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Evaluation of teff (*Eragrostis tef* [Zucc.] Trotter) lines for agronomic traits in Australia

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Abstract

Teff (*Eragrostis tef* [Zucc.] Trotter) is a native cereal crop widely grown in Ethiopia as the main grain for local consumption. With the increasing challenge by climate change, there is a need for alternative cereal crops in Australia. However, despite its early introduction in the 1800's, there is limited information on teff production in the country. The purpose of this study was to evaluate 20 teff lines using the seed supplied by The Australian Tropical Germplasm Centre at Biloela Research Station (Queensland). A replicated glass house pot trial was carried out to test performance of agronomic traits related to yield. The teff lines were propagated in the glasshouse and data was collected over one growing season and analysed for days to flowering, days to maturity, plant height, peduncle length, internode length, leaf width and number of tillers. In addition, plant biomass, grain yield and 100 seed weight were determined. Results of this study displayed variability in teff traits demonstrating the potential for a future breeding program. Lines that exhibited promising outcomes were identified for further screening under field conditions. Teff lines 302136, 302135, 302131, 302126, 302127 and 302129 demonstrated higher performance in grain yield, tillering, panicle length and vegetative biomass signalling increased opportunity for better yield. Higher grain yield was associated with medium and late maturing lines. Regression analysis showed that vegetative biomass was positively correlated with higher grain yield. Further field evaluation and improvement of teff is required before the crop can be considered as an alternative for growers and provide a source of gluten free product for people with coeliac disease.

Keywords: Agronomic traits, *Eragrostis tef*, variety trials, gluten free, lodging.
Abbreviations: UQ_University of Queensland; PL_Peduncle length; IL_Internode length; FIL_First internode length; SIL_Second internode length; PC_principal component

Introduction

Teff (*Eragrotis tef* Zucc. Trotter) is a small-grained cereal grass species that has been grown as a food crop in east Africa for thousands of years (D'Andrea, 2008). It is a self-pollinated warm season annual grass with the advantage of C_4 photosynthetic pathway (Miller, 2010). It is a tetraploid 2*n*=40 plant (Stallknecht et al., 1993; Yu et al., 2006). The vast range of varieties is estimated to be 4000 worldwide (Davison et al., 2011) with great genetic diversity (Ayalew et al., 2011; Ayalneh et al., 2012; Kebebew and Tefera, 2003; Chanyalew et al., 2006), thus leading to increased opportunity to develop cultivars that could be suitably adapted to any country that would invest in teff production.
It is reported that in Ethiopia, believed to be the centre of origin of teff, maximum production occurs at altitudes between 1800 and 2100 m with growing season rainfall of 450-550 mm and a temperature range of 10 to 27 °C (Stallknecht et al., 1993). The temperature range of 10 to 27°C is most suitable to avoid frost (Ketema, 1997), and soil temperature range of 18°C to 27°C and above was recommended in US (Miller, 2008). Being a C_4 plant, the crop responds well to warm temperatures and can be grown in areas experiencing moisture stress as well as in waterlogged areas (Balsamo et al., 2005) as it has the ability to withstand anaerobic conditions better than many other cereals (Ketema, 1997). The crop has been introduced to other countries such as U.S.A, India, Africa, Western Europe and Australia, mainly as a forage crop. Teff seed is very small, ranging from 1–1.7 mm long and 0.6–1 mm diameter with l000 seed weight averaging 0.3–0.4 grams. It requires a firm moist seed bed for good soil moisture-seed contact due to its smaller size. Seeding rate of 15 kg/ha was recommended based on a study conducted by Laekemariam et al., (2012). Teff germinates rapidly when planted at an average depth of 0.3 to 0.6 cm, however, the initial growth is slow until a good root system has been established (Miller, 2010). It is a low input crop and would require as little as 32-46 kg/ha of Nitrogen fertiliser to boost production but excessive application would result in lodging of the crop (Miller, 2010; Nosberg et al., 2009). Teff is reported to be an aggressive crop that can outcompete weeds if well established at the seedling stage but it is recommended to establish new crops in a weed free area with clean seeds (Norberg et al., 2009; Ketema, 1997). Chemical weed control in teff is much under research (Miller, 2010). Harvesting teff for seed production can be accomplished, as long as the combine is seed tight (Miller, 2010). However, lodging which is one of the major problems with teff, can hamper effective use of a combine harvestor thus there is a need to develop a strain that has a better crown to ensure good rooting to prevent lodging, and thicker stems to effectively bear the weight of panicles (Delden et al., 2010). Teff grain yield in the US averages from 0.7 t/ha dryland to 1.4 t/ha irrigated (Stallknecht et al., 1993); 0.8 to 1.5 t/ha (Desta, 2009). In Ethiopia, the national average grain yield of teff is about 1.28 t/ha (Abewa et al., 2014). However, improved varieties of teff produced a grain yield of 1700-2200 kg/ha on farmers' fields and 2200-2800 kg/ha on research fields and well managed large farms (Ketema, 1997; Abewa et al., 2014). Teff has an

attractive nutritional profile, being high in dietary fibre, iron, calcium and carbohydrate and also has high levels of phosphorus, copper, aluminium, barium, thiamine and excellent composition of amino acids essential for humans (Hager et al., 2012; Abebe et al., 2007). Research has also shown that teff is free of gluten (Miller, 2010) and can provide alternative food source for people with celiac disease. Therefore, teff has a great potential to add to the range of cropping options extending farmer's flexibility, sustainability, gluten-free product, profitability and availability to Australian communities of east African origin. In Australia, despite some indication of its early introduction in the 1800's, there is limited information and experience in production of teff (Vennings and McMahon, 2006). Teff growing information and genotypes suitable for Australian environment are not well-known. The purpose of this study was to establish and evaluate teff varieties available in Australia and identify performance of genotypes for traits contributing to greater yield and adaptability. Such information is highly useful for further improvement through breeding. Teff seeds of 20 varieties supplied by the Australian Tropical Germplasm Centre at Biloela Research Station (Queensland) were tested.

Results and Discussion

Days to flowering and maturity

Days to flowering were recorded and the results are presented in Table 1. Maturity dates were divided into two stages of early and late maturity. The early maturing varieties were harvested on days 120 – 126. This included the varieties listed both as early and medium flowering plants. The late varieties were harvested at day 130. Days to maturity fell within the range reported by Assefa et al. (2001) (60-140 days). Sowing time for teff is governed by location and environmental condition of the growing region. In Ethiopia teff is grown during the main cereal cropping seasons between July and November whereas in US teff is planted in spring after the risk of frost has passed (Miller, 2010). Identification of lines of different days to flowering and maturity is useful in adjusting sowing time in order to avoid adverse climatic conditions such as severe frost or extreme heat in summer, particularly during flowering and grain filling. Moreover planting teff line with the appropriate growing period allows effective use of seasonal rainfall.

Plant height, internode length and peduncle length

Plant height, internode length (IL) and peduncle length (PL) of teff are important features that positively contribute to yield on the one hand and negatively to lodging on the other hand. Lodging is a major problem in teff crop and can affect grain yield (Delden et al., 2010). Plant heights of the 20 varieties were significantly different ($P \leq 0.05$; Supplementary Table 1). The tallest variety recorded was 302134 with 69.2 cm height followed by 302121 (67.6) and 302130 (64.2) making them more susceptible to lodging. Lines 302120, 302118 and 302125 recorded very low plant heights of 40.9 cm, 39.4 cm and 37.7 cm respectively. Ashraf et al. (2012) and Chanyalew (2010) indicated that there is a negative and highly significant correlation between plant height and grain yield. This may be attributed to yield loss due to lodging as a result of greater plant height and peduncle length. Generally,

Table 1. Days to flowering for 20 lines.

Early Varieties (50 – 60 days)	Medium Varieties (61 – 70 days)	Late Varieties (71 ≥ 80 days)
302120, 302122, 302125	302117, 302118,302123, 302124, 302126,302128, 302129, 302130, 302131, 302132,302133,	302119,302121, 302127,302134, 302135, 302136

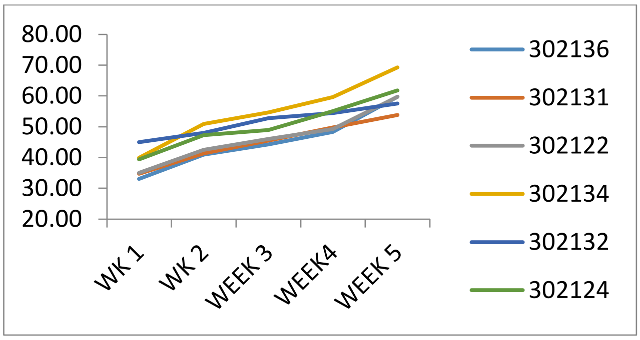

Fig 1. Weekly growth rate (cm) of sampled teff varieties.

greater (PL) were measured for the medium and late maturing lines. There was a statistically significant difference in (PL) between the lines at the 5% level. Peduncle length ranged from 60.4 (line 302121) to 29.9 cm for line 302118 (Supplementary Table 1). First inter-node lengths (FIL) were measured and analysed and result showed that line 302136 was significantly longer (9.6 cm) and lines 302128 & 302120 were shorter with lengths of 4.4 cm and 4.2 cm respectively for the FIL. For the 2nd node length (SIL), lines 302129 and 302124 (18.4 cm & 17.9 cm respectively) were the longest (Supplementary Table 1). The variation between internode lengths (FIL & SIL) was significant ($P \leq 0.05$). First and second internodes were longer for the medium and late maturing lines. Longer SIL have been reported to have a positive correlation with lodging (Delden et al., 2010). A sample of representative lines was used to demonstrate the weekly growth rate (Figure 1). The rate of growth of lines 302136 & 302131 was significantly lower than lines 302132 and 302134 while the rest of the lines were not significantly different from each other from week 3 to week 5 ($P \leq 0.05$).

Tillering, panicle length, 100 seed mass and grain yield

Tillering and panicle length were measured and analysed as they are often closely associated with grain yield. There was no significant difference in tillering among the 20 lines tested ($P \leq 0.05$). Previous studies on teff have indicated that number of tillers per plant, panicle weight and shoot biomass are contributors to yield (Chanyalew, 2010; Tefera et al., 2003). Tiller numbers produced by the different lines were generally higher (11 to 42) than reported by Assefa et al. (2001), (4 to 22) this could be due to abundance of nutrient and water under glass house environment. Panicle lengths were significantly different among lines tested and line 302136 recorded the longest 42.9 cm (Supplementary Table 1). Panicle lengths for all lines were similar to the range reported by Assefa et al. (2001) (10 to 41). Lines that produced longer panicles were mainly from the late maturity group. The lower quartiles of the 20 varieties consisted mostly of early varieties with 302118 having the shortest panicle of 23.3 cm. Line 302136

Table 2. Mean data of the agronomic traits compared using Tukey's significant difference test at 95% level of confidence.

Line	Peduncle(cm)	Panicle (cm)	Tillers (#)	Leaf Width (mm)	1st Node (cm)	2nd Node (cm)	Biomass (g)	100 seeds wts (g)	Grain Yield (g)	Height (cm)
302121	60.4 a*	42.1a	24.4 a	6.0 abc	6.3 ab	16.2 abc	153.2 ab	.03 ab	20.7 abc	59.7 abcd
302134	57.6 ab	38.0 abc	13.3 a	6.3 a	5.9 ab	15.1 abc	133.8 abcde	.04 ab	21.7 abc	57.9 bcd
302120	32.8 fg	26.7 fg	27.1 a	3.5ef	4.2 b	11.2 bc	105.0 e	.0 3b	22.5 abc	53.8 cde
302133	50.2 abc	35.2 bcd	29.3 a	5.3 abcd	7.8 ab	17.1 ab	150.9 abc	.03 b	23.1 abc	63.0 abcd
302123	46.7 bcde	35.2 bcd	28.3 a	5.1 abcd	5.2 ab	11.1 bc	151.8 abc	.04 ab	25.7 abc	59.8 abcd
302128	35.4 defg	29.3 defg	20.1a	4.2 def	4.4 ab	10.6 c	111.1 de	.04 ab	26.7 abc	56.3 cd
302119	43.1 cdefg	34.9 bcde	25.4 a	5.2 abcd	5.7 ab	12.3 abc	143.1 abcd	.04 ab	28.7 abc	64.2 abc
302118	29.9 g	23.3 g	30.6 a	3.4 ef	5.4 ab	12.1 abc	118.8 cde	.04 b	29.4 abc	57.6 bcd
302125	33.6 efg	28.6 efg	28.9 a	3.0 f	5.4 ab	12.3 abc	123.4 bcde	.03 b	30.9 abc	60.9 abcd
302126	46.0 bcdef	33.9 cde	42.3 a	5.9 abc	7.1 ab	16.4 abc	132.0 bcde	.05 a	31.2 abc	37.7 f
302117	48.4 abcd	35.0 bcde	19.2 a	5.3 abcd	6.2 ab	14.7 abc	136.5 abcde	.04 ab	32.3 abc	39.4 f
302130	51.7 abc	35.2 bcd	18 .0a	5.1 abcd	5.4 ab	13.9 abc	150.3 abc	.04 ab	33.3 abc	53.1 de
302127	53.0 abc	42..0a	23.2 a	5.8 abc	8.0 ab	16.4 abc	149.3 abc	.04 ab	35.5 abc	44.2 ef
302122	47.8 abcd	34 cde	24.8 a	4. bcde	5.9 ab	13.9 abc	142.7 abcd	.04 ab	36.9 abc	58.6 abcd
302129	48.1 abcd	38.6 abc	19 .0a	5.1 abcd	7.0 ab	18.4 a	154.8 ab	.04 ab	38.6 ab	56.6 cd
302131	52.56 abc	36.6 abc	24.4 a	5.3 abcd	6.6 ab	14.7 abc	148.9 abc	.03 ab	38.7 ab	40.9 f
302124	51.8 abc	33.0 cdef	11.1 a	4.8 cde	6.9 ab	17.9 a	56.8 f	.04 ab	4.9 c	69.2 a
302135	49.4 abc	40.9 ab	25.7 a	6.2 ab	7.7 ab	16.8 abc	166.1 a	.04 ab	41.0 a	67.6 ab
302136	46.8 bcde	42.9 a	26.2 a	5.8 abc	9.6 a	15.4 abc	155.8 ab	.04 ab	44.3 a	57.6 bcd
302132	50.8 abc	29.2 defg	14.3 a	5.1 abcd	7.2 ab	17.1 ab	66.8 f	.03 b	7.7 bc	61.8 abcd

*Means sharing similar letter (s) are not significantly different at P≤ 0.05.

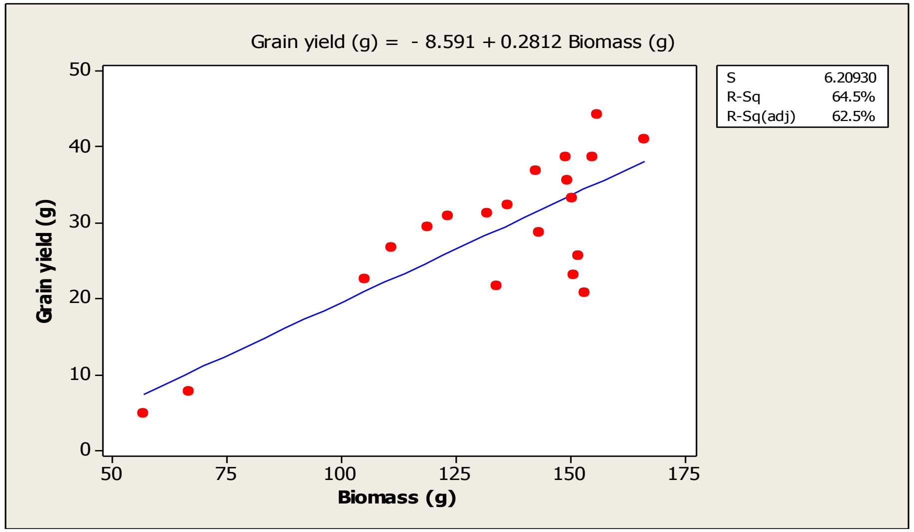

Fig 2. Regression of teff grain yield on biomass.

Table 3. Ambient temperature during teff growing season.

Temperatures (°c)	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Highest daily	25.2	22.7	28.8	32.6	37.8	38.7	39.7
Lowest daily	14.5	16.2	18.0	21.9	19.2	21.8	27.6
Monthly mean	20.7	20.4	23.6	26.7	29.2	30.5	32.0

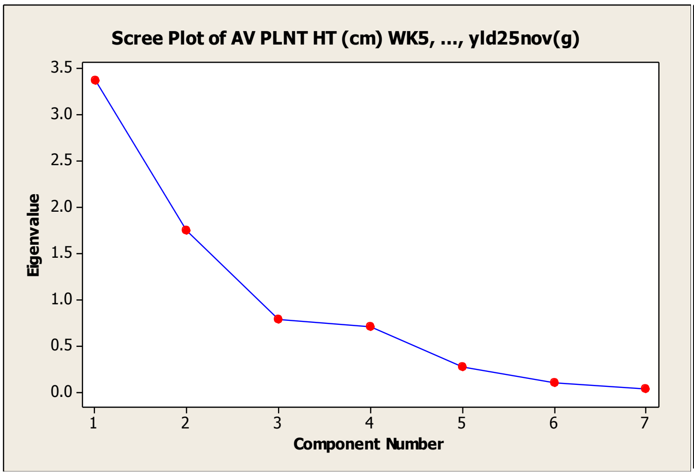

Fig 3. Visualisation of the Eigenvalues as well as the variance explained by each component.

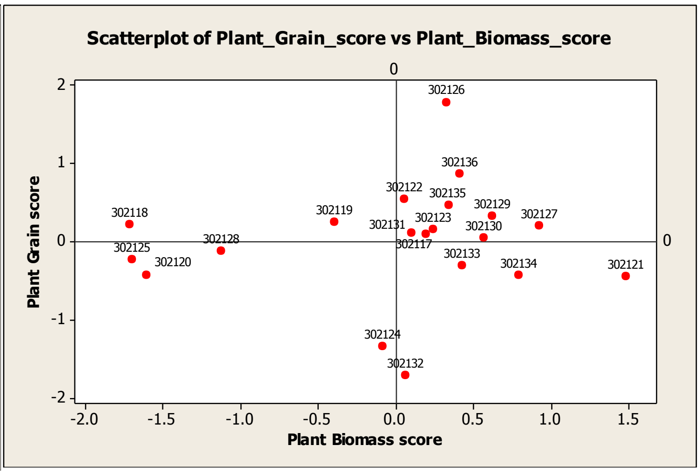

Fig 5. Scatterplot of parameters contributing to grain yield vs those contributing to biomass.

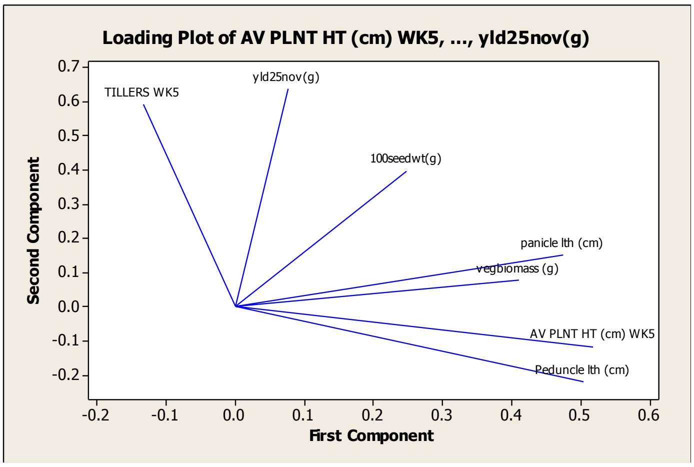

Fig 4. Loading plot for the way the variables contribute to the first 2 dimensions. The 100 seed weight vector is at 45 degrees or so, illustrating its contribution to both dimensions.

Fig 6. Teff plants at grain filling stage in the glasshouse

produced longer panicles as well as higher grain yield confirming the early finding by Assefa et al. (2001). However, longer panicles did not always translate into higher grain yield. Hundred seed mass for the different lines ranged from 0.03 to 0.05. The highest seed mass was recorded from line 302126 (0.05 g) and it was significantly higher than that recorded from many of lines that produced 0.03 g. (Supplementary Table 1). Hundred seed mass has contributions to greater crop yield while other factors such as number of grain spikes and number of productive tillers are also indicated by Ashraf et al. (2012) as being important. Lines 302136 and 302135 recorded the highest grain yield (44.3 g & 41.0 g respectively) while lines 302132 & 302124 produced the lowest grain yield of 7.7 g and 4.9 g, respectively.

Leaf width, vegetative biomass and lodging

Leaf width measurements were significantly different with line 302134 recording the maximum width of 6.3 mm (Supplementary Table 1). With regards to vegetative biomass, results showed that line 302135 (166 g) was significantly higher than the low biomass lines (Supplementary Table 1). Line 302124 exhibited the lowest biomass (57 g) followed by lines 302132 (67 g) and were significantly different from the remaining lines. Biomass provides a reasonable indication of potential yield and accounted for 64.5% of the variation in grain yield (Figure 2). There was no significant difference in lodging among lines possibly due to the fact that plants were kept in the glass house where wind effect was minimal. In addition, as water and nutrients were also maintained at the controlled level in the glasshouse, lodging is likely to be much less than under field conditions. Hundera et al. (2000) found that teff plants with greater shoot biomass and grain yield tend to lodge more easily. He also reported that plants that headed earlier were prone to lodging compared to those that headed later.

Cluster and principal component analysis

Cluster analysis produced a number of smaller groups from which the main ones were selected for principal component (PC) analysis. The principal component analyses demonstrated that the first 2 components (PC1 & PC2) explained 73% of the relationship and were the only ones with significant Eigenvalues, suggesting that the two dimensions are sufficient to explain relationships between the lines (Fig. 3).

Based on the scores, PC1 is driven by height, peduncle length, panicle length and biomass, while the second component (PC2) is driven by tillers and yield. The 100 seed weight contributes to both, but loads more on the second component than the first. The loading plot (Fig. 4) revealed the way the variables contribute to the first 2 dimensions. The 100 seed weight vector is at about 45°, illustrating its contribution to both dimensions. The scatter plot for the two components (Fig. 5) showed the following three main cluster groups of the teff lines.

1) 302118, 302125, 302128 and 302120 where, 302125 and 302120 are from the early maturing lines.

2) 302134, 302124 and 302132. Teff line 302132 produced the lowest vegetative biomass.

3) The rest (14 lines) fall in this group with lines 302126 and 302121 appearing as outliers at the opposite side of the X axis. However these two lines stood out as best performers with Line 302126 showing high performance in parameters contributing to higher grain yield and it also produced the maximum seed weight. Line 302121 was the second tallest among the 20 lines.

Materials and Methods

Plant material and location

A glasshouse trial was conducted at the University of Queensland (UQ), Gatton campus to provide information on varietal characteristics. Seed of 20 varieties of teff were obtained from the Australian Tropical Germplasm Centre at Biloela Research Station in Queensland, Australia. The twenty lines of teff were propagated in the glasshouse in UQ Gatton, Australia (27° 33' S, 152° 20' E) on the 2nd of June and harvested in October 2012.

Growth conditions

Plastic pots with perforated bases, 33 cm in radius and 22 cm in height were filled with the potting media to ensure proper germination of seeds. The potting media consisted of 1 m^3 of composted pine bark and woodchip to which nutrients including 3 kg of osmocote, 2 kg of nutricote, 1.3 kg each of osmoform and coated iron, 1.2 kg of saturaid and 1.3 kg of dolomite were added. About seven seeds were planted in each pot at a depth less than 12 mm. The pots were placed in a controlled environment (Fig. 6) of 84% humidity and temperature of 24°C for three weeks. Thinning was carried out to leave 3 plants per plot. The pots were then transferred to glass house with 70% light and ambient temperature condition as presented in Table 3. The photoperiod from planting to flowering calculated for the location was approximately 10.5 h. A randomised complete block design was used and each variety was replicated three times.

Measurements and data collection

The teff lines were grown from June to October and data collected included days to flowering (from planting to 50% of plants flowering), days to maturity (from planting till 50% of plants showed physiological maturity), plant height (soil level to tip of tallest flag leaf), peduncle length, internode length, leaf width and number of tillers. In addition, plant biomass, grain yield and 100 seed weight were determined. Observations were also carried out on lodging (permanent displacement of plant from crown) of the plants. Measurements were taken on three plants of the same variety in each pot. Vegetative parts of the plant were harvested and oven dried (60°C; 188 hours) to determine biomass. The growth rate of each variety was observed for five weeks and recorded. When the crops reached flowering stage, watering was applied to base of plant only to reduce lodging.

Statistical analysis

Analysis of variance, linear regression based on phenotypic means and cluster analysis were performed using the Minitab 16 statistical package (Supplementary Table 1). Treatment means were compared by Tukey's simultaneous test at the 5% level of significance. The cluster analysis was carried out by first conducting partial correlation using standardized data to determine associations between the parameters measured. This was followed by principal component analysis, which demonstrated that the first 2 components explained 73% of the relationship and were the only ones with significant Eigenvalues, suggesting that the two dimensions are sufficient to explain relationships between the lines. The loading plot and scatter plots were then plotted to demonstrate the clusters formed by the different teff lines.

Conclusion

Teff has a great potential in Australia as a grain crop and it is important to evaluate available lines and identify lines best suited to the climatic conditions. Results of this study displayed variability in teff traits highlighting the potential for future breeding programs. The glasshouse trial produced data that could be used to identify lines with higher potential yield and that would be worthy of further screening under field conditions. Identification of lines of different days to flowering and maturity would be useful in adjusting sowing time in order to avoid adverse climatic conditions in Australia. Teff lines 302136, 302135, 302131, 302126, 302127 and 302129 demonstrated higher performance in grain yield, tillering, panicle length and vegetative biomass signalling great yield potential and could be recommended for further field based research. Most of these traits attributed to higher grain yield were from medium and late maturing lines providing possibilities of extended planting time. Regression analysis showed that vegetative biomass was positively correlated with higher grain yield. Hence, lines that produced higher vegetative biomass such as 302135 may be useful in breeding for higher grain yield. The glass house trial didn't produce great variation in tillering and lodging of the lines examined and this may be a limitation that needs to be further ascertained through field experiments. However, most of the other traits examined resulted in a significant difference among lines providing opportunity for exploitation in teff improvement. Therefore, with further field evaluation and improvement, teff can be considered as alternative cereal crop for growers and also will be beneficial as an additional gluten free source for people suffering from coeliac disease in Australia.

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