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Research Article

Physiological and Molecular Response of Wheat Cultivars to Titanium Dioxide or Zinc Oxide Nanoparticles under Water Stress Conditions

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A field trial was conducted through two successive winter seasons 2017/2018 and 2018/2019 to evaluate the influence of TiO_2 or ZnO nanoparticles on two wheat cultivars (Gimeza 12 and Sids 13) under different water irrigation requirements of 100% (WW) and 75% (WS). The results showed that drought stress decreases morphological parameters, photosynthetic pigments, and wheat yield (ha^{-1}). However, the total soluble sugars, total free amino acids, proline content, and water productivity were increased. Application of TiO_2 or ZnO nanoparticles declines the negative influence of water deficit. Furthermore, SDS-PAGE revealed in Gimeza 12 treated with TiO_2 or ZnO nanoparticles, it stimulates the appearance of some proteins at the MW of 28 kDa in WW and WS, while in WS, it records the new polypeptide at 18 kDa. Moreover, Gimeza 12 treated with nano-TiO_2 led to the disappearance of two bands at 163 and 51 kDa in WS. However, for Sids13, there was no difference between the treatments in WW and WS except in nano-ZnO at WS that disappeared the polypeptides at MWs of 163, 51, and 18 kDa. Primer SRAP results showed that the plants treated with TiO_2 or ZnO nanoparticles had a minor effect at the genomic DNA level, which was illustrated by the appearance or absence of some bands. Besides, the low concentrations of nanoparticles did not damage DNA. On the other hand, one negative marker of −233 bp disappeared in the Gimeza 12 cultivar treated with WS + nano-TiO_2 and was revealed in the other treatments using primer SRAP-2. The results showed that the Gimeza 12 cultivar which had the highest grain yield was more tolerant to drought than the Sids 13 cultivar.

1. Introduction

Water stress is regularly associated with main abiotic stresses such as heat stress and salt stress; thus, this is deliberated as one of the elementary agents accountable for crop yield decrease [1]. So, water preservation is becoming a critical deliberation for agriculture. Water stress produced an opposing influence on plants through decreased growth, nutrient achievement reduction, and water status of plants [2]. During photosynthesis, water stress induced a reduction of photosynthetic efficiency because of the increased accumulation of reactive oxygen species [3]. Thus, plants use various mechanisms to increase overall growth performance for sustaining the yield quality under changeable environmental stresses [4]. Plants enhance the osmoprotectants production (such as total soluble sugar and proline) and antioxidant enzymes, which enables plants to overcome water stress [5].

Nanoparticles (NPs) are minute particles of less than 1000 nm. These particles are very gorgeous compounds to utilize in a biological system. NPs respond with plants producing various morphological and physiological alterations based on the nanoparticles' characteristics. In this regard, Ma et al. [6] showed that the stimulation and reduction have an impact on plant growth and development, based on the size, structure, concentration, and physical and chemical properties of nanoparticles in addition to plant species. Nanotechnology is one of the options that increase the nutritional values of crops, so some engineered nanoparticles (NPs) could be used as a fertilizer.

Titanium has important biological influences on plants. It is useful at lower concentrations but has cytotoxicity and genotoxicity at higher concentrations [7]. Ti-NPs raise the plant's tolerance against environmental stresses and reduce free radicals. It converts O_2 free radicals to O_2 also, alters from Ti^{4+} to Ti^{+3}, and so as to keep its form, it alters O_2 to H_2O_2 and then changes to Ti^{4+} [8]. Nano-TiO_2 improved spinach seed germination and plant growth [9]. The nano-TiO_2 raises the activity of the plant via raising the initial photosystem light energy. This is absorbed through the chloroplast membrane and moved to the photosystem II [10]. TiO_2-NPs can readily catalyze oxidation and regeneration reactions and increase the rate of liberation of whole energy electrons because of the size of the particles is very small [11]. Meanwhile, TiO_2 enhances plants' division and size, so it is recommended as a growth regulator [12].

Zinc oxide nanoparticles (nano-ZnO) play an enormous pivotal function in plants and are generally used in agricultural applications [13]. ZnO-NPs activated the embryogenesis of somatic cells, regenerating plantlets, growth, and progressed tolerance to abiotic stress via improving the synthesis of proline and antioxidant enzymes [14]. ZnO-NPs nanoparticles increased seed germination, plant growth, and development of several plant species [15]. In this connection, Singh et al. [16] showed the beneficial influence of ZnO-NPs at low concentrations in mung bean and tomato, respectively. Zinc as a catalyzer has an activating or building role in many enzymes in plants [17]. Moreover, alteration of endogenous phytohormone levels was induced by nanoparticles which increased stress tolerance [18]. Also, Abdel Latef et al. [19] detected that ZnNPs treatment enhanced growth of lupine plants under salinity stress which regulates hormonal metabolism through modification of tryptophan biosynthesis, thus activating cell division and enlargement.

DNA fingerprinting is a beneficial biomarker examination in the evaluation of the mutagenicity of chemicals and trace metals on plants [20]. Molecular markers like sequence-related amplified polymorphism (SRAP) have been usefully used to reveal alterations in DNA fingerprints that reverberate DNA differences in the genome [21].

Cereal crops such as wheat are one of the most important plants due to their natural genetic variation [22]. It is considered the most important food grain due to its covering approximately 21% of the world's food supply and is an essential source of protein in developing countries [23].

So, the target of our work was to research the effect of nano-TiO_2 or nano-ZnO in mitigating the impact of water stress on growth, yield, some biochemical aspects, and molecular change of two wheat (Gimeza 12 and Sids 13) cultivars.

2. Materials and Methods

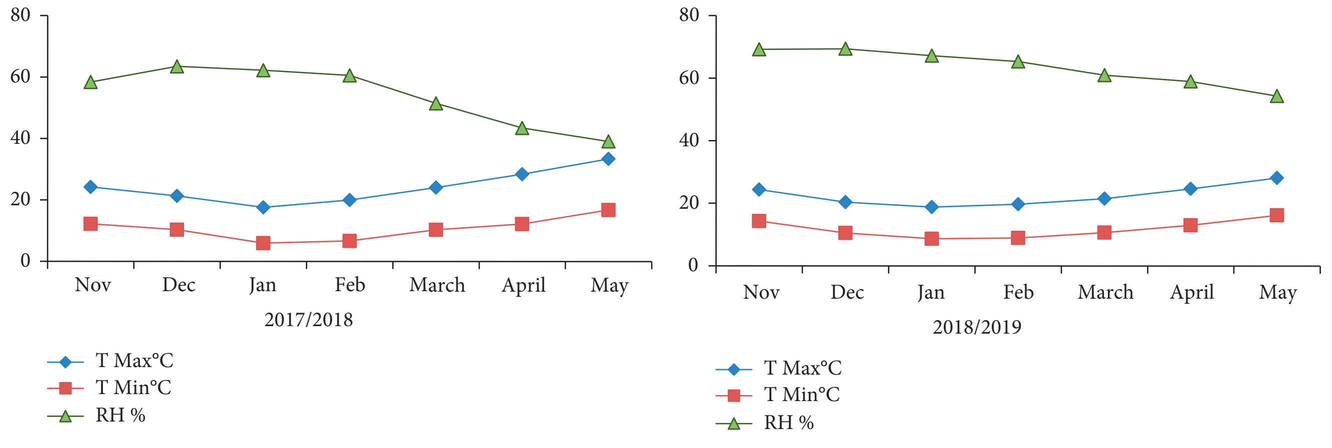
2.1. Materials. Grains of the wheat cultivars (Gimeza 12 and Sids 13) were supplied by the Agricultural Research Centre in Egypt. Titanium dioxide and zinc oxide nanoparticles (NPs) were bought from Sigma-Aldrich Company.

2.2. Experiment Location. Two field experiments were carried out in two successive winter seasons of 2017/2018 and 2018/2019 at the Experimental Farm of National Research Centre Nubaria region, Egypt (30_86′67″ N 31_16′67″ E), with the mean altitude being 21 m above sea level. The farm is classified as an arid or semiarid region. The daytime temperature ranged from 17.61 to 32.4°C with an average of 24.38°C and 18.82 to 28.15°C with an average of 22.52°C, whereas the temperature at night was 6.4 to 16.82°C with an average of 10.7 and 8.7 to 16.23°C with an average of 11.8°C. The relative humidity was in ranges from 39.1 to 62.22 with an average of 54.1% and 58.0 to 69.38% with an average of 64.4% in 2017/2018 and 2018/2019, respectively. Figure 1 shows the climatic data of the experimental site through the two growing seasons. Total precipitation through both successive winter seasons for wheat to be subtracted from the total amount of water calculated 68.1 mm = 680 m^3/ha and 61.7 mm = 617 m^3/ha in 2017/2018 and 2018/2019, respectively.

The sandy soil of the experimental site was conducted at the Experimental Station of the National Research Centre, Al-Nubaria district, El-Beheira governorate, Egypt. Physical and chemical analysis of the sandy soil of the experimental site (Table 1) is determined according to Chapman and Pratt [24]. These results were previously mentioned by Bakry et al. [25] in the same region.

2.3. Experiment Design. The wheat grains were washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 minutes, and washed again with distilled water. The grains were soaked in different rates of nano-TiO_2 or nano-ZnO (5 mg/L and 10 mg/L) for 12 hours before sowing. The experiment was designed in a split-split plot design with four replicates, where the water irrigation requirements (WIR) were 100% (WW) and 75% (WS) occupied the main plots, whereas both wheat cultivars (Gimeza-12 and Sids-13) were randomly assigned in subplots. Meanwhile, the treatments of nano-TiO_2 or nano-ZnO were randomly assigned in sub-sub plots. On 26th November, the grains of wheat were cultivated in two seasons in rows 3.5 m long, and the distance between rows was 20 cm apart. The plot area was 10.5 m^2 (3.0 m in width and 3.5 m in length).

The agricultural practices were carried out as recommended of sowing wheat under sandy soil conditions, and the seeding rate was 140 kg ha^{-1}. Presowing, 360 kg ha^{-1} of



(a) (b)

Figure 1: The data of maximum temperature (TMax°C), minimum temperature (TMin°C), and average relative humidity (RH%), obtained from the weather station installed at the experimental station of the National Research Centre, Nubaria, shows the climatic data of the experimental site during the growing season.

Table 1: Physical, chemical, and nutritional analysis of the experimental soil.

Season	Constant depth (cm)	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture class
2017/	00–30	40.7	44.6	10.7	4	Sandy
2018	30–60	38.2	43	13.8	5	Sandy
2018/	00–30	38.7	42.6	13.7	5	Sandy
2019	30–60	36.5	38.1	17.8	7.6	Sandy

Season	Constant depth (cm)	pH	Electrical conductivity (dS/m)	Coarse sand (%)	Anions (milliequivalents/liter)				Cations (milliequivalents/liter)				CaCo (%)	Organic matter (%)
					$CO_3^=$	HCO_3^-	Cl	$SO_4^=$	Ca^{++}	Mg^{++}	Na_-^+	K^+		
2017/	00–30	7.84	1.17	32	—	0.50	8.40	1.11	1.80	0.90	7.10	0.20	1.00	0.40
2018	30–60	7.89	1.79	27	—	0.60	8.00	1.40	2.10	1.50	6.20	0.20	6.00	0.07
2018/	00–30	7.95	1.59	23	—	0.32	12.70	1.98	4.00	1.80	9.00	0.20	1.90	0.38
2019	30–60	7.85	1.81	25	—	0.45	15.40	2.15	5.60	2.00	10.20	0.20	1.30	0.32

calcium superphosphate (15.5% P_2O_5) was added to the soil. Nitrogen fertilizer was added after plants' emergence in the form of ammonium nitrate 33.5% at a rate of 180 kg/ha^{-1} which was divided into five equal doses before the 1st, 2nd, 3rd, 4th, and 5th irrigation. Potassium sulfate (48.52% K_2O) was divided into two equal doses of 120 kg/ha^{-1} before the 1st and 3rd irrigation. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days according to the amount of water irrigation used.

2.4. Plant Sample.

2.4. Plant Sample. Plant samples were gathered after 75 days from cultivation for measuring morphological parameters (plant height, tiller freshness, and dry weight). Samples of ten plants were gathered for biochemical analysis as photosynthetic pigments, total soluble sugars, proline, free amino acids, protein patterns, and molecular change.

At harvest (after 160 days from sowing), the following wheat characters were recorded on random patterns of ten girded plants in each treatment (grain yield and water productivity (WP) in kg m/m/ha^{-1}.

2.5. Irrigation Water Requirements. Two irrigation water requirements were calculated using the Penman–Monteith equation and crop coefficient according to [26]. The average amount of irrigation water applied with sprinkler irrigation system was 5950 and 4462.5 m^{3}ha^{-1} season^{-1} as (100% (WW) and 75% (WS)) for two seasons of 2017/2018 and 2018/2019, respectively.

The irrigation water requirements were calculated as follows:

$$\mathbf{IWR} = \left[\frac{\mathrm{ET}_0 \times K_c \times K_r \times I}{Ea} + LR\right] \times 4.2, \qquad (1)$$

where we have the following:

IWR = water irrigation quantities (m^3/ha^{-1})
ET$_0$ = evapotranspiration (mm day^{-1})
K_c = crop coefficient
K_r = reduction factor [27]
I = the period between two irrigations, day
Ea = irrigation water efficiency, 90%

LR = leaching requirement = 10% of the total water requirement applied to the treatment

2.6. Water Productivity (WP). WP was calculated according to Howell et al. [28]. Water productivity (WP) is realized as the relation between the grain yield and the quantity of irrigation water. The WP in kg/mm/ha was calculated by the following equation:

$$\mathrm{WP} = \frac{\mathrm{Ey}}{\mathrm{Et}}, \quad (2)$$

where WP is the water productivity (kg/m^3); Ey is the economical yield (kg ha^{-1}); and Et is the total utilized of irrigation water, m^{3}ha^{-1}/season.

2.7. Biochemical Analysis. Photosynthetic pigment content was determined according to the method described by Lichtenthaler and Buschmann [29]. Total soluble sugars were extracted and analyzed according to Prud' Homme et al. [30] and Yemm and Willis [31], respectively. Proline was assayed according to the method described by Bates et al. [32]. The free amino acid was determined according to the method described by Yeman et al. [33].

2.8. Protein Electrophoresis SDS-PAGE. A protein electrophoresis assay was conducted by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was performed according to Laemmli [34] as adapted by Studier [35].

2.9. Molecular Marker

2.9.1. Extraction of Genomic DNA. Bulk samples from young plant leaves of wheat varieties were soaked in liquid nitrogen for DNA extraction using 2% (CTAB) cetyl trimethyl ammonium bromide [36].

2.9.2. Sequence-Related Amplified Polymorphism (SRAP) Analysis. A total of six primers were used to amplify DNA as previously described [37] (manufactured by Bioneer, with new technology certification from ATS Korea). The total reaction mixture was 25 μL and contained 10X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs mixed, 10 pmol primers, 1.25 U Taq polymerase, and about 150 ng of genomic DNA. DNA-PCR amplification was performed in the thermal cycler (Biometra Inc., Germany). The temperature profile was as follows: initial denaturation at 94°C for 3 min; followed by 35 cycles of denaturation temperature 94°C for 5 min; annealing temperature of 37°C for 1 min; extension temperature of 72°C for 1 min; and final extension at 72°C for 5 min.

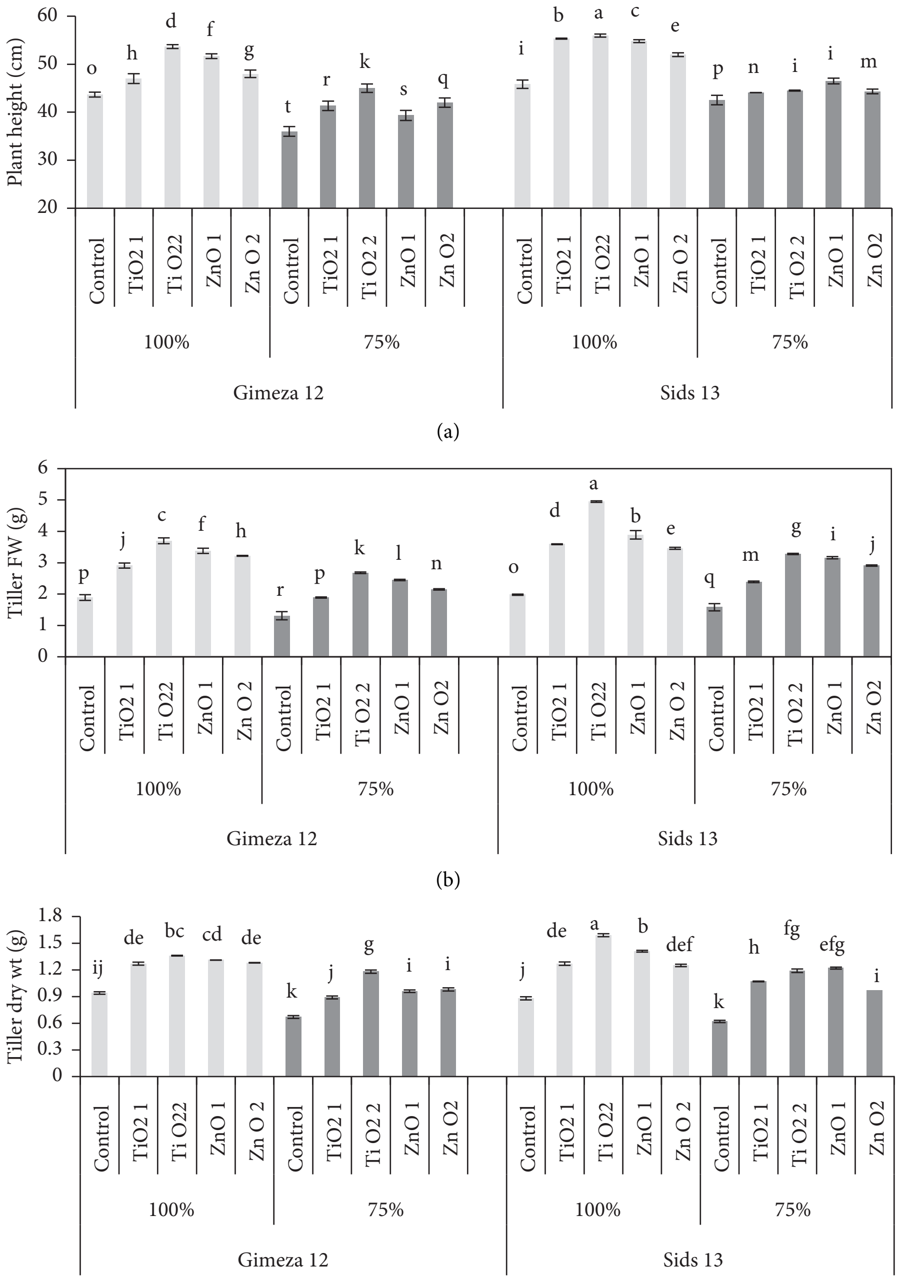
The amplification products were separated on a 1.5% agarose gel containing 1X TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) and 0.5 μg/mL ethidium bromide at 90 V. Gels were analyzed by UVI Geltec version 12.4, 1999–2005 (USA).

2.10. Statistical Analysis. The data were subjected to statistical analysis of variance of the split-split plot design. Since the trend was similar in both seasons, the homogeneity test Bartlet's equation was applied, and the combined analysis of the two seasons was carried out according to the method. Duncan's multiple range test was estimated to compare the means at $P < 0.05$ using SAS software (SAS Institute Inc. 2002; Steel and Torrie [38]).

3. Results and Discussion

3.1. Growth Parameters and Grain Yield of Wheat. The growth parameters, grain yield, and water productivity of both wheat cultivars Gimeza 12 and Sids 13 after treatment with nano-TiO_2 or nano-ZnO (5 and 10 mg/L) and grown under different water irrigation requirements (100% WW and 75% WS) are presented in Figures 2 and 3. All morphological studied traits (height of plant, tiller fresh, and dry weights) were significantly decreased by water stress ($P \leq 0.05$). Also, grain yield/ha was detected as compared to control plants (WW) in both cultivars. Water stress significantly decreased grain yield by 14.8% and 11.5% compared to that of the well-watered Gimeza 12 and Sids 13 cultivars, respectively (Figure 3(a)). Statistical analysis showed that application of nano-TiO_2 or nano-ZnO (5 and 10 mg/L) on water-stressed and well-watered plants induced a significant increase in the morphological parameters, grain yield, and crop water productivity (CWP) of both wheat cultivars (Figures 2 and 3). Particularly, 10 mg L^{-1} TiO_2 and ZnO nanoparticles treatments recorded grain yield of 6.40- and 6.30-ton/ha, respectively, compared to the untreated plant (5.39 kg/ton) in the Gimeza 12 cultivar. While, for the Sids 13 cultivar, the 10 mg L^{-1} of both treatments recorded grain yield of 6.09- and 6.01-ton ha^{-1}, respectively, compared to the untreated plant (4.94 kg/ton). These increments in grain yield of wheat plants in response to application of 10 mg/L of either nano-TiO_2 or nano-ZnO reached 27% and 26% in the Gimeza 12 and 23% and 16% in the Sids 13, respectively, over the control plants under WS. The results also cleared that the Gimeza 12 cultivar surpassed the Sids 13 cultivar in grain yield ha^{-1}. Also, exposure of plants to WS increased significantly the productivity of water as compared with control plants grown under the WW level in two cultivars (Figure 3(b)). Water stress led to a significant increase in WP by 13.6% and 18.0% compared to that of the corresponding WW control in both the Gimeza 12 and Sids 13 cultivars, respectively. Application of nano-TiO_2 or nano-ZnO with various concentrations on wheat plants significantly promoted water productivity at both water levels (WW and WS) as compared with the untreated plant in the Gimeza 12 and Sids 13. The increment in WP due to TiO_2 and ZnO at 10 mg^{-1} reached (18.8 and 16.7%) in the WW control and (27.1 and 26.0%) under the WS treatment in Gimeza 12 and (23.4 and 21.7%) in the WW control and (23.4 and 17.0%) under the WS treatment in Sids 13.

Lack of water from the plant cells due to water-deficits decreases wheat growth and yield production. Water deficiency decreased the plant dry weight and affected on the delivery of carbohydrates to produced grains [39].



(a)

(b)

(c)

Figure 2: Effect of different concentrations of nano-TiO_2 or nano-ZnO (5 and10 mg/L) on plant height (a), tiller fresh weight (b), and tiller dry weight (c) at 75 days from sowing of both wheat cultivars (Gimeza 12 and Sids 13) subjected to different levels of water irrigation requirement (combined analysis of two seasons). TiO_2 1 = 5 mg/L, TiO_2 2 = 10 mg/L, ZnO 1 = 5 mg/L, and ZnO 2 = 10 mg/L.

The enhancement of plant growth is perhaps related to the promoted photosynthetic pigments by n-TiO_2 treatment. Yang et al. [11] stated that TiO_2 nanoparticles increase spinach growth via improving nitrogen metabolism which encourages the absorption of nitrate in plants and accelerates the conversion of inorganic nitrogen into organic nitrogen, thus increasing the fresh and dry weights. Moreover, Jaberzadeh et al. [40] found that TiO_2NPs improved the growth and yield components of wheat plants grown under water deficit. Nano-TiO_2 modulates ROS-dependent signalling pathway(s), so it can regulate plant growth [41]. Also, TiO_2-NPs treatment stimulates plant metabolic activities as a nanonutrient fertilizer and improves biomass production [42]. Moreover, Owolade et al. [43] stated that treatment with nano-TiO_2 improved the seed yield of cowpea (*Vigna unguiculata* (L.)) because of increased photosynthetic rate and the activities of an antioxidant enzyme like catalase and peroxidase. In addition, Shallan et al. [44] proved that treatment with nano-TiO_2 could stimulate the drought tolerance of cotton plants and enhance the yield characteristics.

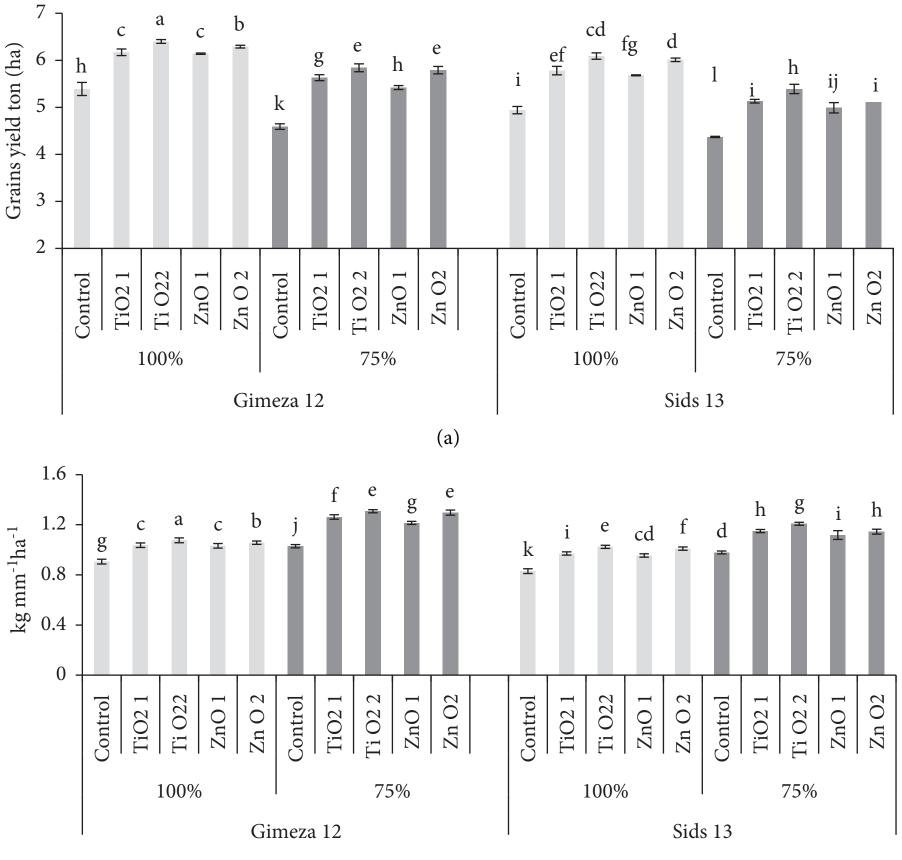


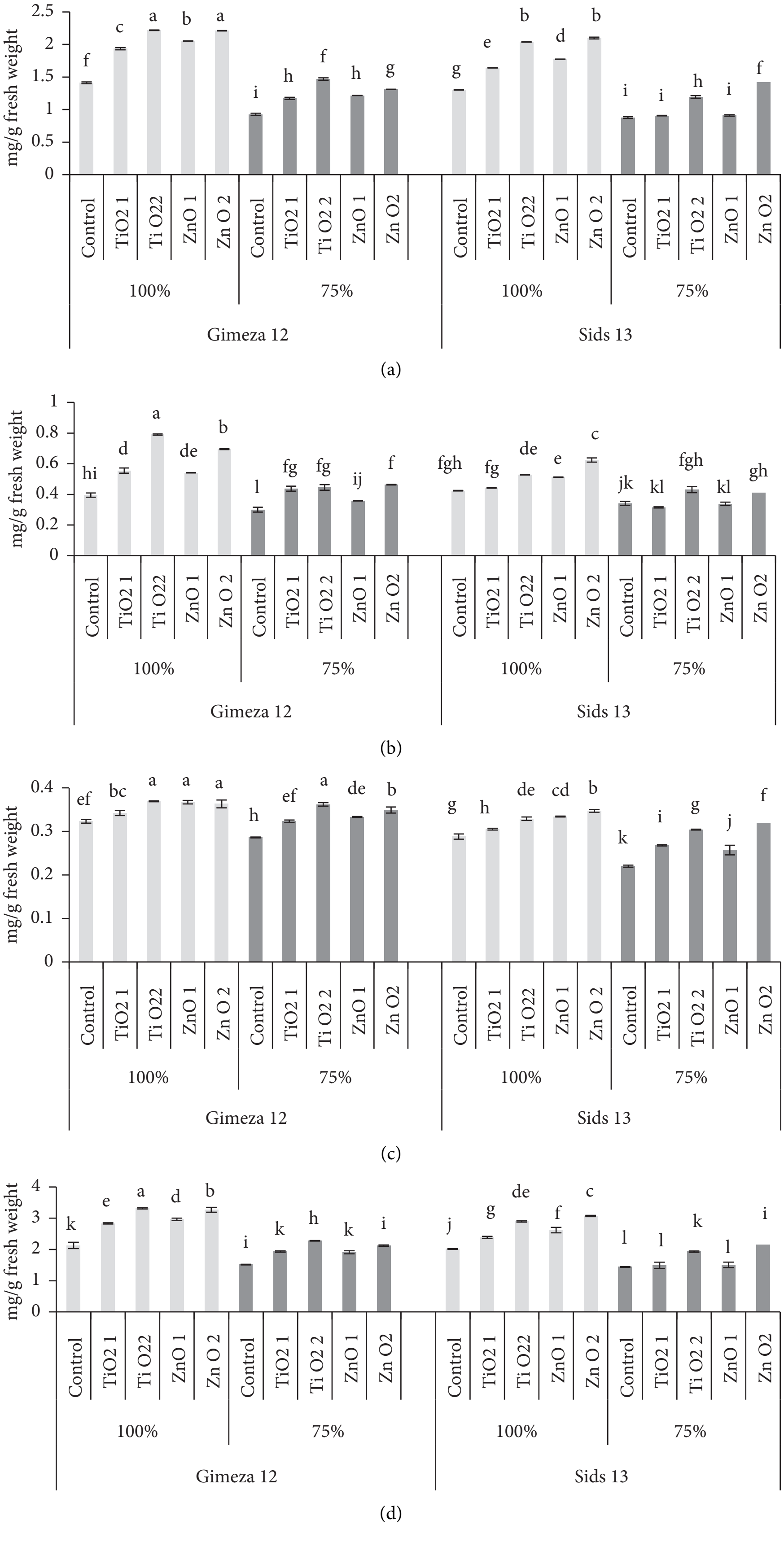
Figure 3: The effect of different concentrations of nano-TiO_2 or nano-ZnO (5 and 10 mg/L) on grain yield (a) and water productivity (b) at harvest of both wheat cultivars (Gimeza 12 and Sids 13) subjected to different levels of water irrigation requirement (combined analysis of two seasons). TiO_2 1 = 5 mg/L, TiO_2 2 = 10 mg/L, ZnO 1 = 5 mg/L, and ZnO 2 = 10 mg/L.

Zinc oxide nanoparticles (ZnONPs) increase plant growth and development in the wheat plant [45]. Zinc has an important role in the regulation of hormone metabolism via alteration in auxin levels through tryptophan biosynthesis and activating cell division and enlargement [46]. Zn induced the enhancement in protein synthesis [47] and scavenged free oxygen radicals. It appears to influence the capability for water uptake and transport in plants and decrease the negative influences of salt stress [48]. Zinc plays a vital role in chlorophyll biosynthesis, carbohydrates, lipids, proteins, as a cofactor in some specific enzymes (DNA and RNA polymerase), and the synthesis of some hormones [47].

The ratio of grain yield to water used is linked with crop water productivity (WP) in general and is inversely proportional with the intensity of water stress. Plants exposed to 75% of WIR caused significant increases in water productivity as compared with control plants (100% WIR) in both cultivars. In this regard, the WP is stimulated under water stress [49]. Through water stress, stomatal closure leads to reduced leaf conductance, photosynthesis, and transpiration. Because of the slight response of leaf conductance to decreased leaf water potential, the more conservative usage of water results in higher WP in water-lacking plants, which might be a mechanism for improving resource use efficacy [50]. WP is an important physiological adaptation mechanism that can progress crop productivity under water deficiency [51]. Stimulation in water productivity in response to various treatments suggests that the plant exploits various mechanisms to reduce water deficits, through decreasing its water consumption and preserving excess biomass [49].

3.2. Photosynthetic Pigments. Exposure of plants to the WS (75%) in the field conditions significantly decreased the contents of photosynthetic pigments as compared with control plants WW (100%) in both cultivars (Figure 4). The impact of TiO_2 or ZnO NPs (5 and 10 mg/L) increased significantly the photosynthetic pigments on wheat plants under various water levels when compared to the corresponding untreated plants. The highest values in total photosynthetic pigments were recorded through the application with 10 $mg{\cdot}L^{-1}$ nano-TiO_2 (55.8% and 55.3%) in response to the Gimeza 12 cultivar and using 10 mg/L nano-ZnO (52.43 and 49.41) in the Sids 13 at WW and WS, respectively. It is notable that the Gimeza12 cultivar surpassed the Sids 13 cultivar in all photosynthetic pigments.

Water stress induced a significant decrease in photosynthetic pigments due to stomata closure and Rubisco inhibition [52]. Carotenoid contents were significantly



(a)

(b)

(c)

(d)

Figure 4: The effect of different concentrations of nano-TiO_2 or nano-ZnO (5 and 10 mg/L) on chlorophyll a (a), chlorophyll b (b), carotenoids (c), and total pigments (d) of both wheat cultivars (Gimeza 12 and Sids 13) subjected to different levels of water irrigation requirement at 75 days from sowing. TiO_2 1 = 5 mg/L, TiO_2 2 = 10 mg/L, ZnO 1 = 5 mg/L, and ZnO 2 = 10 mg/L.

increased in both cultivars under treatment with nano-TiO_2 or nano-ZnO, in combination with WS when compared with control plants. The increased percentage reached 19.8% and 19.2% in the Gimeza 12 and 30.0% and 30.7% in the Sids 13 at nano-TiO_2 or nano-ZnO, respectively (Figure 4). Carotenoids have an important function as a free radical scavenger [53].

Nano-TiO_2 increased aged seeds' vigor and photosynthetic pigments' biosynthesis promotes the activity of ribulose 1, 5-bisphosphate carboxylase (Rubisco) and rises net photosynthesis in parallel to stimulating plant growth and proliferation [11]. They also added that, it promoted the absorbance of light, hurried the conversion and transport of light energy, kept the chloroplasts from senility, and extended the photosynthetic period of the chloroplasts. Moreover, Shallan et al. [44] found that TiO_2 nanoparticles increase photosynthetic pigments in cotton plants under drought stress. Additionally, Ze et al. [54] stated that n-TiO_2 may encourage light absorption by chloroplasts through regulation of genes related to light-harvesting complex II, which is in line with our observation of an increase of soluble sugars upon n-TiO_2 addition (Figure 4).

Tumburu et al. [55] stated that nano-TiO_2-induced genes were generally associated with photosynthetic metabolisms that were proved in a genome-wide transcriptome on *Arabidopsis thaliana* leaves. Also, Ze et al. [54] stated that n-TiO_2 may encourage light absorption by chloroplasts by regulation of genes concerning light harvesting complex II, which is in harmony with our result of an increase of TSS nano-TiO_2 (Figure 4).

In response to nano-ZnO, El Bassiouny et al. [45] observed that when treated with *Triticum aestivumn* L. with low concentrations of nano-ZnO (5 and 10 mg·L^{-1}), significantly raised photosynthetic pigments. In this regard, Raliya and Tarafdar [56] concluded that ZnONPs improved significantly growth, chlorophyll and protein synthesis in clusterbean plants. Zinc has an important role in the synthesis of indoleacetic acid (IAA) from tryptophan and other biochemical reactions required for chlorophyll and carbohydrate metabolism. In this regard, Sun et al. [57] found that, nano-ZnO improved the chloroplast and mitochondria stabilization which preserves the photosynthetic apparatus under water stress.

3.3. *The Change in Organic Solutes.*

The influence of water deficit and different treatments of TiO_2 or ZnO nanoparticles (5 and 10 mg/L) on some metabolites (total soluble sugars (TSS), proline (Pro), and free amino acids (FAA)) in two wheat cultivars (Gimeza 12 and Sids 13) is presented in Figure 5. Under water stress, plants accumulate higher amounts of TSS, Pro, and FAA content in both wheat cultivars by 24.2%, 112.2%, and 22.7 in the Gimeza 12 and 19.7%, 107.7%, and 25.3 in Sids 13, respectively, compared to the corresponding WW control. The application of nano-TiO_2 or nano-ZnO (5 and 10 mg/L) significantly increased ($P < 0.05$) the TSS, Pro, and FAA in WW or WS plants when compared to the corresponding controls in both cultivars. Results in Figure 5 showed that higher concentrations of either nano-TiO_2 or nano-ZnO (10 mg^{-1}) were more efficient in enhancing TSS, Pro, and FAA than lower concentrations under WS in both cultivars Gimeza 12 and Sids 13.

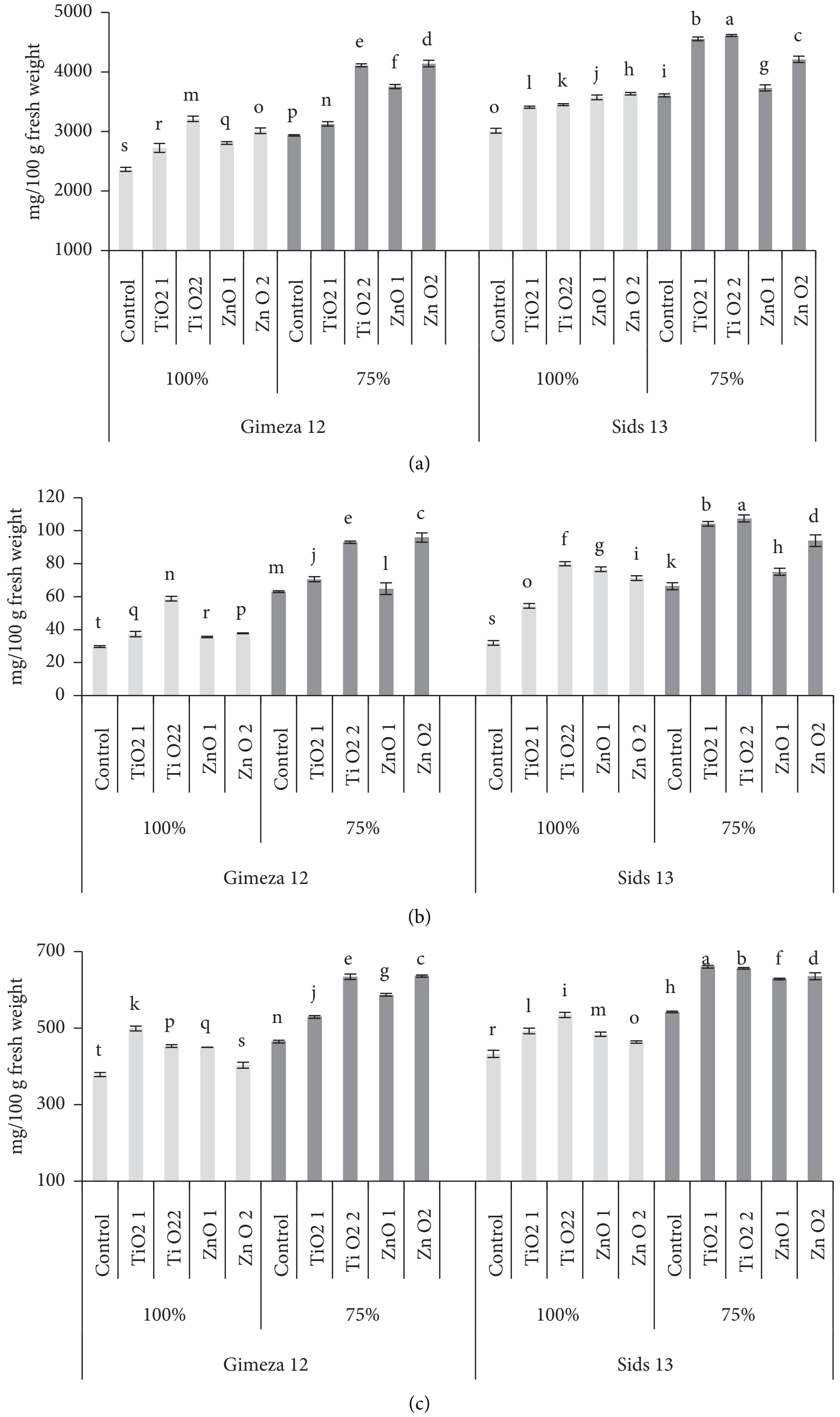
Osmoprotectant accumulation is a regular response of plants exposed to drought [49]. They added that the role of TSS, Pro, and FAA accumulation promote cells tolerance under water stress through stimulation of osmotic pressure in the cytoplasm and relative water content which are important for plant growth and development.

The application of nano-TiO_2 on wheat cultivars under drought stress conditions increased the content of total soluble sugars [58]. Moreover, Khater [59] reported that foliar application of coriander with TiO_2-Nps at 2, 4, and 6 mg/L significantly raised FAA and TSS contents. Shallan et al. [44] found that drought stress with nano-TiO_2 caused an increase in Pro, FAA, and TSS in cotton plants. Also, Mohammadi et al. [60] showed that TiO_2 induced accumulation of proline that is responsible for protecting cell turgor under different stresses, especially drought stress. Moreover, Abdel Latef et al. [61] showed that broad bean plants treated with n-TiO_2 at 10 mg/L led to significant increases in TSS, Pro, and FAA levels under salinity stress.

In this study, the nano-ZnO application might cause increases in TSS, Pro, and FAA concentrations under water stress, which indicates that nano-ZnO can improve osmotic regulation. Amira et al. [62] established that ZnO NPs treatment raised the TSS and Pro concentrations which are associated with the salt tolerance in maize. Abdel Latef et al. [19] and El-Bassiouny et al. [63] stated that zinc oxide nanoparticle treatment augmented the contents of compatible solutes (TSS, Pro, and FAA) in lupine and wheat plants under salinity stress.

3.4. *Protein Electrophoresis SDS-PAGE.*

SDS-PAGE of total protein extracted from wheat leaves treated with nanoparticles is shown in Figure 6 and Table 2. In this study, it was observed that treatment with TiO_2 or ZnO nanoparticles led to a slight increase or decrease in the protein banding pattern as compared with the control. A total number of 13 bands were scored ranging from 8 to 163 kDa; 8 of these were monomorphic (61.54%), while the other five bands were polymorphic (38.46% polymorphism). The highest number of bands was shown in Sids13 in control WW and WS (13 subunits), followed by the plants treated with nano-TiO_2 and nano-ZnO (12 subunits), except for nano-ZnO at WS (9 subunits). However, in Gimeza 12, the control WS plant recorded the two new polypeptides at the molecular weights (MWs) of 28 and 18 kDa and disappeared at 51 kDa as compared with the control WW plant. Moreover, Gimeza 12 treated with TiO_2 and ZnO nanoparticles stimulates the appearance of new bands at the MW of 28 kDa in WW and WS as compared with control WW. While in WS, nano-TiO_2 and nano-ZnO recorded the new polypeptides at 18 kDa not found in the same treatment in WW. Also, Gimeza 12 treated with nano-TiO_2 led to the disappearance of two bands with MWs of 163 and 51 kDa in WS when compared with the control and nano-TiO_2 in WW. On the contrary, at Sids 13, there is no difference between the treatments in WW



(a)

(b)

(c)

Figure 5: The effect of different concentrations of nano-TiO_2 or nano-ZnO (5 and 10 mg/L) on total soluble sugar (a), proline (b), and free amino acids (c) of both wheat cultivars (Gimeza 12 and Sids 13) subjected to different levels of water irrigation requirement at 75 days from sowing. TiO_2 1 = 5 mg/L, TiO_2 2 = 10 mg/L, ZnO 1 = 5 mg/L, and ZnO 2 = 10 mg/L.

and WS except for the nano-ZnO at WS that disappears the polypeptides at MWs of 163, 51, and 18 kDa. Moreover, the protein at Mw 42 kDa was presented only in the control plants at WW and WS in Sids 13. In addition, the increase and decrease in protein bands depend on the wheat cultivar. Data also recorded that Sids 13 cultivar was surpassed by Gimeza 12 with a slight increase in density, intensity, and number of protein bands.

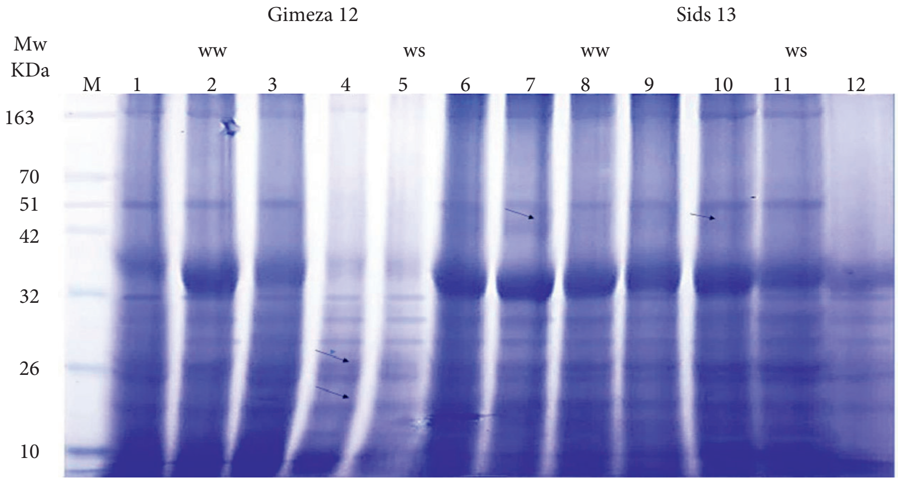


Figure 6: The change of protein bands (marked by arrowheads) in response to different treatments on wheat cultivars (Gimeza 12 and Sids 13) with nano-TiO_2 or nano-ZnO (10 $mg \cdot L^{-1}$) at different water irrigation requirements (WW and WS) at 75 days from sowing. Electrograph of soluble protein pattern by one-dimensional SDS-PAGE. Each lane contains equal amounts of protein extracted from the plant. Protein bands in the gel were visualized by Coomassie Blue stain. Lane M: Marker, Gimeza 12 (lane 1: WW, lane 2: WW + nano-TiO_2, lane 3: WW + nano-ZnO, lane 4: WS, lane 5:WS + nano-TiO_2, and lane 6: WS + nano-ZnO); Sids 13 (lane 7: WW, lane 8: WW + nano-TiO_2, lane 9: WW + NO, lane 10: WS, lane 11:WS + nano-TiO_2, and lane 12:WS + nano-ZnO).

Table 2: The effect of nano-TiO_2 or nano-ZnO (10 mg/L) on protein banding patterns of SDS-PAGE of both wheat cultivars (Gimeza 12 and Sids 13) subjected to different levels of water irrigation requirement at 75 days from sowing (+: present and −: absent).

No.	Mw KDa	Gimeza 12						Sids 13					
		100% WIR			75% WIR			100% WIR			75% WIR		
		C 1	TiO_2 2	ZnO 3	C 4	TiO_2 5	ZnO 6	C 7	TiO_2 8	ZnO 9	C 10	TiO_2 11	ZnO 12
1	163	+	+	+	+	−	+	+	+	+	+	+	−
2	51	+	+	+	−	−	+	+	+	+	+	+	−
3	42	−	−	−	−	−	−	+	−	−	+	−	−
4	36	+	+	+	+	+	+	+	+	+	+	+	+
5	32	+	+	+	+	+	+	+	+	+	+	+	+
6	30	+	+	+	+	+	+	+	+	+	+	+	+
7	28	−	+	+	+	+	+	+	+	+	+	+	+
8	26	+	+	+	+	+	+	+	+	+	+	+	+
9	24	+	+	+	+	+	+	+	+	+	+	+	+
10	20	+	+	+	+	+	+	+	+	+	+	+	+
11	18	−	−	−	+	+	+	+	+	+	+	+	−
12	10	+	+	+	+	+	+	+	+	+	+	+	+
13	8	+	+	+	+	+	+	+	+	+	+	+	+
Bands no. = 13		10	11	11	11	10	12	13	12	12	13	12	9

In leaves of Gimeza 12 and Sids 13 cultivars grown under water deficit, the alteration in protein electrophoretic patterns was recorded with several kinds of alterations. Some protein bands appeared in the synthesis of the novel set of protein bands. In this regard, El-Bassiouny et al. [64] and Sadak et al. [49] approved these obtained results in sunflower and wheat plants under drought conditions, respectively. They added that these proteins have a stimulative osmoprotectant role and a preserving cellular structures. Also, Abdallah [58] suggested that groups of membrane-bound proteins are involved in renovating membrane damage caused by water deficit, managing the permeability of the membrane to water, and influencing water movement through the tissues and cells, consequently maintaining their normal turgidity.

These results were similar to Hajra and Mondal [65] who demonstrated that the *Cicer arietinum* treated with ZnO and TiO_2 NPS increased the protein content. Dubchak et al. [66] reported that silver and titanium NPs have a great surface area in proportion to capacity rate which is responsible for the promotion of their bioactivity, bioavailability, and biochemical metabolism. In this connection, Priyanka and Venkatachalam [67] observed that the treatment of the cotton plant with ZnO nanoparticles improved the total soluble protein (TSP) content compared to the untreated plant. Despite this, the protein content decreased slightly at a higher dose. Similarly, the ZnO NPs at lower doses improved the TSP content in the pea [68]. Moreover, Priyanka and Venkatachalam [67] mentioned that the upregulated expression of proteins protects the plant cells from any

Table 3: SRAP-PCR analysis of two wheat cultivars (Gimeza 12 & Sids 13) treated with nano TiO2 or ZnO (10mg/l) subjected to different levels of water irrigation requirments at 75 days from sowing.

(a) SRAP primers used in this study

Primer's name	Sequence Forward	Reverse
SRAP1	TGAGTCCAAACCGGATA	GACTGCGTACGAATTAAT
SRAP2	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTTGC
SRAP3	TGAGTCCAAACCGGAAT	GACTGCGTACGAATTGAC
SRAP4	TGAGTCCAAACCGGACC	GACTGCGTACGAATTTGA
SRAP5	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTAAC
SRAP6	TGAGTCCAAACCGGTCA	GACTGCGTACGAATTACG

(b) SRAP-PCR analysis of two wheat cultivars treated and untreated with nanoparticles

Primer code no.	Size range of the scorable loci (bp)	Total loci	No. of monomorphic loci	No. of polymorphic loci	% polymorphism	Unique loci	Molecular size of markers (bp)
SRAP-1	90–850	7	3	4	57.14	0	0
SRAP-2	330–495	3	2	1	33.33	1	233-
SRAP-3	250–850	6	5	1	16.66	0	0
SRAP-4	133–361	3	2	1	33.33	0	0
SRAP-5	160–500	5	2	3	60.00	0	0
SRAP-6	125–512	3	2	1	33.33	0	0
Total	90–850	27	16	11	40.74%	1	3.70%

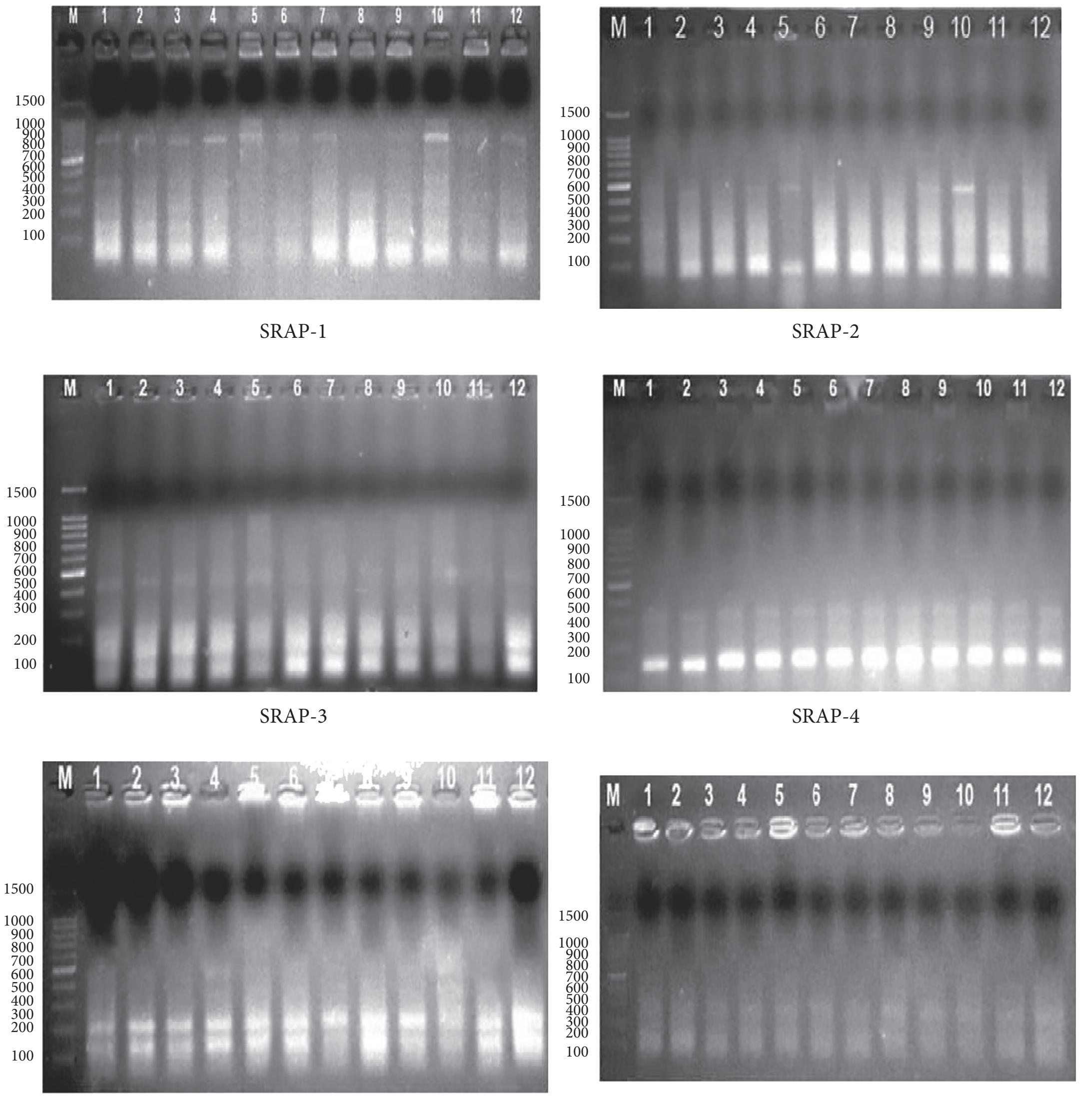
oxidative stress induced by nanofertilizer in the cotton plants. Recently, El-Bassiouny et al. [63] found that nano-ZnO at 5 and 10 mg·L^{-1} could be considered as positive markers at MWs of 51 and 40 kDa and stimulated responsive protein band number and density in wheat cultivars.

3.5. Molecular Markers Analysis

3.5.1. Genetic Diversity Based on SRAP Markers. The experimental design with the two cultivars (Gimeza 12 and Sids 13) was treated with nano-TiO$_2$ or nano-ZnO (10 mg/L). Genomic DNA from the leaves of control and various treatments was amplified by utilizing six SRAP primers. Sequence-related amplified polymorphism (SRAP) is a powerful technique for determining genetic changeability due to its high reproducibility, discriminatory power, and high polymorphism rate in many genetic studies.

A total number of 27 fragments were amplified via six influential SRAP-PCR primers (4.5 loci per primer), ranging from 90 to 850 bp (Table 3 and Figure 7). Sixteen alleles were monomorphic bands (59.25%), whereas 11 loci were polymorphisms (40.74%). The number of alleles per primer differs from three by SRAP-2, SRAP-4, and SRAP-9 to seven by SRAP-1. Primer SRAP-5 obtained the highest number of polymorphisms (60%), followed by primer SRAP-1 (57.14%). Also, primer SRAP-3 recorded the lowest number of polymorphism (16.66%). Except 1 out of the 27 was a unique band (3.70%). One band with a molecular size of −233 bp disappeared in the Gimeza 12 cultivar treated with WS + nano-TiO$_2$ and was revealed in the other treatments using primer SRAP-2. Consequently, this band may be considered a negative marker. It is noted that WS + nano-TiO$_2$ in Gimeza 12 cultivar and WS in Sids 13 cultivar treatments exhibited one band of 850 bp and was absent in the other treatments using primer SRAP-1. On the contrary, one band with a molecular size of 330 bp was absent in WW + nano-TiO$_2$, WW + nano-ZnO in the Gimeza 12 cultivar, WW + nano-TiO$_2$, WW + nano-ZnO, WS + nano-TiO$_2$, and WS + nano-ZnO in the Sids 13 cultivar, respectively, using primer SRAP-1. Also, one band of 290 bp was scored in the treatments WW, WW + nano-TiO$_2$, WW + nano-ZnO, WS in the Gimeza 12 cultivar, WS from the Sids13 cultivar, and was absent with the other treatments. Moreover, with a molecular size of 283 bp, one band from WS + nano-TiO$_2$ and WS + nano-ZnO in the Gimeza 12 cultivar was absent while revealed in the other treatments. One band was revealed with 850 bp in the Gimeza 12 treated with WS and WS + nano-TiO$_2$, and the Sids 13 treated with WS, while absent in the other treatments using primer SRAP-3. In addition, one band of 216 bp was revealed in all treatments of the Sids 13 cultivar and was absent in all treatments of Gimeza 12, using primer SRAP-4. One band with a molecular size of 500 bp disappeared from the WW + nano-ZnO and WS + nano-ZnO treatments of the Gimeza 12 cultivar, WW, WS + nano-TiO$_2$, and WS + nano-ZnO in the Sids 13 cultivar, while appeared with the remaining treatments, using primer SRAP-5. Also, one amplified fragment of 380 bp was absent in the Sids 13 cultivar treated with WW and WS + nano-TiO$_2$, but appeared with the other treatments. In addition, one band with a molecular size of 277 bp disappeared in WW, WW + nano-TiO$_2$, and WW + nano-ZnO of the Gimeza 12 cultivar and WW, WW + nano-TiO$_2$, and WS + nano-TiO$_2$ in the Sids13 cultivar. It was found in all the other treatments, using primer SRAP-5. It is noted that one fragment of 512 bp was exhibited in the Sids 13 cultivar treated at WW + nano-TiO$_2$, WW + nano-ZnO, and WS and was absent in the rest of the treatments, using primer SRAP-6.

Results show that drought WS and 10 mg·L^{-1} of treatments for TiO$_2$ or ZnO NPs have little effect on DNA and are illustrated with the appearance or absence of the bands. Moreover, the Sids 13 cultivar was more affected by treatments than the Gimeza 12 cultivar. The study showed that the low concentration of nanoparticles had not caused DNA damage. In contrast, the high concentration of nanoparticles induced DNA damage. Our results are closely in accordance with those recorded by Al Quraidi



SRAP-1 SRAP-2

SRAP-3 SRAP-4

SRAP-5 SRAP-6

Figure 7: SRAP-PCR profiles using six primers of two wheat cultivars treated and untreated with nanoparticles at 75 days from sowing. Lane M: 100 bp DNA Ladder; Gimeza 12 (lane 1: WW (control); lane 2: WW + nano-TiO_2; lane 3: WW + nano-ZnO; lane 4: WS; lane 5: WS + nano-TiO_2; and lane 6: WS + nano-ZnO); Sids 13 (lane 7: WW (control); lane 8: WW + nano-TiO_2; lane 9: WW + nano-ZnO; lane 10: WS; lane 11: WS + nano-TiO_2; and lane 12: WS + nano-ZnO).

et al. [69], who obtained cytotoxic effects of cucumber plants (*Cucumber sativus*) treated with (50 $mg{\cdot}L^{-1}$, 100 $mg{\cdot}L^{-1}$, and 150 $mg{\cdot}L^{-1}$) CuNPs. Moreno–Olivas et al. [70] used RAPD to evaluate the genotoxicity in *Cucurbita pepo* treated with TiO_2 which established DNA changes in treated versus control conditions. Also, Mattiello et al. [71] assessed the genotoxicity in *Hordeum vulgare* L. treated with cerium oxide (CeO_2) and titanium oxide (TiO_2) using RAPD profiles. López–Moreno et al. [72] used a RAPD assay to find DNA damage and mutations that occurred due to nanoparticles. The appearance of four new bands at 2000 $mg{\cdot}L^{-1}$ and three new bands at 4000 $mg{\cdot}L^{-1}$ treatment of CeO_2 NPs were recorded. Andersen et al. [73] reported that stimulation or inhibition influences on germination and early growth were detected in response to the nanoparticles, based on the species of plant. Gene expression was measured during germination and early seedling proliferation. In *A. thaliana*, TiO_2 and CeO_2 NPs induced several genomic responses as regulation in genes responsible for oxidative stress, which was responsible for early growth and development.

4. Conclusion

Water stress produced an opposing influence on plants through decreased growth, development, photosynthetic efficiency, and water status. The deleterious influences encouraged through a drought can be ameliorated via the application of nano-TiO_2 and ZnO in wheat plants. They enhanced growth, grain yield, and crop water productivity in both wheat cultivars. Also, nanoparticles raised photosynthetic pigments and osmoprotectant content, and some protein bands were improved. It was observed that nano-TiO_2 and ZnO (10 mg/L) were the most efficient in wheat cultivars exposed to water deficits. The results showed that the Gimeza 12 cultivar was more tolerant to drought and had the highest grain yield than the Sids 13 cultivar.

Data Availability

The original contributions presented in the study are included within the article, and further inquiries can be directed to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Prof. Hala M.S. El-Bassiouny and Maha M.S. Abdallah involved in the planning of the work, were responsible for all the physiological and biochemical analysis, and wrote the manuscript. Associate Prof. Heba A. Mahfouze and Magda A. M. El-Enany involved in the planning of the work, were responsible for the biochemical and molecular analysis, and wrote the manuscript. Prof. Bakry A. Bakry involved in the planning of the work, farming of the plants and follow-up, taking samples, and statistical data analysis and wrote the manuscript. All authors read and approved the final manuscript.

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