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ORIGINAL RESEARCH

Photoprotection, Photosynthetic Plasticity and Antioxidant Activity under Drought Stress in *Aloe barbadensis* Miller

Vineet SONI¹, PINKI², Aditi DWIVEDI²

¹Plant Bioenergetics and Biotechnology Laboratory, Department of Botany, Mohanlal Sukhadia University, Udaipur-313001, Rajasthan, India

²Dr. B. Lal Institute of Biotechnology, Malviya Industrial Area, Jaipur-302017 Rajasthan, India

*Corresponding Author email: vineetsonijnu@gmail.com

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ABSTRACT

Aloe barbadensis Miller is adapted to tolerate severe drought and high irradiance levels. Photoprotective, photosynthetic and antioxidative potentials were investigated in plants grown under drought stress. Prolonged periods of drought caused high level accumulation of carotenoids which suggest their role in photoprotection. Due to the presence of strong photoprotective response, plants were found capable to maintain their photosynthetic performance even in severe drought conditions. The high activity of reaction centers, despite their low density in drought stressed plants, indicate strong photosynthetic plasticity in *A. barbadensis*. Water deprivation triggered activities of superoxide dismutase (SOD) and catalase (CAT) to minimize the effects of drought-induced photodamage. The present findings indicate that *A. barbadensis* plants are physiologically quite resistant to extreme water deficit conditions. This study will therefore be useful in transgenics for the development of drought tolerant plants.

KEY WORDS: Drought, Photoprotection, carotenoids, Chlorophyll a fluorescence, Antioxidant enzymes

Introduction

Drought is one of the most important abiotic stresses limiting for plant growth and productivity in most parts of the world. It cause numerous negative effects on plants by a rapid closure of stomata which leads to a reduced CO₂ concentration in leaves that eventually limits photosynthetic activity by direct inactivation of ATP synthase (Tezara *et al*, 1999) and Rubisco enzyme (Carmo-Silva *et al.*, 2012). Levels of light which are optimal for photosynthesis in well-hydrated plants become excessive in plants suffering water deficiency (Luna *et al.*, 2005). Extreme high light conditions lead to photoinhibition, mainly due to oxidative damage of photosystem II (Powles, 1984). Therefore, drought conditions usually provoke photoprotective mechanisms in order to avoid photodamage (Chaves *et al.*, 2009). Recent

studies have been suggested that several strategies are involved in the protection against the photoinhibition. Photorespiration (Park *et al.*, 1996), heat dissipation via xanthophylls cycle (Horton *et al.*, 1996), anthocyanin accumulation (Soni, 2008), and consumption of reducing power via water-water cycle (Asada, 1999) are believed to contribute to reduction and dissipation of excess energy. Plants grown in arid and semi-arid regions frequently encounter drought which ultimately produce deleterious effects on photosynthesis and consequently damage the plant growth. *Aloe barbadensis* Miller, a perennial succulent plant with crassulacean acid metabolism (CAM), is morphologically and anatomically quite resistant to extreme drought condition. Understanding the photoprotective response to drought-induced photoinhibition will be helpful

for improving drought tolerance in various economic important plant species. Therefore present study was carried out to understand the physiological and biochemical basis of drought tolerance in *A. barbadensis* by (1) identifying the mechanism of photoprotection, (2) photosynthetic plasticity and (3) antioxidative defense potential in response to drought stress.

Materials and Methods

Plant Materials

To understand photoprotective, photosynthetic and antioxidative potentials under drought stress, three years old *A. barbadensis* plants, growing in earthen pots, were divided into two sets (each of 30 plants), out of which one set was subjected to drought stress by withholding irrigation, while the second set was watered regularly and served as a control. The experiment was conducted during May-June (summer season) to impose plants under natural drought and high irradiance.

Quantification of total chlorophyll and carotenoid content

To determine the total amount of chlorophyll and carotenoids, 5 g leaf sample was finely homogenized in 30 mL acetone. The homogenate was filtered through two layer cheese cloths, and was centrifuged using the Remi C-24 BL centrifuge at 2500 rpm for ten minutes. The supernatant was separated and the absorbances were read at 400-700 nm on Shimadzu UV-260 spectrophotometer. It was recorded that Chlorophyll-*a* showed the maximum absorbance at 662 nm, chlorophyll-*b* at 646 nm and total carotenoid at 470 nm. the amount of these pigments was calculated as per the method described by Lichtenthaler and Wellburn (1985).

Measurement of photosynthetic performance

Photosynthetic potential of drought stressed and well-hydrated plants of *A. barbadensis* were recorded with a Plant Efficiency Analyzer, PEA (Hansatech Instruments, Kings Lynn, Norfolk, U.K.). Fluorescence transients were induced over a leaf area of 4 mm diameter by a red light (peak at 650 nm) of $3000 \mu\text{molm}^{-2}\text{s}^{-1}$ provided by a high intensity LED array of three light emitting diodes. A total measuring time of one second was used throughout the experiments. The extracted fluorescence data (F_0 , fluorescence intensity at 50

μs , F_m - maximal fluorescence intensity) were used to calculate phenomological fluxes (RC/CS- reaction centers per cross section; ABS/CS-absorbance per cross section; TR/CS-Trapped energy per cross section; ET/CS-electron transport per cross section; DI/CS- dissipated energy per cross section) using the equations of JIP- test (Strasser and Strasser, 1995; Soni and Strasser, 2008).

Catalase (CAT) assay

CAT activity was determined by the method of Teranishi *et al.* (1974). Leaf sample (5 g) was homogenized in ice cold. The reaction mixture (3ml) contains 50 mM phosphate buffer (pH 7.0), 20 mM H_2O_2 , and 0.1 ml of enzyme extract. The reaction was stopped by adding 2 ml of titanium reagent. It was centrifuged at 10,000 rpm to 10 min. The absorbance was read at 410 nm. CAT activity was expressed as enzyme units per gram fresh weight.

Superoxide Dismutase Assay

The activity of SOD was assayed following the method of Kono (1978). Leaf sample (5 g) was homogenized in 50 mM chilled phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm for 10 min at 4°C and supernatant was treated as enzyme extract. The reaction mixture of 2 ml contain 1.3 ml Na-potassium buffer (50 mM, pH 10.0), 500 μl NBT (96 μM), 100 μl Triton X-100 (0.6%) and 100 μl Hydroxylamine-HCl (20 mM, pH 6.0). After 2 min, 70 μl of enzyme sample was added and absorbance was recorded at 540 nm. SOD activity was expressed as enzyme units per gram fresh weight.

Results and Discussion

Plants are exposed to various levels of environmental stresses under natural conditions especially in arid and semi arid region. The regions are characterized by sparse and highly variable precipitation, extreme variation of diurnal and annual temperatures, high wind regime and high evaporation, powerful winds, dust storm during summer and low humidity of the atmosphere. Plants grown in such arid and semi-arid regions are well developed morphologically, anatomically as well as physiologically to survive through such conditions. Drought and high light stress induce photoprotective responses in plants. Present study clearly

indicates the photoprotective role of carotenoids in response of drought-induced-photoinhibition in *A. barbadensis*. Prolonged periods of drought caused high level color changes from green to reddish-brown as a result of carotenoid accumulation have been studied in the sun-exposed needles of *Cryptomeria*, *Metasequoia* and *Thuja* (Czeczuga, 1987; Ida *et al.*, 1991). In present investigation, the relationship between carotenoid accumulation and drought duration was investigated. *A. barbadensis* plants showed a notable induction in total carotenoid content on day 30 of water deprivation (Fig. 2 A). The carotenoids exert photoprotective potential through deactivation of chlorophyll excited states, quenching of singlet oxygen, interception of free radicals and dissipation of the excessive absorbed light energy via xanthophyll cycle. In the present study, drought-

accumulation of carotenoids and turned the leaves color from green to reddish-brown. On the other hand, no color changes were observed in controlled plants (Fig. 1 A-D). The induced accumulation of carotenoids suggests their role in photoprotection. The red pigment has been identified as the xanthophyll rhodoxanthin (Diaz *et al.*, 1990). The results supports the earlier finding (Merzlyak *et al.*, 2005), which specifically describes the photoprotective function of red keto-carotenoid, rhodoxanthin in *Aloe arborescens*. Accumulation of carotenoids in leaves decrease the light intensity reaching the photosynthetic apparatus and prevent the plants from drought-induced photodamage. Some lines of evidence suggest that the accumulation of carotenoids is related with their ability to trap lethal radiation and thus provide photoprotection (Merzlyak and Solovchenko, 2002).

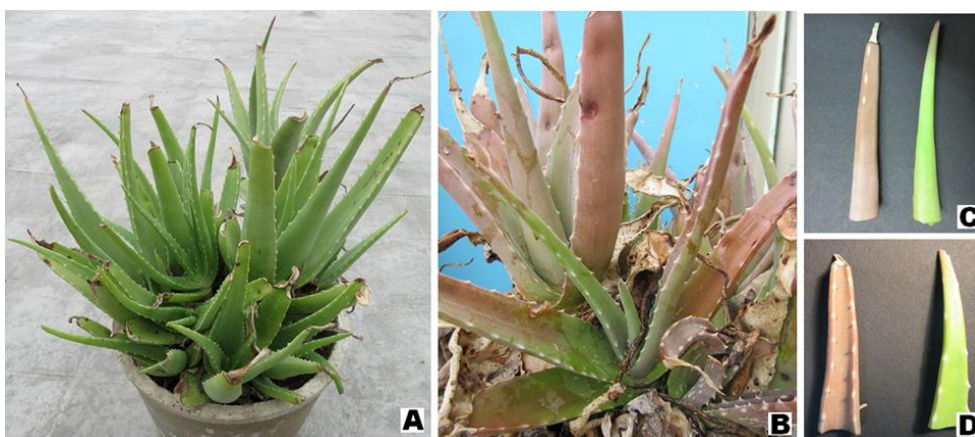


Figure 1: Drought-induced accumulation of carotenoids in leaves of *A. barbadensis*. (A) well hydrated plant, (B) drought stressed plant showing reddish-brown leaves, (C) abaxial, and (D) adaxial views of apical part of leaves of drought stressed (reddish-brown) and controlled (green) plants of *A. barbadensis*

Light-Harvesting Complexes (LHCs) are pigment-protein systems that are responsible for light absorption and excitation energy transfer in plants in light-limiting conditions. In addition to their role in light harvesting, in high-light conditions LHCs are involved in photoprotection, lowering the level of excited states in the membrane through a process known as Non-Photochemical Quenching (Ruban *et al.*, 2012). In present study, total chlorophyll content remained almost same till day 30 day of drought stress in *A. barbadensis* (Fig. 2 B). Thereafter, a drastic reduction in total chlorophyll content was observed (during accumulation of carotenoid pigments in leaves). Chlorophyll degradation may be due to the drought induced activation of chlorophyll

degradation enzymes in *A. barbadensis*. Accumulation of carotenoids and chlorophyll degradation after 30th day of withholding water indicates high level resistance in *A. barbadensis* against drought stress.

Chlorophyll *a* fluorescence analysis, which was carried out to understand the photosynthetic plasticity in *A. barbadensis* under drought stress, indicates that photosynthetic machinery of *A. barbadensis* is quite resistance to water deficit conditions. Drought treatment up to 30 days could not alter photosynthetic performance of *A. barbadensis* (Fig. 2 C). Drought induced harmful symptoms on photosynthetic apparatus was observed on day 15 of drought treatment.

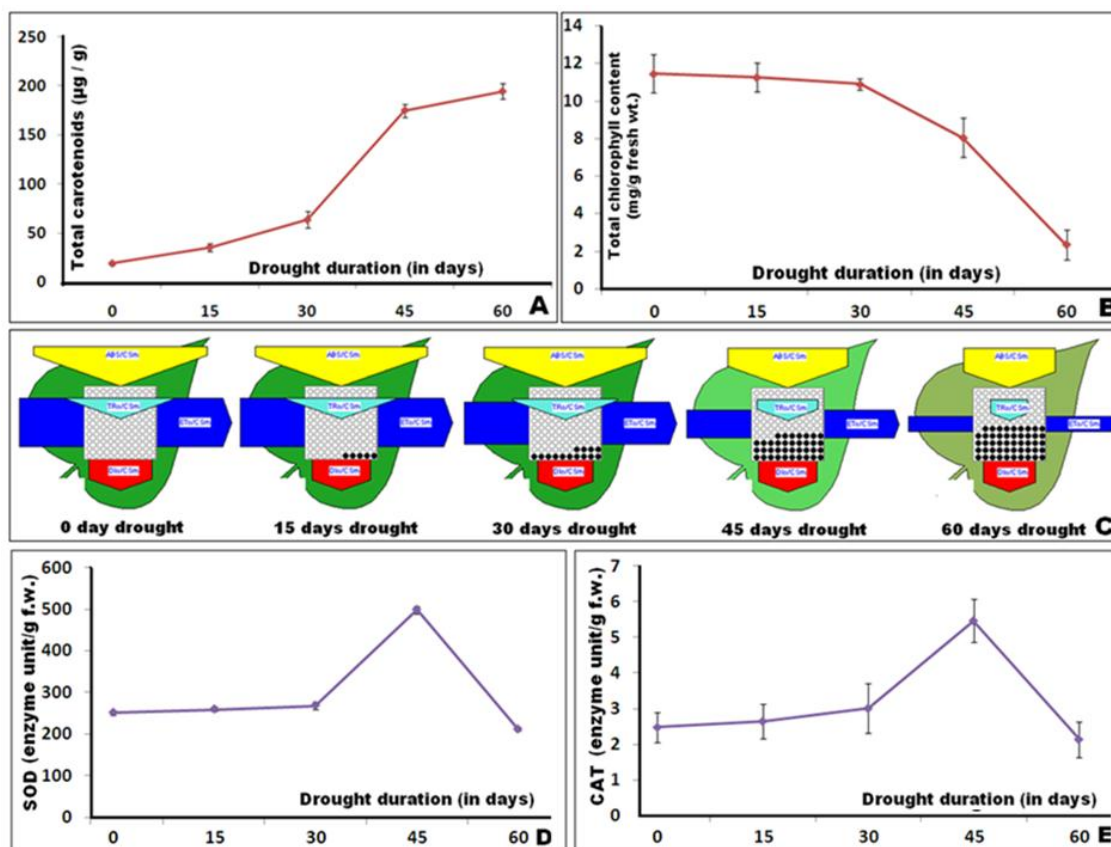


Figure 2: Influence of drought period on (A) total carotenoids and, (B) chlorophyll contents; (C) Leaf model showing various phenomenological fluxes (RC/CS, ABS/CS, TR/CS, ET/CS, DI/CS) under normal and drought stress; (D) SOD, and (E) CAT activities in *A. barbadosis* during water deficit conditions [Bars represent the mean of 10 replicates ± standard error (SE)]

Number of active reaction centers reduced gradually. However, concentration of active reaction centers decline with increasing drought period, but plants were found capable to perform same photosynthetic up to 30 days of drought treatment. It may be due to the presence of drought resistant LHC complexes and enhancement of functional potential of remaining active reaction centers. The high activity of reaction centers, despite their low density in drought stressed plants, indicate that the reaction centers have enhanced their activity to cope up with their meagre number. Similar, drought-induced increase in antenna size of active reaction centers has also been reported in *Chenopodium quinoa* (Fghire *et al.*, 2015) and *Triticum aestivum* (Parihar and Soni, 2016).

A notable reduction in RC/CS and ABS/CS was observed on day 45 of water deprivation. Decline in ABS/CS might be due to the degradation of total chlorophyll contents. It has been

shown that the decline in the rate of photosynthesis in drought stress is primarily due to CO₂ deficiency, as the photochemical efficiency could be brought back to normal after a fast transition of leaves to an environment enriched in CO₂ (Meyer and Genty, 1998). Decline in intracellular CO₂ levels generates ROS including superoxide, hydrogen peroxide (H₂O₂) and hydroxyl radicals. These ROS should be scavenged by the plant as they may cause photo-oxidation.

Plants have also evolved complex biochemical mechanisms to prevent the damage initiated by free radicals. The primary constituents include antioxidant enzymes such as SOD, CAT and free radical scavengers such as carotenoids, ascorbate, tocopherols and glutathione (Price *et al.*, 1994). SOD regulates the cellular concentration of O₂⁻ and H₂O₂. The latter is broken down by CAT. Under moderate stress conditions, the radicals are efficiently scavenged by this

antioxidant defence system. However, in periods of more severe stress in desiccation-sensitive plants, the scavenging system becomes saturated by the increased rate of radical production, and damage is inevitable. Drought stress also induced the antioxidant enzymes SOD and CAT in the leaves of *A. barbadensis*.

Plants exhibited a slight increase in SOD activity up to 30 days of drought period. Thereafter, a gradual reduction in SOD activity was observed (Fig. 2 D). Reduction in SOD activity might be due to the accumulation of carotenoids which decline the concentration of superoxide (O₂⁻) radicals. In addition, accumulation of H₂O₂ under drought also could lower SOD activity.

Similarly, the CAT activity remained almost same till day 30 and increased sharply thereafter in plants subjected to drought stress (Fig. 2 E). A remarkable reduction in CAT activity was recorded on day 45 of water deprivation. The decrease in CAT activity could indicate its inactivation by the accumulated hydrogen peroxide induced by water shortage and could be explained partly by photoinactivation of the enzyme. These observations suggest that oxidative stress resulting from water deficit conditions in *A. barbadensis* could result in the biosynthesis of antioxidative enzymes to counteract the oxidative damage. In conclusion, the present findings suggest that *A. barbadensis* plants are physiologically quite resistant to extreme drought condition. This study will therefore be useful in developing drought tolerant transgenic plants.

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