

# Leaf Photosynthesis, Nitrogen Use Efficiency, and Relationships with Growth and Yield in Teff

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## Research article

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# Abstract

**Background:** Teff (*Eragrostis tef* (Zucc.) Trotter) is an important grain crop, but a paucity of research means that the mean yield is below  $1.5 \text{ t ha}^{-1}$ . Its high genetic diversity has not been exploited to improve its yield potential. Previous efforts at increasing yield were based entirely on phenotypic, morphologic, and agronomic merits. However, photosynthetic capacity has been neglected, so its possible contributions to yield improvements remain unexplored.

**Results:** We grew 15 teff cultivars in a controlled environment to explore variations in photosynthetic capacity and nitrogen use efficiency (NUE), and examined the relationships of gas exchange and NUE before anthesis with growth and yield attributes. Substantial differences were found in leaf photosynthetic rate ( $19 \pm 9 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , mean  $\pm$  SD), stomatal conductance ( $0.11 \pm 0.09 \text{ mol m}^{-2} \text{ s}^{-1}$ ), and transpiration rate ( $2.4 \pm 1.3 \text{ mmol m}^{-2} \text{ s}^{-1}$ ). The cultivars differed significantly ( $P < 0.05$ ) in both NUE ( $190 \pm 227 \text{ g m}^{-2}$ ) and photosynthetic NUE ( $59 \pm 34 \mu\text{mol g}^{-1} \text{ s}^{-1}$ ). On average, the plants partitioned 29% of N to leaf, 28% to panicle, and 13% to stem before anthesis. Yield and biomass production were closely associated with photosynthetic capacity and NUE. Clustering of the cultivars on the basis of photosynthesis, NUE, growth, and yield indicates wide variations in photosynthetic capacity and NUE in the wider teff gene pool that should be exploited.

**Conclusion:** We conclude that leaf photosynthesis and NUE are positively related to yield and biomass production in teff.

## Background

Teff [*Eragrostis tef* (Zucc.) Trotter] is a  $C_4$  (Kebede et al., 1989), self-pollinating, annual cereal (Gugsa et al., 2006; Ketema, 1997) grown mainly in Ethiopia, where it is believed to have originated (Vavilov, 1951). The grain is gluten free (Spaenij-Dekking et al., 2005; Zhu, 2018) and the plant is relatively tolerant to drought (Ketema, 1997; Teklu and Tefera, 2005) and waterlogging (Assefa et al., 2011; Ketema, 1993). Teff supports millions of people as a staple food crop in Ethiopia (Araya et al., 2010; Assefa et al., 2011). Teff straw is a preferred feed for livestock (Assefa et al., 2001b; Assefa et al., 2011; Teklu and Tefera, 2005). Annually,  $3 \times 10^6$  ha of land produces  $5.2 \times 10^6$  t of teff grain in Ethiopia (CSA, 2018). Teff is the main ingredient in the traditional flat bread called “injera”, and is used in bread, pancakes, porridge, and alcoholic beverages in Ethiopia. Teff is grown mainly as a forage crop outside Ethiopia, but demand for the grain is expanding worldwide owing to its nutritional benefits, mainly as it is gluten free and is rich in lysine, iron, and calcium (Baye, 2014; Mengesha, 1966; Paff and Asseng, 2018).

Although teff is a high-value crop, it has received little research attention (Assefa et al., 2001a; Assefa et al., 2011; Paff and Asseng, 2018). As a result, its grain yield remains below  $2 \text{ t ha}^{-1}$  (CSA, 2018). The low yield is attributed to the low yield potential of current cultivars, lack of improved agronomic technologies, and its susceptibility to lodging, exacerbated by a decline in soil fertility and by moisture stress in Ethiopia (Assefa et al., 2001b; Assefa et al., 2011; Paff and Asseng, 2018). Its high genetic diversity has

not been exploited to improve crop productivity (Assefa et al., 2015; Assefa et al., 1999; Assefa et al., 2003; Assefa et al., 2001b; Bai et al., 2000; Paff and Asseng, 2018). Because teff is little known globally, genetic improvement is entirely dependent on the local gene pool (Assefa et al., 2015; Assefa et al., 1999; Assefa et al., 2001b; Assefa et al., 2011; Bai et al., 2000; Teklu and Tefera, 2005). Previous efforts have focused on exploring genetic diversity on the basis of phenotypic, agronomic, and morphological traits (Assefa et al., 2015; Assefa et al., 2001a; Assefa et al., 2011; Chanyalew et al., 2013; Teklu and Tefera, 2005). Although some genetic gain has been achieved, the yield potential of improved teff cultivars remains far below those of most other cereals (Assefa et al., 2011; Teklu and Tefera, 2005). On the other hand, increasing yield potential by enhancing photosynthetic capacity is untouched. Advances in yield and biomass have been made possible by improving leaf photosynthetic rates in wheat (Driever et al., 2014; Makino, 2011) and rice (Alvarez et al., 2015; Makino, 2011; YE et al., 2019). However, leaf-level photosynthesis is not always directly related to crop yield (Driever et al., 2014; Long et al., 2006; Makino, 2011; YE et al., 2019) owing to the complexity of yield formation mechanisms. Leaf photosynthesis can be improved through improved light interception, rubisco activity, mesophyll conductance, and light interception (Long et al., 2006). Exploiting inherent variations in photosynthetic performance of available genetic resources is a key to improving yields. The exploitation of natural variation in leaf photosynthesis has improved yields in several crops, notably wheat (Driever et al., 2014; Makino, 2011) and rice (Makino, 2011; YE et al., 2019). In addition, nitrogen use efficiency (NUE) greatly influences leaf photosynthetic rate by regulating rubisco activity, and natural variation in leaf photosynthetic capacity may be attributed to differences in leaf N content (Driever et al., 2014; Sinclair and Horie, 1989; YE et al., 2019). Thus, quantifying and understanding differences in NUE among existing genetic resources and determining its relationship with photosynthesis and yield-related traits is important.

Crop yield is a function of photosynthetic performance, yet there is no information on variations in the photosynthetic capacity and NUE of teff, governing factors, or potential contributions to yield improvement. Despite the large genetic diversity present in teff, potential variations in photosynthetic capacity and NUE are not well understood. Thus, yields could be increased through enhancing NUE, photosynthetic capacity, or both. Therefore, exploring and characterizing variations in photosynthetic capacity and related traits in teff genetic resources is an important task for improving yields.

The objective of this study was to explore potential variations in photosynthetic capacity and NUE and their possible association with yield and biomass traits of teff. We grew 15 teff cultivars obtained from the Japanese Genetic Resources Center, National Agriculture and Food Research Organization (NARO), in a glasshouse. As this is the first study to analyze variations in photosynthetic capacity and NUE in teff, knowledge gained from this work can be used to reveal variability of such traits within the wider teff gene pool and to improve yields via genetic and agronomic manipulations.

## **Results And Discussion**

### **Leaf photosynthesis**

Cultivars had substantial variations in net photosynthetic rate ( $P_n$ ), with significant differences ( $P < 0.01$ ). Values ranged from  $4 \mu\text{mol m}^{-2} \text{s}^{-1}$  (cv. 3713) to  $38 \mu\text{mol m}^{-2} \text{s}^{-1}$  (cv. 3704), with a mean of  $19.4 \pm 9.0 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Fig. 1). Stomatal conductance ( $g_s$ ) and transpiration ( $T$ ) also differed significantly ( $P < 0.01$ ). The mean  $g_s$  was  $0.11 \pm 0.09 \text{ mol m}^{-2} \text{s}^{-1}$  and the mean  $T$  was  $2.4 \pm 1.3 \text{ mmol m}^{-2} \text{s}^{-1}$ .  $P_n$  was strongly correlated with both  $g_s$  and  $T$  (Fig. 2).  $P_n$  was also positively associated with both aboveground biomass yield (BY) (Fig. 3) and grain yield (GY), despite the low but significant correlation coefficients. Overall, cultivars with better photosynthetic capacity had better growth and yield.

The variation in  $P_n$  among cultivars indicates the presence of natural diversity in photosynthetic capacity within the teff gene pool. The observed variations in growth and physiological traits might be contributing factors, as crop biomass production is governed mainly by photosynthetic capacity (Driever et al., 2014; Mae, 1997; Makino, 2011; Raines, 2011). Until now, no attempt has been made to understand variations in leaf photosynthetic traits and the association with yield and biomass of teff. We found significant variability in leaf  $P_n$ ,  $g_s$ , and  $T$ .  $P_n$  at ambient  $\text{CO}_2$  ( $393 \mu\text{mol mol}^{-1}$ ) under saturated light ( $1000 \mu\text{mol s}^{-1} \text{m}^{-2}$ ) ranged from  $1.5$  to  $37.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The photosynthetic rate of the improved cultivar 'DZ-01-354' ranged from  $27$  to  $31.8 \mu\text{mol m}^{-2} \text{s}^{-1}$  at a range of temperatures (Kebede et al., 1989). In contrast, that of 'DZ-01-196' was  $<4 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Tekalign, 2007). The disparity might be due to the collection of measurements at an early growth stage and the difficulty of gas exchange measurements under non-uniform field conditions. Compared with  $\text{C}_3$  crops, the  $P_n$  values of teff are slightly higher than ranges of wheat (Driever et al., 2014; Makino, 2011) and rice (DA MATTA et al., 2001; Hirasawa et al., 2010). However, the maximum  $P_n$  of teff is generally smaller than that of most other  $\text{C}_4$  crops (Kebede et al., 1989); for instance,  $11.7$ – $42.4 \mu\text{mol m}^{-2} \text{s}^{-1}$  in sorghum (Fernandez et al., 2015),  $23.4$ – $53.8 \mu\text{mol m}^{-2} \text{s}^{-1}$  in maize (DA MATTA et al., 2001), and  $24.0$ – $39.5 \mu\text{mol m}^{-2} \text{s}^{-1}$  in sugarcane (DA MATTA et al., 2001). Despite the complexity, the lower photosynthetic capacity of teff may be attributable to reduced photosynthetic apparatus due to the smaller plant size, lower rubisco kinetics, less efficient  $\text{CO}_2$  fixation mechanism, and less efficient chloroplast electron transport systems than in maize, sorghum, and sugarcane (DA MATTA et al., 2001; von Caemmerer and Furbank, 2016). Stomatal conductance also varied widely among teff cultivars, from  $0.01$  to  $0.95 \text{ mol m}^{-2} \text{s}^{-1}$ . The changes in  $P_n$  were positively correlated with  $g_s$ , suggesting that differences in stomatal behavior might cause variation in the  $P_n$  of teff. This suggests the need to investigate possible differences in stomatal and mesophyll architecture, stomatal density, and stomatal size among teff genetic resources. Significant correlations between  $P_n$  and  $g_s$  have also been reported in rice (Kusumi et al., 2012), wheat (Fischer et al., 1998), and other species (Gago et al., 2016; Radin et al., 1988).

Our results show that leaf  $P_n$  is associated more strongly with BY than with GY. Biomass production is a direct function of photosynthesis, but yield formation is a more complex process that also depends on the efficiency of biomass conversion to grain yield, as in wheat (Long et al., 2006; Parry et al., 2010). This positive relationship substantiates the contribution of leaves to plant- and canopy-level photosynthesis and to biomass- and yield-making processes, as in similar studies of cereal crops (Makino, 2011; Parry et

al., 2010; Raines, 2011; YE et al., 2019). In contrast, other studies reported that leaf-scale photosynthesis is not consistently related with biomass and crop yield (Driever et al., 2014; Long et al., 2006; Makino, 2011). The significant differences in yield among teff cultivars might reflect their marked variation in photosynthetic traits. Genetic variability in yield and yield attributes of various teff germplasms has been reported by several studies (Assefa et al., 2015; Bedane et al., 2015; Teklu and Tefera, 2005; Tesema et al., 2013). Yet, despite yield increases from 0.7 t ha<sup>-1</sup> in 1994 to 1.5 t ha<sup>-1</sup> in 2013 in Ethiopia through selection and hybridization, the yield of teff remains very low owing to limited research (Assefa et al., 2015; Paff and Asseng, 2018; Teklu and Tefera, 2005). Most new teff cultivars, including the famous high-yielding 'Quncho', have been bred to target yield and related agronomic traits. As a result, further yield improvement is unlikely using the same approach, unless breeders include photosynthesis-related traits, which are considered selection criteria for yield improvement in many crops (Long et al., 2006; Raines, 2011; YE et al., 2019). Our findings reveal the presence of untapped natural variations in photosynthetic rates, the potential utilization of which can lead to yield improvements. We might have found more variability if we had included more teff cultivars and wild relatives in this study, but the export of teff genetic resources from Ethiopia is currently banned.

## **N distribution and NUE**

We quantified N contents of leaf (LN), panicle (PN), stem (SN), and seed (SDN) of teff cultivars and determined their relationship with photosynthetic and growth characteristics (Table 2). We found significant variation in LN, SN, and SDN (but not PN) among cultivars. The means were 1.4 ± 0.67 mg LN g<sup>-1</sup>, 1.4 ± 0.54 mg PN g<sup>-1</sup>, 0.81 ± 0.50 mg SN g<sup>-1</sup>, and 2.1 ± 0.55 mg SDN g<sup>-1</sup>. On average, the N distribution ratios were 29% to leaf, 28% to panicle, 13% to stem, and 30% to seed at maturity. Differences in leaf chlorophyll content (Chl) among cultivars were highly significant. Chl ranged from 26.8 µg cm<sup>-2</sup> in cv. 3711 to 49.3 µg cm<sup>-2</sup> in cv. 3704, with a mean of 33.8 µg cm<sup>-2</sup>. There was a strong correlation ( $R^2 = 0.72$ ,  $P < 0.05$ ) between LN and Chl. The variation among cultivars in N distribution ratios among plant parts might be attributable to substantial variation in NUE and P-NUE. Importantly, both BY and P-NUE were positively correlated with NUE, showing that changes in BY and GY are associated with changes in NUE (Fig. 4).

Plant N content is crucial to photosynthesis and to yield- and biomass-making processes, which vary with NUE and with N availability in the soil. Breeding cultivars for high leaf N improved the photosynthesis and yield of wheat (Gaju et al., 2014; Makino, 2011) and rice (Gaju et al., 2014; Hirasawa et al., 2010; Makino, 2011). Varietal differences in plant N and NUE and their relationships with  $P_n$  and yield are not well known in teff (Paff and Asseng, 2018). Pre-anthesis leaf N is the major source of N allocated to the grain (Gaju et al., 2014). We found significant differences in leaf and seed N contents among cultivars under uniform N fertilizer application. Varietal differences in leaf N are known in rice (Hirasawa et al., 2010; Makino, 2011), wheat (Gaju et al., 2014; Makino, 2011), and maize (Gallais and Coque, 2005; Gallais and Hirel, 2004). No data is available on the average N content of teff plant parts, let alone varietal differences in N distribution; however, (Paff and Asseng, 2018) reported values of 4–90 mg N g<sup>-1</sup> in teff

straw and 12–310 mg N g<sup>-1</sup> in teff seed. Their values are much greater than the average N contents we found, of 0.81 mg N g<sup>-1</sup> in stem and 2.1 mg N g<sup>-1</sup> in seed, maybe because they evaluated plants under high N supply. The differences in plant N content detected here indicate the presence of underutilized wide genetic variations in NUE in the teff gene pool. Analyses of QTLs for NUE in teff should include as many cultivars as possible and test under low and high soil N conditions.

Pre-anthesis leaf N determines grain filling and grain number of cereal crops (Makino, 2011; Yoshida, 1981). Grain yield of teff is highly correlated with grain N and total plant N (Balcha et al., 2006). Total plant N in general and leaf N content in particular are proportional to both yield and biomass of wheat (Gaju et al., 2014; Makino, 2011), rice (Makino, 2011; YE et al., 2019), and maize (Gallais and Hirel, 2004). Leaf N status has been used in deciding N management and predicting yields of most cereal crops. Therefore, the dynamics of leaf N at different crop stages is not only a potential input for future N management strategies, but also a good indicator of yield performance in teff. As expected, our results show that Chl was closely associated with leaf N content. We derived the value of Chl from SPAD readings with a general empirical formula (Cerovic et al., 2012), because SPAD measurement is not calibrated for teff. Quantification of Chl would be more accurate if SPAD readings could be calibrated against actual extracted Chl. We expect that the correlation between leaf N and chlorophyll content would be stronger in this case. Mathematical relationships have been established between leaf N, Chl, and SPAD reading and have been used for predicting N requirements of several crops species (Wood et al., 1993), so this should be possible in teff.

However, we found no direct relationship between leaf N and  $P_n$ ,  $g_s$ , and  $T$ , even though leaf N is one of the major factors governing  $P_n$  and stomatal features (Driever et al., 2014; Gallais and Coque, 2005; Kramer and Boyer, 1995; Long et al., 2006; Makino, 2011; YE et al., 2019). Leaf-level photosynthesis is not always directly correlated with leaf N (YE et al., 2019), since it is influenced also by various stomatal and non-stomatal factors (Hirasawa et al., 2010; Kramer and Boyer, 1995). As we sampled the same leaf during leaf N quantification at the same time as measuring gas exchange, this could have contributed to the weak correlation between leaf N and  $P_n$ . We recommend that further studies investigate natural variation in leaf N and NUE among teff cultivars and their interactions with N supply.

## Growth performance

Plant height (PH), biomass (BY), and grain yield (GY) varied widely among cultivars. The mean PH increment was ~7 cm every 10 days. On average, cultivars height increment rate was 0.7 cm day<sup>-1</sup> after planting (Fig. 5), but differences in BY and GY were significant ( $P < 0.05$ ). The means were  $69 \pm 62$  g m<sup>-2</sup> for GY and  $572 \pm 444$  g m<sup>-2</sup> for BY. Both BY and GY were highest in cv. 3704 and lowest in cv. 3708 (Fig. 6). There was a strong correlation ( $R^2 = 0.94$ ;  $P < 0.01$ ) between BY and GY, as previously reported (Assefa et al., 2015); Dargo et al. (2016). Although not statically significant, days to emergency for cultivars was ranged from 4 to 6 days; days to heading ranged from 53 to 60 days; and days to physiological maturity ranged from 138 to 157 days. Better GY is a function of enhanced crop growth that leads to better

biomass production. In contrast, vigorous biomass growth may cause lodging, particularly at high N application, and thus yield reduction (Habtegebrial et al., 2007). Teff is sensitive to root and stem lodging (Habtegebrial et al., 2007). However, lodging was controlled in our study. Similarly, cultivars differed widely in their dry matter allocations to leaves, panicles (significantly), and stems before anthesis (Table 2). The mean dry weight distributions per plant were  $0.25 \pm 0.05$  g (14%) to leaf,  $0.57 \pm 0.33$  g (35%) to panicle, and  $0.77 \pm 0.26$  g (51%) to stem. Limited data is available on dry matter distribution of teff; however, the stem partitioning index was reported as 0.44 (Bediye et al. (1996). Dry matter allocation depends on crop or cultivar type, growth stage, soil nutrient availability, and moisture availability (Marcelis, 1996). Overall, our results show that allocating a higher proportion of dry matter resulted in greater GY. Similarly, performances in BY and GY were closely associated with photosynthetic capacity and NUE.

### Traits association

We analyzed associations among 27 growth, yield, and photosynthetic traits. Among growth and yield traits, total plant dry biomass, total plant N content, panicle weight, panicle N content, and NUE explained most of the variation among cultivars (Fig. 7). Among physiological parameters,  $P_n$  and  $T$  contributed most to the overall variation. Principal component analysis clustered the cultivars into three groups: group 1, cvv. 3704, 3729, 3715, 3712, and 3707; group 2, cvv. 3706, 3727, 3716, 3728, 3710, 3708, and 3703; and group 3, cvv. 3709, 3711, and 3713 (Fig. 7). This clustering indicates that NUE and photosynthesis-related parameters are good descriptors for evaluating teff genetic resources in addition to agronomic criteria. Genetic mapping using diversity array technology clustered cultivars into three groups also: group 1, cvv. 3703, 3713, 3729, 3707, and 3709; group 2, cvv. 3727, 3712, 3716, 3728, 3715, 3708, and 3710; and group 3, cvv. 3711, 3704, and 3706 (Fig. 8). Although the groupings do not match, the results show the presence of variation among the teff cultivars evaluated here. As most of these cultivars were collected from teff improvement fields, they were already selected by breeders for their yield-related merits. However, selection of teff cultivars has never been based on NUE and photosynthetic traits. Therefore, it is to be expected that their genetic differences would not fit the variation observed here among variables related to their photosynthetic and NUE performance. As crop growth and development is a complex process governed by multitudinous genes, it is unlikely to always match genetic and phenotypic observations related to yield and physiology. This is why, for example, improvements in photosynthetic capacity of wheat and rice have been possible without changes in genetic makeup (Makino, 2011). Despite the wide diversity in agronomic and morphologic features of teff, little is supported by genetic information (Assefa et al., 2015).

## Conclusion

The presence of significant variation in leaf photosynthetic rate, NUE, and growth performance among the 15 cultivars evaluated here reveals the potential variability that could be utilized from the huge diversity in teff genetic resources. The significant associations with biomass and yield show that using photosynthetic rate and NUE offers promise for increasing yield. Developing N-efficient cultivars will

greatly help in minimizing the trend to increasing N fertilizer application and thus lodging, which reduces yield. Since we evaluated few cultivars compared with the thousands available, we recommend large-scale characterization and identification of important phenotypic and genotypic traits related to higher photosynthetic rate and NUE.

## List Of Abbreviations' Section

**NUE:** nitrogen use efficiency

**GY:** grain yield

**BY:** biomass yield

**PH:** plant height

**LN:** leaf nitrogen

**PN:** panicle nitrogen

**SN:** stem nitrogen

**SDN:** seed nitrogen

**TPNup:** total plant N uptake

**Chl:** Chlorophyll contents

**Pn:** photosynthetic rate

**gs:** Stomatal conductance

**T:** transpiration (*T*)

## Methods

### Planting materials and growing conditions

The 15 teff cultivars were obtained from the Genetic Resources Center, NARO, Japan. They originated from Ethiopia and were imported into Japan in 1972 (see Table 1 for passport and accession number data). Formal collection and identification of the cultivars were made by NARO. In addition, voucher specimen of this material has been deposited in a publicly available. Further information can be accessed from the website: [https://www.gene.affrc.go.jp/databases-plant\\_search\\_en.php](https://www.gene.affrc.go.jp/databases-plant_search_en.php). Teff is a tropical short-day plant that requires 11–13 h of sunlight and a temperature range of 20–22 °C (Kebede et al., 1989). Hence, it is difficult to grow teff outdoors in Japan, where daylength and temperatures fluctuate throughout the year. So we conducted a greenhouse experiment at the Arid Land Research

Center of Tottori University. The glasshouse was equipped with ceramic metal halide lamps with an average intensity of  $98.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Supplementary lighting was supplied every day at 05:00–07:00 and 16:00–18:00 to meet the average daylength and temperature requirements. During the experiment, the average daily minimum, mean, and maximum temperatures inside the greenhouse were 23.0, 23.4, and 30.9 °C. Seedlings were raised in plastic trays and watered every day. They were transplanted at 30 days (19 January 2018) into rectangular pots with dimensions of 75 cm (length) × 25 cm (width) × 55 cm (depth). Pots were filled with slightly acidic soil that contained 4.18 mg total C  $\text{g}^{-1}$ , 0.23 mg total N  $\text{g}^{-1}$ , 0.15 mg available P  $\text{g}^{-1}$ , 0.215 mg K  $\text{g}^{-1}$ , 0.22 mg Cu  $\text{kg}^{-1}$ , 13.00 mg Fe  $\text{kg}^{-1}$ , 12.8 mg Mn  $\text{kg}^{-1}$ , and 0.27 mg Zn  $\text{kg}^{-1}$ . Each pot had six hills 5 cm apart and two rows 25 cm apart, with two seedlings per hill. Seedlings were planted at a depth of ~3 mm. Each pot received a total of ~10 g of superphosphate (7.7% P; all at planting) and 5 g of ammonium phosphate (21% N; half at planting, half at booting stage). Pots were watered every other day; a water depth of ~5 mm was kept to maintain field capacity. Plants were harvested on 14 June 2018, when >80% of the stands had reached physiological maturity.

### Gas exchange measurements

Plants were grown in a uniform environment and treated equally to avoid the influence of the external environment during leaf gas exchange measurements. Gas exchange was measured with a Li-Cor LI 6400 portable photosynthesis system. Using an infrared gas-exchange system with a 2-cm<sup>2</sup> leaf chamber, we measured fully mature flag leaves during 08:00–12:00 week before anthesis (heading). During measurement, the photosynthetic photon flux density (PPFD) was kept at 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-2}$  by an LED light source with 10% blue and 90% red light. Throughout the measurements, the concentration of CO<sub>2</sub> was  $393.2 \pm 3.6 \mu\text{mol mol}^{-1}$ . The relative humidity was set at ~40% to stabilize the vapor pressure density within the chamber, and the temperature was held at  $29.3 \pm 3.2$  °C. Data were recorded under steady state, 20 min after leaves were inserted into the chamber. Net photosynthetic rate ( $P_n$ ), stomatal conductance ( $g_s$ ), and transpiration rate ( $T$ ) were calculated as described by (Evans and Santiago, 2014).  $P_n$  and  $T$  were calculated from the fluxes of H<sub>2</sub>O and CO<sub>2</sub> between leaf and atmosphere, leaf temperature, air flow rate, atmospheric pressure, irradiance, and leaf area in the chamber.

### Yield and yield attributes

Four plants were randomly harvested from each cultivar before anthesis, and leaves, stems, and panicles were detached and weighed. Detached leaves were scanned on an HP Laserjet scanner and area was calculated in ImageJ software (Schneider et al., 2012). The mean of the four plants was recorded. Leaves, stems, and panicles were oven-dried at 80 °C for 72 h.

Panicles were harvested at full physiological maturity. After air drying for a week, they were threshed, and grain yield was determined. The aboveground dry biomass weight was also determined after oven-drying at 80 °C for 72 h. Grain yield (GY) and aboveground biomass yield (BY) per plant were calculated as ratios to total weight and total number of plants of each cultivar.

## Plant N quantification and NUE

Oven-dried samples were ground for N and C quantification. N and C contents of 500 mg each of leaf, stem, and panicle were determined by combustion in a JM1000CN Macro Corder, J-SCIENCE GROUP. NUE and photosynthetic NUE (P-NUE) were determined as:

$$\text{NUE} = \frac{GY}{\text{TPNup}} \quad \text{eq. 1}$$

$$\text{P-NUE} = \frac{P_n}{LN} \quad \text{eq. 2}$$

where GY is grain yield per plant; TPNup is total plant N uptake (N uptake by leaf + panicle + stem);  $P_n$  is net photosynthetic rate; and LN is leaf N content.

Chlorophyll contents (Chl) of flag, primary, and secondary leaves were estimated with a SPAD-502 Plus chlorophyll meter (Konica Minolta) before anthesis. SPAD readings were converted to surface-based specific units ( $\mu\text{g cm}^{-2}$ ) as (Cerovic et al., 2012):

$$\text{Chlorophyll content (Chl)} = \frac{99 \cdot \text{SPAD}}{144 - \text{SPAD}} \quad \text{eq. 3}$$

## Genotyping

In order to confirm and examine the genetic diversity of the 15 teff cultivars, fresh leaves were collected from one-month-old seedlings and genomic DNA was extracted as illustrated by Edet et al. (2018). NanoDrop2000C Spectrophotometer was used to check the quality and concentration. The purified DNA samples (1  $\mu\text{g}$  for each sample) were sent to Diversity Arrays Technology Pty Ltd (<http://www.diversityarrays.com/>) for genotyping using the DArTseq genotyping platform.

## Data analysis

Data were tested for normality by the Shapiro–Wilk test (Shapiro and Wilk, 1965). All analyses were performed in R v. 3.3.2 software (Team, 2013). Analysis of variance (ANOVA) and mean separation by least significance difference (LSD) (Williams and Abdi, 2010) were used to detect cultivar differences in photosynthetic traits, NUE, and yield-related attributes. Correlations among parameters were tested by Pearson's method (Hauke and Kossowski, 2011) with linear and multiple regression models. Principle component analysis using 15000 SNP markers was performed in TASSEL (Trait Analysis by association, Evolution and Linkage) software to illustrate the genetic diversity in the teff cultivars.

## Declarations

**Ethics approval and consent to participate:** not applicable

**Consent for publication:** not applicable

**Availability of data and materials:** All data generated or analysed during this study are included in this published article

**Competing interests:** The authors declare that they have no competing interests

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### **Authors' contributions**

“FM is the major contributor in running the experiment and writing the manuscript. AT supervised the study, analyzed and interpreted the data. WT supervised the study, analyze and interpreted the data. MS supervised the study, analyzed and interpreted the data. NH supervised the study, analyzed and interpreted the data. MB involved in acquiring the study materials, data collection and analysis. EA supervised the study, analyzed and interpreted the data. MT supervised the study, analyze and interpreted the data. YG helped to analyze the genetic analysis, interpreting the result and writing the manuscript”. All authors have read and approved the manuscript.

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## Tables

Table 1  
Nitrogen partitioning among leaf, panicle, stem, before anthesis and seed at harvest.

Cultivar	Nitrogen content (mg g <sup>-1</sup> )				Dry weight before anthesis (g per plant)		
	Leaf	Panicle	Stem	Seed	Leaf	Panicle	Stem
3704	1.05bc	1.49	0.52bc	2.4a	0.26	1.36a	1.03
3707	0.94c	1.87	0.66bc	2.3a	0.30	0.99ab	1.30
3709	1.15bc	1.02	0.3 cd	2.1ab	0.19	0.38 cd	0.82
3710	1.31b	0.59	0.1c	1.9abc	0.23	0.23d	0.56
3711	0.90c	1.27	0.57bc	1.9abc	0.22	0.51bcd	0.52
3712	1.52b	1.17	0.56bc	1.7c	0.36	0.85ab	1.05
3713	0.75c	1.22	0.54bc	1.8abc	0.20	0.73bc	0.65
3715	1.76ab	0.87	1.7a	1.6c	0.25	0.49bcd	0.62
3726	1.98ab	0.32	1.25ab	1.8abc	0.25	0.44 cd	0.44
3727	2.58a	1.49	1.03ab	1.7c	0.31	0.45 cd	0.84
3728	2.23a	1.3	1.24ab	2.2a	0.22	0.23d	0.51
3729	1.95ab	1.9	0.88bc	2.0abc	0.25	0.63bc	0.90
Mean	1.4	1.4	0.81	2.1	0.25	0.57bcd	0.77
<i>P</i> -values	0.001	0.12	0.03	0.01	0.08	0.02	0.05
CV (%)	7.8	4.5	4.1	4.5	10.1	12.9	10.5

Means with the same letter are not significantly different at 1% or 5% level. CV (%), Coefficient of variation.

Table 2  
Summary of teff cultivars evaluated in this study.

N°	AN	Cultivar	Stock N°	Germination test	
				Percentage	Date
1	3703	Debre Zeit Exp Sta, 73-236	4092	36%	2015/03/09
2	3704	Debre Zeit Exp Sta, 73-241	4093	92%	2015/03/09
3	3706	Debre Zeit Exp Sta, 73-247	4095	92%	2015/03/09
4	3707	Debre Zeit Exp Sta, 73-248	4096	96%	2015/03/09
5	3708	Debre Zeit Exp Sta, 73-252	4097	70%	2015/03/09
6	3709	DZ 01-72, 73-238	4098	79%	1981/09/25
7	3710	DZ 01-257, 73-254	4099	62%	2016/03/16
8	3711	73-77	4100	86%	2015/03/09
9	3712	DZ 01-26, 73-235	4101	98%	2015/03/09
10	3713	DZ 01-27, 73-236	4102	56%	2015/03/09
11	3715	DZ 01-87, 73-239	4104	90%	1992/07/29
12	3726	DZ 01-238, 73-252	4115	100%	2015/03/09
13	3727	DZ 01-323, 73-255	4116	100%	2015/03/09
14	3728	DZ 01-354, 73-256	4117	100%	2015/03/09
15	3729	DZ 01-457, 73-257	4118	98%	2015/03/09
All cultivars originated in Ethiopia.					
AN, accession number.					

## Figures

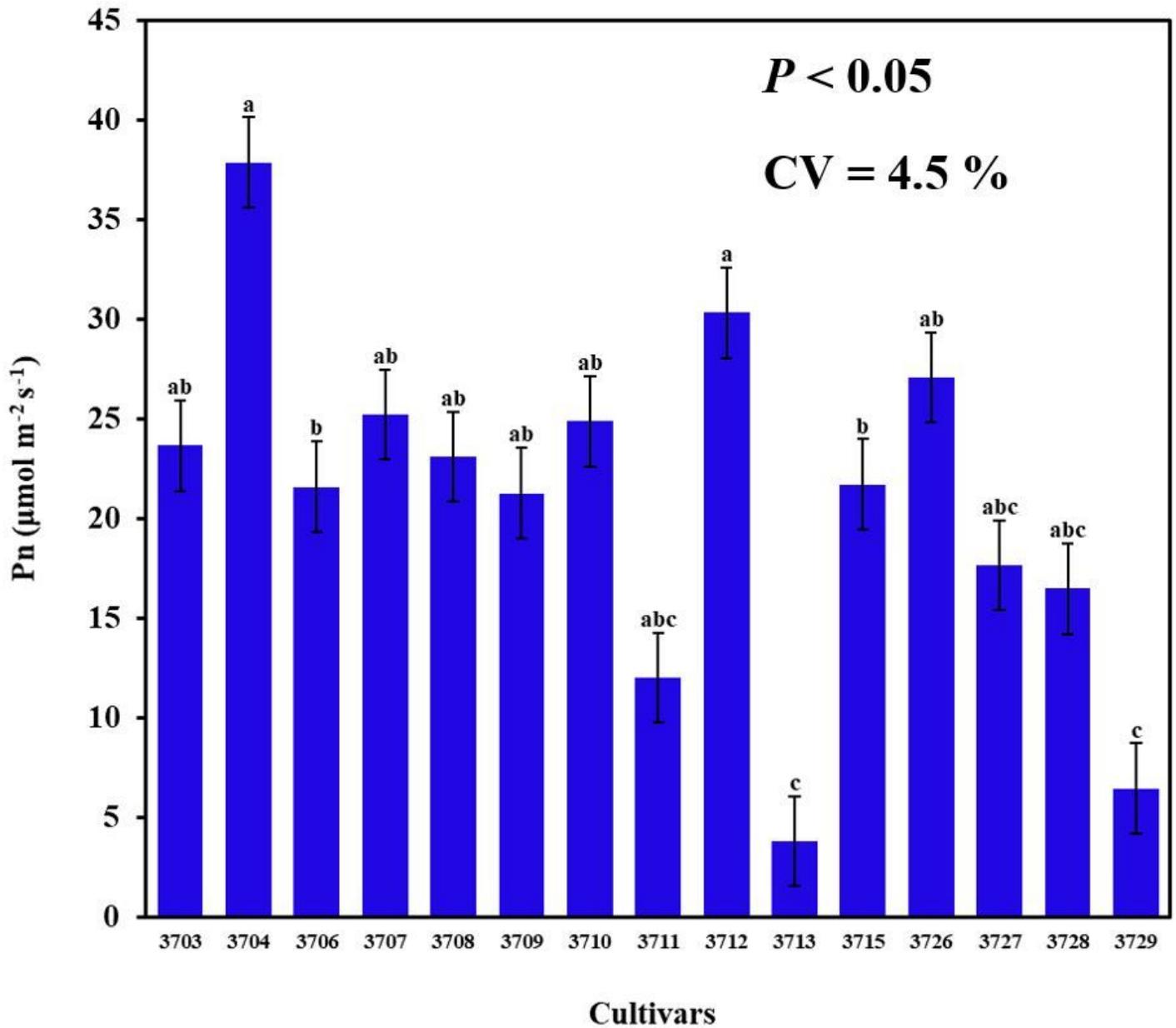


Figure 1

Net photosynthetic rate (Pn) of 15 teff cultivars before anthesis under glasshouse conditions. Means with the same letter are not significantly different at 5% probability level. CV (%), coefficient of variation.

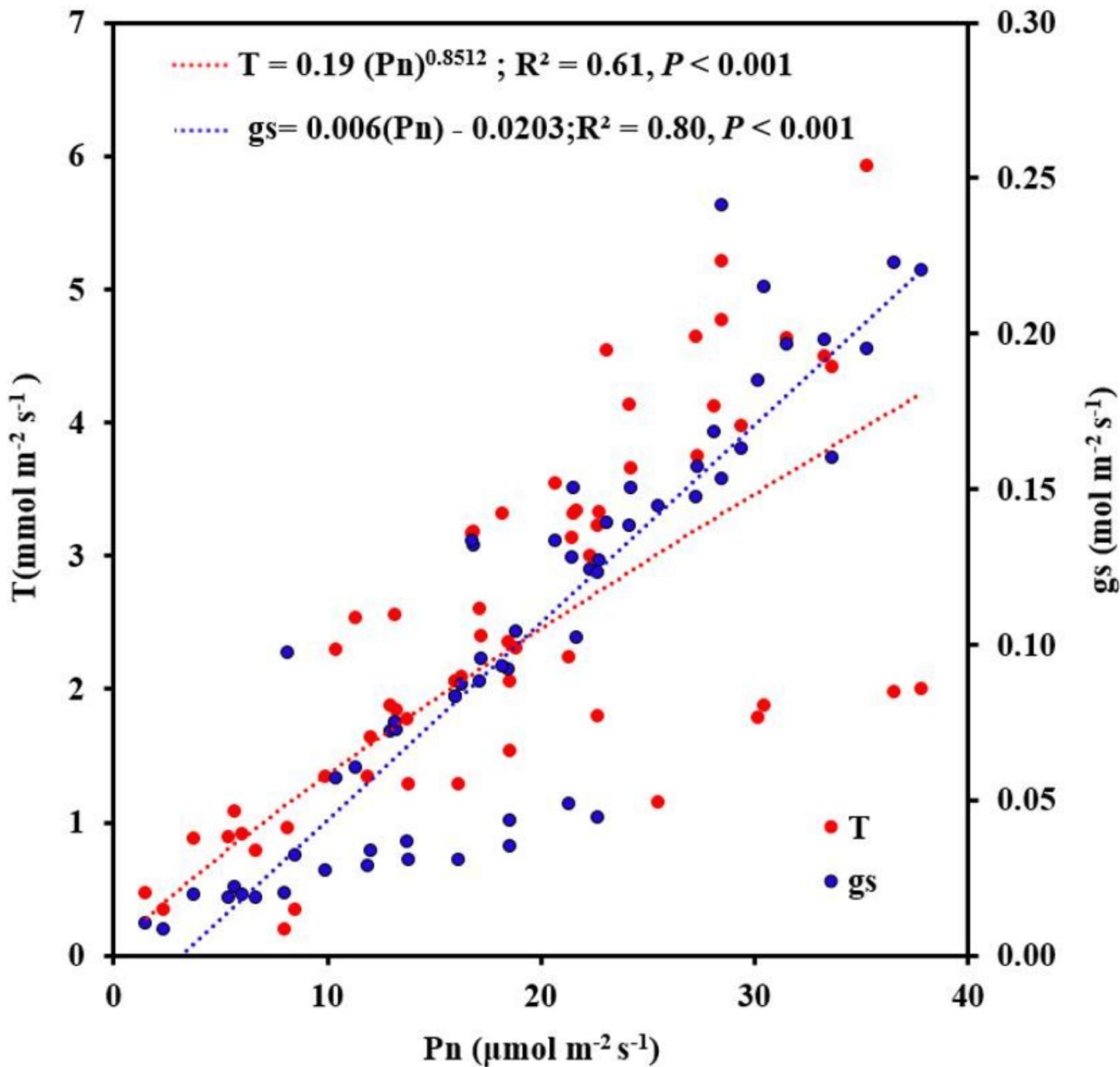


Figure 2

Relationships of net photosynthetic rate (Pn) with stomatal conductance (gs) and transpiration (T) of 15 teff cultivars before anthesis under glasshouse conditions.

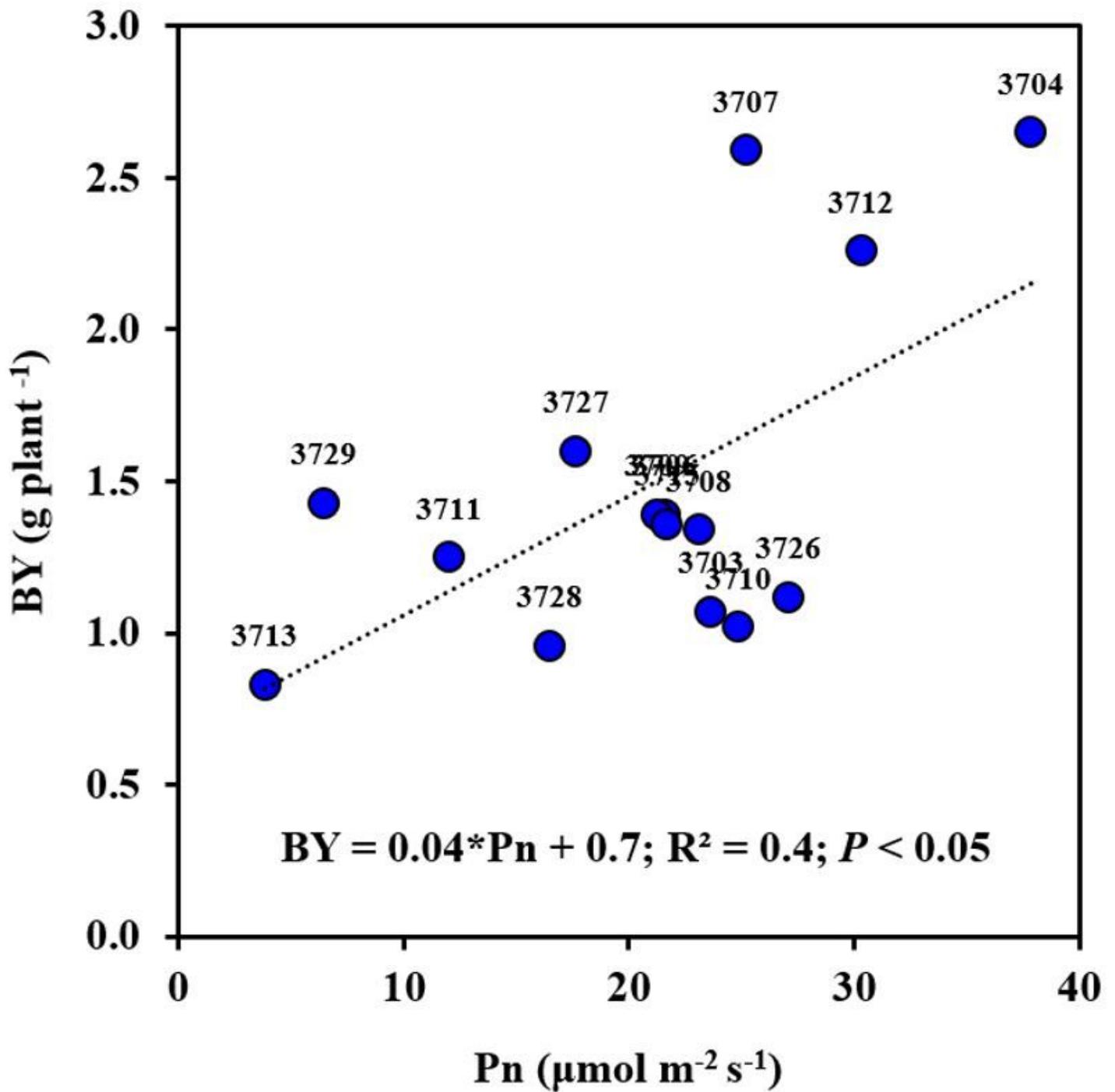


Figure 3

Relationship between net photosynthetic rate (Pn) and dry biomass yield (BY) before anthesis under glasshouse conditions.

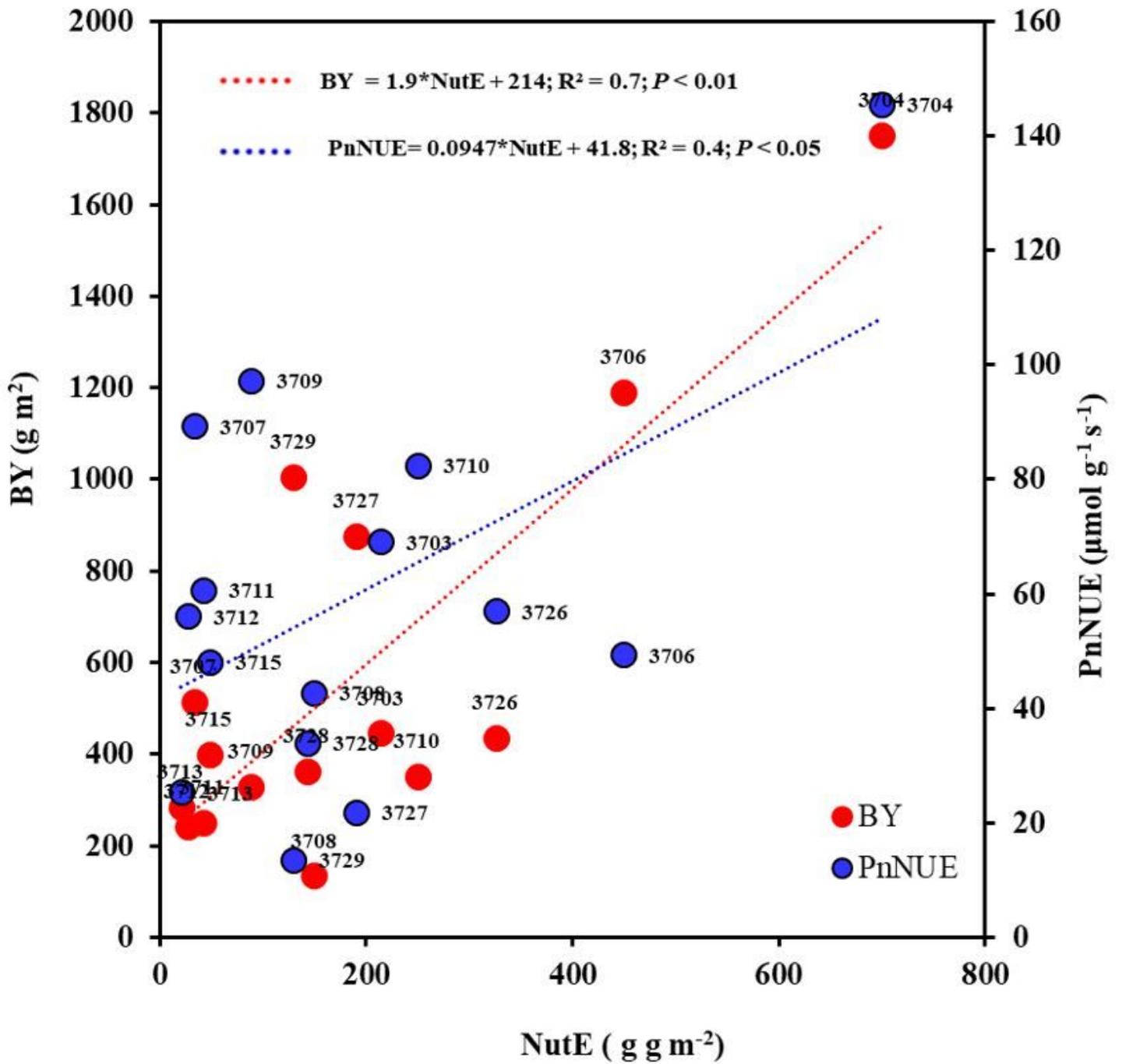


Figure 4

Relationships of NUE with dry biomass yield (BY) and photosynthetic NUE (P-NUE) before anthesis of teff cultivars evaluated under glasshouse conditions.

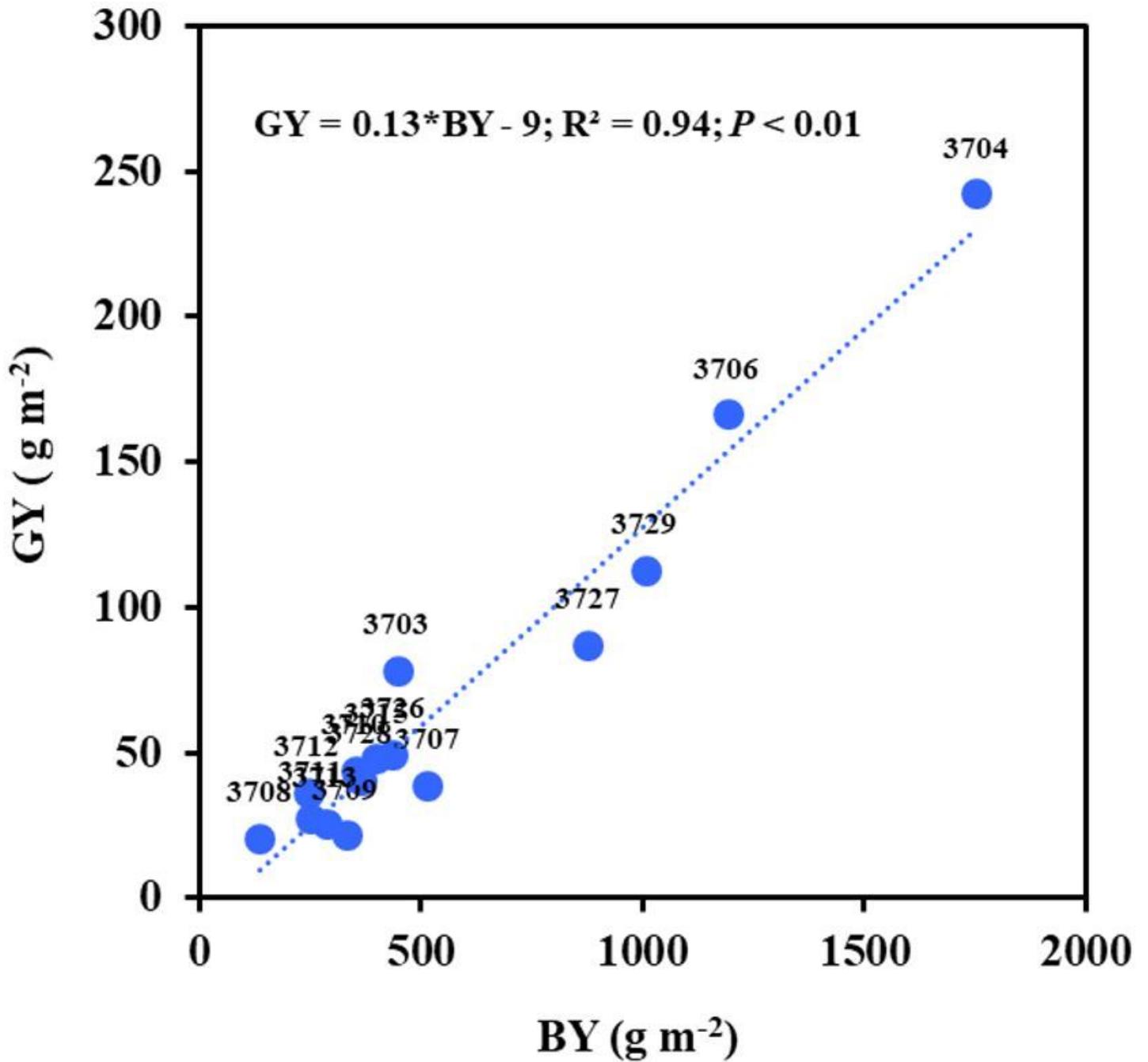


Figure 5

Relationship of grain yield (GY) and biomass yield (BY) at harvest of teff cultivars evaluated under glasshouse conditions.

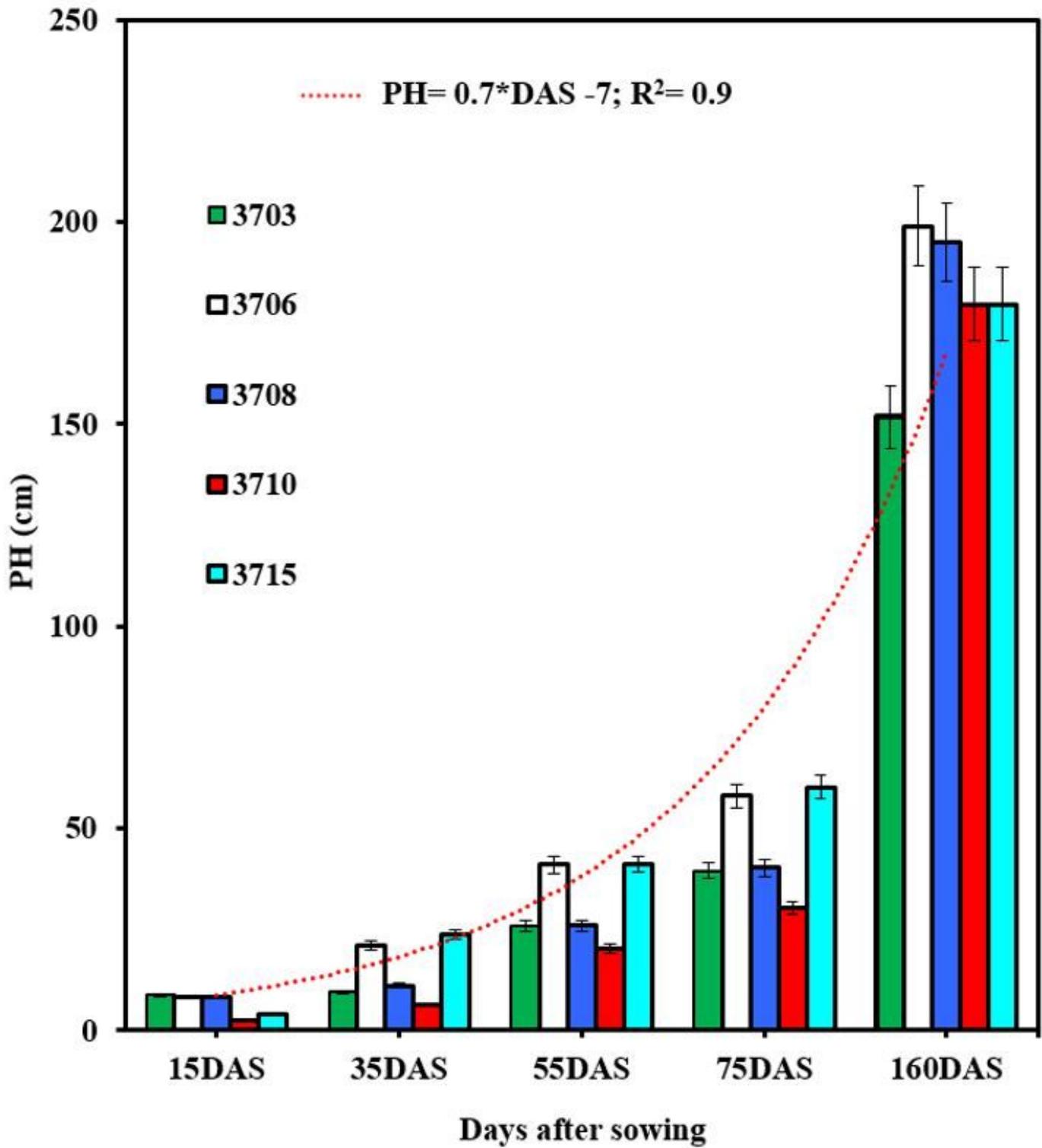


Figure 6

Plant height (PH) increment of 5 teff cultivars measured every 20 days under glasshouse conditions.

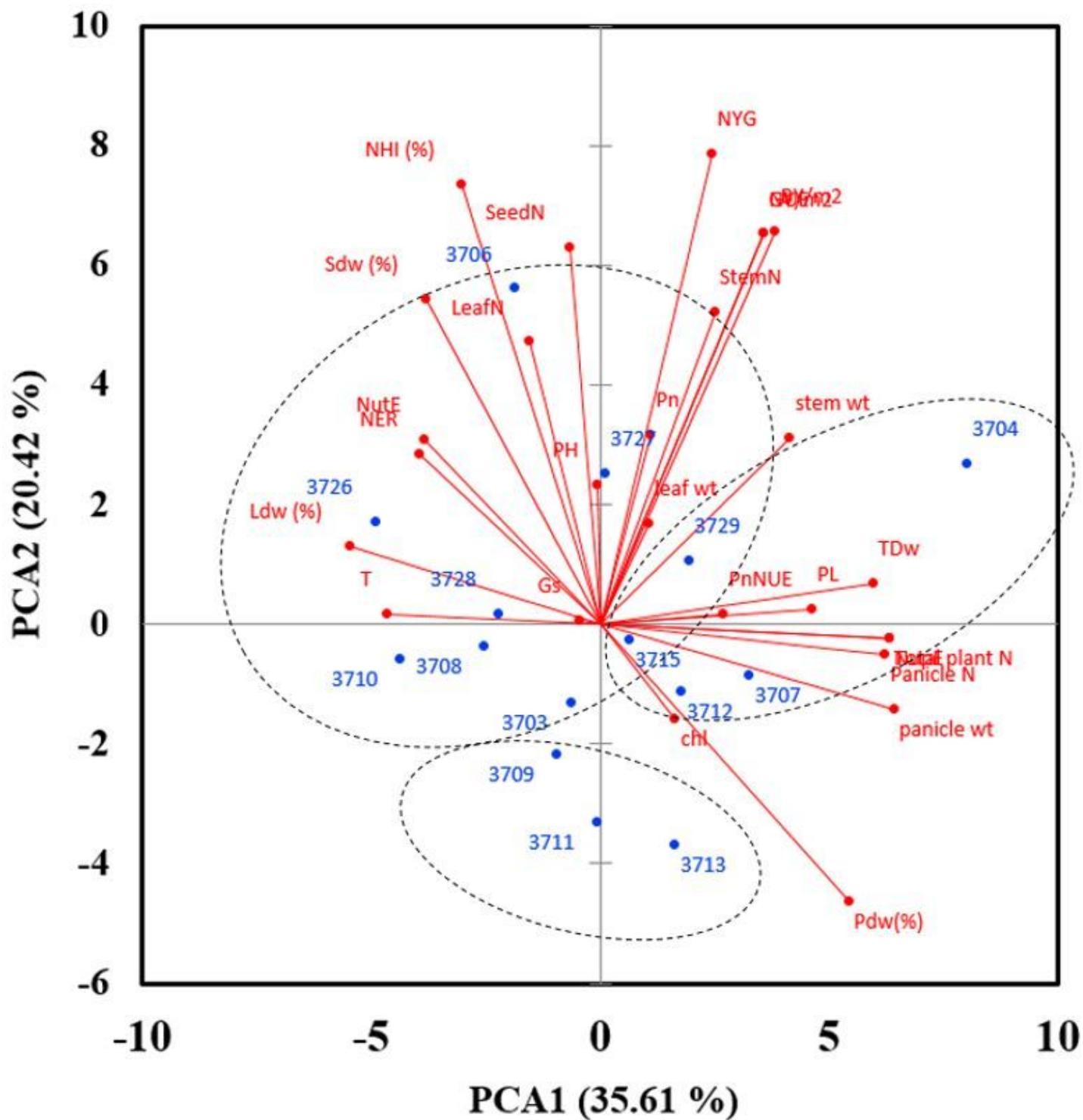


Figure 7

Association of photosynthetic, NUE, and growth traits of 15 teff cultivars evaluated under glasshouse conditions.

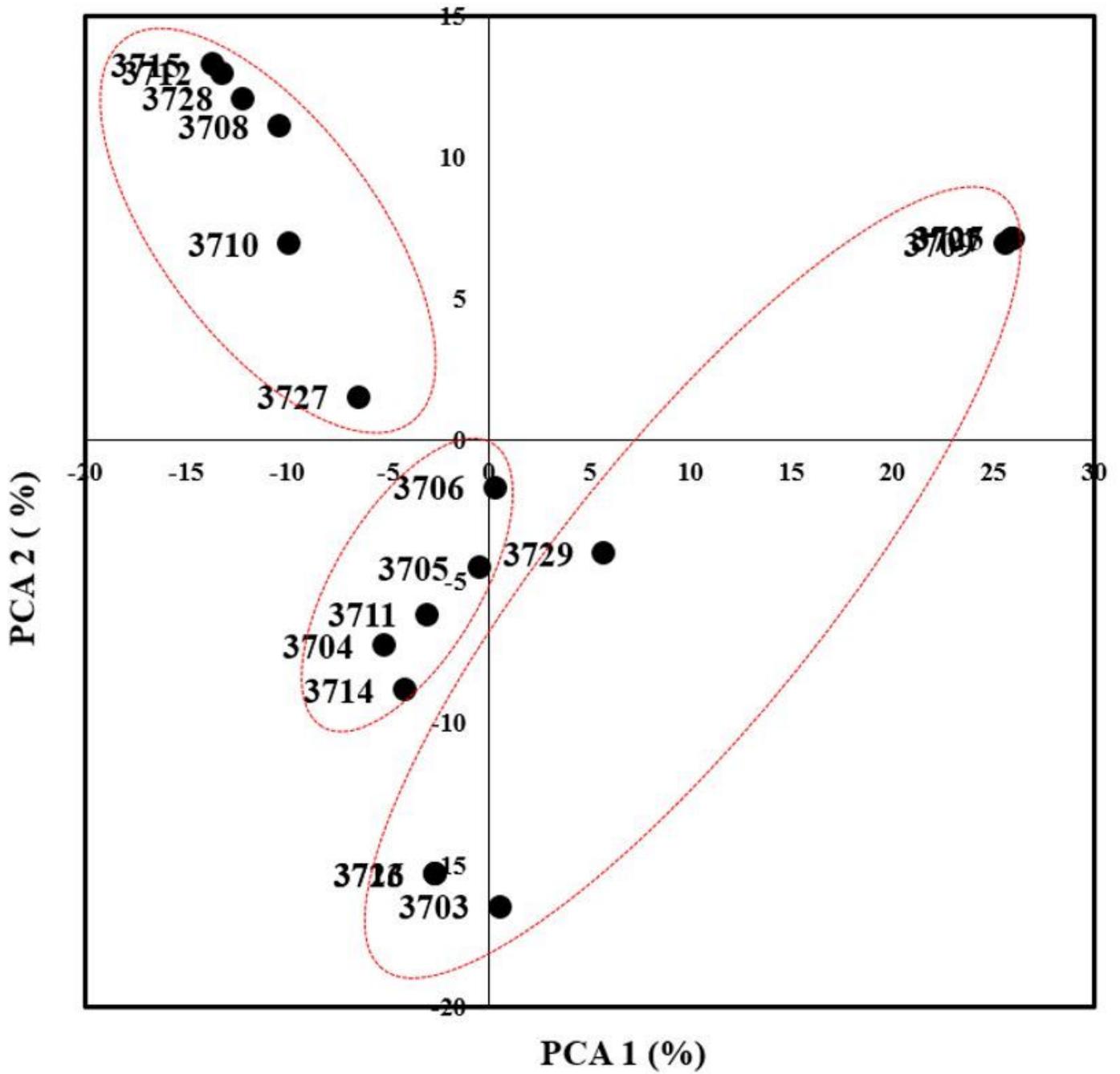


Figure 8

Clustering of 15 teff cultivars based on genetic mapping by diversity array technology.