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Enzymatic Study of Glutamine Synthetase Activity for Validating Nitrogen Use Efficiency in Rice Genotypes

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ABSTRACT

The field experiment was laid out in a split plot design with two nitrogen levels of 60 and 120 kg N ha^{-1} as main plot treatments and twenty six rice genotypes as sub plot treatments and was replicated thrice during 2011and 2012. Glutamine synthetase (GS) is one of the selection criteria to identify nitrogen use efficient cultivars and their use in developing mapping population for high nitrogen use efficiency (NUE). GS activity was more in plants growing at low nitrogen levels than that at high nitrogen levels in rice. Maximum glutamine synthetase activity was recorded in the genotype BPT-5204 when 60 kg N ha^{-1} (0.94 μ mol g^{-1} min^{-1}) was applied at maturity stage as compared to application of 120 kg N ha^{-1}. MTU-1001 recorded maximum NUE in 60 kg N ha^{-1} (83.68) and minimum was recorded in 120 kg N ha^{-1} (45.53). The results showed that the NUE did not increase linearly with the amount of nitrogen application and higher nitrogen levels showed significantly lower NUE values.

Key words*: *Rice, Nitrogen, Glutamine synthetase enzyme and Nitrogen use efficiency

INTRODUCTION

Rice (*Oryza sativa L.*) is the "Global Grain" and world's second most important cereal crop and staple food for more than 60 % of the global population and forms the cheapest source of food and energy. Rice varieties may respond differently to nitrogen application. Cultivars selected under high nitrogen fertilizer application may not be suitable for soils with low nitrogen status. Even after the application of high rates of fertilizer nitrogen to rice, expected yield levels might not be obtained. If plant nitrogen status can be increased without lodging or increasing the incidence of disease, a significant increase in yield requires increased sink capacity, maintenance of high leaf nitrogen content and a longer grain filling duration. Rice varieties differ in their ability to extract soil and fertilizer nitrogen and in its distribution to different plant organs. Understanding nitrogen uptake and assimilation is necessary in any attempt to optimize the efficiency of absorbed nitrogen for grain production[7].

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GS is an important enzyme in nitrogen metabolism. The α-amino group of glutamate is directly involved in both the assimilation and dissimilation of ammonia and is transferred to all other amino acids. It is essential for the nitrogen assimilation and biosynthesis of glutamine. GS catalyzes the ATP depended formation of glutamine from glutamate and ammonia and is considered to be one of the oldest functioning enzyme[1]. It also re-assimilates ammonia released as a result of photo respiration and the breakdown of proteins and nitrogen transport compounds. The GS activity as one of the selection criteria to identify nitrogen use efficient cultivars and their use in developing mapping population for high NUE is in progress.

Nitrogen use efficiency defined as the ratio of grain yield to applied fertilizer nitrogen is a key parameter for evaluating a crop cultivar, and it is composed of nitrogen uptake efficiency and nitrogen physiological use efficiency. Nitrogen uptake efficiency is the ability of the plant to remove nitrogen from the soil as nitrate and ammonium ions, while nitrogen physiological use efficiency represents grain yield relative to nitrogen accumulation[6]. As the amount of nitrogen available from soil and fertilizer is difficult to measure, grain yields can be used for evaluating the NUE and high-NUE cultivars can be defined by their ability to produce higher grain yields than others. The current average NUE in the field is approximately 33% and a substantial proportion of the remaining 67% is lost into the environment, especially in the intensively cropped areas[14]. Unlike other quantitative traits, NUE is one which had produced favourable results showing the effectiveness of this approach in selecting genotypes positively contributing to high nitrogen use efficiency.

MATERIALS AND METHODS

A field experiment was conducted during 2011 and 2012 at College Farm, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad. The experiment was laid out in a split plot design with two nitrogen levels of 120 kg N ha^{-1} [N120] and 60 kg N ha^{-1} [N60] as main treatments, twenty six rice genotypes as sub treatments and the experiment was replicated thrice. The rice genotypes were sown separately in raised bed nursery and thirty day old seedlings were transplanted into 6 m^2 plots by adopting a spacing of 20 X 15 cm and one third dose of nitrogen was applied as basal dose at the time of planting of the crop. Remaining two equal splits of nitrogen was broadcasted at maximum tillering and panicle initiation stages. Phosphorus @ 60 kg P_2O_5 ha^{-1} in the form of single super phosphate and potassium @ 40 kg K_2O ha^{-1} in the form of muriate of potash was applied as basal dose at the time of transplanting. Irrigation and weed management was done in time to time. Plants in one m^2 area were tagged separately.

Estimation of Glutamine Synthetase (GS) activity

Enzyme extraction

The harvested leaf samples from different treatments were taken and ground to a fine powder and then homogenized in an extraction buffer (10 ml/g fresh weight) containing 100 mM Tris-HCl (pH 6.8), 10 mM sucrose, 10 mm EDTA,10 mM β- mercapto ethanol , 10mM KCl and 10 mM $MgC1_2$. The homogenate was centrifuged at 10,000 g for 20 min. The supernatant fraction was used for the assay of GS activity. All operations were carried out under chilled conditions. A unit of enzyme was defined as that amount of the product obtained in 1 μ mol per min at 30°C[9]. GS catalyzes the ATP depended formation of glutamine from glutamate and ammonia.

$$\text{Glutamate} + NH_4^+ + \text{ATP} \xrightarrow[Mn^{++}]{\text{Glutamine Synthetase}} \text{Glutamine} + \text{ADP} + \text{Pi}$$

Determination of glutamine synthetase activity

Crude enzyme preparation (0.8 ml) was added into 2.2 ml reaction solution containing 0.6 ml buffer, 0.6 mol/L MgSO4 (0.2 ml), 1.2 mol/L *L*-monosodium glutamate (0.8 ml, pH 7.0), 0.06 mol/L ATP (0.4 ml), 1:1 mixture solution (0.2 ml) of hydroxylamine (0.7 mol/L) and NaOH (1.0 mol/L). The reaction was conducted at 40°C for 30 min, then stopped by adding stock solution (0.8 ml) with a 1:1:1 proportion of 10% $FeCl_3$, 24% trichloro acetic acid (dissolved in 0.2 mol/L HCl solution), and 50% HCl. Ten minutes later, the OD value was read at 540 nm with spectrophotometer.

Nitrogen Use Efficiency (NUE)

Nitrogen Use Efficiency was calculated by using the following formula:

$$\text{Nitrogen Use Efficiency (NUE)} = \frac{\text{Grain yield (kg ha}^{-1}\text{)}}{\text{N application rate (kg ha}^{-1}\text{)}} \times 100$$

Grain from net plot area was thoroughly sun dried, threshed, cleaned and weight of grains was recorded and expressed in yield per hectare. The data were analyzed statistically wherever the results were significant, the critical difference (CD) was calculated at 5 per cent level of significance (P=0.05)[13].

RESULTS AND DISCUSSION

Glutamine Synthetase (GS) activity

Data on Glutamine Synthetase activity in the leaf of rice genotypes was influenced by nitrogen application at different growth stages (Table 1 and Fig. 1). GS activity gradually increased from basal stage (0.21 µ mol g^{-1} min^{-1}) to panicle initiation stage (0.51µ mol g^{-1} min^{-1}) and maturity stage (0.83 µ mol g^{-1} min^{-1}). Between the treatments, application of 60 kg N ha^{-1} has resulted in mean GS activity of 0.23 µ mol g^{-1} min^{-1} at basal stage which increased upto to 0.54 and 0.84 µ mol g^{-1} min^{-1} at panicle initiation and maturity stages, respectively. While GS activity decreased in 120 kg N ha^{-1} treatment and the values recorded were 0.19, 0.48 and 0.82 µ mol g^{-1} min^{-1} at the basal, panicle initiation and maturity stages, respectively.

Significant differences were also observed among the rice genotypes studied. At basal stage GS activity ranged from 0.17 to 0.27 with a mean of 0.21 µ mol g^{-1} min^{-1}, at panicle initiation ranged from 0.40 to 0.70 with a mean of 0.51 µ mol g^{-1} min^{-1} and at maturity stage ranged from 0.75 to 0.94 with a mean of 0.83 µ mol g^{-1} min^{-1}. GS activity was maximum in genotype BPT-5204 at basal stage (0.27 µ mol g^{-1} min^{-1}), at panicle initiation stage (0.70 µ mol g^{-1} min^{-1}) and at maturity stage (0.94 µ mol g^{-1} min^{-1}), where as minimum values were recorded in genotype Erramallelu at basal stage (0.17 µ mol g^{-1} min^{-1}), Varalu (0.40 µ mol g^{-1} min^{-1}) at panicle initiation stage and Erramallelu (0.75 µ mol g^{-1} min^{-1}) at maturity sage. Study of the activities and expression levels of nitrate assimilatory enzyme in N-efficient and N-inefficient rice genotypes showed that glutamine synthetase play an important role in nitrogen assimilation under low-nitrogen conditions[8]. The interactive effect between nitrogen levels and genotypes was significant for GS activity at panicle initiation stage while at in other two stages the differences were non significant.

The enzyme glutamine synthetase catalyses the conversions of the amino acid into the amide and glutamate into the glutamine, using NH_4^+, ATP and a divalent cation such as Mg^{2+}, Mn^{2+} as a cofactor. Thus, GS is likely to be a key factor controlling plant nitrogen assimilation[1]. The low nitrogen efficient genotypes (LNE), a substantial portion of nitrogen was not re-translocated to the harvested structures and the GS activity in leaves was lower as compared to the high nitrogen efficient genotypes (HNE) [12]. Hence, the ability of the HNE genotypes to harvest more nitrogen and redistribute to grains is due to the well-coordinated system of nitrogen uptake and assimilation. In the LNE genotypes, the GS activity was sufficiently high to maintain a low level of ammonium in leaf tissues irrespective of external nitrogen

supply[4]. It was observed that the GS activity was 2-fold higher in plants growing at low nitrogen levels than that at high nitrogen levels[2]. Therefore, high GS activity in the chloroplasts would be a key point to detoxify ammonium, by incorporating it into amino acids as a sink for ammonium and carbohydrate as well as an excellent parameter for a genetic screening program in order to obtain nitrogen assimilating efficient genotypes.

Nitrogen Use Efficiency (NUE)

Nitrogen Use Efficiency (NUE) largely depends on nutrient balance, water availability, light intensity and cultivated variety. Nitrogen efficient genotype is considered in two different terms: the ability to convert high nitrogen input into yield comparatively better than other genotypes or the ability to realize an above average yield at suboptimal nitrogen level. Rice genotypes showed different nitrogen uptake ability and NUE at different nitrogen level [5, 11].

Data on NUE as influenced by nitrogen supply in rice genotypes has been presented in table 2 and Fig. 2. Significant difference was observed between the treatments and increase in nitrogen level reduced the NUE which ranged from 38.32 to 66.42 with a mean of 52.37. Maximum NUE was recorded at 60 kg N ha^{-1} (66.42) and Minimum NUE was recorded in 120 kg N ha^{-1} (38.32). The results showed that the NUE did not increase linearly with the amount of nitrogen application.

The genotype MTU-1001 recorded maximum NUE (64.61) and Varalu (34.88) recorded the minimum value. Interaction between nitrogen and rice genotypes significantly influenced the NUE values which ranged from 27.29 to 83.68. Among the interactions, the genotype MTU-1001 recorded maximum NUE (83.68) at 60 kg N ha^{-1} where as Varalu recorded minimum NUE (27.29) at 120 kg N ha^{-1}. Rice varieties responded well to higher levels of nitrogen but nitrogen use efficiency was comparatively better in lower levels[3]. Excessive N rate, low nitrogen uptake and irrational application, timing were the key reasons for low nitrogen use efficiency.

Table 1: The influence of nitrogen on glutamine synthetase activity (μ mol g^{-1} min^{-1}) in rice genotypes at different stages of crop

Genotypes		At basal stage			At panicle initiation stage			At maturity stage		
		60 kg N ha^{-1}	120 kg N ha^{-1}	Mean	60 kg N ha^{-1}	120 kg N ha^{-1}	Mean	60 kg N ha^{-1}	120 kg N ha^{-1}	Mean
WGL-14		0.21	0.15	0.18	0.55	0.43	0.49	0.86	0.81	0.84
BPT-5204		0.28	0.26	0.27	0.74	0.65	0.70	0.97	0.92	0.94
WGL-2395		0.21	0.15	0.18	0.45	0.42	0.44	0.88	0.80	0.84
Divya		0.21	0.16	0.19	0.54	0.48	0.51	0.84	0.78	0.81
JGL-11727		0.22	0.20	0.21	0.65	0.50	0.58	0.86	0.82	0.84
Pothana		0.23	0.19	0.21	0.65	0.53	0.59	0.82	0.78	0.80
RNR-C-28		0.25	0.21	0.23	0.53	0.51	0.52	0.79	0.76	0.78
RNR-2354		0.21	0.18	0.19	0.47	0.45	0.46	0.81	0.79	0.80
RNR-2465		0.24	0.17	0.21	0.58	0.44	0.51	0.86	0.82	0.84
JGL-3855		0.23	0.22	0.23	0.49	0.47	0.48	0.82	0.79	0.80
NDLR-7		0.22	0.15	0.18	0.46	0.43	0.45	0.81	0.78	0.80
Surekha		0.22	0.16	0.19	0.49	0.45	0.47	0.81	0.77	0.79
RNR-2458		0.21	0.20	0.20	0.55	0.48	0.52	0.85	0.83	0.84
MTU-1001		0.27	0.23	0.25	0.70	0.64	0.67	0.91	0.88	0.90
Erramallelu		0.19	0.15	0.17	0.47	0.40	0.43	0.76	0.73	0.75
Bhadrakali		0.25	0.20	0.22	0.59	0.51	0.55	0.88	0.82	0.85
JGL-1798		0.23	0.24	0.24	0.60	0.53	0.56	0.89	0.84	0.87
Godavari isukalu		0.22	0.20	0.21	0.48	0.43	0.45	0.78	0.74	0.76
Kavya		0.23	0.20	0.22	0.50	0.44	0.47	0.78	0.75	0.77
MTU-1010		0.26	0.25	0.26	0.68	0.60	0.64	0.90	0.87	0.89
Chittimutyalu		0.20	0.18	0.19	0.46	0.42	0.44	0.81	0.78	0.80
WGL-32100		0.22	0.21	0.22	0.51	0.44	0.48	0.86	0.82	0.84
Varalu		0.21	0.19	0.20	0.44	0.35	0.40	0.80	0.75	0.78
JGL-1470		0.22	0.16	0.19	0.47	0.45	0.46	0.83	0.84	0.83
JGL-3844		0.23	0.21	0.22	0.52	0.47	0.50	0.80	0.78	0.79
JGL-3828		0.22	0.20	0.21	0.47	0.44	0.45	0.80	0.82	0.81
Mean		0.23	0.19	0.21	0.54	0.48	0.51	0.84	0.82	0.82
C.D (5%)	**Treatments (T)**	0.028			0.009			0.007		
	Genotypes (G)	0.030			0.032			0.028		
	T X G	NS			0.046			NS		

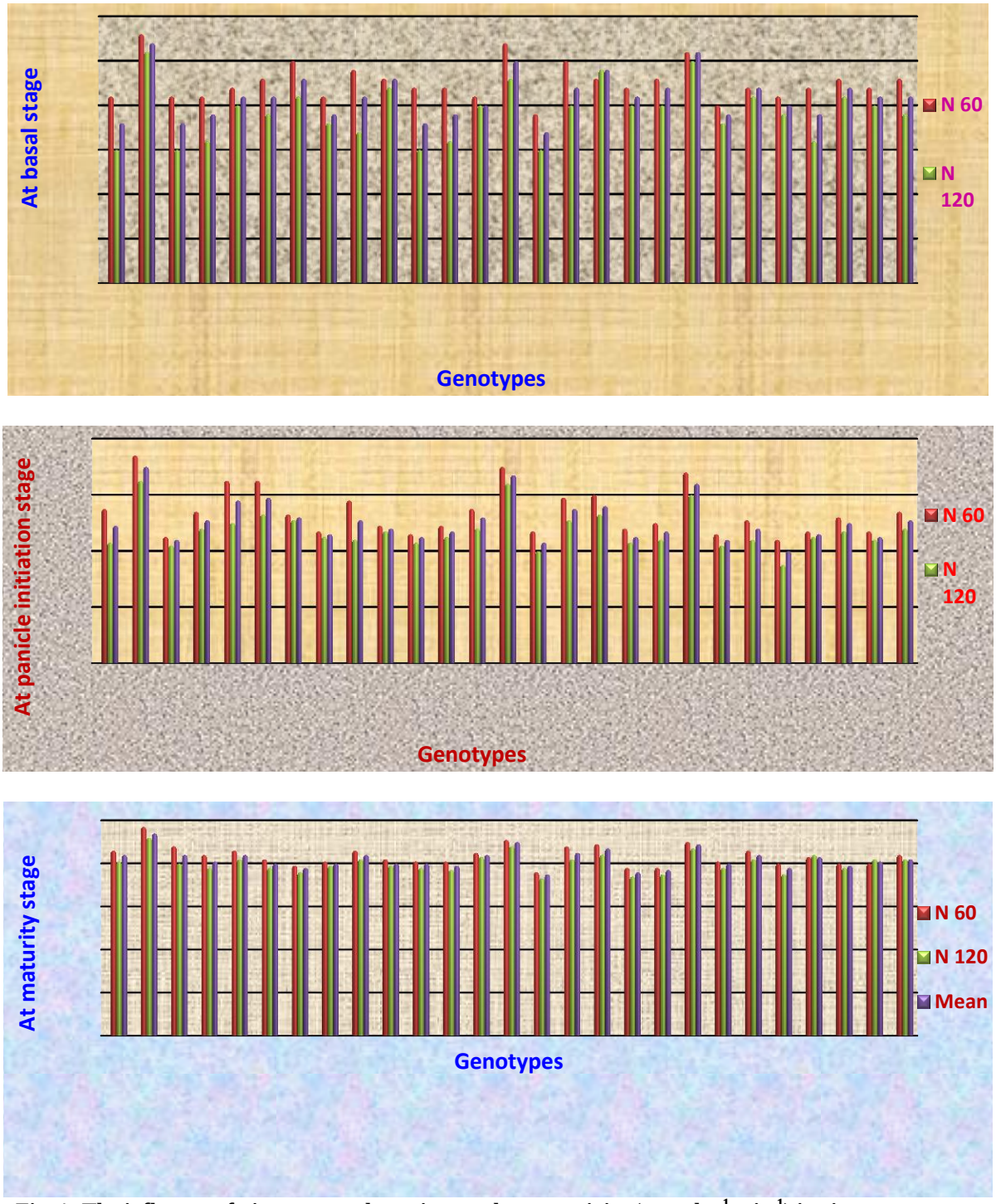

Fig. 1: The influence of nitrogen on glutamine synthetase activity (m mol g^{-1} min^{-1}) in rice genotypes at different crop growth stages

Table 2: The influence of nitrogen on nitrogen use efficiency (NUE) in rice genotypes

Genotypes		Nitrogen Use Efficiency (NUE)		
		60 kg N ha^{-1}	**120 kg N ha^{-1}**	**Mean**
WGL-14		53.33	36.86	45.09
BPT-5204		80.17	41.80	60.98
WGL-2395		82.93	43.99	63.46
Divya		78.99	40.47	59.73
JGL-11727		79.02	41.53	60.27
Pothana		81.15	42.05	61.60
RNR-C-28		70.31	36.68	53.50
RNR-2354		52.47	35.25	43.86
RNR-2465		51.21	33.93	42.57
JGL-3855		62.51	38.49	50.50
NDLR-7		51.22	31.65	41.43
Surekha		43.94	28.81	36.38
RNR-2458		64.11	37.53	50.82
MTU-1001		83.68	45.53	64.61
Erramallelu		48.72	30.34	39.53
Bhadrakali		80.36	41.68	61.02
JGL-1798		81.97	42.84	62.41
Godavari isukalu		68.83	39.77	54.30
Kavya		78.66	40.61	59.64
MTU-1010		83.58	44.48	64.03
Chittimutyalu		69.06	40.13	54.60
WGL-32100		57.42	39.14	48.28
Varalu		42.48	27.29	34.88
JGL-1470		60.81	40.15	50.48
JGL-3844		51.85	36.66	44.26
JGL-3828		68.24	38.62	53.43
Mean		66.42	38.32	52.37
C.D (5%)	**Treatments (T)**	0.855		
	Genotypes (G)	1.446		
	T X G	2.132		

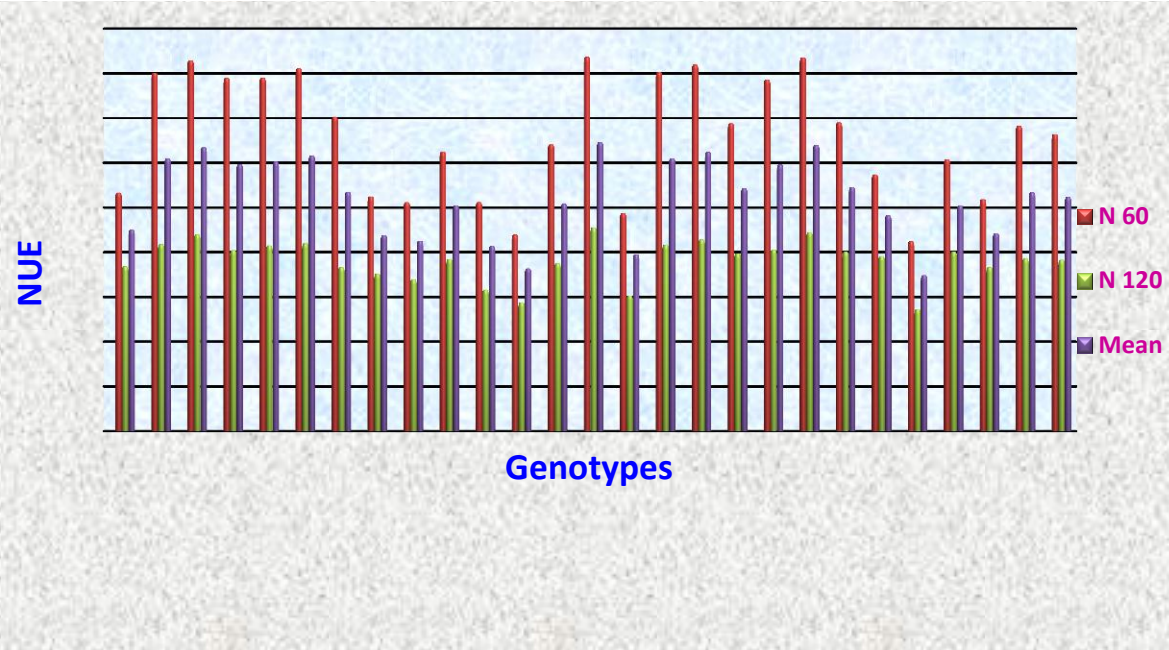

Fig. 2: The influence of nitrogen levels on nitrogen use efficiency (NUE) in rice genotypes

CONCLUSION

Glutamine synthetase (GS) is a key enzyme for nitrogen assimilation, which regulates nitrogen metabolism. Synthesis and transformation of amino acid could be promoted by improving the activity of glutamine synthetase, thus enhancing nitrogen movement in the metabolism. GS activity is one of the selection criteria to identify nitrogen use efficient cultivars and their use in developing mapping population for high NUE. GS activity was more in plants growing at low N levels than that at high N levels in rice. Maximum glutamine synthetase activity was recorded in the genotype BPT-5204 when 60 kg N ha^{-1} (0.94 μ mol g^{-1} min^{-1}) was applied at maturity stage as compared to application of 120 kg N ha^{-1}.

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