



Selection of discrepant maize genotypes for nitrogen use efficiency by a chlorophyll meter

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ABSTRACT - *The objective of the present study was to verify if it is possible to discriminate maize genotypes for their efficiency of nitrogen use by readings obtained with a portable chlorophyll meter. 76 topcross hybrids and 5 previously tested controls were evaluated without sidedress nitrogen fertilization and 81 S₁ families that only differed in relation to nitrogen sidedress fertilization (with and without). Readings were obtained by the chlorophyll meter in the phenological stages of four and eight fully unfolded leaves. Different negative selection pressure was simulated by the chlorophyll meter readings at each phenological stage and the genotypes were screened for grain yield and efficiency of N use. It was verified that the portable chlorophyll meter can be used to eliminate the least productive genotypes before flowering in quick tests, especially in environments without nitrogen sidedress, although the most effective genotypes regarding nitrogen use could not be discriminated this way.*

Key words: *Zea mays* L., mineral stress, nitrogen, portable chlorophyll meter.

INTRODUCTION

Chlorophyll leaf concentration can be used to predict nitrogen concentration in plants, since the quantity of this pigment is positively correlated with the nutrient concentration (Peterson, 1993). Chapman and Barreto (1997) ascribe this positive correlation to the fact that 50 to 70% of the total nitrogen in leaves is integrant of chloroplast-associated enzymes.

The portable chlorophyll meter estimates the chlorophyll leaf concentration in instant measurements (Argenta et al. 2001), which is an advantage over the traditional methods of diagnosing nitrogen concentration. The readings can be obtained quickly without destroying

the leaf (Peterson 1993), sidestepping the phase of sending leaf samples in to laboratories for analyses (Argenta et al. 2001). The maintenance costs of the device are low (Piekielek and Fox 1992). Besides, the chlorophyll meter readings are uninfluenced by “luxury N consumption” since the production potential of a plant is function of the chlorophyll and not of the N content (Blackmer and Schepers 1995).

However, other factors such as cultivar, plant development stage, temperature as well as humidity and sunlight stress also affect the readings. Besides, diseases, mineral deficiency and other types of stress can affect the plant’s ability of producing chlorophyll, altering the leaf coloration (Peterson et al. 1993).

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As the portable chlorophyll meter is affected by so many factors, it is not possible to affirm that a particular reading indicates nitrogen sufficiency. The device has to be calibrated for each plantation, soil, cultivar, and environment to make the readings reliable. For Peterson et al. (1993), the best method of calibrating the meter is to maintain some small, more fertilized control areas than the plantation. The nitrogen quantity applied to these areas must be sufficient to ensure that the reference plants are not deficient. A comparison of the reading means of the control areas with the plantation mean showed the deficiency early enough to correct it and determined the quantity of fertilizer required for this purpose.

According to Balasubramanian et al. (2000), the portable chlorophyll meter can also be used in plant improvement programs for the selection of families better adapted to nitrogen deficiency stress conditions.

The number of families evaluated in maize improvement programs is generally very high. When it is possible to compare the families in evaluation with previously “calibrated” controls by means of readings of the portable chlorophyll meter, one can identify the most effective ones in nitrogen use at an early stage, increasing the gain with selection or avoiding self-pollination of less effective families.

The objective of this study was to verify whether it is possible to discriminate maize genotypes regarding the efficiency of nitrogen use by means of readings obtained with a portable chlorophyll meter.

MATERIAL AND METHODS

A preliminary study aiming at reference readings with the portable chlorophyll meter was carried out with six commercial maize cultivars that were classified for their efficiency of N use and response to N fertilization by the methodology proposed by Fageria and Kluthcouski (1980). Five of them were used as controls in the second experiment, in which 76 top-cross hybrids were evaluated without application of nitrogen sidedress and 81 S₁ families were evaluated with and without nitrogen sidedress.

The experiments were conducted at the experimental station of the Department of Plant Science of the University Federal of Viçosa (UFV) in the county of Coimbra, state of Minas Gerais (lat 20° 45' S, long 42° 51' W, and alt 720 m asl).

The following main soil chemical characteristics were observed at the site of the preliminary study: water

pH = 5.5; P = 5.7 mg (dm³)⁻¹; K = 71 mg (dm³)⁻¹; Ca²⁺ = 2.5 cmol_c (dm³)⁻¹; Mg²⁺ = 1.02 cmol_c (dm³)⁻¹; Al³⁺ = 0.00 cmol_c (dm³)⁻¹; H+Al = 3.96 cmol_c (dm³)⁻¹; SB = 3.70 cmol_c (dm³)⁻¹; t = 3.70 cmol_c (dm³)⁻¹; CTC = 7.66 cmol_c; V = 48.3%. Main soil chemical characteristics at the sites of the other experiments were the following: water pH = 5.00; P = 11.51 mg dm³; K = 72 mg dm³; Ca²⁺ = 1.88 cmol_c dm³; Mg²⁺ = 0.87 cmol_c dm³; Al³⁺ = 0.24 cmol_c dm³; H+Al = 6.53 cmol_c dm³; SB = 2.93 cmol_c dm³; t = 3.17 cmol_c dm³; CTC = 9.46 cmol_c and V = 31%.

In the preliminary study, the design of random split plot blocks was used in three replications; the treatments consisted of five nitrogen doses (0, 30, 60, 120, and 200 kg N ha⁻¹) in the plots and six cultivars (P30F80, BR 201, BR 106, Sol da Manhã, DKB 901 and CMS 39) in the subplots. Each plot contained 12 rows of 4 m, spaced 0.9 m between rows and 0.2 m between plants, with a population of about 55.555 plants ha⁻¹ and each subplot consisted of two 4 m rows.

In the plots that received nitrogen, the application was split in 30 kg N ha⁻¹ on the day of sowing and the rest in sidedress realized when the plants presented four and eight fully unfolded leaves. All plots received 80 kg ha⁻¹ of P₂O₅ and 80 kg ha⁻¹ of K₂O applied in the furrows on the day of sowing. Nutrient sources were the fertilizers simple superphosphate and potassium chloride.

Immediately after female flowering, the leaf below and opposite the first ear was sampled from five plants of each subplot to analyze leaf N, according to Malavolta et al. (1997).

Complementarily, 76 top-cross hybrids and 81 S₁ families were evaluated in experiments of the Maize Breeding Program, using the controls P30F80, DKB 901, CMS 39, Sol da Manhã and BR 106, subdivided as follows: **Experiment I** - S₁ families with 400 kg ha⁻¹ of the formula 8-28-16 plus 90 kg N ha⁻¹ split in two sidedressings (S₁ families with N in sidedress).

Experiment II - S₁ families with 400 kg ha⁻¹ of the formula 8-28-16 (S₁ families without N sidedress).

Experiment III - topcross hybrids and controls with fertilization of 400 kg ha⁻¹ of the formula 8-28-16 at planting (topcross hybrids, without N sidedress).

The experiment had a complete random block design with two replications. The plot consisted of one 3 m row with five plants m⁻¹, representing the approximate population of 55.555 plants ha⁻¹.

The readings of the chlorophyll meter in the two experiments were obtained in the phenological stages of

four and eight fully unfolded leaves and female flowering and before the sidedress applications throughout. The fourth and eight completely expanded leaves and the leaf below and opposite the first ear in each evaluated stage were sampled, respectively, always from the mid third of the leaves between the midrib and the borders, using a Minolta SPAD 502 chlorophyll meter.

Five readings per subplot were realized in the preliminary study and in the experiments of the improvement program, three readings per plot.

Grain yield was measured in kg plot^{-1} and transformed to kg ha^{-1} and the moisture corrected to 13%.

Different pressures of negative selection were simulated by the chlorophyll meter readings at each evaluated phenological stage and the genotypes stratified regarding grain yield and N use efficiency.

RESULTS AND DISCUSSION

By the classification methodology proposed by Fageria and Kluthcouski (1980), the control cultivars CMS 39, BR 201 and DKB 901 were classified as effective and the cultivars P30F80, Sol da Manhã and BR 106 as non-effective in N use. For the use of this methodology, the cultivars were evaluated under two N doses. The lower was determined as critical inferior level, characterized as the lowest applied dose able to express differences among cultivars in relation to yield while the dose under which the cultivars attained at least 80% of their production potential characterizes the critical superior level. In our study, these critical levels were obtained, respectively, by doses of 30 and 120 kg N ha^{-1} (data not shown). After the determination of the critical inferior and superior levels, an index (α) that determines the cultivar response (kg ha^{-1} of grains/ kg ha^{-1} of nutrient) was calculated by dividing the difference of yield among environments by the difference among doses that characterize the critical superior and inferior levels. The productivity of each cultivar at the critical inferior level and its corresponding α were represented in the axes x and y of the Cartesian coordinate system, respectively. The axes' point of intersection was defined by the mean grain yield at the critical inferior level and mean α of all evaluated cultivars. A diagram was obtained which allowed the separation of four quadrants according to the N use efficiency and response to the applied N. The cultivars in the first quadrant were classified as effective and responsive; in the second as non-effective

and responsive; in the third as non-effective and non-responsive, and the fourth as effective and non-responsive.

The mean reading obtained with the chlorophyll meter in the four leaf stages was 16% higher in the treatments that received nitrogen at sowing in comparison to the control without N fertilization, showing that fertilization at sowing favored chlorophyll accumulation in the leaves (Table 1). At the phenological 8-leaf-stage and female flowering, values of the chlorophyll meter readings were almost 22% higher in treatments that received sidedress fertilization in comparison to N application only at sowing (Table 1). At female flowering, the mean value obtained with the chlorophyll meter in treatments that received nitrogen in sidedress was 55.34 and in treatments that did not receive N sidedress it was 43.38. These results show that the adequate N level in the plants was only attained by sidedress fertilizer application once the observed value is close to 55, cited by Argenta et al. (2003) as an adequate reading value at this stage. Small oscillations are permissible though, since Argenta (2001), who worked with two single hybrids, concluded that readings corresponding to an adequate N level were 45.4, 52.1 and 58 at the stages of 3-4 leaves, 6-7 leaves and female flowering, respectively. The values of readings, determined as adequate by Argenta (2001), were higher than in the present study, principally in the stages of four leaves and female flowering. These differences were probably due to climatic and genotypic differences and distinct plant structures, since Argenta worked in a no-till system in succession to a black oat/ common vetch intercrop in the State of the Rio Grande do Sul. Besides, according to the results of the leaf analysis, the cultivars approached the N concentration considered critical, according to Malavolta et al., (1997) to obtain a satisfactory grain yield, when treated with a dose of 60 kg N ha^{-1} (Table 2).

Mean values of the readings obtained with the chlorophyll meter in the cultivars classified as effective by the methodology of Fageria and Kluthcouski (1980) were not always higher than the values of the non-effective ones, even in the treatments where nitrogen was the limiting factor (below 30 kg ha^{-1} N). The mean test did not detect significant differences between the cultivars in the stressful environments at any of the evaluated stages. In relation to the values of the readings realized at the stages of eight leaves and female flowering the cultivars differed only for the treatment 30 kg N ha^{-1}

Table 1. Readings of the chlorophyll meter for six control cultivars at three phenological stages and nitrogen quantity (N) applied until the reading date

Cultivars	N doses (kg ha ⁻¹)				
	0	30	30	30	30
Four Leaves					
BR 201	30.16 a	37.32 a	40.97 a	35.50 ab	37.74 a
DKB 901	31.57 a	36.86 a	35.44 a	38.68 ab	37.60 a
CMS 39	34.25 a	39.10 a	37.71 a	38.15 ab	38.88 a
P30F80	30.67 a	34.84 a	34.67 a	32.71 b	37.86 a
BR 106	27.83 a	35.78 a	36.63 a	34.64 b	36.10 a
Sol da Manhã	32.05 a	38.60 a	38.50 a	43.00 a	41.92 a
Overall mean	31.09	37.08	37.32	37.11	38.35
Eight Leaves					
	0	30	45	75	115
BR 201	39.30 a	41.00 a	47.06 b	49.40 a	53.91 a
DKB 901	43.38 a	42.12 a	51.63 ab	51.42 a	59.82 a
CMS 39	41.18 a	42.03 a	55.55 a	54.56 a	56.37 a
P30F80	41.71 a	42.30 a	48.78 b	51.66 a	54.28 a
BR 106	37.02 a	39.66 a	51.08 ab	49.11 a	54.36 a
Sol da Manhã	38.61 a	42.95 a	52.17 ab	49.48 a	55.34 a
Overall mean	40.20	41.67	51.05	50.94	55.68
Female flowering					
	0	30	60	120	200
BR 201	43.17 a	44.78 a	48.27 b	50.41 a	58.22 a
DKB 901	42.40 a	40.75 a	48.42 b	54.83 a	62.35 a
CMS 39	43.94 a	41.34 a	49.94 ab	58.36 a	57.30 a
P30F80	46.21 a	45.06 a	57.54 a	55.45 a	61.41 a
BR 106	41.36 a	42.41 a	50.98 ab	51.13 a	56.47 a
Sol da Manhã	45.54 a	43.62 a	57.33 a	56.41 a	61.35 a
Overall mean	43.77	42.99	52.08	54.43	59.52

Means followed by the same letter in the vertical did not differ from each other in the Tukey test at 5% probability

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Table 2. Results obtained in the leaf analysis of maize cultivars in five nitrogen doses

Cultivars	N doses (kg ha ⁻¹)				
	0	30	60	120	200
	Dag kg ⁻¹				
P30F80	2.20	1.92	2.82*	2.65	3.11*
BR 201	2.32	2.21	2.62	2.68	3.29*
BR 106	2.10	1.92	2.79*	2.67	3.33*
Sol da Manhã	2.10	2.08	2.78*	2.82*	3.19*
DKB 901	1.95	1.90	2.42	2.53	2.99*
CMS 39	2.25	1.88	2.77*	2.93*	3.32*
Mean	2.15	1.98	2.70	2.71	3.20*

Values equal to or higher than the nitrogen concentration considered critical in maize leaves (2.75 dag kg⁻¹), according to Malavolta et al. (1997)

Table 3. Different pressures of selection (PS) and discarding (PD) of the 81 S₁ families without nitrogen sidedress, number of families considered (NF) according to the pressure, mean reading of the chlorophyll meter (RC) of these families, number of families among the ten most productive (NF 10+), number of families classified as effective (NF Ef) and mean grain yield under each pressure of selection or discarding (YIELD), at three phenological stages

S ₁ families without nitrogen sidedress													
Four Leaves					Eight Leaves					Female flowering			
PS (%)	NF	RC	NF 10+	NF Ef	YIELD	RC	NF 10+	NF Ef	YIELD	RC	NF 10+	NF Ef	YIELD
5	4	38.62	1	2	3091	50.10	2	4	3806	54.40	2	3	3644
10	8	36.49	1	5	3119	49.10	2	5	3160	52.80	4	6	3927
20	16	34.93	1	9	3037	47.70	4	10	3174	51.10	5	11	3610
30	24	34.02	4	15	3379	46.70	8	15	3522	49.90	6	15	3383
40	32	33.32	5	18	3325	45.90	9	19	3505	49.00	7	20	3286
50	40	32.69	5	20	3165	45.20	10	23	3419	48.20	9	24	3309
PD (%)	NF	RC	NF 10+	NF Ef	YIELD	RC	NF 10+	NF Ef	YIELD	RC	NF 10+	NF Ef	YIELD
50	40	26.49	5	16	2746	37.40	-	13	2493	38.80	1	12	2603
40	32	25.89	4	12	2667	36.60	-	11	2527	37.80	-	9	2521
30	24	25.37	2	7	2514	35.60	-	9	2564	36.70	-	6	2549
20	16	24.76	-	3	2224	34.50	-	4	2460	35.20	-	3	2414
10	8	23.87	-	-	1718	33.00	-	1	2155	32.80	-	2	2473
5	4	23.18	-	-	1641	32.00	-	-	1832	31.10	-	-	2309

at sowing plus 30 kg N ha⁻¹ in sidedress and different plants were outstanding in each evaluation period (Table 1).

The mean reading values, obtained with the chlorophyll meter for the S₁ families in cultivation without N sidedress were 31.04, 41.91 and 42.95 for the stages of four leaves, eight leaves and female flowering, respectively. The mean grain yield was 2956 kg ha⁻¹. With N sidedress, these values were 31.33, 49.06 and 53.25 at the same development stages, with a mean grain yield of 4434 kg ha⁻¹.

The values of readings with the chlorophyll meter and the grain yield of the S₁ families tended to increase with the rise of the selection pressure (from 50 to 5%) at the phenological stages of eight leaves and the female flowering and tended to sink with the rise of the negative selection pressure (from 5 to 50%). We observed that values below 30% of negative selection pressure without nitrogen sidedress at the 4-leaf-stage did not discard any S₁ family from among the ten most productive, and for the 8-leaf-stage and female flowering this value reached 50% of the negative selection pressure. The number of effective families that would be discarded generally decreased from the 4-leaf-stage to female flowering (Table 3).

In the evaluation of the S₁ families in an experiment with nitrogen sidedress, the same tendency of higher reading values of the chlorophyll meter were observed with the rise of the selection pressure (from 50 to 5%) and the decrease of the reading values with the increase of the negative selection pressure (from 5% to 50%). This direct relation was however not observed between grain yield and chlorophyll meter reading values. The number of families among the ten most productive and classified as effective, that would be discarded generally decreased from the 4-leaf-stage to female flowering (Table 4).

The reading means of the chlorophyll meter of the topcross hybrids were 38.27, 49.59 and 50.65 for the four and eight-leaf stages and female flowering, respectively.

The chlorophyll meter reading values tended to increase with the increasing selection pressure (from 50 to

5%) and tended to sink with the increase of the negative selection pressure (from 5 to 50%) for the three evaluation stages (Table 5). The increasing relation between the chlorophyll meter reading and grain yield was however only evident for the stages of eight leaves and female flowering. Considering the selection of the ten most productive topcross hybrids by the chlorophyll meter reading values, it was noted that these were dispersed under different pressures of selection and discarding, so selection for this criterion was not effective. On the other hand, analyzing the negative selection pressure at the 4-leaf-stages and female flowering, it was observed that values below 20% did not discard any topcross hybrid that belongs to the top productive group. When considering the number of effective hybrids that would be discarded one notes that the highest number was 12 for the pressure of 50% at the 8-leaf-stage. Considering the reading value of the chlorophyll meter, the ideal moment of discarding topcross hybrids would therefore be the 8-leaf-stage and female flowering. In view thereof, since the least productive genotypes presented lower readings in cycles of intrapopulation selection, the gain with selection for N use efficiency can be increased with the elimination of plants with low chlorophyll meter reading values at the 8-leaf-stage.

The conclusion was drawn that the portable chlorophyll meter was effective to detect nitrogen deficiency; these readings can be used as a quick test to eliminate the least productive genotypes before flowering; the readings were not adequate to classify the most effective genotypes of N use under low nitrogen availability.

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Table 4. Different pressures of selection (PS) and of discarding (PD) of the 81 S₁ families with nitrogen sidedress, number of families taken into consideration (NF) according to the pressure, mean reading of the chlorophyll meter (RC) of these families, number of families among the ten most productive (NF 10+), number of families classified as effective (NF Ef) and grain yield in each pressure of selection or discarding (YIELD), at three phenological stages

S₁ families without nitrogen sidedress													
Four Leaves					Eight Leaves					Female flowering			
PS	NF	RC	NF	NF	YIELD	RC	NF	NF	YIELD	RC	NF	NF	YIELD
(%)			10+	Ef			10+	Ef			10+	Ef	
54	38.40	-	3	4754	54.99	1	2	5333	61.35	-	4	4874	
10	8	37.60	-	6	4760	54.52	1	5	5057	60.54	3	7	5478
20	16	36.17	-	9	4515	53.76	3	11	5117	59.36	6	11	5611
30	24	35.10	2	11	4604	52.98	4	14	5008	58.41	6	13	5031
40	32	34.40	5	16	4883	52.37	7	20	5162	57.69	8	18	4961
50	40	33.90	7	19	4844	51.84	8	23	4947	57.01	9	23	4941
PD	NF	RC	NF	NF	YIELD	RC	NF	NF	YIELD	RC	NF	NF	YIELD
(%)			10+	Ef			10+	Ef			10+	Ef	
50	40	28.76	3	13	4024	46.29	2	9	3921	49.49	1	9	3927
40	32	28.18	3	11	4083	45.71	1	6	3833	48.66	1	7	3843
30	24	27.47	3	9	4213	44.98	1	5	4013	47.99	1	4	3733
20	16	26.54	2	7	4630	44.10	1	3	3968	47.23	1	2	3665
10	8	25.37	1	3	4074	42.54	1	3	4173	45.94	1	1	3511
5	4	24.87	1	2	4168	41.20	1	1	4175	44.64	-	-	2751

Table 5. Different pressures of selection (PS) and of discarding (PD) of the 76 topcross hybrids without nitrogen sidedress, number of hybrids considered (NH) according to the pressure, mean reading of the chlorophyll meter (RC) of these hybrids, number of hybrids among the ten most productive (NH 10+), number of hybrids classified as effective (NH Ef) and grain yield under each pressure of selection or discarding (YIELD), at three phenological stages

Top-crosses without nitrogen sidedress													
Four Leaves					Eight Leaves					Female flowering			
PS (%)	NH	RC	NH 10+	NH Ef	YIELD	RC	NH 10+	NH Ef	YIELD	RC	NH 10+	NH Ef	YIELD
54	44.36	-	4	6256	55.37	-	4	6305	61.97	1	4	7117	
10	8	43.38	2	7	6672	54.88	-	7	5908	60.11	2	8	6843
20	15	42.49	3	11	6211	54.30	2	13	6227	58.48	3	14	6595
30	23	41.61	4	17	6195	53.51	3	20	6374	57.18	4	19	6261
40	30	41.11	6	20	6086	52.83	4	24	6288	56.32	5	23	6137
50	38	40.59	6	25	5962	52.10	4	30	6146	55.40	5	29	6103
PD (%)	NH	RC	NH 10+	NH Ef	YIELD	RC	NH 10+	NH Ef	YIELD	RC	NH 10+	NH Ef	YIELD
50	38	35.45	4	17	4881	45.71	6	12	4696	44.51	5	13	4740
40	30	34.83	2	13	4764	44.87	4	6	4417	43.10	2	6	4373
30	23	34.20	1	8	4499	43.83	2	3	4045	41.30	1	4	4022
20	15	33.27	-	6	4555	42.30	1	1	3562	38.02	-	-	3350
10	8	31.87	-	5	4940	40.19	-	-	2970	35.37	-	-	3310
5	4	29.29	-	2	4371	38.44	-	-	3306	32.48	-	-	3450

Seleção de genótipos de milho eficientes no uso de nitrogênio através do medidor de clorofila

RESUMO - O objetivo do presente trabalho foi verificar se é possível discriminar genótipos de milho quanto à eficiência na utilização de nitrogênio, por meio da leitura obtida com o medidor portátil de clorofila. 76 híbridos top crosses e 5 testemunhas, avaliadas no experimento preliminar, sem adubação nitrogenada de cobertura e 81 famílias S₁ diferenciando-as apenas quanto à adubação nitrogenada de cobertura (sem e com). As leituras do medidor portátil de clorofila foram obtidas nos estádios fenológicos quatro e oito folhas completamente expandidas. “Simularam-se” diferentes pressões de seleção e de descarte pelas leituras do medidor portátil de clorofila em cada estágio fenológico. Verificou-se que o medidor portátil de clorofila pode ser utilizado como teste rápido para eliminar, antes do florescimento, os genótipos menos produtivos, especialmente em ambientes sem adubação nitrogenada de cobertura, porém, não foi adequado para classificar genótipos mais eficientes quanto ao uso do nitrogênio.

Palavras-chave: *Zea mays* L., estresse mineral, nitrogênio, medidor portátil de clorofila.

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