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Effects of low nitrogen on chlorophyll content and dry matter accumulation in maize

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For photosynthesizing plants, studies on the effect of different levels of Nitrogen on chlorophyll could let us know about the overall physiological status of the plant under different environmental conditions. Objective of our study was to understand if there was any difference among the ten recombinant inbred lines (RILs) of IBM population and their parents B73 and Mo17 for chlorophyll content and dry matter accumulation in their response to the N stress. Five IBM genotypes that carry predominantly B73 alleles and five IBM genotypes that carry Mo17 alleles at 5 quantitative trait loci (QTL) regions associated with root traits and N use efficiency from the published Maize Genome Database were evaluated in this study along with their parents. Plants were grown in the Guterman Green House (Cornell University, USA). Two nitrogen treatments (solution with high N contained 65.79 g Ca (NO₃)₂ 4H₂O in 100L of 1X solution making 5.0mM NO₃ and solution with low N contained 2.63 g Ca (NO₃)₂ 4H₂O in 100L of 1X solution making 0.2 mM NO₃) were given. Plant traits like leaf dry weight, stalk dry weight and root dry weight were observed. Chlorophyll content was estimated to measure the effect of different N levels on photosynthetic activity. Genotype with high in B73 composition had relative advantage over Mo17 in chlorophyll content, and dry weight of roots under low N condition. The highest root-shoot ratio under low and high N was observed in IBM056 and IBM153 respectively. Except for IBM153 and IBM337, all other genotypes showed reduced LFS at high N. The result showing lower root-shoot ratio and leaf fraction of shoot (LFS) under the high N treatment suggests that shoot growth increases more than root growth in response to increased N application and that within the shoot, stem growth increases more than leaf growth. Results support the conclusion that under low N condition, shoot growth is retarded than root growth.

Keywords: Nitrogen, Maize, Chlorophyll, Dry matter accumulation, Photosynthesis.

INTRODUCTION

Nitrogen supply has substantial effects on plant growth and development, as it is one of the main constituents of leaf cell components, particularly those associated with the photosynthetic apparatus, including carboxylating

enzymes and membranes proteins (Pandey et al., 2000). N deficiency inhibits plant growth and development, especially in the older leaves near the base of the plant and ultimately they turn yellow and fall off under severe N

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stress condition. Yield is also severely affected by lower grain number and less grain weight which is caused by fewer fertilized ovum, kernel abortion and other changes at physiological and biochemical level (Uhart and Andrade, 1995 a). On the other hand, problems of eutrophication and ground water contamination occur when high amount of N fertilizers are applied to the soil. MacDonald et al., (1997) reported that unfavorable environmental conditions decreased crop yield, but it was not due to less N content in the soil. Plants could perform considerably better even under limited N condition provided that partitioning of N within the plant and efficient utilization of N at the cellular level (Sattelmacher et al., 1994). This emphasizes the fact that plant breeders need to develop varieties and hybrids with high N use efficiency (NUE). For annual crops (like rice, wheat, maize, sorghum etc.), N uptake is regulated by the crop dry mass accumulation under non-limiting nitrogen supply within species and across environments (Lemaire et al., 2007). Tollenaar and Wu (1999) indicated that increased leaf longevity, increased water and nutrient uptake, and greater assimilate supply during grain filling were related to increased low N tolerance in Canadian maize hybrids. N stress cause reduction in leaf area, enhances leaf senescence, and decreases radiation use efficiency (RUE) (Uhart and Andrade, 1995 b). Results of Subedi and Ma (2005) indicated that hybrids with greater yield or NUE were accompanied by greater dry matter production and more N uptake during the grain-filling period. Comparative study between early-senescing maize hybrid and late-senescing maize hybrid, both grown under field conditions with a high fertilisation input involving large quantities of fertilizer showed that leaf senescence occurs independently of the source-to-sink transition at the high level of fertilisation used involving large quantities of fertilizer (Martin et al., 2005). Gastal and Nelson (1994) found that synthesis of Rubisco and other chloroplastic proteins occur largely from recycling of N that was previously incorporated into proteins during cell production and N content was the highest in cell production zone. The relationship between plant photosynthetic capacities, chlorophyll degradation during leaf senescence, and the shift from N assimilation to N remobilization has been investigated in a number of crops by studying the impact of prolonged green leaf area duration on yield of maize (Ma and Dwyer, 1998; Rajcan and Tollenaar, 1999 a, b) and other major crops (Thomas and Smart, 1993; Borrell et al., 2001; Spano et al., 2003). Research is also carried out to identify some of the components responsible for the physiological control of the 'stay-green' phenotype particularly in relation to NUE. For example, in both sorghum and maize, delayed leaf senescence allowed photosynthetic activity to be prolonged, which had a positive effect on the N uptake capacity of the plant. In sorghum this enabled the plant to assimilate more carbon and use more N for biomass production (Borrell et al., 2001), whilst in maize yields

were higher (Ma and Dwyer, 1998; Rajcan and Tollenaar, 1999 a, b). However, further investigation is required to characterize better the physiological and molecular basis of the stay-green phenotype (Verma et al., 2004) in relation to N supply, root N uptake capacity, root architecture, and leaf structure, and to determine whether such a phenotype can be beneficial when N fertilization is reduced (Borrell et al., 2000).

MATERIALS AND METHODS

A list of 260 IBM Recombinant Inbred Lines (RILs) was obtained from the Maize Genome Database (Maize GDB, 2004) (<http://maizegdb.org/>). The RILs were classified into 2 groups based on the constitution of genotype (abundance of B73 and Mo17) in 5 key regions of the genome that had been previously associated with root traits on Chromosome 1 (Liu et al., 2008) and N use efficiency (NUE). The IBM genotypes which have more of B73 in these 5 target regions are classified as IBM RILs with high B73. The IBM genotypes which have more of Mo17 in these regions are classified as IBM RILs with high Mo17. Five genotypes from each group (high B73 and high Mo17) were selected for the experiment along with parental inbreds B73 and Mo17 to compare the difference among the genotypes and the parents.

Fine, medium and coarse sand were mixed well and used to fill the 120 black plastic cylindrical pots to equal depth. The 10 IBM RILs and their parents (B73 and Mo17) were planted in the Guterman Laboratory (greenhouse) (total of 120 plants). Two seeds were sown in each pot and seedlings were thinned to one per pot shortly after germination. Day temperature was about 21.1°C (70°F) and night temperature 15.6°C (60°F). Along with normal sunlight, artificial lights (1000 Watt metal halide lamps) were provided to supplement enough light for plant growth.

Three stock solutions following Engels and Kirby (2001) were prepared: solution A in high and low N versions and solution B. Solution A comprised 10 L of high N and 10 L of low N prepared in 2 different plastic containers (Sen et al., 2015). Iron-EDTA was added in solution A. Solution B contained all other nutrients except Ca (NO₃)₂ 4H₂O and Iron-EDTA and a total of 20 L was prepared in a plastic container. The stock solution was diluted to 10 X solution. Beginning at 8th day after planting, 30 ml solution was applied to each pot and watering was done every other day. At one month after sowing, 30 ml solution was applied every day and continued for 20 days. The plants were harvested at 50 days after planting.

Measurements of dry matter and chlorophyll content

Dry weight

Plants were separated into leaves, stems, and roots and dry weights of each part were measured after drying them in the oven for five days at 60°C.

Root – shoot ratio

Root-shoot ratio was derived by taking the ratio of root and shoot dry weight. Shoot dry weight of a particular plant was obtained by adding leaf and stem dry weight for that plant.

Leaf fraction of shoot (LFS)

LFS was measured by taking the ratio of leaf dry weight and shoot

Table 1. List of IBM RILs selected for screening for nitrogen use efficiency.

IBM RILs with high B73 (high A)	IBM RILs with high Mo17 (high B)
IBM189	IBM055
IBM280	IBM056
IBM284	IBM153
IBM337	IBM236
IBM346	IBM248

Table 2. Sums of squares from analysis of variance for plant traits.

Source	DF	Chlorophyll	Root/Shoot	Leaf/Shoot
Replication	4	0.26	1.76	0.13
Treatment	1	97.82**	0.13**	0.06*
Treatment×Replication	4	0.24	0.1	0.06
Genotype	11	7.68	1.44	0.44
Genotype×Treatment	11	7.87	0.77	0.17
Error(b)	88	7.56	4.55	0.74

*** Significant at $P < 0.05$ or $P < 0.01$, respectively.

Table 3. Mean values for plant traits at low and high nitrogen levels, evaluated for 12 genotypes in 5 replications in the greenhouse.

Nitrogen treatment	Chlorophyll (mg g^{-1})	Root/Shoot	Leaf fraction of shoot (LFS)
Low	0.71	0.72	0.44
High	2.52	0.55	0.38
Std. Error	0.04	0.03	0.01

dry weight.

Chlorophyll

Five leaf disks of 0.25 cm were taken from each plant. Chlorophyll was extracted in 1 ml of 95% (v/v) ethanol/water. The absorbance of chlorophyll at 654 nm wavelength was measured in a spectrophotometer. This wavelength was chosen based on the findings of Wintermanns and de Mots (1965) who reported that chlorophyll has an extinction coefficient of 83.4 L/g at this wavelength.

Experimental design and statistics

A split plot design was used for the experiment with 5 replications. Statistical analysis of various observations was done using the JMP software package.

RESULTS

N treatments significantly affected chlorophyll content, root-shoot ratio, and leaf fraction of shoot (LFS) (Tables 1 and 2). Chlorophyll content was higher for the high N

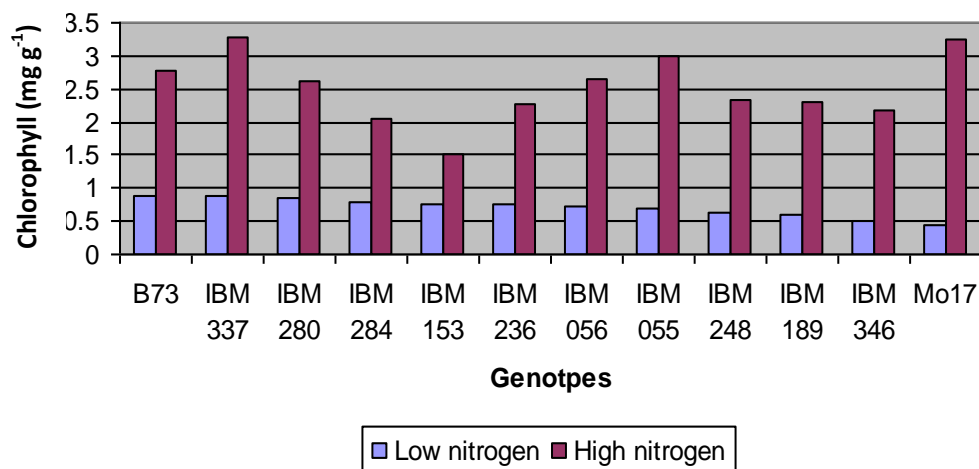
treatment (Tables 3 and 4). Under low N conditions, IBM RILs with high Mo17 at the target QTL regions had higher root-shoot ratio and LFS than the high-B73 RILs (Table 5). Chlorophyll content was increased with increased level of N in all 12 tested genotypes (Figure 1). IBM337 showed the highest chlorophyll content under high and low N conditions (Figure 1). Low root-shoot ratio under high N conditions indicates that the increased level of N promotes shoot growth more than root growth (Table 4). All the genotypes showed decreased root-shoot ratio at high N except the genotype IBM337 (Table 4 and Figure 2). IBM056 had the highest root-shoot ratio under low N and IBM153 had the highest root-shoot ratio under high N (Figure 3). Except for IBM153 and IBM337, all other genotypes showed reduced LFS at high N (Figure 4). IBM337 was extreme in its dry weight partitioning at high N, with high root-shoot ratio, high LFS, and high chlorophyll content at high N. The high N treatment increased LFS and root-shoot ratio also in IBM153, but did not affect its chlorophyll content (IBM153 showed the lowest chlorophyll content at high N of all the genotypes). IBM337 is one of the “high B73” RILs whereas IBM153 is a “high Mo17” RIL.

Table 4. Mean performance of plant traits of 12 genotypes in 5 replications with high B73 and Mo17 under low nitrogen evaluated in green house.

Genotype	Chlorophyll (mg g^{-1})	Root/Shoot	Leaf/Shoot
IBM 055	0.69	0.78	0.51
IBM 056	0.74	1.06	0.51
IBM 153	0.77	0.93	0.48
IBM 189	0.60	0.98	0.48
IBM 236	0.75	0.89	0.49
IBM 248	0.63	0.80	0.49
IBM 280	0.85	0.56	0.34
IBM 284	0.80	0.66	0.46
IBM 337	0.88	0.63	0.37
IBM 346	0.51	0.62	0.32
B73	0.89	0.73	0.50
Mo17	0.45	0.85	0.36

Table 5. Mean performance of plant traits in genotypes with high B73 or high Mo17 under low nitrogen.

Genotype	Chlorophyll (mg g^{-1})	Root/Shoot	Leaf fraction of shoot (LFS)
High in B73	0.73	0.63	0.39
High in Mo17	0.72	0.89	0.50

**Figure 1.** Effect of nitrogen treatment on chlorophyll content of 12 maize genotypes evaluated in 5 replications in the greenhouse.

DISCUSSION

N stress reduces crop photosynthesis by reducing leaf area development and leaf photosynthesis rate and by accelerating leaf senescence (Bänziger et al., 2000; Graham and Vance, 2000). N uptake is positively related to crop growth rate and to biomass accumulation (Gastal and Benaïme, 2002). In this study, the chlorophyll

concentration in the IBM RILs was lower than that in the parents at high N (except for IBM337 and IBM055) but fell between Mo17 (lowest chlorophyll) and B73 (highest chlorophyll) at low N. IBM337 was high in chlorophyll at both N levels, which suggests good potential breeding value since delayed senescence is an important criteria for abiotic stress tolerance. Delayed senescence (or stay-green) was proposed as an indirect selection criterion for

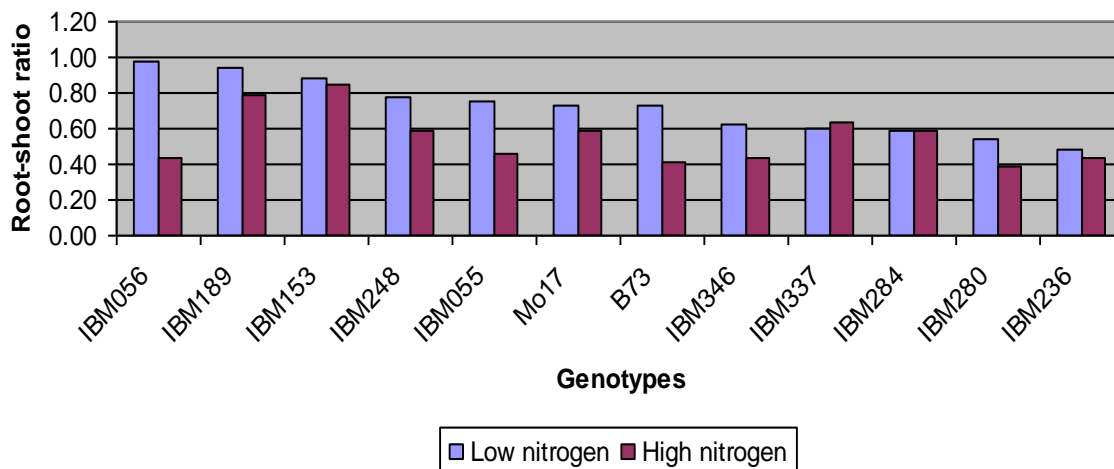


Figure 2. Effect of nitrogen treatment on root-shoot ratio of 12 maize genotypes evaluated in 5 replications in the greenhouse.



Figure 3. Maize genotype IBM236 grown under high nitrogen shows less root growth compared to shoot.

low N tolerance (Moll et al., 1994). Uhart and Andrade (1995a) reported that leaf N decrease has a direct effect on canopy photosynthesis, resulting in more kernel abortion and lower grain number. The IBM RILs with high B73 showed higher chlorophyll content and dry root weight under low N conditions than the IBM RILs with

high Mo17. “Stay green” concept under stress conditions is important for better photosynthetic activities and supplying carbohydrates for developing sink organs (young leaves, kernels, grains) and hence genotypes that had higher chlorophyll content could be further studied for future improvement of low N tolerance. Chlorophyll

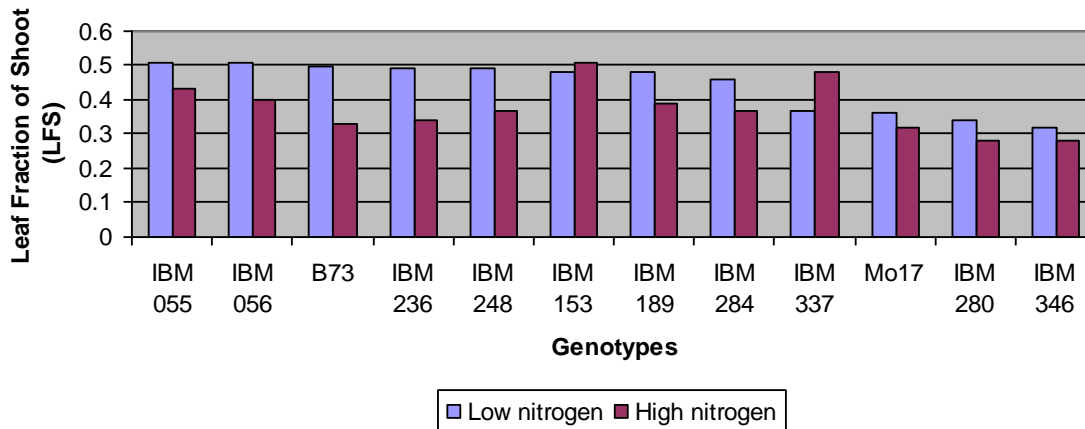


Figure 4. Effect of nitrogen treatment on leaf fraction of shoot of 12 maize genotypes evaluated in 5 replications in the greenhouse.

content of maize leaf can be used effectively in developing recommendations for soil N replenishment (Ványiné et al., 2012).

Costa et al. (2002) and Chun et al. (2005) reported that low N reduced root biomass in all the tested genotypes, which is in accordance with the obtained results showing reduced root dry weight at low N. Root-shoot ratio was higher in N stressed genotypes, which indicates that under low N conditions the shoot growth of the plant is reduced more than the root growth supported by the findings of Amos and Walters (2006). This increased root-shoot ratio at low N may be an adaptive response that conserves plant N while maximizing the ability to acquire more N from the soil and can result from decreased shoot growth with or without increased root biomass (Chun et al., 2005; Yu et al., 2015). The absolute dry root-mass is usually less for plants grown under low N conditions than under normal soil containing sufficient N (Costa et al., 2002; Bänziger et al., 2000), a pattern that is clearly confirmed by root dry weight data from the present study. IBM337 had higher chlorophyll content and high root dry matter content. This establishes the fact that there is direct relationship between higher photosynthetic activity and increase in dry matter content.

Except for the genotypes IBM189 and IBM346 all other genotypes with high B73 composition had higher chlorophyll content. Genotypes with high Mo17 showed more root-shoot ratio than the genotypes with high B73 except the genotype IBM055. The genotypes with high B73 performed better in root dry weight. All the genotypes with high B73 had higher root dry weight than the genotypes with high Mo17 composition. Overall genotypes with high B73 had better performance than the genotypes with high Mo17. Determining the maize chlorophyll content in leaves and maize dry matter content will provide in near future a way to develop maize hybrids with better NUE which can be grown in both resource poor and resource rich environment.

Conflict of Interest

The authors have not declared any conflict of interest.

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