RESEARCH ARTICLE

Estimation of Water Use Efficiency and Osmolyte Accumulation to Evaluate Drought Stress Tolerance in Mulberry

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ABSTRACT

The present study aimed to assess the water deficit stress tolerance in four mulberry genotypes and three varieties. Four months old mulberry plants were subjected to three water regimes *viz.,* 100% PC, 50% PC, and 25% PC for 30 days. Chlorophyll stability index (CSI), intrinsic water use efficiency and osmolyte accumulation (proline content) were estimated before imposing drought stress and 30 days after drought stress. Intrinsic Water Use Efficiency (WUEi) and proline accumulation was increased, while CSI, carbohydrate, and protein contents were found to be decreased as the severity of drought stress progressed. Mulberry genotype, MI-0425 was found to be drought tolerant with higher WUEi (4.13 mmol CO_2 mol^{-1} H_2O) and proline accumulation (8.54 µg g^{-1}). This line also showed lesser protein degradation under severe drought stress. The genotype MI-0613 recorded lower WUEi (3.18 mmol CO_2 mol^{-1} H_2O) and proline content (5.87 µg g^{-1}) under severe drought stress. It also recorded a severe reduction in yield (45.96%) under stress. Hence, MI-0613 was identified as a drought-susceptible genotype. Variety V1 recorded higher CSI (77.68%) and carbohydrate (29.03 mg g^{-1}) and yield (95.48 g $plant^{-1}$) under both moderate and intense water stress treatments. Hence, V1 was found to mitigate drought stress by maintaining higher CSI and carbohydrate content.

Keywords: *Mulberry; Drought; Water Use Efficiency; Osmolyte accumulation; Carbohydrate; Protein*

INTRODUCTION

Mulberry is an economically and traditionally important plant in the sericulture industry. The mulberry foliage yield and its quality depend on soil type, variety, plant nutrients in the soil, agronomical factors and agro-climatic conditions (Sharma *et al*., 2015). The growth and development of silkworm and cocoon crops are mainly influenced by the yield and nutritional quality of mulberry leaf used as feed.

In India mulberry is cultivated under the risk of either intermittent or terminal drought, as 50% of the area under mulberry cultivation falls under arid and semi-arid conditions (Guha *et al*., 2010). Among the districts of Tamil Nadu, mulberry is extensively cultivated in Dharmapuri district in which nearly 22.6% of the area is affected by drought (source: IWMI- South Asia drought monitor 2016-17). Water is an important factor for the mulberry plant because the succulence of the mulberry leaves is depending upon the water availability from the soil.

High biomass-producing mulberry genotypes have a tremendous water demand due to faster growth rate and higher metabolic activities (Susheelamma *et al*., 1990). Being a perennial plant, mulberry suffers from want of water and is susceptible to water stress damages during both the nursery and early plantation stage in the field (Rajat Mohan *et al*., 2015). Water deprivation can arrest the growth and leaf yield performance of elite mulberry genotypes (Guha *et al*., 2010). Moisture stress frequently limits both the quality and yield of a mulberry leaf.

Plants have evolved two major mechanisms for accomplishing water stress, one is drought avoidance and another is drought tolerance. Avoidance depends primarily on specialized adaptations in root and shoots architecture (Aspinall and Paleg, 1981). Water stress tolerance; depends on the result of production and/ or accumulation of compatible osmotic solutes (Ramanjulu and Sudhakar, 2000).

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Direct screening of relatively higher stomatal conductance, photosynthetic rate and Water Use Efficiency (WUEi) under moisture stress may be advantageous in selecting germplasm for drought tolerance (Sharp *et al*., 2004; White *et al*., 2000).

The chlorophyll content is one of the major factors affecting the photosynthetic capacity. Drought stress leads to pigment degradation, resulting in irreversible water deficit damage to the photosynthetic apparatus. Hence, Chlorophyll Stability Index (CSI) is an indication of the abiotic stress tolerance capacity of crop plants. Since CSI is a function of temperature, it is used to correlate the chlorophyll pigments with the drought tolerance or susceptibility of crops. A higher CSI helps the plants to withstand stress through better availability of chlorophyll (Mohan *et al*., 2000). The CSI indicates how well chlorophyll performs under stress conditions (Kumari *et al*.,2004).

Osmotic adjustment is a key mechanism by which plants adapt to water shortages by increasing solute concentration to maintain the water potential to ensure continued uptake of water during the stress period. In addition, osmotic adjustment allows the cell to maintain the turgor, which is essential for plant growth and other physiological processes (Nahar *et al*., 2011). Proline accumulation is the first response of plants exposed to water-deficit stress to reduce injury to cells. Proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Kavi Kishore *et al*., 2005).

Other than an osmoprotectant, proline is regarded as an important non-enzymatic antioxidant playing important roles in stabilizing sub-cellular structures, scavenging free radicals and buffering cellular redox potential under stress conditions (Ashraf and Foolad, 2007). Proline may also act as a storage compound and nitrogen source for rapid growth after stress (Kumar *et al*., 2000). (Pawar *et al*., 2010) suggested that the levels of both osmolytes namely proline and glycine betaine increased simultaneously under water stress conditions and can be used as a drought index.

The quality of the mulberry leaf is determined by its major constituents like water, carbohydrates, proteins, mineral elements, fats, amino acids, and vitamins. Mulberry leaves containing more total sugar, protein, and chlorophyll content are best relished by silkworms (Bongale *et al*., 1995). It has been found that the accumulation of protein in larvae depends largely on the concentration of carbohydrates in the leaves (Ohnuma *et al*., 1997). A huge portion of soluble protein (50 per cent) in leaves is occupied by Rubisco, a prime enzyme for carbon fixation in photosynthesis (Noggle and Fritz, 1986).

Scattered rainfall events during summer rarely meet potential evapotranspiration and expected climate change over the course of the 21st century is likely to increase the frequency of drought events causing plant water stress with which the introduced mulberry species must cope (Guha *et al*., 2014). Hence, the present study was designed with the aim i) To evaluate the drought tolerance in mulberry by estimating the water use efficiency, CSI, and osmolyte accumulation of selected mulberry genotypes under water deficit stress. ii) To quantify the carbohydrate, soluble protein content, and leaf yield of mulberry under drought stress. iii) To correlate the physiological and biochemical traits with leaf yield under drought stress.

MATERIAL AND METHODS

Plant materials and stress treatments:

The present work was carried out from November, 2018 to April, 2019 in the Rain Out Shelter (ROS) at the Department of Crop Physiology, TNAU, Coimbatore. The study comprised four mulberry genotypes (MI-0613, MI-0658, MI-0425, and MI-0535) obtained from CSGRC, Hosur. These genotypes were selected from forty-one mulberry genotypes screened for better yield and other physiological traits under normal conditions at FC & RI, Mettupalayam (Aruna, 2018). Along with the above four genotypes, three mulberry varieties (V1, MR2, and G4) were studied for drought tolerance. The mulberry cuttings of 12-15 cm length with 3 to 4 active buds were planted in pots of size 37× 35cm filled with red loamy soil with a pH of 7.5. The pots were maintained under normal conditions and watered daily for up to 120 days. Crop management and protection measures were taken as per recommendation. After 120 days the pots were kept inside the Rain Out Shelter for inducing drought stress, while a similar area of control was maintained adjacent to the ROS facility. The dimensions of the ROS and the control were 21 m long and 6 m wide. Pots of each genotype/variety were divided into three sets and arranged in the Factorial Completely Randomized block design (FCRD), with three replications. Mulberry genotypes/ varieties were kept as one factor and drought stress treatments were kept as another factor. Drought stress was imposed by the dry-down method (Guha *et al*., 2012). Plants were submitted to three water regimes *viz.* T_1-Control: pots maintained at 100% pot water holding capacity (PC) T_2- moderate drought stress: 50% PC, T_3- intense drought stress: 25% PC. The measured soil water content equivalent to 100% PC was 62.5% (weight basis). Likewise, the soil water contents equivalent to 50% and 25% PC were determined. Water was added to the pots to restore the required level of pot water holding capacity by weight basis. Drought stress was given to the plants for a period of 30 days. All the parameters (CSI, intrinsic water use efficiency, proline content, carbohydrate, and soluble protein content) were assessed at two stages *viz.,* before imposing and thirty days after stress. Leaf yield was recorded at the end of the stress treatment.

Chlorophyll Stability Index (CSI):

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. The 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 25 mL. The OD value was measured at 652 nm.

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

Intrinsic Water use efficiency (WUEi) (mmolCO$_2$ mol^{-1} H$_2$O):

The intrinsic water use efficiency (WUEi) was calculated as Pn/E (Guha *et al*., 2010). Where Pn is the photosynthetic rate and E is the transpiration rate. The Pn and E were measured using the Portable Photosynthesis System (PPS) (Model LI-6400 of LICOR inc., Lincoln, Nebraska, USA) between 10.00 hours to 12.30 hours. Three measurements were taken in the same leaf.

Proline content:

Proline content of the leaf was estimated by (Bates *et al*., 1973) method and expressed as µg g^{-1} of fresh weight.

Total carbohydrate content:

Carbohydrate content in mulberry leaves was measured by the anthrone reagent method (Ranganna, 1998). The carbohydrate content was calculated by the standard sugar solutions (Dextrose L) method and is measured in mg g^{-1}.

Total soluble protein content:

Total soluble protein content was estimated from the leaf samples by the method of (Lowry *et al*., 1951). Soluble protein was estimated from the leaves taken from the middle of the plants and it is expressed as mg g^{-1} fresh weight.

Leaf Yield:

Leaves were harvested from different drought-stressed and control plants and their weights were recorded. The average leaf yield per plant was estimated. The total leaf yield per plant was expressed in grams.

RESULTS AND DISCUSSION

Table 1 represents the genotypic variability of WUEi, CSI, osmolyte accumulation, and carbohydrate and protein content in mulberry genotypes before imposing water stress on 120th days after planting. WUEi of mulberry genotypes varies from 4.46 to 5.62 mmol CO_2 mol^{-1} H_2O. Where MI-0658 recorded higher WUEi followed by MI-0425 and V1. Significant genotypic variation was observed in physiological traits such as WUEi and carbohydrate content. Regarding the CSI, proline, and soluble protein there was no significant genetic variation before imposing water stress. However, V1 recorded higher CSI along with maximum proline accumulation (3.37 µg g^{-1}) even under normal conditions.

Chlorophyll stability index (CSI):

Significant variation in CSI was recorded in mulberry under three water regimes (Table 2). A decreasing trend of CSI was observed in all the mulberry genotypes/varieties exposed to drought stress. All the plants recorded minimum CSI values at intense and moderate water stress compared to their respective control plants. CSI was altered by drought stress and decreased up to 55.35% and 60.05% in MI- 0613 and MI-0658 respectively. While, the drought tolerant V1 (77.68% at 25% PC) recorded the highest CSI under intense water stress, followed by MI-0425 (75.60 % at 25% PC).

A higher CSI value signifies a plant's ability to withstand stress through greater stability of chloroplast membranes leading to higher rates of photosynthesis, more dry matter production, and higher productivity (Mohan *et al*., 2000). Lesser reduction in CSI% was observed in MI-0425 followed by V1, where the reduction percentage was around 10.86% and 11.96%. This higher CSI % in the above-said genotype may be the reason for higher WUEi and drought tolerance. Hence, this genotype was found to maintain membrane stability even under severe water stress. Thimmanaik *et al*., 2002 reported less reduction in CSI% in drought-resistant mulberry, Anantha compared to drought-sensitive M5. The above findings are also supported by the results of Ranjith kumar (2018), where variety V1 recorded a higher CSI percentage when exposed to high-temperature stress.

Intrinsic Water use efficiency (WUEi):

WUEi represents how plants performed best for transpiration under a low water regime. Water stress led to a significant ($p< 0.05$) increase in WUEi in all the mulberry cultivars (Table 2). Drought tolerant MI-0425 exhibited higher WUEi of 4.13 mmol CO_2 mol^{-1} H_2O followed by V1 (4.09 mmol CO_2 mol^{-1} H_2O) at intense water stress. These two genotypes maintained better WUEi under the remaining two water regimes *viz.,* control, and moderate water stress. While, drought-susceptible MI-0613 maintained poor WUEi under control and drought stress conditions. MI-0613 exhibited a minimum increase in WUEi (5.65%) followed by MI-0658 (11.46%). At the same time, MI-0425 ranks first for the same. About 34.09% of the increase in WUEi was observed in MI-0425 followed by V1 (33.22%). Similarly, varieties G4 (32.89%) and MR2 (28.72%) recorded relatively higher WUEi. Similar results were obtained by Guha *et al.,* 2010. He reported drought tolerant V1 (27%) maintained and exhibited a maximum increase in WUEi compared to control and other genotypes under a low water regime (25% PC).

Osmolyte accumulation (Proline content):

Differential changes in proline content of mulberry leaves were observed in all the seven mulberry genotypes/ varieties in both stressed and control plants. Exposure to different drought regimes caused significant ($p<0.05$) changes in proline content (Table 2). An increasing trend was observed in the free proline content of all the genotypes taken for study. An increase in free proline content was higher in intense water stress compared to moderate water stress. Proline content ranged from 4.28 to 4.57 µg g^{-1} fw and 5.87 to 8.54 µg g^{-1} fw both in control and intense water stress respectively. Genotype MI-0425 exhibited the highest proline content of 4.57 µg g^{-1} fw and 8.54 µg g^{-1} fw in control and plants at 25% PC correspondingly. At moderate water stress V1 (6.43 µg g^{-1} fw) recorded higher proline content followed by MI-0425 (6.34 µg g^{-1} fw). Genotype MI-0535 recorded minimum proline content at both control and moderate water stress. While susceptible genotype MI-0613 recorded minimum proline accumulation at 25% PC followed by MI-0535.

A key adaptive mechanism in a large group of crop plants grown under abiotic stresses, including salinity, water deficit, and extreme temperatures is an accumulation of certain organic compounds of low molecular mass, generally referred to as compatible osmolytes (Sakamoto and Murata, 2002). Accumulation of proline under stress shows association with stress adaptation in higher plants (Bartels and Sunkar, 2005). Table 2 shows an increment in the free proline content in all the mulberry genotypes at both levels of drought stress. MI-0425 exhibited the highest level of proline compared to others. However, increment in the proline accumulation was significantly higher in V1 (89.28%, an almost two-fold increase) followed by MI-0425 (86.87%,) compared to its respective control counter-parts. However, at the highest stress level, a lesser increment was found in susceptible genotype MI-0613 (34.32%) followed by MI-0535 (53.74%). The increased accumulation of proline observed in stressed MI-0425 and V1 leaves could afford a better osmotic equilibrium and cell membrane stability during drought stress conditions.

The above results were supported by Guha *et al*., 2012. He reported the highest accumulation of free proline content in drought tolerant V1 exposed to severe water stress (25% PC) under glasshouse conditions. In agreement with the above findings, Ramanjulu (2000) reported elevated proline content in drought tolerant mulberry cultivar S-13 exposed to severe water stress (25% PC). Proline content was increased in three months old mulberry plants exposed to mild (25% PC) to severe (12.5% PC) water stress (RanjithaKumari and Veeranjaneyulu, 1996).

Carbohydrate content:

Invariably in all drought stress treatments (50% PC and 25% PC) carbohydrate content was significantly reduced in all the seven mulberry genotypes. Variety V1 recorded the highest value of carbohydrate content (35.78 mg/g) followed by G4 (34.54 mg/g) in control plants. Figure 1a. shows the variation in carbohydrate content in moderate and intense water stress compared to their respective control plants. Variety V1 recorded maximum carbohydrate content in both moderate and intense water stress followed by MI-0425. At intense water stress the values of carbohydrate content was MI-0613- 20.02mg/g; MI-0658- 20.98 mg/g; MI-0425- 28.97 mg/g; MI-0535- 20.47 mg/g; V1- 29.03 mg/g; MR2- 22.71 mg/g; G4- 27.03 mg/g. Genotype MI-0613 recorded minimum carbohydrate content at 25% PC.

The quality of mulberry leaves mainly depends on the amount of carbohydrate content present in it. A decreasing trend was observed in carbohydrate content in all the genotypes as drought stress progressed (Figure 1a). A sudden decline was observed in all the genotypes at intense water stress compared to control plants. Though the decline was observed in intense water stress the drought tolerant genotypes MI-0425 and V1 recorded a relatively higher amount of carbohydrate with a lesser reduction percentage of 15.98% and 18.87% respectively. At the same time, the susceptible one (MI-0613) recorded the highest reduction percentage of 38.87%. Similar results were obtained by Ranjith Kumar (2018) in 120 days old V1 which recorded the highest carbohydrate content under high-temperature stress conditions.

Total soluble protein:

Similar to carbohydrate content drought stress caused a severe reduction in soluble protein content. A decreasing trend was observed in total soluble protein as drought stress progressed. A severe decline in soluble protein content was observed in intense water stress (25% PC) (Figure 1b). Soluble protein content varies from 25.96 to 33.38 mg g^{-1} and 20.07 to 28.87 mg g^{-1} at moderate and severe water stress respectively. Among all the genotypes, susceptible genotype MI-0613 recorded lower soluble protein content both in moderate and severe water stress. Reduction in protein content affects the quality of mulberry leaves produced which in turn alters the acceptability of silkworm Bombyx mori. A reduction in soluble protein content was observed among genotypes

Table 1. Genetic variability in physiological and biochemical traits in mulberry before imposing water stress.

Mullberry Genotypes/ Varieties	ChlorophyllStability Index (%)	WUE$_i$	Proline (µg/g)	Carbohyrate content (mg/g)	Soluble protein (mg/g)
MI-0613	75 ± 3.30	4.83 ± 0.05	3.04 ± 0.12	28.31 ± 0.41	32.40 ± 0.11
MI-0658	75 ± 1.55	5.62 ± 0.20	3.04 ± 0.14	27.43 ± 0.37	30.97 ± 1.26
MI-0425	78 ± 1.88	5.41 ± 0.08	3.28 ± 0.11	29.78 ± 0.02	32.58 ± 1.55
MI-0535	80 ± 0.65	4.46 ± 0.12	3.02 ± 0.12	27.32 ± 0.87	31.53 ± 0.73
V1	82 ± 2.48	5.13 ± 0.03	3.37 ± 0.16	30.91 ± 0.17	33.87 ± 0.66
MR2	74 ± 0.24	4.59 ± 0.21	3.28 ± 0.14	28.14 ± 1.19	30.33 ± 0.89
G4	73 ± 2.72	4.92 ± 0.05	3.15 ± 0.10	29.67 ± 0.44	32.77 ± 0.42
S.Ed	**NS**	**0.180**	**NS**	**0.879**	**NS**
CD (p<0.05)	**NS**	**0.386***	**NS**	**1.886***	**NS**

Table 2. Effect of drought stress on CSI, WUEi and osmolyte accumulation (Proline content) in mulberry

Genotypes/ varieties	Drought stress treatments	Chlorophyll Stability Index (%)	Water use efficiency (WUE$_i$) ($mmolCO_2\ mol^{-1}\ H_2O$)	Proline (µg/g)
	100% PC	82.45 ± 1.56	3.01 ± 0.15	4.37 ± 0.03
MI-0613	50% PC	60.56 ± 1.06	3.02 ± 0.07	4.83 ± 0.12
	25% PC	55.35 ± 2.39	3.18 ± 0.01	5.87 ± 0.04
	100% PC	78.98 ± 0.16	2.88 ± 0.10	4.30 ± 0.15
MI-0658	50% PC	65.37 ± 1.96	3.02 ± 0.12	5.10 ± 0.17
	25% PC	60.05 ± 1.07	3.21 ± 0.10	7.16 ± 0.06
	100% PC	84.47 ± 2.95	3.08 ± 0.05	4.57 ± 0.07
MI-0425	50% PC	78.05 ± 1.04	3.18 ± 0.03	6.34 ± 0.19
	25% PC	75.30 ± 0.23	4.13 ± 0.05	8.54 ± 0.07
	100% PC	82.05 ± 3.54	2.96 ± 0.13	4.28 ± 0.15
MI-0535	50% PC	70.45 ± 1.97	3.06 ± 0.07	4.76 ± 0.15
	25% PC	68.30 ± 3.51	3.68 ± 0.04	6.58 ± 0.08
	100% PC	88.23 ± 0.88	3.07 ± 0.09	4.48 ± 0.06
V1	50% PC	80.01 ± 0.14	3.14 ± 0.07	6.43 ± 0.01
	25% PC	77.68 ± 0.14	4.09 ± 0.15	8.48 ± 0.03
	100% PC	81.95 ± 2.51	2.89 ± 0.08	4.32 ± 0.03
MR2	50% PC	75.43 ± 2.02	3.10 ± 0.11	5.78 ± 0.14
	25% PC	70.05 ± 1.36	3.72 ± 0.06	7.90 ± 0.20
	100% PC	86.23 ± 2.44	3.04 ± 0.02	4.31 ± 0.01
G4	50% PC	72.45 ± 1.07	3.04 ± 0.16	6.05 ± 0.08
	25% PC	70.77 ± 1.73	4.04 ± 0.02	8.34 ± 0.04
Between subjects	**Genotypes G**	**3.134***	**0.150***	**0.180***
	Treatment T	**2.052***	**0.097***	**0.118***
(p<0.05)	**G× T**	**5.429***	**0.259***	**0.311***

and between treatments. A significant positive correlation was obtained between soluble protein and leaf yield (Table 3). Drought tolerant genotype MI-0425 was found to have a lesser reduction in soluble protein content even under a low water regime. The reduction percentage was around 17.23% followed by V1 with a reduction percentage of 22.04%. Whereas the susceptible MI-0613 was found to have minimum protein content in both moderate (25.96 mg g^{-1}) and intense (20.07 mg g^{-1}) water stress with a higher reduction percentage of 39.02%. While the other genotypes recorded a logical reduction in intense water stress varies from 20.86 to 24.83 mg g^{-1}. The total soluble protein content dropped during stress conditions could be due to protein denaturation and inhibition of protein synthesis. The above findings were agreed with Chaitanya *et al.,* (2001), who observed a significant reduction in RuBPCO of 30% in mulberry cultivar BC2-59 exposed to high-temperature stress. An increase in protein accumulation of 53.9% was observed in salt tolerant mulberry local cultivar exposed to 20 mM NaHCO3 at invitro condition (Ahmad *et al.,* 2007). Heat stress-induced suppression of photosynthesis by mainly decreasing the proportion of soluble protein to total leaf N, adversely affecting the RuBisCO protein and activity (Xu and Zhou, 2006).

Leaf yield:

All water stress treatments (50% and 25% PC) consistently reduced leaf yield in all genotypes/ varieties (Figure 2). A significant reduction in leaf yield was observed at 25% PC compared to control and 50% PC plants. Among all the genotypes, MI-0613 and MI-0658 suffered a greater reduction in leaf yield than V1 which maintained a higher yield under water stress conditions (95.48g/ plant). Whereas in MI-0613 leaf yield was around 53.34 g. A significant positive correlation was obtained between leaf yield with CSI, WUEi, and osmolyte accumulation (Table 3). Hence it is revealed that the genotypes supposed to have higher CSI, WUEi, and proline contents were found to have higher leaf yield under water stress conditions. The economic unit in mulberry cultivation is the leaf. Under drought conditions, the association between leaf yield and its component traits varies significantly (Susheelama *et al.,* 1998). Leaf yield was found to decrease to increase the water stress conditions. Among all the genotypes, variety V1 was found to have a lesser reduction in leaf yield and TDMA.

The reduction in yield of V1 was 12.32% and 20.46% at 50% PC and 25% PC respectively. This was followed by MI-0425 where the reduction percentage was 15.67% and 19.24% at 50% PC and 25% PC respectively. At intense drought stress percentage reduction of leaf, the yield was lower in MI-0425. This is in line with the findings of Guha *et al.,* (2010) who noticed higher yield performance in drought tolerant mulberry variety (V1) when irrigated once a fortnight in a growing season under field conditions. Singhvi *et al.,* (2013) reported a reduction of up to 65.62% in leaf yield in the drought-tolerant mulberry genotype (S-13). The other six mulberry genotypes also had shown yield reduction at 25% field capacity by withholding irrigation. A similar trend was observed by Manjula and Vijayakumari (2017), where field-grown mulberry variety V1 recorded the highest leaf yield under different irrigation schedules like five and seven days.

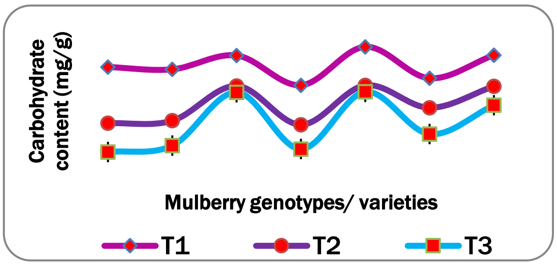

Fig 1a. Impact of drought stress treatments on carbohydrate content in mulberry.

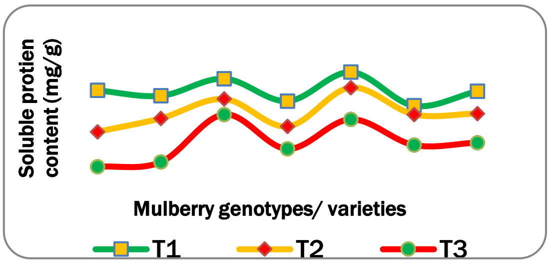

Fig 1b. Impact of drought stress treatments on soluble protein content

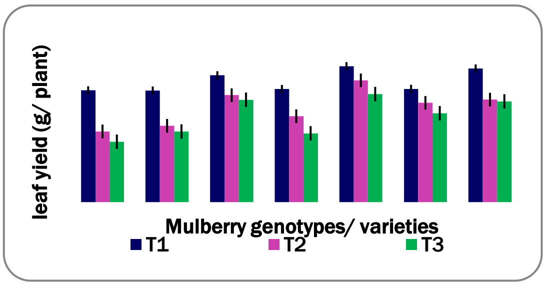

Fig 2. Average leaf yield of mulberry under different levels of drought stress treatments.

Table 3. Correlation between physiological and biochemical traits in mulberry with leaf yield under drought.

	CSI	WUE_i	Proline	Carbohydrate content	Soluble Protein	Yield
CSI	1	0.955**	0.886**	0.829**	0.925**	0.943**
WUE_i	0.955**	1	0.886**	0.899**	0.903**	0.939**
Proline	0.886**	0.886**	1	0.960**	0.936**	0.962**
Carbohydrate content	0.829*	0.899**	0.960**	1	0.912**	0.947**
Soluble Protein	0.925**	0.903**	0.936**	0.912**	1	0.935**
Yield	0.943**	0.939**	0.962**	0.947**	0.935**	1

**** Correlation is significant at (p< 0.01) level**

CONCLUSION

The present study revealed that significant variation was observed in chlorophyll stability index, intrinsic water use efficiency, and osmolyte accumulation of mulberry exposed to various water regimes *viz.,* 100% PC, 50% PC, and 25% PC. Among all the genotypes, MI-0425 and V1 were found to be drought tolerant. The physiological and biochemical basis for drought tolerance in MI-0425 and V1 was due to higher WUEi coupled with higher proline accumulation and CSI at moderate and intense moisture stress. The genotype MI-0613 was identified as drought susceptible due to its lower WUEi and quick degradation and depletion of carbohydrate, protein, and proline content under drought stress. Hence the study indicates that mulberry withstands drought stress by maintaining higher WUEi and CSI. The proline accumulation during stress has contributed to osmotic adjustment thereby maintaining the levels of protein and carbohydrate content.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Originality and plagiarism

We ensure that we have written and submitted only entirely original works, and if we have used the work and/or words of others, that has been appropriately cited.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the MS.

Author contributions

Research grant- nil, Idea conceptualization-DV, Experiments- RD, Guidance - DV, Writing original draft - RD, Writing- reviewing &editing – DV

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