg N ha-1) in the form of urea (46 % N) was applied uniformly in all plots at planting and the remaining half (23 kg N ha-1) was applied as a side dressing in all plots uniformly 45 days after sowing. All agronomic management of the experiment, such as the management of weeding, pests, and disease of the crop, was done according to the recommendation for barley production (EIAR, 2007).

* 1. **Soil Sample and Analyses**

After harvesting the crop, composite soil samples were collected from each plot using an auger to a depth of 0-20cm and brought to the Kulumsa soil laboratory where the samples were processed and analyzed for selected chemical properties, namely pH, exchangeable acidity, available P (av.P), CEC, and exchangeable cations (Ca, K, Na and Mg). The soil pH was measured in 1: 2.5 soils in a water suspension using a pH meter and Taye (1993). Exchangeable acidity was determined following the procedures described by Jones (2001), available P was determined using the Olson method as described by Jones (2001), and CEC and exchangeable cations were determined using the 1.0 M NH4OAc method.

* 1. **Crop Data Collected**
		1. **Agronomic parameters**

Phenological data was collected from the net plot area of ten randomly selected plants. The height of the plant, the number of tillers per plant, the length of the spike, and the number of kernels per spike were recorded from each plot. The height of the plant was recorded from ground level to the top of the spike, excluding awns.

After collecting these data, the crop was harvested and the above-ground biomass yield (AGBY) of the test crop (malt barley) in each plot was dried and weighed; and recorded. The AGBY harvested from each plot was then threshed and the grains were separated from the straw, followed by weighing the grain (GY) and the straw (SY). The seeds were weighed and the moisture content was determined in the laboratory after adjusting the moisture to a level of 12.5 %. The harvest index was calculated as the percentage ratio of grain yield to total dry biomass above ground yield. The weight of a thousand kernels weight was determined using the seed counter and weighting a 1000 seed sample taken from each plot.

The total and effective number of tillers was determined at physiological maturity of the crop by counting two rows of 0.5 m long from the net plot randomly selected and converted to m2. The number of kernels per spike was determined by counting from randomly taken 10 spikes per plot at maturity and averaged to per spike. The weight of 1000 kernels (g) was determined by counting 1000 kernels sampled from each net plot using an electronic seed counter from the net plot and weighed using a sensitive balance, and then the weight was adjusted to 12.5% moisture content. The aboveground biomass (kg ha-1) was determined by weighing the aboveground biomass per net plot area after drying in the sun for five days. The grain yield (kg ha-1) was determined after threshing the sundried above-ground biomass was threshed by weighing using a sensitive balance and the grain yield was adjusted to a moisture content of 12.5%.

* + 1. **Grain Quality Parameters**

Protein content analysis was performed in the Asella Malt Factory laboratory. The weights of thousands of kernels (g) were measured by taking the mass of the thousand kernels counted using a sensitive electronic balance. Hectoliter weight (kg hl-1) was determined on dockage free samples using a standard laboratory hectoliter weight apparatus (grain analysis computer (GAC) as described in the AACC (2000) method. The germination capacity (%) was determined by soaking 200 seeds in 0.3 M H2O and counting the germinated seeds after 48 hours and finally changed to %. Germination energy is the percentage of seeds germinated in 48. The grain protein content (%) and the moisture content (%) were measured using the apparatus (grain analysis computer (GAC) 2100) (processing protein analysis computer) as described in the AACC (2000) method. The sieve test (%) was measured by placing 100 grams of the grain sample at the top of the sieve (>2.8mm,>2.5mm, >2.2mm, and <2.2mm sieve sizes) and the grain was sieved into four fractions in 5 minutes. The four fractions were weighted at each sieve size. The grains were graded by size using slotted sieves (2.8, 2.5 and 2.2mm apertures) following the standard procedure of the Asella Malt Factory laboratory.

* 1. **Statistical data analysis**

Agronomic, grain quality, and post-harvest soil data were subjected to analysis of variance (GLM procedure) using SAS software program version 9.4 (SAS, 2015). The homogeneity of the variances was evaluated using the F-test as described by Gomez and Gomez (1984), and since the F-test showed homogeneity of the variances of the two locations for most of the agronomic parameters, a combined analysis was used for the two locations. Fisher's protected least significant difference (LSD) test at the probability level of 0.05 was used to separate treatment means where there were significant treatment differences.

* 1. **Economic Analysis of Treatments' Effects**

A partial budget analysis was performed to determine the economic feasibility of phosphorus fertilizer and lime for barley production around study areas following procedures described in CIMMYT (1988). The mean grain yield data of barley was used for the economic analysis at the two sites. Furthermore, the grain yields obtained from each treatment were adjusted by 10% to narrow the possible yield gap that may occur due to differences in field management by the researcher and the famers. This is because, generally, researcher-managed fields give higher yield than famer-managed fields.

The average prices of the relevant inputs required to perform the partial budget analyzes were collected from different sources. Consequently, the price of the triple superphosphate fertilizer that was used in this experiment was collected from the agricultural input supply corporation. Consequently, the TSP was 24.00 Ethiopian birr (ETB) kg-1 atthe time of sowing of the crop. The market price of malt barley grain determined by the brewers in the 2017 season was 15.00 ETB kg-1. Furthermore, the market price of lime at the time of sowing of the crop was 1.25 ETB kg-1.

Based on the information above, the gross benefit due to grain yield (GBTGY) was calculated by multiplying the average adjusted grain yield produced by each treatment by the field price of barley grain that farmers receive in the market. The price of the straw was also included in the benefit of the experiment. Total variable costs (TVC), which refer to the sum of all costs that vary for a particular treatment, were calculated for individual treatment based on the amount of fertilizer and lime applied, as well as the labor cost incurred to apply lime and phosphorus. The net benefit (NB) of each treatment was calculated by subtracting the total variable cost of each treatment from the gross benefit (GB) of the same treatment (GB- TVC). The marginal rate of return (MRR) was calculated as the ratio of differences between the net benefits of successive treatments and the difference between the total variable costs of successive treatments.

1. **RESULTS AND DISCUSSION**
	1. **Effects of P and Lime on Selected Soil Chemical Properties After Harvesting**

The main effects of P, lime land location on soil chemical properties after harvest analyzes data of selected soil properties are summarized in **Table 2**. The P fertilizer has significantly improved the avP content of the soil and the amount of P available in the soil has increased significantly with increasing P application rates. Such increases in soil avP after harvest are expected, since the growing crop does not use the entire dose of applied P in one season and the amount of avP remaining in the soil after harvest is proportional to the amount of P applied externally as fertilizer. This result is in agreement with the finding of Temsgen *et al*. (2016) who studied the effects of lime on strongly acid soil (pH<5.0) in central Ethiopia on selected soil physicochemical properties (exchangeable aluminum [from 1.32 Cmol kg−1 to 0.12 Cmol kg−1] and soil pH). But the P fertilizer did not significantly affect the other chemical properties of the soil considered.

However, lime has significantly affected all the chemical properties of the soil analyzed after harvest **(Table 2).** Especially, lime applied at full rate (3 tha-1) significantly increased soil pH followed by 1.5tha-1treatment and it remained unchanged in un-limed control (0tha-1) treatment plot. There was a corresponding significant decrease in the exchangeable acidity content of lime-treated soils. Consistent with this result, Temsgen *et al.* (2016) found that lime applied at rates of 0.55, 1.1, 1.65 and 2.2 t ha−1 increased the pH by 0.48, 0.71, 0.85 and 1.1 units, respectively. These authors also reported that these rates of lime decreased Al3+ by 0.88, 1.11, 1.20 and 1.19 mill equivalents per 100 g of soil, respectively. Furthermore, the application of lime has significantly increased the content of CEC and exchangeable cations of the treated soils compared to the untreated (control) treatment and greater increases in the content of these parameters were recorded with 3tha-1 than 1.5tha-1 **(Table 2).**

**Table 2: Main Effects of P, Lime, and Location on selected soil chemical properties after harvest of malt barley**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **pH** | **Exch. Acidity (Cmol(+)/kg)** | **AvP (ppm)** | **CEC** | **Exh.Ca** | **Exch. K** | **Exch. Mg** | **Exch. Na** |
| **Cmol(+)/kg** |
| **Phosphorus (P)-rates (kg ha-1)** |  |  |  |  |  |
| 0 | 5.67 | 0.17 | 6.34d | 25.68 | 13.61 | 2.44 | 8.56 | 1.5 |
| 10 | 5.73 | 0.15 | 7.81c | 25.59 | 14.07 | 2.46 | 8.91 | 1.4 |
| 20 | 5.75 | 0.16 | 8.99b | 25.32 | 14.01 | 2.45 | 8.49 | 1.2 |
| 30 | 5.78 | 0.16 | 10.14a | 26.01 | 14.23 | 2.45 | 8.61 | 1.3 |
| **LSD (0.05)** | **Ns** | **Ns** | **0.85** | **ns** | **Ns** | **ns** | **ns** | **Ns** |
| **Lime (L) rates (t ha-1)** |  |  |  |  |  |
| 0.0 | 5.34c | 0.27a | 7.35c | 23.63c | 12.14c | 2.04c | 8.20b | 1.21b |
| 1.5 | 5.72b | 0.15b | 8.41b | 25.87b | 14.38b | 2.51b | 8.83a | 1.37ab |
| 3.0 | 6.15a | 0.01c | 9.20a | 27.48a | 14.04a | 2.82a | 8.91a | 1.47a |
| **LSD (0.05)** | **0.11** | **0.057** | **0.74** | **0.73** | **0.47** | **0.20** | **0.59** | **0.17** |
| **Locations (Loc)** |  |  |  |  |  |
| Bekoji | 5.71b | 0.22a | 7.91b | 23.78b | 13.68b | 2.34b | 8.38b | 1.35 |
| Kofele | 5.92a | 0.11b | 8.72a | 27.54a | 14.26a | 2.56a | 8.91a | 1.35 |
| **LSD (0.05)** | **0.093** | **0.046** | **0.61** | **0.59** | **0.39** | **0.16** | **0.48** | **Ns** |
| **CV (%)** | **3.5** | **6.0** | **10.5** | **4.9** | **5.8** | **14.0** | **11.7** | **12.0** |

Means within column followed by the same letter (s) are not statistically different from each other, Ns = statistically nonsignificant

* 1. **The effects of P and lime on the yield component and the yield of malt barley**

The interactions, as well as the main effects of P, lime, and location, significantly (p<0.05) affected the yield and the components of the malt barley yield (**Table 3 and 4**). Plant height (88.6-103.5cm), number of tillers per plant (4.4-6.1), spike length (8.6-9.3cm), number of seeds per spike (27.3-34.7), grain yield (2.85-4.38tha-1) and aboveground biomass yield (7.04-11.01 tha-1) were significantly increased with increasing rates of P (0-30 kgha-1) and lime (0 to 3 tha-1) at both locations, respectively. The highest values of these parameters were obtained at 30kg P ha-1 and the lowest value was obtained from the control treatments. The present result is in line with the finding of Tadesse *et* *al*. (2018) who reported that the application of P has significantly increased the height of the malt barley plant in the Welmera district in central Ethiopia. As far as location by P rate and lime interactions are concerned, the maximum number of tillers per plant (103.4 cm), spike length (9.3cm) and grain yield (4.38tha-1) were produced in Kofele under the P rate of 30 kg ha-1, while the lowest values were recorded from the control treatment in both locations, respectively (**Table 3 and 4**).

Similarly, lime application has significantly increased malt barley growth and yield components, and the highest parameters values of the parameters were obtained with 3tha-1 followed by 1.5tha-1and the least values of plant height, number of tillers per plant, spike length, number of seeds per spike, grain yield and aboveground biomass yield were obtained in plots that were not treated with lime (0 tha-1). The apparent significant improvement in the growth and production components of malt could be due to a reduction in the direct adverse effects of soil acidity (H+ and/or Al3+) and increased availability of nutrients in the lime-treated plots compared to the control. This can be proven by the results of selected soil physicochemical properties after harvest shown in Table 2 where soil pH increased significantly in lime-treated soils compared to control.

The location had also significantly affected malt barley growth and yield components, and therefore significantly higher values of plant height, number of tillers per plant, spike length, grain yield, and aboveground biomass yield were obtained in Kofele than in Bekoji (**Table 3 and 4).** These differences could be attributed to variations in soil fertility and weather variables that can be observed between these locations. For example, the soil of the Kofele location had higher OC, TN, and avP contents than those of Bekoji. Moreover, the location of Kofele is warmer than that of Bekoji, which favors better growth and development of the crop (Berhane *et al.,* 1996).

**Table 3: Main effects of phosphorus and lime application on the growth and yield components of malt barley grown in the Bekoji and Kofele districts**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | **PLH (cm)** | NTPP | **SL (cm)** | **NSPS** |
| **Phosphorus rates (kg ha-1)** |
| 0 | 88.6c† | 4.4d | 8.6b | 27.3c |
| 10 | 93.9b | 4.9c | 8.9a | 30.4c |
| 20 | 103.4a | 5.6a | 9.2a | 33.4b |
| 30 | 103.5a | 6.1a | 9.3a | 34.7a |
| **LSD (0.05)** | **1.38** | **0.19** | **0.31** | **2.28** |
| **Lime rates (t ha-1)**  |
| 0 | 95.3c | 4.9c | 8.8b | 29.7b |
| 1.5 | 97.3b | 5.3b | 8.9b | 31.9a |
| 3.0 | **99.5a** | 5.6a | 9.2a | 32.8a |
| **LSD (0.05)** | **1.19** | **0.16** | **0.26** | **1.97** |
| **Locations**  |
| Bekoji | 95.7b | 4.9a | 8.7b | 31.0 |
| Kofele | 99.0a | 5.7b | 9.2a | 31.9 |
| **LSD (0.05)** | **0.97** | **0.13** | **0.21** | **Ns** |
| **CV (%)** | **7.8** | **8.9** | **5.6** | **10.6** |

*Ns = statistically nonsignificant. The means within a column followed by the same letter (s) are not statistically different from each other.*

The grain yield and the aboveground biomass yield of malt barley were significantly affected by the main effects of P, lime, and location (**Table 4**). The highest GY (4.38 tha-1) and AGBY (11.01 tha-1) were obtained with 30kg Pha-1followed by 20kg P ha-1 and 10kg P ha-1 in that order and the least GY (2.85 tha-1) and BMY (7.04 tha-1) was recorded in the control treatment. These results show that there are insufficient amounts of P (avP) available for optimal malt barley production. This can be further verified by insufficient or below critical soil test values of avP obtained by pre-planting soil sample analyzes of results of both locations. These results are also in line with reports by several researchers that application of P fertilizer significantly increased the yields of several crops, including barley in Ethiopia as P is the second most limiting nutrient in arable land soils of the country (Adamu, 2018; Birhan *et al*., 2016; Wakene *et al*., 2014; Getachew, 2001; Tekalign and Haque, 1991). For example, Adamu (2018) found that fertilizer P applied at 20, 30 and 40 kgha-1 increased GY of malt barley by 52.53, 69.31 and 70.74%, respectively, compared to untreated control treatment. According to the same authors, the corresponding increase in AGBY was 17.7, 24.8 and 21.2% over the control. In a similar study, Wakene *et al*. (2014) reported that the application of P at 10, 20 and 30 kgha-1 has significantly increased AGBY of barely grown in Bore district of southern Ethiopia by 28.7, 49.6 and 60%, respectively, over untreated control treatment. There were also significant differences between lime treatments with respect to their main effects on grain yield and biomass above ground of malt barley (**Table 4).** Consequently, 3tha-1 produced significantly the highest GY (4.18 tha-1) and AGBY (10.63 tha-1) were produced by 3tha-1 and the lowest GY (3.21 tha-1) and AGBY (8.08 tha-1) were obtained in the untreated control plot. This is in line with the findings of several researchers who reported that the application of lime in acid soils significantly and significantly improves barley productivity in the highlands of Ethiopia (Achalu *et al*., 2012; Taye *et al*., 1993; Wassie and Shifraw, 2009). It is also a known fact in the literature that since barley is one of the most sensitive crops to soil acidity, it responds dramatically to lime application when grown in strongly acid soils (Temesgen *et al.,* 2016). Therefore, what differs is that the amount of lime required to be applied for optimum barley production could vary from soil to soil, depending on several factors. For example, Achalu *et al*. (2012) found the highest GY of barley with lime applied at 8 ton ha-1 in east Wolega Zone of western Ethiopia. On the other hand, Tadesse *et al*. (2018) reported the highest GY of barley at lime rate of 6 ton ha-1 in central Ethiopia.

**Table 4: Main effects of the applications of phosphorus and lime GY and AGBY of malt barley grown in the locations Bekoji and Kofele in central and southern Ethiopia.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment** | **GY** | **AGBY** | **HI** |
| **mtha-1** |  |
| **Phosphorus (kgha-1P)-Levels** |
| 0 | 2.85d† | 7.04d | 0.41 |
| 10 | 3.40c | 8.67c | 0.40 |
| 20 | 4.07b | 10.11b | 0.41 |
| 30 | 4.38a | 11.01a | 0.40 |
| **LSD (0.05)** | **1.22** | **5.3** | **ns** |
| **Lime rate (tha-1L)** |
| 0.0 | 3.21c | 8.08c | 0.41 |
| 1.5 | 3.61b | 8.91b | 0.40 |
| 3.0 | 4.18a | 10.63a | 0.39 |
| **LSD (0.05)** | **0.11** | **0.46** | **Ns** |
| **Locations (Loc)** |
| Bekoji | 3.19b | 7.85b | 0.41 |
| Kofele | 4.16a | 10.56a | 0.41 |
| **LSD (0.05)** | **0.09** | **0.37** | **Ns** |
| **CV (%)** | **9.6** | **8.5** | **11.3** |

*Ns = not statistically non-significant; †Means within the column followed by the same letter (s) are not statistically different between each other.*

Similarly, there was a significant difference between the locations in their effects on GY and AGBY of malt barley. Consequently, higher GY (4.16tha-1) and AGBY (10.56 tha-1) were produced in Kofele than in Bekoji **(Table 4**).

In addition to the significant main effects of the treatment factors, P due to the effect of lime interaction was significant (p<0.05) on GY and AGBY of malt barley (**Figure 1 and Figure 2**), which is due to the availability of P, which will increase due to lime application. Consequently, the increasing effect of GY of P was significantly increased when it was applied with lime compared to that without lime and vice versa, indicating a positive or synergistic interaction of P and lime on the GY of malt barley. The highest GY (5 tha-1) was obtained with treatment involving 30kg P ha-1with 3.0 t ha-1 lime which was statistically on par with that obtained with 20kg P ha-1 with 1.5t ha-1 lime indicating that further increase in the application of P or lime or both will no longer significantly increase the GY of malt barley. The current results agree with the findings of several authors (Tadesse *et al*., 2018; Temesgen *et al*., 2016; Taye *et al*., 1993).

* 1. **The effects of P and lime on thousand-kennels weight**

The weight of 1,000 kernels (TKW) of malt was barely significantly (p<0.05) affected only by the main effect of location **(Table 5).** Significantly higher TKW (44.4g) was recorded when the crop was grown at Bekoji than at Kofele (36.5 g) (Table 5). According to the Ethiopian quality standard, the acceptable grain size (TKW) for raw barley is in the range of 25 to 35 g (EQSA, 2006). The results of the present experiment exhibited an acceptable thousand-kernel weight at all P and lime rates. Based on this result, Biadge (2016) obtained significant differences (p<0.05) between the TKW locations where Bekoji produced a greater TKW (51.6 g) than Ankober (48.0 g) and Ankober revealed a greater TKW than Holeta (44.17 g). Shiferaw and Anteneh (2014) also reported that the interaction of P and lime in TKW had a significant effect on thousand grain weight. This is in line with the result obtained by Temesgen (2014) that all lime treatments had higher mean TKW values than without lime.



*\*Bars followed by the same letter (s) are not statistically different from each other at the probability level of 0.05, L0=0tha-1 lime, L1=1 tha-1 lime.5, L2=3 tha-1 lime, P0=0kg P ha-1, P1=10 P ha-1, P2=20 P ha-1, P3=30 P ha-1*

**Figure 1: Effect of phosphorus by lime interaction on the grain yield of malt barley in the Bekoji and Kofele locations**

*\*Bars followed by the same letter (s) are not statistically different from each other at the probability level of 0.05, L0=0tha-1 lime, L1=1 tha-1 lime.5, L2=3 tha-1 lime, P0=0kg P ha-1, P1=10 P ha-1, P2=20 P ha-1, P3=30 P ha-1*

**Figure 2: The phosphate by lime interaction affects the above-ground biomass yield of malt barley over Bekoji and Kofele.**

Lime (L) due to location interaction effects was also significant (p<0.05) on GY and AGBY (**Figure 3 and Figure 4).** The application of lime (3tha-1) has significantly increased GY and AGBY at both locations relative to the unlimed control treatment (**Figure 3 and Figure 4**). However, a significantly higher (4.49 tha-1) GY of malt barley was obtained in Kofele than in the Bekoji location.

*\*Bars followed by the same letter (s) are not statistically different from each other at the probability level of 0.05.*

**Figure 3. Effect of lime interaction and location on the grain yield (t ha-1) of malt barley**

*\*Bars followed by the same letter (s) are not statistically different from each other at 0.05 probability level; L0=0tha-1 lime, L1=1 tha-1 lime.5, L2=3 tha-1 lime,*

**Figure 4: Effect of the lime-location interaction on the aboveground biomass yield (t ha-1) of malt barley.**

* 1. **Grain quality parameters**
		1. **Grain size (sieve size)**

The grain sizes (>2.8mm) and (>2.5mm) of malt were barely significantly (p< 0.05) affected by the interaction of lime rate and location (Table 5). The highest sieve size (>2.8mm [74.2%]) was recorded at Bekoji and the lowest (28.9%) at Kofele when the crop was grown at the rate of 3.0 t ha-1 of lime application (**Table 5).** The size (>2.2mm) of the malt was barely significantly (*P*<0.05) affected only by the main effect of location (Table 5). The size (>2.2mm) significantly higher (20.3%) when the crop was grown in Kofele than in Bekoji (6.3%).

The malt sieve test (<2.2mm) of malt was barely significantly (p< 0.05) affected by the interaction of P rate and location (p<0.05) (Table 5). The highest grain size (<2.2mm) (6.5%) was recorded when the crop was grown at the rate of 20kg P ha-1 at Kofele and the lowest grain size (<2.2mm) (1.5%) was obtained when the crop was grown at the rate of 20kg P ha-1 at Bekoji (Table 5). The highest sieve size, which is statistically far from the highest sieve size (72.0%) was recorded at the Bekoji study site, while the lowest sieve size (31.9%) was recorded at the Kofele study site. According to ESA (2001) and EQSA (2006), percent kernel sample retained on (2.8 mm +2.5 mm) screen sizes should be greater than 65 to 75% while the kernel sample which pass through sieve size <2.2 mm should not be more than 4 to 6%. The current result indicated that the sieve size was in the acceptable range of sieve size. In this study, grain size meets the standard requirement of the Industry according to Ethiopian Barely Business case presentation (EBC) and Ethiopian malt factory (Fox *et al*., 2006) demonstrated fertilizer and environmental effects in improving grain size. Industry standards for large grains are based on the total percentage of grain > 2.5mm (Kefale *et al*., 2016).The present result was in line with the study by Abdisa (2020) and Abebe Assefa (2018), who observed a significant difference between fertilizer and location in the size of the grain sieve.

The grain size is an important parameter of malting quality, because small grains contain less carbohydrate and lead to a low level of malt extract (Zhao *et al*., 2006). Turbidity values were found to be higher on the order of 0-2.5mm >2.5-2.8>2.8mm. The grain sizes that pass through the sieve size of 0-2.5mm were found to be too hazy due to the presence of chaff and the outer hull.

These results are in agreement with the result obtained by Biadge (2016) that the Bekoji varieties had a higher mean grain size than the Ankober varieties and in Ankober had a higher mean grain size of malt barley than Holeta (94.07, 92.87, and 72.62%; respectively) but the requirement in the brewing industry is >80 % must be above the size of the sieve of 2.8 and 2.5mm according to the Ethiopian standard requirement for malt purpose and the current result falls into this category.

* + 1. **Moisture content (MC).**

The main effects of location and P rate were significant (p<0.05) in malt MC. Significantly higher MC (10.5%) was recorded when the crop was grown in Bekoji than in Kofele (10.1 %) **(Table 6).** Similarly, Biadge (2016) reported significant differences (p<0.05) between locations for grain moisture content. The moisture content of malt barley grain was higher in Bekoji than in Holeta and Ankober (13.83, 11.44% and 8.36%, respectively) (Biadge 2016). Moisture levels must be low enough to inactivate enzymes involved in seed germination, as well as to prevent heat damage and the growth of disease microorganisms. According to Fox *et al*. (2003), the maximum reasonable industrial specification of the malt barley moisture content for safe storage is 12.5%, whereas the European Brewery Convention (EBC) standard accepts a moisture content of 12 -13%. In this study, the moisture content was below these standards and in the acceptable range of Asella Malt Factory is 10.5-13.5%.

 Phosphorus at the rate of 0kg ha-1 gave significantly the highest (10.7 %) moisture content (10.7%) and the lowest moisture content (10.1%) was recorded with 30kg phosphorus ha-1, which was statistically at par with 10 and 20 kg phosphorus ha-1 (Table 6). The moisture content of barley malt is one of the characteristics, particularly in relation to safe storage. The increase in moisture content is important during the malting process, as the grain must absorb enough water for the activation of enzymes and the initiation of germination, ultimately affecting the quality of the beer produced from the malt.

The malt moisture content for long shelf-stable storage is recommended to be 8 to 10% (Weston *et al*., 1993). Barley grain for malting over 13.5% moisture does not store well. It is also important to evaluate the moisture content at harvest. Barley should have a moisture content below 14% for long-term storage so that the grain does not mold or germinate prematurely. Barley that has more than 14% moisture should be dried for storage.

* + 1. **Hectoliter weight**

The weight in hectoliters (HLW) of malt was barely significantly (p<0.05) affected only by the main effects of location and lime (Table 6). Significantly higher HLW (71 kg / hl) was recorded when the crop was grown in Bekoji than in Kofele (65 kg / hl) (**Table 6**). According to the Ethiopian quality standard, the acceptable test weight (HLW) for raw barley is in the range of 48 to 62 kg / hL (EQSA, 2006).The results of the present experiment showed an acceptable hectoliter weight at both locations. The result agrees with the result obtained by Biadge (2016) that there are significant differences (p <0.05) between the HLW in two locations. The HLW was relatively higher obtained in Holeta (67.88 kg/hL) and Ankober (67.16 kg/hL) than in Bekoji (62.33 kg/hL). The result is also in agreement with the result obtained by Temesgen (2014) that all lime treatments had higher mean values of HLW compared to those without lime. In the present trial, lime rate at3.0t ha-1 gave significantly the highest (68.9 kg / hL) HLW, while the lowest HLW (67.6 kg/hL) was recorded at 1.5t ha-1, which was statistically at far with 0t ha-1.

* + 1. **Germination capacity**

The malt germination capacity (GC) was significantly (*p*<0.05) affected only by the main effect of location (Table 6). A significantly higher germination capacity (99.0%) was recorded when the crop was grown in Bekoji compared to Kofele with a germination capacity of 98.6 % (Table 6).

One of the key qualities of malting barley is its ability to germinate rapidly and synchronously. Dormancy can interfere with the rapid and uniform germination of barley, thus reducing the resultant quality of malt. Failure of barley grain to germinate at an acceptable level (> 96%) could introduce problems during the malting process. The range of germination capacity (at 72 h) ranged from 73.44% to 100% with an average of 94.31% is the standard for the beer industries. A desirable range of values (>95%) for the germination capacity indicates good seed viability and a good germination process.

* + 1. **Protein Content**

The malt protein content was significantly (p< 0.05) affected by the interaction between the P rate and the location and the main effect of the location (*P*<0.05) (**Table 7**). The highest protein content (14.5%) was recorded when the crop was grown at a rate of 10 kg phosphorus ha-1 at Kofele while the lowest protein (11.0%) was obtained when the crop was grown at a rate of 20 kg phosphorus ha-1 at Bekoji which is due to higher N content of Kofele than in Bekoji. This result is in agreement with the result reported by Kassu *et al.* (2018) that grain protein concentrations of malting barley were very significantly affected by the integrated use of organic and mineral fertilizer. Improved malting barley yields due to the combined application of organic and mineral amendments resulted from positive changes in the soil, including an increase in soil pH, available P and total N, and possibly other macro and micronutrients According to the Ethiopian Standard Authority and the Asella Malt Factory (AMF), the protein level of the raw barley quality standard for malt should be between 9-12.5% (EQSA, 2006). However, the grain protein at the Bekoji site was within the acceptable standard range for malt purposes, while at the Kofele sites it was not within the acceptable range (**Tables 7 and Table 8**).

**Table 5. Main effect of phosphorus rates, lime rates and location on the sieve size test of malt barley at the Bekoji and Kofele locations**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Location** | **Sieve size (%)****(>2.8mm)** | **Sieve size (%)** **(>2.5mm)** | **Sieve size (%)** **(>2.2mm)** | **Sieve size (%)** **(<2.2mm )** |
|  |  |  |  |
| Kofele | 31.9b | 42.3a | 20.3a | 5.5a |
| Bekoji | 72.0a | 19.9b | 6.3b | 1.8b |
| Lime(t ha-1) |  |  |  |  |
| 0 | 52.8 | 52.8 | 12.9 | 3.6 |
| 1.5 | 51.5 | 51.5 | 13.4 | 4.1 |
| 3.0 | 51.6 | 51.6 | 13.5 | 3.2 |
| **P level(kg ha-1)** |  |  |  |  |
| 0 | 51.7 | 32.8a | 12.2 | 3.3 |
| 10 | 51.6 | 32.1ab | 12.7 | 3.6 |
| 20 | 52.3 | 30.1bc | 13.6 | 4.0 |
| 30 | 52.2 | 29.4c | 14.6 | 3.8 |
| Cv(%) | 11.5 | 10.7 | 24.4 | 37.8 |

**The means followed by the same letter within a column are not significantly different at the 5% level of significance.**

**Table 6. Main effect of phosphorus rates, lime rates and location on MC, TGW, HLW, GC,GE and protein content (PC)of malt barley at the Bekoji and Kofele locations.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **MC (%)** | **TGW(g)** | **HLW(kghl)** | **GC (%)** | **GE (%)** | **PC (%)** |
| Kofele | 10.1b | 36.5b | 65.0b | 98.6b | 98.1b | 14.1a |
| Bekoji | 10.5a | 44.4a | 71.3a | 99.0a | 98.4a | 11.1b |
| Lime(t ha-1) |  |  |  |  |  |  |
| 0 | 10.4 | 40.6 | 68.0ab | 98.8 | 98.2 | 12.6 |
| 1.5 | 10.3 | 40.2 | 67.6b | 98.6 | 98.3 | 12.8 |
| 3.0 | 10.3 | 40.5 | 68.9a | 98.9 | 98.3 | 12.6 |
| **P level(kg ha-1)** |  |  |  |  |  |  |
| 0 | 10.7a | 40.7 | 67.9 | 98.8 | 98.1 | 12.5 |
| 10 | 10.3b | 40.9 | 68.3 | 98.6 | 98.2 | 12.6 |
| 20 | 10.2b | 40.2 | 68.5 | 98.9 | 98.2 | 12.8 |
| 30 | 10.1b | 40.0 | 67.9 | 98.8 | 98.4 | 12.7 |
| Cv(%) | 4.4 | 4.3 | 2.7 | 0.6 | 0.6 | 3.4 |

*Means followed by the same letter within a column are not significantly different at the 5% significance level, NS showed significant differences MC = moisture content, TGW = thousand grain weight, HLW = hectoliter weight, GC = gelation capacity, GE = gelation energy.*

**Table 7: The Effect of Lime Interaction and Location on Sieve and Malt Barley Protein Content**

|  |  |
| --- | --- |
| Treatments | Protein content (%) |
| Location | Phosphorus rate (kg ha-1)  |
| Kofele | 0 | 13.8b |
| 10 | 13.9b |
| 20 | 14.5a |
| 30 | 14.4a |
| Bekoji | 0.0 | 11.2c |
| 10 | 11.2c |
| 20 | 11.0c |
| 30 | 11.0c |

*Means followed by the same letter within a column are not significantly different at the 5% level of significance, and NS showed significant differences*.

**Table 8. Standard Quality Parameters of Malt Barley**

|  |  |  |  |
| --- | --- | --- | --- |
| S No. | Parameters  | EBC Standard  | Asela Malt Factory standard |
| 1 | Sieve test (%) |  |  |
| 1.1 | >2.8 mm | 34-45 | 34-45 |
| 1.2 | 2.5mm – 2.8 mm | 40-50 | 40-50 |
| 1.3 | 2.2mm -2.5mm | >5  | >5  |
| 1.4 | <2.2mm | <4  | 7-13 |
| 1.5 | TSR | <4  | 7-16 |
| 2 | GC (%) | 96  | 96 |
| 3 | GE (%) | 96 | 96 |
| 4 | MC (%) | 10.5-13.5 | 10.5-13.5 |
| 5 | PC (%) | 9.0-12.5 | 9.0-12.5 |
| 6 | TKW (gm) | 35-45 | 28-32 |

*TSR=Total sieve rejected; GC = germination capacity; GE = germination energy; MC=moisture content; PC=protein content; TKW=thousand kernel weight*

* 1. **Partial Budget Analysis of Treatment Effects Results**

As the interaction effects of phosphorus and lime on the GY of malt barley were significant, the partial budget analyzes for treatments involving different combinations of P and lime and the results are summarized in **Table 9**. Consequently, all treatment combinations produced higher net benefits (NBT) per hectare of land in Ethiopian Birr (ETB) than those obtained from absolute control treatment. However, only three treatments were used, that is, 30kg P ha-1by 1.5t ha-1lime, 20kg P ha-1 by 3.0t ha-1 lime and 30kg P ha-1 by 3.0t ha-1 lime produced NBTs with acceptable percent marginal rate of return (MRR) of 100%. Among the treatment combinations that produced acceptable MRR, the highest MRR (316.8 %) was produced by 20kg P ha-1 by 3.0t ha-1lime, followed by 30kg P ha-1 by 1.5t ha-1lime (203.8%) and 30kg P ha-1 by 3.0t ha-1lime, (195.2%). In all cases, these results imply that combined applications of P fertilizers and lime rates are economically feasible for the production of malt barley around the study areas **(Table 9).** This is in agreement with the finding of Tadesse *et al*. (2018), who reported that the combined application of P (30 kg P ha-1) and lime (1.5 tonha-1) to be economically viable for the production of malt barley in central Ethiopia.

**Table 9: Results of the partial budget analysis data on the effects of treatment on the GY of the malt barley analyzed at the locations Bekoji and Kofele, central Ethiopia.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **P rate (kg/ha)** | **Lime rate (t/ha)** | **Total****GY**  | **Adjusted** **GY**  | **\*GB** | **FC** | **LC** | **LabC** | **TVC** | **NBT** | **MRR (%)** |
| **ETB ha-1** |
| **kgha-1** |
| 0 | 0 | 2530 | 2277 | 34155 | 0 | 0 | 0 | 0 | 34155 |   |
| 0 | 1.5 | 2890 | 2601 | 39015 | 0 | 1875 | 750 | 2625 | 36390 | 85.1 |
| 0 | 3.0 | 3110 | 2799 | 41985 | 0 | 3750 | 1500 | 5250 | 36735 | 13.1 |
| 10 | 0 | 2970 | 2673 | 40095 | 1200 | 0 | 0 | 1200 | 38895 | D\*\* |
| 10 | 1.5 | 3220 | 2898 | 43470 | 1200 | 1875 | 750 | 3825 | 39645 | 28.5 |
| 10 | 3.0 | 3530 | 3177 | 47655 | 2400 | 0 | 0 | 2400 | 45255 | D |
| 20 | 0 | 4000 | 3600 | 54000 | 1200 | 3750 | 1500 | 6450 | 47550 | 56.7 |
| 20 | 1.5 | 3840 | 3456 | 51840 | 3600 | 0 | 0 | 3600 | 48240 | D |
| 20 | 3.0 | 4030 | 3627 | 54405 | 2400 | 1875 | 750 | 5025 | 49380 | 95.0 |
| 30 | 0 | 4300 | 3870 | 58050 | 3600 | 1875 | 750 | 6225 | 51825 | 203.8 |
| 30 | 1.5 | 4740 | 4266 | 63990 | 2400 | 3750 | 1500 | 7650 | 56340 | 316.8 |
| 30 | 3.0 | 5000 | 4500 | 67500 | 3600 | 3750 | 1500 | 8850 | 58650 | 192.5 |

*\*GB = gross benefit (Birr ha-1), FC = fertilizer cost(Birr ha-1), LC = lime cost(Birr ha-1), Labc = labor cost(Birr ha-1), TVC = total variable cost(Birr ha-1), NBT = net benefit(Birr ha-1), MMR = marginal rate of return; \*\*D = dominated (1USD=30ETB)*

**3.6 CONCLUSION**

The application of P fertilizer and liming has significantly increased the agronomic parameters, the grains quality components of malt barely, and some selected soil chemical properties in both Bekoji and Kofele, suggesting that the soil P content in both locations is insufficient to improve crop productivity and production. Similarly, lime application (3 tha-1) has significantly improved the selected chemical properties of the soils in both locations and also significantly improved the yield of malt barley, indicating the importance of lime application to increase crop productivity in both locations. The application of both P fertilizer (30 kgha-1) and lime (1.5 tha-1) and their combinations produce higher net benefits per hectare compared to the control treatment. The lime and P rates obtained in this study could serve as a reference to increase barley production in the study area and areas with similar ago-ecology that have soil acidity problems. However, economically acceptable net benefits and marginal return rates were obtained from treatments involving 30 kg P ha-1 with 1.5 ton lime. Therefore, the combined application 20 kg P ha-1 and 3.0 ton lime ha-1 is recommended for optimum production with an acceptable quality of malt barley in the study areas.

**Data Availability**

All the necessary data are included in the manuscript. If additional data are required, the corresponding author can be contacted.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest with respect to the publication of this article.

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