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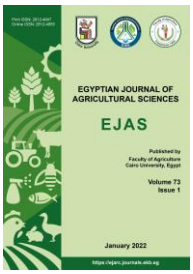

Genotyping of Two Hulled Barley Cultivars Using DNA Barcoding and The Effect of Iron Oxide Nanoparticles (Fe_3O_4) On Growth Enhancement

By

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ABSTRACT

Nanotechnology and DNA barcoding represent a groundbreaking and essential scientific transformation applied in several fields globally. The impact of various Fe_3O_4 concentrations, applied via soaking, spraying, or a combination, on the morphological, physiological, and biochemical traits, as well as antioxidant enzyme activities, in two Egyptian barley cultivars: Giza 130 (salt tolerance) and Giza 136 (salt sensitive) was investigated. DNA barcoding, utilizing trnH-psbA and matK barcodes amplified by PCR and sequenced, distinguished the salt-tolerant from sensitive cultivar. Barcode analysis was performed using DNA Subway, and QR codes were generated for identification. Results showed that the spray method of FeNP application significantly enhanced plant growth compared to other methods. Specifically, spraying barley plants with 50 mg/L FeNPs substantially increased total amino acid and proline content, total sugar, and catalase activity relative to controls. DNA barcoding analysis identified distinct DNA sequences differentiating Giza 130 and Giza 136, and their corresponding QR codes were determined. The genomes of both cultivars exhibited numerous SNP repeats in the trnH-psbA and matK barcoding regions, indicating high polymorphism. This information will aid in identifying salt-tolerant cultivars and advancing breeding programs to develop new high-yield or adaptable barley cultivars with enhanced traits.

Keywords: *Hulled barley, Nanoparticle, Fe_3O_4 NP, DNA barcoding, QR code, trnH-psbA and matK genes.*

1. INTRODUCTION

Barley (*Hordeum vulgare* L.) is a major food cereal crop globally due to its versatile grain with a plentiful nutritional value for humans and livestock. It is also a rich source of minerals, proteins, vitamins, lysine, antioxidants, β-glucan, and fiber, as well as low-fat content (Desta *et al.*, 2024; Vaishnavi and Mamta, 2024). Therefore, it is mainly used for industry and feed due to its high potential as a healthy food source (Ouertani *et al.*, 2022). Consequently, its production and consumption ranked fourth among the top significant crops after maize, wheat, and rice (Ouertani *et al.*, 2022; Desta *et al.*, 2024; Vaishnavi and Mamta, 2024). Barley was domesticated thousands of years ago, and its

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historical significance is demonstrated by the 18,000-year-old remains found in Aswan, Egypt (Vaishnavi and Mamta, 2024). It is often regarded as a poor man's crop owing to its straightforward cultivation process, minimal requirements, and remarkable adaptability to challenging conditions (Desta *et al.,* 2024). Furthermore, barley is cultivated in a diversity of geographical and climatic conditions, reflecting its high adaptability made possible by genetic diversity (Ouertani *et al.,* 2022).

Cultivated barley can be categorized into two distinct types based on the adherence to caryopsis. The first type is pearl barley, or covered barley, where the palea and lemma are firmly attached to the grain. The second type is a naked caryopsis, also known as hulled or hull-less, in which the outer hull is typically removed during its harvest process (Lei *et al.,* 2020; Vaishnavi and Mamta, 2024). Hulled barley's physical characteristics play a crucial role during its harvest, cleaning, processing, and storing, where the hull readily splits from the grain kernel. It gives the food industry an advantage by enabling the omission of a processing step. Consequently, whole grain from hulled barley has a higher nutritional and flavor value than pearled barley, which undergoes an abrasion process to get rid of the hull and bran. Therefore, it has a potential resource for human consumption as well as breeding new healthy food globally (Lei *et al.,* 2020; Desta *et al.,* 2024; Vaishnavi and Mamta, 2024).

Nanotechnology has various applications in diverse sectors, such as biotechnology, agriculture, medicine, and manufacturing. The application of nanotechnology in agriculture recently has a renewed fascination with sustainable agriculture, and it holds promise for sustainable practices (Fu *et al.,* 2023). Nanoparticles (NPs) interact at the molecular level with plants, enhancing nutrient use, resulting in improved seed germination, growth of crops, yield, and quality of products by managing pests and diseases and stress tolerance (Vijayakumar *et al.,* 2022). Plants face abiotic stressors such as drought and salinity, which can affect their growth and induce oxidative stress. Metal oxide nanoparticles, such as iron and zinc oxide, can help alleviate these effects. For example, iron oxide NPs improve water efficiency and chlorophyll in drought-stressed wheat (Manzoor et al. 2023), while zinc oxide NPs boost salinity tolerance in barley by enhancing antioxidant enzyme activity (Aslani *et al.,* 2014; Singh *et al.,* 2022). Iron-based nanoparticles Fe_3O_4 provide an iron supply that promotes the biosynthesis of siderophores, resulting in improved barley growth by improving germination rates and promoting early development. Moreover, iron stimulates the enzyme involved in RNA production, regulates oxidative metabolism, improves water utilization, and enhances the biological process of photosynthesis in barley plants. This leads to enhanced vegetative growth and higher yields (Al-Jubouri *et al.,* 2022; Tombuloglu *et al.,* 2024).

DNA barcoding is a molecular technique that uses an ideally unique, non-coding or coding, standardized DNA sequence from an organism's genome or its organelles to identify or classify an organismal group or distinguish between species (Antil *et al.,* 2023; Letsiou *et al.,* 2024). It focuses on specific genomic regions that show variation between species while remaining conserved within them (Antil *et al.,* 2023). By amplifying the DNA barcode regions, sequencing it, and comparing it to a reference database that contains the pertinent sequences from various species (Letsiou *et al.,* 2024). These barcode regions allow researchers to accurately determine the species identity of an organism (Antil *et al.,* 2023). The main aim of DNA barcoding is to establish a shared community resource of DNA sequences that can be used for organismal identification and taxonomic clarification (Chac and Thinh 2023). Additionally, since biodiversity has been threatened by pollution, deforestation, resource extraction, and human activity, DNA barcoding can be used to preserve rare endemic and endangered species as well as to study the evolution, ecology, and conservation of plants in general. Consequently, barcodes can be used to demonstrate the relationships among species or cultivars and correspond to sequences within the database (Letsiou *et al.,* 2024).

Interestingly, the FDA (US Food and Drug Administration) promotes the use of DNA barcoding for evaluating the quality of herbal products . This approach is effectively utilized in food traceability and authentication, such as in processed foods and dietary supplements (Galimberti *et al.,* 2019; Uncu *et al.,* 2020; Letsiou *et al.,* 2024). Furthermore, it can be utilized for identifying native or local cultivars that enhance the crops or products value and

encouraging the consumption of aromatic plants, fruits, and vegetables cultivated locally (Galimberti *et al.,* 2019; Letsiou *et al.,* 2024). DNA barcoding is also a greatly beneficial tool in forensic investigations, which makes it easy to relate biological samples and crime scenes. Human and non-human DNA analysis has become more significant in criminal investigations. Specifically, the analysis of plant evidence found at crime scenes, especially in cases of corpse transportation, the tracing of a suspect's movement, or the identification of narcotic plants, can play a crucial role in the resolution of criminal cases. DNA barcoding applications in forensic botany boost the precision and effectiveness of investigations, making a substantial contribution to the forensic science field. Finally, ecological and environmental genomic research is a major DNA barcoding application (Letsiou *et al.,* 2024).

This approach was successfully pioneered in animals using a 648-base pair (bp) fragment near the 5′-end of the mitochondrial gene cytochrome c oxidase subunit I (COI). In plants, the mitochondrial genome could not be employed because the mitochondrial genome has evolved differently and because mitochondria from multiple species may exist in a single plant due to plant interbreeding (Skuza et *et al.,* 2019; Antil *et al.,* 2023). Therefore, establishing a standardized DNA barcoding system in plants has been more challenging. DNA barcoding is a relatively new method of identifying plant species using short sequences of chloroplast DNA, including *matK*, encoding maturase K; *rbcL*, encoding the large subunit of RuBisCO; and *trnH-psbA*, a noncoding region, as well as ITS, a nuclear noncoding DNA region, which serve as unique genetic markers for precise species discrimination, producing the best and most dependable results. (Skuza *et al.,* 2019; Antil *et al.,* 2023; Chac and Thinh 2023).

According to our previous study, Soud et al. (2024) demonstrated that the barley cultivars differed in their response to varying levels of salt stress. Giza-130 was more tolerant to salinity, while Giza-136 exhibited the highest sensitivity. Therefore, this study aimed to identify the ideal treatment and concentration of Fe_3O_4 to enhance Egyptian hulled barley development. Consequently, we investigated the effects of different concentrations of Fe_3O_4 by three different nanoparticle treatments (soaking, spraying, and soaking + spraying) on morphological, physiological, and biochemical parameters and antioxidant enzyme activities for these two cultivars of Egyptian barley plants. Furthermore, DNA barcoding was performed to distinguish and classify salt-tolerant and salt-sensitive cultivars of Egyptian hulled barley. This information will facilitate the identification of salt-tolerant cultivars, enhance strategies of saline soil management, and promote programs of breeding that result in the creation of novel adaptable or high-yield barley cultivars with particular improved features.

2. MATERIALS AND METHODS

Plant materials

The seeds of two Egyptian hulled barley cultivars, Giza 130 and Giza 136, were obtained from the Field Crop Research Institute, Agricultural Research Center, Giza, Egypt, as well as the pedigree of these cultivars is presented in **Table 1.** Cultivar seedlings were cultivated in greenhouses, and the young

Table (1): Pedigree of the two Egyptian barley cultivars used

Cultivar	Characteristics
Giza 130	Six rows, Egyptian naked barley accession, precocious, moderately productive in the favorable conditions and tolerant to drought and fungi diseases. It has been selected from the crosses ‘‘Comp.cross’’ 229//Bco.Mr./DZ02391/3/ Deir Alla 106 using the bulk method. ARC- Egypt
Giza 136	Six rows, Egyptian naked barley accession, precocious, moderately productive in the favorable conditions. It is issued from the following cross: PLAISANT/7/CLN-B/LIGEE640/3/S.P-B//GLORIAAR/ COME B/5/FALCONBAR/6/LINOCLN-B/A/S.P- /LIGNEE640/3/S.P-B//GLORIA-BAR/COME B/5/FALCONBAR/6/LINO. ARC- Egypt.

seedlings' leaves were used to isolate DNA.

2.1. Evaluation of the best nanoparticle treatment method for hulled barley

2-1.1. Synthesis of zero iron nanoparticles

Nanoscale zerovalent iron (nano ZVI) was formed by adding a 1:1 volume ratio of $NaBH^4$ (0.8 M) into $FeC1^3.6H_20$ (0.2 M) and mixing the solution vigorously at room temperature for 5 min. Ferric iron was reduced by borohydrate; thus, nano ZVI was formed as the following equation $4Fe^{3+}$ + $3BH^4$- + $9H_20$ -> 4Fe0 + $3H_2B0_3$ + $12H^+$ + 6H2. Nano ZVI was filtrated through 0.45-micron filter paper and thus washed several times with deionized water to remove excessive borohydrate. Nano ZVI was dried by N2 gas and preserved from oxidation by maintaining a thin layer of ethanol on the top of nano ZVI (Sun *et al.* 2006).

2.1.2. Nanoparticle application methods

To study the effect of nanoparticle application methods on Giza130 and Giza136 plant growth, three nanoparticle application methods (spraying, soaking, and spraying + soaking) were applied for four weeks at different concentrations (0, 10, 30, and 50 mgL^1).

2.1.3. Measurement of the improvements in barley plant growth

Samples were collected from control and Fe-nanoparticles-treated plants to determine fresh weights; then, the sample was dried in a forced drying oven (Heraeus-0871, USA) at 80°C for three days. After the drying process, the dry weight of the samples was determined.

2.1.4. Measurement of Biochemical parameters

Total amino acids were determined by the ninhydrin method according to Hamilton and Van-Slyke (1943). Determination of total proline: Concentration was calorimetrically measured in plant samples using a ninhydrin reagent according to Bates *et al.* (1973).Determination of total sugar: Acid hydrolysis of plant material was carried out in a sealed tube using an accurate known sample (0.2 g) and 10.0 ml of 1.0 M H2SO4 solution. The opening sealed tube was put in a boiling water bath for 10 h, after complete hydrolysis, the hydrolysate was neutralized by the addition of barium carbonate, and the precipitate was separated by filtering the solution through the Whatman No. 1 filter paper. After filtration, the clear solution was made up to a known volume. The total sugar was determined in acid using the phenol-sulfuric acid method described by Duobois et al. (1956).

2.1.5. Measurement of antioxidant enzyme activities

The crude extract for peroxidase and catalase assays was extracted according to Sarker and Oba (2018).Estimation of Catalase (CAT) (EC 1.11.1.6):Catalase activity was determined by monitoring the disappearance of H2O2 at 240 nm (@= 40 Mm-1 cm-1) according to the method of Aebi, (1984). The reaction mixture contained 50 mM k-phosphate buffer (pH 7.0), 33 mM H2O2, and enzyme extract. Estimation of peroxidase:Peroxidase activity was determined at 436 nm by its ability to convert guaiacol to tetra-guaiacol (@= 26.6 mM-1 cm-1) according to the method of Polle et al. (1994). The absorbance increase was recorded by adding H_2O_2 at 436 nm for 5 min. All determinations were performed in triplicate and data were represented on a dry weight basis as mean values ± or standard deviations.

2.2. DNA barcoding to distinguish and classify salt-tolerant and salt-sensitive cultivars of hulled barley

2.2.1. DNA extraction

Genomic DNA was isolated from 0.5 g of leaf samples representing the two barley genotypes under investigation using the CTAB method, according to Rogers and Bendich (1985).

2.2.2. Amplify barcode using PCR

The PCR reaction was performed in a total volume of 20 µL containing 2 µL DNA template, 10 µL master mix (GeneDireX, Germany, CAT No. MB208-0110), and 1 µL from each of the forward and reverse primers of the barcoding genes (**Table 2**), and the volume

Table (2): Primers used for DNA barcoding

Gene	Sequences	Annealing temp.	Reference
matk	*matk*390(F)5' - CGATCTATTCATTCAATATITC- 3' *matk*1326(R)5'- TCTAGCACACGAAAGTCGAAGT-3'	48 °C	Skuza et al., 2019
trnH-psbA	*psbA*(F)-5' - GTTATGCATGAACGTAATGCTC-3' *trnH*(R)5' - GCGCATGGTGGATTCACAATCC-3'	55 °C	Skuza et al., 2019

was completed to 20 µL with 6 µL nuclease-free water. The PCR reactions were amplified with the following program: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 1 min, and annealing at 48–55°C for 1 min. The amplified products were analyzed by electrophoresis in 1% agarose, stained by ethidium bromide, and photographed under UV light.

2.2.3. Sequencing analysis

Before sequence analysis, the PCR products of the barcoding region of *Mat-k* and *trnH-psbA* were enzymatically cleaned by adding 3 µL of ExoSAP-IT^{M} PCR product cleaning reagent (Thermofisher, Cat. No. 78200.200, USA) to 5 µL of each amplified PCR product. The mixture was incubated at 37°C for 20 minutes, followed by 80°C for 15 minutes using a GeneAmp PCR 9700 thermocycler (Applied Biosystems). The purified PCR products were then subjected to sequencing using AB Big-Dye 3.1 dye chemistry (Applied Biosystems). For sequencing reactions, 20 µL reaction volumes were prepared, consisting of 2 µL of cleaned PCR product, 1 µL of BigDye Terminator v3.1 Ready Reaction Mix, 2 µL of 5× Sequencing Buffer, 1.6 pmol of forward or reverse sequencing primer, and PCR-grade water. The sequencing reactions were performed for 25 cycles, with each cycle consisting of 96°C for 30 seconds, 50°C for 15 seconds, and 60°C for 4 minutes. The reactions were then held at 4°C using a GeneAmp PCR 9700 thermocycler (Applied Biosystems). The sequencing products were cleaned using Performa® DTR Gel Filtration Cartridges (Edge Bio, Gaithersburg, Cat. No. 42453, CA, USA) according to the manufacturer's protocol and subsequently sequenced using the AB 3500 XL automated DNA sequencers (Applied Biosystems). The sequences were analyzed using the BLAST V2.0 website (http://www.ncbi.nlm.nih.gov/BLAST/). DNA barcoding was generated using the DNA SUBWAY online tool: Fast Track to Gene Annotation and Genome Analysis - DNA Subway. The QR code was generated using the online QR code generator: QR Code Generator: Create your free QR Code | QRFY.

2.2.4. Statistical analysis

Barley plant growth investigations were conducted on at least 5 biological samples. Moreover, each assay was carried out with at least three technical repeats. The results' means are shown as ±SE (standard error).

3. RESULTS

3.1. Assessment of optimal nanoparticle treatment methods for hulled barley

The effects of nanoparticle treatments on the growth improvement of two hulled barley cultivars (Giza 130 and Giza 136) were estimated. One-month-old seedlings were subjected to 10, 30, and 50 mg of FeNPs by three different nanoparticle treatments (soaking, spraying, and soaking + spraying) on two cultivars of hulled barley plants. Seeds germinated under normal conditions (0 mg FeNPs) were used as a control. Comparing the value of the fresh and the dry weight for all the FeNP treatment methods on barley plant growth with different FeNP concentrations was shown in **(Fig. 1).** The results demonstrated that different FeNP concentrations significantly increased plant fresh and dry weight. Additionally, increasing FeNP concentration, especially to 50 mg/L, significantly enhanced plant development compared to the control. Furthermore, the

results indicate that the spray method revealed that plant morphology was

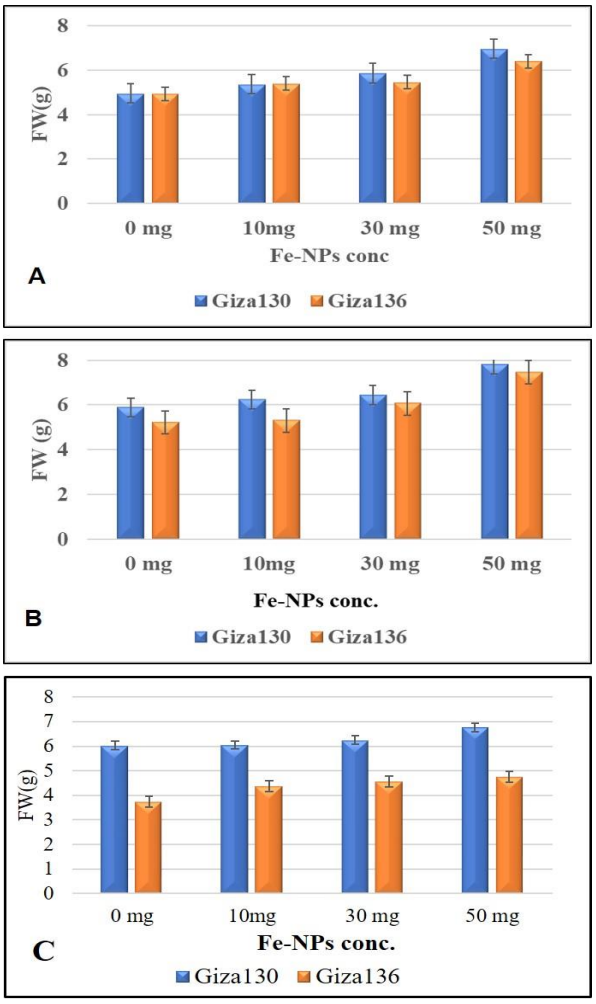

Fig. (1): The effect of different FeNPs treatments on barley plant fresh weight. A- Soaking, B- Spray and C- Spray + Soaking.

significantly influenced by different FeNP concentrations **(Fig. 2).** The visual symptoms of the effect of spraying FeNPs to varying concentrations on barley plant growth included the plant's boosted growth, increased plant size, and greener-than-normal. They became completely healthy and strong after a few days. These symptoms were significantly observed in treated plants with the 50 mg concentration of FeNP.

3.2. Evaluation of spray treatment effects on Hulled barley using biochemical parameters

The changes in total amino acid, total sugar contents, and proline in two barley cultivars sprayed with nanoparticles at different concentrations were determined **(Fig. 3).** The results demonstrated that the spray of barley cultivars with a 50 mg/l concentration of FeNPs significantly increased compared with the control treatment for Giza130 for each total amino acid and proline but slightly increased for total sugar contents. In contrast, the Giza136 cultivar has been slightly increased for each total amino acid and proline, but the total sugar content was significantly increased compared with the control treatment.

3.3. Evaluation of spray treatment effects on Hulled barley using antioxidant enzyme activities analysis

The changes in peroxidase and catalase enzyme activity in two barley cultivars sprayed with FeNPs in different concentrations were assayed **(Fig. 4).** Results from these assays revealed different levels of catalytic activities among different concentrations within the same cultivar. Spraying barley plants with a 50 mg/l concentration of FeNPs has significantly increased peroxidase activity and slightly increased catalase activity compared with the control.

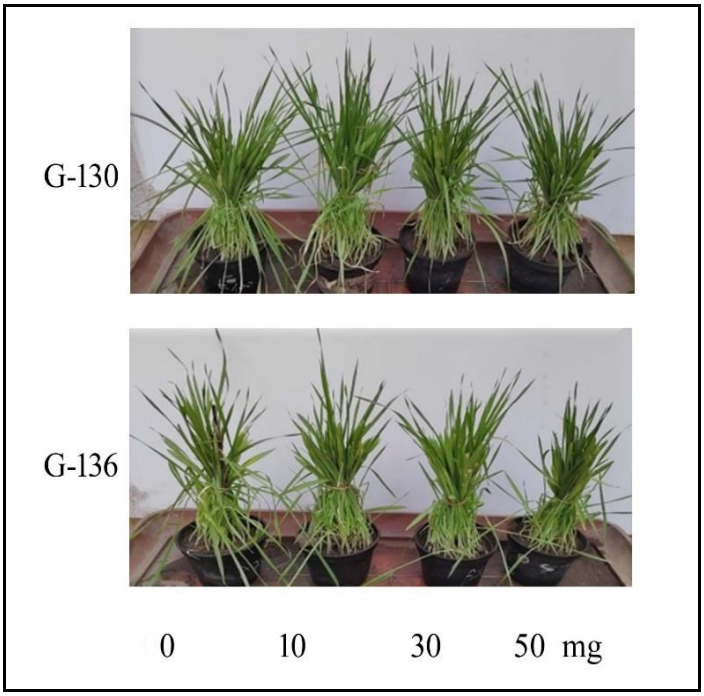

Fig. (2): The effect of spraying FeNPs at different concentrations on barley plant growth.

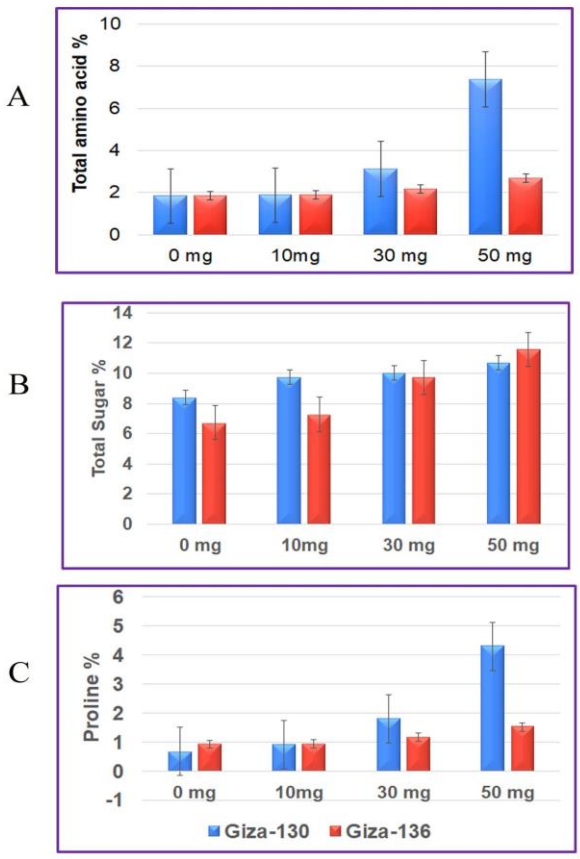

Fig. (3): Change in total amino acid (A), total sugar contents (B), and proline (C) in barley cultivars (Giza130 and Giza 136) sprayed with nanoparticles at different concentrations.

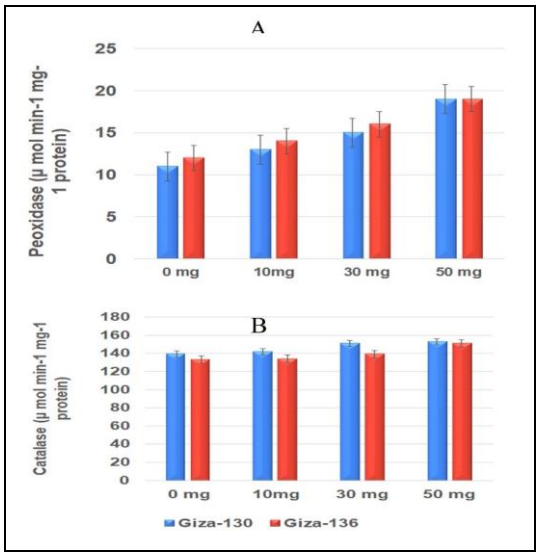

Fig.(4): Change in peroxidase (A) and catalase (B) enzyme activity in barley cultivars (Giza130 and Giza 136) sprayed with FeNPs in different concentrations.

3.4. DNA barcoding

DNA barcoding was performed in this study to distinguish and classify salt-tolerant and salt-sensitive cultivars of hulled barley. Two distinct barcodes were amplified using high-quality genomic DNA from two hulled barley cultivars. The product of PCR analyzed on an agarose gel (1.2%) detected one amplicon for each, ranging in size from 980 bp (*trnH-psbA*) to 1 kb (*matK*), as shown in **(Fig. 5).** These amplicons were sequenced with an automated DNA sequencer (Macrogen Company, Geumcheon-gu Seoul, Seoul, Republic of Korea).

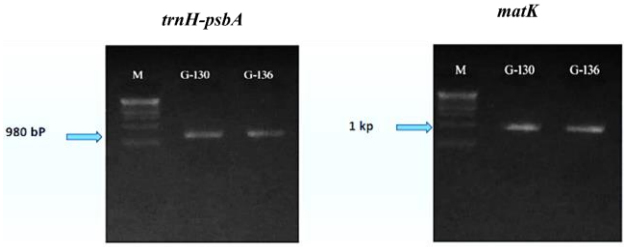

Fig.(5): Amplification of *matk* and trnH-*psbA* barcoding regions from DNA samples prepared from G1iza130 and Giza136

3.5. Data sequence analysis

The AT and GC contents and the average AT% and GC% nucleotide composition were calculated from the sequence data obtained from sequence amplification of selected barley genotypes based on *matk* and *trnH-psbA* markers, as shown in **Table (3).** As presented in **Table (4),** *matK* appeared one amplicon with 1 kb, revealing 7 ORF with sequences ranging from 78 bp coded to 25 a.a. by ORF 7 to 534 bp encoded to 177 a.a. by OFR 2 observed in Giza-130. However,

Giza-136 exhibited 9 ORF with sequences ranging from 78 bp coded to 25 a.a. by ORF 8 to 495 bp coded to 164 a.a. by ORF 1. Similarly, *trnH-psbA* showed one amplicon with 980 bp, revealing 10 ORF with sequences ranging from 81 bp coded to 26 a.a. by ORF 1 to 216 bp encoded to 71 a.a. by OFR 7 observed in Giza130. As well as Giza136 exhibited 10 ORF with sequences ranging from 102 bp coded to 33 a.a. by ORF 2 to 255 bp coded to 84 a.a. by ORF 9.

Table (3): The average AT% and GC% nucleotide composition of selected barley genotypes based on *matk* and *trnH-psbA* markers.

Genotype	Markers	AT	GC	Total length	AT%	GC%
Giza130	*matk*	600	292	879	68.2	33.8
	trnH-psbA	647	540	1187	54.5	45.5
Giza136	*matk*	708	388	1096	64.6	35.4
	trnH-psbA	512	426	938	54.6	45.4

AT and GC contents were calculated from the sequence data obtained from sequence amplification with specific primers

Table (4): Sequences created from two DNA barcodes

Barcoding Name	Cultivars	ORF No.	Strand	Frame	Start	Stop	Length (nt.) bp	Length (a.a)
matK	**Giza 130**	ORF2	+	3	327	860	534	177
		ORF1	+	2	374	742	369	122
		ORF5	-	2	533	533	99	32
		ORF6	-	2	101	>3	99	32
		ORF3	-	1	285	199	87	28
		ORF4	-	1	132	52	81	26
		ORF7	-	3	691	614	78	25
	Giza 136	ORF1	+	1	394	888	495	164
		ORF3	+	3	423	764	342	113
		ORF2	+	2	176	478	303	100
		ORF4	-	1	943	761	183	60
		ORF6	-	2	555	457	99	32
		ORF5	-	1	121	35	87	28
		ORF7	-	3	992	906	87	28
		ORF9	-	3	305	219	87	28
		ORF8	-	3	713	636	78	25
trnH-psbA	**Giza 130**	ORF7	-	1	383	168	216	71
		ORF10	-	3	756	601	156	51
		ORF8	-	2	1036	911	126	41
		ORF9	-	3	981	859	123	40
		ORF4	+	3	216	332	117	38
		ORF3	+	2	980	1093	114	37
		ORF2	+	1	1075	>1185	111	36
		ORF5	+	3	1023	1127	105	34
		ORF6	-	1	1172	1068	105	34
		ORF1	+	1	334	414	81	26
	Giza 136	ORF9	-	3	584	310	255	84
		ORF8	-	3	813	613	201	66
		ORF5	-	1	416	240	177	58
		ORF6	-	2	616	473	144	47
		ORF4	+	2	635	763	129	42
		ORF3	+	2	419	538	120	39
		ORF1	+	1	328	441	114	37
		ORF7	-	2	178	74	105	34
		ORF10	-	3	114	10	105	34
		ORF2	+	2	83	184	102	33

The constructed sequences were compared using BLAST with the available sequences in the National Centre for Biotechnology Information (NCBI) to check for species similarity. *MatK* and *trnH-psbA* were revealed to be effective DNA barcoding regions for species identification.

3.6. Analyzing inter-cultivar relationships

The resulting sequence of each barcoding locus was aligned with another species in the database using the DNA SUBWAY Software, revealing that the two cultivars (Giza130 and Giza 136) are closely related to *Hordeum_ vulgare*. The two cultivars were found in the same main clusters but in distinct subcultures. The dendrogram demonstrated in **(Fig. 6)** of matK that two cultivars were identified in the same main cluster but in distinct subcultures. Furthermore, it is revealed that Giza 130 is closely related to OL 439544.1 Hordeum vulgare while Giza 136 is closely related to OL 505079.1 *Hordeum vulgare*. Moreover, the dendrogram shown in **(Fig. 7)** of trnH-psbA shows that the two cultivars are more closely related to each other.

According to the Plant Working Group, the Consortium for the Barcode of Life (PWG-CBOL), the features of an ideal DNA barcode need to be amplified with ubiquitous primers and high efficiency of amplification and sequencing. Furthermore, the complete sequence of information on the two cultivars is shown in **(Fig. 8),** utilizing the QR code digital technology.

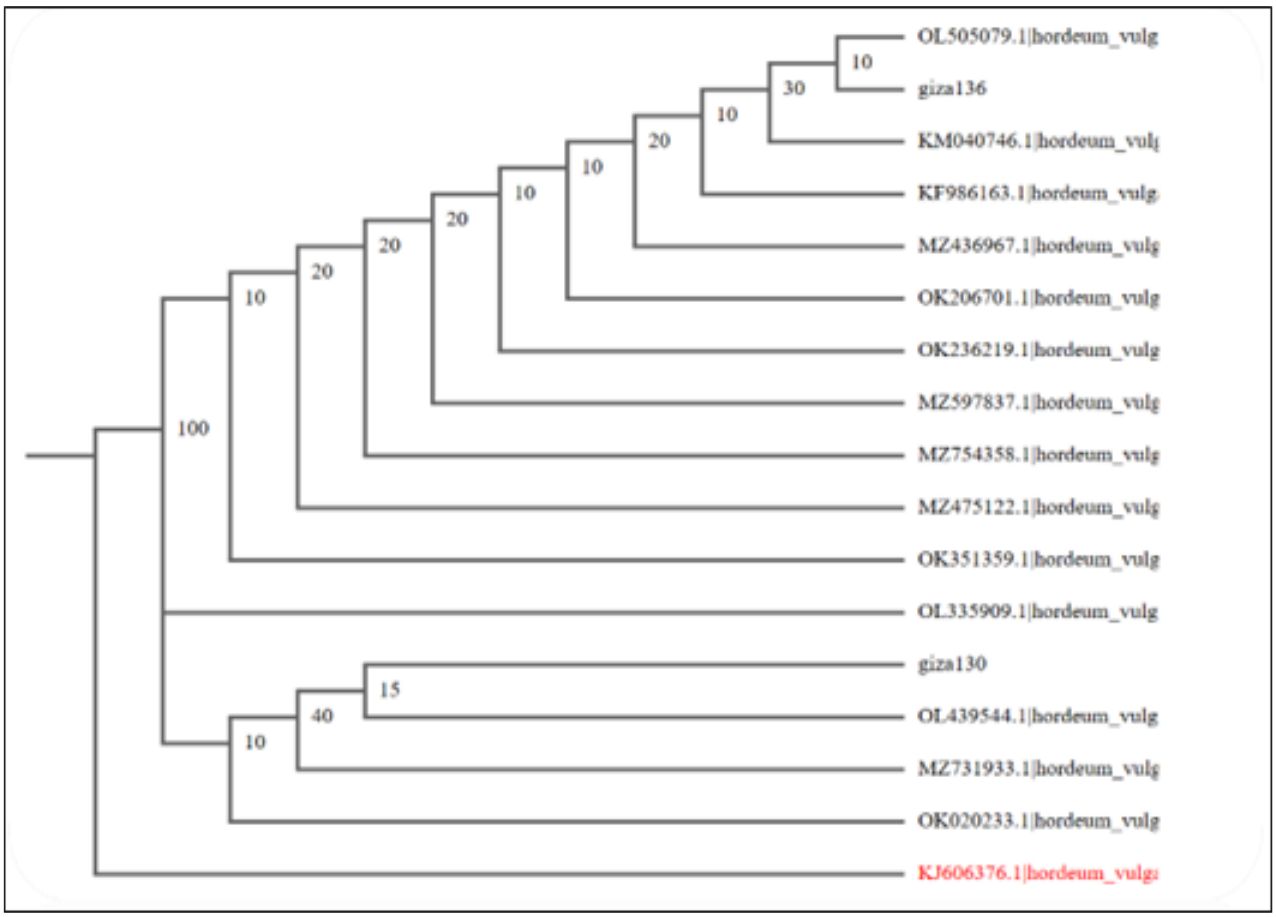

Fig.(6): Phylogenetic relationship between different barley species differentiated based on *matk* genetic marker.

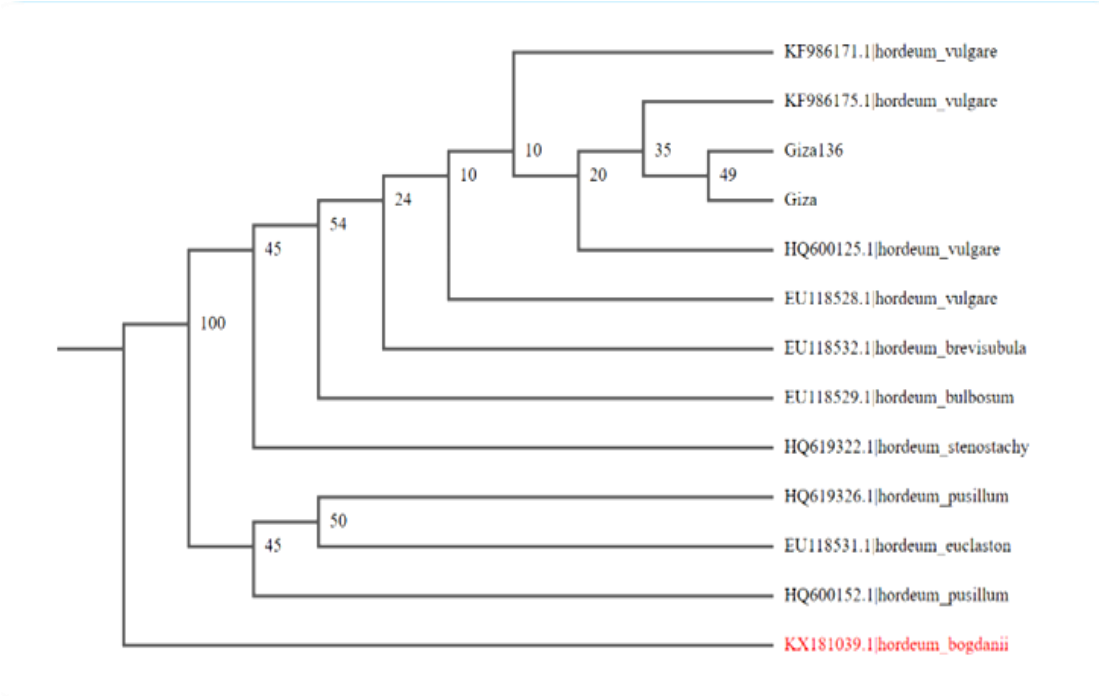

Fig. (7): Phylogenetic relationship between different barley species differentiated based on *trnH-psbA* genetic marker.

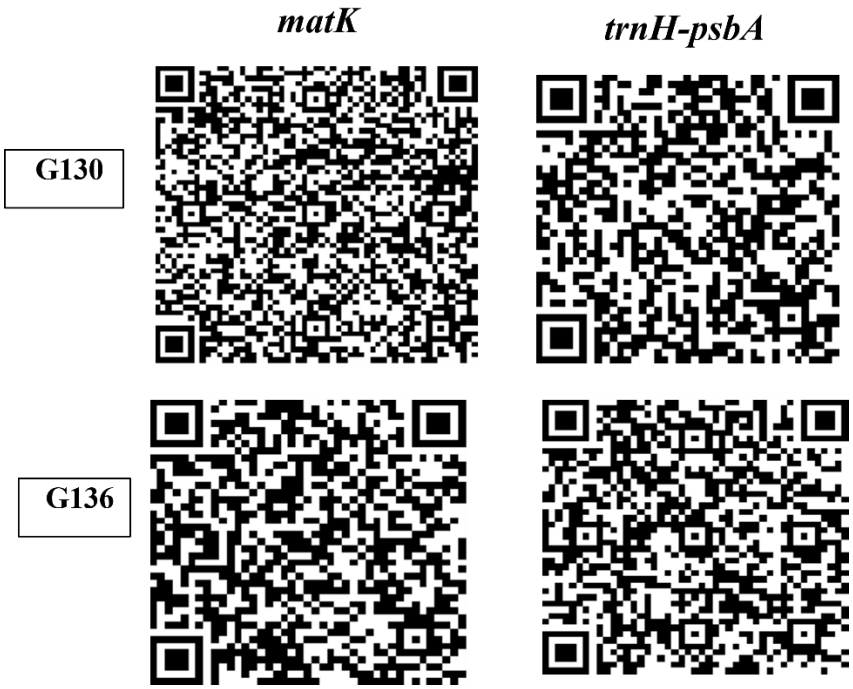

Fig. (8): QR barcode of barley plants, based on *trnH-psbA* and *matK* sequence.

4. DISCUSSION

Nano-biotechnological techniques offer advantages for various aspects of plant biology, such as enhancing seed germination, promoting plant growth, improving nutrient uptake, isolating secondary metabolites, and providing protection against abiotic and biotic stress factors. Moreover, nanotechnology provides valuable prospects for agriculture due to its unique physicochemical properties, including a large surface area, increased reactivity, customizable pore size, and versatile particle shapes (AL-Rawi *et al.,* 2024). Since nanoparticles influence plant growth and development, they can alter the length of shoots and roots, fresh biomass production, and the genome and also affect plant microRNA expression levels (Petrova *et al.* 2021). Therefore, nanoparticles have contributed to tackling the rising challenges of crop production and food security, helping to meet the expanding global food demand (AL-Rawi *et al.,* 2024). Furthermore, iron oxide nanoparticles (FeONPs) have demonstrated multiple positive effects on plant growth and development, including boosting nutrient absorption, increasing stress resistance, and enhancing photosynthesis through interactions with plant cells and soil microorganisms (Razavizadeh *et al.,* 2024). FeONPs offer several advantages over traditional iron sources, including greater solubility, enhanced mobility, improved bioavailability, and higher efficiency (Cao *et al.,* 2022; Shah *et al.,* 2202; Razavizadeh *et al.,* 2024). Previous studies have revealed that applying FeONPs enhances growth and yield by stimulating secondary metabolic gene expression, leading to an increase in flavonoids, essential oils, and phenolic compounds, and improving nutrient availability (Nourozi *et al.,* 2019; Tawfk *et al.,* 2021; Razavizadeh *et al.,* 2024; Smiri, 2024;). To study the effects of FeNP on the growth of barley seedlings, the fresh and dry weight for three different FeNP treatment methods (soaking, spraying, and soaking + spraying) on two cultivars of hulled barley plants (Giza 130 and Giza 136) with three different FeNP concentrations (10, 30, and 50 mg of FeNPs). The plants' morphologic, fresh, and dry weight results confirm that the spray with 50 mg of FeNP concentration significantly improved plant growth compared to the control treatment, which became completely healthy, greener, and stronger after a few days. Similarly, a previous study by Kokina *et al.* (2021) on three different barley genotypes observed a significant increase in the growth of seedlings and enhanced chlorophyll quality after being treated with 20 mg/L Fe_3O_4 NPs. Additionally, Tombuloglu *et al*. (2019) observed a significant improvement in the barley seedling's growth after 3 weeks of treatment with concentrations of Fe_3O_4 NPs up to 250 mg/L. The length of leaves and roots was increased by 27% and 125%, respectively, compared to the control. Petrova *et al.* (2021) studied different effects of the Fe_3O_4 and CuO NPs on the barley varieties; according to their results, Fe_3O_4 NP significantly enhanced lengths of shoots and roots and also fresh biomass, while CuO NP reduced them. Furthermore, nanoparticles chemically and physically can interact with plants depending on their properties (Taran *et al.* 2016, Zohra *et al*. 2021, and Fu *et al.* 2023). Iron oxide nanoparticles (Fe_3O_4) have gained attention in agriculture due to their potential to enhance plant growth and stress tolerance. Their interaction with plant metabolic pathways influences vital biochemical compounds such as amino acids, sugars, and proline, which are crucial indicators of plant health and stress response (Razavizadeh *et al.,* 2024). Consequently, the changes in total amino acid, total sugar contents, and proline in two barley cultivars sprayed with nanoparticles at different concentrations were evaluated. The spray of barley cultivars with a 50 mg/l concentration of FeNPs has significantly increased total amino acid, total sugar contents, and proline compared with the control treatment. This increase indicates improved nitrogen metabolism, photosynthetic activity, and protective compounds under Fe_3O_4 exposure, reflecting the potential role of FeNPs in enhancing stress tolerance and metabolic adjustments (Fu *et al.,* 2023; Razavizadeh *et al.,* 2024).

Normal plant growth and development depend on iron, which also plays critical roles in enzyme reactions, photosynthesis, enhancement of photosystem performance, DNA transcription, RNA synthesis, and auxin activity (Tawfk *et al.,* 2021). Previous research has indicated that nanoparticles can effectively mimic the activity of antioxidant enzymes such as APX, POD, SOD, CAT, and others. Consequently, using

nanoparticles may help enhance plants' ROS homeostasis (Fu *et al.* 2023). In the present investigation, spraying barley plants with a 50 mg/l concentration of FeNPs has significantly increased peroxidase activity and slightly increased catalase activity compared with the control. These results are in agreement with those observed by Sharma *et al*. (2012), who found that treating Brassica juncea with silver nanoparticles raised the levels of antioxidant enzymes such as catalase, ascorbate peroxidase, and guaiacol peroxidase and decreased the activity of reactive oxygen species (ROS). Similarly, Gunjan *et al.* (2014) revealed that the activity levels of antioxidant enzymes such as SOD, GPOX, CAT, and GR rose after treatment with gold nanoparticles of Brassica juncea. Furthermore, Azarin *et al*. (2022) observed that an increase in antioxidant enzyme activity correlates with the protective response of plants as a result of exposure to nano-zinc oxide. The results of the previous biochemical and enzymatic analysis confirmed the results of the morphological analysis that spraying with the 50 mg/l concentration of FeNPs improved the growth of hulled barley plants.

DNA barcoding is frequently employed in plant biology to resolve related taxonomic phylogenies. In this respect, DNA barcoding creates a public database of DNA sequences that can be utilized to identify organisms and for taxonomic clarification, providing valuable insights into cryptic species diversity (Hollingsworth *et al.,* 2011; Chandrasekara *et al.,* 2021). Our previous study by Soud et al. (2024) on three different Egyptian hulled barley cultivars showed that Giza-130 was more tolerant to salinity, while Giza-136 exhibited the highest sensitivity. Notably, this study was conducted in the climate and conditions of the Giza Governorate of Egypt. In this respect, DNA barcoding was performed in this study to distinguish and classify salt-tolerant and salt-sensitive cultivars of hulled barley. DNA barcoding is a relatively recent technology for identifying plant species based on short DNA sequences (Skuza *et al*. 2019). Although there is research employing barcoding on diverse species of plants, there is no similar research in barley.

According to the Plant Working Group—The Consortium for the Barcode of Life (PWG-CBOL), the features of an ideal DNA barcode need to be amplified with ubiquitous primers and high efficiency of amplification and sequencing. Furthermore, the genetic diversity is large enough to identify sequences at the species level, but also conservative enough within individuals of the same species (CBOL Plant Working Group, 2009; Skuza *et al*., 2019; and Letsiou *et al.,* 2024).

The high rate of length divergence can be attributed to the presence of single nucleotide repeats as SNPs, which cause frequent shifts in the reading frame (Abdelaziz et al. 2024). Our results showed that the genomes of two cultivars contain high numbers of SNP repeats. This result supports the PWG-CBOL's recommendation of *trnH-psbA* and *matK* as fundamental barcoding regions for plants, indicating that these markers are highly significantly polymorphic at the interspecific level while relatively preserved at the intraspecific level (Abdelaziz *et al.* 2024 and Letsiou *et al.* 2024).

The QR code (quick response code) is a recent advance in digital technology that can store complex information in a short code (Patil *et al.,* 2020). In this study, the complete sequence information of the two cultivars is shown utilizing the QR code digital technology. This helps researchers to obtain detailed information about plant species. This amazing technology is fantastic for supporting the establishment of a wide range of new research opportunities in the botanical sciences.

Conclusion

Nanotechnology and DNA barcoding have great potential to revolutionize the agricultural sector. Nanotechnology can improve crop productivity and nutritional quality by enhancing nutrient absorption in plants. Meanwhile, DNA barcoding and QR codes are widely utilized for classification, biodiversity assessment, genetic resource conservation, and overcoming the limitations of morphology-based taxonomy. The

findings revealed that foliar application of 50 mg/L Fe_3O_4 nanoparticles significantly improved all examined growth parameters as well as significant increases in total amino acid and proline levels, total sugar content, peroxidase activity, and catalase activity compared to the control. Based on these results, we recommend using a 50 mg/L Fe_3O_4 nanoparticle spray to support plant growth and contribute to breeding programs to develop new high-yield or adaptable barley cultivars with improved characteristics. Furthermore, DNA barcoding was utilized in this study to identify and classify salt-tolerant and salt-sensitive hulled barley cultivars. The results indicated that the genomes of both cultivars contain a high frequency of SNP repeats in the *trnH-psbA* and *matK* regions, which serve as key barcoding markers, demonstrating their high level of polymorphism.

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تحديد النمط الجيني لنوعين من الشعير المقشر باستخدام تقنية ترميز الحمض النووي (DNA barcoding) ودراسة تأثير جزيئات أكسيد الحديد(Fe_2O_3) النانونيه على تعزيز النمو

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ملخص

تُمثل تقنية النانو وترميز الحمض النووي تحولًا علميًا رائدًا وأساسيًا يُطبق في عدة مجالات حول العالم. في هذا الإطار، تهدف هذه الدراسة إلى تحديد أفضل معالجة وتركيز لـ Fe_3O_4 لتحسين نمو الشعير المصري المقشر. بالإضافة إلى ذلك، تم استخدام ترميز الحمض النووي وأكواد QR لتحديد وتصنيف أصناف الشعير المصري المقاومة للملوحة والحساسة لها. تم دراسة تأثير تركيزات مختلفة منFe_3O_4 ، باستخدام ثلاث طرق للمعامله (النقع و الرش و كليهما) على الصفات المورفولوجية والفسيولوجية والكيميائية الحيوية، بالإضافة إلى أنشطة الإنزيمات المضاده للأكسدة، في صنفين من الشعير المصري (جيزة 130 (متحمل للملوحة) وجيزة 136 (حساس للملوحة)). كما تم دراسه التباين الوراثى للصنفين المذكورين عن طريق دراسه تميّز الترميز الشريطي للحمض النووي (DNA barcoding) ،لتسلسلات القواعد لا اثنين من جينات الباركود *trnH-psbA* و *matK* المُضخّمين بواسطة تفاعل البوليميراز المتسلسل (PCR) . أُجري تحليل الباركود باستخدامDNA Subway ، وتم تحديد رموز الاستجابة السريعة (QR codes) للتعريف. أظهرت النتائج أن طريقة رش FeNP حسّنت من نمو النبات بشكل ملحوظ مقارنةً بالطرق الأخرى. وتحديدًا، أدى رش نباتات الشعير بـ 50 ملغم/لتر من FeNPs إلى زيادة ملحوظة في محتوى الأحماض الأمينية الكلية والبرولين، وإجمالي السكريات، ونشاط انزيم الكتاليز مقارنةً بالمعامله الضابطه (Control). حدد تحليل الباركود للحمض النووي تسلسلات قواعد يمكن استخدامها كواسمات جينيه تميز جيزة 130 عن جيزة 136، وتم تحديد رموز الاستجابة السريعة (QR codes) المقابلة لها. أظهرت جينومات كلا الصنفين عن وجود تكرارات أحادية النوكليوتيدات (SNP) عاليه في منطقتي الباركود *rnH-psbAt* و*matK*، مما يشير إلى وجود تعدد أشكال مرتفع. نتائج هذة الدراسة يمكن الاعتماد عليها في تحديد الأصناف المقاومة للملوحة وستخدامها فى استحداث برامج تربية تهدف لتطوير أصناف شعير جديدة عالية الانتاجيه أو قابلة للتكيف مع التغيرات المناخية و ذات صفات مُحسّنة.