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Variations in biochemical attributes of *Cassia tora* L. and *C. auriculata* L. under temperature stress

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Abstract: Plants continuously struggle for survival under various environmental abiotic stress conditions, specifically high temperature. Fourteen-day-old seedlings of *Cassia tora* and *Cassia auriculata* were subjected to differential temperature stress treatments at 30ºC, 37ºC, 42°C and 44°C for 16 h each. Various biochemical parameters viz reducing sugars, total protein, chlorophyll content and antioxidant enzyme system were assessed and found to be increased under high temperature stress. The amount of reducing sugars, total protein and chlorophyll were remarkably increased in both the *Cassia* species at 42ºC. The POX activity was more profound in *C. tora* (0.41 U/mg) than *C. auriculata* (0.24 U/mg) at 42°C. However, the activity of Catalase in both the species recorded a similar effect with a maximum value of 0.39 and 0.43 U/mg in *C. tora* and *C. auriculata* respectively. Similarly, SOD percentage inhibition activity increased significantly at 42°C for *C. tora* and *C. auriculata* showing a noticeable trend of inhibition of 85.23% and 86.89% respectively. Thus it can be concluded that various osmolytes and an efficient antioxidative system play a key role in generating tolerance against temperature stress and maintaining homeostasis to withstand the maximum range for survival at 42ºC in *Cassia* seedlings.

Keywords: *Cassia tora, Cassia auriculata*, Temperature Stress, Reactive Oxygen Species, Antioxidant Enzymes

1. Introduction

Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most of the major plants by more than 50% [1]. Plants in the field are frequently subjected to this stress that adversely affects their growth, development, and productivity [2-4]. Drought and heat stress are the common factors causing the most severe damage [5-8]. Among the ever-changing components of the environment, the constantly rising ambient temperature is considered to be one of the most detrimental stresses. High temperature stress induces morphological [9], anatomical [10] as well as physiological and biochemical changes in plants. It induces the changes in water relations [11-13], accumulation of compatible osmolytes [14, 15], and decrease in photosynthesis [16]. Besides this, high temperature also induces the rapid production and accumulation of reactive oxygen species (ROS) [17, 18]. These high levels of ROS are harmful to all cellular compounds and negatively influence cellular metabolic processes [19]. The detoxification of these ROS is very important and plants have developed complex strategies to deal with them [20]. The plant cells typically respond to increases in ROS levels by increasing the expression and activity of ROS-scavenging enzymes and increasing their production of antioxidants in order to maintain redox homeostasis.

Cassia, an ancient Greek reference to several leguminosae plants [21] is prized for its medicinal virtues and edible quality. Due to its medicinal, agricultural, and economic importance, *Cassia* has drawn the attention worldwide. The two important members of *Cassia* known for their prized, ayurvedic medicinal values are *Cassia tora* L. (Syn. *C. obtusifolia* L.) and *Cassia auriculata* L. *C. tora*, also called ringworm plant, is widely used as a source of medicine for constipation, conjunctival congestion, and blurred vision. The leaves possess hepatoprotective activity

[22]. The paste of the ground, dried root of *C. tora* is used in Ayurveda as a treatment for ringworm and snakebite [23]. It has also proven its worth in piles or haemorrhoids [24]. Besides being used as a medicinal herb, seeds are used as a substitute for coffee bean and mordant for dying and tanning [25]. On the other hand, *C. auriculata* commonly known as "Tanners *Cassia*," is widely used in traditional medicines for curing rheumatism, conjunctivitis, and diabetes. Studies also revealed anti-cancer effect of the leaf extract of *C. auriculata* (*in vitro*) on human breast and larynx cancer [26]. The plant also has anti-peroxidative efficacy in its flower extract [27].

In context to this, much of the reported works related to *C. tora* and *C. auriculata* have been focused on their medicinal aspects and there is a dearth of information on the effect of high temperature stress in the given plants at the biochemical levels. Hence, the present study was conducted to evaluate the effect of high temperature stress tolerance on reducing sugars, total protein, chlorophyll and antioxidant scavenging enzyme system for the two *Cassia* species.

2. Materials and Methods

2.1. Sample Collection and Identification

Disease-free and healthy pods of *C. tora* and *C. auriculata* were collected from the local populations around Bangalore during July 2012 and seeds were separated.

2.2. Preparation of Planting Material

Tetrazolium test for seed viability was conducted to estimate the seed germinability [28]. Seeds were cleaned with running tap water and surface-sterilized by fungicide (Rhedomyl) to prevent fungal contamination. The sterilized seeds were sown in plastic pots (6") filled with red soil, sand and Farm Yard Manure (1:2:1) and allowed them to germinate for 2 weeks in green house. Fourteen-day-old seedlings were subjected to differential temperature stress treatments of 30ºC, 37ºC, 42°C and 44°C for 16 h each. One set of experiment remained at ambient temperature (25±2ºC) as a control. A completely randomized design with three replicates for temperature stress and ten seeds per replicate was taken for the study.

2.3. Biochemical Analysis

Both the stressed and control plants were analyzed for different biochemical parameters, viz. chlorophyll, protein estimation, reducing sugar and antioxidant enzyme assay for Superoxide dismutase (SOD), Peroxidase (POX), and Catalase (CAT) at different temperature treatments.

2.3.1. Chlorophyll and Total Protein Estimation

Chlorophyll (*a*, *b)* and total protein was estimated according to the methods described by Arnon [29] and Lowry *et al*. [30] respectively.

2.3.2. Reducing Sugar Estimation

The reducing sugar was estimated as per the method suggested by Ranganna [31].

2.3.3. Antioxidant Enzyme Extractions and Assay

The antioxidant enzyme activity was conducted for three enzymes, viz. POX, CAT and SOD, at varying temperature stress conditions in both the species following the previous methods [32-34] with few modifications.

2.3.4. Peroxidase (POX) Assay

POX was assayed by the method of Kumar and Khan [32]. Assay mixture of POX contained 2 ml of 0.1 mol/L phosphate buffer (pH 6.8), 1 ml of 0.01 mol/L pyrogallol, 1 ml of 0.005 mol/L H_2O_2 and 0.5 ml of enzyme extract. The solution was incubated for 5 min at 25 °C after which the reaction was terminated by adding 1 ml of 2.5 mol/L H_2SO_4. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 mol/L H_2SO_4 at zero time. The activity was expressed in U/mg protein. One U is defined as the change in the absorbance per 0.1 min per mg protein.

2.3.5. Catalase (CAT) Assay

The activity of CAT was measured according to the method of Chandlee and Scandalios [33]. The assay mixture contained 2.6 ml of 50 mM/L potassium phosphate buffer (pH 7.0), 0.4 ml of 15mM/L H_2O_2 and 0.04 ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in U/(mg protein). One U is defined as 1 mM/L of H_2O_2 reduction per min per mg protein.

2.3.6. Superoxide Dismutase (SOD) Assay

The activity of SOD was assayed as described by Beauchamp and Fridovich [34]. The reaction mixture contained $1.17x10^6$ mol/L riboflavin, 0.1mol/L methionine, $2x10^5$ mol/L KCN and $5.6\ x10^5$ mol/L nitroblue tetrazolium (NBT) salt dissolved in 3ml of 0.05 mol/L Sodium Phosphate buffer (pH 7.8). Three millilitres of the reaction medium was added to 1 ml of enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes in a single row. Illumination was started to initiate the reaction at 30°C for 1 h. Identical solutions that were kept under dark served as blanks. The absorbance was read at 560 nm in the spectrophotometer against the blank. SOD activity is expressed in U/(mg protein). One U is defined as the change in 0.1 absorbance per hour per mg protein.

The percent inhibition of the sample was calculated by using the following formula.

$$\text{Inhibition (\%)} = \frac{(\text{Blank}_{\text{A560}} - \text{Test}_{\text{A560}})}{\text{Blank}_{\text{A560}}} \text{ x } 100$$

2.4. Statistical Analysis

Data was recorded for all the biochemical parameters and were analysed by two way ANOVA and confirming the result

by Tukey's Test using the statistical software (SYSTAT VER. 0.6) to assess the effect of temperature stress on both the species of *Cassia*. All the acquired data was represented by an average of three replicate measurements and standard error. Significance was tested at 5% level.

3. Results

Different biochemical parameters, viz. reducing sugar, total protein, chlorophyll *a* and *b*, and antioxidant enzymes, Peroxidase (POX), Catalase (CAT), and Superoxide dismutase (SOD) were evaluated in two species of *Cassia* at varying temperature treatments. All the biochemical attributes studied in the *Cassia* seedlings exhibited significant responses with increase in temperature stress.

Post hoc comparisons using the Tukey HSD test indicated that the mean score for the control was significantly different from 37ºC and 42ºC. However, the temperature 30ºC did not differ significantly from control. In both the species of *Cassia,* the concentration of reducing sugars, total proteins and level of chlorophyll significantly increased with increase in temperature. Our results suggested that as the temperature was increased from 30ºC to 42ºC, the amount of reducing sugar concentration levels also increased from 0.11-0.71 and 0.09-0.91µg/µl in *C. tora* and *C. auriculata* respectively (Fig-1). Similarly, the amount of total protein content under temperature stressed seedlings in both the *Cassia spp*. was found to be maximum with a value of 4.67 and 4.34 µg/µl at 42ºC (Fig-2). A significant increase in leaf chlorophyll content was observed in both plant species compared with control, and the maximum increase was measured at 42ºC (Fig-3 and 4).

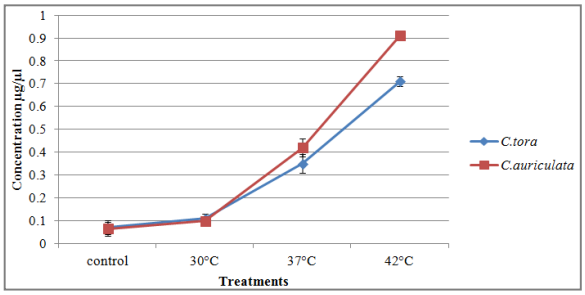

Fig 1. *Effect of different temperatures on reducing sugar content of C. tora and C. auriculata plantlets*

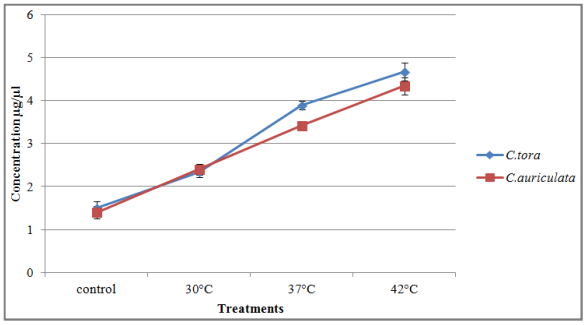

Fig 2. *Effect of different temperatures on protein content of C. tora and C. auriculata plantlets*

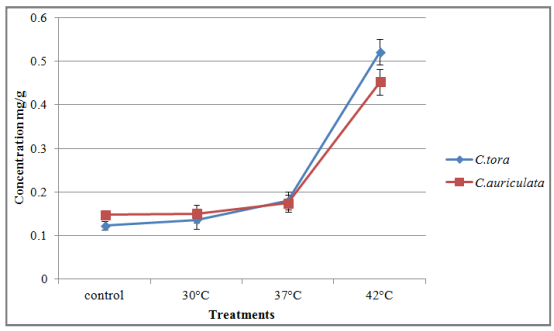

Fig 3. *Effect of different temperatures on chlorophyll a content of C. tora and C. auriculata plantlets*

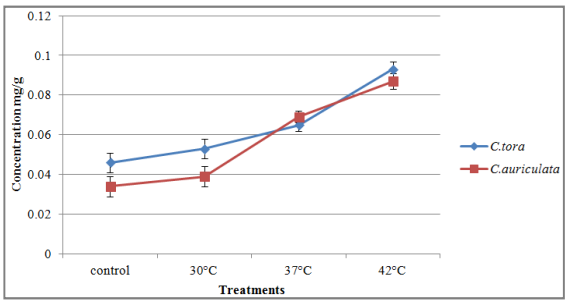

Fig 4. *Effect of different temperatures on the chlorophyll b content of C. tora and C. auriculata plantlets*

Quantitative and qualitative alterations in various antioxidant enzymes and its system are mostly related to the tolerance level of a plant species for a particular stress. Temperature stress in *Cassia* seedlings altered the POX, CAT and SOD activities to a great extend. The gradual increase of temperature stress to 42°C recorded the maximum activity. At 37°C and 42°C, *Cassia* seedlings showed a remarkable increase in the activity for POX and CAT enzymes at high temperature; however, the POX value was just about double in *C. tora* (0.41 U/mg) than *C. auriculata* (0.24 U/mg) at 42°C (Fig-5). However, the activity of Catalase in both the species recorded a similar effect with a maximum value of 0.39 and 0.43 U/mg in *C. tora* and *C. auriculata* respectively. The CAT effect revealed a gradual increase in its activity unlike POX (Fig-6). Total SOD percentage inhibition activity increased significantly at 42°C for *Cassia tora and C. auriculata* showing a noticeable trend of inhibition at 85.23% and 86.89% respectively. Compared to control (unstressed) plants had the minimum value for this enzyme (57%) (Fig-7).

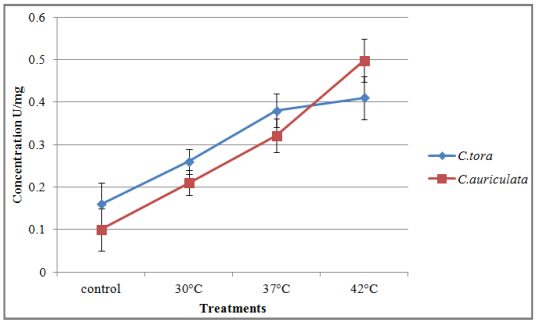

Fig 5. *Effect of different temperatures on POX activity of C. tora and C. auriculata plantlets*

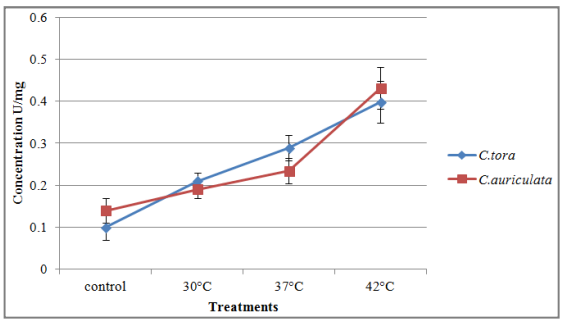

Fig 6. *Effect of different temperatures on CAT activity of C. tora and C. auriculata plantlets*

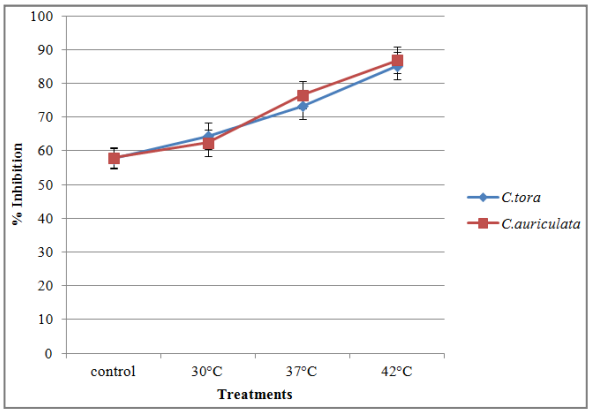

Fig 7. *Effect of different temperatures on SOD activity of C. tora and C. auriculata plantlets*

4. Discussion

In nature, plants resort to various acclimation strategies in response to abiotic environmental stresses like high temperature, dehydration, cold and excessive osmotic pressure [35]. This effect results in series of changes at physiological and biochemical levels like those encoding scavenging antioxidant enzyme system, chlorophyll value, sugar and protein content in a plant. Accumulation of total soluble sugars is a common phenomenon under stress condition [36, 37]. Our results indicates increased reducing sugar concentration levels in both the *Cassia* species with an increase in temperature, which is in agreement with the work done by Nayar *et al.,* [38] in chickpea seedlings. In the present study, *Cassia tora* and *C. auriculata* accumulated more sugars which are effective in maintaining turgor pressure and showing higher osmotic adjustment in the given plants. It has been documented that sucrose plays a positive role in protecting cells from injuries under cold or high temperature in some plants [39] which is seen in both the *Cassia* species.

Under temperature stress, variation in the transcript and enzyme levels of major antioxidant enzymes during stress is well documented. Our result suggests that as the temperature was increased the concentration of total soluble protein levels increased from 30°C to 42°C. The reduction in protein content may reflect delay in protein synthesis which accelerates its degradation. According to the increase in the amount of free amino acids in the protein, the increased values observed in the case of *C. tora* and *C. auriculata* from 30°C to 42°C could be related to the period of active multiplication of plants and consequently higher protein synthesis, thus protecting the seedlings from degradation even at the maximum temperature stress. Similar findings were supported by Isabela et al., [40] in sugarcane seedlings.

Chlorophyll content in plants is an important trait to assess photosynthetic efficiency under varying stressed conditions. High-temperature stress directly or indirectly affects plant photosynthetic functions by changing the structural organization and physicochemical properties of thylakoid membranes [41]. In the present study, an increased level of chlorophyll a and b was observed in Cassia seedlings subjected to heat stress at 42ºC for 16 h. which is in agreement with Gur *et al*. [42] in cotton. The plants exposed to extreme temperatures result in production of reactive oxygen species (ROS) as byproducts, which damage the cellular components [43]. Plants have developed a series of enzymatic and non-enzymatic detoxification systems to counteract ROS and protect cells from oxidable damage [44].The antioxidant enzymes such as SOD, CAT, POX, function in detoxification of superoxide and H_2O_2 [17]. When plants are subjected to various stress, the first ROS scavenging enzyme active and participating in the enzymatic mechanism is SOD. Super oxide dismutases active oxygen radicals into H_2O_2 and plays an important role in cell defense system against ROS [45]. The H_2O_2 generated is further scavenged by other two important enzymes CAT and POX. The protective roles of these antioxidant enzymes in temperature stress have been reported in a number of plants [46]. In the present work, total SOD inhibition percentage, CAT and POX enzymes increased with higher temperature stress. Similar results were found in French bean [47]. Our results are also in agreement with heat stressed mustard suggested by Dat et al, [48]. Increased activity of CAT, POX, and SOD has been suggested as an adaptive mechanism to reduce the H_2O_2 and offer protection against oxidative damage [49] which is significantly observed in *Cassia*.

5. Conclusion

The assessment of the temperature stress on various biochemical attributes in *Cassia tora* and *C. auriculata* seedlings lead us to conclude that all the parameters in consideration revealed a significant effect under temperature stress. The results point out that, high temperature provoked all the biochemical parameters and free radical scavenging enzymes, contributing in making the plant higher temperature tolerant which is essential for the plant growth and its productivity in extreme temperature conditions. This may also permit to understand plant survival capacity following ecological disturbances in the vicinity. Further, the genes playing role in heat tolerance and identification of markers encoding

scavenging properties would be another step towards developing a better responding *Cassia* species under elevated temperature.

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