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Effects of Nitrogen Limitation on Antioxidant Enzymes, Chlorophyll Content and Grain Yield of Rice Genotypes

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TVK, PV, YVR, KNS and RK executed the work, analyzed the data and prepared draft. Authors BS, ISR, MSB and VJ rendered technical help and field work. Author DS Planning, scientific discussions and statistical analysis. Author PRR wrote the protocol. Authors CNN, KS, LVS and DPL revised and edited the manuscript. Author SRV conceived work design and overall supervision. All authors read and approved the final manuscript.

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ABSTRACT

Nitrogen is one of the important elements for rice production. Nitrogen utilization was rapidly increased from the time of green revolution, which resulted in environmental hazards. Increasing rice yield with optimized usage of fertilizers has become important for sustainable agriculture. A field study was conducted to study the effects of nitrogen limitation on antioxidant enzymes, chlorophyll content and yield of four rice genotypes. Nitrogen limitation has increased the antioxidant activity

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and membrane lipid peroxidation of the studied genotypes and reduced leaf chlorophyll, yield components and nitrogen uptake. Grain yield and total dry matter were positively associated with plant nitrogen uptake under both the nitrogen levels. Among the studied genotypes, narendradhan 359 showed relatively higher antioxidant activities, leaf thickness, higher grain yield, while pooja showed low yields under due to limited N conditions with lower grain yields efficiency. Among the studied genotypes narendradhan 359 could be chosen for further yield increment under limited nitrogen conditions.

Keywords: Rice; nitrogen; antioxidant enzymes; grain yield.

1. INTRODUCTION

Rice is considered to be one of the important crops of the world not only for its food source, also for employment generated from cultivation. More than 2.4 billion people depend on rice for livelihood. In India more than 70% of the population is supplemented with 30-76% of the diet requirement from rice, with 44.5 million hectares under cultivation [1]. Before the time of green revolution rice was cultivated in minimal soil fertility conditions. The rice production was increased many folds from period of green revolution to the twentieth century, with the usage of fertilizers [2-3]. Compared to the N, P, K sources used in rice cultivation, nitrogen fertilizers are used in large quantities, as nitrogen being one of the indispensable elements for cellular systems. Nitrogen is present in biosphere in different forms, of all molecular nitrogen (N_2) represents 80%. Nitrogen being an important macronutrient, which is routinely used to increase crop yields even though the efficiency was lower. Increased food production in the world over the past forty years is accompanied by increased use of nitrogen fertilizers by seven fold [4]. Nitrogen is available in soil as mobile forms of NH_4^+ and NO_3^-, crop plants are able to utilize only 30-40% of the applied nitrogen [5] and the remaining is lost to the environment [6], leading to hazardous effects on the environment such as ground water contamination, nitrous oxide emissions. In India nitrogen utilization of agricultural field crops is only around 30% [7] and the remaining amount is lost to the environment. Urea is most widely used as nitrogen source in rice cultivation [8] and its use has augmented significantly during the past 25 years [9]. Besides loss of nitrogen to the environment, cost of cultivation is also becoming a great hindrance as the demand and price of nitrogen fertilizers are increasing. Despite of being environmentally and economically costly, increased application of N fertilizers along with improved cropping methods was the key factor for supplementing sufficient dietary needs to the growing population [10,4]. Increasing crop yields to meet the demand of ever increasing population along with environmentally secure agriculture is prime concern. The present global rice cultivation is concerned about developing nitrogen use efficient cultivars, which could be safer to the environment and farmer for sustainable agriculture. Nitrogen being indispensable to the plant, deficiency could lead to diversified physiological effects. Hence understanding the effects of nitrogen limitation on plant functions could open new chances to understand the key processes to know routes of improving nitrogen deficiency tolerance. Nearly 70% of the leaf nitrogen is allocated to chloroplasts, which are crucial in biological yield production [11] through photosynthesis. Antioxidant enzymes are the defense machinery of the plant system to combat reactive oxygen species produced by various cellular metabolic activities. Extrinsic/Intrinsic factors such as pollutants, pathogens, metals, temperature extremes and nutrient deficiencies causes rise in ROS in cells. Activities of antioxidant enzymes under biotic and abiotic stress were well documented, but relatively less information is available on the activities of AOX under nitrogen limited condition. Hence the present study was aimed at studying antioxidant enzyme responses, chlorophyll content and grain yield under limited nitrogen conditions, which could clear avenues for improvement of rice yield under low nitrogen conditions.

2. MATERIALS AND METHODS

A field experiment was conducted during wet season 2014, at Indian Institute of Rice Research, Hyderabad with four popular rice genotypes (Dhanarasi, NDR359, Pooja and Swarnadhan) to investigate the effect of nitrogen limitation on the activities of antioxidant enzymes, chlorophyll content and grain yield. All the entries were grown under low nitrogen (low N) plot of dimensions 18 m length and 23 m width (without externally added nitrogen / native nitrogen) and recommended N (rec N) plot (@

100 kg N/hec) of dimensions 35 m length and 23 m width. Soil properties of the experimental plots are monitored during the experiment (Table 1).

2.1 Preparation of Enzyme Extract

One gram of leaf sample was extracted in 10 ml of 0.1 M, pH 7.5 phosphate buffer containing 0.5 mM EDTA, followed by centrifugation and the collected supernatant was used as an enzyme source for estimation and all the operation was carried at 4℃ [12].

Table 1. Soil properties of the experimental field

Parameter	Low N	Rec N
pH	7.46	7.42
Electronic conductivity (dS/M)	0.29	0.28
Organic carbon (%)	0.66	0.72
Available N (kg/ha)	233	252
Available P2O5 (kg/ha)	50	61
Available K2O (kg/ha)	721	789

2.2 Estimation of Antioxidant Enzyme Activity

SOD activity was estimated according to the procedure described by Dhindsa [13]. POD activity was assayed according to Castillo [14]. The activities were expressed as unit min^{-1} gram fresh $weight^{-1}$.

2.3 Estimation of Lipid Peroxidation

The level of lipid peroxidation was estimated by measuring Malondealdehyde (MDA) content; following the method of Heath and Packer [15]. The concentration of MDA calculated by using the extinction coefficient of 155 $mM^{-1}cm^{-1}$ for MDA at 532 nm.

2.4 Leaf Chlorophyll Content, Specific Leaf Area (SLA) and Specific Leaf Weight (SLW)

Young fresh leaf blades were collected during early hours of day and grind with chilled 80% acetone and the extract was centrifuged at 4℃ for 5 min x g were then determined with spectrophotometer (Spectrascan UV 2600, Toshniwal Instruments Pvt. Ltd., India) at nanometers of 663.2, 646.8 and 470.0 [16]. Leaf pigment concentrations were calculated according to Lichtenthaler and Wellburn [17]. Specific leaf area (SLA) and specific leaf weight (SLW) were calculated from leaf area and leaf dry weight.

2.5 Grain Yield

Yield attributes were recorded at physiological maturity by harvesting five hills from variety and were done in three replications. Nitrogen content in grain and straw were estimated according to Kjeldahl method [18]. Grain yield efficiency Index (GYEI) was calculated according to Fageria and Baligar [19].

2.6 Statistical Analysis

Analysis of variance was done using Statistix 8.1 (Analytical Software Inc. USA) and statistical significance was determined by performing Fischer's LSD test.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Enzymes and Lipid Peroxidation

Significant differences were observed ($p<0.05$) in SOD and POD activities and lipid peroxidation between treatments (Table 2). N deficiency has resulted in increase in mean SOD and POD activity by 7 and 1.4 folds. Under low N conditions SOD activity was ranged from 37.85 u/min/g FW (swarnadhan) to 18.49 u/min/g FW (pooja). N limited condition resulted in a maximum of 10 fold change in SOD activity in NDR359 and least of 2 folds in pooja. Under recommended N conditions SOD activity was ranged from 10.76 u/min/g FW (pooja) to 3.68 u/min/gm FW (NDR359) with a mean of 5.75 u/min/g FW. Lin et al. [20] reported an increase in SOD activity in leaves of rice seedlings under N deficiency along with cd stress. Peroxide dismutase activity was increased under limited N condition by 1.4 folds in swarnadhan, followed by dhanarasi (1.3 fold), NDR359 and pooja (1.2 folds). Increased peroxidase activity in order to alleviate salt stress was reported by Sreenivasulu et al. [21]. Increase in APX activity was observed in H_2O_2 pre-treated rice seedlings under non heat conditions, which further protected rice seedlings from further Cd stress [22-23]. Neutralization of oxygen free radical produced in the cell are scavenged by superoxide dismutase results in the formation of H_2O_2 [24-27], this resultant H_2O_2 is further degraded into harmless compounds by enzymes such as peoxidase, catalase and other enzymes

[27]. Increase in POX activity might be an indication of plant's capability to cope with the N stress. Subjecting plants to environmental stress results in production of reactive oxygen species, and quenching activity of plant system will be upset resulting in oxidative damage [28-29]. Significant differences were observed in lipid peroxidation *($p<0.05$)* between treatments (Table 3). MDA content was ranged from 2.07 (narendradhan 359) to 2.63 (pooja) and 1.33 (narendradhan 359) to 1.87 (dhanarasi) under low and recommended N conditions respectively. Nitrogen limitation has resulted in 35% increase in malondealdehyde content, indicating greater extent of lipid peroxidation. Increased lipid peroxidation in rice genotypes under N deficient condition was reported by Huang et al. [30].

3.2 Chlorophyll, Leaf Area and Thickness

Significant differences were observed in leaf area, leaf thickness and chlorophyll content between treatments (Tables 4 - 5). Leaf area was ranged from 27.82 cm^{-2} (swarnadhan) to 38.32 cm^{-2} (pooja) and 39.23 cm^{-2} (swarnadhan) to 58.30cm^{-2} (dhanarasi) under low and recommended nitrogen conditions. Leaf thickness was ranged from 0.15 microns (narendradhan) to 0.20 microns (dhanarasi) with a mean of 0.174 microns under low N condition. Reduction in leaf area due to N limitation was more in dhanarasi (51%) and least in pooja (12%) with a mean of 35%. Application of nitrogen resulted in increase of mean leaf area and leaf thickness by 47% and 14% respectively. Similar results were reported by Mhaskar et al. [31], Metwally et al. [32]. Under low nitrogen conditions total leaf chlorophyll ranged from 2.5 (dhanarasi) to 3.53 (narendradhan) mg gm^{-1} fresh weight. A similar trend was observed in chlorophyll A and chlorophyll B under low N conditions (Table 5).

3.3 Yield and Nitrogen Content

Significant differences were observed in panicle number, weight, grain yield and total dry matter between treatments *($p<0.05$)*. Nitrogen limitation resulted in lesser number of panicles (24%) and reduced panicle weight (33%), grain yield (37%), TDM (35%) and HI (5%). Among the genotypes pooja (67%) showed maximum reduction in panicle weight and least reduction was observed in narendradhan 359 (22%) due to N limitation (Fig. 1). Grain yield was greater in narendradhan 359 (323.4 g m^{-2}) and least in pooja (105.8 g m^{-2}) under low nitrogen condition. Under N recommended conditions grain yield was ranged from 320.9 g m^{-2} (swarnadhan) 401.5 g m^{-2} (narendradhan 359). Nitrogen uptake $plant^{-1}$ was reduced by 19% due to N limitation and it was more in swarnadhan (26%) and pooja (23%), followed by dhanarasi (15%) and narendradhan 359 (11%). All the genotypes were showed early flowering stage under N limited conditions, mean flowering was reduced by six days due to N limitation (Fig. 2). Inferior yield characters of genotypes grown under limited N conditions might be due to scarce nitrogen status of the plant. All the genotypes responded well to N application. Mean grain yield was increased by 87% with N application and pooja responded well to N application and lesser response was shown by narendradhan 359. A similar trend was observed by Ebaid and Ghanem [33], Chopra and Chopra [34], Singh c [35], Mhaskar et al. [31], Metwally et al. [32], Swamy et al. [36,37]. Application of nitrogen increased total dry matter, associated with increased nitrogen uptake (Fig. 3). Increased TDM under recommended nitrogen conditions might be associated with the increased leaf area and tillers [38,39] and nitrogen uptake. Narendradhan 359 was grain yield efficient followed by dhanarasi, swarnadhan and pooja (Fig. 4).

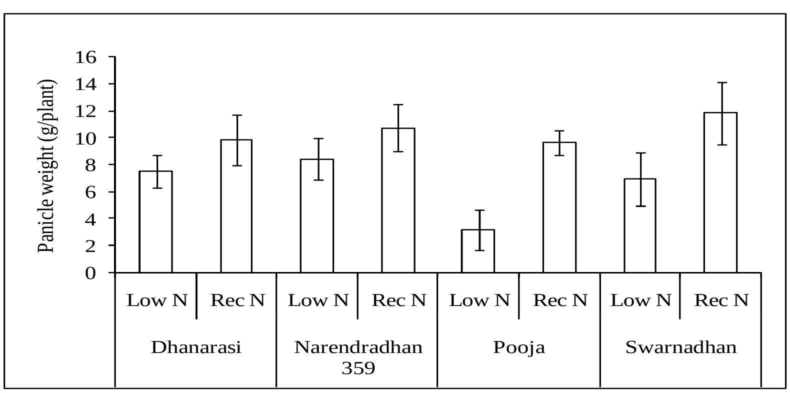


Fig. 1. Effect of nitrogen on panicle weight/plant

Table 2. Super oxide dismutase and peroxidase activities under low and recommended nitrogen levels *(LSD* represents *p<0.05)*

Entry	SOD (U/min/g FW)			POD (U/min/g FW)		
	Low N	**Rec N**	**Mean**	**Low N**	**Rec N**	**Mean**
Dhanarasi	35.85±0.36a	4.005±1.41d	19.927±0.885a	25.05±2.33ab	19.8±4.24cd	22.425±3.285a
Narendradh	35.995±1.69a	3.68±0.65d	19.837±1.17a	18.75±1.48cd	15.9±1.27d	17.325±1.375b
Pooja	18.485±1.76b	10.76±0.81c	14.623±1.285b	23.25±0.64bc	19.95±3.61cd	21.6±2.125a
Swarnadhan	37.845±0.65a	4.545±0.52d	21.195±0.585a	28.55±2.9a	20.55±1.48bcd	24.55±2.19a
Mean	32.044±1.115a	5.748±0.8475b		23.9±1.8375a	19.05±2.65b	
LSD (Treat)		1.3853			2.4148	
LSD (Entry)		1.9592			3.415	
LSD (TxE)		2.7707			4.8295	

Table 3. Lipid peroxidation measured in terms of malondealdehyde content under low and recommended nitrogen levels *(LSD* represents *p<0.05)*

Entry	MDA content in µmol g FW^{-1}		
	Low N	**Rec N**	**Mean**
Dhanarasi	2.14±0.11ab	1.875±0.3bc	2.0075±0.205ab
Narendradh	2.07±0.1ab	1.335±0.54c	1.7025±0.32b
Pooja	2.625±0.05a	1.845±0.16bc	2.235±0.105a
Swarnadhan	2.19±0.23ab	1.67±0.26bc	1.93±0.245ab
Mean	2.2563±0.1225A	1.6813±0.315B	
LSD (Treat)		0.3353	
LSD (Entry)		0.4741	
LSD (TxE)		0.6705	

Table 4. Variation in leaf area and leaf thickness (LSD represents p<0.05)

Entry	Leaf area			Leaf thickness		
	Low N	**Rec N**	**Mean**	**Low N**	**Rec N**	**Mean**
Dhanarasi	28.82±5.18bc	58.3±2.55a	43.56±3.865a	0.205±0.01ab	0.23±0.03a	0.218±0.02a
Narendradhan359	29.25±4.65bc	41.31±9.56bc	35.278±7.105a	0.15±0d	0.165±0.01cd	0.158±0.005c
Pooja	27.82±5.83c	43.77±5.35ab	35.795±5.59a	0.17±0.01cd	0.205±0.02ab	0.188±0.015b
Swarnadhan	38.32±9.86bc	39.225±9.78bc	38.773±9.82a	0.17±0cd	0.19±0.01bc	0.18±0.005b
Mean	31.05±6.38b	45.651±6.81a		0.174±0.005b	0.198±0.0175a	
LSD(Treat)		7.79			0.016	
LSD(Entry)		11.01			0.022	
LSD(TxE)		15.57			0.032	

Table 5. Influence of nitrogen on leaf chlorophyll concentration (LSD represents p<0.05)

Entry	Chlorophyll A			Chlorophyll B			Total chlorophyll		
	Low N	Rec N	Mean	Low N	Rec N	Mean	Low N	Rec N	Mean
Dhanarasi	1.57±0.1d	2.895±0.19a	2.23±0.145a	0.93±0. 07c	2.525±0.07ab	1.7275±0.07a	2.5±0.03c	5.425±0.12a	3.9625±0.075a
Narendradhan359	1.82±0.12cd	2.7±0.07ab	2.26±0.095a	1.71±0.02abc	2.315±0.08ab	2.0125±0.05a	3.525±0.15bc	5.02±0.15a	4.2725±0.15a
Pooja	1.89±0.04c	2.75±0.17ab	2.32±0.105a	1.425±0.18 bc	2.885±0.37a	2.155±0.275a	3.315±0.22bc	5.635±0.55 a	4.475±0.385a
Swarnadhan	1.81±0.14cd	2.455±0.05b	2.13±0.095a	0.97 5±1.34c	1.99±0.07abc	1.4825±0.705a	2.78±1.47c	4.455 ±0.02ab	3.6175±0.745a
Mean	1.77±0.1b	2.7±0.12a		1.26±0.4025b	2.4287±0.14 75a		3.03±0.4675b	5.1337±0.21a	
LSD (Treat)		0.1525			0.6102			0.7039	
LSD (Entry)		0.2156			0.863			0.9955	
LSD (TxE)		0.3049			1.2205			1.4078	

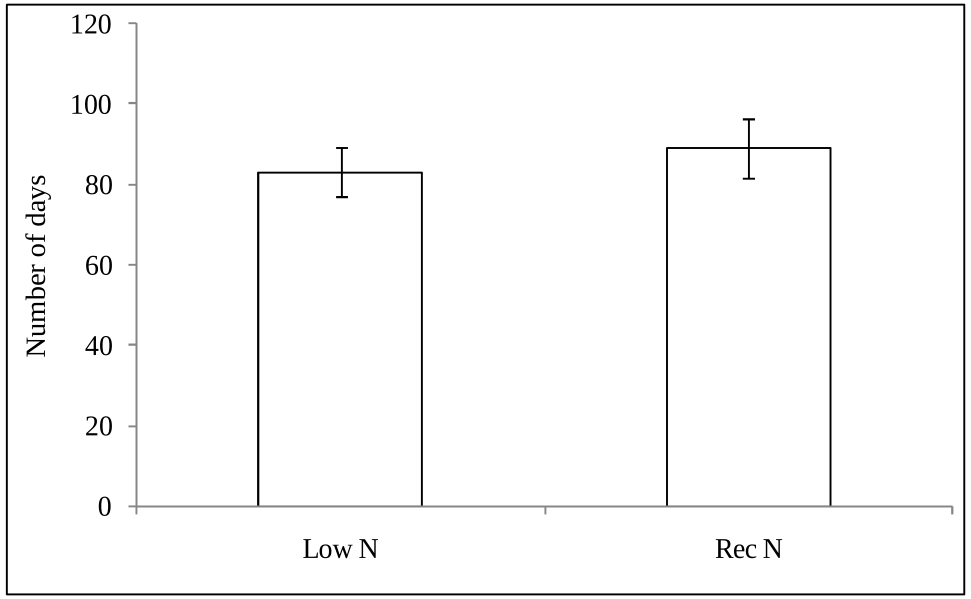
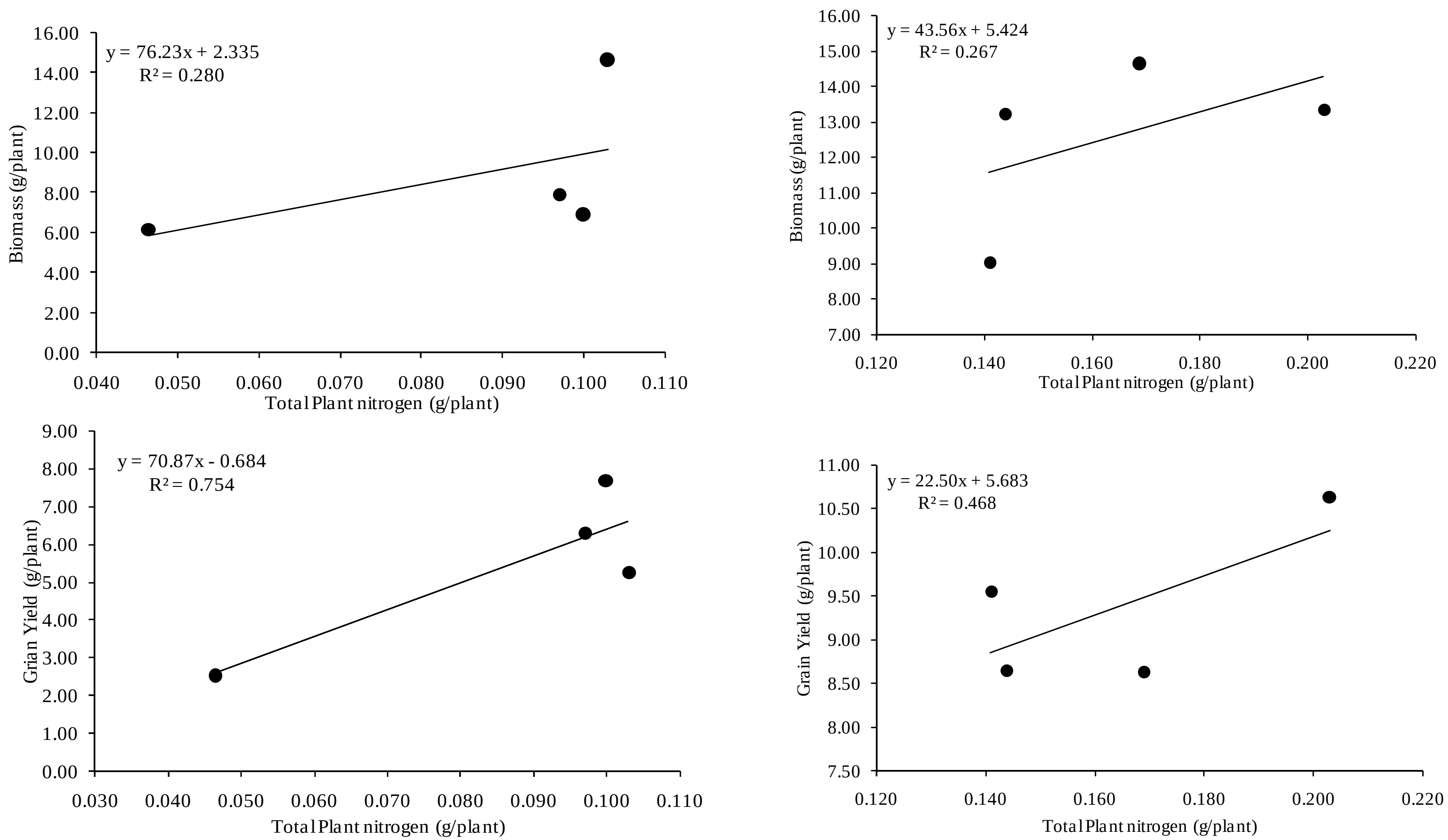


Fig. 2. Effect of nitrogen on days to flowering



Low nitrogen **Recommended Nitrogen**

Fig. 3. Relationship between nitrogen uptake with grain yield and total dry matter

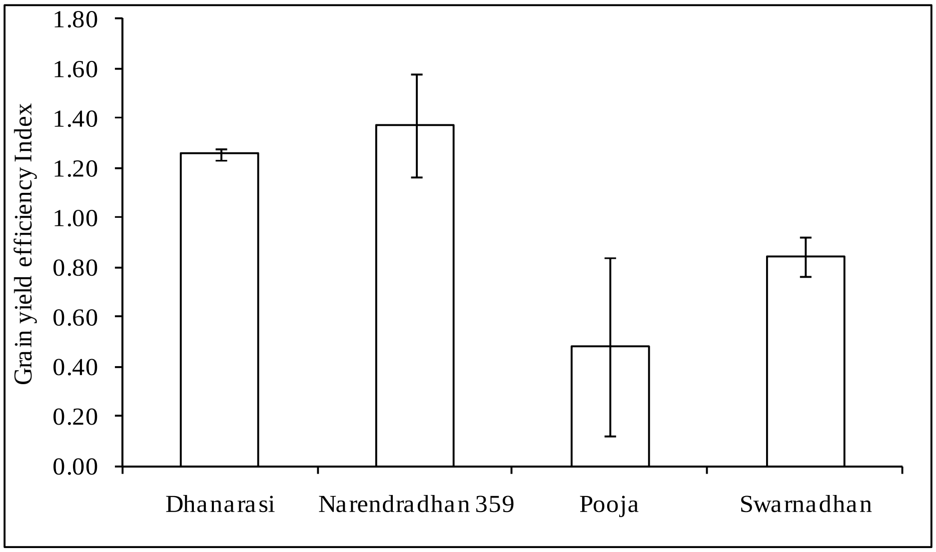


Fig. 4. Variation in grain yield efficiency index (GYEI) of genotypes

4. CONCLUSION

Nitrogen limitation has reduced leaf area, chlorophyll content, leaf thickness and specific leaf area, whereas specific leaf weight was increased upon N limitation. N limitation increased lipid peroxidation, enhanced the activities of antioxidant enzymes and reduced grain yield, nitrogen uptake. Nitrogen uptake was associated with grain yield and total dry matter under both the treatments. Among the tested genotypes, narendradhan 359 showed better tolerance to N limitation in terms of leaf chlorophyll content, leaf thickness, lipid peroxidation and antioxidant activity along with higher grain yield efficiency index, which can be further exploited in breeding programs under low nitrogen conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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