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Research Article

PHYSIO-BIOCHEMICAL ATTRIBUTES, LEAF SPECTRAL TRAITS AND THEIR ASSOCIATION WITH SHADE RESILIENCE IN A MAJOR PULSE CROP LENTIL (*Lens esculenta*)

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Abstract: Various regimes of shade have differentially modulated the physio-biochemical attributes and leaf spectral traits in one of the major pulse crop lentil (*Lens esculenta*) under semi-arid agroclimatic conditions of Central India. There were about 20%, 48% and 69% reductions in the Photosynthetic photon flux density (PPFD) saturated rate of CO_2 assimilation (Amax) under 33% shade, 50% shade and 75% shade respectively in comparison to the open grown lentil. Enzymatic antioxidant activities have differential responses depending upon the level of shades. Peroxidase activity was the highest in open sunlight and progressively declined with the increase of the level of shade being the lowest enzyme activity obtained under 75% shade. A trend was noted in catalase enzyme activity. Lentil crop has exhibited a progressively increasing trend in ascorbate oxidase activity with increasing levels of shade which has importance in acquiring shade-resilience. Among the leaf spectral traits, Normalized Difference Vegetation Index (NDVI) of lentil showed a progressive declining trend with increasing level of shade from open to 75% shade, whereas, Photochemical Reflectance Index (PRI) progressively increased with increasing levels of shade. Interrelationships among the various physio-biochemical attributes and leaf spectral traits have revealed very significant associations and more importantly, the grain yield was positively correlated with NDVI, Amax, ETR, peroxidase and catalse enzyme activities. Physio-biochemical components in association with the leaf spectral traits have been emerged as the major contributing factors for shade resilience in lentil.

Keywords: *Antioxidants, Biochemical attributes, CO_2 assimilation, Leaf spectral traits, Photosynthesis, Shade*

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Introduction

Incident sunlight is one of the major essential requirements for ideal-cum-optimum growth and productivity of crops or plants. Shade is the manifestation of low incident sunlight as it occurs due to many natural and circumstantial situations namely cloud covers, fog, smog, intercropping, multi-strata agriculture and agroforestry for combining trees and crops in the same piece of land [1]. Lentil crop has immense importance being one of the major pulse crops in India [2]. For enhancing pulse crop production to meet the nutritional demand and food security, the accommodation of lentil in various land use systems including agroforestry is gaining momentum [3]. Lentil being light demanding crop, its cultivation under low light or shady environment faces a serious challenge for adopting it in many land use systems for multiple purposes including agroforestry. Thus, limitation due to shade for potential yield of crops is one of the major challenges which acquires a central focus for enhancing the crop production.

To cope with the shade-induced limitation in crop production of lentil, it was felt essential to unravel the various inherent physio-biochemical responses due to various regimes of shade which is still ambiguous for a greater understanding of shade resilience [4]. Various level of leaf function takes place at physiological and biochemical levels in plants growing in a shade which would determine the capacity of the crop plant to show resilience due to shade [5]. In this connection, how the physiological efficiencies and leaf spectral traits are contributing to deal with the shade-induced limitation is not very clear.

Incident sunlight is one of the major physical components to build the light using behaviour of the green leaves. Incident sunlight on leaves meets three different physical phenomena namely light transmission, reflection and absorption by the leaves [6]. Based on these properties and the physiological functioning status of the leaves, various leaf spectral traits are derived for understanding their physiological importance [7].

However, these leaf spectral indices *e.g.* NDVI and PRI can be remarkably modulated under different regimes of shade. Thus, it is very important to unravel their association with the other major physiological attributes to know the mechanistic insights for coping with the limitation of shade.

The pulse crop for nutritional security globally in general and especially in India, is well known [8]. Increasing importance of lentil and being one of the major pulse crops cultivated in the Indo-Gangetic plain including in Central India, lentil was selected for conducting the experiment. Mechanistic insights on the shade resilience in lentil crop remained a research gap for wider perspectives and thus the major objectives were to reveal the role and association of the physio-biochemical attributes and the leaf spectral traits for different levels of shade for understanding their intricacies in conferring shade resilience in the crop.

Materials and Methods

The experiment was conducted in field under simulated shade net houses of varying level of shade and in open field (control) during rabi season in 2013-14 with lentil (*Lens esculenta* variety Mallika) at Central Agroforestry Research Institute, Jhansi. Three shade net houses (25x8x3 m) and each of these shade net houses provided 33%, 50% and 75 % shade of incident sunlight. Different intensity of shade was obtained in each of two separate shade net houses as different category (porosity) of agro-shade net made up of high-density polyethylene (HDPE) was used to cover the respective net house. The crops were grown taking all the standard agronomic practices and plant protection measures. Six uniform plants from the plots (5.8x3.4m) in all four treatments (open, 33% shade, 50% shade and 75% shade) were labelled with tags for conducting physio-biochemical experiments. To get a similar range of PPFD from solar radiation, the measurements were conducted in the morning between 09:30 and 11:30.

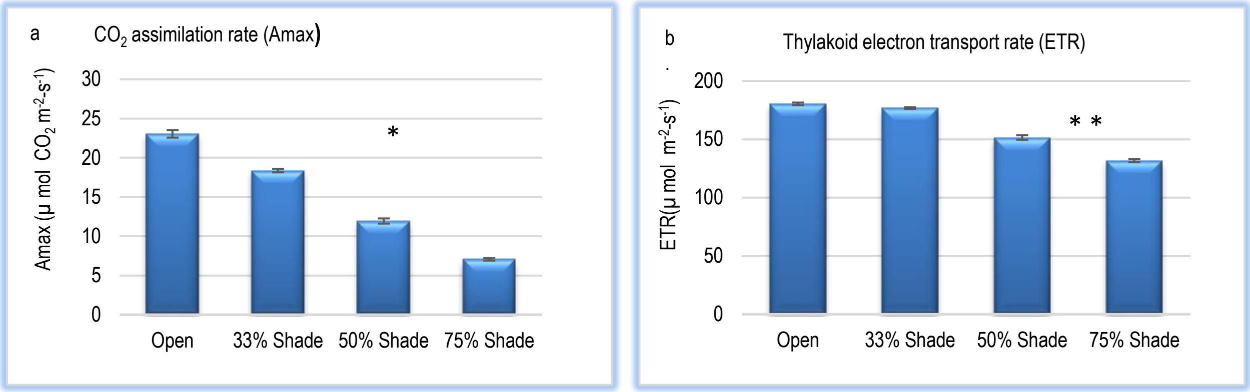

Fig-1 Modulation of CO_2 assimilation (a) and thylakoid electron transport (b) under the impact of various levels of shade on lentil. Vertical bars represent standard errors.
* Significant at 5% level of significance (CD = 0.86). ** Significant at 5% level of significance (CD = 3.58).). CD = critical difference.

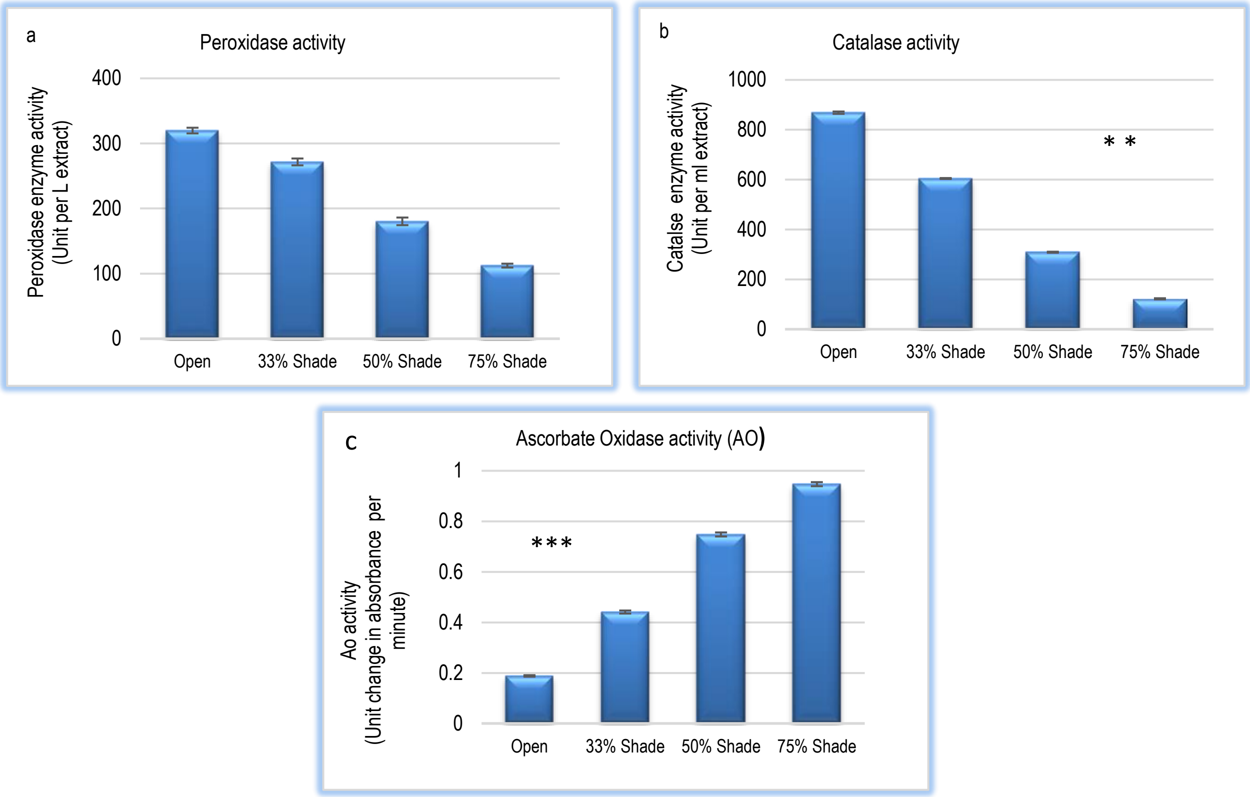

Fig-2 Differential responses in antioxidant enzyme activities (a.Peroxidase b. Catalase and c. Ascorbate oxidase as influenced by various levels of shade on lentil . Vertical bars represent standard errors.
* Significant at 5% level of significance (CD = 10.53). ** Significant at 5% level of significance (CD = 7.90). *** Significant at 5% level of significance (CD = 0.017).). CD = critical difference.

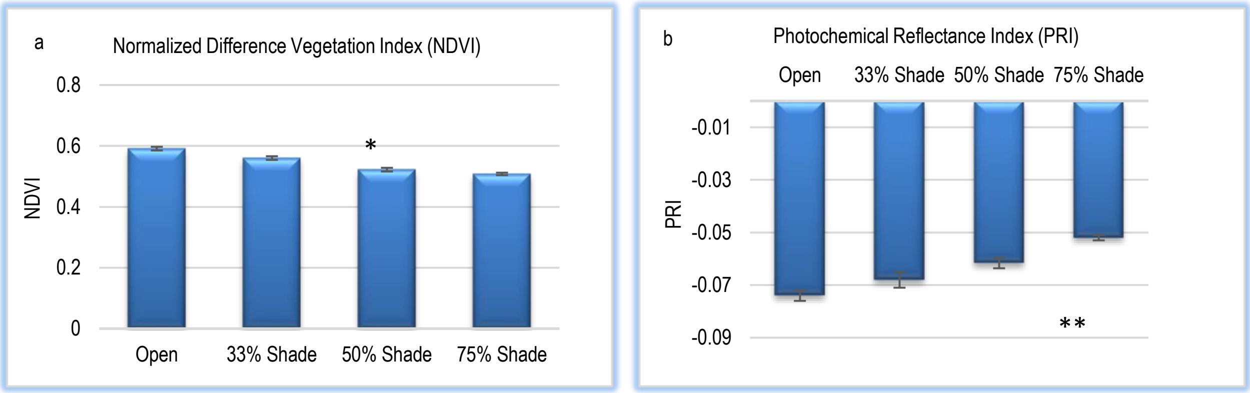

Fig-3 Impact on NDVI (a) and PRI (b) as influenced by various levels of shade on lentil. Vertical bars represent standard errors.
* Significant at 5% level of significance (CD = 0.014). ** Significant at 5% level of significance (CD = 0.005). CD = critical difference.

Simultaneous measurements of net CO_2 assimilation and chlorophyll fluorescence were made on the same leaf surface using a portable photosynthesis system (LI-6400 XT, LI-COR Inc., U.S.A.) and the thylakoid electron transport rate (ETR) was estimated from the chlorophyll fluorescence data (photosystem-2 quantum yield) following refereed protocols [9-10]. All the physio-biochemical measurements and sampling were conducted at physiological maturity with fully expanded leaves when the age of the plants was between 60 and 65 DAS (days after sowing). There were six replications for each of the measurements made.

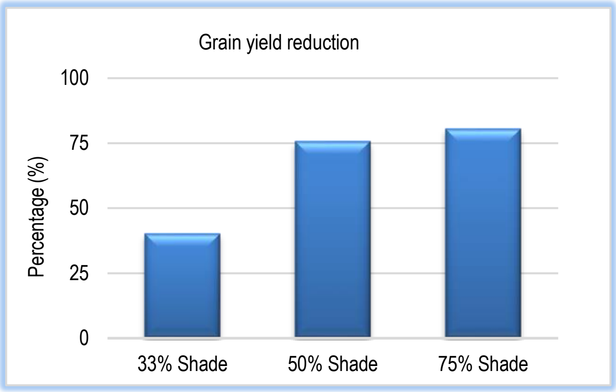

Fig-4 Comparative reduction of grain yield of lentil as affected by various levels of shade

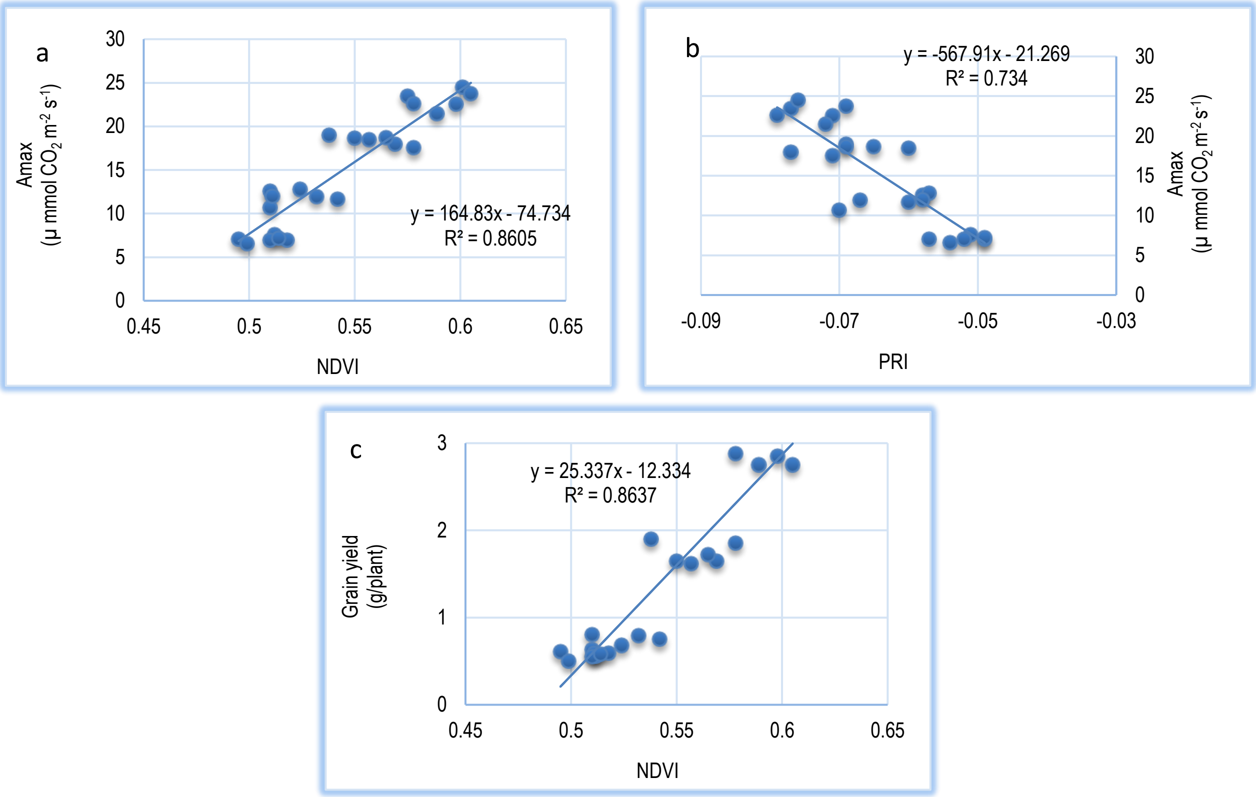

Fig-5 Interrelationship of leaf spectral traits and physiological attributes as influenced by various levels of shade on lentil (a. NDVI vs Amax b. PRI vs Amax and c. NDVI vs grain yield).

Peroxidase enzyme was measured spectrophotometrically by using Phosphate buffer (0.1 M, pH 7.0), Guaiacol solution (20 mM), Hydrogen peroxide solution-(0.042% or 12.3 m M) and enzyme extract from fresh leaves of lentil following the method of Putter (1974)[11]. Catalase enzyme activity was estimated by using Phosphate buffer (0.067 M, pH 7.0) and Hydrogen peroxide by following the method of Luck (1974) [12]. Phosphate buffer (0.1 M, pH 5.6 for assay and Phosphate buffer (pH 6.5) for extraction separately and ascorbic acid in phosphate buffer as substrate solution (pH 5.6) was used for the assay of Ascorbate oxidase (AO) enzyme and the absorbance change was measured at 265 nm in 30 sec intervals for 5 min according to Oberbacher and Vines (1963)[13]. Leaf spectral reflectance was measured using CI-710/720 Miniature Leaf Spectrometer (CID- Bioscience, USA). Measurement of mean reflectance percentage and average data calculation were conducted from 400 nm to 1000 nm wavelength. From these leaf spectral data, Normalized Difference Vegetation Index (NDVI) and Photochemical Reflectance Index (PRI) were derived from the equations mentioned below following the techniques of Gamon *et al.* (1993) [14] and Penuelas *et al.* (1995) [15]. NDVI= (R800-R680)/ (R800+R680) and PRI = (R531−R570)/ (R531+R570) where R stands for the reflectance at the respective wavelength.

Statistical analysis

To find out the significance of the experimental results, analysis of variance (ANOVA) was done following the standard procedure along with computation of standard errors and presenting graphs using MS-Excel software followed by the estimation of the critical difference (CD) at 5% level of statistical significance. CD value has been mentioned in the respective graph's legends.

Results

Different levels of shade have affected the photosynthetic CO_2 assimilation capacity in lentil differentially. Photosynthetic photon flux density (PPFD) saturated rate of CO2 assimilation (Amax) was 23.05 µmol CO_2 $m^{-2}s^{-1}$ in open sunlight and it was 18.37 under the 33% shade in comparison to the open sunlight [Fig-1a]. There were about 48% and 69% reduction in the Amax under 50% shade and 75% shade respectively in comparison to the open grown lentils [Fig-1a]. Thylakoid electron transport rate (ETR) in open sunlight was the maximum as 180.34 µmol $m^{-2}s^{-1}$ and the minimum being 131.93 µmol $m^{-2}s^{-1}$ under the 75% shade [Fig-1b].

Enzymatic antioxidant activities of peroxidase were the highest in open sunlight and progressively declined with the increase of the level of the shade being the lowest enzyme activity obtained under 75% shade [Fig-2a].

Table-1 *Correlation matrix of physio-biochemical and leaf spectral attributes in lentil as revealed under various regimes of shade*

	NDVI	PRI	Amax	ETR	Peroxidase	Catalase	AO	Grain yield
NDVI	1							
PRI	-0.80055	1						
Amax	0.927651	-0.85674	1					
ETR	0.870444	-0.83408	0.960982	1				
Peroxidase	0.91272	-0.881	0.990742	0.973775	1			
Catalase	0.941356	-0.87057	0.99104	0.948083	0.986422055	1		
AO	-0.93544	0.875676	-0.9907	-0.95534	-0.98872837	-0.99852	1	
Grain Yield	0.929338	-0.81969	0.953365	0.865034	0.93743731	0.972706	-0.96758	1

Similar trend was noted with the enzyme activities of catalase [Fig-2b]. However, Ascorbate oxidase (AO) activity progressively increased with the increasing level of shade in comparison to the open sunlight [Fig-2c].

The leaf spectral trait Normalized Difference Vegetation Index (NDVI) of lentil showed a progressive declining trend with increasing levels of shade from open to 75% shade [Fig-3a]. However, the other trait Photochemical Reflectance Index (PRI) of lentil progressively increased with increasing level of shade from open to 75% shade [Fig-3b].

The yield of lentil has been adversely affected by the different levels of shade. There was about 40% reduction of yield under 33% shade in comparison to the open grown lentil [Fig-4]. However, grain yield in lentil declined alarmingly under 50% shade and 75% of shade being about 75% and 80% reduction in comparison to the open grown lentil.

Linear regression between NDVI versus Amax and NDVI versus grain yield were positively correlated [Fig-5a] and [Fig-5c]. Linear regression between PRI versus Amax was negatively correlated [Fig-5b].

Interrelationship among the various physio-biochemical attributes and leaf spectral traits have revealed very significant associations [Table-1]. More importantly, the grain yield was positively correlated with NDVI, Amax, ETR, peroxidase and catalase enzyme activities [Table-1].

Discussion

Reduction in PPFD saturated rate of CO_2 assimilation (Amax) under various levels of shade indicated the limitation of incident light and this has been supported by the reduced photosystem activities through limited thylakoid electron transport. Various levels of shade have impacted the physiological functioning of the leaves and due to shade, the leaves could not achieve maximum photosynthetic carbon gain as it has been noticed in other crops as well [16]. The thylakoid electrons supply also emerged as a limitation under shade and it has been reflected in reduced rate of CO_2 assimilation.

Leaf photosynthetic functions are well connected with leaf cellular antioxidant activities through various antioxidant enzymes [17]. Antioxidant enzymes perform housekeeping functions for maintaining redox balances in the cell [18]. Shade has modified the efficiencies of these enzymes and lentil crop has exhibited its cellular functions through altered enzyme activities under various shade. Shade resilience in lentil is corroborated with the decrease in peroxidase, catalase enzyme activities and whereas, it has balanced the cellular environment with increased ascorbate oxidase. Higher ascorbate oxidase activities indicated a better redox balancing phenomenon as the ascorbate oxidase being one of the components of major antioxidant cascades in green leaves [19]. Various physiological responses in crops are connected with stress resilience or tolerance strategies experiencing stress related defense system [20-22]

Green leaves perform various physiological functions which are invariably dependent on light availability. Incident sunlight on leaves meets three different physical phenomena namely light transmission, reflection and absorption by the leaves [23]. Based on these properties and physiological functioning status of the leaves, various leaf spectral traits are derived for understanding their physiological importance. NDVI clearly indicated its modulation under various regimes of shade in lentil. Decrease in NDVI suggested the changes in its photosynthetic functioning and it has been corroborated with the observed decline in Amax under shade. In many other crops NDVI has been observed as an indicator of various performing ability of the crops under given growing conditions [16]. Photosynthetic carbon gain is associated with leaf photochemical activities [10]. Increase in PRI associated with the decline in electron transport rate clearly suggested the inhibition in photochemical activities. Low or downregulated photochemical events will determine the photosynthetic functions of the plants under various stressful situations [24]. Lentil crop under different level of shades experienced critical physiological limitations and the crop has adapted for shade resilience by responding differently depending upon the degree of shades. The resilience of lentil has been reflected in maintaining the thylakoid electron transport rate (ETR) despite the declining trend in Amax and grain yield in the different levels of shade.

On one hand the linear regression between NDVI versus Amax and PRI versus Amax revealed a good association, on the other, a good association of NDVI versus grain yield indicated its importance. Strong association among the physiological attributes, leaf spectral traits and grain yield has been evidenced through the linear regression and correlation matrix. All the physio-biochemical attributes and leaf spectral traits have shown significant association with the grain yield of lentil which indicated their remarkable association in shade-resilience in crops.

Conclusion

Different physiological components namely selected physio-biochemical attributes along with the leaf spectral traits have emerged as the major contributing factors for shade resilience in lentil. Our findings have also revealed the strong association between the major physiological attributes and leaf spectral traits in lentil which would be very useful for improving shade-resilience in crops.

Application of research

The findings have remarkable importance for use in evaluation, selection and improving shade-resilience in crops.

Research Category: Crop physiology

Abbreviations: Amax-PPFD saturated rate of CO_2 assimilation,
AO-Ascorbate oxidase, ETR- electron transport rate,
NDVI-Normalized Difference Vegetation Index,
PRI-Photochemical Reflectance Index

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Institute: ICAR-Central Agroforestry Research Institute, Jhansi, 284003, India
Research project name or number: Evaluation of shade tolerance of crop species for agroforestry system.

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: ICAR-Central Agroforestry Research Institute, Jhansi, 284003, Uttar Pradesh, India

Cultivar / Variety / Breed name: Lentil (*Lens esculenta*) Variety: Mallika

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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