



## Physiological, Biochemical and Silkworm Bio-assay studies on certain Mulberry cultivars responses to water stress

Obulapathi N., Anandarajulu N., Jhansi Rani T. and S. Thimma Naik\*

Plant Physiology Laboratory, Department of Botany, Sri Krishnadevaraya University, Ananthapuramu -515003, Andhra Pradesh, India.

**Abstract:** Four Mulberry (*Morus alba* L.) cultivars, My-01, BP-01, PP-01, and G4 were studied for their physiological, biochemical and bio-assay responses to 10-days severe water stress conditions. The morphological parameters of shoot weight, leaf weight, root length, root weight, biochemical parameters such as carbohydrates, total chlorophylls, carotenoids, proline content and bio-assay studies of larval weights from the third to the fifth instar, post cocoon parameters of cocoon fresh and dry weight, cocoon shell percentage, filament length, and filament denier were studied in leaves on sixty days drought induction (well water and water stress). The results showed that total morphological parameters, biomolecules such as carbohydrates, protein content, total chlorophylls 30% decreased significantly as the level of all water stress induced leaves decreased four cultivars, proline, and carotenoids increased by 50%. Based on the results of the above study, it was observed that G4 cultivar had significant higher tolerant characteristics was observed in all the parameters studied.

**Keywords:** Mulberry cultivars, *Morus alba*, Silkworm, Water stress, Bio-assay.

### Introduction

Mulberry, the sole food source for the silkworm *Bombyx mori* L. is also economically significant to the sericulture industry. The genus *Morus* consists of a large number of species. The majority of these are found in Asia, especially China and Japan (Shepherd *et al.*, 2006). The most common native species in India is *Morus indica* and the most common exotic species are *Morus multicaulis*, *Morus nigra*, *Morus sinensis* and *Morus phillippinensis* (Sharma *et al.*, 2000).

Significant progress has been made mostly by Central Silk Board in developing and introducing superior mulberry genotypes such as V1, S36, K-2, MR2, S34, and others that are suitable for various agro-climatic zones and growing conditions (Vijayan *et al.*, 2004). Mulberry, as a perennial plant, suffers from lack of water in

the majority of the year. Drought conditions affect almost every state in India particularly in southern states such as Andhra Pradesh, Karnataka and Tamil Nadu the majority of sericulture is intense. Drought conditions influence poor cocoon crop.

A number of researcher studies have reported on the effect of feeding mulberry (*Morus alba* L.) leaves on larval growth, cocoon yield, and other economic characteristics of cocoons. Parpiev *et al.*, (1968) reported that the nutritional value and palatability of one type of leaf over another as a food source for silkworms are more important criteria and the water content of the leaves could be used as one of the criteria for determining their quality.

\*Corresponding Author:

Dr. S. Thimma Naik,

E-mail: [stnaik999@gmail.com](mailto:stnaik999@gmail.com)

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According to Samo-khvalova *et al.* (1972) the food quality had a significant impact on larval growth, cocoon weight, silk yield, and the physical-mechanical properties of silk thread. The nutritional status of mulberry leaf, which influences the economic characteristics of the silkworm crop, is determined by the amount of leaf moisture, total protein, total carbohydrates, and total minerals (Bongale *et al.*, 1991). Various authors have well documented the impact of various agronomical practises on mulberry leaf quality in India (Das *et al.*, 1990). Water scarcity is the world's most significant limiting factor for crop productivity (Jones and Corlett, 1992). Furthermore, water body systems are under severe threat in many parts of the world, with the water table in parts of India declining by 1 million tonnes per year (Somerville and Briscoe 2001). Water scarcity is the most damaging abiotic stress to crop yields, and it is caused by a lack of snowfall and changing rainfall patterns (Toker *et al.*, 2007). Drought severity is determined by the timing, duration, and intensity of the drought (Serraj *et al.*, 2005). Drought tolerance is a sophisticated quantitative trait that is influenced by a number of minor factors and is frequently confounded by phenological differences (Barnahas *et al.*, 2008; Fleury *et al.*, 2010).

Silkworm productivity and growth was essentially based on the quality and quantity of mulberry foliage. In most mulberry-growing areas, productivity and leaf quality are reduced as a result of environmental stress such as drought and high temperatures (Vijayan *et al.*, 1997). Despite significant progress against yield potentials of 60 to 70 tonnes / ha per year, little effort was made to increase leaf quality characters connected to cocoon and silk productivity (Koul *et al.*, 1980; Nataraja *et al.*, 2008; Paul *et al.*, 1992). Leaf quality is determined by a range of factors, including variety, cultivation conditions, pest and disease incidence, harvest methods and water loss techniques (Kishi, 1938;

Jalaja *et al.*, 2008). The leaf to silk inversion rate, always known as feed efficiency, is the most important measurement for evaluating (Khan *et al.*, 2007). The majority of additional crude protein, soluble carbohydrates, and ash in different mulberry genotypes seems to have a major impact on leaf quality (Choudhary *et al.*, 1976). There is evidence that compounds like  $\beta$ -sitosterol and flavonoids (morin-3-O- $\beta$ -D-glucopyranoside, quercitrin) enhance feed-ing development, although secondary metabolites inhibit feeding (Ito,1963). Leaf moisture content, in addition to these quality parameters, is an important determinant of leaf quality. For worms to provide a high ingestion and digestion capacity, leaves should have a moisture content of more than 70% (Rahmathulla *et al.*, 2006). Silkworm growth and cocoon development are also affected by leaf moisture content (Samdur *et al.*, 2003). Reduction in leaf size, changes in leaf orientation and canopy architecture and heavy cuticle depositions are some of the adaptive mechanisms in use by plants to stop water loss from leaf surfaces (Abrahams *et al.*, 1990; Cameron *et al.*, 2006; Fischer *et al.*, 1978; Goodwin *et al.*, 2005; Jarvis *et al.*, 1986; Kosma *et al.*, 2007).

Plants native to arid landshave thicker, waxier cuticles than those native to moist ecosystems and high wax content is often related to drought resistance in crop and tree species (Cameron *et al.*, 2006; Koch *et al.*, 2008). Heavy wax deposits on plant surfaces have traditionally been known to decrease transpiration and increase water retention. Furthermore, many plants have the ability to react to water shortages by increasing epicuticular wax deposition (Cameron *et al.*, 2006; Goodwin *et al.*, 2005; Jenks *et al.*, 2001; Kim *et al.*, 2007; Kosma *et al.*, 2007). In this research, we aimed to examine the physiological, biochemical and bioassay studies on certain Mulberry cultivars responses to water stress conditions.

## Materials and Methods

The experiment was carried out with four Mulberry (*Morus alba* L.) cultivars Viz. MY-01, BP-01, PP-01 and G4 according to the methodology followed by Passioura (1982) and Suseelamma and Datha (1995). Cuttings were collected from eight-months-old healthy plants from the Regional Sericultural Research Station (Central Silk Board-RSRS), Rappthadu, Ananthapuramu, Andhra Pradesh, India. Cuttings of 12 to 15 cm long with 8 to 10 mm diameter and 3 to 4 active buds were planted in earthen pots with 5 kgs of air dried red soil and farm yard manure in a 3:1 ratio. Pots were watered daily for seventy days, maintained in the botanical garden under a natural photoperiod of about 12-13 hr. with a temperature of about  $28 \pm 4^{\circ}\text{C}$ . Seventy day-old plants of each cultivar were divided into 4 sets and arranged in randomized complete Experimental Block Design (REBD). A brief account of the materials used and methods followed in various experiments is presented below. For this study, four varieties of Mulberry MY-1, BP-01, PP-01 and G4 were randomly selected and experimented 10-days with control and drought induced water schedules.

### Total plant height (cm):

Total number of primary branches and height of primary branches was calculated. Total height of the plant was obtained by multiplying the average height of the branches with number of primary branches.

### Number of Leaves per plant:

The samples selected for leaf are determinations were utilized for counting number of leaves per plant.

### Weight of 10 fresh leaves (gm.):

10 leaves were taken in a polythene cover and immediately weight was recorded in the laboratory.

### Leaf moisture percentage (LMP):

The moisture content of the leaves was determined on a dry weight basis according to the protocol followed by Gonzalez and Gonzanlez-Vilar (2001) Drocus and Vivekanandan (1991) and Chaitanya *et al.*, (2002). 10 fresh leaves were harvested randomly from four plants in each variety and fresh weight was taken. The leaves were dried in a hot air oven at  $60^{\circ}\text{C}$  for 72 hours (3 days). Moisture percentage was calculated by using the formula:

$$\text{MP} = \frac{\text{Wt. of fresh leaves} - \text{Wt. of dry leaves}}{\text{Wt. of fresh leaves}} \times 100$$

(Wt. - Weight)

### Relative Water Content (RWC):

Leaf relative water content (RWC) was estimated according to the method of Gonzalez and Gonzanlez-Vilar (2001). RWC was recorded from four leaves of the fully expanded leaf from the top of the main stem from each plant. Harvested leaf and fresh weight were recorded within 15 minutes, then leaf samples were soaked in 20 ml of distilled water for 8 hours and blotted for surface drying and water saturated leaf weight was recorded. The samples were oven dried at  $80^{\circ}\text{C}$  for 48 hr. The leaf's relative water content was calculated using the following formula RWC (Where FW denotes fresh weight, DW denotes dry weight, and TW denotes turgid weighted).

### Determination of Biochemical Parameters :

Biochemical analysis was done according to the modified protocol of Farooq *et al.*, (2012). For this study four plants were selected randomly from both drought induced and control pots and various biomolecules were estimated using standard operating procedures.

### Determination of total carbohydrates (mg/g):

Total carbohydrate was calculated using the anthrone method (Hedge and Hofreiter, 1962). Fresh weight expressed as mg/gram tissue. The calculation was done by using following formula.

Amount of carbohydrates =

$$\frac{\text{Amount of glucose} \times 100}{\text{Volume of test sample}}$$

*Total Chlorophyll estimation (mg / g) :*

The chlorophyll content in leaves was estimated with the method of Arnon (1949). The amount of chlorophyll present in the extract was calculated using the following formula and noted in mg /gram weight of tissue.

*Protein estimation (mg/g)*

Protein content of leaves was estimated by Lowry's method (Lowry et al., 1951).

*Carotenoids (mg/g) :*

The carotenoids in leaves were estimated by the method of Goodwin (1954).

*Ascorbic Acid (mg/g) :*

Ascorbic acid was estimated using the method by Sadasivam and Balasubraminan (1987).

*Free amino acids (µg/g) :*

Free amino acids were estimated following the method suggested by Sadasivam and Balasubramanian (1987).

*Proline content (µg/g) :*

Standard estimation procedure was followed to estimate proline content (Bates et al., 1973).

*Silkworm bioassay studies:*

Silkworm bioassay studies was performed according to the methodology followed by Bari et al., (1985; Machii and Katagiri, (1990); Benchamin and Anantharaman, (1990). The impact of feeding of mulberry leaves grown under water stress conditions on silkworm growth and development was assessed by rearing of silkworm.

*Rearing of silkworm (Bombyx mori L.):*

One dfl of silkworm eggs (Bivoltine double hybrid of (G11 X G19)) were reared on experimental mulberry leaves. They were reared separately fed with drought induced and normal (healthy) leaves of both varieties from

third instar onwards. All the required rearing conditions were maintained in the rearing house. The rearing was carried out by following the rearing practices recommended by Dandin and Kumaresan (2003) and the following parameters were studied.

**(i) Larval weight (g) :**

Larval weight was measured every day after the first feeding in all three instars, beginning with the third instar and continuing until mounting. For this study, ten larvae were selected randomly from control as well as treated trays. Number of cocoons harvested: Cocoons were harvested on fifth day after mounting by ensuring complete development of pupae and the number was recorded.

**(ii) Cocoon weight (g) :**

Cocoons were stifled by keeping them in a hot air oven for three days at 70<sup>o</sup> C. The following cocoon parameters were studied and to assess the quality. The single cocoon weight was assessed as the average of 10 cocoons taken at random for each treatment.

**(iii) Cocoon shell weight (g) :**

The single shell weight was calculated as the average of 10 shells used for cocoon weight assessment.

**(iv) Cocoon shell percentage (%) :**

It is calculated with the formula=

$$\frac{\text{Weight of the cocoon shell} \times 100}{\text{Weight of the entire cocoon}}$$

*Average Filament Length (m):* Average Filament Length was calculated according to the modified method followed by Ruth et al., (2014), Thulasi et al., (2015), Ogunleye and Johnson (2012). The average filament length in meters was calculated after 10 cocoons were cooked and reeled on an epprouvette (circumference 1.125m).

**(ii) Non-breakable filament length (m):**

The total non-breakable filament length was calculated by using the formula Non-breakable silk length = Total filament length 1 + number of breaks

**(iii) Filament Denier:**

Denier, which represents the size of the yarn, is the weight in grams of 9000 meters of the yarn/filament. The denier (size) was calculated using the formula.

$$\text{Diner} = \frac{\text{Weight in gram of filament} \times 9000}{\text{Length in meter of filament}}$$

**Results and Discussion**

The results of this study revealed that drought stress on MY-01, BP-01, PP-01, and G4 varieties causes severe significant changes in development and growth, and also biochemical and silkworm bio-assay parameters, as seen in the table (Table. 1,2,3,4).

**Morphological characters:**

Various parameters were recorded after ten days of drought induction on MY-01, BP-01, and PP-01, G4 varieties to discuss the effect of water stress on the growth and development of mul-berry varieties. Water stress has quite a greater impact on morphological changes, which are the primary factors able to influence leaf yield. The following morphological parameters were observed: total plant height (cm), number of leaves/plants, fresh weight of 10 leaves (g), leaf moisture percentage (%) and relative water content (%), leaf moisture retention percentage (%) (6 & 12 hrs.), leaf yield (kg), shoot fresh weight (g), shoot dry weight (g), root length (g) (cm), All morphological parameters studied in four mulberry cultivars (MY-01BP-01 and PP-01, G4) showed a significant reduction. G4 had the longest shoot length among these cultivars, while MY-01 had the shortest. G4 cultivar leaves have the highest moisture content and moisture retention capacity, while MY-01 leaves get the lowest. MY-01 has the lowest leaf area, water potential,

and relative water content; while G4 has the highest. Drought-induced morphological changes were found to be significant in the G4 variety when compared to control plants in the present research. For this study, four plants were randomly selected: MY-1, BP-01, PP-01 and G4 varieties in control and drought induced water schedules.

**Biochemical analysis:****Total Carbohydrates (mg/g):**

Water stress induced instead of well-watered mulberry leaves of four different mulberry cultivars showed a significant difference in total chlorophyll content. G4 mulberry cultivar leaves had significantly higher levels of total chlorophyll content in both water regimes, whereas MY-01 mulberry cultivar leaves were to have lower levels of total chlorophyll content. The total starch, reducing sugars, but also the total sugars of leaves of four mulberry cultivars were investigated. The greater amount of starch, reducing sugars, and maximum sugars were found in the leaves of G4, followed by MY-01 and BP-01, with the lowest numbers found in the leaves of MY-01. In this study, we looked at water stress induced and well-watered mulberry leaves from four different mulberry cultivars, along with all of the basic metabolic biomolecules (total chlorophylls, carbohydrates, total protein, total starch, and free amino acid) and proline content in the leaves of four different mulberry cultivars. All biochemical parameters of both water regimes were found to be significantly higher in G4 mulberry cultivar leaves, followed by PP-01, BP-01, and MY-01. Lower levels were found in plants of the MY-01. (Cultivar Table II and Figure II).

**Silworm Bio-assay studies :****Cocoon Characters and Silk Quality & quantity:**

Four mulberry cultivars (MY-01, BP-01, PP-01, G4) were studied by feeding silkworm larvae of *Bombyx mori* of bivoltine hybrids fresh mul-

berry leaves and water stress induced mulberry leaves (G11X M98). The first day of the 5<sup>th</sup> instar larvae's life was spent in an environment with an ambient temperature of  $25 \pm 2^{\circ}\text{C}$  and a relative humidity of 60-70%. The cocoon parameters were computed. The quantity and quality of silk are directly proportional to the leaf quality, which influences the health and growth of silkworm larvae as well as their effect on overall cocoon production. The degree and uniformity of mounting vary with mulberry leaf quality, and a higher mounting ratio ensures a better growth rate and silkworm larval weight from the third to the fifth instar. The shell weight percentage indicates how much silk can be reeled from a given number of fresh cocoons, and it varies depending on the age and breed of silkworm. In general, total silk filament lengths range from 600m to 1500m, with only 80% being reliable.

The current study found that both water treatments (well water and water stress) resulted in a significant decrease in larval weight (3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> instar) in the G4 variety when compared to the PP-01, BP-01, and MY-01 varieties were found to be significantly higher in 5<sup>th</sup> day of 5<sup>th</sup> instar larvae reared on G4 mulberry cultivar leaves and lower levels in larvae reared on MY-01 cultivar leaves. G4 variety showed a significant decrease in larval weight fed with leaves of water stress plants (3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> instars) when compared to PP-01, BP-01, and MY-01 varieties.

#### *Parameters for reeling :*

Various post cocoon characteristics such as cocoon yield, cocoon weight, cocoon shell weight, cocoon shell ratio, filament length, filament weight, denier percentage, silk thread length were found to be greater when larvae were fed with control plants and water stress mulberry leaves of highly significant in G4 cultivar, followed by PP-01, BP-01, and MY-01. When larvae were fed MY-01 mulberry cultivar leaves, the

cocoon yield, cocoon weight, cocoon shell weight, cocoon shell ratio, filament length, filament weight, denier percentage and silk thread length were all found to be low. Various post cocoon parameters, such as fresh weight of cocoon, dry weight of cocoon, fresh weight of cocoon shell, dry weight of cocoon shell, and cocoon shell percentage, decreased significantly in Water stress plants when compared to control plants. When compared to PP-01, BP-01, and MY-01, all post cocoon parameters (cocoon yield, cocoon weight, cocoon shell weight, cocoon shell ratio, filament length, filament weight, denier percentage) were highly significant in the G4 variety.

#### *Larval weight :*

In the 10-day drought water schedule, there have been significant reductions in larval weight in all days of drought.

#### *3<sup>rd</sup> instar larval weight :*

The third instar larval weight in Control plants MY-01, BP-01, PP-01, and G4 leaves was 4.85 compared to MY-01, BP-01, PP-01, and G4 the present reduction of 3.45 with 8.25% with MY-01, BP-01, PP-01, and G4 plants was 3.66 with a 20% decrease. Stressed plants were observed in MY-01, BP-01, PP-01, and G4 leaves; G4 cultivar was reduced by 1.60%. The remaining three cultivars have a significantly lower percentage of 2.98, 2.89, and 2.58, respectively.

#### *4<sup>th</sup> instar larval weight :*

The fourth instar larval weight observed in MY-01, BP-01, PP-01, and G4 plants was 12.79 compared to MY-01, BP-01, PP-01, and G4 the current reduction of 9.45 with 18.32 percent with MY-01, BP-01, PP-01, and G4 plants was 35.25 with 30.12 percent decrease. In the fourth instar larval weight was observed in Stress induced plants MY-01, BP-01, PP-01, and G4 leaves; G4 cultivar was only 8.29 percent reduced. The remaining three cultivars get slightly lower percentage of 9.45, 8.90, and 8.95, respectively.

**5<sup>th</sup> instar larval weight :**

The 5<sup>th</sup> instar larval weight observed in MY-01, BP-01, PP-01, and G4 leaves was 34.21 compared to MY-01, BP-01, PP-01, and G4 the present reduction of 28.25 with 15.65% with MY-01, BP-01, PP-01, and G4 plants was 26.58 with a 23.55 percent reduction. Stress-induced plants MY-01, BP-01, PP-01, and G4 leaves were found to have significantly lower larval weight throughout the fifth instar. G4 cultivar was reduced by 21.95 percent. The remaining three cultivars have a significantly lower percentage of 15.61, 17.56 and 24.25, respectively (Table. 3 and Figure. 2).

**Number of cocoons harvested :**

Cocoons were harvested on the fifth day of moulting, and the number of cocoons spun for control but also drought larvae were recorded separately. The number of cocoons harvested in MY-01, BP-01, PP-01, and G4 leaves was 25.12, compared to MY-01, BP-01, PP-01, and G4. The current decrease in plants treated was 18 with 22.70%, and MY-01, BP-01, PP-01, and G4 was 16.71 with 32.56%.

**Cocoon weight (g) :**

Cocoon weight was 14 in MY-01, BP-01, PP-01, and G4 leaves compared to MY-01, BP-01, PP-01, and G4. The present reduction in treated plants was 3.56 with a 35% reduction and 2.37 with a 52.23% reduction in MY-01, BP-01, PP-01, and G4 plants.

**Cocoon shell weight (g) :**

Cocoon shell weight in MY-01, BP-01, PP-01, and G4 leaves was 0.18 compared to MY-01, BP-01, PP-01, and G4. The present reduction in treated plants was 0.16 with 25.23 percent and MY-01, BP-01, PP-01, and G4 was 0.13 with 43.26%.

**Cocoon shell percentage (%) :**

The cocoon shell percentage weight in MY-01, BP-01, PP-01, and G4 leaves was 22.98% when compared to MY-01, BP-01, PP-01, and G4. The present significant decrease in treated plants was 17.42 with 16 with MY-01, BP-01, PP-01, and G4 was 18.02 with 24.98% of decreasing.

**Reeling parameters :**

**Total filament length (m):** Total filament length in MY-01, BP-01, PP-01, and G4 leaves was reduced by 23.06% as compared to MY-01, BP-01, PP-01 and G4.

**Non-breakable filament length (m) :**

Non-breakable filament length was 309.5 in MY-01, BP-01, PP-01, and G4 leaves, compared to MY-01, BP-01, PP-01. The current reduction in treated plants was 176.32 with 38.23% and MY-01, BP-01, PP-01, but also G4 was 118 with 64.25 % decreasing.

**Filament Denier :**

Filament Denier was 3.18 in MY-01, BP-01, PP-01, and G4 leaves when compared to MY-01, BP-01, PP-01. The current reduction in plants treated was 0.61 with 15.21% and MY-01, BP-01, PP-01, and G4 appears to be 1.65 with 42.25% of decreasing (Table. 4 and Figure. 4)

**Table 1:** Morphological characteristics of Four Mulberry cultivars Viz., MY-01, BP-01, PP-01, and G4 (Control and drought)

Mulberry cultivar type	MY-01		G4		BP-01		PP-01	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Root weight (g)	74.4	86.5	98.3	104.2	92.5	54.3	68.2	52.4
Root length (cm)	45.2	46.7	50.1	51.9	32.5	31.0	35.1	38.5
Plant height (cm)	78.2	48.5	97.7	46.3	85.2	25.3	76.2	31.5
Total shoot length (cm)	124.6	80.5	196.5	113.0	121.7	38.5	126.5	36.6
No. leaves shoot	20.0	9.0	24.0	13.0	18.0	5.0	21.0	5.0

Leaf weight (g)	130.2	38.6	154.5	46.5	13.2	16.2	122.3	14.5
S.E m. ±	0.96	0.69	0.87	0.79	0.81	0.74	1.25	1.02
CD at 5 %	NS	NS	NS	NS	NS	NS	S	S

**Table 2:** Effect of Well water and drought stress on biochemical composition in mulberry cultivars Viz., MY-01, BP-01, PP-01 and G4.

Mulberry cultivars	control				stress			
	MY-01	BP-01	PP-01	G4	MY-1	BP-01	PP-01	G4
Carbohydrates (mg/g)	28.70	25.98	32.99	37.27	20.67	19.33	19.29	24.97
Total Chlorophylls (mg/g)	1.48	1.35	1.95	2.04	0.91	0.65	0.83	1.15
Carotenoids (mg/g)	0.55	0.59	0.62	0.66	0.70	0.79	0.73	0.76
Starch (mg/g)	11.54	10.86	11.35	13.44	6.80	5.21	5.88	7.46
Proline (µg/g)	16.03	18.19	14.58	20.55	19.33	22.56	20.95	25.06
S.E m. ±	0.35	0.39	0.42	0.46	0.50	0.59	0.53	0.56
CD at 5 %	NS	NS	NS	S	NS	NS	NS	S

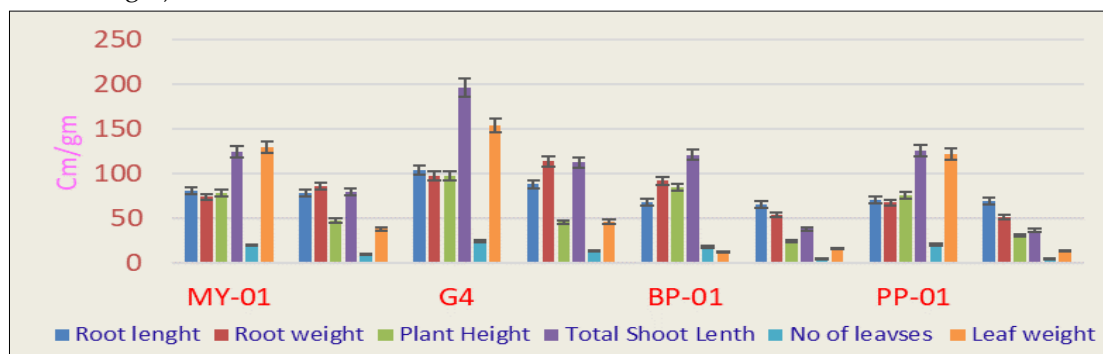
**Table 3:** Effect of Well water and drought stress on larval weight of silkworm *Bombyx mori* L. fed on mulberry cultivars Viz., MY-01, BP-01, PP-01 and G4.

Mulberry cultivars	Control			Stress		
	Weight of larvae in 3 <sup>rd</sup> instar (g)	Weight of larvae in 4 <sup>th</sup> instar (g)	Weight of larvae in 5 <sup>th</sup> instar (g)	Weight of larvae in 3 <sup>rd</sup> instar (g)	Weight of larvae in 4 <sup>th</sup> instar (g)	Weight of larvae in 5 <sup>th</sup> instar (g)
	%					
MY-01	3.47	11.75	22.94	2.98	9.45	15.61
BP-01	4.26	12.32	30.1	2.89	8.90	17.56
PP	4.05	12.65	30.08	2.58	8.95	24.25
G4	4.85	12.79	34.21	3.25	8.29	21.95
S.E m. ±	3.45	8.78	10.02	10.46	5.86	9.89
CD at 5 %	4.24	3.56	5.61	4.85	4.25	4.05

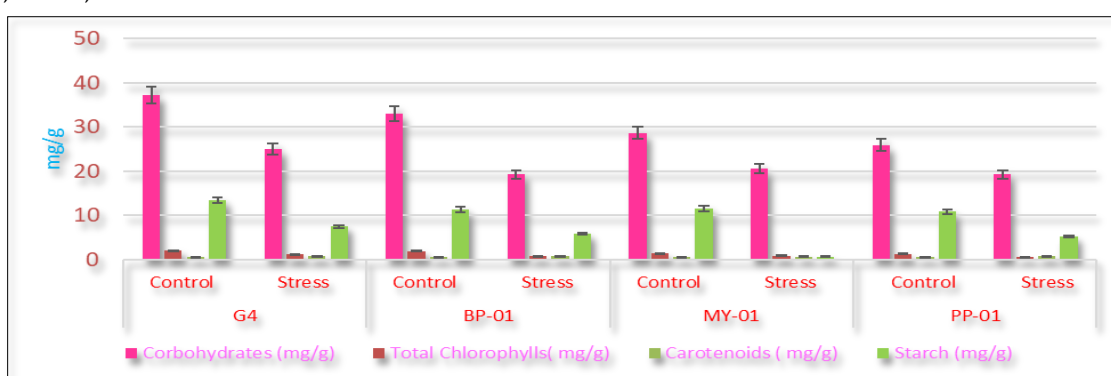
**Table 4:** Effect of Well water and drought stress on Post Cocoon and reeling parameters of silkworm *Bombyx mori* L. fed on mulberry cultivars Viz., MY-01, BP-01, PP-01 and G4.

Mulberry cultivars	MY-01		BP-01		G4		PP-01	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Fresh cocoon weight (g)	0.81	0.66	1.05	0.53	1.18	0.89	0.78	0.60
Dry cocoon weight (g)	0.13	0.10	0.19	0.08	0.40	0.13	0.12	0.11
Fresh cocoon shell weight (g)	0.15	0.11	0.23	0.07	0.14	0.11	0.14	0.09
Dry cocoon shell weight (g)	0.05	0.03	0.09	0.02	0.06	0.04	0.4	0.02
Cocoon shell percentage (%)	18.41	16.42	21.41	13.93	19.49	17.50	16.14	14.59
Total Filament length (MT)	788.3	694	793.6	671.5	827.59	625.1	969.56	724.51
Non- breakable filament length (MT)	232.15	162.18	284.13	176.15	226.12	149.51	309.51	174.14
Filament Denier (d)	2.57	1.78	1.91	1.11	2.11	1.49	3.18	2.57
S.E m. ±	0.55	0.35	0.51	0.29	0.58	0.42	1.25	0.98
CD at 5 %	1.87	1.39	1.89	1.41	1.91	1.45	4.36	2.99

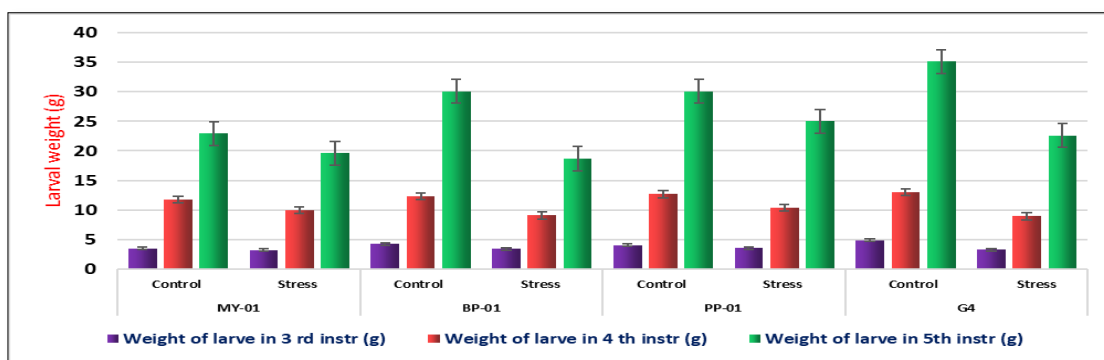
**Figure (1).** Morphological characteristics of four Mulberry cultivars Viz., MY-01, BP-01, PP-01 and G4 (control and drought).



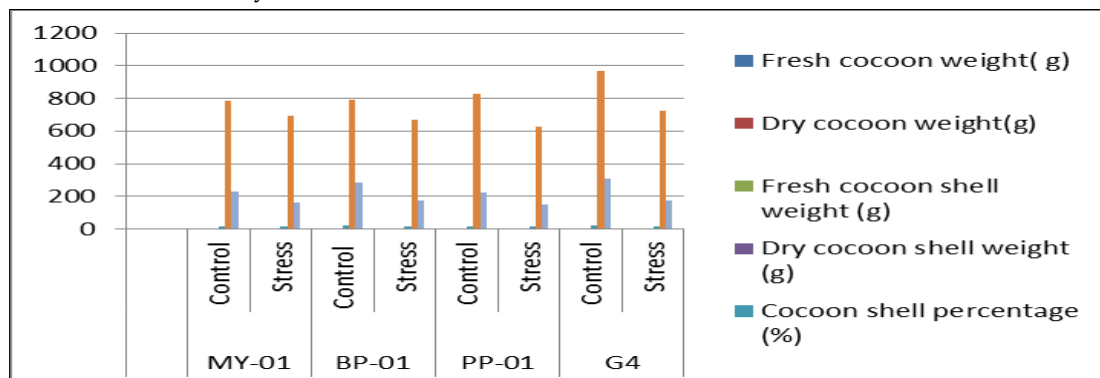
**Figure (2):** Effect of Well water and drought stress on biochemical composition in mulberry cultivars Viz., MY-01, BP-01, PP-01 and G4.



**Figure (3).** Effect of Well water and drought stress on larval weight of silkworm *Bombyx mori* fed on mulberry Cultivars Viz., MY-01, BP-01, PP-01 and G4.



**Figure (4):** Effect of Well water and drought stress on Post Cocoon and reeling parameters of silkworm *Bombyx mori* fed on mulberry Cultivars Viz., MY-01, BP-01, PP-01 and G4.





Pictorial representation of Silkworm rearing bioassay from 3<sup>rd</sup> instr to 5<sup>th</sup> instrs of well water and water stress induced mulberry cultivars (MY -01;BP -01; PP-01& G4) with control and induced stress and Post cocoon harvest.

## Conclusion

According to the findings of this study, drought stress played a significant role in morphological, biochemical and bioassay parameters in drought-induced mulberry plants (MY-01, BP-01 and PP-01, G4). Drought stress induced crop yield deficit, both quantitative and qualitative, affecting silkworm health and cocoon production.

The current study helps to understand the constraints involved in the impact of drought on mulberry varieties, and also in mitigating the conditions for better growth and trying to develop drought-tolerant varieties. Finally, we concluded and after screening (MY-01, BP-01, and PP-01, G4) mulberry cultivars have used morphological, biochemical and bio-assay studies both in environmental conditions (well-watered and water stress induced), all characteristics were higher in the G4 cultivar, followed by MY-01, BP-01, and PP-01. G4 might well be recommended for sericulture activities in drought-prone areas of the Ananthapuramu district of Andhra Pradesh for commercial silkworm rearing purposes at the field level for better cocoon yield as well as the deep growth and development of the sericulture industry.

## Acknowledgment

Authors acknowledge Dr. P. Sudhakar and B. Vijaya Naidu, Regional Sericulture Research Station, Ananthapuramu for providing necessary plant materials and rearing house for screening the results and thankful to Sri Krishnadevaraya University, Anantapuramu for providing research facilities.

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### Cite this article as:

Obulapathi N., Anandarajulu N., Jhansi Rani T. and S. Thimma Naik. Physiological, biochemical, and silkworm bio-assay studies on certain Mulberry cultivars' responses to water stress. *Annals of Plant Sciences*.10.5 (2021) pp. 4206-4218.

 <http://dx.doi.org/10.21746/aps.2021.10.5.1>

Subject Editor : Mitta Mahendra Nath, Andhra Pradesh, India

Source of support: Nil; Conflict of interest: Nil.