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Research Article

Comparison of the Changes in Seed Germination Vigour with Prolonged Postharvest Storage for *Hordeum vulgare* Varieties, Hope and Sierra Malting Barley Varieties, in Zimbabwe

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To ensure production of quality malt that conforms to brewing requirements, the barley used should have minimal postharvest dormancy and be able to germinate rapidly and uniformly. The objective of the study was to compare the changes in seed germination, vigour trends, and storage stability of two-row Zimbabwean commercially grown malting barley varieties (Hope and Sierra), as the postharvest storage time increased. The two varieties were stored for 12 months, and at monthly intervals, germination index, capacity, and energy were determined. In addition, other quality parameters (protein content, screening, moisture content, water sensitivity, and insect damage index) were also assessed. Hope's germination index improved with an increase in storage time from 8.67 at the beginning of the study to 9.18 at the end of the study, while that for Sierra diminished with storage from 9.35 to 6.71. Generally, water sensitivity improved with postharvest storage for both varieties. However, the germination energy for Hope diminished with increased postharvest storage compared to Sierra. Hope variety is the more suitable variety for extended postharvest storage than Sierra. However, Sierra variety is more suitable for early malting postharvest storage.

1. Introduction

Malt is produced by the controlled but limited germination of barley. To produce good-quality malt, the barley employed must be able to germinate rapidly and synchronously [1]. The length of the storage period for barley is very vital to produce good-quality malt. Storage must maintain varietal purity and avoid loss of germinability [2, 3]. During prolonged storage, barley grain will slowly lose its vitality, causing slower germination and even grain death, and will therefore be of less value for the maltster [4–6]. The quality criteria for barley seeds selected for brewing malt production are very precise [7]. The storage time of malting barley will be determined by the amount of barley received from farmers for a particular growing season. The brewing industry has imposed requirements and expects malthouses to deliver materials with high stability of basic seed components and performance characteristics. Malting is the directed manipulation of barley growth to attain a desirable extract and enzyme yield, or in simpler terms, it is the partial conversion of the grain endosperm [8]. This partial conversion results in the synthesis of hydrolytic enzymes whose effect is the incomplete digestion of the cell walls, proteins, and starch of the endosperm, thus increasing grain friability [9–11]. The quality of the malt is of primary significance in the manufacture of beer of excellent quality. To increase the brewing yield and efficiency, malts with high extract values, high enzymatic activity, and good modification are essential. To produce quality malt, which meets the brewer's specifications, the barley employed must have minimal postharvest dormancy and be able to germinate rapidly and uniformly [12]. Thus, the desirable germination performance, which includes high germination index and germination capacity, forms the criterion for selecting malting barley [13–18]. Highly suited for malting is barley, which germinates rapidly and homogenously. The seed quality of barley is of increasing importance due to global climate change. The faster the rate of germination, the shorter the time needed to attain the desirable modifications during malting, the shorter the processing time, and the lower the cost of conversion. As a result, the germination vigour/rate of germination determines the time required for malting and hence the malting efficiency [2]. Generally, the conditions under which barley is stored influence the rate at which it loses its vitality. The rate of quality deterioration also depends on the differences in the genetic backgrounds of the varieties. The relative contributions of the variety to the total variation have been reported to be higher for vigour than for germination [19, 20]. Cereal grains with poor dormancy at harvest are susceptible to preharvest sprouting damage, while long-lasting dormancy can impede activities that depend on rapid germination, such as malting or the emergence of a uniform crop [21]. As such, barley seed vigour is a polygenic trait with importance for field emergence and for malting quality.

In Zimbabwe, Hope and Sierra are amongst the barley varieties used for malting. Different malting barley varieties behave differently during malting. Malting barley varieties possess high levels of several enzyme systems that are necessary to bring about the desired chemical changes within the kernel during malting. Support for breeding higher grain vigour is not only desirable for agronomic activities but also for higher malting values [22]. No systematic scientific study has been conducted to elucidate the trends of changes in seed germination vigour with an increase in storage time for the two Zimbabwean varieties as well as their storage quality stability. It is of importance for malting companies to understand the differences in germination vigour of barley varieties after a prolonged period of storage. The objective of the study was to compare the changes in seed germination vigour of malting barley varieties Hope and Sierra with an increase in postharvest storage time. This data will help provide knowledge of the trends of seed germination vigour for the *Hordeum vulgare* varieties grown in Zimbabwe. The findings will also determine which of the two varieties should be malted first or kept longer in storage depending on the rate at which germination vigour diminishes or improves with an increase in storage time. Such information will be useful to malting companies in contracting farming initiatives to determine quantities of barley to be grown per each variety, so as to avoid growing amounts of barley that cannot be malted in the time frame in which the barley will be retaining high germination vigour.

2. Materials and Methods

2.1. Barley Collection and Storage. Two malting barley varieties (10 kg each), Hope and Sierra, harvested from the winter growing season which was stored in silos at the largest barley processing plant in Zimbabwe, were collected at monthly intervals (for 12 months) in perforated sample bags stored under ambient temperature (22°C–25°C) before analysis.

2.2. Analysis of Barley Varieties. All samples were analysed by standard European Brewery Convention [23] methodology. The experiments were carried out in duplicate.

2.3. Germination Capacity. A random selection of 100 corns was made. The corns were soaked in tap water for about 5 minutes. Thereafter, the corns were longitudinally sectioned and one-half of each corn was discarded. The retained halves of each corn were then placed in test tubes according to the variety. The corns were covered with the 1.0 ml of 2, 3, 5-triphenyl-tetrazolium chloride solution. The test tubes were then placed in a water bath set at 40°C for about 30 min to allow the reaction to proceed. The excess tetrazolium solution was then poured off the half corns, emptied onto a piece of filter paper, and classified as follows:

(a) Corn in which the scutellum or aleurone layer and embryo are unstained is dead or if not dead, it will not modify during malting. (If the scutellum and/or aleurone cells are dead, the enzymes produced by the embryo cannot be translocated to the endosperm for modification.)

(b) Corns, in which the embryo, scutellum, and aleurone are all stained bright pink red, are fully viable.

2.4. Germination Energy. A random selection of 100 corns from the primary samples was done for both varieties. Filter papers (Whatman number 1) were placed in the bottom half of each Petri dish (90 mm diameter). 4 cm^3 of distilled water was pipetted into the Petri dishes on the filter paper, and the 100 corns were then spread evenly on the filter paper. The top half of the Petri dishes were then placed into position, and the corns were then germinated in the dark (thermostat/incubator) at 18°C–21°C for 72 h. Germinated corns were removed at intervals of 24 h. Recording of the total number of grains that would have germinated at 72 h–x% was done. It was useful to note also the grains, which would have germinated at 24 and 48 h for the determination of the germination index as required by the following calculation:

(a) Calculation of % germination energy (x%)

(b) Calculation of the germination index

The germination index, which is an indicator of the rate of seed germination, was calculated from the results of the germination energy as shown in

$$\mathrm{GI} = 10 \times \frac{(n24 + n48 + n72)}{(n24 + 2n48 + 3n72)}, \quad (1)$$

where $n24$, $n48$, and $n72$ are the numbers of germinated kernels at 24, 48, and 72 h, respectively, *2.5. Water Sensitivity.* A random selection of 100 corns from the primary samples was done for both varieties. Filter papers (Whatman number 1) were placed in the bottom half of each Petri dish (90 mm diameter). Thereafter, 8 cm^3 of distilled water was pipetted into the Petri dish, and the 100 corns were then spread evenly on the filter paper. The top half of the Petri dishes were then placed into position, and the corns were then germinated in the dark (thermostat/incubator) at 18°C–21°C for 72 h. Germinated corns were then removed at intervals of 24 h. Recording of the total number of grains that had germinated at 72 h–x% was done.

2.6. Calculation. The water sensitivity was then calculated using the formulae outlined in

$$\%\text{water sensitivity} = \%\text{germination energy} * - x\%. \qquad (2)$$

Here, % germinated in 4 cm^3 – germinated in 8 cm^3 test.

2.7. Moisture Content. Analysis of moisture content was done using a rapid moisture determination instrument (Pfeuffer Lite, Germany). The Pfeuffer Lite grain moisture meter determines the actual internal moisture content of the sample by grinding and appropriately compressing it. A random sample of barley was put into the grinding chamber of the moisture meter. The sample was ground and immediately homogenized on the special grinding surface of the moisture meter instrument's measuring cell. A reading of the moisture content of the sample was recorded from the instrument's digital display unit.

2.8. Protein Content. The nitrogen content was determined using a near-infrared analyzer (Technicon Near-Infrared Analyzer, Bran + Luebbe Germany). The sample was fed into the sample cell, which was then plunged into the infrared analyzer. The % total nitrogen was then recorded. The nitrogen content value was then multiplied with the conversion factor (6.25) to determine the total protein content. The protein content was reported on a dry weight basis.

2.9. Screenings Characterisation. Barley was differentiated by grain size in a shaking machine provided with three sieves having a slot of different widths. A Pfeuffer sieving machine (Pfeuffer, Germany) driven by an electric motor crank was used. The dimensions of the sieves were 43 cm long and 15 cm wide. The sieves were made of hardened brass. The width of the slots of sieve 1 was 2.8 mm, sieve 2 was 2.5 mm, and sieve 3 was 2.2 mm. The speed of shaking was 300 to 320 oscillations per minute, and the total length of the platform was 18 to 22 mm. A 100-gram sample was taken from the primary sample and placed on the top sieve, and the apparatus was set in motion for five minutes. The weights of each fraction were determined and expressed as a percentage of the total weight.

2.10. Insect Damage Index. About 100 grams of barley grains from each variety were weighed using a top pan balance. The barley grains were then spread on a Whatman number 1 filter paper and evaluated under a 60-Watt lamp. The grains which showed signs of insect damage were handpicked and weighed. The insect damage index was determined by expressing the weight of the insect damaged grains as a percentage of the total weight.

3. Results

3.1. Physiological Changes in Malting Barley. The results of the physiological changes that the malting barley varieties underwent during the postharvest stage were analysed by linear regression based on the magnitude of the r values, which measures the strength and direction of correlation between the variables.

Both Hope and Sierra malting barley varieties maintained desirable germination capacity scores, which were above 98%, the expected standard throughout the postharvest storage (Figure 1). In this study, over the storage period, Hope (−0.194) and Sierra (0.393) showed a very weak and weak negative correlation, respectively.

Figure 2 shows that as the storage time increased, both Sierra and Hope malting barley varieties showed strong negative correlations for germination at 4 ml. The decrease in germination energy is significantly pronounced in both varieties with Hope and Sierra having r values of −0.588 and −0.216, respectively. The average germination energy (4 ml) for Hope (97) was slightly below the standard while that for Sierra (98) met the expected standard of 95% (European standard barley specifications). However, of note is that at the end of the storage period, Hope (90) had much lower germination energy than Sierra (98). This was, however, still above the lower rejection of <90.

Both Sierra and Hope barley varieties had their 8 ml germination energy indices decreasing with an increase in storage time (Figure 3). Hope (r: 0.472) variety showed a weak negative correlation, while Sierra (r: 0.617) showed a very moderately strong negative correlation.

For water sensitivity, there was generally an increase in storage time that was characterized by a positive correlation for both malting barley varieties (Figure 4). Hope recorded a weak positive correlation with the r value of 0.378, and on the other hand, the correlation in Sierra was moderately strong as denoted by the r value of 0.588. The varieties generally showed low water sensitivity for most of the twelve-month storage period. For the first 9 months, the water sensitivity for both varieties was below 15%. However, by the 10th month, both varieties had become water sensitive, surpassing the expected limit of >20.

It is shown in Figure 5 that as time of storage increased, seed germination vigour, as indicated by the germination index, did not uniformly change for the two varieties. The trends show a weak positive correlation for Hope as denoted by the r value of 0.213, while Sierra recorded a strong negative correlation with an r value of -0.739. The average germination index over the storage period for Hope (8.03) and Sierra (7.59) was below the expected standard of 8.5 [1]. However, at the end of the storage period, Hope (9.18) had a much higher germination index than Sierra (6.71).

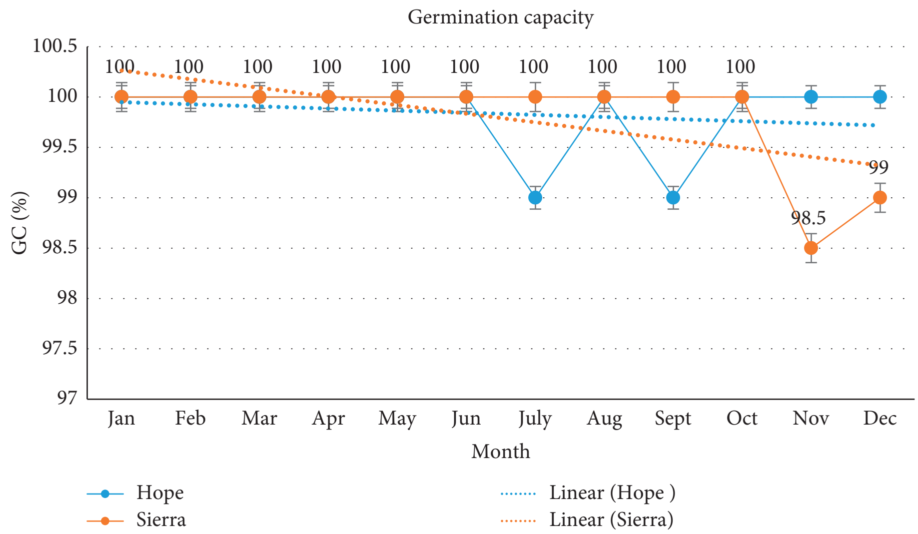

Figure 1: Comparison of germination capacity trends for Hope and Sierra malting barley varieties over the 12-month storage period.

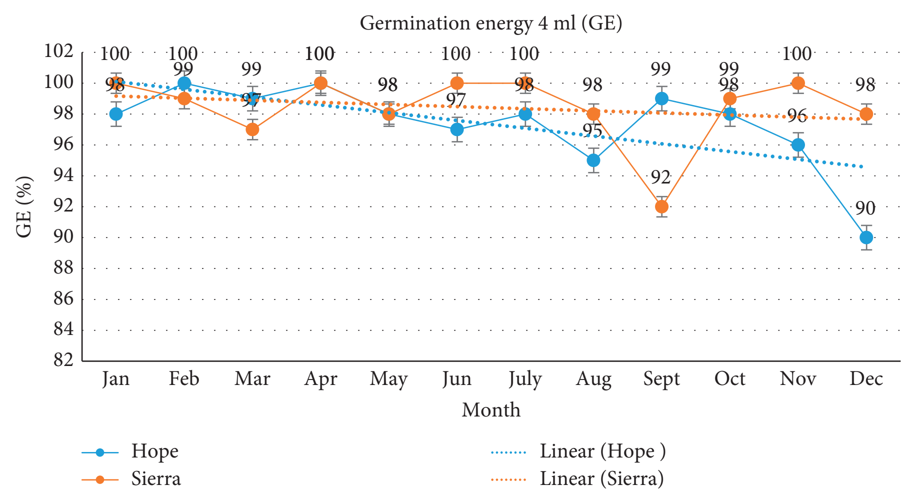

Figure 2: Comparison of germination energy (4 ml) trends for Hope and Sierra malting barley varieties over the 12-month storage period.

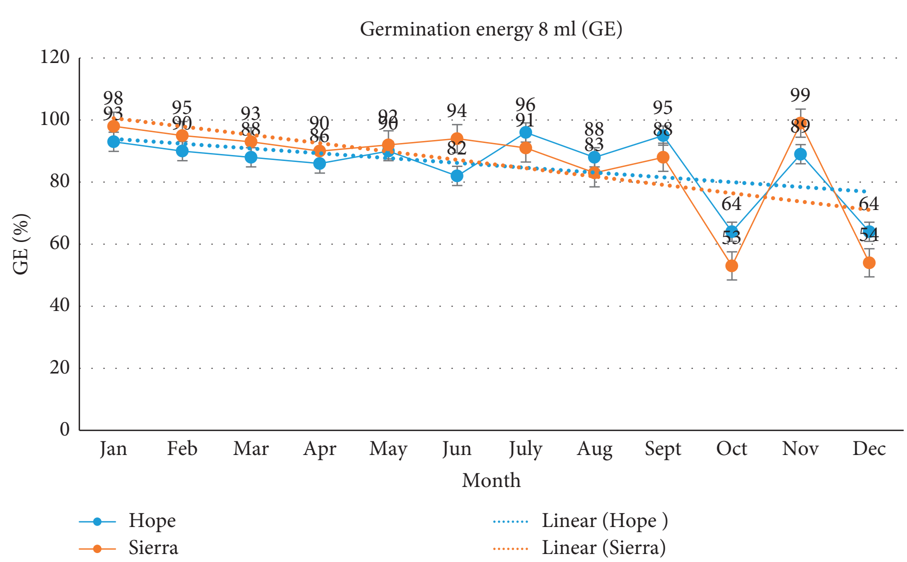

Figure 3: Comparison of germination energy (8 ml) for Hope and Sierra malting barley varieties over the 12-month storage period.

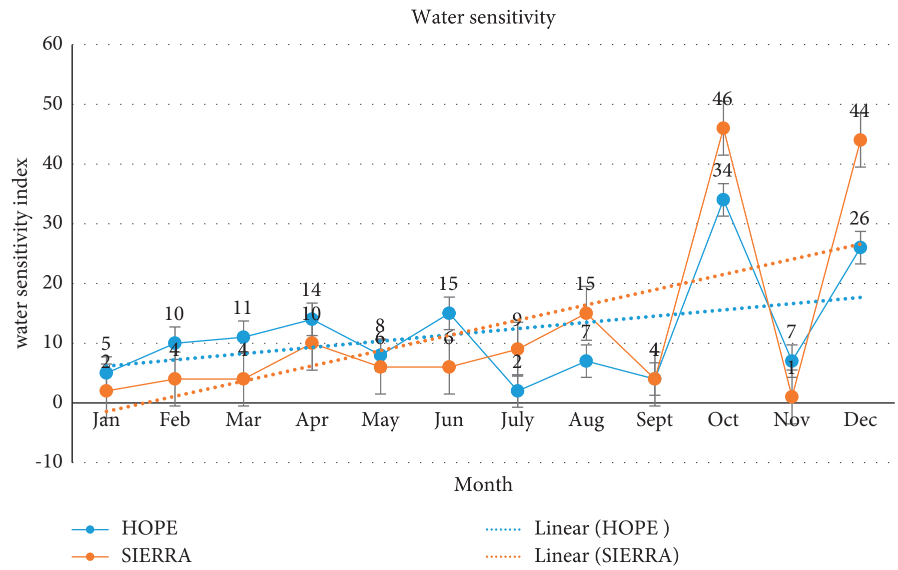

Figure 4: Comparison of water sensitivity trends for Hope and Sierra malting barley varieties over the 12-month storage period.

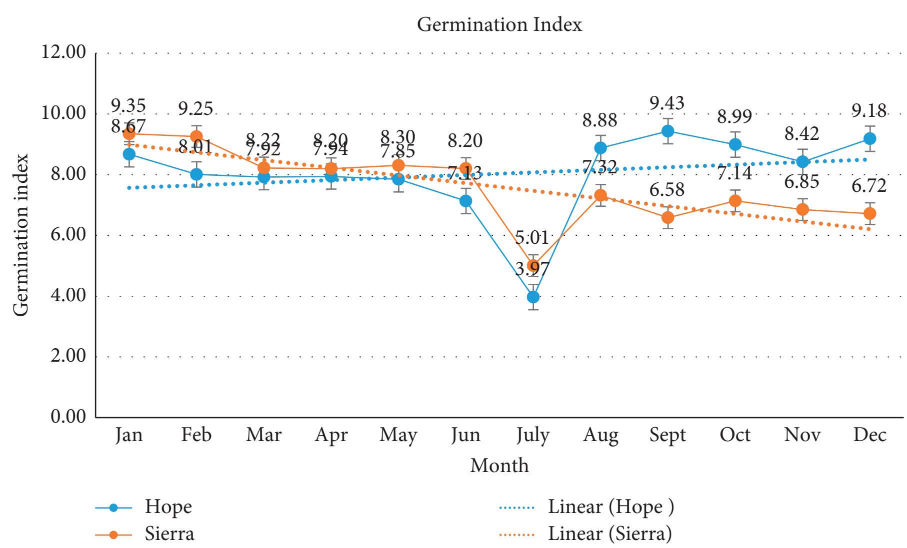

Figure 5: Comparison of the germination index trends for Hope and Sierra malting barley varieties over the 12-month storage period.

Figure 6 shows the progressive moisture content loss for Hope and Sierra varieties. The two varieties recorded a strong negative correlation with correlations of r 0.964 and r 0.953, respectively. During the storage period, the average moisture content for the two varieties decreased by 31.2%. For both varieties, the moisture content was within the acceptable limit of less than 14.5%, expected for barley varieties (European standard barley specifications).

For the protein content shown in Figure 7, both Hope and Sierra recorded a positive correlation with storage time. Sierra had a strong positive correlation (r: 0.82), and Hope had a positive correlation (r: 0.59). The protein content for both varieties remained within the acceptable limits (9.5%–10%) with Sierra (10.6%) having a slightly higher protein content than Hope (10.0%) throughout the storage period.

For screenings > 2.5 mm, the Hope variety had a higher kernel size than Sierra throughout the storage period (Figure 8). The Hope variety had an average percentage of kernel > 2.5 mm of 92.72, which was within the contract standard of >90%. However, Sierra had a lower percentage kernel size which was lower than the contract standard of >90%, although it was well above the lower acceptable limit of >85%. Hope with an r value of 0.289 had a weak negative correlation, while Sierra had a very weak negative correlation (r: 0.046).

For screenings <2.2 mm, both varieties changed in the index values but remained within the expected contract standard of 3% and much less than the threshold limit of>5% (European standard barley specifications). Hope with an r value of 0.631 had a moderately strong positive correlation,

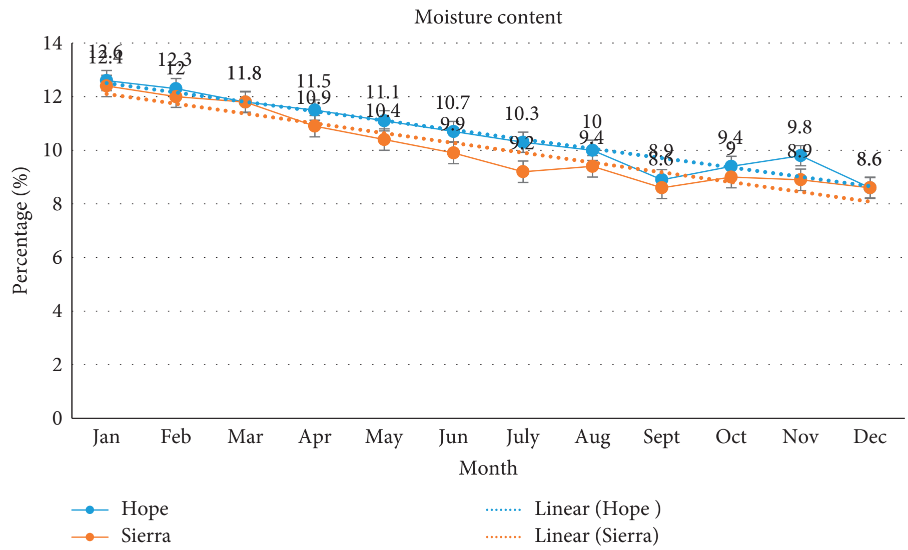

Figure 6: Comparison of moisture content trends for Hope and Sierra malting barley varieties over the 12-month storage period.

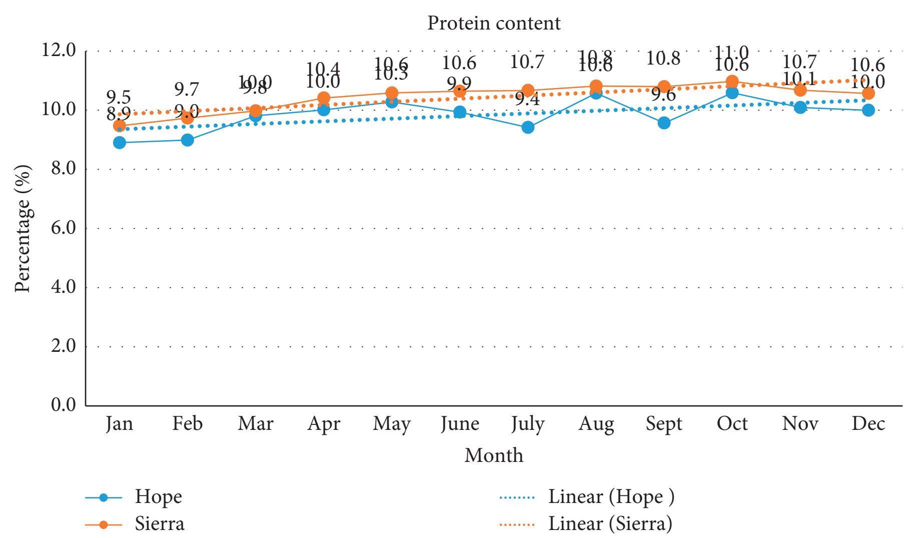

Figure 7: Comparison of protein content trends for Hope and Sierra malting barley varieties over the 12-month storage period.

while Sierra had a strong positive correlation (*r*: 0.786) as shown in Figure 9.

The insect damage index increased during storage (Figure 10), with both varieties having a moderate positive correlation with storage time. The insect damage index of Sierra showed a moderately strong positive correlation (*r* 0.522) and that of Hope showed an *r* value of 0.526. During the first month for the two varieties, the insect damage index had an average of 0.07%, but by the end of the study after twelve months of storage, the average had increased to 0.83%.

4. Discussion

4.1. Germination Capacity. Germination capacity for both Hope and Sierra varieties retained the desired score of above 98% throughout the 12 months (Figure 1). Germination capacity is one of the main properties which influence the quality properties of barley seeds in relation to the percentage of seeds that result in healthy and normally growing plants [7, 24]. It is normally described according to the class of barley. According to classes I and II, the germination capacity of barley should not be lower than 99% and 98% for class III [7, 25]. The germination capacity score indicates the likely potential of barley to germinate when exposed to the conditions necessary for germination. Thus, based on the germination capacity obtained in this study, barley could be categorized into class III. In this case, both varieties demonstrated desirable germination capacity, which is the ability to germinate or, in other terms, grain viability, for twelve months of postharvest storage.

4.2. Germination Energy. Germination energy of scores less than 98% were observed for both varieties (Figure 2). Both varieties had their extent of germination decreasing with an

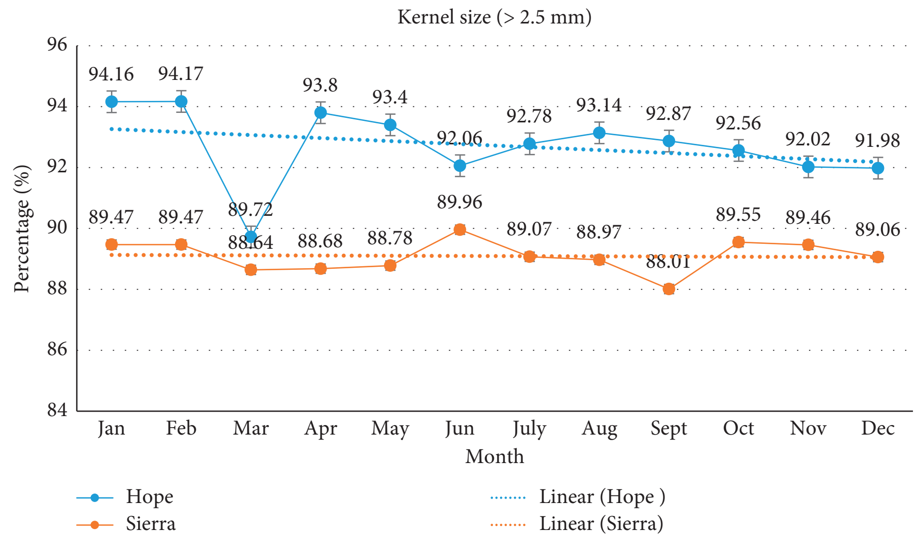

FIGURE 8: Comparison of >2.5 mm kernel size for Hope and Sierra malting barley varieties over the 12-month storage period.

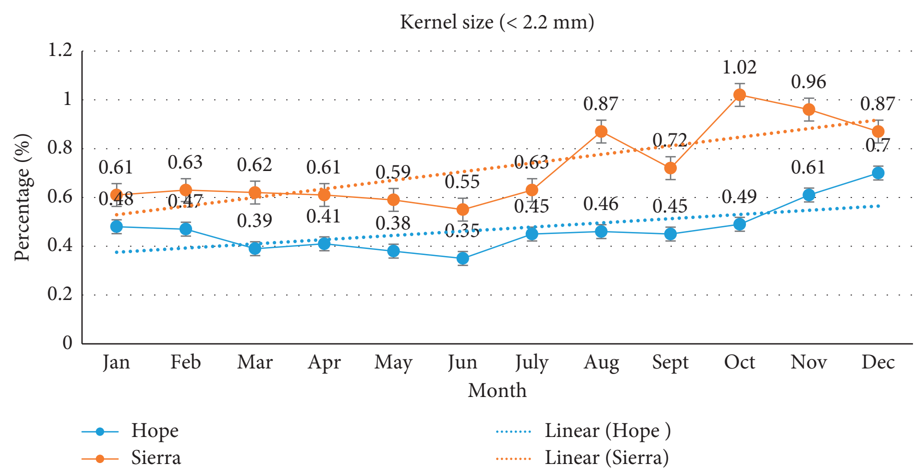

FIGURE 9: Comparison of <2.2 mm kernel size for Hope and Sierra malting barley varieties over the 12-month storage period.

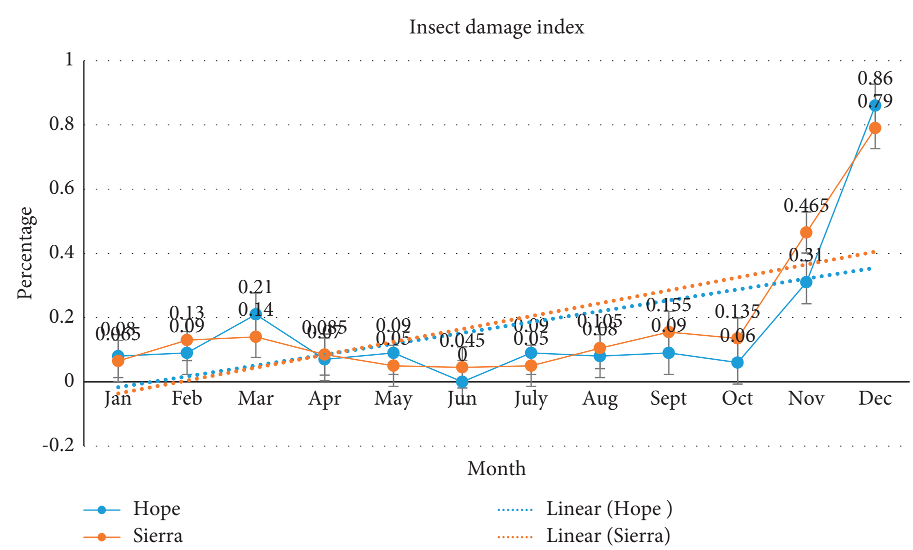

FIGURE 10: Comparison of the insect damage index trends for Hope and Sierra malting barley varieties over the 12-month storage period.

increase in storage time. The loss of the desirable ability to germinate was seen to be more pronounced in Hope, which had an r value of −0.216, than the r value of −0.588 for Sierra. Differences between the germinating abilities of the barley varieties are substantial. In addition to physiological differences and storage conditions, Briggs [8] also noted that differences in inherent varietal attributes (influenced mainly by the genetic makeup) also affect germination energy and recovery from dormancy. There are many other possible factors that may be influencing the changes in germination, enzyme production, and malt quality with barley storage. Changes in the rate and extent of water uptake, the quantity of endogenous hormones, and the aleurone response to hormones may all be associated with the observed changes during the storage of barley [1].

4.3. Water Sensitivity. The Sierra variety becomes more water-sensitive with an increase in the storage period than Hope (Hope, r: 0.378; Sierra, r: 0.588) (Figure 4). The sensitivity of the seed to water is assessed based on the effect of two volumes of water (4 and 8 mL) on the germination energy [6]. Barley water sensitivity is generally classified as low sensitivity (10%), higher sensitivity (11–25%), sensitivity (26–45%), and high sensitivity (over 45%) [7, 26]. As the grain ripens, the optimum amount of water for germination declines from 4 ml to about 3 ml/dish. In this study, at the end of the postharvest storage period, Sierra and Hope can be classified as sensitive, as evidenced by the values of 26% and 44%, respectively. The mechanisms primarily responsible for water sensitivity remain not clearly understood; however, microorganisms present in grains among other factors have been known to play a role [27]. Henceforth, the differences in the water sensitivity behavior of Hope and Sierra may be due to the structural differences in the properties of their pericarp [2].

4.4. Germination Index. The 4 ml germination energy samples showed that both malting barley Hope and Sierra varieties had their malting ability decreasing as storage time increased (Figure 5), but for the germination index, which is the measure of the rate of germination/germination vigour, the varieties performed differently. When the germination index is used as a physiological parameter in barley, it can help accurately distinguish the germination rates among barley varieties [28]. Hope had its germination index improved as storage time increased, implying an improvement in germination vigour of the variety with storage. The germination index for Hope increased by 5.9% from 8.67 to 9.18 during the storage period. Previous studies on barley varieties have also shown an increase in germination index with storage [1]. The increase in germination index during storage was closely associated with large increases in enzyme activities during malting. This observation could possibly explain the increase in germination index in the Hope variety in this study. However, on the other hand, the seed germination vigour for Sierra gradually diminished with time as indicated by the strong negative correlation (r: 0.739). Germination vigour significantly ($p < 0.05$) decreased with storage time. The differences in germination performance are common among different varieties due to inherent differences in genetic composition, grain physiology, and growing conditions [8].

4.5. Moisture Content. Moisture content is the parameter mainly used to assess barley storage stability. The moisture for both varieties generally showed a downward trend over the storage period. The decrease in moisture content is attributed to the atmospheric conditions in silos, in which the grain was stored. The moisture equilibration between the seeds and environmental air could have resulted in a decrease in moisture content as storage period progressed. Previous studies have documented well the increase or decrease in grain moisture in relation to relative humidity and temperature of storage air [29, 30].

4.6. Protein Content. Sierra has a naturally higher protein content (10.81) than Hope (10.19), and this difference was maintained throughout the postharvest period storage, with Sierra having a strong positive correlation (r: 0.702) and Hope a weak positive correlation (r: 0.383) for the protein content as shown in Figure 2. Of importance was the fact that the protein content for the two varieties for the storage period was within the expected standards. Higher protein content has been shown to increase the speed of germination of seeds [31].

4.7. Kernel Size. In a related study, Belay and Fetene [32] concluded that, as the moisture content of barley increases, the dimension of grain also increases. However, the apparent increase in % of small kernels (<2.2 mm) recorded in Figure 9 may be due to slight moisture reduction during storage, making the smaller kernels thinner. This was supported by findings observed earlier where there was a decrease observed in the moisture content of both varieties (Figure 6). A decrease in moisture content leads to shrinkage of barley kernels and a subsequent change in mass. It would have been expected that Hope, whose moisture content loss trend line indicates a stronger negative correlation (r: 0.966) than that of Sierra (r: 0.941), would have a screening index signifying a stronger positive correlation than Sierra. However, this was not the case in this study, and this can be chiefly explained due to the fact that Sierra as one of its varietal physiological characteristics is naturally less plump and any slight moisture loss will significantly amount to an increase in the percentage of screening (undersized grains).

4.8. Insect Damage Index. For the insect damage index, both varieties recorded moderate positive uphill correlations, meaning that insect infestation increased gradually with time, almost at the same degree in both varieties. The trend observed in both grains could be due to the fact that the varieties had the same initial moisture ranges and were stored under the same conditions. Degree of insect infestation is mainly attributed to moisture content and storage conditions [33]. Generally, grains with moisture content below 5% are not prone to fibrous micromycetes or insect pests [34]. Most damaging insects, including the granary weevils, which were observed in this research, have their growth restricted by moisture content below 9% [35]. The grain was stored at relatively safe storage moisture (less than 13%) to avoid a proliferation of microbes such as fungi and molds; however, the moisture was not low enough to hinder the growth of damaging insects such as weevils.

5. Conclusion

The barley varieties Hope and Sierra are both relatively stable and generally maintain desirable postharvest storage parameters under ambient temperature conditions. Both varieties maintain desirable germination capacity above the expected standard of 98% during storage. The germination capacities (4 ml) were above the expected limit for grain acceptance. However, the germination capacity (8 ml) decreased with extended postharvest storage. For water sensitivity, the varieties generally showed relatively low values for most of the twelve-month storage period with increases observed more at the end of the storage period. The germination index (germination vigour) of Hope improved with an increase in postharvest storage under ambient temperature conditions, while that of Sierra diminished. Hence, for brewers in Zimbabwe, the Hope variety is relatively more suitable for extended postharvest storage since it maintains a more desirable germination vigour performance than Sierra. The Sierra variety is more suitable than Hope for early malting postharvest stage as it has a higher germinative index within the first six months.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The work presented is an adaptation of the dissertation by Milford Mwazha which he did while studying at the Midlands State University.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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