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Effect of zinc oxide nanoparticles on the growth, genomic DNA, production and the quality of common dry bean (*Phaseolus vulgaris*)

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ABSTRACT

Zinc as an essential micronutrient for plant metabolism was prepared as zinc oxide nanoparticles (ZnO-NPs), where their structure and morphology were investigated by X-ray diffraction and transmission electron microscope. A suspension solution of ZnO-NPs was used as a foliar fertilizer for common bean with the concentration 0, 10, 20, 30 and 40 ppm, during two successive seasons 2016 and 2017, to study there on the production and the quality of common bean. Also, the Genotoxic effect of ZnO-NPs on genomic DNA of common bean by RAPD-PCR and on the expression of some genes encoding proteins by SDS-PAGE was studied. It was found that the best values of common bean plant criteria i.e. the number of branches and leaves per plant, fresh and dry weight of branches and leaves, as well as the highest yield of seeds (2.41 and 2.48 ton/ha during 2016 & 2017, respectively) were obtained with 30 ppm of ZnO-NPs. The RAPD analysis showed that the ZnO-NPs (20 and 30 ppm) effect on the plant genome and on the expression of some genes encoding proteins. Also, the SDS-PAG analysis represented those unique proteins at the molecular weight 78 KDa appeared in the plant treated by 30ppm ZnO-NPs these proteins may play an important role in enhancement the agro-morphological criteria of the plant.

1. Introduction

The dry common bean (Phaseolus vulgaris L.), is the majority essential food legume for direct eating in the world. Among the major food crops, it has one of the maximum levels of difference in growth habit, seed characteristics (shape, volume, and color), ripeness, and adaptation. The dry common bean seeds are the economic part of the bean plant. They are estimated in the developing world because they have good dietetic properties and a prolonged storage life about two year with moisture content lower than 10% at 8 °C (Aparicio-Fernández et al., 2005; Palilo et al., 2018). It plays an important function in human nutrition for that the reason it considers the source for protein, carbohydrates, vitamins and elements thus it is considered as one of the most vital vegetable crops cultivated in Egypt for the domestic market and export market (Abdel-Hakim et al., 2012; Boutraa, 2009).

The world population is increasing every year, so agricultural productivity must be increasing. This can be done either by expanding the area of the cultivated land or by increasing the productivity of plants. Nano fertilizers are the resent and most technically advanced way of supplying mineral nutrients to plants compared to conventional fertilizers. It supplies nutrient required for the plant, therefore, minimizes leaching and improves fertilizer efficiency. Nanoparticles (NPs), that defined as particles have a size range of 1–100 nm, were used in the industrial and agriculture fields (Jayarambabu et al., 2014; Srilatha, 2011). It can be synthesized by various methods such as sol-gel method (Toutorski et al., 2003), chemical vapor deposition (Purica et al., 2002), homogeneous precipitation (Taubert et al., 2002), thermal decomposition (Yang et al., 2004) or hydrothermal synthesis (Pimentel et al., 2015). It causes various morphological and physiological changes when interacting with plants, depending on the chemical structure, size, reactivity, and most significantly the dose at which they are useful (Elizabath et al., 2017; Khodakovskaya et al., 2012).

Zinc is considered one of the most important micronutrients for plants, whereas, there are limited studies conducted to investigate its beneficial effect on plant growth (Lin and Xing, 2007). Even though there is a small amount of Zn require for plants (5–100 mg/kg) but if

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enough of this element is not available, the plants suffer physiological stress resulting from the incompetence of several enzyme systems and other metabolic actions interrelated to Zn (Baybordi, 2006). Fageria et al. (2002) recorded that zinc plays an essential role in carbohydrate and proteins metabolism plus it controls plant growth hormone. Also, it is required for the production of tryptophan which is the precursor of indole acetic acid (IAA), as well as it has an active role in the production of an essential growth hormone auxin (Alloway, 2004).

Fewer studies have been done on the beneficial effect of ZnO-NPs on plant growth, additionally, the effect of zinc NPs varied according to the type of the plant (Lin and Xing, 2007). Mahajan et al. (2011) found that ZnO-NPs affect the growth of mung (*Vigna radiata*) and gram (*Cicer arietinum*) seedlings. The highest effect was found at 20 ppm for mung and 1 ppm for gram seedlings. While, Prasad et al. (2012) illustrated that when peanut treated with ZnO-NPs as a foliar application it improved the germination, root and shoot growth, and the pod yield than that treated with chelated $ZnSO_4$. In this trend, ZnO-NPs application increases growth characteristic, photosynthesis and biomass of wheat plant (Munir et al., 2018).

Many genotoxic studies have been carried out on mammalian and human cell, but studies on plants in this field are rare. Nowadays, studies on the genotoxic effects of NPs on plants are appearing. Genotoxicity describes the property of chemical agents that damage the genetic information within a cell causing mutations, induced by nanoparticles in plants (Remédios et al., 2012). Randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) is used for DNA analysis in the field of genotoxicity in different plants as a sensitive method, capable of detecting variations in genome profiles (Kekec et al., 2010; Liu et al., 2009). The genotoxic and phytotoxic studies represent that ZnO and CuO -NPs at 2000 and 4000 mg/l, respectively, effects on buckwheat (Fagopyrum esculentum) seedling (Lee et al., 2013).

Zinc is an important micronutrient for plant metabolism. This study aims to increasing the efficiency of zinc by using zinc oxide nanoparticles, as well as established the effects of different concentrations of ZnO-NPs as a foliar application on plant growth, yield and chemical composition of common bean plant. Also, the genotoxic effect of ZnO-NPs on genomic DNA of common dry bean using the molecular marker (RAPD-PCR) and on the expression of some genes which encoding into proteins by SDS-PAGE was investigated.

2. Materials and methods

2.1. Materials

Seeds of common bean (Nebraska) were provided from Agricultural Research Centre, Ministry of Agricultural and Land Reclamation. Superphosphate and potassium sulfate are obtained from El-Naser Company for intermediate chemicals, Egypt. Ammonium sulfate was supplied from Abu Qir Fertilizers Company, Egypt. Sodium hydroxide and zinc acetate were obtained from Sigma-Aldrich Company.

2.2. Methods

2.2.1. Preparation of ZnO nanoparticles

ZnO-NPs prepared by dissolving 3.942g zinc acetate and 1.44 g NaOH in one litter ethanol and refluxed at 70 °C for 2 h. Zinc acetate was converted to zinc oxide which dispersed in an alcohol medium, where it was clear and transparent. ZnO-NPs can be obtained and purified by adding DI-water and then separated from the dispersion by centrifugation at 5000 rpm for 10 min then dried in the oven at 60 °C for 24 h. The calcination of the powder was conducted at 500 °C for 2h to obtain ZnO-NPs (Chen et al., 2008).

2.2.2. Experiment layout

The field was cleaned, plowed, leveled and divided into plots at the mid of February. Seeds were sown on the first week of March at rate 120 kg/ha during two seasons 2016 and 2017 in clay soil in Shebin El-Kom, El-Monifia governorate, Egypt, and then sown at rate of two seeds per hill and 30 cm distance between hills on one side of ridge (60 cm at distance between ridges). Organic manure (47.6 m^3/hectare) were added during the soil preparation with calcium superphosphate at rate 476 kg/ha (15.5% P_2O_5) + 238 kg/ha agricultural sulfur +119 kg/ha potassium sulphate (48% K_2O) + 119 kg/ha ammonium sulfate (20.6% N) before the first irrigation (after seeds germination) ammonium sulfate (20.6% N) at rate 238 kg/ha were applied, then potassium sulphate (48% K_2O) 119 kg/ha + ammonium sulfate (20.6% N) 238 kg/ha were supplied before the second irrigation.

2.2.3. Experiment treatments

Common bean plants were sprayed with ZnO-NPs after 20 days after sowing with 0, 10, 20, 30 and 40 ppm concentration. The experiment was set in a completely randomized block design with five replicates for each particular treatment.

2.3. Characterization of ZnO-NPs

2.3.1. X-ray diffraction (XRD)

The XRD pattern of ZnO-NPs was carried out on a Diano X-ray diffractometer using CoK_α radiation source energized at 45 kV and a Philips X-ray diffractometer (PW, 1930 generator, PW, 1820 goniometer) with CuK radiation source ($\lambda = 0.15418$ nm). The basal spacing (dL) was calculated from the (001) reflection via the Bragg's equation.

2.3.2. Transmission electron microscope (TEM)

The morphological and particles size of ZnO-NPs were demonstrated by using TEM model JEM-1230, Japan, operated at 120 kV, with a maximum magnification of 600×10^3 and a resolution until 0.2 nm. A drop of an aqueous dispersion of the nanomaterial was placed on a carbon-coated copper grid and allowed to dry in the air before characterization.

2.4. Agro-morphological criteria

Five plants of common bean plants were randomly taken from each experimental plot at vegetative stage (after 45 days from seeds sowing) for measuring the vegetative growth parameters i.e. plant length, root length, number of leaves and branches per plant, fresh and dry weight of leaves and branches.

2.5. Yield and its components

The plants were harvested to determine the number of pods/plant, weight of pods, seeds/plant, seed index (100 seed weight), shelling percentage, shoot residues per plant, weight of seeds and shoot residues (ton/ha and kg/ha, respectively) at harvesting stage (after 90 days from sowing).

2.6. DNA extraction and RAPD-PCR analysis

RAPD technology is rapid, reproducible and does not require specific knowledge of the DNA sequence, which makes it ideal for examining and estimating the genomic variation in study the mutagenicity or genotoxicity (Atienzar and Jha, 2006). The plant genomic DNA was isolated from common dry bean plant by using Gene Jet Plant Genomic DNA purification Mini Kits (Thermo scientific K0791). The extracted plant DNA was quantitated using a NanoDrop 1000 spectrophotometer (Thermo Scientific) and diluted to 50 ng/μl then used as PCR a template.

RAPD-PCR was performed by using seven random primers such as A-12 (5'-TCGGCGATAG-3'), OP-A08 (5'-GTGACGTAGG-3'), OP-B10 (5'-

CTGCTGGGAC-3'), OP-C19 (5'-GTTGCCAGCC-3'), OP-H07 (5'-CTGCAT CGTG-3'), OP-H12 (5'-ACGCGCATGT-3') and OP-H18 (5'- GAATCGG CCA-3'). PCR amplification for isolated DNA was performed in 0.2 ml PCR Eppendorf containing (25 µl) consisted of 12.5 µl Dream Taq green PCR Master Mix 2X (Thermo scientific K1081), 1 µl primer 10 pmol (Metabion, German) and 1 µl Template DNA (50 ng/µL) then completed to 25 µl by water (nuclease-free). Thermocycler (Bio-Rad) was programmed as follows: 94 °C for 5 min (one cycle) then 94 °C for 1 min, Tm°C for 45sec and 72 °C for 45sec (35 cycles) then 72 °C for 5min (one cycle) then held at 4 °C. Then 100 bp DNA Ladder H3 RTU (GeneDirex, Cat No. DM003-R500) and 5 µl of DNA amplified PCR product were loaded in each well of agarose and then placed in 1X TAE buffer (1%) and run at 100 V for about 2 h. The gel was photographed by gel documentation (Bio-Rad) and was analyzed by Total Lab program to find out the molecular size of each band.

2.7. SDS-PAGE protein banding patterns

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is considered a successful tool to determine the mutagenicity resulted from accumulative pollution such as chemicals also it described a genetic structure which correlated with the produced variation due to genotoxic of genetic material resulted by environmental pollutants (Oppong-Konadu et al., 2005; Salimi, 2013). SDS-PAGE was performed according to the method of Laemmli (1970), and described by Tsugama et al. (2011).

The water-soluble proteins (W·S·P) were extracted from the leaves of these plants in two different growth stage (seedling and flowering stage). The marker of used protein is BLUltra Prestained Protein Ladder (GeneDirex, Cat No. PM001-0500). Protein fractionations were performed exclusively on vertical slab gel (19.8 cm × 26.8 cm x 0.2 cm) using the electrophoresis apparatus manufactured by Cleaver, UK. The images were captured by digital camera (Sony, made in Japan) and transferred directly to the computer and then the protein bands were analyzed by Total Lab program to find out the molecular weight of each band and that to find the effect of different concentrations ZnO-NPs on gene expression of different genes responsible for formation of soluble proteins in common dry bean.

2.8. Chemical analysis

2.8.1. Photosynthetic pigments (chlorophyll a, b, total chlorophyll and carotenoids)

Photosynthetic pigments were determined in fresh leaves (45 days from sowing). The chlorophyll (a and b) and carotenoid contents were determined after extraction in 85% acetone and measured spectrophotometrically at 663 and 644 nm and 452, respectively, using SHIMADZU 240 UV/VIS spectrophotometer. The amount of these pigments was calculated using the equations proposed by Jiang et al. (2018).

2.8.2. Proximate analysis, minerals and amino acids determination

Fresh samples of common bean (leaves and seeds) were dried in the oven at 60 °C till constant weight, and then dried sample was taken for proximate analysis (100g dry weight basis) and minerals determination.

2.8.2.1. Proximate analysis. The proximate composition was determined by using the standard methods of the AOAC (Abd El-Aziz et al., 2019; Jayant et al., 2018). Ash was measured by ashing the samples at 550 °C for 6 h in a muffle furnace. The moisture content was calculated by drying the samples at 105 °C in the oven until constant weight was achieved. The crude protein content ($N \times 6.25$) was determined by the Kjeldahl method. The crude fiber of experimental diets was determined by acid digestion (1.25%) followed by alkali digestion (1.25%). The ether extract was estimated by the solvent extraction method. The energy value was calculated using the Atwater factor method [(9x fat) + (4 x carbohydrate) + (4 x protein)] as described by Nwabueze (2007).

2.8.2.2. Minerals determination. Plant samples were ground and digested with H_2SO_4-H_2O_2. The concentration of phosphorus was determined by spectrophotometer, whereas zinc, copper, iron and manganese in the digested solutions were determined by atomic absorption. Potassium was determined by flam spectrophotometer. While the nitrogen, in the digested solutions, was determined by the Kjeldahl method (Hao et al., 2018; Nielsen, 2010).

2.8.2.3. Amino acids analysis. Amino acids were determined by HPLC according to Moran-Palacio et al. (2014). The samples were digested with HCl at 150 °C in oven for 6 h, the obtained mixtures were filtrate with syringe filter and after evaporation the samples were dissolved in a sodium citrate buffer (0.1 mol/l, pH 2.2). Sample derivation was achieved adding o − phthalaldehyde 7.5 mM to the sample on citrate buffer. The HPLC method precision and accuracy was evaluated using external and internal standards.

2.9. Statistical analysis

All data were subjected to statistical analysis according to the procedures reported Kobata et al. (2018). The data obtained were subjected to analysis of variance (ANOVA) and analyzed for statistically significant differences using LSD test at 5% level.

3. Results and discussion

3.1. Characteristics of zinc oxide nanoparticles

The prepared ZnO-NPs had a particles size less than 20 nm (Fig. 1a). In addition, the ZnO-NPs possessed main peaks at 2θ = 32°, 34.4°, 36.4°, 47.7° and 56.7° corresponding to (100), (002), (101), (102) and (110) planes, respectively (Fig. 1b). All peaks were appropriated to ZnO-NPs structure (Chen et al., 2008). The crystal size of ZnO-NPs was 8 nm, where it calculated from the full width at half maximum (FWHM (β)) of the diffraction peaks using Debye - Scherer's equation (Kumara et al., 2014).

$$d = \frac{K\lambda}{\beta \cos(\theta)}$$

Where (d) is the average crystalline dimension perpendicular to the reflecting phases, (k) is Scherer's constant (0.92), (λ) is the X-ray wavelength = 1.5403, (β) is FWHM intensity of a Bragg reflection excluding instrumental broadening, and (**θ**) the Bragg's angle.

3.2. Vegetative growth criteria

The results show that the concentration 30 ppm was significantly to improve the growth, yield quality and quantity of common bean. Where, the plants sprayed with 30 ppm ZnO-NPs had the highest plant length (61.0 & 61.9 cm), root length (20.0 & 20.7 cm), number of leaves/plant (45.7 & 49), branches per plant (5.0 & 5.1), fresh weight of plant (227.2 & 242.3 g) and dry weight of plant (40.3 & 44.8 g) during two seasons 2016 and 2017, respectively, as compared to other concentrations (Table 1).

This effect might be due to the efficiency of nanoparticles on enhancement the expression of some genes encoding proteins (Table 3) which stimulate some metabolic processess that reflected on increasing plant growth criteria (Table 1). These results were in harmony with those obtained by Lin and Xing (2007), who observed that the application of ZnO-NPs (2 mg/l) improved root elongation of germinated radish (*Raphanus sativus*) and rape (*Brassica napus*) seeds over those of the control, while metallic Zn-NPs (2 mg/l) enhanced growth of ryegrass (*Lolium perenne*) seedlings. Jayarambabu et al. (2014) showed that ZnO-NPs with different concentration enhanced plant growth shoot of

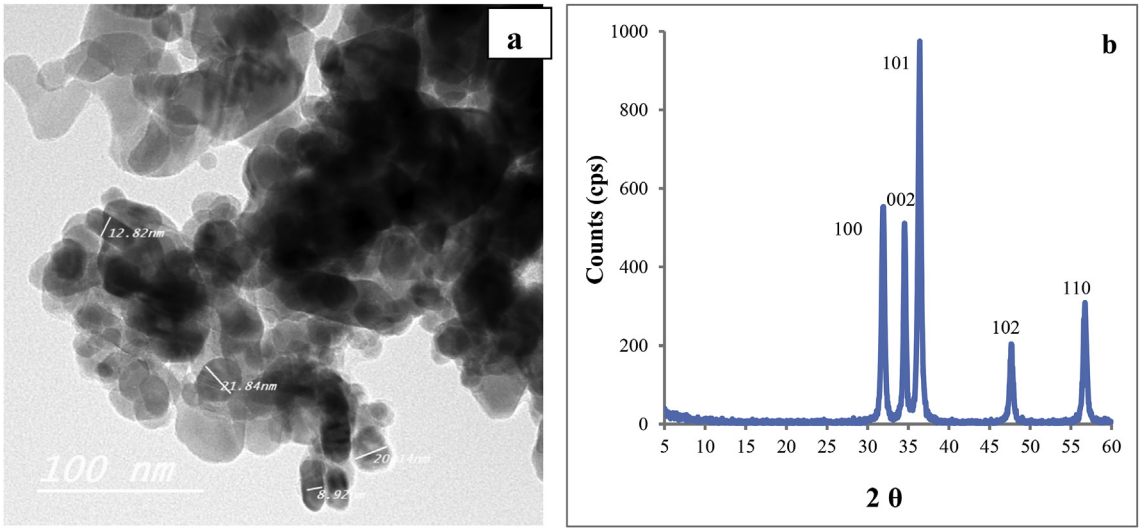

Fig. 1. (a) TEM image and (b) XRD patterns of ZnO-NPs.

mung bean as compared with control. Since the concentration 20 mg of ZnO-NPs gave the highest shoot length as compared with other concentration. It was noted that the low concentration of ZnO-NPs improves the plant shoot development and plant growth hormone. Laware and Raskar (2014) investigated that onion plants treated with 20 and 30 mg/l of ZnO-NPs lead to improving the growth, flowered earlier by 12–14 days and significantly improved seed fruit per umbel than the control. These results could be discussed by Fageria et al. (2002), who recorded that zinc plays important role in carbohydrate and proteins metabolism as it controls plant growth hormone i.e. (IAA). Also, these results might be due to increasing the photosynthetic pigments with 30 ppm ZnO-NPs (Table 5).

3.3. Yield and its components

The yield and its components were significantly increased with enhancement the ZnO-NPs concentration (Table 2). The common bean plants treated with 40 ppm ZnO-NPs gave the highest numbers of pods/plant (32.7 & 32.0), the weight of shoot residues per hectare (804.0 & 869.6 kg/ha) and shelling percentage (96.3 & 97.2) during seasons 2016 and 2017, respectively. On the contrary, the highest values of number of seeds/plant (90.0 & 92.7), seed index (45.3 & 46), and weight of seeds per plant (43.8 & 45.0 g) and per hectare (2.41 & 2.48 ton/ha) were obtained with 30 ppm of ZnO-NPs during seasons 2016 & 2017, respectively, and then followed by ZnO-NPs at the concentration 20 ppm. This effect showed that the 30 ppm of ZnO-NPs was sufficient as co-enzyme to cell differentiation for stimulation plant growth, pods and seeds formation of common bean plant.

These results might be due to enhancing the plant growth of common bean plants with a concentration of 30 ppm ZnO-NPs (Table 1). Similar results were found before by Elizabath et al. (2017), who showed that the foliar application of ZnO-NPs increased the yield of carrot plant (ton/ha) with 150 ppm of ZnO-NPs application. Also, Khanm et al. (2017) found that the use of ZnO-NPs (400 ppm) by applying any methods like seed priming, seed priming + foliar spray and flour spray has a great positive effect on physiological and yield parameters as compared to zinc sulphate (800 ppm). The effect of Zinc NPs varied according to the duration and concentration (Faizan et al., 2018).

3.4. Effect of ZnO-NPs on genomic DNA using RAPD-PCR marker

The total numbers of bands results from samples DNA amplification using A-12 primer were six bands ranged from 1795 to 425 bp. The three monomorphic bands were detected at molecular size 1275, 825 and 535 bp while the three polymorphic bands were detected at molecular size 1795, 640 and 425 bp, these polymorphic bands considered a +ve unique bands (Marker bands) for sample treated with 30 ppm

Table 1
Effect of ZnO-NPs concentrations on vegetative growth criteria of common bean plants during two successive seasons 2016 and 2017.

Vegetative growth characters		Concentration											
		0 ppm (control)		10 ppm		20 ppm		30 ppm		40ppm		LSD at 5%	
		2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Plant length (cm)		53.3	53.7	53.7	54.0	59.3*	59.9*	61.0*	61.9*	58.0	58.6	**5.49**	**4.43**
Root length (cm)		12.3	12.7	17.5**	17.7*	14.7*	15.1*	20.0*	20.7*	14.3	14.7	**2.33**	**2.31**
No. leaves/plant		29.3	29.7	37.8	37.3	34.0	35.0	45.7*	49.0*	33.7	36	**9.85**	**8.10**
No. branches/plant		4.7	4.7	4.7	5.0	4.7	5.0	5.0	5.1	4.7	4.8	**0.65**	**0.73**
Fresh weight (g)	Leaves	90.1	90.3	98.7	99.7	96.7	98.3	155.9*	160*	102.2	105.3*	**15.21**	**12.16**
	Branches	36.3	36.4	37.5	38.3	45.1*	45.7*	71.3*	73.0*	43.0	43.7	**4.43**	**4.66**
	Roots	4.7	4.8	6.1*	6.1*	7.3*	7.5*	8.4*	9.3*	5.8*	6.1*	**1.21**	**1.15**
	Plant	126.4	131.6	136.2*	144.1*	141.8*	151.5*	227.2*	242.3*	145.2*	155.1*	**5.12**	**7.79**
Dry weight (g):	Leaves	15.2	16.3	16.8	17.7	15.9	17	25.7*	26.7*	17.5	19*	**2.70**	**2.44**
	Branches	6.3	6.7	6.3	7	9.4*	10*	14.6*	15.0*	8.0*	8.7*	**1.69**	**1.59**
	Roots	1.4	1.6	1.8*	2.1*	2.0*	3.0*	2.5*	3.1*	1.7	2.5*	**0.38**	**0.39**
	Plant	21.5	24.6	23.1*	26.8*	25.3	30.0*	40.3*	44.8*	25.5*	30.2*	**1.58**	**3.0**

* The significant corresponding to control.

Table 2
Effect of ZnO-NPs concentrations on yield and it's components of common bean plant during two successive seasons 2016 and 2017.

Yield and its components		Concentration											
		0 ppm (control)		10 ppm		20 ppm		30 ppm		40 ppm		LSD at 5%	
		2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
No./plant:	Pods	19.7	20.1	25.3	26.3	29.3*	30.0*	31.3*	31.7*	32.7*	32.0*	6.37	6.66
	Seeds	55.3	56.0	70.0	73.3	88.7*	90.3*	90.0*	92.7*	83.3*	85.0*	**19.65**	**17.64**
Weight (g)/plant:	Pods	45.7	46.0	45.5	47	45.3	45.7	48.2	49.7	35.0	35.3	**6.96**	**6.96**
	Seeds	26.2	26.3	33.0*	34.7*	40.2*	40.7*	43.8*	45.0*	33.7*	34.3*	**3.30**	**2.69**
	Shoot residues	10.2	10.7	11.8*	12.7*	12.8*	13.8*	14.5*	15.8*	14.7*	15.9*	**1.35**	**1.30**
Seed index (100 seeds weight (g))		37.3	38.7	38.5	39.8	41.0	43.0	45.3*	46.0*	41.8	42.8	**8.01**	**6.15**
Shelling percentage		57.3	57.1	72.5*	73.8*	88.7*	89.1*	90.9*	90.5*	96.3*	97.2*	**1.46**	**1.49**
Seeds yield (ton/ha)		1.44	1.45	1.82*	1.91*	2.21*	2.24*	2.41*	2.48*	1.85*	1.89*	**0.18**	**0.15**
Shoot residues (kg/ha)		557.8	585.3	645.3*	694.5*	700.0*	754.5*	793.0*	864.0*	804.0*	869.6*	**15.58**	**16.00**

* The significant corresponding to control.

ZnO NPs.

In primer OP-A08, the total numbers of bands were eight (three monomorphic and five polymorphic) that ranged from 1400 to 155 bp. A unique band at molecular size 965 bp was appeared for sample treated with 10 ppm ZnO-NPs, while in 10, 20 and 30 ppm treatments appeared band at molecular size 1400 bp and absent in control and 40 ppm of ZnO-NPs. There were bands at molecular size 600 and 480 bp absent in control but present in all ZnO-NPs treatments (10, 20, 30 and 40 ppm), there was band present in control while absent in both treatment 10 and 40 ppm at molecular size 700 bp. In primer OP-B10, the total numbers of bands were six ranged from 1140 to 400 bp. The three monomorphic bands were obtained at molecular size 860, 615 and 400 bp while the bands at molecular size 1140, 700 and 500 bp were polymorphic bands, these polymorphic bands were absent in control but present in all samples treated by ZnO-NPs.

In the case of OP-C19, the total numbers of bands were ten ranged from 2090 to 290 bp. Since some bands appeared at different molecular size 2090, 1000 and 374 bp for that treated with 20 and 30 ppm ZnO-NPs only, while the band at molecular size 1810 bp present only in common bean treated by10, 20 and 30 ppm. The band at molecular size 830 bp was absent in control (0 ppm) but was present in other treatments (10, 20, 30 and 40 ppm). The primer OP-H07 had a total number of bands six ranged from 1065 to 190 bp, that hadn't any polymorphic band. The total numbers of bands were seven ranged from 1560 to 400 bp for primer OP-H12, where there is one polymorphic band at molecular size 1560 bp which present in all treatments (10, 20, 30 and 40 ppm) but absent in control.

Finally, in primer OP-H18, there were eleven bands ranged from 2475 to 195 bp. Since, two + ve unique bands at molecular size 2475 and 1790 bp were present only in concentration 20 ppm, while the bands at molecular size 1405 and 1155 bp were absent in treatments 30 and 40 ppm (Fig. 2 & Table 3). The presence of these new bands may exhibit alterations in the priming sites leading to new annealing conditions in addition to homologous recombination which lead to the appearance of new bands (Atienzar and Jha, 2006). Our plants were more sensitive to the effects of ZnO-NPs as more band alterations were observed when compared to the control. The only difference between the treated and control plant was the presence or absence of ZnO-NP which support that the changes in the DNA were caused by the effect of the ZnO-NP.

It was observed that the more effective concentrations on the plant genome were 20 and 30 ppm compared to control. From field experiment, the best values of common dry bean plant criteria i.e. number of branches and leaves per plant, fresh and dry weight of branches and leaves were obtained with ZnO-NPs at a concentration of 30 ppm, this indicates that the concentration of ZnO-NPs (30 ppm) considered as a good mutagenic or a good genotoxic agent.

There were some bands at the same molecular size present in concentrations 20 and 30 ppm but disappeared in the highest concentration (40 ppm) of ZnO-NPs, this might be explained by the highest concentration of ZnO-NPs act as Genotoxic agent causing DNA damage in the priming sites, this was agreement with Ghosh et al. (2016). They observed that at high concentration of ZnO-NPs, the root meristems *of Allium cepa* cells lost the membrane integrity, the chromosome aberrations increased, and the DNA strand was breaking. While, in *Vicia faba* and *Nicotiana tabacum*, the content of ROS increased as well as both ZnO-NPs and ROS react with genomic DNA and lead to DNA strand break or damage.

3.5. Effect of ZnO-NPs on protein banding patterns

Genotoxic effect of ZnO-NPs on the genomic DNA of common dry bean cause a great effect on expression of some genes encoding certain proteins, it could be caused turn on or turn off the expression of some genes (Fig. 3). There were unique proteins at molecular weight 78 and 27 KDa at the concentrations 30 and 40 ppm of ZnO-NPs, respectively, in the flowering stage. In the seedling stage, there was a unique protein at molecular weight 38 KDa only at the concentration 10 ppm of ZnO-NPs. There were some proteins at molecular weight 37 KDa at 10 and 20 ppm of ZnO-NPs in both seedling and flowering stages while in the seedling stage, there are proteins at molecular weight 27 KDa at the concentrations of ZnO-NPs 30 and 40 ppm. This occurs due to the effect of ZnO-NPs treatment on the expression of some genes causing to it turn on to encode proteins while in the control this gene turns off. In contrast, the application of ZnO-NPs may cause turn off to some genes while expressed in the control sample (0 ppm), lead to the absence of some proteins under ZnO-NPs treatments while presence in control condition. Such as the proteins at molecular weight 68, 60, 36, 35 and 33 KDa absent only in the concentration of ZnO-NPs 10 ppm in both the seedling and the flowering stages. The proteins at molecular weight 230 KDa were absence in 10, 20 and 30 ppm in the seedling stage while in the flowering stage was absence in 10, 30 and 40 ppm. The proteins at molecular weight 98 KDa were absence in 10, 30 and 40 ppm in the seedling stage while in the flowering stage was absence in 10 ppm only. The proteins at molecular weight 51 KDa were absence in 20 and 40 ppm in flowering stage only (Table 4).

Zinc plays an important role in the metabolism and protein synthesis, in the regulation of gene expression, as well as enzyme and hormone activities (Faiz et al., 2015; Zhao et al., 2014). The disappearance of some bands of soluble proteins of common dry bean might be due to the inherited effects of ZnO-NPs which depend on the mutational event at the regulatory genes that prevent or attenuate transcription (Gottschalk and Wolff, 2012).

Some plants are capable of taking up and accumulating engineered nanomaterials (ENs). The interaction of plant cells with the ENs leads to the modification of plants' gene expression and associated biological

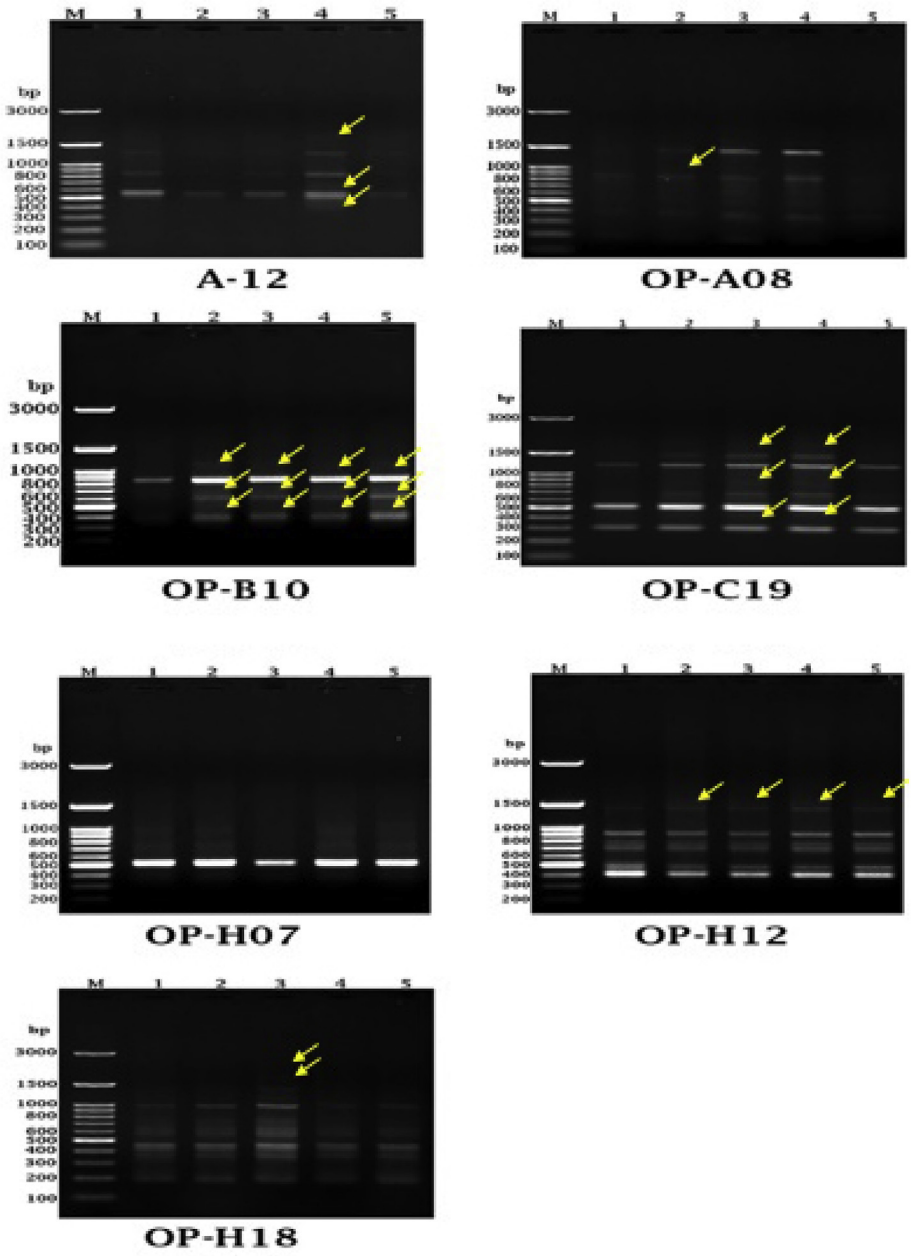

Fig. 2. The effect of different concentrations of ZnO-NPs on genomic DNA for common dry bean. M: DNA ladder 100 bp, 1: control (0 ppm), 2: 10 ppm, 3: 20 ppm, 4: 30 ppm, 5: 40 ppm.

pathways, which eventually affect plants' growth and development (Khiew et al., 2011). The interactions of nanomaterial with plants have not been fully elucidated. There are different and often conflicting reports on the absorption, translocation, accumulation, biotransformation and toxicity of NPs on various plant species.

3.6. Chemical analyses

3.6.1. Photosynthetic pigments

The leave pigments were significantly increased with ZnO-NPs application compared with the control. Chlorophyll *a* significantly improved with ZnO-NPs as a foliar application in both seasons with increasing ZnO-NPs concentration up to 40 ppm, while the chlorophyll *b*, total chlorophyll and carotenoids increases up to 30 ppm to be 0.177, 0.226 and 0.195 at season 2016 and 0.177, 0.228 and 0.196 at season 2017, respectively (Table 5). The appearance of new bands of common bean DNA (Table 3) caused an increasing the photosynthetic pigments in the leaves. In addition, this effect might due to that the presence of zinc is essential for protochlorophyllide formation. Since the zinc is the most essential elements to synthesis chlorophyll. Furthermore, the ratio of glutamic and glycine were increased in the plants that sprayed with 30 ppm ZnO-NPs (Table 8). These amino acids are responsible for the basic metabolism in the formation of plant tissues and the synthesis of chlorophyll. Also, this might be due to metal nanoparticles are powerful amplifiers of photosynthetic effectiveness that in parallel cause light absorption by chlorophyll, as it causes the transfer of energy from chlorophyll to nanoparticles (Mingyu et al., 2007; Mohsenzadeh and Moosavian, 2017). Similar results found before by Gokak and Taranath (2015) who designate the effect of four concentrations of ZnO nanoparticles (50, 100, 200 and 500 ppm) on the *Abelmoschus Esculantus* (L.). They found that chlorophyll *a*, chlorophyll *b* and total chlorophyll content in the leaves were higher with a concentration 100 ppm of ZnO-NPs. Narendhran et al. (2016) found that ZnO-NPs application was positive in crop growing and improving photosynthesis pigment of

Table 3
The effect of different concentrations of ZnO-NPs on genomic DNA of common dry bean.

RAPD primers	Total no. of bands	Mono-morphic bands	Poly-morphic bands	(+ve) Unique bands	(-ve) Unique bands	Mw (bp)	1	2	3	4	5
A-12	6 (1795 - 425 bp)	3	3	3	0	1795	–	–	–	+	–
						640	–	–	–	+	–
						425	–	–	–	+	–
OP-A08	8 (1400 - 155 bp)	3	5	1	2	1400	–	+	+	+	–
						965	–	+	–	–	–
						700	+	–	+	+	–
						600	–	+	+	+	+
						480	–	+	+	+	+
OP-B10	6 (1140 - 400 bp)	3	3	0	3	1140	–	+	+	+	+
						700	–	+	+	+	+
						500	–	+	+	+	+
OP-C19	10 (2090 - 290 bp)	5	5	0	1	2090	–	–	+	+	–
						1810	–	+	+	+	–
						1000	–	–	+	+	–
						830	–	+	+	+	+
						374	–	–	+	+	–
OP-H07	6 (1065 - 190 bp)	6	0	0	0	–	–	–	–	–	–
OP-H12	7 (1560 - 400 bp)	6	1	0	1	1560	–	+	+	+	+
OP-H18	11 (2475 - 195 bp)	7	4	2	0	2475	–	–	+	–	–
						1790	–	–	+	–	–
						1405	+	+	+	–	–
						1155	+	+	+	–	–

+: present -: absent.

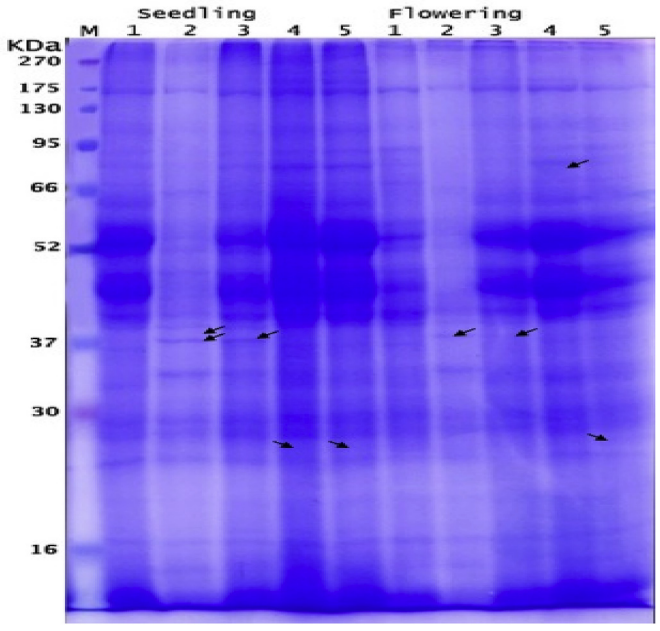

Fig. 3. SDS-PAGE banding patterns of water soluble proteins for common dry bean under the effect of different concentrations of ZnO-NPs in seedling and flowering stage.
M: protein marker, 1: control (0 ppm), 2: 10 ppm, 3: 20 ppm, 4: 30 ppm, 5: 40 ppm.

Sesamum indicum. Also, Faizan et al. (2018) concluded that ZnO-NPs on tomato plants enhanced the antioxidant systems and speeded up proline accumulation that could provide stability to plants and reinforce the photosynthetic efficiency.

The appearance of new bands (Table 3) caused an increasing the photosynthetic pigments in the leaves of common bean.

3.6.2. Proximate components

The application of ZnO-NPs with a concentrations 30 and 40 ppm was significantly increased the proximate components (moisture, protein, ash and energy (kcal/g protein)) of common bean leaves during two seasons 2016 and 2017 (Table 6a). ZnO-NPs as a foliar fertilizer on common bean plants with a concentration 10 ppm gave the highest percentage of carbohydrates, fiber and energy (kcal/g carbohydrates) to be 40.3%, 12.0% and 161.2, respectively, during both seasons in the leaves followed by ZnO-NPs concentration 20 ppm (Table 6a). On the other hand, the best percentage of protein (21.7 & 21.6%), lipid (4.8 & 4.7%), ash (20.8 & 20.9%), energy from protein (86.9 & 86.4 kcal/g) and energy from lipid (43.5 & 42.3 kcal/g) during two seasons 2016 & 2017, respectively, were obtained with ZnO-NPs concentration 40 ppm as compared to other concentrations. It can summarize that use leaves of common bean plants for feeding animals as a result of increasing nutritional value.

The proximate components (moisture %, and protein %) of common bean seeds were significantly increased with increasing ZnO-NPs concentration up to 40 ppm (Table 6b). The highest percentage of fiber content in seeds showed with 10 ppm ZnO-NPs. The best value of carbohydrates (41.7 & 41.8%) and energy from carbohydrates (166.7 & 167.1 kcal/g) during the two seasons 2016 and 2017, respectively, in seeds were obtained by 20 ppm ZnO-NPs. On the contrary, the total energy in seeds was 279.3 and 280.3 during season 2016 and 2017, respectively, with ZnO-NPs concentration 30 ppm.

These results were in agreement with Fageria et al. (2002) who showed that the zinc plays a vital role in carbohydrate and proteins metabolism. Also, Gokak and Taranath (2015) found that the best value of protein in the leaves of okra plant was 48.88 mg/g with 100 ppm ZnO-NPs and the least content was obtained by 200 ppm ZnO-NPs. On the contrary, the carbohydrate content was lower in the leaves with 500 ppm ZnO-NPs as compared to control. In addition, Kisan et al. (2015) indicated that the spinach plants sprayed with 1000 ppm ZnO-NPs showed the best percentage of protein, fiber, lipid, while the content of ash enhanced with 100 ppm ZnO-NPs.

Table 4
Electrophoretic of W·S.P. banding patterns of common dry bean under the effect of ZnO-NPs in seedling and flowering stage.

No. of bands	Mw (KDa)	Seedling stage					Flowering stage				
		0 ppm	10 ppm	20 ppm	30 ppm	40 ppm	0 ppm	10 ppm	20 ppm	30 ppm	40 ppm
1	**230**	+	-	-	-	+	+	-	+	-	-
2	**98**	+	-	+	-	-	+	-	+	+	+
3	**78**	-	-	-	-	-	-	-	-	+	-
4	**68**	+	-	+	+	+	+	-	+	+	+
5	**60**	+	-	+	+	+	+	-	+	+	+
6	**51**	+	+	+	+	+	+	+	-	+	-
7	**38**	-	+	-	-	-	-	-	-	-	-
8	**37**	-	+	+	-	-	-	+	+	-	-
9	**36**	+	-	+	+	+	+	-	+	+	+
10	**35**	+	-	+	+	+	+	-	+	+	+
11	**34**	+	+	+	-	-	+	+	+	-	-
12	**33**	+	-	+	+	+	+	-	+	+	+
13	**27**	-	-	-	+	+	-	-	-	-	+
14	**23**	-	-	-	-	-	+	+	+	+	+

+: present -: absent.

Table 5
Effect of ZnO-NPs concentrations on photosynthetic pigments of common bean plants during two successive seasons 2016 and 2017.

Concentration	Photosynthetic pigments							
	Chlorophyll *a* mg/g F·W		Chlorophyll *b* mg/g F·W		Total chlorophyll mg/g F·W		Carotonoids mg/g F·W	
	2016	2017	2016	2017	2016	2017	2016	2017
0 ppm (control)	0.039	0.042	0.071	0.078	0.110	0.120	0.130	0.132
10 ppm	0.045*	0.049*	0.108*	0.109*	0.153*	0.157*	0.143*	0.146*
20 ppm	0.052*	0.053*	0.126*	0.128*	0.178*	0.181*	0.151*	0.154*
30 ppm	0.049*	0.050*	0.177*	0.177*	0.226*	0.228*	0.195*	0.196*
40 ppm	0.064*	0.065*	0.144*	0.147*	0.208*	0.211*	0.172*	0.174*
LSD at 5%	**0.003**	**0.003**	**0.002**	**0.004**	**0.004**	**0.004**	**0.005**	**0.002**

F·W = Fresh Weight.
* The significant corresponding to control.

Table 6a
Effect of ZnO-NPs concentrations on proximate components of common bean leaves during two successive seasons 2016 and 2017.

Proximate components	Concentration											
	0 ppm (control)		10 ppm		20 ppm		30 ppm		40 ppm		LSD at 5%	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Moisture%	7.3	7.4	6.7	6.8	7.1	7.1	8.7*	8.6*	9.0*	9.0*	0.35	0.36
Carbohydrate%	39.5	39.6	40.3	40.3	36.8	37.0	33.4	33.3	32.7	32.8	**2.24**	**2.21**
Protein%	18.2	18.2	18.5	18.4	21.2*	21.3*	21.4*	21.4*	21.7*	21.6*	**0.31**	**0.25**
Lipid%	4.8	4.8	4.1	4.2	4.3	4.4	4.6	4.6	4.8	4.7	**0.40**	**0.44**
Fiber%	11.9	12.0	12.0	12.0	11.9	12.1	11.1	11.0	11.0	11.1	**0.61**	**0.59**
Ash%	18.4	18.0	18.4	18.3	18.7	18.2	20.7*	21.1*	20.8*	20.9*	**2.16**	**2.30**
Total Energy (Kcal/g)	273.4	274.7	272.1	272.6	270.6	272.8	260.7	260.3	261.1	259.9	**8.37**	**8.09**
Energy (Kcal/g carbohydrates)	157.7	158.5	161.2	161.1	147.1	148.0	133.7	133.3	130.7	131.2	**8.94**	**8.82**
Energy (Kcal/g protein)	72.7	72.9	74.0	73.7	84.8*	85.2*	85.6*	85.6*	86.9*	86.4*	**1.25**	**1.01**
Energy (Kcal/g lipid)	42.9	43.2	36.9	37.8	38.7	39.6	41.4	41.4	43.5	42.3	**3.56**	**3.92**

* The significant corresponding to control.

3.6.3. Minerals

The different treatment of ZnO-NPs caused a significant effect on minerals content in the leaves and the seeds of common bean plants during two seasons 2016 and 2017 (Table 7). Foliar application of ZnO-NPs concentration 10 ppm on common bean plants showed the best phosphorus and copper content (0.34% & 13.4 ppm, respectively, in season 2016) of the leaves and (0.43% & 11.1 ppm, respectively, in season 2016) of seeds, on a dry weight basis. The manganese content in the leaves and seeds were increased by increasing the concentration of ZnO-NPs up to 30 ppm to be 55.5 and 9.1, respectively, during season 2016. The increment of nitrogen, iron and zinc content in leaves and seeds were significant with foliar application of 40 ppm ZnO-NPs. On the contrary, traditional agriculture appeared the best content of potassium in common bean leaves (3.24 & 3.25%) and seeds (2.32 & 2.36%) during two seasons 2016 and 2017, respectively. These results might be due to the effect of ZnO- NPs on minerals in the leaves and seeds of common bean plants depend on ZnO- NPs concentration. Since, the nutrients in nano-size were more available to nanoscale plant pores, and more efficiency (Abd El-Aziz et al., 2019).

3.6.4. Effect of ZnO-NPs concentration on amino acids of the common bean seeds

The ZnO-NPs with different concentration effected on the content of amino acids Aspartic (ASP), Threonine (THR), Serine (SER), Glutamic

Table 6b
Effect of ZnO-NPs concentrations on proximate components of common bean seeds during two successive seasons 2016 and 2017.

Proximate components	Concentration											
	0 ppm (control)		10 ppm		20 ppm		30 ppm		40 ppm		LSD at 5%	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Moisture%	6.4	6.4	5.5	5.6	6.2	6.1	6.9*	7.0*	7.2*	7.2*	1.16	1.10
Carbohydrate%	39.2	39.3	39.1	39.3	41.7*	41.8*	39.4	39.5	36.0	36.1	**2.49**	**2.19**
Protein%	20.2	20.2	21.2*	21.3*	18.9	19.0	21.2*	21.1*	21.3*	21.4*	**1.89**	**1.46**
Lipid%	4.1	4.0	3.6	3.6	3.8	3.9	4.1	4.2	4.4	4.3	**0.64**	**0.67**
Fiber%	13.1	13.2	13.2	13.3	11.9	11.8	10.6	10.6	12.9	13.0	**0.63**	**0.70**
Ash%	17.0	16.9	17.4	16.9	17.5	17.4	17.8	17.6	18.2	18.0	**3.09**	**2.76**
Total Energy (Kcal/g)	274.5	274.0	273.6	274.9	276.5	278.3	279.3	280.3	268.8	268.7	**12.15**	**9.66**
Energy (Kcal/g carbohydrates)	156.8	157.2	156.4	157.2	166.7*	167.1*	157.6	158.1	144.0	144.4	**9.95**	**8.77**
Energy (Kcal/g protein)	80.8	80.8	84.8	85.2	75.6	76.0	84.8	84.4	85.2	85.6	**7.56**	**5.83**
Energy (Kcal/g lipid)	36.9	36.0	32.4	32.4	34.2	35.1	36.9	37.8	39.6	38.7	**5.77**	**6.02**

* The significant corresponding to control.

Table 7
Effect of ZnO-NPs concentrations on minerals of common bean leaves and seeds during two successive seasons 2016 and 2017.

Minerals	Concentration											
	0 ppm (control)		10 ppm		20 ppm		30 ppm		40 ppm		LSD at 5%	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
leaves												
N %	2.91	2.91	2.96	2.94	3.40*	3.41*	3.41*	3.42*	3.48*	3.46*	**0.06**	**0.08**
P %	0.24	0.28	0.34*	0.35*	0.30*	0.32*	0.28	0.30	0.22	0.25	**0.04**	**0.03**
K%	3.24	3.25	3.07	3.10	2.91	2.95	2.66	2.72	2.82	2.89	**0.06**	**0.13**
Fe ppm	611.8	612	736.2*	736*	665.0*	665.3*	704.8*	704.8*	847.3*	847.5*	**0.49**	**0.44**
Zn ppm	18.3	18.3	19.6*	19.7*	19.3*	19.2*	20.0*	20.2*	23.9*	23.7*	**0.54**	**0.59**
Cu ppm	8.7	8.7	13.4*	13.6*	10.1*	10.1*	9.2*	9.1*	9.4*	9.6*	**0.10**	**0.18**
Mn ppm	44.1	44	45.3*	45.4*	52.5*	52.6*	55.5*	55.5*	50.0*	50.1*	**0.18**	**0.15**
Seeds												
N %	3.23	3.23	3.39	3.41*	3.02	3.04	3.39	3.38	3.41	3.42*	**0.51**	**0.16**
P %	0.32	0.36	0.43*	0.45*	0.35	0.41*	0.30	0.33	0.22	0.24	**0.04**	**0.03**
K%	2.32	2.36	2.16	2.22	2.08	2.14	1.99	2.02	2.16	2.21	**0.07**	**0.12**
Fe ppm	25.4	25.3	79.0*	79.2*	38.7*	38.6*	45.2*	54.0*	82.7*	82.8*	**0.48**	**0.50**
Zn ppm	18.9	19	20.3*	20.3*	19.0	19.2	20.5*	20.4*	20.6*	20.4*	**0.56**	**0.49**
Cu ppm	9.1	9.1	11.1*	11.1*	10.8*	10.8*	10.5*	10.6*	9.6*	9.7*	**0.09**	**0.14**
Mn ppm	4.9	5.1	5.3*	5.4*	7.3*	7.2*	9.1*	9.2*	5.1	5.2	**0.19**	**0.26**

* The significant corresponding to control.

Table 8
Effect of ZnO-NPs concentration on the percentage of amino acids in the common bean seeds.

Amino Acid	Concentration				
	0 ppm (control)	10 ppm	20 ppm	30 ppm	40 ppm
ASP	2.19	2.02	2.32	2.46	2.46
THR	0.79	0.73	0.85	0.94	0.85
SER	0.93	0.91	1.06	1.19	1.03
GLU	3.19	3.08	3.47	3.74	3.61
GLY	0.72	0.68	0.68	0.76	0.73
ALA	0.96	0.95	0.91	1.04	1.02
VAL	1.27	0.99	1.28	1.42	1.39
ILE	1.05	0.81	0.88	1.03	0.91
LEU	1.56	1.43	1.54	1.73	1.65
TYR	0.87	0.7	0.8	0.88	0.89
PHE	1.2	1.1	1.22	1.32	1.31
HIS	0.61	0.58	0.64	0.68	0.68
LYS	1.35	1.3	1.41	1.53	1.5
ARG	1.2	1.17	1.15	1.43	1.32
PRO	0.58	0.73	0.56	0.44	0.61
CYC	0.32	0.42	0.31	0.33	0.36
METH	0.24	0.23	0.24	0.28	0.28

(GLU), Glycine (GLY), Alanine (ALA), Valine (VAL), Isoleucine (ILE), Leucine (LEU), Threonine (TYR), Phenylalanine (PHE), Histidine (HIS), Lysine (LYS), Arginine (ARG), Proline (PRO), Cystine (CYC) and Methionine (METH) in the seeds of common bean plant (Table 8). Where the amino acids THR, SER, GLU, GLY, ALA, VAL, LEU, PHE, HIS, LYS, ARG and METH showed the highest value in the seeds of common bean treated by 30 ppm ZnO-NPs, while ASP and TYR increased with increasing the concentration of ZnO-NPs up to 40 ppm. In addition, the amino acids PRO and CYC show the highest value with the concentration 20 ppm of ZnO-NPs, while the ILE was the highest in the control. GLU and ASP were surpassed on the other amino acids. These common bean seeds content increases were due to metabolism translocation from source to sink that led to increasing both amino acids GLU and ASP. It was obtained that the content of GLU and ASP in common bean seeds was surpassed than other amino acids; these may be due to metabolism translocation from source to sink. These results may be due to nanoparticles interact with plants causing various morphological and physiological changes, depending on the properties of NPs and also, efficacy of NPs as their chemical structure, size, surface covering, reactivity and most significantly the dose at which they are useful (Elizabath et al., 2017; Khodakovskaya et al., 2012).

Olkhovych et al. (2016) reported that *Piston Stratiotes* L. plants incubation with ZnO-NPs (89 mg/l) had a decrease in the total content of amino acids by about 15% and also, the content of leucine, methionine,

phenylalanine, proline and tyrosine. The significant reduction in the contents of amino acids might adversely affect plants adaptive reactions related to the synthesis of stress proteins. While, Bhattacharya and Gupta (2005) found that the nanoparticles cause vary of cell metabolism and changing the intensity of biochemical reactions that have a significant impact on plant resistance for different unfavorable environments.

4. Conclusions

It can be generally concluded that the application of ZnO-NPs at a concentration of 30 ppm can be recommended for producing of common bean plant. The best values of common bean plant criteria (plant length, number of leaves and branches/plant, and fresh weight of plant), seeds yield (2.41 & 2.48 ton/ha in seasons 2016 & 2017, respectively) and shoot residues (793 & 864 kg/ha in seasons 2016 & 2017, respectively) as well as the total energy for common bean seeds (279.3 & 280.3 kcal/g in seasons 2016 & 2017, respectively) recorded with a concentration 30 ppm of ZnO-NPs. Also, the amino acids content were increased with increasing ZnO-NPs concentration up to 30ppm except proline amino acid. The content of glutamic and aspartic amino acids in the seeds was higher than other amino acids. In addition, the RAPD analysis represented that the more effective concentration on the plant genome were 20 and 30 ppm of ZnO-NPs compared to control and effect on the expression of some genes encoding proteins or enzymes. Furthermore, the SDS-PAG analysis showed unique proteins at molecular weight 78 KDa in concentration 30 ppm this protein may play an important role in enhancement the agro-morphological criteria of common dry bean.

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