Effect of light stress on peroxidase, succinate dehydrogenase and total chlorophyll content in *Andrographis paniculata*

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SUMMARY

Andrographis paniculata*, a medicinal herb was grown in two different light intensities *i.e.* 1.44x10^3-2.24x10^{3}ìmole.photons.m^{-2}sec^{-1} and 0.24x10^3-0.96x10^3 ìmole.photons.m^{-2}sec^{-1} for a period of three different growth stages *i.e.* vegetative, flowering and fruiting to evaluate its response towards peroxidase (POD), succinate dehydrogenase (SDH) activity and total chlorophyll content. Increased activity of POD was observed in the plants grown in higher light intensity. On the contrary higher light intensity proved to be detrimental for the activity of SDH and total chlorophyll content which was recorded higher in lower light intensity. From the present study it can be suggested that higher light intensity is acting as a stressful condition for *A. paniculata.

Key words : *Andrographis paniculata*, Peroxidase (POD), Succinate dehydrogenase (SDH), Total chlorophyll content, Light stress

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Higher plants have the ability to respond towards the amount of light available during their growth. Light plays an important role in the environment, controlling the process associated with dry matter accumulation and thus contribute to the plant growth (Vilela and Ravetta, 2000). Different environmental stresses like light, high temperature, salinity and many other cause oxidative effect and high production of different oxygen reactive species (ROS) in plants which is dangerous for membrane lipids, proteins, chloroplasts, enzymes and nucleic acids (Asad, 1994,1992, Shah *et al.*, 2001). The plants have a defense system against oxidation which includes catalase, peroxidase, superoxide mutase and many other components (Sairam *et al.*, 2002). Peroxidases (POD) that trigger the conversion of H_2O_2 to water and oxygen are part of the enzymatic defense of the plant cells (Gulen and Eris, 2003). Succinate dehydrogenase (SDH, Succinate: ubiquinone oxidoreductase) is a part of complex II of the electron transport chain (ETC) in mitochondria. It converts succinate to fumarate and ubiquinone to ubiquinol. SDH activity has often been used to determine the senescent tissues as well as damaged plant tissues induced by environmental stresses (Kang *et al.*, 1996). Many plant species exhibit acclimatization of their photosynthesis apparatus to varying light intensity. Several external and internal factors affect chlorophyll metabolism. Light is considered one of the factors associated with chlorophyll metabolism (Brand, 1997). Excessive light intensity has a destructive effect on photosynthetic pigments leading to inhibition of photosynthesis (Ferus and Arokosiova 2001). Hence, the quantitative determination of chlorophyll in different experimental plant material is specially recommended as a valuable characteristic of light harvesting capacity under stress.

Andrographis paniculata (Family: Acanthaceae) grown widely in tropical area of Asia and commonly known as Kalmegh is medicinally important crop. It has been extensively used for the treatment of fever, diarrhea, inflammation, sore throat and hepatitis (Shah *et al.*, 2007). The plant has been studied for its antifertility, antidiabetic and hypotensive activity (Kumaran *et al.*, 2003). *A. paniculata* was reported to contain pharmacologically active diterpene lactone like andrographolide, neoandrographolide and deoxydihydroandrographolide (Shah *et al.*, 2007). Andrographolide is the main component and is believed to be the active constituent for biological activities and represents as an identity indicator for the plant (Aromdee *et al.*, 2005).

The present study deals with the effect of light stress on metabolism of the plant in field condition, as they are often subjected to fluctuating light intensities at its different growth stages which have a profound effect on the plant metabolism.

MATERIALS AND METHODS

Two months old plantlets of *A. paniculata* were brought from Anand Agricultural University, and planted in experimental plots of size 10x10 ft with 50x 50 cm spacing between two plantlets. The plantlets were then allowed to grow in two different light intensity conditions *i.e.*, Sunlight $1.44x10^3$- $2.24x10^3$ ìmole.photons.m^{-2}.sec^{-1} and shade $0.24x10^3$-$0.96x10^3$ ìmole.photons.m^{-2}.sec^{-1} for a period of one month. For all the three types of comparative assays, leaves of three different growth stages *i.e.* vegetative, flowering and fruiting were selected, as they are exposed to maximum stress condition during change in light intensities.

Fresh leaf materials were sampled from both types of plant and were used for assay.

Sample preparation for stress related enzymes:

Leaves (1 g) were homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) by using a prechilled pestle and mortar. The homogenate was centrifuged at 10,000 g for 20 min and the supernatant was used as enzyme source for the assay of peroxidase (POD), and succinate dehyrogenase (SDH) activity.

Peroxidase (POD) activity:

POD activity was determined spectrophotometrically based on the oxidation of guaiacol in the presence of H_2O_2. The assay mixture contained 0.1M potassium phosphate buffer (pH 7.5), 4mM guaiacol as donor, 3mM H_2O_2 as substrate, and 0.1ml crude enzyme extract. The total reaction was placed in quartz cuvette and the optical density was recorded at 30 seconds intervals for 3 min at 420nm. The level of enzyme activity was determined by measuring the difference in optical density and expressed in µ mol $min^{-1}g^{-1}$FW.

***Succinate dehydrogenase (SDH) activity*:**

The enzyme mixture consisted of 2ml of 0.2M sodium succinate, 1ml of phosphate buffer, 1ml of TTC (Triphenyl tetrazolium chloride) and 2ml of enzyme extract. The mixture was incubated in a water bath at 30^0C. At various time intervals, 7ml of acetone was added to stop the reaction. The mixture was centrifuged at 2000 *g* for 30min and the supernatant was measured at 460nm. Standard curve was plotted against sodium sulphite (Copper and Beevers, 1969).

Total chlorophyll content:

One gram leaf sample was macerated in 80% acetone. The sample was then centrifuged at 6000 rpm for 5 minutes and finally made into a volume of 5ml with acetone (80%). The optical density of the sample was measured at 645 and 663nm with spectrophotometer. Chlorophyll content (mg g^{-1} FW) was estimated according to Arnon (1949)

RESULTS AND DISCUSSION

Effect of light on peroxidase (POD) activity:

POD activity was recorded maximum in the leaves of all the three stages grown under higher light intensity. The maximum activity of POD was recorded in the leaves of the plants grown under higher light intensity in fruiting stage followed by flowering and then vegetative stage. It showed a maximum activity of 97.22 µ mol $min^{-1}g^{-1}$FW in higher light intensity while those in lower light intensity showed a level of 55.77 µ mol $min^{-1}g^{-1}$FW (Fig 1). The irradiance at higher light intensity increased the POD activity by 70 %.

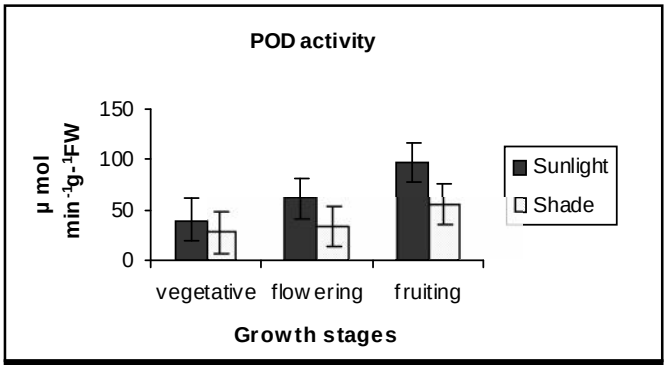

Fig. 1 : Effect of light intensity on POD activity

Effect of light on succinate dehydrogenase (SDH) activity:

The activity of SDH was observed maximum in the leaves of the plants grown under lower light intensity. Out of the three stages maximum activity was observed in the vegetative stage, where the activity was 0.032 units/ mg protein compared to those in higher light intensity where it was 0.006 units/mg protein (Fig 2). In other two stages the activity of SDH showed similar trend. The activity of dark grown plants was about eight fold higher than that of light grown plants. The SDH activity showed a decline in values from vegetative to fruiting stage.

Effect of light on chlorophyll content:-

The total chlorophyll content was found higher in the leaves of the plants in the vegetative stage grown under lower light intensity compared to those in higher. The plants grown in higher light intensity in the vegetative stage showed 9.25 mg.g^{-1}FW of chlorophyll content while

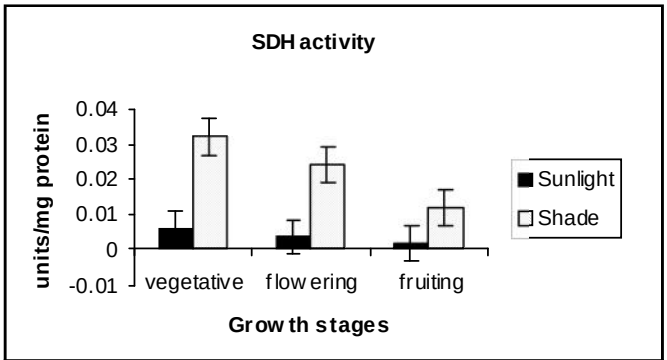

Fig. 2 : Effect of light intensity on SDH activity

those in shade showed 18.47 mg.g^{-1}FW (Fig 3). The results indicated that direct light had a detrimental effect on the total chlorophyll content of the plants.

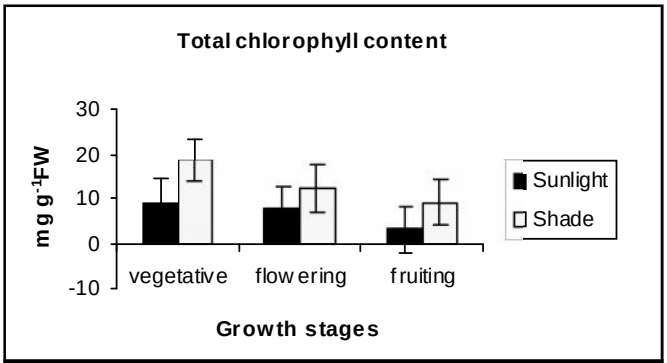

Fig. 3 : Effect of light intensity on total chlorophyll content

In present study irradiance of different light intensities changed the total chlorophyll content, POD activity and SOD activity drastically at different growth stages.

The leaves of *A.paniculata* showed maximum POD activity in the plants grown in higher light intensity in the fruiting stage compared to those in lower light intensity. The higher activity of POD in the fruiting stage may be due to the fact that during aging changes in the oxidative metabolism takes place in plant tissues which increase the concentration of ROS, which react with protein, lipids and other cellular components and cause their dysfunction and degradation. To prevent this action enzymatic components of the defence mechanism of the plants produce peroxidases (Lepedus *et al*., 2005). The results confirm the findings of Chen *et al*. (2002) where POD activity was enhanced in light treated mungbean. Similar results were also reported by Jose and Prue (1977) and Koornneef *et al*. (1980) where higher light intensity increased POD activity in radish and *Arabidopsis thaliana*, respectively. A possible mechanism that may be involved in the resistance to many types of stress is the increased activity of the antioxidant pathway (Mazorra *et al.*, 2002 and Gulen and Eris, 2003). High contents of antioxidant enzymes have been found in response to abiotic stress and they may have a role in the acquisition of tolerance of plants to different environmental stress. According to Sreenivasulu *et al*. (1999) a regulated balance between oxygen radical production and destruction is required, if metabolic efficiency and function are to be maintained in stress conditions. A constitutively high anti oxidant capacity under stress conditions can prevent damage and correlate with plants resistance to that particular stress. Thus, the mechanisms that reduce oxidative stress are expected to play an important role in imparting tolerance in plants under stress conditions. The increase in the POD activity, using guaiacol as an artificial substrate under the stress conditions indicates the formation of large amounts of H_2O_2 in plants, which indicates the effective scavenging active oxygen species production of certain secondary metabolites to withstand during stressful condition (Chaitanya *et al.*, 2002).

The present results emphasize the inverse relation between light and SDH activity. The decrease in SDH activity in flowering stage followed by fruiting indicates that the enzyme is age dependent and decreases with respect to aging (Kang *et al.*, 1996). The work correlates with work of Popov *et al*. (2007) where the SDH activity was much lower in the leaves of the *Arabidopsis* grown in higher light intensity. According to Filippova *et al*. (1989) the photosynthetic ETC is a source of energy in green plants under the light. Under these conditions, the role of Krebs cycle in the energy system of the plant cell is reduced to the minimum and the activity of enzymes involved in it is significantly suppressed because in presence of light the energy is generated by the photosynthesis and the catabolic role of the Krebs cycle is much lower. Under higher light intensity the primary role of the Krebs cycle is not to provide energy but rather to generate components necessary as intermediates for the constructive metabolism. Changes in the SDH activity affect not only the rate of TCA functioning but also the balance between photosynthesis, respiration and photorespiration in the plant cell. The regulation of the enzyme systems by changing gene expression is widely studied. Norichito and Masaki (2000) explained that phytochrome system is one of the mechanisms for light regulation of gene expression of SDH. SDH is involved in both the TCA cycle and the EC. In eukaryotic organisms, the SDH complex is composed of four polypeptides *i.e.* SDH1 which is covalently bound to FAD,

while SDH2 carries three Fe-s clusters. (Lemire and Oyedotun, 2002 andYankovskaya *et al.*, 2003). The fact that SDH is encoded by a large number of genes suggests that this enzyme plays an important role in the regulation of plant metabolism (Popov *et al.*, 2007). It is important to study the relationship of important processes such as photosynthesis, photorespiration and respiration in the plant cell. Among other things, the phytochrome system is involved in the regulation of these processes (Igamberdiev, 2001). The phytochrome system was previously shown to take part in the regulation of the activity of the key enzyme involved in plant respiration, in the light the activity of SDH enzyme was suppressed possibly due to the effect of the active form of phytochrome (Popov *et al.*, 2007).

The detrimental effect of higher light intensity on total chlorophyll content in the leaves of plants was shown in the present work. The decrease in the total chlorophyll content in different growth stages reveals that aging affects the chlorophyll content by affecting the photosynthetic electron transport in chloroplasts (Lepedus *et al.*, 2005). Similar work was reported by Baig *et al.* (2005) in grasses and legumes. This negative correlation between chlorophyll and light intensity for *A. paniculata* may be due to the fact that the leaves with high chlorophyll content do not photosynthesize more rapidly because they lack the enzymes or coenzymes which are the product of light reaction for reducing the available CO_2. Stitt (1986) reported that the differences in the rate of photosynthesis between the light- grown and shade- grown plants are often associated with altered ribulose-1, 5-biphospahe carboxylase/oxygenase content under saturating CO_2 concentration. Chlorophyll is constantly synthesized and destroyed (photo oxidation) in the presence of light. Under intense radiation, the degradative process is very active. The leaf chlorophyll concentration seems to increase under low light condition (Brand 1997). Kramer and Koslowisk (1979) revealed that the leaf chlorophyll levels are controlled by light. In elevated radiation intensities, chlorophyll molecules are susceptible to photo oxidation and the equilibrium is reached in lower radiation levels. Thus, leaves in lower light intensity have higher chlorophyll levels than leaves grown under higher light intensity. Similar results were obtained by Alvarenga *et al*. (1998, 2003) where *Guarea guidonia* and *Croton urucurana* shown higher chlorophyll content in the plants grown under lower light intensity. This acclimatization is characterized by significant alterations in the relative distribution of resources among the component parts of the photosynthetic apparatus.The species adaptive plasticity to solar radiation depends on the adjustment of the photosynthetic apparatus, in order to render radiant energy conversion in carbohydrates highly efficient and, consequently, to promote higher growth.

The knowledge of the effect of light stress on *A. paniculata* serves as an important index to understand metabolism of the plant at different light intensity.

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