International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 24-03-2021, Accepted: 25-04-2021, Published: 11-05-2021
www.botanyjournals.com
Volume 6, Issue 3, 2021, Page No. 125-131

# Impact of drought on the characteristics attributes in the varieties of *Momordica Charantia* L

**Jayaraj MS, S Suhara Beevy**
Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India

**Abstract**
Drought stress is the most well-known natural stress, considered a genuine threat to crop production. A better understanding of the mechanisms related to this abiotic stress is crucial in designing experiments to improve crop plants. The present investigation analyzed the drought tolerance in two varieties of *Momordica charantia* belongs to the family Cucurbitaceae, *viz*, *Momordica charantia* var. *charantia* (Cultivar) and *Momordica charantia* var. *muricata* (Wild) in terms of physiological and biochemical characters. The experiment was carried out in the greenhouse, using a completely randomized design, in the control and test groups. The control group was maintained by daily irrigation, while the test group was subjected to drought stress by withholding irrigation for 3, 5, 7, 9 and 11days duration, each treatment with five replicates. Analysis of physiological characters revealed a stability in the amount of chlorophyll content and relative water content (RWC) in the wild variety compared to that of the cultivar. Moreover, the amount of Proline, total phenol, total protein and MDA together with the activity of APOX, Catalase, showed a significant increase in the wild variety. The study noticed the presence of an effective antioxidative system and secondary metabolites which conferred drought stress tolerance in *M. charantia* var. *muricata.*

**Keywords:** *Momordica charantia*, wild variety, drought stress, antioxidants

## Introduction

Environmental stresses are the most critical factors responsible for the reduction in plant growth and productivity worldwide. Drought stress is an essential factor that affects crop productivity. Plant sensitivity to drought is a complex phenomenon that depends on various physiological and biochemical aspects. Prolonged drought stress causes damage to the photosynthetic apparatus and its components, mainly the chlorophyll pigments (Nayyar & Gupta 2006) [22]. To overcome this adverse condition, plants adopt physiological and biochemical strategies like ABA production (Abscisic acid) and accumulation of compatible molecules like sugar and Proline. During drought, plants develop enzymatic and non-enzymatic antioxidant systems to protect cells from oxidative damage (Mittler 2002) [20]. The primary antioxidant enzymes that play a crucial role in scavenging the ROS are CAT(Catalase), SOD (Superoxide dis mutase), PPO (Polyphenol oxidase), GPOD (Guaiacol peroxidase), and APOX (Ascorbate peroxidase) (Sgherri *et al.* 2000) [30]. *Momordica charantia* L., one of the most important vegetable crops cultivated in tropical regions of Indian subcontinent including Kerala, belongs to the family Cucurbitaceae. It is commonly known as bitter gourd, karela, balsam pear, or bitter melon, used as a vegetable in ancient Indian, Chinese, and African pharmacopoeia. Two popular varieties in India include *M. charantia* var. *charantia* (Cultivar) with large fusiform fruits and *M. charantia* var. *muricata* (wild) (Chakravarthy 1990) [7] with small (6-10cm) round fruits which contain a high amount of protein, fat, carbohydrate, and minerals (i.e., iron, calcium, vitamin) compared to the variety M. *charantia* var *muricata*. Beevy & Bai (Personal Communication) have suggested the drought tolerance capacity of the wild variety which can be a potential source of biotic or abiotic stress resistance. However, reports are not yet available on the influence of various factors in drought tolerance of the varieties of *M. charantia* var. *muricata*. The present study was carried out to compare the physiological and biochemical changes in the varieties of *M. charantia* under water deficit condition.

## Materials and methods

Materials for the present study included two varieties of *M. charantia viz, M. charantia* var. *charantia* and *M. charantia* var. *muricata*. The seeds of the variety, *M. charantia* var. *charantia* were procured from the College of Agriculture, Vellayani, Thiruvananthapuram, and the wild variety *muricata* collected from the Department of Botany, University of Kerala, Kariavattom. The effects of drought stress on two varieties of *M. charantia* were studied under controlled conditions in a greenhouse (temperature: 28 ± 2°C and relative humidity: 60 ± 5%) at the Department of Botany, University of Kerala. The experiment was carried out in polythene bags (120×40 cm size) and were filled with a 5.5kg soil mixture containing topsoil, river sand, and animal manure in the ratio 1:2:1, respectively. For proper aeration, holes were made in each bag, one opposite to the other. Ten seeds each of uniform size of the two varieties were sown. Twelve days after germination, the seedlings with the same height and number of leaves were selected from each variety and planted in different bags. They were arranged in a complete randomized design with five replications. The plants were thinned to one plant per bag and maintained under controlled conditions in the greenhouse. The plants were then divided into test and control groups, and the control group was maintained by daily irrigation while the test group was subjected to drought stress by withholding irrigation for 3, 5, 7, 9 and 11days. After stress treatment leaves were collected, flash frozen in liquid nitrogen and stored in -80$^{0}$C until analysis. Physiological parameters like relative water content, estimation of pigments and the biochemical parameters like Proline, total carbohydrate, phenol, lipid peroxidation, and antioxidant assay include peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APOX) was carried out.

### Physiological parameters

**Estimation of relative water content (RWC)- Castillo (1996)** [6]

The topmost entire expanded leaves excised from each test and control plants on the day of stress (3day,5day,7day,9day,11day and control) were used for the estimation. The fresh weight (FW) of 5-10 leaf discs (around 1.5cm diameter) was taken immediately and immersed in double-distilled water, and incubated under normal light and room temperature for 4 hrs. The leaflets were taken out, thoroughly wiped with blotting paper to remove the water on the leaf surface, and weighed to obtain the turgid weight (TW). Then the samples were dried in hot air oven at 80°C for 24 hours and weighed to determine the dry weight. RWC was calculated using the formula.

$$\text{RWC \%} = \left[ \frac{\text{Fresh Weight} - \text{Dry Weight}}{\text{Turgid Weight} - \text{Dry Weight}} \right] \times 100$$

**Estimation of pigments (Arnon 1949)** [3]

Total chlorophyll (TC) content was determined using the method described by Arnon (1996) [3]. Fresh leaves (250mg) of the plants were homogenised in 10ml 80% acetone. The extract was centrifuged at 5000 rpm for 10 minutes and the supernatant was collected. Absorbance of the supernatants was measured at 470, 646 and 663 nm using1700 UV–Vis Spectrophotometer (Shimadzu, Japan). The total chlorophyll content in each sample, expressed in mg/g fresh weight was calculated using the formula:

chl.a mg $g^{-1}$ = $(12.7 \times A_{663})$-$(2.6 \times A_{663})$ ml acetone $mg^{-1}$
chl.b mg $g^{-1}$ = $(22.9 \times A_{646})$-$4.68 \times A_{663})$ml acetone $mg^{-1}$

Total chlorophyll mg $g^{-1}$ = (7.05× chl.a) +18.09× chl.b) ml acetone $mg^{-1}$

Carotenoid mg $g^{-1}$ = $(1000 \times A_{470)}$- $(1.9 \times A_{663})$ - $(63.14 \times A_{646})$/214

W= weight of the fresh leaf (g)
V= volume of the extract (ml)
A = is the absorbance as 470,646 and 663nm.

**Estimation of Proline (Bates *et al.* 1973)** [4]

The amount of free Proline was estimated, following the method of Bates *et al.* (1973) [4]. Fresh leaf tissues (100 mg) of the control and treated samples were collected and was homogenized in 10 ml of 3% w/v Sulphosalicylic acid, and the residue was removed by centrifugation at 12000 rpm at 5min. The resulting 2ml supernatant was mixed with 1ml acidic ninhydrin [1.25g ninhydrin in 30 ml glacial acetic acid, 20 ml 6M phosphoric acid] solution and 1 ml glacial acetic acid in a test tube; thereafter, the reaction mixtures were put in a water bath at $100^0$c for 60 min to develop colours. The reaction was terminated by incubating the mixtures in ice for 5 min. Then 2ml Toluene was added and mixed vigorously and kept at room temperature for 20 to 30 min to separate chromophores. The optical density was measured at 520 nm using 1700 UV–Vis Spectrophotometer. Free proline content [l mol/g fresh weight (F. WT)] in leaf tissues was calculated from a standard curve made by using 0–100 l g L-proline.

### Biochemical parameter`

**Protein -Bradford method (1976)** [5]

For protein, estimation of 100mg plant sample was homogenized with 5 ml phosphate buffer saline (PBS). 5ml diluted dye-binding solution was added to each tube, mixed well and allowed the colour to develop for at least 5 minutes; the red dye turns blue. Read the absorbance at 595nm. The standard curve was plotted using the standard protein, and the amount of protein was calculated using the standard curve.

**Estimation of Rate of Lipid Peroxidation (Health and Packer 1968)** [13]

The level of lipid peroxidation was measured by determining malondialdehyde (MDA) content using Heath and Packer's standard procedure (1968) [13]. The fresh leaf samples (500mg) were homogenized in 3ml of 0.1%(w/v) trichloroacetic acid (TCA). This homogenate was centrifuged for 15minute at 10000g. in $4^0$c. 0.5ml of the supernatant and 3mlof the 0.5% thiobarbituric acid (TBA) prepared in 20% trichloroacetic acid (TCA) was mixed, and the resultant mixture was heated to $95^0$c for 25 min in a water bath. The reaction was stopped by cooling the sample in an ice water bath for 10 min, and the absorbance of the supernatant was measured at 532 and 600 nm using UV-1700 UV–Vis Spectrophotometer (Shimadzu) with 1% Thiobarbituric acid in 20% Trichloroacetic acid as control.
(µmol MDA g/FW) = 6.45 (OD532−OD600) −0.56 OD450

**Phenol (Malik and Singh 1980**) [19]

Total phenol content was determined by Folin – Ciocalteau reagent method described by Malik and Singh, 1980 [13]. 0.5 gm of fresh samples were weighed and ground in 10 times volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was saved, and the residue was re-extracted with 5 times the volume of 80% ethanol, centrifuged, and the supernatants were pooled. The supernatant was evaporated to dryness, and the residue was dissolved in a known volume of distilled water (5ml). Different aliquots (0.2-2ml) were pipetted out into test tubes, and the volume was made up to 3 ml with distilled water. Then add 0.5 ml Folin-Ciocalteau reagent; after 3 minutes, 2ml of 20% sodium carbonate solution was added to each tube, mixed thoroughly and placed in a boiling water bath for exactly 1 minute, then cooled and measured the absorbance at 650 nm against a reagent blank. A standard curve was prepared using different concentrations of catechol. The concentration of phenol in the test sample was calculated from the standard curve and expressed as mg phenol /100 g material.

### Enzyme extraction and antioxidant enzyme assays

**Enzyme Extraction**

The frozen leaves (0.5g) of the treated and control plants were homogenized in mortar and pestle with 5.0 ml of 100 mM potassium phosphate buffer at pH 7.0 under ice-cold conditions. The homogenate was centrifuged at 15,000 rpm for 20 min, and the supernatant was used for the analysis of antioxidative enzyme activities.

**Guaiacol peroxidase (POD)**

Guaiacol Peroxidase was estimated according to the method given by Putter (1974) [27]. The reaction mixture comprised of 3.0 ml of 0.1 M phosphate buffer, 50 µl of 20 mM guaiacol solution, 100 µl enzyme extract and 30 µl of 12.3 mM $H_2O_2$ solution. The rate of formation of GDHP (guaiacol dehydrogenation) was followed spectrophotometrically at 436 nm. (UV-1700 visible spectrophotometer, SHIMADZU. Japan)

**Calculations**

One unit of enzyme activity is defined as the amount of enzyme catalyzing the formation of 1.0 µM of GDHP/min./g FW.

$$\text{Unit activity (unit/min/g FW)} = \frac{\text{change in abs./minute} \times \text{total volume (ml)}}{\text{Ext.coefficient} \times \text{vol.of sample taken(ml)}}$$

Where, extinction coefficient = 25 $mM^{-1}$ $cm^{-1}$

$$\text{Specific activity (m mol UA/mg protein)} = \frac{\text{unit activity (units/min/gFW)}}{\text{Protein content (mg/gFW)}}$$

**Ascorbate peroxidase** (APOX)

The APOX enzyme was estimated, according to Nakano and Asada (1981) [22]. 600 µl enzyme extract was added to the reaction mixture containing 1.5ml of phosphate buffer 300 µl ascorbate, 600 µl $H_2O_2$. A decrease in absorbance was measured at 290 nm.

**Calculation**

$$\text{Unit activity (Units/min/g FW)} = \frac{\text{change in abs./minute} \times \text{Total volume (ml)}}{\text{EXt.coefficient} \times \text{vol.of sample taken (ml)}}$$

Where, Extinction coefficient = 2.8 $mM^{-1}cm^{-1}$

$$\text{Specific activity (mol UA/mg protein)} = \frac{\text{Unit Activity (Unit/min/gFW)}}{\text{protein Content(mg/gFW)}}$$

**Catalase (CAT)**

The activity of catalase was determined according to the methods of Aebi (1983) [1]. 300µl enzyme extract was added to the reaction mixture containing 1.5ml phosphate buffer and 1.2 ml of hydro5gen peroxide. The absorbance was recorded at 240nm with a 1700 UV visible Spectrophotometer. The rate of decomposition of $H_2O_2$ was determined after absorbance decreased at 240nm.

**Calculation**

$$\text{Unit activity (Units/min/g FW)} = \frac{\text{change in abs./minute} \times \text{Total volume (ml)}}{\text{EXt.coefficient} \times \text{vol.of sample taken (ml)}}$$

Where, Extinction coefficient = $6.93 \times 10^{-3} mM^{-1}cm^{-1}$

$$\text{Specific activity (mol UA/mg protein)} = \frac{\text{Unit Activity (Unit/min/gFW)}}{\text{Protein Content(mg/gFW)}}$$

**Total protein** was assayed according to the Bradford (1976) [5] method using bovine serum albumin as a standard.

**Statistical Analysis**

The results were reported as the mean ± SE of three separate measurements. The data were also statistically analyzed by the ANOVA followed by Duncan's multiple range test using SPSS (IBM Statistics version 25). P values< 0.05 were considered as significant.

**Results**

**Relative water content (RWC)**

Relative water content (RWC) is considered as one of the important factors to screen drought-tolerant varieties. The control plants of the two varieties showed more than 90% relative water content (Table1). However, the relative water content decreased with an increase in the period of water stress. The wild variety showed a high percentage of water content, i.e., 72.16% and 70.51%. Compared to that of the cultivar (61.33% and 61.633%) during 9days and 11 days of stress, respectively.

**Chlorophyll pigment**

The results showed that the chl a, chl b and total chlorophyll content of both the varieties were decreased as the drought stress was imposed progressively (Fig.1). As compared to control, drought stress slightly declined the production of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl a+b), chlorophyll a/b ratio. Imposition of different days of drought stress (3, 5, 7, 9 and 11 days, respectively) caused a remarkable decrease in chlorophyll content in the variety *M. charantia* var. *charantia*, whereas the wild variety var. *muricata* retained relatively high chlorophyll content when exposed to different days of water stress.

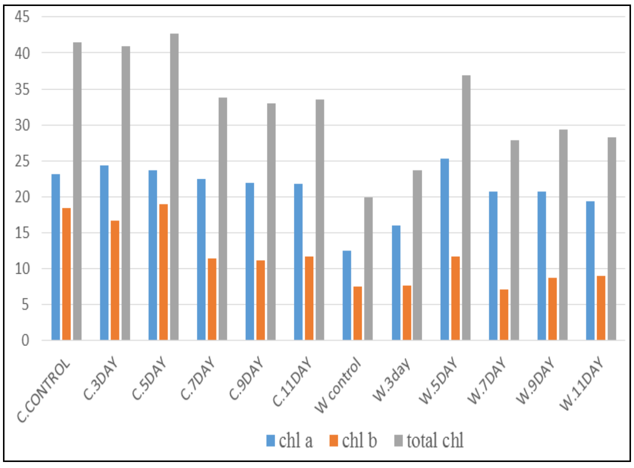


**Fig 1:** Chlorophyll (Chl a and b) content, total chlorophyll (Chl (a + b) leaves for each water stress treatment (the control group treatment, 3, 5, 7, 9 and 11 days) C: cultivar W: Wild

**Proline**

Proline is the most important osmolyte produced during the stress condition. The quantity of Proline produced in control and treated plants in the two varieties are shown (Table1). The amount of proline content in the control plant of wild and cultivar was more or less similar, whereas, in the wild treated plants, it varied from 3.168 to 3.558 u $g^{-1}$ Proline level was found to be increased (3.168, 3.249, 3.222, 3.244, 3.341, 3.558) with respect to the increase in the period of drought, but the cultivar showed only a slight increase.

**Protein**

Imposition of different water stress treatment to both varieties of Momordica charantia showed a significant increase in protein content (Table 1). As compared to the control increase in protein was observed in the wild variety. The protein content significantly increased ($p<0.05$) on 9th and followed the 11th day stressed wild *Momordica charantia* (1.19, 1.23). But a significant decrease was observed in the cultivar *M. charantia*. Wild variety had the highest amount of protein content under water deficit condition as compared with the cultivar.

**Lipid peroxidation**

Lipid peroxidation in the two varieties of *M. charantia* is given in (Table1). The MDA level in the leaves of wild and cultivated varieties of *M. charantia* was significantly influenced by the severity of water deficit though, at the beginning of the experiment, no change was observed in MDA content in the wild and cultivar plant. The rate of lipid peroxidation and MDA content was found to increase with respect to increasing the duration of stress. However, the MDA content was found to be higher in the cultivar.

**Phenol**

Total phenolic (TPC) content during the stress period in the wild and cultivated varieties are given in (Table 1). In normal irrigation regimes, the phenolic content was higher in *M. charantia* var. *charantia* while it was lower in *M. charantia* var. *muricata*. Under drought stress, TPC was increased in wild and cultivated varieties, whereas its increment rate was different in different treatments. A significant difference in TPC was observed between control and treated plants of the two varieties of *M. charantia* var. *charantia* in (Table 1). The highest amount of TPC (1.337) was observed in the 11day stressed wild variety; however, TPC content was found to be higher in the wild variety than the cultivar during drought stress.

**Table 1:** Effect of drought stress on biochemical parameter of two varieties of *Momordica charantia* L.

| Sample | RWC | Protein | Proline | Lipid peroxidation | Phenol |
|---|---|---|---|---|---|
| *C.CONTROL* | 92.722±1.920$^{a}$ | 0.729±0.052$^{cb}$ | 3.032±0.014$^{e}$ | 1.029±0.128$^{bc}$ | 0.569±0.145$^{c}$ |
| *C.3DAY* | 75.000±0.941$^{cd}$ | 0.683±0.024$^{c}$ | 3.147±0.017$^{d}$ | 1.294±0.075$^{abc}$ | 0.498±0.102$^{c}$ |
| *C.5DAY* | 71.808±2.603$^{de}$ | 0.729±0.074$^{cb}$ | 3.210±0.017$^{cd}$ | 1.340±0.045$^{abc}$ | 0.462±0.085$^{c}$ |
| *C.7DAY* | 65.080±3.428$^{efg}$ | 0.760±0.040$^{cb}$ | 3.191±0.010$^{cd}$ | 1.478±0.379$^{abc}$ | 0.454±0.007$^{c}$ |
| *C.9DAY* | 63.334±0.892$^{fg}$ | 0.858±0.043$^{abc}$ | 3.192±0.017$^{cd}$ | 1.543±0.263$^{abc}$ | 0.373±0.063$^{c}$ |
| *C.11DAY* | 61.634±0.555$^{g}$ | 0.918±0.100$^{abc}$ | 3.188±0.041$^{cd}$ | 1.618±0.188$^{a}$ | 0.350±0.055$^{c}$ |
| *W. CONTROL* | 96.02±0.704$^{a}$ | 1.206±0.215$^{a}$ | 3.168±0.034$^{cd}$ | 1.327±0.071$^{abc}$ | 0.427±0.058$^{c}$ |
| *W.3DAY* | 83.06±2.739$^{b}$ | 1.031±0.197$^{ab}$ | 3.249±0.026$^{c}$ | 1.336±0.130$^{abc}$ | 0.425±0.024$^{c}$ |
| *W. 5DAY* | 83.17±2.885$^{b}$ | 1.220±0.134$^{a}$ | 3.222±0.026$^{cd}$ | 1.353±0.043$^{abc}$ | 0.567±0.068$^{c}$ |
| *W. 7DAY* | 80.91±4.212$^{bc}$ | 1.082±0.080$^{ab}$ | 3.244±0.016$^{c}$ | 1.272±0.069$^{abc}$ | 0.923±0.238$^{b}$ |
| *W. 9DAY* | 72.16±3.567$^{de}$ | 1.191±0.093$^{a}$ | 3.341±0.045$^{b}$ | 1.014±0.081$^{bc}$ | 1.401±0.134$^{a}$ |
| *W. 11DAY* | 70.51±2.154$^{def}$ | 1.238±0.175$^{a}$ | 3.558±0.031$^{a}$ | 1.000±0.063$^{c}$ | 1.337±0.093$^{a}$ |

**Guaiacol peroxidase (GPOX)**

Guaiacol peroxidase (GPOX) measured in leaf extracts of control and stressed plants during the period of stress in the two varieties of *M. charantia* are shown in Fig.2. Significant ($P<0.05$) increase in the enzyme activity was observed in response to drought stress on 3rd, 5th, 7th, 9th and 11th days in the wild variety *M. charantia* var. *muricata*. The GPOX activity ranged from 0.006 to 0.022. Maximum activity was found in the 11th day stressed wild *M. charantia* (0.022), whereas the amount was meagre (0.010). As compared to control, the wild *M. charantia* varieties showed higher GPOX activity.

**Ascorbate peroxidase (APOX)**

The ascorbate peroxidase content was increased substantially with an increase in drought stress level. A significantly higher level of ascorbate peroxidase was found in the wild *Momordica charantia* var. *muricata* under water deficit condition in comparison with the control plants (Fig.2). The highest ascorbate peroxidase content (1.269) under drought stress was observed in the 11day stressed wild variety. It was noticed that water-stressed plants had a significantly high amount of ascorbate as compared to the control.

**Catalase (CAT)**

Catalase is one of the essential antioxidant enzymes produced during drought stress or water deficit. The study noticed that the amount of catalase activity differed significantly ($P<0.005$) among the control and treatment groups (Fig.2). The wild variety (*M. charantia* var *muricata*) showed high catalase activity, contrary to the cultivar.

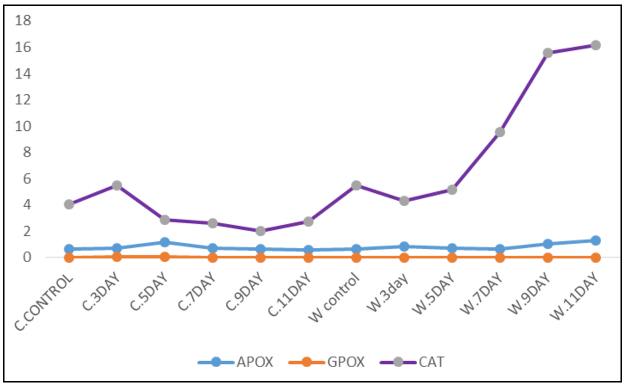


**Fig 2:** Dynamics of antioxidant enzymes in the control and test varieties of *Momordica charantia* L. C: Cultivar, W: wild

**Discussion**

Drought is important abiotic stress, limiting plant growth and productivity in plants, especially in vegetable crops. The present investigation analyzed water deficit's effect in the morphological, physiological and biochemical processes in the herbaceous vegetable crop, *M. charantia*, which needs more water for their normal metabolic processes.

Relative water content (RWC) of the leaves has been proposed as a better indicator of water stress than other growth biochemical parameters of plants. The present investigation noticed a decrease in the relative water content in the cultivar *M. charantia* var *charantia,* whereas the wild variety maintained its water content. Moreover, wild variety showed a higher percentage of RWC % (76% to 85%) compared to the cultivar. Plants having higher yields under drought stress should have high RWC (Hassanzadeh *et al.* 2009) [12].

Pigments such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoid were decreased during different days of stress interval in the cultivated variety, whereas increment in the amount of pigment was observed in the wild during the period of stress, particularly on 9$^{th}$ and 11$^{th}$ day of stress. High chlorophyll content helps to increase light absorption and thereby increase the amount of photosynthate that in turn increase the yield in the wild variety. Earlier studies revealed that high chlorophyll and carotenoid content has also been associated with stress tolerance in plants (Pastori & Trippi 1992 [25]; Sairam 1994 [29]; Kraus *et al.* 1995 [17]). The low-level pigment content found in the cultivar in the present study may be due to the decrease in the relative water content.

Proline is an important osmolyte accumulated during the period of drought. A higher amount of Proline was accumulated in the wild variety *M. charantia* var *muricata* during 9$^{th}$ and 11$^{th}$ day of stress compared to the cultivar. The increase in the amount of Proline indicates its tolerance to water stress. An increased amount of proline accumulation during the drought period was reported in rice, pea and maize (Alexieva *et al.* 2001[2]; Sánchez Francisco 1998 [30]; Choudhary *et al.* 2005 [9]). According to Solanki & Sarangi (2014) [35], the Proline is used as a dependable index for screening drought tolerance in plants.

The present study revealed high protein content in the leaf of wild variety than the cultivar. The production of a high amount of protein may be of help to overcome drought stress. The same result was shown in the *Sesamum indicum* L. (Bray 1994) [6]. Palva (1994) [24] reported that a particular protein, probably associated with stress tolerance, is synthesized in response to water stress. Enhanced level of protein was also reported in *Cassia fistula* (Sinhababu & Kar 2002) [34]

In the present investigation, the amount of MDA was increased with respect to the increase in the period of stress. According to Peever and Higgins (1989) [26], the final product of lipid peroxidation is MDA. A higher amount of MDA represents a higher degree of membrane damage. Under osmotic stress, the amount of MDA content evaluation is a physiological indicator of drought tolerance (Luo *et al.* 2008) [18]. The present study revealed that the MDA content was meagre in the sensitive cultivar variety than the tolerant wild genotype. The same result was observed in the salt-tolerant genotype of wheat (Sairam *et al.* 2005) [29] and in drought-tolerant sesame (Fazeli *et al.* 2007) [11]. The higher level of MDA (malondialdehyde) content in the leaf of wild varieties indicated better protection against oxidative damage.

Phenol is another important antioxidant. Increased antioxidant activity during drought correlates with total phenolic content in blueberry (Ehlenfeldt & Prior 2001) [10]. Phenolic content showed remarkable variations in both wild and cultivated varieties of *Momordica* during the period of stress. Many kinds of plant phenolic have been considered to be the main lines of cell acclimation against stress in plants (Rivero *et al.* 2001) [28].

According to Mittler (2002) [21], the peroxidase enzyme involved in growth, development and senescence, is widely distributed in plant tissues. In the present investigation, POD levels were found to increase when days of drought increased compared to the control, and the level of increment was higher in the wild variety than in the cultivar. Higher POD content in the drought-tolerant plant helps to protect themselves against oxidative stress (Fazeli *et al.* 2017) [11]. When a plant is exposed to drought stress number of antioxidant enzyme level was increased, this elevated activity can be correlated with an increase in drought tolerance (Srivalli *et al.* 2003 [36], & *Hojati et al.* 2011 [15]). During the period of water stress, the level of POD activity was increased in *Arabidopsis thaliana* as reported by Jung (2004) [16] and Shao *et al.* (2007) [33] in wheat.

Ascorbate peroxidase is an antioxidant enzyme, which reduces the effect of ROS during drought or oxidative stress. In the present investigation, APX activity was found higher in the wild varieties of *M. charantia* than in the cultivar. Higher activity of APX indicates its effective removal of $H_2O_2$ during the period of drought in the tolerant Brassica napas cultivar (Mirzai *et al*. 2013) [20]

Catalase plays an essential role in oxidative stress. In the present study, catalase was found to increase in both the varieties of stressed plants, but the amount of the enzyme was meagre in the cultivar compared to the wild. The higher the catalase activity during drought stress indicates its ability to decompose $H_2O_2$ in the stressed condition. Sánchez-Rodríguez (2010) [31] and Wang *et al*. (2009)[37] reported the same in the stressed plants of Tomato and Alfalfa. The physiological and biochemical characterization indicated higher drought tolerance of the wild variety of *M. charantia* than the cultivar.

## Conclusion

Drought stress is a serious issue facing all over the world which negatively affects the agricultural sector, limiting the production of crop and their quality. The loss of crop productivity under water stress is directly correlated to the physiological and biochemical processes at the cellular and molecular levels of plants. Analysis of the physiological and biochemical attribute of drought tolerance in the wild and cultivated varieties of *M. charantia* revealed that the two varieties differ in their tolerance potential against drought stress. Accumulation of phenolics, Proline and higher efficiency of the antioxidant system eventually resulted in higher RWC by wild variety indicating its drought tolerance capacity. Hence, the study emphasized the significance of physiological and biochemical markers to evaluate drought tolerance potentials of the wild genotype of *M. charantia* thereby widening its possibilities for crop improvement programmes.

## Reference

1. Aebi HE. Catalase. Methods of enzymatic analysis, 1983.
2. Alexieva V, Sergiev I, Mapelli S, Karanov E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell & Environment,2001:24(12):1337-44.

3. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant physiology,1949:4(1):1.
4. Bates LS, Waldren RP, Teare ID. Rapid determination of free Proline for water-stress studies. Plant and soil,1973:39(1):205-7.
5. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry,1976:7:72(1-2):248-54.
6. Bray EA. Alterations in gene expression in response to water deficit. Stress-induced gene expression in plants,1994:21:1-23.
7. Castillo FJ. Antioxidative protection in the inducible CAM plant *Sedum album* L. following the imposition of severe water stress and recovery. Oecologia,1996:107(4):469-77.
8. Chakravarty HL. Cucurbits of India and their role in the development of vegetable crops. Biology and Utilization of the Cucurbitaceae (DM Bates, RW Robinson, and C. Jeffrey, eds.). Cornell University Press, Ithaca, NY,1990, 325-34.
9. Choudhary NL, Sairam RK, Tyagi A. Expression of $\Delta^1$-pyrroline-5-carboxylate synthetase gene during drought in rice (*Oryza sativa* L.).
10. Ehlenfeldt MK, Prior RL. Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry. Journal of Agricultural and Food Chemistry. 2001:49(5):2222-7.
11. Fazeli F, Ghorbanli M, Niknam V. Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars. Biologia Plantarum,2007:51(1):98-103.
12. Hassanzadeh M, Ebadi A, Panahyan-e-Kivi M, Eshghi AG, Jamaati-e-Somarin S, Saeidi M *et al*. Evaluation of drought stress on relative water content and chlorophyll content of sesame (*Sesamum indicum* L.) genotypes at early flowering stage. Research Journal of Environmental Sciences,2009:3(3):345-50.
13. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of biochemistry and biophysics,1968:125(1):189-98.
14. Hildebrandt S, Steinhart H, Paschke A. Comparison of different extraction solutions for the analysis of allergens in hen's egg. Food chemistry,2008:108(3):1088-93.
15. Hojati M, Modarres-Sanavy SA, Karimi M, Ghanati F. Responses of growth and antioxidant systems in *Carthamus tinctorius* L. under water deficit stress. Acta physiologiae plantarum,2011:33(1):105-12.
16. Jung S. Variation in antioxidant metabolism of young and mature leaves of *Arabidopsis thaliana* subjected to drought. Plant Science,2004:166(2):459-66.
17. Kraus TE, McKersie BD, Fletcher RA. Paclobutrazol-induced tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity. Journal of Plant Physiology,1995:145(4):570-6.
18. Luo Y, Tang H, Zhang Y. Production of reactive oxygen species and antioxidant metabolism about strawberry leaves to low temperatures. Journal of Agricultural Science,2011:3(2):89.
19. Malik CP, Singh MB. Plant enzymology and histo-enzymology.
20. Mirzai M, Moeini A, Ghanati F. Effects of drought stress on the lipid peroxidation and antioxidant enzyme activities in two canola (*Brassica napus* L.) cultivars.
21. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends in plant science,2002:7(9):405-10.
22. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and cell physiology,19811:22(5):867-80.
23. Nayyar H, Gupta D. Differential sensitivity of C3 and C4 plants to water deficit stress: association with oxidative stress and antioxidants. Environmental and Experimental Botany,2006:58(1-3):106-13.
24. Palva ET. Gene expression under low temperature stress. Stress induced gene expression in plants,1994:21:103-30.
25. Pastori GM, Trippi VS. Oxidative stress induces high rate of glutathione reductase synthesis in a drought-resistant maize strain. Plant and cell Physiology,1992:33(7):957-61.
26. Peever TL, Higgins VJ. Electrolyte leakage, lipoxygenase, and lipid peroxidation induced in tomato leaf tissue by specific and nonspecific elicitors from *Cladosporium fulvum*. Plant physiology,1989:90(3):867-75.
27. Pu¨tter J Peroxidase. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Academic Press, New York,1974, 685-690
28. Rivero RM, Ruiz JM, Garcıa PC, Lopez-Lefebre LR, Sánchez E, Romero L. Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. Plant Science,2001:160(2):315-21.
29. Sairam RK. Effect of moisture-stress on physiological activities of two contrasting wheat genotypes. Indian Journal of Experimental Biology,1994:32:594-.
30. Sánchez FJ, Manzanares M, de Andres EF, Tenorio JL, Ayerbe L. Turgor maintenance, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in response to water stress. Field crops research,1998:59(3):225-35.
31. Sánchez-Rodríguez E, Rubio-Wilhelmi M, Cervilla LM, Blasco B, Rios JJ, Rosales MA, Romero L, Ruiz JM. Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. Plant science,2010:178(1):30-40.
32. Sgherri CL, Maffei M, Navari-Izzo F. Antioxidative enzymes in wheat subjected to increasing water deficit and rewatering. Journal of Plant Physiology,2000:157(3):273-9.
33. Shao HB, Chu LY, Wu G, Zhang JH, Lu ZH, Hu YC. Changes of some anti-oxidative physiological indices under soil water deficits among 10 wheat (*Triticum aestivum* L.) genotypes at tillering stage. Colloids and Surfaces B: Biointerfaces,2007:54(2):143-9.
34. Sinhababu A, Kar RK. Response of four fuel-wood yielding seedlings to water stress. Indian journal of plant physiology,2002:7(1):88-91.
35. Solanki JK, Sarangi SK. Effect of drought stress on proline accumulation in peanut genotypes. International journal of advanced research,2014:2(10):301-9.

36. Srivalli B, Sharma G, Khanna-Chopra R. Antioxidative defense system in an upland rice cultivar subjected to increasing intensity of water stress followed by recovery. Physiologia Plantarum,2003:119(4):503-12.
37. Wang WB, Kim YH, Lee HS, Kim KY, Deng XP, Kwak SS. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. Plant physiology and Biochemistry,2009:47(7):570-7.