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# Alleviation The Deleterious Impact of Water Stress on *Sorghum Bicolor* (L.) Plants Using Biostimulant Humic Acid

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## ABSTRACT

This investigate was conducted during the summer seasons of 2021 and 2022 to study the beneficial role of humic acid as biostimulant for reducing the harmful effect of water deficit on sorghum plant. Results show that water deficit at 75% FC and 50% FC caused decreases in vegetative growth parameters; grains yield and yield components, carbohydrate content of the yielded grains accompanied by increases in total soluble sugar and proline of the dry leaf tissues and protein content of the yielded grains. Regarding the interaction between water deficit and humic acid treatments, it was noted that humic acid at 50 mg/L and 100 mg/L markedly increased vegetative growth parameters, total soluble sugar and protein of the dry leaf tissues, grains yield and yield components, carbohydrate content and protein content of the yielded grains of sorghum grown under 95% FC, 75% FC and 50% FC relative to corresponding controls. It was noted that humic acid at 50mg/L and 100mg/L significantly increased weight of the yielded grains/plant by 11.46% and 34.50% respectively in plant irrigated with 95%FC, and by 17.25% and 24.37% respectively in plants irrigated with 75% FC and by 9.16% and 38.50% respectively in plants irrigated with 50% FC relative to corresponding controls. Clearly, humic acid treatment at 100mg/L was more effective than humic acid at 50mg/L under all conditions.

***Keywords:*** *Sorghum bicolor*, biostimulant, humic substance, water deficit

## Introduction

Nowadays, due to the scarcity of water or the low quality of the available water, agricultural food production is limited in many places of the world (Turhan and Kuscu 2020; Forotaghe *et al.,* 2021). Water deficit is considered as one of the most destructive abiotic factors, affecting 64% of the global land area and ultimately threatening agricultural productivity all over the world (Ma *et al.,* 2020; Tyagi *et al.,* 2022). Since, drought leads to a 9 -10% decline in global cereal production (Lesk *et al.,* 2016). In the future, drought has been identified as one of the major global problems that caused dramatically effect on different agricultural activities particularly in arid and semiarid regions and lead to impair plant performance and thereby reducing crop productivity and quality (deOliveira *et al.,* 2022; Ma *et al.,* 2022). Plants behave differently to stress depending on the species of plant, and its stage of development, along with the duration and degree of severity the water shortage. Water stress inhibits plant growth, due to the limited amount of water in the soil, causing transpiration to exceed the amount of water absorbed, and leading to a considerable reduction in the potential for water and cell turgor which causes significant alterations at the physiological and biochemical processes (Ma *et al.,* 2020; Ma *et al.,* 2022; Ramadan *et al.,* 2023; Raza *et al.,* 2023).Water stress triggered physiological modifications in plants, including a decrease in cell water potential and closure of stomatal, leading to reduced availability of $CO_2$ for the plants and seriously affecting photosynthetic process, yield of crops and their nutritional quality (Saddiq *et al.,* 2021).According to studies by Ma *et al.,* (2020), Ahluwalia *et al.,* (2021) and others, drought stress inhibits cell expansion by causing cellular dehydration, decrease

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in water potential, transpiration and nutrient uptake. Moreover, water stress leads to secondary stress, called oxidative stress, during which the excessive accumulation of reactive oxygen species, such as hydrogen peroxide ($H_2O_2$), superoxide ($O^{2-}$), and hydroxyl radicals ($OH^-$), damages membrane permeability, initiates lipid peroxidation, and degrades both nucleic acids and proteins (Singh *et al.,* 2022). Under normal conditions, ROS are produced at low concentration from oxidative processes such as photosynthesis and respiration without any negative effect on plants. In stressful circumstances, levels of reactive oxygen species increased as a measure of the oxidative damage induced by the stress (Foyer 2002). Overproduction of ROS caused series of damages to plant metabolism, such as degradation of photosynthetic components, lowering the nutrient uptake, deactivation of proteins and enzymes, and disruption of the structure and permeability of the cell membrane via lipid peroxidation (Ma *et al.,* 2022; Vishnupradeep *et al.,* 2022). To adapt to harsh environmental conditions, plants have developed a number of mechanisms, as well as signaling and acclimation strategies (Zandalinas *et al.,* 2020). These mechanisms include increasing water uptake by developing large and deep root systems, reducing water loss by accumulating osmolytes, and preventing membrane disintegration (Kaya *et al.,* 2018; Kiran *et al.,* 2019). Likewise, plants' ability to withstand drought is favorably connected with maintain a high level of enzymatic and non-enzymatic antioxidants (Ramadan *et al.,* 2023; Raza*et al.,* 2023). Antioxidants enzymes as catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) are effectively scavenge reactive oxygen species. Moreover, reactive oxygen species are controlled by plants using non-enzymatic antioxidant metabolites such flavonoids, proline, and phenolics to prevent oxidative stress (Zandalinas *et al.,* 2020; Ma *et al.,* 2020; Ma *et al.,* 2022). Water shortage impacts sorghum growth and development from germination to reproductive and grain-filling stages as well as the physicochemical characteristics of the plants, leading to in a significant decrease in grain quality and quantity (Bobade *et al.,* 2019; Abreha *et al.,* 2022).

Biostimulants are a viable eco-friendly strategy, used to deal with environmental issues and satisfy the requirement for developing sustainable/modern agriculture (Lau *et al.,* 2022).

Humic acid (HA) is biostimulant that has a significant impact on plant growth, yield, and resistance to a biotic stress. Moreover, foliar application of HA is eco-friendly, inexpensive, and does not pollute the soil or the air (Suddarth *et al.,* 2019; Altaf*et al.,* 2023). Humic acid contains organic carbon (C) nitrogen (N), oxygen (O), hydrogen (H), and sulfur (S) (Ampong *et al.,* 2022). So, humic acid is considered as one of the used organic mineral fertilizers. In addition, these elements of humic acid are necessary for plant growth and developmental stages, and act as a co-factor in a variety of biological processes such as the production of proteins, nucleic acids and photosynthetic pigments (Danesh-Talab *et al.,* 2014; Ghania *et al.,* 2015). The two functional groups that are most prevalent in humic acid are the phenolic (OH) and carboxylic (COOH) groups (Nardi *et al.,* 2021). These two functional groups are mainly responsible for humic acid functions as enhancing physical and chemical properties of the soil (Nardi *et al.,* 2021). Humic acid enhances the properties of soil, as aggregation, permeability, aeration, water-holding capacity, and availability of micronutrients in the soil (Sharif *et al.,* 2002). Humic acid could be applied directly to plants at low doses to increase growth, nutrient availability, and yield (De Azevedo *et al.,* 2019; Altaf *et al.,* 2023) by influencing the mechanisms involved in hormone fluxes, water and nutrient uptake, photosynthesis, synthesis of protein, and respiration of cell (Berbara *et al.,* 2014; Matuszak-Slamani *et al.,* 2022). Humic acid has a hormone-like activity (DeMelo *et al.,* 2016; Yang *et al.,* 2021) and efficiently contributes to the synthesis and function of a number of enzymes (Mikkelsen 2005). So, humic acid is also believed to play a pronounced impact in improving plant resistance to drought stress and enhancing the productivity of the crops under limited water conditions (Karaman *et al.,* 2017 Kaya *et al.,* 2018; Altaf *et al.,* 2023). In addition, foliar spraying of humic acid boosted maize production via minimizing the effects of water scarcity throughout both the vegetative and reproductive stages of plants (Gomaa *et al.,* 2014). According to Haghighi *et al.,* (2012 a), humic acid detoxifies $H_2O_2$ to mitigate the oxidative damage to photosynthesis during drought.In addition, humic acid significantly reduced the accumulation of reactive oxygen species (ROS) and stimulated antioxidative enzymes (El-Bassiouny et al.2014; García *et al.,* 2016;Altaf *et al.,* 2023).

Sorghum (*Sorghum bicolor* (L.) Moench), especially in dry and semi-arid locations where drought stress is a significant limiting factor, provides food for about 500 million people in developing countries. Although sorghum is believed of being tolerant, water stress is still significantly hampers its output and nutritional quality across the major growing locations (Sarshad *et al.,* 2021; Abreha *et al.,* 2022).It provides protein, fiber rich, and gluten-free nutrition (Impa *et al.,* 2019). In addition to human nutrition, it is utilized as a source of feedstock and for the creation of bioethanol (Mathur *et al.,* 2017).

This work aimed to study the beneficial impact of humic acid as biostimulant for decreasing the deleterious role of water stress on sorghum plant.

## 2. Materials and Methods

Two pot experiments were conducted at Botany greenhouse, National Research Centre, Egypt (30° 3' 0" N / 31° 15' 0" E), during the summer seasons of 2021 and 2022.

### 2.1. Experimental design

Sorghum grains (cv. Dorado) were purchased from Agriculture Research Center, Egypt. The chosen grains that had the same size and color were thoroughly cleaned with distilled water before being sterilized for about two minutes with a solution of 1% sodium hypochlorite and thoroughly washed again with distilled water. Each plastic pot contained 10.0 kg of clay soil mixed with sand soil in a ratio of 3:1(v:v) respectively. Four uniform air dried sorghum grains were sown in each pot along a central row at a depth of 30 mm. The pots were arranged in a split plot design with six replicates. The plants received regular irrigation at 95% of field capacity (FC) before water stress treatments were applied. The humic acid (0, 50 and 100 mg/L) was applied at 40 and 50 days after sowing in both seasons. Three irrigation levels were used (95%FC, 75%FC and 50%FC) as normal irrigation, moderate irrigation and sever irrigation respectively. After 40 days from sowing, the plants were subjected to water stress treatments.

### 2.2. Data collection

During vegetative growth stage, plant samples were taken randomly from each treatment to estimate the growth criteria (plant height (cm), number of leaves/plant and dry weight of both leaves and stem/plant). Moreover, at harvest stage, plant height, panicle height, panicle weight, straw weight, weight of grains/ plant and 100- grains weight were recorded.

### 2.3. Chemical analysis

Total soluble sugars of dry leaf tissues and yielded grains were estimated by the method of Mecozzi (2005). Proline content of dry leaf tissues was determined by Kalsoom *et al.,* (2016). Total carbohydrate content of the yielded grains was determined according to Albalasmeh *et al.,* (2013). Polysaccharides were calculated by the difference between total carbohydrate content and total soluble sugars. Total protein of the yielded grains was determined according to Pedrol and Tamayo (2001).

### 2.4. Genetics analysis

DNA of sorghum under study was isolated fresh and young leaves using CTAB (Khater *et al.,* 2022). A total of 10 ISSR primers were tried, but 5 ISSR primers ($(AC)_8$ T, $(CT)_8$ TG, $(CT)_8$ GC, $(CT)_8$ AC and $(CA)_6$ GT) with positive results were used in this study. However, the primers were chosen upon the production of distinct and reproducible bands in PCR reactions. Moreover, PCR procedures were done as described by Khater *et al.* (2022).

### 2.6. Statistical analysis

Analysis of variance was used to statistically examine the average data of two seasons. According to Silva and Azevedo (2016), the differences in means were calculated by the least significant differences (LSD) at the 5% level.

## 3. Results

Tables (1) shows that water deficit at 75% FC and 50% FC caused non significant decreases in plant height and leaves number /plant accompanied by significant decreases in dry weight of leaves /plant (43.92% and 50.82% respectively) and dry weight of stem (11.78% and 10.16% respectively) relative to well watered plant (95% FC).

Regarding the interaction between water deficit and humic treatments, it was noted that humic acid at 50 mg/L and 100 mg/L increased all investigated growth parameters of sorghum plant grown under 95% FC, 75% FC, 50% FC relative to corresponding controls.

Sorghum plants irrigated either with well watered (95% FC) or moderate water deficit at 75% FC showed significant increases in both dry weight of leaves/plant and dry weight of stem due to humic acid at 50 mg/L and 100 mg/L as compared with corresponding controls. Whereas, sorghum plant grown under sever water deficit at 50% FC showed significant increases in dry weight of leaves and stem by using humic acid at 100 mg/L relative to corresponding control. Humic acid treatment at 100mg/L was more effective than humic acid at 50mg/L under all conditions. Since, 100mg/L humic acid significantly increased dry weight of leaves/plant by 28.45%, 73.89%, 42.13% in sorghum plant irrigated with 95% FC, 75% FC and 50% FC respectively relative to corresponding controls. Likewise, 100mg/L humic acid significantly increased dry weight of stem by 24.79%, 29.95%, 16.28% in sorghum plant irrigated with 95% FC, 75% FC and 50% FC respectively relative to corresponding controls.

**Table 1:** Interaction effects among water stress and humic acid on vegetative growth characters of sorghum (*sorghum bicolor* L. Moench) plants.

| Water stress | Humic acid (mg/L) | Plant height (cm) | Number of leaves /plant | Dry weight of leaves/plant (g) | Dry weight of stem(g) |
|---|---|---|---|---|---|
| **95% FC** | 0 | 125.33 | 6.00 | 3.62 | 2.46 |
| | 50 | 133.67 | 6.00 | 4.38 | 2.71 |
| | 100 | 150.67 | 6.33 | 4.65 | 3.07 |
| **75% FC** | 0 | 118.00 | 5.33 | 2.03 | 2.17 |
| | 50 | 123.00 | 5.67 | 3.15 | 2.64 |
| | 100 | 125.67 | 5.67 | 3.53 | 2.82 |
| **50% FC** | 0 | 118.33 | 5.00 | 1.78 | 2.21* |
| | 50 | 124.67 | 5.33 | 2.32 | 2.27 |
| | 100 | 131.67 | 5.33 | 2.53 | 2.57 |
| **LSD at 5%** | | **9.76** | **1.51** | **0.73** | **0.23** |

Figure 1 illustrates that water stress at 75%FC and 50% FC caused non significant increases in total soluble sugars accompanied by significant increases in proline content of dry leaf tissues relative to those irrigated with well watered (95% FC).

Regarding interaction between water deficit and humic acid treatments, it was noted that humic acid at 50 mg/L and 100 mg/L caused increases in total soluble sugar and proline under all conditions of water irrigation relative to corresponding controls.

In details, humic acid at 50mg/L and 100mg/L caused non significant increase in total soluble sugar in sorghum plant irrigated with 95%FC or 75% FC relative to corresponding controls. Whereas, sorghum plant irrigated with 50%FC, humic acid at 50mg/L and 100mg/L significantly increased total soluble sugar in dry leaf tissues by 39.84% and 64.06% respectively relative to corresponding control. Meanwhile, humic acid at 100mg/L significantly increased proline content of dry leaf tissues by 19.54% in plants irrigated with 95%FC and by 14.43% in plants irrigated with 75%FC and by 12.65% in plants irrigated with 50%FC relative to corresponding controls.

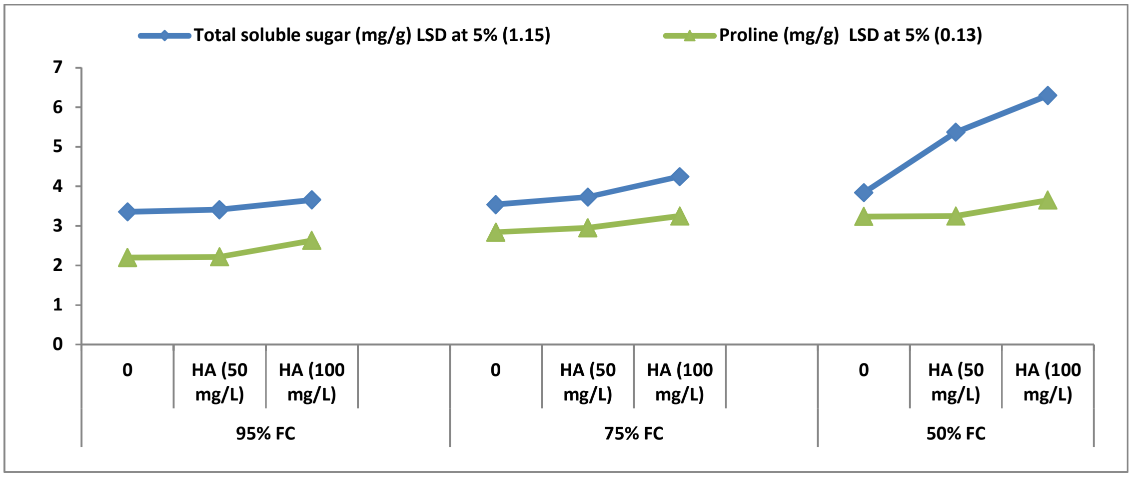


**Fig. 1:** Interaction effects among water stress and humic acid on total soluble sugar and proline of dry leaf tissues of sorghum (*sorghum bicolor* (L. ) Moench)plants**.**

Table 2 shows that water deficit at 75%FC and 50% FC caused significant decreases in grains yield/plant and yield components relative to those irrigated with 95% FC. Since irrigation with 75%FC and 50% FC significantly decreased grains weight /plant by 9.19% and 31.89% respectively relative to well irrigated plant.

Both humic acid at 50mg/l and 100mg/l significantly increased grains weight /plant and yield components in plant irrigated with 95%FC, 75%, and 50% FC relative to corresponding controls.It was noted that humic acid at 50mg/l and 100mg/l significantly increased weight of gains/plant by 11.46% and 34.50% in plant irrigated with 95%FC, and by 17.25% and 24.37% in plants irrigated with 75%FC and by 9.16% and 38.50% in plants irrigated with 50% FC relative to corresponding controls. So, the effect of 100mg/L humic acid was more pronounced than 50mg/L humic acid in enhancing the sorghum yield under normal condition or water deficit conditions.

**Table 2:** Interaction effects among water stress and humic acid on grain yield and yield components of sorghum (*Sorghum bicolor* L.) Moench).

| Water stress | Humic acid (mg/L) | Panicle height (cm) | Panicle weight (g) | Straw weight/plant (g) | weight of grains/plant (g) | Weight of 100 grains (g) |
|---|---|---|---|---|---|---|
| **95% FC** | 0 | 15.83 | 12.52 | 16.27 | 8.81 | 3.71 |
| | 50 | 16.00 | 13.84 | 17.24 | 9.82 | 4.00 |
| | 100 | 17.00 | 14.81 | 28.82 | 11.85 | 4.17 |
| **75% FC** | 0 | 13.33 | 10.64 | 15.23 | 8.00 | 3.10 |
| | 50 | 15.00 | 11.99 | 16.56 | 9.38 | 3.34 |
| | 100 | 16.17 | 12.22 | 17.24 | 9.95 | 3.35 |
| **50% FC** | 0 | 13.33 | 6.91 | 13.42 | 6.00 | 2.40 |
| | 50 | 13.67 | 9.72 | 15.82 | 6.55 | 3.10 |
| | 100 | 15.67 | 10.63 | 17.31 | 8.31 | 3.22 |
| **LSD at 5%** | | **1.51** | **1.28** | **0.86** | **0.52** | **0.22** |

Figure 2 shows that water stress at 75% FC and 50% FC significantly reduced total carbohydrate content accompanied by significant increases in protein content of the yielded grains relative to well watered sorghum plant (95% FC).

Humic acid at 50mg/L and 100mg/ L significantly increased total carbohydrate and protein content in well irrigated plant or those irrigated with 75%FC and 50% FC relative to corresponding controls. The effect of humic acid at 100 mg/L was more pronounced than that of 50mg/ L.

In plants irrigated with 95% FC, 100mg/L humic acid significantly increased carbohydrate content from 70.32% to 84.71% and protein content from 8.80% to 12.50% in the yielded grains. In pants irrigated with 75% FC, 100mg/l humic acid significantly increased carbohydrate content from 65.21% to 75.96% and protein content from 9.30% to 12.50%. In pants irrigated with 50% FC, 100mg/l humic acid significantly increased carbohydrate content from 65.02% to 73.90% and protein content from 9.55% to 10.50%.

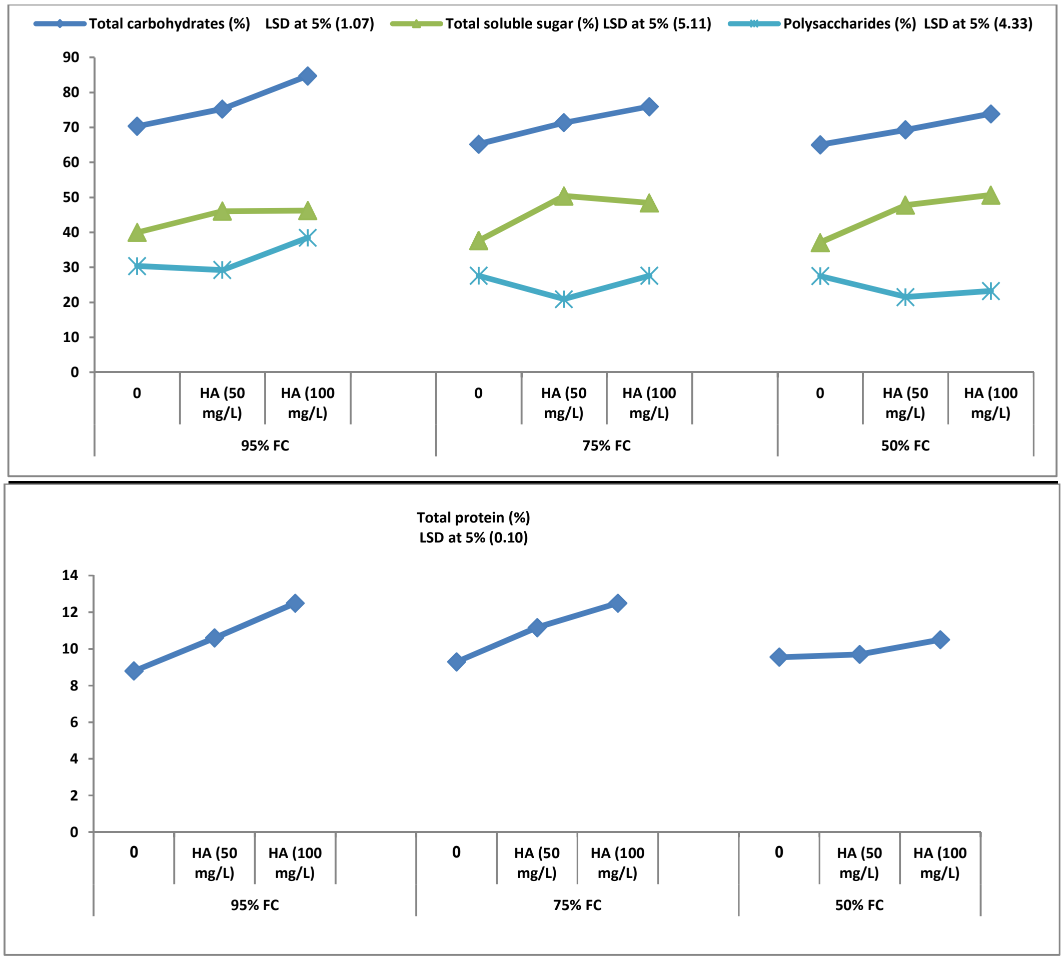


**Fig. 2:** Interaction effects among water stress and humic acid on carbohydrate and protein content of the yielded of grain of sorghum (*Sorghum bicolor* L.) Moench).

Table (3) and Figure (3) illustrated the interaction effects among water stress and humic acid on sorghum plants under study using 5 ISSR primers. It was noticed that 93 bands (Total bands (TB)) with molecular weights ranged from 65.27 – 1480.18 bp were detected. Moreover, these total band were distributed between 45 polymorphic bands (PB) with an average 9.00, 40unique bands (UB) with an average 8.00 and 8 monomorphic bands (MB) with an average 1.6, so, polymorphism percentage (PB%) was 91.14%.

However, the highest level of polymorphism was observed with both primers (CT)$_8$ TG and (CA)$_6$ GT) that showed (100%) polymorphism, while the lowest polymorphism was 73.33% with primer (CT)$_8$ AC). (Table 3)

Moreover, every primer detected a different number of bands with different ranges of molecular weights and polymorphism as follow:

- 21 bands with molecular weights ranged between (121.35 – 1489.18bp) were detected using ((AC)$_8$ T) primer, and these repressible bands were distributed as 3 (MB), 9 (UB) and 9 (PB) with 85.72% polymorphism.
- 17 bands with molecular weights ranged between (65.27 – 464.51bp) and 100.00% polymorphism were detected using (CA)$_6$ GT) primer, and distributed as 0 (MB), 6 (UB) and 11 (PB).
- 15 bands with molecular weights ranged between (121.13 – 1282.636bp) and 86.67% polymorphism were detected using (CT)$_8$ GC) primer, and distributed as 2 (MB), 7 (UB) and 6 (PB).

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- 18 bands with molecular weights ranged between (117.82–1134.42bp) and 83.33% polymorphism were detected using($(CT)_8$ AC) primer, and distributed as 3 (MB), 7 (UB) and 8 (PB) and at the end detected (Table 5).
- On the other hand, the highest total amplified bands (22), polymorphic bands (11), unique (11) and polymorphism% (100.00%), respectively, were scored with ($(CT)_8$ TG) IS-02 primer with molecular weights ranged between (122.99 – 1342.84bp) (Table 3 and Figure 3).

**Table 3:** Effect of water stress and humic acid on reproducible DNA fragments of sorghum plants using ISSR molecular markers.

| Primers | Marker weights (bp) | Amplified bands | | | | PB % |
|---|---|---|---|---|---|---|
| | | TBV | MB | UB | PB | |
| **$(AC)_8$ T** | **1489.18 –121.35** | 21 | 3 | 9 | 9 | 85.72 % |
| **$(CT)_8$ TG** | **1342.84 – 122.99** | 22 | - | 11 | 11 | 100.00 % |
| **$(CT)_8$ GC** | **1282.63 – 121.13** | 15 | 2 | 7 | 6 | 86.67 % |
| **$(CT)_8$ AC** | **1134.43 – 117.82** | 18 | 3 | 7 | 8 | 83.33 % |
| **$(CA)_6$ GT** | **464.513 – 65.27** | 17 | - | 6 | 11 | 100.00 % |
| **Total** | | **93** | **8** | **40** | **45** | **-** |
| **Average** | | **18.6** | **1.6** | **8.0** | **9.0** | **91.14 %** |

Table (4) represent a general idea about the reproducible bands detected using previous five ISSR primers. However, Table 4 draw the attention to number, size, type and conjugative reproducible bands that were detected by each primer separately. Moreover, there were some bands which have the same molecular weight and these called polymorphic bands and this conjunction due to the effect of the treatments.

**Table 4:** Effect of water stress and humic acid on molecular weights of reproducible DNA fragments of sorghum plants using ISSR molecular markers.

| MW | 95% FC | | | 75% FC | | | 50% FC | | | PB% |
|---|---|---|---|---|---|---|---|---|---|---|
| | Humic ($0mgL^{-1}$) | Humic ($50mgL^{-1}$) | Humic ($100mgL^{-1}$) | Humic ($0mgL^{-1}$) | Humic ($50mg L^{-1}$) | Humic ($100 mgL^{-1}$) | Humic ($0 mgL^{-1}$) | Humic ($50mgL^{-1}$) | Humic (100mg L-1) | |
| | | | | | **(AC)8 T** | | | | | |
| **1489.18** | - | - | - | - | - | + | + | + | - | **PB** |
| **1080.67** | + | + | + | + | + | + | + | + | + | **MB** |
| **827.27** | - | - | - | - | - | - | + | - | - | **UB** |
| **787.72** | + | - | - | + | + | - | - | - | - | **PB** |
| **760.15** | - | + | - | - | - | - | - | - | - | **UB** |
| **683.09** | - | - | - | - | - | + | + | + | + | **PB** |
| **544.30** | + | + | + | + | + | + | + | + | + | **MB** |
| **427.96** | + | + | - | - | + | - | - | - | - | **PB** |
| **409.32** | - | - | - | - | - | + | + | - | - | **PB** |
| **364.56** | - | - | - | + | - | - | - | - | - | **UB** |
| **348.68** | - | + | - | - | - | - | - | - | - | **UB** |
| **339.49** | - | - | - | - | + | + | - | - | - | **PB** |
| **329.07** | - | - | - | - | - | - | + | - | - | **UB** |
| **321.82** | - | - | - | - | - | - | - | + | + | **PB** |
| **297.03** | + | - | - | - | - | - | - | - | - | **UB** |
| **258.73** | - | - | + | - | + | - | - | - | - | **PB** |
| **237.74** | - | - | - | - | - | - | + | + | + | **PB** |
| **228.40** | - | + | - | - | - | - | - | - | - | **UB** |
| **208.93** | - | - | - | + | - | - | - | - | - | **UB** |
| **158.52** | + | + | + | + | + | + | + | + | + | **MB** |
| **121.35** | + | - | - | - | - | - | - | - | - | **UB** |
| | | | | | **(CT)8 TG** | | | | | |
| **1342.84** | + | - | - | - | - | - | - | - | - | **UB** |
| **1214.88** | - | - | - | - | - | - | - | + | - | **UB** |
| **1161.17** | - | - | + | - | + | + | + | - | + | **PB** |
| **1013.85** | + | + | + | + | - | - | - | - | - | **PB** |
| **935.19** | - | - | - | - | - | + | + | - | - | **PB** |
| **770.42** | + | - | - | - | - | - | - | - | - | **UB** |
| **717.57** | - | - | - | - | - | - | - | - | + | **UB** |
| **672.68** | - | - | - | + | - | + | - | - | - | **PB** |
| **668.35** | - | - | - | - | - | - | + | - | - | **UB** |
| **649.20** | - | + | + | - | + | - | - | - | - | **PB** |
| **589.24** | - | - | - | - | - | - | - | + | - | **UB** |
| **498.13** | + | + | + | + | + | - | - | - | - | **PB** |
| **382.21** | - | + | + | - | + | + | + | + | + | **PB** |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **297.08** | - | - | - | - | - | + | + | - | - | **PB** |
| **276.70** | + | + | + | + | + | - | - | - | - | **PB** |
| **250.34** | - | - | - | - | - | - | - | + | - | **UB** |
| **243.16** | - | - | - | - | - | - | - | - | + | **UB** |
| **223.57** | - | - | - | - | - | + | + | - | - | **PB** |
| **196.47** | + | - | - | - | - | - | - | - | - | **UB** |

**Table 4:** cont.

| **MW** | **95% FC** | | | **75% FC** | | | **50% FC** | | | **PB%** |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Humic ($0mgL^{-1}$)** | **Humic ($50mgL^{-1}$)** | **Humic ($100mgL^{-1}$)** | **Humic ($0mgL^{-1}$)** | **Humic ($50mgL^{-1}$)** | **Humic ($100mgL^{-1}$)** | **Humic ($0mgL^{-1}$)** | **Humic ($50mgL^{-1}$)** | **Humic ($100mgL^{-1}$)** | |
| | | | | | **(AC)8 T** | | | | | |
| **168.25** | - | + | + | - | + | - | + | + | + | **PB** |
| **128.27** | - | - | - | - | - | - | - | - | + | **UB** |
| **122.99** | + | - | - | - | - | - | - | - | - | **UB** |
| | | | | | **(CT)8 GC** | | | | | |
| **1282.63** | + | + | + | + | + | + | - | - | - | **PB** |
| **1225.33** | - | - | - | - | - | - | - | - | + | **UB** |
| **836.10** | - | - | + | - | - | - | + | + | - | **PB** |
| **802.08** | - | - | - | + | - | - | - | - | - | **UB** |
| **750.50** | - | + | - | - | - | - | - | - | - | **UB** |
| **594.72** | + | + | + | + | + | + | + | + | + | **MB** |
| **409.19** | - | - | - | - | + | + | + | + | + | **PB** |
| **397.46** | - | - | - | + | - | - | - | - | - | **UB** |
| **381.29** | + | + | - | - | - | - | - | - | - | **PB** |
| **365.77** | - | - | + | - | - | - | - | - | - | **UB** |
| **328.32** | - | - | - | + | - | - | - | - | - | **UB** |
| **239.42** | + | + | + | + | + | + | + | + | + | **MB** |
| **185.06** | - | - | + | + | - | - | - | - | - | **PB** |
| **129.46** | + | + | + | - | + | + | + | + | + | **PB** |
| **121.13** | - | - | - | + | - | - | - | - | - | **UB** |
| | | | | | **(CT)8 AC** | | | | | |
| **1134.436** | + | + | + | + | + | + | + | + | + | **MB** |
| **882.976** | - | - | + | - | - | - | - | - | - | **UB** |
| **855.746** | - | - | - | - | + | + | + | + | - | **PB** |
| **842.448** | - | + | - | + | - | - | - | - | - | **PB** |
| **685.105** | + | - | - | - | - | - | - | - | - | **UB** |
| **674.459** | - | - | - | + | - | - | - | - | - | **UB** |
| **663.978** | - | - | - | - | - | - | - | - | + | **UB** |
| **604.424** | - | + | + | + | + | + | + | + | + | **PB** |
| **470.447** | - | - | - | - | - | + | + | + | - | **PB** |
| **429.595** | + | + | + | + | - | - | - | - | - | **PB** |
| **421.597** | - | - | - | - | + | - | - | - | - | **UB** |
| **350.456** | - | - | - | - | - | + | + | + | + | **PB** |
| **324.059** | + | + | + | + | + | - | - | - | - | **PB** |
| **275.349** | + | + | + | + | + | + | + | + | + | **MB** |
| **210.985** | + | - | - | - | - | - | + | + | + | **PB** |
| **188.485** | - | - | + | - | - | - | - | - | - | **UB** |
| **158.656** | + | + | + | + | + | + | + | + | + | **MB** |
| **117.820** | - | - | + | - | - | - | - | - | - | **UB** |
| | | | | | **(CA)6 GT** | | | | | |
| **464.513** | + | - | + | + | + | + | + | - | - | **PB** |
| **358.599** | - | - | - | - | - | + | - | - | - | **UB** |
| **355.300** | - | - | - | - | - | - | - | + | + | **PB** |
| **330.996** | - | - | - | - | - | - | + | - | - | **UB** |
| **318.001** | + | - | + | + | - | - | - | - | - | **PB** |
| **281.132** | - | + | - | - | - | - | - | - | - | **UB** |
| **238.780** | - | - | - | - | - | - | - | - | + | **UB** |
| **196.656** | - | - | - | - | + | + | + | + | + | **PB** |
| **187.197** | - | + | - | - | - | - | - | - | - | **UB** |
| **177.646** | + | - | + | + | - | - | - | - | - | **PB** |
| **138.414** | - | - | - | - | + | + | - | - | - | **PB** |
| **132.980** | + | + | + | + | - | - | - | - | - | **PB** |
| **118.289** | - | - | - | - | - | - | + | + | + | **PB** |
| **98.629** | - | + | + | + | - | + | - | - | - | **PB** |
| **92.166** | - | - | - | - | - | - | + | - | + | **PB** |
| **81.731** | + | - | - | - | - | - | - | - | - | **UB** |
| **65.271** | - | + | - | - | - | - | - | + | - | **PB** |

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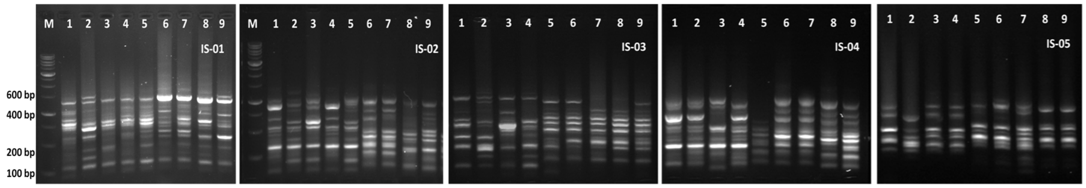


**Fig. 3:** Profile of reproducible DNA fragments of sorghum plants using ISSR molecular markers.

M=DNA Marker; 1= 95% FC with Humic (0$mgL^{-1}$); 2= 95% FC with Humic (50$mgL^{-1}$); 3= 95% FC with Humic (100$mgL^{-1}$); 4= 75% FC with Humic (0$mgL^{-1}$); 5= 75% FC with Humic (50$mgL^{-1}$); 6= 75% FC with Humic (100$mgL^{-1}$); 7= 50% FC with Humic (0$mgL^{-1}$); 8= 50% FC with Humic (50$mgL^{-1}$); 9= 50% FC with Humic (100$mgL^{-1}$)

## 4. Discussion

Water stress reduced vegetative growth parameters of sorghum (Table 1), increased total soluble sugars and proline content of dry leaf tissues (Figure 1), reduced grains yield and its components (Table 2), reduced carbohydrate content and increased protein content of the yielded grains (Figure 2).

The reduction in vegetative growth parameters under water deficit may be attributed to several cell membrane alterations, which have a detrimental effect on the plant's overall biomass (Farooq *et al.,* 2009). Since, biomass is one of the most important indicators of the growth and development of plants under stress. These inhibitions became more prominent as duration, severity, and frequency of the drought stress increased. Since, Dawood and Sadak (2014) showed that moderate and severe drought stress decreased shoot dry weight/plant by 14.56% and 42.19% respectively and root dry weight/plant by 25.6% and 30.4% respectively relative to control plants. The water deficit affects various aspects of plant growth, with the most obvious signs of water stress being expressed by the reduction of plant size, leaf area (Kramer 1983), cell differentiation, cell division, cell elongation and, finally dry matter production (Manavalan *et al.,* 2009). Likewise, dehydration caused by water stress also decreased the amount of carbon dioxide that could enter into closed stomata, which in turn decreased the availability of photosynthetic resources and plant output (Daszkowska-Golec and Szarejko 2013). Sheteiwy *et al.* (2021) reported that water stress significantly decreased plant height, fresh weight, pods weight/plant, and weight of 100 soybean seeds when compared to the well-watered plants. The decrease in the photosynthetic pigments under water deficiency stress conditions may be attributed to breakdown of chloroplast and suppression of its biosynthesis by increasing the production of reactive oxygen species, degradation of the precursor to chlorophyll, and increased chlorophyllase activity (Rezaei-Chiyaneh *et al.,* 2021).

It is worthy to mention that when plants exposed to drought, they accumulated osmoprotectants that can modify plant cell osmotics, and increasing the plant's resistance to stress (Rady *et al.,* 2018). Additionally, these substances are actively contributed to the reduction of oxidative stress imposed by environmental constraints (Van Oosten *et al.,* 2017; Forotaghe *et al.,* 2021). These omoprotectants as soluble sugars, free proline, and soluble protein maintained a certain turgor pressure, that decreased excessive water loss due to water stress (Suddarth *et al.,* 2019; Semida *et al.,* 2020). Dawood and Sadak (2014) Sadak *et al.,* (2019) mentioned that moderate or severe drought stress significantly increased total soluble sugars, free amino acids, and proline of canola and quinoa leaves respectively as compared with normal irrigated plants. Moreover, drought stress (50% FC) significantly increased free amino acids and proline by 8.11 and 17.26 % relative to control (Dawood *et al.,* 2019). In addition, Pirzad *et al.,* (2011) has shown that the drought stress induced conversion of hexoses and other carbohydrates as sucrose and starch to simple sugars. These sugars are known to protect plants from dehydration and act as a precursor for the production of energy and carbon materials. Proline is speculated as multifunctional molecule in plant development that accumulate under stress conditions (Qayyum *et al.,* 2011; Pirzad *et al.,* 2011). It acts as a suitable osmolyte, radical scavenger substance and source of energy for re-growth after stress situations (Szepesi and Szőllősi 2018).

Drought negatively impacted on crop growth and productivity (Pour-Aboughadareh *et al.,* 2019). Since, insufficient water supply reduced growth due to leaf abscission of leaves and a lack of accumulation of photosynthates and subsequently decreased the maize yield (Zhang *et al.,* 2018). Moreover, insufficient water supply at the vegetative and reproductive stages decreased the grain yield ofmaize by up to 25% and 50%, respectively (Mi *et al.,* 2018). Drought at 75% FC and 50% FC significantly reduced canola seed yield /plant by 24.76% and 55.24% respectively relative to control plants (Dawood and Sadak 2014). Likewise, drought stress (50% FC) significantly reduced sunflower seed yield by 21.13% as compared to control (Dawood *et al*., 2019). El-Awadi *et al.* (2021) added that water deficit condition decreased soybean seed yield by 37.53% and carbohydrate content by 5% relative to well-watered condition. According to Machiani *et al.* (2023), moderate and severe drought stress reduced the dry matter yield of thyme (*Thymus vulgaris* L.) by 27% and 40%, respectively. During vegetative growth stages, drought stress decreased leaf area and photosynthesis, non-structural carbohydrates stored in the stem, and grain weight(Rashidi 2005).Drought stress at 75% FC and 50% FC considerably reduced carbohydrate contents accompanied by drastically increases in protein content in comparison to control plants (Dawood and Sadak 2014).These reductions in carbohydrates are extremely important because they have an immediate relationship to physiological processes like photosynthesis, nutrient transport, and respiration (Habib *et al.,* 2020) that may be explained by a decrease in photosynthetic pigments and a decrease in the activity of calvin cycle enzymes (Ashraf *et al.,* 2013). Ali and Alqurainy (2006) stated that the main cellular constituents susceptible to damage under drought stress are lipids of cell membranes, proteins, and nucleic acids through increasing level of free radicals. While, drought stress reduced the carbohydrate content (including sucrose and starch) of the grain (Barnabás *et al.,* 2008), and increased the protein content(Flagella *et al.,* 2010).Insufficient water supply reduced synthesis of carbohydrate of different crops leading to lower grain yield (Selim *et al.,* 2019). In addition, water deficit increased protein of flour due to increase the accumulation rates of grain nitrogen (Guttieri *et al.,* 2005).

Humic acid treatments reduced the deleterious effect of water stress on sorghum growth and productivity. Since, humic acid treatments increased vegetative growth parameters of sorghum (Table1), increased total soluble sugars and proline content of dry leaf tissues (Figure 1), increased grains yield and its components (Table 2) and increased carbohydrate content and protein content of the yielded grains (Figure2) even grown under water stress.

Firstly, it is worthy to mention that humic acid have the ability to stimulate the antioxidant enzymatic system (catalases, peroxidases, superoxide dismutases) in charge of converting reactive oxygen species into harmless species and increasing plant resistance to abiotic stresses (Forotaghe *et al.,* 2021). The increases in plant growth and grains yield due to humic acid may be attributed to its role in increasing the permeability of the cell membrane (Noroozisharaf and Kaviani 2018), which can promote absorption of water and improve the nutrient uptake and utilization (Ali *et al.,* 2020). The adequate amount of nutrients are necessary to produce protein, chlorophyll, and nucleic acids, and boosting the capability of plants to carry out photosynthesis and supplying metabolites required for cell division and elongation (Heidari and Minaei 2014, Morozesk *et al.,* 2017, Ding *et al.,* 2021, Sible *et al.,* 2021, Matuszak-Slamani *et al.,* 2022, Altaf *et al.,* 2023). Likewise, humic acid application on maize plants enhanced the nitrogen content of cells, which in turn increased cell division and elongation and enhanced the plant growth parameters (Ayas and Gulser 2005). Moreover, humic acid enhanced uptake of nutrient from the soil to leaves and translocation of those nutrients from the leaves to seeds, thus enhancing yielded seeds without using any energy and without any loss in respiration, biosynthesis of enzymes and, nucleic acids and overall, plant dry weight (Ulukan 2004). Eyheraguibel *et al.* (2008) stated that humic acid treatment increased stem length by 72.5% and dry weights of leaf and stem of maize by 53% and 100% respectively. Recent research has shown that humic acid enhanced the auxin, cytokinin and gibberellins, thereby improving plant growth (Olaetxea *et al.,* 2020; van Tol de Castro *et al.,* 2021). Furthermore, humic substance improved photosynthesis under drought conditions by enhancing electron transport rate, gas exchange, and chlorophyll content in *Brassica napus* (Lotfi*et al.,* 2018). Humic acid application enhanced osmolytes as soluble sugar and proline in plants, by maintaining water absorption and cell turgor which is an adaptation strategy for plants under water stress (Azevedo and Lea 2011; El-Bassiouny *et al.,* 2014). Humic acid at 5% significantly increased soybean seed yield, yield components and total carbogydrate content of the yielded seeds (Dawood *et al.,* 2019). There are several reports regarding the influence of humic acid on the transport of glucose across cell membranes that increased the amount of carbohydrates in the leaves of grasses (Tan 2003, Ahmed *et al.,* 2013), and enhanced the soluble proteins and free proline level on rice (Muscolo *et al.,* 2007). Humic acid can participate effectively in producing more carbohydrate that positively reflects on plant productivity and quality (Canellas and Olivares 2014). Humic acid foliar application improved the nitrogen content in the seed and straw, pods number per plant, and the seed index of faba bean plants grown under water stress (Ayman *et al.,* 2018). Haghighi *et al.,* (2012 b) mentioned that application of humic acid increased nitrate concentration and nitrate reductase in a dose-dependent manner, and thereby increased the nitrogen content in plant (Hatami 2017). Humic acid improved yield of *Cucumis melo* by providing appropriate conditions for increasing nitrogen content of plant and increasing the production rate of nitrogenous organic compounds as amino acids and protein, and consequently increased the growth rate and biomass production (Kiran *et al.,* 2019).

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**Declaration of competing interest**
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