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Comparative adaptive traits in green gram (*Vigna radiata* L.) and soybean (*Glycine max* L.) as influenced by varying regimes of shade

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Abstract

Study of shade adaptive traits of understorey crops is of utmost importance for their potential use in agroforestry system. The present study was conducted to decipher shade-adaptive traits through comparative changes in leaf pigments, epicuticular wax level and protein profiles in leaves of green gram and soybean grown in three different regimes of shade (33%, 50%, and 75% shade) and without shade (open sunlight). Chlorophyll a (Chl a), Chl b and total Chl progressively increased, whereas, Chl a/b ratio gradually decreased with increase in shade intensity in both the crops. Anthocyanin, wax level and soluble protein of leaves gradually decreased with increase in shade. Green gram showed higher anthocyanin than soybean in all the light environments. Wax level of leaf in green gram was higher in open than soybean, whereas, it was comparable in 33% shade in both the crops. Wax level of leaf in green gram decreased more in deep shade (50% shade and 75% shade) than soybean indicating more shade resilience of soybean. The rate of shade-induced reduction of leaf protein content in soybean was less than green gram. Protein profiling through SDS-PAGE clearly revealed degeneration of proteins in shade around ~55 kDa which indicated to the RUBISCO (Ribulose bisphosphate carboxylase-oxygenase) proteins. Both the crops revealed their shade adaptation mainly through alteration in epicuticular wax, pigments and proteins mostly associated with LHCP (Light harvesting complex protein) complex and towards RUBISCO protein which assume significance for their use in crop improvement programme in searching shade tolerant crops for agroforestry system.

Key words: Agroforestry, Anthocyanin, Chlorophyll, Epicuticular wax, LHCP, Protein profiling, RUBISCO, Shade tolerance.

Abbreviations: Chl: Chlorophyll, **DMSO:** Dimethyl sulphoxide, **HDPE:** high density poly ethylene, **LHCP:** Light Harvesting Complex Protein, **PPFD:** Photosynthetic photon flux density, **RBD:** Randomized block design, **RH:** Relative humidity, **RUBISCO:** Ribulose bisphosphate carboxylase-oxygenase, **SDS-PAGE:** Sodium dodecyl sulphate Polyacrylamide gel electrophoresis

Introduction

Shade is one of the major constraints for productivity of agroforestry system as the understorey crops do not get sufficient solar radiation due to tree canopy depending upon the trees adopted in any given agroforestry system (Canham *et al*., 1994; Newaj *et al.* 2005). Solar light has an immense impact on crop development and production and plants often attempt to acclimatize their external as well as internal variation with different levels of sun light (Moore, 1991; Chow *et al*., 1990). Plants which appears to be adapted to low light intensity make use of available light energy and these plants invest a greater portion of this towards the synthesis and maintenance of light harvesting machinery than do sun grown plants (Anderson, 1986). Severe reduction of light imposes a great limitation to plant growth and productivity and this holds importance for agroforestry system also. The amount of the limitation differs with resilience or shade tolerance of the crops. Low availability of solar radiation due to many physical phenomena in the atmosphere such as climate change is now getting wide attention (Valladares, 2003). This would also affect the crops like green gram and soybean grown in semi arid region of Central India including under agroforestry practices.

Comprehensive studies on biochemical traits of crops have relevance to decipher if there is any modification or acclimatization in crop with reference to their light limiting conditions. This may help immediately or in future to understand the differential responses of the crops with their changing light environments (Boardman *et al*., 1974). Adaptation of crops takes place at biochemical levels which maintains their stability with external environment growing in low light (Syvertsen and Smith, 1984). These changes maximize light interception and tend to increase carbon gain at low solar irradiances through more efficient investment in photosynthetic machinery (Alam *et al.*, 2011). Among the major crops in semi arid region of Central India, green gram and soybean are also important crops which are vulnerable to the implications of changing light during rainy season. Hence, it is important to comprehensively study on biochemical traits of the two crops *i.e.,* soybean and green gram at different intensity of shade in comparison to open field grown plants to have mechanistic insights. With this background, experiments were conducted to examine the effects of different regimes of shade on green gram and soybean with reference to their biochemical attributes in search of shade-adaptive traits in a semi-arid region of Central India which assumes much importance in the context of climate change implications and use in agroforestry system.

Materials and Methods

Plant material and growth conditions: The study was conducted at National Research Centre for Agroforestry, Jhansi (25° 27′ N, 78° 35′ E, 271 above MSL) in the semi-arid tract of central plateau of India during kharif (rainy) season in the years 2007 and 2008. Green gram (*Vigna radiata* L. variety PDM-54) and soybean (*Glycine max* L. Merill variety JS-335) were grown in four different light environments *i.e.,* three different intensity of shade (33%, 50%, 75%) and in open field without shade. The crop was grown in randomized block design (RBD) replicated thrice having plot size each of 5.8×3.4 m. Different intensity of shade was obtained in each of three separate shade net houses (25× 8× 3 m) with different category (porosity) of agro-shade net made up of high density poly ethylene (HDPE). Soil in the experiment site was black having a mean pH 7.03. All the standard agronomic practices including irrigation and plant protection measures were followed. The biochemical traits were investigated in nine plants from each treatment.

Photosynthetic photon flux density (PPFD) in each shade and in open field was monitored during crops season of 2007 and 2008 using Steady State Porometer (LI-1600, LICOR, U.S.A) along with Relative humidity (RH) and air temperature (Ta). The average microclimatic data was taken from July to September during both the years. The average PPFD of both the years in open was around 1200 to 1300 $\mu molm^{-2}s^{-1}$ from July to September. Similarly, during same duration in open field average relative humidity was around 55% to 65%. In 75% shade, average relative humidity of both years was higher than open by about 10 to 12%. In open field average air temperature was around 33 to 36°C, which was higher than 75% shade by about 2° to 4° C.

Chlorophyll (Chl) of leaves was determined using acetone and DMSO (Dimethyl sulphoxide) solvents (Arnon, 1949) and absorbance was recorded at two wavelengths *viz.* 663 nm and 645 nm by spectrophotometer. Anthocyanin in leaf was estimated using methanol and HCl solvents (Kho *et al.,* 1977) and absorbance was recorded at wavelength of 530 nm by spectrophotometer (Lambda 25, Perkin Elmer, U.S.A.). Leaf epicuticular wax level was estimated as per the gravimetric technique of Silva Fernandes *et al.* (1964) using chloroform solvent extraction. The amount of wax was calculated against fresh weight. Leaf soluble protein was estimated using spectrophotometer as per the technique of Bradford (1976).

SDS-PAGE of protein: Leaf protein banding pattern was analyzed using SDS-PAGE (Sodium dodecyl sulphate Polyacrylamide gel electrophoresis) following the technique of Laemmli (1970) having 12–15 % gradient of polyacrylamide. One gram of clean leaves for each sample was ground in a pre-cooled mortar and pestle with liquid nitrogen and grinding buffer. The extract was centrifuged at 4°C at 10,000 rpm for 45 min. The supernatant was mixed with sample buffer as ratio of 500µl 1:1 (v:v). Sample buffer contained 0.2M Tris-HCl (pH 6.8), 10mM 2-mercaptoethanol, 10% (w/v) SDS, 20% (w/v) glycerol, 0.05% (w/v) bromophenol blue and sucrose. Electrophoresis was carried out at 30 mA for 4 hours with running buffer (25mM Tris buffer, 200mM glycine and 0.1% (w/v) SDS). Gels were stained in methanol/acetic acid/ water (4:1:5, v/v/v) containing 0.1 % (w/v) Comassie brilliant blue R and de-stained in methanol/acetic acid/ water (4:1:5, v/v/v).

Statistical analysis: The numeric data for consecutive two years were analyzed through ANOVA using SYSTAT to compare the significance of means of four light environments (control and three regimes of shade).

Results and Discussion

Chlorophyll (Chl) estimation: Chl a, Chl b and total Chl gradually increased with increasing shade, whereas, Chl a/b ratio decreased with increasing shade in both the crops (Table 1). In open, total Chl was higher in soybean than green gram, whereas, in 33% shade total Chl was similar. In 50% shade, total Chl was higher in green gram than soybean, whereas, in 75% shade total Chl was similar in both the crops (Table 1).

Progressive increase in the Chl content with increasing shade intensity was accompanied by relative increase in Chl b more than Chl a as depicted by a decrease of Chl a/b ratio. Relative increase in accessory pigment Chl b is adaptive response of plants to variable PPFD (Anderson *et al*., 1988). Chl b is a pigment associated with distal antennae of LHCP II, the relative changes in this pigment could indicate a change in the distal antenna size (Misra, 1995). The increase in the distal antenna size under shade grown leaves probably increases the relative radius of solar energy interception in a chloroplast. Higher light harvesting efficiency of crops for agroforestry system would be important for better productivity (Jefferson and Pennacchio, 2005).

Table 1. Effect of varying regimes of shade on chlorophyll (Chl) content (mg/g fresh wt.)

Crops	Light environment	Chl a	Chl b	Total Chl	Chl a/b
Green gram	Open	2.42	0.62	3.04	3.90
	33% shade	2.85	0.80	3.65	3.70
	50% shade	3.29	1.09	4.38	3.08
	75% shade	3.43	1.24	4.67	2.91
	CD at 5%	0.27	0.14	0.34	0.41
Soybean	Open	2.68	0.76	3.44	3.90
	33% shade	2.89	0.80	3.69	3.74
	50% shade	3.12	0.94	4.06	3.49
	75% shade	3.45	1.15	4.60	3.09
	CD at 5%	0.35	0.16	0.42	0.71

Anthocyanin, wax level and soluble protein in leaf: Anthocyanin, wax level and soluble protein of leaf gradually decreased with increase in shade in both the crops (Table 2). Green gram showed higher anthocyanin content than soybean in all the light environments. The reduction of anthocyanin with decrease in sunlight indicates that colouring and antioxidant were also reduced in decreasing sunlight.

Wax level of leaf in green gram was higher in open than soybean, whereas, it was comparable in 33% shade in both the crops. However, wax level of leaf in green gram was less in 50% shade and 75% shade than soybean (Table 2). Epicuticular layer of plants is mainly covered by a waxy deposit which plays a major role in water balance and the behaviour of leaves in plant growth depending on microenvironment (Mcwhorter, 1993). Our results indicate that in high light, the deposition of wax was higher than in low light. Soluble protein in leaf was higher in soybean than green gram in all light environments. Thus higher soluble protein under shade in soybean could be well linked for its shade-resilience under agroforestry system. Soybean showed much higher content of soluble protein in leaf in 50% shade and 75% shade than in green gram.

SDS-PAGE protein banding pattern: Major differences were observed in protein profiling through gel electrophoresis under varying regimes of shade in comparison to the open grown crops. Major differential expressions were noted around ~55 kDa and ~26 kDa in both the crops. Protein around ~55 kDa mostly corresponds to large sub-unit of RUBISCO (Ribulose bisphosphate carboxylase-oxygenase) and the proteins at low molecular weight region of ~26 kDa most likely to be related to LHCP (Light Harvesting Complex Protein). In soybean protein bands around ~55 kDa and ~26 kDa were thicker than in green gram.

The low level of soluble proteins in shade grown leaves might be due to decrease of Rubisco synthesis, a major soluble protein in leaves (Anderson *et al*., 1988, Misra, 1995). This is supported by SDS-PAGE analysis of leaf proteins which strongly suggests the reduction of ~55 kDa in shade grown leaves corresponding to the RUBISCO in both the crops. The reduction of proteins in soybean was less than in green gram under varying shade, most probably due to higher content of soluble proteins as mentioned above.

Correlation of all parameters: All biochemical parameters exhibited positive correlation with each other except total chlorophyll which showed negative correlation with all parameters in both the crops (Table 3). The negative correlation clearly indicates that total Chl increased whereas anthocyanin, wax level and anthocyanin decreased with increase in shade intensity indicating their adaptive significance (Tables 1 and 2).

Table 2. Effect of varying regimes of shade on anthocyanin, wax level and soluble protein of green gram and soybean leaves.

Crops	Light environment	Anthocyanin (Abs. /g fresh wt.)	Wax level (mg/g fresh wt.)	Soluble Protein (mg/g fresh wt.)
Green gram	Open	2.20	1.34	136.72
	33% shade	2.09	0.84	118.77
	50% shade	1.75	0.61	107.74
	75% shade	1.46	0.50	101.94
	CD at 5%	0.20	0.38	2.95
Soybean	Open	1.90	1.08	161.60
	33% shade	1.58	0.84	147.75
	50% shade	1.26	0.76	138.25
	75% shade	0.82	0.63	133.15
	CD at 5%	0.27	0.26	2.30

Table 3. Correlation coefficient matrix of leaf biochemical traits of green gram and soybean grown under varying regimes of shade or without shade (open).

	Total chlorophyll	Anthocyanin	Wax level	Soluble protein
Green gram				
Total chlorophyll	1.000			
Anthocyanin	-0.442*	1.000		
Wax level	-0.367*	0.177**	1.000	
Soluble protein	-0.780*	0.629*	0.477*	1.000
Soybean				
Total chlorophyll	1.000			
Anthocyanin	-0.433*	1.000		
Wax level	-0.385*	0.430*	1.000	
Soluble protein	-0.507*	0.685*	0.335*	1.000

*, ** Values are significant at 1% and 5% level respectively.

More biosynthesis of chlorophyll pigments as expected would down-regulate the synthesis of other pigments like anthocyanin. This has been reflected in the negative correlation of their contents in the leaf. Thus it is clear that the response of the crops through the biochemical traits studied under varying regimes of shade hold much significance in understanding the shade-adaptable features of the crops.

Conclusion

The findings suggested that green gram and soybean revealed their shade adaptation through alteration in epicuticular wax, pigments and proteins associated with LHCP complex and towards RUBISCO protein which assume significance for their use in crop improvement programme in searching shade tolerant crops. Further, the identified shade adaptive traits would be useful for assessing crops for their higher adaptability under shade or low light environment in agroforestry system.

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