International Scholars Journals

African Journal of Soil Science ISSN 2375-088X Vol. 12 (1), pp. 001-004, January, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Enzymatic adaptations in nitrogen metabolism during rice germination and early seedling growth across genotypes

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Accepted 16 April, 2023

Seeds of three rice (*Oryza sativa* L.) genotypes Viz. IR-42 (intolerant), USAR-1 and NDR-501(Tolerant) were germinated in Petri-dishes containing aqueous solution of $NaHCO_3$ at pH of 8.5, 8.8, and 9.2 at 30+1°C in seed germinator. Seed kept for germination on distilled water served as control. Nitrate reductase (NR), Nitrite reductase (NiR) and Glutamine synthetase (GS) activities were assayed in embryonic axis after 72, 96 and 120 h, respectively after soaking. Nitrate reductase (NR) and nitrite reductase were higher in USAR-1 fallowed by NDR-501 and IR-24 respectively under normal condition. Glutamine synthetase activity on the other hand, was maximum in NDR-501 and minimum in IR-24. Activities of the entire enzyme increased with growth stage but decreased progressively with increasing level of alkalinity, though the magnitude of reduction was more in intolerant genotype IR-24. Enzymes activity in the tolerant genotype under normal as well as alkaline condition was higher and might be due to their better equipped salt tolerant mechanism. Glutamine synthetase being one of the key regulatory enzymes of nitrogen metabolism. It linked with the molecular basis of salt tolerance in rice genotypes and providing certain degree of protection against hostile environment.

Key words: Alkalinity, glutamine synthetase, nitrate reductase, nitrite reductase, and rice.

INTRODUCTION

Soil alkalinity is one of the major problems in part of India as well as world for rice production. About 434 m.ha rice area of world is affected by soil alkalinity (Wang et al., 2012). Soil alkalinity and salinity are the complex and two different abiotic stresses which is caused by high amount of Na_2CO_3 or $NaHCO_3$ and NaCl or $NaSO_4$ respectively.

Alkaline soil is more destructive for plant growth and development due to high pH and hard physical structure in comparison to saline soil. Alkali stress precipitates many minerals and restricts the absorption of Cl^-, NO_3^- and H_2PO_4 and disturbs the ionic balance of the cells (Yang et al., 2008; Wang et al., 2012).

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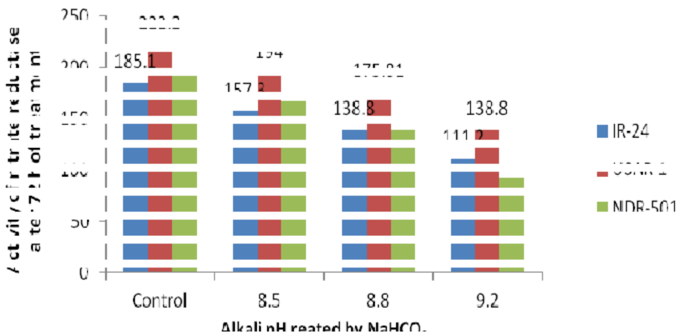

Figure 1. Effect of alkalinity on nitrate reductase activity (µ moles of nitrite produced h-[1] mg-[1] protein) in germinating embryonic axis of rice genotypes after 72 h.

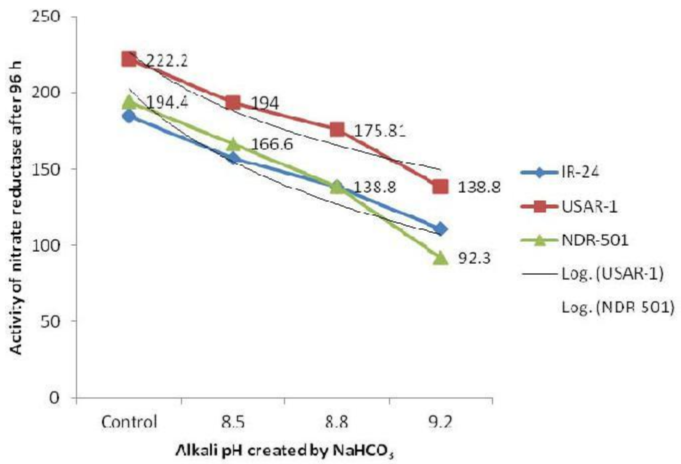

Figure 2. Effect of alkalinity on nitrate reductase activity (µ moles of nitrite produced h-[1] mg-[1] protein) in germinating embryonic axis of rice genotypes after 96 h.

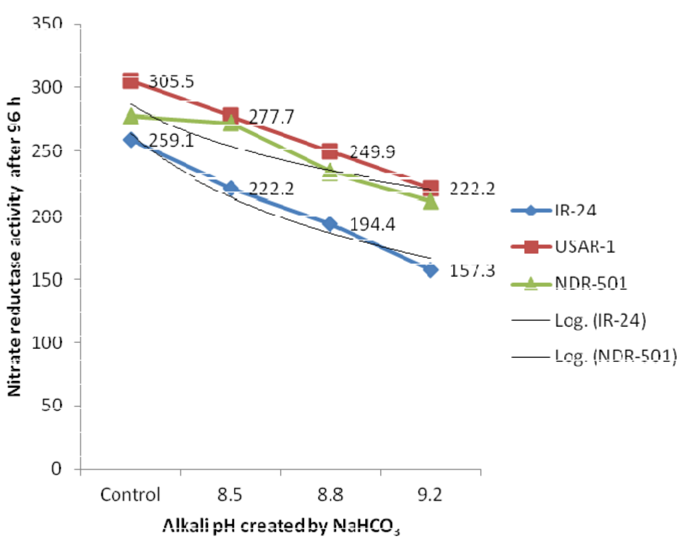

Figure 3. Effect of alkalinity on nitrate reductase activity (µ moles of nitrite produced h-[1] mg-[1] protein) in germinating embryonic axis of rice genotypes after 120 h.

Plant growth under alkaline condition is affected primarily by restricted root development. Plant survival and growth in such condition is the result of adaptive process such as ion transport and accumulation of compatible solutes (Yang et al., 2007). Many of these compatible solutes are nitrogenous compounds like amino acids, betaines, proline etc. Thus, proper and efficient nitrogen metabolism is crucial for salt tolerance (Munns and Tester, 2008).

Nitrogen metabolism disturb owing to high pH of the substrate and poor soil physical condition. Nitrogen metabolism being one of the key processes in the growth and development of plant needs particular attention especially at the enzymatic level. Enzymes like nitrate reductase and glutamine synthetase are the key rate limiting steps in nitrogen metabolism (Wang et al., 2012). Variability in nitrate and nitrite reductase, glutamine synthetase and glutamate dehydrogenase activity (Rakova et al., 1978) has been reported under saline condition in a number of crops. However, studies pertaining to the activity of these enzyme under alkaline condition are very few and inconclusive. The present study was, therefore undertaken using three rice cultivars differing in salt tolerance for assessing the enzymatic behavior in endosperm and embryonic–axis of germinating seeds under alkaline conditions.

MATERIALS AND METHODS

An experiment was conducted with three rice genotypes namely IR-24 (intolerant) USAR-1 and NDR-501(tolerant) under growth cabinet. The solutions of different levels of alkalinity viz. 8.5, 8.8 and 9.2 were prepared by 50 mM amount of $NaHCO_3$ in distilled water. Ten seeds were placed in each petri dish containing 10 ml solution of desired alkalinity level. Control treatment contained 10 ml distilled water. The petri dishes were placed in seed germinator in darkness for germination at 30+1°C. Enzyme analysis was done at 72, 96 and 120 h, respectively after sowing. Nitrate reductase (NR) and Nitrite reductase (NiR) activities were assayed according to the method of Jaworski (1971) and Ferari and Verner (1971) respectively. Glutamine synthetase (GS) was assayed by the modified r-glutamyl transferase method of Elliot (1955).

RESULTS AND DISCUSSION

A sharp progressive decrease in nitrate reductase (NR) activity in embryo-axis of all the three rice genotypes were apparent due to increasing levels of alkalinity (Figures 1, 2 and 3). NR activity in embryo-axis decreased sharply under salt stress condition at 72 h showing 79.2% reduction at pH 9.2 in salt intolerant genotype IR-24 while 62.8 and 52.8% in NDR-501 and USAR-1 respectively.

Nitrate reductase activity under alkaline medium greatly reduced due to unavailability of substrate for enzymatic reaction. Absorbed sodium disturbs the cells homeostasis of Na^+/K^+ ratio and cell environment for nitrate reductase activity (Wang et al., 2012). Decreased NR activity in rice

Table 1. Effect of alkalinity on nitrite reductase activity (µ moles of nitrite reduced min^{-1} mg^{-1} protein) in germinating embryonic axis of rice genotypes.

Genotypes	Control	pH			Mean
		8.5	8.8	9.2	
		72 h			
IR-24	88.9	74.0	51.8	44.4	64.8
USAR-1	111.1	88.9	74.0	59.2	83.3
NDR-501	103.7	811.4	59.2	51.8	74.0
Mean	101.2	81.4	61.7	51.8	
	Genotypes	**Alkalinity**	**Interaction**		
CD at 5%	8.2	9.4	16.3		
		96 h			
IR-24	133.3	118.5	103.7	74.1	107.4
USAR-1	162.9	133.3	111.1	96.3	125.9
NDR-501	140.7	133.3	88.9	66.7	107.4
Mean	145.7	128.1	101.2	79.0	
	Genotypes	**Alkalinity**	**Interaction**		
CD at 5%	7.8	9.0	16.0		
		120 h			
IR-24	177.8	163.0	133.3	111.1	146.3
USAR-1	214.8	200.0	177.8	155.6	187.0
NDR-501	192.6	148.1	133.3	118.5	148.1
Mean	195.1	170.4	148.1	128.4	
	Genotypes	**Alkalinity**	**Interaction**		
CD at 5%	7.1	8.2	14.2		

varieties under sodic conditions has also been reported by Dwivedi et al. (1982). Pandey and Lal (2003) also reported decreased activity of nitrate reductase in wheat. Lower NR activity under alkaline condition could possibly be due to reduced uptake of nitrate by the tissues under alkali condition and leading to lower induction of enzyme activity (Wang et al., 2012).

Like nitrate reductase, nitrite reductase (NiR) activity in salt sensitive genotype IR-24 was also lower than that of salt tolerant genotypes USAR-1 and NDR-501, which maintained the same trend even under alkaline condition at all the stages of seedling growth (Table 1). Nitrite reductase activity increased with time period in embryo axis in all the treatment irrespective of genotypes. Once again the salt sensitive genotypes IR -24 showed greater reduction in (NIR) activity than salt tolerant genotypes USAR-1 and NDR-501.

NiR activity is affected due to poor activity of NR under alkaline environment. High Na^+ content in and outside the cell environment changes ion balance and reduces the activity of enzyme involves in nitrogen metabolisms (Munns and Tester, 2008). Decrease in NiR activity under salinity condition has also been reported by Wang et al. (2012).

Glutamine synthetase activity in the embryo axis decreased significantly due to increase in alkalinity levels in three rice genotypes (Figures 4, 5 and 6) although the magnitude of reduction was higher in salt susceptible genotype IR-24 (46%) at pH 9.2 against 30 and 32% in USAR-1 and NDR-501 respectively. Enzyme activity in embryo axis was lower at 72 h but increased gradually at 96 and 120 h period in all the treatment.

In nitrogen metabolism, nitrate is reduced to nitrite by nitrate reductase and nitrite to ammonium by nitrite reductase. Ammonium compound from this reduction and other sources is converted into organic compound by glutamine synthetase (GS) or alternate glutamate dehydrogenase (GDH) pathway (Shi et al., 2010). But the decrease in glutamine synthetase, nitrate reductase and nitrite reductase activities under alkaline condition is an indication of disturbed nitrogen metabolism in rice due to high sodium and bicarbonate ions (Kusano et al., 2011; Rakova et.al., 1978). Lower GS activity under alkaline condition is an accumulation of ammonia and amino acid especially glutamate which was toxic in higher concentration. Accumulation of glutamate, alanine, asparagines, and cysteins under salt stress was shown by Strogonov (1973).

Differential behavior of nitrate reductase, nitrite conferring salt tolerance to rice. Glutamine synthetase

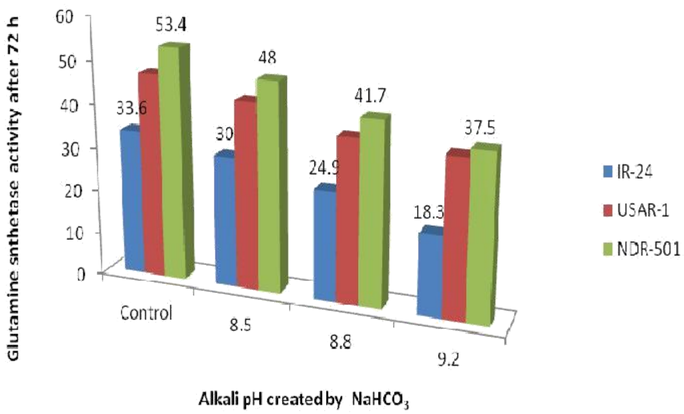

Figure 4. Effect of alkalinity on glutamine synthetase activity (µ moles glutamyl hydroxamate produced g^{-1} fresh weight h^{-1}) in germinating embryonic axis of rice genotypes after 72 h.

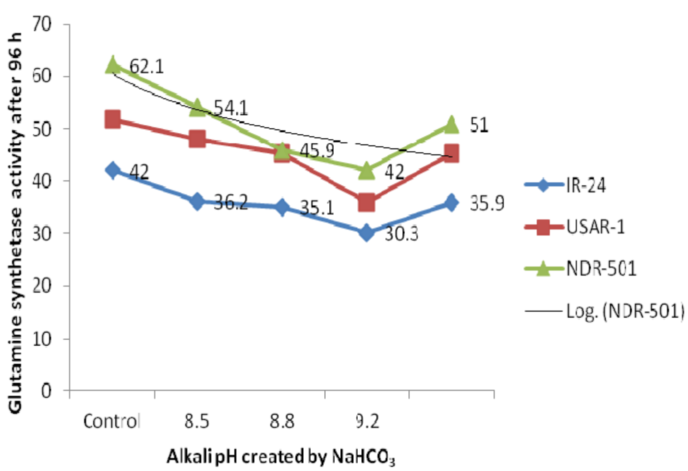

Figure 5. Effect of alkalinity on glutamine synthetase activity (µ moles glutamyl hydroxamate produced g^{-1} fresh weight h^{-1}) in germinating embryonic axis of rice genotypes after 96 h.

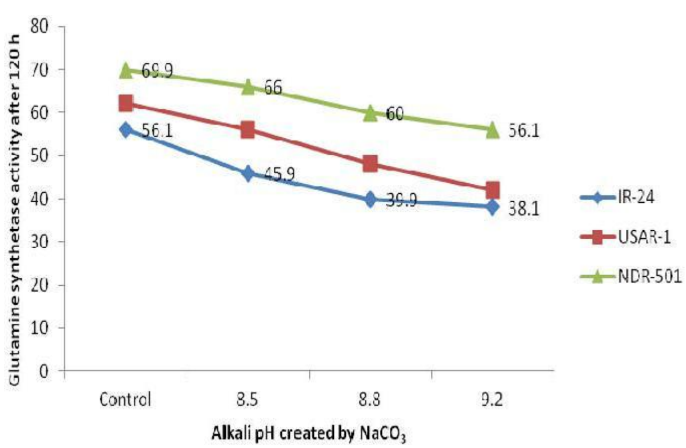

Figure 6. Effect of alkalinity on glutamine synthetase activity (µ moles glutamyl hydroxamate produced g^{-1} fresh weight h^{-1}) in germinating embryonic axis of rice genotypes after 120 h.

reductase and Glutamine synthetase in rice genotype differing in salt tolerance indicates their possible roles in being key regulatory enzyme of nitrogen metabolism might be playing some useful roles in inducing salt tolerance to USAR-1 and NDR-501 genotypes which maintained relatively higher GS activity even under alkaline condition (Figures 4, 5 and 5) . In conclusion, it appears that genotypic differences do exist in different enzyme activities but their measurement at two or three stages during early seedling stage alone may not correlate with the growth and yield of plant.

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