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Quantifying the antioxidant enzymes, NRase activities and yield traits to assess drought tolerance in mulberry

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Abstract
The present study was under taken to assess the drought tolerance in four mulberry genotypes and three varieties. 120 days old mulberry plants were subjected to three water regimes viz., 100% PC, 50% PC, 25% PC for 30 days. Enzyme activities were quantified before and 30 days after drought stress. MI-0425 was found to be drought tolerant with higher catalase (5.6 µg H_2O_2 $min^{-1}g^{-1}$) and peroxidase (4.42 Δ 430 nm g^{-1} min^{-1}) activities at moderate and severe drought stress. V1 recorded higher NRase activity both in moderate and intense water stress. Severe reduction in yield and TDMA was observed in the genotypes that recorded less enzyme activities. Susceptible MI-0613 and MI-0658 showed lesser enzyme activities with higher yield reduction (45. 96% and 36.70%). V1 recorded higher yield under drought stress. MI-0425 showed lesser reduction in yield and TDMA. Hence the study has identified MI-0425 and V1 to be drought tolerant.

Keywords: Mulberry, drought tolerance, nitrate reductase, anti oxidant enzymes, leaf yield

Introduction
Sericulture is an agro based cottage industry and mulberry is the sole food crop to silkworm *Bombyx mori*. The full potential of mulberry crop production is often decreased by the limitations on physiological and biochemical characteristics imposed by various environmental stresses (Manjula *et al.*, 2015) [21]. Among the abiotic stresses, drought is one of the world's major natural hazards which occur in almost every climatic region, which is the silent threat to rural economy as agriculture is the immediate victim impacting crop area, crop production and farm employment. Being a perennial plant, mulberry suffers from want of water and susceptible to water stress damages during both nursery and early plantation stage in the field (Rajat Mohan *et al.*, 2015) [28]. Water stress alters the physiological and biochemical characters of mulberry, which results in yield reduction. Water deprivation can arrest the growth and leaf yield performance of elite mulberry genotypes as a consequence of severely down regulated photosynthesis and carbon assimilation (Guha *et al*., 2010) [17]. In general biomass production is directly proportional to the supply and use of water.

Nitrate reductase activity is vital for the metabolic and physiological status of plants. Nitrate reductase is used as a biomarker of plant stress since nitrate reductase activity decreases in plants exposed to water limitation (Azcon *et al*., 1996) [7]. Nitrate reductase activity in water stressed leaves can be attributed to the decreased nitrogen metabolism. Decline in nitrate reductase (NR) activity has often been shown to decline when water status is lowered (Barathi *et al*., 2001) [8].

Drought induces oxidative stress in various higher and lower plants (Kotresha *et al* 2007)[19]. This oxidative stress creates deleterious effects on both primary and secondary metabolism of plants (Terman and Brunk 2006) [34]. Several physiological, biochemical and molecular responses in crops help them to adapt to adverse environmental conditions (Arora *et al*., 2002) [5]. To mitigate the oxidative damage, plants have developed a complex defense mechanisms viz., antioxidant enzymes such as catalase and peroxidase (Ahmad *et al.*, 2005) [2]. Catalase and peroxidase can scavenge $H_2O_2$and removes the bulk of H_2O_2. The superoxide produced during stress conditions are scavenged by antioxidant enzymes.

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) [17]. 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). Only few varieties are available which could perform well with the limited inputs. Hence it is very essential to screen out the potential mulberry variety which can perform well under the water stress condition with higher yield. So, the present study was designed with an aim to (i)To identify drought tolerant mulberry genotypes/ varieties by assessing antioxidant enzyme systems (ii) To find out the variation in Nitrate reductase activity and (iii) To correlate the physiological traits with yield under drought stress.

Materials 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)[6]. Along with the above four genotypes three mulberry varieties (V1, MR2 and G4) were studied for drought tolerance (Table 1). 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.

Table 1: List of mulberry genotypes/ varieties taken for study

Sl. No	Genotypes / Varieties	Origin
1.	MI-0613- Chandrapuri	Jammu & Kashmir
2.	MI-0658- Roop Nagar	Himachal Pradesh
3.	MI-0425- Deharadun local-11	Tamil Nadu
4.	MI-0535- Araku Local-2	Himachal Pradesh
5.	V1- Victory 1	Karnataka
6.	MR2- Midew Resistant2	Tamil Nadu
7.	G4	Karnataka

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) [16]. 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 enzyme activities were assessed at two stages viz., before imposing and thirty days after stress. Yield and yield traits were recorded at the end of the stress treatment.

Enzyme activities

Nitrate reductase activity was estimated as per Nicholas *et al*., (1976) [24] method. The leaves were pooled from three replications. The enzyme activity was expressed as µmol NO_2 g^{-1} h^{-1}. Catalase activity was assayed from the rate of H_2O_2 decomposition extinction coefficient of 39.4 mmol as measured by the decrease in the absorbance at 240 nm, following the procedure of Aebi (1974) [1]. The catalase activity is expressed as µg H_2O_2 $min^{-1}g^{-1}$. Peroxidase activity (change in OD value at 430 nm g^{-1} min^{-1}) was estimated by Perur (1962) [27] and Angelini *et al*., (1990) [4] method. The change in absorbance in minutes was used to calculate the enzyme activity.

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.

Total dry matter production

The plants were first shade dried and then oven dried at 72ºC for 48 hours. The dry weight of the whole plant at maturity (170 days) were recorded and expressed as g $plant^{-1}$.

Statistical analysis

Data on enzyme activities studied during the analysis were subjected to an analysis of variance as per the methods suggested by Gomez and Gomez (2010) [15]. An ANOVA was performed for each variable in this experiment to determine whether there were differences among the mulberry genotypes. A Pearson correlation analysis between enzyme activities and yield parameters was worked out.

Results

Enzyme activities

Enzymes activities in mulberry genotypes observed before imposing drought stress are presented in Table 2. The genotype MI-0425 recorded highest catalase and peroxidase activities before imposing stress followed by V1. NRase activity was found to be higher in V1 compared to other genotypes.

Table 2: Enzyme activities in mulberry genotypes/ varieties before imposing drought stress (120th day after planting)

Mullberry genotypes/ varieties	Enzyme activities		
	Nitrate Reductase (µg NO_2 g^{-1} h^{-1})	Catalase (µg H_2O_2 $min^{-1}g^{-1}$)	Peroxidase (Δ 430 nm g^{-1} min^{-1})
MI-0613	11.95 ± 0.37	2.24 ± 0.03	1.82 ± 0.03
MI-0658	11.78 ± 0.20	2.25 ± 0.04	1.78 ± 0.04
MI-0425	12.94 ± 0.26	2.68 ± 0.06	2.01 ± 0.02
MI-0535	11.97 ± 0.30	2.28 ± 0.08	1.75 ± 0.03
V1	12.96 ± 0.50	2.54 ± 0.09	1.95 ± 0.01
MR2	12.54 ± 0.53	2.24 ± 0.05	1.87 ± 0.08
G4	12.32 ± 0.06	2.31 ± 0.02	1.90 ± 0.07
S.Ed	0.501	0.079	0.065
CD (0.05)	1.076*	0.169*	0.138*

Nitrate reductase content

All mulberry genotypes compared to their corresponding control counterparts differed significantly ($p< 0.05$) within genotypes and treatments and exhibited an apparent decline in NRase content (Fig 1). At intense water stress level (25% PC), NRase declined maximum in all the genotypes/ varieties, and the lowest amount of NRase was found in genotype M I-0613 (7.56 µg NO_2 g^{-1} h^{-1}) followed by MI-0658 (9.47 µg NO_2 g^{-1} h^{-1}). However V1 recorded the highest amount of NRase content (13.12 µg NO_2 g^{-1} h^{-1}) followed by genotype MI-0425 (12.98 µg NO_2 g^{-1} h^{-1}) compared to other genotypes at the most intense drought regime (25% PC). A similar trend was observed for both V1 and MI-0425 at drought regime of 50% PC.

Catalase and Peroxidase activity

Exposure to different drought regimes caused substantial changes in antioxidant scavenging enzyme activity, causing significant difference within genotypes and among treatments. Alteration in antioxidant enzyme activities obtained from all seven genotypes/ varieties of three water regimes (100%, 50% and 25% PC). In variably, all the genotypes/ varieties showed increased enzyme activity at moderate water stress regime (50% PC) compared to their respective control and further decrease was observed at the intense water stress level (25% PC). Genotype MI- 0425 showed maximum catalase activity of 5.6 µg H_2O_2 $min^{-1}g^{-1}$ followed by V1 (5.52 µg H_2O_2 $min^{-1}g^{-1}$) at moderate water stress. The genotype MI-0613 and MI-0658 showed minimum peroxidase activity at moderate water regimes (2.97 Δ 430 nm g^{-1} min^{-1}). MI-0425 had highest peroxidase activity at 50% water stressed condition (4.42 Δ 430 nm g^{-1} min^{-1}). Over all MI-0425 ranked highest among all the genotypes in enzyme activity under drought stress, where as Mi-0613 scored the lowest for the same.

Leaf yield

Table 3: Yield and yield traits in mulberry genotypes/ varieties exposed to drought stress.

Mulberry genotypes/ varieties	Leaf yield (g/ plant)			TDMA (g/ plant)		
	T1	T2	T3	T1	T2	T3
MI-0613	98.70 ± 4.6	62.45 ± 1.3	53.34 ± 2.1	45.35 ± 0.1	33.20 ± 1.5	20.25 ± 0.5
MI-0658	98.50 ± 2.6	67.45 ± 1.5	62.35 ± 0.9	46.10 ± 0.1	35.70 ± 1.7	22.47 ± 0.5
MI-0425	112.00 ± 1.5	94.45 ± 3.6	90.45 ± 0.5	50.35 ± 1.8	45.75 ± 0.9	36.78 ± 0.1
MI-0535	99.85 ± 1.3	75.86 ± 0.4	60.78 ± 3.1	46.75 ± 2.4	36.45 ± 0.2	25.40 ± 0.8
V1	120.00 ± 3.2	107.56 ± 1.7	95.45 ± 0.9	52.50 ± 2.0	47.50 ± 1.3	38.65 ± 0.2
MR2	100.00 ± 2.8	87.68 ± 0.9	78.56 ± 4.1	48.60 ± 1.9	44.43 ± 2.3	35.43 ± 1.2
G4	118.00 ± 1.2	90.56 ± 1.4	88.97 ± 2.0	48.90 ± 0.4	40.60 ± 0.7	32.30 ± 0.4
V (P<0.05)	3.817*			2.057*		
T	2.499*			1.347*		
V×T	6.611*			3.562*		

All water stress treatments (50% and 25% PC) consistently reduced leaf yield and TDMA, in all genotypes/ varieties (Table 3). 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 thanV1 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 yield and antioxidant enzyme activities and NRase. Similarly, significant difference was observed in total biomass/ plant of all the genotype compared to control. Among all variety V1 recorded highest TDMA (52.50, 47.50 and 38.65) followed by MI-0425 (50.35, 45.75 and 36.78) at 100%, 50% and 25% PC respectively.

Table 4: Correlation of leaf yield under drought stress and enzyme activities in mulberry genotypes/ varieties.

Physiological/ yield parameters	Nitrate reductase	Catalase	Peroxidase	Total dry matter accumulation	Leaf yield
Nitrate reductase	1	0.962**	0.989**	0.977**	0.959**
Catalase	0.962**	1	0.982**	0.930**	0.952**
Peroxidase	0.989**	0.982**	1	0.952**	0.939**
Total dry matter accumulation	0.977**	0.930**	0.952**	1	0.959**
Leaf yield	0.959**	0.952**	0.939*	0.959**	1

Discussion

This study demonstrated that the mulberry genotype MI-0425 and the variety V-1 subjected to water limited conditions manifested significant drought tolerance relative to other genotypes studied (MI-0613, MI-0658, MI-0535, MR_2 and G4). Variety V1 and genotype MI-0425 showed maintenance

of better yield and biomass accumulation with increasing NRase and antioxidant enzyme activity at all water stress regimes. The remaining genotypes exhibited higher degree of plasticity in enzyme activities and severe loss in biomass and yield. When exposed to intense of moderate drought regimes.

NRase is an important enzyme for nitrogen assimilation and protein synthesis in plant cells. It is highly sensitive to heat and drought stress condition. A decreasing trend was observed in the NRase activity as drought intensity increases. Reduction in the NRase activity was more in MI-0613 with 46.31% decrease over control at 25% PC where as in the tolerant genotype MI-0425 the decreasing percentage was around 15.05%. This is in line with the findings of Nirmalkumar *et al*., 2017 [25], where a reduction in NRase content was observed in rice genotypes exposed to combined heat and drought stresses NR activity decreases in wheat under water deficit (Dwivedi *et al*., 2012) [14]. Correia *et al*., (2005) [12] stated that, maintenance of NRase activity has an imperative role in the tolerant genotypes for nitrogen assimilation and protein synthesis, which ultimately leads to improved productivity under heat and drought.

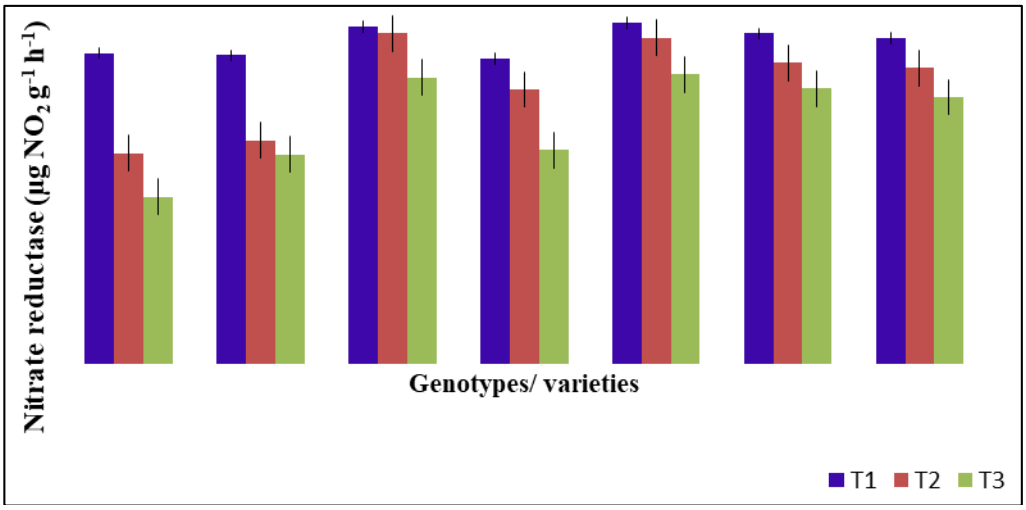

Fig 1: Influence of different levels of drought stress on Nitrate reductase activity in mulberry genotypes/ varieties

Tolerance to heat and drought stress in crop plants has been associated with an increase in antioxidant enzyme systems (Almeshlmani *et al*., 2016) [3]. Similar to the above hypothesis, the tolerant genotype MI-0425 was found to have high antioxidant enzyme activity such as catalase and peroxidase. MI-0425 recorded highest catalase activity with an increasing percentage of 53.85% over control in moderate water regime. Whereas the susceptible genotype MI-0613 was found have less increase in catalase activity of 13.92% over control. These results coincide with the findings of Reddy *et al*., (2005) [29] who reported, an increase in catalase activity in mulberry varieties S13 and $BC_2$59 when plants were exposed to water and heat stress. Similarly Kotresha *et al*., (2007) [19], reported an increased catalase activity in mulberry cultivars S13, S1635 and V1 when exposed to drought and temperature stress.

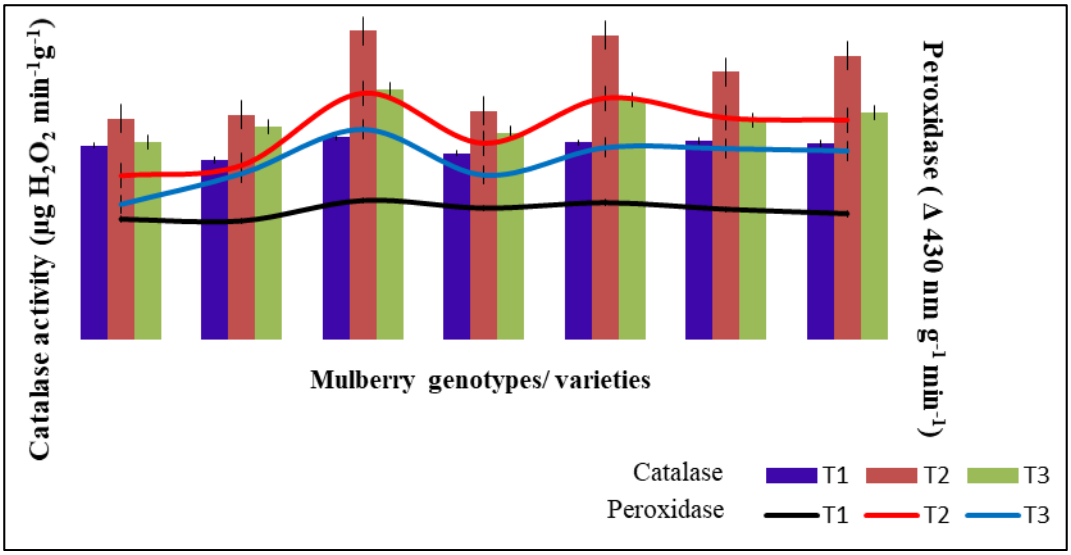

Fig 2: Alteration in antioxidant scavenging enzymes in mulberry genotypes/ varieties exposed to different levels of drought stress

Similar trend was observed in peroxidase activity. The genotype MI-0425 recorded maximum peroxidase activity with an increase in 77.38% at 50% PC. Increase in the activity of antioxidant enzymes is related to increase in stress tolerance (Sairam *et al*., 2002) [31]. Irrespective of all thegenotypes/ varieties taken for the study showed that an increased peroxidase activity with increasing degree of water stress. Similar finding of increased level of antioxidant enzyme activity reported by Chaitanya *et al*., (2001) [11] in mulberry cultivars exposed to water and heat stress.

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) [33]. 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) [17] 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) [32] 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) [20], where field grown mulberry variety V1 recorded highest leaf yield under different irrigation schedules like five and seven days.

TDMA is the reflection of the biological yield in mulberry varieties. Significant reduction was observed in TDMA when the genotypes/ varieties were exposed to water stress. At intense water stress the genotype MI-0613 had 55.05% reduction in TDMA. Where as in V1 and MI-0425 TDMA was around 26.38% and 26.95% respectively. Similar to the above findings, Paul and Quaiyuum (2015) [26] noticed a reduction in dry weight of five mulberry varieties subjected to 25% PC in pot culture experiments. Singhvi *et al*., (2013) [32] also noted reduction in TDMA in mulberry genotypes at 25% field capacity by withholding irrigation. In addition to the above, Misra *et al*., (2012) [22], reported a reduced TDMA in one year old mulberry variety S-1635 irrigated once in a month under glass house condition. TDMA was found to be reduced in mulberry genotypes exposed to sever salinity stress imposed by1.00% of NaCl (Vijayan *et al.,* 2010) [36].

Conclusion

To conclude, the involvement of enzymatic antioxidants in potentiating antioxidative defense system and ameliorating oxidative damage have significant implications in mulberry in relation to drought stress tolerance. These biochemical markers play a major role in the screening of drought tolerance in mulberry genotypes/ varieties. Further this may lead to the development of mulberry genotypes which provide better yield under water stress condition for the drought prone area for the betterment of sericulture farmers.

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