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Research Article

COMPARATIVE ANALYSIS OF HEAT TREATMENTS ON MORPHOLOGY OF SELECTED CASSIA SPECIES

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ABSTRACT

Plants are often subjected to unfavourable environmental conditions - abiotic stresses that play a major role in determining the productivity and distribution of the plant species across different types of environment. These stresses elicit complex cellular responses that help in understanding plant abiotic responses at the morphological, biochemical and molecular level. The present study was focused on two species (*Cassia tora* and *C.auriculata)* and their morphological response to the temperature. Germination percentage, leaf area index, fresh and dry weights of the leaf, root and shoot were studied in detail at different temperature treatments exposed for number of days. High level of temperature treatments showed a significant effect on all the parameters. Results suggest that with an increase in temperature till 37ºC the physiological activities of both the species are increased with respect to control and gradually decreased at 42ºC. For percent germination there was a gradual decrease in germination from control to 42ºC in both the species. On the other hand, prolonged exposure of high temperature had a negative effect on few physiological parameters such as shoot length and root fresh weight. Others parameters such as shoot dry weight, root length, leaf dry and fresh weight, and LAI had a positive effect on temperature treatment up to 37ºC. Thus, it can be concluded that the optimum temperature at different levels of physiological activities for C. tora and C. auriculata is 37ºC.

Keywords: *Cassia tora, Cassia auriculata, Percent germination, Leaf area index*

INTRODUCTION

The Cassias are among the 2,500 species in 180 genera of *Caesalpinaceae* subfamily of leguminosae. *Cassia* is an ancient Greek reference to several leguminosae plants[1] prized for its medicinal virtues along with 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*.

Various parts of *C.tora* plant are used for various purposes. As a source of medicine, it is used for constipation, conjunctival congestion and blurred vision. The leaves possess hepatoprotective activity [2]. The paste of the ground, dried root is used in Ayurveda as a treatment for ringworm and snakebite[3]. It has also proven its worth in piles or haemorrhoids[4]. The juice extracted from its leaves is given in conditions like skin ailments, rashes and allergies. The leaf decoction is very widely used as an anti-obesity agent. Seeds of the plant are known for hypolipidemic activity[5,6].

Emodin, is a naturally occurring anthraquinone present in the roots and barks of *C. tora* as an active ingredient. The biological property of emodin molecule offers a broad therapeutic window, which in future may become a member of anticancer[7]. Besides being used as a medicinal herb, seeds are used as a substitute for coffee bean. Seeds are also used as a mordant for dying[8,9,10].

On the other hand, *C. auriculata* L., commonly known as "Tanners Cassia," is a shrub found throughout southern, western and central India[11]. The bark and leaves of the plant give relief against rheumatism, eye diseases, gonorrhoea, diabetes and gout[12]. Studies have revealed anti-cancer effect of the leaf extract (*in vitro*) on human breast and larynx cancer[13]. The plant also has anti-peroxidative efficacy in its flower extract[14]. The flower possesses antihyperlipidaemic effect in addition to antidiabetic activity[15]. The leaf extract of *C. auriculata* exhibits a significant broad spectrum activity against *B. subtilis* and *S. Aureus[*16].

Evaluation of antibacterial activity of flower extracts is also observed against a wide range of microorganisms such as *Staphylococcus aureus*, *E. faecalis, B. subtilis, S. typhi, V. cholerae,* and *K. Pnemoniae[*17]. Thus, various parts of the plant have been reported to cure diseases such as leprosy, asthma, and gout[4], and have also been used in the treatment of skin infections.

Both the plants (*C.tora* and *C. auriculata*) have the capacity to adapt successfully to a wide range of habitat; being wild, they can easily be grown but a number of environmental fluctuations such as abiotic stresses (high temperature, salinity, and pH) represent the major constraint to their adaptability. These abiotic stresses are serious threats to the plants and result in the deterioration of the environment. Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major plants by more than 50%[18]. Hence, elucidating the various changes in plant response to stress and their role in acquired stress tolerance is thus of practical and basic importance. In conjunction to this, most of the reported works related to *C. tora* and *C. auriculata* limit to their medicinal and few physiological descriptions. However, no work has been reported on the plant response to abiotic stress in relation to a comparative study of both the species under its morphological analysis with varying temperature stress.

Therefore, to improve the reliability and adaptability of *C. tora* and *C. auriculata* against abiotic stresses, studies of induced changes was carried out, which may help in understanding the ability of plants to tolerate and withstand the environmental stresses that often occur during acclimation processes.

MATERIALS AND METHODS

Sample collection

Disease-free and healthy pods of *C. tora* and *C. auriculata* were collected from the local populations and seeds were separated. The quality of seeds and authenticity of particular variety was certified from plant breeder prior to experimentation.

Preparation of Planting Material

Tetrazolium Test: Seed Viability Test

Tetrazolium test, commonly known as the TZ test for seed viability, was conducted to estimate seeds germinability[19].

Surface Sterilization of Seeds

Seeds are cleaned with running tap water. Thereafter, seeds are surface-sterilized by fungicide (Rhedomyl) to prevent fungal contamination during the study.

Seed Sowing

The sterilized seeds are sown in plastic pots (6") filled with red soil, sand, and Farm Yard Manure (1:2:1) and allow them to germinate for 2 weeks in green house. Fourteen-day-old seedlings were subjected to varying temperature stress treatments at varying intervals (viz. 0, 7, and 14 days).

Stress Treatment Parameters

A completely randomized design with three replicates for different stress treatments and ten seeds per replicate for each stress was taken for the study.

Temperature Stress

Pots with 14-day-old seedlings are subjected to differential temperature viz. 30ºC, 37ºC, and 42ºC for 16 h each. One set of experiment remained at ambient temperature (25 ±ºC) as a control.

Morphological Analysis- Plant Growth Analysis

After completing the stress treatment process, all plants are carefully uprooted and washed with running tap water followed by distilled water and subjected to morphological investigations or analysis.

Germination for treated and control plants is determined by a radical emergence of 2 mm and germination percentage is calculated by using the following formula [20]:

$$\textbf{Germination \%} = \frac{\textbf{No. of seeds germinated}}{\textbf{Total no. of seeds taken for germination studies}} \times 100$$

Leaf area is measured using a graph paper. Fresh and dry weight of leaves, stem, and roots is determined in grams. Length of the sample is measured in centimeters. The dry weight of the samples is recorded by transferring them into hot air oven at 100ºC for 1 hour followed by vacuum dry at 80ºC. To determine the fresh weight, a 0.1gm leaf sample was cut into small pieces. The leaf samples were floated in freshly deionized water for 12 h and weighed thereafter to determine the fully turgid weight. To determine the dry weight of the leaves, the leaf samples were oven-dried at 80ºC for 3 days [19].

Statistical Analysis

Data was recorded for morphological 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 on both the species of Cassia. All the acquired data are represented by an average of three replicate measurements and standard error. Significance is tested at 5% level.

RESULTS

Morphological Analysis

Morphological analysis was conducted for three different abiotic stresses. A total of 11 different morphological parameters, viz. germination %, shoot and root length, shoot and root fresh as well as dry weight, leaf fresh dry weight, leaf area index, and leaf turgid weight, were evaluated with a different number of treatments and days interval (0, 7, and 14 days) in two species of *Cassia*.

Temperature stress

A completely randomized design, with three replicates for temperature stress and 10 seeds per replicate was taken for the study. Fourteen-day-old seedlings were subjected to differential temperature, viz. 30ºC, 37ºC, and 42ºC for 16 h each. The observations were recorded for 0 day, 7 day, and 14 day intervals. One set of experiment remained at ambient temperature (25 ± 2ºC) as a control. The study was also focused to check for the comparative analysis between the two *Cassia* species(*C. tora* and *C. auriculata***).**

Temperature stress on Percent Germination in *C. tora* and *C. auriculata*

A two-way analysis of variance (ANOVA) between the number of days and treatments was conducted to compare the effect of temperature on germination percentage in both the species of *Cassia* separately. All effects were statistically significant at the 0.05 significance level. There was a significant effect of amount of heat treatment and the number of days on percent germination in both the species remembered at the $p < 0.05$ level. *Cassia tora* showed significance to the different temperatures [$F(3,6) = 11.1597$, $p = 0.0072$] and to the number of days [$F(2,6) = 39.352$, $p = 0.0004$]. *Cassia auriculata* also showed a significance to the different temperatures [$F(3, 6) = 4.9773$, $p = 0.0045$] and to the number of days [$F(2, 6) = 17.1991$, $p = 0.00328$].

Post hoc comparisons using the Tukey HSD test were done to point out the significance in species for the heat treatments and the number of days at [$Q_{crit} = 4.34$, d$f = 6$, n=3]. There was significance between the control and 7 days, whereas there was a gradual decrease in germination percentage as the number of days increased to 14 days. A germination percentage was found to increase at 30ºC and then decreased thereafter on increasing the temperature to 42ºC. By contrast, there was a significant result observed between control and the seeds exposed to 14 days, and germination percentage decreased gradually at 42ºC when compared with the control.

Taken together, these results suggest that high levels of heat treatments do have an effect on the percent germination. Specifically, our results suggest that as the temperature was increased, the percent germination values decreased from control to 42ºC.

Table 1: Percent Germination

	C.tora			*C.auriculata*		
	0 DAYS	**7 DAYS**	**14 DAYS**	**0 DAYS**	**7 DAYS**	**14 DAYS**
CONTROL	4.333 ± 0.05	4.0 ± 0.012	1.333 ± 0.01	7.66 ± 0.23	5.66 ± 0.12	3.0 ± 0.03
T1-30°C	7.33 ± 0.254	5.0 ± 0.043	2.667 ± 0.04	7.33 ± 0.051	4.33 ± 0.03	2.66 ± 0.01
T2-37°C	7.667 ± 0.03	5.33 ± 0.04	3.33 ± 0.05	5.0 ± 0.02	4.33 ± 0.04	2.33 ± 0.03
T3-42°C	4.33 ± 0.23	4.0 ± 0.03	1.33 ± 0.02	3.667 ± 0.03	3.33 ± 0.02	2.0 ± 0.01

Fig 1: Table showing the values of germination percentage of both the species at different temperature treatments and duration in days. All the values are average of triplicates. Germination percentage is expressed in % ± s.d

Effect of temperature on shoot length, shoot fresh weight and dry weight.

A two-way ANOVA between the number of days and treatments was conducted to compare the effect of temperature on shoot length, shoot fresh weight and shoot dry weight in both the species of *Cassia* separately. All effects were statistically significant at the 0.05 significance level.

There was a significant effect of amount of heat treatment and the number of days on shoot length in both the species remembered at the $p < 0.05$ level. *Cassia tora* showed a significance to the different temperatures [$F(3,6) = 21.222$, $p = 0.0013$] and to the number of days [$F(2,6) = 29.9737$, $p = 0.0008$]. *Cassia auriculata* also showed a significance to the different temperatures [$F(3,6) = 9.03009$, $p = 0.0012$) and to the number of days [$F(2, 6) = 16.8021$, $p = 0.00348$]. *Post hoc* comparisons using the Tukey HSD test were done to point out the significance in species for the heat treatments and the number of days at [$Q_{crit} = 4.34$, df = 6, n=3]. (Table 2)

Table 2: Table showing the values of Root length, Root fresh weight and Root dry weight of the two species at different temperature treatments. All the values are average of triplicates. (F.W: Fresh weight; D.W: Dry weight); Root length measured in cm ±s.d, fresh and dry weights measured in gm ±s.d.

	ROOT	*C.tora*				*C.auriculata*			
		CONTROL	T1-30°C	T2-37°C	T3-42°C	CONTROL	T1-30°C	T2-37°C	T3-42°C
0 days	Length	3.867 ± 0.03	4.46 ± 0.1	4.83 ± 0.12	3.2 ± 0.12	5.033 ± 0.2	4.93 ± 0.12	4.7 ± 0.1	4.63 ± 0.11
	F.W	0.017 ± 0.12	0.020 ± .1	0.030 ± 0.1	0.010 ± 0.12	0.023 ± 0.12	0.023 ± .11	0.013 ± 0.03	0.003 v 0.12
	D.W	0.010 ± 0.02	0.013 ± 0.12	0.017 ± 0.11	0.003 ± 0.11	0.000	0.023 ± 0.03	0.013 ± 0.1	0.003 ± 0.11
7 days	Length	3.80 ± 0.11	4.067 ± 0.11	4.467 ± 0.01	3.125 ± 0.03	4.33 ± 0.02	4.36 ± 0.12	3.967 ± 0.12	3.733 ± 0.11
	F.W	0.018 ± 0.1	0.023 ± 0.1	0.033 ± 0.3	0.017 ± 0.1	0.027 ± 0.03	0.023 ± 0.1	0.017 ± 0.05	0.013 ± 0.1
	D.W	0.007 ± 0.11	0.010 ± 0.07	0.017 ± 0.1	0.003 ± 0.03	0.027 ± 0.05	0.023 ± 0.1	0.017 ± 0.09	0.013 ± 0.08
14 days	Length	4.16 ± 0.1	4.667 ± 0.03	4.767 ± 0.02	4.167 ± 0.08	3.53 ± 0.09	3.3 ± 0.09	3.0 ± 0.03	3.067 ± 0.03
	F.W	0.019 ± 0.06	0.028 ± 0.03	0.041 ± 0.03	0.023 ± 0.1	0.027 ± 0.03	0.027 ± 0.05	0.023 ± 0.12	0.021 ± 0.2
	D.W	0.010 ± 0.06	0.017 ± 0.09	0.017 ± 0.07	0.007 ± 0.03	0.027 ± 0.05	0.027 ± 0.1	0.023 ± 0.03	0.021 ± 0.03

Table 3: Table showing the values of Shoot length, Shoot fresh weight and shoot dry weight of the two species at different temperature treatments. All the values are average of triplicates. (F.W: Fresh weight; D.W: Dry weight). Shoot length measured in cm ± s.d, fresh and dry weights measured in gm ±s.d.

	SHOOT	*C.tora*				*C.auriculata*			
		CONTROL	T1-30°C	T2-37°C	T3-42°C	CONTROL	T1-30°C	T2-37°C	T3-42°C
0 days	Length	5.367 ± 0.03	5.8 ± 0.03	5.8 ± 0.04	4.86 ± 0.03	5.73 ± 0.04	5.7 ± 0.03	5.067 ± 0.21	4.8 ± 0.12
	F.W	0.060 ± 0.12	0.070 ± 0.12	0.07 ± 0.1	0.053 ± 0.11	0.063 ± 0.12	0.073 ± 0.2	0.057 ± 0.12	0.050 ± 0.21
	D.W	0.010 ± 0.1	0.013 ± 0.1	0.017 ± 0.1	0.003 ± 0.12	0.013 ± 0.1	0.017 ± 0.12	0.010 ± 0.2	0.007 ± 0.1
7 days	Length	4.533 ± 0.01	5.1 ± 0.03	5.53 ± 0.1	3.83 ± 0.12	5.367 ± 0.02	5.26 ± 0.02	4.33 ± 0.12	4.267 ± 01
	F.W	0.057 ± 0.1	0.063 ± 0.03	0.073 ± 0.1	0.047 ± 0.1	0.053 ± 0.1	0.053 ± 0.23	0.043 ± 0.13	0.043 ± 0.10
	D.W	0.010 ± 0.11	0.013 ± 0.03	0.017 ± 0.2	0.012 ± 0.02	0.017 ± 0.04	0.017 ± 0.03	0.010 ± 0.1	0..07 ± 0.01
14 days	Length	5.633 ± 0.03	6.03 ± 0.04	6.23 ± 0.1	5.33 ± 0.1	4.467 ± 0.02	4.46 ± 0.04	4.5 ± 0.12	3.833 ± 0.12
	F.W	0.057 ± 0.03	0.073 ± 0.1	0.083 ± 0.1	0.053 ± 0.12	0.047 ± 0.12	0.050 ± 0.1	0.043 ± 0.12	0.037 ± 0.11
	D.W	0.013 ± 0.2	0.017 ± 0.1	0.023 ± 0.1	0.003 ± 0.1	0.017 ± 0.1	0.017 ± 0.1	0.007 ± 0.03	0.003 ± 0.2

Table 4: Table showing the values of leaf area index, leaf fresh weight, leaf dry weight and leaf turgid weight of the two species at different temperature treatments. All the values are average of triplicates. (LAI: Leaf area index; .W: Fresh weight; D.W: Dry weight; T.W: Turgid weight). Leaf area index measured in mm² ± s.d , fresh and dry weights expressed in gm ± s.d.

	Leaf	*C.tora*				*C.auriculata*			
		CONTROL	T1-30°C	T2-37°C	T3-42°C	CONTROL	T1-30°C	T2-37°C	T3-42°C
0 days	LAI	2.667 ± 0.05	3.33± 0.01	3.667 ± 0.1	2.33 ± 0.5	3.0 ± 0.03	2.667 ± 0.9	2.0 ± 0.04	2.0 ± 0.06
	F.W	0.117 ± 0.03	0.143 ± 0.06	0.167 ± 0.02	0.093 ± 0.04	0.160 ± 0.05	0.157 ± 0.07	0.120 ± 0.03	0.103 ± 0.03
	D.W	0.017 ± 0.05	0.023 ± 0.1	0.027 ± 0.03	0.027 ± 0.03	0.023 ± 0.01	0.027 ± 0.02	0.013 ± 0.02	0.007 ± 0.02
	T.W	0.097 ± 0.03	0.110 ± 0.03	0.143 ± 0.04	0.090 ± 0.02	0.150 ± 0.04	0.137 ± 0.02	0.087 ± 0.02	0.080 ± 0.02
7 days	LAI	3.0 ± 0.07	3.33 ± 0.02	4.33 ± 0.03	2.33 ± 0.01	3.33 ± 0.03	3.33 ± 0.02	2.33 ± 0.07	2.33 ± 0.02
	F.W	0.113 ± 0.03	0.140 ± 0.04	0.147 ± 0.03	0.090 ± 0.12	0.160 ± 0.02	0.160 ± 0.01	0.123 ± 0.02	0.097 ± 0.07
	D.W	0.017 ± 0.04	0.023 ± 0.06	0.023 ± 0.12	0.013 ± 0.03	0.030 ± 0.1	0.027 ± 0.02	0.017 ± 0.02	0.017 ± 0.03
	T.W	0.10 ± 0.05	0.107 ± 0.05	0.140 ± 0.03	0.083 ± 0.02	0.143 ± 0.03	0.101 ± 0.03	0.072 ± 0.02	0.068 ± 0.03
14 days	LAI	2.33 ± 0.1	3.0 ± 0.06	3.33 ± 0.05	2.0 ± 0.02	3.667 ± 0.02	3.66 ± 0.02	2.667 ± 0.01	2.33 ± 0.05
	F.W	0.117 ± 0.03	0.137 ± 0.02	0.147 ± 0.03	0.090 ± 0.1	0.157 ± 0.02	0.147 ± 0.04	0.120 ± 0.04	0.123 ± 0.04
	D.W	0.010 ± 0.06	0.010 ± 0.05	0.017 ± 0.02	0.003 ± 0.03	0.013 ± 0.02	0.020 ± 0.03	0.010 ± 0.04	0.07 ± 0.03
	T.W	0.080 ± 0.1	0.090 ± 0.04	0.117 ± 0.03	0.060 ± 0.01	0.150 ± 0.06	0.153 ± 0.04	0.123 ± 0.03	0.083 ± 0.04

There was also a significant effect of amount of heat treatment and the number of days on shoot fresh weight in both the species remembered at the $p < 0.05$ level. *Cassia tora* showed significance to the different temperatures [$F(3,6) = 56.5$, $p = 0.0086$] and to the number of days [$F(2,6) = 6.5$, $p = 0.0315$]. *Cassia auriculata* also showed a significance to the different temperatures [$F(3,6) = 13.257$, $p = 0.0046$] and to the number of days [$F(2,6) = 27.857$, $p = 0.0009$]. *Post hoc* comparisons using the Tukey HSD test were done to point out the significance in species for the heat treatments and the number of days at [$Q_{crit} = 4.34$, df = 6, n = 3]. There was a significance between the 7-day and 14-day exposure. The shoot fresh weight gradually increased from control to 37ºC and thereafter decreased at 42ºC. In *C. auriculata,* there was a significant effect found between control and 7 and 14 days. However, there was no effect found between 7 and 14 days. The significant effect was found between control and 42ºC and between 30ºC and 37ºC and between 37ºC and 42ºC. (Table 2)

There was a significant effect of amount of heat treatment and the number of days on shoot dry weight in both the species remembered at the $p < 0.05$ level. *Cassia tora* showed a significance to the different temperatures [$F(3,6) = 24$, $p = 0.0096$] and to the number of days [$F(2,6) = 2.6$, $p = 0.1537$]. *Cassia auriculata* also showed a significance to the different temperatures [$F(3,6) = 30.6667$, $p = 0.0049$] and to the number of days [$F(2,6) = 1$, $p = 0.42187$]. (Table 2)

Effect of temperature on Root length, Root fresh weight and Root dry weight.

A two-way ANOVA between the number of days and treatments was conducted to compare the effect of temperature on root length, root fresh weight and root dry weight in both the species of *Cassia* separately. All effects were statistically significant at the 0.05 significance level.

There was a significant effect of the amount of heat treatment and the number of days on root length in both the species remembered at the $p < 0.05$ level. *Cassia tora* showed a significance to the different temperatures [$F(3,6) = 14.549$, $p = 0.0036$] and to the number of days [$F(2,6) = 5.9978$, $p = 0.0371$]. *Cassia auriculata* also showed a significance to the different temperatures [$F(3,6) = 17.504$, $p = 0.0022$] and to the number of days [$F(2,6) = 267.48$, $p = 0.0014$]. (Table 3)

Post hoc comparisons using the Tukey HSD test were done to point out the significance in species for the heat treatments and the number of days at [$Q_{crit} = 4.34$, d$f = 6$, $n = 3$]. There was a significant effect on the heat treatments and on the days of exposure on root length in both the species.

There was also a significant effect of amount of heat treatment and the number of days on root fresh weight in both the species remembered at the $p < 0.05$ level. *Cassia tora* showed a significance to the different temperatures [$F(3,6) = 34.067$, $p = 0.0036$] and to the number of days [$F(2,6) = 12.565$, $p = 0.0072$]. *Cassia auriculata* also showed a significance to the different temperatures [$F(3,6) = 8.8606$, $p = 0.0012$] and to the number of days [$F(2,6) = 5.7438$, $p = 0.0403$]. (Table 3)

Post hoc comparisons using the Tukey HSD test were done to point out the significance in species for the heat treatments and the number of days at [$Q_{crit} = 4.34$, d$f = 6$, $n = 3$]. There was a significant effect of the heat treatments and the days of exposure on root fresh weight in both the species. Both the species responded similarly to the days of exposure, and the effect was found between the control and 14 days of exposure.

There was a significant effect of amount of heat treatment on root dry weight in both the species remembered at the $p < 0.05$ level. However, there was no such significance found on the days of exposure. *Cassia tora* showed significance to the different temperatures [$F(3,6) = 34.37$, $p = 0.0035$]. *Cassia auriculata* also showed a significance to the different temperatures [$F(3, 6) = 13.375$, $p = 0.0045$]. (Table 3)

Effect of temperature on Leaf area index, leaf fresh weight, leaf dry weight and leaf turgid weight.

A two-way ANOVA between the number of days and treatments was conducted to compare the effect of temperature on leaf area index, leaf fresh weight, leaf dry weight and leaf turgid weight in both the species of *Cassia* separately. All effects were statistically significant at the 0.05 significance level.

There was a significant effect of amount of heat treatment and the number of days on leaf area index in both the species remembered at the $p < 0.05$ level. *Cassia tora* showed a significance to the different temperatures [$F(3,6) = 40.272$, $p = 0.0022$] and to the number of days [$F(2,6) = 10.090$, $p = 0.0120$]. *Cassia auriculata* also showed a significance to the different temperatures [$F(3,6) = 46.8571$, $p = 0.0014$] and to the number of days [$F(2,6) = 21$, $p = 0.00195$]. (Table 4)

Post hoc comparisons using the Tukey HSD test were done to point out the significance in species for the heat treatments and the number of days at [$Q_{crit} = 4.34$, d$f = 6$, n=3]. There was a significant effect of the heat treatments on leaf area index in both the species. Both the species responded similarly to the heat treatments.

There was also a significant effect of amount of heat treatment on leaf fresh weight in both the species remembered at the $p < 0.05$ level. *Cassia tora* showed a significance to the different temperatures [$F(3,6) = 88.4337$, $p = 0.0023$]. *Cassia auriculata* also showed a significance to the different temperatures [$F(3, 6) = 23.090$, $p = 0.0010$]. (Table 4)

There was a significant effect of amount of heat treatment and the number of days on leaf dry weight in both the species remembered at the $p < 0.05$ level. *Cassia tora* showed a significance to the different temperatures [$F(3,6) = 40.272$, $p = 0.0022$] and to the number of days [$F(2,6) = 10.090$, $p = 0.0120$]. *Cassia auriculata* also showed a significance to the different temperatures [$F(3,6) = 46.8571$, $p = 0.0014$] and to the number of days [$F(2,6) = 21$, $p = 0.00195$]. (Table 4)

A two-way ANOVA between the number of days and treatments was conducted to compare the effect of temperature on leaf turgid weight in both the species of *Cassia* separately. All effects were statistically significant at the 0.05 significance level. There was a significant effect of the amount of heat treatment and the number of days on leaf turgid weight in both the species remembered at the $p < 0.05$ level. *Cassia tora* showed a significance to the different temperatures [$F(3,6) = 153.914$, $p = 0.0045$] and to the number of days [$F(2,6) = 60.771$, $p = 0.0001$]. *Cassia auriculata* also showed a significance to the different temperatures [$F(3,6) = 18.808$, $p = 0.0018$] and to the number of days [$F(2,6) = 5.899$, $p = 0.03831$]. *Post hoc* comparisons using the Tukey HSD test were done to point out the significance.

DISCUSSION

A total of 11 different morphological parameters, viz. percentage germination, shoot and root length, shoot and root fresh as well as dry weight, leaf fresh dry weight, leaf area index, and leaf turgid weight, were evaluated with a different number of treatments and days interval (0, 7, and 14 days) in two species of *Cassia*. The effect of both, treatments and number of days, was studied and discussed as follows:

Effect of temperature stress on percent germination in C.tora and C. auriculata

Results clearly indicated that the interaction between temperature and duration of exposure was significant in both the species of *Cassia* seedlings. A high level of heat treatment showed a decrease in percent germination at the values ranging from control to the minimum at 42°C. The maximum percent germination resulted in control and 30°C with a 7-day exposure. The optimum temperature for *C. tora* is 25°C[1] which is in consent with our results.

Exposure of seedlings at 42°C for 14 days resulted in poor germination and was lower than the control which could be attributed to a detrimental effect on the seeds due to long duration of exposure to high temperature. The exposure to dry heat treatment as revealed for seedlings of *C.tora* and *C. auriculata* is the survival strategy of differentiation in heat requirements for germination. The range of temperature in the soil allows the seed of each legume, whose germination is promoted by thermal treatments to find their specific heat requirements[21]. High temperature and prolonged exposure result in poor seed germination which has also been reported in *Swartzia madagas cariensis* (snake bean), a reported medicinal plant [22], *Onopordum acanthium* [23], and *Vitex agnus castus* L.[24]

Effect of temperature stress on shoot length, shoot fresh and dry weight in C. tora and C. auriculata

There was a significant decrease in shoot length with an increase in temperature in both the species of *Cassia*. A gradual decrease in shoot length was observed in *C. tora* with respect to control till 37°C and further at 42°C resulted in a significant decrease in shoot length. The duration of exposure of high temperature did not have any effect on *C. tora*.

In contrast to this, *C. auriculata* could withstand a high-temperature stress comparable to control until 30°C followed by a significant decrease in shoot length. The duration of exposure in this species has shown the effect. This can be due to the fact that the high temperature causes considerable damage leading to shoot and root inhibition[25,26,27]. The damage or modification may be direct as on existing physiological processes or indirect in altering the pattern of development of a plant. Major impact of high temperatures is observed on the shoot length resulting in premature death of plants [28]. Our results are in consent with the study done in sugar cane plants grown under high temperature exhibiting reduction in shoot length[29,30] in *Brassica juncea* L. where exposure of plants to elevated temperature at 40°C inhibited plant growth with respect to shoot length compared to that of control. Similar results were also observed in French bean[31].

On the other hand, high levels of heat treatments showed an effect on the shoot fresh weight in both the species of *Cassia*. As the temperature was increased, the shoot fresh weight increased from control to 30°C and then gradually decreased at 42°C. The days of exposure also showed the same response between 7 day and 14 days.

Our results are in agreement with those of [31]as seen in French bean and in *B. juncea*[30]. This can be justified by the fact that the brief exposure of plants to heat stress can accelerate senescence and seed weight and reduce yield[32]. This phenomenon is a result of the plant tending to divert resources to cooperate with the heat stress at the expense of decrease in shoot length, shoot fresh weight, and ultimately plant growth and development. This effect is well illustrated in both the species of *Cassia*.

Shoot dry weight gradually increased at 37°C then decreased beyond control in *C. tora* whereas in *C. auriculata* the shoot dry weight increased till 30°C and then decreased beyond this temperature. These results were in consent with French bean and Indian mustard[30].

On the contrary, there was no effect on days of exposure in shoot dry weight. This is not in agreement with[33] as shown in Rough Stalk Blue grass where shoot dry weight decreased with an increase in temperature at 33°C after 11-day exposure.

Effect of temperature stress on root length, root fresh weight and root dry weight in C. tora and C. auriculata

Temperature and water stress affected the ability of seedlings to develop a root system rapidly after germination, which is critical for successful plant establishment. In the present study in *C. tora* and *C. auriculata*, the root length slowly decreased as the temperature was increased and met with the control at 42°C specifically in *C. auriculata.*

These results were not in agreement with the early root grown in barley cultivars where the optimum temperature for root length was 21°C and 27°C regardless of water stress levels[34] as compared to two *Cassia* species, *C. tora* and *C. auriculata*, where the root length can withstand the temperature till 37°C. It is also reported that the root elongation varies among different genotypes of legume species[35,36].

Both the species of *Cassia* had an effect on the root fresh weight at high levels of heat temperatures. In *C. tora* root fresh weight gradually increased at 37°C and then decreased when temperature was 42°C, whereas in *C. auriculata* the root fresh weight gradually decreased at 42°C. In both the species of *Cassia*, there was a gradual decrease in root fresh weight with an increase in temperature at 42°C. These results were in consent with the work done elsewhere[31] in French bean where the seedlings of *Phaseolus vulgaris* showed reduction in root growth and fresh mass with an increase in temperature. Similar results were also observed in *Brassica juncea*[30] L., and in sugar cane leaves[37].

Our results suggested that an increase in temperature proved to have an inhibitory effect on the root dry weight in *C. tora* and *C. auriculata* which is in conformity with in sugar cane leaves [31] in *Phaseolus vulgaris*, and in *Brassica juncea* L[30].

Effect of temperature stress on leaf area index, leaf fresh and dry weight and leaf turgid weight in C. tora and C. auriculata

In the present study, it was observed that increased temperature stress reduced leaf area index, leaf water potential, and plant growth and ultimately led to an overall decrease in plant yield. These effects are congruent with in *Phaseolus vulgaris*[31]. This phenomenon is a result of the plant tending to divert resources to cooperate with heat stress at the exposure of photosynthesis leaf area index and ultimately plant growth and development. The process of leaf expansion is partly controlled by genetic constitution (adaptation) and partly by the environmental conditions such as temperature, radiation, nitrogen supply, etc. [38,39].

Our results were not in consent with that of in *Fagus sylvatica[*40] where leaf area index increased with an increase in temperature. This behavior reflects the conflicting demands of competitive and stress-tolerating strategies on plant physiology and morphology of the given species.

On the other hand, in both the species of *Cassia* with gradual increase of temperature, the leaf fresh weight was decreased which reveals that the high temperature has an inhibitory role for leaf fresh weight in *C. tora* and *C. auriculata*. Similar results were found in French bean[31]. The results of leaf dry weight also showed a similar effect like leaf fresh weight where a significant decrease in dry weight was observed at 42°C in both the *Cassia* species. These results were in consent with the study done by in *Phaseolus vulgaris*[31].

For both the species, leaf turgid weight gradually decreased with an increase in temperature maximum at 42°C which was also observed by in *Phaseolus vulgaris*[31].

Form the above study it was concluded that leaf fresh weight, dry weight, and turgid weight were very much correlated with one another in both the species of *Cassia*. The higher-temperature maximum at 42°C acts as an inhibitory effect for *C. tora* and *C. auriculata*.

CONCLUSION

High level of temperature treatments showed a significant effect on all the parameters. Results suggest that with an increase in temperature till 37ºC the physiological activities of both the species are increased with respect to control and gradually decreased at 42ºC. For percent germination there was a gradual decrease in germination from control to 42ºC in both the species. On the other hand, prolonged exposure of high temperature had a negative effect on few physiological parameters such as shoot length and root fresh weight. Others parameters such as shoot dry weight, root length, leaf dry and fresh weight, and LAI had a positive effect on temperature treatment up to 37ºC. Then the effect was gradually decreased with an increase in temperature in both the species. Thus, it can be concluded that the optimum temperature at different levels of physiological activities for C. tora and C. auriculata is 37ºC.

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