

Full Length Research Paper

## Saved barley (*Hordeum vulgare*) seed quality in mid-altitudes and high-lands of Southern Ethiopia

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Samples of saved barley (*Hordeum vulgare*) seed were collected at sowing and threshing time from mid-altitudes and highlands of southern Ethiopia in 2009, and were examined in the laboratory and field condition to determine the status of seed quality. The seed samples included farmers' cultivar 'Horsiso' (two-rowed), 'Nuro' and 'Melo' (six-rowed). This study revealed that saved barley seeds were not vulnerable to insect pests. However, *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Helminthosporium*, *Penicillium*, *Trichoderma*, *Trichothecium* spp. and *Ustilago hordei* were detected. The seed samples collected at sowing showed lower standard germination (SG) and higher electrical conductivity (EC). All cultivars had SG above 80%, nevertheless field emergence index (FEI) showed 15.22 up to 37.35%, which is less than the ideal plant population. This indicates differences among cultivars in seed quality deterioration during storage. SG was positively correlated ( $r = 0.678$ ) with field emergence (FE); EC showed a negative correlation with FE ( $r = -0.347$ ) and SG ( $r = -0.233$ ). In conclusion, farmers' saved seed was found to be low in quality. Therefore, farmers' seed management practices need to be improved to retain the seed quality and enhance productivity.

**Key words:** Field emergence index, fungi, germination, seed health.

### INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important staple food crops in mid-altitudes and highlands of Ethiopia. Barley grain is used for the preparation of different foodstuffs in the country, such as malt products, *injera*, porridge, *qolo*; and local drinks, such as *tela*, *borde*, and beer. The straw is used as animal feed, especially during the dry season. It was cultivated on around one million hectares in the peasant private holdings in 2007/2008 in the main cropping season and the grain yield was 1376 kg per hectare (CSA, 2008). Barley is cropped twice a year: in the major season, which relies on June to September '*meher*' and March to May '*belg*' rainfall season. Diseases, insect pests, weed competition low-yielding varieties, and unimproved farming system are among the most important factors

that reduce grain yield of barley in Ethiopia.

The extent of the formal seed system, particularly for barley is negligible in mid-altitudes and highlands of southern Ethiopia. The previous report indicated that informal seed system in Ethiopia was estimated at 80 to 90% (Bishaw et al., 2008). Moreover, commercial seed producers, as businessmen, they are mainly looking for profits so that they focus on a hybrid open-pollinated and other profit-generating crops only. Hence, they may not satisfy the demand for seed of unprofitable crops. Therefore, the informal seed system needs to be supported by better farming practices, especially for self-pollinated crops like barley. In addition, farmers use traditional way of farming practices. For instance, farmers do not keep barley seed separately, but simply draw the

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seed from the large quantity of grain stored for food and sow such a seed by broadcasting. Thus, the status of saved barley seed quality in mid altitudes and highlands of southern Ethiopia is not known. A seed lot possesses a high field planting value may lose slowly or rapidly its planting value during storage depending on storage conditions (Thomson, 1979).

In general, informal seed system and traditional way of farming system might be among most determinant factors for the barley grain yield reduction in the study area. Thus, to prioritize the determinant factors for the barley grain yield reduction and take appropriate measures, determination of saved barley seed quality status is among crucial actions. Therefore, the present study was conducted in Bore and Sora districts in southern Ethiopia in 2009 to assess the status of saved barley seed quality.

## MATERIALS AND METHODS

Farmers' saved barley seed samples were collected from mid-altitudes and highlands of southern Ethiopia in 2009. The study was conducted in Bore district and Sora district. Formerly Bore district and Sora district were administered in the name Bore district. In this study Bore district refers both Bore and Sora districts. The altitude of the study area ranges from 1450 to 2900 m.a.s.l. The annual temperature ranges from 10.1 to 20°C. Barley (cultivar 'Nuro') is likely the dominantly grown crop in the district.

### Seed sampling

Criteria-based purposive sampling was used to select the peasant associations (PAs). Eleven PAs each in the highland and in the intermediate categories were selected based on the accessibility and the extent of barley production. Four farmers from each 10 PAs and five farmers from one PA a total of 45 farmers were randomly selected in the highlands of the district. Similarly, 45 farmers were selected from the intermediate agro-ecology of the district, making ninety randomly selected farmers in the district as a whole.

All barley cultivars in the study area were distinctly named and grown for food. The six rowed barley, named 'Nuro,' is the dominantly grown cultivar in the district. 'Horsiso' (two-rowed, mostly used as roasted grain, locally called (*qolo*) and 'Melo' (six-rowed) were the second predominantly grown cultivars in the highland and in the intermediate areas, respectively. Since *Nuro* was dominantly grown in both highland and intermediate agro-ecologies, it was selected for seed quality assessment along with *Horsiso* and *Melo*. But this cultivar was evaluated as *Nuro* (highland) and *Nuro* (intermediate) to differentiate the site of production. Thus, 23 seed samples of *Horsiso* and 22 seed samples of *Nuro* were collected from the 45 farmers selected in the highland area of the district. Likewise, 23 seed samples of *Melo* and 22 seed samples of *Nuro* were collected from 45 selected farmers in the intermediate area. *Nuro* cultivar seed samples collected from the highland and intermediate were separately bulked and analyzed. This was because the cultivar was grown at different agro-ecologies and also the seed management practices were expected to be different at different agro-ecologies. During seed sample collection, one kilogram of farmers' saved barley seed was taken from each selected PA and farmer. Seed sampling was made from different parts (store depths and points) of each seed container. A total of 90 kg seed samples (that is, 45 kg seed

from each of highland and intermediate agro-ecologies) were collected at the time of sowing. Similarly, the same amount of seed sample was collected, at the time of threshing, from farmers addressed earlier in each agro-ecology. Thus, a total of 180 kg of seed samples was collected in the 2009 main cropping season. The same cultivars were bulked and eight composite samples were made from two cultivars each from highland and intermediate agro-ecologies at the time of sowing and at threshing. The seed samples of the highland and the intermediate cultivars separately collected from both agro-ecologies at the time of sowing and threshing were also separately examined and analyzed. Sub-sampling for obtaining working sample was made using rotary seed divider after mixing completely.

### Insect pest assessment

Four replications of 100 seeds, each were taken at random as working samples and each was examined under the stereoscopic microscope for the presence of any insect pests. The injured seeds were counted and expressed in percentage.

### Disease causing fungi examination

Seed-borne microorganisms were examined using the seed washing method (ISTA, 2008). The working seed samples were immersed in water containing Tween 20 as a wetting agent, and were shaken with a mechanical shaker to remove fungal spores and hyphae intermingled with or adhering to the seed surfaces. The supernatant was then separated by centrifugation (3000 revolution per minute for ten minutes) and removed by pouring off and the extracted material (residue) was examined under the compound microscope.

Seed-borne microorganisms were also examined using the agar plate method (ISTA, 2008). Seeds were surface-sterilized in 10% solution of commercial sodium hypochlorite (NaOCl), laundry bleach, for three minutes and rinsed several times with sterile distilled water. Then 15 seeds were evenly placed on potato dextrose agar (PDA) in nine cm diameter Petri-dish and incubated at 25°C with 12 h alternating cycle of light and darkness for seven consecutive days. Then, the associated microorganisms were observed under the compound microscope and identified.

### Disease-causing bacteria examination

Twenty-five seeds were taken at random from each cultivar seed samples collected at sowing and threshing time. Seeds were surface-sterilized in 10% solution of commercial sodium hypochlorite (NaOCl) laundry bleach for five minutes, rinsed several times with sterile distilled water, placed on sterile paper towels to dry and incubated on PDA medium at 28°C for five days. Then the bacterial colonies were sub-cultured on standard media, namely nutrient glucose agar (NGA) and yeast extract-dextrose (YDC) containing CaCO<sub>3</sub>. Colonies were directly visually examined for their color and also used for further tests. King's medium B agar (KB) and D-1 agar were used as selective media. Gram-staining and anaerobic growth tests (Hugh and Leifson, 1953) were also employed to identify seed-associated disease causing bacteria at the genus level.

### Seed moisture content determination

Constant-temperature oven method was used to determine the moisture content (MC) of the seeds. All materials and procedures of

the International Seed Testing Association (ISTA, 2008) including 0.2% replicates tolerance was applied. The MC as percentage by weight was calculated to one decimal place for each seed sample using the formula:

$$MC = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

$M_1$  is the weight in grams to three decimal places of the container and its cover;  $M_2$  is the weight in grams to three decimal places of the container, its cover and seed sample before drying, and  $M_3$  is the weight in grams of three decimal places of the container, its cover and seed sample after drying.

### **The standard germination**

Farmers' saved barley seeds were tested to determine the maximum germination potential of farmers' saved barley cultivar seeds and to estimate the field planting value. Seed germination testing procedures and seedling evaluation were employed as specified by ISTA (2008). Four hundred seeds were randomly taken and seeded in four replicates (two paper towel rolls containing fifty seeds each making a replicate) with 100 seeds each. Seed samples collected at harvesting time were preheated at 30°C with free air circulation for seven days before placing under prescribed germination conditions to break seed dormancy. However, seed samples collected at sowing time were not preheated. The seeds were germinated between papers (BP) in towel rolls. The adjacent seeds were spaced sufficiently far apart. Towel rolls and the transparent plastic bags were loosely wrapped to allow sufficient air circulation around the seeds. The sown seeds were then incubated in germination cabinet at 20°C and 95% relative humidity for seven days. The plastic bags were illuminated from artificial light sources in the cabinet for eight hours per day.

Normal seedlings were daily removed starting from the fourth day. Badly decayed seedlings were removed but abnormal seedlings with other defects were allowed to remain on the substrate until the final count. Finally, seedlings were categorized into normal seedlings, abnormal seedlings, ungerminated seeds and dead seeds. The percentage of the number of normal seedlings, abnormal seedlings, ungerminated seeds and dead seeds were calculated to the nearest whole number. To check the reliability of a test result, the average percentage of the replicates were calculated and compared with tolerance ranges.

### **Electrical conductivity test**

Four replicates of fifty seeds each were soaked into a 200 ml Erlenmeyer flask containing 75 ml of distilled and deionized water. The flasks were covered with paraffin to reduce evaporation and placed in an incubator at 25°C for 24 h. The distilled and deionized water was used as a control for the test. The electrical conductivity of the seed leachates was measured to determine seed vigor using electrical conductivity meter and expressed as  $\mu\text{S cm}^{-1}\text{g}^{-1}$  according to seed vigor testing handbook (AOSA, 1983).

### **Field emergence index (FEI)**

All barley seed cultivars collected at the time of sowing and threshing from both agro-ecologies were used for field emergence test. Seed samples collected at the time of threshing were stored for three months until seeds fully developed. Four replications of one hundred seeds each were sown in the Haramaya University research field. Emerged seedlings were daily counted until no more

seedlings emerged. The field emergence index for each seed sample was calculated on the basis of the number of emerging seedlings following the procedure used by Egli and Tekrony (1995; 1996):

$$FEI = \frac{FE}{SG} \times 100$$

Where: FEI = Field emergence index; FE = mean seedling field emergence; SG = mean standard germination (SG).

The higher the value in FEI, the better the field conditions would be until FEI becomes equal to one, implying that it is ideal when FE is equal to SG.

### **Data analyses**

Statistical analysis system (SAS version 9.0) software of the general linear model (GLM) procedure was applied to calculate seed quality data. The results of the statistical analyses were declared significant at  $P = 0.05$ . Mean separation was carried out using the least significant difference (LSD). Analyses of correlation coefficients were calculated using statistical package for social science (SPSS) software version 16.

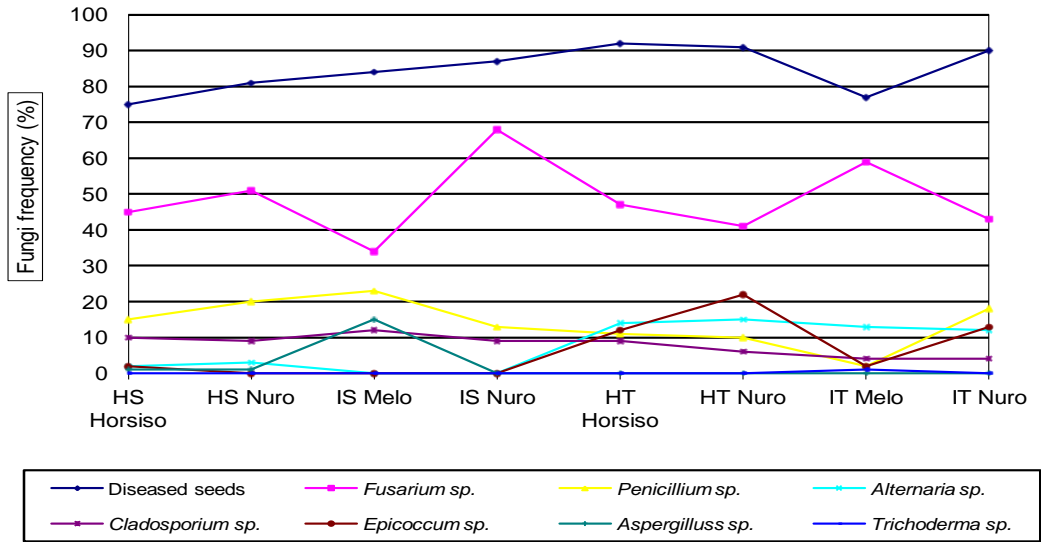
## **RESULTS**

The examination of seed for evidence of insect pests, pathogens and seed vigor elucidates the status of seed quality and performance of crop stand establishment in the field. Healthy seed with high physical purity, germination, and vigor implies the production of healthy seedlings, good crop stand establishment and thereby high yield. Prior seed health testing and other seed quality test results are indicators for the field establishment potential of the seed.

All examined saved barley cultivars seed samples revealed no evidence of insect pests infestation and seed damage. However, Fungi found the abundant microflora in tested barley seed samples. The fungi genera found at various levels of incidence in association with examined barley cultivars (Figure 1). The disease incidence difference could be due to differences in moisture content and seed management including differences in seed storage conditions.

*Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Helminthosporium*, *Penicillium*, *Trichoderma*, and *Trichothecium* spp. were seed microflora isolated from barley seeds (Figures 2 to 10). Plates of Microflora Isolated from Saved Barley Seed; plate size 6.25 by 6.25 centimeter.

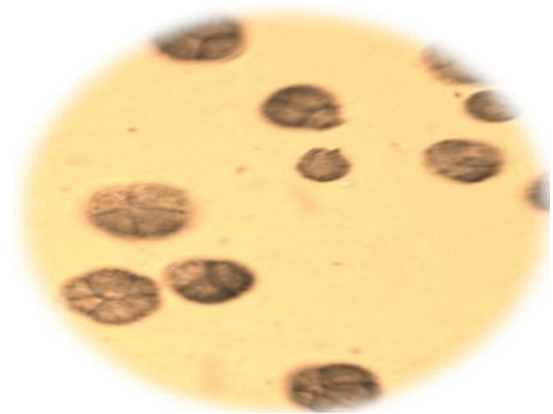
In addition, washings of all seed samples taken at sowing and threshing time revealed infection by *Ustilago hordei*, which is the cause of covered smut of barley. Seed moisture content determination revealed that all seed samples collected at the time of sowing had a moisture content below 12.5%, cited which is the maximum level in the Ethiopian seed quality standard (certified seed D class), whereas samples collected at



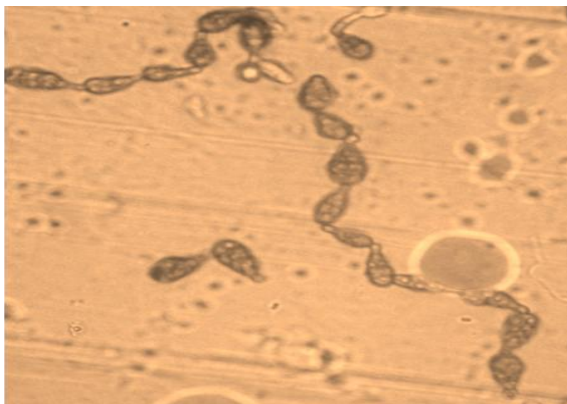
**Figure 1.** Incidence of fungi isolated from farmers' saved barley seed. HS and IS, samples collected at sowing time from highland and intermediate agro ecologies, respectively; HT and IT, samples collected at threshing time from highland and intermediate agro ecologies, respectively.



**Figure 2.** Microbes grown on agar plates.



**Figure 4.** *Epicoccum sp.*



**Figure 3.** *Alternaria sp.*



**Figure 5.** *Helminthosporium sp.*



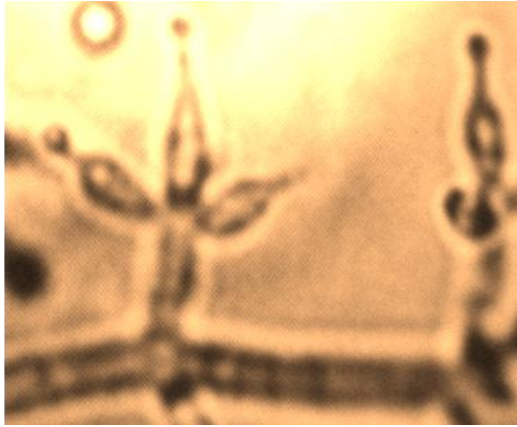


Figure 6. *Trichoderma* sp.



Figure 9. *Aspergillus* sp.

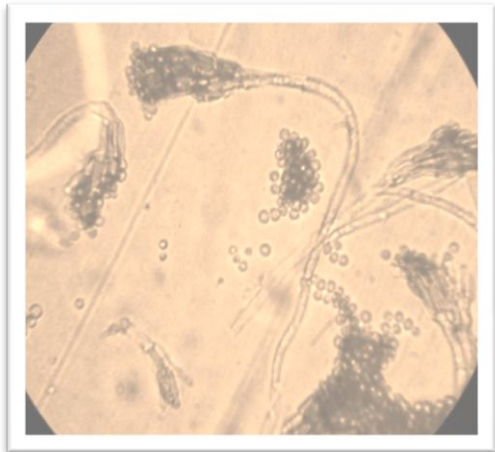


Figure 7. *Penicillium* sp.



Figure 10. *Fusarium* sp.



Figure 8. *Trichothecium* sp.

threshing time from both highland and intermediate agro-ecologies showed higher moisture content than the Ethiopian seed quality standard (Table 1). The more the seed moisture content, the more will be the seed quality deterioration.

## DISCUSSION

Among various genera of fungi isolated from farmers' saved barley seed, *Fusarium* was the predominantly found disease-causing microorganism (Figure 1). The co-occurrence of seed microflora was observed in all seed samples except in *Horsiso* and *Melo* cultivar seed

**Table 1.** Mean moisture content, germination and field emergence of farmers' saved barley seed.

Sample †	MC (%)	SG (%)	EC ( $\mu\text{ cm}^{-1}\text{ g}^{-1}$ )	FE (%)	FEI (%)
HS <i>Horsiso</i>	11.97 <sup>d</sup>	89 <sup>a</sup>	52.72 <sup>a</sup>	59.0 <sup>bc</sup>	66.29
IS <i>Melo</i>	11.31 <sup>f</sup>	83 <sup>b</sup>	30.90 <sup>cd</sup>	52.0 <sup>d</sup>	62.65
IS <i>Nuro</i>	11.61 <sup>ef</sup>	90 <sup>a</sup>	33.31 <sup>bc</sup>	76.0 <sup>ab</sup>	84.44
HT <i>Horsiso</i>	13.64 <sup>a</sup>	92 <sup>a</sup>	30.40 <sup>cd</sup>	65.0 <sup>c</sup>	70.65
HT <i>Nuro</i>	13.53 <sup>a</sup>	91 <sup>a</sup>	19.09 <sup>f</sup>	67.0 <sup>bc</sup>	73.63
IT <i>Melo</i>	12.61 <sup>c</sup>	90 <sup>a</sup>	27.12 <sup>de</sup>	58.0 <sup>dc</sup>	64.44
IT <i>Nuro</i>	13.02 <sup>b</sup>	92 <sup>a</sup>	23.39 <sup>ef</sup>	78.0 <sup>a</sup>	84.78
Mean	12.42	89.63	31.63	64.38	
CV (%)	1.09	3.48	10.13	9.94	
SE $\pm$	0.22	0.69	1.77	1.81	

Figures followed by the same letter in the column are not statistically different from each other at 0.05 probability level. † HS and IS, samples collected at sowing time from highland and intermediate agro ecologies, respectively; HT and IT, samples collected at threshing time from highland and intermediate agro ecologies, respectively; mean MC, moisture content; mean SG, Standard germination; mean EC, electrical conductivity; mean FE, field emergence; FEI, field emergence index; CV, Coefficient of variation; .SE, Standard error of means.

samples collected at the time of sowing, and *Nuro* (intermediate) collected at threshing time. Profuse development of fungi could be an indication that the seed is not of good quality (ISTA, 2008).

*Nuro* cultivar collected from intermediate agro-ecology at sowing and threshing time was positive for *Xanthomonas* and *Pseudomonas* spp. Similarly, *Melo* seed taken at threshing was found to be positive for same bacteria. This indicated that bacterial infection was not common in all barley seed samples as compared to fungal infection. This observation is consistent with the previous finding (Mundt and Hikle, 1976).

The seed moisture content above the standard could contribute to seed quality deterioration during storage time. This study showed that the higher the moisture content, the lower was the field emergence. This is due to increase in fungal growth under the moist condition (Harpper and Lynch, 1981).

The seed samples collected at sowing showed a lower SG and higher EC than samples collected at the threshing time when the same cultivar was considered. However, all seed samples had germination above 80%, which is the minimum seed quality standard of Ethiopia (Table 1). Nevertheless, SG does not always indicate the potential of seed lot performance in less optimal field conditions (Hampton and Tekrony, 1995). The higher EC value implies reduction in seed vigor due to an increment of membrane permeability (Vieira and Krzyzanowski, 1999). Samples of a cultivar collected at threshing time revealed better field emergence than samples taken at the time of sowing. Moreover, FEI of the cultivars showed 15.22 up to 37.35% less than the ideal plant population in the field (Table 1). This fact could be explained by the differences among cultivars in seed quality deterioration during storage.

In this study, it was found that SG was significantly and positively ( $r = 0.678$ ) correlated with FE, whereas EC

showed a non-significant negative ( $r = -0.347$ ) correlation with FE and SG ( $r = -0.233$ ). This result is consistent with previous reports on barley and other crops (Kim et al., 1989; Krzyzanowski, 1999).

In general, farmers' saved seed was low in quality because of high seed moisture content at the time of threshing and traditional seed management practices, leading to profuse growth of disease-causing fungi and bacteria. As a result, farmers' saved seed loses their germination and field emergence. This observation is consistent with seed germination reduction due to competition between micro-organisms and growing seed for oxygen (Mathews and Collins, 1975).

In conclusion, farmers' seed management practices required to be improved to reduce incidence of disease causing micro-organisms and seed infection, and thereby to enhance seed planting value and productivity. Further research required on seed storage methods and their contribution for the barley seed quality reduction.

## ACKNOWLEDGEMENT

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