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In-Depth Field Characterization of Teff [*Eragrostis tef* (*Zucc.*) *Trotter*] Variation: From Agronomic to Sensory Traits

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Abstract: Teff is an important food crop that serves to prepare Injera-flat-bread. It is cultivated worldwide and is particularly susceptible to lodging. A diverse collection of teff [*Eragrostis tef* (*Zucc.*) *Trotter*] populations was characterized for a wide range of traits, ranging from agronomic to final Injera sensory parameters, under well-irrigated Mediterranean spring conditions. The populations tested were collected from single plants presenting lodging resistance at the site of collection and their traits were characterized herein. An early type of lodging was observed, which was most likely triggered by a fast and sharp inflorescence weight increase. Other populations were 'strong' enough to carry the inflorescence during most of the grain-filling period, up to a point where strong lodging occurred and plants where totally bent to the ground. Three mixed color seed populations were established from a single plant. These were separated into 'white' and 'brown' seeds and were characterized separately under field conditions. The newly 'brown' populations appear to be the result of a rather recent non-self (external) airborne fertilization from a dark pollen donor. Some of these hybrids were found to be promising in terms of Injera sensory traits. The population of these studies might serve as breeding material. Integration between a wide range of parameters and the correlations obtained between agronomic and sensory traits might improve our ability to breed towards a "real world" better end-product.

Keywords: crop breeding; lodging-resistance; teff [*Eragrostis tef* (*Zucc.*) *Trotter*]; injera; sensory evaluation; productivity; chlorophyll

1. Introduction

Teff [*Eragrostis tef* (*Zucc.*) *Trotter*], commonly referred to as teff, is an annual self-pollinated, allotetraploid ($2n = 4x = 40$) warm season crop belonging to the Poaceae (grass) family [1,2]. It is a major food crop native to Ethiopia and Eritrea for the production of a range of traditional foods and beverages including Injera (flatbread).

Teff is a C_4 plant that has high chlorophyll a/b ratios and utilizes CO_2 very efficiently during photosynthesis. Teff is adapted to a range of growing environmental conditions [3]. Teff grain also presents excellent storage properties. Therefore, it plays an important role in food security in Eastern Africa and in combating global climate change [4].

In recent years, teff is becoming popular in the health-food markets of developed countries due to its attractive nutritional properties and gluten-free nature. The inability to separate the bran from the seed makes teff flour rich in fiber and thus has health benefits as an anti-oxidative and improves the hemoglobin level in the human body [4,5].

Despite teff's versatility in adapting to extreme environmental conditions, teff is susceptible to lodging, which can drastically reduce yield and grain quality, and complicates harvesting [5]. Lodging can limit productivity directly by reducing the photosynthetic capacity due to changes in sun/shade architecture. Lodging also limits the use of high input Nitrogen fertilizer to boost yield.

Lodging is a process through which the shoot cereals are displaced from vertical orientation (upright position) and settle in a permanent horizontal position [6]. It is a complex phenomenon that is influenced by many factors, including wind, rain, geography, landscaping, soil type, crop history, agricultural system, and disease [5].

Stem lodging results from bending or breaking of the lower culm internodes, and root lodging results from a failure in root soil integrity [7]. Lodging is worsened by the use of fertilizers, reducing the yield potential of teff. The problems of lodging can be reduced by decreasing plant height, however, yield might be reduced when plants are shortened too much with dwarfing genes or plant growth regulators [5]. Hence, it was suggested to target traits other than height for further improvement in lodging resistance in teff.

Teff has weak stems that easily succumb to lodging caused by wind or rain [1]. Various attempts were made to develop lodging-resistant teff cultivars but presently no cultivar with reasonable lodging resistance has been obtained [1]. Despite lodging being the greatest cause for yield loss in teff, its genetic and physiological control is understudied in terms of molecular breeding techniques and biotechnology [5].

Ethiopia is teff's origin and the center of its biodiversity, harboring landraces with a wide array of phenotypic diversity, wild progenitors, and related wild species. The genetic diversity of teff is represented in a very large collection of accessions [8] from across its cultivation range at the Ethiopian Institute of Biodiversity. There has been a major increase in the collection size over the last decades, which demonstrates the presence of both a wide diversity of germplasm in Ethiopia, as well as the commitment of institutes and individuals to collect and preserve these germplasms for future use [1].

The genetic diversity in teff was also discovered by using a range of molecular markers [4] and its genome has been sequenced [9]. Great genetic diversity in yield, lodging index, and stem strength related traits was recorded in teff [10,11].

Phenotypic variability in teff was recorded in—grain yield, grain color and size, days to panicle emergence, days to maturity (21 to 81 and 50 to 140, respectively), number of grains/plant (9000–90,000), plant height (20–156 cm), number of tillers/plant (5–35), and culm diameter (1.2–5 mm) [10,11].

Teff breeding should target the improvement in the following traits—grain yield, shoot biomass, lodging resistance, grain size and color, grain coat properties, nitrogen-use efficiency, osmotic adjustment root depth, tolerance to drought, salinity, and acidity, nutritional values, physicochemical, and palatability [1]. Variability for the culm internode diameter is a key factor for improved lodging resistance [4].

Overall, there is a limited amount of research on the genetic basis for the processing and nutritional quality of teff and its components as food [11]. Therefore, it is necessary to assess the genetic diversity of this crop for potential improvement of agronomic as well as food-processing traits [4].

The preparation of Injera, the Ethiopian sourdough type flat bread, involves fermentation processes of the teff flour [12]. The fermentation preparation consists of two stages of natural fermentation, which last for about 24 to 72 h, depending on ambient temperatures [13]. Good quality Injera will have uniformly-spaced honeycomb-like "eyes" or holes, and no blind spot (flat area with no holes) on its surface. The major factor that decrease Injera quality result from inadequate fermentation. Good quality Injera becomes soft and pliable in texture, which enables the consumer to wrap and pick up sauce in the Injera with fingers [5]. In Ethiopia, people prefer their Injera to be white [5].

Texture is determined by touch and refers to the degree of fluffiness, roughness, smoothness, hardness, or softness.

Four phenotypes of seed coat color (grain color) were documented in teff—dark brown, medium brown, yellowish-white, and grayish-white. However, the dark and medium brown are difficult to differentiate so they are both included as brown. A duplicate gene pair is known to be involved in seed color inheritance, with simple dominance and additive gene effects. Tests of independence showed that lemma and seed color are inherited independently [14].

There is scarce documentation on seed size and seed coat in teff [1]. Depending on the varieties, the color of teff grain can be ivory, light tan to deep brown or dark reddish-brown to purple [1,5]. Based on people's preference for their consumption, white teff is the most expensive, while in terms of benefit, red teff is more nutritious and gains acceptance by the health-oriented consumers in Ethiopia and worldwide [5].

Different teff varieties have different mineral concentrations. Red teff has a higher content of iron and calcium than mixed or white teff varieties, and in contrast, white teff has a higher copper content than the red and mixed teff varieties [5].

Thirteen teff plants were found among commercial teff plots in Israel (cultivar name White) and were selfed. These plants were randomly distributed in the plots and looked different from the general population in terms of lodging (Figure S1). A single panicle was collected from these thirteen Teff plants for further proliferation and study. These plants were propagated off-season in a greenhouse (Figure S2), and from these thirteen F1, eleven F2 populations were selected for further detailed analyses under field conditions.

In a teff plot, we found plants that looked different from the surrounding population. First, and most striking, was the lodging resistance presented by these single plants under the prevailing environmental conditions, compared to the surrounding plants. Secondly, these plants, randomly distributed within the plot, were different from the common white cultivar in stem diameter, leaf size, phenology, and inflorescence coloration. From each of these plants a single panicle was collected for further characterization.

The objective of this study was to characterize the relations between lodging and other agronomic and sensory traits in the newly discovered populations.

2. Materials and Methods

2.1. Plant Material

Thirteen teff populations were established from a single plant found among commercial teff plots in Israel (cultivar Israeli gene bank name White). These plants were randomly distributed in the plots and looked different from the general population in terms of lodging (Figure S1). A single panicle was collected from these thirteen Teff plants for further proliferation and study. These plants were propagated off-season in a greenhouse (Figure S2), and from these thirteen F1, eleven F2 populations were selected for further detailed analyses under field conditions. These populations were deposited to the Israeli seed bank and included (Some are detailed in Table 1)—44A-163-B (pure brown) and 44B-163-W (pure white). The three populations—53-1, 53-2, and 53-3 were also included, and were found to be a mixed color after a generation of propagation (excluding 53-1 which was already mixed when collected) as detailed in Table 1. Each of the three exhibited a different proportion of brown/white seeds after one cycle of propagation (Table 1), threshed as a bulk, and then separated into white and brown manually. We also harvested two plants separately from the greenhouse pot of 53-2, in order to establish a single plant selection according to lodging resistance score, to create 53-2-1-W and 53-2-2-W. As a control we used one of the commercially grown cultivars in Israel, refereed herein as 'White'.

Table 1. List populations, average grain weight (AGW) of the initial seed set, seed color (W for white and B for brown). The phenotype of plant that grew in the greenhouse includes: AGW (mg), grain yield (g/pot), stems per pot, grain per stem (g), days from sawing to panicle emergence (DSP in days), maximal plant height (MPH in cm), and the proportion of brown out of white seeds, and out of total, quantified in grams.

Line/Population	Initial Collected Single Plant/Cultivar	AGW Initial Set (mg)	Brown/White	Brown/Total	AGW Greenhouse (mg)		GY (g/pot)	Stems/pot	Grain/Stem (g)	DSH	MPH (cm)
White	White Teff	0.23			0.31	BC	22.83	74	0.33	48.75	181
44A-163-B	44A-163-B	0.36			0.39	AB	24.94	35	0.71	56.00	182
44B-163-W	44B-163-W	0.27			0.30	BC	18.27	27	0.68	56.00	147
53-1-W	53-1-M	0.60			0.50	A	25.41	58	0.44	56.00	197
53-1-B			0.064	0.060	0.40	AB					
53-2-W	53-2-M	0.41			0.40	AB	27.22	42	0.65	56.00	190
53-2-B			0.105	0.095	0.22	C					
53-2-1-W *											
53-2-2-W **											
53-3-W	53-3-M	0.48			0.32	BC	28.49	48	0.59	56.00	200
53-3-B			0.015	0.015	0.28	BC					

* initiated from a single seed descend. ** Undetectable light brown might exist. W, white seeds. B, brown seeds. Different letters indicate significant differences at $p < 0.05$.

2.2. Pot Propagation and Phenotyping

Seeds from a single plant for each of the populations were sowed in small pots in the first stage (August 2018), and then transplanted into 10 L pots in a greenhouse (31°55′45.23″ N 34°51′56.27″ E) located at ARO, Bet Dagan, Israel. Pots were well irrigated using a dripping system (Figure S2).

Although statistics were unavailable during this propagation cycle due to the small number of plants, several traits were scored per pot (Table 1). Maximal plant height (MPH) was measured from the pot surface to the top of the panicle. Grain was threshed manually and grain yield (GY) was measured in dry weight (g) per pot. Stems were counted for each pot and grain per stem was calculated. Days from sowing to panicle emergence (DSP) was evaluated as the date when over 50% of the plants in the pot were at or after panicle emergence. Plants where harvested at maturity, weighed, and threshed manually. Average grain weight was roughly assessed by the calculation of the mean value of 10 seeds.

2.3. Field Trial

A total of eleven populations (Table 1) were subjected to a field trial within the growing season under well-irrigated conditions. The trial was held at the ARO located at Bet Dagan, Israel. Seeds that were propagated in the greenhouse were sown at March 7th, 2019, where each experimental unit was comprised of a 0.9 m × 2 m plot (Figure S3). Raised beds were implemented and irrigation and fertilizer were applied using a dripping system. Populations had between 3–5 replicate plots, except for the brown populations 53-1-B, 53-2-B, and 53-3-B, because their seed yield was insufficient for more than a single replication. Seeds for each plot were sown in a row, alongside the dripping system in six rows. Seeds for each row were placed within a tube that was used to evenly distribute the seeds between rows. These seeds were weighed in advanced and were sown at a rate of 2.4 g per 1.8 m^2 plot as a base line (common commercial rate ranges from 0.15–0.5 kg/acre). Since AGW was different between population lines, this proportion was used to standardize the seeding rates (relative to the white cultivar) for each line. Express® herbicide (active ingredient tribenuron-methyl) was applied (0.1% with BB-5) 13 days after sowing (DAS), when the plants emerged.

2.4. Field Trial Phenotypic Measurements

2.4.1. Total Dry Biomass and Grain Yield

At full grain maturity and after the plants were fully dried, all aboveground biomass was harvested and weighed to determine total dry matter (TDM) for each plot. Grain was then threshed using Wintersteiger thresher apparatus. Grain was weighed to determine grain yield per plot (GY), and the Harvest index (HI) was calculated as the ratio between GY and TDM.

2.4.2. Early Ground Cover

Early growth cover (EGC%) was measured at 33 DAS. Coverage Tool [15] was used to quantify ground coverage by canopy, in percent, for each plot. A set of photos was taken vertically from above the plots, under the same settings (operator shoulder height of camera from the ground, light, same camera, Figure S4). Then, the canopy colors were sampled to be taken into account (foreground), whereas the bare soil brown shades were not selected (background).

2.4.3. Chlorophyll

Chlorophyll a and b were measured at 42 DAS, from the youngest fully extended leaf blade. Leaf samples were weighed and then placed in DMF for 72 h. Absorbance of the extracted pigments was measured using a spectrophotometer (UV-VIS recording spectrophotometer, UV-2401PC) at 645 nm and 663 nm. The photosynthetic pigment content was expressed in mg per gram-fresh weight leaf tissue (mg/g^{-1} FW), as adapted from [16].

2.4.4. Stem Width

Stem width projection was measured at 70 DAS (at or after stem elongation phase, Figure S5) for the first lowest node that bore one leaf (upper) and, the most basal internode (lower, below Figure S5).

2.4.5. Phenology

Days from sowing to panicle emergence (DSP) was recorded based on daily inspection, and was evaluated as the date when over 50% of the plants in the plot were at/after panicle emergence. Days from panicle emergence to maturity (DPM) was recorded at maturity (upon plants desiccation). Days from sowing to maturity (DSM) was recorded as well.

2.4.6. Average Grain Weight

Average grain weight (AGW) was measured by first weighing the seeds (~60 mg). Next, these seeds were spread over a white sheet and photographed. For each plot, the image was thresholded using 'coverage tool' [15] (by sampling seeds to account, Figure S6). Then, the threshold images were analyzed using Image J's 'particle count' option. Finally, AGW was calculated as the seed weight divided by number of seed for each plot.

2.4.7. Seed Length and Spikelet Imaging

Seed length was measured using a Leica MZFLIII fluorescence stereomicroscope. About 12 seeds were measured for each plot, and the mean value was recorded. Spikelet images for each line were recorded using Leica MZFLIII stereomicroscope as well.

2.4.8. Height and Lodging Measurements

A lodging index can be computed as the weighted average lodging scores according to a 0–5 scale. The Caldicott and Nuttall method [17] calculated the index as follows: Lodging index = [Sum (lodging score × the relative area for the score)]/5.

Average plant height in each plot was measured (Figure S7a,b) from 53 DAS up to 77 DAS (eight times). Height (H) was measured at three different locations along the plot, from the ground up to the maximal plant height (which could not be absolutely defined as the panicle tips). As panicles emerged (and during grain filling) the fraction of the plot that exhibited lodging was scored (F), as well as the height of the lodged fraction (LH). *p*, plant heights were averaged as follows:

$$\text{Avg plot plant height} = (\text{Erect fraction} \times \text{H}) + (\text{Lodged fraction} \times \text{LH})$$

Maximal plot height (MPH) was the maximal height the plot had reached, regardless of the lodging fractions.

2.4.9. Injera Sensory Trial

In this experiment we used the bulked grain samples (from our ARO field trail) from each line. These samples were passed through a 0.85 mm sieve (standard test sieve, Fisher Scientific Company, Hampton, NH, USA) and grain were further cleaned using the Selecta machine (Machinefabriek BV, Enkhizen, The Netherlands). The samples were then ground to flour by Pashut (https://www.pashutli.co.il/) grinding services, which can grind relatively small grain samples (300 g in our case), followed by a final manual sieving through a kitchen sieve. Within a week from grinding, sensory evaluation was conducted and the flour was stored in sealed plastic bags, at 4 °C.

A total of 14 flour samples, including 11 lines grown in the ARO field experiment, with an additional three samples bought in local markets (two white and one brown flour samples) were included in the test. The flow diagram of the Injera preparation is described in Figures S8 and S9.

All Injera flat bread were prepared similarly, using the same quantities, proportions, and apparatus. The sensory evaluation was conducted 8 h after baking the Injera. In this study, a panel of 14 Ethiopian judges (eight males and six females, 27–73 years old) was used to assess the degree of consumer acceptance/satisfaction on the Injera prepared from the different populations and controls. Those judges are part of a religious community that share a synagogue. The judges were requested to taste the samples and rate various characteristics on a five-point scale (1- "Strongly dislike"/lowest, and 5- "Like very much"/highest). The traits that were evaluated were—general appearance, color (color preference), odor (odor preference), odor intensity (odor strength), texture (softness), acidity (strength), and flavor (taste preference).

2.5. Statistics

The JMP version 12.0 statistical package (SAS Institute, Cary, NC, USA) was used. Results are presented as least square means and standard errors. Coefficients of correlation (r) was calculated and are presented as a heat map. Principal component analysis (PCA) for all studied traits was conducted. PCA was based on a correlation matrix and presented as bi-plot coordinates of the populations (PC scores).

3. Results

3.1. Off-Season Phenotyping and Line Segregation

The subjected teff populations were propagated in 10 L pots in the greenhouse off-season. Each line originated from a single panicle of a single plant. The initial collected seed set AGW (0.23–0.60 mg) as well as AGW scored from the greenhouse (0.22–0.50 mg) are presented in Table 1. MPH ranged from 147 to 200 cm. The white cultivar and the pure brown cultivar 44A-163-B presented similar heights. The white cultivar had more stems per pot and panicle emergence was about a week earlier than the rest of the populations under this condition.

Three populations that had mixed color seeds were separated as detailed in Table 1. The proportion between the brown to white seeds was tripled from 0.021 to 0.064, within a generation of prorogation. Whereas in 53-2 and 53-3, no dark brown seeds were detected upon collection. However, after a second round of propagation in the field, dark brown seeds were found in those populations (0.105 and 0.015 for 53-2 and 53-3, respectively).

3.2. Phenotyping

Analysis of variation for most traits showed a significant line effect (ANOVA in Table 2). Within the agronomic and plant physiological parameters tested, a very wide range of values was found for EGC and Chlb/gFW (higher than 3 folds, Tables 3 and 4). A 2–3 fold range was recorded for—TDM, GY, HI, LSD, Chla/gFW, Chl/gFW, Chl a/b ratio, and AGW. A low range of values (under 2 folds) was found for—USD, DSP, DPM, DSM, SL, and MPH. ANOVA analyses of all sensory traits (except for 'Odor intensity') reveled a significant effect of variations between the populations (Table 2).

Table 2. Analyses of variance of the different studied traits for the studied populations as well as the commercial cultivar 'white" under field conditions.

ANOVA	Source	DF	MS	Prob > F
Total DM	Model	10	0.34015	0.0008
	Error	20	0.06516	
	C. Total	30		
GY	Model	10	0.24667	<0.0001
	Error	20	0.00378	
	C. Total	30		
HI	Model	10	0.01458	<0.0001
	Error	20	0.0008	
	C. Total	30		
EGC	Model	10	108.137	0.0253
	Error	18	37.827	
	C. Total	28		
USD	Model	10	0.00463	0.0118
	Error	20	0.00142	
	C. Total	30		
LSD	Model	10	0.00371	0.0015
	Error	20	0.00078	
	C. Total	30		
EGC	Model	10	108.137	0.0253
	Error	18	37.827	
	C. Total	28		
USD	Model	10	0.00463	0.0118
	Error	20	0.00142	
	C. Total	30		
LSD	Model	10	0.00371	0.0015
	Error	20	0.00078	
	C. Total	30		
DSH	Model	10	46.6935	<0.0001
	Error	20	1.95	
	C. Total	30		
DHM	Model	10	57.586	0.03
	Error	20	21.5167	
	C. Total	30		
DSM	Model	10	119.4215	0.0007
	Error	20	22.1667	
	C. Total	30		
AGW	Model	10	1.84×10^{-8}	0.00147
	Error	20	3.86×10^{-9}	
	C. Total	30		
Seed length	Model	10	14513	0.00081
	Error	20	2765	
	C. Total	30		
Spikelet length	Model	10	2111806	0.00238
	Error	33	580102	
	C. Total	43		

Table 3. LS means for TDM (Total Dry Matter, g/plot), GY (Grain Yield, g/plot), HI (Harvest Index), EGC (Early Ground Cover), LSD (Lower Stem width), USD (Upper stem width) for the studied populations and the commercial cultivar 'white' under field conditions.

Line/Population	TDM (kg/plot)			GY (kg/plot)			HI			EGC %			USD (cm)			LSD (cm)		
White	1.74	0.10	DE	0.50	0.03	A	0.29	0.01	A	15.2	2.75	D	0.22	0.02	D	0.18	0.01	D
53-3-W	2.42	0.13	AB	0.25	0.03	C	0.10	0.01	E	21.6	3.08	CD	0.33	0.02	A	0.24	0.01	B
53-3-B	1.27	0.26	E	0.35	0.06	BC	0.27	0.03	A	11.0	6.15	D	0.25	0.04	BCD	0.18	0.03	BCD
53-2-W	2.20	0.15	ABC	0.24	0.04	C	0.11	0.02	E	19.1	3.55	CD	0.26	0.02	CD	0.21	0.02	BCD
53-2-B	2.16	0.26	ABCD	0.59	0.06	A	0.27	0.03	A	37.4	6.15	AB	0.27	0.04	BCD	0.15	0.03	D
53-2-2-W	2.51	0.18	AB	0.32	0.04	BC	0.13	0.02	DE	21.9	4.35	BCD	0.33	0.03	AB	0.31	0.02	A
53-2-1-W	2.54	0.26	AB	0.36	0.06	BC	0.14	0.00	CDE	23.6	6.15	ABCD	0.33	0.04	ABC	0.24	0.03	ABC
53-1-W	2.52	0.13	A	0.33	0.03	BC	0.13	0.01	DE	28.6	3.55	ABC	0.30	0.02	ABC	0.22	0.01	BC
53-1-B	1.78	0.26	CDE	0.41	0.06	AB	0.23	0.03	B	40.2	6.15	A	0.24	0.04	BCD	0.19	0.03	BCD
44B-163-W	2.41	0.13	AB	0.39	0.03	B	0.16	0.01	CD	21.8	3.08	CD	0.29	0.02	BC	0.22	0.01	BC
44A-163-B	2.14	0.13	BC	0.41	0.03	B	0.19	0.01	BC	22.5	3.08	CD	0.25	0.02	CD	0.20	0.01	CD

Different letters indicate significant differences at $p < 0.05$.

Table 4. LS means for Chla/gFW (Chlorophyll a), Chlb/gFW (Chlorophyll b), Chl/gFW (Total Chlorophyll), and Chl a/b ratio, for the studied population under field conditions.

Line/Population	Chla/gFW			Chlb/gFW			Chl/gFW			a/b Ratio		
White	136.2	14.8	B	49.2	17.4	B	185.4	48.8	B	2.97	0.35	AB
53-3-W	122.7	18.1	B	34.9	8.7	B	157.5	48.8	B	3.50	0.43	AB
53-3-B	226.9	36.3	A	145.4	17.4	A	372.1	24.4	A	1.56	0.85	AB
53-2-W	118.2	20.9	B	32.6	7.1	B	150.7	48.8	B	3.60	0.49	A
53-2-B	107.9	36.3	B	39.5	8.7	B	147.4	24.4	B	2.73	0.85	AB
53-2-2-W	120.4	25.6	B	38.4	8.7	B	158.7	19.9	B	3.14	0.60	AB
53-2-1-W	164.6	36.3	AB	47.7	10.1	B	212.3	24.4	B	3.45	0.85	AB
53-1-W	143.6	18.1	AB	48.8	12.3	B	192.4	34.5	B	2.91	0.43	AB
53-1-B	162.6	36.3	AB	105.4	17.4	A	267.9	24.4	AB	1.54	0.85	B
44B-163-W	165.1	18.1	AB	56.9	17.4	B	221.9	28.2	B	3.17	0.43	AB
44A-163-B	136.0	18.1	B	46.0	8.7	B	182.0	48.8	B	3.41	0.43	AB

Different letters indicate significant differences at $p < 0.05$.

3.3. Biomass Production

A range of GY was observed in the current study. The white commercial cultivar and pure brown 44A-163-B were ranked relatively high in GY and HI, and low in TDM, with the former showing a significantly higher GY compared to the latter (Table 3). Interestingly, the brown populations (53-1-B, 53-2-B, and 53-3-B) exhibited a similar pattern and were also ranked higher in GY and HI and lower in TDM, as compared to most of their white counterparts (Table 3). The best performance in terms of GY was the white cultivar. Second was 44B-163-W, which was not significantly different from the pure brown 44A-163-B, as well as from the other brown populations.

3.4. Early Growth Cover and Chlorophyll Measurements

Early growth cover (Table 3 and Supplementary Figure S4) of the white commercial cultivar and 53-3-B was low as compared to other populations (such as 53-1-W) at 33 DAS. 53-1-B and 53-2-B exhibited the highest values of EGC.

Leaf chlorophyll content was measured at 42 DAS (a, b, and total Chl). Both the commercial white cultivar and pure brown 44A-163-B line had a very similar midrange value (Table 4). Both 53-3-B and 53-1-B contained the highest Chl (b, and total Chl) among the evaluated populations. Whereas 53-2-B exhibited the opposite patterns in terms of Chl levels. Interestingly, all three brown populations presented a low Chla/Chlb ratio.

3.5. Phenology

The difference in panicle emergence time ranged between 53 to 64 DAS, the white commercial cultivar being the earliest to enter the reproductive stage at 53 DAS (Table 5), along with 53-1-B. In general, the brown populations exhibited earlier heading, compared to their white-counterparts. 53-3-W was the latest to head and had the highest DSP, DPM, and DSM within the collection. While the white commercial cultivar was significantly earlier than brown 44A-163-B, their grain filling period (indicated by DPM) was not statistically different. Both the pure brown 44A-163-B and the white cultivar exhibited the lowest DSM in the collection.

3.6. Stem Phenotyping

Stem width was measured at 70 DAS during stem elongation and the lower and upper basal stem widths were measured (Table 3). The brown populations (as well as the white commercial cultivar) tend to group as having a narrower stem (low USD and LSD) than the white populations (Table 3). The single plant selected 53-2-2-W exhibited the highest LSD in the collection.

3.7. Plot Plant Height Dynamics and Lodging

Maximal plot plant height of the white commercial cultivar and two of the brown populations, 53-1-B and 53-2-B, was lower compared to the other white populations (Table 5). The dynamics of plant height, which was documented in detail from 53 DAS to 87 DAS (Figure 1, Table 5), revealed distinct patterns among the studied populations.

White, the earliest to head, started exhibiting lodging three days after panicle emergence. However, this lodging was later revealed to be essentially different from the lodging observed in other populations. The main differences were that while the white commercial cultivar was relatively uniform in its lodging across the plot (Figure S7b), as well as relatively static/stable in terms of the plot's height throughout the grain filling period (at around 35 cm above ground), the other populations did not lodge so soon after panicle emergence and their lodging was not uniform across the plot. For example line 44A-163-B started lodging between 70–77 DAS (Figure 1 and Figure S7b), and the plot's height reached around 50 cm above ground. Other populations, like 44-B-163-W exhibited strong lodging between 70 and 77 going from a plot height of 77 cm to 27 cm in seven days (Figure S7b). This line was among the tallest populations and its heavy panicles filling seemed to bend the entire plant downwards.

Some of the populations, such as 53-2-2-W, exhibited a relatively prolonged period of erect posture during grain filling, before lodging (Figure S7b), which was not severe.

Table 5. LS means for plant height in centimeter, measured along the reproductive phase (53–77 DAS). MPH (Maximal Plot Height), DT < 75% in 11 lines under field conditions, as well as ANOVA for each of the traits.

Line/Population	53			56			60			63			67		
White	50.67	1.80	A	63.06	1.66	A	36.00	3.01	C	32.98	3.15	C	35.84	3.84	CD
53-3-W	40.75	2.21	C	47.08	2.04	CD	59.50	3.68	AB	62.97	3.86	A	73.67	4.71	A
53-3-B	38.00	4.42	CD	45.00	4.07	CD	60.00	7.37	AB	70.33	7.72	A	76.00	9.42	AB
53-2-W	44.00	2.55	BC	53.22	2.35	BC	62.33	4.25	AB	60.31	4.46	A	71.69	5.44	AB
53-2-B	55.00	4.42	A	58.00	4.07	AB	56.00	7.37	AB	49.00	7.72	BC	54.08	9.42	ABC
53-2-2-W	48.50	3.12	ABC	57.17	2.88	AB	69.00	5.21	A	65.51	5.46	A	66.59	6.66	AB
53-2-1-W	50.00	4.42	ABC	56.00	4.07	BC	70.00	7.37	AB	68.40	7.72	A	64.60	9.42	AB
53-1-W	46.25	2.21	ABC	54.50	2.04	B	59.75	3.68	AB	60.34	3.86	A	57.54	4.71	B
53-1-B	50.00	4.42	ABC	60.33	4.07	AB	49.00	7.37	BC	36.20	7.72	BC	25.85	9.42	D
44B-163-W	34.00	2.21	D	41.58	2.04	D	56.25	3.68	AB	64.58	3.86	A	73.09	4.71	A
44A-163-B	50.50	2.21	AB	62.83	2.04	A	64.00	3.68	AB	53.27	3.86	AB	67.95	4.71	AB
Line/Population	**70**			**75**			**77**			**MPH (cm)**			**DT < 75%**		
White	30.43	5.27	C	27.48	5.67	C	28.04	6.61	B	61.59	2.48	D	60.00	4.39	C
53-3-W	63.83	6.45	A	67.27	6.94	A	59.98	8.10	A	71.03	3.51	ABC	72.33	2.53	AB
53-3-B	78.00	12.90	A	61.80	13.88	AB	35.17	16.20	AB	78.00	6.08	AB	77.00	4.39	A
53-2-W	66.17	7.45	A	69.00	8.01	A	59.78	9.35	A	76.45	3.51	A	72.33	2.53	AB
53-2-B	46.50	12.90	BC	52.50	13.88	ABC	46.67	16.20	AB	58.00	6.08	CD	60.00	1.96	C
53-2-2-W	52.41	9.12	AB	47.92	9.81	ABC	48.42	11.46	AB	66.59	4.30	ABCD	65.00	3.10	BC
53-2-1-W	45.80	12.90	BC	31.65	13.88	BC	32.60	16.20	AB	64.60	6.08	ABCD	67.00	4.39	ABC
53-1-W	51.47	6.45	AB	45.43	6.94	BC	39.71	8.10	AB	65.75	3.04	BCD	65.50	2.19	BC
53-1-B	24.75	12.90	BC	30.17	13.88	BC	23.50	16.20	AB	60.33	6.08	BCD	63.00	4.39	BC
44B-163-W	65.00	6.45	A	36.60	6.94	BC	26.94	8.10	B	75.34	3.04	A	75.50	2.19	A
44A-163-B	59.56	6.45	A	53.51	6.94	AB	52.01	8.10	A	69.66	3.04	ABCD	61.50	2.19	C

ANOVA

	Source	DF	MS	Prob > F
53	Model	10	109.33	0.0005
	Error	20	19.52	
	C. Total	30		
56	Model	10	176.10	<0.0001
	Error	20	16.58	
	C. Total	30		
60	Model	10	348.07	0.0002
	Error	20	54.26	
	C. Total	30		
63	Model	10	476.46	<0.0001
	Error	20	59.66	
	C. Total	30		
67	Model	10	718.17	<0.0001
	Error	20	88.68	
	C. Total	30		
70	Model	10	632.19	0.0054
	Error	20	166.43	
	C. Total	30		
75	Model	10	676.29	0.0103
	Error	20	192.63	
	C. Total	30		
77	Model	10	532.08	0.0857
	Error	20	262.48	
	C. Total	30		

Different letters indicate significant differences at $p < 0.05$.

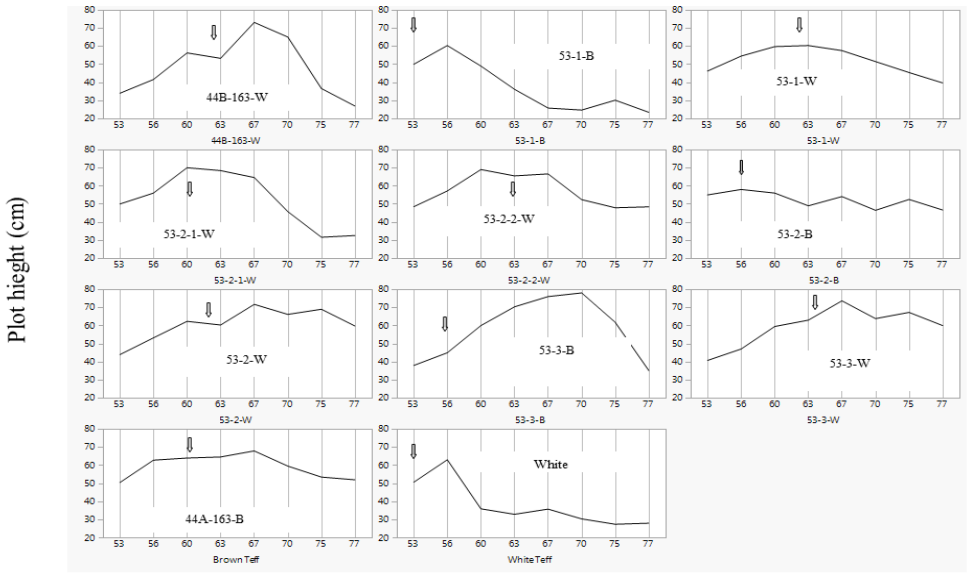

Figure 1. Plot height (cm) averaged for each genotype as a function of days after sawing (DAS). The arrows indicate averaged date of panicle emergence under field condition.

3.8. Seed and Spikelet Phenotyping

AGW of the brown segregated populations 53-1-B and 53-3-B was significantly higher, as compared to their white counterparts (Table 6). The commercial cultivar exhibited midrange values of AGW. A similar pattern was obtained for SL, where this time all three brown segregated populations exhibited significantly higher averaged values, as compared to their white counterparts (Table 6).

Table 6. LS means for: AGW (Average Grain Weight), SL (Seed length), SpL (Spikelet length), DSP (Days from Sowing to Panicle emergence), DPM (Days from Panicle emergence to Maturation), and DSM (Days from Sowing to Maturation) for the studied populations and commercial cultivar 'White" under the field conditions for the studied populations.

Line/Population	DSP			DPM			DSM			AGW (g)			Seed Length (µm)			Spikelet Length (µm)		
White	53.50	0.57	E	35.83	1.89	BC	89.33	1.92	D	0.42	0.0004	A	1176	21	BC	6855	341	AB
53-3-W	64.00	0.70	A	46.00	2.32	A	110.00	2.35	A	0.34	0.0003	BC	1054	26	D	4945	381	C
53-3-B	56.00	1.40	DE	40.00	4.64	ABC	96.00	4.71	BCD	0.48	0.0005	AB	1340	53	A	6291	440	B
53-2-W	62.00	0.81	ABC	36.00	2.68	BC	98.00	2.72	BC	0.47	0.0005	A	1171	30	BC	6700	440	B
53-2-B	56.00	1.40	DE	40.00	4.64	ABC	96.00	4.71	BCD	0.38	0.0004	ABC	1243	53	ABC	7051	440	AB
53-2-2-W	63.00	0.99	AB	37.00	3.28	BC	100.00	3.33	B	0.32	0.0003	C	1251	37	AB	5933	440	BC
53-2-1-W	60.00	1.40	BCD	36.00	4.64	ABC	96.00	4.71	BCD	0.25	0.0002	C	1161	53	BCD	6819	288	B
53-1-W	62.25	0.70	AB	35.25	2.32	BC	97.50	2.35	BC	0.30	0.0003	C	1215	26	B	6613	341	B
53-1-B	53.00	1.40	E	43.00	4.64	AB	96.00	4.71	BCD	0.51	0.0005	A	1341	53	A	5882	440	BC
44B-163-W	62.25	0.70	AB	34.25	2.32	BC	96.50	2.35	BC	0.49	0.0005	A	1129	26	CD	7986	440	A
44A-163-B	60.00	0.70	C	31.00	2.32	C	91.00	2.35	CD	0.32	0.0003	C	1188	26	BC	6714	341	B

Different letters indicate significant differences at $p < 0.05$.

Coloration of the Spikelet lemma was also documented (Figure 2). Whit's lemma were gray-purplish and highly transparent. 44A-163-B exhibited a dark-purple coloration and was transparent as well. 44B-163-W exhibited bright pink lemma with whitish-gray outer borders and veins. The brown seeded line of 53-1 exhibited much less pink coloration (mainly at the outer borders), compared to their white counterparts, which had bright pink lemma with white gray outer borders and veins. The second brown line of 53-2 exhibited purple coloration at the outer border of the lemma, as compared to their white counterparts which were pink—not purple. The third brown line of 53-3 also exhibited purple coloration at the outer borders of the lemma, as compared to the white counterparts, which had whitish-gray lemma (similar to white in terms of coloration).

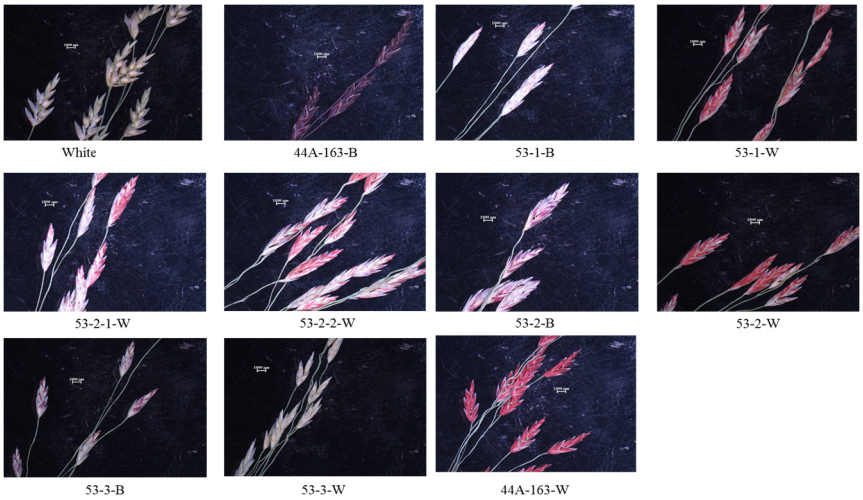

Figure 2. Images of the Spikelets of each of the 11 populations characterized in the field experiment.

3.9. Injera Sensory Evaluation

The sensory evaluation acceptability trials of Injera made from 14 flours samples of the 11 populations grown in the ARO field experiment with additional three commercial samples bought in local markets are presented in Table 7 and Figure S9.

Table 7. LS means for sensory traits—appearance (look preference), color (color preference), odor (odor preference), odor intensity (odor strength), texture (softness), acidity (strength), and flavor (taste preference) for the 14 flour samples from the field experiment, as well as 3 market flours.

Line/Population	Appearance			Color			Odor			Odor Intensity			Texture			Acidity			Flavor		
White	3.57	0.31	ABC	3.77	0.30	ABCD	2.71	0.38	ABCDE	2.71	2.71	BC	3.71	0.34	A	2.93	0.38	ABC	2.79	0.33	ABCD
Market I- W	4.08	0.32	AB	4.08	0.30	ABC	2.92	0.39	ABCD	2.54	2.54	BC	3.92	0.36	A	2.00	0.40	BCDE	2.69	0.35	ABCD
Market II-W	3.69	0.32	ABC	3.62	0.30	BCD	2.92	0.39	ABCD	3.23	3.23	AB	3.62	0.36	AB	2.00	0.40	BCDE	2.92	0.35	ABCD
Market -B	3.92	0.32	AB	3.85	0.30	ABCD	3.23	0.39	ABC	2.38	2.38	BC	3.92	0.36	A	1.69	0.40	DE	3.15	0.35	ABC
53-3-W	3.23	0.32	BCD	3.31	0.30	CDE	2.08	0.39	DE	3.23	3.23	AB	2.00	0.36	E	2.54	0.40	ABCD	2.00	0.35	DEF
53-3-B	4.13	0.30	A	4.47	0.28	A	3.73	0.36	A	2.40	2.40	BC	3.64	0.34	AB	2.14	0.38	BCDE	3.50	0.33	A
53-2-W	4.00	0.32	AB	4.08	0.30	ABC	2.62	0.39	BCDE	3.08	3.08	ABC	3.46	0.36	ABC	2.85	0.40	ABC	2.38	0.35	BCDE
53-2-B	2.54	0.32	DE	2.54	0.30	EF	2.23	0.39	CDE	2.85	2.85	ABC	3.08	0.36	ABCD	2.46	0.40	ABCD	1.69	0.35	EF
53-2-2-W	3.92	0.32	AB	4.08	0.30	ABC	2.77	0.39	ABCDE	2.92	2.92	ABC	3.54	0.36	ABC	3.38	0.40	A	2.31	0.35	BCDEF
53-2-1-W	1.58	0.34	F	1.75	0.31	DF	1.75	0.41	EF	3.92	3.92	A	2.67	0.37	BCDE	1.91	0.43	CD	1.36	0.38	F
53-1-W	2.92	0.32	CDE	3.62	0.30	BCD	3.00	0.39	ABCD	2.77	2.77	BC	2.46	0.36	DE	3.08	0.40	AB	2.23	0.35	CDEF
53-1-B	2.15	0.32	EF	3.31	0.30	CDE	2.85	0.39	ABCDE	2.62	2.62	BC	3.00	0.36	ABCD	2.85	0.40	ABC	3.00	0.35	ABC
44B-163-W	4.38	0.32	A	4.15	0.30	AB	3.77	0.39	A	2.46	2.46	BC	2.62	0.36	CDE	2.46	0.40	ABCD	3.08	0.35	ABC
44A-163-B	3.00	0.32	CDE	3.23	0.30	DE	3.69	0.39	AB	2.08	2.08	C	3.85	0.36	A	3.08	0.40	AB	3.23	0.35	AB

Different letters indicate significant differences at $p < 0.05$.

The sensory evaluation scored values for all sensory attributes were sampled 8 h after baking the Injera by a panel of 14 Ethiopian judges, to assess the degree of consumer acceptance of Injera prepared from different populations.

44B-163-W and 53-3-B were significantly preferable in terms of Injera appearance, color, and odor, which significantly differed from some of the populations, but not from the commercial ones. In terms of Injera appearance, color, and odor, 53-2-1-W was significantly the least preferable. 53-2-1-W also presented the highest (unpleasant) odor intensity, compared to 44A-163-B, which had the lowest odor intensity.

Both 44B-163-W and 53-3-B exhibited a relativity low odor intensity (which is apparently preferable). The texture of all market samples as well as that of the White was generally ranked higher than the rest of the populations. The acidity level of 53-2-2-W was the highest among the collection while the market samples were generally less acidic in taste The highest ranked in terms of flavor were 53-3-B, 44A-163-B (around 3.2), and the lowest were 53-2-B and 53-2-1-W (around 1.6).

3.10. Principal Component Analysis

Principal component analysis (PCA) was conducted for all studied traits (Figure 3). Two components were extracted using eigenvalues > 1 to ensure meaningful implementation of the data by each factor. The PCA of the 11 lines extracted two major principal components (eigenvalues > 1) that accounted collectively for 56% of the variance between the populations. Principal component 1 (PC1, *X*-axis) explained 37% of the data set variation, and PC2 (*Y*-axis) explained 19% of the data set variation.

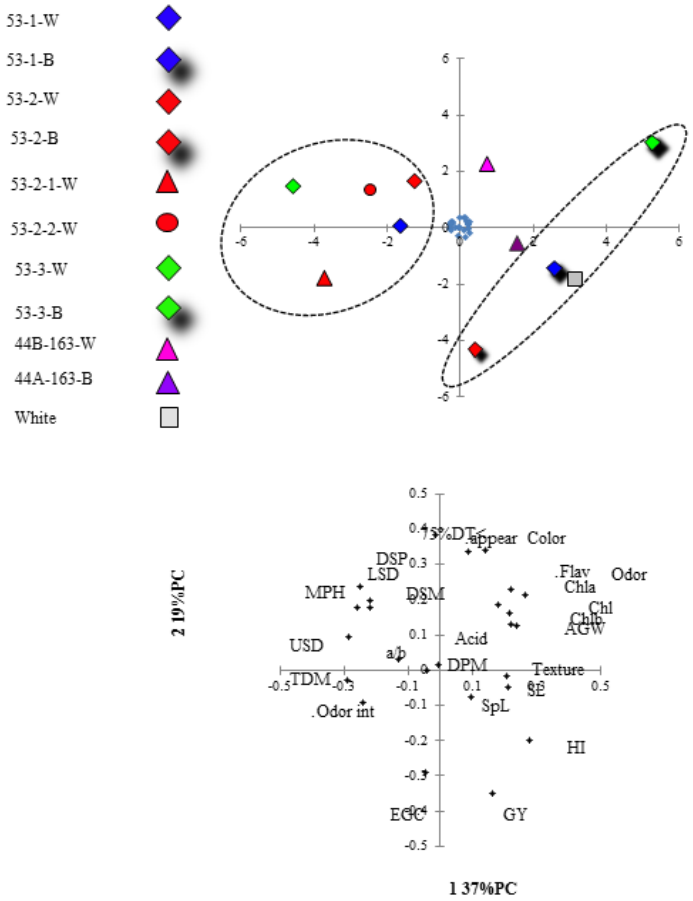

Figure 3. Principal component analysis (PCA) (based on correlation matrix) of continuous plant traits: TDM (Total Dry Matter),GY (Grain Yield), HI (Harvest Index), EGC (Early Ground Cover), LSD (Lower Stem width), USD (Upper stem width), Chla/gFW (Chlorophyll a), Chlb/gFW (Chlorophyll b), Chl/gFW (Total Chlorophyll), Chl a/b ratio, DSP (Days from Sowing to Panicle emergence), DPM (Days from Panicle emergence to Maturation), DSM (Days from Sowing to Maturation), AGW (Average Grain Weight), SL (Seed length), SpL (Spikelet length), MPH (Maximal Plot Height), and DT < 75% are recorded on the 11 lines. Elipses represent the grouping trend of the white/brown segregating lines. Biplot vectors are trait factor loadings for PC1 and PC2. The dots and labels in the bottom panel represent the strength (by distance from the axis center) and their direction relative to each other, each explain some of the total variance according to the different strengths.

Both the correlations and the PCA showed a negative association between the two components representing reproductive variables (GY and HI) and MPH, DSP, DSM, DT < 75%, LSD ($r = -0.8$ **, −0.69 *, −0.62 *, −0.64 * with GY, respectively). Along the axis of association, white as well as the brown segregants were the highest yielding and had the lowest MPH, DSP, DSM, DT < 75%, LSD.

Injera color and appearance were grouped and were negatively associated ($r = -0.61$ *) with EGC. Another group which was obtained was negatively associated with TDM and odor intensity,

which included the traits—Chl Tot, Chlb, flavor, and AGW (r = −0.71 *, −0.8 **, −0.6 *, −0.64 *, with TDM respectively).

3.11. Correlations

Figure 4 shows the heat-map correlation matrix obtained for the studied traits. TDM was found to be significantly negatively correlated with Chlb/gFW (r = −0.8 **) and Chl/gFW (−0.71 **), and positively correlated with USD (0.79 **) and LSD (0.63 *). GY was negatively correlated with MPH (−0.8 **) and with LSD (0.63 *). The correlation between Chla/gFW and Chlb/gFW was found strongly significant (0.88 ***). MPH was correlated with TDM, USD, and LSD (0.66 *, 0.68 *, and 0.74 **).

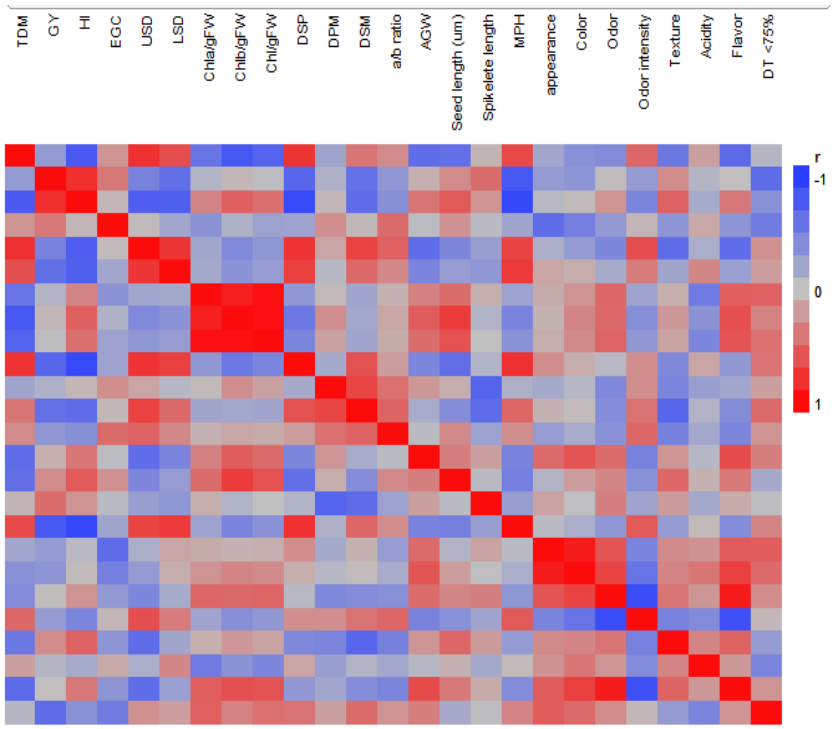

Figure 4. Heat map of Coefficients of correlation (r) for the measured traits.

Low DSM or DSP were correlated with increased GY (r = −0.62 * and −0.69 * respectively) and SL (−0.65 * and −0.63 *).

In the sensory parameters evaluated, flavor was found to be positively correlated with odor (0.9 ***) and color (0.7 *), and negatively with odor intensity (−0.86 ***). Odor and odor intensity were negatively correlated (−0.86 ***), and color and appearance were positively correlated (0.90 ***). Odor and color were positively correlated (0.7 *).

Flavor was positively correlated to Chlb/gFW (0.6 *) and negatively with TDM (−0.61 *) and USD (−0.64 *). AGW was found to be positively correlated with odor intensity and negatively with acidity (−0.73 ***). Texture was found to be negatively correlated (−0.64 *) with USD, and USD was positively correlated with odor intensity.

4. Discussion

The teff populations evaluated in this study exhibited a wide phenotypic variation (Supplementary Figure S7a) was comparable to previous literature reports [8,18–20]. For example, the GY range in the current field experiment was equivalent to 1.3–2.7 t/ha, where the national average farmer's yield was around 1 t/ha, and 2.5 t/ha under experimental conditions in Ethiopia [4,18,19,21]. Teff has a potential for yielding 4.6–5 t/ha if lodging can be resolved [22]. The harvest index values previously

reported [23] were of a similar range to our field experiment (Table 3). In addition, the phenological values (Table 6) were in accordance with the previously reported ranges for teff cultivation [24,25].

Within the genetic material tested, no correlation was found between TDM and GY, whereas in others, a positive [5] or negative correlation was reported [26]. However, when analyzing the white and brown separately (and excluding white which appeared to be much different) there appeared to be some degree of correlation (r = 0.6 and 0.7 for the white and black, respectively), yet not statistically significant. We found correlation between GY and HI (0.8 ***) that was in agreement with previous reports [27].

Plant height was previously reported to range between 74 and 116 cm [18]. Under our field conditions, within the growing season, MPH was 58–78 cm (Table 5), and in the greenhouse off-season, MPH was 180–200 cm (Table 1), which was more than double the field growth but with a narrower range across populations. Differences in day length and other environmental factors might account for these differences. Some of the populations were ranked similarly in both experiments (Tables 1 and 6) in terms of MPH.

Since there are two duplicate genes for grain color in teff, which are known to be dominant [14], the small fraction of the brown seeds found within the seeds propagated from the initial collected panicle (0.064, 0.105 and 0.015 for 53-1, 53-2, 53-3, respectively, Table 1, Figure 5) could only be explained by an external foreign pollination. The increase in brown seed ratio in 53-1 from the first collected generation to the greenhouse next-generation, was from 0.021 to 0.064—as would be expected from the segregation of heterozygosity of the grain color loci (A/a). The small fraction of A/a in the collected panicle would be expected to triple (a total of 1 A/A and 2 A/a) over the course of a single generation. The data also support the hypothesis that the brown populations are half-siblings (hybrids) to their white counterparts; these half-siblings share the maternal side but differ in the paternal one. It is very likely that these hybridizations were most probably wind-driven. As opposed to 53-1, no dark brown seeds could be detected within the grain of the collected panicles of these two populations, so there must have been undetectable light brown seeds that were in a heterozygous state A/a and were later segregated.

Figure 5. Color segregation in seeds and suggested genotypes.

The brown populations in this study (pure and segregating) exhibited an overall advantage over the white populations in terms of directing their biomass towards grain production (Table 3, PCA Figure 3, excluding the case of the 'White'). White, the pure brown 44A-163-B, and some of the segregating brown populations were the highest ranked for GY (Table 3, PCA Figure 3).

A clear pattern emerges that all brown hybrids were earlier to flower (along with the white, Table 6) as compared to their white half-siblings. Therefore, it is possible that the pollen donor/s was/were a relatively early flowering type. Additionally, the significant differences in plant height, observed between 53-2-W and 53-2-B (76 cm vs. 58 cm, Table 6) might indicate that the pollen donor in this case had a shorter stature than the maternal line.

53-3-B, which originated from the segregation of the mixed color line 53-3 into brown and white seeds (Table 1), was especially interesting. This line exhibited relatively low TDM and high HI (Table 3), low EGC (Table 3), and high Chl levels (Table 4). In contrast, this line was also relatively tall, as indicated by its high MPH (Table 5) and thin stems (Table 3). In terms of sensory evaluation, 53-3-B was the most promising line with its preferable taste, smell, and appearance (Table 7).

Our hypothesis was that each of the three half-siblings originated from a different pollen donor and was strengthened by the large variations in Chl levels between the three half-siblings. 53-2-B had the lowest Chl levels, and 53-1-B and 53-3-B exhibited the highest levels among the collection (Table 4 and Figure 3). Interestingly, 53-2 which presented the lowest Chl levels had the highest GY among the three. Another result was the grouping of the traits—Chl Tot, Chlb, flavor, and AGW that was negatively correlated with TDM (Figure 3). This was in agreement with the literature that suggest that a smaller plant might contain denser leaves and more chloroplast and Chl per g FW of leaf tissue [28]. The positive correlation obtained between flavor and Chl might be indirect, however, these correlations might have importance for future breeding programs as initial phenotypes for selection.

Results from the six crosses of parental populations derived from the Ethiopian gene bank collected from diverse climate and elevation, differing in lemma color (purple, red grey, and yellowish-white), showed that at least four pairs of genes control the inheritance of lemma color in teff [14], with dominance complementary and epistatic gene actions. Berhe [14] suggested the following model. C is a gene for basic anthocyanin color; P1 and P2 are duplicate genes responsible for development of purple lemma color in the presence of dominant C (either P1 or P2 alone); p1 and p2 are genes responsible for the red lemma color in the presence of dominant C; G is gene for the gray lemma color, visible only when the dominant C is absent; g is gene for yellowish-white lemma color in the absence of C and G [14].

Following the genetic model of lemma color [14], it appeared that both the maternal line of 53-2 and 53-3 had the basic p1 or p2 genes in the background of the dominant C gene, thus, resulting in red lemma color, and that the hybridization with an unknown brown donor introduced a P1 or P2, thus, resulting in purple lemma color. There seems to be differences that might have come from maternal differences between 53-2 and 53-3 in lemma color, because 53-3-W was not red like 53-2-W, but rather was gray (Figure 2).

In this work we characterized in detail the lodging phenomena in the studied teff populations. The simplified scoring system for lodging [29], which is commonly used, does not take into account at which growth stage lodging starts, nor the uniformity of lodging within a plot. Therefore, we chose to document plot-height over the course of the reproductive period (Figure 1), as well as to calculate the days to 75% lodging in a plot. This detailed inspection and documentation explained mechanisms related to teff lodging (Figure 1, Figure S7a,b), which were very much context-dependent in terms of environmental conditions. With respect to lodging, the lodging resistance—the reason for collecting these populations to begin with (Supplementary Figure S1)—it appeared that since the collected plants grew randomly as single plants within a homogeneous lodging with inclined genetic population, and occurred at very low-density planted areas, they showed a lodging-resistant phenotype. In well-irrigated, well-fertilized and low-stress conditions of our experiments, a complete lodging-resistant line was not found, and lodging seemed to be a flash-mob phenomenon where several plants start a lodging movement that sweeps the reset of the field. However, we showed that

our in-depth documentation and interpretation of lodging here might be useful for future breeding of lodging resistance characteristics in teff.

High yielding populations tend to lodge at harvest time [30]. The lack of variation in lodging resistance might be a result of unfavorable associations of lodging resistance with productivity promoting traits like plant height, panicle length, grain, and shoot biomass [20,29]. Improvement of lodging-related traits—like culm length, overall-height, and diameter of the culm internodes—through breeding is expected to be a demanding task due to their relatively low heritability and lack of reliable genetic advance-estimates [31]. Therefore, increasing our understanding of the lodging phenomena and its phenotyping can improve our ability to breed for high yielding lodging-resistant cultivars. This study showed that the nature of lodging was variable in terms of timing and strength (Figure S7a). In terms of timing, we observed an early type of lodging that was most likely triggered by the fast inflorescence weight increase exhibited by the white (Figure S7b) and 53-1-B (Figure 1). Other populations were 'strong' enough to carry the inflorescence during most of the grain-filling period, like 44A-163-B and 44B-163-W (Figure S7b). Therefore, the rate at which panicle increased in weight, prior and throughout grain filling, appeared to vary and might be important from a breeding perspective.

Surprisingly, white, which was the first to lodge, was mostly stable in plot-height once lodged (around 35 cm above ground surface) during the entire grain filling and was the best yielding line. In white, despite stem weakness and the plant being bent towards the ground, the panicles were mostly above ground level. This pattern created a medium level of lodging that appeared to be different from the strong lodging where the plant was heavier and was totally bent to the ground (Figure S7b: 44B-163-W and 53-2-2-W).

Despite the large number of studies screening teff populations [11,19,20,32] there are only few that include in-depth characterization of different populations. In addition, there are hardly any studies that present agronomic as well as sensory traits, side by side, to analyze possible links between them. The processing, eating, and nutritional quality of food products might be greatly influenced by teff variety. Therefore, it is necessary to assess the genetic diversity of this crop for potential improvements of agronomic, as well as edible traits. New breed varieties must be subjected to sensory analysis, for consumer acceptance, to make the research efforts commercially meaningful [4]. We report a significant genotypic effect on most of the sensory traits evaluated (Table 7). The effectiveness of flour grinding was found to be crucial for texture, across all market samples. The white cultivars were ranked higher than the rest of the populations. The size of the grain might also effect Injera acidity and odor, as AGW was found to be positively correlated with odor intensity and negatively with acidity. There is a need to explore the variability of flower grinding parameters as well.

The current study growing conditions (pots and field experiments) were not favorable to observe the lodging resistance that was observed during the original seed collection. The experiment did not replicate the specific environmental context of a single seed developing in a low-density planted area and was not surrounded by a homogenous population of the plots from which it was collected. A wide genotypic variance was found in the current study for stem width and plant height. However, under an abundance of water and nutrient and, at a high plant density, a thick stem did not ensure lodging resistance. It appeared, however, that under low density or some sort of environmental stress might lead to increased stem lignification and hardening, allowing the plant to carry the grain load at an erect posture. Future experimentation to test this hypothesis might include combinations of agro-technics implementations like seed-coverage to enlarge seeds, using a mixture of teff populations, reducing sowing density to reduce plant density, and the introduction of controlled stresses like water deficiency and salinity. It is clear that late maturing, thick stem, and tall teff varieties possess deeper root systems than early maturing populations of shorter height [25]. Therefore, a combination that includes populations that are characterized by thick stems with stress can improve lodging resistance. Integration between a wide range of parameters and the correlations obtained between agronomic and sensory traits might improve our ability to breed towards a "real world" better end-product.

5. Conclusions

The present study empathize the need for an in-depth exploration of lodging resistance as well as Injera sensory parameters within the population presented here, followed by their genetic stabilization, for the befit of future breeding.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/8/1107/s1. Figure S1: Two examples of teff plants that didn't exhibit lodging in original plot locations. From these plants, and from other plants spotted, a single spike was collected for establishing the current seed collection. Figure S2. Populations collection propagated in the greenhouse off season. Each population originated from a single spike was sawn in on pot. Figure S3: A field experimental plot design as well as an overview of the field at early (24 DAS) growth stages. Figure S4: Early growth cover evolution of plots in the field experiment. Images were analyzed using the *Coveragetool* (ref). Figure S5: Projected stem width of the first lowest node which bears one leaf ('Upper') and, b) the most basal internode ('Lower', below a), measured at 70 DAS. Figure S6: As a part of the process of average grain weight (AGW) evaluation, seeds were spread over a white sheet and photographed. For each plot the image was threshold using '*coveragetool*' (by sampling seeds to account) to be further processed by Image J's particle count tool. Figure S7: (a) A general overview of the ARO filed experiment which shows the wide phenotypic variance of the genetic material. (b) A survey on one of the: a) White, b) 44A-163-B, c) 44B-163-W, and d) 53-2-2-W plots at: 63,70,77,87 days after sawing at the ARO field experiment. The dashed boxes is distinctive of the subjected plot. Figure S8: Flow diagram of Injera preparation. Figure S9: Images of Injera flat beard made from of each of the 11 populations characterized in the field experiment as well as three market controls.

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Abbreviations

AGW	(Average Grain Weight), g
Chl	a/b ratio
Chl/gFW	(Total Chlorophyll/gram Fresh Weight)
Chla/gFW	(Chlorophyll a/g Fresh Weight)
Chlb/gFW	(Chlorophyll b/g Fresh Weight)
DAS	(Days After Sowing), days
DPM	(Days from Panicle emergence to Maturation)
DSM	(Days from Sowing to Maturation)
DSP	(Days from Sowing to Panicle emergence),
DT < 75%	(Days to reach 75% of the plants lodging in a plot)
EGC	(Early Ground Cover), %
GY	(Grain Yield), kg/plot
HI	(Harvest Index)
LSD	(Lower Stem width), cm
MPH	(Maximal Plot Height), cm
SL	(Seed length), µm
SpL	(Spikelet length), µm
TDM	(Total Dry Matter), kg/plot
USD	(Upper stem width), cm

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