NITROGEN ASSIMILATION EFFICIENCY IN MAIZE GENOTYPES UNDER AMMONIA STRESS[1]

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ABSTRACT- Plant growth, free ammonia, soluble sugar, glutamine synthetase (GS), nitrate reductase (NR) activities in leaves, and rhyzosphere pH of four maize genotypes were compared in the presence of NH_4^+ or NO_3^- forms of N in sand: vermiculite culture. In the presence of nitrate, all of the maize genotypes tested exhibited similar values for all the parameters analysed, except NR activity. This enzyme tended to be inverse to plant growth, being higher in leaves of maize Pioneer 3230 that exhibited lower plant growth, indicating not a good parameter for nitrogen assimilation efficiency determination. In the presence of ammonium as a sole source of nitrogen, the maize genotypes can be easily screened in terms of plant growth and nitrogen assimilation responses. GS activity in leaves of maize treated with NH_4^+ was positively related to plant growth and the accumulation of free ammonia decreased as GS activity increased, indicating these two physiological parameters as the key factors for N assimilation efficiency. Soluble sugar content in shoot tissue was significantly reduced in NH_4^+ treated plants due to high requirement of carbon skeletons for NH_4^+ incorporation into amino acid.

Additional index terms: glutamine synthetase, nitrate reductase, free ammonia, soluble sugar and plant breeding.

EFICIÊNCIA DE ASSIMILAÇÃO DE NITROGÊNIO EM GENÓTIPOS DE MILHO SOB ESTRESSE DE AMÔNIA

RESUMO- Crescimento de planta, amônia livre, açúcares solúveis, atividades da glutamina sintetase (GS), nitrate redutase (NR) nas folhas e pH de rizosfera em quatro genótipos de milho foram comparados na presença de NH_4^+ e NO_3^- como forma de N em cultura hidropônica com areia: vermiculita. Na presença de nitrato, todos os genótipos de milho testados exibiram semelhantes valores para todos os parâmetros analisados, exceto a atividade da NR. Esta enzima teve uma tendência inversa ao crescimento da planta, com alta atividade foliar no Pioneer 3230 que exibiu um menor crescimento de planta, indicando não ser este um bom parâmetro de eficiência na assimilação de nitrogênio. Na presença de amônio como única fonte de N, os genótipos de milho podem ser facilmente separados em termos de crescimento da planta e resposta de assimilação de nitrogênio. A atividade da GS em folhas de milho tratado com NH_4^+, foi positivamente correlacionada com o crescimento da planta e a acumulação de amônia livre decresceu à medida que a atividade de GS aumentou, indicando estes dois parâmetros fisiológicos como fatores chave na eficiência de assimilação de N. O teor de açúcares solúveis no tecido da parte aérea foi significativamente reduzido em plantas supridas com NH_4^+ devido ao alto requerimento de esqueleto de carbono para incorporação de NH_4^+ em amino ácido.

Termos adicionais para indexação: glutamina sintetase, nitrato redutase, amônia livre, açúcar solúvel e fitomelhoramento.

INTRODUCTION

Ammonium is an important form of nitrogen fertilizer. Most of this nitrogen is converted to nitrate by nitrifying bacteria in the soil, and nitrate then is transported to the plant shoot, where it is reduced to ammonia and assimilated into amino acids. Under different environment stress conditions, such as acidic soils, aluminum toxicity, dry or waterlooged soils, low temperature, low nitrogen, and other limiting factors for nitrification, ammonium is the major nitrogen source (Middleton & Smith, 1979; Rhodes, 1987). In addition to that, with the increasing use of nitrification inhibitors (*e.g.* nytrapirin), ammonia assimilation in plants may become much more important in agriculture (Prasad et al., 1971; Tsai et al., 1978; Rhodes, 1987).

From ammonia, all organic forms of nitrogen must be elaborated, so that NH_3 assimilation must be regarded as an extremely important step in plant nitrogen metabolism. It has been reported that plants under different environmental stress have exhibited similar pattern of biochemical metabolism responses compared with ammonia stress (Glass, 1989; Rhodes, 1987).

By judicious use of ammonia and nitrification inhibitors, economic advantages might be gained in the growth and development of certain agronomic plants as compared to the more common nitrate nutrition. Actually, plants adapted to acid soils, *i.e.*, many tree species or those adapted to low soil redox potentials such as blueberry, paddy rice, have preference for ammonium (Blevins, 1989; Magalhães & Huber, 1989).

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It has been mentioned that the efficiency of ammonia assimilation in high plants is directly related with stress tolerance (Rhodes, 1987). The differences in terms of ammonia assimilation have been observed not only among plant species (Magalhães & Huber, 1989; Magalhães & Huber, 1991a), but also in different maize genotypes (Magalhães et al., 1990; Magalhães & Huber, 1991b). An understanding of nitrogen interaction with plant growth and metabolism is important in order to increase the efficiency of fertilizer amendments for crop production.

This study addresses several of the important questions of ammonium assimilation, by evaluating the effect of nitrogen forms and ammonium stress on Plant growth. N-assimilating enzymes activities, soluble sugar and free ammonia accumulation in tissue of different maize genotypes. Also, the objective of this study, is to verify the possibility of the utilization of biochemistry parameters in plant breeding programs to obtain nitrogen assimilating efficient maize genotypes.

MATERIAL AND METHODS

An experiment was carried out in controlled greenhouse at 24- 28°C and 14h photoperiod at 1150 μmol m^{-1} s^{-1} (photosynthetic active radiation). The maize genotypes used were BR 201 double hybrid, Pioneer 3230, Nitroflint (NF), and Nitrodent (ND).

Maize plants were grown for three weeks in a 5 L black plastic pot, with 1:1 (v/v) sand: vermiculite, sterilized and treated with N-serve (Nitrapyrin), 20 mg/L in the substrate mixture. Plants were irrigated with a modified Hoagland solution, 1000 mL/pot/day, with either NO_3^-, or NH_4^+ form of nitrogen. The NO_3^- modified nutrient solution contained 2.0 mM K_2SO_4, 2.0 mM $MgSO_4$, 1 mM KH_2PO_4, 5 mM $Ca(NO_3)_2$, 25 μM H_3BO_3, 2.0 μM $MnSO_4$, 4,0 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM Na_2MoO_4, 50 μM KCl, and 50 μM Fe-DTPA. N was supplied as $(NH_4)_2SO_4$ in the NH_4^+ solution and $Ca(NO_3)_2$ was replaced by $CaCl_2$. The pH was adjusted to 5.7 with NaOH. The experimental design was completely randomized, with 5 plants per pot and 4 replications per treatment.

At harvest, the plants were washed with distilled water, blotted dry and weighed. One g shoot sample was placed in 10 mL methanol for determination of free ammonia. One g leave sample was placed in a styrofoam container with ice for NR assay and two g leaf sample were frozen in liquid nitrogen for subsequent ammonia assimilating enzyme assay. Two g sample of fresh roots without washing were placed in 2.5 mL distilled water for subsequent determination of rhizosphere pH, and one g was used to estimate root length. The rest of plant tissue, separated root and shoot, were dried in a forced air oven at 70 °C. Shoot was ground to pass a 40 mesh screen in a Wiley mill for soluble sugar analyses. Free ammonia was determined by phase separating the methanol extract in 5 ml chloroform and 6 ml distilled water. The upper aqueous phase was evaporated to dryness, redissolved in 2 mL H_2O and assayed for ammonia by phenol- hypochlorite reaction as previously described (Magalhães, 1991). Glutamine synthetase activity was assayed as described by Anghinoni et al. (1988). Nitrate reductase activity was assayed according to Lillo (1983). Root length was measured by using the intersect method of Tennant (1975). Rhizosphere pH was estimated according to procedure described by Silva (1984) and soluble sugar was determined by using 10 ml hot 80% ethanol extraction and antrona reagent (method adapted from Dubois et al., 1956).

RESULTS AND DISCUSSION

In the presence of NO_3^-, the genotypes exhibited similar values for all the parameters analyzed, except nitrate reductase activity (Table 1). This enzyme activity tended to be inversely related to plant growth, showing higher activity in maize genotypes that exhibited lower plant growth. Accordingly, NR does not appear to be a good parameter to indicate nitrogen assimilation efficiency. Although NR studies have been widely reported (Magalhães et al., 1974; Kleinhofs et al, 1989), this enzyme activity in higher plants not always is related to plant growth or nitrogen assimilation (Machado et al., 1990, 1992). In addition to that, under environmental stress conditions, such as acidic soils, aluminum toxicity, dry or water-logged soils, low temperature (Middleton & Smith, 1979), as well as the increasing use of nitrification inhibitors (*e.g.* nitrapyrin), ammonium is the major form of nitrogen source (Prasad et al., 1971). In such approach, the enzyme nitrate reductase becomes not important for nitrogen assimilation in plants, since from ammonia all the organic forms of nitrogen must be elaborated and N is not reoxidized in higher plants to provide NO_3^- as the substrate to NR.

On the other hand the glutamine synthetase activity in leaves of maize treated with NH_4^+ (Table 1), was positively related to plant growth, with a concomitant decreasing in free ammonia accumulation as GS activity increased. These observed results together indicate the two physiological parameters (GS activity and free ammonia in green tissue) as the key factors for N-assimilating efficiency as has been previously reported in different plant species (Magalhães & Huber, 1989).

The data in Table 1 also show significant differences in growth responses among the genotypes treated with NH_4^+, but not with NO_3^-, indicating NH_4-nitrogen for better screening maize for N-assimilation efficiency. It is important to show that the data presented to GS activity to the ND, NF and P3230 genotypes in the two forms of nitrogen, were well related with the data presented by Machado et al. (1992) in field conditions. This corroborated the importance of this physiological parameter and the possibility of utilizing it early in plant breeding programs to obtain nitrogen assimilating efficient maize genotypes. Actually, GS activity in maize leaves has shown to be related to plant growth and inversely related to free ammonia accumulation in green tissue. As free ammonia accumulate in green tissue, disrupt various aspects of plant metabolism leading to uncoupled photophosphorilation, inhibition of ATP formation, reduced CO_2 fixation within the chloroplast

TABLE 1- Plant growth, rhizosphere pH, free ammonia, and nitrogen assimilating enzyme activities in green tissues of maize genotypes grown with two forms of nitrogen.

Parameter	N-FORMS/GENOTYPES								
	NH_4^+				NO_3^-				L.S.D.
	BR 201	NF	ND	P3230	BR 201	NF	ND	P3230	(0.05)
Shoot FM (g/pot)	115.39*	103.52	99.46	82.32	167.66	164.68	168.73	140.23	21.73
Shoot DM (g/pot)	8.51	6.84	6.32	5.69	13.67	12.53	12.99	11.32	3.70
Root DM (g/pot)	1.50	1.34	0.95	0.70	2.61	1.88	1.88	1.84	0.58
Rhizosphere pH	4.51	4.64	4.77	4.97	5.59	5.77	5.61	5.74	0.20
GS (nmol/gFW/min)	110.25	104.00	98.25	87.00	90.00	91.00	81.75	67.50	17.38
Free NH_4^+ (nmol/gFW)	1062	1102	992	1546	416	395	370	456	184
NR (nmol NO_2^-/gFW/h)	844	1101	833	869	895	1372	1339	1686	409

GS - Glutamine Synthetate NR - Nitrate Reductase

* Significant at 5% of probability by the Tukey test.

(Ikeda & Yamada, 1981; Puritch Barker, 1967) and thus resulting in low availability of carbon skeletons.

The data presented in Table 2 showed a drastic effect of NH_4^+ in reducing soluble sugar in shoot of all the maize genotypes tested as compared to nitrate. Once the ammonia assimilating enzyme does not incorporate ammonia in amino acids, and free ammonia accumulate in green tissue, decrease in production and availability of carbon skeletons appears to be the most severe consequence of NH_4^+ toxicity. Reports that plants well supplied with carbohydrate are better able to utilize NH_4^+ (Barker & Mills, 1980; Givan, 1979; Reisenauer, 1978), are consistent with these ideas. By providing carbon skeleton exogeneously, Magalhães et al. (1993) monitored ammonia assimilation into amino acids in both roots and shoots of tomato seedlings supplied with $^{15}NH_4$ labeled nitrogen in the presence and absence of alfa-keto-Glutarate (αKG). The pools of amino acids increased 2-fold and the amino acids accumulated in response to αKG, exhibited an isotope abundance 2-fold higher, accompanied by a sharp decrease in free ammonia in tissue which clearly indicate carbon skeleton as a key limiting factor for ammonia assimilation.

TABLE 2- Soluble sugar in shoot of maize genotypes grown with two forms of nitrogen.

	Soluble sugar in shoot (% D.M.)		
Genotype	NO_3^-	NH_4^+	mean
NF	2.28	1.45	1.87
ND	2.12	1.78	1.95
BR 201	2.85	1.58	2.22
P3230	3.04	1.93	2.49
Mean	2.58	1.68	2.13
LSD (0.05)		0.45	

It appears reasonable to conclude that high GS activity in the chloroplasts would be a key point to detoxify ammonia by incorporating it into aminoacids as the sink for ammonia and carbohydrate as well as an excellent biochemistry parameter for a genetic screening program in order to obtain nitrogen assimilating efficient maize genotypes.

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