

PHOTOSYNTHETIC RESPONSE TO TEMPERATURE
IN ERAGROSTIS TEF (ZUCC.) TROTTER

By

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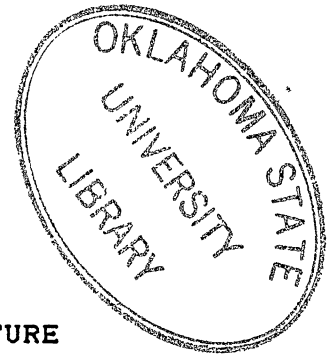
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CHAPTER I

INTRODUCTION

Tef, Eragrostis tef (Zucc.) Trotter, a tropical annual grass species, is one of the most important food crops in Ethiopia. It occupies the largest cultivated land area of any single crop in the country, which is over 30% of the land area under cereal production. However, in other parts of the world it is used as a forage grass. Its presence is reported in Kenya, Burma, and Pakistan; and it is also cultivated for forage in India, Australia, and in Orange Free State and Transvaal, South Africa (1,14).

The Ethiopian farmer harvests and uses all aerial parts of the tef plant. The grain is used for human food and the straw is used for cattle feed and as a binder in building mud houses. Tef grain has the highest mineral content of all the major cereals grown in the country. The grain also has a high fiber content and is competitive with wheat in its protein content (1). It can be stored for long periods without storage pest problems.

Tef is adapted to a great diversity of climatic conditions and soil types in Ethiopia and this is one reason why it is widely grown. There are many different tef varieties which are cultivated under a wide range of acidic and

alkaline soil types and wide range of altitudes. In areas which are warmer and have relatively less rainfall, early maturing varieties of tef are usually grown. In the cooler and wetter areas late maturing varieties are predominant.

Some agronomic studies have been made on tef. However, there is very little information on its physiological response to environmental factors and apparently no information on its photosynthetic behavior. Since temperature is one of the major ecological variables that determine the distribution of plants (2) and photosynthesis is strongly affected by temperature (5,12,13,19,24) it is important to investigate the photosynthetic characteristics of different tef varieties under different temperature regimes.

Therefore, this study was designed to: (1) investigate the gas exchange characteristics of contrasting tef varieties at various leaf temperatures and (2) determine the optimum leaf temperature for tef photosynthesis.

CHAPTER II

LITERATURE REVIEW

Leaf temperature, light, carbon dioxide concentration, plant water status, water vapor pressure difference between the leaf and the surrounding air (15,19,52,55) and plant nutrition (19) are some of the factors which affect the rate of photosynthesis in a plant. Net photosynthesis, or carbon dioxide exchange rate (CER), is dependent on the resistance to CO₂ transport into the leaf and on the carboxylation reaction of photosynthesis (48). Several studies have indicated that increased resistance to CO₂ diffusion could be caused by varying levels of the factors mentioned above (6,15,16,19,35,54).

The components of the resistance to CO₂ diffusion in the gaseous phase are located in the boundary layer of the leaf and in the leaf itself. The leaf resistances arise from the cuticle and the stomata which result in stomatal resistance (r_s'). The transport of CO₂ to the sites of carboxylation and the biochemistry associated with carboxylation are considered as an additional source of resistance, which is termed the mesophyll (49) or residual resistance (r_r') (16). Increased r_r' is generally associated with an increase in the accumulation of internal

substomatal CO_2 concentration (C_i), since less CO_2 is transported to the sites of carboxylation.

Numerous experiments have indicated that photosynthesis is strongly affected by temperature (5,12,13,24,53). Woldge and Dennis (53), in experiments with ryegrass and white clover leaves indicated that measurement temperature showed a striking effect on the photosynthetic rate of both species. The rates increased steeply from the lowest temperature, 5°C , up to at least 18°C . Van Driessche and Conner (52) made a similar study in the laboratory with brigalow (Acacia harpophyl) and found that temperature strongly influenced net photosynthesis in phyllodes. Net photosynthesis in the phyllodes brigalow attained a maximum value at about 26°C , and decreased to 50% of the maximum at 16°C and at 35°C when the measurement was made at an irradiance of $1170 \mu\text{mol m}^{-2}\text{s}^{-1}$ (52). Temperature influenced r_s also, which decreased from about 15 s cm^{-1} at 15°C to a minimum of 5.5 s cm^{-1} at 26°C and then increased again to 15 s cm^{-1} at about 37°C (52).

Much of the biochemical part of photosynthesis is enzyme mediated and is temperature-dependent (18). Low and high temperatures may inhibit photosynthesis by directly affecting enzymatic activity and by lowering of CO_2 diffusion rates to the sites of carboxylation (18,26). The activity of photosynthetic enzymes declines at sub-optimal and supra-optimal temperatures for photosynthesis (26,49). If plants are exposed to temperatures outside the normal

physiological ranges there will likely be irreversible damage to the enzymes, pigment-protein complexes, reaction centers, and membranes which help the photosynthetic process to function (2,46). The proteins in these structures may be denatured and lose conformation (49). Since many of the reactions of the photosynthetic process are associated with membranes, changes in membranes caused by temperature stress might be expected to have significant effect on photosynthesis (2).

The optimum temperature range for photosynthesis depends on the plant species. C_4 species (plants in which the first detectable product of photosynthesis is the C_4 compound malic or aspartic acid) tend to show higher optimum temperatures for photosynthesis at normal atmospheric CO_2 concentrations than C_3 species (plants in which the first detectable product is the C_3 compound 3-phospho-D-glyceric acid) (2,48). Optimum leaf temperature for photosynthesis for C_4 species is usually between 30 and 45°C. For C_3 species the temperature optimum for photosynthesis is usually 15 to 25°C (25,46). C_4 plants generally show a higher photosynthetic rate than C_3 plants at their respective optima. The maximum net photosynthetic rate for C_3 plants is generally 10 to 30 $\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$ and for C_4 plants 25 to 55 $\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$ (32,37). However, the C_3 desert annual Cammossonia claviformis has shown a maximum rate of about 60 $\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$ (37) and the C_4 grass Pennisetum typhoides has exhibited a maximum rate of about

$63 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, which is so far the highest rate reported for any plant (39). There are a few species with characteristics intermediate between C_3 and C_4 photosynthetic types such as Panicum milioides, Panicum schenkii (6,8) Panicum decipimes (28) and Moricandia arvensis (28). The intermediate nature of these species was based on reduced O_2 inhibition of net photosynthesis, low CO_2 compensation concentration and high concentration of chloroplasts and mitochondria in bundle sheath cells compared to C_3 species.

C_4 species are photosynthetically more efficient than C_3 species at their respective optimum temperatures for photosynthesis because they possess a mechanism and anatomy for increasing the concentration of CO_2 available for the Calvin cycle, which is localized in the bundle sheath cells of C_4 species (47). Also O_2 competes with CO_2 for the RuBP carboxylase:oxygenase enzyme, so an increase of CO_2 in the bundle sheath cells of C_4 plants reduces the reaction of RuBP carboxylase:oxygenase with O_2 resulting in little photorespiration (32). The mesophyll cells of C_4 plants fix CO_2 into 4-carbon acid compounds with the help of the enzyme phosphoenol pyruvate (PEP) carboxylase. The 4-carbon acids are transferred to the bundle sheath cells, decarboxylated, and the resulting CO_2 is then refixed by the enzyme ribulose bis-phosphate (RuBP) carboxylase in the Calvin cycle (46). One of the advantages of the C_4 system is that a small amount of CO_2 released from photorespiration in the bundle

sheath cells is refixed by PEP carboxylase in the cytoplasm of the outer mesophyll cells (47). Therefore, increase in CO_2 concentration in bundle sheath cells would consequently raise net CO_2 fixation rates of C_4 plants (47).

The effect of temperature on photosynthesis is influenced by light intensity, CO_2 concentration, leaf age, growth temperature, plant water status, humidity and plant nutrition and these factors can directly affect the photosynthetic apparatus or indirectly affect stomatal conductance ($1/r_s'$, G_s') (2,8,15,17). Stomata control the resistance to the diffusive transfer of water vapor and CO_2 between the leaf and the ambient air and can affect the CO_2 concentration in the internal substomatal air spaces (49).

Studies on the response of stomata to temperature have yielded differing results. There are reports which indicate that with increasing temperature G_s' increases (13,21,24) whereas others have reported G_s' decreases (20,27).

Berry and Bjorkman (2) stated that stomatal response to temperature is strongly influenced by factors other than temperature, of which the internal plant water status and the water vapor pressure difference between the leaf and the surrounding air (VPD) are very important. They also stated that in many instances, closure of stomata with increasing temperature results from a stomatal response to increased VPD, which normally results when the leaf temperature is increased. Coyne and Bradford (15), in their study on Caucasian bluestem, indicated that stomatal closure at high

temperatures might have resulted from low leaf water potential. However, Even-Chen et al. (24) found that stomata remained open even when the leaf temperature was increased to 47°C in "French" prune (Prunus domestica) L. Cv. Agen trees maintained under a non-limiting soil water supply. They stated that photosynthesis was greatly reduced, indicating an insensitivity of the guard cells to internal CO₂ levels. In the absence of water stress and high VPD, stomata may tend to respond in concert with the changing photosynthetic demand for CO₂ (2), or stomata may remain open or even increase aperture when leaves are heated to temperatures that caused damage to the photosynthetic apparatus (41).

Increased VPD can result in reduced photosynthesis (15,16,17,38). Morison and Gifford (38) examined the effect of VPD on two C₃ and two C₄ grasses. Photosynthesis in all four species was not affected by change in VPD from 0.5 to 1.4 KPa, but a VPD larger than 2 KPa caused a lower photosynthesis in the C₃ species, which they presumed to be as a result of the onset of water stress at high evaporation rates (38).

In most of the studies made on effect of VPD on stomata it was found that stomatal conductance to water ($1/r_s$, G_s) decreased with increases in VPD (2,15,17,38). Morison and Gifford in their study on the two C₃ and two C₄ grasses (38) found that G_s responded markedly to VPD. They observed that with a VPD larger than 0.9 KPa there were only small in-

creases in transpiration because of decreased G_s . Measurements in wheat and barley at a constant temperature (25°C) showed that VPD above 1 KPa resulted in a depressed photosynthesis, a large increase in r_r and a small decrease in G_s (31). There are few cases where G_s was not sensitive to VPD. However, Raschke and Kuhl (43) found that stomata of leaf sections of Zea mays did not close in response to a change from moist to dry air.

Water-use efficiency (WUE), which is the amount of CO_2 fixed per unit of water lost, is tightly coupled to CO_2 since both water vapor and CO_2 pass simultaneously through the stomatal pores (47). Species having the C_4 photosynthetic pathway are generally considered to have higher WUE than C_3 species because C_4 plants are more efficient at taking CO_2 from air than C_3 plants, so C_4 plants lose less water per CO_2 fixed and the biochemical basis for their efficiency is the efficient carboxylation of CO_2 , which is attributed to PEP carboxylase (47).

The photosynthetic response of leaves to temperature is sensitive to light intensity. Under rate-saturating light intensities, C_4 plants have a more notable temperature response than C_3 plants and the optimum temperature for photosynthesis in C_4 plants is usually higher than in C_3 plants (35). Net photosynthesis in single leaves of C_4 species is saturated only at photon flux densities above full sunlight ($>2,000 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$) (11,39) while in C_3 species it is saturated at photon flux densities of

one-quarter of full sunlight, or less (11). As light intensity is lowered, the temperature response curve becomes flatter and broader (15,35,53). At low temperatures the light intensities required to saturate photosynthesis are lower than at high temperatures and if light intensity is reduced it has little effect on photosynthesis until the light intensity becomes limiting at that temperature (2,53). There is a rise in photosynthesis in bright light with increased temperature up to the optimum temperature and this was mainly attributed to a fall in the r_r' (35,53). However, there may also have been a small decrease in r_s' with increased temperature (53), and this was also associated with a decrease in the C_i .

Increased CO_2 concentration in the atmosphere increases the rate of photosynthesis curvilinearly in C_3 plants (55) whereas C_4 plants are affected little (2). At high CO_2 levels, the temperature optimum for photosynthesis is increased in C_3 plants probably because photorespiration is inhibited (23,55). In contrast to C_3 plants, C_4 plants are more efficient in fixing low levels of CO_2 and atmospheric levels of CO_2 are saturating for C_4 photosynthesis (31).

The nutrient regime in which the plant has been grown can also affect the temperature response of photosynthesis. Doley and Trivett (19) observed that nutrient deficiency caused a depression on photosynthesis. They measured photosynthesis in plants with both adequate and inadequate nutrition, at various temperatures, and at an irradiance of

2030 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$. The photosynthesis was higher in the plants with adequate nutrition than in those without adequate nutrition at all temperatures. A close relationship was found between leaf nitrogen and photosynthesis, which might be because RuBP carboxylase comprises the major portion of soluble protein in chloroplasts and plays a key role in photosynthetic carbon metabolism (45). Plants with the C_4 pathway of CO_2 assimilation utilize N more efficiently than C_3 species (50). C_4 plants invest only 10 to 25% of their leaf nitrogen supply in RuBP carboxylase in contrast to C_3 plants which invest 40 to 60%, and the presence of the efficient CO_2 trap, PEP carboxylase, allows C_4 plants to use less RuBP carboxylase (47).

In summary, it has been found that temperature has a marked effect on photosynthesis (5,12,13,24). Plants show considerable differences in their photosynthetic response to temperature and many of these differences reflect adaptation of the different species to environments with different temperature regimes (2), generally C_4 species being adapted to higher temperature conditions (warm season plants) and C_3 species adapted to lower temperature conditions (cool season plants) (46).

CHAPTER III

MATERIALS AND METHODS

Plant Culture

Plants of an early maturing tef (Eragrostis tef) variety (Red Dabi) and a late maturing variety (DZ-01-354) were grown in pots in a growth chamber. The growing media was a mixture of clay soil, sand, and peatmoss in the proportion of 3:1:1 parts, respectively, and the pot volume was 3 liters. Light was provided by 96-inch Cool-white fluorescent lamps and 60W incandescent lamps for 12 h each day, which provided photosynthetic photon flux density (PPFD) of about $600 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$. Average day and night temperatures were 25°C and 15°C , respectively. The plants were watered every other day and 40 ml of full strength Hoagland's nutrient solution was applied to each pot weekly.

Photosynthesis and Transpiration Determination

All gas exchange measurements were made on recently fully emerged leaves when the plants were about seven weeks old. Carbon dioxide exchange rates (CER) of the two varieties were determined at six temperatures with a PPFD of

about $1800 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$ and ambient CO_2 concentration of $330 \mu\text{ll}^{-1}$. All CER and transpiration (E) measurements were made using a stirred, temperature and humidity controlled reaction chamber (cuvette) described in detail by Bingham et al. (4) and Coyne et al. (14). Humidity was measured in the reaction chamber with a small condensation dew-point hygrometer (General Eastern 1111D, Watertown, MA) and CO_2 was monitored by diverting the chamber exhaust through the sample cell of a differential infrared gas analyzer (IRGA- Horiba PIR-2000-R, Irvine, CA). Leaf temperature was measured with a copper-constantan (ANSI Symbol T, 0.025 cm diameter) thermocouple and PPFD with a quantum sensor (Li-Cor LI-190SB, Lincoln, NE). For leaf temperature measurements the thermocouple was in contact with the underside of the leaf being measured. The six temperatures for the measurement of gas exchange were 18, 24, 30, 36, 42 and 48°C . The same leaf was exposed to all six temperatures by starting at the lowest temperature and increasing to the highest, keeping the water vapor pressure gradient from leaf to air (VPD) relatively constant at 3 KPa for each measurement. Measurements were made on one leaf from each plant for each variety per replication. Gases of known CO_2 concentration, mixed with dry air (-15°C), were passed through the cuvette until the CO_2 output was at a steady state of $330 \mu\text{ll}^{-1}$. Cuvette conditions (leaf temperature, dew point, IRGA output and pressure transducers) were monitored using a computer-interfaced data acquisition

system.

CER and E were calculated on a leaf area basis from the amount of added gases required to keep the desired steady state conditions inside the chamber (4). After calculating CER and E, stomatal conductance to water vapor (G_s) and CO_2 (G_s') along with internal substomatal CO_2 concentration (C_i) were calculated. The ratio of CER to E was used to measure water-use efficiency (WUE). Calculation of G_s , G_s' , and C_i were based on the following equations:

$$E = (e_i - e_a)G_s$$

where e_i and e_a were the vapor pressure of water inside the leaf (assumed to be vapor saturated) and in the air, respectively.

$$CER = (C_a - C_i)G_s' \text{ and } G_s' = G_s / 1.6$$

where C_a = ambient CO_2 concentration.

Leaf water potential (WP) was estimated prior to and after measurements by using leaf cutter psychrometers (J.R.D. Merrill, Logan, UT) as described by Johnson et al. (30) to determine if any change in leaf water status occurred during the measurement period. When a leaf was initially inserted into the cuvette, a corresponding leaf from the same position and of similar physiological age from an adjacent culm was used to determine initial WP. Each leaf psychrometer removes 0.24 cm^2 leaf disc and two samples were taken from each leaf. The psychrometers were then placed in a water bath at 30°C for 2 h before reading wet bulb depression. The samples were read using a Wescor HP-115

Water Potential Data System (Logan, UT). Leaf WP was calculated from these readings by using calibration equations derived for each psychrometer. The final leaf WP was determined on the enclosed leaf immediately upon its removal from the cuvette and at the same time a corresponding leaf was used to determine if there was a difference in WP between the leaves outside the chamber and the leaf in the chamber after the measurement period.

Leaf Anatomy

Pieces of tef leaves were killed and fixed in FPA (formalin-propionic acid-alcohol), dehydrated and infiltrated with a graded Zirkle's modified TBA (t-butyl alcohol) series, embedded in Paraplast embedding media, and sectioned at 10 to 12 μm using a rotary microtome. Cross-sections were mounted on slides and stained with a safranin-fast green combination.

Statistical Analysis

Two experiments each with four replications were conducted. Data obtained on CER, E, G_s , C_i , and WUE were analyzed in a split-plot with variety as main plot and temperature as subplot. Regression analysis was made on CER vs C_i , E vs VPD, and WUE vs VPD. A third degree polynomial model in temperature was fitted to the CER data to estimate the optimum leaf temperature for CER. The equation used

in this model was:

$$y = b_0 + b_1 t + b_2 t^2 + b_3 t^3$$
$$t_{\text{opt}} = b_2 + \sqrt{\frac{b_2 - 3b_1 b_3}{(-3b_3)}}$$

where $Y = \text{CER}$ and b_0 , b_1 , b_2 and b_3 are coefficients, and $t = \text{temperature}$.

CHAPTER IV

RESULTS

Although the results were similar, a combined analysis of the two experiments indicated that there was a significant temperature by experiment interaction, so each experiment was considered separately for all the factors measured. Because significant differences were not observed between the two varieties for all the factors measured in each experiment, except for CER in Expt. I, the results for varieties were averaged within each experiment.

CER increased steadily with increased temperatures from 18 to 42°C and then declined sharply (Fig. 1). The average maximum observed values for CER were $25 \mu\text{mol m}^{-2}\text{s}^{-1}$ in Expt. I and $29 \mu\text{mol m}^{-2}\text{s}^{-1}$ in Expt. II. There was about three fold increase in CER as temperature increased from 18 to 42°C (Fig. 1). CER response to temperature in the two varieties was similar in Expt. II, but the variety effect was significantly different at the 5% level in Expt. I. at 48°C. Dabi showed a higher CER than DZ-01-354 at the highest temperature (48°C), having a rate of $17.2 \mu\text{mol m}^{-2}\text{s}^{-1}$ in Expt. I and $12.3 \mu\text{mol m}^{-2}\text{s}^{-1}$ in Expt. II, while the rates for DZ-01-354 were 7.1 and $6.9 \mu\text{mol m}^{-2}\text{s}^{-1}$ in Expt. I and II, respectively. A third degree polynomial

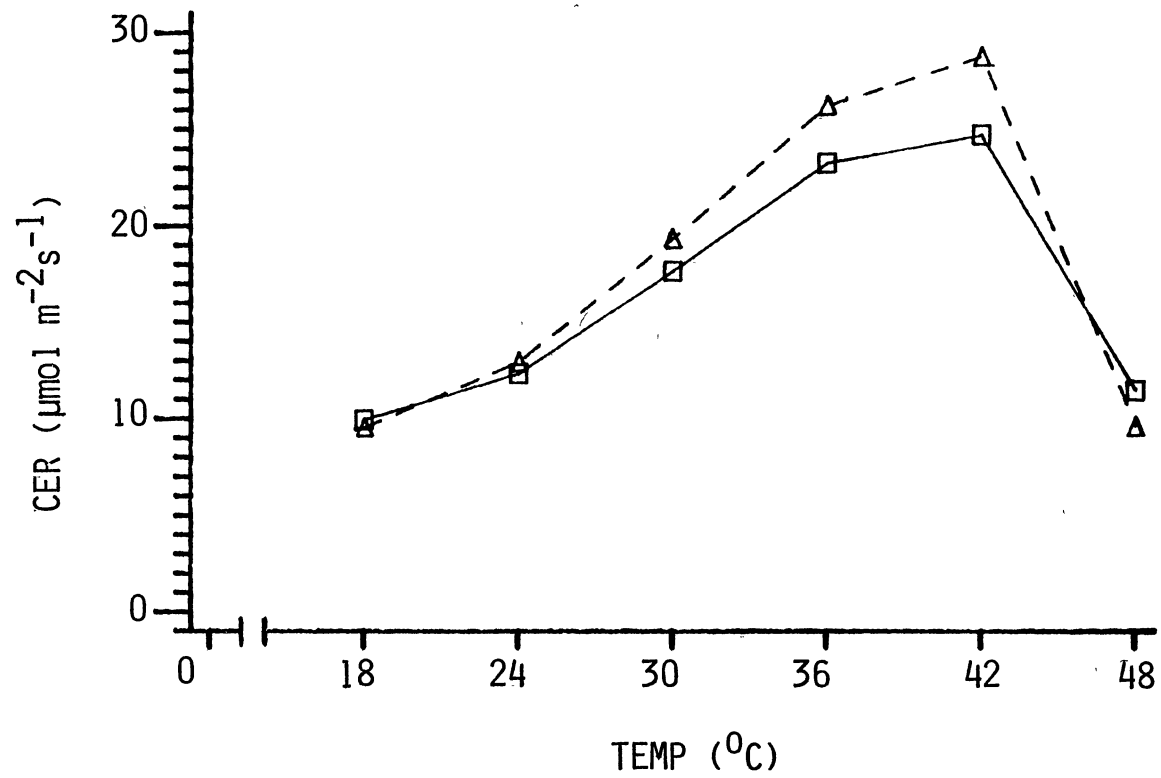


Fig. 1. Response of CO₂ exchange rate (CER) to leaf temperature in Expt. I (■) and in Expt. II (▲) in two tef varieties.

model relating CER to temperature indicated that the optimum temperature for CER was about 39°C.

Temperature had a highly significant effect on G_s (Fig. 2), but did not show consistent results in the two experiments. G_s increased with increased temperature in Expt. I then declined at 48°C (Fig. 2). In Expt. II G_s was higher at all measurement temperatures than in Expt. I and declined at 48°C.

C_i showed a strong negative correlation with CER ($r = -0.936$ for Expt. I and -0.978 for Expt. II) (Fig. 3). As CER increased and reached its maximum, C_i decreased steadily. As temperature was increased further to 48°C, CER declined and C_i increased (Fig. 4).

Values of E showed a response to temperature very similar to that of G_s with temperature showing a highly significant effect on E . As with G_s , E increased with an increase in temperature up to 42°C and then declined in Expt. I, but in Expt. II E increased with increased temperature and did not decline (Fig. 5).

The two varieties exhibited similar WUE and showed a response curve to temperature with a maximum value near 36°C for all measurements made (Fig. 6). Temperature showed a highly significant effect on WUE. WUE showed an increase with increased temperature from 18 to 36°C and then declined at 42 and 48°C. At the lowest (18°C) and the highest (48°C) measurement temperatures there was some difficulty keeping the water vapor pressure difference from leaf to air (VPD)

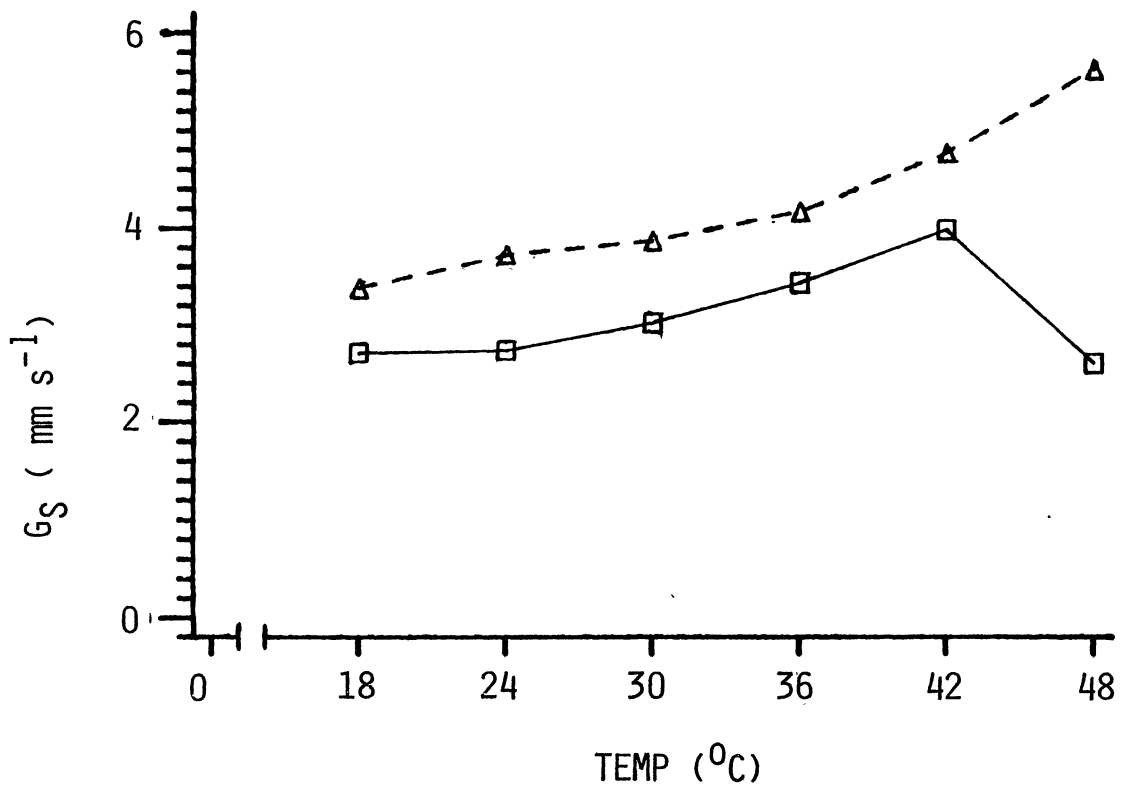


Fig. 2 Response of stomatal conductance (G_s) to temperature in Expt. I (■) and Expt. II (▲) in two Stef varieties.

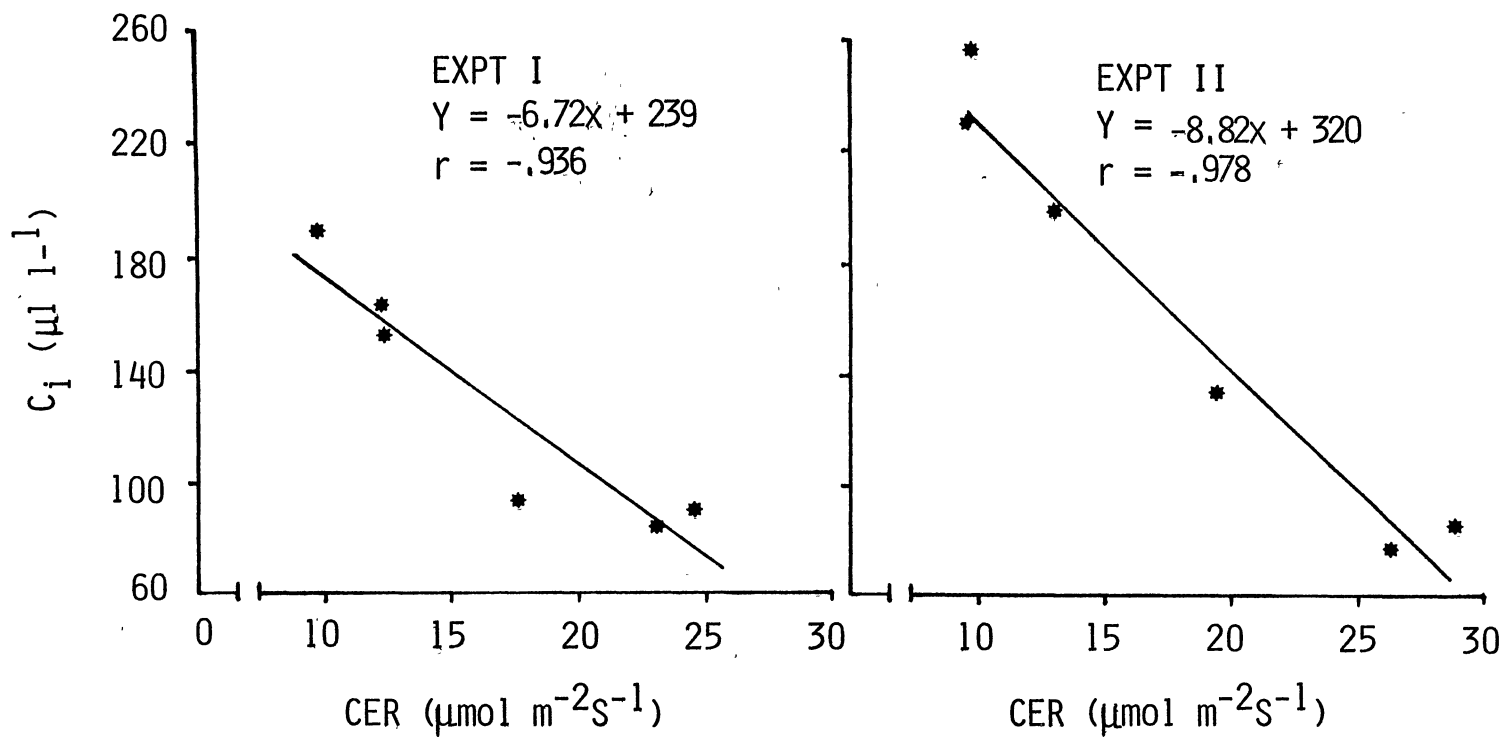


Fig. 3. The relationship between CO_2 exchange rate (CER) and internal substomatal CO_2 concentration (C_i) in two tef varieties with increase in temperature from 18°C to 48°C . Values of r significant at the 0.01 level of probability.

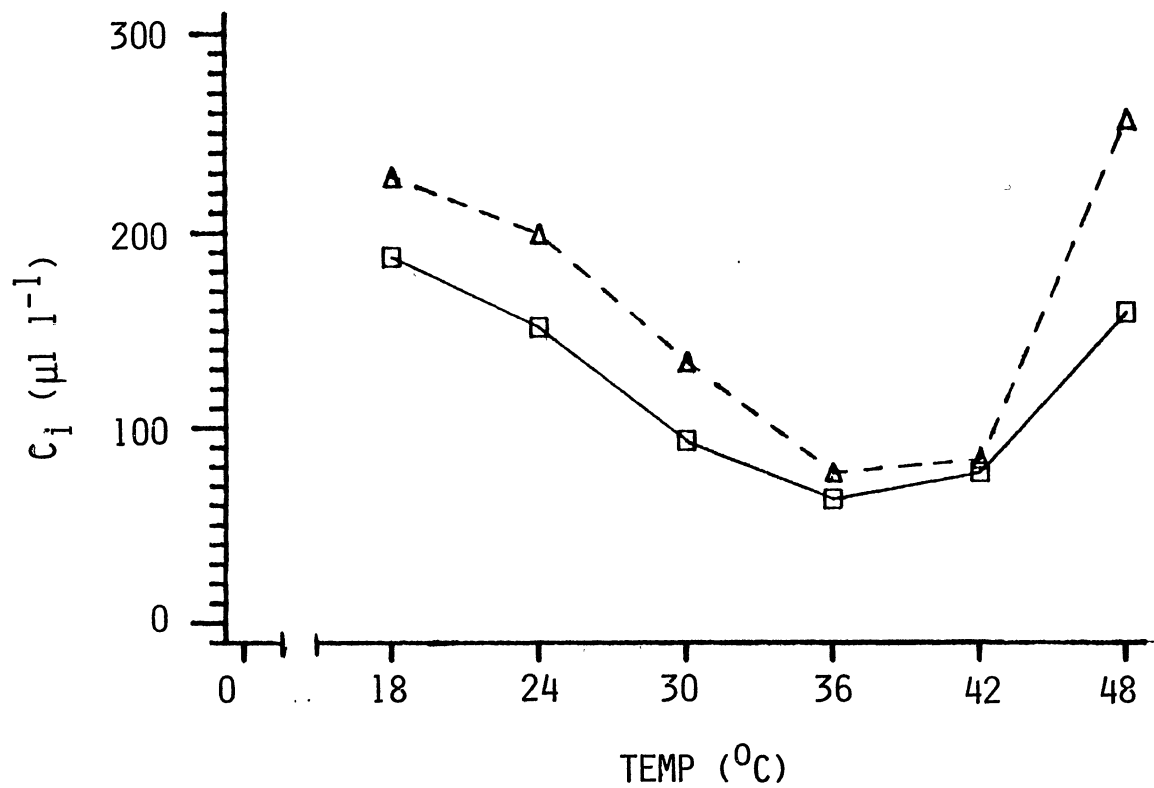


Fig. 4. Response of internal substomatal CO₂ concentration (C_i) to leaf temperature in Expt. I (—□—) and Expt. II (---△---) in two teff varieties.

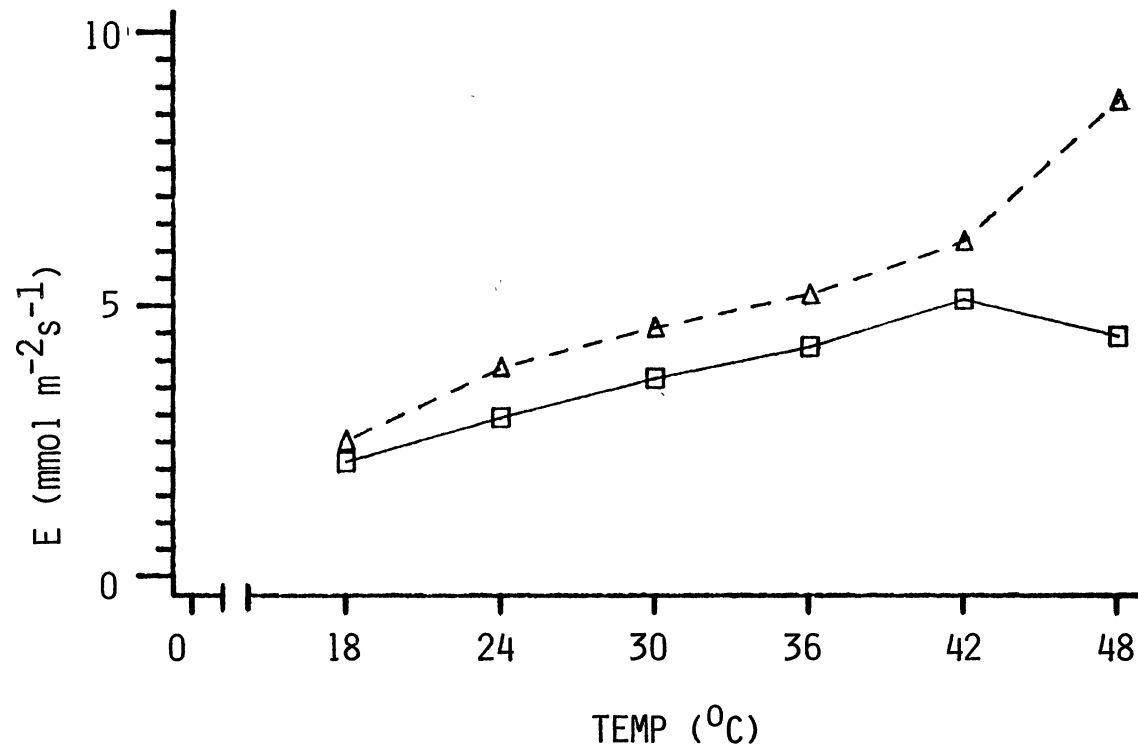


Fig. 5. Response of transpiration (E) to leaf temperature in Expt. I (□-□) and Expt. II (△-△) in two tef varieties.

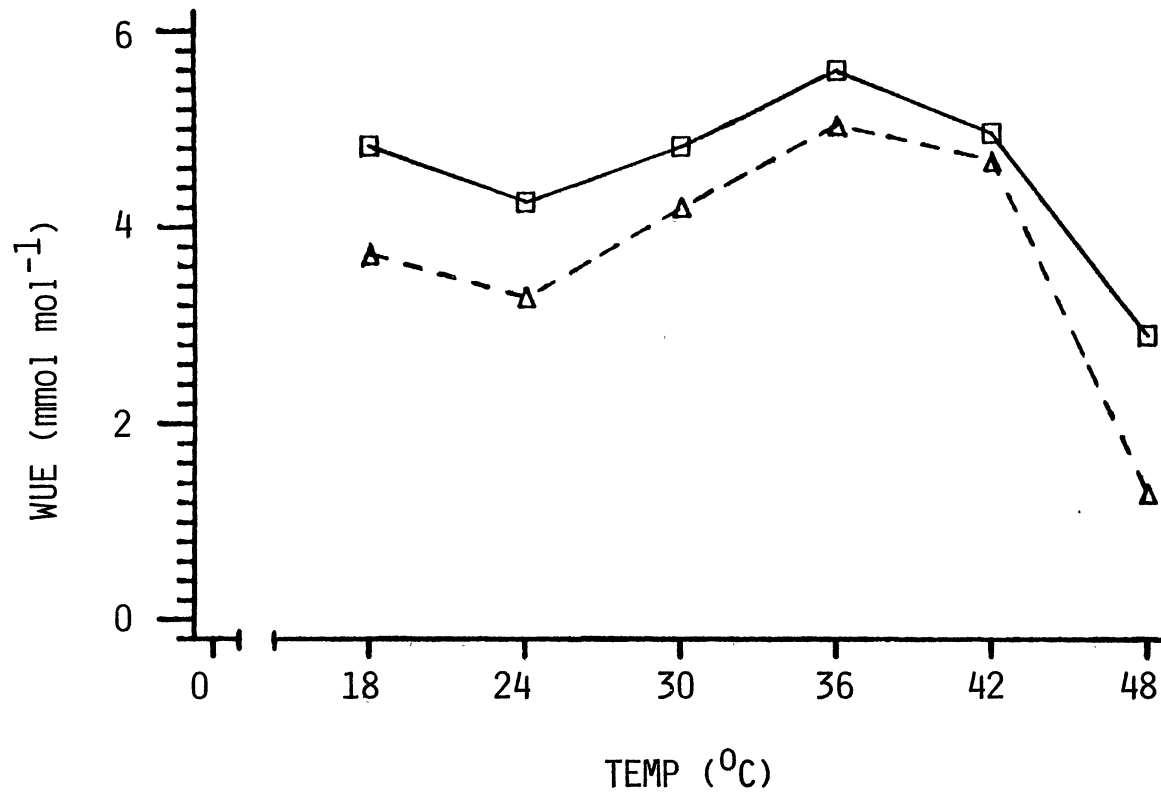


Fig. 6. Response of water use efficiency (WUE) to leaf temperature in Expt. I (□-□) and Expt. II (△-△) in two tef varieties.

constant at 3 KPa. It was as low as 1.9 KPa at 18°C and as high as 4.1 KPa at 48°C and this difference was statistically significant (see appendix). Nevertheless, regression analysis made on WUE and VPD indicated that there was no significant correlation between VPD and WUE ($r = -.503$ in Expt. I and $-.349$ in Expt. II), indicating that temperature effects on WUE were not simply a result of changing VPD.

Leaf WP during the CO₂ exchange measurement period was lower in the plants in Expt. I than in the plants in Expt. II. The mean initial WP was -0.64 MPa in Expt. I and -0.47 MPa in Expt. II, and the final WP was -1.45 MPa in Expt. I and -0.95 in Expt. II. Statistical analysis showed that there was significant difference between the initial and the final leaf WP in both experiments (see appendix).

The leaf anatomy study made on leaf sections of both varieties showed vascular bundles that were surrounded by bundle sheath cells in a circular manner (Fig. 7). Thus tef displayed Kranz anatomy, which is a characteristic of C₄ plants.

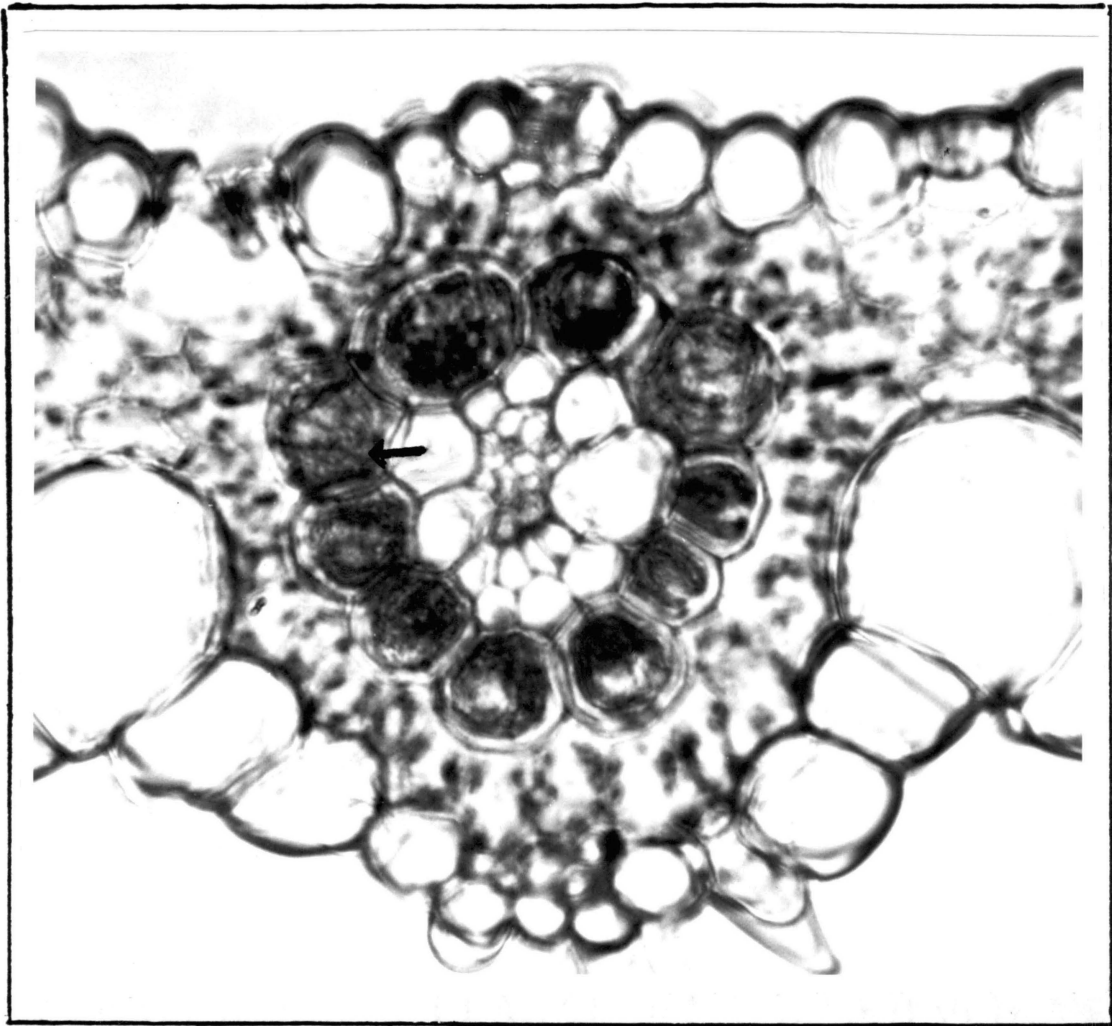


Fig. 7. Tef (Eragrostis tef) leaf anatomy. Note the conspicuous bundle-sheath cells, pointed by the arrow.

CHAPTER V

DISCUSSION

Maximum CER was reduced by low and high temperatures and the depression in CER at these temperatures was likely caused by thermal stress, affecting the biochemical reactions of the photosynthetic apparatus. Fitter and Hay (25) have indicated that in C_4 plants a reduction in CER at sub-optimal and above optimal temperatures was due to a reduced capacity of specific rate-limiting enzymes, such as phosphoenol pyruvate (PEP) carboxylase and ribulose-bisphosphate (RuBP) carboxylase. As temperature increases to the optimum for CER, the activation of these two enzymes increases, giving maximum CER (25,40). Further increase in temperature causes inactivation of the enzymes and an abrupt fall in CER is observed (25). Other than the inactivation of the enzymes, it is indicated that high temperature damages chloroplast components which results in reduction of CER (25). Then the low activity of the enzymes in fixing CO_2 at low and high temperature could have resulted in increased residual resistance (r_r'), that is, increased resistance to carbon dioxide transport between the internal substomatal air spaces and the chloroplasts in the mesophyll cells (3,16,51). In other studies it was found that r_r'

appeared more limiting than stomatal resistance (r_s') at low and high temperatures in C_4 species (15,34). This might also be true for tef in this study since a reduction in the capacity of the CO_2 concentrating mechanism to function well at low and high temperatures was likely reflected in C_i values. It would be expected that low C_i would result if the CO_2 concentrating mechanism was working optimally and transporting the maximum amount of CO_2 to the bundle sheath cells. Since C_i was lowest when CER was highest, and C_i was negatively correlated with CER, the results support this idea.

Since tef is a tropical grass species and a closely related species, Eragrostis pilosa, is a C_4 species (46), tef was assumed to be a C_4 species. The literature indicates that for C_4 species the optimum leaf temperature for CER ranges from 30 to 45°C (25,46), and maximum CER is generally 25 to 55 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (39). In this study, tef's optimum leaf temperature for CER was found to be within the range reported for other C_4 plants, but its maximum CER was at the lower range of the rates obtained from C_4 species. The photosynthetic response of the two varieties to temperature together with the Kranz leaf anatomy provided evidence that tef is a C_4 species.

Some studies have indicated that there is a linear relationship between CER and G_s' measured at constant temperature (54). Wong et al. (54) observed this linear relationship at constant temperature with varying ambient

CO_2 concentration, irradiance and mineral nutrition, and the C_i was observed to be constant. But in this study, it appeared that there was an independent temperature effect on stomata with increasing temperatures. Also, C_i was not constant perhaps because a linear relationship between CER and G_s did not exist.

Studies on the stomatal response to temperature have yielded contradictory results. Some reports indicate that stomata close with increasing temperatures (20,29), while others indicate that they may tend to open with increasing temperatures (13,21,24). It was also indicated that in many instances in which stomata tend to close with increasing temperature, the closure probably resulted from a reduction in internal plant water status or from a stomatal response to VPD (2). In this study, G_s increased as measurement temperature increased, except at 48°C in Expt. I, when G_s declined. There was some difficulty keeping the VPD constant at 3 KPa at the lowest and the highest measurement temperatures as indicated earlier. However, an experiment was conducted on the effect of VPD on G_s in tef and G_s did not decrease with increases in VPD from 2.6 up to 5.2 KPa (results not shown). It was indicated earlier that the plants in Expt. I showed a lower final leaf WP at the highest measurement temperature than the plants in Expt. II. The reduction in WP was associated with a lower G_s at 48°C in Expt. I than in Expt. II. It is likely that a reduction in WP in the leaf was associated with a loss of

turgor in the guard cells and resulted in a decrease in stomatal aperture, decreasing G_s in Expt. I.

In the absence of water stress and high VPD, stomatal response to temperature showed different results in various studies (35,36,38). Raschke (42) indicated that at high temperatures stomata of well watered plants are insensitive to CO_2 and the stomata are open even if CO_2 evolves from the leaf tissue. In Expt. II of this study, where the leaf WP was relatively high, the stomata remained open even when C_i increased at the highest temperature (Fig. 3). It was also evident that, because of the high C_i , the sharp decline in CER that took place at $48^{\circ}C$ was not caused by stomatal closure, which implied that G_s' did not appear to have a prominent role in determining the CER above optimal temperatures. A loss of stomatal sensitivity to CO_2 at high temperatures may be advantageous as it not only reduces the danger of overheating by promoting latent heat loss, but also helps keep the leaf nearer the optimum temperature for CER (24). The danger is of course, that the plant will use limited soil water too quickly, promoting water stress.

However, G_s' might have affected CER in some situations in this study. G_s' in Expt. I was lower than in Expt. II which might be related to the lower leaf water status in Expt. I than in Expt. II, as indicated by the lower initial and final leaf WP in Expt. I. There is usually a close linear relationship between leaf WP and CER (19). It was explained earlier that reduced leaf WP could have caused a

decrease in G_s . Therefore, one possible reason why CER was generally lower in Expt. I than in Expt. II might be the decrease in G_s .

The maximum WUE values in the two tef varieties ranged between 5 and 7 mmol mol⁻¹, which is similar to that observed in studies made on other C₄ grasses (15). It appears that there was a drop in WUE at 24°C observed in both experiments, suggesting that there was less CER per E at 24°C than at 18°C, but this was not significant. Even if statistical analysis indicated that there was no correlation between WUE and VPD, VPD might be involved in some of the reductions in WUE at the highest temperatures. Since E showed a significant positive correlation with VPD, increased VPD might have caused reduced WUE by increasing E. Nevertheless, the major cause for the reduction of WUE at the higher temperatures appeared to be the decline in CER.

A plant's capacity for high WUE may be an important characteristic, especially in areas where moisture is a limiting factor. In this study it was hypothesized that the variety Dabi, which is usually grown in the warmer and drier areas of Ethiopia, would have a higher WUE than the variety DZ-01-354, which is grown in the cooler and wetter areas. However, based on the estimation of WUE as the ratio of CER to E, the two varieties were not significantly different in their WUE across temperatures. Dabi is an early maturing variety and that may help explain its adaptation to warmer and drier areas. The earliness would help Dabi to complete

its life cycle before the dry season comes without necessarily having a greater WUE than DZ-01-354. However, given a large number of tef genotypes, important genetic variation for WUE may exist in tef as observed for other C₄ grasses (17).

CHAPTER VI

SUMMARY AND CONCLUSIONS

In this study some basic information on photosynthetic characteristics of tef was collected for the first time. The two varieties studied, grown in different environments in Ethiopia, were not generally different in their photosynthetic response to temperature. The leaf temperature optimum for CER, based on the results of the two varieties, was found to be about 39°C, which is characteristic of C₄ species. The CER achieved at the optimum leaf temperature was 25-29 μmol m⁻²s⁻¹ and this is at the lower range of the maximum CER reported for C₄ plants. Kranz type leaf anatomy and the photosynthetic response to temperature of the two varieties indicated that tef is a C₄ species. CER and C_i showed a highly negative correlation as temperature was varied from 18 to 48°C. C_i showed a decrease with increase in CER as temperature progressed to the optimum and then showed an increase as CER declined above the optimum temperature. Thus, the rate of diffusion and transport of CO₂ from the bundle sheath cells might have been reduced at low and high temperatures. G_s showed an increase as measurement temperature increased from 18 to 48°C in Expt. II, but in Expt. I it declined at 48°C which was probably

due to a reduced leaf water status. The two varieties were similar in their WUE. The maximum WUE observed in these two tef varieties was similar to that obtained from other C_4 grasses.

Further investigations need to be done in order to describe tef's photosynthetic behavior. No work has been done on how factors such as illuminance, ambient carbon dioxide concentration, moisture stress and nutrition affect tef's photosynthesis. This study was made only on two varieties. Study on the photosynthetic efficiency of a larger number of tef varieties might uncover differences in CER and WUE that could be exploited in a plant improvement program.

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APPENDIXES

Table 1. Means for CO₂ exchange rate (CER), water-use efficiency (WUE = CER/E), conductance to water (G_s), internal substomatal CO₂ concentration (C_i) and transpiration (E) at six temperatures in Expt. I and Expt. II in two tef varieties.

Temp °C	Expt. I				
	CER μmol m ⁻² S ⁻¹	WUE mmol mol ⁻¹	G _s mm S ⁻¹	C _i μl l ⁻¹	E mmol m ⁻² S ⁻¹
18	9.94	4.82	2.75	187	2.13
24	12.34	4.26	2.75	152	2.94
30	17.65	4.82	3.05	94	3.66
36	23.28	5.60	3.45	81	4.23
42	24.74	4.96	4.00	89	5.11
48	12.16	2.95	2.70	160	4.51
LSD _{.05}	2.81	0.95	0.53	38.69	1.33

Temp °C	Expt. II				
	CER μmol m ⁻² S ⁻¹	WUE mmol mol ⁻¹	G _s mm S ⁻¹	C _i μl l ⁻¹	E mmol m ⁻² S ⁻¹
18	9.50	3.72	3.40	229	2.51
24	12.92	3.28	3.75	199	3.86
30	19.33	4.20	3.90	134	4.59
36	26.23	5.04	4.20	77	5.20
42	28.76	4.66	4.75	85	6.13
48	9.59	1.30	5.65	256	8.75
LSD _{.05}	2.43	0.64	0.69	22.2	1.35

Table 2. Mean water vapor pressure difference between the leaf and the air in the measurement chamber (VPD) at six measurement temperatures in Expt. I and Expt. II in two tef genotypes.

Measurement temperature (°C)	VPD (KPa)	
	Expt. I	Expt. II
18	1.89	1.78
24	2.73	2.58
30	3.10	3.00
36	3.12	3.03
42	3.41	3.12
48	4.10	3.77
LSD _{.05}	0.50	0.39

Table 3. Mean leaf water potentials (WP) during CO₂ exchange measurement period in Expt. I and Expt. II in two tef genotypes.

	WP in KPa	
	Expt. I	Expt. II
Initial	-0.64	-0.47
Final	-1.46	-0.95
Auxiliary	-1.21	-0.81
LSD _{.05}	0.17	0.07

Table 4. Mean leaf nitrogen (N) levels in g of N per kg of leaf dry weight in Expt. I and Expt. II in two tef genotypes during CO₂ exchange rate (CER) measurement.†

Experiment	Leaf N concentration gN kg ⁻¹
I	39.4 ± 3.4
II	35.8 ± 4.4
LSD.05	NS

†Leaf nitrogen determination.

Leaf nitrogen was determined by using a modification of the Kjeldahl procedure (7). Ten to 50 mg samples were weighed and placed in 75 ml nitrogen digestion tubes. The samples were digested in 7.0 ml conc. H₂SO₄ for 45 min. at room temperature. Potassium sulfate (0.9 g), CuSO₄.5H₂O (0.09 g) and Se metal (9 mg) were then added and the samples were digested at 180 C for 1.5 h and at 380 C for an additional 2.5 h. Then the digests were brought to 50 ml with H₂O and analyzed for NH₄⁺. NH₄⁺ was determined by using the method of Cataldo et al (10). One ml of the digest was analyzed for NH₄⁺ by adding 0.2 ml of a solution containing 85 mM Na₂ EDTA and 0.2 mM methyl red at pH10, 10 to 20 drops of 3N NaOH, 1 ml of a solution containing 0.11 M phenol and 0.3 M Nanitroprusside and 1 ml of a solution containing 26 mM Na₂HPO₄.7H₂O, 84 mM

$\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ and 7.0 mM NaOCl. Final volume was brought to 10.0 ml with H_2O and absorbance at 625 nm was measured after 3 h.

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