NITRATE REDUCTASE AND GLUTAMINE SYNTHETASE ACTIVITIES IN S_1 ENDOGAMIC FAMILIES OF THE MAIZE POPULATIONS SOL DA MANHÃ NF AND CATETÃO

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ABSTRACT - The possibility of improving nitrogen use efficiency in maize was investigated using S_1 endogamic families of the populations Sol da Manhã NF and Catetão. A simple 10 X 10 lattice design was adopted and the trials carried out at the experimental field of MITLA AGRÍCOLA LTDA, in Uberlândia, State of Minas Gerais, during the 1994/95 planting season. Based on grain production figures, the three best and three worst performing S_1 endogamic families were selected for this study. These were pooled to form four sub-populations denominated NFB, NFR (the best and worst families, respectively, of the Sol da Manhã NF variety), CATB and CATR (the best and worst families, respectively, of the Catetão variety). Each of these sub-populations was evaluated under greenhouse conditions. The experimental design was factorial with treatments arranged in randomized blocks. Sample replicates consisted of pots with four plants. Feeding with modified Hoagland's nutrient solution began on the seventh day after sowing. The study involved four nitrogen regimes, where varying proportions of NO_3^- and NH_4^+ were formulated, such that the nutrient solution contained the following mixtures: 75% NO_3^- : 25% NH_4^+; 25% NO_3^- : 75% NH_4^+; 50% NO_3^- : 50% NH_4^+ (all high N mixtures) and 5% NO_3^- : 5% NH_4^+ (low N mixture). Twenty-five days after planting, the activities of the enzymes nitrate reductase and glutamine synthetase (transferase and synthetase assays) were determined for the leaves using the third topmost expanded leaf of the four plants in each pot. The data show that glutamine synthetase (transferase assay) and nitrate reductase activities were efficient in discriminating the S_1 endogamic families and could therefore be useful biochemical parameters in breeding programs seeking nitrogen use efficiency.

ADDITIONAL INDEX TERMS: Corn, nitrogen efficiency, nitrogen forms, nitrogen uptake

ATIVIDADE DAS ENZIMAS NITRATO REDUTASE E GLUTAMINA SINTETASE EM FAMÍLIAS ENDOGÂMICAS S_1 DAS POPULAÇÕES DE MILHO SOL DA MANHÃ NF E CATETÃO

RESUMO - Com o objetivo de verificar as possibilidades do melhoramento genético em milho para o uso eficiente do nitrogênio, famílias endogâmicas S_1 das populações Sol da Manhã NF e Catetão foram avaliadas em látices simples 10 X 10, no campo experimental da MITLA AGRÍCOLA LTDA, em

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Uberlândia, MG, no ano agrícola de 1994/95. Com base nos dos dados de produção de grãos, escolheram-se as três melhores e as três piores famílias endogâmicas S_1. Essas famílias foram reunidas, formando quatro sub populações denominadas NFB (melhores famílias da variedade Sol da Manhã NF), NFR (piores famílias da variedade Sol da Manhã NF), CATB (melhores famílias da variedade Catetão) e CATR (piores famílias da variedade Catetão). Essas sub-populações foram avaliadas sob condições de casa-de-vegetação. O delineamento experimental utilizado foi o fatorial com disposição dos tratamentos em blocos ao acaso. A parcela experimental constituiu-se de vasos com quatro plantas. Colocou-se solução nutritiva modificada de Hoagland nos vasos a partir do sétimo dia. Neste estudo, trabalhou-se com quatro regimes de nitrogênio, variando as formas de NO_3^- e NH_4^+ e tivemos então, quatro soluções de Hoagland modificada com as seguintes proporções: 75% NO_3^- : 25% NH_4^+; 25% NO_3^- : 75% NH_4^+; 50% NO_3^- : 50% NH_4^+ e 5% NO_3^- : 5% NH_4^+. Aos vinte e cinco dias após o plantio, foram determinadas as atividades das enzimas nitrato redutase e glutamina sintetase (método da transferase e sintetase) nas folhas, utilizando-se a terceira folha desenvolvida de cima para baixo das quatro plantas do vaso. As atividades da glutamina sintetase (reação da transferase) e nitrato redutase foram eficientes para discriminar as famílias endogâmicas S_1 e podem ser utilizadas como parâmetros bioquímicos em programas de seleção genética à visando eficiência no uso de nitrogênio.

TERMOS ADICIONAIS PARA INDEXAÇÃO: Eficiência em nitrogênio, formas de nitrogênio, absorção de nitrogênio.

INTRODUCTION

The selection of genotypes with higher capacity to take up and use nitrogen efficiently is a useful strategy for increasing the nitrogen (N) utilization efficiency of maize, increasing yield and reducing environmental problems (Machado, 1997).

The optimization of grain yield depends on several factors, such as the efficiency of carbon and nitrogen mobilization to and accumulation in the grains. A better understanding of the genetic and biochemical mechanisms underlying such processes is necessary (Sodek, 1989).

Nevertheless, the task of obtaining genotypes efficient in N use is highly complex, since the metabolism of N is affected by a diversity of environmental factors (Machado and Magalhães, 1995). N uptake and assimilation can be visualized as metabolic events mediated by carriers and enzymes that are interrelated (Cacco *et al.*, 1983), and each of which is under genetic control. Different genotypes show a wide range of nitrogen and total organic matter accumulation when they encounter similar levels of N. There is interest, therefore, in studying physiological, biochemical, morphological and agronomic processes in order to better understand these genotypic differences (Murulli and Paulsen, 1981; Jelenic and Sukalovic, 1983; Sherrard *et al.*, 1984; Hageman and Lambert, 1988).

A wide range of responses to the use of nitrogen fertilizer has been observed among maize genotypes (Balko and Russel, 1980a, 1980b; Tsai *et al.*, 1984; Smiciklas and Below, 1992; Bänziger *et al.*, 1995; Lafitte and Edmeades, 1995; Rizzi *et al.*, 1995) and N-carriers (NO_3^- and NH_4^+), (Magalhães and Huber, 1991; Magalhães *et al.*, 1993; Magalhães and Machado, 1995; Beuchamp *et al.*, 1976; Chevalier and Schrader, 1977; Moll and Kamprath, 1977; Pollmer *et al.*, 1979; Reed *et al.*, 1980; Jackson *et al.*, 1986; Mollaretti *et al.*, 1987).

Physiological and biochemical parameters can be of value in the selection of efficient genotypes for higher nitrate content (Mollaretti *et al.*, 1987), higher nitrate reductase activity (Cregan and Van Berkum, 1984; Feil *et al.*, 1993), greater N mobilization from leaves and stems to the grain (Beuchamp *et al.*, 1976; Eghball and Maranville, 1993) and higher glutamine synthetase activity (Machado *et al.*, 1992; Magalhães *et al.*, 1993; Machado and Magalhães, 1995; Machado, 1997).

In the present study, endogamic S_1 families were evaluated under field and greenhouse conditions with different levels and carriers of N, in order to identify biochemical parameters that might be useful in breeding programs seeking genotypes more efficient in the use of N.

MATERIAL AND METHODS

Three hundred endogamic S_1 families were used in this study, consisting of 200 of the open pollinated variety Sol da Manhã NF and 100 of the Catetão variety. The Sol da Manhã NF variety is a population of hard and semi-hard grains with an orange coloured endosperm segregating for white and whose germplasm is predominantly Cateto, Eto and Duros do Caribe (Machado, 1997). The Catetão variety is a population of hard grains with orange coloured endosperm and a predominance of Cateto germplasm (Machado, 1997).

The endogamic S_1 families of the Sol da Manhã NF varieties were evaluated using a 10 x 10 simple Lattice design at the Embrapa Agrobiologia and Mitla Agrícola Ltda experimental fields in Seropédica, Rio de Janeiro and Uberlândia, Minas Gerais State (Brazil), respectively, during the 1994/95 growing season. The endogamic S_1 families of the Catetão were evaluated using a 10 x 10 simple lattice design at the Mitla Agrícola Ltda experimental field. Fertilizer application was carried out according to the chemical analysis of the soil, with 400 kg/ha of the formula 4-26-20 + 2 of zinc, using as sources of NPK, ammonium sulphate, superphosphate and potassium chloride. Additional N in the form of urea (40 kg/ha) was applied 45 days after planting. The experimental units consisted of a three meter row with one meter apart. Twenty-five seeds were planted in each row and 30 days later the rows were thinned, leaving 17 plants per row. The following characteristics were recorded: 1) number of plants; 2) grain weight; 3) grain moisture.

Based on the field data for grain yield, the three best and three worst S_1 endogamic families were selected for the two varieties Sol da Manhã NF and Catetão. The grains of these families were pooled to form 4 sub-populations, denominated NFB, NFR (the best and worst families, respectively, of the Sol da Manhã NF variety), CATB and CATR (the best and worst families, respectively, of the Catetão variety).

The sub-populations were then evaluated under greenhouse conditions. A factorial design was used, with treatments arranged in randomized blocks. Replicates consisted of 4 plants in a 3-litre pot of vermiculite. The pots were irrigated twice a week with 250 ml of modified Hoagland solution from the seventh day onwards. The nutrient solution was formulated from the stocks I and II (Tables 1 and 2) to produce 4 combinations of NO_3^- and NH_4^+: a) High N: 75% NO_3^- : 25% NH_4^+; 25% NO_3^- : 75% NH_4^+; 50% NO_3^- : 50% NH_4^+; b) Low N: 50% NO_3^- : 50% NH_4^+. High and Low levels were 100 mg N/week and 10 mg N/week respectively.

The plants (twenty one days old) were analysed for nitrate reductase and glutamine synthetase activities (transferase and synthetase assays) in the leaf extracts, using the third (from the top) fully expanded leaf from each of the 4 plants in the pot. Leaf fresh weight was also determined.

In vivo determination of nitrate reductase activity

The *in vivo* nitrate reductase activity (NR) was determined according to Reed *et al.* (1980). The method involves infiltration of the tissue with a nitrate solution and measurement of the nitrite produced after it diffuses out into the medium.

In the greenhouse experiment, leaf segments of approx. 10 x 10 mm were cut from the third topmost expanded leaf in quantities sufficient to total 0.4 g. The material was always collected between 10:00 and 12:00 h.

TABLE 1 - Composition of two stock solutions, denominated solution I (100% NO_3) and solution II (100% NH_4).

Macronutrients	Concentration (mg.L^{-1})	
	Solution I	Solution II
$Ca(NO_3) . 4 H_2O$	1180	-
KNO_3	505	-
$K H_2 PO_4$	136	136
$Mg SO_4$	492	492
$(NH_4)_2 SO_4$	-	990
$CaCl_2. 2 H_2O$	-	735
$K_2 SO_4$	-	-
$CaSO_4. 2 H_2O$	-	-
KCl	-	372.5
$Fe SO_4, 7 H_2O$	24.1	24.1
EDTA	25.1	25.1
Micronutrients		
H_3BO_3	2.04	2.04
$MnCl_2, 4 H_2O$	2.34	2.34
$ZnSO_4, 7 H_2O$	0.88	0.88
$CuSO_4, 5 H_2O$	0.20	0.20
$Na_2 MO O_4, 2 H_2O$	0.26	0.26

Leaf segments were weighed and placed in glass vials measuring 30 mm in diameter and 60 mm in height, containing infiltration medium in the proportion of 1:10 (w/v). This medium consisted of 0.1 M phosphate buffer pH 7.5 with 1% n-propanol and 0.1 M potassium nitrate. A plastic cylinder with a nylon net at one end was introduced into the assay vessels to keep the leaf tissue submersed. The vessels were placed under vacuum (approx. 5 mm Hg) for 2 min, then the vacuum was rapidly released and the procedure repeated. The vessels were transferred to a water-bath and incubated in the dark under constant agitation at 32 ^oC. At 10 and 40 min intervals aliquots (0.2 ml) of the reaction mixture were removed and mixed with 1.8 ml of distilled water; 1.0 ml of 1% (w/v) of sulphanilamide in 1.5 N HCl and 1.0 ml of 0.02% (w/v) of N-(1-naphthyl)-ethylenediamine-dichloride. The amount of nitrite produced was determined colorimetrically reading the absorbance at 540 nm. Activity was expressed in micromoles of nitrite formed per hour, per gram fresh weight (μmoles NO_2^- h^{-1} g. FW^{-1}).

TABLE 2 - Concentration of nutrients in solutions I (100% NO_3^-) and II (100% NH_4^+).

Nutrients	Nutrient concentration (mg.L^{-1})	
	Solution I	Solution II
$N-NO_3$	210	-
$N-NH_4$	-	210
Ca	200	200
K	234	234
P	31	31
Mg	48.6	48.6
S	64	304
Cl	-	527.5
Fe	4.85	4.85
Mn	0.67	0.67
B	0.36	0.36
Zn	0.20	0.20
Cu	0.05	0.05
Mo	0.11	0.11

Determination of glutamine synthetase activity

- *Extraction*

Leaf material was harvested in the same way as described for nitrate reductase. A total of 0.5 g of leaf segments was macerated in a pre-cooled mortar and pestle with 10 volumes of extraction buffer at 0 to 4 ^oC, in an ice bath. The extract was maintained at low temperature (around 0 ^oC) throughout. The extraction buffer was made up of 0.1 M imidazole-HCl, pH 7.8, containing 1 mM DTT (dithiothreitol). Half a gram of leaves were washed, blotted dry and macerated in 0.3 ml of the extraction buffer. After maceration, the extract was filtered through cheesecloth and centrifuged at 15,000 x g for 15 min at 2 ^{o}C . An aliquot (1 ml) of the supernatant was dessalted on a Sephadex G-25 column (1.5 x 12 cm) previously equilibrated with extraction buffer. The protein fraction was collected in 2 ml and used in the synthetase assay. For the transferase assay an aliquot was taken from the crude extract before dessalting.

- *Glutamine synthetase assay*

Glutamine synthetase activity was assayed by the method of Rhodes *et al.* (1975). Activity may be measured by the synthetase reaction (GSS) through the formation of γ-glutamyl hydroxamate from glutamate and hydroxylamine (which substitutes for ammonia), according to the reaction:

$$\text{glutamate} + \text{ammonia} + \text{ATP} \xrightarrow{Mg^{++}} \text{glutamine} + \text{ADP}$$

The transferase reaction (GST), which is the inverse of the synthetase reaction, is the other glutamine synthetase activity that can be evaluated. In this reaction, the enzyme is incubated with glutamine and hydroxylamine, and the product γ-glutamyl hydroxamate determined:

$$\text{glutamine} + \text{hydroxylamine} \xrightarrow[\text{NaAsO}_4,\ Mn^{++}]{\text{ADP}} \gamma\text{-glutamyl hydroxamate} + \text{ammonia}$$

The assay mixture used for the synthetase reaction was 500 mM glutamate, 60 mM hydroxylamine, 200 mM $MgSO_4$, 80 mM ATP and 50 mM imidazole buffer, pH 7.4. For the transferase reaction the assay consisted of 65 mM glutamine, 17 mM hydroxylamine, 33 mM sodium arsenate, 4 mM $MnCl_2$, 1.7 mM ADP and 100 mM imidazole buffer, pH 6.8.

The reaction was started by the addition of enzyme (0.2 ml) to a final volume of 1 ml. The assays were incubated in a water-bath at 32 ^{o}C for 30 minutes when the reaction was stopped by the addition of 1 ml of Ferguson and Sims (1971) reagent, consisting of 0.67 N HCl, 0.20 M TCA and 0.37 M $FeCl_3$. The resulting precipitate was removed by centrifugation at 1500 x g for 3 min. and the supernatant read at 535 nm. The data are expressed as μmoles of product formed per hour per gram of tissue, based on the standard value of 0.34 for 1 μmol of glutamyl hydroxamate under the assay conditions described (Mori, 1981).

RESULTS AND DISCUSSION

Field Data

Grain yield for the 200 S_1 families of the Sol da Manhã (NF 1 and NF 2) evaluated at the two locations and for the 100 families of the Catetão varieties evaluated at one location only (Uberlândia), denominated CAT, is shown in Table 3 and 4. The F test revealed significance at the 1% level between non-adjusted treatments for NF 1 and CAT at Uberlândia, and between NF 1 and NF 2 at Seropédica. For the adjusted treatments, the same significant differences were found, except for NF 2 at Seropédica. The lattice design was more efficient than randomized blocks in the NF 1 and CAT trials at Uberlândia, equally efficient in the NF 2 trials at Uberlândia and NF 1 at Seropédica, and less efficient in the NF 2 trials carried out at Seropédica.

In the NF 2 trial the data were analysed using a randomized blocks design and consequently only the non-adjusted means of the lattice trial were considered. The coefficients of variation had percentage values of 26.58; 37.61 and 40.96 for the NF 1, NF 2 and CAT trials, carried out at Uberlândia, and of 26.94 and 28.90 for the NF 1 and NF 2 trials, carried out at Seropédica.

Table 4 contains the value ranges for grain yield in tons/ha, for the NF 1, NF 2 and CAT trials carried out at Uberlândia and for the NF 1 and NF 2 trials, carried out at Seropédica. The means for the 20 best and 20 worst families are also presented for all trials.

TABLE 3 - Analysis of variance for grain yield among 200 endogamic S_1 families of the Sol da Manhã NF variety arranged in two 10x10 lattices, denominated NF1 and NF2, at two locations, Uberlândia, MG and Seropédica, RJ, and among 100 endogamic S_1 families of the Catetão (CAT) variety, evaluated at a single location (Uberlândia) in a 10x10 lattice.

		Mean Squares				
		Uberlândia			Seropédica	
SOURCE	DF	NF1	NF2	CAT	NF1	NF2[1]
Replicates	1	6.808	3.726	0.344	8.242	7.566
Treatments						
• non adjusted	99	2.843**	2.108	1.873**	1.563**	1.153**
• adjusted	99	2.513**	1.770	1.463**	1.469**	-
Replicates in blocks	18	2.754	2.996	2.583	0.896	0.467
Error						
• effective	81	0.921	1.716	0.695	0.516	-
• random blocks	99	1.169	1.837	0.969	0.552	0.570
• within blocks	81	0.817	1.580	0.611	0.476	0.593
Lattice efficiency: (compared to randomized blocks)		126.9	107.1	139.3	106.9	-
C.V. (%):		26,58	37,61	40,96	26,94	28,20

[1] The lattice of the NF2 trial, carried out at Seropédica was less efficient than randomized blocks and therefore only the non-adjusted means were considered. ** Significant at 1 % by the F test.

TABLE 4 - Value ranges for grain yield in tons/ha for the 200 endogamic S_1 families of the Sol da Manhã NF variety, arranged in two 10x10 lattices, denominated NF1 and NF2, at two locations, Uberlândia, MG and Itaguaí, RJ, and 100 endogamic S_1 families of the Catetão variety (CAT) evaluated at Uberlândia, as means of the 20 best and 20 worst families.

	Uberlândia			Seropédica	
	NF1	NF2	CAT	NF1	NF2
Range	1,09 - 6,33	1,42 - 5,96	0,55 - 5,80	1,09 - 4,76	0,90 - 5,60
Mean for the 20 best families	5,13	4,94	3,31	3,91	3,65
Mean for the 20 worst families	1,97	2,34	1,03	1,55	1,59
Overall mean	3,61	3,48	2,03	2,66	2,67
LSD (at 5% by Duncan's test)	1,90	2,60	1,65	1,42	1,49

The ranges observed for the S_1 families evaluated in the NF 1, NF 2 and CAT trials at Uberlândia were from 1.09 to 6.33; 1.42 to 5.96 and 0.55 to 5.80 tons/ha, respectively. For the Seropédica trials, ranges from 1.09 to 4.76 and 0.90 to 5.60 tons/ha were observed for NF 1 and NF 2 , respectively.

The wide range found for the families used in this investigation indicates that promising possibilities exist for selection with regard to N-use-efficiency in future breeding programmes.

Greenhouse Experiment

An analysis of variance of the data is shown in Table 5, with respect to glutamine synthetase activity (transferase assay - GST), glutamine synthetase activity (synthetase assay - GSS), nitrate reductase activity (NR) and fresh weight (FW), where significance at the 1% level was obtained in the F test for the parameters GSS, NR and FW, for replicates, S_1 families (A) and levels of N (B). GST had significance at the 5% level for S_1 families and at 1% for levels of N. Interaction was significant at the 1% level (A X B), with GSS. The coefficients of variation were: 19.90; 22.06; 34.42 and 14.53%, for GST, GSS, NR and FW, respectively.

Table 6 contains data for glutamine synthetase activity (transferase assay -GST) in the sub-populations NFB, NFR, CATB and CATR, subjected to the four N regimes. For all N regimes it was found that GST was slightly higher in the sub-population NFR when compared to NFB (not significant). The CATB population had higher GST activities than CATR, on all N-combinations. Overall, the values found for GST activity in the sub-populations were 187, 205, 219 and 184 µmol h^{-1} g^{-1} for NFB, NFR, CATB and CATR, respectively. With regard to the means for each N regime, a significantly higher value (235 µmol h^{-1} g^{-1}) was found for GST on the N4 regime (low N) but no other significant difference was found for the remaining regimes.

Table 7 contains the data for glutamine synthetase activity measured by the synthetase reaction (GSS), in the four sub-populations NFB, NFR, CATB and CATR for the four N regimes. On the N1 regime, the sub-population CATR had significantly higher activity compared to NFB, NFR and CATB. Activities were 153, 213, 200 and 285 µmol h^{-1} g^{-1} for NFB, NFR, CATB and CATR, respectively. In the N2 regime, the variety NFB had a much higher GSS activity compared to NFR (222 *vs.* 122 µmol h^{-1} g^{-1}). The sub-populations CATB and CATR in the N2 regime behaved similarly to the N1 regime, with values of 191 and 213 µmol/h/g. In the N3 and N4 regimes, the GSS activity was superior in the sub-population NFR compared to NFB, and similar between the sub-populations CATB and CATR.

Table 8 contains data for nitrate reductase (NR) in the sub-populations NFB, NFR, CATB and CATR, subjected to the four N regimes. The overall means for the sub-populations were 0.96; 1.00; 0.69 and 0.57 µmol h^{-1} g^{-1} for NFB, NFR, CATB and CATR, respectively. Activity of NR in the NFB and NFR sub-populations was significantly greater than in CATB and CATR. Overall means for N regimes produced a significantly higher value for N1 compared to the others. The N4 regime had the lowest NR activity.

Table 9 contains data for fresh weight in grams/5 plants. The overall means were 62.24; 69.72; 62.91 and 52.97 g/5 plants, for NFB, NFR, CATB and CATR, respectively. The fresh weight of the NFR sub-population was significantly higher when compared with the remaining sub-populations, and the value for CATR was significantly lower. No significant differences were found for the overall means for N regime, except for the N4 regime whose value was significantly lower.

Data concerning glutamine synthetase activities (synthetase assay) among the four sub-populations under study is shown in table 7. Lower activities were found depending on the level of N, in contrast to the transferase reaction data. It was possible to differentiate the genotypes with regard

TABLE 5 - Analysis of variance for the enzyme activities of glutamine synthetase via the transferase (GST) and synthetase reaction (GSS), of nitrate reductase (NR) and for fresh weight (FW), in a greenhouse trial with four sub-populations and four N regimes at Campinas, in 1996.

		Mean Squares			
FV	DF	GST	GSS	NR	FW
Replicates	3	640	15281^{**}	1.40^{**}	1635^{**}
Sub-populations (A)	3	4287^{*}	10205^{**}	0.70^{**}	756^{**}
N regimes (B)	3	9518^{**}	63036^{**}	1.69^{**}	11537^{**}
A X B	9	2649	5276^{**}	0.103	32
Error	45	1566	1565	0.077	81
C.V. (%)		19.90	22.06	34.42	14.53

* Significant at 5% by the F test; ** Significant at 1% by the F test

TABLE 6 - Leaf Activity of Glutamine Synthetase in µmol.g^{-1}.h^{-1} (GST - transferase assay) in four contrasting sub-populations for yield, denominated NFB, NFR, CATB and CATR, where NFB and NFR are superior and inferior S_1 families, respectively, of the Sol da Manhã NF variety, and CATB and CATR are the superior and inferior S_1 families of the Catetão variety, subjected to the 4 specified N regimes. Trial: Campinas, 1996.

	Nitrogen Regimes				
	N_1	N_2	N_3	N_4	
Sub-populations	75% NO_3 :25% NH_4	25% NO_3: 75% NH_4	50% NO_3 : 50% NH_4	5% NO_3 : 5% NH_4	Mean
NFB	186	193	172	196	187 B
NFR	208	202	201	208	205 AB
CATB	193	200	210	272	219 A
CATR	155	154	162	265	184 B
Mean	185 b[1]	187 b	186 b	235 a	

[1]Means given different letters differ from each other by Duncan's test at 5%.

TABLE 7 - Leaf activity of Glutamine Synthetase in µmol.$g^{-1}.h^{-1}$ (GSS - synthetase assay) in four contrasting sub-populations for yield, denominated NFB, NFR, CATB and CATR, where NFB and NFR are superior and inferior S_1 families, respectively, of the Sol da Manhã NF variety, and CATB and CATR are the superior and inferior S_1 families of the Catetão variety, subjected to the 4 specified N regimes. Trial: Campinas, 1996.

	Nitrogen Regimes				
	N_1	N_2	N_3	N_4	
Sub-populations	75% NO_3 :25% NH_4	25% NO_3: 75% NH_4	50% NO_3 : 50% NH_4	5% NO_3 : 5% NH_4	Mean
NFB	153 Bb[1]	222 Aa	192 Bab	62 Ac	157
NFR	213 Ba	122 Bb	207 ABa	102 Ab	161
CATB	200 Bab	191 Ab	254 ABa	100 Ac	186
CATR	285 Aa	213 Ab	258 Aab	89 Ac	211
Mean	213	187	228	88	

[1]Means given different letters differ from each other by Duncan's test at 5%. Upper case letters distinguish between the means of sub-populations within each nitrogen regime while lower case letters distinguish the effect of nitrogen regimes within each sub-population.

TABLE 8 - Leaf Activity of Nitrate Reductase (NR) in µmol.$g^{-1}.h^{-1}$ for four contrasting sub-populations for yield, denominated NFB, NFR, CATB and CATR, where NFB and NFR are superior and inferior S_1 families, respectively, of the Sol da Manhã NF variety, and CATB and CATR are the superior and inferior S_1 families of the Catetão variety, subjected to the 4 specified N regimes. Trial: Campinas, 1996.

	Nitrogen Regimes				
	N_1	N_2	N_3	N_4	
Sub-populations	75% NO_3 :25% NH_4	25% NO_3: 75% NH_4	50% NO_3 : 50% NH_4	5% NO_3 : 5% NH_4	Mean
NFB	1.44	0.91	1.19	0.28	0.96 A
NFR	1.22	1.05	1.29	0.45	1.00 A
CATB	0.95	0.79	0.68	0.35	0.69 B
CATR	0.69	0.67	0.65	0.26	0.57 B
Mean	1.08 a[1]	0.86 b	0.96 ab	0.34 c	

[1]Means given different letters differ from each other by Duncan's test at 5%.

to N-form. A comparison of NFB with NFR reveals that under a regime in which nitrate predominates, the sub-population NFR had greater activity than NFB, the same being found for CATB and CATR. The lower GS activities in the superior populations may be due to a greater GS activity in the roots or even to a better energetic economy. In regimes where ammonium predominated, the sub-population NFB presented higher GS activity compared to NFR, and there were no differences between CATB and CATR.

A better understanding of tropical environmental conditions for maize production, especially concerning environmental stress, is essential for the determination of N forms in the soil (Paterniani, 1990). It is known that the majority of soil N is converted into nitrate by soil nitrifying bacteria and that nitrate subsequently may be transported to the shoot where it is reduced to ammonia and utilized in the synthesis of amino acids (Magalhães *et al.*, 1993). Under environmental stress conditions such as acid soils, insufficient or excess water, high or low temperature, and nutrient deficiency, the nitrification process is limited and ammonia becomes the predominant form of N (Magalhães *et al.*, 1993; Magalhães and Machado, 1995).

In plant breeding it is difficult to control the above situations especially to know just how much nitrate or ammonia is present in selection trials. Thus, four different N regimes (at two levels) were adopted which might simulate field situations. Studies on the efficiency in the use of NH_4^+ proposes the determination of free ammonium in the leaf mesophyll as a selection parameter (Alfoldi *et al.*,1992; Magalhães and Machado, 1995), while others propose the use of enzymes of N assimilation as an auxiliary biochemical criterion in breeding programmes (Hageman and Lambert, 1988; Eichelgerger *et al.*, 1989a,b; Feil *et al.*, 1993; Machado *et al.*, 1992; Magalhães *et al.*, 1993, among others).

Several investigations have been carried out elsewhere, and three are outstanding in this regard: first, the classical work carried out at Illinois, using the divergent recurrent selection method, aiming for greater and smaller nitrate reductase activity (Sherrard *et al.*, 1986; Eichelberger *et al.*, 1989a,b); second, the work of Kamprath *et al.* (1982) and Moll *et al.* (1994), using reciprocal recurrent selection techniques and recurrent selection within full sib families, showing that the former selection technique was more efficient for obtaining higher grain weight, dry matter and total N accumulation; third, the work of Lafitte and Edmeades (1994), using the divergent recurrent selection technique with full sib families in order to study the relationship between grain yield and secondary aspects aimed at N use efficiency.

The Sol da Manhã NF variety, from which the NFB and NFR sub-populations were obtained, underwent eight selection cycles in low N level conditions. The high GS activity found in the NFB sub-population may indicate that N-efficient plants are those that have mechanisms for the efficient use of ammonia, through the GS/GOGAT system. This is in agreement with several studies found in the literature (Magalhães and Huber, 1989a,b; 1991; Magalhães and Fernandes, 1993; Machado and Magalhães, 1995; Magalhães and Machado, 1995).

When ammonia predominates, GS (synthetase reaction) would appear to be an important biochemical parameter for use in breeding programmes (Table 7). Similar results were obtained by Magalhães *et al.* (1993), who found that GS activity in the leaves of NH_4^+-treated maize was positively related to plant growth and to diminished levels of free ammonia, indicating a key role for GS in nitrogen assimilation.

With the 50% NO_3^-: 50% NH_4^+ regime, no differences were found between the sub-populations under study for GS activity. With regard to the overall mean values for N regime it may be seen that at high levels of N (100 mg N), the 50% NO_3^-: 50% NH_4^+ regime produced higher values whereas the 25% NO_3^-: 75% NH_4^+ regime produced lower values for GS activity, showing that the regime with equal proportions of nitrate and ammonia leads to the highest GS activities. Several studies report the superiority

TABLE 9 - Fresh weight in grams/5 plants (mean of 4 replicates) in four contrasting sub-populations for yield, denominated NFB, NFR, CATB and CATR, where NFB and NFR are superior and inferior S_1 families, respectively, of the Sol da Manhã NF variety, and CATB and CATR are the superior and inferior S_1 families of the Catetão variety, subjected to the 4 specified N regimes. Trial: Campinas, 1996.

	Nitrogen Regimes				
	N_1	N_2	N_3	N_4	
Sub-populations	75% NO_3 :25% NH_4	25% NO_3: 75% NH_4	50% NO_3 : 50% NH_4	5% NO_3 : 5% NH_4	Mean
NFB	70,05	77,17	79,20	22,55	62,24 B
NFR	78,25	87,20	86,50	26,92	69,72 A
CATB	77,22	76,87	76,32	21,25	62,91 B
CATR	63,00	64,97	67,32	16,60	52,97 C
Mean	72,13 a[1]	76,55 a	77,38 a	21,83 b	

[1]Means given different letters differ from each other by Duncan's test at 5%.

of an equal mixture of nitrate and ammonia for N acquisition and translocation in plants and for increasing the number of grains per cob (Below and Gentry, 1992), prolificacy (Pan *et al.*, 1984; Smiciklas and Below, 1992), productivity and the accumulation of total plant N in maize (Smiciklas and Below, 1992; Gentry and Below, 1993). Such studies suggest that the N mixture leads to increased productivity from events occurring at different stages of plant development (Gentry and Below, 1993), and that the N mixture may benefit different physiological processes like enzyme activity, dry matter partition and phytohormone production (Salsac *et al.*, 1987).

The nitrate reductase activity (NR) shown in Table 8 is evidently under the influence of both N regime and N level. Higher values were found for higher N and for regimes where nitrate predominates (75% NO_3^-: 25% NH_4^+) and lower values for low N levels (5% NO_3^-: 5% NH_4^+). These data are in agreement with those found in the literature. Fernandes and Rossiello (1995), in an extensive revision of the subject, came to the conclusion that NR is affected and activated by its substrate (NO_3^-) and a constant flow of NO_3^- is necessary to maintain the enzyme activity. They also comment that the flow of nitrate is more important than the endogenous level for NR activity. It should be noted that NR activity under the 75% NO_3^-: 25% NH_4^+ regime was not statistically higher than for the 50% NO_3^-: 50% NH_4^+ regime.

Among the sub-populations studied, it may be noted that for all three regimes with the high levels of nitrogen, the NR activities were higher in the NFB and NFR sub-populations. The superiority of these sub-populations over CATB and CATR in this respect may be due to the selection for efficient use of N to which the Sol da

Manhã NF variety was subjected. Among sub-populations, NFB presented the highest NR activity for the regime where nitrate predominates and under the low N regime it had a lower value than NFR. In general, the sub-populations presented lower activities of NR for the regime where NH_4^+ predominates. This is in agreement with the data of Li *et al.* (1995), which show that NH_4^+, glutamine and other amino acids (final products of nitrate assimilation) are potential inhibitors of NR.

Comparing the data in Tables 7 and 8 it may be seen that the NFB sub-population had higher GS activity (synthetase reaction) under the high NH_4^+ regime and higher NR activity under high NO_3^- regime. Such data are important indicators that the mechanisms of efficiency are related to the predominant forms of mineral nitrogen available. Under tropical conditions, an efficient variety should have efficient mechanisms for assimilation of both forms of mineral nitrogen since either may predominate under different conditions (Balko and Russel, 1980a,b; Tsai *et al.*, 1984; Smiciklas and Below, 1990; Magalhães *et al.*, 1993; Rizzi *et al.*, 1995).

Table 9 shows the data for fresh weight for which no difference between high and low N regimes was observed. The NFB sub-population had a lower weight compared with NFR and the contrary was observed between the CATB and CATR sub-populations. There was a tendency for the NFB sub-population to accumulate less fresh weight, probably as a result of its efficiency process, but the data are not conclusive.

With regard to breeding parameters, the data of Tables 7 and 8 suggest that GS and NR activity may be used, but these should be evaluated under both high nitrate and high ammonia regimes. One would consider efficient those sub-populations that produce higher values for GS under the ammonia predominant regime and high NR under the nitrate predominant regime. These data are associated with the yield potential of the S_1 endogamic families that made up the sub-populations, as may be seen in Table 4.

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