

## Research Article

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# Impacts of High-Temperature Stress on Physiological Parameters of Mulberry Varieties/Genotypes



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## ABSTRACT

Extreme variations during hot summers cause damage to the intermolecular interactions needed for proper growth, thus impairing plant development. Temperature variations occur naturally during the growth of plants. In this study, irrespective of the mulberry varieties, the photosynthetic rate was considerably decreased under high-temperature stress. Closure of stomata is a common heat-induced feature in many crops. When the mulberry plants were exposed to high-temperature stress of 40°C stomatal conductance was considerably decreased. Greater reduction was observed in G2 and G4 at both 7<sup>th</sup> and 14<sup>th</sup> day after stress. The percent reduction in G4 at the 14<sup>th</sup> day after stress was 52.2% and G2 at 14<sup>th</sup> day after stress was 52.63% with the values 0.19 cm s<sup>-1</sup> and 0.18 cm s<sup>-1</sup>. Similarly, the Transpiration rate has significant impact on the physiological and biochemical processes of the plant system because it alters the leaf temperature which in turn affects many processes. The varieties G2 and G4 showed lower transpiration rates under stress conditions, whereas, the variety V1 recorded higher transpiration rates when exposed to 40°C. The chlorophyll stability index on the 14<sup>th</sup> day after stress showed that the variety V1 recorded the highest of 79.85% followed by MR2 at 75.64% and S36 at 69.75%.

**Keywords:** High-temperature stress, Transpiration rate, stomatal conductance, chlorophyll stability index

## INTRODUCTION

Mulberry (genus, *Morus*), is an important crop used for yielding foliage and is the primary food for silkworm, *Bombyx mori* L. The mulberry foliage yield and its quality depend on soil type, the variety available, plant nutrients in the soil, agronomical factors and agro-climatic conditions. Quality mulberry leaf is essential for the success of sericulture and it is an important component in sericulture as the cocoon and leaf yield per unit area has a direct impact on cocoon yield. Mulberry thrives under a varied climate ranging from temperate to tropical. The ideal range of temperature for normal growth of mulberry is 24-28°C.

Tamil Nadu is one of the major states of India with great potential development for mulberry crops. The state has ten agro-climatic regions suitable for growing a variety of mulberry all around the year in Tamil Nadu. At present, the total area under mulberry cultivation is 43,935.75 acres and the major districts growing mulberry are Krishnagiri, Dharmapuri, Salem, Erode, Coimbatore, Tiruppur, Vellore, Namakkal, Dindigul, Tirunelveli and Theni. An average increase of at least 0.2°C per decade is projected from now onwards. The increase in the levels of the greenhouse gasses is becoming a major cause of global warming.

Mulberry is cultivated under semi-irrigated conditions, and

hence gets exposed to high temperature stress during summer in Tamil Nadu. Heat stress due to high ambient temperature is a serious threat to crop production. High temperature influences the growth and metabolic activity of mulberry. High temperature stress induces several physiological, biochemical and molecular responses in mulberry plant. High temperature causes injury to cell membrane, lipid metabolism and bleaching of pigments. In the chlorophyll molecules of a leaf, light energy can stimulate photosynthesis, huge levels of energy is dissipated as heat, or reemitted as light, that is, chlorophyll fluorescence, and these three processes occur in competition. By measuring the yield of chlorophyll fluorescence, changes in the efficiency of photochemistry and heat dissipation can be obtained (Krishnan *et al.*, 2011).

Environmental stresses have a direct impact on the photosynthetic apparatus, essentially by disrupting all major components of photosynthesis including the thylakoid electron transport, the carbon reduction cycle, and the stomatal control of the CO<sub>2</sub> supply (Allen and Ort, 2001). The intensity of the greenness in terms of chlorophyll index can be measured using SPAD meter. SPAD values can be used for evaluating the response of the plant species to high temperature stress in the field (Hawkins *et al.*, 2009).

The photosynthetic rate of the leaf under given environmental condition is a function of various physiological and biochemical process involved during diffusion of CO<sub>2</sub> from atmosphere into chloroplast and subsequent enzyme reaction. Alterations in various photosynthetic characters are good indicator of crop thermo tolerance as they shows correlation with growth and yield.

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Closure of stomata is common heat induced features in many crops (Shrivastava et al., 2012). Stomatal conductances are inhibited by heat stress in plants due to decrease in the activation state of Rubisco (Prasad et al., 2004). Transpiration rate has significant impact on the physiological and biochemical processes of plant system because of the fact that it alters the leaf temperature which in turn affects many processes.

Not many studies have been explored in these aspects to understand the physiological basis underlying high temperature stress tolerance in mulberry. The mulberry varieties were imposed to high temperature stress under temperature controlled open top chamber to assess tolerance/susceptibility.

## MATERIALS AND METHODS

A pot culture experiment was carried under temperature controlled Open Top Chambers (OTCs) and various physiological and biochemical traits associated with control and high temperature stress were measured before imposing stress (on 120<sup>th</sup> day after planting), 7 days after exposing to a temperature of 40<sup>o</sup> C and 14 days after exposing to a temperature of 40<sup>o</sup> C at OTCs. Based on the survey of literature, it was found that mulberry could tolerate a maximum temperature of 35-37<sup>o</sup> C without any penalty on the leaf yield. Again, the last two years weather data on Tamil Nadu has revealed that day time temperature of upto 40<sup>o</sup> C was recorded. Hence, the day time (9.00 a.m – 1.00 p.m) was fixed as high temperature stress treatment for the current study. Five commercially cultivated mulberry varieties of Tamil Nadu were used in this experiment (Table 1).

**Table 1: List of five mulberry varieties used in the present study**

Sl.No	Mulberry varieties	Parentage/Origin
1.	V1	S 30 x C 776
2.	G2	<i>Morus multicaulis</i> x S 34
3.	G4	<i>Morus multicaulis</i> x S 13
4.	MR2	Open pollination of unknown origin
5.	S36	Chemical mutagenesis – Ethyl methane sulphonate treatment of Berhampore local variety.

The mulberry cuttings were grown in pots of size 37 x 35 cm with one plant per pot. Three sets of 20 pots one control and one for high-temperature stress for 7 days and the third set for high temperatures stress upto the 14<sup>th</sup> day were maintained for each variety. The plants were grown normally under ambient condition until 120 days. Then, the pots were shifted to control and high temperature chambers for treatments. In high temperature chamber the temperature was maintained at 40<sup>o</sup> C from 9:00 hrs upto 13:00 hrs. Crop management and protection measures were taken as per recommendation. Observations taken during the experiment viz., before stress, 7<sup>th</sup> day after stress and 14<sup>th</sup> day after stress.

Chlorophyll index was recorded using a portable chlorophyll meter (Minolta SPAD 502). The Minolta SPAD-502 measures chlorophyll content as ratio of transmittance of light at wavelength of 650 nm and 940 nm. Five readings were taken from each replication and the average values computed using method described by (Minolta, 1989) and (Monje and Bughree, 1992).

Chlorophyll fluorescence measurements were recorded by using Junior Pulse Amplitude Modulation Fluoro meter (PAM wincontrol-3.16, Germany) following the method advocated by (Lu et al., 2001). Measurements were made between 9.00 hours to 12.00 hours on intact leaves, which were dark adapted for 30 minutes prior to measurement. The minimal fluorescence level (Fo) with all PS II reaction centers open was assessed by measuring the modulated light, which was sufficiently low (< 0.1 μmol m<sup>-2</sup> s<sup>-1</sup>) not to induce any significant variable fluorescence. The maximal fluorescence level (Fm) with all PS II reaction centers closed. These were determined by a 0.8 s saturating pulse at 8000 μmol m<sup>-2</sup> s<sup>-1</sup> in dark adapted leaves (Lu et al., 2001). Using light and dark fluorescence parameters, the maximal efficiency of PS II photochemistry in the dark-adapted state, Fv/Fm = (Fm-Fo) / Fm (Van Kooten and Snell, 1990) was calculated.

Leaf gas exchange measurements were performed using Portable Photosynthesis System (PPS) (Model LI-6400 of LICOR Inc., Lincoln, Nebraska, USA) equipped with a halogen lamp

(6400-02B LED) positioned on the cuvette. Totally, three measurements were taken in the same leaf. Leaves were inserted in a 3 cm<sup>2</sup> leaf chamber. All the measurements were taken at a constant flow rate of 500 ml min<sup>-1</sup> and CO<sub>2</sub> concentration of C.380 μmol mol<sup>-1</sup> under a PAR of 1000 μmol m<sup>-2</sup> s<sup>-1</sup>. The readings were taken between 10.00 hours to 12.30 hour, using PPS system, the following gas exchange parameters were recorded and the values expressed as in parentheses.

Photosynthetic rate (Pn: μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>)

Transpiration rate (E: mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)

Stomatal Conductance (gs: cm s<sup>-1</sup>)

Based on Koloyereas (1958) protocol chlorophyll stability index was estimated. The third leaf was selected for estimating CSI. The leaf samples were taken early in the morning. Sample size of 250 mg was taken and homogenized using 80 per cent acetone. The sample was then centrifuged at 3000 rpm for 10 min. The supernatant was collected and made up to 25ml. The OD value was measured at 652 nm.

$$\text{CSI (\%)} = \frac{\text{Total chlorophyll content (treated)}}{\text{Total chlorophyll content (control)}} \times 100$$

## RESULTS AND DISCUSSION

Mulberry varieties viz., V1, G2, G4, MR2, and S36 were examined for high-temperature stress tolerance by exposing them to 40<sup>o</sup> C in Open Top Chambers (OTCs). Observations were recorded on various physiological traits at three stages viz., before imposing stress (120<sup>th</sup> DAP), 7<sup>th</sup> DAS (127<sup>th</sup> DAP), 14<sup>th</sup> DAS (134<sup>th</sup> DAP). Data on various parameters and yield traits at the end of the experiment are described here.

Chlorophyll index of the varieties before imposing the stress ranged from 34.27 to 40.25 (Table 2). There was no significant difference among the varieties for the trait on 120<sup>th</sup> day after planting. The highest reduction in the chlorophyll index was found at 14<sup>th</sup> day after stress compared to 7<sup>th</sup> day after stress. At 7<sup>th</sup> day after stress, the variety G4 showed the highest reduction of 13.22 % (from 35.1 to 31.0) (Fig 1.). At 14<sup>th</sup> day after stress, the variety MR2 recorded lesser reduction of 5.37 % (from 43.1 to 40.9) followed by V1 and S36. The higher reduction percentage

was noted in G2 and G4 respectively. In the present study, MR2 recorded higher SPAD values in control, 7<sup>th</sup> day and 14<sup>th</sup> day after high temperature stress. Among the varieties, G4 recorded lowest chlorophyll index when exposed to heat stress. The reduction in the SPAD values under heat stress may be fact that stress blemishes chlorophyll content by causing internal modification in the thylakoid membrane. Similar findings were reported by Djanaguiraman *et al.* (2011) in soya bean leaves exposed to high-temperature stress. The decrease in the SPAD values was noted in *Brassica juncea* in response to high-temperature stress (Hayat *et al.*, 2009).

Chlorophyll fluorescence values of the varieties V1, G2, G4, MR2 and S36 were recorded before imposing high-temperature stress and after the 7<sup>th</sup> DAS and 14<sup>th</sup> DAS. The values of chlorophyll fluorescence were on par with each other in the varieties taken for the study (Table 2). Compared to the control, stress brought about the reduction in chlorophyll fluorescence. On 7<sup>th</sup> day after stress, there was less reduction in chlorophyll fluorescence in all varieties. At the 14<sup>th</sup> day after stress, larger reduction was observed. The variety V1 recorded lesser reduction (from 0.96 to 0.76) followed by MR2 (from 0.93 to 0.72) and S36 (from 0.89 to 0.60) (Fig.2). The photosynthetic apparatus, photosystem II plays a key role in response to leaf photosynthesis to environmental stress, especially to high temperature stress. PS II is highly labile its activity is greatly decreased under heat stress. A considerable decrease in chlorophyll fluorescence was observed under high-temperature stress. In the present study, V1 showed higher chlorophyll fluorescence values compared to other varieties under high-temperature stress. The maximum quantum efficiency of PS II (Fv/Fm) was more stable in control plant than stress plant. The highest (Fv/Fm) values indicate that the variety has highest efficiency in protecting their photosynthetic apparatus under heat. Similarly, under drought conditions a significant drawdown in chlorophyll fluorescence was recorded in the susceptible mulberry genotypes, particularly in Bogurai followed by DD (Guha *et al.*, 2010). In the present study, it was found that maintenance of chlorophyll, chlorophyll fluorescence and chlorophyll stability index are all integrated parameter that can maintain normal physiology of mulberry varieties under high temperature stress. In line with the above hypothesis, a positive correlation was found between chlorophyll fluorescence and chlorophyll stability index under both control and stress treatments (Fig.3).

At 120<sup>th</sup> day after planting, the Chlorophyll Stability Index of the varieties taken for the study was observed. There was no huge difference in chlorophyll stability index among the varieties (Table 2). The values were on par with each other in the varieties taken for the study. The 14<sup>th</sup> day stress was found to be more deleterious compared to 7<sup>th</sup> day stress. The chlorophyll stability index was less in V1 at 7<sup>th</sup> day after stress (80 %) over control which was 86 %. At 14<sup>th</sup> day after stress, V1 recorded highest chlorophyll stability index of 79.85% followed by MR2 with 75.64% and S36 with 69.75%. While G2 and G4 recorded lowest chlorophyll stability index of 59.63 % and 52.89 % respectively (Table 3). Chlorophyll stability index is an indication of the stress tolerance capacity of plants. A high CSI value means that the stress did not have much effect on chlorophyll contents of plants. A higher CSI helps the plants to withstand stress through better availability of chlorophyll (Mohan *et al.*, 2000). The primary effect of heat stress at the cellular level is to affect the integrity of membrane which in turn leads to disruption of cellular compartment ultimately destructing chlorophyll contents.

The current research revealed that, certain varieties like V1 recorded optimal CSI even after 14 days of high temperature stress. Similar to the above findings, Sudhakar *et al.* (2000) reported that lesser extent of cell membrane injury was observed in mulberry variety S-1 when exposed to 150 mM NaCl stress.

The photosynthetic rate of the varieties before imposing stress ranged from 30 to 34, (Table 2) there was no significant variation among the varieties for the parameter on 120<sup>th</sup> day after planting. The photosynthetic rate brought about greater variation under stress conditions than control. The highest variation was observed at 14<sup>th</sup> day after stress compared to the 7<sup>th</sup> day after stress (Table 4). The photosynthetic rate in G2 and G4 varieties reduced from 28.60 to 19.67 and 28.10 to 17.31 respectively at 7<sup>th</sup> day after stress. The variety V1 showed lesser reduction from 34.11 to 26.46 even at 14<sup>th</sup> day after stress.

The Photosynthetic rate of the leaf under given environmental condition is a function of various physiological and biochemical process involved during diffusion of CO<sub>2</sub> from atmosphere into chloroplast and subsequent enzyme reaction. Alterations in various photosynthetic characters are good indicator of crop thermo tolerance as they shows correlation with growth and yield. Irrespective of the varieties, the photosynthetic rate was considerably decreased under high temperature stress. It corroborates with earlier reports of Gesch *et al.* (2003) who stated that the photosynthetic rate declined to extent of 15-25% under day and night temperature of 40/30°C. Similarly, Yu *et al.* (2013) has observed a reduction of upto 60 % in mulberry when the mulberry plants when the day/night temperature was maintained at 34.5°C – 40.5°C. Interestingly, the tolerant variety V1 recorded comparable photosynthetic rate even under stress condition. G4 recorded lesser photosynthetic rate which indicates that the variety is highly susceptible to high temperature stress in the present study. Similarly, Guha *et al.* (2010) observed larger reduction in photosynthetic rate in mulberry genotypes viz., DD, Bogurai and PNG under water deficit conditions. The main biochemical constituent in mulberry is the protein which was found to be enhanced by higher photosynthetic rate.

At 120<sup>th</sup> day after planting, the Stomatal conductance of the varieties was recorded. Not much difference in the conductance of the varieties was observed. The conductance ranged from 0.38 cm s<sup>-1</sup> to 0.49 cm s<sup>-1</sup> (Table 2). But, there was a significant difference between the varieties when exposed to stress treatments. Among the varieties tested, lesser reduction in stomatal conductance was noted in variety V1 (25.64%) on 14<sup>th</sup> day after stress (Table 5). The greater reduction was observed in G2 and G4 at both 7<sup>th</sup> and 14<sup>th</sup> day after stress. The percent reduction in G4 at 14<sup>th</sup> day after stress was 52.2% and G2 at 14<sup>th</sup> day after stress was 52.63% with the values 0.19 cm s<sup>-1</sup> and 0.18 cm s<sup>-1</sup>. Closure of stomata is common heat induced features in many crops (Shrivastava *et al.*, 2012). In the present study, when mulberry plants were exposed to high-temperature stress of 40°C stomatal conductance was considerably decreased. The variety G4 recorded the lowest stomatal conductivity. Even under high temperature stress, variety V1 showed comparable conductance. Similar results were obtained by Greer *et al.* (2012) in *Vitis vinifera* leaves which showed a 15 - 30% reduction in stomatal conductance under a high temperature of 45°C. Yu *et al.* (2013) reported no significant reduction in stomatal conductance in mulberry genotypes when exposed to combined stress of salinity and high temperature. Whereas, Guha *et al.* (2010) reported a decline in stomatal conductance in mulberry genotypes Jhorpakarai and S-1 under

drought conditions in the field where mulberry genotypes were irrigated once in fortnight only. Genotypes V1 and S-13 showed higher stomatal conductance and proved to be a drought tolerant genotype.

Transpiration rate has significant impact on the physiological and biochemical processes of plant system because it alters the leaf temperature which in turn affect many process. Before imposing high temperature stress, the Transpiration rate was recorded for all the varieties taken for the study. The values ranged from 7.58 to 8.16 (Table 2). The varieties exhibited a significant difference in transpiration rate under the stress condition. There was a reduction in the transpiration rate with an increase in days of stress. The reduction was high at 14<sup>th</sup> day after stress compared to 7<sup>th</sup> day after stress. At 7<sup>th</sup> day after stress, the variety V1 has reduction of 17.86% (from 8.30 to 7.11). At 14<sup>th</sup> day after stress, the varieties G2 and G4 have showed a percent reduction of 61.9 % (from 7.79 to 4.81) and 67.6% (from 7.56 to 4.51) respectively (Table 6). The variety V1 has showed lesser reduction of 41.32% (from 8.31 to 5.88) at 14<sup>th</sup> DAS followed by MR2 48% (from 8.01 to 5.41) and S36 of

54.3% reduction (from 7.81 to 5.06). In the present study, high-temperature stress decreased the transpiration rate. The varieties G2 and G4 showed lower transpiration rate under stress conditions, whereas, the variety V1 recorded higher transpiration rate when exposed to 40°C. This finding is contradictory to the findings of Weerakoon *et al.* (2008) who reported that lower transpiration under high temperature is one of the avoidance mechanisms in plants. The higher transpiration rates in the variety V1 might have brought about the transpiration cooling of the canopy which might have led to stress tolerance. In line with the above hypothesis Crawford *et al.* (2012) reported that transpiration rates lead to canopy cooling in *Arabidopsis* plants. Guha *et al.* (2012) observed a drastic reduction in the transpiration rate in mulberry genotypes with an average decline of 70%. Varieties V1 and S-13 maintained higher transpiration rates under low water regimes. Yu *et al.* (2013) noticed the decrease in transpiration rate in four mulberry varieties exposed to combined salinity and high-temperature stress.

**Table 2. Genetic variation in morphological, physiological traits, and membrane integrity parameters of mulberry varieties at 120<sup>th</sup> day after planting (Before imposing stress)**

Sl. No	Traits/ Parameters	Varieties					S.Ed	CD *p<0.05
		V1	G2	G4	MR2	S 36		
1.	Chlorophyll index	39.23±1.89	36.02±1.57	34.27±1.23	40.25±2.11	38.40±1.75	<b>0.473</b>	<b>1.00</b>
2.	Chlorophyll fluorescence(Fv/Fm)	0.88±0.04	0.79±0.00	0.76±0.01	0.85±0.02	0.84±0.03	<b>0.005</b>	<b>0.012</b>
3.	Chlorophyll stability Index (%)	85±2.05	69±1.56	68±1.23	80±1.89	75±1.67	<b>0.590</b>	<b>1.25</b>
4.	Photosynthetic rate(μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	34±0.28	30±0.15	30±0.12	33±0.25	32±0.23	<b>0.432</b>	<b>0.921</b>
5.	Stomatal conductance(cm s <sup>-1</sup> )	0.49±0.11	0.39±0.05	0.38±0.03	0.46±0.08	0.41±0.07	<b>0.011</b>	<b>0.025</b>
6.	Transpiration rate(mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	8.16±0.05	7.61±0.03	7.58±0.02	7.98±0.04	7.90±0.03	<b>0.038</b>	<b>0.081</b>

The values are expressed in Mean ± Standard deviation

**Table 3. Changes in Chlorophyll Stability Index (%) in mulberry varieties exposed to high-temperature stress for two weeks intervals.**

Mulberry Varieties	7 <sup>th</sup> DAS		14 <sup>th</sup> DAS	
	Control	Stress	Control	Stress
V1	86.0±1.19	80.0±1.23	87.25±1.99	79.85±1.58
G2	71.0±1.25	60.0±1.42	72.64±1.58	59.63±1.39
G4	70.0±1.56	58.0±1.37	70.98±1.61	52.89±2.01
MR2	82.0±1.87	76.0±1.89	83.02±2.02	75.64±1.89
S36	78.0±0.99	70.0±1.47	79.24±1.86	69.75±1.74
P=CD(0.05) Variety	0.28**		0.16**	
Treatment	0.18**		0.10**	
V X T	0.40**		0.23**	

The values are expressed in Mean ± Standard deviation; \* - Significant, \*\* - Highly significant; V- Variety, T- Treatment

**Table 4. Changes in Photosynthetic rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) in mulberry varieties exposed to high-temperature stress for two weeks interval**

Mulberry Varieties	7 <sup>th</sup> DAS		14 <sup>th</sup> DAS	
	Control	Stress	Control	Stress
V1	35.13±0.15	31.07±0.13	34.11±0.06	26.46±0.20
G2	30.73±0.09	24.31±0.08	28.60±0.07	19.67±0.15
G4	30.61±0.13	24.02±0.15	28.10±0.12	17.31±0.11
MR2	34.16±0.31	29.34±0.21	33.08±0.13	24.43±0.08
S36	31.97±0.15	26.01±0.07	31.01±0.09	21.07±0.17
CD at p ≤ 0.05	V	0.173**		0.425**
	T	0.109**		0.268**
	V X T	0.245**		0.601**

The values are expressed in Mean ± Standard deviation; \* - Significant, \*\* - Highly significant; V - Variety, T - Treatment

**Table 5. Changes in Stomatal conductance ( $\text{cm s}^{-1}$ ) in mulberry varieties exposed to high-temperature stress for two-week intervals.**

Mulberry Varieties	7 <sup>th</sup> DAS		14 <sup>th</sup> DAS	
	Control	Stress	Control	Stress
V1	0.54±0.03	0.38±0.04	0.49±0.02	0.39±0.00
G2	0.43±0.02	0.31±0.05	0.40±0.04	0.19±0.02
G4	0.41±0.03	0.24±0.01	0.38±0.01	0.18±0.01
MR2	0.50±0.02	0.37±0.01	0.43±0.01	0.31±0.01
S36	0.45±0.01	0.36±0.03	0.42±0.03	0.30±0.02
P=CD (0.05) Variety	0.0177**		0.0156**	
Treatment	0.0112**		0.0098**	
V X T	0.0251*		0.0220**	

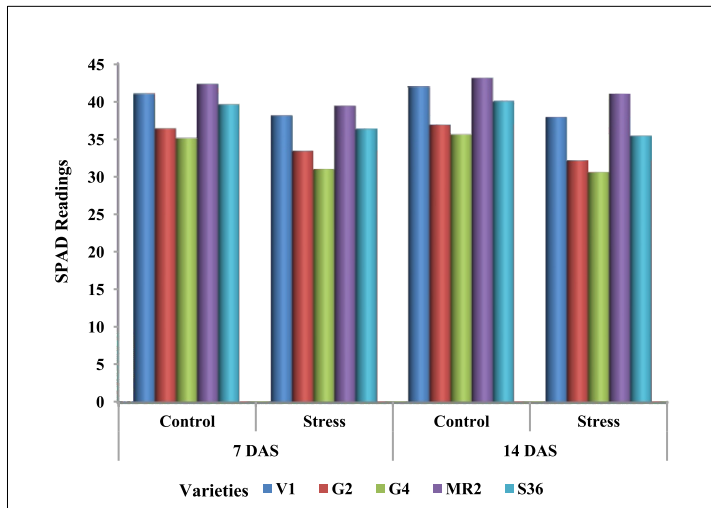
The values are expressed in Mean ± Standard deviation; \* - Significant, \*\* - Highly significant; V - Variety, T - Treatment

**Table 6. Changes in Transpiration Rate ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) in mulberry varieties exposed to high-temperature stress for two weeks intervals.**

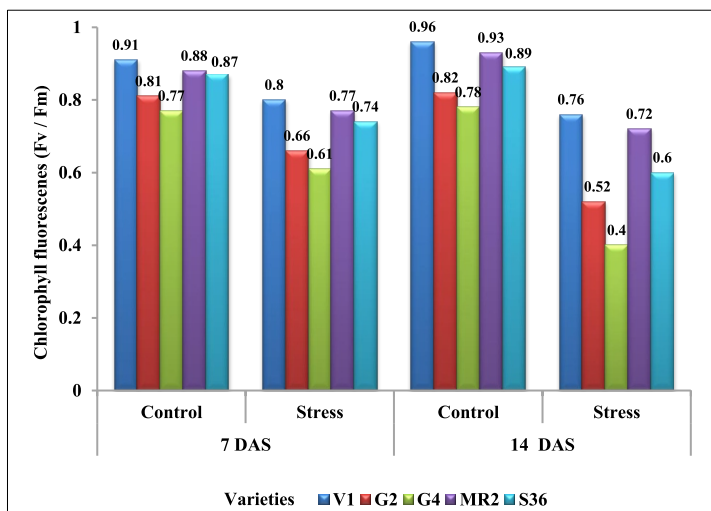
Mulberry Varieties	7 <sup>th</sup> DAS		14 <sup>th</sup> DAS	
	Control	Stress	Control	Stress
V1	8.38±0.11	7.11±0.12	8.31±0.21	5.88±0.02
G2	7.94±0.14	6.81±0.15	7.79±0.19	4.81±0.09
G4	7.81±0.09	6.63±0.11	7.56±0.07	4.51±0.06
MR2	8.13±0.07	7.02±0.08	8.01±0.14	5.41±0.18
S36	7.99±0.06	6.96±0.03	7.81±0.31	5.06±0.23
P=CD(0.05) Variety	0.0450**		0.0442**	
Treatment	0.0284**		0.0279**	
V X T	0.0636*		0.0625**	

The values are expressed in Mean ± Standard deviation; \* - Significant, \*\* - Highly significant; V - Variety, T - Treatment

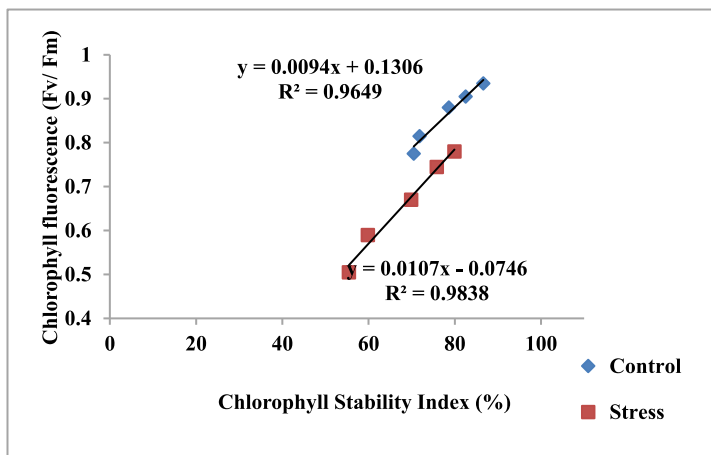
**Fig. 1. Changes in Chlorophyll index in mulberry varieties exposed to high-temperature stress for two weeks interval**



**Fig. 2. Impact of high-temperature stress on Chlorophyll fluorescence (Fv/Fm) values in mulberry varieties maintained at OTCs**



**Fig. 3. Correlation between Chlorophyll fluorescence and Chlorophyll stability index in mulberry varieties under control and high-temperature stress**



## CONCLUSION

The chlorophyll index measured using the SPAD meter showed a decreasing trend as days of stress increased. After 14 days of stress, the variety MR2 showed lesser reduction in chlorophyll index of 5.37% compared to control, whereas, the variety G4

recorded a higher reduction of 13.22% compared to control. The chlorophyll fluorescence values also decreased under high-temperature stress conditions. The variety V1 showed comparable fluorescence values on 7<sup>th</sup> and 14<sup>th</sup> day after stress by overcoming the heat induced damage which indicates its tolerant capacity to high temperatures. Variety V1 and MR2 recorded good chlorophyll stability index under high temperature stress while G2 and G4 showed the lowest CSI and high lipid peroxidation (MDA content) showing its susceptibility to high-temperature stress. The ability of the genotype to maintain a higher CSI under heat is a desirable characteristic for tolerance. Gas exchange parameters viz., photosynthetic rate, stomatal conductance, and transpiration rate were decreased under high-temperature stress in all the varieties. The variety V1 at all the stages maintained gas exchange parameters by avoiding stress conditions through transpiration cooling which mitigates heat stress. From this study, it is clearly observed that the variety V1 has a high heat stress tolerant capacity followed by varieties MR2 and S36.

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