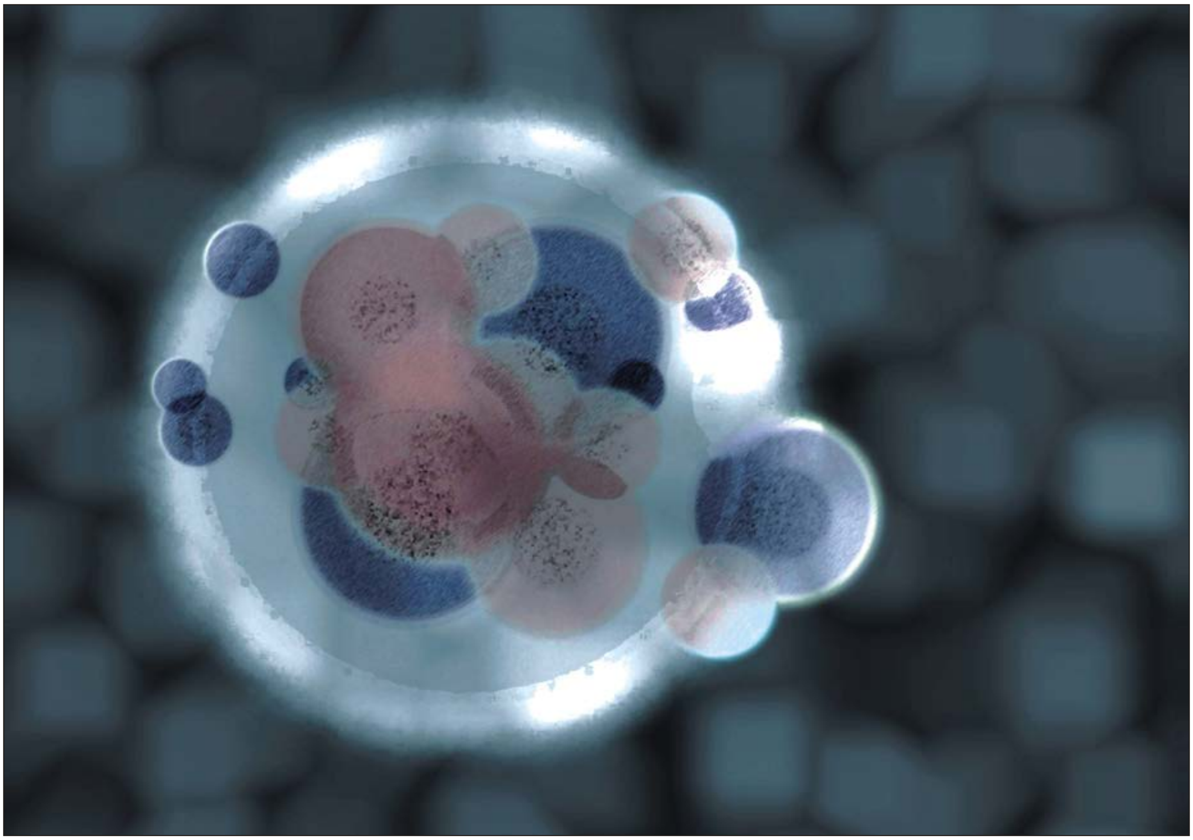

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Physiological and Morphological Adaptations in Two Rice Varieties Cultivated Under Ammonium and Light Deficiency

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Abstract: In this study, two rice varieties were evaluated: Piauí and IAC-47. Piauí is adapted to Humid Tropic environmental conditions, where there is N deficiency and low light since rice is planted. IAC-47 is an improved variety. This research aimed to characterize physiological and morphological adaptations observed during vegetative growth under low ammonium-N nutrition and low light in both varieties. To analyze it, developmental parameters, soluble sugars content, amino-N, NH_4^+, total-N and pigments were compared between varieties. The assay was carried out in controlled growth chamber conditions, at 24°C and 12/12 h light/dark periods (200 $\mu E\ m^{-2}\ sec^{-1}$). Rice plants were grown in nutrient solution until 26 Days After Germination with a modified Hoagland and Arnon. All plants received two treatments with NH_4^+-N 0.1 and 1.0 mM, pH 5.5. Under these conditions, Piauí plants presented longer roots, chlorophyll a+b enhances, soluble sugars levels reduction and free amino-N content increases, what would be important for its adaptation to low N and low light environments, found in Humid Tropic rice culture. In spite of this, IAC-47, selected for high nutrient and light availability, presented typical senescence symptoms.

Key words: *Oryza sativa*, IAC-47, Piauí, N-nutrition, soluble fractions, total-N, pigments

INTRODUCTION

About half planet population, more than three billion of people depend on rice for their nutrition (Santos *et al*., 2003). Only Asia produces and consumes 90% of all world rice production. Out of Asian continent, Brazil represents the greatest cereal producer (Pereira *et al*., 2007).

Due to the culture widespread, rice is vulnerable to great temperature amplitude, with intense solar radiation offer variability, mainly because of photoperiod variation, as well as it suffers differential water offer. It is good to point out that climate variations of such importance also influences soils origin, what directly causes nutrient availability and adaptations in culture system (Fernandes and Rossiello, 1995).

Certainly, Nitrogen availability is the most limiting fertility index, which turns Nitrogen fertilizers into the great responsible for production expenses (Ferraz *et al*., 1997). Also, it represents serious environmental risks (Rodrigues and Garrido, 2005).

Stress effects, such as N deficiency, can be observed in fresh and dry weight data, in roots and shoots developmental relation, also in total-N accumulation, soluble fractions and in photosynthetic pigments quality analysis in several plants such as corn and rice (Santos *et al*., 2003; Majerowicz *et al*., 2002). Some of these parameters are even more representative when stress is caused by ammonium used as N source (Fernandes and Rossiello, 1995). In many cultivated areas, this cation appears as the single mineral Nitrogen source for plants (Fan *et al*., 2007).

Specially in this research, two rice varieties were evaluated: Piauí and IAC-47. Piauí is adapted to Humid Tropic environmental conditions, where there is N deficiency and low light since the culture begins and IAC-47 is an improved variety. Piauí plants are known for their best Nitrogen Use Efficiency (NUE), what could be related to assimilatory efficiency, once were not these plants improved under high N levels environmental

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pressure, such as it happened to IAC-47. On the other hand, IAC-47 does show greater N absorption capacity (Ferraz *et al*., 1997).

This research aims to characterize physiological and morphological adaptations happened during vegetative growth, under low ammonium-N nutrition and low light in rice IAC-47 and Piauí varieties. To analyze it, developmental parameters, soluble sugars content, amino-N, NH_4^+, total-N and pigments were compared between varieties.

MATERIALS AND METHODS

This study was carried on at Universidade Federal Rural do Rio de Janeiro, Seropédica campus, Brazil, between 2005 and 2006. Two rice (*Oryza sativa* L.) varieties were used: Piauí and IAC-47. Rice plants were cultivated in a growth chamber condition, at 24°C of average temperature and with 12/12 h light/dark periods (200 $\mu E\ m^{-2}\ sec^{-1}$). The experimental design was a Randomized Completely Block with 2 rice varieties (Piauí and IAC-47) and 2 treatments (NH_4^+-N 0.1 and 1.0 mM) and 3 replications. Means were compared using t-test.

Two rice (*Oryza sativa* L.) varieties were used: Piauí and IAC-47. Rice plants were grown in nutrient solutions until 26 Days After Germination (DAG). A modified Hoagland and Arnon (1950) solution was used at ¼ of its ionic strength. At four DAG it was changed to ½ of its ionic strength and kept so up to 8 DAG. After that, plants were grown in a full strength solution up to the final harvest at 26 DAG. During 26 days, all plants received two treatments with NH_4^+-N 0.1 and 1.0 mM, pH 5.5.

At 26 DAG, plants were harvested and, after distillated water washing, plants were dried, weighted and seminal root was measured. A shoot and a root one gram samples were retrieved from each treatment for maceration into 80% ethanol 20.00 mL and after that, each crude extract suffered chloroform partition for soluble sugars content (Yemm and Willis, 1945), amino-N (Yemm and Cocking, 1955) and NH_4^+ (Mitchell, 1972) determinations.

A shoot one gram sample was taken from each treatment for maceration into pure cold acetone. Samples were stocked under 4°C for three days. After that, crude extract was filtered and its absorbance was measured with 661.6, 644.8 and 470.0 nm, respectively for Chlorophyll a, Chlorophyll b and Carotenoids (Majerowicz *et al*., 2002). Pigments contents were calculated by following formula (Lichtehthaler, 1987):

$$\text{Chlorophyll a } (\alpha) = [(11.24\ Y) - (2.04\ Z)]\ 50$$
$$\text{Chlorophyll b } (\beta) = [(20.13\ Z) - (4.19\ Y)]\ 50$$
$$\text{Chlorophyll a + b } (\gamma) = [(7.05\ Y) + (18.09\ Z)]\ 50$$
$$\text{Carotenoids} = [(50000\ \theta):214]-[(1.9\ \alpha):214]-[(63.14\ \beta):214]$$

Where:
Y = Absorbance at 661.6 nm
Z = Absorbance at 664.8 nm
θ = Absorbance at 470.0 nm

Shoots and roots samples were retrieved from each treatment for drying under 60°C for 3 days for dry weight determination. The material was grinded and a 0.200 g sample was taken to Kjeldahl digestion (Tedesco, 1983).

RESULTS AND DISCUSSION

Improved and adapted to Humid Tropic rice plants present different development deleterious effects while exposed to low nitrogen and low light (Rodrigues *et al*., 2004). Actually, fresh weight did not differ at both varieties in 0.1 mM NH_4^+-N treatment (Table 1). Plants under low N presented reduced development. Plants grown with 0.1 mM N-NH_4^+ showed N deficiency in leaves, such as older leaves yellowness, as well as other senescence characteristics (data not shown).

Plants grown with low ammonium had a shoot/root reason near 1 (Table 1). This result was also found by Baptista *et al.* (2000) in Bico Ganga variety cultivated with low N. They found great root development together with NH_4^+ absorption. However, in 1.0 mM NH_4^+-N plants, shoots grew more than roots (Table 1). It is known that radicular length is related to water and nutrients absorption potential (Zonta *et al.*, 2006).

When varieties fresh weight in the same treatment were analysed, it was clear IAC-47 roots and shoots higher development at both treatments (Table 1). In fact, IAC-47 growth during vegetative stages in treatments with low N can be harmful for older developmental stages, when cellular constituents are needed in these stages, as it was observed by Ferraz *et al.* (2001) in improved varieties.

Comparing roots fresh weight variations between treatments in the same variety, it was observed a small weight enhancement in IAC-47 1.0 mM. However, there was a significative dry weight accumulation in shoots. When NH_4^+-N concentration was elevated 10 times, it was observed a 61% enhancement in Piauí fresh weight and 89% in IAC-47.

There was longer seminal root in 1.0 mM NH_4^+-N Piauí plants, more than IAC-47 under the same treatment (Table 1). Roots length was always smaller in 1.0 mM NH_4^+-N treatment, when compared to 0.1 mM NH_4^+-N (Table 1). These data were confirmed by these plants radicular visual inspection (data not shown). Great radicular growth was observed in other rice varieties under low N concentration, particularly when N source was ammonium (Baptista *et al*., 2000; Mendonça *et al*., 2005).

Generically, roots main axis lengthening cannot be considered radicular growth, but only spatial changes. It happens because, in certain limiting conditions, radicular growth pattern included intense lateral axis growth (Zonta *et al*., 2006). So, differences between root length and fresh weight in 1.0 mM NH_4^+-N treatment shall be explained by distinct IAC-47 root architecture. These roots were thinner and hairy root (data not shown). Same result was found by França *et al*. (1999) when compared IAC-4440 and Comum Branco radicular area. Improved variety (IAC-4440) had thinner roots and wider specific area. However, radicular area was not reflected in N accumulation, what could be due to a compensatory radicular influx mechanism (França *et al*., 1999).

Baptista *et al*. (2000) showed that Bico Ganga variety, such as Piauí, was adapted to Humid Tropic conditions. When it was grown in NH_4^+-N concentrations lower than 4.3 mM it presented better radicular development, accompanied to NH_4^+ absorption enhances. Despite what was observed in Agulha variety, that presented less radicular development and NH_4^+ absorption capacity only under high nitrogen (Baptista *et al*., 2000).

Ammonium effects over radicular growth can be noticed because of this ion relationship with carbon metabolism. For radicular development, about 44% fixed carbon should be sinked by this organ. A quarter of this is used in cellular respiration and the remaining carbon is directed to tissue growth (Zonta *et al*., 2006).

Both varieties and treatments mass variation (Table 1) was observed in 26 DAG shoots. IAC-47 plants presented greater development in both treatments. In spite of this, differences were markedly clear between treatments for different varieties. It could have been due to water concentration differences in 1.0 mM NH_4^+ plants, what must have influenced development differences between treatments (Table 1).

Despite differences between treatments in the same variety, fresh weight profile was repeated in dry weight analyses (Table 1). This result confirmed the hypothesis that water concentration in 1.0 mM NH_4^+ plants did not influence organic matter accumulation and salt deposits.

It is certain that plant morphologic variations, such as fresh weight reduction, seminal root length alteration, as well as root/shoot modifications (Table 1) are results of metabolic dynamics related in other works (Baptista *et al*., 2000; Hirel *et al*., 2005). In spite of this, parameters such as radicular area enhances were not reflected in plants N accumulation, as observed by França *et al*. (1990). It could be due to a compensatory radicular influx mechanism.

In this study, Piauí and IAC-47 soluble fractions, photosynthetic pigments and N accumulation were analyzed, so that metabolic interactions around ammoniacal nutrition could be better understood. Total Nitrogen was higher in 1.0 mM NH_4^+ plants (Table 2). In both varieties, NH_4^+-N levels were proportional to total-N, either in shoots and roots. Between 0.1 mM NH_4^+-N plants, there was no significative varietal difference, what confirmed by Fernandes (1990) findings after working with Cana Roxa, an adapted variety and IR-8, the improved one. In 1.0 mM NH_4^+-N, Piauí presented higher total-N levels (Table 2).

In Table 2, it can be observed that total-N and free amino-N were increased in shoots. In case of ammoniacal nutrition, roots, rather than only function as an absorption site, would be an important assimilatory organ. In this particular condition, free amino-N high level in shoots (Table 2) in all treatments could be a senescence protein disruption result motivated by low N and low light (Silveira and Machado, 1990; Fernandes and Rossiello, 1995; Buchaman-Wollaston, 1999).

Piauí shoots free amino-N were never lower than those found in IAC-47 (Table 2). In review, Fernandes and Rossiello (1995) showed that amino-N accumulation could be due to synthesis disruption or to increase in protein

Table 1: Piauí and IAC-47 rice varieties vegetative parameters. Plants were cultivated in a growth chamber condition at 24°C and with 12/12 h light/dark periods (200 µE m^{-2} sec^{-1}), under 2 treatments (NH_4^+-N 0.1 and 1.0 mM)*

	Vegetative parameters					
	Fresh weight (g)				Dry/fresh weight	
Varieties/Treatments	Root	Shoot	Root/ shoot	Seminal root (cm)	Root	Shoot
Piauí 0.1 mM NH_4^+-N	7.85^{B}	7.98^{B}	0.98^{a}	23.33^{a}	0.13	0.16*
Piauí 1.0 mM NH_4^+-N	7.46^{Bb}	12.88^{Ba}	0.56^{b}	19.50^{Ab}	0.10^{b}	0.13^{Ba}
IAC-47 0.1 mM NH_4^+-N	10.03^{A}	9.99^{A}	1.00^{a}	23.10^{a}	0.12^{b}	0.18^{a}*
IAC-47 1.0 mM NH_4^+-N	12.36^{Ab}	18.86^{Aa}	0.66^{b}	16.67^{Bb}	0.10^{b}	0.14^{Aa}

*: (a, b) significant at the 5% level between treatments in the same variety. (A, B) significant at the 5% level between varieties in the same treatment. (*) significant at the 5% level between treatments in the same variety

Table 2: Piauí and IAC-47 rice varieties total-N mg×g^{-1} DW (Dry Weight); Amino-N µmol×g^{-} FW (Fresh Weight); Soluble sugars mg×g^{-} FW and NH_4^+ µmol×g^{-1} FW In shoot and root. Plants were cultivated in a growth chamber condition at 24°C and with 12/12 h light/dark periods (200 µE m^{-2} sec^{-1}), under 2 treatments (NH_4^+-N 0.1 and 1.0 mM)†

Varieties/Treatments	Total-N (mg×g^{-1} DW)	Amino-N (µmol×g^{-1} FW)	Soluble sugars (mg×g^{-1} FW)	NH_4^+ (µmol×g^{-1} FW)
Shoot				
Piauí 0.1 mM NH_4^+-N	9.08	8.55^{a}	6.78^{Ba}	1.57^{b}
Piauí 1.0 mM NH_4^+-N	20.89^{Aa}	11.97^{Aa}	3.33^{Bb}	2.07^{Aa}
IAC-47 0.1 mM NH_4^+-N	8.58	7.92^{a}	11.02^{A}	1.69^{a}
IAC-47 1.0 mM NH_4^+-N	17.06^{B}	8.02^{Ba}	8.24^{A}	0.80^{Bb}
Root				
Piauí 0.1 mM NH_4^+-N	8.79	4.53^{Ab}	0.72^{B}	0.95^{a}
Piauí 1.0 mM NH_4^+-N	18.24^{Ab}	5.32^{Ab}	0.75^{B}	0.72^{b}
IAC-47 0.1 mM NH_4^+-N	8.78	3.56^{Bb}	1.62^{A}	1.12
IAC-47 1.0 mM NH_4^+-N	15.07^{B}	4.10^{Bb}	1.79^{A}	0.82

†: (a, b) significant at the 5% level between treatments in the same variety. (A, B) significant at the 5% level between varieties in the same treatment

degradation. In both cases, data pointed out an enhanced nutrient remobilization efficiency in Piauí plants grown with low N (Ferraz *et al*., 2001), specially in 1.0 mM treatment.

However, Piauí soluble sugars were lower in shoots and roots (Table 2). This profile was better observed in 1.0 mM NH_4^+-N treatment. This result justified free amino-N content. Under these conditions, Piaui plants would be actually assimilating ammonium-N. However, due to protein synthesis disruption, there was an increase in free amino-N levels.

Soluble sugars decrease in high ammonium treatment was observed by Fernandes and Rossiello (1995) as a way for immediate NH_4^+ assimilation by plants. Low soluble sugars levels in Piauí plants could also point out carbohydrate stock that may be later used in development stages, what would be important for grain protein yield (Ferraz *et al*., 2001).

As Piauí plants are adapted to low fertility, these plants seem to deal well with ammonium deficiency. These plants would present less efficiency in assimilating N as external N concentrations increase. Results in Table 2 show that 1.0 mM NH_4^+-N Piauí plants kept high ammonium levels in shoots, despite assimilation reflected in free amino-N high concentrations as well as soluble sugars sink.

It is important to observe that ammonium metabolism problems may appear after an absolute increase in nutrient availability or due to a cetoacid carbon skeleton depletion, causing NH_4^+ enhances (Souza and Fernandes, 2006). In this case, high soluble sugars for immediate N assimilation would not be sufficient for plants. Above all, it is necessary to increase biochemical pathway that convert sugars into cetoacids.

Once ammonium movement among organs is quite low, it is possible that high free amino-N in shoots under the same conditions (Table 2) could stimulate deamination pathways, motioned specially by GDH activity, that produces ammonium and keeps α-ketoglutarate levels in shoots (Dubois *et al*., 2003).

Although based in adaptive characteristics, it is possible to understand enhances at free ammonium levels in 0.1 mM IAC-47. Those plants, selected for high N levels, would present smaller assimilatory pathway enzymes activities under low N.

Low N and low light favoured free amino-N accumulation (Table 2) without protein synthesis, due to energetic deficiency. This physiological condition decreases dry weight, as described by Fernandes and Rossiello (1995). After that, Piauí plants could increase substract stock for grain protein production (Ferraz *et al*., 2001). A great chance to allow this metabolism destiny would be reducing sugar oxidation in roots.

Table 3 shows photosynthetic pigments content obtained from both varieties and treatments. Those pigments are recognized as the most powerful markers for N acquisition by plants (Majerowicz *et al*., 2002). IAC-47 grown with 1.0 mM NH_4^+-N presented higher pigments concentration than those grown with 0.1 mM. This is mainly because of chlorophyll b increase. Actually, 0.1 mM IAC-47 did also present varietal difference: total chlorophyll and chlorophyll b contents were smaller than Piauí grown under the same concentration. Chlorophyll content reduction is a characteristic senescence symptom (Silveira and Machado, 1990; Buchaman-Wollaston, 1999). According to Majerowicz *et al*. (2002), in maize, photosynthetic pigments content analysis allowed observations of different plants nutritional stages, but did not evaluate varietal differences.

Lima *et al*. (2004) working with BRS Bojurú and IAS 12-9 Formosa rice varieties, resistant to salt stress, found carotenoid, a, b and total chlorophyll contents equal to Table 3 levels. However, they did not observe significative difference between varieties. This was only

Table 3: Piauí and IAC-47 shoots pigment content μg x g^{-1} FW (Fresh Weight). Plants were cultivated in a growth chamber condition at 24°C and with 12/12 h light/dark periods (200 μE m^{-2} sec^{-1}), under 2 treatments (NH_4^+-N 0.1 and 1.0 mM)‡

	Pigments content (μg×g^{-1} FW)			
Varieties/Treatments	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Carotenoids
Piauí 0.1 mM NH_4^+-N	860.4	833.0^{A}	1693.4^{Aa}	253.2
Piauí 1.0 mM NH_4^+-N	853.8	771.2	1625.0^{b}	258.3
IAC-47 0.1 mM NH_4^+-N	831.8	490.5^{Bb}	1322.3^{Bb}	263.0
IAC-47 1.0 mM NH_4^+-N	868.2	728.0^{a}	1596.2^{a}	256.5

‡: (a, b) significant at the 5% level between treatments in the same variety. (A, B) significant at the 5% level between varieties in the same treatment

found when they compared these plants with BRS Agrisul variety, the one more sensitive to salt stress, in which all chlorophyll contents were higher.

It should be detached that, though chlorophyll b is not a photosynthetic energy transduction directly linked pigment, but a light spectra amplification pigment, Lima *et al*. (2004) found that in rice, b and total chlorophyll contents were higher than 33%.

In IAC-47 grown with 1.0 mM NH_4^+-N as in Piauí both treatments, b chlorophyll was higher than 50% from chlorophyll a+b. It seemed that this accessory chlorophyll increases was related to low light (200 μE m^{-2} sec^{-1}). Specially Piauí plants presented chlorophyll b contents enhanced, as a signal of a varietal characteristic to absorb light better under low energetic levels. According to Rodrigues and Garrido (2005), the same environmental condition happens from December to July, when insolation is reduced in Humid Tropic, to which Piauí plant is well-adapted.

CONCLUSION

Piauí plants presented morphological adaptations, such as longer roots; as well as physiological adaptations, after chlorophyll a+b enhances, soluble sugars levels reduction, together with free amino-N content increases. These characteristics show adaptation to low N and low light environments, found in Humid Tropic rice culture. In spite of this, IAC-47, selected for high nutrient and light availability, presented typical senescence symptoms under experimental conditions.

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