POLYPHASIC CHLOROPHYLL FLUORESCENCE ANALYSIS OF PHOTOSYNTHETIC ADAPTATION IN *COMMIPHORA WIGHTII* TO THE HARSH NATURAL CONDITIONS OF ARID ENVIRONMENT

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Abstract. Succulent plants are well adapted to survive in harsh conditions of arid and semi-arid environments. Understanding the biophysical basis of plant adaptation under such adverse conditions will be helpful in the development of transgenic plants against various abiotic stresses. Therefore, the present study was carried out to assess the mechanism of maintaining photosynthetic performance in *Commiphora wightii* (*C. wightii*) growing under harsh natural conditions of arid environment of Rajasthan, India. The membrane and phenomenological models represent high photosynthetic adaptability in *C. wightii* plants. In wild and control plants of *C. wightii*, the quantum yield of primary photochemistry was observed 0.82 and 0.62 respectively. Present results clearly indicate that the *C. wightii* are well developed physiologically to survive in harsh arid environment by manipulating their photosynthetic performance.

Key words: chlorophyll florescence, JIP test, abiotic stress, *Commiphora wightii*.

INTRODUCTION

Abiotic stress has been long recognized as a major factor limiting plant productivity. In arid and semi-arid environments, plants are exposed to various abiotic stress conditions such as drought, heat, high light intensity etc. All these environmental stress factors combinedly produce deleterious effects on photosynthetic apparatus especially the photosystem II (PS II) and consequently damage the plant growth and development. One of the primary responses of plants to water deficit is stomatal closure. This response minimizes water loss, but also lowers the intercellular CO_2 concentration causing drastic reduction in photosynthesis process in C3 plants (group of plants which synthesize 3-carbon compound as first product during CO_2 fixation) [3, 4]. In addition, reduced

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transpiration increases leaf temperature and, consequently, the incidence of heat stress. Rubisco activate the protein that facilitates the release of sugar phosphates from Rubisco ceases to function properly at elevated temperatures, inactivating Rubisco [5]. Heat also induces the dissociation of the manganese-stabilizing 33 kDa protein from the PSII RC complex followed by release of the manganese atoms [17]. High light (beyond what is needed for maximum photosynthesis) also lead to photoinhibition. Understanding the biophysical basis of plant adaptation under arid and semi-arid environments will be helpful in developing selection strategies for improving plant varieties against various abiotic stresses.

Commiphora wightii (Arnott) Bhandari (Burseraceae) is a critical endangered succulent medicinal plant species of India, Pakistan and South Africa [10]. The plant is quite adapted to adverse climatic conditions of arid and semi-arid environments. In this work, several biophysical parameters have been used to understand the mechanism of photosynthetic adaptation in *C. wightii* plants growing in natural habitats under extreme arid conditions.

MATERIALS AND METHODS

PLANT MATERIAL AND GROWTH CONDITIONS

C. wightii plants were propagated during raining season using 2 cm thick stem cuttings at natural habitat near the Jaipur, Rajasthan, India (26.92 N, 75.82 E). Biophysical studies through chlorophyll fluorescence analysis were carried out during summer (May) after three years of successfully acclimatization of plants in natural habitats (wild plants). During summer, the habitat is characterized by extreme variation of diurnal temperatures (day 46–50 °C; night 32–36 °C), high wind regime and high evaporation, dust storm and low humidity of the atmosphere (9–14%). The plants propagated through stem cuttings in green house conditions (30 °C; 50% humidity) were watered regularly and served as a control.

CHLOROPHYLL *A* FLUORESCENCE MEASUREMENTS

Chlorophyll *a* fluorescence of intact dark-adapted leaves of *C. wightii* was measured using a Plant Efficiency Analyser, PEA (Hansatech Instruments, Kings Lynn, Norfolk, U.K.). For providing the dark conditions leaf clips were placed on the leaves and then shutter plates were closed for 60 minutes. Fluorescence transients were induced over a leaf area of 4 mm diameter by a red light (3000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) provided by a high intensity LED array of three light emitting diodes. A total measuring time of one second was used throughout the experiments.

Table 1

Description of the chlorophyll fluorescence parameters used in the text

Data extracted from the chlorophyll a fluorescence (O-J-I-P) transient

$F_0 \cong F_{50\mu s}$	minimal fluorescence at the O step of O-J-I-P (at t = 0), when all PS II reaction centers are open
F_M	maximal fluorescence, when all PS II reaction centers are closed
$F_{100\mu s}$	fluorescence at 100 µs
$F_{300\mu s}$	fluorescence at 300 µs
$F_J \equiv F_{2ms}$	fluorescence at the J-step (2 ms) of O-J-I-P
$F_I \equiv F_{30ms}$	fluorescence at the I-step (30 ms) of O-J-I-P
$M_0 \equiv 4(F_{300\mu s} - F_0)/(F_M - F_0)$	approximated initial slope (in ms^{-1}) of the fluorescence transient
$V_J = (F_J - F_0)/(F_M - F_0)$	relative variable fluorescence at the J-step

Quantum efficiencies or flux ratios

$\Phi_{P0} = TR_0/ABS = [1 - (F_0/F_M)] = F_V/F_M$	maximum quantum yield for primary photochemistry (at t = 0)
$\psi_0 \equiv ET_0/TR_0 = (1 - V_J)$	probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-

Specific energy fluxes (per Q_A-reducing PSII reaction center – *RC*)

$ABS/RC = M_0(1/V_J)(1/\Phi_{P0})$	absorption flux per reaction center
$TR/RC = M\cdot(1/V_J)$	trapped energy flux per reaction center
$ET/RC = M\cdot(1/V_J)\cdot\psi_0$	electron transport flux per reaction center
$DI/RC = (ABS/RC) - (TR/RC)$	dissipated energy flux per reaction center

Phenomenological energy fluxes (per excited cross section – *CS*)

ABS/CS	absorption flux per cross section
$TR/CS = \Phi_{P0}(ABS/CS)$	trapped energy flux per cross section
$ET/CS = \Phi_{P0}\ \psi_0\ (ABS/CS)$	electron transport flux per cross section
$DI/CS = (ABS/CS) - (TR/CS)$	dissipated energy flux per cross section

Density of reaction centers

$RC/CS = \Phi_{P0}(V_J/M_0)$	density of reaction centers (Q_A-reducing PSII reaction centers)

Fluorescence signals were detected using a photocell after passing through a near-infrared (NIR) short pass filter.

The chlorophyll *a* fluorescence transient O-J-I-P was analyzed according to the JIP-test [15]. The extracted and technical parameters, specific fluxes (per reaction center), phenomenological fluxes (per cross section), quantum efficiencies or flux ratios, density of reaction centers and performance indexes were calculated using equations of JIP-test (Table 1).

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

Mature green leaves (5 leaves/plant) were used to measure photosynthetic performance. Experiment was conducted with 20 plants per growth condition. The data were analyzed statistically by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

A sharp difference was observed between the photosynthetic performance of wild and control plants (Fig. 1A). Two intermediate peaks: F_J and F_I were observed between F_0 and F_M forming a typical O-J-I-P curve in wild as well as in control plants (Fig. 1B). A drastic reduction in active PS II *RC*s was noted in wild plants growing under natural habitat. The density of active PS II *RC*s per cross section (*RC/CS*) was observed 312% higher in the control plants than the wild plants. The membrane and phenomenological models depict the differences in the photosynthetic performance of wild and control plants of *C. wightii* (Fig. 2). The average antenna size (*ABS*/*RC*) of wild plants was about 138% larger than the control plants of *C. wightii*. Due to the presence of many inactivated PS II *RC*s, *DI*/*RC* was found 5 times higher in the wild plants. The light trapping (*TR*/*RC*) and electron transfer (*ET/RC*) efficiencies per reaction center were respectively 84% and 50% higher in the wild plants.

The phenomenological models represent the higher *ABS/CS, TR/CS, ET/CS* and chlorophyll contents (denoted by the intensity of leaf's green colour) in the control plants as compared to the wild plants. In wild and control plants of *C. wightii* the quantum yield of primary photochemistry ($\Phi_{P0} \cong F_V/F_M$) was observed 0.82 and 0.62 respectively.

Stresses are a natural part of forest ecosystem and are, in many respects, a positive force in shaping adaptive capacities [9]. The aim of this work was to understand the mechanism of maintaining the photosynthetic performance under extreme arid conditions in *C. wightii* plants. Presence of a 'K' peak between O and J peaks forming O-K-J-I-P chlorophyll *a* fluorescence transient in an indicator of one or other type of stress in all the plants studied so far [14]. Appearance of 'K'

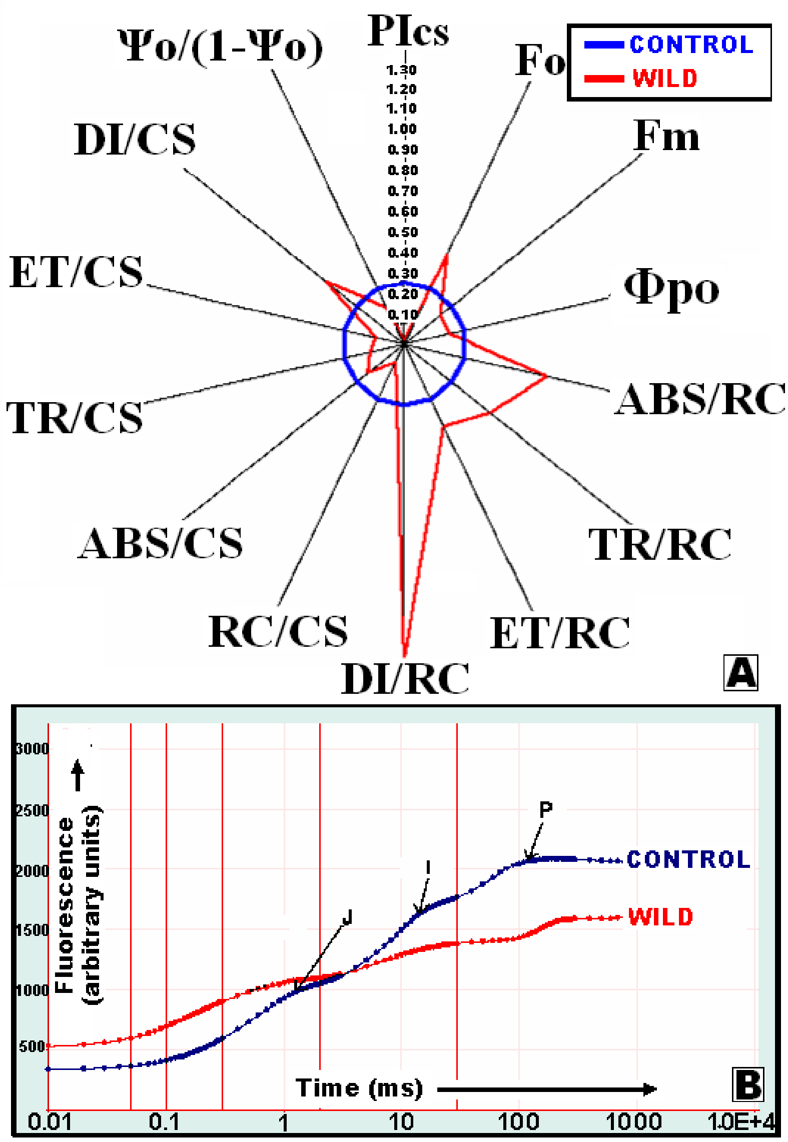

Fig. 1. (A) Radar plot showing relative values of various photosynthetic parameters and (B) polyphasic chlorophyll fluorescence O-J-I-P induction in wild and control plants of *C. wightii*; values represent the mean of 100 replicates. Red and blue lines indicate various parameters of photosynthesis in wild and control plants of *C. wightii*. Pics indicates performance index per cross section.

peak was observed in various plants subjected to a variety of stress conditions, such as heat stress [6, 7, 8, 12, 16] or drought stress [6]. Moreover, the O-K-J-I-P transient is also exhibited by different higher plants growing naturally in ecosystems with dry and hot environment, like leaves of *Cycus revoluta* and *Permelia* sp., as well as fruits of *Juniperus* sp. [11]. Several reports [11, 13] suggested that the K-step is related to the inactivation of the oxygen-evolving-complex (OEC). The absence of the prominent 'K' peak in the *C. wightii* plants growing under harsh arid conditions clearly indicates that the oxygen evolving complex is not adversely affected by the extreme arid conditions.

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Although the density of active reaction centers (RC/CS) was low in plants growing under natural harsh environmental conditions, the activity of active reaction centers was very high which maintained the photosynthetic performance under environmental stresses. The high activity of reaction centers, despite their low density in wild plants, indicate that the reaction centers have enhanced their activity to cope up with their meagre number. Similar results were also observed in various plant species such as *Spirodela polyrhiza* [1] and *Anabaena* [2]. Chlorophyll *a* fluorescence analysis of wild plant of *C. wightii* indicates that the plants growing naturally in arid regions are quite resistant to extreme environmental conditions and are well developed physiologically to survive through such conditions by maintaining their photosynthetic performance. These plants create heat sinks to cope up with the unfavorable environmental conditions and increase the efficiency of remaining active reaction centers to maintain the photosynthetic performance and to survive such adverse climatic conditions.

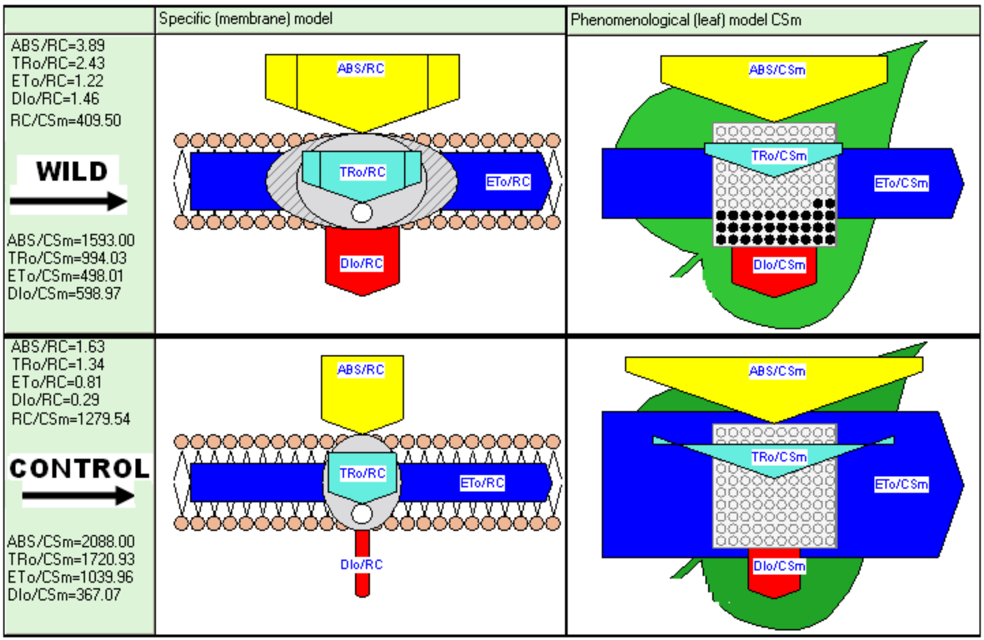

Fig. 2. Thylakoid membrane and leaf phenomenological models showing specific (*ABS/RC*, *TR/RC*, *ET/RC*, *DI/RC*) and phenomenological fluxes (*ABS/CS*, *TR/CS*, *ET/CS*, *DI/CS*) in wild and control plants of *C. wightii* (black dots in leaf model represent inactive PS II *RC*s).

CONCLUSIONS

Present studies were carried out to understand the photosynthetic adaptations in *C. wightti* plants growing under adverse arid environment of Rajasthan, India. Photosynthetic analysis shows that wild plant of *C. wightii* are physiologically quite resistant to survive in adverse climatic conditions by enhancing the activity of their active reaction centers and creating an efficient heat sink system to prevent photodamage. The present studies will be helpful in the development of transgenic plants against various abiotic stresses of arid and semiarid environment.

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