Genetic Variation in Barley Enables a High Quality Injera, the Ethiopian Staple Flat Bread, Comparable to Tef

Addis Abraha, Anne Kjersti Uhlen, Fetien Abay, Stefan Sahlstrøm, and Åsmund Bjørnstad*

ABSTRACT

Injera, a thin flat bread and a staple in Ethiopia, is made from fermented dough primarily from tef [Eragrostis tef (Zuccagni) Trotter], Barley (Hordeum vulgare L.) and sorghum [Sorghum bicolor (L.) Moench] are cheaper sources for injera but are considered to be of inferior quality. This study investigates varietal differences in injera quality and the possibility to improve barley injera quality by breeding. Eleven barley varieties and tef were evaluated for injera quality through participatory sensory evaluations and flour compositional analyses. Significant differences in sensory injera quality were observed where at least two barley varieties (Haftusene and Himblil, released in 2011) were found to have injera quality equal to tef. Partial least squares regression was used to build models to predict injera sensory quality from pasting properties. These models allowed the separation of Haftusene and Himblil from varieties with lower quality. To investigate if the high injera quality of Himblil was heritable, it was crossed to the intermediate quality Saesa, and 14 F3:7 families were evaluated. The evaluation suggests transgressive segregation for injera sensory quality and flour properties. Some families matched tef in overall quality over four testing environments. The family S×H-T182, derived from the Saesa × Himblil (S×H) cross and officially released in 2012 as a high injera quality variety, is a major achievement for barley breeding in Ethiopia.

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Abbreviations: ⍺*, redness; β*, yellowness; L*, whiteness; PLSR, partial least square regression; PPO, polyphenol oxidase; RCBD, randomized complete block design; RVA, Rapid Visco Analyzer; S×H, Saesa × Himblil; TKW, thousand kernel weight; WAC, water absorption capacity; WSI, water solubility index.

Injera is a thin, flat leavened bread made from fermented dough of different cereals such as tef, barley, or sorghum and is the traditional staple food in Ethiopia (Gebrekidan and Gebrehiwot, 1982; Yetneberk et al., 2004). A good injera is soft, with uniformly distributed gas holes on its top surface and nonsticky top and bottom surfaces, is supple (rolls easily), and has a slightly sour taste (Parker et al., 1989; Yetneberk et al., 2004; Zegeye, 1997). The small gas holes on the surface of injera are due to the fermentation and baking process, facilitated by gas-producing Enterobacte-riaeae present in the dough and starter culture. As the pH of the fermenting dough falls below 5.8, other bacteria such as Lactobacil-lus spp. contribute to the slightly sour taste (Parker et al., 1989). The appearance, size, and distribution of gas holes on the injera surface and its taste and texture all impact the preference and acceptability of injera. Stone milling of grains for injera preparation can damage starch granules, depending on grain texture, hardness, and milling energy. More damaged starch granules have

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greater water absorption capacity (WAC) and are more easily hydrolyzed than undamaged granules, which can affect the pasting properties and quality of end products (Karkalas et al., 1992; Mariotti et al., 2006). There is no available information on the role of barley starch, starch pasting properties, protein, and β-glucan on barley injera quality or if varietal differences may exist.

In the household, injera is commonly baked and consumed while the next dough is fermented. Thus, the process of injera making is continuous. The initial step in the process is mixing sifted flour with water and knead well by hand to make a thick dough. Starter liquid saved from prefermented sourdough is added to enhance fermentation at room temperature for 3 to 4 d. Hot boiling water of the required amount is then added to the fermented dough and mixed to dilute the batter. Approximately 0.65 L of batter is poured on to a hot (168°C) clay griddle oven, covered tightly, and baked for 2.5 min. Commonly, injera is served with a variety of stews or sauces made from vegetables, pulses, meat, or their combinations.

Although tef grain is at least twice as expensive as other cereals, injera from tef is most preferred and consumed daily by the majority of people (Gebrekidan and Gebrehiwot, 1982; Parker et al., 1989; Yetneberk et al., 2004; Zegeye, 1997). This is due to its softer texture, preferred taste, and especially its color, ranging from purple to “very white,” the latter fetching at least 20% higher price that normal “white” types (personal observations). Recently, a successful “very white” tef cultivar, Quncho, has been released from a participatory varietal selection program (Assefa et al., 2011). Due to high domestic grain prices, exports of tef from Ethiopia are banned, but some fresh injera made from tef is being exported to the Ethiopian diaspora. Although this positively impacts foreign currency earning, it further increases the price of tef making injera unaffordable for low income citizens that depend on it as a daily staple. Considering the high price of tef grain in the local market and its low yield potential, searching for a less expensive grain such as barley as a substitute to make injera with comparable quality has become very important.

In the Tigray region in northern Ethiopia, barley is widely cultivated with an average yield of 1.4 t ha⁻¹ (CSA, 2009). It is the most dependable crop under low input and semiarid growing conditions, and farmers emphasize different traits such as grain yield, maturity, and value for injera and other end uses when choosing varieties (Abay et al., 2008). This has fostered an interest in developing barley varieties with improved injera quality. There are indications that injera quality may vary between cultivars. The recently released variety Himblil, for example, was selected for its superior quality injera. The origin of Himblil is interesting in that the wife of the farmer breeder, Mr. Kahsay Negash, noticed good injera quality in the landrace from which it was extracted (Abay et al., 2008; Abay and Bjørnstad, 2009). It is then of interest to validate these observations and to see if injera quality is heritable. For the latter end, Saesa, an early maturing and drought tolerant cultivar with acceptable injera quality, was crossed with Himblil, a late maturing, higher yielding, and high quality cultivar. There are no previous investigations of genetic variation in barley for injera making quality. Therefore, the objectives of the present study were to (i) compare a set of nine commonly grown barley varieties with Himblil and two known starch mutants (waxy and high amylose) for sensory injera and grain quality, (ii) evaluate 14 F₁₇ families from the Saesa × Himblil (S×H) cross, and (iii) analyze injera sensory quality in both sets of genotypes in relation to grain composition and physical grain quality.

**MATERIALS AND METHODS**

This report comprises the following three experiments all involving testing for sensory injera quality: (i) Exp. 1 in which 11 diverse barley genotypes and tef were tested in one location, (ii) Exp. 2 in which 14 F₁₇ families, parents, and tef were tested in one of the locations, the same as in (iii) Exp. 3, in which the quality of four selected F₃, families and parents grown in four locations were tested.

**Barley Varieties**

Two sets of barleys were evaluated in one group of representative barley varieties and 14 F₁₇ families from the Himblil × Saesa cross. In Exp. 1, nine barley varieties adapted to high- and midaltitude areas of Tigray (Abay, 2007) including Himblil (released in 2011) were grown in a field trial with two replicates in a randomized complete block design (RCBD) at Korem (altitude 2500 m and rainfall 649 mm) in southern Tigray during the 2008 main (summer) season. The plot size was 4 by 5 m (20 m²). Except for Demhay, all barleys were hulled, and their description is given in Supplemental Table S1. In addition, grain samples of two barley starch mutants, the high amylose line STS-211 and the waxy variety Cindy, were included in the injera tests. These grain samples were obtained from field trials grown at the Norwegian University of Life Sciences, Ås, Norway.

In Exp. 2, 14 S×H families developed to the F₃, generation, the two parents Saesa and Himblil, the check variety Sihu-may, and tef were used. The 14 F₁₇ families had been originally selected for agronomic performance on farm in 2009 but not for injera quality. All genotypes were grown in an off-season field trial in 2011 using a RCBD and two replicates at Adishehu in southern Tigray. A plot size of 3 by 4 m (12 m²) was used. The field was irrigated uniformly once every 2 wk until the plants reached the grain filling stage. Experiment 3 was conducted to confirm results from some of the lines used in Exp. 2 and grown at several locations during the main season. Three of the 14 S×H families, now candidates for variety release, were grown at four locations (Adishehu, Korem, Hagereselam, and Atsbì) during the main summer season in 2011 in 10 by 10 m (100 m²) single plots including the parents and a check (S×H-S82) in 2012.
in a circular motion from outside toward the center and covered with a tightly fitting lid. Two injera samples were baked from each dough type. The average baking time to make one injera was 2 min and 40 s at 168°C. The baked injera was taken out of the oven and placed on a flat tray to cool at room temperature and then evaluated within 2 h. In Exp. 2 and 3, the same injera preparation procedures were applied for the evaluation of lines in 2011 and 2012, all at Atsbi. Injera from tef obtained in local markets was always included for comparison. In Exp. 1 and 2, the same white tef sample was used; in Exp. 3, a different and purple-seeded tef sample was used.

Evaluation of Injera Sensory Quality
A total of 20 panelists (12 men and eight women, 10 at each site, from 36 to 72 yr old) participated in the injera evaluations (Fig. 2). They were informed in group discussions on how the sensory evaluation and scoring would be conducted. The injera samples were to be evaluated on a scale of 1 to 5 (1 represents very poor, 2 represents poor, 3 represents acceptable, 4 represents very good, and 5 represents excellent), one trait at a time. The range within a given sensory attribute was demonstrated and explained before the tests. Two injeras from each variety or family were then arranged on a flat tray on a table at ambient temperature (around 20°C). Individual evaluators were asked to take a small piece of injera sample twice and score the taste and then new samples were provided for mouth feeling, texture, top surface gas holes

Figure 1. Procedures in barley injera preparation.

Injera Preparation
For Exp. 1, two injera making experiments were conducted in 2010, at Maichew in southern and at Atsbi in eastern Tigray. The injera making process presented in Fig. 1 followed local procedures, with controlled uniform amounts of water, dough making, and baking. For uniformity, a single person prepared and baked the dough at each site. Flour (1150 g) from each variety and 1070 mL of water was used to make thick dough and the dough was thoroughly kneaded by hand for 6 min. At Maichew, 50 mL of prefermented sourdough of barley was added as a liquid starter to each sample to enhance fermentation while at Atsbi starter culture was not used. The dough was left to ferment at ambient room temperature (20°C). On the fourth day, 120 mL of water was added and thoroughly mixed to enhance fermentation and left overnight. On day five, 1200 mL of hot boiling water was added to the dough, which was thoroughly mixed and thinned to a uniform level. A batter of 650 mL was poured onto hot circular clay oven (60 cm in diameter, heated by firewood)
(size and distribution), color, suppleness, and overall ranking (as an independent score, not an average of the others). A bottle of natural spring water was provided to each evaluator for rinsing his or her mouth after scoring each sample. The response of each evaluator was recorded on a questionnaire sheet. Injera sensory evaluations in Exp. 1, 2, and 3 were performed in this manner.

**Physical Grain Analyses, Milling, and Flour Color**

Kernel hardness was determined in Exp. 1 using a single kernel characterization system (SKCS 4100; Perten Instruments NA) by analyzing 200 kernels from each replicate of barley. Thousand kernel weight (TKW) (in grams) was measured by counting a sample of 1000 kernels using a numerical seed counter. For milling each variety, 3 kg of grain from two replicates were separately cleaned, sun dried, and then milled using an attrition type (disc) stone mill. During the milling process maximum care was followed to avoid any mixing of flour samples. The flour samples were sieved uniformly with 1-mm sieve size before baking and lab analysis.

Flour color was measured using a Hunter LabScan XE spectrophotometer (Hunter Labs; http://www.hunterlab.com) based on the reading values L*, a*, and b* color system. The color reading values indicate degree of whiteness (L*), redness (a*), and yellowness (b*), respectively.

**Compositional Analysis of Barley Flour Samples**

Dry weight of the barley flour was determined in 2.5 g of flour using the Halogen HB43-S moisture analyzer (Mettler-Toledo Intl. Inc.) at 160°C for 7 min. Total starch was analyzed using the Megazyme total starch assay kit procedure (amylglucosidase and ρ-amylase method) as described by McCleary et al. (1994). The apparent amylose content was determined by iodine test as described by Knutson (1986) and expressed as percent of total starch. Damaged starch after milling was analyzed by enzymatic treatment of fungal ρ-amylase and amylglucosidase using the Megazyme starch damage assay procedure and expressed as percentage of flour weight.

Total β-glucan content was analyzed using Megazyme assay mixed linkage kit (Megazyme International Ireland Ltd.) based on the McCleary method (McCleary and Codd, 1991). Total N content was determined by the combustion method (Bremmer and Mulvaney, 1982) using an elemental analyzer, model CHN-1000 (Leco Corporation). Protein content was calculated by multiplying percent N × 6.25 and was expressed as percentage of dry weight. Ash content was analyzed according to AACC International method 08-01 (AACC International, 2000). Water absorption capacity and water solubility index (WSI) of the flour samples were measured by centrifugation method according to Anderson et al. (1969). Three grams of flour were dispersed in 30 mL of distilled water at 30°C in preweighted centrifuge tube and stirred constantly for 30 min in a vortex mixer. The slurry was centrifuged at 3000 × g for 15 min at room temperature (18°C) (Eppendorf centrifuge 5810 Hamburg). The supernatant was carefully poured into preweighted evaporating dish and dried at 135°C for 3 h in Heraeus drier (Kendro 63505). The same sample was used to calculate WAC and WSI. Water absorption capacity = weight of gel sediment (g)/weight of dry sample (g), and WSI (%) = water soluble matter (g) × 100/dry sample (g).

Starch pasting properties were measured using Rapid Visco Analyzer model RVA-4 (Newport Scientific Pty. Ltd.) according to AACC International recommendations (Croisbie and Ross, 2007). Flour samples (3.5 g on 14% moisture) were mixed with 25 mL of distilled water in a canister and using a stirring plastic paddle the suspension was mixed continuously during the programmed heating and cooling cycles of the Rapid Visco Analyzer (RVA). The standard profile (RVA standard version 1 [STD1]) was followed with Termocline for Windows 3 software (Newport Scientific Pty. Ltd.). The sample suspension was heated with initial temperature 50°C increased to 95°C over 6 min, held at 95°C for 3 min, and cooled to final temperature 50°C in 4 min. The following parameters were measured: peak viscosity (maximum hot paste viscosity), trough viscosity (the lowest viscosity after peak), breakdown viscosity (difference between peak and trough), setback viscosity (viscosity change from trough to final viscosity), final viscosity (viscosity at the end of the RVA run after heating and cooling phases), peak time, and pasting temperature.

**Data Analysis**

Analysis of variance was performed using GenStat Discovery Edition 3 (VSN International, 2012). In the model of data analysis varieties or entries were considered as fixed and replications as random effects. Sensory scores were analyzed as raw data (distributions were wider and less normal in the variety trial in Exp. 1 than among the progenies in Exp. 2 but unaffected by normalization). Simple correlations were analyzed using Minitab software (Minitab, 2011). Partial least square regression (PLSR) was used to analyze the relationships between the physical and chemical analyses (x variables) and injera sensory quality attributes (y variables) among the different barley varieties or entries. The x variables were divided in three sets: all variables, “pasting variables,” and “physical and chemical variables” (the ones not related to pasting). The data were standardized and analyzed using the Unscrambler X version 10.1 software (Computer-Aided Modelling, 2011). Results are displayed in biplots of scores and loadings for the first and second PLSR factors (Fig. 3A) or as regression plots (Fig. 3B). Only results obtained after full cross-validation are reported.

**RESULTS**

**Injera Sensory Quality**

**Variation in Injera Quality in a Diverse Set of Genotypes (Experiment 1)**

The two independent replicate evaluations in Exp. 1 gave consistent results ($r > 0.58–0.91$, $p < 0.001$) between the scores of sensory quality traits. The most consistent were overall ranking ($r = 0.91$), texture, taste and mouth feeling ($r = 0.89$), gas holes ($r = 0.77$), and color ($r = 0.68$) whereas suppleness showed least consistency ($r = 0.58$). This gave confidence that the method was reproducible and that panels were competent, in spite of the minor differences in

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and were similar to tef in most parameters (except color for Himblil scored much lower due to purple glume color; Haftusene and tef were white). (ii) A second group (Atsa, Burguda, Rie, Saesa, and Tselimoy) ranged from acceptable to very good quality (0.5–1 unit below the best). All had white injera color, except the black-seeded Tselimoy that

injera preparation. The reproducibility was also evidenced by the consistent rankings of Himblil, Saesa, and tef throughout all experiments (Tables 1, 2, and 3).

There were significant differences ($p < 0.001$) among the varieties. Overall four groups may be recognized: (i) Himblil and Haftusene had very good to excellent scores and were similar to tef in most parameters; (ii) A second group (Atsa, Burguda, Rie, Saesa, and Tselimoy) ranged from acceptable to very good quality (0.5–1 unit below the best). All had white injera color, except the black-seeded Tselimoy that
received a very low color score (2.0). (iii) A third group of lines, Demhay, Sihumay, and STS-211 was ranked as acceptable (overall rate 3.0) due to small nonuniform gas holes, medium to rough texture, low suppleness, rough to sticky mouth feeling, and slightly sour taste. Particularly Demhay was sensitive to baking heat and resulted in sticky texture, sour taste, and few gas holes.

As may be gleaned from Table 1, most of the sensory parameters were highly correlated ($r$ values from 0.88–0.95). The exception was color ($r = 0.14–0.41$). The correlations were slightly lower if tef was excluded. Without Cindy only texture and mouth feeling ($r = 0.92$), texture and taste ($r = 0.88$), and suppleness and taste ($r = 0.84$) remained in this range. The weakest correlation was gas holes and taste ($r = 0.62$) and no traits were related to color. These relationships were also evident in principal component analysis plots (not shown). Evidently, Cindy is a very different genotype and contributed to the strength of the correlations in Exp. 1.

### Variation in Injera Quality among Selected F$_{3:7}$ Families from a Biparental Cross (Experiment 2)

In spite of a narrower range in most sensory quality parameters, they varied significantly ($p < 0.001$) among the entries. The parents differed significantly in overall score, and in individual traits, the scoring was consistent with Exp. 1 with Himblil as the better parent except for color although not always significantly different. Transgressive values were observed in all parameters, in some cases significantly exceeding the better parent Himblil. In most cases the parental and progeny means were not or only marginally different. In overall score $S \times H$-T182, $S \times H$-S122, $S \times H$-S105, and $S \times H$-T155 excelled due to soft texture, smooth mouth feeling, slightly sweet taste, evenly distributed gas holes, and medium suppleness. The overall injera quality scores of these lines were comparable to tef and equal to or slightly better than Himblil. Tef, however, always ranked best in taste. Three of the best preferred lines have blue aleurone color while $S \times H$-S122 had a white aleurone, indicating little impact on sensory parameters. The lines $S \times H$-S106, $S \times H$-S82, and $S \times H$-S88 had low sensory scores (Table 1).
The relationships between the parameters differed markedly in the S×H families from genotypes in Exp. 1. While taste was positively correlated with mouth feeling ($r = 0.73$) and suppleness ($r = 0.71$), the other relationships were weaker. Even if Cindy was excluded, the correlations were stronger. From Table 1 it may be easily seen that even genotypes such as Demhay or Tselimoy exceeded the range in Exp. 2.

**Variation in Injera Quality across Environments (Experiment 3)**

The average ranking of genotypes in this experiment (Table 2) was consistent with the previous experiment and revealed significant differences ($p < 0.01$). The families S×H-T182 and S×H-S105 again surpassed Himblil in overall sensory score and matched tef. The S×H-T182 family was considered equal to tef in taste and excelled particularly in color where tef received an unusually low score due to the use of a purple variety. Most importantly, the marked environmental differences did not impact sensory quality (right side of Table 3), which is promising in terms of injera quality of Himblil and the further breeding for injera quality. This is supported by the consistent differences between Saesa and Himblil across years (seasons) in Table 1.

**Modeling Injera Quality from Physical and Chemical and Sensory Traits**

**Experiment 1**

The barley varieties differed significantly in most traits including glume and aleurone color and row type (Supplemental Table S1). The former affected flour color and the latter impacted TKW and kernel hardness ($p < 0.001$). The hulless Demhay had the hardest kernels. In general, two-rowed varieties had lower kernel hardness than the six-rowed types, except Rie. There were also significant differences ($p < 0.01$) in total starch, amylose, $\beta$-glucan, protein content, damaged starch, and WSI. Total starch varied from 51.2 to 59.7%, lowest in Himblil, Haftusene, and Saesa, which were also lowest in WSI. The high amylose variety STS-211 behaved as expected and was also high in $\beta$-glucan and protein content. The waxy Cindy had (as expected) the lowest levels of amylose but was intermediate in terms of waxiness. It was also on top in $\beta$-glucan, protein content, WAC, and WSI.

Starch pasting (viscosity) properties also differed significantly ($p < 0.001$) (Supplemental Table S2). Atsa, Burguda, Himblil, and Saesa had higher peak viscosity, trough, breakdown viscosity, setback, and final viscosity than the other varieties whereas Cindy, Demhay, Sihumay, and STS-211 were at the other end. Tef had the highest starch, damaged starch, and ash but the lowest $\beta$-glucan content and WSI. Its pasting properties were in the lower range of barley varieties although not as deviating as Cindy.

With a few exceptions, most traits showed weak or no correlations. Protein was positively correlated ($p < 0.05$) with $\beta$-glucan, WSI, and starch. Thousand kernel weight had a negative correlation ($p < 0.05$) only with kernel hardness and damaged starch but was positively correlated with most of the starch pasting traits. The later showed significant ($r > 0.8–0.9$, $p < 0.01$) positive correlations and with the sensory quality traits except pasting temperature (much weaker). These had no correlation with color. Deleting tef or Cindy made no major difference in this regard.

Relationships among the physical and chemical and/or pasting properties (as the observable variables $x$) and sensory variables (as the predicted variables $y$) were assessed by PLSR. The pasting properties could be used to predict sensory quality reasonably well (a cross-validated $R^2$ of 45.8%) (Fig. 3A), separating the acceptable (Demhay, Tselimoy, and STS-211) and the unsuitable Cindy from the very good or excellent. The predictions were best for taste ($R^2 = 82.9\%$) (Fig. 3B) and acceptable for texture and did not work for color. However, no

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**Table 2. Sensory injera quality parameters of the four selected F$_{3:7}$ families and parents from the Saesa × Himblil (S×H) cross (Exp. 3).**

<table>
<thead>
<tr>
<th>Variety</th>
<th>Surface gas holes</th>
<th>Color</th>
<th>Texture</th>
<th>Mouth feeling</th>
<th>Injera taste</th>
<th>Suppleness</th>
<th>Overall rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>S×H-T182</td>
<td>4.3a†</td>
<td>4.4a</td>
<td>4.4a</td>
<td>4.1a</td>
<td>4.5a</td>
<td>4.1a</td>
<td>4.6a</td>
</tr>
<tr>
<td>S×H-S106</td>
<td>3.6bc</td>
<td>3.8bc</td>
<td>4.1ab</td>
<td>3.7a</td>
<td>3.8b</td>
<td>3.7bc</td>
<td>3.8bc</td>
</tr>
<tr>
<td>S×H-S105</td>
<td>3.8bc</td>
<td>4.2ab</td>
<td>4.2a</td>
<td>3.8a</td>
<td>4.1ab</td>
<td>3.9ab</td>
<td>4.3ab</td>
</tr>
<tr>
<td>S×H-S82</td>
<td>3.3c</td>
<td>3.3cd</td>
<td>3.5bc</td>
<td>3.5b</td>
<td>3.6c</td>
<td>3.5c</td>
<td>3.5c</td>
</tr>
<tr>
<td>Himblil (parent 1)</td>
<td>4.0ab</td>
<td>3.1d</td>
<td>4.2a</td>
<td>3.8a</td>
<td>4.1ab</td>
<td>3.7bc</td>
<td>4.1b</td>
</tr>
<tr>
<td>Saesa (parent 2)</td>
<td>3.5c</td>
<td>3.6c</td>
<td>3.8b</td>
<td>3.6a</td>
<td>3.7b</td>
<td>3.5c</td>
<td>3.7bc</td>
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<tr>
<td>Mean</td>
<td>3.75</td>
<td>3.73</td>
<td>4.03</td>
<td>3.75</td>
<td>3.97</td>
<td>3.73</td>
<td>4.00</td>
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<tr>
<td>LSD (0.05)</td>
<td>0.35</td>
<td>0.36</td>
<td>0.36</td>
<td>0.40</td>
<td>0.43</td>
<td>0.27</td>
<td>0.40</td>
</tr>
<tr>
<td>CV (%)</td>
<td>6.2</td>
<td>6.6</td>
<td>6.1</td>
<td>7.2</td>
<td>7.3</td>
<td>4.9</td>
<td>6.7</td>
</tr>
<tr>
<td>Tef§</td>
<td>4.1</td>
<td>3.4</td>
<td>4.3</td>
<td>4.3</td>
<td>4.5</td>
<td>3.8</td>
<td>4.5</td>
</tr>
</tbody>
</table>

†Mean data from the four sites: Adishehu, Korem, Hagereselam, and Atsbi.
‡Means followed by different letters are significant at these probability levels: a indicates $p < 0.05$, b indicates $p < 0.01$, c indicates $p < 0.01$, d indicates $p < 0.001$, and e indicates $p < 0.0001$.
§Tef is included as a check.
model was able to predict differences among those with or close to very good quality. Deleting Cindy did not affect the relationships when only pasting properties were included while prediction based on physical and chemical traits depended entirely on including Cindy. When all x variables were included in the model, 69.2% of the taste variation could be explained. The variables total starch, peak time, and trough accounted for 59.3%. In all cases, only one principal component was significant. In other words, it appears possible to identify poor and acceptable quality barleys by some of the dough pasting properties.

**Experiment 2**

The parents in the S×H cross showed a number of differences compared to Exp. 1 (Supplemental Table S3). Most pronouncedly, the parental differences and level in TKW and total starch were much greater. Himblil was high in β-glucan, as before, but the superiority in protein content was not significant. The tef sample was the same as before, and the differences from Table 1 indicate sampling variation, notably in pasting time. However, its distinctness from barley was largely similar. The most conspicuous features of the Supplemental Table S3 are the significant differences between the F3:7 families and the high frequencies of transgressive values in most traits. Except for damaged starch, most F3:7 families exceeded the better parent. This is also reflected in the parental vs. progeny mean differences. Of particular interest are the wide ranges within the row types. Most of the two-rowed lines had higher TKW, β-glucan, and protein, except for the six-rowed lines S×H-S122 and S×H-S126 that had high values exceeding some two-rowed families. The differences in flour color reflect the segregation of grain color genes.

The starch pasting properties (peak viscosity, trough, breakdown, setback, final viscosity, peak time, and pasting temperature) showed highly significant differences \(p < 0.001\) among the S×H families but not between the parents, suggesting transgressive segregation. The range was clearly lower than in Exp. 1.

Correlation analyses of physical and chemical traits in the S×H families (excluding parents and tef) showed that protein was positively correlated with β-glucan \((r = 0.89, p = 0.0001)\), TKW \((r = 0.70, p = 0.002)\), and WSI \((r = 0.91, p < 0.001)\) but negatively correlated \((r = -0.83, p < 0.001)\) with WAC.

Most pasting traits were moderately or weakly correlated, except those between peak and final viscosity and peak viscosity and trough. Again pasting temperature and color were not correlated with other parameters. In other words, the strong positive trait correlations among the varieties included in Exp. 1 were not present in the progeny families. The same applied to PLSR analysis. The sensory data \((y)\) were poorly predicted by the physical and chemical as well as pasting properties.
Experiment 3

Average values of the families and the parents confirmed the outstanding TKW of S×H-T182 as well as improvements in total and damaged starch and β-glucan while there were no differences in protein content and flowering time (Supplemental Table S4). Himbil had the highest grain yield (3539 kg ha\(^{-1}\)) followed by S×H-T182, but the remaining lines and Sae.sa did not differ in grain yield. Water absorption capacity and WSI showed no variation in Exp. 3. Relatively narrow differences were observed in most of the parameters may be due to the few selected candidate varieties in this experiment. Starch pasting properties also varied significantly (\(p < 0.05\)) among the selected lines in peak viscosity, trough, setback, and final viscosity. Notably, pasting temperatures were closer to the data in Exp. 1. This also applied to most traits in tef although the distinctness from barley remained.

DISCUSSION

This study demonstrates that barley has a potential to supplement tef as the main ingredient in the national staple bread of Ethiopia. To realize this potential the traits involved need to be incorporated in breeding programs. This again requires accurate screening techniques and genetic variation in the breeding gene pool.

This study has also shown that screening may be achieved through participatory methods involving end users. Our results were highly repeatable and the participants in our project quickly identified the merits of S×H-T182. Its release as a variety in 2012 was based on data presented here (and agronomic data not included).

Tef currently fetches at least twice the market price of barley. In comparative agronomic trials (CSA, 2009) barley yields 27% more than tef although they often fill different agroecological niches. That Himbil and S×H-T182 may fetch an additional price premium as a “tef quality barley” is not unlikely if the popular preconceptions about barley for injera can be overcome.

Whole scale village sensory testing is an expensive way of screening samples in a breeding program. On the other hand, PLSR analysis presented in Exp. 1 suggests that low injera quality genotypes may be discarded by screening for pasting properties of flour. This approach should be validated in a larger set of genotypes. To separate the acceptable from the “very good” and excellent injera quality the traditional method of injera baking must be used. For breeding, a screening technique for up scaled sensory testing based on small size injeras should also be developed.

The lack of predictive ability in barley genotypes with injera quality scores from 3.5 to 4 and above calls for a closer analysis of the fermented products and the fermentation process. This may open the possibility to replace sensory testing by further compositional information. The lack of predictive ability of the examined pasting or grain quality parameters can also be inferred from our tef data. In general, tef had pasting quality values like the acceptable barley genotypes Demhay or STS-211. Although the consistently higher levels of total and damaged starch are noteworthy, barley may have similar injera scores.

Although there are no previous studies on barley injera quality, our results are in agreement with the findings of Yetneberk et al. (2004) on sorghum varietal sensory quality or for the Sudanese sorghum flat bread Kisra (Ejeta, 1982). These studies also indicated that sensory quality showed predominantly varietal differences, with less impact from environmental factors.

In injera from tef (Parker et al., 1989), starch plays a major role in the formation of surface gas holes. Poor quality injera with less gas holes were also reported in some sorghum varieties (Gebrekidan and Gebrehiwot, 1982; Yetneberk et al., 2004, 2005). They indicated that the poor quality injera in sorghum was due to sticky texture, a bitter taste, and high tannin content. The authors of the studies involving sorghum did not mention if the varieties were waxy or not. In this study, Cindy’s unsuitability for injera may be due to the higher water holding capacity and the stickiness of waxy starch. Therefore, this poor quality implies that waxy barley varieties, in general, may not suitable for injera. The same may be true for high amylose barleys such as STS-211. In any case, stocks such as Cindy or STS-211 may be valuable in later research to identify factors that affect injera quality.

Although variation in injera color was significant, the overall quality ranking was less affected by color differences. Rather, injera texture, taste, mouth feeling, gas holes (size and distribution), and suppleness were given more focus by the evaluators. In spite of its purple glumes, injera from Himbil was rated as very good or excellent by most of the evaluators. The limited importance attached to color was confirmed by the high scores of the black colored injera made from variety Tselimo, similar to white colored injera genotypes. Black-seeded barley varieties are preferred for malting and local drinks due to the color (Abay et al., 2008; Eticha et al., 2008). This does not imply, however, that color may not become more important. Now that pure white barley for injera has become available with the release of S×H-T182, it may become as valuable as the most expensive tef. The activity of polyphenol oxidase (PPO) and total polyphenol content has been reported to significantly correlate with discoloration potential in barley grain products (Quinide-Axtell et al., 2004). Polyphenol oxidase activity also causes undesirable darkening and discoloration of wheat (Triticum aestivum L.) noodles (Baik and Ullrich, 2008) where He et al. (2007) have developed functional markers for this high and low PPO. Therefore, PPO effects on barley injera should be investigated in further studies.
That two of the nine local genotypes tested in Exp. 1 had tef-like quality is very promising and indicates that high injera quality may be frequent in barley. The same is indicated by the analysis of the 14 F₃:₇ families from a Himblil × Saesa cross. Although these families were selected for agronomic merit from a total of 198 families, their outstanding performance calls for a closer genetic analysis. A recombinant inbred line population for quantitative trait loci mapping of various traits has been developed and the production of mapping data is under way. From the present data, injera quality appears to be a highly heritable trait. The families, although theoretically >99% homozygous, contained considerable heterogeneity, which must have blurred the sensory differences, for example, color. Since one quarter of the total additive variance is expected to be within families (Mather and Jinks, 1983), the range expected in recombinant inbred variance is expected to be within families (Mather and Jinks, 1983), the range expected in recombinant inbred lines will be greater than that observed in the F₃:₇ families. The differences between family and parental means in a number of grain quality traits further indicate that additive × additive epistatic interactions may play a role in the transgressive patterns observed (Longin et al., 2012). This indicates that the parents come from distinct gene pools. Indeed, the highland late maturing variety type is represented by Himblil and the early drought prone lowland two-rowed landrace Saesa represents varieties adapted to the two major agroecologies of Tigray. According to farmer observations, two-rowed barleys are early whereas six-rowed types are late. This has, therefore, aroused keen interest in the relatively early six-row families in the S×H cross plots. The recently released variety S×H-S106 is such a genotype. As shown by Tshaye et al. (2012), the S×H cross is not transgressive when it comes to earliness, but the row type has much less influence on the earliness than the phenotypic impression given by currently grown cultivars. Thus, Mendelian genetics in well adapted germplasm may have much to offer for farmers in Tigray.

**Supplemental Information Available**

Supplemental material is available at http://www.crops.org/publications/cs.

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**References**


