Measurement of Instantaneous Nitrogen Use Efficiency among Pearl Millet Genotypes

G. Alagarswamy, J. C. Gardner, J. W. Maranville,* and R. B. Clark

ABSTRACT

Nitrogen is often a limiting factor in pearl millet [*Pennisetum glaucum* (L.) R. Br.] production. Genotypes are known that differ in their response to N. In this study four pearl millet genotypes, which were previously identified as extremes in N use efficiency (total above ground biomass/unit of N absorbed) in the field, were compared in nutrient solution culture for their response to N supply and the instantaneous measurement of photosynthetic N efficiency (μmol CO_2 g^{-1} N s^{-1}). The latter component possibly contributes to N use efficiency. N-efficient genotypes, 'Souna B' and 700112, and N-inefficient genotypes, 'BK560' and 'BJ104', were grown at four N levels containing 60, 120, 180, and 240 mg N $plant^{-1}$. Specific leaf area (cm^2 g^{-1}) was calculated from an accompanying growth analysis. Leaf CO_2 exchange rate was measured on several leaves as they became fully expanded. Photosynthetic N efficiency was derived using values of leaf N concentration. Nitrogen-efficient genotypes maintained thicker leaves (316.1 vs. 332.5 cm^2 g^{-1}) and were generally less responsive in terms of leaf N concentrations and leaf CO_2 exchange rate (CER) to the N available in solution as compared to the N-inefficient genotypes. Souna B, the most efficient genotype, maintained a stable CER across all N levels. Photosynthetic N efficiency was similar for all genotypes except BK560, which was 10 to 15% less efficient. The small difference in photosynthetic N efficiency among genotypes coupled with relatively high photosynthetic N efficiency values of BJ104 (N-inefficient genotype) suggests that differences in instantaneous measures of N use efficiency occurring during photosynthesis offer little explanation for overall differences in N use efficiency among these genotypes in a previous field study. Nitrogen use efficiency was more related to the partitioning of N resources available into additional leaf area.

Additional Index Words: ***Pennisetum glaucum*** **(L.) R. Br., Nitrogen response, Photosynthesis, Leaf nitrogen content, Leaf area.**

ASSUMING SUFFICIENT SOIL WATER is available, N is the factor most often limiting crop production (Goh and Haynes, 1986). With present cropping systems, crop recovery of applied N has been reported from 8 to 75% (Goh and Haynes, 1986). Excessive and inefficient use of fertilizer N in developed countries is also becoming a chronic environmental problem (Olson et al., 1973). As crop production increases on a worldwide basis, the use of fertilizer N will become increasingly influenced by availability, cost, and environmental impact rather than prescribed amounts for maximum productivity. Given these circumstances, emphasis should be placed upon increasing the efficiency of fertilizer N use.

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Both fertilizer application methods and crop selection to improve the plants' ability for fertilizer recovery are being extensively studied to improve fertilizer N recovery. Another approach for decreasing the need for fertilizer N is the possible selection of crop plants for efficiency of conversion of the total N recovered into yield. Defining total above ground biomass per unit of N absorbed as N use efficiency (NE), differences among and within some crop species have been demonstrated. Genotypic differences in N uptake and utilization have been found in wheat (*Triticum aestivum* L.) (Cox et al., 1985), corn (*Zea mays* L.) (Chevalier and Schrader, 1977), and sorghum [*Sorghum bicolor* (L.) Moench] (Maranville et al., 1980). Saric and Krstic (1984) found more potential differences in NE among cultivars within a species than across several crop species. Based on these findings, it seems possible not only to identify efficient crop species but to isolate and develop a cultivar with a reduced fertilizer N requirement.

Pavlik (1983) found N efficient species of European Beachgrass (*Ammophila areenaria*) to have 30 to 40% greater CO_2 uptake per unit leaf N (CER/N) than N inefficient species American Dunegrass [L. Mollis (Trin.) Pilger]. Among *Panicum* species differing in carbon fixing pathways, *P. maximum* with C_4 carbon fixing has also been reported to have a greater CER/N than C_3 species (Brown and Wilson, 1983). Thus, differences in photosynthesis rate at similar amounts of leaf N have been suggested as a potential mechanism contributing to NE. This trait, CER/N, has been termed the instantaneous measurement of N use efficiency (Field et al., 1983).

Alagarswamy and Bidinger (1987) assessed the differences for NE among 20 diverse pearl millet genotypes from a field study. These genotypes differed little in the total N uptake, but considerable difference existed in the amount of biomass produced, hence NE. Differences in CER/N as described by Pavlik (1983) and Bolton and Brown (1980) could possibly explain the differences in NE found among these genotypes.

Pearl millet is the principal cereal crop in many areas of the semi-arid tropics, especially where soil fertility is marginal. Development of cultivars with higher NE could have a large impact in many such production areas where N is limiting and use of fertilizer N is minimal or not possible. The objective of the present study was to compare the relationship among N supply, leaf N concentration, and photosynthesis rate among N efficient and N inefficient genotypes as an aid to identifying the underlying physiological mechanism(s) responsible for differences in NE.

MATERIALS AND METHODS

The pearl millet genotypes used were based on the field results from Alagarswamy and Bidinger (1987) and included the N efficient genotypes Souna B and 700112, and two N inefficient genotypes, BK560 and BJ104 (Table 1). Plants

were grown at Lincoln, NE in solution culture under greenhouse conditions of natural light and day length plus a daytime supplement of 450 μmol m^{-2} s^{-1} photosynthetic photon flux density at the canopy surface from metal halide lamps. Temperatures were maintained at 33 ± 3° C daytime and 26 ± 2° C nighttime. Relative humidity was not directly controlled and fluctuated from 20 to 50% with outdoor ambient conditions. Since plants from each genotype were desired with a range of leaf N concentrations, a preliminary study was conducted to determine the quantities of N needed in nutrient solution that would result in a range of leaf N. Four solutions were deemed necessary, supplying approximately 60, 120, 180, and 240 mg N $plant^{-1}$. Nutrient solution compositions can be found in Table 2. Twenty-four uniform seedlings (21 for an accompanying growth analysis and three for gas exchange measurements) from each genotype were transplanted into each of the solutions. Each plant was individually potted, randomly assigned to one of three replications, and arranged in a randomized complete block design within the greenhouse. No further nutrients were supplied to plants, and only distilled water was added as necessary to maintain solution volume at 2 L pot^{-1}.

Leaf areas were determined by destructive sampling and passing the green leaves through a LiCor portable area meter; LI-COR, Lincoln, NE. Eight days after transplanting and at weekly intervals thereafter, 12 plants per genotype were measured corresponding to one plant per block per N level treatment combination. Sampling was terminated 43 d after transplanting.

Gas exchange was measured by the LI-6000 portable photosynthesis system using the 0.25-L plexiglass leaf chamber. Measurements were made between 1100 to 1500 h with a minimum of 1200 μmol m^{-2} s^{-1} photosynthetic photon flux density supplied by natural light and metal halide lamps and at air temperatures of 33 ± 3° C. Approximately 20 cm^2 of leaf at mid-length was placed in the chamber, and CO_2 exchange measurements (CER) began when the closed system that contained the leaf segment reached 300 μL L^{-1} CO_2.

Table 1. Origin and field greenhouse-measured N use efficiency for each genotype.

Genotype	Origin and breeding	N use efficiency	
		Field†	Greenhouse
		g g^{-1} N	
Souna B	Mali, open-pollinated cultivar	123a*	98a
700112	Nigeria, breeding line	114a	83a
BK50	India, F_1 hybrid	95b	94a
BJ104	India, F_1 hybrid	93b	80a

* Means within a column followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.
† Alagarswamy and Bidinger, 1987.

Table 2. Composition of the four nutrient solutions used to study N efficiency in pearl millet.

Compound 1 *M* solution	Total mg N-$plant^{-1}$			
	60	120	180	240
	mL $plant^{-1}$			
$NH_4H_2PO_4$	1.08	2.16	3.24	4.32
$Ca(NO_3)_2$	1.61	3.21	4.82	6.42
KH_2PO_4	12.60	10.00	9.50	3.60
$CaCl_2$	6.70	5.10	3.50	1.90
$MgSO_4$	1.50	1.50	1.50	1.50
Micronutrients†	2.00	2.00	2.00	2.00
FeHEDTA	4.00	4.00	4.00	4.00

† Micronutrient and FeHEDTA solutions prepared according to Clark (1982).

Gas exchange measurements were made on the most recent fully expanded leaf (Dwyer and Stewart, 1986) to reduce interplant variability and compare leaves of similar physiological age and N sink strength among cultivars. The first leaf emerging from the coleoptile was identified as leaf number and subsequent leaves were numbered sequentially. Gas exchange was measured on leaf 8, 9, 10, and the leaf that was most recently fully expanded 49 d after transplanting.

After CER was determined, the portion of the leaf enclosed by the chamber, without the mid rib, was subjected to a Kjeldahl digest, and organic N concentration was determined colorimetrically with Nessler's reagent. Leaf NO_3^- concentration was periodically measured but considered insignificant in magnitude to justify continued analysis. Specific leaf areas (cm^2 g^{-1}) were calculated from plants harvested at midday for an accompanying growth analysis and were used to convert CER from a leaf area basis (μmol CO_2 m^{-2} s^{-1}) to a leaf dry weight basis (μmol CO_2 g^{-1} s^{-1}). Specific leaf area from the entire green canopy closely paralleled the specific leaf area of the leaf used for CER measurements since the plants were in early vegetative stages of growth with the last fully expanded leaf the most significant contributor to total leaf area.

The integration of data from all leaves and solution N levels was accomplished by regression analysis relating CER and leaf N concentration for each genotype. The effect of genotype, solution N level, and leaf number upon leaf N concentration and CER was determined by analysis of variance.

RESULTS AND DISCUSSION

Statistical Analyses

To compare with field data, NE was calculated from the last harvest of the growth analysis made 43 d after transplanting (Table 1). Cultivars were ranked somewhat similarly; however, differences among greenhouse grown plants were not yet statistically different after such a short period of growth.

Results of the analysis of variance for determining the influence of genotype, N level in the nutrient so-

Table 3. Analysis of variance for leaf N (g kg^{-1}) and CER (μmol CO_2 g^{-1} s^{-1}) values in pearl millet.

Source of variation	df	Trait	
		Leaf N	CER
		F values	
Genotype (G)	3	32.6**	6.1**
Solution N level (N)	3	25.1**	7.2**
G × N	9	5.3**	2.7**
Leaf (L)	3	45.5**	43.8**
G × L	9	1.4	1.6
N × L	9	0.9	0.7
G × N × L	27	0.3	0.4

*,** Significant at the $P = 0.05$ and 0.01 probability levels, respectively.

Table 4. Mean leaf N concentration and CER as influenced by leaf number in pearl millet.

Leaf number	Leaf N concentration	CER
	g kg^{-1}	μmol CO_2 g^{-1} s^{-1}
8	30.7	0.535
9	27.1	0.483
10	22.6	0.352
>10	18.4	0.230
LSD (0.05)	2.1	0.056

lution, and leaf number upon leaf N concentration (g kg^{-1}) and CER (μmol CO_2 g^{-1} s^{-1}) are summarized in Table 3. Inverse relationships between leaf number and mean leaf N concentration and between leaf number and mean CER were observed (Table 4), and these were consistent among all genotypes and solution N levels. As leaves emerged they were progressively larger with lower leaf N concentration and lower CER. This supports the published data from several studies summarized by Bhagsari and Brown (1986).

Genotype differences in the instantaneous measurement of NE, CER/N, were compared using regression analysis. The CER increased linearly as leaf N concentration increased for all genotypes (Fig. 1). The slope of the linear regression of CER versus leaf N was significantly ($P < 0.01$) lower for BK560, being 0.014 ± 0.002 compared to the average slope of the other genotypes, 0.0180 ± 0.002. Steeper slopes indicate a higher photosynthetic N use efficiency for Souna B, 700112, and BJ104 than BK560.

Effect of Specific Leaf Area

The N efficient genotypes differed ($P < 0.01$) from N inefficient genotypes for specific leaf area. Mean specific leaf areas were 316.1 cm^2 g^{-1} for efficient genotypes and 332.5 cm^2 g^{-1} for the inefficient genotypes. Among other possibilities, differences in specific leaf area have been associated with changes in leaf thickness, especially in response to N supply (Gulmon and Chu, 1981; Gardner et al., 1987). Microscopic examinations of leaf cross sections from Souna B and BJ104 from a field study (data not shown) would support this assumption. Stronger correlations have been found between photosynthesis and leaf N content when expressed on a leaf weight basis rather than a leaf area basis because of differences in genotypic adjustment of specific leaf area to N (Gulmon and Chu, 1981; Field et al., 1983). Given these circumstances and that the primary objective of this study was to compare genotypes for their rate of photosynthesis given a fixed N mass, efficiency results are expressed in mass units.

Response to Increasing N Supply

With greater N supply, leaf N concentration and CER usually were greater; however, the magnitude and type of response were dependent upon genotypes (Table 5). Souna B kept similar leaf N concentrations and CER despite a fourfold increase in N supply by investing in additional leaf area, especially during early growth (Fig. 2). Souna B, and to a lesser extent 700112, were developed out of landrace gene pools and were locally adapted to low input agriculture where maximum expansion and exposure to the limited resources available are adaptive traits (Danbroth and El Bassam, 1983). In contrast, BK560 responded to the lowest N level by producing small, slow growing plants that kept leaf N and CER at relatively high levels. At higher N levels, BK560 had greater vigor but still limited leaf area, resulting in leaves with a higher concentration of N. Genotypes such as BK560 and BJ104 are the result of extensive breeding efforts within a selection environment of high fertility and minimum stress.

Increased N fertilizer rates are usually associated with declining NE (Hocking et al., 1984). Field investigations by Alagarswamy and Bidinger (1987) of the

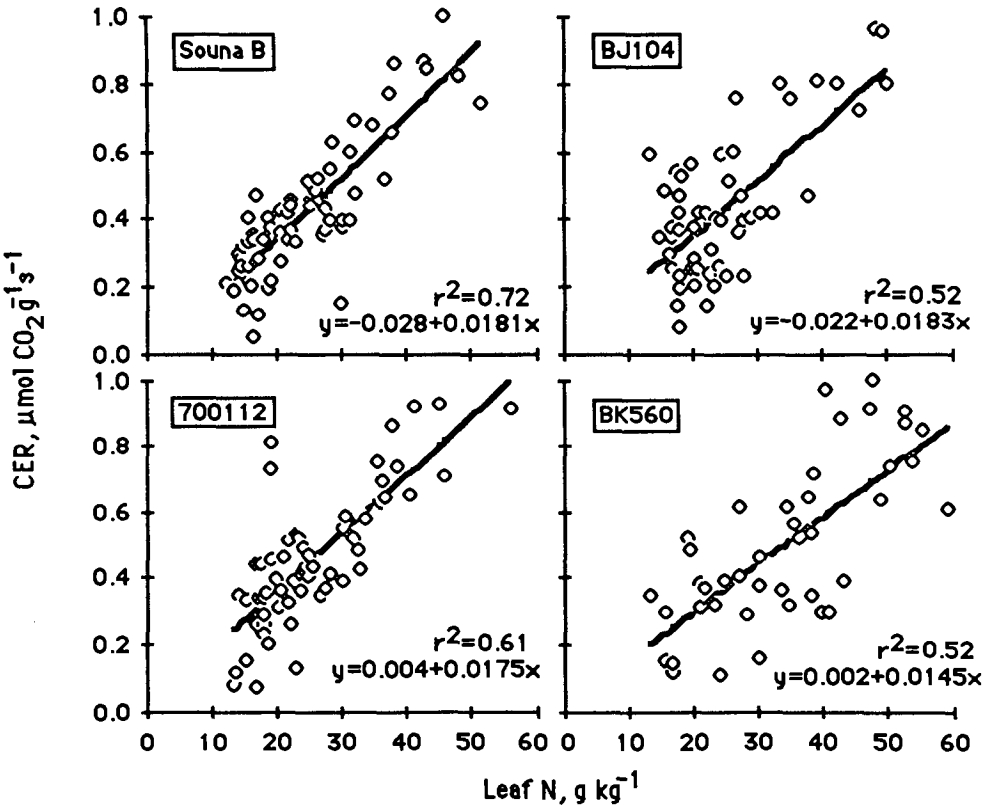

Fig. 1. Relationship between leaf N concentration and CER for N efficient (left) and N inefficient (right) pearl millet genotypes.

Table 5. Mean leaf N content and CER as affected by genotype and N level in the nutrient solution.

Genotype	N level	Leaf N conc.	CER
	mg N plant^{-1}	g kg^{-1}	μmol CO_2 g^{-1} s^{-1}
Souna B	60	19.5	0.349
	120	20.9	0.351
	180	21.2	0.361
	240	22.3	0.351
700112	60	19.8	0.304
	120	20.7	0.351
	180	23.6	0.430
	240	28.6	0.433
BJ104	60	19.8	0.284
	120	21.6	0.304
	180	24.1	0.589
	240	26.8	0.405
BK560	60	31.1	0.540
	120	22.1	0.375
	180	30.8	0.495
	240	42.4	0.509
LSD (0.05)		4.0	0.106

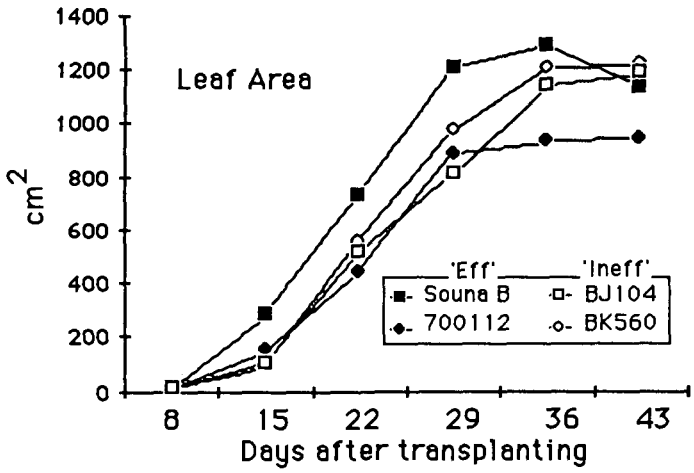

Fig. 2. Mean accumulative leaf area by genotype from growth analysis.

genotypes used in this study grown with both 20 and 100 kg ha^{-1} of fertilizer N revealed a similar trend among all genotypes. The rate of NE decline with increasing availability of N differed among genotypes. Souna B was reduced in NE by 24% while BJ104 declined by 34% from low to high fertility regimes. The ability of Souna B to maintain stable leaf N concentrations across a wide range of fertility levels may reduce its decline in NE. Within the range of N supplied in this study, visual differences among the genotypes were obvious. At low solution N levels, only slight differences in leaf color among genotypes were noticeable, with Souna B appearing slightly darker green. At high solution N levels, however, Souna B appeared obviously lighter and almost chlorotic in comparison to the other genotypes.

Bolton and Brown (1980) suggested the leaf N concentration may commonly limit photosynthesis and hence productivity in field crops. These data contradict this hypothesis. Souna B maintained the lowest leaf N concentration but was most productive in terms of leaf area development in the greenhouse and total biomass yield in the field (Alagarswamy and Bidinger, 1987).

Photosynthetic Nitrogen Efficiency

It is evident that extra N put into the leaves at higher solution N levels by the N-inefficient genotypes did not contribute entirely to higher photosynthetic rates. The reduced photosynthetic N efficiency of BK560 illustrates that genotypic differences exist for CER/N; however, they are not of the magnitude to account for the large differences in NE found in the field (Alagarswamy and Bidinger, 1987). Variability among genotypes most likely exists for the proportion of leaf N allocated to active and storage forms of photosynthetic enzymes and other leaf proteins depending upon the availability of N. These in turn affect CER/N, NE, N translocation, and ultimate yield.

The small magnitude of CER/N differences found, coupled with the relatively high CER/N of the other N-inefficient genotype, BJ104, suggest that instantaneous measurement of N use efficiency in the greenhouse could not distinguish genotypes that have been proven N efficient in the field. Further, it appears that CER/N was not a major mechanism responsible for differences in NE. The differences between N efficient and N inefficient genotypes were more associated with increasing leaf area with a low N concentration rather than limiting leaf area to maintain a high N concentration. These N and carbohydrate partitioning relationships will be explored further in growth analysis of these genotypes.

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