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Sorghum Responses to Foliar Spraying With Zinc under Water Regime Stress

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Abstract

This study was conducted during the summer seasons of 2019 and 2020 at the greenhouse of National Research Centre; Giza, Egypt, to study the effect of foliar spraying with zinc in improvement negative effects of irrigation water regime in Sorghum. Three levels of water regime (1, 1.5 and 2 Liter) were used interacting with three concentrations of zinc as foliar spraying (00, 50 and 100 ppm). Generally, obtained results showed significant increment in all vegetative growth criteria, yield and chemical constituents by increasing both quantity of irrigation and zinc spraying. Moreover, using high concentrations of zinc leaded to reduce and alleviate the harmful effect of irrigation water deficiency. Inter simple sequence repeats (ISSR) molecular marker was applied using three different ISSR primers, and results recorded 61 amplified bands with different molecular weights ranged from 183 – 1278 bp detected by these primers. Moreover, these amplified fragments were distributed between polymorphic bands (37 bands) with an average 12.33, unique bands (9 bands) with an average 3 and monomorphic bands (15 bands) with an average 5, so, polymorphism percentage was 60.82%.

Keywords: Sorghum, *Sorghum bicolor*, Zinc, Water regime

1. Introduction

Sorghum (*Sorghum bicolor* L.) is a major crop ranked the fourth place in the worldwide production of cereals after wheat, maize and rice **[1]**. It has versatility and unique ability of abiotic stress tolerance. In Egypt sorghum is usually cultivated in the southern governorates, where the environmental conditions are relatively favorable for its production **[2]**.

However, one of a biotic stress is drought which not only effects on physiological and biochemical properties of plants; but it limits growth and development of plants and reduces its production **[3]**.

Moreover, ROS (reactive oxygen species) accumulation has been increased as response to drought stress **[4]**, and this resulted as a reaction of activation of antioxidant enzymes SOD (superoxide dismutase), POD (peroxidase), and CAT (catalase) contributed to ROS accumulation in tolerant cells. **[5,6]**.

Zinc (Zn) is an essential plant micronutrient that contributes in many physiological functions; protein and carbon hydrate synthesis **[7]**. Moreover, drought stress leads to decrement in zinc levels and consequently, restriction of root growth **[8]**, so that, utilization of (Zn) under drought conditions will effects on yield and quality of plant. Zinc acts on to reducing the harmful effects of drought by regulating stomata and ionic balance in crops **[9, 10]** and also has protective effects on photo oxidative damage caused by ROS **[11]**.

Inter simple sequence repeats (ISSR) analysis was used in *Sorghum bicolor*, L. Moench to study the genetic diversity and variation among different sorghum genotypes Dema *et al*. **[12]**. Also, Basahi **[13]** determined these genetic variations in some

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sorghum genotypes that grown in some parts of Saudi Arabia and Yemen and Khaled, *et al.* **[14]** in Egypt.

This study aimed to investigate the effectiveness of zinc (Zn) foliar applications in mitigate and improvement the negative effects of irrigation water regime on physiological traits and yield components in Sorghum.

2. Materials and Methods

Two pot experiments were established during the summer seasons of 2019 and 2020 at the greenhouse of National Research Centre; Giza, Egypt, to study the alleviation of negative effects of irrigation water regime on *Sorghum bicolor* (Cv Dorado) using foliar spraying with Zinc.

Experimental design:

The pots in these experiments were arranged in split plot design with three replications. Three irrigation treatments were used (1, 1.5 and 2L water). Moreover, the three irrigated treatments were interacted with three concentrations of zinc as foliar spraying (0, 50 and 100 ppm). The zinc spraying treatments were applied at 40 and 50 days after sowing in both seasons. Tween 60 was mixed as a wetting agent.

Data collection

To determine the growth criteria (plant height (cm), number of leaves/plant and dry weight of both leaves and stems) at vegetative stage, five plants were taken at random from each plot, and TSS% (Total soluble solids) were determined in fresh juice using Refractometer (Shibuya-0-32, Japan). Moreover, plant height, panicle height, panicle weight, straw weight, weight of grains and 100- grains weight were recorded at harvest stage.

Chemical analysis:

FAA (Free amino acids) was determined using ninhydrin colorimetric method defined by Rosein **[15]** as modified by Selim *et al.* **[16]**. Total nitrogen content was determined by the Kjeldahl method Peach and Tracey **[17]** and then, the total protein percentage was calculated by multiplying the total nitrogen value in 6.24 protein factor according to the following equation:

Total protein (%) = Total Nitrogen (%) × F

Where, F is the protein factor that equals 6.25.

TC (Total carbohydrates) was extracted according to Smith *et al.* **[18]**. Moreover, total carbohydrates were determined colorimetrically by the phenol-sulfuric acid method as described by Dubois *et al*. **[19]**.

DNA extraction and PCR procedure

Fresh young leaves of sorghum plants were collected and immediately ground to fine powder in extraction buffer using CTAB (cetyltrimethyl ammonium bromide) protocol Porebski *et al.* **[20]**.

The PCR assays were performed for ISSR markers in a 15 µl volume containing 1µl DNA (40 ng), 7.5µl Master Mix (Gene Direx one PCR TM), 1µl template DNA and 1µl primer. Three different primers were used (Table 1). The amplification reaction consisted of an initial denaturation step at 94°C for 7 min, followed by 35 cycles of 30 sec. at 94° C (denaturation), 30 sec. at 48° C (annealing) and 2 min at 72° C (extension) followed by a final extension step at 72°C for 5 min. Amplification products were electrophoresed on 1.5% agarose in 1× TBE buffer. The gels were stained with ethidium bromide and documented using gel documentation system.

Polymorphism percentage was calculated according to this equation:

Polymorphism percentage (PB%) = (UB + PB) / Total bands

Where:- UB = Number of unique bands, PB = Number of polymorphic bands

Statistical analysis:

Data were statistically analyzed, Means, analysis of variance and LSD at 5% level were determined according to Silva and Azevedo [21-22].

Table 1: ISSR primers and their sequences used used in this study.

	Primer Code	Primer Sequence (5\ ⟶ 3\)	
1	IS- 01	(AC)8 T	AGA GAG AGA GAG AGA GT
2	IS- 02	(GA)8 T	GAG AGA GAG AGA GAG AT
3	IS- 03	(TC)8 A	TCT CTC TCT CTC TCT CA

3. RESULTS AND DISCUSSION

There were significantly increment in all studied growth characters (plant height, number of leaves/plant and dry weight of leaves and stem per plant) of sorghum (*Sorghum bicolor* L. Moench) plants at vegetative stage by increasing the quantity of irrigation water up to 1.5 liter (the maximum values), where the minimum values were at 1.0 liter. Fig. (1a.b).

On the other hand, TSS% measurements was affected by deficiency of irrigation water quantity, whereas, it decreased by increasing the quantity and the treatment with 1 liter was the highest one and then significantly decreased gradually and recorded the lowest one with the treatment 2 liter.

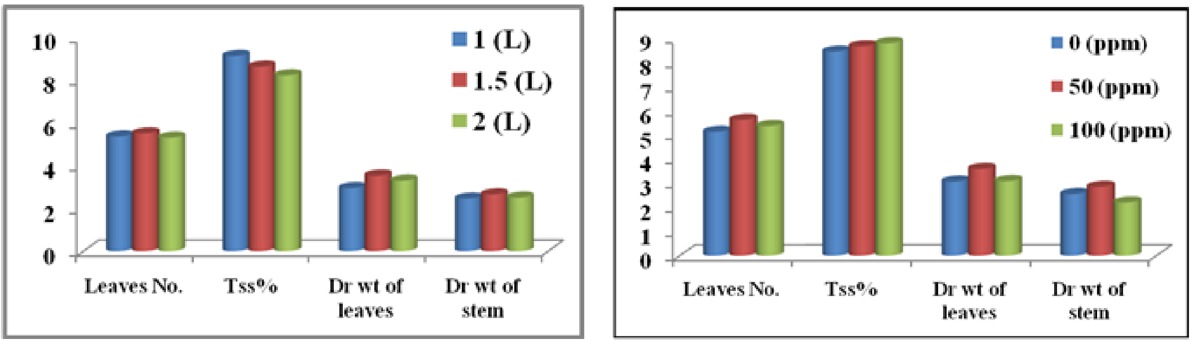
Kramer and Boyer **[23]** studied the damage in cell division and enlargement due to the water stress. Moreover, in fenugreek plants grown under water stress conditions have a lower stomatal conductance in order to converse water. Thus, CO_2 fixation is decreased and photosynthetic rate reduced, consequently, caused in less assimilate production for growth plant **[24, 25]**. On the other hand, Ahmed *et al.* **[26]** Al-Chammaa *et al.* **[27]**; Ahmed *et al.* **[28]** found that one skipping one irrigation at certain developmental growth stages of plant caused a significant decrement in growth characters.

With regards to the effect of foliar spraying by zinc with different concentrations (0, 50 and 100 ppm) on vegetative characters and chemical constituents of sorghum. Fig (1) illustrated that all studied characters have been increased as a response to increasing zinc concentration to 50 ppm and then decreased at 100 ppm, except TSS% that improved and increased by increasing zinc concentrations and reached its maximum with the treatment 2 liter Fig. (1). Rajput **[29]** and Devendra *et al*. **[30]** reported that grain and straw yield of wheat significantly increased by 5 kg ha^{-1} Zn as a foliar spray and 20 kg ha^{-1} as basal over control. Moreover, Hassan *et al.* **[31]** stated that under drought stress conditions, Zn application improves all plant growth, physiological and interacts with plant hormones to stimulates the antioxidant enzymes that improved the harmful effects of drought stress.

Data in Table (2) indicated that the negative effects of drought (decrease irrigation water quantity) conditions have been alleviated by using foliar spraying with zinc. Moreover, all studied parameters were improved and significantly increased in case of interaction of water quantity and since concentrations where the best interaction was 1.5 liter water with 50 ppm zinc. While as, in TSS%, the best interaction was 1 liter water with 100 ppm zinc (Table 2). Wang *et al.* **[32]**, concluded that Zn application affects positively on photosynthesis and growth under sufficient moisture supply not under water stress.

However, there were highly significant effects of both drought and foliar spraying by zinc on chemical constituents of sorghum at vegetative stage, whereas, all studied chemical characters were increased by increasing both irrigation water quantity and zinc concentrations up to 1.5 liter and 50 ppm, respectively (Fig 2 a,b). With respect of, the interacting foliar spraying by zinc with water irrigation quantity, it obviously notes that utilizing zinc played an important role in decreasing the harmful effects of water deficiency in irrigation, such as, all recorded data in Table (3).

At harvest stage, it was noticed that all studied yield criteria were improved by increasing water quantity and zinc concentrations to 1.5 liter and 50 ppm, respectively (Fig 3a,b and Tables 4).

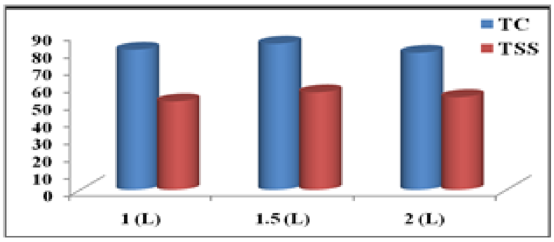


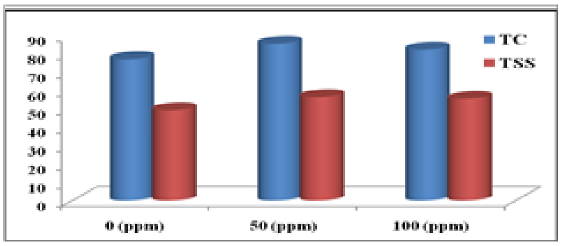
A **B**

Fig. (1): Interaction effects of water regime **(A)** and zinc **(B)** concentrations on vegetative growth characters in sorghum. (Average of 2019 and 2020 seasons).

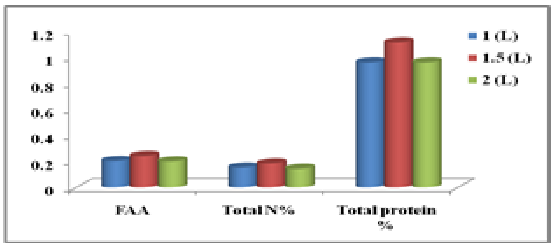
Table (2): Interaction effects of water regime and zinc concentrations on vegetative growth characters of sorghum. (Average of 2019 and 2020 seasons).

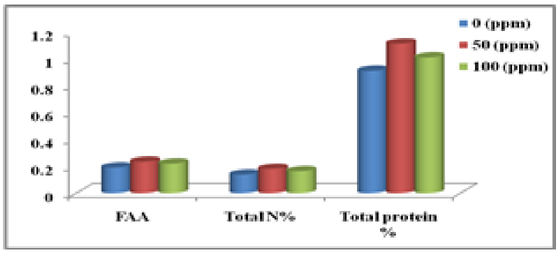
Water regime (Litre)	Zinc Conc. (ppm)	Plant Height (cm)	Leaves /plant	TSS%	Leaves DW. gm/plant	Stem DW. gm/plant
1 (L)	0 (ppm)	116.33	5.33	8.98	2.66	2.38
	50 (ppm)	129.67	5.47	9.16	3.38	2.81
	100 (ppm)	125.00	5.27	9.25	2.81	2.18
1.5 (L)	0 (ppm)	122.33	5.67	8.34	3.19	2.74
	50 (ppm)	133.00	5.67	8.67	3.95	2.97
	100 (ppm)	129.00	5.17	8.88	3.45	2.26
2 (L)	0 (ppm)	126.33	5.00	8.09	3.39	2.57
	50 (ppm)	129.00	5.93	8.23	3.47	2.79
	100 (ppm)	126.67	4.97	8.34	3.05	2.18
LSD 5% level		2.89	0.178	0.048	0.053	0.047





A B



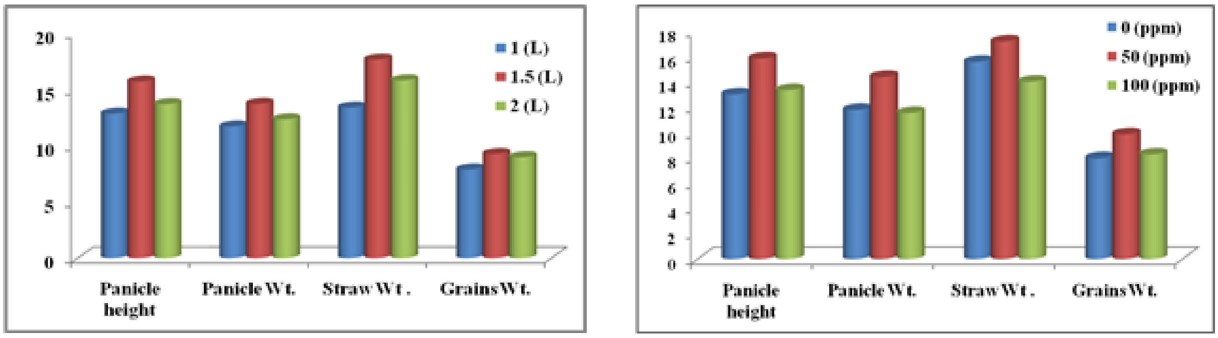


A B

Fig. (2): Effect of water regime **(A)** and zinc **(B)** concentrations on chemical characters of sorghum plants. (Average of 2019 and 2020 seasons).

Table (3): Interaction effects of water regime and zinc concentrations on Chemical constituents of sorghum. (Average of 2019 and 2020 seasons).

Water regime (Litre)	Zinc Conc. (ppm)	TC	TSS	FAA	Total N%	Total protein %
1 (L)	0 (ppm)	82.09	47.22	0.180	0.130	0.810
	50 (ppm)	81.26	53.47	0.230	0.180	1.130
	100 (ppm)	79.46	52.59	0.200	0.150	0.940
1.5 (L)	0 (ppm)	77.55	51.27	0.200	0.160	1.000
	50 (ppm)	89.06	59.58	0.260	0.200	1.190
	100 (ppm)	86.75	57.97	0.260	0.190	1.150
2 (L)	0 (ppm)	71.42	49.05	0.190	0.120	0.92
	50 (ppm)	85.95	56.15	0.210	0.160	1.020
	100 (ppm)	80.30	55.42	0.200	0.150	0.940
LSD 5% level		3.28	0.051	0.047	0.034	0.29



A **B**

Fig. (3): Effect of water regime **(A)** and zinc **(B)** concentrations on some yield characters of sorghum plants. (Average of 2019 and 2020 seasons).

Table (4): Interaction effects of water regime and zinc concentrations on growth characters of sorghum at harvest stage. (Average of 2019 and 2020 seasons).

Water regime (Litre)	Zinc Conc. (ppm)	plant height	panicle height	Panicle wt	straw wt	wt of grains	wt of 100 grains
1 (L)	0 (ppm)	73.00	11.50	10.43	13.12	6.78	3.18
	50 (ppm)	81.50	14.50	13.73	15.01	9.61	3.61
	100 (ppm)	81.00	12.50	10.86	11.95	7.21	3.19
1.5 (L)	0 (ppm)	91.50	15.00	12.95	17.00	8.80	3.23
	50 (ppm)	106.00	17.50	16.03	19.33	10.12	3.61
	100 (ppm)	103.50	14.50	12.09	16.57	8.80	3.29
2 (L)	0 (ppm)	73.50	12.50	11.93	16.60	8.26	3.26
	50 (ppm)	74.00	15.50	13.36	17.21	9.79	3.31
	100 (ppm)	65.50	13.00	11.64	13.49	8.76	3.08
LSD 5% level		2.36	1.72	0.053	1.14	0.093	0.033

Paul *et al.* **[33]** recorded that wheat yield and yield components were significantly affected by using Zn foliar spraying under water stress conditions.

Poornima and Koti **[34]** reported that application of nano ZnO recorded more yield and growth of sorghum as compare to bulk ZnSO4. Moreover, this might be due to that Zinc acts as an activator of enzymes in plants and is directly involved in the biosynthesis of auxin, which produces more cells and dry matter that in turn will be stored in seeds as sink.

Assad *et al.* **[35]** showed that drought stress affected negatively on morphological and yield-related traits of sorghum, while it effected positively on some quality criteria such as total soluble carbohydrate, crude protein, and proline contents.

Ramadan *et al.* **[36]** studied the effect of foliar applications of plant growth regulators, micronutrients or osmoprotectants for stimulating drought tolerance in maize plants under drought stress. Furthermore, they found that foliar applications enhanced the significant reduction in morphological characters, yield and its components that resulted under drought stress conditions.

The results found in table (5) and Fig.(4) revealed that there were 61 amplified bands with different molecular weights ranged from 183 – 1278 bp detected by the used primers (Table 1). Moreover, these amplified fragments were distributed between polymorphic bands (37 bands) with an average 12.33, unique bands (9 bands) with an average 3 and monomorphic bands (15 bands) with an average 5, so, polymorphism percentage was 60.82%. Moreover, there were variability in number of amplified bands that detected by each primer (Table 6), so it varied between 19 and 22 from total of 61 alleles were amplified with an average of 12 polymorphic alleles per primer. ISSR primer number 3 produced a higher percentage of polymorphic fragments (70%).

Table (5): ISSR markers, total number of fragments and fragments sizes detected by each primer in sorghum plants under study.

Primers	Primer Sequence	Marker size (bp)	Amplified bands				PB %
			TAF	MB	UB	PB	
IS- 01	(AC)8 T	1278 –191	22	6	4	12	54.55 %
IS- 02	(GA)8 T	969 – 183	19	5	3	11	57.90 %
IS- 03	(TC)8 A	1245 – 228	20	4	2	14	70.00 %
	Total		61	15	9	37	-
	Average		20.33	5	3	12.33	60.82 %

TAF= Total amplified fragments, MB = Number of monomorphic bands, UB = Unique bands, PB = Number of polymorphic bands, PB% = Polymorphism percentage

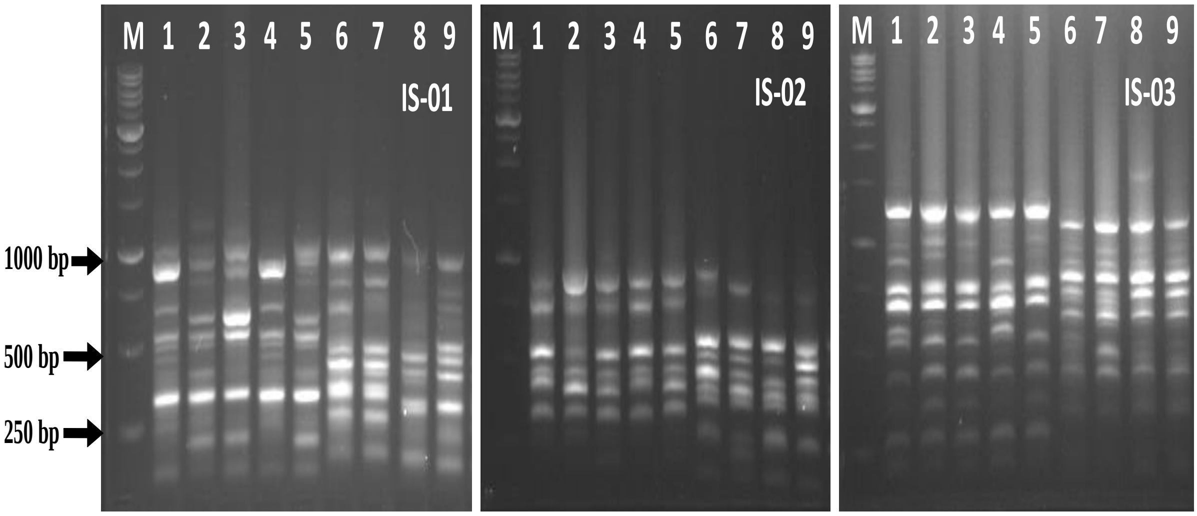


Fig. (4): PCR amplification profile generated with three ISSR primers from genomic DNA treated sorghum under study.

Mejri *et al*. **[37]** concluded that, appearance or disappearance of different DNA bands with variation of their intensity as well, might be connected with structural rearrangements in DNA caused by different types of DNA damages (breaks, transpositions, deletions, etc).

However, Taher *et al.* **[38]** reported that poly (AG)-anchored primers were more polymorphic and reproducible than other di-nucleotides and tri-nucleotides motifs. Meanwhile, Song *et al.* **[39]** and Sofalian *et al.* **[40]** proposed, the %P values were higher when the motifs comprise three to five nucleotides in wheat genotypes. Khaled *et al.* **[14]**.

Table (6): Molecular weight of DNA bands detected by ISSR primers in treated sorghum plants with foliar spraying by zinc.

Primer	MW (bp)	1	2	3	4	5	6	7	8	9	Polymorph.
IS-01	1278	1	1	0	0	0	0	0	0	0	PB
	1060	0	0	0	0	1	0	0	0	0	UB
	1022	0	0	1	0	0	0	0	0	0	UB
	986	0	0	0	0	0	1	1	1	1	PB
	930	1	1	1	1	1	0	0	0	0	PB
	882	1	0	0	0	0	0	0	0	0	UB
	820	1	1	1	1	1	1	1	1	1	MB
	757	0	0	0	0	1	1	1	1	0	PB
	686	0	0	0	1	0	1	0	0	1	PB
	637	0	1	1	0	0	0	0	0	0	PB
	605	0	0	0	0	1	0	0	1	0	PB

	548	1	1	1	1	1	0	0	0	0	PB
	505	1	1	1	1	1	1	1	1	1	MB
	471	1	1	1	1	1	1	1	1	1	MB
	431	1	0	0	0	0	0	0	0	0	UB
	416	0	0	0	0	0	0	1	1	1	PB
	396	0	1	0	1	1	0	0	0	0	PB
	348	1	1	1	1	1	1	1	1	1	MB
	304	0	0	0	0	0	1	1	1	1	PB
	239	1	1	1	0	0	0	0	0	0	PB
	203	1	1	1	1	1	1	1	1	1	MB
	191	1	1	1	1	1	1	1	1	1	MB
IS-02	969	1	1	1	1	1	1	0	1	0	PB
	885	1	1	1	0	0	0	0	0	0	PB
	847	1	1	1	1	1	1	1	1	1	MB
	819	1	0	0	0	0	0	0	0	0	UB
	691	0	0	0	0	0	0	1	1	0	PB
	672	1	1	1	1	1	0	0	0	0	PB
	617	1	0	0	0	0	0	0	0	0	UB
	586	0	0	0	0	1	0	0	0	0	UB
	543	0	0	0	0	0	1	1	1	1	PB
	488	1	1	1	1	1	1	1	1	1	MB
	453	0	1	0	0	0	1	1	1	0	PB
	412	0	0	0	0	1	1	1	1	1	PB
	393	1	1	1	1	1	1	1	1	1	MB
	375	0	0	0	0	0	1	0	1	1	PB
	332	0	0	0	0	0	1	1	1	0	PB
	306	1	1	1	1	1	1	1	1	1	MB
	279	0	0	1	1	1	0	0	0	0	PB
	255	1	1	1	1	1	1	1	1	1	MB
	183	0	0	0	0	0	1	1	1	1	PB
IS-03	1245	0	0	0	1	1	0	0	0	0	PB
	1202	1	1	1	1	1	1	1	1	1	MB
	1166	1	0	0	0	0	0	0	0	0	UB
	951	0	0	1	0	0	0	0	1	1	PB
	924	1	0	0	1	0	0	0	0	0	PB
	885	1	1	1	1	1	1	1	1	1	MB
	809	1	1	1	0	0	1	1	1	0	PB
	787	0	0	0	1	1	1	1	1	1	PB
	724	0	0	0	0	1	1	1	1	0	PB
	704	1	1	0	0	0	0	0	0	0	PB
	686	0	0	1	0	0	0	0	0	1	PB
	608	1	1	1	1	1	1	1	1	1	MB
	551	0	0	0	0	0	1	1	1	0	PB
	522	0	1	1	0	0	0	0	0	0	PB
	480	0	0	0	0	0	0	1	1	1	PB
	439	1	1	1	1	1	1	1	1	1	MB
	374	1	0	0	0	0	0	0	1	0	PB
	310	1	0	0	0	0	0	0	0	0	UB
	286	0	0	1	0	1	1	1	1	1	PB
	228	1	1	1	1	1	0	0	0	0	PB

MB = Monomorphic bands, UB = Unique bands, PB = Polymorphic bands.

4. Conclusion

Overall results showed that irrigation water regime negatively influenced morphological and yield-related traits of sorghum and chemical constituents. In addition, using zinc with high concentrations leaded to reduce and alleviate the harmful effect of irrigation water deficiency on all plant characters.

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