J. Curr. Crop Sci. Technol., 2022; https://doi.org/10.29321/MAJ.10.000662(online first draft)

RESEARCH ARTICLE

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

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Received : 20th December, 2021
Revised : 28th January, 2022
Revised : 04th February, 2022
Accepted : 11th February, 2022

ABSTRACT

The present study was under taken to assess the drought 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, 25% PC for 30 days. Chlorophyll Stability Index, intrinsic Water Use Efficiency and osmolyte accumulation (proline content) were estimated before imposing drought stress and 30 days after drought stress. WUEi and proline accumulation was increased, while CSI, carbohydrate and protein contents were found to be decreased as 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 at severe drought stress. The genotype MI-0613 recorded lower WUEi (3.18 mmol CO2 mol^{-1} H_2O) and proline content (5.87 µg g^{-1}) under severe drought stress. It also recorded 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) and yield (95.48 g/plant) under both in 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 of sericulture industry. The mulberry foliage yield and its quality depends on soil type, variety, plant nutrients in soil, agronomical factors and agroclimatic conditions (Sharma *et al*., 2015). Growth and development of silkworm and cocoon crop are mainly influenced by 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 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 susceptible to water stress damages during both nursery and early plantation stage in 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 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 shoot 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). 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).

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 for 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 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 in order 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 condition and can be used as a drought index.

The quality of 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 silkworm (Bongale *et al* 1995). It has been found that 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 21st century is likely to increase 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 an 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 during November, 2018 to April, 2019 in the Rain Out Shelter (ROS) at Department of Crop Physiology, TNAU, Coimbatore. The study comprised of 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 the pH of 7.5. The pots were maintained under normal condition and watered daily 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 genotypes/varieties were divided into three sets and arranged in the Factorial Completely Randomized block design (FCRD), with three replications. Mulberry genotypes/ varieties kept as one factor and drought stress treatments were kept as another factor. Drought stress was imposed by dry down method (Guha *et al*., 2012). Plants were submitted to three water regimes viz. T1-Control: pots maintained at 100% pot water holding capacity (PC) T2- moderate drought stress: 50% PC, T3- 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 was 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 protien 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. 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.

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

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 photosynthetic rate and E is transpiration rate. The Pn and E was measured using Portable Photosynthesis System (PPS) (Model LI-6400 of LICOR inc., Lincoln, Nebraska, USA) between 10.00 hours to 12.30 hour. Totally, 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 anthrone reagent method (Ranganna, 1998). The carbohydrate content was calculated by 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, 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$_2$O. 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 in before imposing water stress. However, V1 recorded higher CSI along with maximum proline accumulation (3.37 µg g^{-1}) even under normal condition.

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 value 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 highest CSI under intense water stress, followed by MI-0425 (75.60 % at 25% PC).

A higher CSI value signifies a plants 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 maintained 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 higher CSI percentage when exposed to high temperature stress.

Intrinsic Water use efficiency (WUEi):

WUEi represents how plants performed best for transpiration under 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 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 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 maximum increase in WUEi compared to control and other genotypes under 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 free proline content of all the genotypes taken for study. 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 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 large group of crop plants grown under abiotic stresses, including salinity, water deficit and extreme temperatures is 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 significant increment in the free proline content in all the mulberry genotypes at both the levels of drought stress. MI-0425 exhibited highest level of proline compared to others. However, increment in the proline accumulation was significantly higher in V1 (89.28%, almost two fold increase) followed by MI-0425 (86.87 %,) compared to its respective control counter parts. However at the highest stress level, 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.

These above results are supported by Guha *et al*., (2012). He reported highest accumulation of free proline content in drought tolerant V1 exposed severe water stress (25% PC) under glass house 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 significantly reduced in all the seven mulberry genotypes. Variety V1 recorded highest value of carbohydrate content (35.78 mg/g) followed by G4 (34.54mg/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- 20.98mg/g; MI-0425- 28.97mg/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 amount of carbohydrate content present in it. A decreasing trend was observed in carbohydrate content in all the genotypes as drought stress progressed (Fig 1a). Sudden decline was observed in all the genotypes at intense water stress compared to control plants. Though decline was observed in intense water stress the drought tolerant genotypes MI-0425 and V1 recorded relatively higher amount of carbohydrate with lesser reduction percentage of 15.98% and 18.87% respectively. At the same time the susceptible one (MI-0613) recorded highest reduction percentage of 38.87%. Similar results were obtained by Ranjith kumar (2018) in 120 days old V1 which recorded highest carbohydrate content under high temperature stress condition.

Total soluble protein:

Similar to carbohydrate content drought stress caused severe reduction in soluble protein content. Decreasing trend was observed in total soluble protein as drought stress progressed. 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. Reduction in soluble protein content was observed among genotypes and between treatments. Significant positive correlation was obtained with soluble protein and leaf yield (Table 3). Drought tolerant genotype MI-0425 was found to have lesser reduction in soluble protein content even under low water regime. The reduction percentage was around 17.23% followed by V1 with the reduction percentage of 22.04%. Whereas the susceptible MI-0613 found to have minimum protein content in both moderate (25.96 mg g^{-1}) and intense (20.07 mg g^{-1}) water stress with higher reduction percentage of 39.02%. While the other genotypes recorded 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 was agreed with Chaitanya *et al*., (2001), who observed significant reduction in RuBPCO of 30% in mulberry cultivar BC2-59 exposed to high temperature stress. Increase in protein accumulation of 53.9% was observed in salt tolerant mulberry local cultivar exposed to 20mM 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). 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 greater reduction in leaf yield than V1 which maintained higher yield at water stress condition (95.48g/ plant). Where as in MI-0613 leaf yield was around 53.34 g. Significant positive correlation was obtained between leaf yield with CSI, WUEi and osmolyte accumulation (Table 3). Hence it is clearly revealed that the genotypes suppose to have higher CSI, WUEi and proline content found to had 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 vary significantly (Susheelama *et al*., 1998). Leaf yield was found to decrease to increasing the water stress conditions. Among all the genotypes, variety V1 was found to have lesser reduction in leaf yield and TDMA. 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 yield was lower in MI-0425. This is 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 upto 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. Similar trend was observed by Manjula and Vijayakumari (2017), where field grown mulberry variety V1 recorded highest leaf yield under different irrigation schedules like five and seven days.

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

Genotypes/ varieties	*Drought stress treatments*	*Chlorophyll Stability Index (%)*	*Water use efficiency (WUE$_i$) (mmolCO$_2$*	*Proline (µg/g)*

			mol^{-1} H_2O)	
MI-0613	*100% PC*	*82.45 ± 1.56*	*3.01 ± 0.15*	*4.37 ± 0.03*
	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*
MI-0658	*100% PC*	*78.98 ± 0.16*	*2.88 ± 0.10*	*4.30 ± 0.15*
	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*
MI-0425	*100% PC*	*84.47 ± 2.95*	*3.08 ± 0.05*	*4.57 ± 0.07*
	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*
MI-0535	*100% PC*	*82.05 ± 3.54*	*2.96 ± 0.13*	*4.28 ± 0.15*
	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*
V1	*100% PC*	*88.23 ± 0.88*	*3.07 ± 0.09*	*4.48 ± 0.06*
	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*
MR2	*100% PC*	*81.95 ± 2.51*	*2.89 ± 0.08*	*4.32 ± 0.03*
	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*
G4	*100% PC*	*86.23 ± 2.44*	*3.04 ± 0.02*	*4.31 ± 0.01*
	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 (p<0.05)	***Genotypes G***	***3.134****	***0.150****	***0.180****
	Treatment T	***2.052****	***0.097****	***0.118****
	G× T	***5.429****	***0.259****	***0.311****

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

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.*

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

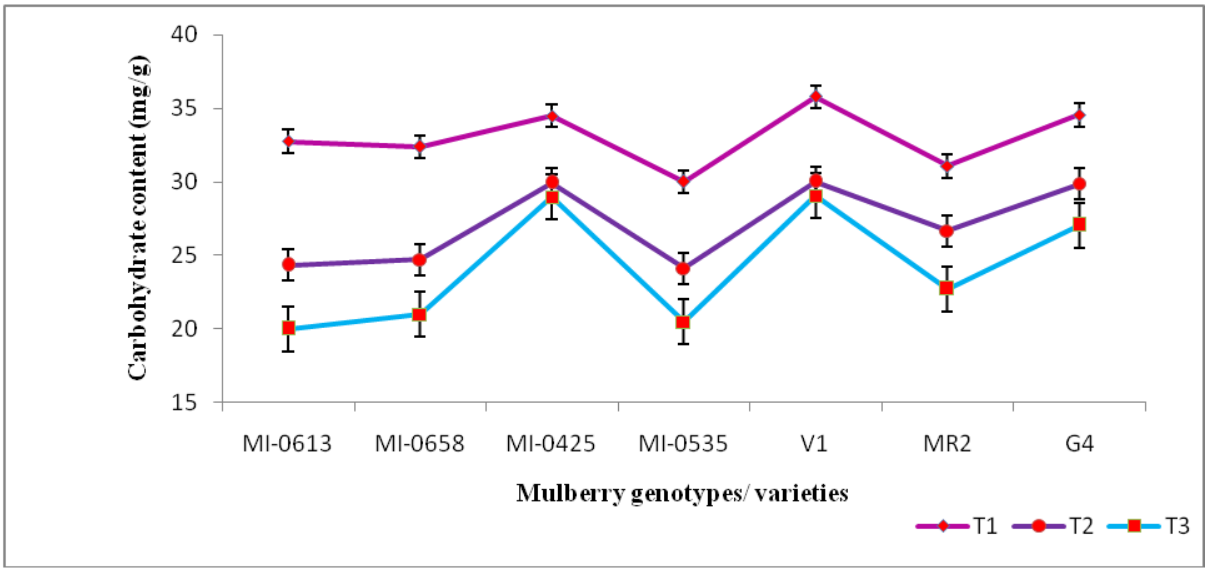


Fig 1b. Impact of drought stress treatments on soluble protein content

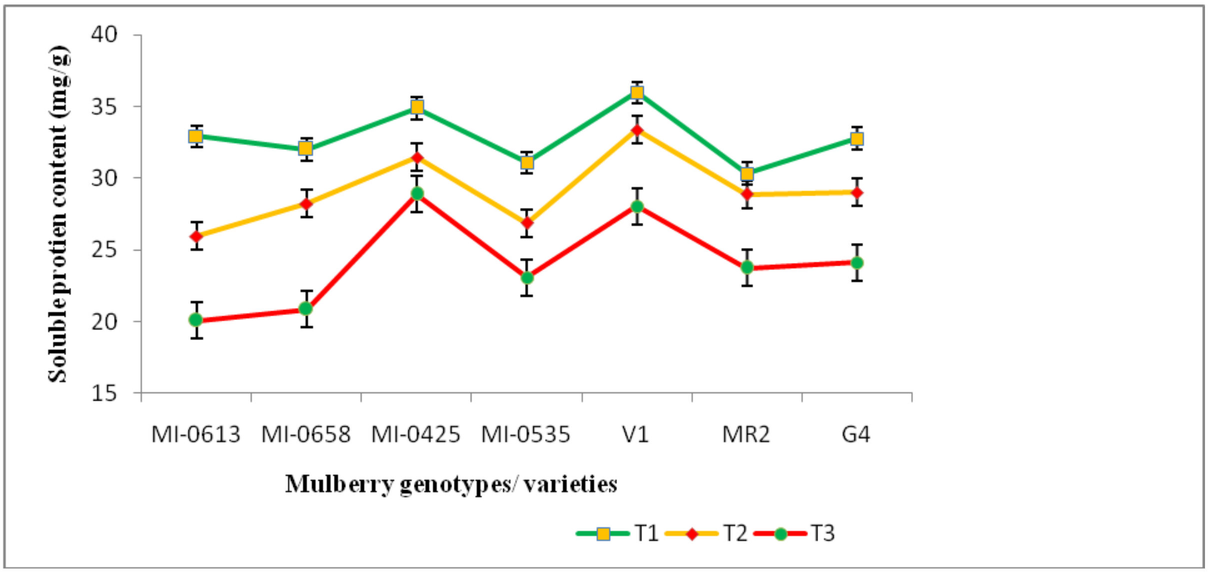
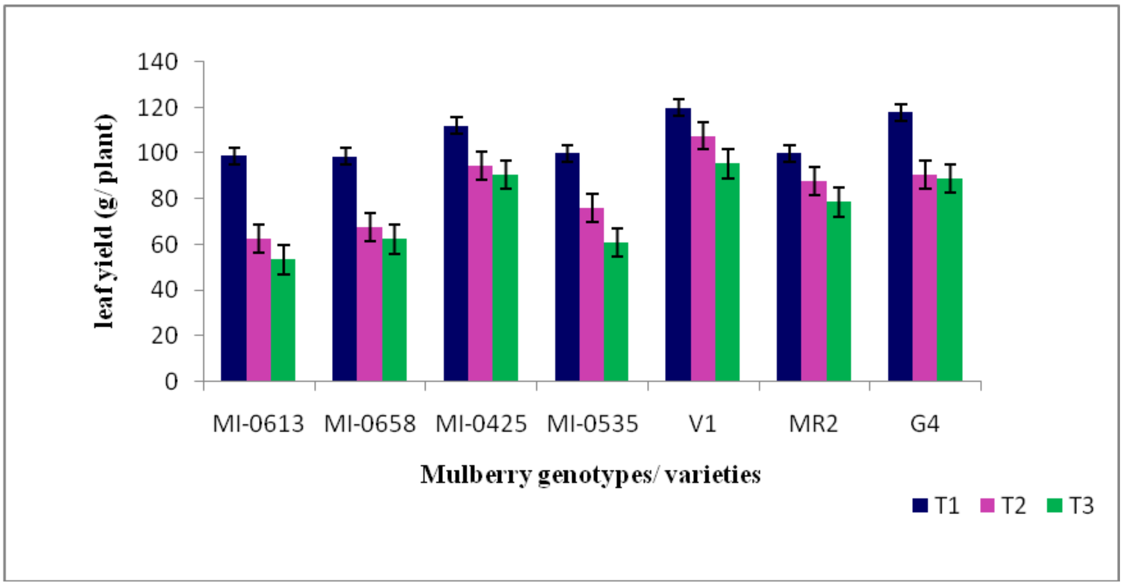


Fig 2. Average leaf yield of mulberry under different levels of drought stress treatments.



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 was found to be a 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 clearly indicates that, mulberry withstand drought stress by maintaining higher WUEi and CSI. The proline accumulation during stress have contributed to osmotic adjustment there by maintain the levels of protein and carbohydrate content.

Funding and Acknowledgment

No funding

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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