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Zinc Accumulation in Wheat, and How It's Affected by Genetics and Sulphate of Zinc

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Abstract

The significant prevalence of zinc insufficiency in people makes it crucial to increase zinc (Zn) content in wheat grains on a worldwide scale. Grain-based diets high in zinc help combat the global issue of zinc deficiency. Inter Simple Sequence Repeats (ISSR) and Start Codon Targeted Polymorphism (SCoT) were used to analyes the genetic diversity of 10 Egyptian wheat cultivars in order to find the ones with the highest levels of built-in Zn bio-fortification. The level of polymorphism detected by SCoT was greater than that detected by ISSR. To clarify the influence of Zn foliar treatment on wheat grain production in 2019 and 2020, under conditions of zinc deficiency, Zn foliar application (5 g/L as $ZnSO_4.7H_2O$) was administered at two physiological phases (tillering and milking). Foliar treatment greatly raised the zinc content of the granules. A grain production boost of 50% was seen with a Zn content of 55 ppm. Zinc was bio-fortified in the Giza-168, Gemiza-7, and Gemiza-10 cultivars. This means that foliar treatment of $ZnSO_4$ may be a useful and cost-effective method for achieving agricultural bio-fortification.

Keywords: Triticum Aestivum; ISSR; SCoT; Foliar application; Zinc sulphate; biofortification

1. Introduction

Global concern exists over human micronutrient deficits [1]. The highest-priority countries in Africa for enhancing zinc accumulation in wheat grain are Egypt and Morocco [2]. 30% of Egyptian students have poor diets and lack essential micronutrients like iron, zinc, and vitamin A [3].

A crucial food crop, hexaploid or common wheat (*Triticum aestivumL.*, 2n = 6x = 42, AABBDD) will become even more crucial as the world's population rises [4]. Common wheat has a relatively low genetic diversity compared to its two donor species as well; the majority of the genetic variation seen in tetraploid wheat is not present in the readily available hexaploid germplasm [5]. Wheat grains are relatively low in essential micronutrients particularly Zn and Fe [1] because wheat verities cannot realize their full potential in Zn absorption and accumulation in grains [6]. Egyptian government reported that 3.6 million/ feddan have been planted and the average wheat yield reached about 9.7 million tonnes in Egypt [7]. Globally, more than 30% of soil is low in plant-available Zn [8], for example in Egypt the availability of micronutrients especially Zn, Mn, and Fe in different soil types is mostly insufficient [9] as it was affected by environmental and soil factors such as high pH, high concentrations of Ca, Mg, and P in soil solution [10]. Thus, there are various methods for reducing micronutrient deficiencies in crops, including food diversity, food supplements, food fortification, and biofortification, which is the process of raising the concentration of micronutrients in the edible part of strategic crops like maize, wheat, and sweet potatoes [1]. There are two methods of adding micronutrients to food: a) agronomic biofortification using fertilization of the soil and foliar spray, and b)

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genetic biofortification using plant breeding or genetic engineering [11]. Malnutrition can be decreased by choosing plants that contain a high concentration of micronutrients and growing them using traditional agronomic biofortification techniques [12]. Because of this, enhancing Zn accumulation in wheat grains depends greatly on understanding the genetic diversity among wheat cultivars. Various marker methods have been used to explore the genetic diversity and relationships of species, including DNA-based markers such as random amplified polymorphic DNA (RAPD) [13], inter-simple sequence repeats (ISSR) [14], chloroplast DNA markers, nuclear sequences [15]. The start codon targeted polymorphism (SCoT) method, which uses a single primer to anneal to the flanking regions of the translation initiation codon (ATG) on both DNA strands, is a novel, straightforward, and trustworthy gene-targeted marker system [16].

SCoT markers with high polymorphism and high effectiveness have been successfully applied in oak [18], ramie [19], Dendrobium [20], durum wheat [21], and bread wheat [22]. On other hand, foliar application of Zn had a positive effect on wheat grain yield [23] and improved Zn content of both the entire grain and endosperm while soil applications of Zn were not so effective [24], its efficiency depends on many factors like the time of foliar Zn application [25]. In the case of wheat, when foliar Zn was sprayed to wheat after the blooming stage, as opposed to applications made before the flowering period [26] it was demonstrated that the highest Zn content in grains was reached [27].

The current study's goal was to examine genetic variation among ten wheat cultivars using ISSR and SCoT genetic markers. Furthermore, the results from the two distinct markers were compared, and the ability of these cultivars to accumulate Zn in grains was evaluated by employing Zn foliar treatment at two different physiological phases (tillering and milking).

Table 1
Names, pedigree and origin of ten bread wheat genotypes used in the study

NO	Genotypes	Pedigree	Origin
1	Sakha-94	Opata/Rayon//Kauz.	Egypt
2	Giza-168	MIL/BUC//Seri CM93046-8M-0Y-0M-2Y-0B	Egypt
3	Gemmiza-7	CMH 74A.360 / SX // SERI 8213 / AGENT CGM4611-2GM-3GM-1GM-0GM	Egypt
4	Gemmiza-10	Maya 74 “S”/On//1160-147/3/Bb/4/Chat”S” /5/ctow.	Egypt
5	Gemmiza-11	B0W"S"/KVZ"S"//7C/SERI 82/3/GIZA168/SAKHA61.CGM7892-2GM—1GM-2GM-1GM0GM	Egypt
6	Misr-1	OASIS/SKAUZ//4*BCN/3/ 2*PASTOR. CMSS00Y01881T -050M-030Y-030M-030WGY-33M-0Y--0EGY	Egypt
7	Misr-2	SKAUZ/BAV92.CMSS96M 03611S-1M-010SY-010M-010SY-8M-0Y-0EGY	Egypt
8	Shandaweel-1	SITE//MO/4/NAC/TH.AC// 3*PVN/3/MIRLO/BUC. CMSS93B00567S-72Y010M-010Y-010M-0HTY-0SH	Egypt
9	Sids14	Bow’’s’’/Vee’’s’’//Bow’s’/ Tsi/3/BAN	Egypt
10	Gemmiza-12	OTUS/3/SARA/THB//VEE. CCMSS97Y00227S-5Y-010M-010Y-010M-2Y-1M-0Y-0GM	Egypt

2. Materials and Methods

2.1. Plant materials

In this study, ten wheat genotypes from the Agricultural Research Center (ARC), Giza, Egypt, were gratefully provided (Table 1).

2.2. DNA extraction

After seven days, complete DNA was extracted from the leaves of the germinated plants using the CTAB (Cetyl Trimethyl Ammonium Bromide) technique [27].

2.3. ISSR analysis

Ten ISSR markers described by Rogers and Bendich [28] (2x), and 2 ul of primers from (Table 2) make up the PCR reaction mixture, which has a total volume of 25 ul. Amplifications were performed for the PCR reaction at

95 °C for 5 min, followed by 35 cycles of primer annealing for 40 s at 72°C, elongation at 72 °C for 2 min, and final extension at 72 °C for 10 min.

Table 2
SCoT and ISSR primers names and sequences

P.NO	Primmer name	Seq5\ -------3\	Annealing Tem°C	Primmer name	Seq5\ -------3\	Annealing Tem°C
1	SCOT-24	CACCATGCTACC GACCAT	45	UBC-810	GAGAGAGAGAGAGAGAT	50
2	SCOT-13	ACGACTGGCACC GATCG	39	HB-13	GAGGAGGAGGC	50
3	SCOT-14	ACGACATGGCGA CCACGC	47	UBC-811	GGAGAGAGAGAGAGAAC	50
4	SCOT-34	ACCATGGCTACC ACTGCA	52	UBC-834	GAGAGAGAGAGAGAGAGAGA T	50
5	SCOT-52	ACAATGGCTACC ACTGCA	52	UBC-835	AGAGAGAGAGAGAGAGYC	50
6	SCOT-66	ACCATGGCTACC AGCGAC	56	TA-1	AGAGAGAGAGAGAGAGAGAG C	50
7	SCOT-70	ACCATGGCTACC AGCGCGC	47	UBC-818	CACACACACACACAG	50
8	SCOT-71	CCATGCCCTACC ACTACCC	49	UBC-823	TCTCTCTCTCTCTCC	50
9	SCOT-77	CCATGGCTACCA CTACCC	45	UBC-817	CACACACACACACAA	50
10	SCOT-26	ACCATGGCTACC ACCGTC	45	UBC-814	CTCTCTCTCTCTCTCTA	50

2.4. SCoT analysis

Ten SCoT primers were selected according to Singh *et al*. [1] for genotyping assays (Table 2). The PCR reactions were performed in a 10 µl volume including 1 µl of extracted DNA (50 ng/µL), 0.5 µl of each primer (2 µM/µL), 5 µl GeneDireX® One PCR™ (cat.no. MB203-0050) master mix, and 3 µl st. ddH_2O (sterilized double-distilled water). The ideal PCR procedure included a5-minute initial denaturation at 94 °C, 35 cycles of DNA denaturation at 94 °C occurring for 30 s, primer annealing at 50 °C occurring for 45 s and at 72 °C occurring for 2 min, and a final extension at 72 °C occurring for 7 min. Following electrophoresis, all of the PCR findings were placed into 2% agarose gels stained with ethidium bromide, and a Biometra UV star transilluminator was used to view them.

3. Data Scoring and Statistical Analysis

By measuring the total of polymorphic bands from binary data, the percentages of polymorphism were calculated. ISSR and SCoT bands were manually graded as present ("1") or absent ("0") to estimate similarity among all the evaluated samples. The tree diagram was created by clustering the similarity data using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) approach using Systat ver. 7 (SPSS Inc. 1997 SPSS Inc.3/97 standard version) [31]. Pairwise comparison across cultivars was performed using the Dice coefficient. The polymorphism information content (PIC), marker index (MI), and resolving power (RP) of the markers were determined to assess their informativeness in distinguishing between genotypes. PIC was computed using the formula in Yang *et al.* [32], while MI was calculated using Anderson *et al.* [33]. Each primer's RP was determined based on Varshney *et al.*[17].

3.1. Field experiment

This experiment aimed to study the effect of foliar application of Zn at different physiological stages (tillering and milking) on Zn accumulation in wheat grains. Based on the combined data obtained from the ISSR and SCoT analysis, seven genetically distant commercial genotypes (Sakha-94, Giza-168, Gemmiza-7, Gemmiza-10, Gemmiza-11, Misr-1, and Misr-2) were selected and planted in the field. The experiment was designed in a split-split-plot design with three replicates. Two Zn treatments (without Zn and 5 g/L $ZnSO_4.7H_2O$) foliar spray [17]. Zn concentrations in selected cultivars before sowing are presented in (Table 3). The foliar spray was applied at two different physiological stages: tillering stage, and milking stage, and sprayed twice at tillering and milking stages [36]. Randomly placed grain plots were placed on the experiment site, and the soil was levelled with a wooden leveller, ploughed with a chisel plough, and divided into experimental units (plots). Each allotment is 10.5 m2 in size (3.0 m long and 3.5 mwide).

Table 3: Zinc content (ppm) in wheat grains before sowing in both seasons

Genotypes	Sakha-94	Giza-168	Misr- 1	Misr -2	Gemiza -7	Gemiza -10	Gemiza -11
1st season	29	26	32	30	22	29	30
2nd season	27	25	30	29	23	27	32

Values are means of three replicates (n=3)

Table 4: Physical and chemical characteristics of soil (0-30 cm depth) before sowing

Characteristics	Physical properties			Macronutrients					Micronutrients			
	Texture	pH	EC (dS/m)	P	K	Ca	Mg	Na	Zn	Fe	Cu	Mn
	Clay	1.04 vL	0.95 vL	1.3 vL	28 M	31.3 vL	66 vL	35 L	0.75 L	2.1 vL	6.5 vH	6 L

Data are means for the first and second season (n=3), M=medium, L=low, vL=very low, H=high, vH=very high [38].

Wheat grains of 60 kg wheat grains/feddan were sowed on 23 December in the (2019/20) and (2020/21) seasons [37]. Wheat plants in all treatments were flowed and irrigated at sowing.

3.2. Soil analysis

After the completion of the soil preparation, and before to the application of fertilizer, a representative soil sample was obtained from the testing area (0-30 cm depth). The soil sample was allowed to air-dry, then crushed in a wooden mortar, and then placed through a sieve with a pore size of 2 millimeters so that its physical and chemical characteristics could be analyzed. The results of the soil analysis are shown in (Table 4), and the values have been evaluated in line with the limits specified in Ankerman and Large [38]. According to Jackson [39], an assessment of the soil's physical and chemical qualities should be performed prior to planting seeds.

At a rate of 70 units per feddan of ammonium nitrate, the suggested amount of nitrogen was supplied to the soil. The nitrogen was spread out over three applications: 1/3 during sowing, 1/3 at the first irrigation, and the last 1/3 at the second irrigation. Before sowing, the soil received 200 kg/fed of full-dose superphosphate (15.5% P_2O_2) fertilization.

At maturity, (140 days after sowing) the plants were harvested plant samples were taken to determine the following characteristics:

- Grain yield, ardb / fed. (One ardb = 150 kg of grains).
- Grain Zinc concentration (ppm).

Determination of Zinc concentration in grains:

An atomic absorption spectrophotometer was used to measure the amount of zinc in the shoot and root digest (GBC Scientific Equipment Pty Ltd A.C.N. 005 472 686.). Using certified standard reference materials that were received from Germany's National Center of Standard Materials, grain zinc concentrations (mg Zn/grain^{-1}) were calculated, and measurements were checked for accuracy.

Statistical analysis:

The results were subjected to a variance analysis. According toSnedecor [40], differences between treatment means were assessed using the LSD test with a 0.05 level of significance.

4. Results

4.1. SCoT analysis

Start codon targeted (SCoT) analysis was carried out to investigate the genetic variations between the cultivars used. All of the employed primers produced PCR products with a variety of band sizes (Fig.1). Scot-24 produced the most bands (9 bands), whereas Scot-26 was the least productive as a primer (one band). There were 65 bands in total, with each primer having anywhere from 1 to 9 bands. Scot primers (14, 24, and 77) revealed 100% of the polymorphism, while Scot primers revealed 86% of the polymorphism (66, 70, and 71). Scot24 had a PIC value of 0.00, SCoT-77 had a PIC value of 0.39, and

each primer had an average PIC value of 0.23 (Table 5).

Cluster analysis of SCoT analysis:

Based on jaccard's similarity index the ten cultivars were grouped into two clusters (Fig.2). Gemiza-10 in cluster I while Cluster II consisted of two clades the first contain the cultivar Shandweel 1 and the second divided into Four subclades the cultivars Giza-168, Misr-2 and Misr-1 respectively, the fourth subclades divided into two clades Gemiza-7 and the second clade divided into three clades (Gemiza-11 and Sids-14) into first and third subclades while the third clade contains Gemiza-12 and Sakha-94.

4.2. ISSR analysis

Overall bands count, polymorphism, and percentage of polymorphism were provided in (Table 6), 43 bands were present in all primers (Fig.3), with 3 to 8 bands produced by each primer. UBC-823 produced the most bands (8 in all), whereas UBC-(810,834 and 814), TA-1, and HB-13 primer produced the least bands (3 bands). The percentage of polymorphism that the various primers revealed ranged from 100% for UBC-823 primer to 67% for UBC-814 primer. PIC values were between 0.10 and 0.37 (for UBC-814) and averaged 0.19 for each primer. The ISSR marker's (RP) value is 2.79. ISSR primer (MI) was at (Table 6).

Cluster analysis of ISSR analysis:

The ten cultivars were divided into two clusters (Fig.4). The first cluster contain Shandweel-1 cultivar and the second was divided into two clades, the first clade had Gemiza-12 cultivar while the second clade was divided into three subgroups one subclade contains Sakha-94 cultivar while the second subgroup divided to two clades (Gemiza-10 and Sids-14). The third clade contains only Misr-1 cultivar while Giza-168 and Misr-2 were more relative.

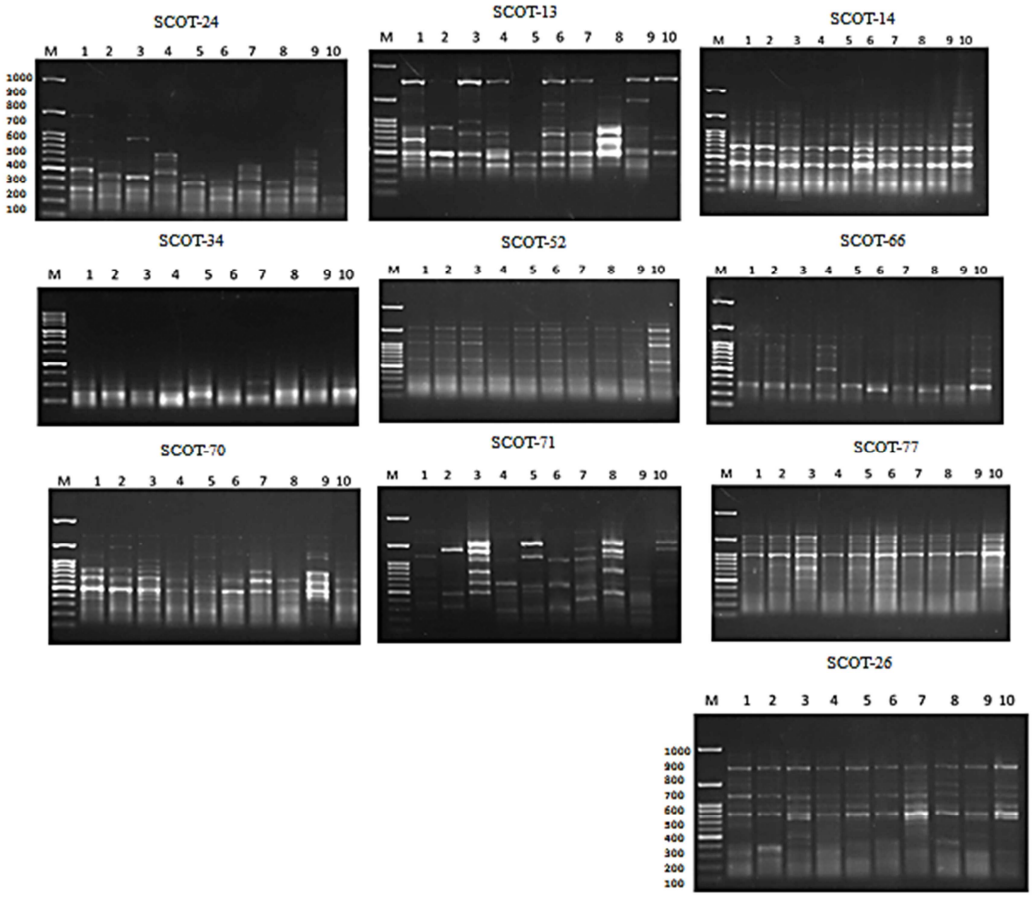

Fig. 1: SCoT profiles demonstrated polymorphism among the 10 wheat cultivars. Lanes 1-10; Misr-1,Misr-2,Giza-168,Gemiza-7, Gemiza-10, Gemiza-11, Gemiza-12, Sakha-94, Sids-14 and Shandweil-1, respectively. M refers to DNA marker of 100 pb ladder.

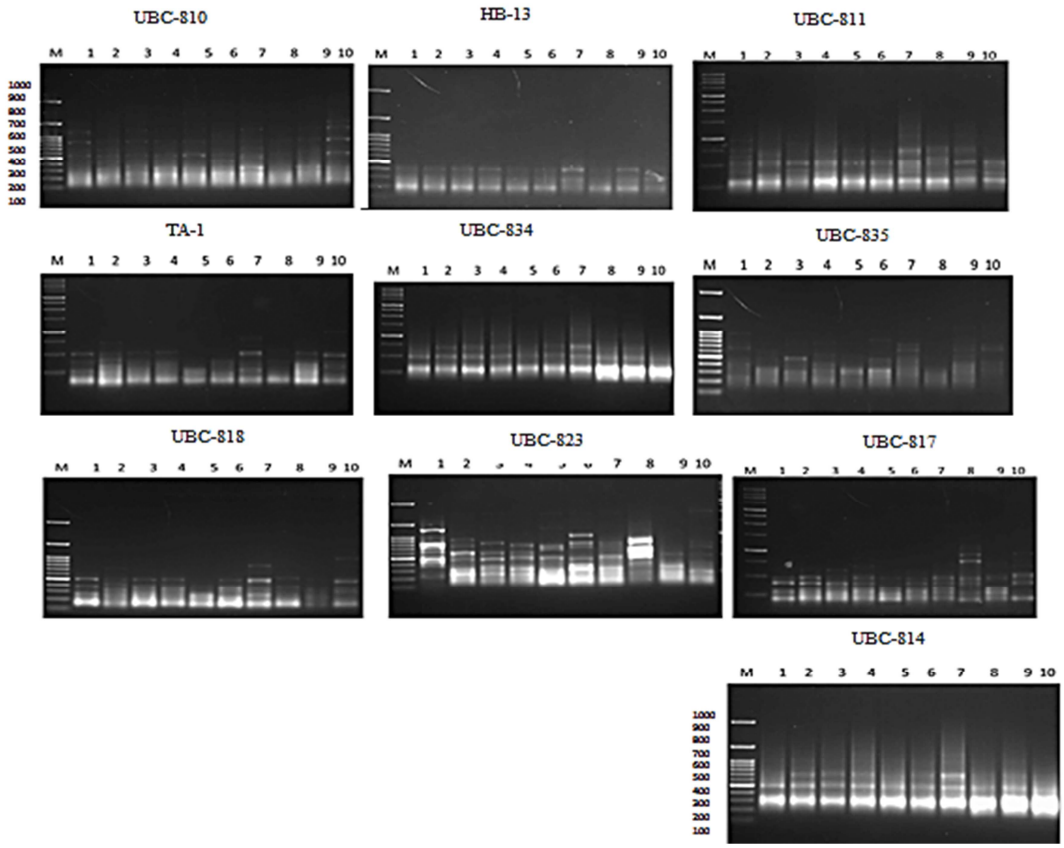

Fig. 2 : ISSR profiles demonstrated polymorphism among the 10 wheat cultivars. lanes 1-10; Misr-1,Misr-2,Giza-168,Gemiza-7, Gemiza-10, Gemiza-11, Gemiza-12,Sakha-94, Sids-14 and Shandweil-1, respectively. M refers to DNA marker of 100 pb ladder.

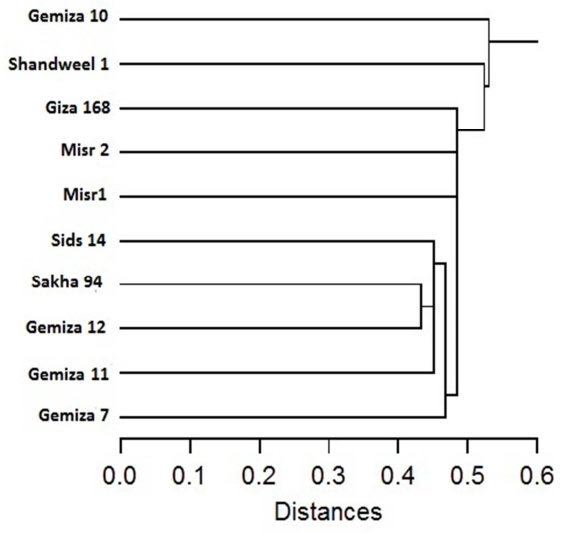

Fig. 3: Dendrogram based on Jaccard' s similarity coefficients scored from SCoT data using UPGMA algorithm between the eight wheat cultivars.

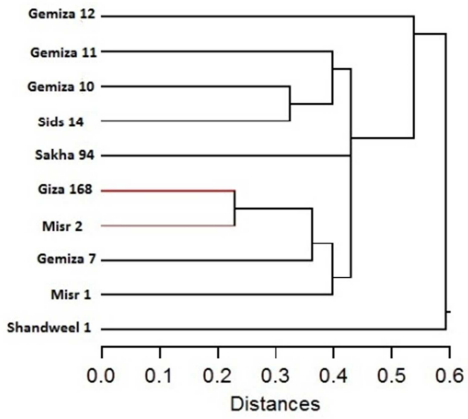

Fig. 4: Dendrogram based on Jaccard's similarity coefficients scored from ISSR data using UPGMA algorithm between the ten wheat cultivars.

4.3. Phylogenetic Relationship Based on Amplified SCoT and ISSR Fragments

The data from ISSR and SCOT analysis combined to obtain a more realistic phylogenetic tree (Fig. 5).The phylogenetic tree divided the ten wheat cultivars into three major subgroups and two major clusters. The cultivar Shandweel-1 was the only one in the first cluster. The Misr-1 cultivar is found in the first subgroup, the Gemiza-11 cultivar is found in the second subgroup, and the remaining five cultivars are found in the third subgroup, which is central.

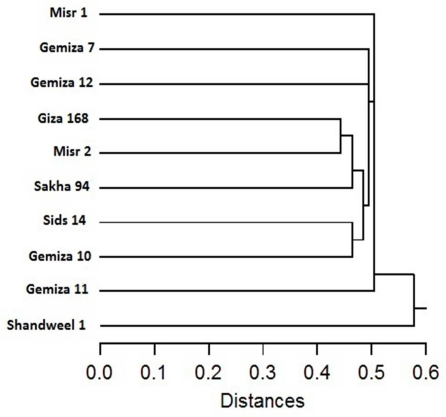

Fig. 5 : Dendrogram based on Jaccard's similarity coefficients scored from SCoT and ISSR data using UPGMA algorithm between the ten wheat cultivars.

Table 5: SCoT primers names and polymorphism percentage

P.NO	Primmer name	TNB	NPB	NMB	NUB	P (%)	M %	Pic	MIC	RP
1	**SCOT-13**	7	5	2	2	71	29	0.11	0.12	6.12
2	**SCOT-14**	5	5	0	2	100	0	0.16	0.05	2.30
3	**SCOT-24**	9	9	0	0	100	0	0.37	0.03	3.67
4	**SCOT-26**	1	0	1	0	0	100	0.00	0.12	1.67
5	**SCOT-34**	8	7	1	2	88	13	0.25	0.08	4.73
6	**SCOT-52**	8	7	1	1	88	13	0.26	0.07	5.06
7	**SCOT-66**	7	6	1	2	86	14	0.27	0.08	3.67
8	**SCOT-70**	7	6	1	1	86	14	0.30	0.05	2.90
9	**SCOT-71**	7	6	1	2	86	14	0.22	0.06	3.83
10	**SCOT-77**	6	6	0	2	100	0	0.39	0.03	2.94
	Total	**65**	**57**	**8**	**14**	**87**	**13**	**0.23**	**0.07**	**3.69**

(NMB) number of monomorphic bands, (NPB) number of polymorphic bands, (TNB) total number of bands, (NUB) number of unique bands unique band (PPB), percentage of polymorphic bands, (PIC)polymorphism information content, (RP) resolving power, (MI) marker index

Table 6: ISSR primers names and the extent of polymorphism

P.NO	Primmer name	TNB	NPB	NMB	NUB	P (%)	M (%)	PIC	MIC	Rb
1	**UBC-810**	3	3	0	1	100	0	0.37	0.02	1.33
2	**HB-13**	3	3	0	1	100	0	0.35	0.04	2.78
3	**UBC-811**	7	6	1	3	86	14	0.15	0.06	2.72
4	**UBC-834**	3	2	1	1	67	33	0.04	0.12	3.44
5	**UBC-835**	4	3	1	1	75	25	0.11	0.07	2.79
6	**TA-1**	3	2	1	2	67	33	0.09	0.13	3.83
7	**UBC-818**	4	3	1	3	75	25	0.11	0.08	2.67
8	**UBC-823**	8	8	0	0	100	0	0.40	0.02	1.61
9	**UBC-817**	5	4	1	2	80	20	0.17	0.06	2.67
10	**UBC-814**	3	2	1	1	67	33	0.10	0.13	4.06
	Total	**43**	**36**	**7**	**15**	**82**	**18**	**0.19**	**0.07**	**2.79**

(NMB) number of monomorphic bands, (NPB) number of polymorphic bands, (TNB) total number of bands, (NUB) number of unique bands unique band (PPB), percentage of polymorphic bands, (PIC)polymorphism information content, (RP) resolving power, (MI) marker index.

4.4. Effect of Zn foliar application on wheat grain yield

Both seasons (2019 and 2020) revealed that foliar Zn application during the different physiological stages had a substantial effect on yield as well as its component (Table 7), In comparison to the control, Zn treatments intensely increased wheat grain yield and biological yield. The twice foliar spray of Zn at the tillering and grain filling stages was the most effective treatment on grain yield as increased by (50% to 80%) to control. All cultivars showed high significance ($p < 0.05$) differences between treatments as Giza-168, Gemiza-7, and Gemiza-10 had the highest grain yield (19 to 22 ardb/fed) when we applied Zn foliar spray at milking stages and also spray twice at tillering and milking stages, although Zn foliar at stem elongation slightly increased the grain yield by 30 % in most cultivars.

4.5. Effect of Zn foliar application on grain zinc concentration

The obtained results in both seasons revealed that foliar Zn application at different phonological stages had a significant effect on yield and its components. Data presented in (Table 8) showed a significant difference between all cultivars and treatments. Furthermore, the results indicated that applying Zn foliar spray at the milking stage increased Zn concentration in all cultivars in both seasons. Giza-168, Gemiza-7, and Gemiza-10 had high grain Zn concentrations in the second season (55, 56, and 50 ppm) respectively, while applying at tillering stage showed slightly increasing in Zn concentration by (2% to 10%) compared to the control. In addition, spraying Zn fertilizer twice at tillering and milking stage increased Zn concentration in all cultivars by 80%. In this case there were a variation in grain Zn concentration and grain yield between all wheat cultivars. With this in mind the complex attribute of grain yield and grain Zn concentration is influenced by the impacts of genotype (G), environment (E), and their interactions (GEI). The GEI effect is significant for breeders because it reflects yield variance that cannot be explained by the individual G and E effects [41].

5. Discussion

Plant breeding programmers are depending on using efficient and low-cost analytical approaches for assessing genetic variations in numerous genotypes [21] and [42].One of these approaches are ISSR and SCoT markers that provide more reliable diversity information and excellent tools for researching the genetic variation[43], the two markers produced encouraging results and grouping in the current experiment [44]. These markers offer more thorough and varied information regarding the genetic diversity

of Egyptian wheat accessions and within them [45]. Some plants, including snake melons [46], and sponge gourds [47] have produced dendrogram that don't match up when different markers are used to createthem.

Table 7: Effect of Zn foliar application at different wheat physiological stages on wheat grain yield (ardb/fed)

Treatment	Control		Spray 45 day		Spray 65 day		Spray 65 and 45 day	
Genotypes	S^{st}	S^{nd}	S^{st}	S^{nd}	S^{st}	S^{nd}	S^{st}	S^{nd}
Misr-1	14.6 ± 0.6	13.8 ± 0.2	16.6 ± 0.3	17.8 ± 0.1	19.0 ± 0.0	18.0 ± 0.0	19.33± 0.3	21.0 ± 0.0
Misr-2	14.0 ± 0.0	14.8 ± 0.1	18.6 ± 0.3	19.4 ± 0.1	19.0 ± 0.0	22.0 ± 1.0	21.0± 0.5	22.3 ± 1.2
Sakha-94	14.0 ± 0.0	15.0 ± 0.3	19.0 ± 0.0	20.4 ± 0.1	19.0 ± 0.5	21.0 ± 0.0	19.6 ± 0.3	19.0 ± 0.6
Gemiza-11	13.3 ± 0.3	13.7 ± 0.2	18.0 ± 0.5	17.8 ± 0.4	21.3 ± 0.3	19.3 ± 0.9	21.3 ± 0.3	21.0 ± 0.6
Gemiza-10	14.0 ± 0.0	14.4 ± 0.2	18.3 ± 0.3	17.8 ± 0.4	21.0 ± 0.5	22.0 ± 0.0	21.3± 0.3	21.0 ± 0.0
Giza-168	14.0 ± 0.0	14.1 ± 0.2	17.6 ± 0.3	15.1 ± 0.2	20.6 ± 0.3	19.3 ± 0.3	20.3 ± 0.6	20.7 ± 0.9
Gemiza-7	14.6 ± 0.3	13.6 ± 0.1	19.0± 0.0	17.0 ± 0.3	20.3 ± 0.3	20.3 ± 0.3	20.00± 0.0	19.0 ± 0.0

Values are means ± Stander error (n=3), s for season

Table 8: Effect ofZn foliar application at different wheat physiological stages on grain Zn concentration (ppm)

Treatment	Control		Spray at 45 day		spray at 65 day		spray at 45 and 65 day	
Cultivars	S^{st}	S^{nd}	S^{st}	S^{nd}	S^{st}	S^{nd}	S^{st}	S^{nd}
Misr-2	21.3 ± 0.6	24.6 ± 0.3	28.0 ± 0.5	28.0 ± 0.5	41.6 ± 1.4	40.0 ± 0.5	51.3 ± 1.2	44.3 ± 0.3
Gemiza-11	22.6 ±1.4	29.0 ± 0.5	32.6 ± 0.6	32.6 ± 0.6	42.6 ± 1.7	34.3 ± 1.8	34.0 ± 0.5	32.6 ± 0.8
Gemiza-10	21.3 ± 0.3	26.0 ± 1.0	26.6 ± 0.3	26.6 ± 0.3	37.0 ± 2.0	50.0 ± 0.5	35.3 ± 1.4	41.3 ± 0.8
Giza-168	21.6 ± 1.6	25.3 ± 0.8	27.0 ± 0.5	27.0 ± 0.5	39.0 ± 2.0	55.6 ± 1.4	29.3 ± 0.3	55.3 ± 0.8
MISr-1	21.6 ± 0.6	27.3 ± 0.3	26.3 ± 0.6	26.3 ± 0.6	37.0 ± 1.5	33.6 ± 1.2	38.6 ± 1.2	53.6 ± 0.8
Sakha-94	19.6 ± 0.8	25.3 ± 0.3	30.3 ± 0.8	30.3 ± 0.8	35.0 ± 1.7	50.3 ± 0.8	41.6 ± 0.8	41.6 ± 1.3
Gemiza-7	24.6 ± 0.8	22.3 ± 0.8	27.6 ± 0.3	27.6 ± 0.3	28.0 ± 0.5	56.3 ± 0.3	28.6 ± 0.3	46.0 ± 1.7

Values are means ± Stander error (n=3)

5.1. SCoT analysis

This research shows that SCoT primers have a greater RP than ISSR primers. Genetic corrosion in grown wheat provides an excellent reason for assessing genetic variety among various cultivars and figuring out the possibility of increasing plant material efficiency [48], which may finally lead to enhanced food production [49] and [50] Intriguingly, the cultivar Gemiza-10 was in the first group of the created dendrogram based on SCoT markers, while the other cultivars were placed in the second group [51]. These findings support those made by Zhang *etal.* [52] who examined variance across 53 Chinese genotypes of Elymus sibiricus. The dendrogram separated the genotypes into three main groups and two minor groupings. Also, Abulela *et al.* [22] studied the genetic diversity between ten Egyptian bread wheat cultivars by SCoT markers and reported that SCoT marker generate cultivar specific markers with 23% which were similar to these results.

5.2. ISSR analysis

When compared to SCoT markers, the dendrogram based on ISSR markers also separated wheat cultivars into two major groups. Because each marker targets a different genomic sequence, the

polymorphism and cluster analysis between the two markers varies. A similar finding has been reported in earlier studies, which emphasizes the significance of ISSR and SCoT markers in detecting polymorphism and determining precise genetic links Al-Khayri *etal.* [53] and Özlem *et al.* [54] reported that ISSR markers provided greater recurrence, polymorphism, and the ability to distinguish across bread wheat cultivars.

5.3. Effect of zinc foliar application on wheat grain yield

Grain yield, followed by grain Zn absorption, was the most significant variable for explaining changes in grain Zn concentration [55].The time of Zn foliar application affected directly the grain yield as it increased by 50% when Zn sprayed twice at tillering and milking stages as Zn foliar spray at tillering stage increase the number of Spikes in m^2 [56] while Zn foliar spray at milking stage increasing number of grains per spike by increasing of fertile spikelet per spike and 1000 grain weight [3]. The current findings are corroborated by Cakmak [57], who demonstrated that foliar treatment of micronutrients at the tillering, jointing, and booting stages improves wheat production. Microelements effectively increased photosynthesis rate and photo-assimilated translocation to the grain by improving enzymatic activity. Durum wheat [27] and bread wheat all indicated positive effects of Zn application on grain yield and agronomic parameters. These findings are consistent with those of Zoz *et al.* [57] who indicated that Zn plays a crucial role in biomass production. Furthermore, Mosanna *et al.* [58] demonstrated that Zn nano-chelate soil and foliar spray during the grain-filling stage increased maize pigment content and biological yield (75 and 54%, respectively). Therefore, pot and field trials yielded differing results for wheat cultivars and zinc application quantities. This could be due to variations in genetic make-ups and responses differently to various zinc application methods [59].

5.4. Effect of Zn foliar application on grain zinc concentration

Zn concentration increased in wheat cultivars by more than 50% after sowing in both seasons. According to Liu *et al.* [60], foliar zinc application is better because it can boost yield characteristics and grain zinc content by up to 80%. Ram *et al.* [61] reported that an increase in wheat production and grain zinc content at the same time, this approved with this results in both seasons (2019/20 and 2020/21) which revealed that foliar Zn application at different phonological stages had a significant effect on yield and its components. These findings are supported by Hao *et al.* [62], who found that foliar micronutrient administration at tillering, jointing, and booting stages aids in improving wheat output in agreement with these findings, Nazir *et al.* [63] who demonstrated that in the field experiments, increasing the pool of Zn in the vegetative tissue during the reproductive growth stages (for example, by spraying foliar Zn fertilizers) epitomizes a crucial field practice in maximizing Zn accumulation in grain. Spraying Zn at the milking stage greatly increased Zn concentration in the grains [64]. Genetic and agronomic factors affect the accumulation of trace elements [65]. For example, Lina *et al.* [66] reported that foliar Zn application significantly increased Zn concentration and predicted bioavailability in both whole grain and flour of wheat, while Ning *et al.* [67] stated that foliar Zn application alone or in combination with soil Zn application significantly increased Zn concentration in wheat grain. Furthermore, Nikolic *et al.* [68] reported that foliar zinc application at the early milk stage of grain filling raises the zinc concentration in wheat grain.

For Zn accumulation in grain, Zn must first be remobilized from shoots and then continue to accumulate in shoots during the grain-filling stage [69]. The internal remobilization of stored Zn inside plants and root acquisition has generally been linked to variations in Zn accumulation in grains.

6. Conclusion

Specific molecular markers like ISSR and SCoT were effective tools to study the genetic diversity between Egyptian wheat cultivars. There was variation between wheat cultivars in their ability to accumulate Zn in grains under Zn deficiency soils. Gemiza-7, Giza-168 and Gemiza-10 not only had the highest grain yield but also accumulated Zn effectively in their grains. Therefore they are most suitable cultivars for Zn biofortification breeding programs.

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