

Effect of Nitrogen Nutritional Stress on Some Growth Parameters of *Zea mays* L. and *Vigna unguiculata* (L.) Walp.

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Abstract

This study investigated the responses of maize (*Zea mays* L.) and cowpea (*Vigna unguiculata* L. Walp.) seedlings growth parameters to nitrogen nutritional stress. This was with a view to determining whether nitrogen nutritional stress would retard or enhance maize and cowpea growth, partly, wholly or not at all through its effect on biomass accumulation and some morphological parameters. Germination of seeds was done using treated sand in sixty plastic pots. A group of the seedlings was nutrient stressed by administering 200 ml of complete nutrient solution minus nitrogen (-N) while the other groups were fed with five times (X5N) and ten times (X10N) the optimal concentration of nitrogen and the last regime was fed with full nutrient solution (FN). The effects of optimal concentration and nitrogen stress on the growth rates (as measured by their fresh and dry weight) were studied. The result of the growth analysis showed that there was increase in shoot height with supraoptimal concentrations of nitrogen treatments (X10N and X5N) while there was a decrease in shoot height with minus nitrogen (-N) regimes. The observed higher biomass (dry matter yield) under the FN regimes in both *Zea mays* and *Vigna unguiculata* were attributed to optimal nutrient assimilation rate.

Keywords: biomass, metabolism, morphological parameters, optimal, stress

Introduction

A biological stress is defined as any change in environmental conditions that might reduce or adversely change a plant growth or development (Levitt, 1980). Any change in the environment that results in plant response that is less than the optimum might be considered stressful (Salisbury and Ross, 1991). In plants, water and nutritional deficiency, high salinity and extreme temperature are some of the most studied stress factors (Lambers and Jones, 1998). In both natural and agricultural conditions, plants are frequently exposed to unfavorable environments that results in some degrees of stress (Hasegawa *et al.*, 2000). Water deficit, heat stress, chilling and freezing, salinity, oxygen and nutrient are major stress factors restricting plant growth such that biomass or agronomic yield at the end of the season express only a fraction of the plant's genetic potential (Bray and Bailey-Serres, 2000). In addition, stress plays a major role in determining how soil and climate limit the distribution of plant species (Shinozaki, 2000). Thus understanding the physiological process that underlies stress injury and the adaptation and acclimation mechanisms of plants to environmental stress is of immense importance to both agriculture and the environment (Hong and Vierling, 2000). Nutrient stress can result either from the form in which the nutrient exist, the process by which they become available to the plant; content of soil solution

and soil pH (Evans, 1989). The demand by a plant for a given nutrient changes with time because it is influenced by changes in all other environmental factors that control plant growth which include other nutrients, water, radiation, temperature and age among others (Hartung *et al.*, 1998). Nutrient stress can be evaluated as the proportion by which the growth rate of the plant or crop is limited by that nutrient under the prevailing conditions. It affects all aspects of growth and so should be quantified in terms of growth parameters such as dry weight and biomass accumulation (Pollock and Cairns, 1991). The present study is expected to provide more definite information on the effect of nitrogen nutritional stress on some aspects of the primary metabolic activities of plants.

Nitrogen is one of the important nutrients required for normal plant growth and a close relationship has been found between plant growth and nitrogen supply (Chandra and Mishra, 1991; Vanhan, 1990). However continuous cultivation of crops in addition to adverse environmental factors make the arable soils deficient of nitrogen along with the other important nutrient and the crops grown on such soils exhibit very destructive deficiency symptoms such as poor growth, chlorosis, necrosis of leaves and disorders in many physiological and biochemical characteristics (Bray and Bailey-Serres, 2000). Because of considerable uptake and utilization of nitrogen nutrient, its deficiency frequently occurs in most soils (Ashraf

and McNelly, 1994; Marschner, 1995). In existing literature, nitrogen deficiency is known to effectively presume metabolic process in plants. For instance, it was presumed that nitrogen deficiency leads to disruption of the fine structures of chlorophyll and instability of the pigment protein complex (Reddy and Dakora, 2007). An increase in the nitrogen supply not only delays senescence and stimulates growth, but also change plant morphology in a typical manner, particularly if the nitrogen availability is high in the rooting medium during early growth stages (Graham and Vance, 2000). In several species, it has been observed that local NO_3 application induces root proliferation due to an increased growth of laterals (Zhang and Forde, 2000). Yoshida and Tadano (1989), found typical nitrogen induced changes in leaf morphology in rice. The length, width, and area of the leaf blades increase, but the thickness decrease. In addition, the leaves become increasingly droopy, an effect that interferes with light interception (Lindo *et al.*, 1982). In cereals, the enhancement of stem elongation by nitrogen increases the susceptibility to lodging. This change in shoot morphology is less distinct with ammonium than with nitrate-nutrition (Sommer and Six, 1982), and is presumably related to nitrogen-induced changes in the phytochrome balance. The response of leaf photosynthesis to irradiance is largely dependent on the leaf nitrogen content (Sachs, 1996). Photosynthetic enzymes including large amounts of Ribulose biphosphate carboxylase oxygenase (Rubisco) and, to a lesser extent, light harvesting complex proteins, represents a large proportion of total leaf nitrogen (Evans, 1989; Field and Mooney 1986). Nitrogen supply increases the leaf area of plants and canopies to a greater extent than on leaf and canopy photosynthesis (MacDonald, 1986). The increase in leaf area of plants and canopies is brought about by a large effect of nitrogen supply on the expansion of individual leaves and on branching, or tillering in grasses (Gastal, 2002; Vos and Biemond, 1992). However, a number of other species do show a significant effect of nitrogen on the rate of leaf appearance (Cruz and Boval, 2000). In all instances, the impact of nitrogen on leaf expansion rate of grasses was related more to the effect of nitrogen on cell production than cell elongation rate (Drew and Morgan, 2000). In dicots, early studies conducted showed that the impact of nitrogen supply on leaf growth was mostly due to an increased cell growth rate, because a large final cell size was observed and nitrogen supply seemed to increase leaf water potential (Radin and Ackerson, 2001).

Nutritional deficit especially nitrogen triggers a number of response that are expressed by changes in the shoot: root ratio and in the metabolism of leaves and reserve organs (Roberts *et al.*, 1992). The reduction of cell division and cell expansion, leaf production and photosynthesis are evident effects of nitrogen deficiency (Chapin *et al.*, 1988).

Materials and methods

Soil treatment and germination of seedlings

Seedlings of *Zea mays* L. ('SWANI') and *Vigna unguiculata* L. ('Ife Brown' variety) were utilized in the experiment. Soil was treated by soaking sand in 1N hydrochloric acid for one hour to eliminate microbes and solubilize mineral elements which might be present in it. The acid was drained off and the sand washed with tap water and then distilled water until the pH of the decantable water was between 6 and 7, which was optimal for the germination and growth of the seedlings. The washed sand was air dried and transferred into sixty plastic pots (about 24 cm in diameter and 21 cm in depth) each with four holes of approximately 4 mm in diameter bored at the bottom to enhance drainage during the course of the experiment. Ten seeds were planted in each pot and after germination thinned to five. The plants were exposed to approximately eight hours of sunlight daily. Each bowl was supplied with 200 ml of distilled water in the morning and evening during the first 6 days after planting. After germination and on the 7th day after planting, the pots were divided into four nutrient regimes each containing fifteen pots, each pot containing five seedlings. The experiment was repeated and carried out under the same condition.

Nutrient solution composition and application of nutrient regimes

This was prepared according to the modified Long Ashton Formular (Hewitt, 1952). The nutrient solution composition were given the subscripts; FN, X5N, X10N, and -N.

Weight Analysis

Weight analyses were carried out on ten seedlings harvested at random (sampling procedure) from each nutrient regime. The plants were carefully uprooted, blotted dry, weighed fresh and then placed inside a labeled envelope and kept in a Gallenkamp drying oven set at 80°C to dry constant weight.

Measurement of physical parameters

A meter rule was used to measure the following parameters: leaf length, leaf width and shoot height from soil level to the terminal end and the number of leaves per plant were noted. The fresh weight was taken after which the plants was dried at 80°C in a Gallenkamp oven until a constant weight was achieved. After cooling, the dry weight was determined. The dried samples were separated into leaves, shoots and roots and their different dry weights determined. These were kept for further analysis.

Growth Analysis

The following plant growth parameters were determined from the data obtained from the physical parameters (Osei-Yeboah and Jiang, 1988)

Leaf Area (LA)

$$LA=L \times W \times 2.325$$

The unit of LA is cm², L and W are leaf length and width respectively while 2.325 is the correction factor.

Leaf Area Ratio (LAR)

$$LAR=LA/W_s$$

LAR accounts for the total surface area used for assimilation power unit of plant biomass present. The unit is cm²g⁻¹, W_s is plant dry weight.

Leaf Weight Ratio (LWR)

$$LWR=W_1/W_2$$

LWR defines assimilation in terms of leaf density: the unit is g⁻¹, W₁ is the leaf dry weight.

Root Shoot Ratio (RSR)

$$RSR=W_3/W_2$$

Root shoot ratio defines the method of assimilate partitioning, W₂ and W₃ are shoot and root dry weight respectively, the unit is g⁻¹

Statistical analysis

A two way analysis of variance (ANOVA) was performed using Statistical Analysis System (SAS) software version 9.1 (SAS, 2003). The data were first tested between normality and assumption of constant variance. Post hoc testing was carried out using Duncan Multiple Range Test (DMRT) to separate the significance means at 0.05, confidence limit (alpha level) for the mean.

Results

The heights of the shoots of the cowpea and maize plants recorded similar pattern of growth for a greater part of the experimental period (Fig. 1a and 1b). There was no significant difference ($p>0.05$) in the control (FN). However, X10N was significantly different.

In line with the pattern of growth in cowpea regimes (Fig. 2a), the leaf biomass of the maize plants increased gradually for a greater part of the experimental period (Fig.

2b). Meanwhile, there were lags in peak periods of leaf biomass on the 45th day in FN and on the 52nd day in X10N and -N regimes respectively. Also, there was no significant difference ($p>0.05$) in the control (FN). However, X10N was significantly different.

The shoot biomasses in maize and cowpea plants follow a similar linear pattern and were approximately equal throughout the experimental period (Fig. 3a and 3b). Again, results of the ANOVA showed that there was no significant difference ($p>0.05$) in the control (FN). However, X5N and X10N were significantly different.

The root biomass of FN and X5N increased rapidly during the course of the experiment with both reaching their peak on the 45th day (Fig. 4a). These particular patterns were also recorded in the maize regimes during this period (Fig. 4b). There was no significant difference ($p>0.05$) in the control (FN), X10N and -N treatments. However, X5N was significantly different.

The number of leaflets/plant in all treatments followed a regular linear pattern for a greater part of the experimental period (Fig. 5a). In the maize seedlings grown under the different treatments, such patterns of growth were also recorded during the period of the experiment (Fig. 5b). There was no significant difference ($p>0.05$) in the control (FN), X10N and -N treatments. However, X5N was significantly different.

The leaf area ratio (LAR) of X5N and X10N respectively alternated between rapid increase and decrease for a greater part of the experimental period (Fig. 6a). However, in FN and X5N, LAR maintained a similar plateau throughout the experimental period. In the same vein with cowpea plants, the maize LAR in X10N and -N treatments increased till the 45th day after which they decreased for the remaining part of the experimental period (Fig. 6b).

There was no significant difference ($p>0.05$) in the control (FN), X10N and X5N treatments. However, -N was significantly different.

The leaf weight ratio (LWR) of all regimes decreased for a greater part of the experimental period (Fig. 7a). In contrast to LWR in cowpea, maize LWR was inconsistent

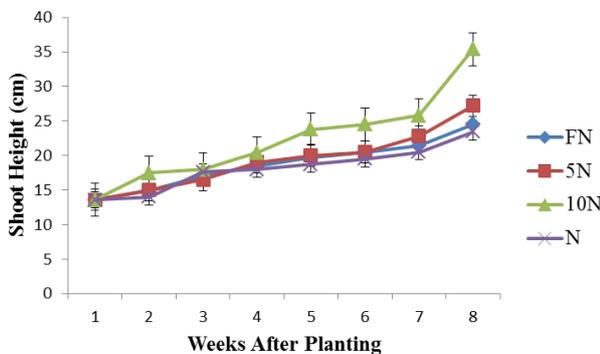


Fig. 1a. The effect of nitrogen nutritional stress on the shoot height of *Vigna unguiculata* measured at different periods

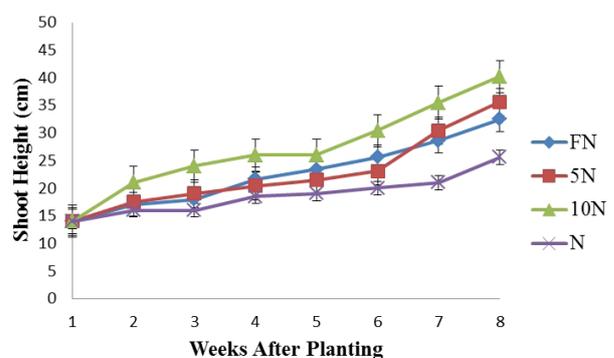


Fig. 1b. The effect of nitrogen nutritional stress on the shoot height of *Zea mays* measured at different periods

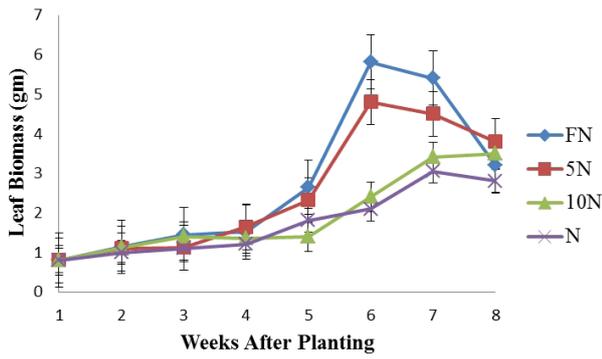


Fig. 2a. The effect of nitrogen nutritional stress on the leaf biomass of *Vigna unguiculata* measured at different periods

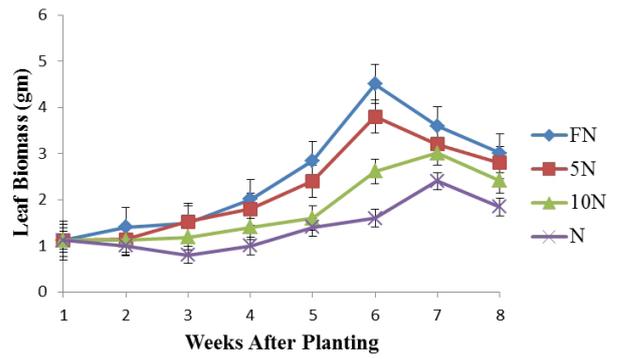


Fig. 2b. The effect of nitrogen nutritional stress on the leaf biomass of *Zea mays* measured at different periods

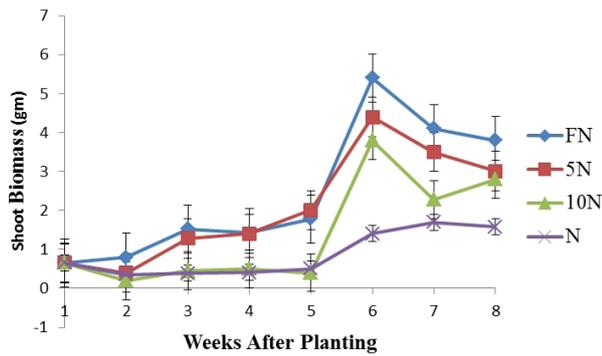


Fig. 3a. The effect of nitrogen nutritional stress on the shoot biomass of *Vigna unguiculata* measured at different periods

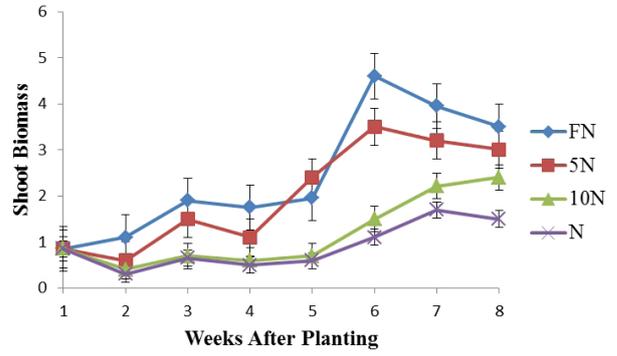


Fig. 3b. The effect of nitrogen nutritional stress on the shoot biomass of *Zea mays* measured at different periods

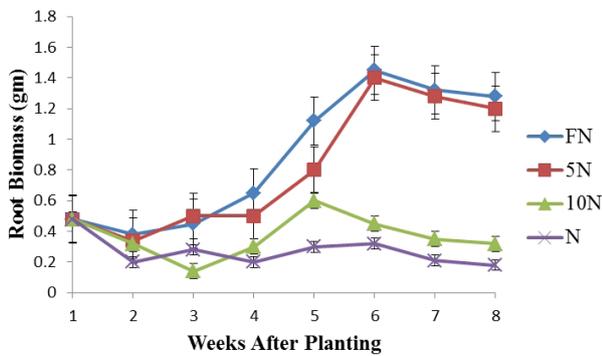


Fig. 4a. The effect of nitrogen nutritional stress on the root biomass of *Vigna unguiculata* measured at different periods

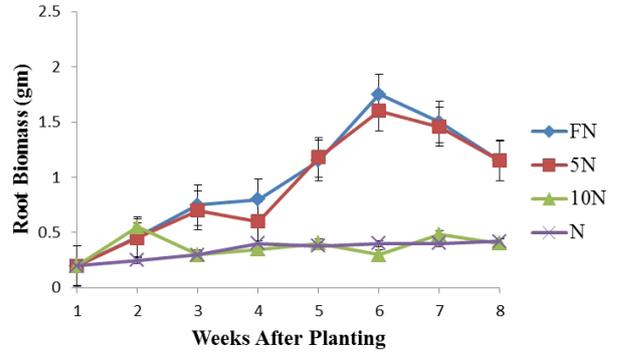


Fig. 4b. The effect of nitrogen nutritional stress on the root biomass of *Zea mays* measured at different periods

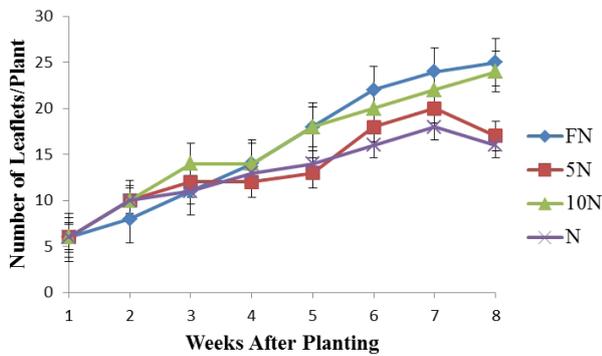


Fig. 5a. The effect of nitrogen nutritional stress on the number of leaflets/plant of *Vigna unguiculata* measured at different periods

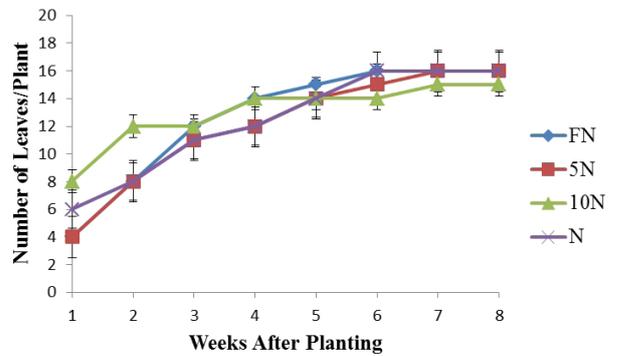


Fig. 5b. The effect of nitrogen nutritional stress on the number of leaves of *Zea mays* measured at different periods

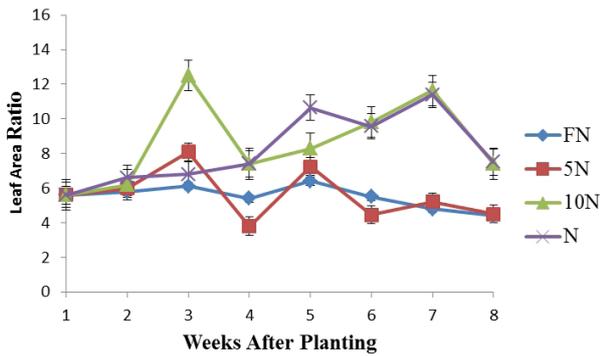


Fig. 6a. The effect of nitrogen nutritional stress on the leaf area ratio of *Vigna unguiculata* measured at different periods

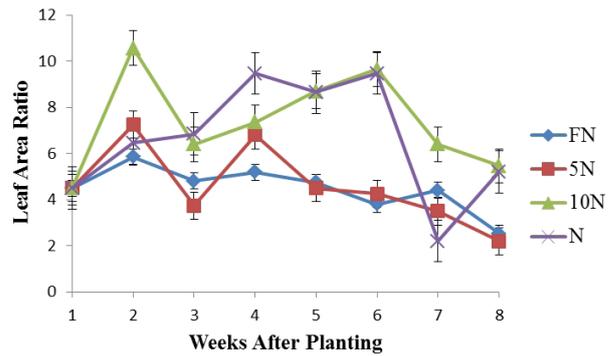


Fig. 6b. The effect of nitrogen nutritional stress on the leaf area ratio of *Zea mays* measured at different periods

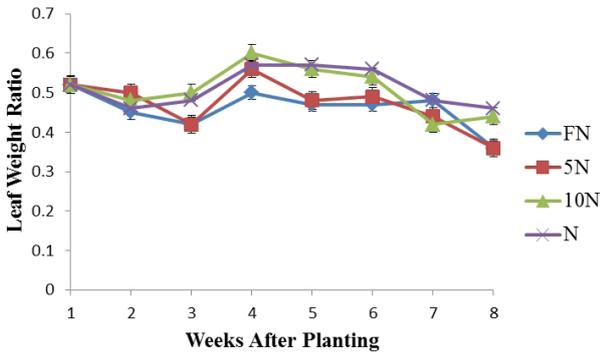


Fig. 7a. The effect of nitrogen nutritional stress on the leaf weight ratio of *Vigna unguiculata* measured at different periods

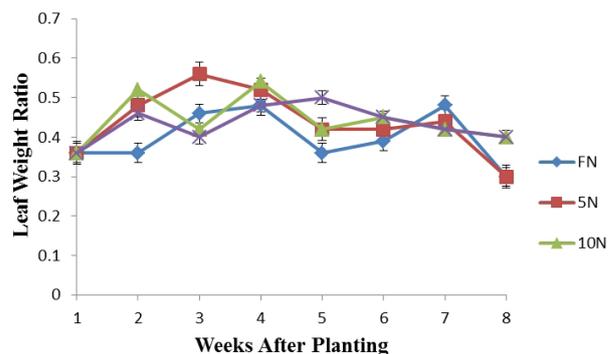


Fig. 7b. The effect of nitrogen nutritional stress on the leaf weight ratio of *Zea mays* measured at different periods

in growth patterns as the LWR of all the regimes alternated between increase and decrease throughout the experimental period (Fig. 7b).

Discussion

In the cowpea regimes, the X10N regime had the highest shoot height. The plants can be said to have devoted more of their nutrient for stem extension as apical dominance were more pronounced in them than in X5N regime (Thomas and Raper, 1983). This was in agreement with the results of Bouma and Nielsen (2000) and Bonifas *et al.* (2005) that plants growing in supraoptimal concentration of nutrient respond to nutrient stress by devoting more of their available carbon to shoot growth resulting in elongated stems. The FN plants had adequate nutrient supply and so do not require extra carbon for root growth; this invariably led to the normal plant height and short peduncles observed in this regime. The observed higher biomass (dry matter yield) under the FN regime can be attributed to optimal rate of photosynthesis, adequate nutrient and greater leaf surface area. Adequate nutrient supply increased the dry matter production in an optimal condition; a situation that corroborated the findings of Peace and Grubb (1982) and Thompson *et al.* (2000) that higher dry weight was attributed to optimal leaf expansion rates. The lowering of the shoot biomass under low

nutrient supply may not be unconnected with the reduction in the production of photosynthates as more carbon was diverted to root growth from both stem and leaf tissues (Morgan and Smith, 1981). The production of more leaves under the optimal nutrient concentration (FN) may be a mechanism evolved by cowpea plants to increase total surface area for photosynthesis due to reduced leaf area (Morgan and Smith, 1981). In the -N regime, there was increase in leaf abscission and reduction in the number of leaves produced due to the fact that the extra carbon needed for greater root growth as a result of low nutrient supply was taken from their non-assimilatory tissue as well as leaf tissue (Peace and Grubb, 1982) and so stem component production was proportionately less in nutrient stressed plants. The trend in the leaf area ratio showed that the X10N regime had a higher leaf area ratio than the X5N regime as a consequence of nutrient addition; more leaves which led to a higher surface area for photosynthesis and so a proportionate high dry matter in plant tissues. There was more or less a direct correlation between the leaf weight ratio and nutrient application. Also, Singh (1978) found that leaf weight ratio increased with increasing nutrient application indicating that photosynthesis is partitioned more to the leaves.

Nitrogen limitation resulted in a reduction in shoot growth and photosynthetic capacity in maize (Foyer and Lethoeneau, 1990). However, this was at variance with

the observation of Tischer and Giffen (2000), that an increase in nitrogen supply not only delays senescence and stimulates growth but also changes plant morphology in a typical manner, particularly if the nitrogen availability is high in the rooting medium during the early growth, shoot elongation is enhanced and root elongation inhibited, a shift which is unfavorable for nutrient acquisition and water uptake in later stages. Nitrogen acquisition is highly related to crop growth rate and to biomass accumulation (Gastal and Bemaire, 2002). Depending on the plant species, developmental stage and organ, the nitrogen content required for optimal growth varies between 2 and 5% of the plant dry weight. The biomass accumulation (dry weight) of an organ may reflect, among other things the rate of movement of solute into the organ (Renalto *et al.*, 1997). The biomass accumulation in the FN regime could therefore indicate optimal acquisition and uptake of nitrogen for efficient metabolic activities. Renalto (1997) emphasized the importance of nitrogen in the distribution of proteins in the leaves.

Conclusions

This study has to a larger extent emphasized the importance of biomass in dictating the growth pattern of other morphological parameters. It also contributed to the relationship between nitrogen nutritional stress, metabolic activities and photosynthetic apparatus in the species under study.

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