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(Research Article)

Low light induced physiological changes determining yielding abilities in *Brassica juncea* (L.)

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Abstract

Physiological traits were studied in selected *Brassica juncea* genotypes and released varieties during two winter seasons (2017-19) under the low light stress to identify the shade insensitive genotypes /varieties. The experiment was laid in randomized block design with two treatments comprising shading with nets which cuts 25-30% of sunlight for 30 days and control/ no shading with nets or open sunlight. Observations were recorded at two stages after removal of low light stress imposed by nets i.e. 10 (S1) and 30 (S2) days. Our comprehensive studies revealed that chlorophyll b synthesis improved unlike other photosynthetic pigments which were significantly reduced after 10 days (S1) and relatively greater decline was observed after 30 days (S2). The photochemical efficiency of PSII (Fv/Fm) was reduced in response to shade. The effects of shade was profound on leaf traits like leaf area, specific leaf area, specific leaf weight, leaf water retention and relative water content which declined with low light stress while relative saturation deficit, water saturation deficit increased at both the stages (S1 and S2). The studied traits exhibited positive correlation with seed yield. Promising cultivars showed minimal reduction in physiological traits in response to low light stress.

Keywords: Chlorophyll; Chlorophyll fluorescence; *B juncea*; SPAD; Seed yield

1. Introduction

Rapeseed mustard is the third most important source of edible oilseeds in the worldafter oil palm and soybean (Goel et. al, 2018) which experiences low light stress due to fog-haze events, cloudy and rainy weather. Rapid economic growth and the speed of urbanization have caused emissions of air pollutants from automobiles and industrial exhausts. The net radiation reaching the earth's surface has progressively declined with the changing climatic conditions (Díaz-Torres et. al, 2017). These environmental changes may directly affect the growth and crop productivity. To cope up with changing environmental conditions, crops will have to synchronize with the alterations in the physiological changes (Alam et. al, 2018).

Light plays a major role in photosynthetic capacity; it not only provides the driving force for assimilation but also affects the leaf structure and function. Shade or low light stress is one of the major constraints for crop productivity, where the plants need to adjust photosynthesis, pigment biosynthesis and physiological traits as per the available sunlight (Singh et.al, 2012) with considerable modulation in the performance and structure of the photosynthetic apparatus along with blockage in the energy transport from PSII to PSI, reduction in the leaf thickness, which ultimately leads to low chlorophyll fluorescence (Wu et. al, 2017). Leaf pigments were adversely affected by the changing light intensity (Feng et. al, 2019). However, shading eventually limited the seed yield in *B. juncea* (Kaur, 2018).

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The prime objective of the present investigation was to identify the tolerant Indian mustard genotypes to low light stress based on identified physiological traits, along with the association studies with seed yield. These genotypes can be grown in the intensive cropping system and agroforestry.

2. Material and methods

2.1. Experimental design

The field experiment was conducted in the winter season for two years (2017-18 and 2018-19) at the research farm of Oilseeds section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India. Five mustard genotypes (PHR-126, PBR-450, PBR-396, PBR-464 and PBR-561) along with the released varieties (Kranti and Varuna) were selected based on their earlier field performance. Each genotype was sown in paired rows of row length 2.5 m and spacing of 30 cm in randomized block design (RBD) with three replications in loamy soil. Treatments consisted of: (i) Lowlight stress imposed by shading with nets which cuts 25-30% of natural light (ii) Control/unshaded plots which received full sunlight. Shading was done for one month commencing from mid December to mid January in both the years (2017-19). 3rd and 4th leaf of main raceme was selected to record various physiological traits at two stages i.e., 10 (S1) and 30(S2) days after removal of low light stress imposed by nets. Chlorophyll fluorescence was recorded at S2 stage only.

2.2. Chlorophyll fluorescence

Chlorophyll fluorescence in intact plants was measured with a Multi-mode Chlorophyll Fluorometer (OS-30p, Opti-Sciences, Inc., Hudson, NY, USA) by cutting the light with PAR clip of the 3rd and 4th fully open leaf on the main raceme. The selected leaves were dark-adapted with PAR clips for around 30 minutes before fluorescence measurements. Observations were recorded between 12:00 pm to 2:30 pm. Fluorometer protocols were followed as per OS-30p user's guide. Chlorophyll fluorescence parameters were recorded as explained by Sulewska et al. (2019).

- Fo- minimum fluorescence
- Fm- maximum fluorescence
- Fv/Fm- maximum photochemical efficiency of photosystem II
- Fv - variable fluorescence was calculated by Fv= (Fm-Fo)

2.3. SPAD-values

The greenness in intact plants was measured by Minolta SPAD 502 Chlorophyll Meter (Japan) with caution that midrib should not come under sensor area of the instrument.

2.4. Photosynthetic pigments

The designated leaf samples weighed 0.1 g followed by placing them in the vials containing 5 mL of dimethyl sulphoxide (DMSO). The chlorophyll and carotenoid content were estimated by the method of Hiscox and Israelstam(1979) and Kirk and Allen (1965). The concentration of Chl a, b total Chl and carotenoids were calculated by using Arnon's equations.

Chl a (mg/g FW) = 12.7 × A663 – 2.69 × A645 × [Volume / 1000 × Weight (g)]

Chl b (mg/g FW) = 22.9 × A645 – 4.68 × A663 × [Volume / 1000 × Weight (g)]

Total Chl (mg/g FW) = 20.2 × A645 + 8.02 × A663 × [Volume / 1000 × Weight (g)

$$\text{Carotenoids (mg/g FW)} = \frac{1000 \times A_{480} - 1.29 \times \text{Chl a} - 53.78 \times \text{Chl b}}{220} \times [\text{Volume} / 1000 \times \text{Weight (g)}]$$

2.5. Relative water content

Five leaf discs were excised, weighed (FW) and added in a vial containing 10mL of distilled water for 4 h. Saturated weight (SW) of the discs was recorded and were oven dried for dry weight (DW). Formulae of Weatherley (1950) and Barrs (1968) were used to compute relative water content (RWC), relative saturation deficit (RSD) and water saturation deficit (WSD).

$$RWC = [(FW–DW)/(SW–DW)] \times 100$$

$$RSD = [(SW–FW)/ (SW)] \times 100$$

$$WSD = [(SW–FW)/ (SW–DW)] \times 100$$

2.6. Leaf traits

Leaf area (cm^2) along with the length and width (cm) were measured by leaf area meter AM 300 (Bioscientific Ltd.). These leaves were oven dried at 60-70˚C for 48 h for dry matter (DM). Specific leaf area (SLA) and specific leaf weight (SLW) were calculated as per the formulae of Poorter et. al, (2010).

$$SLA= LA\ (cm^2)/DW\ (g)$$

$$SLW= DW\ (g)/LA\ (cm^2)$$

2.7. Leaf water retention

Leaf water retention (LWR) was estimated by the method of Sangakkara et. al, (1996). Fresh weight (FW) of leaf samples were taken and then kept undisturbed for 4 hours under shade. The loss of water was recorded after 4 hrs by taking their weight. These leaf samples were oven dried (60°-70°C) for 48 hrs for dry weight (DW).

$$LWR\ (\%) = [1– \{(FW – \text{Weight after 4 hrs})/ (FW)\}] \times 100$$

2.8. Seed yield

Seeds obtained after threshing of the dried produce per plot which were then cleaned, dried and weighed for seed yield per plot which was converted on area basis to $kgha^{-1}$.

2.9. Statistical analysis

Analysis of variance was carried out for all the data sets using SPSS statistical software. The treatment values (main effect as well as interaction means) were presented mean ± standard error (SE) and were compared based on least significant differences (LSD) at $P \leq 0.05$ using Duncan multiple range test (DMRT). OPSTAT software was used for correlation analysis.

3. Results

Chlorophyll fluorescence varied significantly under control and shade and interactions were significant too (Table 1). Fo values are related to chlorophyll fluorescence of PSI receptors and considering significant Fo differences among the genotypes revealed variable efficiency of the receptor chlorophylls (Sharma 2015). In the open sunlight, Fv values were higher in the studied genotypes, indicator of reduced state of electron acceptor Quinone (Q) i.e., normal electron transfer. However, low light stress lowered Fv values implying oxidized state of Q, signifying disruption of normal electron transfer during photolysis of water at PSII. Dark adapted values of maximum quantum yield or photochemical efficiency of PSII (Fv/Fm) of the leaves in Brassica germplasm is around 0.780 in the normal light environment and in the present investigation PSII (Fv/Fm) efficiency of the studied genotypes showed significant variations. Decline in the photochemical efficiency of PSII at S2 stage was recorded. However, Kranti had maximum Fv/Fm values under both control and shade revealing lesser damage to PSII whereas Varuna was not affected by low light stress.

Low light stress significantly affected the SPAD values of *B. juncea* genotypes at both the stages (Fig.1). SPAD values decreased with low light stress to the tune of8.5% (S1) and 6.5% (S2) under shade over control. PBR-464 registered minimal reduction of 2.2% at S1 while PBR-396 of 2.1% at S2 stage.

Concentrations of Chl a, total Chl and Chl a/b decreased in all the seven genotypes/varieties in the present study however decline was more for the photosynthetic pigments at S1 stage then S2 when estimated (Table 2). The most significant decrease was in Varuna for Chla (42.7%) and total Chl (32.6%). PHR-126 showed least reduction of 2.2% (Chl a) and 1.8% (total Chl) trailed by Kranti for Chl a (9.3%) and total Chl (2.7%) at S1 stage. At S2 stage, Chla was reduced to a lesser extent in Kranti (10%) while total Chl in PHR-126(1.9%) followed by again Kranti (3.8%). Mean while, Chl b, which absorbs diffuse light with a short wavelength increased to protect against low light stress. Compared with control, Chl b content increased considerably under low light stress at S1(16.7%) and S2 stage (37.5%),suggesting a direct link of chlorophyll with the changes in available light intensity specially diffuse light. Subsequently, Chl a/b decreased due to low light stress by 35.0% (S1) and 58.1% (S2) over control.

Table 1 Chlorophyll flourescence (average of 2 years) 30 days after removal of nets in *B. juncea*

Genotypes	F_o			F_m		
	Control	**Low light stress**	**Red (%)**	**Control**	**Low light stress**	**Red (%)**
Kranti	50.8±0.6^{bcde}	49.5±0.6^{de}	2.5	191.5±0.3^{b}	173.0±0.7^{d}	9.7
Varuna	52.3±0.3^{bc}	51.5±0.3^{bcd}	1.4	163.3±0.9^{gh}	160.8±0.8^{hi}	1.5
PHR-126	56.0±0.5^{a}	52.5±0.4^{b}	6.3	194.8±0.6^{a}	152.0±0.9^{j}	22.0
PBR-450	49.5±0.7^{de}	49.3±0.5^{de}	0.4	167.3±0.8^{ef}	159.0±0.5^{i}	4.9
PBR-396	51.5±0.3^{bcd}	50.3±0.3^{cde}	2.4	190.5±0.7^{b}	170.3±0.4^{de}	10.6
PBR-464	50.8±0.4^{bcde}	50.0±0.7^{de}	1.5	164.8±0.4^{fg}	160.5±0.6^{hi}	2.6
PBR-561	51.5±0.5^{bcd}	49.3±0.9^{e}	4.4	179.3±0.2^{c}	160.3±0.3^{i}	10.6
Mean	51.8 ± 0.5	50.3 ± 0.4		178.8 ± 0.7	162.3 ± 0.6	
CD (p= 0.05)	S= 0.471, G= 0.882, S×G= 1.247			S= 0.691, G= 1.292,S×G= 1.828		

Genotypes	F_v			F_v/F_m		
	Control	**Low light stress**	**Red (%)**	**Control**	**Low light stress**	**Red (%)**
Kranti	140.8±0.3^{a}	123.5±0.6^{c}	12.3	0.735±0.002^{a}	0.714±0.002^{b}	2.9
Varuna	111.0±0.8^{g}	109.3±0.4^{g}	1.6	0.680±0.002^{e}	0.680±0.003^{e}	0.0
PHR-126	138.8±0.4^{a}	99.5±0.2^{h}	28.3	0.712±0.003^{bc}	0.655±0.002^{f}	8.12
PBR-450	117.8±0.2^{e}	109.8±0.3^{g}	6.8	0.704±0.001^{c}	0.690±0.001^{de}	2.0
PBR-396	139.0±0.5^{a}	120.0±0.5^{d}	13.7	0.730±0.002^{a}	0.705±0.004^{bc}	3.4
PBR-464	114.0±0.9^{f}	110.5±0.8^{g}	3.1	0.692±0.004^{d}	0.688±0.002^{de}	0.5
PBR-561	127.8±0.7^{b}	111.0±0.7^{g}	13.1	0.713±0.004^{bc}	0.693±0.001^{d}	2.8
Mean	127.0 ± 0.5	111.9 ±0.5		0.709 ± 0.002	0.689 ± 0.002	
CD (p= 0.05)	S= 0.487, G= 0.912, S×G= 1.290			S= 0.002, G= 1.004,S×G= 1.005		

Least Squares-means with the different letters are significantly different (P<0.05)

Table 2 Photosynthetic pigments (mg g^{-1} FW) at 10 (S_1) and 30 (S_2) days after removal of nets in *B. juncea* (average data of 2 years)

Genotypes	Chlorophyll a			Chlorophyll b			Total Chlorophyll			Chlorophyll a/b		
	Control	Low light stress	Red (%)	Control	Low light stress	Inc (%)	Control	Low light stress	Red (%)	Control	Low light stress	Red (%)
S_1												
Kranti	1.85±0.03^{a}	1.68±0.01^{bc}	9.3	0.32±0.02^{bc}	0.44±0.01^{a}	26.1	2.17±0.02^{a}	2.11±0.03^{ab}	2.7	5.77±0.17^{abc}	3.83±0.05^{ef}	33.7
Varuna	1.66±0.01^{c}	0.95±0.03^{i}	42.7	0.33±0.02^{bc}	0.40±0.02^{a}	14.4	2.00±0.03^{c}	1.35±0.01^{g}	32.6	4.95±0.15^{cd}	2.41±0.03^{h}	51.4
PHR-126	1.48±0.02^{e}	1.44±0.02^{e}	2.2	0.34 ±0.03^{b}	0.35±0.04^{bc}	2.0	1.82±0.05^{d}	1.78±0.02^{d}	1.8	4.30±0.05^{de}	4.21±0.09^{de}	2.1
PBR-450	1.56±0.04^{d}	1.34±0.04^{f}	14.2	0.25±0.01^{d}	0.32±0.03^{bc}	22.2	1.81±0.02^{d}	1.66±0.04^{e}	8.3	6.23±0.02^{a}	4.16±0.17^{de}	33.2
PBR-396	1.47±0.02^{e}	1.06±0.01^{h}	27.9	0.30±0.04^{bc}	0.34±0.05^{bc}	5.9	1.79±0.04^{d}	1.38±0.03^{g}	23.6	4.40±0.07^{de}	3.30±0.05^{fg}	24.9
PBR-464	1.73±0.05^{b}	1.18±0.03^{g}	32.0	0.31±0.05^{bc}	0.39±0.02^{a}	17.5	2.06±0.01^{bc}	1.57±0.01^{f}	23.6	5.34±0.08^{bc}	3.00±0.02^{gh}	43.9
PBR-561	1.46±0.01^{e}	0.92±0.05^{i}	37.0	0.26 ±0.01^{d}	0.29±0.01^{cd}	6.5	1.70 ±0.03^{e}	1.21±0.05^{h}	28.8	5.97±0.06^{ab}	3.13±0.05^{fgh}	47.5
Mean	1.60 ±0.03	1.22 ±0.04		0.30 ±0.03	0.36 ±0.02		1.91 ±0.04	1.58 ±0.02		5.28 ±0.09	3.43 ±0.06	
CD (p=0.05)	S=0.014, G= 0.026, S×G= 0.037			S= 0.010, G= 0.020, S×G= 0.028			S= 0.017, G= 0.031, S×G= 0.045			S= 0.175, G= 0.328, S×G= 0.464		
S_2												
Kranti	1.59±0.02^{b}	1.43±0.04^{c}	10.0	0.29±0.00^{fg}	0.39±0.02^{d}	22.5	1.89±0.02^{b}	1.82±0.03^{c}	3.8	5.26±0.09^{ab}	2.62±0.05^{e}	50.3
Varuna	1.41±0.04^{c}	0.73±0.01^{i}	48.0	0.32±0.02^{e}	0.52±0.01^{a}	38.7	1.73±0.04^{d}	1.26±0.01^{j}	27.3	4.37±0.07^{d}	1.39±0.01^{g}	68.1
PHR-126	1.39±0.01^{cd}	1.20±0.02^{e}	14.3	0.30±0.01^{ef}	0.47±0.01^{b}	36.1	1.69±0.01^{e}	1.66±0.04^{f}	1.9	4.69±0.12^{c}	2.57±0.06^{e}	45.2
PBR-450	1.37±0.01^{d}	1.11±0.02^{f}	18.5	0.25±0.03^{h}	0.43±0.03^{c}	43.0	1.61±0.05^{g}	1.55±0.02^{h}	4.1	5.53±0.14^{a}	2.55±0.08^{e}	53.6
PBR-396	1.40±0.04^{c}	0.83±0.01^{h}	40.8	0.27±0.04^{g}	0.44±0.03^{c}	38.5	1.67±0.03^{ef}	1.27±0.05^{j}	24.0	5.15 ±0.07^{b}	1.87±0.02^{f}	63.6
PBR-464	1.64±0.03^{a}	0.96±0.03^{g}	41.5	0.31±0.02^{ef}	0.52±0.05^{a}	40.8	1.94±0.04^{a}	1.47±0.02^{i}	24.1	5.34±0.07^{ab}	1.85±0.02^{f}	65.4
PBR-561	1.42±0.01^{c}	0.69±0.02^{j}	51.3	0.32±0.01^{e}	0.41±0.04^{d}	21.2	1.74±0.03^{d}	1.10±0.02^{k}	36.9	4.44±0.06^{cd}	1.71±0.02^{fg}	61.6
Mean	1.46 ±0.02	1.00 ±0.03		0.30 ±0.02	0.48 ±0.02		1.75 ±0.03	1.48 ±0.02		4.97 ±0.09	2.08 ±0.04	
CD (p=0.05)	S=0.007, G= 0.014, S×G= 0.021			S= 0.003, G= 0.007, S×G= 0.010			S= 0.006, G= 0.012, S×G= 0.017			S= 0.057, G= 0.106, S×G= 0.151		

Lower carotenoid content in the shaded leaves was estimated at both the stages in comparison with sun-exposed leaves (Fig.1).However, Kranti was least affected by shade at S1 (5.8%) and S2 (3.4%) whereas PBR-561 registered maximum reduction of 31.9% at S1 and 47.9% at S2 stage.

Plants under low light stress exhibited lower leaf RWC (Table 3). Compared with the control treatment, RWC under shade significantly decreased by 18.7% (S1) and 16.4% (S2) while both the saturation deficits increased i.e., RSD (34.6%) and WSD (33.4%) at S1 whereas 27.8 and 30.6% respectively at S2 stage. At S1 stage, PBR-450 showed least reduction in RWC (11.0%) whereas minimum increase in RSD (18.8%) and WSD (17.2%) was in PBR-396. However at S2 stage, only variety Varuna registered least reduction in RWC (1.3%) and had minimum increase of 4.7% (RSD) and 2.9% (WSD).

Table 3 Relative water content and related traits at S1 and S2 stages in *B. juncea*(average data of 2 years)

S_1									
Genotypes	**RWC (%)**			**RSD (%)**			**WSD (%)**		
	Control	**Low light stress**	**Red (%)**	**Control**	**Low light stress**	**Inc (%)**	**Control**	**Low light stress**	**Inc (%)**
Kranti	78.3±0.4^{a}	53.7±0.7^{g}	31.4	19.6±0.2^{e}	43.5 ±0.3^{a}	54.9	21.7±0.4^{g}	46.3±0.4^{a}	53.2
Varuna	75.9±0.6^{ab}	66.7±0.6^{cd}	12.1	21.7±0.1^{e}	30.9±0.6^{cd}	29.7	24.1±0.5^{fg}	33.3±0.6^{de}	27.7
PHR-126	71.2±0.5^{bc}	54.1±0.9^{g}	23.9	26.0±0.7^{de}	42.7±0.9^{a}	39.1	28.8±0.9^{ef}	45.9±0.2^{a}	37.1
PBR-450	74.0±0.9^{ab}	65.9±0.4^{cd}	11.0	23.8±0.8^{e}	31.7±0.5^{cd}	24.8	26.0±0.2^{fg}	34.1±0.7^{de}	24.0
PBR-396	64.0±0.7^{de}	56.5±0.2^{fg}	11.7	33.0±0.4^{c}	40.7±0.3^{ab}	18.8	36.0±0.1^{cd}	43.5±0.8^{ab}	17.2
PBR-464	71.9±0.2^{abc}	57.0±0.8^{efg}	20.7	25.6±0.6^{de}	39.8±0.2^{ab}	35.9	28.1±0.4^{efg}	43.0±0.1^{abc}	34.7
PBR-561	75.3±0.8^{ab}	61.4±0.7^{def}	18.4	22.6±0.3^{e}	36.0±0.7^{bc}	37.1	24.7±0.8^{fg}	38.6±0.6^{bcd}	35.9
Mean	72.9 ± 0.2	59.3 ± 0.4		24.6 ± 0.5	37.9 ± 0.4		27.1 ± 0.3	40.7 ± 0.5	
CD (p=0.05)	S= 1.565, G= 2.928, S×G= 4.140			S= 1.469, G= 2.748, S×G= 3.887			S= 1.565, G= 2.928, S×G= 4.140		
S_2									
Kranti	72.0±0.3^{a}	49.6±0.5^{g}	31.2	25.2±0.2^{g}	47.3±0.4^{a}	46.6	28.0±0.8^{g}	50.4±0.4^{a}	44.5
Varuna	70.3±0.6^{a}	69.4±0.4^{a}	1.3	26.9±0.9^{g}	28.3±0.7^{fg}	4.7	29.7±0.4^{g}	30.6±0.5^{g}	2.9
PHR-126	64.6±0.2^{bc}	55.3±0.9^{ef}	14.3	32.0±0.4^{ef}	41.6±0.2^{bc}	23.0	35.4±0.6^{ef}	44.7±0.3^{bc}	20.6
PBR-450	68.7±0.1^{ab}	61.4±0.8^{cd}	10.7	28.3±0.5^{fg}	35.4±0.5^{de}	20.0	31.3±0.9^{fg}	38.6±0.9^{de}	19.0
PBR-396	61.4±0.5^{cd}	51.1±0.1^{g}	16.8	35.4±0.8^{de}	45.6±0.3^{ab}	22.3	38.6±0.2^{de}	48.9±0.6^{ab}	21.1
PBR-464	69.2±0.7^{a}	54.4±0.2^{ef}	21.4	28.1±0.3^{fg}	42.4±0.8^{bc}	33.7	30.8±0.5^{g}	45.6±0.8^{bc}	32.5
PBR-561	71.0±0.8^{a}	57.5±0.6^{de}	19.0	26.4±0.7^{g}	39.5±0.7^{cd}	33.2	29.0±0.3^{g}	42.5±0.2^{cd}	31.8
Mean	68.2 ± 0.4	57.0 ± 0.3		28.9 ± 0.5	40.0 ± 0.4		31.8 ± 0.3	43.0 ± 0.5	
CD (p=0.05)	S= 0.984, G= 1.842, S×G= 2.605			S= 0.926, G= 1.734, S×G= 2.452			S= 0.984, G= 1.842, S×G= 2.605		

Least Squares-means with the different letters are significantly different (P<0.05) Red: Reduction Inc: Increase

Leaf area at both the stages was significantly affected in studied genotypes/varieties and decreased with low light stress (Table 4). PHR-126 had maximum leaf area at S1 stage under control(145.4 cm^2) and low light stress (106.3 cm^2) while at S2 stage, higher leaf area was registered for PBR-561 in control (117.6 cm^2) and PBR-396 under low light stress (88.3cm^2).Under the normal sunlit condition, the average SLA was more at both the stages (0.40 and 0.53 $cm^2 mg^{-1}$) respectively. However, when the plants were grown under nets for shade, the SLA declined by45% (0.22 cm^2 mg^{-1}) at S1 and by 49.1% (0.27 cm^2 mg^{-1}) at S2 stage. Average SLW was maximum in plants grown under control condition at S1 (3.89 mg cm^{-2}) and S2 (3.23 mg cm^{-2}) stage and was reduced to 2.43 at S1 and 1.99 mg cm^{-2}at S2forshade grown plants. Significant differences existed among genotypes for SLW. Under control, Kranti (4.65 mg cm^{-2}) showed higher SLW at

S1 and PBR-561 (4.10 mg cm-2) at S2stage. However, under shade, PBR-561 (3.04 mg cm^{-2}) exhibited higher SLW at S1 andPBR-464 (2.59 mg cm^{-2}) at S2 stage. Conclusively, under control and shade PBR-561 had higher SLW irrespective of the studied two stages.

Table 4 Effect of low light stress on leaf area and related traits (average data of 2 years) at S_1 and S_2 stages in*. B juncea*

S_1									
Genotypes	**Leaf area (cm^2)**			**SLA (cm^{-2}mg)**			**SLW (mg cm^{-2})**		
	Control	**Low light Stress**	**Red (%)**	**Control**	**Low light stress**	**Red (%)**	**Control**	**Low light stress**	**Red (%)**
Kranti	118.5±1.8^{de}	89.1±2.0^{gh}	24.8	0.41±0.01^{cd}	0.20±0.02^{i}	51.6	4.65±0.01^{b}	2.50±0.01^{j}	46.1
Varuna	122.8±1.6^{cd}	68.7±0.3^{i}	44.0	0.47±0.01^{a}	0.21±0.02^{hi}	55.2	3.91±0.02^{a}	2.21±0.02^{l}	43.4
PHR-126	145.6±1.9^{a}	106.3±2.3^{f}	27.0	0.44±0.02^{b}	0.23±0.01^{gh}	48.8	3.66±0.01^{e}	2.26±0.02^{k}	38.1
PBR-450	128.1±1.5^{bc}	84.6±1.3^{h}	33.9	0.42±0.03^{bc}	0.24±0.02^{g}	42.6	3.83±0.03^{d}	2.09±0.01^{m}	45.5
PBR-396	102.0±2.2^{f}	92.9±1.0^{g}	8.9	0.39±0.01^{d}	0.22±0.01^{ghi}	44.1	3.26±0.02^{g}	2.13±0.03^{n}	34.7
PBR-464	114.0±1.5^{e}	64.7±0.5^{i}	43.3	0.36±0.02^{e}	0.17±0.02^{j}	53.7	3.48±0.01^{f}	2.78±0.01^{i}	20.0
PBR-561	132.7±3.2^{b}	86.9±0.9^{gh}	34.6	0.32±0.01^{f}	0.13±0.01^{k}	59.8	4.43±0.01^{c}	3.04±0.02^{h}	31.5
Mean	123.4 ± 2.0	84.7 ± 1.2		0.40 ± 0.02	0.22 ± 0.02		3.89 ± 0.01	2.43 ± 0.02	
CD (p=0.05)	S= 1.564, G= 2.927, S×G= 4.139			S= 0.004, G= 0.008, S×G= 0.011			S= 0.004, G= 0.008, S×G= 0.011		
S_2									
Kranti	108.1±0.5^{bc}	82.8±1.9^{g}	23.3	0.68±0.01^{a}	0.33±0.03^{g}	50.6	2.05±0.01^{h}	1.52±0.01^{m}	25.8
Varuna	102.6±1.1^{d}	65.0±1.3^{i}	36.6	0.58±0.03^{b}	0.25±0.03^{hi}	57.3	3.13±0.02^{e}	1.84±0.01^{l}	41.3
PHR-126	104.4±1.0^{cd}	84.5±0.8^{fg}	19.1	0.51±0.01^{d}	0.23±0.02^{ij}	54.3	3.61±0.02^{b}	1.98±0.02^{j}	45.1
PBR-450	107.2±0.7^{bcd}	73.5±1.8^{h}	31.4	0.53±0.02^{cd}	0.27±0.01^{h}	49.1	3.59±0.01^{c}	2.06±0.01^{h}	42.5
PBR-396	96.4±1.3^{e}	88.3±1.7^{f}	8.4	0.46±0.02^{e}	0.35±0.03^{g}	24.1	2.94±0.01^{f}	2.01±0.01^{i}	31.6
PBR-464	109.9±0.6^{b}	51.2±1.3^{j}	53.5	0.40±0.01^{f}	0.26±0.02^{h}	34.2	3.19±0.03^{d}	2.59±0.02^{g}	18.9
PBR-561	117.6±1.5^{a}	85.9±1.2^{fg}	27.0	0.54±0.02^{c}	0.21±0.02^{j}	60.9	4.10±0.01^{a}	1.94±0.01^{k}	52.6
Mean	106.6 ± 1.0	75.9 ± 1.4		0.53 ± 0.02	0.27 ± 0.02		3.23 ± 0.02	1.99 ±0.02	
CD (p=0.05)	S= 1.133, G= 2.120, S×G= 2.998			S= 0.005, G= 0.011, S×G= 0.015			S= 0.004, G= 0.007, S×G= 0.011		

Least Squares-means with the different letters are significantly different (P<0.05)

Mean LWR was higher at S1 (77.2%) with a slight difference at second stage S2 (74.1%) in plants grown under normal light condition. However low light stress of one month reduced LWR by6.9% (S1) and 8.5% (S2)over control (Fig. 1). At S1 stage, among the studied genotypes, PBR-561 registered minimal decline of 1.3% while PBR-396 the maximal of 13.1% in LWR. At S2 stage, minimal decrease of LWR was in Kranti (0.5%) and PBR-464 (0.7%) while maximum inPHR-126 (18.9%).

Table 5 Correlation coefficients of physiological traits at S_1 stage under control (below the diagonal) and low light stress (above the diagonal)

Traits	Chl a	Chl b	T Chl	CAR	SPAD	LL	LW	LA	SLA	SLW	LWR	RWC	RSD	WSD	SY
Chl a	1	0.489	0.989**	0.887**	-0.146	0.223	0.077	0.417	0.433	-0.225	-0.23	-0.558	0.565	0.558	0.128
Chl b	0.315	1	0.612	0.828**	-0.019	-0.033	-0.530	-0.323	0.178	-0.126	-0.234	-0.315	0.318	0.315	-0.500
T Chl	0.965**	0.549	1	0.946**	-0.136	0.204	-0.013	0.310	0.416	-0.216	-0.231	-0.546	0.553	0.546	0.016
CAR	0.961**	0.405	0.963**	1	-0.127	0.119	-0.217	0.048	0.309	-0.14	-0.222	-0.507	0.512	0.507	-0.200
SPAD	-0.761*	0.337	-0.585	-0.676*	1	0.4	0.227	-0.027	0.542	-0.527	-0.520	0.156	-0.181	-0.156	-0.219
LL	-0.374	-0.089	-0.389	-0.445	0.442	1	0.826**	0.391	0.210	-0.119	0.175	0.072	-0.078	-0.072	-0.401
LW	-0.483	-0.268	-0.524	-0.511	0.379	0.928**	1	0.555	-0.012	0.113	0.257	0.047	-0.055	-0.047	0.038
LA	-0.321	-0.168	-0.355	-0.35	0.283	0.919**	0.981**	1	0.335	-0.257	-0.288	-0.497	0.508	0.497	0.609
SLA	0.175	0.556	0.298	0.320	0.334	0.279	0.126	0.175	1	-0.965**	-0.497	0.039	-0.036	-0.039	0.039
SLW	0.389	-0.324	0.227	0.219	-0.493	0.365	0.261	0.317	-0.146	1	0.452	-0.177	0.171	0.177	0.001
LWR	0.049	-0.367	-0.074	-0.016	-0.128	0.256	0.111	0.060	0.166	0.695*	1	0.613	-0.601	-0.613	-0.427
RWC	0.570	-0.198	0.418	0.413	-0.562	0.432	0.312	0.416	0.111	0.854**	0.463	1	-0.999**	-0.999**	-0.518
RSD	-0.575	0.166	-0.43	-0.421	0.543	-0.444	-0.319	-0.426	-0.137	-0.846**	-0.453	-0.999**	1	0.999**	0.523
WSD	-0.570	0.198	-0.418	-0.413	0.561	-0.432	-0.312	-0.416	-0.111	-0.854**	-0.463	-0.999**	0.999**	1	0.518
SY	-0.893**	-0.030	-0.786*	-0.777*	0.801**	0.259	0.398	0.275	-0.073	-0.445	-0.146	-0.697*	0.693*	0.697*	1

*Significant at 5%, ** Significant at 1%, Chl- Chlorophyll, T Chl- Total chlorophyll, CAR- Carotenoids, LL- Leaf length, LW- Leaf width, LA- Leaf area, SLA- Specific leaf area, SLW- Specific leaf weight, LWR- Leaf water retention, RWC- Relative water content, RSD- Relative saturation deficit, WSD- Water saturation deficit, SY- Seed yield

Table 6 Correlation coefficients of physiological traits at S_2 stage under control (below the diagonal) and low light stress (above the diagonal)

Traits	Chl a	Chl b	T Chl	CAR	SPAD	LL	LW	LA	SLA	SLW	LWR	RWC	RSD	WSD	SY
Chl a	1	-0.378	0.983**	0.875**	0.409	0.141	-0.027	0.143	0.347	-0.325	-0.477	-0.499	0.499	0.499	0.120
Chl b	0.375	1	-0.206	-0.084	0.336	-0.219	-0.093	-0.767*	-0.314	0.610	-0.134	0.506	-0.503	-0.506	-0.513
T Chl	0.974**	0.570	1	0.909**	0.505	0.115	-0.048	-0.002	0.310	-0.237	-0.512	-0.409	0.410	0.409	0.011
CAR	0.934**	0.084	0.854**	1	0.286	-0.093	-0.287	-0.151	0.410	-0.296	-0.252	-0.383	0.394	0.383	-0.204
SPAD	-0.312	0.211	-0.228	-0.479	1	0.075	-0.010	-0.188	0.189	0.235	-0.519	0.102	-0.116	-0.102	0.059
LL	-0.605	0.037	-0.549	-0.793*	0.395	1	0.913**	0.352	-0.598	-0.468	-0.181	0.462	-0.463	-0.462	-0.290
LW	-0.517	0.106	-0.454	-0.700*	0.174	0.956**	1	0.276	-0.815**	-0.157	-0.382	0.383	-0.387	-0.383	-0.145
LA	0.294	0.432	0.354	0.103	-0.281	0.31	0.394	1	0.222	-0.650	-0.008	-0.392	0.394	0.391	0.569
SLA	-0.024	0.139	0.032	-0.081	0.375	0.187	0.176	0.132	1	-0.268	0.274	-0.532	0.535	0.532	0.371
SLW	-0.549	0.056	-0.494	-0.659	-0.052	0.671*	0.662	0.407	-0.450	1	-0.379	0.019	-0.035	-0.019	0.157
LWR	-0.904**	-0.138	-0.837**	-0.966**	0.502	0.810**	0.692*	0.018	0.237	0.611	1	0.356	-0.345	-0.356	-0.347
RWC	0.424	0.475	0.491	0.215	0.249	0.140	0.087	0.725*	0.552	-0.102	-0.021	1	-0.999**	-0.999**	-0.747*
RSD	-0.412	-0.465	-0.478	-0.200	-0.266	-0.167	-0.114	-0.717*	-0.574	0.108	0.006	-0.999**	1	0.999**	0.732*
WSD	-0.424	-0.475	-0.491	-0.215	-0.249	-0.140	-0.087	-0.725*	-0.552	0.102	0.021	-0.998**	0.999**	1	0.747*
SY	-0.645	-0.295	-0.648	-0.534	-0.314	0.305	0.426	-0.303	-0.371	0.485	0.367	-0.811**	0.805**	0.811**	1

*Significant at 5%, ** Significant at 1%, Chl- Chlorophyll, T Chl- Total chlorophyll, CAR- Carotenoids, LL- Leaf length, LW- Leaf width, LA- Leaf area, SLA- Specific leaf area, SLW- Specific leaf weight, LWR- Leaf water retention, RWC- Relative water content, RSD- Relative saturation deficit, WSD- Water saturation deficit, SY- Seed yield

Table 7 Correlation coefficients of chloro flourescence at S_2 stage under control (below the diagonal) and low light stress (above the diagonal)

Traits	F_0	F_m	F_v	F_v/F_m	SY
F_0	1	-0.519	-0.631	-0.796*	-0.259
F_m	0.495	1	0.991**	0.929**	0.197
F_v	0.368	0.990**	1	0.971**	0.220
F_v/F_m	-0.028	0.854**	0.918**	1	0.238
SY	0.580	0.583	0.529	0.343	1

*Significant at 5%, **Significant at 1%, F_o- Minimum flourescence, F_m- Maximum flourescence, F_v- Variable flourescence, F_v/F_m- Photochemical efficiency of PSII, SY- Seed yiel

Seed yield was reduced under low light stress by 15.8% over control and ranged from 1159.9 (Kranti) to 1680.**3** kg ha^{-1} (Varuna) in control whereas from 595.7 (Varuna) to1602.0 kg ha^{-1}(PBR-450) under low light stress. PBR-396 suffered minimal yield decline of 1.8% and maximum of 51.1% in Varuna (Fig.2).

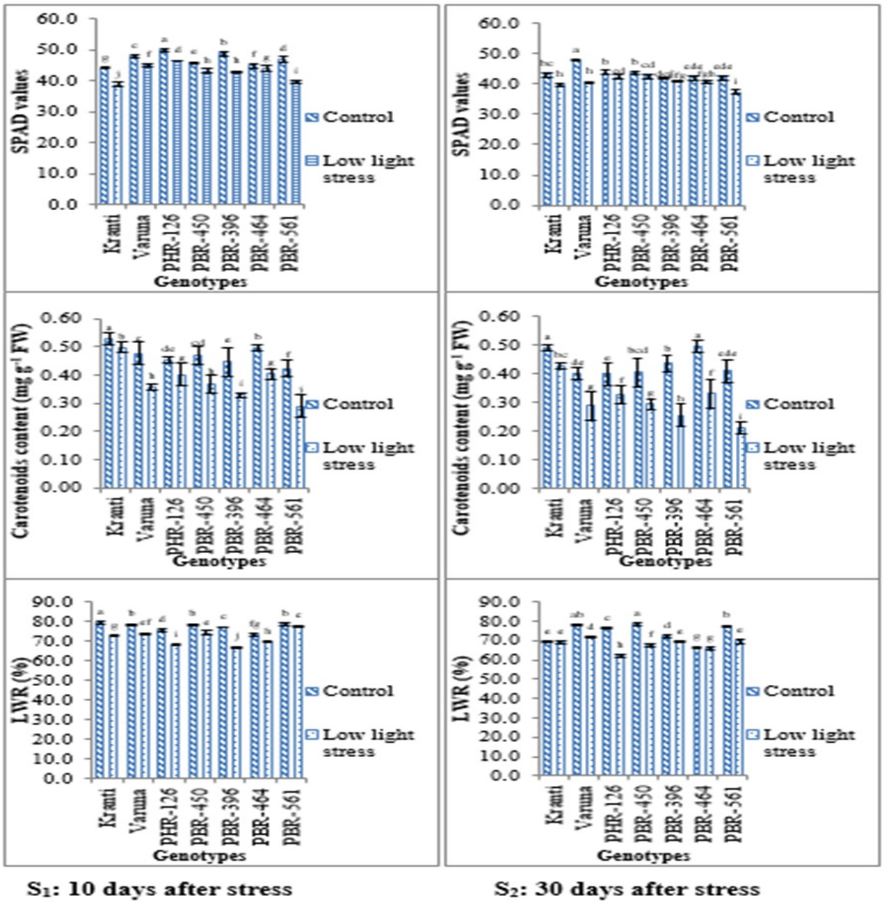

Figure 1 Variation in SPAD, carotenoids and LWR at S_1 and S_2 stages in *B. juncea* (average data of 2 years)

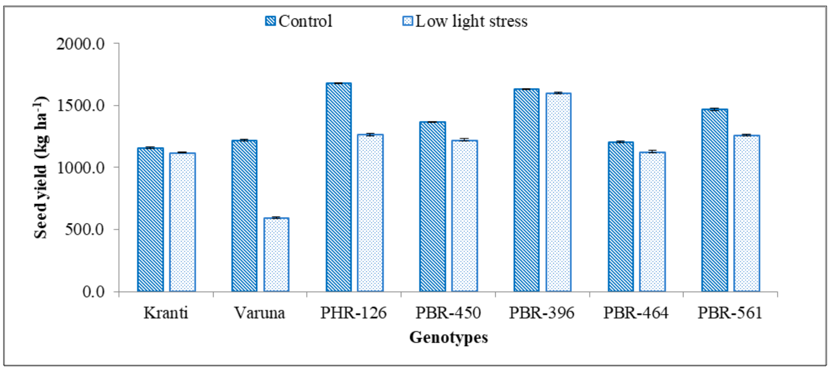

Figure 2 Effect of low light stress on seed yield in *B. juncea* (average data of 2 years)

Correlation between different traits under control and low light stress at S1 stage is depicted in Table 5. At S1 stage, significant and negative correlation existed between seed yield and chlorophyll a in control (-0.893**) but was positive (0.128) under low light stress. Negative association occurred between seed yield and chlorophyll b under both control (-0.030) and low light stress (-0.500). Seed yield had negative correlation with total chlorophyll (-0.786*) and carotenoids (-0.777*) under control and also under stressed condition (-0.200). Significant positive association of seed yield with SPAD existed in control (0.801**) while it was negative under low light stress (-0.219). Seed yield was negatively correlated with RWC (-0.697*) however association was positive with RSD (0.693*) and WSD (0.697*) in control and similar trend was witnessed under shade. Highly positive correlation existed between carotenoids and chlorophyll a (0.961**), total chlorophyll (0.963**) in control along with chlorophyll a (0.887**), chlorophyll b (0.828**) and total chlorophyll (0.946**) in shaded plants. Negative correlation was found between SPAD and chlorophyll a (-0.761*) and carotenoids (-0.676*). Leaf area in the controlled plants had positive and significant association with length (0.919**) and width (0.981**) along withthe shade plants for leaf length (0.391) and width (0.555). Shaded plants had significant negative association with SLA and SLW (-0.965**) and similar relationship existed between the RWC with RSD (-0.999**) and WSD (-0.999**) under both the treatments. However, strong positive association was registered between RSD and WSD (0.999**) under control and low light stress. Association between SLW with LWR (0.695*) and RWC (0.854**) was significant in control whereas positive with LWR (0.452) but negative with RWC (-0.177) with 25-30% shading. Significant and negative association existed between SLW with RSD (-0.846**) also with WSD (-0.854**) in sunlit plants but stress weakened this relationship.

Photosynthetic pigments had negative association under control and low light stress except for chlorophyll a (0.120) and total chlorophyll (0.011) as shown in Table 6. Seed yield and RWC in control (-0.811**) and shaded (-0.747*) had significant negative correlation. However, under both treatments, seed yield had positive association with RSD and WSD. Significant positive correlation existed between chlorophyll a and total chlorophyll (0.974**), carotenoids and chlorophyll a (0.934**) and carotenoids and total chlorophyll (0.854**) in the sunlit plants and similar positive association was also found between these pigments under low light stress. In controlled plants, leaf length had significant correlation with SLW (0.671*) and LWR (0.810**) whereas negative association with SLW (-0.468) and LWR (-0.181) in shaded plants. Leaf width with LWR (0.692*) and leaf area with RWC (0.725*) had positive association in control which however was negative with imposed low light stress. A negative correlation existed between RWC with RSD (-0.999**) and WSD (-0.998**) under control and likewise with RSD (-0.999**) and WSD (-0.994**) with low light stress. RSD and WSD had significant positive association under stressed (0.999**) and control (0.999**) plants. Sunlit plants had positive association with studied chloro fluorescence parameters i.e., Fm (0.583), Fv (0.529), Fv/Fm (0.343) and the association was weakened in the shaded condition (Table 7).

4. Discussion

Environmental conditions significantly affected photosynthetic activity of Brassicas, as Fv/Fm values were lower in the wet year than in the dry year, which indicated photoinhibition in plants in response to environmental fluctuations (Sharma, 2015). Low light stress significantly reduced the chlorophyll fluorescence. Significant high values of Fv/Fm in soybean have also been reported under high light as compared to low light according to Feng et. al, (2019). Higher quantum yield of 9% (Fv/Fm) with high light intensity indicated the role of light in improving the chlorophyll fluorescence and photosynthetic capacity of soybean leaves. Similar findings have been reported by Khalid et. al, (2019) for maintaining optimum growth and development under changing light conditions.

Chlorophyll pigment is a vital index for the development of chloroplast and the capacity for photosynthesis (Zhang et. al, 2020). Total chlorophyll and SPAD values were higher in the control plants as compared to the shaded/stressed plants. Our findings are consistent with those of Dong et. al, (2018) in wheat where higher SPAD and total chlorophyll was reported in relation to black polythene screens over iron nets. SPAD values were higher in the sole soybean crop than its intercropping with maize (Raza et. al, 2019).SPAD values increased with increased light intensity in Rosa hybrid as reported by Fanourakis et. al, (2019).

Our results indicated significant increase in chlorophyll b content in all the cultivars. Earlier Yao et. al, (2017) have also reported increased chlorophyll b under low light stress which further decreased chl a/b ratio in rape (*Brassica napus*). This is the shade tolerance response which optimizes the light capture and utilization, including the increase in Chl b content and reduction of chl a/b ratio (Wu et. al, 2017). Study from our laboratory by Kaur, (2018) revealed reduced chlorophyll a and total chlorophyll but enhanced chlorophyll b content in *B. juncea* and *B. napus* (unpublished) with imposed low light stress. However, according to Mishra and Chaturvedi, (2019) photosynthetic pigments increased gradually with decreasing light intensity in *B. juncea*. In a recent study of Perkasa et. al, (2020) in soybean showed considerable increase in chlorophyll b and total chlorophyll content with 55% shading. Carotenoid content in the studied cultivars was significantly affected by imposed 25-30% shading. Decreased carotenoid content with decreased light intensity has already been reported by Yao et. al, (2017) in rape seedlings, sweet pepper(Sui et. al, 2012) and tomato (Shu et. al, 2016). Carotenoid content declined by 5.8% in *B. juncea* with shade (Kaur, 2018) but an average increase of 20% with high light intensity ensured chlorophyll protection in soybean by Feng et. al,. (2019). Recently, a study on *Arachis hypogea*, revealed that shading increased the Chl content but reduced the Chl a/b ratio (Wang et. al, 2021).

Our results are corroborated with the observations of Golezani et. al, (2013) in soybean where RWC decreased with shading probably due to decrease in temperature and transpiration. RWC decreased under low light intensity in rose (Fanourakis et. al, 2019) and. *B. juncea* (Kaur, 2018). Reverse trend in RSD and WSD has been reported in our study which are in accordance with the findings of (Kaur, 2018) and with other abiotic stresses in mustard viz., moisture stress (Rhythm, 2020) and heat stress [Kaur, 2020; Priya, 2020].Light limiting conditions inhibits photosynthesis which directly affects the leaf area and related leaf traits. Leaf area decreased in the present investigation. Results of Wu et. al, (2017) in soybean reported similar decline in the total leaf area under low light stress than the normal light condition and were later endorsed by Fanourakis et. al, (2019) in *Rosa hybrid* longer leaf length but smaller width in shaded soybean plants has been reported by Perkasa et. al, (2020) as compared to non-shaded plants. Shading reduced SLA in Indian mustard (Kaur, 2018). Decrease in SLW has also been reported in *B. juncea* by16.7% (Kaur, 2018) and in soybean with the increased shading levels (Jumrani and Bhatia, 2020).Studies in *B. juncea* from our laboratory revealed a significant effect of abiotic stresses on leaf water retention (LWR). Moisture stress decreased water retention capacity (Chowdhury et. al, 2017) in soybean and recently in Indian mustard (Rhythm, 2020). Water retention capacity was higher in sunlit *B. juncea* genotypes (Kaur, 2020) and declined with imposed low light stress. Similar trend under terminal heat stress has been reported by Priya (2020).

Sharma, (2015) reported positive correlation of maximum fluorescence with photochemical efficiency of PSII (0.726**) in different Brassica species. Our results are corroborated with the earlier study of Kaur and Sharma, (2015) where a significant correlation existed between physiological traits and seed yield under different moisture regimes of *B. juncea*. A negative correlation existed between SLA and SLW and also SLA with LWR and seed yield in moisture stressed *B. juncea*. Correlation coefficients of chlorophyll and carotenoids showed parallelism with findings of Majidi et. al, (2015) in Brassica species under both non-stress and severe abiotic stress conditions. Seed yield had positive association with SPAD under moisture stress (Rhythm, 2020) and late sown conditions (Priya, 2020) in *B. juncea*

Abbreviations:

PS II – Photosystem II
RWC – Relative water content
RSD – Relative saturation deficit
WSD – Water saturation deficit
LA – Leaf area
SLA – Specific leaf area
SLW – Specific leaf weight
LWR – Leaf water retention
Red – Reduction; Inc –Increase

5. Conclusion

The significant effects of low light stress on oilseed crops have been extensively investigated, but rarely scientists have studied the effect of shade in *B. juncea* under field conditions to identify shade insensitive varieties/ germplasm under changing climatic conditions. Our study revealed the impact of low light stress imposed by nets which cuts the incoming solar radiations by 25-30% for one month, significantly affected the physiological characteristics thereby decreasing the potential yields. However, variations existed in the genotypes for the studied traits to imposed low light stress. The promising cultivars PBR-396 and PBR-464 suffered lesser decline in the physiological traits and maintained relatively higher RWC, LWR in response to low light stress. Leaf traits played a vital role in the light harvesting capacity under shade with increased chlorophyll b imparting tolerance manifested in the form of differential genotypic responses. The elite genotypes can be grown in the intercropping and agro forestry trails tosustain the growing need for edible oil.

Compliance with ethical standards

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Disclosure of conflict of interest

No potential conflict of interest was reported by the authors.

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