Journal of Agricultural Science and Technology B 11 (2021) 61-72
doi: 10.17265/2161-6264/2021.02.002

Effect of Low Light Stress on Photosynthetic Pigments and Antioxidative Enzymes in Field Grown Indian Mustard (*Brassica juncea* L.) Genotypes

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Abstract: Oilseed Brassicas have prominent place after soybean and groundnut. More than 90% of the area under oilseed Brassicas is occupied by the Indian mustard (*Brassica juncea*) because of its relative tolerance to biotic and abiotic stresses as compared to other oilseed Brassica species. Light plays key a role in net primary productivity and is necessary for plant growth, morphogenesis and several physiological processes. The present investigation aimed to assess the effect of low light stress on photosynthetic traits and antioxidative enzymes in *Brassica juncea* genotypes. Shading was imposed with nets from mid-December to mid-January which cut 25%-30% of natural sunlight. Shading period coincided with the onset of flowering. The results showed that chlorophyll *b* and antioxidant activities of superoxide dismutase (SOD), guaiacol-peroxidase (G-POD) and catalase (CAT) increased under low light stress at two stages of investigations (10 and 30 days after removal of nets). With shading treatment, soil plant analysis development (SPAD) chlorophyll meter values, chlorophyll *a*, total chlorophyll, chlorophyll *a*/*b* ratio, carotenoid and protein content decreased significantly while malondialdehyde content increased due to damages of plant cells. This study provides valuable information for further deciphering genetic mechanism and improving agronomic traits in Indian mustard cultivated under optimal light requirements.

Key words: Photosynthesis, antioxidative enzymes, chlorophyll, carotenoid, malondialdehyde, Brassicas.

1. Introduction

The genus Brassica belongs to the family *Brassicaceae* that involves various important crops used as oilseed, condiments and vegetables all over the world. Brassica oilseeds are mainly grown for their oil and seed meal [1] throughout India. However, scarcity of oil leads to import of oil to fulfill the rising demands. In order to achieve this, there is need to enhance the oilseed production with the changes in climatic conditions like light, temperature, rainfall, wind etc. [2].

Photosynthesis is the basic phenomenon of crop growth and yield production. Photosynthesis is a process used by plants and other organisms to convert light energy into chemical energy that can later be released to fuel the organisms activities (energy transformation) [3]. Crop productivity, the end result of crop development pattern and process physiology is greatly influenced by several intrinsic and extrinsic factors including environment and management practices. Light plays key role in primary productivity [4]. Light does not only act as the driving force for photosynthesis, but also affects the structure and function of photosynthetic apparatus. Plant population, spatial arrangement, canopy structures and crop development stages are also influenced by light intensity [5]. Light is essential for plant growth, morphogenesis and several physiological processes [6]. Plants grown under low light are more vulnerable to photo-inhibition as compared to plants grown under high light intensity [7]. The SPAD meter determines the relative amount of chlorophyll/greenness of the leaf [8].

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Chlorophyll is an important pigment involved in absorbing, transmitting and converting solar energy into electrochemical energy. Chlorophyll and carotenoid content decreased due to photosynthetic damage while chlorophyll *b* increased to resist low light stress [9]. Antioxidative metabolism plays an important role in protecting plants from a wide variety of environmental stresses, such as drought, extreme temperatures, pollutants, ultraviolet radiation and high levels of light [10, 11]. Tolerant varieties of rice to low light thus could regulate osmotic stress and detoxify reactive oxygen species (ROS) to maintain water potential in cells thereby reducing the opposing effects of low light on physiological mechanisms in plants which could increase tolerance to different stresses [12]. Studies in purple pak-choi (*B. compestris* ssp. *Chinensis Makino*) resulted in significant changes of antioxidant enzyme activities under low light stress [9]. Hence, it was essential to evaluate the photosynthetic pigments and antioxidative enzymes related with the performance of Indian mustard (*B. juncea*) genotypes to low light stress with changing climatic conditions.

2. Materials and Methods

2.1 Plant Material and Treatments

The field experiment was conducted at the research farm and biochemical estimations were carried out in laboratories of Oilseeds Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Experiment was conducted in randomized block design with three replications under irrigated conditions for two crop seasons (2015-2017). Seven genotypes of *Brassica juncea* comprising of released varieties (RLC-3, PBR-357, PBR-210 and RLM-619) and promising entries in pipeline (PBR-422, PHR-1 and PHR-2) were sown in the first week of November according to the recommended package and practices. Shading was done with the nets which cut 25%-30% of natural sunlight and the treatment commenced from mid-December to mid-January which lasted for 30 days. Observations were recorded at two stages after removal of nets i.e. 10 days and 30 days and were represented as mean values of the two years.

2.2 Relative Pigment Levels

Chlorophyll and carotenoid contents were assayed by using Hiscox and Israelstam method [13]. Leaf samples of 0.1 g were placed in vial containing 10 mL of dimethylsulphoxide (DMSO). Vials were then kept into the boiling water bath at 65 ℃ for 30 minutes. Absorbance was recorded at 645 nm and 663 nm. The concentrations of chlorophyll *a*, chlorophyll *b* and total chlorophyll were calculated by using Eqs. (1)-(3):

$$\text{Chlorophyll } a \text{ (mg g}^{-1}\text{ FW)} = 12.7 \times A_{663} - 2.69 \times A_{645} \times \frac{\text{Volume}}{1000 \times \text{Weight}} \quad (1)$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1}\text{ FW)} = 22.9 \times A_{645} - 4.68 \times A_{663} \times \frac{\text{Volume}}{1000 \times \text{Weight}} \quad (2)$$

$$\text{Total chlorophyll (mg/g}^{-1}\text{ FW)} = 20.2 \times A_{645} + 8.02 \times A_{663} \times \frac{\text{Volume}}{1000 \times \text{Weight}} \quad (3)$$

Absorbance was again measured at 480 nm in UV-spectrophotometer to estimate the carotenoid content by using Eq. (4):

$$\text{Carotenoids (mg g}^{-1}\text{FW)} = 1000 \text{ x} A_{480} - 1.29 \text{ x Chla} - 53.78 \text{x Chlb/220 x Volume /1000 x weight} \quad (4)$$

2.3 Antioxidative Enzymes Activity Assay

Enzymes were extracted at 4 ℃ to minimize denaturation. A total weight of 0.15 g samples and enzyme were extracted with 0.1 M sodium phosphate buffer (pH 7.5) containing 1% PVP. The extracts were centrifuged at 10,000 g for 20 min. Supernatant was used for enzymatic estimation. Superoxide dismutase (SOD) activity was determined by Marklund and Marklund method [14]. For estimation, 1.5 mL of 0.1 M Tris-HCl buffer (pH 8.2), 0.5 mL of 6 mM ethylene diamine tetra acetic acid (EDTA), 1 mL of 0.6 mM

pyrogallol solution and 0.1 mL of enzyme extract were added in a cuvette. Absorbance was recorded at 420 nm after an interval of 30 seconds up to 5 minutes. Guaiacol-peroxidase (G-POD) was assayed by Shannon *et al.* [15]. The reaction mixture contained 3 mL of 0.05 M guaiacol prepared in 0.1 M sodium phosphate buffer (pH 6.5), 0.1 mL of enzyme extract and 0.1 mL of 0.8 M H_2O_2. The reaction was initiated by adding H_2O_2 and rate of change in absorbance was recorded at 470 nm for 5 minutes at an interval of 30 seconds. Catalase (CAT) was estimated by Chance and Maehley method [16]. In spectrophotometric cuvette, 1.8 mL of 50 mM sodium phosphate buffer (pH 7.5) and 0.2 mL of enzyme extract were added. The reaction was initiated by adding 1 mL of H_2O_2. Utilization of H_2O_2 was recorded at intervals of 30 seconds for 5 minutes by measuring the decrease in absorbance at 240 nm.

2.4 Qualification of Malondialdehyde and Soluble Proteins

Malondialdehyde was estimated according to Bernheim method [17]. A total of 0.2 g tissue, homogenized with 2 mL of 5% trichloroacetic acid (TCA) and centrifuged at 10,000 rpm for 15-20 minutes.For estimation, 1 mL of thiobarbutyric acid (TBA) reagent was added to 1 mL of supernatant. The mixture was heated for 30 minutes over a water bath at 95 ℃. The samples were centrifuged at 10,000 rpm for 10-15 minutes. Absorbance of the filtrate was read at 532 nm and 600 nm on UV-spectrophotometer using TBA reagent as blank. Total soluble protein was determined through Lowry *et al.* method [18]. The concentration of protein samples was calculated from the standard curve of bovine serum albumin (BSA) (10-70 μg).

2.5 Statistical Analysis

SAS (version 9.3 for windows) was used to perform analysis of variance (ANOVA). The physiological variables are presented as the mean ± standard deviation (SD), with minimum of three replications. Differences between the control and shading were considered significant at $p = 0.05$.

3. Results and Discussion

3.1 Analysis of Relative Pigments Levels

Low light stress had significant impact on relative values of SPAD, chlorophyll and carotenoid content of Indian mustard (Tables 1-3). At two stages of investigation SPAD values were reduced significantly by low light stress. Mean SPAD values indicated a decline of 4.8% and 10.2% after 10 and 30 days of treatment respectively (Table 1). Minimum reduction was observed in PHR-2 (1.4%) followed by RLC-3 (1.5%) at 10 days after removal of nets. SPAD values were reduced to comparable extent in PBR-422, PBR-357 and PBR-210. At 30 days after treatment, minimum decline of SPAD value was 4.7% in PBR-210 and comparable in PBR-422 and PBR-357 and also in RLM-619 and PHR-1. SPAD values did not vary much in PHR-1, PHR-2 and PBR357 and also in PBR-422 and RLM-619 with no shading as observed 10 days after net removal. SPAD values declined considerably at 30 days after shading except in PHR-1, PBR210 and RLM-619.

Chlorophyll content was significantly reduced after exposure to low light stress. Shade reduced mean chlorophyll content by 7.5% and 19.8% at two stages respectively (Fig. 1). Chlorophyll *a* content was lowered more significantly under shading than in the natural sunlight and was comparable in all genotypes except for PHR-2 (1.47%) and RLC-3 (1.51%) at 10 days after removal nets. However, maximum reduction was recorded in RLC-3 (41.9%) and minimum in PHR-2 (6.2%) after 30 days of net removal. Mean of chlorophyll *b* content enhanced significantly by 5.7% and 23.5% when estimated after 10 and 30 days of treatments (Fig. 1). Chlorophyll *b* increased by 21.2% in PHR-2 and the increase was comparable in RLC-3 and PBR-422 at 10 days after

Table 1 Effect of shading on SPAD value 10 and 30 days after removal of nets in Indian mustard genotypes.

Genotypes	SPAD value					
	10 days after treatment			30 days after treatment		
	Control	Shaded	Red (%)	Control	Shaded	Red (%)
RLC-3	41.2 ±0.1	40.6 ±0.1	1.5	40.7 ±0.2	36.7 ±0.2	9.8
PHR-1	43.1 ±0.3	40.0 ±0.1	7.2	42.9 ±0.3	39.7 ±0.2	7.5
PHR-2	43.0 ±0.2	42.4 ±0.1	1.4	43.2 ±0.2	35.1 ±0.4	18.8
PBR-422	43.7 ±0.2	41.2 ±0.3	5.7	41.0 ±0.2	36.3 ±0.3	11.5
PBR-357	43.0 ±0.3	40.8 ±0.0	5.1	42.0 ±0.3	37.2 ±0.1	11.4
PBR-210	45.4 ±0.1	42.9 ±0.3	5.5	44.3 ±0.4	42.2 ±0.2	4.7
RLM-619	43.8 ±0.4	40.6 ±0.2	7.3	41.3 ±0.5	38.1 ±0.3	7.7
Mean	43.3 ±0.2	41.2 ±0.2		42.2 ±0.3	37.9 ±0.2	
CD (p = 0.05)		S = 0.25 G= 0.46 S × G = 0.66			S = 0.31 G = 0.59 S × G = 0.83	

removal of nets. Increased trend was also noticed 30 days after shading treatment to the tune of 60.5% (PBR-210) and 7.8% in PHR-1. Impact of shading was quite evident as per treatment mean which led to decline in total chlorophyll by 7.1% and 19.3% at two respective stages (Fig. 1). Decline in greenness/pigments was to variable extent in the genotypes being 13.1% (PHR-2) after 10 days and 37.7% (RLC-3) after 30 days of nets removal.

Mean chlorophyll *a*/*b* decreased by 5.5% and 14.2% with shading at the two studied stages (Fig. 1). At the first stage of investigation, the least decline was noted at 0.3% in RLM-619 and maximum 10.5% in PHR-1 (10.5%) while 1.6% in PHR-1 and 30.5% in PHR-2 at second stage after removal of nets.

Carotenoids were affected significantly by shading only at second stage i.e. 30 days after removal of nets. However, genotypes suffered decrease in accessory pigments/carotenoids due to shading as indicated by mean decline of 6.4% and 17.9% at 10 and 30 days after treatment. Low light reduced carotenoids to equal extend in PHR-1 and PBR-422 and RLM-619 and PBR-357 at first stage while in PHR-1 and PBR-357 and also in PHR-2 and RLM-619 at second stage as evident from the recorded average of the genotypes.

Overall, genotypes possessed higher chlorophyll pigments along with the carotenoids at first stage (10 days) except for chlorophyll *a* under control, chlorophyll *b* content under both treatments i.e. control and shaded conditions as well as total chlorophyll.

3.2 Antioxidant Enzyme Activity

Low light stress resulted in significant changes of enzymatic activities of G-POD, CAT and SOD (Fig. 2). Genotypes possessed higher antioxidative activities under control and corresponding increase was witnessed with shading at first stage of investigation. Antioxidative activities were comparatively lower at second stage, however, enzyme activities increased with shading treatment even at the second stage. SOD activity increased by 21.8%, G-POD activity by 24.5% and CAT activity by 25.3% at 10 days after removal of nets. Similar pattern was observed 30 days after removal of nets where SOD activity increased by 35.9%, G-POD activity by 40.1% and CAT activity by 14.8% at 30 days after removal of nets.

Lesser decline in the antioxidative enzyme activities was observed in PHR-1 and PHR-2 for SOD, PHR-2 and PBR-210 for G-POD at first stage while PBR-357 and PBR-210 for CAT at the second stage of the net removal (Tables 4 and 5).

3.3 Malondialdehyde and Soluble Protein Analysis

Malondialdehyde content (MDA) increased significantly by 48.0% and 38.0% with shading at two stages after

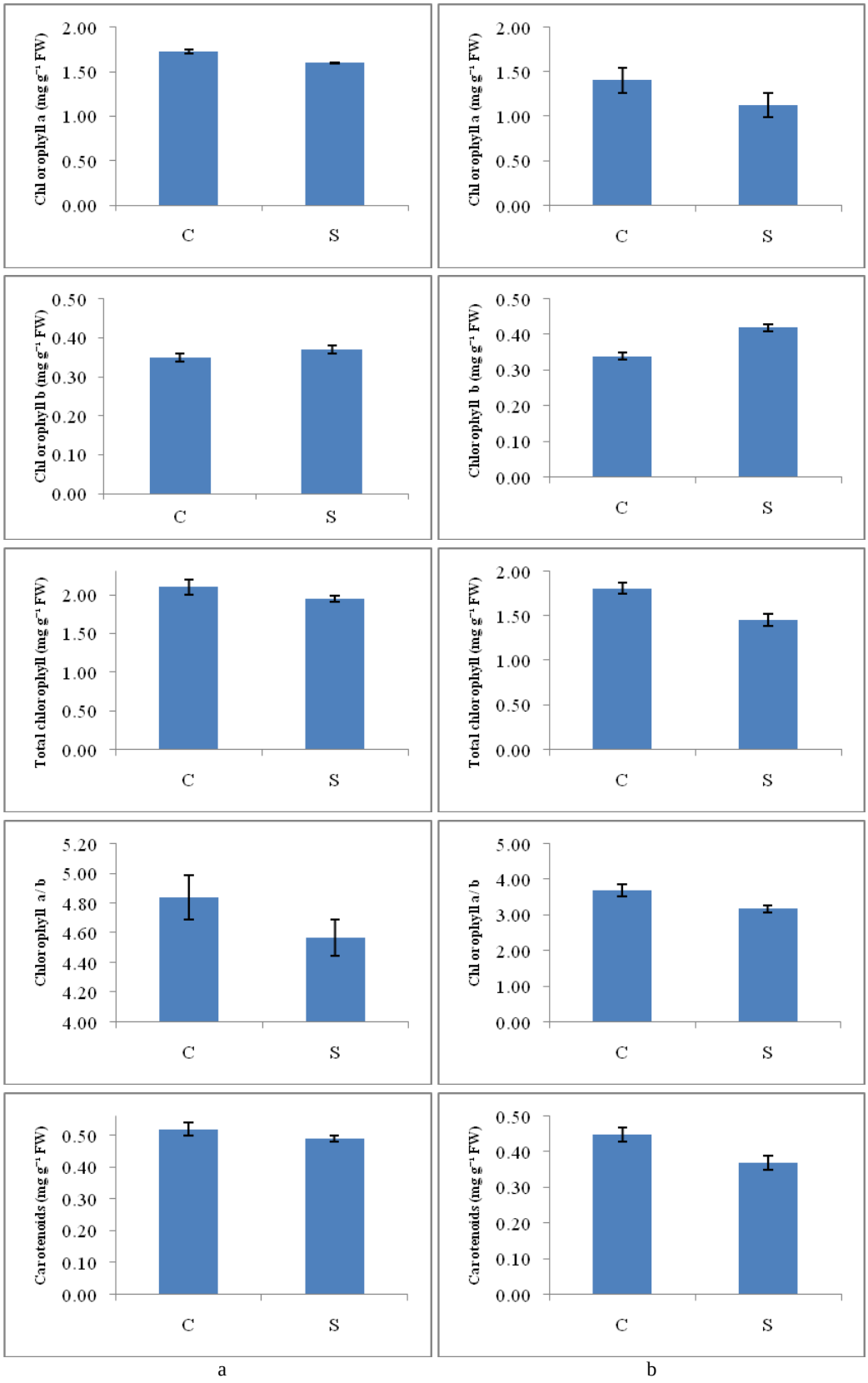

Fig. 1 Mean photosynthetic pigments in *Brassica juncea* genotypes 10 days (a) and 30 days (b) under control (C) and shading (S).

Table 2 Effect of shading on photosynthetic pigments (mg g^{-1} FW) 10 days after removal of nets in Indian mustard genotypes.

Genotypes	Chlorophyll *a*			Chlorophyll *b*			Total chlorophyll			Chlorophyll *a*/*b*			Carotenoids		
	Control	Shaded	Average	Control	Shaded	Average	Control	Shaded	Average	Control	Shaded	Average	Control	Shaded	Average
RLC-3	1.59±0.01	1.43±0.01	1.51	0.31±0.00	0.33±0.00	0.32	1.91±0.02	1.74±0.12	1.83	4.87±0.05	4.76±0.19	4.82	0.47±0.01	0.44±0.01	0.45
PHR-1	1.83±0.01	1.74±0.00	1.78	0.37±0.01	0.40±0.01	0.38	2.20±0.10	2.13±0.04	2.16	4.93±0.17	4.41±0.09	4.67	0.55±0.01	0.53±0.01	0.54
PHR-2	1.56±0.01	1.37±0.00	1.47	0.28±0.00	0.34±0.00	0.31	1.90±0.05	1.65±0.11	1.77	4.94±0.06	4.64±0.16	4.79	0.47±0.01	0.42±0.03	0.44
PBR-422	1.81±0.00	1.73±0.01	1.77	0.38±0.00	0.40±0.00	0.39	2.19±0.11	2.13±0.01	2.16	4.78±0.18	4.31±0.07	4.55	0.55±0.02	0.53±0.03	0.54
PBR-357	1.78±0.00	1.70±0.01	2.74	0.36±0.01	0.38±0.01	0.37	2.15±0.06	2.06±0.06	2.11	4.81±0.21	4.74±0.12	4.77	0.53±0.01	0.51±0.02	0.52
PBR-210	1.68±0.01	1.56±0.01	1.62	0.32±0.01	0.38±0.01	0.35	2.06±0.07	1.89±0.13	1.97	4.84±0.15	4.47±0.17	4.66	0.51±0.03	0.47±0.02	0.49
RLM-619	1.88±0.01	1.67±0.00	1.78	0.36±0.01	0.41±0.01	0.38	2.28±0.06	2.02±0.03	2.15	4.70±0.09	4.69±0.15	4.70	0.55±0.03	0.50±0.01	0.53
Mean	1.73±0.01	1.60±0.01		0.35±0.01	0.37±0.04		2.10±0.10	1.95±0.04		4.84±0.15	4.57±0.12		0.52±0.02	0.49±0.01	
CD (p = 0.05)	S = NS G = 0.01 S × G =0.02			S = 0.01 G = 0.02 S × G = 0.03			S = NS G = 0.16 S × G = 0.22			S = 0.15 G = NS S × G = NS			S = NS G = 0.04 S × G = NS		

Table 3 Effect of shading on photosynthetic pigments (mg g^{-1} FW) 30 days after removal of nets in Indian mustard genotypes.

Genotypes	Chlorophyll *a*			Chlorophyll *b*			Total chlorophyll			Chlorophyll *a*/*b*			Carotenoids		
	Control	Shaded	Average	Control	Shaded	Average	Control	Shaded	Average	Control	Shaded	Average	Control	Shaded	Average
RLC-3	1.10±0.01	0.64±0.02	0.87	0.24±0.01	0.31±0.01	0.28	1.42±0.03	0.88±0.04	1.15	3.60±0.17	2.69±0.09	3.15	0.35±0.01	0.29±0.01	0.32
PHR-1	1.53±0.04	1.44±0.03	1.48	0.38±0.01	0.41±0.01	0.39	1.94±0.07	1.62±0.09	1.78	3.82±0.09	3.76±0.18	3.79	0.48±0.01	0.42±0.01	0.45
PHR-2	1.09±0.04	0.85±0.02	0.97	0.30±0.01	0.34±0.01	0.32	1.39±0.06	1.19±0.04	1.29	3.74±0.05	2.60±0.14	3.17	0.35±0.01	0.34±0.07	0.35
PBR-422	1.41±0.08	1.04±0.02	1.23	0.31±0.01	0.42±0.01	0.37	1.83±0.17	1.35±0.03	1.59	3.56±0.14	3.40±0.11	3.48	0.47±0.02	0.36±0.01	0.41
PBR-357	1.67±0.02	1.49±0.03	1.58	0.44±0.01	0.49±0.04	0.47	2.11±0.10	1.98±0.02	2.05	3.98±0.40	3.06±0.08	3.52	0.52±0.02	0.41±0.02	0.46
PBR-210	1.86±0.02	1.29±0.03	1.57	0.38±0.01	0.61±0.00	0.49	2.47±0.08	1.67±0.12	2.07	3.54±0.07	3.47±0.12	3.51	0.64±0.03	0.43±0.02	0.53
RLM-619	1.22±0.03	1.12±0.04	1.17	0.33±0.01	0.37±0.00	0.35	1.55±0.03	1.50±0.06	1.52	3.71±0.12	3.28±0.15	3.50	0.36±0.01	0.36±0.02	0.36
Mean	1.41±0.03	1.13±0.04		0.34±0.01	0.42±0.01		1.81±0.06	1.46±0.07		3.71±0.17	3.18±0.10		0.45±0.02	0.37±0.02	
CD (p = 0.05)	S = 0.04 G = 0.07 S × G = 0.1			S = 0.01 G = 0.03 S × G = 0.04			S = 0.08 G = 0.16 S × G = 0.22			S = 0.17 G = 0.33 S × G = 0.46			S = 0.02 G = 0.04 S × G = 0.06		

Table 4 Effect of shading on enzyme activity (superoxide dismutase, guaiacol-peroxidase and catalase) 10 days after removal of net in Indian mustard genotype.

Genotypes	SOD (units min^{-1} g^{-1} FW)			G-POD (ΔOD min^{-1} g^{-1} FW)			CAT (ΔOD min^{-1} g^{-1} FW)		
	Control	Shaded	Average	Control	Shaded	Average	Control	Shaded	Average
RLC-3	126.5 ±2.6	144.3 ±1.8	135.4	75.9 ±1.4	106.6 ±1.4	91.3	48.4 ±0.2	61.0 ±0.4	54.7
PHR-1	87.9 ±2.0	96.1 ±1.8	92.0	78.3 ±1.5	117.6 ±0.6	97.9	35.2 ±1.3	51.0 ±0.2	43.1
PHR-2	149.0 ±4.1	161.8 ±1.8	155.4	86.6 ±1.9	99.6 ±2.1	90.6	39.5 ±0.4	49.7 ±0.9	44.6
PBR-422	180.3 ±4.6	203.4 ±0.6	191.9	74.3 ±1.5	92.4 ±0.5	78.4	58.2 ±0.2	71.3 ±0.4	64.8
PBR-357	188.5 ±0.9	207.6 ±0.8	198.0	91.2 ±1.5	100.4 ±1.2	95.8	59.6 ±0.1	76.5 ±0.4	68.1
PBR-210	108.1 ±0.7	169.6 ±3.1	138.8	90.8 ±1.5	101.4 ±1.2	91.1	56.6 ±0.3	69.9 ±0.4	63.2
RLM-619	130.5 ±2.1	200.1 ±3.9	165.3	83.1 ±.04	103.6 ±1.3	88.4	67.5 ±1.6	77.9 ±0.4	72.7
Mean	138.7 ±2.4	169.0 ±2.0		82.9 ±1.4	103.1 ±1.2		52.1 ±0.6	65.3 ±0.4	
CD (p = 0.05)	S = 2.74 G = 5.13 S × G = 7.25			S = 1.53 G = 2.87 S × G = 4.06			S = 0.71 G = 1.33 S × G = 1.88		

Table 5 Effect of shading on enzyme activity (superoxide dismutase, guaiacol-peroxidase and catalase) 30 days after removal of net in Indian mustard genotypes.

Genotypes	SOD (units min^{-1} g^{-1} FW)			G-POD (ΔOD min^{-1} g^{-1} FW)			CAT (ΔOD min^{-1} g^{-1} FW)		
	Control	Shaded	Average	Control	Shaded	Average	Control	Shaded	Average
RLC-3	83.4 ±1.1	101.1 ±1.1	92.2	36.9 ±0.7	55.0 ±0.5	58.7	34.8 ±0.2	41.3 ±0.6	38.1
PHR-1	57.7 ±1.8	79.8 ±1.1	68.8	43.5 ±1.0	66.0 ±0.6	60.0	34.5 ±0.8	38.6 ±0.7	36.6
PHR-2	63.1 ±1.1	95.2 ±1.2	79.1	59.4 ±0.5	78.9 ±0.8	68.5	34.8 ±0.2	41.3 ±0.5	38.1
PBR-422	94.4 ±1.0	122.3 ±1.0	108.3	63.1 ±1.1	76.9 ±2.8	66.5	44.7 ±0.6	53.3 ±0.8	49.0
PBR-357	97.0 ±0.3	126.1 ±1.7	111.6	59.3 ±0.7	78.1 ±1.5	69.1	52.0 ±0.3	56.6 ±0.3	54.3
PBR-210	75.9 ±1.7	111.5 ±2.7	93.7	59.1 ±0.5	74.8 ±2.0	68.6	48.0 ±0.7	52.6 ±0.6	50.3
RLM-619	90.4 ±0.9	128.0 ±0.4	109.2	55.6 ±0.5	77.2 ±1.3	61.1	49.9 ±0.5	59.3 ±0.6	54.6
Mean	80.3 ±1.1	109.1 ±1.3		53.9 ±0.7	72.6 ±1.3		42.7 ±0.5	49.0 ±0.6	
CD (p = 0.05)	S = 1.44 G = 2.69 S × G = 3.80			S = 1.33 G = 2.48 S × G = 3.52			S = 0.49 G = 0.91 S × G = 1.29		

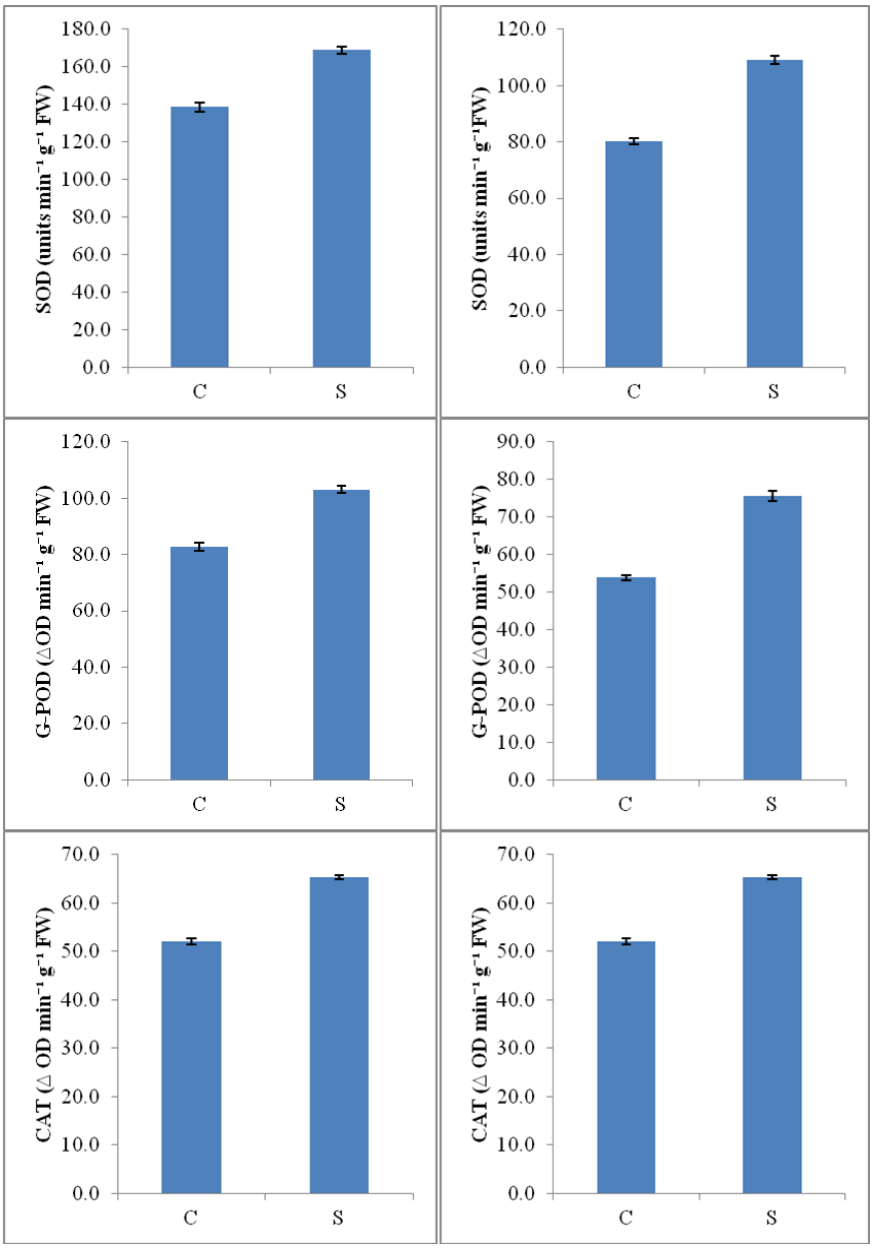

Fig. 2 Mean antioxidative enzymes in *Brassica juncea* genotypes 10 days (a) and 30 days (b) under control (C) and shading (S).

removal of nets. Minimum increase in MDA content was in PBR-422 (18.0%) and maximum in PHR-2 (95.5%) at 10 days, while PBR-422 (3.7%) and RLM-619 (66.0%) at 30 days after removal of nets (Table 6). Mean TSP decreased by 14.5% and 10.4% after 10 and 30 days of net removal (Table 7). TSP (total soluble protein) content was reduced to 8.9% in RLC-3 and 22.9% in PHR-2. Reduction in TSP varied from 0.7% (RLC-3) to 22.0% (PBR-422).

Photosynthetic pigments along with the accessory pigments play an important role in photosynthesis as they can capture and transfer light energy. Therefore, the contents of the pigment directly affect the photosynthetic efficiency. In our study, SPAD values, chlorophyll *a*, total chlorophyll and chlorophyll *a*/*b* decreased while chlorophyll *b* content increased when

Table 6 Effect of shading on malondialdehyde after removal of nets in Indian mustard genotypes.

Genotypes	MDA (μmol MDA g^{-1} FW)					
	10 days after treatment			30 days after treatment		
	Control	Shaded	Average	Control	Shaded	Average
RLC-3	21.1±0.4	30.5±2.0	25.8	27.0±1.0	35.5±1.1	31.3
PHR-1	14.9±0.3	26.9±1.1	20.9	17.5±1.8	28.3±1.0	22.9
PHR-2	13.2±0.2	25.8±2.0	19.5	21.4±1.0	31.6±1.7	26.5
PBR-422	29.8±2.7	35.1±1.9	32.5	30.6±0.6	31.7±1.9	31.2
PBR-357	22.4±0.1	29.4±1.3	25.9	25.4±1.2	39.1±1.3	32.2
PBR-210	22.0±0.3	33.3±2.3	27.7	29.8±0.7	38.2±0.8	34.0
RLM-619	15.1±0.6	24.0±1.7	19.6	17.7±1.5	29.4±0.6	23.5
Mean	19.8±0.7	29.3±1.7		24.2±1.1	33.4±1.2	
CD (p = 0.05)	S = 1.47 G = 2.76 S × G = NS			S = 1.34 G = 2.51 S × G = 3.55		

Table 7 Effect of shading on total soluble protein after removal of net in Indian mustard genotypes.

Genotypes	Total soluble proteins (mg g^{-1}FW)					
	10 days after removal of net			30 days after removal of net		
	Control	Shaded	Red (%)	Control	Shaded	Red (%)
RLC-3	62.1±0.5	56.5±1.8	8.9	55.0±0.3	54.6±1.1	0.7
PHR-1	72.7±0.4	59.4±1.3	18.2	55.5±0.8	48.4±0.8	12.7
PHR-2	75.0±0.6	57.8±0.9	22.9	52.5±1.2	45.4±0.6	13.4
PBR-422	61.9±0.8	54.8±2.0	11.4	52.7±0.8	41.2±0.9	22.0
PBR-357	67.1±0.9	56.7±0.9	15.5	54.8±1.1	49.5±0.8	9.7
PBR-210	66.9±0.7	58.1±1.6	13.2	54.2±0.3	48.4±0.4	10.7
RLM-619	62.1±0.7	56.5±0.8	9.1	51.3±0.2	49.0±0.7	4.6
Mean	66.8±1.3	57.1±0.7		53.7±0.7	48.1±0.8	
CD (p = 0.05)	S = 1.14	G = 2.14	S × G = 3.03	S = 0.81	G = 1.51	S × G = 2.14

Indian mustard genotypes were exposed to low light stress. Decline in SPAD values has also been reported in rice by Yang *et al.* [19]. However, contradictory results have been noticed by Restrepo [20] in rice where SPAD and leaf chlorophyll content was higher under low irradiance than in rice leaves exposed to full sunlight conditions. According to Burkey and Wells [21] the chlorophyll *a*/*b* was higher for leaves exposed to full sunlight or high irradiance growth chamber conditions relative to shade or low irradiance acclimated soybean leaves. Shao *et al.* [22] reported that in *Anoectochilus roxburghii* chlorophyll *a*, chlorophyll *b* and total chlorophyll significantly decreased under 50% irradiance conditions and this was in accordance with the earlier findings by Schiefthaler *et al.* [23] in *Scheffera arboricola*. Leaf chlorophyll as reported by Makus and Lester [24] was higher in leaves grown at higher light levels as compared to leaves exposed to lower light level in *B. juncea*. Gregoriou *et al.* [25] suggested that in olive (*Olea europa* L.), chlorophyll *b* content increased while chlorophyll *a*/*b* decreased under shading conditions. Chlorophyll content decreased due to inactivation of photosynthetic system in *Tetrastigma hemsleyanum* plants with 50% shading [26], subterranean clovers with 90% shading [27], in soybean [28] and in *Torreya grandis* with shading of 75% [29]. The amount of the antenna pigments in light-harvesting complex II improved because of enhanced chlorophyll *b* content that ultimately enabled the leaves to catch light effectively in the blue fraction of light [30]. Low chlorophyll and carotenoid contents were observed by Zhu *et al.* [8] with low light stress in purple pak-choi (*Brassica compestris*)

due to photosynthetic damage however chlorophyll *b* content increased which could reduce the damage to some extent. Carotenoids play a critical role in light collection and protect plants from high light stress. These also save plants from photooxidative damage and UV-radiation [31].

Oxidative stress is activated under biotic and abiotic stresses and results in the abundant production of ROS. Present study revealed increase in antioxidative enzyme activities particularly SOD, G-POD and CAT at both the stages of assay under 25%-30% shading. Bano and Nosheen [32] suggested that ABA significantly increased the activities of SOD and POD in wheat cultivars under drought stress. Under different levels (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl, antioxidative enzymes activity increased in *B. juncea* varieties (RH-30 and Varuna) as reported by Wani *et al.* [33]. According to Weng *et al.* [34] in different wheat genotypes SOD, POD and CAT activities increased while MDA content decreased under drought stress. Under shading, antioxidative enzyme activity increased and osmotic regulation for low light tolerant varieties of rice could help to maintain the scavenging of ROS [3]. After 40 days of shading POD, SOD and MDA levels were significantly higher in 30%, 20% and 5% irradiance than in 50% irradiance while CAT activity remained low in *A. roxbughii* plants as reported by Shao *et al.* [35]. All antioxidative enzyme activities increased under 25% and 75% light intensity because low light stress produced ROS and increased the activity of antioxidative enzymes which could lead to damages to some extend [9].

Low light stress causes damage of plant cells to different levels and to various degrees. One type involves the destruction of membrane leading to increased cell permeability. Lipid peroxidation products are considered useful and reliable indication of oxidative damages. In genotypes of *Brassica juncea* L. MDA content increased by 47.9% and 38.0% while protein content decreased by 14.5% and 10.4% after 10 and 30 days of net removal. Our studies are in confirmation with those of Zhu *et al.* [9] where increased MDA content was found in purple pak-choi indicating the degree of lipid peroxidation in cell memberane due to low light treatment.

Shading modified the physiological and antioxidative enzyme activity in *B. juncea* genotypes at two stages after removal of nets. Content of chlorophyll *b*, SOD, POD and CAT activities increased to improve the photosynthetic efficiency while SPAD and protein content decreased. Low light stress inflicted damages by enhancing the permeability of the membranes as indicated by increased MDA content to variable degrees in the genotypes under the present investigation.

4. Conclusions

Our studies emphasize the physiological changes under low light stress in Indian mustard (*Brassica juncea*) in subtropical and semi-arid conditions. The time of shading coincided with the flowering and the sink establishment. Chlorophyll *b* enhanced the photosynthetic efficiency by capturing more energy to improve the utilization efficiency. Low light stress induced the generation of ROS along with lipid peroxidation causing membrane damage to variable extent. However, increased antioxidative activities at both the stages could scavenge the ROS and lower the damages. PBR-422 showed better performance under low light stress due to higher photosynthetic pigments, antioxidative enzymes and less damage to membrane.

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