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IRAP-PCR Technique for Determining the Biodiversity between Egyptian Barley Cultivars

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THE GENETIC diversity of some Egyptian barley cultivars was assessed using twelve primers from the inter-retrotransposons amplified polymorphism (IRAP) marker system. In addition, moisture content, crude, lipids, ash, and crude fiber contents were measured in the studied cultivars. The obtained results showed that the average protein content was 11.19%, and the highest one was recorded for Giza135 cultivar with an average value of 12.3% to a lesser extent in Giza 129 (8.7%). Sixty-three IRAP bands, scores of 33.3% and 66.7% of monomorphic and polymorphic bands were recorded, respectively. The polymorphism heterozygosity index values IRAP marker traits were ranged as H (0.04–-0.47), PIC (0.04–0.36), E (7.50–11.75) HAV (0.00–0.01), MI (0.04–0.07), D (0.04–0.61), and R (0.5.–6.86). The proportion of appearing polymorphism was 63%. This multiplicity was used to measure the difference between the studied cultivars; however, there were no unique bands in all cultivars for each IRAP primer. There was a similarity between the PCA, heat map, and dendrogram distributions of Giza123, Giza124, Giza125, and Giza126. The Bioplot analysis divided the cultivars into four groups. The data revealed a significant difference, among the surveyed varieties of barley cultivars in Egypt, at the molecular level.

Keywords: Barley cultivars, Biodiversity, Dendrogram, IRAP-PCR.

Introduction

Barley (*Hordeum vulgare* L.) is one of the major crops grown on a grand scale in the Coastal Region of North Egypt and in the newly reclaimed soils characterized by a lack of fresh water and salinity stress (El-Sherif & Ibrahim, 2020; Ewida et al., 2021). It is primarily used for animal forages, but it is increasingly being used as a human food because of its nutritional and health benefits in most countries that use hull-less barley (Noaman et al., 2011). Molecular markers are used for biodiversity, characterization, and genetic mapping molecular breeding and molecular genetics are terms used in the recent definition of plant breeding (Muhammad et al., 2018; Elghamery et al., 2021). Molecular plant breeding is an interdisciplinary science revolutionizing crop improvement in the twenty-first century (Lörz & Wenzel, 2005; Varshney et al., 2006; Eathington et al., 2007; Mumm, 2007; Adeyemo et al., 2020; El-Badan et al., 2021). As a result, the most crucial aspects of molecular breeding are the detection and characterization of genetic markers (Stephen & Mumm, 2008; Voosen, 2009; Khatab et al., 2021). Molecular genetics frequently apply an "investigative technique" to detect the structure and function of a gene in a plant's genome using genetic screens by one and multi-molecular markers (Waters, 2013; Alberts, 2014).

It has been a long time since the first-generation DNA molecular marker, the Restriction Fragment Length Polymorphism technique was introduced in 1980 until the introduction of the inter-

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retrotransposon amplified polymorphism (IRAP) molecular marker in 2010 (Boronnikova & Kalendar, 2010; Yang et al., 2015). Transitional elements (TEs) are a cluster of repetitive sequences (SanMiguel et al., 1996; Miller & Capy, 2004). IRAP is a multiplex and dominant technique (Kalendar et al., 1999).

Thomas et al. (2017) discovered that barley genes make up just about 20% of the genome, with TEs accounting for the remaining 80%, and that the TE fraction comprises at least 350 distinct families. However, only fifteen high-copy TE families account for 50% of the genome; however, all other TE families possess low or moderate copy numbers. Class I retrotransposons and class II DNA transposons have been described based on their transposition mechanisms (Bourque et al., 2018). LTR-lacking retrotransposons and long terminal repeat (LTR)-containing are divided into two classes. The scale of LTR retrotransposons ranges from a few kb to 15kb (Finnegan, 1989; Kumar & Bennetzen, 1999; Miller & Capy, 2004). In five LTRs, the promoter is present, and in three LTRs, the transcriptional terminator is present (Schulman & Kalendar, 2005). In plant genomes, both LTR and non-LTR retrotransposons can exceed high copy numbers (Kumar & Bennetzen, 1999). LTR retrotransposons are also excellent sources of molecular markers because of their ubiquity, abundance, dispersion, and dynamism in plant genomes (Kalendar & Schulmann, 2006; Poczai et al., 2012). Kalendar & Schulman (2006) and Kalendar et al. (1999) clarified the IRAP-PCR technical steps in the lab protocols, PCR, and data management. Saulius et al. (2016) investigated the chemical composition of spring barley varieties, finding that crude protein content was higher in spring barley varieties than in winter varieties, ranging between 10.35% DM and 12.38% DM. Crude fat content varied between 1.09% DM and 2.00% DM in both spring and winter barley varieties, whereas crude ash content varied between 1.94% DM and 2.40% DM. We used many molecular parameters to differentiate between Egyptian barley varieties at the molecular level, during this time. This research shows a molecular parameter dependent on the differentiation of fixed locations of DNA caused by TEs, implying that the variance is because of mutations. This study aims to distinguish Egyptian barley cultivars using a combination of IRAP-PCR and chemical composition techniques.

Materials and Methods

Plant materials

Barley Research Department, Agricultural Research Center, provided grains of twelve barley cultivars. The names of these cultivars are listed in Table 1.

Chemical Composition

Moisture content, ash, lipids, crude protein (N x 5.7), and crude fiber contents were detected according to the A.O.A.C. (2005) procedures. The nitrogen-free extract was determined according to the method described by Van Soest et al. (1991).

IRAP marker

To evaluate genetic diversity in barley, we have used the IRAP marker method. DNA was extracted from young leaves of the twelve cultivars (10-day old seedlings) using the modified CTAB (cetyl trimethyl ammonium bromide, Sigma-Aldrich CAS No. 57-09-0) method according to (http\\www.primerdigital.com/DNA). Twelve IRAP primers were designed based on the most abundant transposon groups in barley (Huck, Ji, Opie, Grandle-4, and Tekey) using the fast PCR software (http://primerdigital.com/fastpcr.html). Primers were designed to fit a long terminal repeats (LTR) sequence at either its 3′ or 5′ end with the primer oriented. The amplification direction is adjacent to the nearby end of the LTR, using conserved terminal regions of transposable elements, which are relatively abundant in the whole genome. The available barley genome sequence provided a great quantity of information used for primers design. (http://archive.barleygdb.org/sequencing_project.php) and (http://ensembl.gramene.org/Zea_mays/Info/Index).All universal precautions were followed in designing the tested primers. The codes and sequence of the tested primers are shown in Table 2. The primers that gave scorable amplifications are marked with (*). PCR amplifications were carried out using a Bio-Rad 3.03 version thermocycler. Thirty primers were used (Table 2). The reaction started with a hot start Taq polymerase at 95°C for 3min, and then amplifications were performed for 35 cycles with denaturation at 95°C for 20sec, annealing at ~55ºC for 30sec, and extension at 72ºC for 90 seconds. The reaction mixture (20µL) contained 3µL sterilized MQ H_2O, 2.0µL buffer, 0.4µL dNTPs, 2.0µL primer, 1µL $MgCl_2$, 0.2µL Taq

polymerase, and 0.2µL template DNA. PCR products were detected on agarose gel (1.2%) in 1X THE buffer (2.4g Tris-base, 4.76g HEPES, 1mL 0.5M EDTA, dissolved in MQ-water, and brought to the last volume of 100mL) at a constant voltage of 70 volts. Electrophoresis Gene Ruler™ DNA ladder solution (Thermo-Scientific - Fermentas, Canada) 1000–10000 base range 25ng/µL was used. Gels were stained with ethidium bromide (0.5mg/mL) solution and stored at room temperature. A high-quality gel solution with high sensitivity and resolution using a second-harmonic-generation green laser (FLA-5100 imagine system Fuji photo Film GmbH., Germany) was adopted. Data were scored using MEGA 5.10 (Molecular Evolutionary Genetics Analysis) version 7 (http://www.megasoftware.net). The method applied is based on cluster analysis expressing the relationships of the studied cultivars as distance percent in a cluster tree and similarity matrix. The scored data were analyzed using NTSYs-pc version 2.11, and a dendrogram expressing the relationships of studied cultivars was achieved using the un-weighted pair-group approach of arithmetic means.

Results and Discussion

Many factors, including the chemical composition, explained the genetic variation between the barley varieties. We measured the differences at the molecular level through many molecular parameters according to the type of molecular marker. We used this molecular marker as a new method for differentiating barley varieties. This work focused on the characterization of molecular parameters and their effect on the strength of the molecular parameter (Table 3). Therefore, this work focused on the characterization of the molecular parameter and its effect on the strength of the molecular parameter (Table 3). The parameter of IRAP was found to measure molecular teacher traits (Amiryouse et al., 2018). First, heterozygosity index (H) is defined as an individual's probability of being heterozygous in the population. Polymorphic Information Content (PIC) Probability that the genotype marker of an offspring can allow the deduction without crossing over. Subsequently, the two markers of the affected parents were received **(**Amiryouse et al., 2018).

TABLE 1. Origin, release of year, and names of the Egyptian barley cultivars and chromosome number is 2n=2x=14

No.	Cultivar	Kind	Pedigree	Origin and year of release	Rowed
G1	Giza 123	Naked*	Giza117 / FAO86	Egypt-1998	Six
G2	Giza 124	Naked	Giza 117/Bahtim 52// Giza 118/ FAO86	Egypt-1998	Six
G3	Giza 125	Naked	Giza 117/Bahtim52// Giza 118/ FAO86(2)	Egypt-1995	Six
G4	Giza 126	Naked	Baladi Bahteem/S D729-Por12762-BC	Egypt-1995	Six
G5	Giza 127	Naked	WI2291/Bags//Harmal-02	Egypt-1995	Two
G6	Giza 129	Hull-less **	Deir Alla106/Cel//As 46/Aths*2	Egypt-2003	Six
G7	Giza 131	Hull-less	CM67-B/CENTENO/CAM-B/ROW 906.73/4/GLORIA-BAR-COME-B/5/ FALCON-BAR/6/LINO	Egypt-2003	Six
G8	Giza 132	Naked	Rihane-05//As 46/Aths*2 Aths/Lignee 686	Egypt-2006	Six
G9	Giza 134	Naked	Alanda-01/4/WI2291/3/Api/CM67//L2966-69	Egypt-2019	Six
G10	Giza 135	Hull-less	ZARZA/BERMEJO/4/DS4931//GLORIA-BAR/COPAL /3/SEN/5/AYAROSA	Egypt-2019	Six
G11	Giza 136	Hull-less	PLAISANT/7/CLN-B/4/S.P-B/ LIGNEE640/3/S.P-B / GLORIA-BAR/ COME-B/5/FALCON-BAR/6/LINO	Egypt-2019	Six
G12	Giza 2000	Naked	Giza117/Bahteem52// Giza118/ FAO86 / 3/ Baladi16/ Gem.	Egypt-2003	Six

TABLE 2. IRAP primer names, sequences, GC%, Fragment range (FR) monomorphic band (MB), total bands (TB), polymorphism band (P) polymorphism percentage (P%), polymorphism heterozygocity index (H), information content (PIC), effective multiplex ratio (E), arithmatic mean of H (HAV), Discriminating power (D), marker index (MI) and resolving power (RP), as revealed by IRAP profiles in 12 Egyptian Barley cultivars

Code	Sequence	GC%	FR(bp)	TB	M	P	%P	H	PIC	E	H.av	MI	D	R
680	5CTTACAAATATGGATGGAGGAGTTACC3	41.4	100-900	4	3	1	25	0.04	0.04	11.75	0	0.01	0.04	0.5
708	5TGTGCCCCGCGATGAGAATCAAAATGG3	53.8	200-1500	10	1	9	90	0.47	0.36	7.5	0	0.03	0.61	5.4
713	5GTGGGTTTGGCCGGGTGTTAG3	63.6	200-1150	7	5	2	20	0.34	0.28	9.43	0	0.04	0.38	5.14
727	5TCGCACTAGTCCCTGAGGAGACGATA3	53.8	200-1250	6	0	6	100	0.41	0.33	8.5	0.01	0.05	0.5	6.33
815	5AGGATCTCTAGACTGGCCCCCAC3	59	200-1200	7	3	4	57	0.41	0.32	8.57	0	0.04	0.49	6.86
823	5CTGCACGCACCCGGTGATGC3	70	300-800	3	1	2	67	0.24	0.21	10.33	0.01	0.07	0.26	2.67
829	5CTACAAACCTCTGCGCTTGGAG3	54.5	200-1000	4	1	3	75	0.41	0.33	8.5	0.01	0.07	0.5	6
813	5TGTCACGCCCAAGATGCGATTCTATC3	50	200-1000	6	1	5	83	0.42	0.33	8.33	0.01	0.05	0.52	4.67
1368	5CATGTTGGTCTGTGAGGGCAG3	50	500-800	2	1	1	50	0.15	0.14	11	0.01	0.07	0.16	2
4383	5GGAAGGTGGGCATCGGGCTG3	70	200-1500	5	2	3	60	0.36	0.29	9.2	0.01	0.05	0.42	4.8
4384	5TGCCTCTAGGGCATATTTCCAACAA3	55	200-1500	5	1	4	80	0.46	0.35	7.8	0.01	0.06	0.58	6.8
831	5AATTTCCCGGTCGGTCACC3	57.9	200-1000	4	2	2	50	0.33	0.28	9.5	0.01	0.07	0.38	5
	Total			63	21	42		4	3			1	5	56
	Mean			5.3	2	4	63	0.34	0.27	9.2	0.01	0.051	0.404	4.68

TABLE 3. Chemical analysis of protein, fiber, fat, and ash percentage

No.	Cultivar	Protein %	Fiber %	Fat %	Ash %
1	Giza 123	10	7.3	3.68	2.48
2	Giza 124	11	7.09	3.63	2.61
3	Giza 125	12	6.82	3.81	2.43
4	Giza 126	12	6.47	3.88	2.43
5	Giza 127	9.9	4.23	4.21	2.01
6	Giza 129	8.7	3.56	4.22	1.78
7	Giza 131	11.6	1.53	3.43	1.66
8	Giza 132	10.5	7.13	3.15	2.15
9	Giza 134	12.2	6.92	3.07	2.46
10	Giza 135	12.2	3.41	2.68	2.37
11	Giza 136	12.3	3.18	2.91	2.05
12	Giza 2000	11.9	6.73	2.92	2.45
Min		8.7	1.53	2.68	1.66
Max		12.3	7.3	4.22	2.61
Average		11.19	5.36	3.47	2.24

Chemical Composition

We analyzed the chemical composition of the seed powder of twelve cultivars of barley to determine the quality of the food and the feed for the studied cultivars. The studied cultivars differed in their characteristics, e.g., protein, fat, fiber, and ash In the protein trait percentage, the average of the trait was 11.19%, and Giza 129 was the lowest in content, while Giza 135 was the most in the protein content, 12.2 %. In the quality of fibers, the general average was 5.36%, and the lowest varieties were Giza 131, which contained 53% while the highest of the varieties was Giza 123, and it contained 7.3%. In the characteristic of fats, the general average was 3.47%, and the least cultivar was Giza 135, which was brief. This shows the inverse relationship between the fat content and the protein content in Giza 129 cultivar. In ash content, the average was 2.24, the lowest ash content Giza 131 cultivar this was brief. The highest ash content was Giza 124, and this shows the positive relationship between the ash and fiber contents.

IRAP-primer molecular marker traits

Results from IRAP-PCR products and analysis involved 12 primers of the IRAP 63 bands, 21 monomorphic bands, and 42 polymorphic bands (Table 3). The proportion of appearing polymorphism was 63%. This multiplicity was used to measure the difference between the studied cultivars, but there were no unique bands in all cultivars for each IRAP primer. The TE were constituted 80% between barley DNA. In the first primer IRAP-680, the GC percentage showed the lowest (41.4%) of the used prefixes. This IPAP-680 primer produced four bands that matched 100–900 (bP) and had a 25% polymorphism ratio, meaning one band polymorphism of the four bands. Primer IRAP-680 was given a ratio of (H) to the amount of (0.04), the least of its production in all primers in this study. The same value was also PIC, which is a reflection in the height of the top of (E) for (11.75), and the value of marker index (MI) was lower. These values and the ratio of (D) were the lowest value in the primers, and (R) was the lowest value as well. The second molecular primer, IRAP-708, had a GC ratio of 53.8%, which gave ten bands with a polymorphic ratio of 90%, meaning that there was only one primer that had an appearance. This primer, IRAP-708, with the highest values was given in H (0.47), PIC (0.36), D (0.61). Since they are like (E) HAIV, MI, and R values were 7.50, (0.00), 0.03, and 5.40, respectively.

In the third molecular primer, IRAP-713, the percentage of% GC was to a certain extent, during which it produced seven bands ranging from 200 to 1150 (PB), and the percentage of polymorphism was 20% i.e., two bands between the seven bands. While the primer, IRAP-713 gives H, PIC, E 0.34, 0.28, 9.43, respectively. In other terms, the ratio of MI, D, R 0.04, 0.38, 5.14. In the fourth, primer IRAP-727, the ratio of GC was 53.8 to follow six bands ranging between 200 and 1250bP and by 100% estimated polymorphism, which is the highest percentage in all IRAP primers used in the study. The category values H, PIC, and E are slightly higher than the rest of all primers, as are R, D, and MI values at 6.86, 0.49, and 0.04, respectively. In the fifth primer IRAP-815, the GC% was 59% of those given seven bands ranging from 1200 to 200 with ratios of a polymorphism 57% and H, PIC, and E, which were 0.41, 0.32, 8.57, respectively, and the highest percentage was given in R 6.86 giving 0.49, 0.04 for D and MI, respectively.

In the sixth primer, IRAP-823, the GC% ratio among the highest proportions was 70%, which gave three bands ranging between 300 and 800 (bp), and the polymorphism was 67%, and the characteristics of the molecular initiator H, PIC, E were 0.24,0.21,10.33 (Fig.1). The E ratio is high, and the ratio is (R). Low 2.67 and D ratio of 0.26 while MI. 0.07 and the ratio of HAV is 0.01 G. primer No. 7 IRAP-829, the ratio of GC was 54.5%, which ranged between ratios estimated at polymorphism 75%, and the molecular marker traits were H, PIC, E 0.41, 0.33, 8.50 while the HAV traits were, MI, R, and D were 0.01, 0.07, 0.50, and 6.00, respectively.

In the eighth primer, which is IRAP-813, the GC ratio was 50%. IRAP-813 produced six bands, and the polymorphism ratio was 82%. The characteristics of the molecular marker H, PIC, and E were 0.42, 0.33, and 8.33, respectively. The rest of the traits were HE, MI, D, R was medium. In the primer No. 9 IRAP-1368, the percentage of GC was 50%, which produced two bands, the polymorphism was 50%, and the rest of the characteristics of primer IRAP-1368 were other than traits. The value of E was the high value (11). In primer No. 10 IRAP-4383 the percentage of GC was 70%. This primer produced five bands with a polymorphism rate of 60%, so the character of the primer was R 4.80, D 0.42, and E 9.20.

Primer No. 11 IRAP-4384 had a percentage of GC of 55%. Ranging from 200 to 1500bp where the number of bands was five bands with polymorphism of 10% and the high value of (H) was in this primer, and the R ratio was also quite high. The last primer was IRAP-831 with a percentage of GC of 57.9%. This primer produced four bands of polymorphism ratio of 50% and was among the 200 to 10000 and R which reaches a value of 5.08. The results agree with Shehata et al. (2015) where they found that retrotransposon-based techniques (IRAP, RAP, and REMAP) showed a greater number of bands more than those nonretrotransposon-based (RAPD and ISSRs) techniques which make them a useful approach as molecular markers. Campbell et al. (2011) determined the IRAP system demonstrated an average of one hundred and 21 bands per primer, with 15 polymorphisms of which nine were original non-parental bands. Furthermore, Elframawy & EL-Bakatoushi (2017) introduced 96 bands of >100–1500 bp, of which 84 were polymorphic, as well as the highest MI, was observed with the primer Nikita (3.93) and the lowest with the primer LTR2 (1.05) with an average MI of 2.28 per primer.

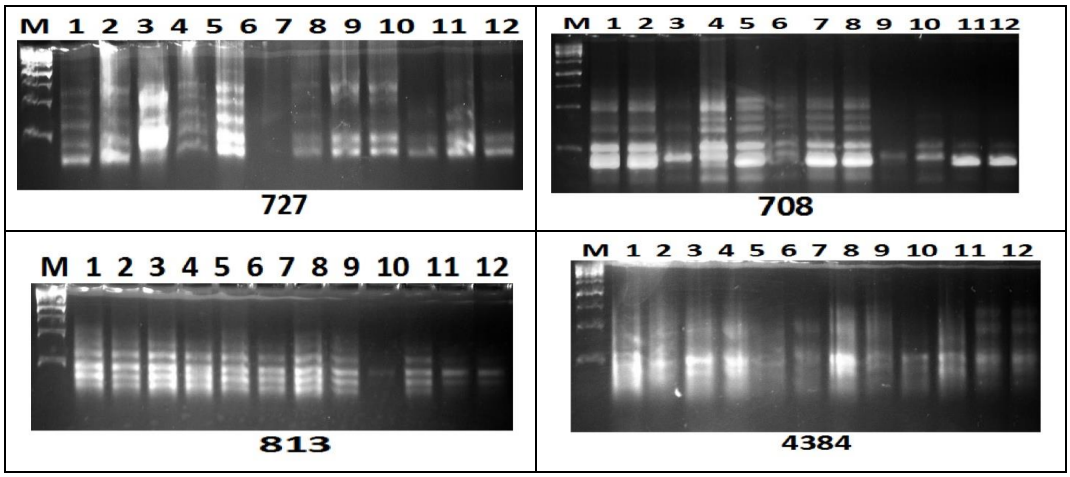

Fig. 1. Agarose gel electrophoresis PCR amplicons for IRAP primers showing the polymorphism of IRAP markers [DNA size marker 1ng/5uL 10Kb plus DNA ladder (lane M) was used as molecular size standards in bps. Lanes from 1 to 12 refer to the sampling code of the studied cultivars]

IRAP markers and cultivar analysis

Dendrogram

The profile and distribution of the genetic relatives in the dendrogram showed that the cultivars were distributed in three clusters (Table 4). The first cluster contains the genotype of Giza2000 and Giza136, while the genotype Giza134 is a single cluster. The second cluster includes four cultivars, and upscale is distributed in two groups; the repentance group contains the genotypes Giza131 and Giza132, whereas the second group contains the genotypes Giza135 and Giza129. In the third and last cluster, four genotypes fall into two independent groups, and the first group includes the cultivars Giza125, Giza126, and the other group was with the cultivars, Giza3, 12th, Giza124 (Fig. 2 and Table 4). These findings agree with Campbell et al. (2011) who noted that retrotransposon-based marker systems, such as IRAP, rely on retrotransposons such as BARE-1 as a precise tool for the detailed description of mutation profiles. Barely accessions are clustered according to their pedigree and caryopsis character (hulled or naked caryopsis). This study shows the efficiency of IRAP markers as a genetic tool for selecting suitable accessions for breeding programs (Elframawy & EL-Bakatoushi, 2017; Ahmed & Al-Sodany, 2020). Cheraghi et al. (2018) determined average PIC values for REMAP and IRAP markers were 0.38. The marker system of retro-elements produced 76 alleles in the range of 1000–3000bp. Kalendar et al. (1999) obtained a polymorphism percentage between 53% and 83% with a polymorphism average of 67% in a genetic evaluation study of *Hordeum vulgare* L. using five selected primers of REMAP.

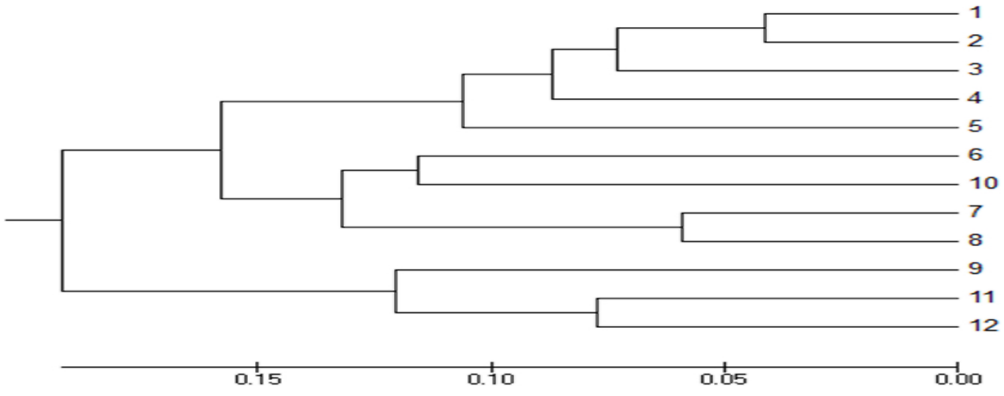

Fig. 2. Cluster tree illustrating the genetic distance, based on the analysis of 12IRAP primers for 12 Egyptian cultivars using the Euclidean distance and the UPGMA algorithm in the PAST software

TABLE 4. Genetic dissimilarity among twelve Egyptian barley cultivars based on IRAP banding pattern

	G.123	G.124	G.125	G.126	G.127	G.129	G.131	G.132	G.134	G.135	G.136	G2000
Giza 123												
Giza 124	**0.08**											
Giza 125	0.19	0.1										
Giza 126	0.21	0.15	0.15									
Giza 127	0.27	0.17	0.21	0.19								
Giza 129	0.34	0.37	0.41	0.38	0.23							
Giza 131	0.31	0.29	0.34	0.31	0.21	0.19						
Giza 132	0.34	0.31	0.36	0.25	0.23	0.34	0.12					
Giza 134	0.45	0.43	0.34	0.41	0.43	0.45	0.43	0.41				
Giza 135	0.41	0.34	0.29	0.31	0.29	0.23	0.21	0.31	0.29			
Giza 136	0.43	0.36	0.36	0.34	0.31	0.38	0.27	0.38	0.23	0.19		
Giza 2000	**0.56**	0.48	0.48	0.41	0.43	0.41	0.34	0.31	0.25	0.29	0.15	

Principal Component Analysis (PCA)

The PCA analysis divided the genotypes into four different groups from each other, and it was in the first quadrant Giza123, Giza124, Giza125, and Giza126. The second quadrant contained only Giza127 and Giza129 cultivars because of their strong link to each other. The third quadrant was the genotypes Giza131, Giza132, and G135 lie with each other, leaving one-quarter of the cultivar's distribution, which is the last quarter, in which the genotypes Giza134, Giza136, and Giza2000 are located together (Fig. 3). The study confirmed the data by Shitian et al. (2020), which explored the three-dimensional PCA of the 58 Asian bamboo genetics, based on the variance-covariance conditions, displayed 6.5%, 7.9%, and 13% of the total divergence based on IRAP marker for the 1st, 2nd, and 3rd component axes, respectively.

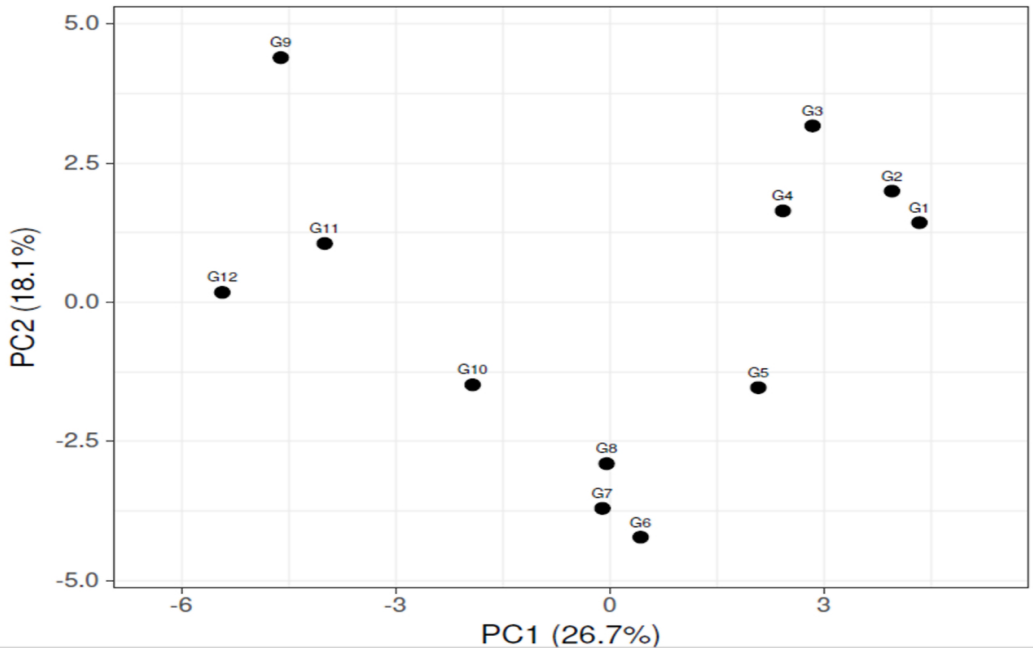

Fig. 3. Principle component analysis (PCA) scatter diagram illustrating the genetic diversity expressed by the grouping of the 12 Egyptian barley cultivars based on their analysis of IRAP marker Polymorphism and by blotting the first two principle component using PAST software

Multivariate heat map

In the heat map, the occupations were distributed into three clusters. The first cluster contains three groups, including the first groups containing Giza131 and Giza132 cultivars No. 8, whereas the cultivar Giza135 fell into a single group. The third group includes cultivars Giza127 and Giza129, whereas the second cluster includes genotypes in two groups. The first group contains Giza131 and Giza132 cultivars. The second group contains Giza135 cultivars and Giza129. Finally, in the third and last cluster, four cultivars fall into two independent groups., The first group includes Giza125 and Giza126 cultivars, and the other group contains Giza123, and Giza124 cultivars (Fig. 4). Manosh et al. (2010) compared with the result obtained with IRAP, RAPD, ISSR, and REMAP markers. They found that IRAP and REMAP can be reliable molecular markers for diversity study, fingerprinting, and mapping citrus and its relatives.

There is a similarity between the PCA distributions and the heat map distributions, and the dendrogram distributions in the difference distribution. In PCA, the cultivars Giza123, Giza124, Giza125, and Giza126 are in one quadrant. It is the same in the heat map as well as in the dendrogram, in the same cluster. Likewise, cultivars Giza136 and Giza2000 fall into the three-way subdivisions PCA, heat map, and dendrogram. Cultivars Giza126 and Giza135 occurred in PCA in a different group, while in the same cluster in the heat map and dendrogram. Giza127 and Giza129 cultivars fall together in one cluster in the heat map, the dendrogram, and the PCA; cultivars Giza131 and Giza132 are located together in the PCA, heat map, and dendrogram. This indicates that the results match the three analyzes (Figs. 2, 3, and 4). Table 4 shows that the lowest percentage difference was between Giza2000 and Giza12, showing that the percentage difference showed Giza123 and Giza124. Kalendar & Schulman (2014) demonstrated the primary methods of REMAP, IRAP, SSAP, and RBIP and detected all polymorphic sites in which the retrotransposon DNA is combined into the genome. Marker approaches exploiting these methods can be developed cost-effectively, particularly in the absence of complete genome sequence data. The data revealed distributions between the varieties based on the molecular characterization. The varieties were distributed among the first group affected by a specific set of molecular markers IRAP4384, IRAP1368, and IRAP829. This group of taxa was Giza2000, Giza136, and Giza134. The second group included the rest of the Egyptian cultivars Giza124, Giza131, Giza127, Giza132, Giza123 Giza129, and Giza125. The phytochemistry was not affected by the cultivar's distribution. Distribution of cultivars based on the type of marker used and its effect on the genetic diversity of barley cultivars. The results were concentrated, as shown in Fig. 5, in two groups that make up most of the varieties, which is equivalent to the effect of primer quality on the genetic diversity.

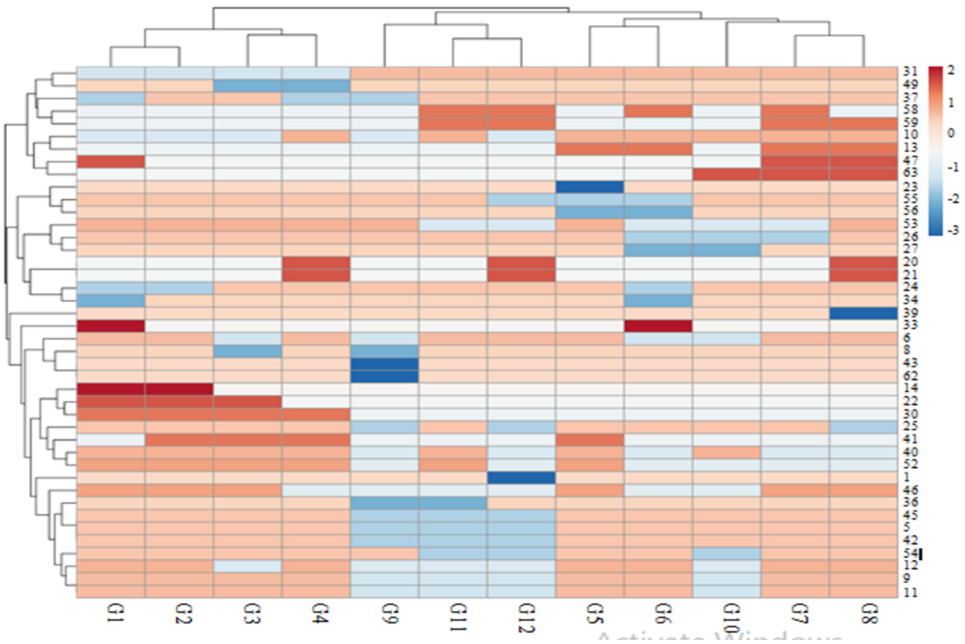

Fig. 4. Multivariate heat map illustrating the genetic diversity of twelve Egyptian barley cultivars based on the 12 IRAP markers constructed using the module of heatmap of R software

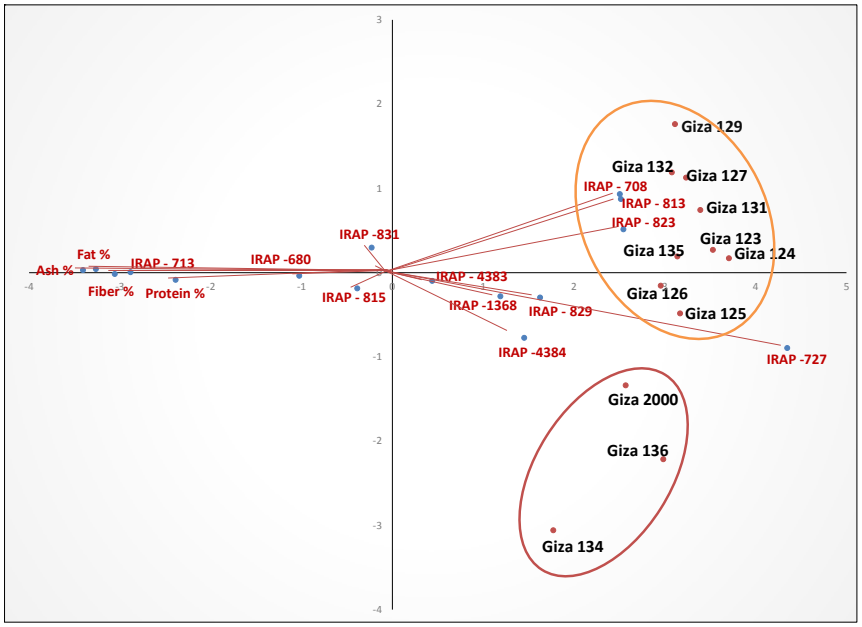

Fig. 5. Bioblot analysis based on the characteristics of chemical analysis and molecular characterization by means 12 IRAP markers using the module of Bioplot of SASTAT 13.2 software

Conclusion

We assessed the genetic diversity of some Egyptian barley cultivars. Moisture content, crude, lipids, ash, and crude fiber contents were measured in the studied cultivars. The multiplicity was used to measure the difference between the cultivars. There was a similarity between the PCA distributions and the heat map distributions. Marker approaches exploiting these methods can be developed and are costly-effective. The data showed a significant difference at the level of molecular detection among all the collected varieties of barley cultivars in Egypt.

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استخدام تقنية IRAP-PCR في تقييم التنوع الحيوي بين أصناف الشعير المصرية

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تم تقدير التنوع الوراثي بين أصناف الشعير المصرية عن طريق 12 بادئ جزيئي من المعلم الجزيئي الـــ IRAP بالإضافة الى الصفات الكيماوية للأصناف الوراثية مثل نسبة الرطوبة، والدهون، والرماد، والبروتين في الاصناف تحت الدراسة. أظهرت النتائج المتحصل عليها أن متوسط نسبة البروتين كانت 11.9%، وقد احتوى الصنف جيزة 135 على اعلى نسبة بروتين 12.3%، بينما احتل الصنف جيزة 129 المرتبة الأخيرة بين الأصناف لإحتوائه على نسبة 8.7%. أنتجت البادئات الجزيئية الإثنى عشر 63 حزمة جزيئية. كما أظهرت البادئات من هذا المعلم الجزيئي نسبتي 33.3%، و66.7% وحيدة المظهر ومتعدد المظهر على التوالي. كما أظهرت الصفات المعلوماتية المختلفة لسلوك المعلم الجزيئيIRAP مع الأصناف المصرية تتراوح نسبة التعدد المظهري المختلط بين (0.004 إلى 0.47) وأيضا نسبة المعلوماتية PIC (0.36 – 0.04)، وكذلك E (11.75 – 7.50)، وأيضا قيمة H.av (0.00 الى 0.01). كما أوضحت قيمة MI مدى بين قيمة (0.04 – 0.07)، و قيمة D (0.04 – 0.61)، وقيمة R (0.5 – 6.86). كما أظهرت النسبة العامة للتعدد المظهري 63%، وهذا يوضح الكفاءة في التفريق بين الأصناف على الرغم من عدم وجود حزم فريده في كل المعلمات الجزيئية المستخدمة مع الأصناف تحت الدراسة. على الجانب الآخر فقد تم أستخدام نسبة التشابه في تحليل ال PCA وكذلك توزيعات الخريطة الحرارية والتي قسمت الأصناف المصرية إلى أربع مجموعات. كما وزعت الشجرة الوراثية أصناف جيزة 123، وجيزة 125، وجيزة126 . كما أظهر تحليل BioPlot توزيع الأصناف المصرية في اربع مجموعات أيضا.