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MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES IN CERTAIN MULBERRY CULTIVARS INDUCED WATER STRESS.

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Abstract: Photosynthesis is important in metabolic pathways and is influenced by environmental stress. Drought stress is one of the environmental factors that alter several regulatory mechanisms, resulting in yield loss in crop plants. Using the foregoing background information, four Mulberry (Morus alba L.) genotypes were chosen, namely MY-01, BP-01, PP-01, and G4 and experiments were carried out in potted plants under natural photoperiod conditions under normal and water stress conditions. Morphological and Physiological parameters such as plant height, no of leaves, no of branches, leaf area, leaf yield and photosynthetic rate, stomatal conductance, transpiration rates, relative water content, and plant growth and yield parameters were measured in control and drought induced plants during the experiment. Water stress conditions significantly influenced the altering of all photosynthetic, plant growth, and yield parameters in water stressed conditions compared to watered conditions, according to the results, all of the varieties in the order had significantly lower photosynthetic rates. The most important crop in the sericulture industry is mulberry (Morus alba L.). In this study, we show that water scarcity characteristics can be finely controlled by leaf gas exchange characteristics in 1-year-old field-grown mulberry genotypes (Selection-MY-01, BP-01, PP-01, and G4) subjected to water stress by withholding water for 10 days. Significant differences in net photosynthetic rates (P_n), stomatal conductance and transpiration rate, and intercellular CO_2 have been observed among four mulberry genotypes. Among the four genotypes, G4 significantly higher rates in Morphological-physiological characters of both water treatments (control and during drought stress (DS).

Keywords: well water, drought stress, Mulberry, photosynthesis.

INTRODUCTON

The current scenario of global climate change is altering precipitation patterns in many parts of the world, with the predicted result of more frequent and prolonged severe drought events (IPCC 2013). Drought is known to affect various physiological processes, resulting in decreased photo-assimilation, which ultimately affects plant growth and survival (Pou *et al*. 2013, Nolf *et al*., 2015). Drought-tolerant (DT) mesophytic plants, on the other hand, have shown better photosynthetic performance even under prolonged drier conditions, which is associated with higher survivability, growth rates, and yields than drought-stressed (DS) plants. As a result, determining specific mechanisms conferring DT that could be targeted for various yield improvement programmes under water deficit regimes is critical. Furthermore, DT can affect plants' ability to survive and recover from drought, which varies between plant species as well as genotypes within the same species (Martorell et al. 2014). Among the many factors that contribute to DT, the efficiency of water transport through stems or leaves, are critical because the hydraulic compatibility between plant and environment under water stress (WS) conditions determines plant survival (Pivovaroff et al. 2014).

Previous research has shown that increased negative water potential during severe drought boosts stomatal closure cavitation (Brodribb and Cochard 2009). As a result, keeping plant water potential within the threshold range can help to avoid extensive embolism and drought-induced mortality (Martorell *et al*. 2014). Plant mortality under severe drought conditions can be explained by two interdependent mechanisms. The first is the water deficit failure hypothesis, which states that decreased soil water supply combined with increased evaporative demand causes extensive cavitation in conduits, preventing water flow and leading to plant tissue desiccation and cellular death (Brodribb and Cochard 2009, Choat 2013, Urli *et al*., 2013). The second mechanism is carbon starvation and stomatal closure to prevent hydraulic failure, which significantly reduces photosynthetic carbon uptake and may eventually be linked to reduced synthesis as well as increased depletion of stored carbon reserves (Sala *et al.*, 2010). Recent research, however, has shown that the hydraulic failure hypothesis played a significant role in tree mortality in water-stressed environments (Nardini *et al*., 2013, Pou et al. 2013, Quirk *et al*., 2013). Furthermore, changes in hydraulic conductivity under water stress were found to be positively related to changes in leaf gas exchange characteristics (Hubbard *et al*., 2001, Cochard *et al*., 2002). In addition to the foregoing, plants can refill embolized vessels during recovery with sufficient soil water availability following prolonged drought periods, and this recovery can be completed in a matter of hours rather than the longer time intervals required to grow new active xylem (Brodribb and Cochard 2009). Plants that are less susceptible to embolism formation during severe drought are classified as DT, while plants that are more susceptible to embolism formation are classified as DS (Costa *et al*., 2004, Martorell *et al*., 2014).

Mulberry (Morus alba L.) was chosen as a model plant in this study because different genotypes of mulberry have shown varying responses to WS, including tolerance and susceptibility. As a result, comparing the responses of different DT mulberry genotypes may be a better way to identify the traits that confer DT mechanisms. Previous research on drought responses in mulberry genotypes focused primarily on changes in photosynthetic leaf gas exchange physiology, biomass yields, and anti-oxidant defence mechanisms (Guha *et al*., 2010a, 2010b, Guha and Reddy 2013). However, little is known about how WS affects hydraulic dynamics and photosynthetic assimilation patterns in different DT mulberry genotypes. Three mulberry genotypes with varying DT were chosen for this study to investigate changes in hydraulic dynamics as well as photosynthetic leaf gas exchange physiology during progressive drought and recovery. We also looked at how the expression of both leaf and root AQPs changed in different DT mulberry genotypes during progressive drought stress and recovery.

The availability of water resources affects the entire nutrient statuses listed above, either directly or indirectly. Several studies have found that water stress conditions affect stomatal conductance, transpiration rate, and photosynthesis rate. There is very little information available about how decreasing and increasing photosynthesis rates, stomatal and transpiration rates vary under water stress conditions among mulberry genotypes. There is a scarcity of literature on various mulberry cultivars' physiological changes under water stress conditions (Dandin *et al*., 2003; Chaitanya *et al*., 2003; Ramanjulu *et al*., 1998; Thimma Naik *et al*., 2002). Furthermore, there is a scarcity of data on the physiological growth responses and leaf yield contributing traits of mulberry under water stress conditions. The ability to maintain key physiological processes such as photosynthesis during water stress is an indicator of long-term productivity under water scarcity. It is critical to identify certain morpho-physiological traits that are directly associated with drought stress tolerance in order to understand the different factors controlling and determining the growth and leaf yield of mulberry under water deficit. As a result, the study was carried out to discover what was happening in leaf physiology and morphology, plant growth, and yield modifications under stress conditions in Andhra Pradesh's tropical region.

MATERIALS AND METHODS:

The experiment was carried out at the Department of Botany, Sri Krishnadevaraya University Ananthapuramu, Andhra Pradesh, in 2016-19. Mulberry cultivars such as (MY-01, BP-01, PP-01, and G4), were chosen for the study. Cuttings of 12-15 cm long and 8-10 mm in diameter with 3 to 4 active buds were prepared from 6 months old matured shoots of the above varieties and planted in earthen pots containing 5 kg of air dried red loamy soil and farmyard manure (FYM) in a 3:1 proportion. The initial soil reaction (pH & EC) and soil organic carbon were determined prior to filling the soil as pH-7.45, EC-0.058 ds/m, and OC 0.280 percent. The experimental design was a randomised block design (RBD) with treatment plots (Chaturvedi and Sarkar, 2000). The earthen potted plants of various varieties described above were kept in the university's botanical garden for one year under natural photoperiod exposure using standard procedures (Dandin *et al*., 2003).

Treatment application: Before beginning treatments, the potted plants were pruned to a height of 10-12cms, and 750g of FYM (i.e. 1.50kg in two splits /yr.) was applied in each pot, followed by irrigation. Chemical fertiliser's viz. 28:14:4 kg /plant/crop of ammonium Sulphate (AS), Single Super Phosphate (SSP), and Muriate of Potash (MOP) among the entire treatment pots, a five-crop schedule with a 70-80-day gap/pruning schedule was maintained throughout the year (Dandin *et al*., 2003). The treatments were given on two levels: irrigation regimes with well-watered as well as Control and water-limited with stress conditions as treatment during all seasons, but irrigation in the form of control and treating was meticulously imparted under the prevailing drought stricken conditions in 2016 and 2019. Drought stress i.e. water stress (50 percent water of the FC) and non-stress i.e. control (80 percent water of the FC) watering treatments were applied to the plants at 10 days after emergence (DAE) and maintained throughout the growing season. The tested genotype G4 was chosen as a check plant among all the varieties because it has been recommended to the Southern part of India as a high-yielding and most suitable variety for irrigated cultivation (Dandin *et al.*, 2003; Kotresha *et al.*, 2007). The photosynthetic measurements were taken with an IRGA Analysis instrument (model IRGA-LICOR 6400-20, UK). Photosynthetic rate, stomatal conductance, transpiration rate, chlorophyll content, and relative leaf water content (RLWC) were measured using a Soil Plant Analysis Development (SPAD) instrument during photo-synthetically active radiation (PAR) at peak photosynthetic time (08:00–09.00 hours) and ranged from 1400 to 50 mol $m^{-2}s^{-1}$ after 45 days of pruning in the 5th and 6th leaves (Rao et al., 1991). After 70 days of pruning, plant growth and leaf yield parameters such as plant height, number of branches, number of leaves, leaf area, and leaf yield were measured (Satpathy *et al.*, 1992). Depending on the edaphic and climatic conditions, control pots were irrigated twice per week (with a frequency of 20-24 irrigations) during each growing season, whereas stressed watering pots were irrigated once every two weeks. The replicated data's mean values were subjected to ANOVA and are shown in Tables 1 and 2.

RESULTS:

The results show that all of the mulberry varieties considered for the study, such as MY-01, BP-01, PP-01, and G4, showed significant physiological and morphological differences between the normal (well watered) and stress (limited watered) conditions. The photosynthetic rate was found to be lower in all varieties under stress conditions when compared to normal watering conditions (control). G4 21.45, BP-01 18.15 and MY-01 16.25 as compared to control of G4 17.56.2, MY-01 13.25, BP-01 15.35 and PP-01 16.31, (Table. 1 and Fig. 1) respectively. Similar trends were observed in all other physiological parameters such as stomatal conductance, intercellular CO_2 concentration, and relative water content across all varieties, with the exception of transpiration rate, which showed increased levels of transpiration rate under stress conditions compared to normal watering (Table. 1 and Fig. 1 Image 1) Among the varieties, the G4 variety had the highest levels of photosynthetic rate under normal watering (Control-22.2 mol $CO_2/m^{-2}s^{-1}$) and stressed conditions 17.6 followed by MY-01, BP-01, and PP-01 9.79 to 7.40, respectively (Table. 1 and Fig. 1 Image 1). In terms of stomatal conductance (SC), the G4 variety had the highest level (4.05-2.56 followed by the BP-2.55 -1.21, the PP-01 2.82-1.42 and the MY-01 variety had the lowest level 1.96-1.23(Table. 1 and Fig. 1 Image 1). In terms of transpiration rate (TR), G4 had the highest 7.75-5.58 followed by MY-01 5.79-4.22, BP-01 5.35-3.56 and PP-01 5.89-4.16 (Table. 1 and Fig.1). G4 had the highest intercellular CO_2 concentration 268.91-159.65 followed by MY-01 217.61-111.52, BP-01 227.34-151.12 and PP-01 209.65-123.25 (Table. 1 and Fig. 1 Image 1). in that order. The moisture content of the leaf (RWC) was highest in G4 (81.5-72.4 percent), followed by MY-01 (70.6-68.6 percent), BP-01 (69.8-66.8 percent), PP-01 (75.1 to

68.4 percent) (Table. 1 and Fig. 1, Image 1). Drought stress, according to Kawamitsu *et al.,* (2000), reduced the rate of photosynthesis in an intertidal algae and a land plant. Purwanto (2003) also found that as water stress increased, photosynthetic rate decreased. Drought-induced photosynthetic reduction was caused by a decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence, and an associated decrease in food production (Wahid and Rasul, 2005). Reduced photosynthesis under stress could be attributed to reduced stomatal conductance and decreased photosynthate transpiration (Nagy and Galiba, 1995). Drought stress can reduce photosynthesis by reducing leaf area, closing stomata, and decreasing the activity of dehydrated protoplasmic machinery.

Drought stress had a similar effect on transpiration rate as it did on photosynthesis. Water stress reduced the rate of transpiration in all six mulberry genotypes. Water stress conditions influenced mulberry plant growth and yield parameters in all of the varieties in the order listed above. The study concluded that water stress conditions not only impair physiological parameters but also have a negative impact on plant growth and yield parameters, and that this information can be used to screen mulberry genotypes for water stress tolerance. The stress conditions caused by reduced irrigation level and frequency of irrigation have further influenced the reduced levels of plant growth and yield parameters in all high-yielding Mulberry cultivars Increased leaf yield was observed in the G4 variety (786 g/plant) with decreased yield due to stress conditions (659 g/plant), followed by MY-01 (782-0.658), PP-01 (638-601), and BP-01 (689g to 595 g/plant) when compared to all mulberry varieties. A similar pattern was observed for all other plant growth and yield parameters such as plant height, number of branches per plant, number of leaves per plant, and leaf area (Table 2 & Fig. 2, Image 1). Several researchers reported changes caused by drought stress conditions, such as changes in photosynthetic pigments and components (Anjum *et al.,* 2003), damaged photosynthetic apparatus (Fu and Huang, 2001), and decreased activities of Calvin cycle enzymes, all of which are important factors in crop yield reduction (Monakhova and Chernyadev, 2002). The reduced reduction in photosynthesis under stress conditions was obviously beneficial for maintaining better growth, so the G4 variety has become the farming community's choice of cultivation for enhanced quality leaves and increased silkworm cocoon production in the southern part of India.

DISCUSSION:

According to the findings of the preceding study, water is essential for plant growth, and without it, any biological activity will suffer, and wilting of the plant is a symptom of a plant on the verge of dying. Mulberry leaves with more than 90% moisture in chawki leaves are fed to chawki worms up to the third mould, and later the leaves with more than 70% leaf moisture are fed to silkworms until the life cycle is completed by farming the cocoons. The silkworms cannot compromise any changes in moisture content or nutritional aspects of the leaves. As a result, breeders must always pay close attention when selecting mulberry varieties for recommendation, unless they are resistant to drought tolerance by maintaining all of the required quality parameters even under limited water resources, and are only screened for the benefit of silkworm rearing. As a result, mulberry varieties such as G4, MY-01, BP-01, and PP-01 were recommended for irrigated regions, while G4 was recommended for low water with limited nutritional status and rainfed regions, naming them resource constraint varieties for the benefit of sericulturists.

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Table 1: Influence of stress conditions on the plant physiological activities of different mulberry varieties.

Mulberry variety	Photosynthetic Rate (μ mol CO_2/m-^{2}s-^{1})		Stomatal Conductance (μ mol H_2O/m-^{2}s-^{1})		Intercellular CO_2 concentration		Transpiration Rate (μ mol CO_2/m-^{2}s-^{1})		Relative leaf water content (RWC %)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
MY-01	16.25	13.25	1.96	1.23	217.61	111.52	5.79	4.22	70.6	68.6
BP-01	18.15	15.35	2.56	1.21	227.34	151.12	5.35	3.56	69.6	66.8
PP-01	19.68	16.31	2.82	1.42	209.65	123.25	5.89	4.16	75.1	68.4
G4	21.45	17.56	4.05	2.56	268.91	159.65	7.75	5.58	81.5	72.4
SE m ±	0.75	0.52	0.29	0.21	6.58	4.45	0.38	0.32	4.26	3.58
CD at 5 %	NS	NS	NS	NS	S	S	NS	NS	S	S

Table 2: Influence of stress conditions on the plant growth and yield parameters of different mulberry varieties.

Mulberry variety	Plant height (cm)		No. of branched/ plant		No. of leaves/ plant		Leaf area (cm^2)		Leaf yield/ plant (gm)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
MY-01	89	87	2	2	25	15	163.4	144.3	782	658
BP-01	96	91	2	2	30	28	150.7	142.4	689	595
PP-01	99	97	1	1	26	22	158.7	142.4	638	601
G4	115	113	3	2	37	35	193.7	184.5	786	659
SE m ±	3.25	2.89	0.008	0.005	1.57	1.02	5.13	4.62	15.25	12.36
CD at 5 %	NS	NS	NS	NS	NS	NS	S	S	S	S

Fig 1: Influence of stress conditions on the plant physiological activities of different mulberry cultivars.

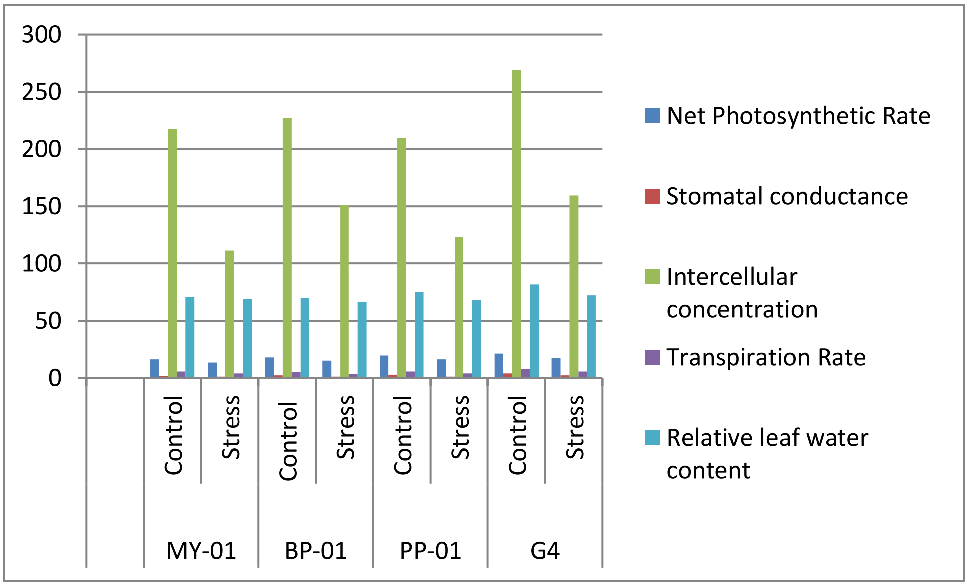
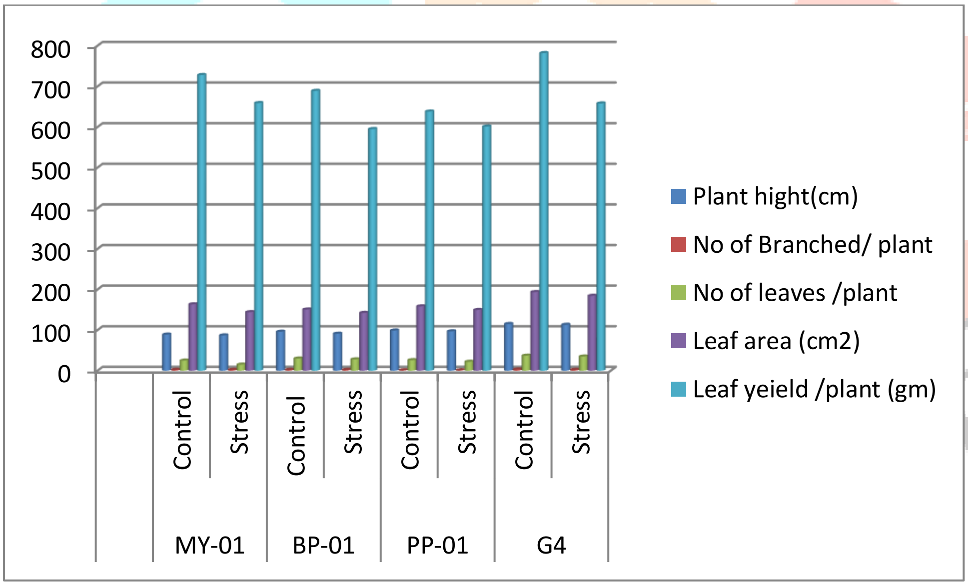


Fig2. Influence of stress conditions on the plant growth and yield parameters of different mulberry cultivars.



MULBERRY PLANTAION AT BOTANICAL GARDEN
DEPARTMENT OF BOTANY SK UNIVERSITY

WELL WATER (CONTROL)
MULBERRY CULTIVARS

WATER STRESS (DROUGHT STRESS)
INDUCED MULBERRY CULTIVARSMULBERRY

MEASURMENTS OF PHOTOSYNTHESIS,
WELL WATER MULBERRY CULTIVARS

WATER STRESS INDUCED PHOTOSYNTHESIS
MEASUREMENTS MULBERRY CULTIVARS

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