



Genotype by Environment Interactions in Barley (*Hordeum vulgare* L.) Cultivars for Nutritional Quality Assessment

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ABSTRACT

In current study twenty-five barley genotypes were grown under RCBD (randomized complete block design). Barley flour was analyzed for proximate composition, β -glucan content, soluble and insoluble dietary fiber. Based on the results of nutritional quality best line (4158) was selected for the preparation of wheat flour supplemented bread. The sensory evaluation of bread was carried out to assess its suitability for consumers. The data obtained from all the experiments was subjected to statistical analysis by CRD. The results indicated that the highest moisture content (13.47%), protein content (13.93%), fat content (3.39%), fiber content (7.08%), ash content (2.67%) and NFE (71.54%) were observed in lines 4220, 4158, 4149, 4193, 4233, 4220 respectively. Similarly, significant differences for β -glucan (4.99%), total dietary fiber (16.62%), soluble (6.23%) and insoluble dietary fiber contents (10.36%) were observed in barley line 4193, 4233, 4168 and 4233, respectively. The bread prepared with the addition of 5% flour to wheat flour was liked most by the judges after the control bread. The current study showed significant potential of flour to be used by baking industry for the preparation of bread and other food products by the addition of flour.

INTRODUCTION

Cereal grains are the main source of human nutrition. They provide almost half of the calories consumed by the peoples (Loskutov & Khlestkina, 2021). Barley is one of the major cereal and first cultivated grains ever, which grows mainly in temperate climate globally. Barley and other entire grains have gained fame in recent years because of their impressive therapeutically benefits (Rosentrater & Krishnan, 2006). The changes in the relative genetic performance of different genotypes across different environments are described as GEIs (genotype by environment interactions). As the selection and evaluation of top genotypes is facilitated by GEIs, therefore, GEIs (genotype by environment interactions) can have an impact on breeding progress and breeders invariably may encounter different GEIs when they are testing different varieties across a number of different environmental factors (Beriso & Asefa, 2020). Using genetic resources to solve production restriction can be difficult, due to lack of understanding that how different environmental factors and multiple characteristics interact to control crop performance. Rodriguez, Rau, Papa, & Attene (2008) reported that the analysis of GEIs is critical in breeding of barley and in many other aspects of barley research. In Mediterranean locations, GEIs is a concerned issue because barley growth can be affected by the drought and high temperatures, as well annual changes in climate factors. As a result, in order to discover and assess the primary factors that cause genotype adaptation, experimental research must be conducted across several environment trials.

In Pakistan, after rice-wheat and maize, barley is the fourth most important cereal crops (Naeem et al. 2021). Only the inedible outer shell of hulled barley has been removed by minimal processing, leaving the bran and germ intact.

Barley is a significant source of beta glucan. Beta glucan has the functional properties to ameliorate different lifestyle related disorders like diabetes prevalent globally (Khan et al., 2021).

Barley flour can be used to supplement wheat flour in the preparation of supplemented flour bread. It is sometimes mixed with wheat flour to make composite flour, which is used to make variety of breads with improved nutritional properties. The main aim of a plant breeder when

he is working in crop improvement programs is to generate cultivars with high yield potential in order to sustain high agricultural production. In many aspects of the plant breeding programs the study of GEI is vital for selecting stable genotypes and high-yield. When conducting agricultural performance trials across various and unexpected conditions, it tends to be a difficult issue among plant breeders and agronomist (Hongyu, García-Peña, de Araújo, & dos Santos Dias, 2014). As a result, the understanding on the causes of GEI will aid in the breeding program of the respected crop and the developed genotypes are expected to have wider adaptation and perform well on a variety of environmental conditions.

MATERIALS AND METHODS

Field Trial

Twenty-five barley genotypes were grown during 2019-2020 at the field area of COMSATS University Islamabad (CUI), Vehari Campus, Pakistan. After harvesting the grains of each line, they were collected in separate bags and stored in a ware house where they were spread over the sun so that its moisture content reduces to the optimum level. After 4 to 5 days the thrashing of the barley lines (BLs) were started in the lab. The grains of barley were collected and stored in small packets for further analyses.

Preparation of Raw Material

The milling process of barley includes three steps: cleaning, dehulling and milling. Cleaned barley was dehulled by passing each line through de-huller which separated inner grains of each line. The lighter barley was separated and grains were taken for milling through Quaderumate Senior Mills.

Proximate Analysis

In each sample, the moisture content was calculated based on the method No. 44 of AACC (2000) while, for crude protein content analysis, the Kjeldahl's method No 46-10 of AACC (2000) was used. Nitrogen content were measured following steps of digestion, distillation and titration and using the formula as

$$\text{Nitrogen (\%)} = \frac{\text{Vol. of 0.1 N H}_2\text{SO}_4 \text{ used} \times 0.0014 \times 250}{\text{Weight of sample} \times \text{Vol. of sample (10 ml)}} \times 100 \dots 1)$$

$$\text{Crude Protein Content} = \text{N\%} \times 5.70 \dots 2)$$

AACC (2000) technique No. 30-25 was used to determine crude fat content using the Soxhlet device. The dry samples were weighted for 5 g and collected in separate thimbles and placed in a Soxhlet extraction tube. The crude fat was calculated using the following equation;

$$\text{Crude Fat (\%)} = \frac{\text{Wt. of petroleum ether extract residue}}{\text{Wt. of sample (g)}} \times 100 \dots\dots 3)$$

The crude fiber content were measured after fat extraction of the samples. AACC (2000) method No. 32-10 was used for this purpose. During this assay, each 2 g fat free flour sample was boiled for 30 minutes with 1.25% H_2SO_4 . After boiling the mixture was filtered and 2-3 washings were done to make it acid free. After that it was boiled with base 1.25% NaOH for 30 minutes, and then again washed with water. The alkali free sample was dried for 3-4 hours in an oven at 100°C until it obtained a consistent weight. The samples for crude fiber content analysis were then placed in a muffle furnace at a temperature of 550°C for consistent 4 hours. The crude fiber content were analysed by calculating the difference in weight by the following formula.

$$\text{Crude Fiber (\%)} = \frac{\text{Loss in weight on ignition}}{\text{Wt. of sample (g)}} \times 100 \dots\dots\dots 4)$$

For the determination of ash contents in flour samples method no 08-01 of AACC (2000) was used. To calculate the ash contents, the formula given below was used.

$$\text{Ash (\%)} = \frac{\text{Wt. of ash in sample (g)}}{\text{Wt. of sample (g)}} \times 100 \dots\dots\dots 5)$$

The barley flour was tested for NFE using the methods outlined in the AACC (2000). The flour samples were tested for β -glucan extraction based on AACC Method No. 32-23.01.

Development of Supplement Flour Bread

The bread was prepared using AACC (2000) straight dough method No. 10-10B. To reduce the amount of wheat flour in supplemented breads, barley flour was added at a rate of 5%, 10%, and 15%. In a Hobart A-200 Mixer, the dough was prepared by mixing all the ingredients for a total time of 5-10 minutes which was then fermented at 30 °C, for 180 minutes and 75% relative humidity. After 120 and 150 minutes, the first and second punches were completed. The dough was formed and panned into 100 g test pan. The dough was proofed at a temperature of 35 °C while given

relative humidity of 85% for 45 minutes. Thereafter, the bread was then baked for 13 minutes at 232 °C.

Sensory Evaluation of Bread

The sensory scores for internal characteristics (IC) (grain, taste, aroma, crumb color, mastication and texture) and for external characteristics (EC) (volume, symmetry break and shred, crust color, and evenness of bake) were carried out on each prepared bread made from different treatments by fully trained judges panel as described by Holtekjølén, Bævre, Rødbotten, Berg, & Knutsen (2008).

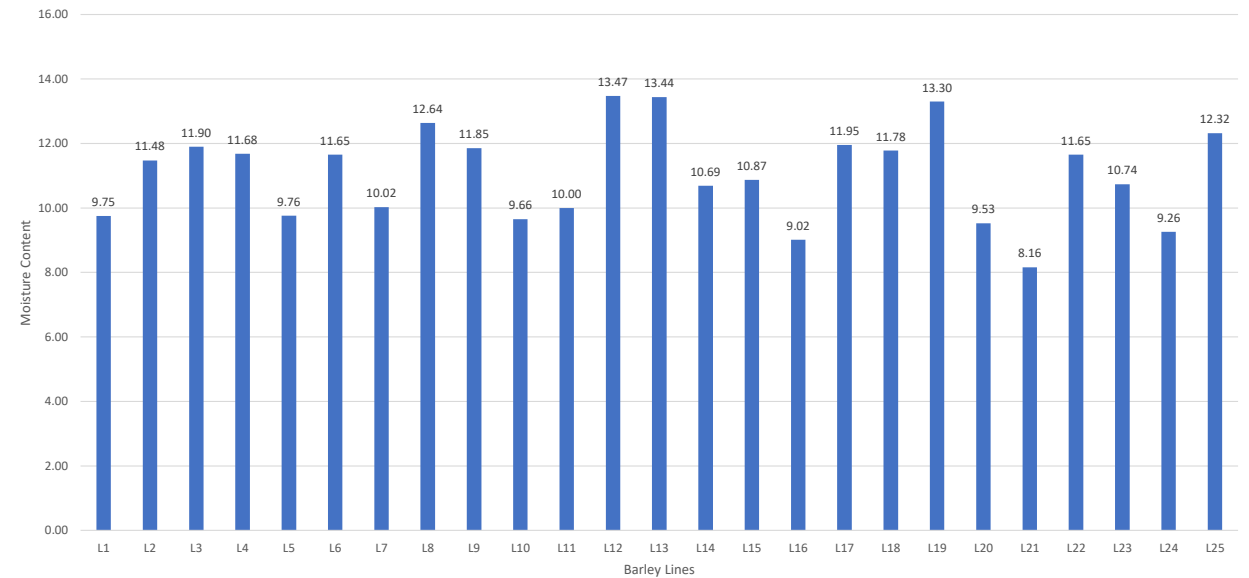
Statistical Analysis

Co-Stat-2003 (Cohort v-6.1) Statistical Package was used to analyse the data for each parameter. The experiment was carried out using a randomised complete block design (RCBD) and the level of significance determined using analysis of variance. In addition, the significant ranges in several cultivars were statistically analysed by the DMRT (the Duncan's multiple range test) to investigate the various quality parameters of the results obtained.

RESULTS AND DISCUSSION

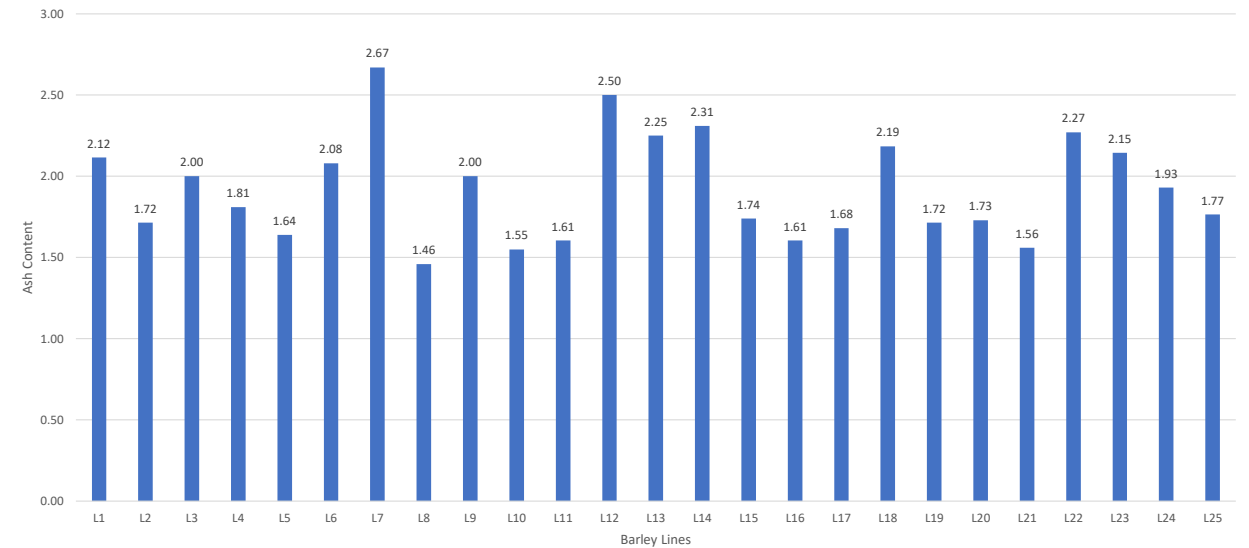
Effect of Proximate Composition in Different Barley Lines (BLs)

The moisture content ranged from 8.16 to 13.47% among barley lines. Fig. 1 indicated that barley line 4220 had the maximum moisture content of 13.47%, while barley line 4236 had the lowest moisture level of 8.16%. The statistical results showed significant effect of genotype by environment on the ash content of different barley lines. The ash content of different barley lines ranged from 1.46 to 2.67%, as presented in Fig. 2. The ash content was found significantly higher in the barley line 4233, followed by 2.50% and 2.31% and the lowest was observed in barley line 4199. The fat content also showed significant difference with the range of 3.39 (line 4149) to 1.45% (line 4206) as shown in Fig. 3. Loaf volume and swelling of starch granules can be reduced at lower concentration of fat. Contrariwise, gas cells is stabilized at higher fat content and promote greater loaf volume (Watanabe, Arruda, Kitzberger, & Coelho, 2019). Likewise, the statistical results showed significant effect of genotype by environment on the fiber content of different barley lines.



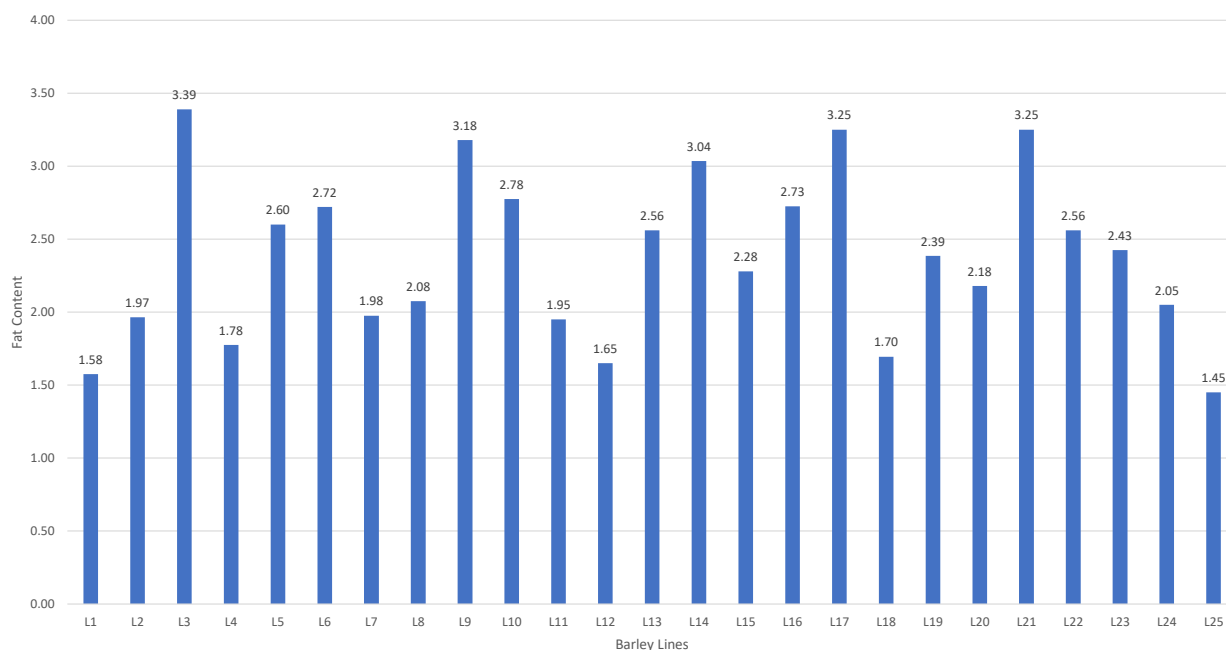
Remarks: L1-4177; L2-4158; L3-4149; L4-4181; L5-4234; L6-4182; L7-4233; L8-4199; L9-4227; L10-4237; L11-4193; L12-4220; L13-4204; L14-4219; L15-4168; L16-4176; L17-4224; L18-4140; L19-4223; L20-4215; L21-4236; L22-4231; L23-4192; L24-4167; L25-4206

Fig. 1. Moisture content in different Barley lines as influenced by GEIs.



Remarks: L1-4177; L2-4158; L3-4149; L4-4181; L5-4234; L6-4182; L7-4233; L8-4199; L9-4227; L10-4237; L11-4193; L12-4220; L13-4204; L14-4219; L15-4168; L16-4176; L17-4224; L18-4140; L19-4223; L20-4215; L21-4236; L22-4231; L23-4192; L24-4167; L25-4206

Fig. 2. Ash content different Barley lines as influenced by GEIs.

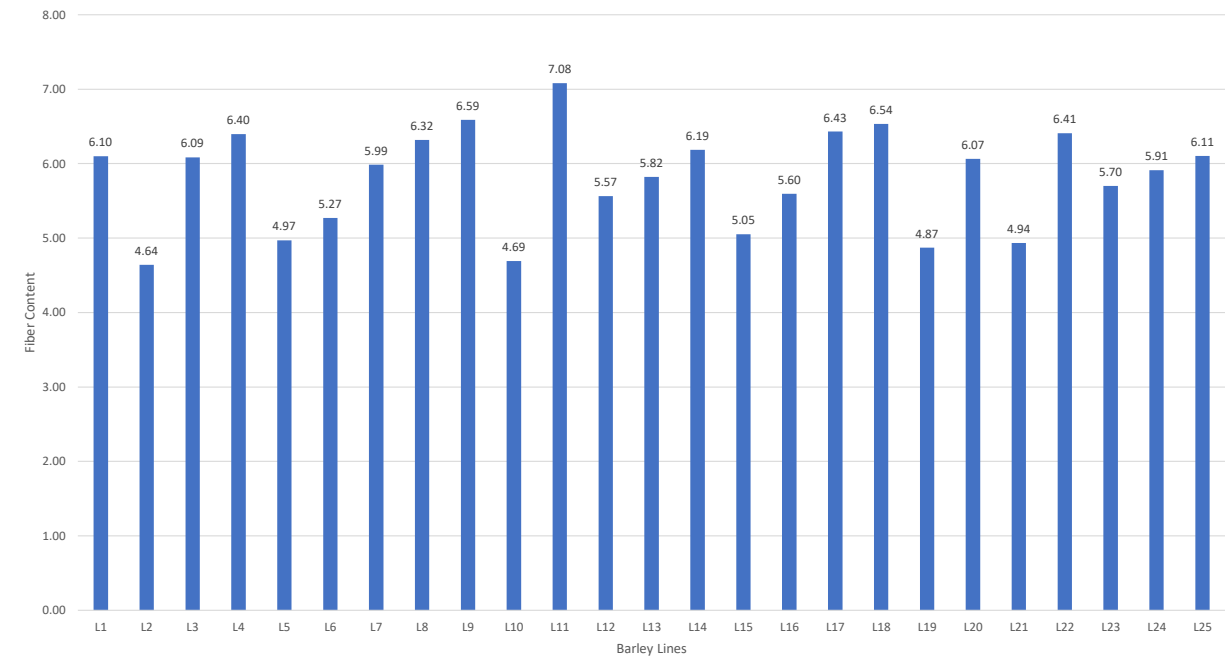


Remarks: L1-4177; L2-4158; L3-4149; L4-4181; L5-4234; L6-4182; L7-4233; L8-4199; L9-4227; L10-4237; L11-4193; L12-4220; L13-4204; L14-4219; L15-4168; L16-4176; L17-4224; L18-4140; L19-4223; L20-4215; L21-4236; L22-4231; L23-4192; L24-4167; L25-4206

Fig. 3. Fat content in different Barley lines as influenced by GEIs.

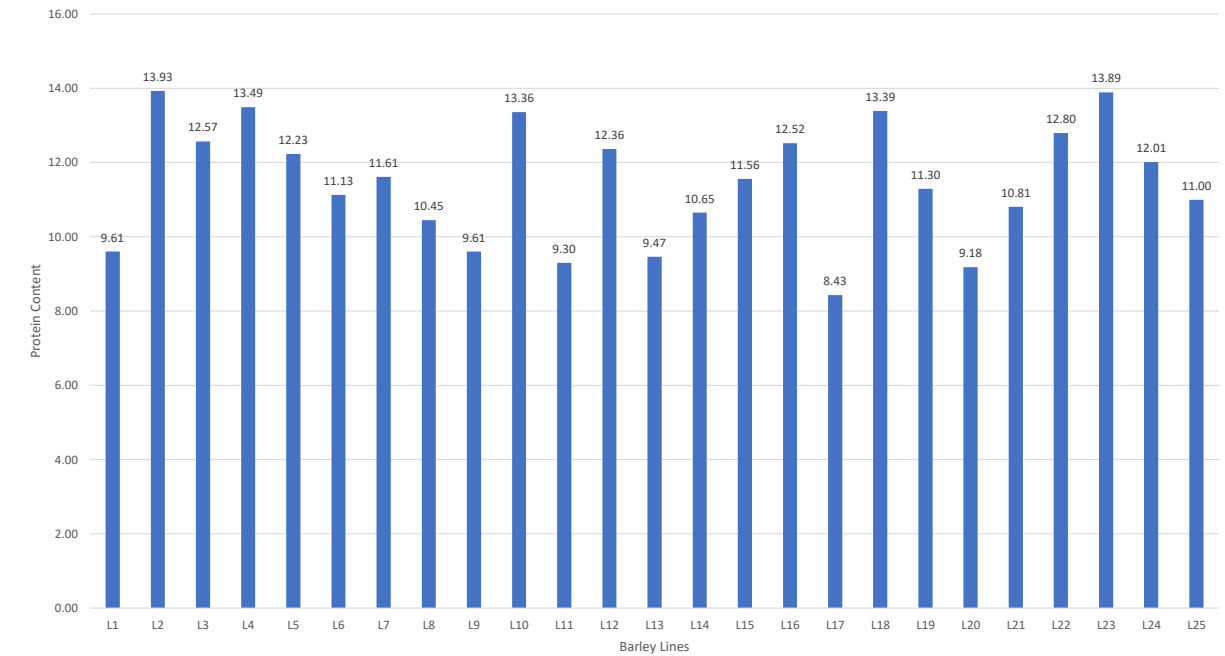
The results described in Fig. 4 showed that fiber content was found significantly the highest 7.08% in barley line 4193, followed by 6.59% and 6.43% and the lowest with the value of 4.64% dietary fiber was noticed in barley line 4158. The protein content of several barley lines ranged from 8.43 to 13.93 %, according to the results shown in Fig. 5. The results described that the highest protein contents was found as 13.93% in barley line 4158, followed by 13.89% and 13.49%. However, the lowest protein content-observed in barley line 4224. The significant effect of genotype by environment was also observed in NFE of different barley lines. The results described in Fig. 6 showed that the NFE content ranged from 71.54 to 64.08% in different barley lines. The NFE content was found significantly higher in barley line 4236, followed by 71.39% and 70.89%. The least NFE content was observed in barley line 4149. The significant statistical results for soluble dietary fiber contents of different barley lines are depicted in Fig. 7. Barley line 4168 showed significantly higher soluble dietary fibre content at 6.23% followed by 6.08% and 5.79 %. However, the lowest 4.19% soluble dietary fiber contents were recorded in barley line 4177.

The statistical results showed significant effect of genotype by environment on the insoluble nutritional fiber content of different barley lines. The results described in Fig. 8 indicated that the insoluble dietary fiber content ranged from 10.36 to 6.77% in among barley lines. The insoluble dietary fiber content was found significantly higher in barley line 4233 at 10.36%, followed by 10.23% and 10.13% while the least 6.77% was observed in barley line 4136. The statistical results showed significant effect on the total dietary fiber content of different barley lines. In several barley lines, the total dietary fiber content ranged from 16.62 to 7.19 %, as presented in Fig. 9. The total dietary fiber content was found highest in barley line 4233, followed by 16.38% and 15.39% and the lowest was found in line 4149 with the value of 7.19%. The statistical results showed significant effect of genotype by environment on the contents of β -glucan among barley lines. The results described in Fig. 10 showed that the the contents of β -glucan ranged from 2.54 to 4.99% in different barley lines. The contents of β -glucan was found highest in barley line 4193, followed by 4.58% and 4.52%. While the contents of β -glucan was observed the lowest at 2.54% in barley line 4158.



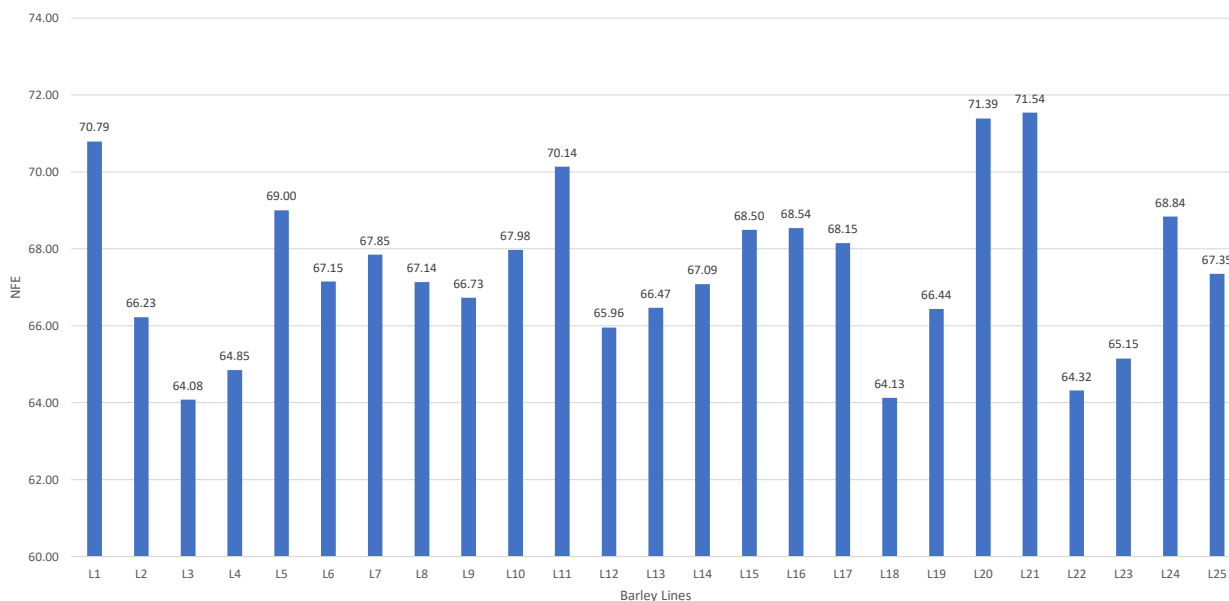
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Fig. 4. Fiber content in different Barley lines as influenced by GEIs.



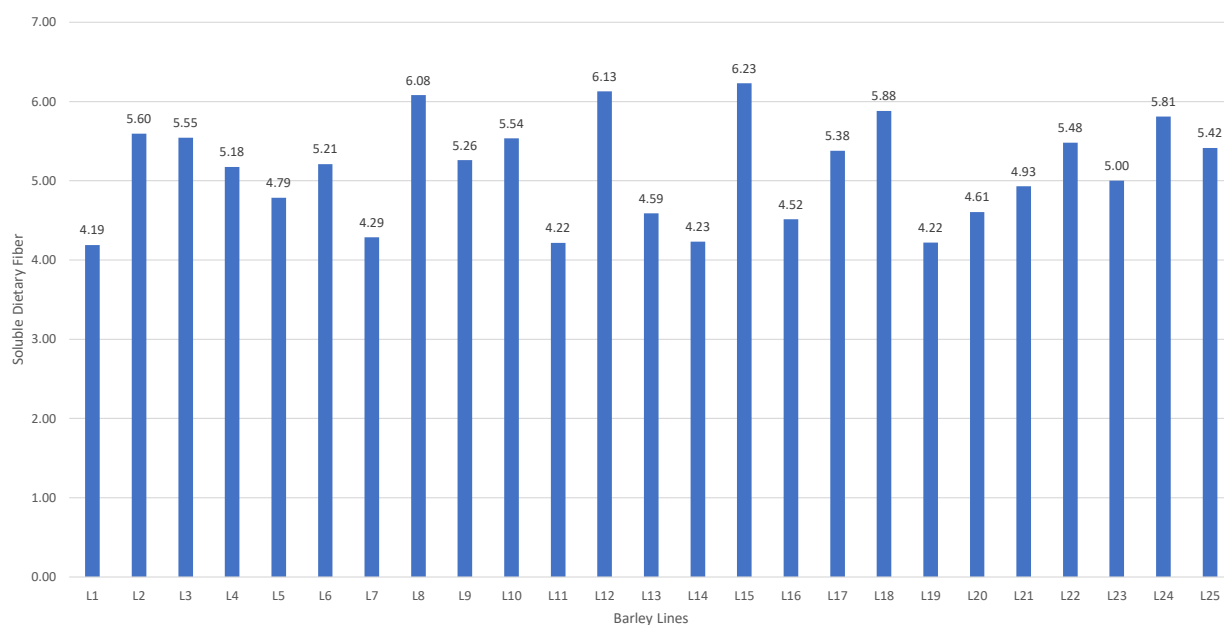
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Fig. 5. Protein content in different Barley lines as influenced by GEIs.



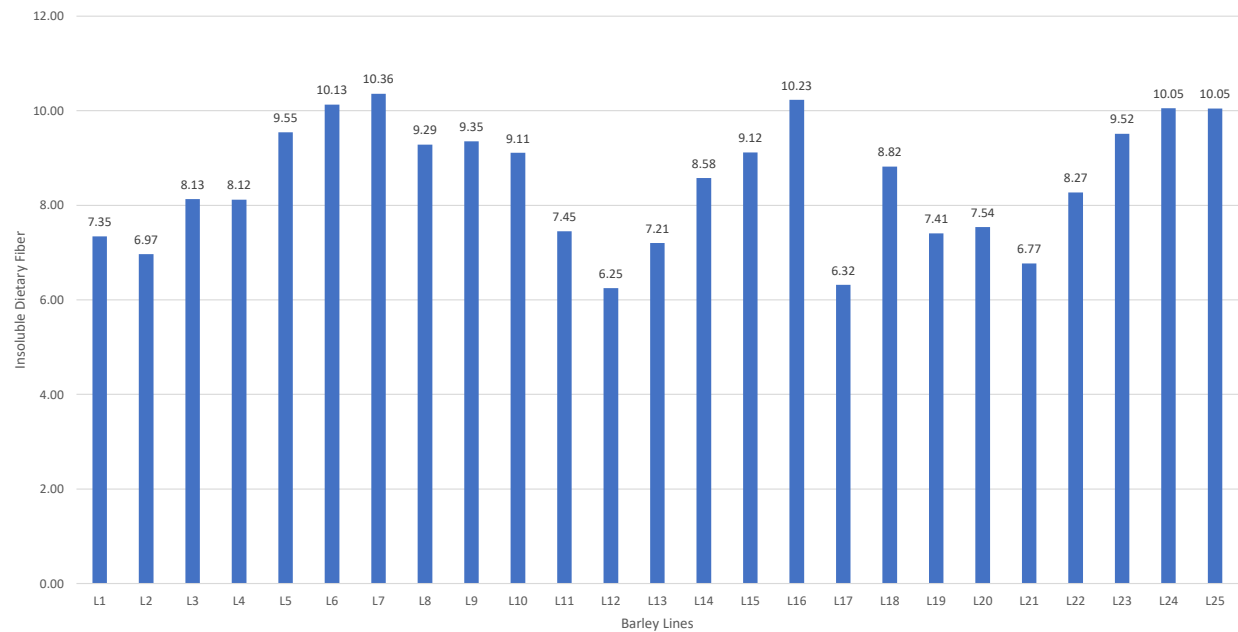
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Fig. 6. Nitrogen free extract of different Barley lines as influenced by GEIs.



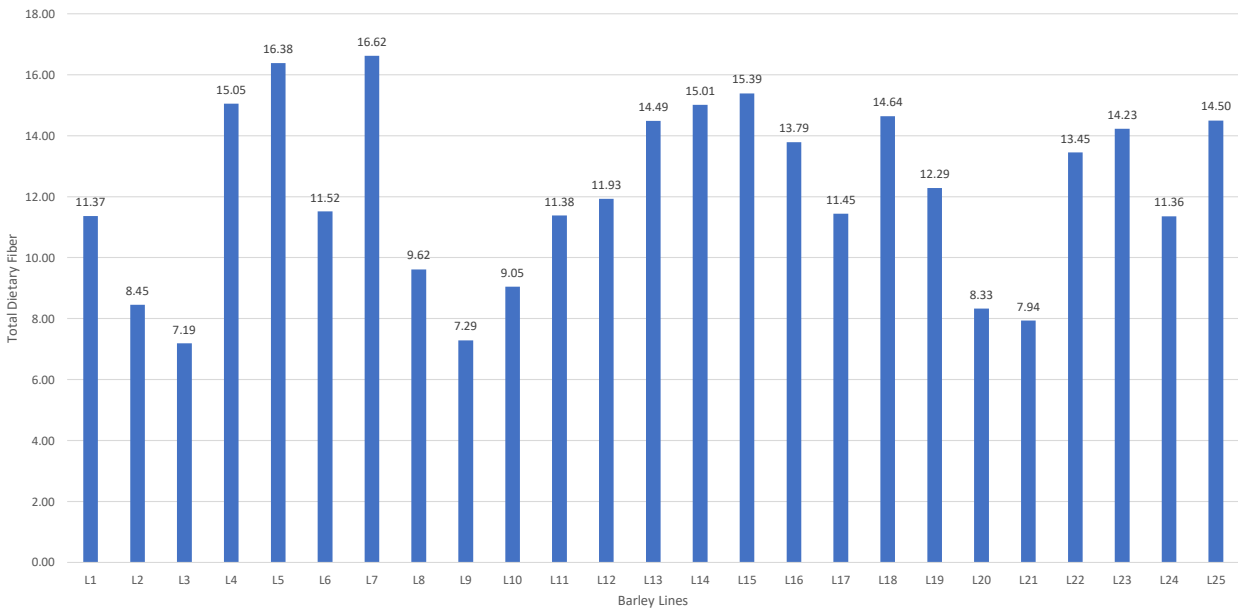
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Fig. 7. Soluble dietary fiber content in different Barley lines as influenced by GEIs.



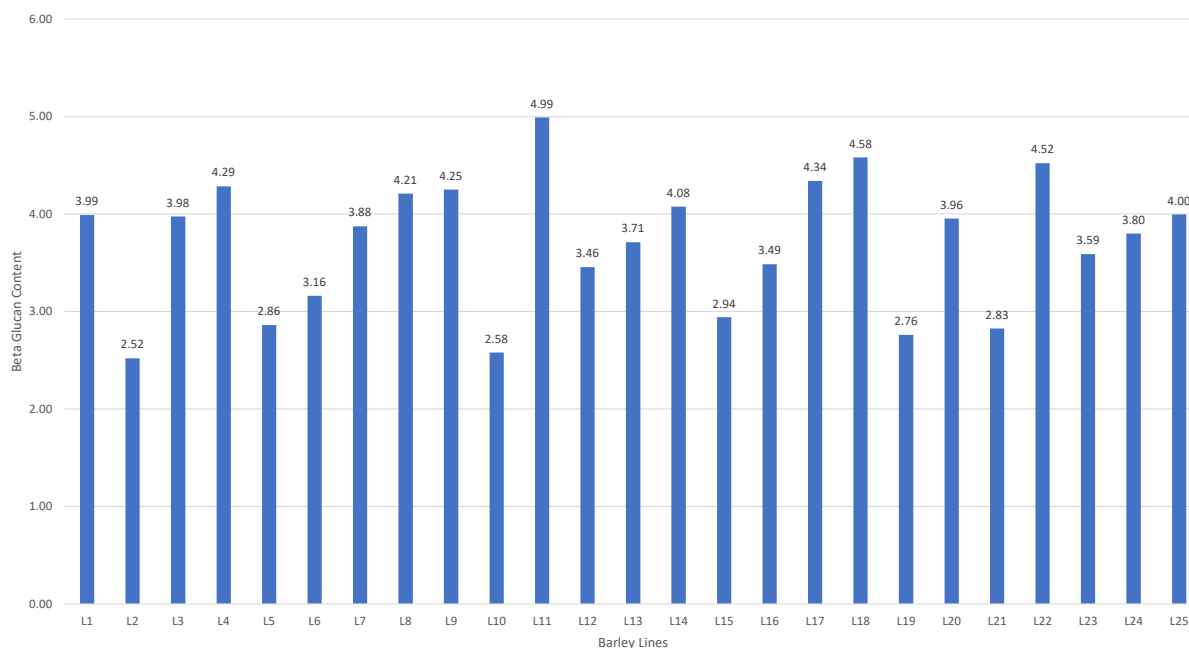
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Fig. 8. Insoluble dietary fiber content in different Barley lines as influenced by GEIs.



Remarks: L1-4177; L2-4158; L3-4149; L4-4181; L5-4234; L6-4182; L7-4233; L8-4199; L9-4227; L10-4237; L11-4193; L12-4220; L13-4204; L14-4219; L15-4168; L16-4176; L17-4224; L18-4140; L19-4223; L20-4215; L21-4236; L22-4231; L23-4192; L24-4167; L25-4206

Fig. 9. Total dietary fiber content in different Barley lines as influenced by GEIs.



Remarks: L1-4177; L2-4158; L3-4149; L4-4181; L5-4234; L6-4182; L7-4233; L8-4199; L9-4227; L10-4237; L11-4193; L12-4220; L13-4204; L14-4219; L15-4168; L16-4176; L17-4224; L18-4140; L19-4223; L20-4215; L21-4236; L22-4231; L23-4192; L24-4167; L25-4206

Fig. 10. β -glucan content in different Barley lines as influenced by GEIs.

Sensory Evaluation of Barley Supplemented Bread

The results of sensory analysis explains that the panel of expert judges assigned different scores to the external characteristics (volume, symmetry, crust color, break and shred and evenness of bake) and internal characteristics (texture, taste, grain, aroma, mastication and crumb color) for all the samples. The score given to the breads for external characteristics are depicted in Fig. 11 and for internal characteristics are depicted in Fig. 12. The highest scores for were observed in breads prepared with control sample without barley flour supplementation. However, the bread prepared with the addition of different concentrations of barley flour were also found satisfactory and showed good results after the control bread.

The study of genetics of phenotypic responses to changing environments remains elusive (Huang, Carbone, Lyman, Anholt, & Mackay, 2020). In the previous studies we have found out the strong effect of environment and variety on the antioxidant potential of wheat varieties (Nadeem et al., 2021). Highly significant GEI was identified for barley grain yield in dryland conditions (Ahakpaz et al., 2021). The moisture content of barley lines is dependent on

genetic make-up of varieties, meteorological factors and agronomic conditions experienced throughout growing period. There is a pronounced difference observed in moisture content in barley lines that may be due to the change in the weather pattern in this location especially humidity present in the atmosphere. These results are comparable to the research work done by previous researchers who reported moisture content from 14.23 to 9.55% in barley (Tamm, Jansone, Zute, & Jakobson, 2015).

The presence of variations in the ash contents could be to the differences in the genetic makeup of different lines. The ash content of a product generally denotes the concentration of mineral contents present. Although a high ash level is beneficial for high nutrition content but, it is also related with a dark flour color, poor baking quality, and very low milling yield, all of which are qualities that limit the triticale in adoption of processed food products qualities (Watanabe, Arruda, Kitzberger, & Coelho, 2019). These results are similar to the research work done by previous researchers who reported ash range from 1.72 to 2.01% (Alijošius et al., 2016).

There are significant variations recorded in fat content of 25 barley lines. The change in fat content may be due to the change in viscosity

of the grain that was found among barley lines. Such variations are due to varietal differences (genotypes) as reported by previous researchers. These results are also similar to the findings of other researchers who reported the range of fat content from 1.57 to 2.73% in different barley lines (Svihus

& Gullord, 2002). There is a significant difference in fiber content and variation is recorded in barley fat content. Non genetic and genetic variables are significantly responsible for the diversity in fiber content, although it is also dependent on shape, grain size and bran thickness.

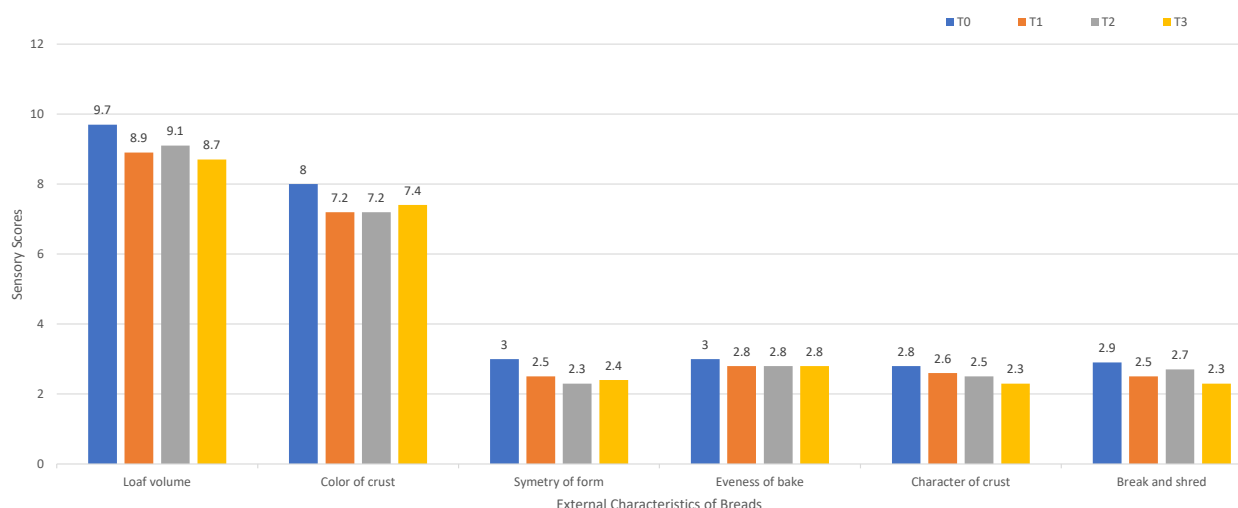


Fig. 11. Sensory scores for external characteristics of barley supplemented breads.

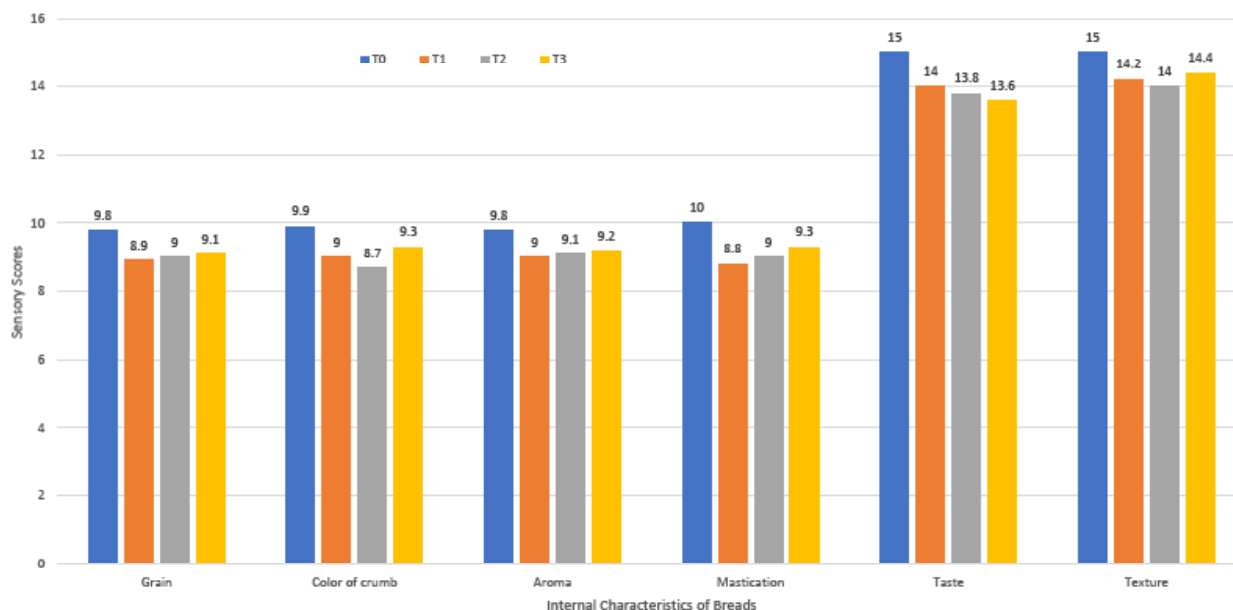


Fig. 12. Sensory scores for internal characteristics of barley supplemented breads.

There is a significant variation in protein content due to the change in temperature and available nitrogen in the soil. These findings are comparable to research work of Tamm, Jansone, Zute, & Jakobsone (2015) who have found that fat content in barley ranges from 13.33 to 18.55%. The change in β -glucan content in different barley genotypes may be due to increase in soil temperature in the growing period and low soil pH. It is possibility that the change in NFE content is related to antioxidants that are not evenly distributed in barley grains. Our findings are similar to those of Gupta, Abu-Ghannam, & Gallagher (2010) who investigated barley for brewing characteristics changes throughout malting, brewing and applications of its by-product and recorded range from 60.32 to 67.57 %.

The interactions of GEIs with the dietary fiber β -glucan which is one of the decisive elements in the final usage of barley (*Hordeum vulgare* L.) grain is remain unknown. Choi, Esser, & Murphy (2020) stated that the content of dietary fiber β -glucan was affected by genetics and non-genetic variables. They are primarily responsible for the variations in total soluble and insoluble dietary fibre content, which is also influenced by bran thickness, grain size and shape. The more bran content will contribute to higher the dietary fiber. The varied dietary fiber contents among barley lines may be due to their genetic makeup differences. Salinity influence grain yield and quality among all genotypes. As salinity level increased, the wheat genotypes showed the increase of sensitivity to salt in respect to the growth and chemical parameters (Nadeem et al., 2020). The dietary fiber content may also be related to flour extraction rate. The more more bran is scraped will contribute to higher dietary fiber content.

Sensory Characteristics of Breads

The sensory test uses the concepts of statistical analysis and experimental designs and is a scientific discipline. It uses human senses to analyze and score consumer products. This model required a assessment of a panel of differnet jugdes who scores the product by using their senses according to the given standards. Bread baking is a complex process that involve role of ingredients to bring about desired physical and chemical changes in bread (Raheem, Liu, & Li, 2019). After sensory results it is significantly possible to make final assessment and get complete insights about the

developed products by applying different statistical techniques to the results. Worldwide, bread is being used as an ideal product. Bread is also considered a functional food since it is used by human beings as an important element of their daily diet. No any other food can substitute the consumption of bread. In many countries, bread is consumed in various forms. Texture, aroma, appearance, and flavor are sensory attributes which are used to characterise the quality of breads. The different sensory parameters of the studied breads as assessed by the judges were rated to be significantly varied across different barley lines in this investigation. The differences in the chemical composition of barley lines may be responsible for the observed changes in the assigned scores to various attributes particularly the contents of crude protein, as seen in earlier sections.

CONCLUSION

The findings from this study provide information and guidelines regarding the use of superior quality barley lines which can be utilized by different stake holders. The breeder also can use best bread quality barley lines in their future breeding program to produce and introduce new varieties of barley with the aim of production of best quality breads. The baking industry can utilize this research and information to maximise the future potential for best quality of bread for highly supplemented nutritious bread manufacturing, as well as in wheat breeding initiatives.

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