



Effect of Low Light Stress on Leaf Chlorophyll a, b, a+b, a/b, Catalase, Peroxidase, SOD and Yield of Long duration Rice Varieties (*Oryza sativa* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Light has a significant role in growth and development of plants because of its crucial role in photosynthesis and photo morphogenesis. If the amount of light intensity reaches the plants is reduced than the optimum level then it creates low light stress for plants and this problem is identified in the eastern and north-eastern region of India which is the major rice belt in our country. Therefore the present experiment was conducted during Kharif 2019 at plot no-4, Block-V of

Department of Crop Physiology and Biochemistry, NRRI, Cuttack to study the Low light effect on the biochemical changes and grain yield of long duration rice cultivars. In the present research, 9 long duration rice varieties along with 2 check varieties were exposed to 75% light and 50% light condition in comparison to control (100% light) to know the leaf chlorophyll behaviour and yield during *kharif* (July–November, 2019). Plants were grown in field condition with shade installation done 15 days after transplanting to impose low light stress in plants. Among the varieties, Swarnaprabha was found with the highest total chlorophyll content in 100% L (2.311 mg g⁻¹ fresh weight), 75% L (2.705 mg g⁻¹ fresh weight) and 50% L (3.684 mg g⁻¹ fresh weight) at 50% flowering stage. Similarly, Swarnaprabha was recorded with the highest chlorophyll at 7 days after 50% flowering. In both the cases, low light induced more chlorophyll in plants than normal light. Among the antioxidant enzymes, Peroxidase and Catalase exhibited an increased activity under low light stress, whereas Superoxide dismutase (SOD) exhibited decreased activity in low light stress. Besides, higher yield was recorded in normal light condition than 75% light and 50% light condition. Among the varieties Nasati Sali leads with higher yield in 100% (5.10 t ha⁻¹) and 75% (4.27 t ha⁻¹) light condition. On the other hand Swarnaprabha (3.05 t ha⁻¹) having highest yield at 50% light intensity.

Keywords: *Catalase; chlorophyll; peroxidase; rice; superoxide dismutase.*

1. INTRODUCTION

Light is the main energy source used by the plants to produce the photosynthesates through the process called 'photosynthesis' [1] and is an environmental signal used to trigger growth and structural differentiation in plants. Photosynthesis plays a pivotal role in providing energy and food for survival of the plants as well as animals [2]. Therefore, it is very much essential for the existence of all life on the earth. In photosynthesis, chlorophyll pigments play a major role in light energy perception [3] and fulfil the primary energy requirement of the process. Photosynthesis indirectly controls the translocation of photosynthates by keeping a balance between source production and sink requirement, Size of grain, quality of grain and ultimately the yield [4]. On the other hand, the light regulating morphogenesis in plants is known as photomorphogenesis [5] i.e. the light-regulated plant development. Photo-receptors like phytochrome and cryptochrome plays a key role in light perception at particular wavelength to induce photomorphogenic effect in plants [6].

In the absence of light, a negative value is observed in the net photosynthesis rate because of the releasing CO₂ through dark respiration [7]. Shade tolerance refers to the capacity of a given photosynthetic organism to tolerate low light levels and it is typically characterized by a set of morphological and physiological traits such as decrease in growth rate, light compensation point, dark respiration rate, net photosynthetic rate and chlorophyll a/b ratio, increase in quantum yield, chlorophyll content (both area⁻¹

and dry mass⁻¹ basis) and carbohydrate storage together with many other traits [8]. Chlorophyll accumulation in leaf under low light stress gives a positive impact on leaf photosynthesis and plant biomass production [9]. In South-East Asia and China, rice is mainly grown as a *kharif* crop and it frequently faces a poor light intensity problem at various growth stages. Light, the most important environmental factors, plays a critical role in plant development and metabolism [10, 11]. Additionally, light is crucial for photomorphogenesis and photosynthesis. Low light is a widespread abiotic stress in case of plant breeding and cultivation because of the insufficient availability of light during cloudy weather, snowfall etc. Substantially low light was shown to affect the agronomic traits and physiological processes, which includes photosynthesis and antioxidant characteristics, carbon and nitrogen fixation in plants [12-17]. In addition to slow growth rate, decreased leaf weight and number of flower buds, low light stress also causes reduced sugar and starch contents in eggplant, grape and rice [18]. Continuous cloudy days or rainfall during critical stages of growth, such as panicle differentiation or grain-filling stages, often induce decreased grain yield and quality [14,19, 20]. Reduction in the number of spikelet and enhanced spikelet sterility is the most prominent symptoms under low light intensity. Rice plant requires about 1500 bright sunshine (BSS) h for the period from transplanting to maturity. Instead, prevalence of only about 800–900 BSS hours during August–December in places like North-Eastern region of India hampers the physiological efficiencies, and ultimately the productivity of

winter rice crop [21]. It's because, solar radiation in tropics is one of the major climatic factors limiting grain yield in rice, therefore the present study was designed to understand the leaf chlorophyll changes under 75% and 50% light intensity in comparison to 100% light intensity and yield performance of 11 long duration rice varieties under decreased light intensity. Yield reduction in shaded conditions is more (32.2%–65%) than normal light conditions due to reduction in number of viable pollen and productive tillers [22-24]. Therefore the present experiment was designed to study the Low light effect on the leaf chlorophyll, antioxidant enzymes (Catalase, Peroxidase, SOD) and grain yield of long duration rice cultivars.

2. MATERIALS AND METHODS

A field experiment was conducted during *kharif* (July–November of 2019) in Plot number V6 at National Rice Research Institute, Cuttack. Sample collection was done at different stages of plant growth from field for better interpretation of research data.

2.1 Chlorophyll Estimation

Fully expanded leaf from the top of rice plant were collected from a field of normal light, 75% light and 50% light conditions at 50 % flowering stage and 7 days after 50% flowering stage and were kept in a beaker containing water to prevent the leaf from rolling. Chlorophyll content was estimated by the acetone method by Arnon [25]. The collected leaf was chopped and 25 mg of leaf samples was weighed and kept in a 10 mL test tube containing 80% acetone and incubated for 48 h at 4°C. The absorbance was recorded at 663 and 645 nm for chlorophyll using UV visible spectrophotometer (UV-Vis, Shimadzu, Japan). Chlorophyll A, B, total chlorophyll content, and chlorophyll a/b ratio were quantified using the following formula and expressed in mg g⁻¹ fresh weight:

$$\text{Chlorophyll } b \text{ (mg/g fresh weight)} = \frac{[22.9(A_{645}) - 4.68(A_{663})] \times \frac{\text{volume}}{\text{weight}}}{1} \quad (1)$$

$$\text{Chlorophyll } a \text{ (mg/g fresh weight)} = \frac{[12.7(A_{663}) - 2.69(A_{645})] \times \frac{\text{volume}}{\text{weight}}}{1} \quad (2)$$

$$\text{Chlorophyll } \frac{a}{b} = \frac{\text{Chlorophyll } a}{\text{Chlorophyll } b}$$

$$\text{Total chlorophyll } \left(\frac{\text{mg}}{\text{g}} \text{ fresh weight} \right) = \text{chlorophyll } a + \text{chlorophyll } b \quad (3)$$

2.2 SOD Activity

In this assay, around 0.2 gm of leaf tissue was taken and was homogenized in 1.2 mL of 0.2 M potassium phosphate buffer (pH 7.8 with 0.1 mM EDTA). The samples were centrifuged at 15,000×g for 20 min at 4°C and the supernatant was removed and is suspended in 0.8 mL of the same buffer. The combined supernatant was stored in ice and was used for NBT assay. In NBT assay, 200 µL of reaction buffer along with the sample was added and finally, 2 µL of riboflavin was added. As soon as riboflavin was added, the NBT changes its colour to purple. After the addition of riboflavin, the sample is kept under light conditions and another batch in dark conditions. After 10-15 mins, the absorbance is taken at 560 and 600 nm, and the SOD enzyme activity was expressed as unit i.e. mg. gm⁻¹ protein (the amount of enzyme utilized to inhibit 50% of NBT reduction was calculated as 1 unit) [26].

2.3 Peroxidase Activity (POD)

Plant supernatant was used as an enzyme extract in peroxidase assay. 2 mL phosphate buffer was added and shaken thoroughly and the absorbance was recorded in each 30 sec. the interval for 1 minute at 436 nm and expressed in units, degradation of one micro-molar H₂O₂ in one minute at 436 nm was calculated as one unit, and expressed in mg. gm⁻¹ protein [27].

2.4 Catalase Activity

In this assay, 20 µL supernatant was mixed with 3 mL phosphate buffer where plant supernatant was used as enzyme extract. After adding 10 µL of H₂O₂ sample was shaken thoroughly and the absorbance was recorded in each 30 sec. the interval for 1 minute at 240 nm and expressed as mg. gm⁻¹ protein [28].

2.5 Yield

During Harvest, 10 hill samples were taken randomly from each treatment. Grain was separated and dried in hot air oven and the dry weight of the grains was taken with help of weighing machine. After that yield was calculated, it was expressed in t ha⁻¹.

3. RESULTS AND DISCUSSION

3.1 Chlorophyll

Chlorophyll a, chlorophyll b, chlorophyll a+b, and chlorophyll a/b were recorded at 50% flowering stage and 7 days after 50% flowering stage and presented in Tables 1 and 2 respectively.

3.1.1 Chlorophyll a

At normal light conditions, the chlorophyll-a pigment was found highest in Swarnaprabha (1.807 mg g⁻¹ fresh weight) followed by IR-8 (1.682 mg g⁻¹ fresh weight), while, the least chlorophyll a was found in Torabali (0.976 mg g⁻¹ fresh weight) during *khariif* season of year 2019.

The amount of chlorophyll a was enhanced significantly in 75% light intensity and 50% light intensity as compared to 100% light intensity. Although, all the varieties showed an increased chlorophyll a content at 75% and 50% light intensity, Swarnaprabha leads with highest chlorophyll content of 2.080 mg g⁻¹ fresh weight and 2.807 mg g⁻¹ fresh weight respectively. At 7 days after 50% flowering plant showed a similar trend with enhanced chlorophyll level with Swarnaprabha as the leading variety content at 100% and 75% light intensity (1.802 mg g⁻¹ fresh weight and increase in chlorophyll b is much higher than chlorophyll a (Table 2) and the photosynthetic pigment shift under low light is one of the major reasons for it [29].

Table 1. Effect of low light stress on chlorophyll a, b, a+b and a/b (mg g⁻¹ fresh weight) at 50% flowering stage

Light intensity (L)	Variety (V)	Chl-a	Chl-b	Chl(a+b)	Chl-a/b
		2019	2019	2019	2019
L1 (NL)	Swarnaprabha	1.807	0.504	2.311	3.6
	IR-8	1.682	0.544	2.227	3.097
	Nalini Sali	1.584	0.448	2.032	3.539
	Sagara Sali	1.008	0.304	1.312	3.32
	Getw Sali	1.136	0.337	1.473	3.375
	Sali Bahan	1.27	0.399	1.669	3.184
	Kola Bordhan	1.363	0.539	1.901	2.532
	Moimonsingia	1.307	0.566	1.873	2.306
	Na Sali	1.093	0.26	1.353	4.212
	Nasati Sali	1.054	0.303	1.358	3.49
	Torabali	0.976	0.31	1.286	3.145
L2 (75% L)	Swarnaprabha	2.08	0.625	2.705	3.329
	IR-8	1.993	0.651	2.644	3.062
	Nalini Sali	1.602	0.484	2.086	3.313
	Sagara Sali	1.376	0.429	1.806	3.206
	Getw Sali	1.944	0.666	2.61	2.92
	Sali Bahan	1.849	0.655	2.504	2.828
	Kola Bordhan	1.859	0.765	2.623	2.446
	Moimonsingia	1.572	0.637	2.209	2.465
	Na Sali	1.358	0.446	1.804	3.045
	Nasati Sali	1.231	0.437	1.668	2.815
	Torabali	1.288	0.395	1.682	3.261
L3 (50% L)	Swarnaprabha	2.807	0.877	3.684	3.202
	IR-8	1.934	0.695	2.629	2.783
	Nalini Sali	1.68	0.502	2.182	3.354
	Sagara Sali	2.377	0.739	3.117	3.212
	Getw Sali	1.778	0.594	2.371	2.995
	Sali Bahan	2.326	0.795	3.121	2.924
	Kola Bordhan	1.843	0.763	2.606	2.414
	Moimonsingia	2.552	0.984	3.536	2.593
	Na Sali	1.672	0.534	2.206	3.136
	Nasati Sali	1.844	0.63	2.473	2.929
	Torabali	1.714	0.519	2.233	3.31
CD (0.05) L	0.068	0.031	0.099	0.059	
CD (0.05) V	NS	NS	NS	NS	
L×V	NS	NS	NS	NS	

Table 2. Effect of low light stress on chlorophyll a, b, a+b and a/b (mg g⁻¹ fresh weight) at 7 days after 50% flowering stage

Light intensity	Variety	Chl-a	Chl-b	Chl(a+b)	Chl-a/b
		2019	2019	2019	2019
L1 (NL)	Swarnaprabha	1.802	0.534	2.336	3.377
	IR-8	1.472	0.488	1.96	3.018
	Nalini Sali	1.364	0.375	1.74	3.823
	Sagara Sali	1.217	0.354	1.571	3.639
	Getw Sali	1.399	0.428	1.827	3.274
	Sali Bahan	0.789	0.202	0.991	3.915
	Kola Bordhan	1.159	0.333	1.491	3.489
	Moimonsingia	1.053	0.335	1.388	3.155
	Na Sali	0.956	0.321	1.277	3.12
	Nasati Sali	0.679	0.195	0.874	3.488
L2 (75% L)	Torabali	1.321	0.379	1.7	3.488
	Swarnaprabha	3.176	1.066	4.242	2.98
	IR-8	1.897	0.662	2.559	2.866
	Nalini Sali	2.789	0.881	3.67	3.174
	Sagara Sali	1.332	0.402	1.734	3.319
	Getw Sali	1.559	0.537	2.095	2.903
	Sali Bahan	1.334	0.389	1.724	3.436
	Kola Bordhan	1.78	0.563	2.342	3.163
	Moimonsingia	1.635	0.547	2.182	2.992
	Na Sali	2.111	0.693	2.804	3.049
L3 (50% L)	Nasati Sali	1.34	0.416	1.756	3.225
	Torabali	2.048	0.733	2.781	2.797
	Swarnaprabha	2.107	0.73	2.836	2.888
	IR-8	1.624	0.572	2.195	2.843
	Nalini Sali	2.11	0.679	2.789	3.106
	Sagara Sali	1.185	0.438	1.624	2.704
	Getw Sali	1.503	0.525	2.028	2.862
	Sali Bahan	1.2	0.39	1.59	3.073
	Kola Bordhan	1.577	0.532	2.109	2.966
	Moimonsingia	1.373	0.475	1.848	2.895
	Na Sali	1.49	0.501	1.991	2.976
	Nasati Sali	1.309	0.43	1.739	3.046
	Torabali	1.979	0.666	2.645	2.974
	CD (0.05) L	0.079	0.036	0.105	0.272
	CD (0.05) V	NS	NS	NS	NS
	LxV	NS	NS	NS	NS

3.1.2 Chlorophyll b

In the present experiment Moimonsingia at 100% light intensity leads with 0.566 mg g⁻¹ fresh weight of chlorophyll b followed by variety Kola Bordhan (0.539 mg g⁻¹ fresh weight) at 50% flowering stage. Whereas, in 75% light intensity Kola Bordhan performed better with highest chlorophyll b content of 0.765 mg g⁻¹ fresh weight. The increasing trend of chlorophyll b was also followed in 50% light intensity with leading chlorophyll content in Moimonsingia (0.984 mg g⁻¹ fresh weight). During 7 days after 50% flowering highest chlorophyll b content was found in 75% light intensity in Swarnaprabha (1.066 mg g⁻¹ fresh weight) and at the same time chlorophyll b value was found reduced in 100% light

intensity in Swarnaprabha (0.534 mg g⁻¹ fresh weight). This indicates the increased rate of chlorophyll b in 75% light intensity than 100% light intensity. (Table 2)

3.1.3 Chlorophyll (a+b)

Total chlorophyll (a+b) pigment was recorded highest in normal light intensity in Swarnaprabha (2.311 mg g⁻¹ fresh weight). The total chlorophyll content increase gradually from normal light to 75% light intensity and 50% light intensity. The tolerant check swarnaprabha showed highest total chlorophyll in 75% light (2.705 mg g⁻¹ fresh weight) and 50% light intensity (3.684 mg g⁻¹ fresh weight) during 50% flowering of the crop. A similar trend was obtained during 7 days after

50% flowering with highest chlorophyll value in Swarnaprabha during all the 3 treatments. Both 75% light intensity and 50% light intensity found with more chlorophyll (a+b) (i.e. 4.242 mg g⁻¹ fresh weight and 2.836 mg g⁻¹ fresh weight, respectively) than 100% light intensity (2.336 mg g⁻¹ fresh weight) (Table 2). The marked increase in chlorophyll (a+b) content in plant during 75% and 50% light intensity indicates the ability of the plants to maximise light harvesting capacity under low light condition and the efficient use of light that captured by the plant leaf by reduce the respiration cost to maintain the plant under low light stress [30,31,32].

3.1.4 Chlorophyll (a/b)

At normal light conditions, chlorophyll a/b pigment was the highest in Na-Sali (4.212) followed by variety Swarnaprabha (3.600), and the least chlorophyll a/b was found in Moimonsingia (2.306) during *kharif* 2019. In comparison to normal light condition 75% light condition showed decreased chlorophyll a/b content with highest chlorophyll a/b in Swarnaprabha (3.329). At 50% light intensity highest value of chlorophyll a/b content is also lower than 100% light condition with highest value in Nalini Sali (3.354). This result was supported by Ren et al. [33] according to them the decrease in chlorophyll a/b under low light intensity is due to increased content of chlorophyll b in low light intensity. The similar trend was followed for chlorophyll a/b ratio at 7 days after 50% flowering with highest chlorophyll a/b at normal light intensity in Sali Bahan (3.915). The chlorophyll a/b ratio decreases gradually in 75% light and 50% light with highest chlorophyll a/b value in Sali Bahan (3.436) and Nalini Sali (3.106) respectively (Table 2). A similar trend was seen in the research of Hidema et al. [34]. They found a constant decrease in chlorophyll a/b during 20% light treatment in comparison to normal light. The decrease in chlorophyll a/b is due to the higher rate of enhancement of chlorophyll b than chlorophyll a under low light intensity and it is the consequence of chlorophyll shift in rice [35].

3.2 Superoxide Dismutase (SOD)

The Amount of SOD in rice leaves was expressed in units and presented in Fig. 1. Among the varieties, Nasati Sali (1.75 mg gm⁻¹ protein) recorded the highest and Na Sali and

IR-8 (1.35 mg gm⁻¹ protein) recorded the lowest SOD content under normal light conditions and at par with each other. A similar trend among the varieties were seen in 75% light intensity, whereas at 50% light intensity Swarnaprabha (1.362 mg gm⁻¹ protein) had the highest SOD content and IR-8 (0.68 mg gm⁻¹ protein) having low SOD content. The SOD activity decreased under low light stress shows an irresponsive behaviour of SOD enzyme under stress. As reduction in anthocyanin synthesis was accompanied by a decline in antioxidants during low light stress [36].

3.3 Catalase Activity

The amount of catalase in rice leaves was expressed in units (mg gm⁻¹ protein) and presented in Fig. 2. Among the varieties, Swarnaprabha (0.20 mg gm⁻¹ protein) recorded the highest catalase activity under normal light conditions and 75% light intensity, whereas at 50% light intensity Kola Bordhan (0.39 mg gm⁻¹ protein) had the highest catalase activity and Nasati Sali (0.21 mg gm⁻¹ protein) having low catalase activity. Increased catalase activity under low light stress may be a indication for light responsiveness of this enzyme which will vary from genotype to genotype [37]. According to Zhu et al. [36] for minimizing the negative effect of low light the elevation of antioxidative enzyme activity is necessary as it helps in maintenance of the ROS.

3.4 Peroxidase Activity

The amount of peroxidase in rice leaves was expressed in units and presented in Fig. 3. Among the selected varieties, Swarnaprabha (0.0006 mg gm⁻¹ protein) recorded the highest and IR-8 (0.0003 mg gm⁻¹ protein) recorded the lowest peroxidase content under normal light conditions and the same trend was observed during 75% light intensity, whereas at 50% light intensity Swarnaprabha (0.0008 mg gm⁻¹ protein) having the highest peroxidase content and both IR-8 and Na Sali having low peroxidase content (0.0004 mg gm⁻¹ protein).

Peroxidase activity shows a similar trend with the catalase activity by showing increased peroxidase effect under low light stress. Similar result was obtained by Kusvuran et al. [16] and they established a positive relation between different abiotic stresses and increased antioxidant activity.

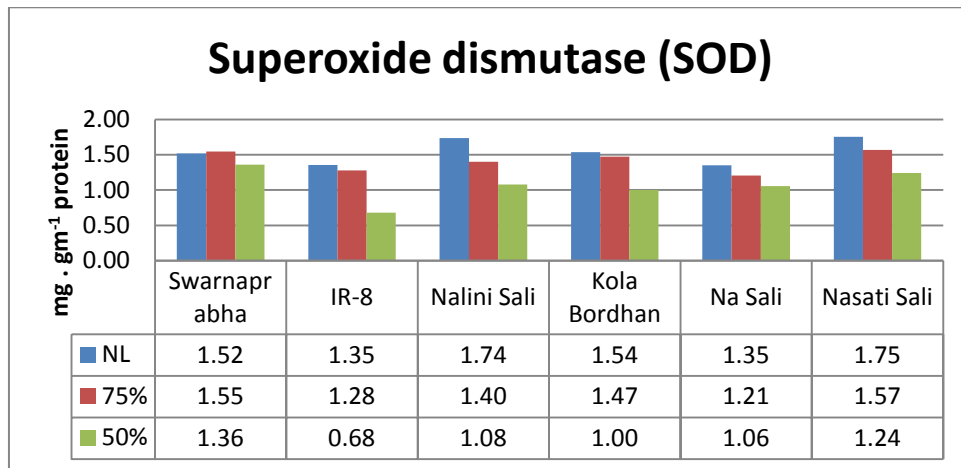


Fig. 1. Effect of low light on Superoxide dismutase (SOD) activity

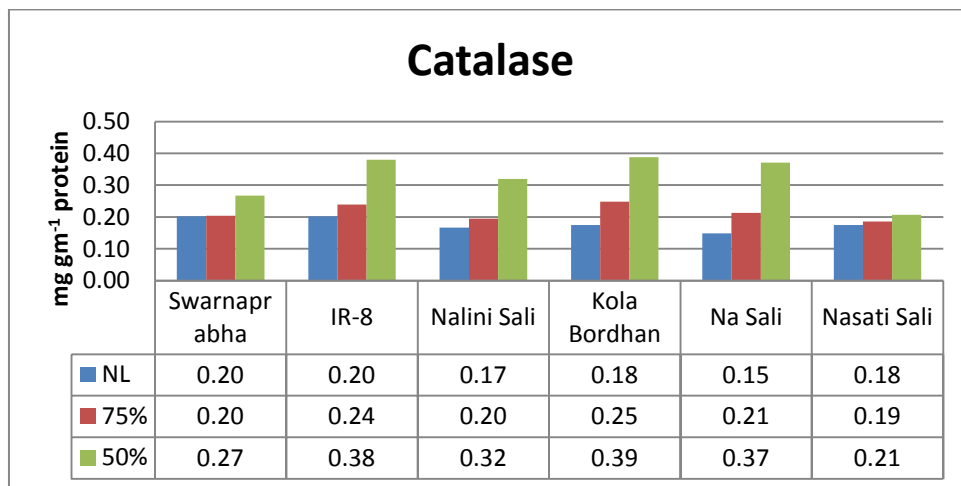


Fig. 2. Effect of low light on Catalase activity

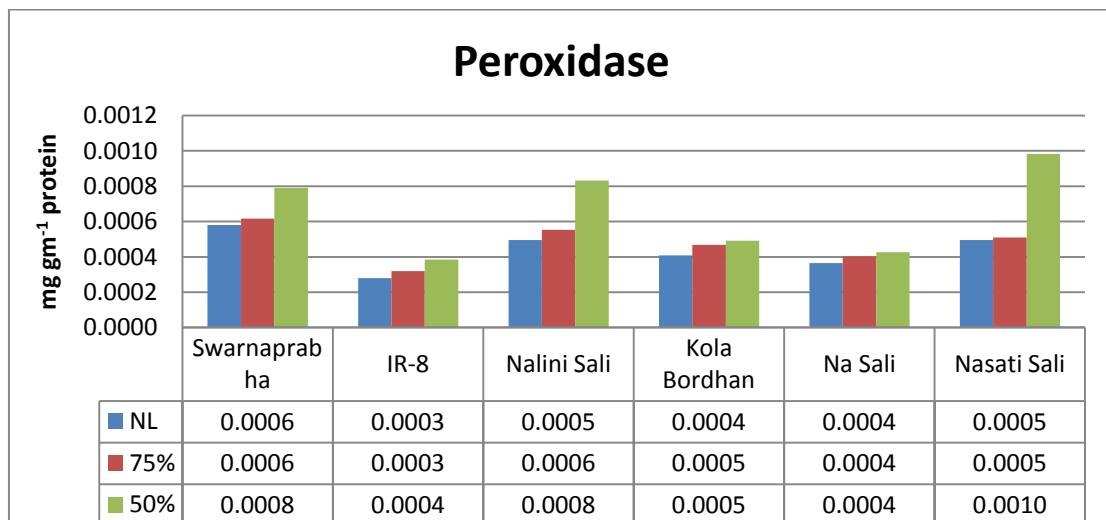


Fig. 3. Effect of low light on Peroxidase activity

Table 3. Interaction effect of light and varieties on grain yield during *Kharif* 2019-20

Grain Yield (t ha ⁻¹)	Normal light	75%light	50% light
Swarnaprabha	4.89	4.26	3.05
IR-8	3.52	2.29	1.16
Nalini Sali	4.44	4.27	2.19
Sagara Sali	4.74	3.00	1.35
Getw Sali	2.51	2.44	0.90
Sali Bahan	2.49	2.09	1.41
Kola Bordhan	3.77	2.25	1.19
Moimonsingia	4.14	2.55	2.09
Na Sali	1.96	1.53	0.90
Nasati Sali	5.10	3.68	2.37
Torabali	4.72	4.10	2.18
	L in G	G in L	
SE(m)±	0.30	0.29	
CD(0.05)	0.59	0.58	

Table 4. Correlation between chlorophyll and yield under low light stress at 7 days after 50% flowering

	Chl-a	Chl-b	Chl(a+b)	Chl-a/b	Yield
Chl-a	1				
Chl-b	0.983107	1			
Chl(a+b)	0.998846	0.990762	1		
Chl-a/b	-0.41059	-0.55754	-0.45049	1	
Yield	0.06335	-0.03797	0.036944	0.525452	1

3.5 Grain Yield

The grain yield of 11 varieties with three treatments is presented in Table 3. Grain yield under low light intensity was significantly reduced than normal light (100% light) condition. In 100% light intensity, among the eleven varieties, Nasati Sali performed best with 5.10 t ha⁻¹ grain yield. A decreasing trend was seen in 75% and 50% light intensity. At 75% light intensity both Nasati Sali and Swarnaprabha found with the maximum yield of 4.27 t ha⁻¹ and 4.26 t ha⁻¹ respectively. At 50% light intensity grain yield reduced drastically with highest grain yield in Swarnaprabha (3.05 t ha⁻¹) during *kharif*, 2019. The yield reduction at low light intensity is mainly due to reduction in grain weight [24]. According to Hairmansis et al. [23] 55% shade enhanced the blooming time, the height of plant and spikelet sterility but reduce number of productive tillers and Yield.

3.6 Correlation between Chlorophyll and Yield

Although chlorophyll does not have any direct correlation at the initial stage of plant growth, but at the later stage the chlorophyll a, a+b and a/b having a positive correlation with yield. At the

same time chlorophyll b having negatively correlated to yield. Therefore increase in chlorophyll b under low light stress may causes reduction in plant yield by affecting stomatal conductance. According to Dey et al. [38], low light stress declines rate of photosynthesis due to reduced mesophyll thickness and simultaneous increase in chlorophyll b influences stomatal conductance under low light condition. The correlation of yield with chlorophyll a, b, a+b and a/b at 7 days after 50% flowering is represented in Table 4.

4. CONCLUSION

The research showed that low light stress causes significant changes in plant and reduces the yield of plants by bringing the physiological and biochemical changes during different stages of plant growth. Antioxidant activity affected under low light stress and varies from varieties to varieties. Light responsive antioxidant enzymes (Catalase and Peroxidase) show a more increased activity in susceptible ones under low light stress than tolerant ones. As the tolerant varieties affected less and maintain the rice production by inducing the physiological and biochemical changes, it is a wiser decision to

grow the short duration rice varieties with low light tolerant properties (e.g. Swarnaprabha) are highly recommended for low light prevailing areas.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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