Indian J. of Agroforestry Vol. 18 No. 1 : 96-101 (2016)

Characterizing the thermotolerance properties of *Albizia procera* in temporal and seasonal scale through physio-biochemical traits and spectral indices

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ABSTRACT: *Albizia procera* is well recognized for timber, nutritious fodder for livestock and reforestation tree species with agroforestry importance. To reveal the inherent thermotolerance properties of *A. procera* through physio-biochemical traits and spectral indices, experiments were conducted under temporal and seasonal scale *viz*. pre-winter, winter and post-winter seasons. Effects of temporal and seasonal variation have been conspicuously noticed in physiological traits. Canopy temperature depression (CTD) increased with increase in atmospheric temperature. In winter season at low atmospheric temperature rate of CO_2 assimilation (A_{max}) and thylakoid electron transport rate (ETR) decreased. There were prominent effects of seasonal and temporal variations on leaf level spectral indices like Normalized Difference Vegetation Index (NDVI) and Photochemical Refelectance Index (PRI) as well. During pre-winter and post-winter season, NDVI and PRI were higher than in winter period. Thus, an overall impact of increasing temperature through temporal variation has been reflected on photosynthetic apparatus, intercellular biochemical reactions and spectral traits. Correlations among various traits indicated that NDVI and PRI may be useful to understand the physiological status of plants under such conditions if judiciously used. Our results demonstrated the inherent thermotolerance properties of *A. procera* which may help to understand the mechanistic insights involved in coping with elevated temperature under changing climate scenarios. To our knowledge, the association of PRI and NDVI in *A. procera* in the context of thermotolerance is being reported for the first time which is noteworthy.

***Key words*:** Canopy temperature depression, malondialdehyde, NDVI, PRI, rate of CO_2 assimilation and thermotolerance.

Received on: 31/05/2016 Accepted on: 14/06/2016

1. INTRODUCTION

To meet the increasing demand of food, fodder and fuel opting agroforestry is one of the important additional avenues. In this context, tree fodder assumes much importance. *Albizia procera* is an important tree species which plays crucial role for its multiple values including timber and its leaves as fodder. This tree is observed to be conventionally grown in agroforestry system depending upon its availability. There are good characteristics of this species for wide adaptability including in agroforestry mainly in agrisilvicultural practices. This tree grows well in wide range of agroclimate *e.g.* semiarid to highly humid areas where the annual rain fall is about 1000 mm to 4000 mm and it has fast growing habit with proven carbon sequestration potential (Newaj *et al*., 2012). In the context of climate change, increase in atmospheric temperature is one of the major challenges to meet. High air temperature beyond a threshold limit has been widely noted to be detrimental to agricultural crops and this holds equal importance for tree species as well. However, much emphasis to understand the impacts of climate change on tree species has not been given until recently. Thus, it is essential to know the thermal responses of the tree species towards better preparedness for the future.

Being perennial, trees experience seasonal extremes and it is important to know the characteristic responses of the trees in respect to seasonal trends in ambient conditions. These would help to assess their inherent traits when it will be subjected to environmental extremes. There are two extremes namely winter with low temperature and summer with extremely high atmospheric temperature in central India. These would provide a basis for evaluating the physiological responses at temporal scale *viz.* pre-winter, winter and post-winter seasons (Vargas and Cordero 2013). In addition to carbon assimilatory functions, leaf spectral indices have many advantages for quantifying intrinsic traits linking important physiological functions. These measurements being non-destructive and rapid, thereby only minimally affecting the plants to understand the thermotolerance ability and carbon gain over diverse spatial and temporal scales (Field *et al*., 1995; Gamon *et al*., 1995).

Several environmental factors including temperatures often lead to decreased photosynthetic yield in plants. Photosynthesis and photochemistry of photosystem II (PSII), is one of the most heat-sensitive processes in plants (Berry and Bjorkman 1980; Bilger *et al*., 1987). Denaturation of PSII proteins, an increase in the lipid fluidity of the thylakoid membrane or an alteration of protein–lipid interaction may occur when the temperature approaches the thermal limit of PSII (Berry and Bjorkman 1980; Yordanov 1992). In addition to genetic differences in heat tolerance among species and cultivars (Shabala 1996; Epron 1997), the thermal limit of photosynthetic processes varies with many environmental factors. Both long-term adaptations and short-term adjustments to moderately elevated temperature have been reported and probably involve changes in membrane organization and composition (Berry and Bjorkman 1980).

Earlier studies suggested that experiments and investigations are required to understand the responses expressed at different temporal or seasonal scales for better understanding the physiology behind it (Harte *et al.,* 1995; Dunne *et al.,* 2004). For this, reflectance indices such as NDVI (Normalized Difference Vegetation Index) and PRI (Photochemical Reflectance Index) would be used as an estimator energy use efficiency of plants (Gamon *et al*., 1995). For characterising the thermotolerance properties of any tree species it is essential to have reference set of trends with respect to seasonal and temporal variation under ambient condition. In this direction, experiments were conducted to study the seasonal and temporal responses in young seedlings of *A. procera* under ambient conditions to find out its inherent adaptive trends for environmental and seasonal extremes.

2. MATERIALS AND METHODS

Location of the experiment was at Central Agroforestry Research Institute, Jhansi, Uttar Pradesh, India, situated at 25° 27' N latitude and 78° 35' E longitude, 271 m above mean sea level. Experiment was conducted in the seedlings of *Albizia procera* by growing them in polythene bags (size 60cm × 45cm) having mixture of black soil and farm yard manure (FYM) in the ratio of (1:1) under ambient conditions and various measurements were done in temporal and seasonal scale namely Pre-winter (September to November), winter (December to February) and post-winter (March and April). The mean air temperature in pre-winter was 28.2 ºC, in mid-winter the average air temperature decreased up to 6.8 ºC whereas, in post-winter seasons the mean air temperature was 29.8 ºC.

Simultaneous measurement of photosynthetic traits

A portable photosynthesis system (LI-6400XT, Licor, U.S.A.) attached with a leaf chamber fluorometer (LCF-6400-40) was used for simultaneous measurements of chlorophyll fluorescence and gas exchange of plants. Measurement of photosynthetic traits were simultaneously conducted on intact mature leaves (top most) attached to about one year old plants. Various chlorophyll fluorescence parameters were recorded for the estimation of different components of photochemical events and electron transport rate across PSII (ETR) of the leaf following the standard techniques (Schreiber *et al*., 1998). All the measurements were made in six independent seedlings grown in polybags under ambient conditions taking care of all standard practices.

Measurement of leaf spectral reflectance indices

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. Photochemical reflectance index (PRI) was calculated from leaf and canopy reflectance using 531 nm as the xanthophyll waveband and 570 nm as the reference waveband (Gamon *et al*., 1993; Alam *et al*., 2013). Normalized difference vegetation index (NDVI) was derived from average reflectance in the near-infrared wavebands (800 nm) and in a narrow red waveband (680 nm).

Measurement of malondialdehyde (MDA) concentration

MDA was estimated according to the method of Heath and Parker (1968). Materials used for the study of malondialdehyde were potassium phosphate buffer- 50m M (7.0 pH), sodium dodecayl sulphate (0.8%), 2-thiobarbituric acid (0.8%), acetic acid 20% (3.5 pH) and enzyme extract (from fresh leaf). The MDA was assayed spectrophotometrically by taking absorbance at 535nm and 600nm.

Statistical analysis

The data obtained were subjected for ANOVA (analysis of variance) to compare the significance of means through a statistical software (SYSTAT-11) followed by calculation of LSD (Least significant difference).

3. RESULTS AND DISCUSSION

There were clear trend of temporal and seasonal responses of *A. procera* seedlings. Differential trend in leaf temperature with respect to ambient air temperature were noted through infrared thermometer and canopy temperature depression (CTD) were estimated (Table 1). CTD has been recognized as one such trait and is used for assessing the plant response to environmental stress (Balota *et al*., 2007). High CTD was used to distinguish between the stress-avoidant and the stress sensitive genotypes and was suggested as a selection criterion for improving the tolerance to stress (Alam *et al*., 2014; Kashiwagi *et al*., 2008). Higher CTD of *A. procera* indicated its relatively better inherent thermotolerance capacity.

Table 1. Physiological indices of *Albizia procera* in seasonal scale

Seasonal scale	A_{max}	ETR	CTD	MDA	NDVI	PRI
Pre-winter	17.36 ±0.29	82.97 ±0.75	2.14 ±0.13	1.185 ±0.04	53.26 ±0.0001	-2.32 ±0.001
Winter	11.75 ±0.15	55.28 ±0.583	1.60 ±0.35	0.635 ±0.02	48.59 ±0.005	-4.58 ±0.0007
Post-winter	23.71 ±0.30	107.58 ±1.166	2.62 ±0.13	1.393 ±0.01	57.55 ±0.008	-3.80 ±0.001
CD (0.05)	0.72	2.39	0.44	0.07	0.37	0.44

A_{max} = maximum rate of CO_2 assimilation [μ mol m^{-2} s^{-1}]; ETR= thylakoid electron transport rate [μ mol m^{-2} s^{-1}]; CTD= canopy temperature depression [°C]; MDA= malondialdehyde concentration [μ mol kg^{-1} fr. wt.]; NDVI= normalized difference vegetative index [%]; PRI= photochemical reflectance index [%].

The major determinants for the temporal and seasonal variation were the CO_2 assimilatory functions and related photosynthetic traits (Fig. 1). These were mainly expressed at the level of leaf gas exchange as well as chloroplast photochemical activities. It has been observed through CO_2 assimilatory rate versus photosynthetic photon flux density (PPFD) response analysis, rate of CO_2 assimilation (A_{max}) of *A. procera* was higher at relatively high atmospheric temperature in pre-winter and post-winter season (Table 1). This was mainly due to increase in thylakoid electron transport rate (ETR) under such condition (Sharkey 2005). This is due to increase in PSII activity (effective quantum yield of PSII) under such conditions which resulted in increased ETR. At the same time, the quantum of light necessary to saturate the rate of CO_2 assimilation was much higher in case of pre-winter and post-winter season. The rate of CO_2 assimilation (A_{max}) was higher during pre-winter and post-winter season than winter season. This is because of optimum (favorable) temperature prevailing during pre-winter and post-winter season (28ºC to 30ºC). This clearly supported the thermotolerance property of photosynthetic apparatus of the plants, which is a major determining factor for differential photosynthetic functions. Photochemical reactions are well correlated with the efficiency of net CO_2 assimilation and quantum yield of CO_2 fixation (Berry and Bjorkman 1980), which ultimately affected entire photosynthetic apparatus of the plants in respect to temporal and seasonal changes as well. There was

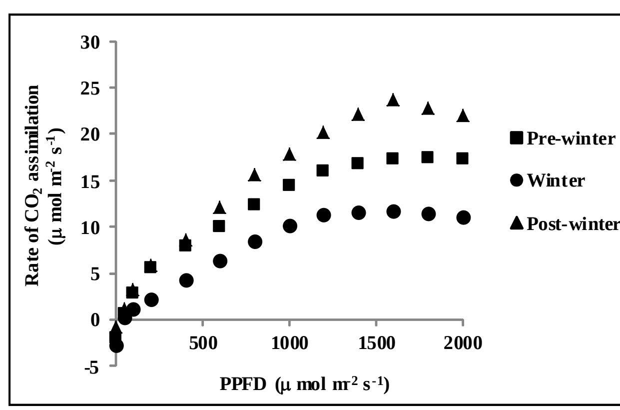

Fig. 1. PPFD (Photosynthetic photon flux density) versus rate of CO_2 assimilation (A) of *Albizia procera* plants under temporal variation of pre-winter, winter and post-winter seasons.

a rapid decrease in photosynthetic rate at temperatures above 30 °C in various tree species (Vann *et al*., 1994). But in *A. procera* the increase in rate of CO_2 assimilation during post-winter season with prevalent relatively higher atmospheric temperature suggested towards its intrinsic capacity for thermotolerance.

Malondialdehyde (MDA) concentration is an indicator of lipid peroxidation in cell. It has been noted in present experiment that with increase in atmospheric temperature (in pre-winter and post-winter season) there were an increasing trend in MDA concentration in leaves of *A. procera* as observed in pre-winter and post-winter time period (Table 1) and it suggests that lipid peroxidation is interconnected with thermal response (Holaday 1992).

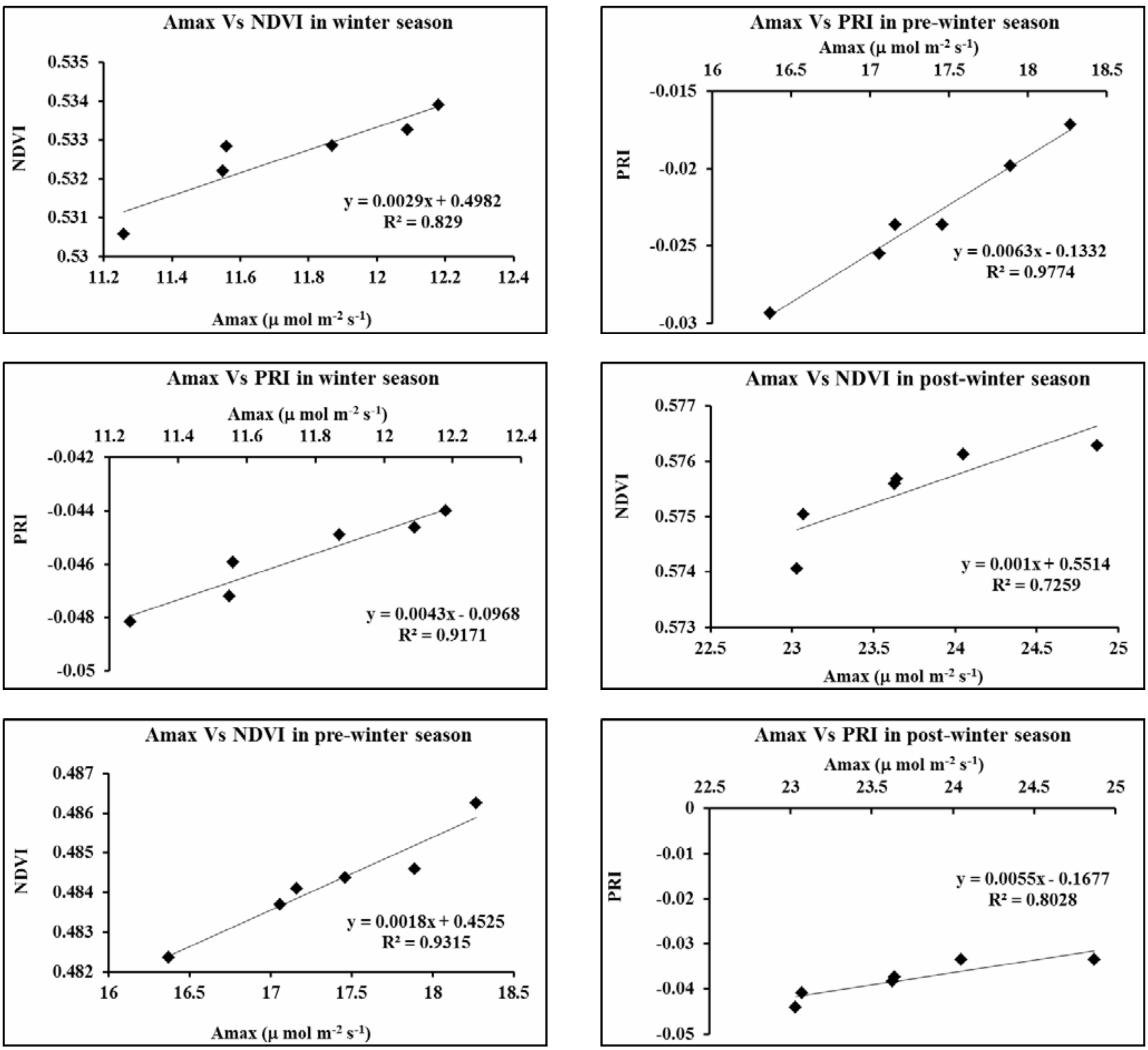

Fig. 2. Normalized Difference Vegetation Index (NDVI) versus rate of CO_2 assimilation (A_{max}) and Photochemical Reflectance Index (PRI) versus rate of CO_2 assimilation (A_{max}) of *Albizia procera* plants under temporal variation of pre-winter, winter and post-winter seasons.

Some important spectral indices such as NDVI and PRI at temporal and seasonal scale were also evaluated. NDVI and PRI showed higher values in pre-winter and post-winter season than winter. Zhang (2013) also observed similar seasonal changes in evergreen broadleaf trees. Our results deciphered the relationship of PRI and NDVI with photosystem-II photochemical efficiency. PRI are closely tied to PSII photochemical efficiency (Gamon *et al*., 1997), and also correlates with total activity of PSII photochemical efficiency of leaves. Strong correlations between spectral indices and photosynthetic traits were observed in our present experiment (Fig. 2). From these results, it has been clearly manifested that plants of *A. procera* could perform better under high environmental temperature. Probably, for the first time to our knowledge, we are reporting the association of PRI and NDVI in *A. procera* in the context of thermotolerance.

The electron transport which is an important component of photosynthesis is susceptible to irreversible high temperature damage. The PRI is sensitive to the relative concentration of xanthophyll cycle pigments and thus, is closely related to PSII efficiency as well as to photosynthetic light-use efficiency in conditions when photoelectron transport and carboxylation are well coupled (Gamon *et al*., 1995 and 1997). The relationships between photochemical efficiency and PRI and/or between net CO_2 uptake and PRI have also been examined in present study and found a good correlation between both the traits (Fig. 2). The fraction of incident photosynthetically active radiation (PAR) absorbed by plants is either converted to new biomass or is respired. Thus PAR is directly correlated with the photosynthetic capacity of the plants. According to the theoretical studies (Asrar *et al.,* 1984; Sellers 1987) and empirical studies (Kumar and Monteith 1981; Hatfield *et al.,* 1984 and Bartlett *et al.,* 1990) NDVI is closely related to the PAR intercepted or absorbed by the tree canopy.

Our results provide evidences and reference scenarios for coordinated regulation of CO_2 assimilation, thylakoid electron transport across PSII and spectral indices at temporal and seasonal scale in *A. procera*. These findings can help to better understand the mechanistic insight of plants under elevated temperature stress for optimizing tree-physiological traits. We also deciphered that physiological adjustments are more important in modifying CO_2 fixation capacity during periods of photosynthetic downregulation as observed in temporal variations evidently correlated with photosynthetic and spectral traits for the tree species.

ACKNOWLEDGEMENT

The financial support as extended by the ICAR through ongoing project National Innovation on Climate Resilient Agriculture (NICRA) at ICAR-Central Agroforestry Research Institute is gratefully acknowledged.

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