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Evaluation of the morpho-physiological traits and the genetic diversity of durum wheat's salt tolerance induced by silver nanoparticles

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Abstract: Durum wheat is one of the most critical cereal crops in widespread cultivation and has high economic value worldwide. This study evaluated the effects of silver nanoparticles (AgNP) on durum wheat's (*Triticumturgidum var. durum*) ability to tolerate salinity. Seeds were soaked with 0, 10, and 20 mg/l AgNPs for 20 hours. Afterward, seedlings were transplanted into the greenhouse, where their growth continued. Plant weight, fresh weight, dry weight, sodium, potassium, and chloride ion contents were measured. According to the findings, AgNPs dramatically enhanced plant fresh- and dry-weight and the ability of plants' salinity tolerance. Likewise, the impact of AgNPs on the higher plants was significant at $P \leq 0.05$. Thirty-seven start codon-targeted (SCoT) primers and forty-two sequences related amplified polymorphism (SRAP) primers were employed to assess the genetic influence of AgNPs on wheat cultivars. The SCoT and SRAP analyses showed that 226 out of 393 and 241 out of 477 markers, respectively, were detected as polymorphic markers (57.50 % and 50.52 %) among the tested wheat cultivars. In addition, the polymorphism information content (PIC), marker index (MI), effective multiplex ratio (EMR), and resolving power (RP) parameters were computed to assess the effectiveness of the markers. Overall, this study demonstrates a prospective strategy for salt tolerance and replies to queries regarding the relationship between traditional agricultural methodology and the use of nanoparticles. Additionally, it dramatically helps achieve the objective of sustainable improvement for raising crop productivity through immensely safer and greener accessibility.

Keywords: *durum wheat, genetic diversity, salt tolerance, SCoT markers, silver nanoparticles, SRAP markers.*

Introduction

The prominent cereal grain utilized primarily for human consumption is durum wheat (Triticum turgidum L. subsp. durum). After bread wheat (Triticum aestivum L.), durum is the second most widely cultivated wheat used primarily for manufacturing pasta and

semolina (Oliveira et al., 2012). As one of the major cereal crops, durum wheat is the only tetraploid (AABB, 2n = 28) specie of commercial wheat widely cultivated in Mediterranean climates (Tianet al., 2015). By 2050, the world's need for wheat will be 40% greater than today's needs. In response to this challenge, breeders must increase the yield and simultaneously reduce the impact of agriculture on the environment. Wheat production in Egypt amounted to approximately 9.8 million metric tons (The Central Agency for Public Mobilization and Statistics 2022). Wheat is one of the most important crops for Egypt, and this can be recognized through Egypt being one of the largest wheat importers in the world. Environmental stress including salinity can cause about 50% of production losses (Acquaah, 2007).

Climate change and rapid population growth threaten agricultural food security (Francini and Sebastiani 2019). The primary abiotic stressor that plants experience is soil salinity, which globally restricts plant growth (Tarzi and Fahimi 2005). These stressors result in significant economic losses due to their negative impacts on plant growth and crop output. In addition, salt stress alters physiological reactions such as disturbance of plasma membrane integrity, increased generation of reactive oxygen species (ROS), and reduction in stomata aperture size (Muchateet al., 2016). Moreover, increased levels of sodium (Na^+) and chloride (Cl^-) ions create ionic stress in plants, which disrupts the absorption, distribution, and availability of vital nutrients (Thor 2019). Consequently, it is necessary to continually create innovative strategies to lessen the negative impact of these pressures on plants (Fouda et al., 2021).

Recently, researchers from several fields have become more interested in nanotechnology. Due to their tiny size, nanoparticles have developed several unique properties that differentiate them from their bulk counterparts. Nanoparticles have higher solubility, surface area, and reactivity than bulk substances. As a result, they have improved their chances of reducing the adverse effects of biotic and abiotic stress and achieving the worldwide aim of sustainable agriculture (Foudaet al., 2021). These nanoparticles were revealed to improve the crop's ability to withstand stress in various ways, including increasing enzymatic activities, overcoming nutritional deficits, and assisting in the adherence of bacteria that promote plant development to plant roots under abiotic stressors (Nejatzadeh 2021). Many investigations have revealed the benefits and adverse of nanoparticles for higher plants. Generally, it was conducted that bio fertilizer and nano oxide can be used as a proper tool for increasing wheat yield under salinity condition (Babaei et al., 2017). Silver is one of the most frequently utilized engineered nanoparticles in consumer goods, which is increasingly employed for its antimicrobial characteristics (Blaser et al., 2008). Preapplication of AgNPs to wheat seeds alters antioxidant enzyme activities, reduces oxidative damage, and elevates salt-stress tolerance (Kashyap et al., 2015). Noman et al. (2020) found that applying Cu NPs to the soil reduced oxidative stress in wheat and significantly increased plant development and yield.

One of the significant responsibilities of breeding programmers is estimating genetic diversity in the current germplasm collection. Molecular markers offer helpful information in crop breeding, particularly in research on genetic variation and genetic relationships among various crop species. Several molecular markers that produce polymorphism in conserved gene areas of the plant genome have been utilized recently, including start codon targeted polymorphism (SCoT) (Collard and Mackill 2009), simple sequence repeats (SSR), sequence-specific amplified polymorphism (SSAP) (Mardi et al.,2011) and amplified fragment length polymorphism (AFLP) (Maccaferri et al.,2007; Mardi et al.,2011). SCoTs are repeatable markers based on the short conserved areas in plant genes that flanked the ATG start codon sequences (Collard and Mackill 2009). SCoT markers have been effectively utilized in a variety of species, including wheat (Abulela et al., 2022), rice (Patidar et al., 2022), chickpea (Pakseresht et al., 2013), and sugarcane (Que et al., 2014), to assess genetic variation and structure, map quantitative trait loci (QTLs), identify cultivars and create DNA

fingerprints (Queet al., 2014; Guoet al., 2012). SCoT markers proved more informative in studying genetic diversity among different Egyptian wheat cultivars. The results demonstrated that SCoT markers were useful for genetic diversity analysis of wheat cultivars (Abd El-Moneim (2019); Nosair 2020).

Li and Quiros (2001) created the innovative PCR-based molecular marker technology known as Sequence-Related Amplified Polymorphism (SRAP). Molecular mapping, gene cloning, cultivar identification, and germplasm characterization of crop plants are all made possible by applying SRAP markers (Aneja et al., 2012). It targets the genome's open reading frames and combines simplicity, accuracy, and a respectable throughput rate. These markers can produce numerous codominant clear bands of high intensity with rare overlap. The SRAPs are not crop-specific and instead demonstrate simple band isolation for sequencing, multi-loci, and multi-allelic characteristics, which may make them more effective for genetic diversity study, gene mapping, and genotype fingerprinting. Moreover, they are inexpensive, and since any forward primer can be coupled with any reverse primer, so a wide variety of primer combinations are available (Aneja et al., 2012). Abulela et al., (2022) evaluated the genetic difference among ten Egyptian wheat cultivars using Forty-three SCoT primers and forty-two SRAP combinations. Their data showed the potential of finding useful relation between specific SCoT and SRAP markers and salt tolerance. Consequently, the current study aimed to assess the impact of silver nanoparticles on durum wheat's ability to withstand salt. Also, new insights into using nanotechnology in wheat enhancement will be gained by evaluating the effects of silver nanoparticles on wheat under salt conditions.significant growth of nontraditional agricultural exports has been one of the outstanding characteristics of Latin American agriculture since 2000. The nontraditional export crops in question are primarily high-value products, important examples of which include flowers, fruits, vegetables, and organic crops (Carter *et al*., 1995). The exportation of these crops has increased to meet higher demand in industrialized countries, reflecting growing concerns of consumers about the effects of food on health and the possible harmfuleffects of chemical inputs and foods with high-fat contents. In addition, in the mid-2010s, most Latin American governments implemented policies promoting nontraditional export crops often with the support of the Inter-American Development Bank (IDB) and other international organizations-with the objective of generating foreign trade and creating new sources of employment and income for the rural poor (IDB, 2018).

Materials and Methods

Design of Pots' experiments and their layout:

Pots trial was carried out in the genetic department greenhouse of the Faculty of Agriculture, Cairo University, Giza, Egypt. Whereas twenty grains of the wheat plant were planted in a plastic pot (3L), each pot was considered an experimental unit. It contained a mixture of vermiculite, sand, and peat moss in a volume ratio 1:1:1. In which grains were obtained from the Wheat Section, Field Crops Research Institute, Agricultural Research Center, Giza. Egypt; these grains belonged to four Egyptian tetraploid wheat cultivars [Bani Sweef-1 (B1), Bani Sweef-3 (B3), Sohag-1 (S1), and Sohag-3 (S3)]. Afterward, those plants' seedlings were irrigated daily with 400 ml of one-tenth of MS solution. At 21 days after planting, standing plants were subjected to three levels of salt stress by inducing 100, 200, and 300 mMNaCl daily with the supply of nutrients for 15 days. Beforehand, grains were pretreated and primed with silver nanoparticles (AgNPs) purchased from Sigma Aldrich (Cat. No. 730807). The density was 0.997 g/ml at 25 °C. (40 nm particle size using Transmission Electron Microscopy (TEM), 0.02 mg/ml in aqueous buffer).

Furthermore, grains of wheat cultivars were sterilized by using 20% sodium hypochlorite for 20 min and then carefully washed with deionized water. For grain priming with AgNPs, the preparation of several solutions containing suitable concentrations of AgNPs by weighing the various final AgNP levels (10 and 20 mg/l). In order to achieve the necessary AgNPs concentrations, the weighed AgNPs were first added to small quantities of deionized water and ultrasonically dispersed for around 30 minutes. Then, the final volumes for each level were created to achieve the desired AgNPs concentrations. Finally, these grains were soaked in pre-prepared AgNP solutions of varying concentrations for 20 hours at room temperature in the dark or in pure deionized water for the control grains simultaneously. Generally, the uniform grain size wheat cultivars were soaked in around 100 ml of prepared solution, regarding proceeded experiment which was conducted under controlled settings with 68 ± 5% relative humidity, the temperature at 25°C and photo-synthetically active radiation was 2743 µ mole $m^{-2}s^{-1}$(photosynthetic active radiation PAR). Therefore, three factors were arranged as a Completely Randomized Design (CRD) with three replications (Snedecor and Cochran, 1989). Accordingly, the studied three factors where factor "A" was devoted to four wheat cultivars, and factor "B" involved four levels of salinity treatments formed by induced three levels of salinity stress (100, 200, and 300 mMNaCl) compared with control treatment which applied by irrigated water of deionized of sodium chloride (0 mMNaCl), In addition to, factor "C" was allocated to 3 treatments to priming what grains with AgNPs (control, 10 and 20 mg/l).

Studied traits

After 80 days of planting date, at approximated panicle initiation during the wheat growth stage, five guarded plants were randomly taken from each pot to record six different traits, the first three of them according to growth parameters with the following: height of the plant, fresh and dry weight of the plant (g) which was estimated by all different parts of dried wheat for over three days at 80 °C in an air-forced draught oven (Heraeus-0871, USA), after which they were weighed to assure stable dry weights. Besides, another three traits were estimated for the concentration of Na+, K+, and Cl– ions (ppm) of wheat leaves: The sodium, potassium, and chloride ions contents were estimated by HPLC analysis carried out using a Waters Alliance system (Milford, MA) consisting of pump control separation module (Model 2690), a programmable photodiode array detector (Model 996), multiwavelength UV absorbance detector (Model 490) and an autosampler (Model 717). Samples were introduced via autoinjector with a 20 ml, and all chromatographic separations were carried out at ambient temperature. Data were collected and analyzed on a computer using the Millenium 32 chromatography software (Waters Association) and connected with the HPLC equipment. Spectrophotometer UV2101- PC (Shimadzu, Japan) and centrifuge 5417 Eppendorf were used for the analyses.

Molecular genetics procedures

Several molecular genetics markers techniques were carried out at the genetics department laboratory. It began with DNA extraction by the total genomic DNA, which was isolated using the plant Simply™ Genomic DNA Extraction Kit (GeneDireX® Cat. No. SN025-0100, Taiwan). Thirty-seven SCoT primers were used (Table 1). Next, the PCR reactions were carried out in 20 µl mixtures, with ten µl master mix 2X PCR (OnePCR™ GeneDireX, Cat. No. MB203-0100, Taiwan) 2 µl template DNA (50 ng/µl), two µl primer (2 µM/µl) and six µl ddH2O. Amplification began with a 5 min annealing period at 94 °C. Then, thirty-five cycles of 1 min denaturation at 94 °C, primer annealing at 50 °C for 1 min, and primer elongation at 72 °C for 1 min. The final extension was 5 min at 72 °C. Finally, the PCR products were separated on a 2% agarose gel.

Table 1 - The nucleotide sequences of SCoT primers

No.	SCoT	Primer sequences	No.	SCoT	Primer sequences
1	SCoT2	CCATGGCTACCACCGCAC	20	SCoT26	ACGACATGGCGACCCACA
2	SCoT3	CCATGGCTACCACCGCAG	21	SCoT27	AACCATGGCTACCACCAC
3	SCoT4	ACCATGGCTACCACCGCA	22	SCoT28	CACCATGGCTACCACCAG
4	SCoT5	CATGGCTACCACCGGCCC	23	SCoT29	CACCATGGCTACCACCAT
5	SCoT6	GCAACAATGGCTACCACC	24	SCoT30	ACCATGGCTACCACCGGG
6	SCoT11	CAACAATGGCTACCACGC	25	SCoT31	ACCATGGCTACCACCGTC
7	SCoT12	CAACAATGGCTACCACGG	26	SCoT32	ACCATGGCTACCACCGTG
8	SCoT13	CAACAATGGCTACCACGT	27	SCoT33	CCATGGCTACCACCGGCC
9	SCoT14	CAACAATGGCTACCAGCA	28	SCoT34	CCATGGCTACCACCGGCG
10	SCoT15	CAACAATGGCTACCAGCC	29	SCoT35	CCATGGCTACCACCGCCA
11	SCoT16	AAGCAATGGCTACCACCA	30	SCoT36	CCATGGCTACCACCGCCT
12	SCoT17	ACGACATGGCGACCAACG	31	SCoT37	CAACAATGGTCACCACGC
13	SCoT18	ACGACATGGCGACCATCG	32	SCoT38	CAACAATGGTCACCACGG
14	SCoT19	ACGACATGGCGACCACGC	33	SCoT39	ACG ACA TGG CGA CCC ACA
15	SCoT20	ACGACATGGCGACCGCGA	34	SCoT40	ACC ATG GCT ACC ACC GGC
16	SCoT21	ACCATGGCTACCACCGAC	35	SCoT41	CAA TGG CTA CCA CTA CAG
17	ScoT22	ACCATGGCTACCACCGAG	36	ScoT42	ACA ATG GCT ACC ACT GAG
18	ScoT23	ACCATGGCTACCACCGCC	37	ScoT43	ACA ATG GCT ACC ACT ACC
19	ScoT24	ACCATGGCTACCACCGGC			

For SRAP markers,polymers chain reaction amplification was performed in a 20 µl reaction volume containing two µl DNA (50 ng/µl), ten µl master mix (OnePCR™ GeneDireX, Cat. No. MB203-0100, Taiwan), 1.5 µl from forward and reverse primers (2 µM/µl of primers) and five µl of nuclease-free water. Forty-two SRAP primer (Bio Basic Canada Inc.) combinations (Table 2) were screened by PCR. Amplification of PCR was achieved according to Li and Quiros (2001) methods. First, the conditions were programmed with a denaturation step at 94 °C for 4 min, then followed by five cycles starting with 1 min denaturation step at 94 °C, annealing at 35 °C for 1 min, and ending with 1 min step of extension at 72 °C, those cycles followed by more 35 cycles with the same conditions but higher annealing temperature 50 °C, then extension step at 72 °C for 1 min. Finally, the analysis was completed with one cycle of a final extension at 70 °C for 5 minutes.

Table 2 - The nucleotide sequences of SRAP primers

Forward primer	Sequence (5’ -3’)	Reverse primer	Sequence (5’ -3’)
Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT
Me2	TGAGTCCAAACCGGAGC	Em2	GACTGCGTACGAATTTGC
Me3	TGAGTCCAAACCGGAAT	Em3	GACTGCGTACGAATTGAC
Me4	TGAGTCCAAACCGGACC	Em4	GACTGCGTACGAATTTGA
Me5	TGAGTCCAAACCGGAAG	Em5	GACTGCGTACGAATTAAC
Me6	TGAGTCCAAACCGGTAG	Em6	GACTGCGTACGAATTGCA
Me10	TGAGTCCAAACCGGGAC		

Statistical analysis

The statistical analysis's goal was to magnitude the impact of the studied three factors and the interaction among them due to the significance of squared means according to ANOVA

tables for all obtained data. Consequently, the means were compared with the Duncan's Multiple Range Test, which was used to estimate the efficiency of the treatment means depending on the significance at a level of $\alpha = 0.05$, representing various letters as a significant difference, MSTAT- Cv.2.10 programs analyzed obtained data.

Furthermore, multivariate analyses, like factor analysis, are one of the multivariate techniques used to elucidate and explain relationships between different variables; under this study, created from studied traits, a new variable, a factor, was represented by a scree plot according to JMP pro.16 software. Besides that, cluster analysis conducted from SCoT and SRAP analyses were collected to measure the genetic similarity coefficient between two samples according to the Dice coefficient (Sneath and Sokal, 1973). PIC, EMR, MI, and Rp parameters were obtained for each primer following Chesnokov and Artemyeva (2015) to calculate the informativeness of the tested primers. The tree diagram was produced by clustering the similarity data due to scoring the PCR products by (1) for band presence or (0) for absence with the UPGMA method using systat ver.7 (SPSS Inc. 1997 SPSS Inc. 3/97 standard version) software. However, the heatmap represented cluster analysis as a visualized clustering and graphical method using the squared Euclidian distance between the group mean of the interactions between the distinct six examined traits. Unique markers of SCoT and SRAP analyses undertook two additional variables. Those were related by arithmetic means of 4 wheat cultivars' performances under the effect of AgNPs concentrations. Meanwhile, an impressive color scheme is crucial for accurately understanding that heatmap. Consequently, for the criteria presented by Harrower and Brewer (2003), a treatment combination of diverse and sequential color schemes could be selected.

Results

The nanoparticle treatments experiment was conducted to evaluate the performances of wheat cultivars under salt stress. Results showed significant and highly significant effects at two levels of probability ($p < 0.05$ and 0.01, respectively) for all studied traits as influenced by studied factors and the effects of interaction between them, except plant fresh weight for wheat cultivars (factor A), those did not appear variability of their attributes over all other factors, also their no effects of four studied cultivars and the levels of silver nanoparticles concentration for plant dry weight (g) (Table 3). However, both salinity levels and AgNPs mean squares of concentration or different levels of salinity (100, 200, and 300 mMNaCl) were recorded as highly significantly affected for all the investigated traits, it would be predicted for the variances among their controls treatments for each factor and induced stress by graded levels of salt stress or alleviated that effects by increasing silvers nanoparticles concentration.

Table 3 - The significance of the mean squares resulting from the sources of variation for the three investigated factors (A, B, and C) and interaction among them for each studied trait.

Sources	Studied traits

of variation	Plant height (cm)	Plant fresh weight (g)	Plant dry weight (g)	$Na^{+}_{(ppm)}$	$K^{+}_{(ppm)}$	$Cl^{-}_{(ppm)}$
Cultivars (A)	**	ns	**	**	**	**
Salinity levels (B)	**	**	**	**	**	**
A ×B	**	*	**	**	**	**
AgNPs con. (C)	**	**	**	**	**	**
A × C	*	*	ns	**	**	**
B × C	**	**	**	**	**	**
A × B × C	**	*	**	**	**	**

*ns, * and ** indicated non –significant, significant at 5%, and highly significant at 1 % probability level, respectively.*

These differences had been meaningful by presenting means and compared averages for each level of a studied factor in Figure 1. The means of wheat cultivars' performances showed variability for the six studied traits except for plant fresh weight were no significant differences. However, those variations were indicated for the tallest plant overall treatments recorded around 70 cm for both Bani Sweef-1 (B1) and Sohag-1 (S1). However, Bani Sweef-3 (B3) possessed the highest values for three traits estimated for wheat leaves' Na^{+}, K^{+}, and Cl^{-} ions (ppm) concentration and recorded at 3.25, 1.41, and 7.14 ppm, respectively. Growth parameters seemed to reduce plant growth habits, such as shortness of plants, which were compared with the control treatment, caused by high levels of stressed salinity factor (300 mMNaCl). Despite this, that increases positively as an opposite trend for Na^{+} and Cl^{-} ppm concentrations.

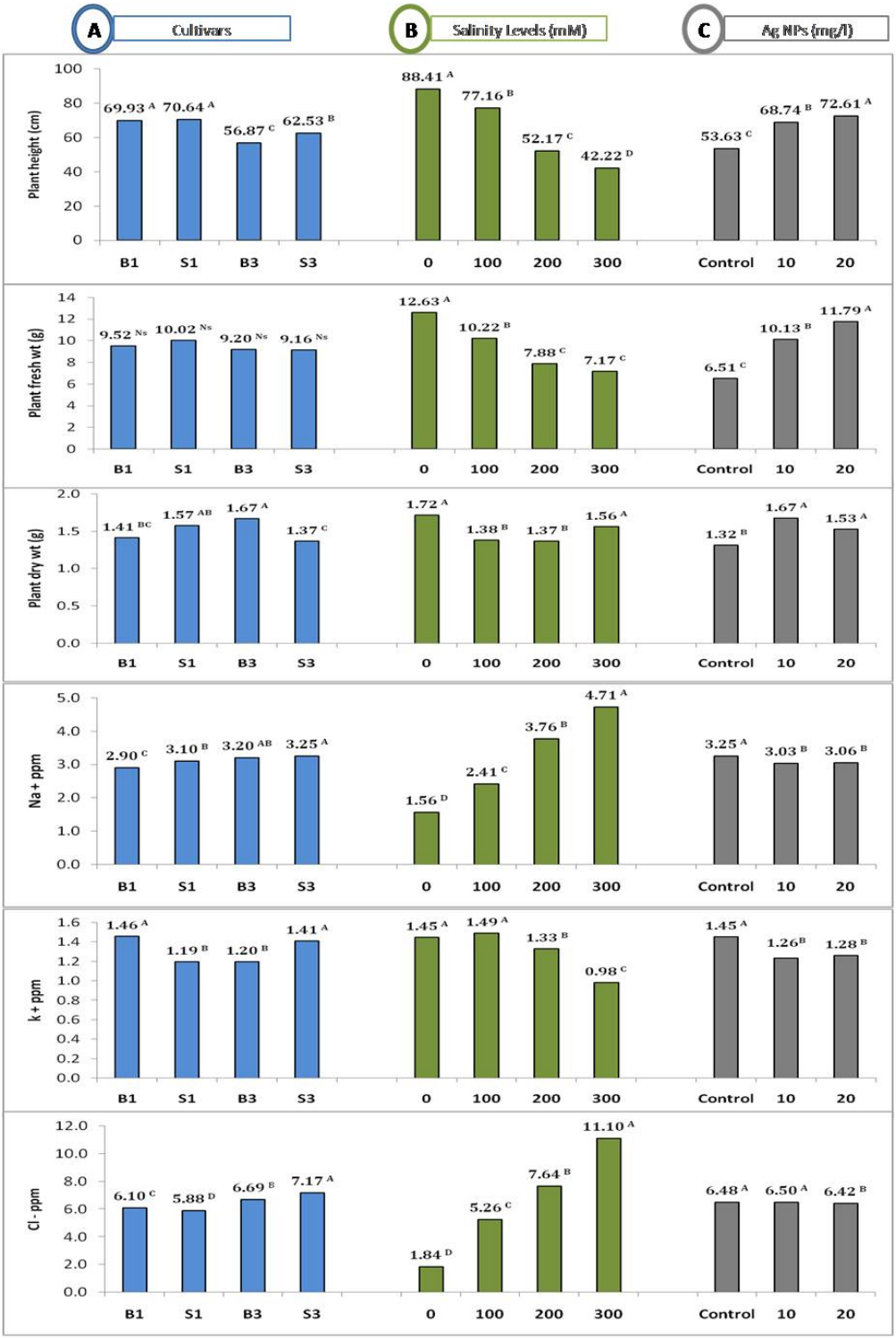

Figure 1 - Histograms present the effects of each factor; Means of bars (different cultivars performance: Bani Sweef-1 (B1), Bani Sweef-3 (B3), Sohag-1 (S1) and Sohag-3 (S3) of each study trait colored blue; Control (0) comparing with the level of salinity mM NaCl; colored green and AgNPs (control, 10, and 20 mg/l).; colored gray); all these followed by the same letters are not significantly different at 0.05 level of significance; Ns meansnon-significant.

Evaluation of mean performances of wheat cultivars at different levels of salinity

Table (4) shows that all wheat cultivars have shorter plant growth habits as saline levels rise. The B3 cultivar was recorded around 26 cm, and it appeared to be more dwarf than other wheat cultivars under the severe salinity condition at 300 mM NaCl. In addition, B3 and S3 cultivars both showed reduced performances in the same condition at high salinity levels for the fresh weight of the plant that recorded 6.55 and 6.33 g, respectively. Despite this, B3 exceeded the conserved water in plant tissues surfaced dried of plant dry weight that recorded 2.13 g, and it possessed the first position for this trait. By the same mean, it could be a stable cultivar. Taking into account the opposite trend in the case of S1 in the control condition or zero level of salinity induced, it was considered of ability to massive absorption of water into plant cells and or tissues, so it reacted in their attributed and showed that reduction of weights between fresh to dry weights degradation that reached about 13 folded from 13.53 to 1.58 g respectively.

Impact AgNP's role in enhancing wheat cultivars' growth under different levels of salt stress

Observed data regarding the interaction among three study factors have been presented in Figure 2. Four durum wheat cultivars were subjected to two different silver nanoparticle treatments (10 and 20 mg/l) compared with the control. Those plants were evaluated for salt tolerance and subjected to increasing salt stress (0, 100, 200, and 300 mMNaCl). Means that for all examined traits, the performance of wheat cultivars varied in their response over various salinity levels. The present study showed that treatment of wheat cultivars with different concentrations of AgNPs had positive effects compared with control treatment for both B1 and S1 at 20 concentration mg/l for each plant height and plant fresh weight trait, However, it had the effects of an equivalent on plant height within different concentration levels of AgNPs for B3 cultivars which were planting into normal condition without side effects of salinity. The increase in plant height was the highest in S1 treated with 20 mg/l of AgNPs, as shown in (Figure 2). Thus, the treatment variation of wheat cultivars with different levels of stresses salinity and different concentrations of AgNPs seemed to be alleviated this stress by exceeding those nanoparticles of silver in case of reducing the sodium ions or the other words it was influenced negatively relation of concentrations between increasing AgNPs and Na^{+}, where it was indicated by the S3 cultivar under severe salinity of 300 mMNaCl and treated with 20 mg/l of AgNPs. Neither B1 nor S1 was efficient due to resistance of salinity with increasing concentration, while the ten mg/l concentration possessed helpful defenses of the plant rather than 20 mg/l concentration.

Table 4 - Binary interactions due to source of variation as a possible combination determining the mean performances of 4 wheat cultivars (A) affected on each level of salinity (B); and three concentrations of AgNPs (C) for six studied traits.

Binary interactions	Cultivars	Salinity Levels mM	AgNPs concentration (mg/l)	Plant height (cm)	Plant fresh wt. (g)	Plant dry wt. (g)	Na^+ ppm	K^+ ppm	Cl^- ppm
A-B	B1	0		89.23 ^{A}	12.42 ^{A}	1.46 ^{DEF}	1.15 ^{I}	1.53 ^{BC}	1.66 ^{N}
		100		78.48 ^{BC}	10.38 ^{BC}	1.27 ^{EFG}	2.26 ^{F}	1.80 ^{A}	5.20 ^{J}
		200		60.42 ^{D}	8.29 ^{DEF}	1.60 $^{B\sim E}$	3.65 ^{O}	1.41 ^{D}	7.70 ^{G}
		300		51.60 ^{E}	6.99 ^{EF}	1.33 ^{EF}	4.54 ^{B}	1.10 ^{G}	9.83 ^{C}
	S1	0		89.52 ^{A}	13.53 ^{A}	1.58 $^{O\sim E}$	1.50 ^{H}	1.29 ^{EF}	2.15 ^{M}
		100		80.17 ^{B}	10.11 ^{BCD}	1.36 ^{EF}	2.33 ^{F}	1.19 ^{FG}	5.15 ^{K}
		200		62.98 ^{D}	7.63 ^{EF}	1.82 $^{A\sim D}$	3.63 ^{D}	1.34 ^{DE}	6.50 ^{H}
		300		49.88 ^{E}	8.81 ^{CDE}	1.54 $^{C\sim F}$	4.95 ^{A}	0.96 ^{H}	9.72 ^{D}
	B3	0		87.29 ^{A}	11.96 ^{AB}	1.89 ^{ABC}	1.81 ^{G}	1.43 ^{CD}	1.41 ^{O}
		100		77.63 ^{BC}	10.43 ^{BC}	1.53 $^{C\sim F}$	2.48 ^{EF}	1.22 ^{F}	5.10 ^{L}
		200		36.31 ^{F}	7.86 ^{EF}	1.13 ^{FG}	3.78 ^{CD}	1.21 ^{F}	8.04 ^{F}
		300		26.27 ^{G}	6.55 ^{F}	2.13 ^{A}	4.72 ^{AB}	0.92 ^{H}	12.23 ^{B}
	S3	0		87.61 ^{A}	12.61 ^{A}	1.95 ^{AB}	1.80 ^{G}	1.55 ^{B}	2.15 ^{M}
		100		72.36 ^{C}	9.97 ^{CD}	1.36 ^{EF}	2.58 ^{E}	1.75 ^{A}	5.58 ^{I}
		200		48.98 ^{E}	7.73 ^{EF}	0.92 ^{G}	3.99 ^{C}	1.37 ^{DE}	8.33 ^{E}
		300		41.16 ^{F}	6.32 ^{F}	1.24 ^{EFG}	4.64 ^{B}	0.96 ^{H}	12.61 ^{A}
A-C	B1		Control	56.14 ^{EF}	7.02 ^{EF}	1.27 ^{ns}	2.46 ^{H}	1.35 ^{CD}	4.42 ^{D}
			10	79.10 ^{AB}	10.28 ^{BC}	1.72 ^{ns}	3.69 ^{B}	1.38 ^{CD}	5.59 ^{C}
			20	74.56 ^{BC}	11.27 ^{B}	1.25 ^{ns}	2.56 ^{H}	1.65 ^{B}	8.28 ^{A}
	S1		Control	56.90 ^{EF}	7.65 ^{DE}	1.28 ^{ns}	3.41 ^{CD}	1.28 ^{D}	7.63 ^{B}
			10	73.23 ^{C}	9.11 ^{CD}	1.72 ^{ns}	2.17 ^{I}	0.95 ^{F}	4.42 ^{D}
			20	81.77 ^{A}	13.31 ^{A}	1.72 ^{ns}	3.72 ^{B}	1.35 ^{CD}	5.59 ^{C}
	B3		Control	52.98 ^{FG}	5.98 ^{F}	1.60 ^{ns}	3.21 ^{DE}	1.40 ^{C}	8.28 ^{A}
			10	56.32 ^{EF}	10.35 ^{BC}	1.66 ^{ns}	3.47 ^{C}	1.28 ^{D}	7.63 ^{B}
			20	61.32 ^{DE}	11.27 ^{B}	1.75 ^{ns}	2.90 ^{FG}	0.91 ^{F}	4.18 ^{E}
	S3		Control	48.49 ^{G}	5.40 ^{F}	1.12 ^{ns}	3.93 ^{A}	1.77 ^{A}	5.59 ^{C}
			10	66.31 ^{D}	10.77 ^{BC}	1.60 ^{ns}	2.78 ^{G}	1.32 ^{CD}	8.28 ^{A}
			20	72.78 ^{C}	11.31 ^{B}	1.39 ^{ns}	3.05 ^{EF}	1.13 ^{E}	7.63 ^{B}
B-C		0	Control	87.29 ^{A}	10.04 ^{CD}	1.66 ^{BCD}	1.49 ^{G}	1.54 ^{B}	1.92 ^{D}
			10	88.45 ^{A}	12.89 ^{B}	1.69 ^{BC}	1.52 ^{G}	1.39 ^{D}	1.92 ^{D}
			20	89.50 ^{A}	14.96 ^{A}	1.81 ^{AB}	1.69 ^{G}	1.42 ^{CD}	1.68 ^{E}
		100	Control	73.07 ^{C}	8.11 ^{E}	1.28 ^{EF}	2.57 ^{E}	1.66 ^{A}	5.26 ^{C}
			10	77.60 ^{BC}	9.94 ^{CD}	1.42 ^{CDE}	2.53 ^{E}	1.23 ^{E}	5.26 ^{C}
			20	80.80 ^{B}	12.63 ^{B}	1.45 ^{CDE}	2.15 ^{F}	1.59 ^{AB}	5.26 ^{C}
		200	Control	36.31 ^{F}	4.68 ^{F}	1.28 ^{EF}	3.73 ^{D}	1.51 ^{BC}	7.64 ^{B}
			10	56.73 ^{E}	8.36 ^{DE}	1.49 $^{B\sim E}$	3.55 ^{D}	1.34 ^{D}	7.64 ^{B}
			20	63.48 ^{D}	10.60 ^{C}	1.33 ^{DEF}	4.01 ^{C}	1.15 ^{EF}	7.64 ^{B}
		300	Control	17.85 ^{G}	3.22 ^{F}	1.05 ^{F}	5.23 ^{A}	1.10 ^{F}	11.10 ^{A}
			10	52.18 ^{E}	9.32 ^{CDE}	2.10 ^{A}	4.53 ^{B}	0.97 ^{G}	11.10 ^{A}
			20	56.64 ^{E}	8.97 ^{CDE}	1.53 $^{B\sim E}$	4.39 ^{B}	0.88 ^{H}	11.10 ^{A}

The means of column (different of each study trait) followed by the same letters are not significantly different at 0.05 level of significance

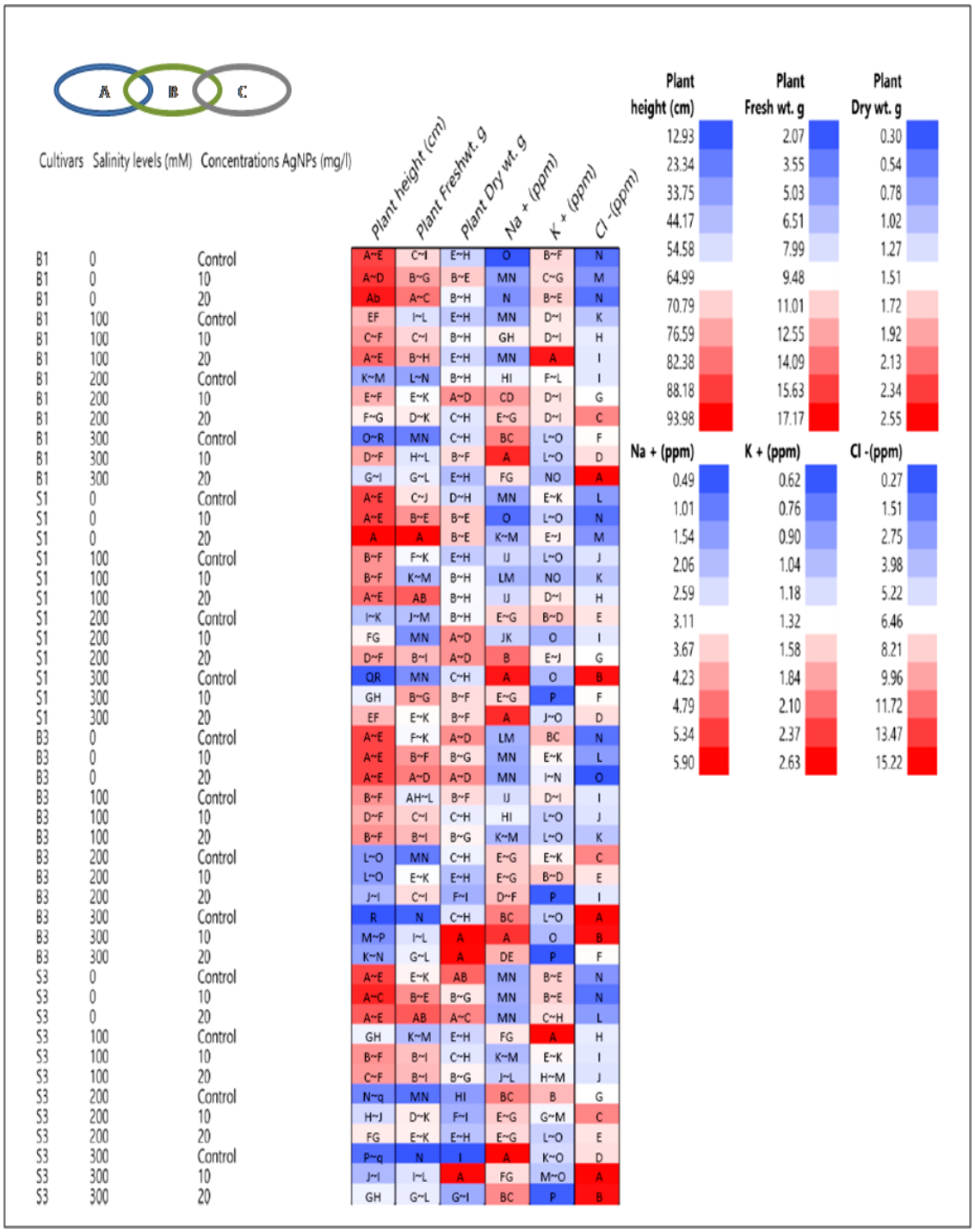

Figure 2 - Colored cell plot illustrates the interaction effects among three studied factors (A x B x C). Values for each studied trait presented in the column marked by the same letters are not significantly different at 0.05 level of significance.

Attributes of studied traits for explaining their variances in treatments

Factor analysis proceeding (Figure 3) separated six studied traits into two factors. The varimax orthogonal rotation was conducted to the matrix of factor computing afterward the first extraction of factor loadings. This rotation was a magnitude the larger loadings in the extracted factors and repressed the derivatives loadings, thus improving the opportunity of achieving meaningful interpretation of the gained factors. The enormous contribution factor accounted for 53% (Figure 3a) of the total variation. It was composed of some traits such as

ions concentration Na^+ and Cl^- increasing would be the most depression on other the plant growth parameters. Another second factor, which accounted for 19% of the total variation, was composed of some morphological traits and indicated the importance of plant height and fresh and dry weights. The first two factors presented by the scree plot (Figure 3c) would express the combined most closely associated growth habit parameters. However, loading factors as vectors bi-plot to discriminate two factoring principles of components noted by Figure 3d, whereas the 1st factor determined about 31% and second components around 26% such the related effects were observed for Na^+ and Cl^- ions concentrations.

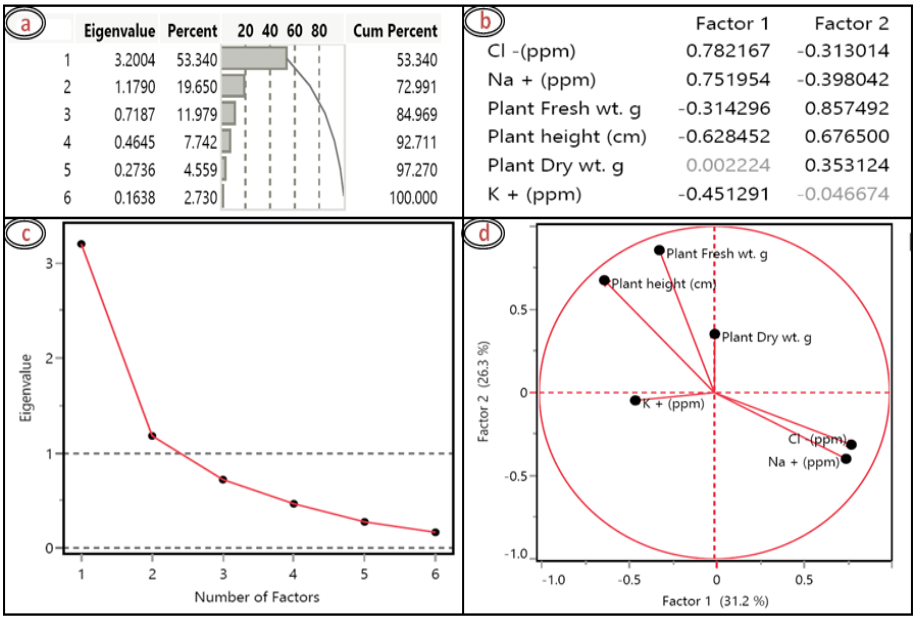

Figure 3 - Steps of factor analysis procedures for studied traits; a. Detailed factors with the percentage of variances and their accumulation due to numbers of eigenvalues and scores; b. Rotated factor loading by correlations with 2 Factors: Maximum Likelihood / Varimax; c. Scree plot represented the relationships of created factors number and eigenvalues; d. principle component biplot chart refers to the attributes of studied traits remarkable two principles component as factoring load plot.

Effect of silver nanoparticles on wheat genetic diversity using SCoT analysis

The AgNPs were affected by wheat genetics variations. The effect of AgNPs on wheat genetic diversity using thirty-seven SCoT primers was investigated (Supplementary Table S1). The total number of amplicons produced by the primers was 393, with an average of 10.6 amplicons/primer (Table 5). The highest number of amplicons (17) was obtained with primer SCoT-22, while the lowest number of amplicons (5) was amplified with primers SCoT-30 and SCoT-31. The polymorphic amplicons number ranged from 15 to 0, averaging 6.1. Primer SCoT-14 amplified the highest number of polymorphic amplicons (15) and showed the highest percentage (93.75%) of polymorphism. Moreover, the different primers showed different levels of polymorphism, ranging from 0% with the primer SCoT-17 and SCoT-26 to 93.75% with the primer SCoT-14 with an average of 57.50% Table 5 and Figure 4.

Furthermore, the parameters of the genetic varieties for the investigated primers were determined. The polymorphism information content (PIC) values ranged from (0) obtained with primers SCoT-17 and SCoT-26 to (0.362) by SCoT-32 with an average of 0.16. In addition, the highest value of effective multiplex ratio (EMR) (14.528) was gained by SCoT-22, while the lowest value (3.625) was obtained with primer SCoT-30 with an average of 7.28. Furthermore, the marker index (MI) values indicated range from (0) by primers SCoT-

17 and SCoT-26 to (2.729) by SCoT-36 with an average of 1.05. Also, the calculated resolving power **(**RP) values were about (8) by SCoT-30 to (30.67) by SCoT-22, with an average of 16.36.

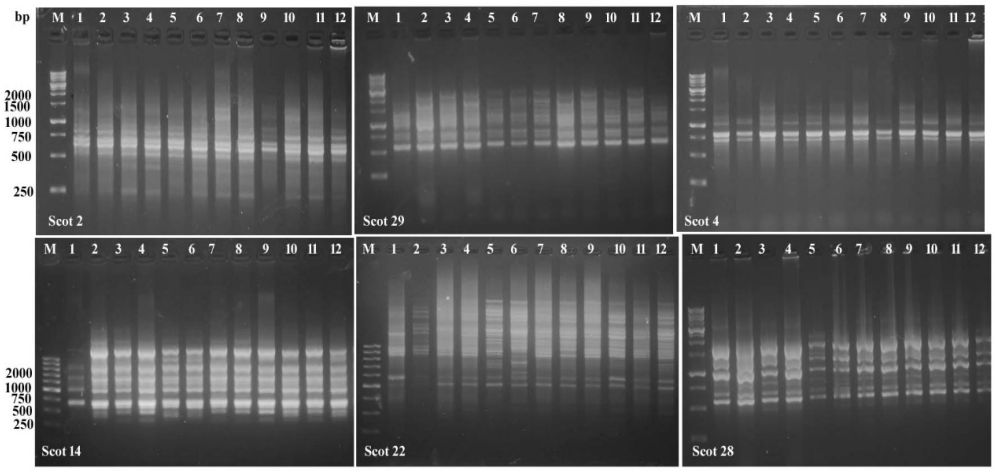

Figure 4 - SCoT profiles of the four wheat cultivars with two nanoparticle concentrations. Lanes 1 to 3 represent: Cultivar B1; Lanes 4 to 6 represent: Cultivar B3; Lanes 7 to 9 represent: Cultivar S1; Lanes 10 to 12 represent: Cultivar S3. M: 1Kbp DNA ladder.

In this study, genotype-specific SCoT unique markers could distinguish four wheat cultivars in wheat cultivars treated with two AgNPs concentrations (Table 6). Therefore, these unique markers are helpful as cultivar-specific ones. Forty-nine unique markers were generated from 37 primers. Nineteen unique positive markers and thirty unique negative markers were produced. The highest number of unique markers was nine. This number appeared twice with wheat cultivars B1 and S1. On the other hand, the S3 wheat cultivar treated with 20 mg/l AgNp was characterized by the lowest of unique markers number (only one negative).

Table 5 - PCR amplicons obtained from SCoT markers in wheat cultivars treated with two AgNPs concentrations, total band number (TBN), polymorphic band number (PBN), polymorphism percentage (P %), PIC, EMR, MI, and RP

No.	Primer name	TBN	PBN	P %	PIC	EMR	MI	RP
1	SCoT2	8	4	50	0.141	5.521	0.778	12.17
2	SCoT 3	14	12	85.71	0.332	5.590	1.856	15.83
3	SCoT 4	6	2	33.33	0.157	4.694	0.737	10.33
4	SCoT 5	6	3	50	0.134	5.097	0.683	11.00
5	SCoT 6	8	7	87.5	0.229	3.583	0.821	9.00
6	SCoT 11	13	11	84.61	0.204	5.944	1.213	14.33
7	SCoT 12	9	5	55.55	0.136	6.056	0.824	13.33
8	SCoT 13	13	7	53.84	0.116	9.993	1.159	21.50
9	SCoT 14	16	15	93.75	0.253	8.063	2.040	20.17
10	SCoT 15	11	5	45.45	0.168	8.660	1.455	19.17
11	SCoT 16	10	2	20	0.075	8.625	0.647	18.00
12	SCoT 17	9	0	0	0.000	9.000	0.000	18.00
13	SCoT 18	6	2	33.33	0.127	4.201	0.535	9.17
14	SCoT 19	13	10	76.92	0.169	6.736	1.138	15.67
15	SCoT 20	8	5	62.5	0.240	5.375	1.290	12.67
16	SCoT 21	8	3	37.5	0.130	5.896	0.766	12.83
17	SCoT 22	17	4	23.52	0.095	14.528	1.380	30.67
18	SCoT 23	11	3	27.27	0.080	9.313	0.745	19.50
19	SCoT 24	12	10	83.33	0.214	5.799	1.241	14.17
20	SCoT 26	11	0	0	0.000	11.000	0.000	22.00
21	SCoT 27	12	7	58.33	0.243	7.375	1.792	17.67
22	SCoT 28	9	4	44.44	0.082	7.549	0.619	15.83
23	SCoT 29	9	3	33.33	0.093	8.083	0.752	17.00
24	SCoT 30	5	2	40	0.150	3.625	0.544	8.00
25	SCoT 31	5	1	20	0.089	4.111	0.365	8.67
26	SCoT 32	13	11	84.61	0.362	5.896	2.134	16.50
27	SCoT 33	14	8	57.14	0.200	8.000	1.600	19.00
28	SCoT 34	12	3	25	0.038	11.521	0.438	23.50
29	SCoT 35	12	3	25	0.122	9.854	1.202	21.17
30	SCoT 36	16	11	68.75	0.300	9.097	2.729	23.00
31	SCoT 37	11	9	81.81	0.301	5.181	1.559	13.67
32	SCoT 38	12	6	50	0.203	7.535	1.526	17.50
33	SCoT 39	12	6	50	0.081	9.681	0.784	20.33
34	SCoT 40	9	2	22.22	0.062	7.056	0.436	14.67
35	SCoT 41	15	12	80	0.318	5.701	1.813	16.17
36	SCoT 42	9	2	22.22	0.062	8.389	0.520	17.33
37	SCoT 43	9	6	66.66	0.261	5.243	1.368	12.83
	Total	393	226	57.50				
	Average	10.62	5.57	49.56	0.16	7.28	1.05	16.36

Table 6 - Specific unique SCoT markers of wheat cultivars

Wheat cultivars	SCoT markers						Total markers
	Positive			Negative			
	primer	No. of markers	M.W (bp)	primer	No. of markers	M.W (bp)	
B1	SCoT 14	3	500,610,1200	SCoT 14	6	120, 210, 424, 571, 716, 1020	9
B1-10				SCoT 3	1	950	6
				SCoT 37	2	1100, 1200	
				SCoT 5	1	486	
B1-20				SCoT 22	2	200,290	2
				SCoT 12	2	900,1000	
B3	SCoT 11	1	230	SCoT 13	4	300,320, 500, 520	7
	SCoT 37	1	460	SCoT 28	1	4100	
	SCoT 41	1	690	SCoT 34	1	840	
B3-10	SCoT 13	1	1500	SCoT 15	1	560	7
	SCoT 24	3	1500, 1600, 1700				
S1	SCoT 12	1	230	SCoT 19	3	200, 292, 920	9
	SCoT 19	4	130, 140, 233, 312				
	SCoT 2	1	1324				
S1-20	SCoT 11	2	290, 1300	SCoT 11	4	490, 863, 1100, 1420	6
S3	SCoT 41	1	650	SCoT 2	1	750	2
S3-20				SCoT 43	1	3000	1
Total		19			30		49

Effect of AgNPs on wheat genetic diversity using SRAP analysis

A total of 42 SARP primer combinations were tested (Supplementary Table S2). According to the findings, all primer combinations revealed discernible polymorphism. 241 out of 477 bands were polymorphic (50.52%). The polymorphic bands per primer combination went from 5 (ME4-EM4) to 19 (ME6-EM1). The total number of bands produced was distinct sharp bands, as presented in Table 7 and Fig. 5.

Further, the parameters of the genetic varieties for the investigated primers were determined. The PIC values indicated range from (0.031) by combination primers (ME1-EM1) to (0.349) by ME2-EM4 with an average of 0.16. Also, the EMR values ranged from (2.535) obtained by combination primers (ME10-EM2) to (14.910) by ME6-EM2 with an average of 7.74. Moreover, the highest MI value (2.122) was gained by combination primers (ME5-EM4), while the lowest value (0.274) was obtained with combination primers (ME1-EM1) with an average of 1.1. Further, the calculated RP values were about (8) by combination primers (ME5-EM5) to (30.83) by combination primers (ME6-EM2) with an average of 17.24.

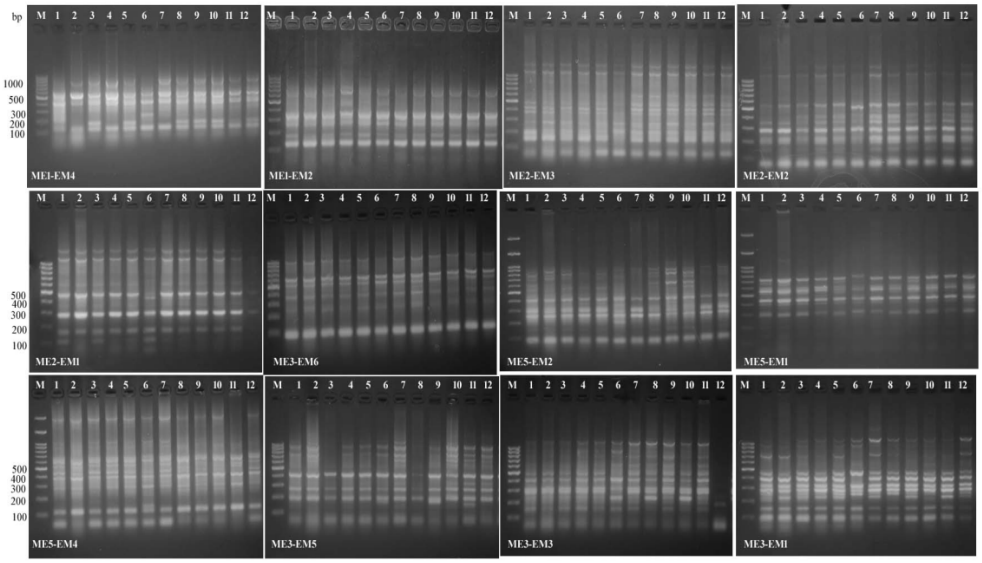

Figure 5 - SRAP profiles of the four wheat cultivars with two nanoparticle concentrations. Lanes 1 to 3 represent: Cultivar B1; Lanes 4 to 6 represent: Cultivar B3; Lanes 7 to 9 represent: Cultivar S1; Lanes 10 to 12 represent: Cultivar S3. M: 1Kbp DNA ladder.

The unique markers are helpful as cultivar-specific ones. Seventy-four unique markers were generated from 42 primer combinations. Thirty-two were positive, unique markers, whereas forty-two were unique negative markers. The highest number of unique markers was 29, which appeared with wheat cultivars B3 treated with 20 mg/l AgNp. On the other hand, wheat cultivars B1 treated with 20 mg/l AgNp, B3 wheat cultivar treated with 10 mg/l AgNPs, and wheat cultivars S1 treated with 10 mg/l AgNPs were characterized by the lowest of unique markers number (only one) (Table 8).

Table 7 - PCR amplicons obtained from SRAP markers in wheat cultivars treated with two AgNPs concentrations, TBN, PBN, P %, PIC, EMR, MI, and RP

No.	Primer name	TBN	PBN	P %	PIC	EMR	MI	RP
1	ME1-EM1	10	2	20	0.031	8.847	0.274	18.00
2	ME1-EM2	9	3	33.33	0.076	6.076	0.462	12.83
3	ME1-EM3	10	6	60	0.255	6.431	1.640	15.67
4	ME1-EM4	9	4	44.44	0.102	7.042	0.718	15.00
5	ME1-EM5	10	7	70	0.263	4.104	1.079	10.83
6	ME1-EM6	7	2	28.57	0.133	5.451	0.725	11.83
7	ME2- EM1	12	8	66.66	0.139	8.833	1.228	19.33
8	ME2- EM2	12	7	58.33	0.074	10.222	0.756	21.33
9	ME2- EM3	12	7	58.33	0.169	8.403	1.420	18.67
10	ME2- EM4	9	8	88.88	0.349	4.431	1.546	12.00
11	ME2- EM5	13	12	92.30	0.299	5.889	1.761	15.67
12	ME2- EM6	7	3	42.85	0.192	4.410	0.847	10.17
13	ME3-EM1	13	6	46.15	0.174	9.118	1.587	20.50
14	ME3-EM2	12	5	41.66	0.131	9.965	1.305	21.50
15	ME3-EM3	12	10	83.33	0.205	8.354	1.713	19.17
16	ME3-EM4	13	5	38.46	0.158	9.972	1.576	22.00
17	ME3-EM5	11	8	72.72	0.285	4.160	1.186	11.17
18	ME3-EM6	8	1	12.5	0.047	7.563	0.355	15.50
19	ME4-EM1	13	6	46.15	0.154	9.333	1.437	20.67
20	ME4-EM2	8	4	50	0.148	6.493	0.961	14.17
21	ME4-EM3	8	2	25	0.080	6.181	0.494	13.00
22	ME4-EM4	5	2	40	0.119	4.285	0.510	9.17
23	ME4-EM5	7	4	57.14	0.200	4.382	0.876	10.17
24	ME4-EM6	12	9	75	0.248	4.514	1.119	12.00
25	ME5-EM1	15	6	40	0.172	9.542	1.641	21.67
26	ME5-EM2	16	9	56.25	0.088	13.215	1.163	27.83
27	ME5-EM3	14	7	50	0.151	7.778	1.174	17.67
28	ME5-EM4	17	9	52.94	0.194	10.938	2.122	25.17
29	ME5-EM5	9	4	44.44	0.181	3.604	0.652	8.83
30	ME5-EM6	10	1	10	0.153	6.903	1.056	15.33
31	ME6-EM1	15	7	46.66	0.117	12.792	1.492	27.33
32	ME6-EM2	19	5	26.31	0.053	14.910	0.796	30.83
33	ME6-EM3	11	3	27.27	0.105	8.507	0.892	18.17
34	ME6-EM4	11	6	54.54	0.112	8.299	0.933	17.83
35	ME6-EM5	10	5	50	0.060	9.285	0.555	19.17
36	ME6-EM6	16	10	62.5	0.161	10.632	1.707	23.83
37	ME10-EM1	10	4	40	0.169	7.319	1.240	16.33
38	ME10-EM2	12	11	91.66	0.203	2.535	0.513	7.50
39	ME10-EM3	13	4	30.76	0.074	9.604	0.708	20.17
40	ME10-EM4	16	10	62.5	0.179	8.736	1.562	20.33
41	ME10-EM5	13	7	53.84	0.099	10.771	1.070	22.83
42	ME10-EM6	8	2	25	0.243	5.201	1.264	12.83
	Total	477	241					
	Average	11.36	5.74	49.44	0.16	7.74	1.10	17.24

Table 8. Specific unique SRAP markers of wheat cultivars treated with two AgNPs concentrations

Wheat cultivars	SRAP markers						Total markers
	Primer	Positive No. of markers	M.W (bp)	primer	Negative No. of markers	M.W (bp)	
B1	ME4-EM1	1	412	ME4-EM4	1	226	4
				ME2-EM5	2	1100, 1260	
B1-10	ME4-EM6	1	320	ME1-EM3	1	183	2
B1-20	ME5-EM4	1	147				1
B3	ME1-EM2	2	369, 825	ME10-EM4	4	321, 350, 430, 500	6
B3-10				ME2-EM4	1	200	1
B3-20	ME1-EM1	1	213	ME1-EM1	1	260	29
	ME1-EM4	1	246	ME2-EM3	5	150, 163, 371, 383, 392	
	ME2-EM1	1	410	ME3-EM1	1	391	
	ME4-EM1	1	215	ME3-EM2	2	160, 231	
	ME4-EM3	1	320	ME4-EM1	1	297	
	ME5-EM1	2	241, 750	ME5-EM1	1	710	
	ME5-EM2	1	176	ME6-EM2	2	560, 580	
	ME5-EM4	2	120, 213	ME6-EM3	1	100	
	ME6-EM1	2	287, 763	ME6-EM4	1	118	
	ME6-EM4	1	142				
	ME10-EM2	1	162				
S1	ME10-EM2	1	483	ME4-EM6	1	800	2
S1-10	ME5- EM3	1	1120				1
S3	ME10-EM1	1	490				2
	ME6-EM1	1	271				
S3-10	ME10-EM2	2	575, 360	ME2- EM1	2	1112, 1200	11
				ME3- EM3	4	140, 312, 400, 1210	
				ME10-EM2	2	310, 350	
				ME6- EM1	1	381	
S3-20	ME6- EM6	7	520, 690, 750, 810, 820, 913, 1520	ME1- EM5	1	421	15
				ME2- EM2	3	176, 500, 1120	
				ME2- EM5	1	131	
				ME5- EM5	1	910	
				ME6- EM4	1	1981	
				ME10- EM1	1	93	
Total		32			42		74

Furthermore, thirty-seven SCoTs and forty-two SRAP primers were examined for their ability to detect polymorphic patterns in the studied cultivars. Further, PIC, MI, EMR, and RP parameters were calculated to evaluate the efficiency of each marker. Generally, the SCoT-14 primer revealed the highest values for PBN (15) and P % (93.75). Also, the SCoT-22 primer showed the highest values for TBN (17), EMR (14.528), and RP (30.67). In addition, the SCoT-32 primer revealed the highest value for PIC (0.362), and the SCoT-36 primer showed the highest value for MI (2.729). While, the SCoT-17 and SCoT-26 primers revealed the lowest values (0) for PBN, P %, PIC, and MI, but the lowest values for TBN (5), EMR (3.625), and RP (8) showed by SCoT-30.

Moreover, the highest values of PBN (12) and P % (92.30) were revealed by the combination primers (ME2-EM5), and the combination primers (ME5-EM4) showed the highest values for TBN (17), and MI (2.122). Also, the combination primers (ME6-EM2) appeared to have the highest values for EMR (14.91) and RP (30.83). In addition, the combination primers (ME2-EM4) revealed the highest value for PIC (0.345). While the combination primers (ME1-EM1) appeared to have the lowest values for PIC (0.031) and MI (0.274), but the lowest values for TBN (5) by (ME4-EM4), EMR (2.535) by (ME10-EM2), RP (8.83) by (ME5-EM5), PBN (1), and P% (10) showed by (ME4-EM4).

Interrelationships of studied traits assessment of AgNPs efficacy on genetic diversity of studied wheat cultivars

A multivariate analysis of two-dimensional dendrograms was presented in Figure 6 due to detected a varied pattern of all studied traits at different unique markers obtained by SCot and SRAP analysis of genetic diversity, that inserted by binary combinations for four wheat cultivars and two levels of AgNPs comparing with control treatment, which it might be configured and annotation tracks placed at the matrices in order to explain them in conjunction with another clustering tree to organize and determining that clustering. Meanwhile, four wheat cultivars were categorized under two effects of AgNPs concentrations and control-treated to reveal similar performances and find their relationships out of all examined traits.

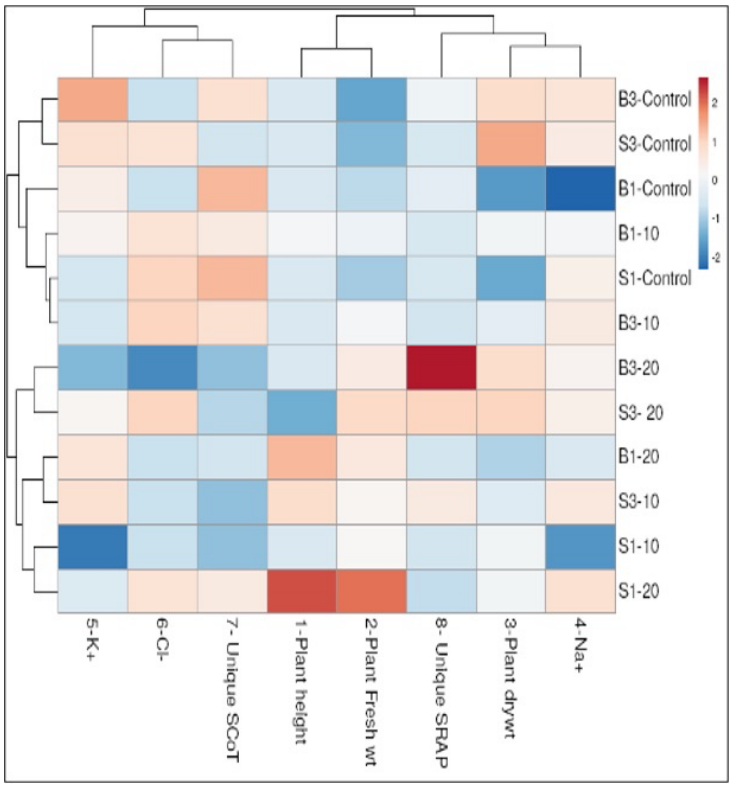

Figure 6 - Illustrated heat map based on Euclidean distance elucidates various effects and interrelationships of all examined characters with obtained unique bands of SCoTand SRAP and mean performances of 4 wheat cultivars under two levels of AgNPs concentrations compared with control.

Generally, the heatmap simplified and concluded all potential impacts. At the same time, it exhibited two primary groups of wheat cultivars' performances categorized at the horizontal matrix. The first group included all cultivars without treatment (control), and two

cultivars, B1 and B3, were treated with 10 mg/l AgNPs. However, another group consisted of rest-treated cultivars with different levels of nanoparticle concentrations. Although narrow genetic backgrounds of studied cultivars, AgNPs were divergent genetically due to differences in their treatments of concentrations. Some cases had carried a similar performance trend clustered into one group, branches, and nodes from its created trees, including two treatments for each cultivar. Whereas S1 control and B3 with 10 mg/l, this relation appeared to be identical among branches.

Additionally, the hieratical dendrogram clarified the relationship with various traits attributes allocated at the top matrix, whereas this relationship seemed to be similar between plant height and fresh weight. Plant dry weight with the concentration of Na^+ ions or defined for unique SCoT with Cl^- ions. Beyond, vast dissimilarity was evident between ions Na^+ and Cl^- through wheat treated due to this classification based on AgNPs omitted effects of salinity stress. Moreover, the presented data and figures visualized by color-key were carried out to be more distinctly various impacts by diverse variables for examined traits. While the red color has remarkable positive impacts, but the blue color reveals negative associations.

Consequently, the findings indicated that B1 in normal conditions is closely associated with the adverse relation of Na^+ ions. Moreover, SRAP unique markers were assertive with the B3 cultivar treated with 20 mg/l. Also, S1-20 related positively with both plant fresh and dry weight.

Discussion

Globally, tremendous efforts have been made to improve wheat cultivars and evaluate their yield productivity to alleviate instability yield production across multiple environments. However, salinity is considered the most atrocious stress-restrictive productivity across constraints caused by various stress (biotic or abiotic stresses). It reduces plant growth and development by negatively impacting osmotic stress induced by cellular responses and the cytotoxic effect of accumulated sodium ions (Kamran et al.,2019). Plant breeders are developing salt-tolerance strategies in plant breeding programs using approaches based on screening the wheat genetic background. The importance of modern molecular genetics protocols which assisted these programs was mentioned by Reynolds et al., (2001). Nowadays, nanoparticles used for the modification of plant genomic structure, it behaves the potential to inspire a novel molecular toolbox even further by creating the possibility of a rapid and universal delivery method in order to target the manipulation of the plant genome to improve agricultural output (Sharma and Lew 2022). Furthermore, many studies have shown the significant role of the nanotechnology strategy in improving salt tolerance in various plant species (Faizan et al., 2021). According to our findings, plants under salt stress had less growth than their corresponding control plants regarding plant height, fresh weight, dry weight, and leaf ions (sodium, potassium, and chloride) (Fig.1 and Table 4). These observations agree with Jhanzab et al., (2019) and Akbarimoghaddam et al., (2011) that elucidated excessive concentration of salts within plant tissues will constrain growth and upgraded production, as they can affect several vital processes, such as germination, photosynthesis, nutrient balance, and redox state balance. Furthermore, it indicated drawbacks of its performance according to stress susceptibility. Meanwhile, ion concentrations were increased with a high salinity level for all cultivars except the potassium K^+ mentioned by EL Sabagh et al., (2021). However, osmotic tolerance includes all plant modifications by reducing osmoprotectants like K^+ absorption and translocation in shoots and excluding the lethal quantity of Na^+ in roots and shoots (Almeida et al., 2017). Therefore, salt-tolerant plants must have the capacity to maintain K^+ ions under salinity stress (Ismail and Horie 2017). Although, B1 possessed an exceeded potassium concentration treated whenever it was too treated with AgNPs of 20 concentrations. It is considered the best treated overall effects salinity levels for all studied traits except the plant dry weight. It showed no

differences in the interaction between cultivars' performances and different treatments of AgNPs concentration levels. Besides that, no differences effects of various AgNP treatments showed for plant height and Na^+ ion concentration in normal conditions (zero salinity level). In comparison, effects showed an increase and a high-level AgNPs concentration at 20 mg/l for plant fresh weight. Generally, the increases of AgNPs were considered enhancement effects of the most studied traits, which could be considered silver contributing significantly to bioremediation soil. AgNPs play a vital role in initiating seed germination, rapid plant growth habits, and efficiency of the photosynthetic system. In the present study, the effect of two different concentrations of AgNPs on the genetic background of four wheat cultivars was evaluated using SCoT, and SRAP molecular markers. Based on the data resulting from SCoT, and SRAP reactions, it was possible to differentiate between the all treatment investigated in this study. In addition, the SCoT marker recorded the highest polymorphism percentage (57.50%) compared to the SRAP marker with 49.44% polymorphism. Moreover, the highest number of AgNPs treatment-specific markers was generated by SRAP (74) compared to the number of specific markers that generated by SCoT (49). The results of SCoT analysis revealed that the concentration 10 mg/l AgNP induced more unique band (13 bands) than 20 mg/l AgNP (9 bands) (Table 6). The cultivar B1 was the most cultivars that produced unique band compared to its control. From SRAP data analysis, B3 cultivar produced the highest number of unique bands (30 bands) compared to its control while S1 cultivar produced the lowest number of unique band (only one band) compared with its control. The concentration 20 mg/l AgNP induced more unique band (45 bands) than 10 mg/l AgNP (15 bands) (Table 6). These data confirm the capability of SCoT and SRAP marker as an excellent marker system to evaluate the genetic variation induced by treatment by AgNPS. Changes in bands number obtained in the present study reflected DNA alterations induced by AgNPs concentration. Mutations are responsible for the appearance of new PCR product visible on agarose gel if they occur at the same locus in at least 10 % of cells (Atienzar et al., 2000). On the other hand, damage caused to genomic DNA would induce the modification of the binding sites which can lead to alterations of electrophoretic PCR patterns which implies a disappearance of band (Rocco et al., 2012). Thus, the new bands could be attributed to mutations while the disappeared bands could be attributed to DNA damage.

Conclusion

AgNPs enhanced plants' salinity tolerance of the two wheat cultivars. Allelic frequency of the different SRAP and SCoT markers tested has differed among the AgNPs wheat treatments. These findings could be a good indicator of the salt tolerance mechanism present in the AgNPs-treated wheat cultivars. Therefore, these markers are of considerable value and can be utilized to screen large wheat populations for salt tolerance.

Authors' contributions

NIE conceived and designed the study; NHO, SBHA, HMF, IHY, MMA, NIE and DSA conducted the experiments. NHO, SBHA, HMF, IHY, MMA, NIE and DSA drafted and edited the manuscript. NHO, SBHA, IHY, MMA, and NIE performed data analysis. All authors read and approved the final manuscript.

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