Effects of Nitrogen on Biochemical indices of Some Winter Rice (*Oryza sativa* L.) Crop under Low Light Condition

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Abstract— *Light is a critical natural resource for growth and development of rice crop. Rice grown in North East India especially Assam during kharif season (June-December) sufferes from natural low bright sunshine (800-900 hours i.e 50% of normal). On the otherhand, Nitrogen becomes a limiting nutritional factor. Because rice crop exhibits lower N use efficiency (≈33%) in subtropical regions (viz., North east India) due to heavy rainfall causing its loss by leaching or ammonia volatilization that contributes environmental pollution too. It's inquisitive to understand the physiological and biochemical changes in rice crop brought about by nitrogen under low light conditions. Therefore, a dose response study of N (0, 50, 100 kgha^{-1}) on eight winter (kharif) rice genotypes (Aki Sali, Senduri Sali, Rong Salpana, Bodumoni Sali, Kati Sali, Bordubi Sali, IR-8 (low light susceptible), and Swarna Prabha (low light tolerant) was performed applying N in splits as basal. In the study, 50 Kg Nha^{-1} was optimal in regulating most of the plant biochemical traits under low light condition. As such, Chlorophyll contents, NR activity, N content, NUE, Carbohydrate contents (Starch and Reducing sugar) were maximum at the optimum N level as compared to the control under low light condition.*

Keywords— Choloriphyll, Carbohydrate, Low light, Nitrogen, NUE, NR activity, PPFD.

I. INTRODUCTION

Rice (*Oryza sativa* L.) is the world's single most important food crop, being the primary food source for more than one-third of the world's population (Shaiful-Islam *et al.,* 2009) as concerned to food security (Jianxin et al., 2023). About 90% of all rice grown in the world is produced and consumed in the Asia region (Viraktamanth, 2007; Bandumula, 2018). In India, it is estimated that rice demand in 2030 will be 142.2 million tons (Goyal and Singh, 2002).

Among the different classes of rice, Kharif rice (Winter rice) accounts for nearly 72% of the total area (Yoshida, 1972; Anonymous, 1987). More than half of the area (55%) under rice cultivation is rain-fed, and 80% of this rain-fed rice area lies in eastern and NE India. Apart from being vulnerable to the vagaries of monsoon along with other biotic and abiotic stresses, occurrence of low light intensity is also a considerable factor. The north eastern region of India covers about 60% of total rice-cropped area with a 48% of the total production only. Numerically about 55% rice cultivation is rain-fed, and 80% of this rain-fed rice area is distributed in eastern and NE India (Adhya *et al.,* 2008; Dutta et al., 2017).

In Assam, rice among the cereals is the single most predominant staple food crop covering about 75% of the gross cropped area. Rice grown in Assam during Kharif season is ensured by sufficient water in the soil by natural precipitation. Rice crop is well adopted to the existing soil water condition, and survives in varying depth of water on the surface of the soil during the peak monsoon season. Kharif rice is characterised by its tropical agro climatic environment of low sunshine hours, high temperature and humidity. It's grown in different land situations like uplands, medium lands and low lands with varying conditions of water availability. Due to extreme diversity in growing conditions in the state, the situation as a whole is not

favourable for higher productivity (Sengupta and Dasgupta, 1978). Rice plant requires about 1500 bright sunshine (BSS) hours for the period from transplanting to maturity. Instead, prevalence of only about 800-900 BSS hours (PPFD<500 $\mu Ms^{-1}m^{-2}$) during August to December in places like Northeastern region of India not only hampers the physiological efficiencies but also renders nutritional imbalance by retarding nitrogen uptake and ultimately the productivity of winter rice crop (Bharali *et al.*,1994; Bharali et al., 2020). It's because, solar radiation in tropics is one of the major climatic factors limiting grain yield in rice (Vergara *et al.*,1976). Low light intensity acts as a stress and determines rice productivity in tropical and subtropical climate. Lower incident radiation is mainly responsible for lower productivity rather than temperature in tropical and subtropical zones (Venkateswarlu and Visperas, 1987; Bormudoi and Bharali, 2016).

Nitrogen (N) at below or above the optimum concentration limits growth and development. Nitrogen is one of the integral constituents of compounds such as amino acids, proteins, RNA, DNA and several phytohormones (Wang and Schjoerring, 2012). In rice, nitrogen is required at early and mid tillering stages to maximize panicle number and to optimize filled spikelets at reproductive stage (Sathiya and Ramesh, 2009). Nitrogen use efficiency (NUE) in cereal crops is generally 33% only (Raun and Johnson, 1999). Rice grain requires N for protein, which is transported directly from the soil or from remobilization during canopy senescence. Under low light intensity, N acquisition is not a limiting factor but its utilization efficiency might be the major reason for reduced productivity. Adequate supply of nitrogen is required to maintain targeted crop yield, but its application costs highly to both the farmers and the environment (Frink *et al.,* 1999). Consequently, there is considerable interest in decreasing fertilizer N inputs by improving plant N use efficiency (Garnett *et al.,* 2009). In general, crops demand higher nitrogen to enrich NUE and carbon gain (Tashiro *et al.,* 1980), and even under abiotic stress situation (Torenpi and Bharali, 2018, Torenpi and Bharali, 2019). Moreover, it is imperative to understand how efficient the nutrient assimilation should be in the presence of external factors e.g. light intensity. However, information on the response of kharif rice genotypes to nitrogen under low light stress condition is scanty, and it deserves further investigation.

II. MATERIALS AND METHODS

A pot experiment (23rd July-6th December, 2016) in rice with eight genotypes *viz.*, Aki Sali, Senduri Sali, Rong Salpona, Bodumoni Sali, Kati Sali, Bordubi Sali, IR 8 (LL susceptible) and Swarna Prabha (LL tolerance), replicated twice following three factorial CRBD (completely randomized block design) was conducted during the winter (kharif) season of Assam at the Department of Crop Physiology, Assam Agricultural University, Jorhat. The experimental site is geographically positioned at 26°45′ N latitude, 94°12′ E longitude having an elevation of 87 m above mean sea level. The crop growing season received higher total rainfall (917.1mm), lower cumulative bright sunshine (745.5 hours), and higher monthly mean RH (67-99%).

In the pot culture, thirty days old seedlings were transplanted on the earthen pots (diameter: 35cm, height: 30cm; capacity: 6.5Kg soil). A mixture of sandy loamy soil with FYM @5tha^{-1} (≈0.50$gpot^{-1}$) was used to fill the pot. Nitrogen @ 0, 50, 100kg Nha^{-1} was applied in splits. 50% of Nitrogen along with whole doses of phosphorous (SSP) and Potassium (MOP) was applied as basal under both light regimes. Rest of Nitrogen was applied at maximum tillering stage under the natural and reduced light conditions. A constant water supply (2-3cm) was ensured from transplanting till post flowering stage in each of the light regimes.

Shade net (UV Stabilised HDPE tapes, white x white-25-35% shading (size: 6.5 m x 3 m) was fitted in a bamboo frame at 1.5m above the ground surface. The light treatment was given from the transplanting to harvesting stages of the crop. Light intensities below (I_b) and above (I_a) the crop canopies were measured using a Lux Meter (HTC TM) at maximum tillering and panicle initiation stages of the crop. The mean values of I_b and I_a in terms of $\mu Ms^{-1}m^{-2}$ (PPFD: Photon Flux Density = Lux X 0.0185 as per Dhopte et al., 1989; Hershey, 1991) were in the range of 401.81 $\mu ES^{-1}m^{-2}$ to 848.44 $\mu ES^{-1}m^{-2}$ and 156.08 $\mu ES^{-1}m^{-2}$ to 364.67 $\mu ES^{-1}m^{-2}$ under normal and shaded conditions respectively during maximum tillering to maturity stage of the crop (Table 1).

TABLE 1
Variation of Light Intensity (PPFD ($\mu ES^{-1}m^{-2}$) at different growth stages of rice crop

Stages of the Crop→	**Maximum Tillering stage**			**Panicle initiation stage**		
Light regimes ↓	I_a (above canopy)	I_b (below canopy)	Mean	I_a (above canopy)	I_b (below canopy)	Mean
Normal light:(NL)	1020.11	336.68	678.39	675.08	191.157	433.11
Low light (≈35% of NL): (LL)	478.52	250.82	364.67	343.04	119.93	231.49
Mean	749.32	293.75	521.53	509.06	155.54	332.3
Stages of the Crop→	**Flowering stage**			**Maturity stage**		
Light regimes ↓	I_a	I_b	Mean	I_a	I_b	Mean
Normal light:(NL)	1193.71	335.6	764.65	408.83	158.47	283.65
Low light (≈35% of NL): (LL)	503.18	176.65	339.92	394.8	153.69	274.24
Mean	848.44	256.12	552.28	401.81	156.08	278.945

2.1 Biochemical Parameters:

Chlorophyll content in leaves was estimated by 'Non Maceration' method (Hiscox and lsraelstam, 1979). 50mg of leaf slices (size ≈1cm^2) was suspended in test tubes containing 2ml of dimethyl sulfoxide (DMSO). The tubes were then incubated at 65°C for 20min in a hot water bath. The supernatant was decanted, then another 3 ml of DMSO was added to the residue and incubated at 60°C for 20 minutes. The supernatants were pooled, and the volume was made up to 10ml by adding DMSO. Absorbance (Optical density: OD) of the extract read at 645 and 663nm in a UV spectrophotometer were used to calculate Chlorophyll contents as Chl a (mgg^{-1} f.w.) = [12.7 (A_{663}) – 2.69 (A_{645})] x V/1000xW; Chl b (mgg^{-1} f.w.) = [22.9 (A_{645}) – 4.68 (A_{663})] × V/1000xW; and Total chlorophyll (mg g^{-1}f.w.) = [20.2 (A_{645}) + 8.62 (A_{663})] × V/1000xW, Where, V = final volume of extract (ml), W = weight of the samples taken (g), A_{663} = OD value at 663 nm, A_{645} = OD value at 645 nm, f.w. =Fresh weight (g)

In vivo **Nitrate Reductase** (NR) activity was estimated following the method of Keeper *et al.* (1971). Leaf samples were collected in ice bucket, brought to laboratory and cleaned properly with distilled water and wiped out excess water using filter paper. 0.20g of punched leaf (size 0.20mm) from leaf blade was taken in 50ml Erlenmeyer flask containing 2.5ml of 0.1 M phosphate buffer (pH 7.5) and 2.5ml of 0.1 M potassium nitrate. To this, 0.3g leaf sample was added in culture tubes, kept in a vacuum desiccators and infiltration was carried out for 2 minutes. A blank was run simultaneously using 2.5ml of 0.1 M phosphate buffer and 2.5ml of 0.1 M potassium nitrate without the leaf samples. The tube was incubated in water bath for 30 minutes at temperature of (33±2°C). The reaction was stopped by immersing the tubes containing the reaction mixture in boiling water for 15 minutes. After cooling the tube, 0.2ml of reaction mixture was taken in a test tube to which 1ml of sulphanilamide and 1ml of NEDD solution were added and kept for 15 minutes till the pink colour developed in the solution. 1% sulfanilamide solution was prepared with 3N HCl, whereas 0.02% N- (1-napthyl)- ethylene diamine dihydrochloride was prepared with distilled water. Finally, the sample solution was used for measuring absorbance at 540nm in spectrophotometer. The NR activity was calculated from the standard curve of nitrite assay and expressed as µmoles NO_2 formed g^{-1}fresh tissue weight hr^{-1}.

2.2 Estimation of Nitrogen content in rice:

The modified Kjeldhal method (Jackson, 1973) was used to determine total Nitrogen content, which is based on catalytic conversion of organic nitrogen into ammonia and its subsequent estimation by acid base titration. 500mg of oven dried samples were digested in a 100cm^3 Kjeldahl flask. Added the same amount of salt mixture (K_2SO_4 or Na_2SO_4 with $CuSO_4.5H_2O$ and metallic selenium @ 50:10:1 ratio) and 3 ml of concentrated H_2SO_4. Each tube was heated gently and then at increasing intensity up to 400°C after the initial vigorous reaction subsided. On continued heating for about 1-1.5 hour, when the digest become clear, allowed 30 minutes to cool it. The digested samples were diluted with 10ml of distilled water, mixed thoroughly and allowed the sample to cool again. Blank digestions were also carried out.

Automatic analyses of nitrogen were undertaken using the Kjeltech (Kelplus DISTYL-EM). The digested sample along with three rinses with distilled water was transferred into the micro-Kjeldahl distillation tube. The machine was calibrated to add automatically all the reagents to the digested sample e.g. 10ml of the 40 per cent NaOH to it. A 200-ml flask was prepared containing 10ml of 4% boric acid reagent and three drops of mixed indicator (0.3g of bromo cresol green and 0.2g methyl red in 400 ml of 90% ethanol). Placed the flask under the condenser of the distillation apparatus, and made sure that the tip of the condenser outlet was beneath the surface of the solution in the flask. Allowed the steam from the boiler to pass through the sample, distilling off the ammonia into the flask containing boric acid and mixed indicator solution for about 7 minutes. The tip of the condenser outlet was washed with distilled water. Then, titrated the solution of boric acid and mixed indicator containing the 'distilled off' ammonia with the standardized 0.1 N HCl. The reading was noted down each time and calculations were done as:

$$\text{Total N (\%) in sample} = \frac{[\text{(Sample titre - Blank titre) x normality of HCl x 14 x 100}]}{\text{Sample weight } (g)} \times 1000 \quad (1)$$

Nitrogen Use Efficiency (NUE) of the rice crop was calculated as:

NUE (%) = Grain yield (g plant^{-1}) × Grain N% (Goodroad, and Jellum, 1988) (2)

2.3 Estimation of Carbohydrates (sugar and starch) contents in leaf and grain:

For Reducing Sugar, one mililiter of the aliquot from digested sample was taken in test tube and made to a uniform volume of 2ml with distilled water. It was then mixed with 1.0ml of Somogyi`s copper reagent (Oser, 1979), heated in boiling water bath for 12 minutes, and then cooled in running tap water. One ml of arsenomolybdate reagent was added to it, and volume was made to 10ml. Absorbance was recorded in a spectrophotometer at a wavelength of 530nm. A blank and two freshly prepared glucose standards were also included with each set of samples. The sugar content was calculated from a standard curve drawn from freshly prepared glucose solutions and expressed in mg g^{-1} dry weight.

Starch content was determined by Anthrone Method (Mc Cready *et al*., 1950). The dry residue left after sugar extraction was powdered and known amount of it was hydrolysed by boiling with 10ml of 1N HCL in a glycerin bath at 112-115°C for 30 minutes. The residue was repeatedly washed with distilled water until a negative test of starch by iodine was obtained. The extract was collected and final volume was made upto 100ml. An aliquot (0.5-1.0ml) of the above extract was made to uniform of 2.5ml with distilled water. It was then mixed thoroughly with 10ml of freshly prepared anthrone reagent (100mg of anthrone in 100ml of chilled concentrated sulphuric acid) in a cold water bath. The tubes then kept in a boiling water bath for 15 minutes and cooled in running tap water. Absorbance was measured at 620nm in a spectrophotometer. A blank and two freshly prepared glucose standards were also included with each set of samples. Starch content was calculated by multiplying the glucose values by 0.9 and expressed in mg g^{-1}d.w.

All data for each character were analysed by Fisher's method of analysis of variance (Panse and Sukhatme, 1978). Significance or non-significance of variance due to the treatment effects was determined by calculating the respective 'F' values. The standard error of the means (S.Ed.) and critical differences were calculated as follows:

1) $\text{S.Ed } (\pm) = \sqrt{\frac{2 \times \text{error mean square}}{\text{Pooled number of replication}}}$

2) CD = S.Ed. × $t_{0.05}$, where, t = tabulated value of 't' at 5% probability level for appropriate error degrees of freedom.

III. RESULTS AND DISCUSSION

The present investigation revealed how biochemical traits of eight rice genotypes are regulated differently by nitrogen levels under low light stress condition.

3.1 Light intensities above and below canopy of the plant:

It was apparent from the results that light intensity gradually declined from panicle initiation to harvesting stage in both the low light and normal light regimes at canopy level i.e. above and below canopies. Varietal differences in light attenuation were observed below and above the canopy levels under both the light regimes **(Table 1)**. A pioneering work (Hoover *et.al.*,1934) on utilization of incident solar radiation by crops of different structures confirmed that about 20-25% of incoming radiation is reflected by plants, and a value of 20% will be assumed although it will vary with plant and the stage. Similarly, the present finding also tallies with the finding of Pandey and Seetharaman (1980) who indicated that low light intensity reduced rice yield

particularly when light intensity at the reproductive and ripening stage are low. Yoshida and Parao (1976) opined that the solar radiation requirement of rice crop differs from one growth stage to another.

3.2 Effect of low light on Nitrate reductase (NR) activity in leaf tissues at flowering stage of rice crop:

Data presented in **Table 2 (a)** indicate that there were significant differences of NR activity due to nitrogen only at flowering stage. Among the N treatments under low light condition, 50kg Nha^{-1} showed higher NR activity at flowering stage (9.90%). On the other hand, the variety Senduri Sali (27.93%) and Bordubi Sali (14.94%) had higher NR activity at 50kg N ha^{-1} as compared to control. Similarly, under normal light, Swarna Prabha (24.88%) and Bordubi Sali (57.18%) showed the higher NR activity at 50kg N ha^{-1}as compared to control. Murty *et al.* (1976) compared NR activity among several rice varieties under low light intensity. Lower activity of NR in leaf tissues of plant is an indication of the poor efficiency of varieties for nitrogen assimilation (Sarkar *et al.*, 1991). A decrease in NR activity under darkness or shade was attributed to lack of reducing energy (Beevers and Hageman, 1972; Wells and Hageman, 1974) or due to production of NR inhibitor (Jolly and Tolbert, 1978).

TABLE 2 (a)
Variation of Nitrate Reductase (NR) activity in leaf at flowering stage of rice crop under different light and nitrogen regimes

Varieties	NR activity (µmoleNO_2 formed g^{-1}f.w.hr^{-1})							
	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	2.025	2.225	1.675	1.97	2.455	2.36	1.55	2.12
Senduri Sali	1.625	2.255	2.23	2.03	1.9	2.3	2.19	2.13
Rong salpona	2.01	1.76	2.395	2.05	2.5	1.655	2.265	2.14
Bodumoni Sali	2.005	2.215	1.815	2.01	2.425	2.295	1.895	2.2
Kati Sali	1.685	2.27	2.38	2.11	1.885	2.2	2.595	2.22
Bordubi Sali	2	1.74	2.305	2.02	2.405	1.53	2.135	2.02
IR- 8	2.175	2.285	1.805	2.08	2.29	2.285	1.78	2.11
Swarna Prabha	1.77	2.22	2.36	2.11	1.675	2.23	1.84	1.91
Mean	1.91	2.12	2.12		2.19	2.1	2.03	
	Light (L) treatment	Nitrogen (N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.124	0.152	0.248	0.215	0.351	0.43	0.608	
CD(0.05)	NS	0.306	NS	NS	NS	NS	NS	

Nitrate reductase, the first in a series of enzymes that reduces nitrate to ammonia, has been shown to be sensitive to light (Zucker,1972). Light has a stimulatory effect on NR activity and reduction in irradiance lowered NR activity (Nicholas *et al.*,1976). It resulted in a decrease in the extractable enzyme content (Hageman *et al.*, 1961). The presence of nitrate in the supply medium is necessary for maintenance of NR activity where ambient concentration of nitrate is more important than the tissue nitrate content (Ezeta and Jackson, 1975; Aslam and Oaks,1976). Several plant species accumulate NO_3^- as a result of an excess of uptake over reduction, the accumulation is most frequently present under low light conditions (Blom-Zandstra and Lampe,1985; Cantliffe,1972).

3.3 Effect of low light on nitrogen use efficiency of rice crop:

Data presented in Table 2 (b) reveal that nitrogen use efficiency (NUE) in grains varied significantly due to Light, nitrogen and varieties. Nitrogen is a very important nutrient for plant growth and development. The estimation of NUE in crop plants is crucially needed to assess the fate of applied nitrogen and their role in improving maximum economic yield through efficient

absorbed or utilization by the plant. The diminishing trend of NUE at higher N rates pointed out that rice plants are unable to absorb or utilize N at higher rates or the rate of N uptake by plant cannot keep pace with the loss of N (Fageria and Baligar, 2005). Excessive nitrogen input and improper timing of N application lead to the poor NUE in rice production and cause problems such as environmental pollution, increased production cost, grain yield reduction, and could even lead to global warming (Peng *et al*.,2010). It was clear from our results that there were significant variations of NUE among the varieties. However, it was observed that under low light condition and at 50kg Nha^{-1}, the variety Senduri Sali (9.09%) maintained higher NUE as compared control. A lower NUE in plants was observed under 100kg Nha^{-1} treatment for Swarna Prabha (2.29%) and Senduri Sali (2.33%). Under normal light condition, at 50kg Nha^{-1} treatment, the variety Senduri Sali (12.97%) maintained the higher NUE.

TABLE 2 (b)

Variation of Nitrogen Use Efficiency (NUE) in grain of rice crop under different light and nitrogen regimes

Varieties	NUE (%)							
	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	45.92	47.79	44.23	45.98	51.61	55.54	50.02	52.39
Senduri Sali	58.01	67.1	55.68	60.26	64.44	77.41	62.52	68.12
Rong salpona	19.41	20.71	18.45	19.52	23.14	25.39	22.35	23.62
Bodumoni Sali	13.94	16.74	15.29	15.32	15.47	19.25	17.33	17.35
Kati Sali	22.18	23.43	22.47	22.69	24.93	27.69	25.54	26.05
Bordubi Sali	26.41	29.9	26.71	27.67	32.04	38.23	32.66	34.31
IR- 8	15.46	17.13	15.27	15.95	16.38	18.58	16.27	17.07
Swarna Prabha	31.14	35.02	33.43	33.19	34.8	40.49	37.7	37.66
Mean	29.05	32.22	28.94		32.85	37.82	33.05	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.757	0.927	1.513	1.311	2.14	2.621	3.707	
CD(0.05)	1.523	1.865	3.045	2.637	4.307	5.275	7.459	

Campbell and Davison (1979) suggested that inefficient use of N is associated with excessive vegetative growth. Decline in NUE can be attributed to lesser interception of light or increase in evapotranspiration that could result from excessive vegetation (Pearman *et al*.,1977). The present finding is close confirmation with the findings of Ladha *et al*. (1998). It's clear from our result that the crop varieties grown with 50kg N ha^{-1} had the higher increment of NUE as compared to control under the both low and normal light conditions i.e 3.17%, 4.97% respectively. Nonetheless, the magnitude and nature of N losses vary depending on the timing, rate and method of N application, source of N fertilizer, soil chemical and physical properties, climatic conditions and crop status (Zhu,1997). Decreases in N uptake efficiency at higher N rates have also been reported by Mae *et al*. (2006). Reasonable N fertilizer application can improve nitrogen use efficiency in rice (Miao *et al*., 2011). NUE is relatively low in rice as major part of N applied to rice is released as gaseous N, effecting environment and reducing economic efficiency of applied N (Hakeem *et al.,* 2012). Nitrogen utilization efficient genotypes can absorb and accumulate higher N, which influence growth and yield under low N condition (Mi et al., 2007).

3.4 Chlorophyll contents of rice crop:

In the study, chlorophyll 'a' varied non significantly among the light regimes, nitrogen and varieties at maximum tillering stage (Table 3a), but it was significant at panicle initiation (Table 3b) and flowering stages (Table 5a). Overall, low light increased

the Chlorophyll 'a' content in almost all the varieties as compared to normal light condition. Chlorophyll 'b' varied significantly among the Light treatments only at maximum tillering stage (Table 4a). At panicle initiation stage (Table 4b), and flowering stage (Table5b), chlorophyll 'b' varied non significantly among the light, nitrogen and variety. Total chlorophyll differed non significantly among the light, nitrogen and varieties at maximum tillering stage (Table 6a). However, there were significant effects of light and varieties on total chlorophyll contents, but nitrogen treatments could not bring significant effects at panicle initiation stage (Table 6b). Total chlorophyll was variable significantly among the the light regimes as well as nitrogen levels at flowering stage (Table 7). Overall, low light increased the total chlorophyll contents in almost all the varieties as compared to normal light condition**.**

TABLE 3
Variation of Chlorophyll 'a' in leaf of rice crop under different light and nitrogen regimes
(a) Chlorophyll 'a' (mgg-1f.w.) at Maximum tillering stage

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	2.39	2.96	2.44	2.59	2.07	1.43	1.56	1.68
Senduri Sali	2.41	3.08	3.09	2.85	1.71	1.56	1.67	1.64
Rong salpona	2.16	3.06	3.31	2.84	2.06	1.74	1.53	1.77
Bodumoni Sali	2.62	2.43	3.01	2.68	2.02	1.76	1.52	1.76
Kati Sali	2.38	2.51	4.04	2.97	1.16	1.84	1.92	1.64
Bordubi Sali	2.02	2.04	2.77	2.27	1.95	1.58	1.29	1.61
IR- 8	2.52	2.46	3.21	2.73	2.04	1.34	1.69	1.69
Swarna Prabha	2.12	3.83	2.91	2.95	1.48	2.16	2.1	1.91
Mean	2.32	2.79	3.09		1.81	1.67	1.66	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.135	0.165	0.27	0.234	0.382	0.468	0.661	
CD(0.05)	NS	NS	NS	NS	NS	0.941	NS	

Chlorophyll is the main pigment responsible for photosynthesis. Generally, the leaves of shade plants are thinner, their chloroplasts are larger and richer in chlorophyll than the leaves of sun plants (Kirk and Tilney-Bassett 1967; Govindjee et al., 2019). Nitrogen is not only the constituent of key cell molecules such as amino acids, nucleic acid, chlorophyll, ATP and several plant hormones, but also the pivotal regulator involved in many biological processes including carbon metabolism, amino acid metabolism and protein synthesis (Cai *et al*., 2012; Hanson *et al.*, 1981). Thus the processes like protein synthesis, role of nucleic acids and chlorophyll synthesis are related to nitrogen. Nitrogen is part of the enzymes associated with chlorophyll synthesis and the chlorophyll concentration reflects relative crop N status and yield level (Blackmer and Schepers, 1995).

From the present finding it was clear that at maximum tillering stage, under the low light condition, 100kg Nha^{-1} (24.91%) showed the higher increment of Chl'a' as compared to control. Similarly, under the normal light condition, the same dose of nitrogen showed the higher (9.03%) reduction of Chl'a' content as compared to control. At panicle initiation stage, under the both low and normal light conditions, 100kg Nha^{-1} (1.85% and 14.67%) showed the higher reduction of Chl'a' content as compared to control. Again at flowering stage, under the both low and normal light conditions, 100kg Nha^{-1} (9.47% and 25.29%) showed the higher increment of Chl'a' content as compared to control.

TABLE 3
Variation of Chlorophyll 'a' in leaf of rice crop under different light and nitrogen regimes
(b) Chlorophyll 'a' (mgg^{-1}f.w.) at Panicle initiation stage

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	4.04	4.77	4.8	4.53	3.79	3.53	3.17	3.49
Senduri Sali	4.35	4.36	2.86	3.85	4.02	3.23	2.55	3.26
Rong salpona	3.45	3.68	5.24	4.12	2.7	1.98	2.78	2.48
Bodumoni Sali	5.41	5.51	3.81	4.91	1.94	1.62	1.73	1.63
Kati Sali	3.4	4.19	3.91	3.83	2.64	1.97	1.87	2.16
Bordubi Sali	2.85	2.42	3.8	3.02	3.22	2.65	2.58	2.81
IR- 8	3.68	3.08	3.27	3.34	2.36	3.68	3.38	3.14
Swarna Prabha	3.56	2.53	2.51	2.86	3.14	2.48	2.68	2.76
Mean	3.84	3.81	3.77		2.97	2.58	2.59	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.148	0.182	0.296	0.257	0.419	0.513	0.726	
CD(0.05)	0.298	NS	0.597	NS	0.844	1.033	1.461	

TABLE 4
Variation of Chlorophyll 'b' in leaf of rice crop under different light and nitrogen regimes
(a) Chlorophyll 'b' (mgg^{-1}f.w.) at Maximum tillering stage

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	1.09	1.02	1.59	1.23	0.34	0.31	0.32	0.32
Senduri Sali	1.82	1.28	1.57	1.56	0.85	0.85	0.69	0.79
Rong salpona	1.69	1.47	1.76	1.64	1.01	0.61	0.66	0.76
Bodumoni Sali	1.21	1.76	1.19	1.38	0.8	0.48	1.05	0.78
Kati Sali	1.51	1.82	1.37	1.57	0.28	0.34	0.84	0.48
Bordubi Sali	1.72	1.07	1.69	1.49	0.63	0.68	0.8	0.7
IR- 8	1.65	1.26	1.78	1.56	1.03	0.71	0.69	0.81
Swarna Prabha	3.21	4.02	4.2	3.81	0.92	0.77	1.83	1.17
Mean	1.74	1.71	1.89		0.73	0.59	0.86	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.222	0.271	0.443	0.384	0.627	0.767	1.085	
CD(0.05)	0.466	NS	NS	NS	NS	1.544	NS	

TABLE 4
Variation of Chlorophyll 'b' in leaf of rice crop under different light and nitrogen regimes
(b) Chlorophyll 'b' (mgg^{-1}f.w.) at Panicle initiation stage

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	2.86	2.84	1.89	2.53	1	1.57	1.67	1.41
Senduri Sali	2.87	1.93	1.86	2.22	1.07	1.04	0.82	0.97
Rong salpona	2.26	2.6	2.91	2.59	1.15	2.32	1.65	1.7
Bodumoni Sali	3.89	3.34	2.58	3.27	0.32	0.82	0.61	0.58
Kati Sali	2.07	2.47	2.07	2.2	1.54	1.33	1.26	1.37
Bordubi Sali	1.42	1.47	2.62	1.84	0.8	1.08	0.78	0.88
IR- 8	1.63	2.03	2.34	2	0.98	1.11	0.91	1
Swarna Prabha	1.73	2.22	2.43	2.12	0.85	1.35	1.07	1.09
Mean	2.34	2.36	2.33		0.96	1.32	1.09	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.116	0.142	0.233	0.201	0.329	0.403	0.57	
CD(0.05)	NS	NS	NS	0.405	NS	NS	NS	

TABLE 5
Variations of Chlorophylls in leaf at flowering stage of rice crop under different light and nitrogen regimes
(a) Chlorophyll a (mgg^{-1}f.w.)

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	2.25	3.17	3.72	3.04	2.13	2.12	1.95	2.06
Senduri Sali	2.24	3.51	5.87	3.87	1.77	4.07	3.81	3.21
Rong salpona	4.42	3.98	2.84	3.74	2.38	3.53	4.23	3.38
Bodumoni Sali	3.81	4.43	4.86	4.36	3.28	2.61	4.69	3.52
Kati Sali	4.68	3.12	3.09	3.63	2.58	3.76	4.57	3.63
Bordubi Sali	1.07	1.74	1.37	1.39	2.12	2.18	2.27	2.19
IR- 8	4.45	4.19	4.11	4.25	3.65	2.32	3.37	3.11
Swarna Prabha	3.12	3.18	2.93	3.07	2.72	3.18	2.7	2.86
Mean	3.25	3.41	3.59		2.57	2.97	3.44	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.116	0.142	0.231	0.2	0.327	0.4	0.566	
CD(0.05)	NS	0.285	NS	0.403	0.658	0.806	NS	

TABLE 5
Variations of Chlorophylls in leaf at flowering stage of rice crop under different light and nitrogen regimes
(b) Chlorophyll b (mgg^{-1}f.w.)

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	1.61	0.96	1.06	1.21	0.57	1.41	0.4	0.79
Senduri Sali	1.41	1.76	2.16	1.77	0.41	1.34	0.65	0.8
Rong salpona	1.55	2.6	1.54	1.9	0.63	0.81	0.8	0.74
Bodumoni Sali	1.24	1.31	2.07	1.54	0.42	0.88	1.26	0.85
Kati Sali	2.16	2.33	1.92	2.13	0.43	1.28	1.1	0.94
Bordubi Sali	0.54	0.67	0.72	0.64	0.7	1.22	1.32	1.08
IR- 8	1.3	1.5	0.97	1.25	0.66	0.62	0.66	0.65
Swarna Prabha	0.88	0.63	2.64	1.38	0.79	0.89	0.53	0.74
Mean	1.33	1.47	1.63		0.57	1.05	0.84	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.095	0.116	0.19	0.164	0.268	0.329	0.465	
CD(0.05)	NS	NS	NS	0.331	NS	NS	NS	

TABLE 6
Variation of Total chlorophyll in leaf of rice crop under different light and nitrogen regimes
(a) Total chlorophyll (mgg^{-1}f.w.) at Maximum tillering stage

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	3.48	3.98	4.03	3.83	2.41	1.74	1.88	2.01
Senduri Sali	4.23	4.36	4.66	4.42	2.56	2.41	2.46	2.47
Rong salpona	3.85	4.53	5.07	4.48	3.04	2.45	2.29	2.59
Bodumoni Sali	3.83	4.19	4.2	4.07	2.88	2.44	2.57	2.63
Kati Sali	3.89	4.33	5.41	4.54	1.44	2.18	2.36	1.99
Bordubi Sali	3.74	3.11	4.46	3.77	2.58	2.26	2.1	2.31
IR- 8	4.17	3.72	4.99	4.29	2.97	2.05	2.38	2.46
Swarna Prabha	5.33	7.85	7.11	6.76	2.4	2.93	3.23	2.85
Mean	4.07	4.51	4.99		2.53	2.31	2.41	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.367	0.45	0.734	0.636	1.039	1.272	1.799	
CD(0.05)	NS	NS	NS	NS	NS	2.56	NS	

TABLE 6
Variation of Total chlorophyll in leaf of rice crop under different light and nitrogen regimes
(b) Total chlorophyll (mgg^{-1}f.w.) at Panicle initiation stage

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	6.9	7.61	6.69	7.06	4.79	5.1	4.84	4.91
Senduri Sali	6.22	5.79	4.22	5.41	5.29	4.28	3.47	4.34
Rong salpona	5.71	6.28	8.14	6.71	3.85	4.3	4.43	4.19
Bodumoni Sali	9.3	8.86	6.39	8.18	2.26	1.85	2.35	2.15
Kati Sali	5.47	6.66	5.97	6.03	4.18	3.3	3.13	3.53
Bordubi Sali	4.27	3.89	6.42	4.86	4.02	3.73	3.36	3.7
IR- 8	5.31	5.11	5.61	5.34	3.34	4.79	4.29	4.14
Swarna Prabha	5.29	4.75	4.94	4.99	3.99	3.83	3.75	3.85
Mean	6.06	6.11	6.05		3.96	3.89	3.7	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.209	0.255	0.417	0.361	0.59	0.722	1.022	
CD(0.05)	0.42	NS	0.839	NS	1.187	1.454	2.056	

TABLE 7
Variation of Total Chlorophyll at flowering stage of rice crop under different light and nitrogen regimes

Varieties	Total Chlorophyll (mgg^{-1}f.w.)							
	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	3.86	4.13	4.78	4.25	2.7	3.53	2.36	2.86
Senduri Sali	3.65	5.27	8.03	5.65	2.48	5.04	4.46	3.99
Rong salpona	5.97	6.58	4.38	5.64	3.01	4.34	5.03	4.12
Bodumoni Sali	5.05	5.74	6.93	5.9	3.7	3.49	5.95	4.38
Kati Sali	6.84	5.45	5.01	5.76	3.01	5.04	5.67	4.57
Bordubi Sali	1.61	2.41	2.09	2.04	2.82	3.4	3.59	3.27
IR- 8	5.75	5.69	5.08	5.5	4.31	2.94	4.04	3.76
Swarna Prabha	4	3.81	5.57	4.46	3.51	4.07	3.23	3.6
Mean	4.35	4.88	5.23		3.19	3.98	4.28	
S.Ed (±)	0.172	0.211	0.345	0.299	0.345	0.597	0.844	
CD (0.05)	0.347	0.425	NS	0.601	NS	1.201	NS	

In case of Chl'b' at maximum tillering stage, the higher increment was observed at 100kg Nha^{-1} (7.93% and 15.11%) under the low and normal light condition respectively as compared to control. At PI stage, under the both low and normal light condition,50kg Nha^{-1} (0.84% and 27.27%) nitrogen treatment showed the higher increment of Chl'b' content as compared to control. At flowering stage, under low light condition, 100kg Nha^{-1} (18.40%) showed the higher increment of Chl'b' content as compared to control. Similarly, under normal light condition, 50kg Nha^{-1} (45.71%) showed the higher increment of Chl 'b' content as compared to control

In case of total chlorophyll at maximum tillering stage, under the low light condition, 100kg Nha^{-1} (18.43%) showed the higher increment. Similarly, under normal light condition, 50kg Nha^{-1} (9.52%) showed the higher reduction as compared to control. At panicle initiation stage, under the low light condition, 50kg Nha^{-1} (0.81%) showed the higher increment, and 100kg Nha^{-1} (7.03%) showed the higher reduction of total Chl content as compared to control under normal light condition. At flowering stage, the higher increment of total chlrophyll content was observed at 100kg Nha^{-1} (16.28% and 25.46%) under the low and normal light condition as compared to control.

Murchie and Horton (1998); Yamazaki *et al.* (1999) reported that the leaves grown in low irradiance have lower rates of photosynthesis due to a low content of photosynthetic component per unit leaf area. The changes also occur at the single chloroplast level, the ratio of PSII to PSI has been shown to vary according to irradiance level. Plants grown under low light conditions have more peripheral light-harvesting complexes per PSII reaction center, and a higher amount of Rubisco per unit chlorophyll, and Cytochrome b/f complex per unit chlorophyll (Murchie and Horton, 1998; Beneragama and Goto, 2010). Liu *et al*. (2006) observed that the Ribulose bisphosphate carboxylase (Rubisco) activity in chloroplasts declines dramatically under low light conditions. Chl a and Chl b are important pigments which are involved in the absorption and transmission of solar energy (Wang, 2011 and Zhang *et al.*,2014). Variation in chlorophyll content produced in response to low light among varieties has been reported (Zhu *et al*., 2008; Liu *et al.*, 2009). Ren *et al*. (2002) suggested that tolerant varieties capture as much solar energy as possible under low light conditions through increased leaf area and higher chlorophyll 'b' content. It is also reported that low light negatively affects stomatal conductance (fewer stomata are produced per square millimeter), and results in enhanced concentrations of intercellular CO_2 in rice leaves (Meng *et al*., 2002; Yang *et al*., 2011). Restrepo and Garcés (2013) showed that the leaf chlorophyll content (SPAD readings) was higher in rice leaves under low irradiance. The chlorophyll concentration increased being more prominent in the Chl'b' fraction, leading to a lower proportion of Chl a to Chl b (Janardhan and Mutty 1980; Murty *et al*., 1976; Venkateswarlu *et al*., 1977). Venkateswarlu *et al*. (1977) and Hidema *et al*. (1991) showed that leaf chlorophyll content was lower in full sun conditions than in low irradiance environments. Differences in the leaf chlorophyll content among the light treatments could be the response of the plant to increase its protection against excess light, since plants show an increase in the xanthophyll cycle pool size under full light conditions (Bilger *et al*.,1995) and a regulated loss of chlorophyll and pigment proteins per chloroplast (Anderson,1986) as compared to other N-treatment.

3.5 Effect of low light on Nitrogen content in leaf tissue at different stages and in grain at harvest stage of rice crop:

There were no significant differences of nitrogen content due to the Light, Nitrogen and varieties at maximum tillering (Table 8a) and panicle initiation stages (Table 8b). At maximum tillering stage, among the N-treatments, the highest nitrogen content in plants was observed in case of 50 kg Nha^{-1} (2.17%), and the lowest was shown by 100 kg Nha^{-1} (1.27%) under low light condition. Similarly, under normal light condition, 2.21%. and 1.29% N were recorded. Among the N-treatments, nitrogen content was the highest in case of 50 kg Nha^{-1} (2.57%), and the lowest was in 100 kg Nha^{-1} (1.76%) under low light condition. The similar trend was found under normal light condition i.e 2.98% and 1.81% at 50& 100KgNha^{-1} respectively. Data presented indicate that there were no significant differences of nitrogen content due to the treatments of Nitrogen and varieties but it was significant because of the Light treatments at flowering stage (Table 9a). At this stage, Among the N-treatments, the highest nitrogen content was observed in case of 50 kg Nha^{-1} (2.15%), and the lowest was in 100 kg Nha^{-1} (1.82%) under low light condition. The similar trend was found under normal light condition i.e 2.19% and 1.82% at 50&100 KgNha^{-1}. At harvest stage (Table 9b), there were significant differences of N-contents.

TABLE 8
Variation of Nitrogen content in leaf of rice crop under different light and nitrogen regimes
(a) Nitrogen content (%) at maximum tillering stage

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	1.36	2.23	1.4	1.66	1.46	2.27	1.43	1.72
Senduri Sali	1.44	2.14	1.35	1.64	1.43	2.18	1.36	1.66
Rong salpona	1.33	2.18	1.25	1.58	1.33	2.15	1.28	1.59
Bodumoni Sali	1.45	2.32	1.42	1.73	1.53	2.43	1.46	1.8
Kati Sali	1.26	1.95	1.2	1.47	1.31	2.04	1.21	1.52
Bordubi Sali	1.48	2.26	1.35	1.69	1.5	2.31	1.37	1.72
IR- 8	1.24	2.16	1.08	1.49	1.27	2.11	1.1	1.49
Swarna Prabha	1.26	2.18	1.16	1.53	1.34	2.23	1.14	1.57
Mean	1.35	2.17	1.27		1.39	2.21	1.29	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.106	0.129	0.211	0.183	0.299	0.366	0.517	
CD(0.05)	NS	NS	NS	NS	NS	NS	NS	

TABLE 8
Variation of Nitrogen content in leaf of rice crop under different light and nitrogen regimes
(b) Nitrogen content (%) at Panicle initiation stage

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	1.81	2.81	1.78	2.13	1.84	2.89	1.8	2.17
Senduri Sali	2.14	2.45	2.12	2.23	2.18	3.5	2.17	2.62
Rong salpona	1.78	2.72	1.67	2.05	1.82	2.75	1.73	2.1
Bodumoni Sali	1.7	2.71	1.58	1.99	1.74	2.75	1.62	2.03
Kati Sali	1.81	2.08	1.82	1.9	1.89	3.06	1.82	2.26
Bordubi Sali	1.98	2.11	1.81	1.96	1.77	3.15	1.89	2.27
IR- 8	1.57	2.81	1.58	1.98	1.66	2.83	1.6	2.03
Swarna Prabha	1.84	2.93	1.79	2.18	1.91	2.94	1.84	2.23
Mean	1.82	2.57	1.76		1.85	2.98	1.81	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.123	0.15	0.245	0.212	0.347	0.425	0.6	
CD(0.05)	NS	NS	NS	NS	NS	NS	NS	

TABLE 9
Variation of Nitrogen content in leaf of rice crop under different light and nitrogen regimes
(a) Nitrogen content (%) in leaf at flowering stage

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	1.84	2.24	1.83	1.97	1.9	2.2	1.85	1.98
Senduri Sali	1.92	2.29	1.83	2.01	1.95	2.28	1.88	2.04
Rong salpona	1.91	2.34	2.08	2.11	2.03	2.38	1.95	2.12
Bodumoni Sali	1.78	2.17	1.67	1.87	1.81	2.2	1.72	1.91
Kati Sali	1.84	2.12	1.81	1.92	1.87	2.18	1.83	1.96
Bordubi Sali	1.86	1.98	1.73	1.85	1.85	2.05	1.77	1.89
IR- 8	1.76	2.04	1.71	1.83	1.81	2.11	1.72	1.88
Swarna Prabha	1.95	2.08	1.91	1.98	1.99	2.14	1.84	1.99
Mean	1.85	2.15	1.82		1.9	2.19	1.82	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.044	0.054	0.088	0.076	0.125	0.153	0.216	
CD(0.05)	NS	NS	NS	NS	NS	NS	NS	

TABLE 9
Variation of Nitrogen content in leaf of rice crop under different light and nitrogen regimes
(b) Nitrogen content (%) in grain at harvest

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	3.22	3.34	3.12	3.22	4.23	4.83	4.12	4.39
Senduri Sali	4.21	4.79	4.08	4.36	3.25	3.38	3.16	3.26
Rong salpona	1.76	1.82	1.71	1.76	1.8	1.92	1.75	1.82
Bodumoni Sali	1.11	1.33	1.24	1.22	1.15	1.38	1.29	1.27
Kati Sali	1.74	1.8	1.77	1.77	1.73	1.83	1.77	1.78
Bordubi Sali	2.55	2.87	2.59	2.67	2.59	2.92	2.63	2.71
IR- 8	1.91	2.02	1.88	1.93	1.93	2.05	1.92	1.96
Swarna Prabha	2.72	2.94	2.93	2.86	2.74	3.1	2.97	2.94
Mean	2.4	2.61	2.41		2.42	2.67	2.45	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.035	0.043	0.071	0.061	0.1	0.123	0.173	
CD(0.05)	0.071	0.087	0.142	0.123	0.201	0.247	0.349	

It was observed that there were no significant changes in nitrogen content in leaf tissues of the varieties under low light condition at maximum tillering to flowering stages of the rice crop. However, light treatments brought significant changes in N content in grain at harvest stage (Table 9b). Low light condition results in lesser amount of N allocated to panicles as compared to ambiant under natural light (Liu *et al*.,2014). At maximum tillering stage, under the low light condition both IR-8 and Swarna Prabha (0.92%) showed the higher increment of nitrogen content at 50Kg Nha^{-1}. But IR-8 (0.16%) and Aki Sali (0.04%) showed the higher reduction of N- content at 100Kg Nha^{-1} as compared to control. Similarly, under normal light condition, Bodumoni Sali (0.9%) showed the higher increment at 50kg Nha^{-1} and Swarnaprabha (0.2%) experienced the higher reduction of N-content at 100Kg Nha^{-1} as compared to control. At PI stage, under the 50kg Nha^{-1} nitrogen treatment, IR-8 (1.24%) maintained the higher increment of N- content, and Bordubi Sali (0.17%) had the higher reduction of N-content at 100kg Nha^{-1} under low light treatment, similarly under normal light condition, Bordubi Sali (1.38%) maintained the higher increment of N- content at 50kg Nha^{-1} nitrogen treatment, Bordubi Sali (0.12%) and Bodumoni Sali (0.12%) showed the higher increment and reduction of N- content at 100kg Nha^{-1} nitrogen treatment as compared to control. The source of N under low light intensity shifts towards NH_3 to optimize energy available for biosynthesis (Poolman *et al*.,2013). At flowering stage Rong Salpona (0.43%) exhibited the higher increment of N-content in leaf tissues at 50kg Nha^{-1} nitrogen treatment, and Bordubi Sali (0.13%) exhibited the higher reduction of N-content in leaf tissues at 100Kg Nha^{-1} level under low light condition. Similarly, under normal light condition, Bodumoni Sali (0.39%) exhibited the higher increment of N-content in leaf tissues at 50kg Nha^{-1} nitrogen treatment, and Swarna Prabha (0.15%) exhibited the higher reduction of N-content in leaf tissues at 100Kg Nha^{-1} level.

Likewise, under low light condition at harvest stage, in rice grain, the higher increment and reduction were observed in the variety of Aki Sali (0.58%), (0.13%) under N-treatment of 50Kg Nha^{-1} and 100Kg Nha^{-1} respectively as compared to control. Similarly under normal light condition, the same variety had the higher (0.6%) increment and reduction (0.11%) of grain nitrogen under N-treatment of 50Kg Nha^{-1} and 100Kg Nha^{-1} as compared to control.

Light intensity may also affect rice grain nitrogen as protein levels under low light condition (Ren *et al*., 2003). Glutamine synthetase (GS) and glutamate synthase (GOGAT) catalyze the assimilation of NH_4^+, which plays an important role in N metabolism in higher plants (Miflin and Lea, 1976). Some studies have confirmed that the GS/GOGAT cycle in rice grains only plays a limited role in N metabolism in rice grains (Yamakawa and Hakata,2010; Liang *et al*., 2011). The source of N under low light intensity shifts towards NH_3 to optimize energy available for biosynthesis (Poolman *et al*., 2013). Sahu and Murty (1976) also opined that nitrogen uptake at flowering is relatively high in wet season, and is reduced only after flowering. Greater accumulation of nitrogen, especially soluble N occurs in panicle during anthesis, and at a juvenile stage of grain development. Low light intensity influences the amount of nitrogen utilized for grain production (Pandaraju and Deb, 1976). Low light also decreases the amount of nitrogen (N) transported from culm and sheaths to panicles, which triggers N in leaves and culm-sheaths as well as a decrease in panicles while compared with the total amount of N in aboveground (leaves + culm + sheaths + panicles). The results showed that the amount of N allocated to panicles under low light conditions is lesser than that under natural light, and the amount of N used for the development of leaves and culm-sheath increases under low light conditions (Ren *et al*., 2003). Fageria (2003) reported that in cereals including rice, N accumulation is associated with dry matter production and yield of shoot and grain.

3.6 Effect of low light on Carbohydrate (Starch and Reducing sugar) contents in leaf tissue at different stages and in grain at harvest stage of rice crop

Data indicated that there was no significant impact of light on starch content, but starch content varied significantly due to nitrogen and varieties at maximum tillering, panicle initiation (Table 10ab), flowering (Table 11ab) and havest stages (Table 12ab) of the crop. Overall, low light reduced the carbohydrate content in leaf tissue at different stages and grain at harvest (Table 13ab) as compared to normal light condition. Low light primarily attributed to an insufficient supply of assimilates and decrease activity of a soluble starch branching enzyme involved in starch synthesis in grains (Tashiro and Ebata, 1975; Miizuno *et al*.,1992; Li T G *et al*., 1997; Ren *et al*., 2003).The reduction in carbohydrate content in leaf by reduced light is due to impairment of dry matter production at panicle initiation and even more reduction of it after flowering for partitioning into the developing grains at harvest (Janardhan *et al*., 1980). Rice quality is formed mainly through the synthesis and accumulation of starch and protein (Cai *et al*., 2004; Liu *et al*., 2008). Under conditions of sugar deprivation, substantial physiological and biochemical changes occur to sustain respiration and other metabolic processes (Journet *et al*., 1986; Ren *et al*., 2003; Wang *et al*., 2013). Low light during grain filling stage gives rise to significant decreases in rice grain amylose levels, suggesting that low light severely impacts rice starch pasting viscosity. Low light during the grain-filling stage results in a decreased supply of carbohydrates to grains as well as a decrease in starch synthase activity in grains, which directly inhibits grain filling and

enhances the occurrence of chalky rice (Tashiro *et al*., 1980; Li *et al*., 2006).Shading treatment during the mid-tillering or heading stages can markedly decrease photosynthesis rate in rice leaves, which leads to less soluble carbohydrate available for transport to the grain of rice (Yang, 2014).

TABLE 10

Variation of Carbohydrates (Starch & Reducing sugar) in leaf at Maximum tillering stage of rice crop under different light and nitrogen regimes

(a) Starch content (mgg^{-1}d.w.)

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	12.52	13.02	12.03	12.52	13.89	14.79	13.66	14.11
Senduri Sali	18.78	19.26	18.23	18.75	20.67	22.22	21.08	21.32
Rong salpona	17.65	18.77	16.89	17.77	20.78	20.19	18.67	19.88
Bodumoni Sali	17.89	18.92	17.07	17.96	19.11	20.14	19.16	19.47
Kati Sali	14.56	15.88	14.28	14.91	16.43	17.5	16.55	16.83
Bordubi Sali	16.62	17.66	16.23	16.83	18.88	19.76	18.2	18.94
IR- 8	15.81	16.04	15.42	15.75	17.62	18.55	17.98	18.05
Swarna Prabha	18.01	19.06	17.89	18.32	20.19	20.64	19.3	20.04
Mean	16.48	17.32	16.01		18.44	19.22	18.07	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.229	0.281	0.458	0.397	0.648	0.794	1.123	
CD(0.05)	NS	0.565	0.922	0.799	NS	1.598	2.259	

TABLE 10

Variation of Carbohydrates (Starch & Reducing sugar) in leaf at Maximum tillering stage of rice crop under different light and nitrogen regimes

(b) Reducing sugar content (mgg^{-1}d.w.)

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	3.13	4.39	3.02	3.51	5.14	6.73	5.09	5.65
Senduri Sali	3.58	4.79	3.67	4.01	5.68	6.93	5.16	5.92
Rong salpona	4.04	5.13	4.23	4.46	5.32	7.24	6.69	6.42
Bodumoni Sali	4.76	5.55	4.65	4.98	6.92	7.82	6.09	6.94
Kati Sali	4.83	5.98	4.34	5.05	6.72	7.7	6.67	7.03
Bordubi Sali	3.89	4.78	3.23	3.96	5.48	6.83	5.27	5.86
IR- 8	3.24	4.66	3.15	3.68	5.57	6.77	5.51	5.95
Swarna Prabha	5.04	6.49	4.89	5.47	6.81	8.22	5.56	6.86
Mean	4.06	5.22	3.89		5.95	7.28	5.75	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.233	0.285	0.465	0.403	0.658	0.806	1.14	
CD(0.05)	NS	0.573	NS	0.811	NS	1.621	NS	

TABLE 11
Variation of Carbohydrates content in leaf at Panicle initiation stage of rice crop under different light and nitrogen regimes
(a) Starch content (mgg^{-1}d.w.)

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	21.19	22.34	21.02	21.51	23.67	24.46	23.18	23.77
Senduri Sali	24.45	25.78	24.03	24.75	26.42	28.2	26.06	26.89
Rong salpona	26.33	26.78	25.78	26.29	28	28.48	26.51	27.66
Bodumoni Sali	26.09	27.09	26.29	26.32	28.06	29.34	27.5	28.3
Kati Sali	27.24	28.89	26.32	27.75	29.61	31	29.36	29.99
Bordubi Sali	23.29	24.56	27.75	25.2	25.13	26.21	25.32	25.55
IR- 8	22.47	23.95	23.65	23.35	24.91	25.52	24.55	24.99
Swarna Prabha	27.77	28.99	26.84	27.86	30.03	30.65	29.59	30.09
Mean	24.85	26.04	24.52		26.97	27.98	26.51	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.25	0.306	0.5	0.433	0.708	0.867	1.225	
CD(0.05)	0.503	0.616	NS	0.872	1.424	1.744	2.466	

TABLE 11
Variation of Carbohydrates content in leaf at Panicle initiation stage of rice crop under different light and nitrogen regimes
(b) Reducing sugar content (mgg^{-1}d.w.)

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	5.84	6.77	5.78	6.13	6.67	7.77	7.17	7.2
Senduri Sali	7.23	8.44	7.19	7.62	8.72	9.25	9.28	9.08
Rong salpona	5.05	6.23	4.89	5.39	6.56	8.77	6.73	7.35
Bodumoni Sali	6.67	7.03	6.23	6.64	8.16	8.45	7.49	8.03
Kati Sali	7.01	6.89	7.12	7.01	8.41	8.46	8.42	8.43
Bordubi Sali	6.29	6.78	6.11	6.39	7.81	8.33	8.51	8.22
IR- 8	4.88	4.95	4.69	4.84	6.41	7.25	7.35	7.01
Swarna Prabha	6.78	7.08	6.46	6.77	8.26	8.13	8.78	8.39
Mean	6.21	6.77	6.05		7.62	8.3	7.96	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.146	0.178	0.291	0.252	0.412	0.505	0.714	
CD(0.05)	NS	NS	0.586	0.508	NS	1.015	NS	

TABLE 12

Variation of Carbohydrates content in leaf at flowering stage of rice crop under different light and nitrogen regimes

(a) Starch content (mgg^{-1}d.w.)

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	17.12	17.79	17.02	17.31	16.28	19.21	17.27	17.59
Senduri Sali	18.03	19.77	17.69	18.49	19.31	21.85	18.23	19.79
Rong salpona	13.34	14.29	15.03	14.22	15.42	17.31	16.03	16.25
Bodumoni Sali	16.67	17.03	17.44	17.04	17.04	19.22	19.34	18.53
Kati Sali	17.89	18.88	17.03	17.93	20.05	21.12	18.28	19.82
Bordubi Sali	14.52	15.22	14.44	14.72	16.14	16.24	16.32	16.23
IR- 8	12.02	13.44	12.23	12.56	14.24	16.02	14.31	14.85
Swarna Prabha	17.26	18.29	17.11	17.55	20.42	20.34	19.18	19.98
Mean	15.85	16.83	15.99		17.36	18.91	17.37	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.247	0.303	0.495	0.428	0.7	0.857	1.212	
CD(0.05)	0.498	0.61	0.996	0.862	NS	1.724	2.439	

TABLE 12

Variation of Carbohydrates content in leaf at flowering stage of rice crop under different light and nitrogen regimes

(b) Reducing sugar content (mgg^{-1}d.w.)

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	2.67	3.44	2.57	2.89	3.65	5	4.22	4.29
Senduri Sali	3.39	4.78	3.31	3.82	4.2	5.77	3.9	4.62
Rong salpona	2.98	3.89	2.78	3.21	4.81	5.3	4.61	4.91
Bodumoni Sali	3.01	4.59	3.09	3.56	4.98	6.32	4.64	5.31
Kati Sali	3.42	4.46	3.45	3.77	5.24	6.04	6.07	5.78
Bordubi Sali	2.88	3.51	3.02	3.13	4.87	5.1	4.99	4.99
IR- 8	2.12	2.89	2.34	2.45	3.79	4.23	3.76	3.92
Swarna Prabha	3.28	4.67	3.12	3.69	5.75	5.79	5.7	5.75
Mean	2.96	4.02	2.96		4.66	5.44	4.73	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.176	0.215	0.352	0.304	0.497	0.609	0.861	
CD(0.05)	NS	NS	NS	0.613	NS	1.225	NS	

TABLE 13
Variation of Carbohydrates content in Grain at harvest of rice crop under different light and nitrogen regimes
(a) Starch content (mgg^{-1}d.w.)

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	39.23	40.23	39.03	39.49	40.15	41.21	38.93	40.1
Senduri Sali	43.42	45.05	42.82	43.76	45.14	46.16	43.14	44.81
Rong salpona	37.29	38.88	37.11	37.76	36.15	40.09	38.15	38.13
Bodumoni Sali	41.67	43.23	40.08	41.66	40.07	45.22	39.89	41.73
Kati Sali	42.11	43.02	41.96	42.36	45.26	45.05	42.13	44.15
Bordubi Sali	39.99	40.87	40.05	40.3	42.14	43.94	41.17	42.41
IR- 8	34.28	35.76	35.14	35.06	36.2	37.69	36.2	36.7
Swarna Prabha	40.67	41.22	41.07	40.98	42.27	44.29	40.27	42.27
Mean	39.83	41.03	39.77		40.92	42.95	39.98	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.33	0.404	0.659	0.571	0.932	1.142	1.615	
CD (0.05)	NS	0.812	1.327	1.149	1.149	2.298	3.249	

TABLE 13
Variation of Carbohydrates content in Grain at harvest of rice crop under different light and nitrogen regimes
(b) Reducing sugar content (mgg^{-1}d.w.)

Varieties	Low light				Normal light			
	T_0	T_1	T_2	Mean	T_0	T_1	T_2	Mean
Aki Sali	10.33	12.04	10.22	10.86	12.28	13.99	12.24	12.83
Senduri Sali	15.38	16.13	15.21	15.57	17.83	18.32	17.77	21.31
Rong salpona	11.23	12.92	11.02	11.72	13.37	14.84	13.02	13.74
Bodumoni Sali	14.86	15.44	14.23	14.84	16.85	17.82	16.49	17.05
Kati Sali	14.98	15.37	14.45	14.93	16.78	17.48	16.72	16.99
Bordubi Sali	12.46	13.72	12.31	12.83	14.33	15.79	14.5	14.87
IR- 8	11.12	12.82	10.95	11.63	13.06	14.89	13.55	13.83
Swarna Prabha	14.78	15.69	14.31	14.92	16.39	17.38	16.63	16.8
Mean	13.14	14.26	12.83		16.36	16.31	15.11	
	Light (L) treatment	Nitrogen(N) treatment	Varieties (V)	L × N interaction	L × V interaction	N × V interaction	L×N ×V interaction	
S.Ed (±)	0.41	0.502	0.82	0.71	1.159	1.42	2.008	
CD (0.05)	NS	NS	NS	1.428	NS	2.857	4.04	

From the investigation, it was observed that under the both light treatments 50kg Nha^{-1} produced higher carbohydrate content as compared to control. At maximum tillering stage, under the low and normal light treatments, 50kg Nha^{-1} showed the higher (4.84% and 4.05%) increment of starch. Similarly at panicle initiation and flowering stages, 50kg Nha^{-1}showed the higher increments (4.56% and 5.82%) of starch in leaf tissue under low light condition. Likewise under normal light condition, the same nitrogen treatment showed the higher increment (3.06% and 8.19%) of starch content as compared to control. Again, at

harvest stage i.e in grain, the higher increment of starch content was found in at 50kg Nha^{-1} (2.92% and 4.72%) under the low and normal light condition as compared to the control respectively. Likewise, at maximum tillering, panicle initiation and flowering stages, 50kg Nha^{-1} exhibited the higher increment (22.22%,8.27% and 26.36%) of reducing sugar content in leaf tissue under low light condition respectively. Under normal light condition also, the same nitrogen level exhibited the higher increment (18.26%,8.19%,14.33%) of reducing sugar as compared to control. Again, at harvest stage i.e in grain 50kg Nha^{-1} treatment showed the higher increment (7.85%) under low light condition, but 100kg Nha^{-1} reduced the highest (8.27%) per cent of reducing sugar as compared to control.

Tian *et al*. (2006) reported that under low light condition, starch, amylose and sucrose contents decreased, but ADP-glucose pyrophosphorylase (ADPGPPase) activity showed a little change. They also found soluble starch synthase activity and granule bound starch synthase activity decreased, while soluble starch branching enzyme (SSBE, Q-enzyme) activity and granule bound starch branching enzyme (GBSBE, Q-enzyme) activity increased. It is a widely recognized fact that in rice plants the smaller the nitrogen supply at the heading stage, the greater is the carbohydrate accumulation (Matsushima, 1957). Tian *et al*. (2006) also reported that under low light condition, starch, amylose and sucrose contents decreased, but ADP-glucose pyrophosphorylase (ADPGPPase) activity showed a little change. Li *et al*. (2005) reported that low light during the grain-filling stage results in a decreased supply of carbohydrates to grains as well as a decrease in starch synthase activity in grains, which directly inhibits grain filling and enhances the occurrence of chalky rice.

IV. CONCLUSION

In the study, significant reductions in grain N-content (0.01-0.08%), grain starch (0.16-5.23%), grain sugar (12.60-36.86%) and NUE (1.12-7.86%), were estimated under low light condition. The varieties Senduri Sali, Kati Sali and Swarna prabha performed well under low light condition. Low light increased chlorophyll a (3.49-66.80%), chlorophyll b (34.36-73.98%) and total chlorophyll (19.28-73.71%) in the varieties as compared to normal light condition as a measure of tolerance to low light intensity. Overall, the variety Senduri Sali exhibited its highest biochemical performance in terms of grain N- content (4.36%), NUE (60.26%) under low light condition. The variety is charcterised especially for higher NUE (67.10%) at 50$KgNha^{-1}$ under low light condition.

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