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Effect of PEG induced drought stress on Genetic diversity using SDS-PAGE and ISSR markers for Egyptian barley varieties

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Abstract

Seven barley accessions were selected based on their morphological and agronomic diversity in a randomized complete design with three replications to examine the impact of drought stress on germination of barley cultivars. Seven Egyptian barley varieties, designated as Giza126 (G1), Giza127 (G2), Giza 128 (G3), Giza130 (G4), Giza2000 (G5), Giza133 (G6), and Giza134 (G7), were the initial factors in the controlled experiment. Two irrigation treatments using PEG (6000) solutions at concentrations of 50% and 56 %, respectively, were administered as the second factor, while the third treatment was a simple water control. The effects of genotype, PEG-induced osmotic potentials, and their interactions all considerably changed the examined parameters. Osmotic potential decreased seed germination and growth metrics; however, significant genotypic differences were found. The best cultivars in terms of yield parameters were G3, G5, and G6, which showed tolerance to drought stress, while G1, G2, and G4, G7 cultivars showed less tolerance. Genetic diversity across barley types was investigated using ISSR and SDS-PAGE. Seven barley cultivars from various origins were chosen in order to maximise genome coverage, and they were examined using six chosen ISSR markers. There were 63 amplified bands altogether, 30 polymorphic allelic variants (ranging from 3 to 8 per locus with an average of 5) and 33 monomorphic allelic variations (ranging from 4 to 7 per locus with an average of 5.5) that were found. The calculated genetic distance using ISSR data ranged from 0.813 to 0.972. The two groupings on the dendrogram based on genetic distance estimation are typically congruent with the currently available genetic data. The fact that they had a common ancestor and were grouped together further supported their genetic link. The patterns of SDS-protein banding showed a range of variance that revealed a total of 18 bands with various molecular weights, only 14 of them were polymorphic. All varieties displayed a wide range of genetic diversity, as shown by the ISSR and SDS-PAGE results. These data were supported by growth parameters, which we ranked as drought-stress tolerant, and showed them to be excellent candidates for selective breeding for specific traits and enlarging genetic base.

Keywords: *Genetic diversity, SDS-PAGE, ISSR, Polyethylene Glycol, Barley.*

1. Introduction:

Various molecular weights of the polymer polyethylene glycol (PEG) are created. For controlled drought stress in nutrient solution cultures, greater molecular weights PEG (4000 to 8000) was frequently utilised in physiological investigations during the 1970s and 1980s. For PEG of various molecular weights, several articles also presented theoretical or observed concentration-osmotic potential relationships (**Money, 1989; Michel, 1983; Michel and Kaufmann, 1973**). Users' experience suggested that these correlations could vary somewhat depending on the lot or supplier of the particular PEG being used. Therefore, it is advisable to check the true osmotic potential of the PEG solution culture. One of the most important and historically significant cereal crops is barley (*Hordeum vulgare* L.).

After maize, rice, and wheat, its grain production ranks fourth globally (**FAO, 2019**). Because it is simple to grow, has minimal requirements, and is very tolerant of severe circumstances, barley is frequently referred to as a poor man's crop. According to certain sources, barley has existed for 11,000 years (**Riehl, 2019**).

Al-Sayaydeh *et al.* 2019 research also discovered that there were substantial variances in all evaluated attributes across genotypes, settings, and interactions. Furthermore, (**Sharma, *et al.*, 2014**) we identified and selected accessions with respectable yield and performance for additional ancillary attributes using Euclidean distances based on non-hierarchical cluster analysis to divide all accessions into several groups. Programmes to improve barley can use hybridization with the potential breeding lines. Among the barley cultivars, the 20% PEG level of drought caused the greatest loss. Giza 134, Giza 127, and Giza 126 were the best cultivars in terms of germination features, which show their tolerance to drought stress. Giza 130, 135, and 2000 cultivars were moderately tolerant, while the rest cultivars are less tolerant. After the stress, nearly all cultivars had high intensity protein bands at 27 and 78 kDa (**Hellal *et al.*, 2018**). Potassium may have helped barley plants respond to drought stress by slowing the movement of their stomata. Plants growing in drought conditions exhibited induction or suppression in the synthesis of a small number of

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polypeptides, according to SDS-PAGE examination. When it came to the appearance of new bands in the protein profile, Giza 126 performed the best. The five genotypes were examined using the ISSR-PCR approach to identify some molecular markers linked to drought tolerance (**Akladious and Abbas 2014**). In order to directly detect variations between and within genetic materials at the DNA level, molecular markers are a crucial tool (**Bahieldin *et al.*, 2012**). They offer a reliable assessment of genetic similarity that is not frequently areached using morphological data alone. Based on field traits and molecular criteria, a comparison can also be made to ascertain how genetically distinct the Egyptian cultivars are from one another. Numerous agricultural species have benefited from the intersimple sequence repeats (ISSRs) approach (**Reddy *et al.*, 2002; Tanyolac, 2003**).

Identification of genetic variants, morphological features, and genetic analysis of ISSR, SSR, and RAPD are useful techniques. These findings demonstrate a large ratio of variation in sorghum. This work could serve as a guide for future research on sorghum and aid in the understanding of species and breeding initiatives (**Heiba et al., 2023**).

Because random markers have the advantage of using no specific sequence information for primer synthesis, ISSRs show the specificity of microsatellite markers. As a result, they have been widely used for cultivar identification in various crops (**Chahidi *et al.*, 2007; Hailu *et al.*, 2005; Marotti *et al.*, 2007**). Additionally, (**Guasmi *et al.*, 2012**) it was discovered that ISSR primers varied in terms of the proportion of polymorphism, resolving power (Rp), and band in formativeness (Ib); the rate of polymorphism was 66.67%, the Rp ranged from 0.74 to 1.16, and the average Ib ranged from 0.24 to 0.39, suggesting that ISSRs are reliable molecular markers that can distinguish between Egyptian cultivars. The results showed that RAPD primers produced 49 bands with an 87.75% polymorphism proportion and a size range of 0.1-3 kb. 43 polymorphic bands with distinct patterns bands were found for RAPD. The UPGMA Dendrogram was divided into three groups by morphological characteristics and RAPD analysis (**Rashad *et al.*, 2023**).

Figuring out how different media compositions affect the embryogenic responses and regrowth of different barley genotypes. Using biochemical and molecular genetics investigations of protein, isozymes, and RAPD-PCR, the three barley genotypes El-kasr, G126, and G130 were successfully examined for the presence of somaclonal variation (**Rashad *et al.*, 2020; Rashad *et al.*, 2023**). The ability of RAPD markers to pinpoint connections between various genotypes to show how molecular level yield parameters are related. It is crucial to identify the diversity of barley in order to ensure that it can be produced sustainably and used to its maximum potential by barley breeders working with gene banks. This can be achieved through comprehensive phenotyping and genotyping of the barley collections using state-of-the-art molecular, biochemical, and physiological approaches (**Merwad *et al.*, 2020**). Genetic variants can be discovered using ISSR analysis and morphological traits (**Shata *et al.*, 2021**). ISSR markers are better at identifying genetic variation among the tested cultivars of Alfalfa than SSR marker analysis. The ISSR marker is more accurate and provides more information than the SSR marker (**Heiba *et al.*, 2022**).

With an 87.5% success rate, ISSR primers generated 37 bands, with sizes ranging from 100 to 2000 bp. proportion of polymorphism. For ISSR, the polymorphic information content PIC was 0.74. According to morphological features, the UPGMA Dendrogram was separated into two clusters, and ISSR analysis is a useful method for identifying genetic variants (**Rashad *et al.*, 2023**). Due to its high nutritional content and low glycemic index, barley is enjoying a resurgence in popularity worldwide (**Ullrich 2011**).

One of the first plants that humans domesticated was barley (***Hordeum vulgare***), and as a result, genetic variation arose in its genome and contributed to genetic diversity in its wild ancestors (**Ghaderi *et al.*, 2003**). In recent years, it has been proposed that the population diversity of barley can be resolved using biochemical techniques achieved with molecular markers. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is one of the biochemical techniques that is frequently used in studies of plant genetic diversity because it is straightforward, requires little time and labour, and is effective (**Abou-Ellail *et al.*, 2014; Ahmed *et al.*, 2010; Masoumi *et al.*, 2012; Radwan *et al.*, 2013; Sadia *et al.*, 2009**).

The goal of the current study was to use sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for protein profiles to analyse the effects of PEG-induced drought stress on genetic diversity and connections among seven barley genotypes.

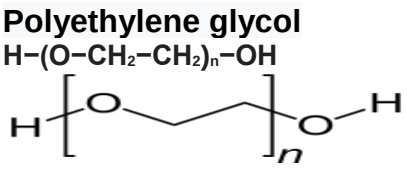
2. Materials and methods:

During the years 2022–2023, the inquiry was conducted on the Nubaria experimental field. Laboratory tests have been carried out at the National Research Centre in Egypt's Laboratory of Microbial Genetics Department and Genetics and Cytology Department of the Biotechnology Research Institute. Only plants that fully matched the UPOV phenotypic descriptions were employed for biometric analysis, protein extraction, and DNA extraction.

The first experiment In order to create the representative core sample of the species names Giza126, Giza127, and Giza128 in agriculture, seven accessions of barley were chosen based on their morphological and agronomic diversity.Giza130, Giza2000, Giza133, and Giza134, respectively. In earthen pots filled with sand and supplemented, 15 seeds of each accession were sowed at a depth of 1 cm. The experiment was set up in three replications with a completely randomised

design, and irrigation was carried out for a week using tap water.

The second experiment According to **Michel and Kaufinan (1973)**, two irrigation treatments were administered using polyethylene glycol (6000) solutions of 50% and 56% concentration, respectively. These solutions were made by adding 3.3 g/l of PEG 6000 and 3.75 g/l of distilled water, respectively.



The third experiment Samples were gathered from the most recent leaves after a month of irrigation that was treated exclusively with water control.

2. 1. Electrophoretic analysis of protein by SDS-PAGE

SDS-PAGE was used to analyse the quantitative and qualitative characteristics of the total proteins in order to identify genetic variation among six genotypes of barley. This approach was used in accordance with **Laemmli, 1970**, with modifications made by **Studier, 1970**.**2.2. Protein sample extraction:** On seedlings cultivated in greenhouses for 15 to 20 days, electrophoresis was performed. Each genotype received a sample of ten plants. A leaf sample weighing 0.5 g was obtained, pulverised in a cold mortar and pestle using liquid nitrogen, and then combined with 2 mL of extraction buffer made up of 1M TrisHCl, pH 8.0, 250 mM NaCl, and 0.25 mM EDTA. The samples were put into Ependorf tubes, put in the fridge for the night, vortexed for 15 seconds, and then centrifuged at 13,000 rpm for 10 minutes at 4°C. After gathering the pellets, the previous stages were twice repeated. Each pellet was then given 1 mL of water soluble protein buffer (SDS 10 mM b mercaptoethanol, 1M TrisHCl, 0.25 mM EDTA) and thoroughly mixed before being placed in the fridge overnight. The material was then vortexed and centrifuged for 10 min. while being cooled at 13,000 rpm. The water soluble protein extract, or supernatant, was transferred to a fresh Ependorf tube.

2.3. Separation of protein samples: Before adding the sample, each tube received 10 mL of bromophenol blue and 50 mL of the water-soluble protein fraction, which had been cooked in a water bath for 10 minutes. Using a micropipette, a volume of 15–20 L was added to each well, depending on the amount of protein in the sample, and control wells were loaded in accordance with the previously reported procedures (**Laemmli, 1970**). On one lane of each gel, low-molecular-weight standard proteins from Sigma Chemical Co. (St. Louis, MO) were produced in accordance with the manufacturer's instructions. Gels were gently shaken until the gel background became transparent and the polypeptide bands were easily discernible.

Instead of using constant voltage electrophoresis in our investigation, we used constant current electrophoresis.15 L of sample were loaded. Starting current was 30 mA; when the bromophenol blue marker reached the edge of the stacking and separating gels, current changed to 50 mA. Following electrophoresis, protein bands were stained with 12% (w/v), trichloro acetic acid solution containing 5% (v/v) ethanol and 0.05% (w/v), Coomassie Brilliant Blue R-250 (Sigma), and then distained in solution consisting of 9% methanol and 2% acetic acid. At 4°C, relative electrophoresis was carried out until the blue marker touched the gel's bottom. The gels were scanned by the Bio-Rad versa DOC3000 gel imaging system and seen through a transilluminator. From BIOMATIK, "Broad range protein marker" was introduced.

2.4. DNA extraction and ISSR reaction conditions:

DNA extraction: using DNA kit methods.

PCR products were visualized by UV-transilluminator and photographed by the Biometra - Bio Documentations gel documentation system using a DNA ladder of 100 bp. The amplified bands were scored as (1) for the presence and (0) for the absence of all examined wheat varieties in accordance with the gel analyzer protocol. For the purpose of identifying polymorphism among the seven barley cultivars, a set of six primers is shown in Table 1. These primers were created at ISSR-PCR, which followed the instructions provided by **Williams *et al.*, (1990)** with a few minor adjustments.

Inter-simple sequence repeats (ISSRs) are used in PCR-ISSR assays to detect ISSRs. 20 ml of the reaction mixture (PCR buffer 1X, MgCl2 2.5 mM, dNTPs 1 mM, primer 10 pmol, Taq polymerase 1 unit, genomic DNA 50 ng; 38 cycles of 56 °C: 1 min annealing, 2 min extension, and 10 min final extension at 72 °C) will be standardised. A 1.4% (w/v) agarose gel will be used for the analysis of the ISSR-PCR results. **Heiba *et al.* (2022)** will use a gel documentation system (SyngeneTM) to take photographs of the gels and a 1Kb and 100bpDNA ladder (Ferments Life Sciences) to measure the size of the amplified bands.

2.5. ISSR-PCR analysis: According to **Zietkiewciz *et al.* (1994)**, ISSR primers will be used in PCR experiments. The ISSR-PCR reaction of several samples will be carried out using the extracted DNA. 2 l of genomic DNA, 1 l of the primer, 2.5 l of 10X Taq DNA polymerase reaction buffer, 1.5 units of Taq DNA polymerase, and 200 mM of each dNTP will be included in the reaction mixture in a 25 l container. A DNA amplification Thermo cycler (PTC-100 PCR version 9.0 from M J Research-USA) will be used to carry out the amplifications. The apparatus is set up to operate under the following conditions: a 5 min denaturation step at 94°C, followed by 35 cycles of 30 s at 94°C, 90 s at the annealing temperature (determined by each primer), and 90 s at 72°C.

Only reproducible products will be used in the amplifications, which will be carried out at least twice, for the purposes of conducting further data analysis.

2.6. Gel electrophoresis: The ISSR amplification products will be separated on 1.5% agarose gels using a DNA ladder (1Kb) in 1X TAE buffer. As per **Sambrook *et al.*, (1989)**, the gels will next be identified by staining with ethidium bromide. The gel documentation system, Biometra - Bio Doc.

Analyse, will be used to image and document the PCR results.

2.7. Data analysis:

For data analysis, only distinct, unambiguous, and reproducible bands will be taken into account. Every band will be treated as a separate locus.

According to the Gel analyser programme, which is used to find positive and negative markers, data will be evaluated as (1) for presence and (0) for absence for each of the seven barley samples. The SPSS programme version 10 (**Norman *et al.*, 1975**) will produce the similarity coefficients in order to create a dendrogram using the unweight pair group technique with arithmetical average (UPGMA).

2.8. Statistical Analysis

A statistical analysis of the data was performed in accordance with **Gomez and Gomez (1984)**. At the 5% and 1% levels of probability, differences between treatment means were compared using the least significant differences (LSD).

3. Results

3.1. Yield parameters

According to Table 1, PEG had a substantial impact on plant dry weight, plant height, number of spikes, spike length, number of kernels per spike, 100-grain weight, and grain yield. Significant variations ($p < 0.01$) were across genotypes in terms of plant dry weight; genotype G3 produced high dry weight at 50, whilst genotypes G6 and G7 produced the lowest dry weight at 21.25. Additionally, the genotype G5's plant height was measured at 67.5, but the genotype G3's was measured at 40 after PEG treatment. The genotype G3 had the most spikes observed (16.5), while the genotype G4 had the fewest (4.25). Spike length varied significantly ($p < 0.01$) between genotypes; genotype G2 produced the largest spikes, measuring 16.50, whereas genotype G7 produced the lowest spikes, measuring 11.25. Additionally, PEG was used more effectively in G2 at both levels of drought stress, increasing the number of spikes per spike to 23.75, whereas G5 only used PEG at the severe degree of drought stress, lowering the parameter to 6.25. The genotypes used showed substantial variations in the quantity of grains per spike ($p < 0.01$). The grain weight is a crucial component of yield and significantly influenced the grain yield of barley.

Drought stress and soil nutrients have a significant impact on the weight of 1000 grains. PEG has a considerable impact on grain weight in 1000 grains. For the 1000-grain weight, there were significant differences ($p < 0.01$) across the five genotypes. G2 yielded the most weight of 1,000 grains (130 g), whereas G7 yielded the lowest weight of 1,000 grains (70 g). With the exception of the decrease in these parameters shown in G4, G7, PEG administration generally considerably boosted the yield characteristics of the drought-stressed plants as evidenced by the trait data (Fig. 1). As a result of the administration of PEG in comparison to the corresponding controls, there were also notable differences across genotypes in their responses to drought stress conditions. The study's findings revealed that plants with the genotypes G3, G6, and G5 were reacting to drought stress the best.

Table (1): Some agronomic traits as affected by PEG and drought stress of barley genotypes with irrigation treatments

Interaction	Interaction	**Plant dry weight**	**Plant height (cm)**	**Number of spikes**	**Spike length (cm)**	**Number of kernels/spike**	**100-grain weight**	**Grain yield (g)**
Control	G1	133.75**	86.00**	22.25**	21.25*	22.50**	5.88*	325.00**
	G2	120.00*	80.75*	29.00**	18.00*	18.00*	6.48*	255.00*
	G3	142.50**	92.25**	21.00**	36.25**	33.25**	7.06**	365.00**
	G4	66.25*	73.25*	12.00*	16.50*	16.25*	5.15*	105.00*
	G5	151.25**	116.00**	26.50**	20.50**	20.00**	7.22**	304.75**
	G6	115.00*	86.75*	23.00**	23.25**	23.50**	7.31**	400.00**
	G7	82.50*	84.00*	17.75*	19.00*	19.00*	6.66*	175.00*
Stress	G1	25.00*	47.75**	8.00*	16.25**	16.00**	3.22*	80.00
	G2	35.00**	50.50**	11.00**	16.50**	23.75**	4.60**	130.00**
	G3	50.00**	40.00*	16.50**	13.75**	15.25**	4.92**	110.00**
	G4	22.50*	47.00**	4.25*	12.25*	10.50*	4.12**	75.00*
	G5	37.50**	67.50**	5.50*	13.50**	6.25*	4.24**	105.00**
	G6	21.25*	52.75*	6.75*	15.75**	12.00*	3.93*	95.00*
	G7	21.25*	45.50*	4.50*	11.25*	14.50*	3.70*	70.00*
LSD 5%		20.14	11.79	4.72	4.99	4.05*	0.37	6.99
LSD 1%		27.01	15.81	6.33	6.69	5.43	0.50	9.38

Levels of significance are represented by at * = $P < 0.05$; ** = $P < 0.01$ and ns = non-significant ($P > 0.05$).

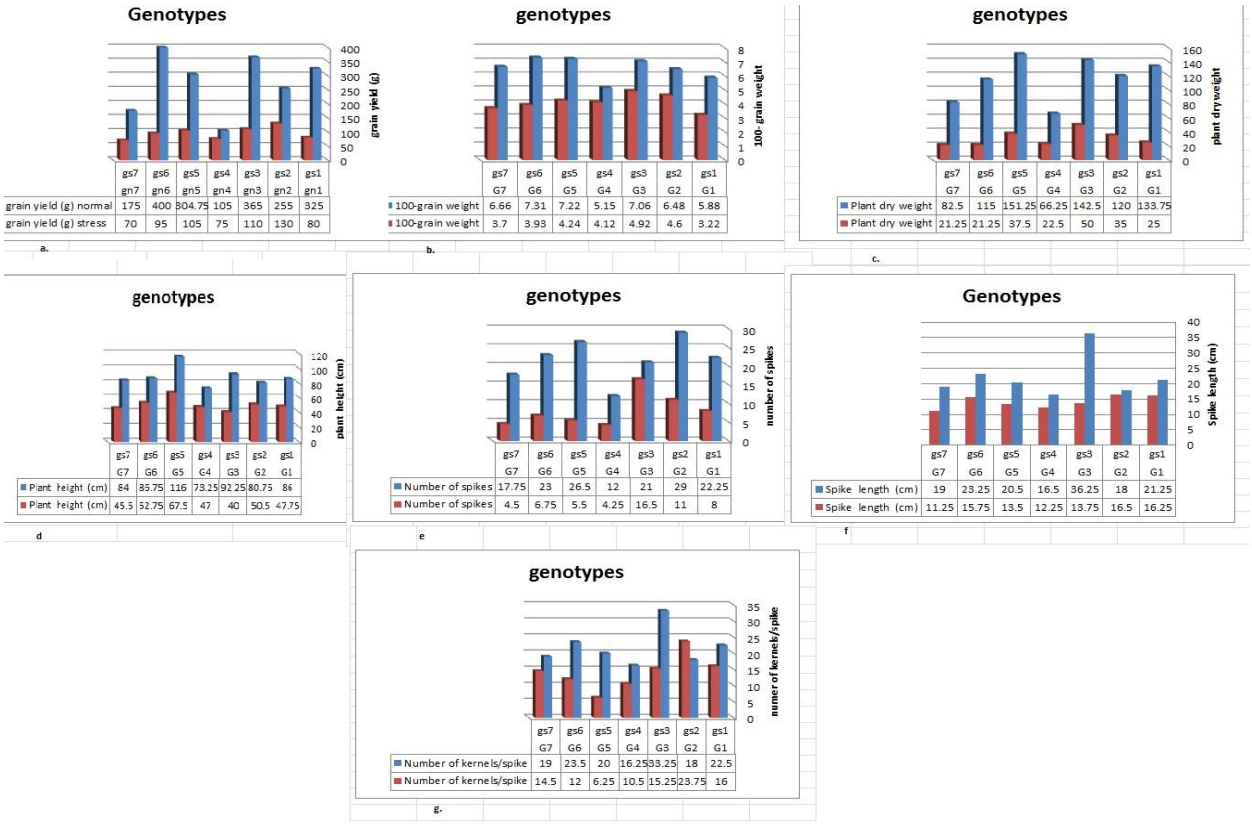


Figure1. Histograms of seven characters among seven barley cultivars.

3.2. SDS-PAGE protein banding pattern

The seven barley genotypes were subjected to SDS-PAGE analysis to determine protein patterns implicated in drought stress response in an effort to comprehend the molecular basis of drought tolerance, as shown in table 2 and figure 2. Figure 2 shows the SDS-PAGE for total proteins from seven genotypes. With molecular weights (MWs) ranging from 245 to 17 KDa, a total of 18 bands were found, 14 of which were polymorphic with 77.78% polymorphism. Regarding protein banding patterns, all genotypes showed four similar (monomorphic) bands with MWs of (245, 155, 75, and 25). The genotype with the most bands (12) was genotype number seven, and the genotype with the fewest bands (5) was genotype number four. However, genotypes (2 and 3) and (5 and 6) have the same amount of bands (7 and 6, respectively). In genotypes No. 1, 2, and 7, eight distinct bands with MWs (110, 100, 70, 63, 58, 20, and 17 KDa) were detected. From genotype No. 1, five distinct bands with MWs of 100, 63, 58, 20, and 17 emerged. While genotype No. 2 had one distinct band, measuring 90 kDa, and genotype No. 7 had two distinct bands, measuring 110 and 60 kDa. These bands might be thought of as distinctive marks.

These findings concur with those of **Ahmed *et al.* (2010)**; **Imamjomeh and Zarghani1 (2011)** suggested that the high polymorphism found in the SDS-PAGE results may have resulted from a genetic diversity evaluation based on the total seed protein. According to **Shehata (2004)**, the electrophoresis separation of water soluble protein reveals that the examined wheat cultivars have a wide range of genetic differences. These findings were in line with those of **Chen *et al.* 1996** discovered variations in soluble protein electrophoresis patterns and hypothesised that these variations may be successfully used to distinguish between wheat and barley varieties.

3. 3. Analysis of banding patterns

Gels were shot while Quantity One 4.5.1 (Bio-Rad) software was used to identify and analyse protein bands. Protein bands' relative mobility was used to measure their molecular weight. All the bands under investigation were categorized as having monomeric prolamins (indicated by +) or not (represented by -).

3.4.Molecular markers by using ISSR analysis

In order to identify molecular markers for drought resistance, six oligonucleotide primers were employed to create ISSR-PCR fingerprints of the seven barley genotypes seeded under PEG and drought stress. The ISSR-1, ISSR-2, ISSR-3, ISSR-4, ISSR-5, and ISSR-6 primers were among them. With the various primers, there were significant differences in the quantity and size of the amplified products. Table (3)

contains the ISSR-PCR findings for the genotypes of barley under investigation.

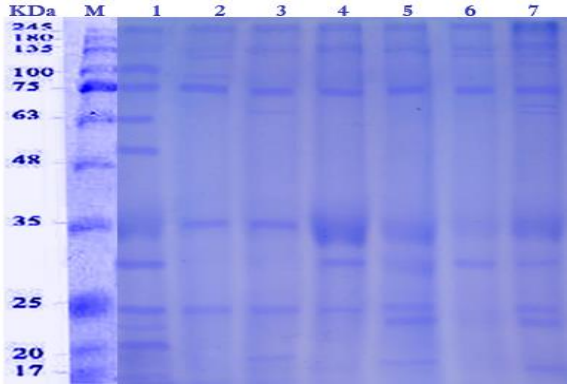


Figure (2). Protein profile of various barley genotypes' leaves as influenced by PEG treatment and drought stress.

Table (2): The impact of PEG administration on the protein patterns of the leaves of the barley genotypes under investigation that was grown under drought stress.

No	MS	Giza126	Giza127	Giza128	Giza130	Giza2000	Giza133	Giza134
1	245	+	+	+	+	+	+	+
2	155	+	+	+	+	+	+	+
3	120	_	+	_	_	_	+	+
4	110	_	_	_	_	_	_	+
5	100	+	_	_	_	_	_	_
6	90	_	+	_	_	_	_	_
7	75	+	+	+	+	+	+	+
8	70	_	_	_	_	_	_	+
9	60	_	_	+	_	_	_	+
10	63	+	_	_	_	_	_	_
11	58	+	_	_	_	_	_	_
12	35	_	+	+	_	_	_	+
13	30	+	_	_	+		+	+
14	25	+	+	+	+	+	+	+
15	23	+	_	_	_	+	_	+
16	20	+	_	_	_	_	_	_
17	18	_	_	+	_	+	_	+
18	17	+	_	_	_	_	_	_
Total		11	7	7	5	6	6	12

MW=Molecular weightbandAbsence of band= - band += Presence of

This table makes it evident that these primers produced 30 polymorphic bands in the study samples, with a polymorphism proportion of 47.62%. Figures (3) showed the ISSR profile created by the ISSR-1 primer, which had a polymorphism rate of 61.54%. The amplified fragments produced by this primer ranged in size from 180 to 1050 bp.
In this profile, there were five monomorphic bands found. These can be regarded as a genotype-positive sign. Ten bands were formed by primer ISSR-2, with the amplified fragments it produced having sizes between 200 and 900 bp. Nine bands with a 55.56% polymorphism percentage were produced with the ISSR-3 primer. This primer revealed four monomorphic bands, and under extreme drought stress, nine bands with molecular sizes between 110 and 720 bp were discovered. 10% polymorphism in ten bands was achieved by primer ISSR-4. In this profile, seven monomorphic bands were found. Nine bands with a 44.44% polymorphism percentage were generated by primer ISSR-5. This primer captured five monomorphic bands and four polymorphic ones in the profile.

Twelve bands were created with the ISSR-6 primer, with a polymorphism rate of 58.33%. In this profile, there were five monomorphic bands found. The data obtained showed that the primers ISSR-1 and ISSR-6 amplified the most bands,

whilst ISSR-3 and ISSR-5 amplified the fewest bands. According to these findings, the barley genome has more ISSR-1 and ISSR-6 repetitions than ISSR-3 and ISSR-5 repeats. The ISSR-1 primer showed the greatest amount of polymorphism (61.54%), whereas ISSR-2 and ISSR-4 primers showed the least amount (30%). Out of all the bands, a total number of bands were found which is known as marker-assisted selection. Among them, were G3, G5, and G6-specific and were picked up by the ISSR-1 and ISSR-6 primers under extreme drought stress conditions. Additionally, under extreme drought stress conditions, bands were distinctive for the moderately tolerant genotypes (G1, G2), and two of them were found in G4, G7 by the primers ISSR-3 and ISSR-5. Because it has several advantages over utilising simply conventional markers, the use of PCR-based molecular marker technology in breeding programmes and cultivar identification is thus gaining favor.

In this case, genetic diversity was investigated using the Inter-simple sequence repeat (ISSR) approach and DNA fingerprinting to determine the phylogenetic relationships between the different species of barley (**Rashad *et al.*, 2020; Guasmi *et al.*, 2012; Lamine *et al.*, 2015**). Using ISSR-based genetic diversity analysis, high polymorphism was discovered between Egyptian barley collection accessions; this finding was also shown for rice (**Gorji *et al.*, 2011**) and orange (**Izzatullayeva *et al.*, 2014**). ISSR markers, which were occasionally even more successful than SSR markers, were frequently used to differentiate between accessions. The ISSR method was recommended in earlier and more recent studies as an effective tool for genotypic evaluation in a variety of plant species, according to many publications (**Vaja *et al.*, 2016**).

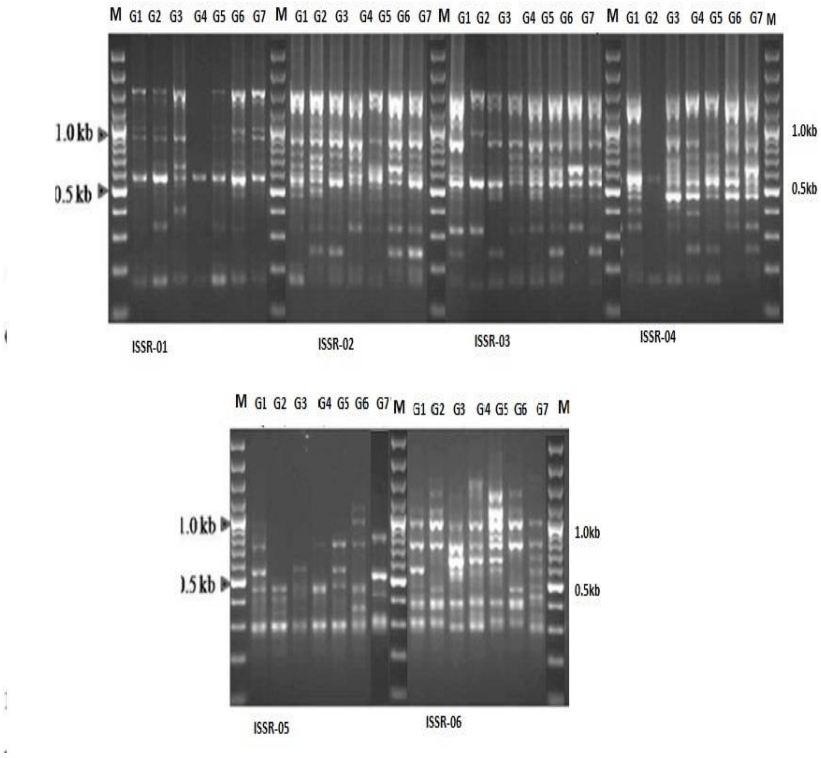


Figure (3): Amplify DNA using six ISSR primers for seven Egyptian barley cultivars (Giza126 (G1), Giza 127 (G2), Giza 128 (G3), Giza130 (G4), Giza2000 (G5), Giza133 (G6) and Giza134 (G7)).

Table (3): ISSR analyses of barley cultivars; Sequences, Total Number of Bands (TB), Monomorphic Bands (MB), Polymorphic Bands (PB) and Percentage of Polymorphism (%P).

Primer	Sequences	TB	MB	PB	%P	Fragment size
ISSR-1	5'-AGAGAGAGAGAGAGAGC-3'	13	5	8	61.54	**180-1050**
ISSR-2	5'-AGAGAGAGAGAGAGAGG-3'	10	7	3	30	**200-900**
ISSR-3	5'-ACACACACACACACACT-3'	9	4	5	55.56	**110- 720**
ISSR-4	5'-ACACACACACACACACG-3'	10	7	3	30	**200-830**
ISSR-5	5'-GTGTGTGTGTGTGTGTG-3'	9	5	4	44.44	**160-560**
ISSR-6	5'-CGCGATAGATAGATAGATA-3'	12	5	7	58.33	**200-1000**
Total		63	33	30	-	-
Average		10.5	5.5	5	47.62%	

3.5. Genetic diversity and relationships

Figure 4 depicts the Dendrogram obtained from the UPGMA cluster analysis of six ISSR markers from seven different types of *Hordeum*. On the phenogram, there were two different clusters with genetic similarity scores ranging from (0.4 to 2.4). Cluster A has five genotypes, which were divided by G3 at a phylogenetic distance of (1.6). G2, G1 were clustered together at a taxonomic distance of 1.4, whereas G6, G5, which are separated by sub-clusters in Cluster A, were grouped together at a phylogenetic distance of (1, 1.2), respectively. A taxonomic distance of (2) separated Cluster B, G4 and G7 from the remainder. This can be explained by the evolution of these genotypes in different agroclimatic zones, which indicates large levels of variation in response to selection pressure as described by numerous authors (**Allel *et al.*, 2016**). The morphological dendrogram (Fig. 4) illustrates how cluster analysis revealed a slight link between the regional origin of genotypes and their separation.

Geographic distribution has some bearing on group structure, as seen by the dendrogram created in this study using UPGMA from ISSR based on the genetic similarity matrix. Combining genes from the same region, such as G1 and G2, G7 and G4, or both, as seen in (Fig. 4). The G5 and G4 genotypes had maximum genetic similarity coefficient values of 99% and 98%, respectively, indicating a considerable level of genetic closeness. The lowest closeness ratio of 80% was recorded by the towed accessions G7 and G1, as indicated in Table (5).

Molecular markers have also been used to describe wheat as adhering to this pattern of geographically related grouping (**Strelchenko *et al.*, 1999**). Our findings, however, debunk past studies on barley and Aegilops that assert that the molecular clustering of barley does not reflect its origin (**Owuor *et al.*, 2003; Mahjoub *et al.*, 2009**). Furthermore, 37 polymorphic bands were discovered by 7 ISSR primers, suggesting a high level of polymorphism (87.5%), and the results revealed highly polymorphic profiles. 60 percent of Moroccan barley's polymorphism is lower than average (**Dakir *et al.*, 2002**). Our results revealed 37 polymorphic bands, as shown in (Table 4).

Table (4). the percentages of seven different barley cultivars that are comparable based on ISSR data.

Case	G1	G2	G3	G4	G5	G6	G7
G1	1.000						
G2	0.970	1.000					
G3	0.941	0.971	1.000				
G4	0.912	0.943	0.972	1.000			
G5	0.925	0.957	0.958	0.986	1.000		
G6	0.831	0.866	0.870	0.899	0.912	1.000	
G7	0.813	0.818	0.824	0.824	0.836	0.923	1.000

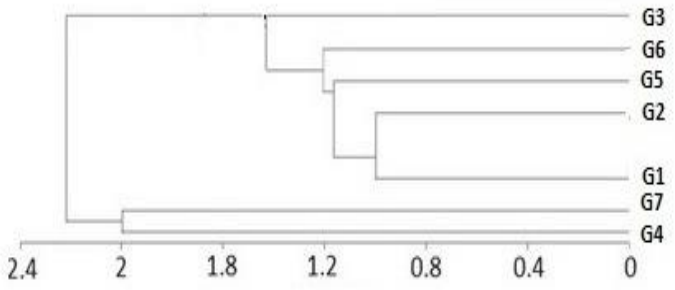


Fig 4: Dendrogram among seven barley cultivars using UPGMA.

4. Discussion

4.1. Yield parameters

How many seeds have sprouted? The percentage of barley cultivars under study whose seeds germinated after being treated to polyethylene glycol (PEG) is shown in Table 1 and Fig. 1. According to the findings, PEG has a much greater impact on yield characteristics than well-watered plants do. Following the application of PEG to drought-stressed plants, the obtained findings showed a considerable improvement in yield metrics (plant dry weight, plant height, number of spikes, spike length, number of kernels/spike, 100-grain weight, and grain yield). G1, G2, and G4, G7 cultivars demonstrated poorer tolerance for drought stress while G3, G6, and G5 cultivars performed best in terms of the yield parameter.

One of the major factors affecting plant development and productivity is drought. Because this tension lowers germination rate and percentage and ultimately delays establishment of plantlets, it can have an impact on many aspects of plant metabolism and growth (**Prisco *et al.*, 1992**). The data showed that increasing PEG% was associated with a decrease in germination rate, with decreasing percentages of 24%, 49%, 81%; 25%, 46%, 77%; and 19%, 42%, 45% at PEG% 5%, 10%; 20% after 3,5; and 7 days, respectively, as compared to untreated ones. Therefore, it is evident that PEG% had a deleterious impact on the seeds that had already begun to germinate, however this impact was lessened by the time the germination test was complete.

Additionally, the percentage of germination that has decreased as a result of the addition of PEG could be ranked as follows: Compared to the untreated control treatment, 20% (57.7) > 10% (25.0) > 5% (9.5%). In locations where plant

establishment has failed owing to drought, trait modification linked to germination is significant (**Bayoumi *et al.* 2008**). Germination is one of the stages that is vulnerable to drought stress. The germination process is regulated by hormonal and environmental factors. In addition to other parameters, light, oxygen, temperature, and water availability all play significant roles (**Finch *et al.*, 2001**). According to the data available, increasing PEG% was linked to decreasing Yield parameters and decreasing values as compared to the control, respectively. Additionally, results showed that increasing PEG level resulted in reductions of PEG of 50% and 56%, respectively, in comparison to the untreated therapy. At lower potential levels, the seedlings had thinner and longer roots than the control treatment, and as the stress increased up to roughly 1.2 MPa, the root length decreased even more. **Jamshidi (2006)** studied safflower genotypes under water stress and reported.

4.2. SDS-PAGE protein banding pattern

According to **Amini et al. (2007)**, the presence and disappearance of some protein bands indicates that the drought stress caused some proteins to rise and others to decrease. The emergence of novel protein bands in response to drought stress levels raises the possibility that these proteins are to blame for the induction of drought resistance in various barley genotypes (**Zoro *et al.*, 2006**). The genes responsible for protein synthesis may have been entirely suppressed due to stress, which is one explanation for the loss of some protein bands during drought stress. As a result, the developing tissue had lost its capacity to produce these proteins in response to stress. Another possibility is that the stress-induced inhibition of the genes led to partial suppression rather than full reversal of the inhibition was not achieved (**Amal, 2005**).

The most stable genotypes (Giza 123 and Giza 126) appear to express or inhibit bands the least. In Giza 126, just a small number of genes were directing protein expression, or gene expression was more stable under drought conditions. (**Amini *et al.*, 2007**). Other explanations include the possibility that many mRNA molecules are not translated, or that changes in enzyme activity or protein levels can happen without any discernible changes in transcript (**Amini *et al.*, 2007**). Our findings therefore suggested that the seven barley genotypes' altered levels of protein synthesis on a quantitative and qualitative level may be related to stress injury or tolerance mechanisms as compatible cytoplasmic solutes in the cytoplasm's osmotic potential with vacuoles during drought stress.

There has been a major accumulation of the processes by which drought stress may cause the development of certain polypeptides in drought-stressed plants. These polypeptides, known as osmotin, were specific to tobacco cells because they were being gradually adjusted osmotically to desiccation stress when they were synthesised and accumulated (**Amal, 2005**). In both drought-tolerant and drought-sensitive cultivars of sorghum, **Wood and Goldsbrough (1997)** found that drought-induced expressions of a few genes. Additionally, both cultivars vulnerable to and tolerant of drought had their gene expression controlled by the drought (**Zoro *et al.*, 2006**).

4.3. Molecular markers by using ISSR analysis

ISSR analysis is used to identify molecular markers. According to **Sonante and Pignone (2001)**, ISSR markers have been employed to assess genetic variation within collections of farmed plants. Accessions might be distinguished thanks to the polymorphisms produced by ISSR. In order to distinguish between genotypes that are closely related to one another, ISSR markers have been employed to identify cultivars in many different plant species (**Zhao *et al.*, 2006**). The use of the ISSR method for gene tagging and marker-assisted selections is growing in popularity. Results indicated that banding patterns and agronomic traits can be correlated using ISSR primers as informative markers. To successfully tag the desired gene, plant breeders and molecular biologists must work together effectively.

The distinctive bands created by the primers may act as a distinctive phenotypic marker for drought tolerance. However, this requires additional research utilising more primers. These fingerprints may be cultivar-specific markers that can be used to inform the design of barley crosses, hence improving the management and conservation of barley germplasm. This idea has been supported by a number of researchers who claim that molecular markers have a number of advantages over the conventional phenotypic markers that plant geneticists have previously had access to. By doing selection not directly on the characteristic of interest but on molecular markers connected to that trait, they provide significant potential for increasing the efficiency of conventional plant breeding (**Negussie & Pretorius, 2012**).

According to **Durán and Vega (2004)**, RAPD and ISSR markers both contribute a sizable number of polymorphic markers that may be beneficial for detecting lentil genotypes, saturating genetic maps, and marker-assisted selection. The genotypes of barley that were investigated in this study displayed substantial genetic diversity. Therefore, intra-population improvement programmes should concentrate on choosing particular plants from these groups that have desired features. On the other side, a useful criterion for germplasm enhancement programmes is the genetic separation between genotypes.

Inheritance research on biotic and abiotic stress tolerance and resistance is helpful in developing the best breeding strategy based on local needs. Priority should be given to molecularly tagging genes for resistance to biotic and abiotic challenges

so that they can be used in realistic breeding programmes with higher selection efficiency and greater accuracy.

5. Conclusion

In seven barley genotypes, this study examined the physiological and biochemical indicators linked to drought stress. The study's findings revealed that plants of the genotypes G3, G5, and G6 responded to drought stress the best when PEG was present. The outcomes also suggested that several drought-induced proteins played a direct or indirect role in cellular adjustments to stress. These proteins from the seven diverse barley genotypes would help with future research into the molecular detection of changes in gene expression of barley genotypes under drought stress and the control of drought tolerance and sensitivity in plant cultivars.

In conclusion, alterations in protein synthesis were caused by PEG and drought stress. Barley genotypes under drought stress were found to accumulate proteins, which may act as a barrier against additional dehydration damage. More genotypic variants were identified by ISSR; however more primers are required for additional research. Nevertheless, this study's findings offer several ISSR molecular markers linked to the productivity of different barley genotypes. By using marker-assisted selection, they could be utilised to improve breeding programmes that attempt to increase the plant's tolerance to drought. This study's ISSR can be applied in future research to identify stress-tolerant barley genotypes or other field crops.

Conflicts of interest

The authors declare there are no conflicts of interest.

Author's contribution

SER, EAE design the work, SER, EAE conduct the experimental work and the paper was prepared by SAAH and SER. Each author contributed to the serious debate, data analysis, and text revision.

6. References

[1] Money N.P. (1989). Osmotic pressure of aqueous polyethylene glycols. Relationship between molecular weight and vapor pressure deficit. Plant Physiol.91:766-769.

[2] Michel B.E. and Kaufmann M.R. (1973). The osmotic potential of polyethylene glycol 6000. Plant Physiol. 51:914-917.

[3] Michel B.E. (1983). Evaluation of the Water Potentials of Solutions of Polyethylene Glycol 8000. Plant Physiol. 72:66–70.F.A. Hellal, H.M. El-Shabrawi, M. Abd El-Hady, I.A. Khatab, S.A.A. El-Sayed, Chedly Abdelly (2018). Influence of PEG induced drought stress on molecular and biochemical constituents and seedling growth of Egyptian barley cultivars. Journal of Genetic Engineering and Biotechnology, Volume 16, Issue 1, Pages 203-212, ISSN 1687-157X,

[4] FAO (2019). FAOSTAT. Food and Agriculture Organization of the United Nations.. Available online: https://www.fao.org/faostat/en/#home (accessed on 30 October 2021).

[5] Riehl, S. (2019). Barley in archaeology and early history. In Oxford Research Encyclopedia of Environmental Science; Oxford University Press: Oxford, UK. [Google Scholar]

[6] Al-Sayaydeh, R.; Al-Bawalize, A.; Al-Ajlouni, Z.; Akash, M.; Abu-Elenein, J.; Al-Abdallat, A. (2019). Agronomic Evaluation and Yield Performance of Selected Barley (*Hordeum vulgare* L.) Landraces from Jordan. Int. J. Agron. 1–12. [Google Scholar] [CrossRef][Green Version]

[7] Sharma, A.; Joshi, N.; Cheema, B.; Jindal, M.; Singh, S (2014). Assessment of genetic diversity in barley (*Hordeum vulgare* L.). J. Res. Punjab Agric. Univ., 51, 105–108. [Google Scholar]

[8] Hellal F.A., El-Shabrawi H.M., Abd El-Hady M., Khatab I.A, El-Sayed S.A.A., Abdelly C. (2018), Influence of PEG induced drought stress on molecular and biochemical constituents and seedling growth of Egyptian barley cultivars. Journal of Genetic Engineering and Biotechnology, Volume 16, Issue 1, Pages 203-212, ISSN 1687-157X,

[9] Akladious, S. & Abbas, S. M. (2014). Inter simple sequence repeat (ISSR) markers and some physiological attributes of barley (*Hordeum Vulgare* L.) genotypes to drought and potassium nutrition. Journal of Animal and Plant Sciences. 24. 620-633.

[10] Bahieldin, A.; Ramadan, A.M.; Gadalla, N.O.; Alzohairy, A.M.; Edris, S.; Ahmed, I.A.; Shokry, A.M.; Hassan, S.M.; Saleh, O.M.; Baeshen, M.N. (2012). Molecular markers for salt tolerant wild barley *Hordeum spontaneum*. Life Sci. J., 9, 5838–5847. [Google Scholar]

[11] Reddy, M.P.; Sarla, N.; Siddiq, E. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica, 128, 9–17. [Google Scholar] [CrossRef]

[12] Tanyolac, B. (2003). Inter-simple sequence repeat (ISSR) and RAPD variation among wild barley (*Hordeum. vulgare* subsp. spontaneum) populations from west Turkey. Genet. Resour. Crop Evol., 50, 611–614. [Google Scholar] [CrossRef]

[14] Chahidi, B.; El-Otmani, M.; Jacquemond, C.; Tijane, M.h.; El-Mousadik, A.; Srairi, I.; Luro, F. (2007). Use of morphological and physiological characters, and molecular markers to evaluate the genetic diversity of three clementine cultivars. C. R. Biol., 331, 1–12. [Google Scholar] [CrossRef] [PubMed]

[15] Samy A. A. Heiba a, Ibthal Salah Eldemerdash a and Shimaa E. Rashad *b (2023). Evaluation of biological control of sorghum strains using *Bacillus thuringiensis* and *Pseudomonas aeruginosa* under drought stress. Journal of advanced zoology. In press [16] Marotti, I.;

Bonetti, A.; Minelli, M.; Catizone, P.; Dinelli, G. (2007). Characterization of some Italian common bean (Phaseolus vulgaris L.) landraces by RAPD, semi-random and ISSR molecular markers. Genet. Resour. Crop Evol., 54, 175–188. [Google Scholar] [CrossRef]

[17] Guasmi, F.; Elfalleh, W.; Hannachi, H.; Feres, K.; Touil, L.; Marzougui, N.; Triki, T.; Ferchichi, A. (2012). The use of ISSR and RAPD markers for genetic diversity among south tunisian barley. ISRN Agron., 1–10. [Google Scholar] [CrossRef][Green Version]

[18] Rashad S. E., El- Demerdash I. S., Abdel-Rahman H. M., EL-Enany, M. A. M, Heiba S. A. A. (2023). Screening of genetic diversity in Seventeen sunflower (Helianthus annuus L.) genotypes for oil content and the early flowering rate using RAPD markers

[19] Rashad S. E.*1, F. M. Abdel-Tawab 3, Eman M. Fahmy3, Saker, M. M2. (2020). Somaclonal variation from mature embryo explants of some Egyptian barley genotypes. Egypt. J. Genet. Cytol.,; 49:103-121.

[20] Sozan Eid El-Abeid1 ,Samy A. A. Heiba2, Ibthal S. El-Demerdash2, ,Maha Haredy3, Shadia Sabry4 and Shimaa E. Rashad*5 (2023). Detected genetic markers for three varieties of Rice (Oryza sativa L.) under Nano- particles. journal of advanced zoology. *In press*

[21] Merwad M.A.1; E.A.M. Mostafa1; N.E. Ashour1; M.M.S. Saleh1; Ibthal S. El- Demerdash2 and Shimaa E. Rashad*3.(2020). Horticultural Studies And Genetic Relationship Via Dna Fingerprinting Using RAPD Markers Between Sewi Date Palm And Two Superior Seeded Females. Plant Cell Biotechnology And Molecular Biology,; 22 (59&60): 56-66.

[22] Shata S. M. 1,* , Said W. M. 1 , Abdel-Tawab F. M. 2 , Kamal L. M. 2. (2021). Morphological and Quantitative traits of phylogenetic relationships of some barley (*Hordeum vulgare* L.) accessions in Egypt.- Journal of Scientific Research in Science; 38, (1): 16-35

[23] Heiba1 S. A.A., Rania Tawfick Ali1, Hamdy M. Abdel-Rahman*1 and Shimaa E. Rashad2. (2022). Detected molecular markers for Alfalfa (Medicago sativa) using ISSR and SSR under Egyptian conditions. International Journal of Latest Technology in Engineering, Management & Applied Science (IJLTEMAS); XI, XI, 2278-2540

[24] Rashad S. E., El- Demerdash I. S., Abdel-Rahman H. M., EL-Enany, M. A. M, Heiba S. A. A. (2023). Enhancement of some barley (*Hordeum vulgare* L.) resistance for nematode (Heterodera avenae) using DNA fingerprinting analysis. Egyptian Pharmaceutical Journal, *in press*.

[25] Ullrich S. E. (2011). Significance, adaptation, production, and trade of Barley.In Barley: production, improvement, and uses, 1st edition (ed. S. E. Ullrich), pp. 3–13. Wiley-Blackwell, Oxford, UK..

[26] Ghaderi, M., P. Vojdani and A. Gerami. (2003). Genetic diversity in the Iranian landraces of barley and its relation to geographical and climatic variation. Journal of Agricultural Science and Technology, 9: 50-70.

[27] Abou-Ellail, M., M. E.-S. Hattem, A. M. Mohamed, I. A. Usama, A. G. Hassan and A. E. E. (2014). Appraisal of biochemical and genetic diversity of mango cultivars using molecular markers. African Journal of Biotechnology, 13: 2796-2806..

[28] Ahmed, M. F., M. Iqbal, M. ShahidMasood, M. AshiqRabbani and M. Munir. (2010). Assessment of genetic diversity among Pakistani wheat (Triticumaestivum L.) advanced breeding lines using rapd and sds-page. Electronic Journal of Biotechnology, 13.

[29] Masoumi, S. M., D. Kahrizi, H. Rostami-Ahmadvandi, J. Soorni, S. Kiani, A. Mostafaie and K. Yari. (2012). Genetic diversity study of some medicinal plant accessions belong to Apiaceae family based on seed storage proteins patterns. Molecular Biology Reports, 39: 10361-10365.

[30] Radwan, S. A., A. S. El-Koly and R. H. Sammour. (2013). Genetic variation among accessions of Lathyrus inconspicuous (L.) as revealed by SDS Polyacrylamide Gel Electrophoresis / Analizagenetskevariabilnostiakcesijgrahorja (Lathyrus inconspicuous (L.) s SDS poliakrilamidnogelskoelektroforezo. ActaagriculturaeSlovenica, 101:21-30.

[31] Sadia, M., S. A. Malik, M. A. Rabbani and S. R. Pearce. (2009). Electrophoretic characterization and the relationship between some Brassica species. Electronic Journal of Biology, 5: 1-4..

[32] Laemmli, U.K., 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T. Nature., 227: 680-689.

[33] Studier, F.W., 1973. Analysis of bacteriophage T, early RNAs and proteins of slab gel. J. Mol. Bio., 79: 237.-

[34] Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. Nov 25; 18(22): 6531-5. doi: 10.1093/nar/18.22.6531. PMID: 1979162; PMCID: PMC332606.

[35] Zietkiewciz, E.,Rafalksi, A., Labuda, D (1994). Genome fingerprinting by simple sequence repeat (SSR)-Anchored Polymerase Chain Reaction Amplification. Genomics. 20, 176-183.

[36] Sambrook, J., Fritsch, E. R., & Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual (2nd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

[37] Norman N, H (1975). Spss Statistical Package for the Social Sciences, Second Edition. New York: McGraw-Hill Book Co., Journal of Advertising, 5:1, 41-42, DOI: 10.1080/00913367.1976.10672624

[38] Gomez K. A. and Gomez A. A. (1984). Statistical procedures for agricultural research. John wiley & sons. P. 180.

[39] Ahmed, M.F., M. Iqbal, M.S. Masood, M.A. Rabbani and M. Munir, (2010). Assesment of genetic adversity amonge Pakistani wheat (Triticumaestivum L.) advanced breeding lines using RAPDand SDS-PAGE . Electronic Journal of Biotechnology, 13(3):1-10.

[40] Imamjomeh A.., and Zarghani1, E., (2011). Assesmnt of Genetic Diversity In High-Molecular-Weight Glutenin

Subunits And Relationship To Breed-Making Quality In Common Wheat. Trakia Journal of Sciences, 9(1): 37-42.

[41] Shehata, M.M., (2004). The role of seed proteins and RAPD-PCR in genotyping variabilities of some wheat (Triticumvulgare L.,) cultivars. Pakistan Journal of Bilological Science, 7(6): 984-994.

[42] Chen, D.M., L.F. Filippis and L.F. De Filippis, (1996). Application of genomic DNA and RAPD-PCR in genetic analysis and fingerprinting of various species of wheat and woody trees. Aust. Forest., 59: 46-55.

[43] Rashad S. E.*1, F. M. Abdel-Tawab 3, Eman M. Fahmy3, Saker, M. M2.(2020) Transformation system of mature embryo of some Egyptian barley genotypes. Egypt. J. Genet. Cytol.; 49:89-102.

[44] Lamine M, A Mliki, (2015). Elucidating genetic diversity among sour orange rootstocks: a comparative study of the efficiency of RAPD and SSR markers, applied biochemistry and biotechnology; 175: 2996-3013

[45] Gorji AM, P Poczai, Z Polgar, J Taller (2011). Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato, American journal of potato research; 88: 226-237

[46] Izzatullayeva V, Z. Akparov, S. Babayeva, J. Ojaghi, M. Abbasov (2014). Efficiency of using RAPD and ISSR markers in evaluation of genetic diversity in sugar beet, Turkish Journal of Biology; 38: 429-438

[47] Vaja KN, HP Gajera, ZA Katakpara, SV Patel, BA Golakiya (2016). Biochemical indices and RAPD markers for salt tolerance in wheat genotypes, Indian Journal of Plant Physiology; 21: 143-150

[48] Allel D, A Ben-Amar, M Lamine, C Abdelly (2016). Relationships and genetic structure of North African barley (*Hordeum vulgare* L.) germplasm revealed by morphological and molecular markers: Biogeographical considerations. South African Journal of Botany, 2017; 112: 1-10.

[49] Strelchenko P, O Kovalyova, K Okuno. (1999). "Genetic differentiation and geographical distribution of barley germplasm based on RAPD markers." Genetic resources and crop evolution; 46: 193-205.

[50] Owuor ED, A Beharav, T Fahima, VM Kirzhner, AB Korol, E Nevo, (2003). Microscale ecological stress causes RAPD molecular selection in wild barley, Neve Yaar microsite, Israel, Genetic Resources and Crop Evolution; 50: 213-224

[51] Mahjoub A, MS El Gharbi, K Mguis, M El Gazzah, NB Brahim, (2009). Evaluation of genetic diversity in Aegilops geniculata Roth accessions using morphological and RAPD markers, Pakistan journal of biological sciences: PJBS; 12: 994-1003

[52] Dakir EH, ML Ruiz, P García, MP de la Vega, (2002). Genetic variability evaluation in a Moroccan collection of barley, *Hordeum vulgare* L., by means of storage proteins and RAPDs, Genetic Resources and Crop Evolution; 49: 619-631

[53] Prisco JT, Baptista CR, Pinheiro EJL (1992). Effect of seed pretreatment on seed germination under water stress condition. Revta Brasil Bot;15:31–5.

[54] Bayoumi TY, Eid MH, Metwali EM (2008). Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. Afric J Biotech; 7: 2341–52.

[55] Finch SWF, Phelps JRA, Whalley WR, Rowse HR (2001). Seed reserve-dependent growth responses to temperature and water potential in carrot (Daucus carota L.). J Exp Bot; 252:218–9.

[56] Jamshidi M (2006). The effect of environmental stress on safflower genotypes. In: The 9th congress on Iranian agronomy and plant breeding. Shahid Beheshti , University.

[57] Amini F, Ehsanpour A, Hoang Q, Shin J. Protein pattern changes in wheat under in-vitro salt stress. Russ. J. plant physiol. 2007: 54(4): 446- 471. 33.

[58] Zoro I, Maquet A, Wathelet B, Baudion J. Genetic control of alcohol dehdrogenase, mlate dehydrogenase and phosphoglucomutase isozymes in lime bean (Phaseolus Lunatus L.). J. Plant breeding 2006: 116 (2): 181-85.183. 34.

[59] Amal A, Mohamed, E. Two-dimensional electrophoresis of soluble proteins and profile of some isozymes isolated from maize plant in response to NaCl. Research Journal of Agriculture and Biological Sciences, 2005: 1(1):38-44. 35.

[60] Wood AJ, Goldsbrough PB. Characterization and expression of dehydrins in water-stressed Sorghum bicolor. Plant Physiol 1997: 99:144- 152

[61] Sonante G, Pignone D. Assessment of genetic variation in a collection of lentil using molecular tools. Euphytica 2001:120:301-307. 38.

[62] Zhao WG, Zhang JQ, Wangi YH, Chen TT. Analysis of Genetic Diversity in Wild Populations of Mulberry from Western Part of Northeast China Determined by ISSR Markers. Genetics and Molecular Biology 2006: 196-203. 39.

[63] Negussie T, Pretorius ZA. Lentil rust: Present status and future prospects. Crop protection 2012: 32: 119-128. 40.

[64] Durán Y, Vega MP. Assessment of genetic variation and species relationship in a collection of Lens using RAPD and ISSR. Span J Agric Res 2004:2(4): 538-544