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Phenotypic Characterization, Genetic Variation and Yield Performance of Some Egyptian Barley (*Hordeum vulgare* L.) Genotypes

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Abstract

Seventeen promising lines of barley (*Hordeum vulgare* L.) were grown in two consecutive growing seasons to evaluate the morphological, genetic variation and yield performance under field experimental conditions compared with three varieties (Giza 2000, Giza 132 and Giza 133). Twenty genotypes were distributed in a randomized complete blocks design in three replications. Twenty nine morphological traits were described using UPOV (The International Union for the Protection of New Varieties of Plant) Guidelines. Results showed that most of the morphological and yield characters were very different among all genotypes. Meanwhile, the heading data and maturity date characters varied among the lines comparing to their check varieties. The seed yield components had the highest mean values in lines 1 to 7, as the seed index had the lowest weight in two lines and reached the superior weight in 10 lines. The molecular characterization was carried out using 15 anchored-ISSR primers. The selected ISSR primers amplified a reliable banding pattern with the studied barley genotypes. The total percentage of polymorphism was 84.5%, recorded for 240 polymorphic bands out of 284 total bands. The fifteen used ISSR primers were able to characterize 14 lines and 3 check varieties with total 35 unique markers. The highest genetic similarity was 0.84, and the lowest similarity index revealed 0.60. The resulted phylogenetic dendrogram clustered the twenty genotypes into two main clusters, where the check varieties grouped together with 0.75 similarity. The most noted observation was that the high level of genetic homogeneity and the high similarity matrix (ranged from 0.64 to 0.84 similarity) recorded for within and between genotypes. Based on the barley yield traits, out of ten revealed Eigen values, the first three PCs assigned 69.67% variation for all studied traits. These results concluded that 17 lines may be considered as genetic resources for indirect and direct selection criteria in breeding program to improve the grain barley quality and quantity.

Keywords: Barley, Phenotyping, Agronomical traits, ISSR, PCA.

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Introduction

Barley (*Hordeum vulgare* L.) is one of the most important crop species world widely subjected to considerable intensive genetic improvements. Barley is diploid (2n = 2x = 14), mainly self-fertilizing species (Bennett and Smith, 1976), with a large genome with about 5 Gb genome size (International Barley Genome Sequencing Consortium, 2012). Barley grown under saline and drought conditions (Kovacevic *et al.*, 2018). Its grains used for food and feed, meanwhile, the straw has a serious role in roughage. Various ways could be used to increase cereal production, such as cultivating larger production areas, improving agricultural practices, and manipulating promising cultivars (Al-Tabbal and Al-Fraihat, 2011).

The new populations can be simply introduced among different regions as well as improving novel breeding lines (Ifftikhar *et al.*, 2009). Identification of superior genotypes with the desired characteristics have a critical role in plant breeding programs, especially in selecting criteria that is useful to produce improved novel varieties. Analysis of variability among the studied traits and their associations, are the fundamentals for crop improvement strategies (Mary and Gopalan, 2006). The global barley production increased by 30.2% in the last decade. In Egypt, the barley production increased by 59.5% , although its cultivated areas were decreased by 53.2% during the period from 2009 to 2019. The main reason for decreasing local Egyptian production is the low rate comparing to other main winter crops, such as wheat and forage crops (FAO, 2020). Identification of crop varieties had become increasingly important to the documentation of genetic resources and to the protection of the breeders' rights. Morphological characterization is the pillar of genetic diversity, crop germplasm, and breeding programs. A high variability level was observed within and among barley populations (Gupta *et al.*, 2009). Two types of barley are classified based on the kernel numbers in the head. The first type called *Hordeum vulgare* with six-row, which is a commonly cultivated variety. The second one is two-row, known as *Hordeum distichum* which is also known as *Hordeum* (Katz *et al.*, 2003). Barley shares similar morphological traits as wheat and rye. It has two rooting systems; the first starts when seedling roots developed to a tillering stage, the second begins when the growing reached deeper as crown roots, it helps in absorption of the water and nutrients. The plant height trait average reached one meter and the plant stem is ranged from five to seven joints that separated by the nodes where the leaves is grown. The leaves are narrow, linear lanceolate and consist of ligule, auricles, blade and sheath (Newman, 1991). Ahmed *et al*. (2021, 2018 and 2014) and Ashrei *et al.* (2018) investigated the genetic diversity of barley, flax, faba bean and lupine genotypes using different methods, such as molecular markers, morphological traits and yield components, which result's indicated that the variation existed within the genotypes for most of characters and could be investigated using these different techniques.

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Assessment of the genetic diversity among barley genotypes and their yield performance is necessary for the future usage in breeding programs to improve stability and grain yield (Al-Sayaydeh *et al.,* 2019). Several biochemical techniques have been used to complement morphological characterization of barley cultivars, most of them based on isoenzymes or seed storage proteins (Canci *et al*., 2003; Laugesen *et al*., 2007). Nevertheless, characterization with these types of markers were not sufficient for barley varieties, as the low levels of allelic variation in many traits which is dominated by enzymatic loci, the high degree of genetic relationship among the different varieties, and the high degree of polymorphism within varieties. The advent of the polymerase chain reaction (PCR) favored the development of different molecular techniques, such as random amplified of polymorphic DNA (RAPD), simple sequence repeats (SSR or microsatellites), sequence-tagged sites (STS), random amplified microsatellite polymorphism (RAMP), and inter simple sequence repeats (ISSR) (Wu *et al*., 1994). ISSR markers, which involve PCR amplifications of DNA using a primer, composed of a microsatellite sequence anchored at 3’ or 5’ end by 2–4 arbitrary nucleotides, used to assess genetic diversity and polymorphism (Qian and Hong, 2001). ISSRs have been used for cultivar identification for potatoes (Prevost and Wilkinson, 1999), wheat (Nagaoka and Ogihara, 1997), bean (M´etais *et al.,* 2000), and barley (Tanyolac, 2003 and Fern´andez *et al.,* 2002). Guasmi *et al*. (2012) explained that the ISSR and RAPD molecular markers were preferable in the genetic studies of barely specimens. Principal component analysis (PCA) is a multivariate analysis considered as a useful tool used to establish genetic relationship among genotypes, used to study the correlation among variables (Gurrieri *et al.,* 2001). Certainly, the visual result biplot graph of principal components analysis would be a useful tool to discover the interrelationships among yield and morphological traits.

The overall aims of the present study are 1) evaluating yield and its components, 2) identification of morphological traits and molecular markers for 17 promising barley lines and 3 check varieties grown under the prevailing Egyptian environmental conditions.

Materials and methods

Plant Materials

New 17 promising barley lines (recorded from Line1 to Line17) were studied along with 3 commercial check varieties (Giza 2000, Giza 132 and Giza 133). These twenty barely genotypes are presented in Table 1. Genotypes were obtained from the Barley Research Department, Field Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

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Table 1 Line name and pedigree of the studied barley genotypes

No.	Cross Name
Line 1	C .C 89 /6/Cen/Bglo'S'/5/Baca'S'/3/AC253//CI05761/4/Mari/ Aths*2//M-Att-73-337-1
Line 2	C .C 89 /6/Cen/Bglo'S'/5/Baca'S'/3/AC253//CI05761/4/Mari/ Aths*2//M-Att-73-337-1
Line 3	Giza 125/5/Alanda-01/4/WI2291/3/Api/CM67//L2966-69
Line 4	ACSAD 1164/3/Mari/Aths*2//M-Att-73-337-1/7/GIZA121/ CI06248/4/Apm/B65//11012-2/3/Api/ CM67//Ds/Apro/5/Srs-04/6/Can/Bgla"S"
Line 5	Aths/Lignee 686/5/ACSAD618/5/M9878/CARDO//QUUINA/3/ CHAMICO/4/CIRU
Line 6	JLB70-20/Sen"s"//Rihane-03
Line 7	Lignee527/Chn-01//Gustoe/5/Alanda-01/4/WI2291/3/Api CM67//L2966-69
Line 8	Giza 118 /6/Ager//Api/CM67/3/Cel/wi2269//Ore/4/Alanda/5/ CompCr229//As46/Pro/3/Srs
Line 9	Giza 118 /4/Rhn-03/3/Mr25-84/Att//Mari/Aths*3-02
Line 10	Giza 118 /4/Rhn-03/3/Mr25-84/Att//Mari/Aths*3-02
Line 11	ACSAD 1164/3/Mari/Aths*2//M-Att-73-337-1/4/Aths/ lignee686 /3/Deir Alla 106//sv.Asal/ Attiki /4/Cen/Bglo."S"
Line 12	ACSAD 1164/3/Mari/Aths*2//M-Att-73-337-1/4/Aths/ lignee686/3/Deir Alla 106//sv.Asal/Attiki/4/Cen/Bglo."S"
Line 13	Aths/Lignee 686/5/Apm/RL/4/Api/EB489-8-2-15-4//por/
Line 14	C .C 89/4/AwBlack/Aths//Rhn-08/3/Malouh
Line 15	Giza 121/6/Alanda-01/5/CI01021/4/CM67/U.Sask.1800// Pro/CM67/3/DL70
Line 16	Giza 123/6/Alanda-01/5/CI01021/4/CM67/U.Sask.1800// Pro/CM67/3/DL70
Line 17	ACSAD 1182/4/Arr/ESP//Alger/Ceres362-1-1/3/WI/5/ Alanda/Hamra//Alanda-01
Giza 2000	Check variety, Egypt
Giza 132	Check variety, Egypt
Giza 133	Check variety, Egypt

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Field Experiments

Genotypes were sown in the 2nd half of November during two successive seasons 2020/2021 and 2021/2022 in the Giza Research Station, ARC**,** in a randomized complete block design (RCBD) with three replicates, in plots area 10.5 m^2 (3x3.5 m^2). Seeds were on rows, and fertilized with the recommended dose of fertilizer (NPK 100:70:50) kg/ha. Weeds were removed by hand prior to the flowering stage, and all standard agricultural practices were followed for raising the crop.

a) Morphological Characteristics

The identification of the studied morphological characteristics were conducted using UPOV (The International Union for the Protection of New Varieties of Plant) 2018 barley descriptor. The studied twenty nine characters were plant growth habit, lowest leaves hairiness of leaf sheaths, flag leaf anthocyanin coloration of auricles, flag leaf intensity of anthocyanin coloration of auricles, plant frequency of plants with recurved flag leaves, flag leaf glaucosity of sheath, time of ear emergence (first spikelet visible on 50% of ears), awns anthocyanin coloration of tips, awns intensity of anthocyanin coloration of tips, ear glaucosity, ear attitude, plant: length (stem, ear and awns), ear number of rows, ear shape, ear density, ear length (excluding awns), awn length (compared to ear), rachis length of first segment, rachis curvature of first segment, sterile spikelet attitude (in mid-third of ear), median spikelet length of glume and its awn relative to grain, grain rachilla hair type, grain husk, grain anthocyanin coloration of nerves of lemma, grain speculation of inner lateral nerves of dorsal side of lemma, grain hairiness of ventral furrow, grain disposition of lodicules, kernel color of aleurone layer and seasonal type were recorded. The decimal code for the growth stage of legume according to Tottman *et al.* (1987) was also used to standardize the growing stages of varieties during the morphological description and identification.

b) Agronomical Characters

At the harvest stage, ten guarded plants were randomly sampled from the central row to estimate and measure: plant height (Plth) (cm), No. of tillers per each plant in m^2, No. of spikes per each plant in one m^2, No. of grains per spike (No. g/s), No. of days to heading (HD), No. of days to maturity (MD), seed index, biological yield (ton/ha) (BY), grain yield per plant (GY) (ton/ha) and straw yield (SY) (ton/ha).

c) Molecular Characterization

The twenty barley genotypes were characterized based on DNA level using fifteen anchored-ISSR markers. The healthy young shoots were subjected to the DNA isolation using

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Qiagen DNeasy plant kit (Chatsworth, CA), and adjusted to 50 ng/µl using Biophotometer (Eppendorf). Fifteen robust ISSR primers were selected to characterize the twenty barley genotypes (Table 2). The ISSR primers synthesized by HVD Corporation (Germany). The ISSR-PCR reactions carried out in 25µl, composed of Emerald Amp Max (2X ready mix), 20 pmol oligonucleotide, and 50 ng genomic DNA. The reactions were performed on Eppendorph Master Cycler (Germany) as followed: 35 cycles, started with initial step at 95°C for 5 min, followed by 35 cycles, as denaturation step at 94°C for 1 min, annealing step (Ta) for 1 min, and extension step at 72°C for 1 min, and a final extension step at 72°C for 10 min. Ladder DNA used was Thermo 100 bp plus. The amplified reactions were visualized on 2% agarose gel, stained by ethidium bromide, and photographed by Alpha Innotech gel image system.

Table 2 ISSR primers used to characterize the twenty barley genotypes

Primer name	Primer sequence	Annealing Temperature Ta (ºC)	Primer name	Primer sequence	Annealing Temperature Ta (ºC)
17899-B	$(CA)_6$ GG	42°C	841	$(GA)_8$ TC	53 °C
AW-3	$(GT)_7$ AG	42 °C	842	$(GA)_8$ TG	53 °C
ISSR-34	$(AG)_8$ TG	53 °C	809	$(AG)_8$ G	53 °C
ISSR-35	TCGA $(CA)_7$	53 °C	ISSR-4	CGA $(CA)_7$	53 °C
834	$(AG)_8$ CT	53 °C	W-7	$(CT)_8$ GG	49 °C
8	$(CA)_8$ GAC	50 °C	16	CGTC $(AC)_7$	49 °C
17	CAGC $(AC)_7$	50 °C	W844	$(CT)_8$ TG	50 °C
15	GGTC (AC)7	50 °C			

d) PCA Analysis:

The principal component (PC) analysis was applied on the collected morphological and yield components data to show the relationships between all traits of the genotypes.

Statistical Analysis

Analysis of variance using randomized complete block design (RCBD) was computed for all yield characters using MSTAT-C software (version 21), to assess variations among barley genotypes at 0.05 significance level.

The ISSR scorable bands were scored as (1) and (0) for presence and absence. Molecular dendrogram and similarity matrix were calculated using Phoretix 1D Pro software (non-linear

dynamics). PCA analysis was performed using Minitab software package to determine the relationships among phenotypic and yield characters of the genotypes.

Results and Discussion

Simple statistical analysis of the obtained data showed a range of variability among the studied barley genotypes in the evaluation of the morphological and yield performance under field experiment.

a) Morphological Characterization

The results indicated that the lowest leaves: hairiness of leaf sheaths and flag leaf: anthocyanin coloration of auricles were absent, flag leaf: intensity of anthocyanin coloration of auricles was very weak, flag leaf: glaucosity of sheath was absent or very weak, ear: number of rows was more than two, grain husk was present, grain speculation of inner lateral nerves of dorsal side of lemma was absent or very weak, grain: hairiness of ventral furrow was absent, kernel: color of aleurone layer was strongly colored and Seasonal type was spring type of all barley lines and check varieties.

Results represented in Table 3 and Fig 1 shows the most morphological traits which had variation among genotypes under study. Plant growth habit was varied among all genotypes (such as erect, semi erect, intermediate and semi prostrate). Also, plant: frequency of plants with recurved flag leaves for all barley genotypes was between absent or very low, low and medium. Time of ear emergence (first spikelet visible on 50% of ears) was between early and medium average in all genotypes, while awns: anthocyanin coloration of tips recorded as absent and present. Awns: intensity of anthocyanin coloration of tips was very weak in all genotypes, except 2 in lines (Lines 1 and 15) which were medium, and Lines 8 and 11 were weak, Giza 2000 check variety was strong, while Giza 132 check variety and Line 5 were very strong. Ear: glaucosity for all genotypes was recorded absent or very weak and weak only. However, ear attitude was assigned in four categories (erect, semi erect, semi-recurved and horizontal) for all genotypes. Plant length character (stem, ear and awns) varied in all genotypes, and recorded as medium, long and very long.

The resulted characterization showed narrow variation among barley genotypes for ear: shape, which ranged between fusiform, parallel and tapering. Sixteen genotypes were dense, and four genotypes (lines 4, 15, Giza 2000 and Giza 132) were medium for ear: density character. Also, ear length (excluding awns) was short or long in all genotypes, except Giza 2000 and Giza 132 which were medium. The awn length trait (compared to ear) was long in 14 genotypes, and in 4 genotypes (lines 1, 3, 9, 15, Giza 2000 and Giza 133) were medium. Results showed wide

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variation among barley genotypes for rachis length of first segment character from short, medium and long. Results for morphological data are demonstrated in Table 3.

Concerning, sterile spikelet: attitude (in mid-third of ear), median spikelet: length of glume and its awn relative to grain, grain: rachilla hair type, grain husk and grain anthocyanin coloration of nerves of lemma (Table 3 and Fig 1). The results of these traits revealed that all barley genotypes were different in all characters, recorded as weak, medium and strong for rachis curvature of first segment, 15 genotypes were divergent, whereas, Lines 3, 4, 5, 15 and Giza 133 were parallel to weakly divergent for sterile spikelet: attitude (in mid-third of ear) character. Also, median spikelet length of glume and its awn relative to grain categorized in to 3 categories (short, long and equal) in all barely genotypes. Moreover, grain: rachilla hair type was recorded as short and long for all the studied barley genotypes. Data indicated that the grain anthocyanin coloration of nerves of lemma was assigned as absent or very weak, weak, medium, strong and very strong for all barley genotypes. The recorded traits indicated that the grain disposition of lodicules was clasping and frontal in the different genotypes.

Table 3 Morphological characters of significant variation for the studied barley genotypes

No	Character	Class	Percentage	Genotypes
1	Visual Assessment by a Single observation of a group of plants or parts of plants			
	Plant growth habit	Erect	50	Lines 7, 9, 11, 12, 13, 14, 16, Giza 2000, Giza 132 and Giza 133
		Semi erect	30	Lines 1, 6, 8, 10, 15 and 17
		Intermediate	10	Lines 3 and 4
		Semi prostrate	10	Lines 2 and 5
		Prostrate	00	
2	Plant frequency of plants with recurved flag leaves	Absent or very low	5	Line 7
		Low	55	Lines4, 6, 8, 12, 13, 14, 15, 16, 17, Giza132 and Giza 133
		Medium	40	Lines 1, 2, 3, 5, 9, 10, 11and Giza 2000
		Strong	00	
		Very Strong	00	

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Visual Assessment by a Single observation of a group of plants or parts of plants				
3	Time of ear emergence (first spikelet visible on 50% of ears)	Very Early	00	
		Early	35	Lines 3, 5, 7, 8, 15, Giza 132 and 133
		Medium	50	Lines 1, 2, 4, 6, 12, 13, 14, 16, 17 and Giza 2000
		Late	15	Lines 9, 10 and 11
		Very late	00	
4	Awns anthocyanin coloration of tips	Absent	65	Lines 2, 3, 4, 6, 7, 9, 10, 12, 13, 14, 16, 17 and Giza 133
		Present	35	Lines 1, 5, 8, 11, 15, Giza 2000 and Giza 132
5	Awns intensity of anthocyanin coloration of tips	Very weak	65	Lines 2, 3, 4, 6, 7, 9, 10, 12, 13, 14, 16, 17 and Giza 133
		Weak	10	Lines 8 and 11
		Medium	10	Lines 1 and 15
		Strong	5	Giza 2000
		Very strong	10	Lines 5 and Giza 132
6	Ear glaucosity	Absent or very weak	35	Lines 3, 4, 6, 7, 12, 14 and 15
		Weak	65	Lines 1, 2, 5, 8, 9, 10, 11, 13, 16, 17, Giza 2000, Giza 132 and Giza 133
		Medium	00	
		Strong	00	
7	Ear attitude	Erect	10	Lines 10 and 11
		Semi erect	50	Lines 1, 2, 6, 7, 8, 9, 12, 15, 17 and Giza 133
		Horizontal	35	Lines 4, 5, 13, 14. 16, Giza 2000 and Giza 132
		Semi-recurved	5	Lines 3
		Semi-drooping	00	

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Visual Assessment by a Single observation of a group of plants or parts of plants				
8	Plant length (stem, ear and awns)	Short	00	
		Medium	30	Lines 3, 9, 12, Giza 2000, Giza 132 and 133
		Long	55	Lines 1, 2, 5, 6, 8, 11, 13, 14, 15, 16 and 17
		Very long	15	Lines 4, 7 and 10
9	Ear shape	Tapering	40	Lines 2, 5, 7, 8, 10, 11, 16 and Giza 132
		Parallel	35	Lines 3, 4, 6, 9, 14, 17 and Giza 133
		Fusiform	25	Lines 1, 12, 13, 15 and Giza 2000
10	Ear density	Sparse	00	
		Medium	20	Lines 4, 15, Giza 2000 and Giza 132
		Dense	80	Lines1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17 and Giza 133
		Very dense	00	
11	Ear length (excluding awns)	Short	25	Lines 1,2,6,7,8,10, 11, 12,14, 16,17 and Giza133
		Medium	8	Giza 2000 and Giza 132
		Long	67	Lines 3, 4, 5, 9, 13 and 15
12	Awn: length (compared to ear)	Very short	00	
		Short	00	
		Medium	70	Lines 1, 3, 9, 15, Giza 2000 and Giza 133
		Long	30	Lines 2, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 16, 17 and Giza 132

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Visual Assessment by a Single observation of a group of plants or parts of plants				
13	Rachis length of first segment	Short	25	Lines 6, 7, 8, 15 and 16
		Medium	40	Lines 1, 2, 5, 11, 13, 14, 17 and Giza 133
		Long	35	Lines 3, 4, 9, 10, 12, Giza 2000 and Giza 132
14	Rachis curvature of first segment	Very weak	00	
		Weak	35	Lines 2,6, 10, 11, 15, 17 and Giza133
		Medium	40	Lines 1, 3, 7, 8, 9, 13, 14, and Giza132
		Strong	25	Lines 4, 5, 12, 16 and Giza 2000
15	Sterile spikelet attitude (in mid-third of ear)	Parallel to weakly divergent	25	Lines 3, 4, 5, 15 and Giza133
		Divergent	75	Lines 1 2, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, Giza2000 and Giza 132
16	Median spikelet length of glume and its awn relative to grain	Shorter	15	Lines 3, 11 and Giza132
		Equal	50	Lines 5, 6, 7, 9, 10, 12, 13, 14, 15 and Giza133
		Longer	35	Lines 1, 2, 4, 8, 16, 17 and Giza2000
17	Grain rachilla hair type	Short	55	Lines 3, 4, 5, 7, 9, 12, 13, 14, 17 Giza 132 and 133
		Long	45	Lines 1, 2, 6, 8, 10, 11, 15, 16 and Giza2000
18	Grain: disposition of lodicules	Clasping	35	Lines 1, 2, 8, 10, 16 and Giza 132
		Frontal	65	Lines 3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 15, 17, Giza 2000 and Giza 133
19	Grain anthocyanin coloration of nerves of lemma	Absent or very weak	60	Lines 1, 2, 3, 5, 6, 7, 8, 9, 10, 13, 16 and 17
		Weak	5	Lines 15
		Medium	15	Lines11, 12 and Giza 133
		Strong	10	Lines 4 and 14
		Very strong	10	Giza 2000 and Giza 132

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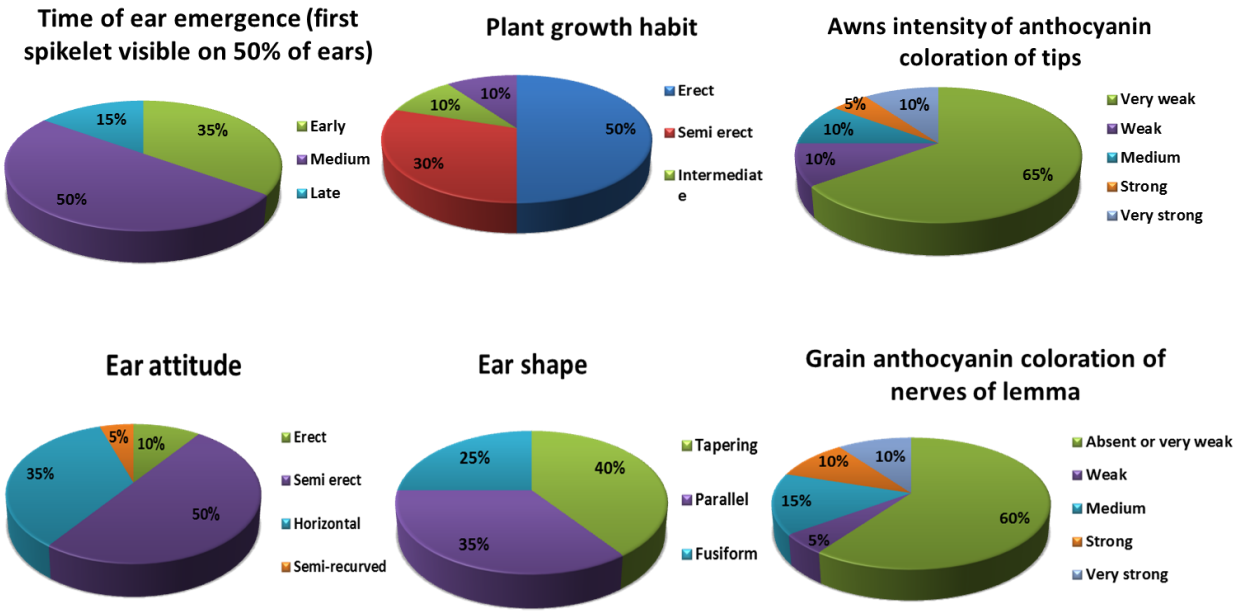

Fig. 1 Frequency distribution of barley morphological traits

b) Agronomical Characters

Comparing the new promising barley lines to the standard check varieties pointed that the promising genotypes were different in their average response with respect to the studied traits. Also, the three check varieties had fluctuated averages among the studied parameters. According to the evaluated characters, their mean values were illustrated into 10 figures. These figures contained the indirect selectable traits, which had the direct selectable traits, for improving grain yield.

Figures 2 to 6 showed the fluctuated variances between the novel lines and the three checks varieties (Giza 2000, Giza 133, and Giza 132). Fig 2 demonstrated the plant height, it was found that the Line7 had the superior tall character, meanwhile, Lines 3, 10, 12 and 17 were recorded as the shortest lines compared to the three checks varieties. Lines 4, 6, 7 and 8 were superiors, while, Lines 11, 12 and 13 had the lowest numbers with respect to their three checks check varieties in No. of tillers and spike (per m^2) traits as illustrated in Fig 3. The heading date and maturity date characters recorded lowest values in Lines 8, 9,10, 12, 13, 15 and 16 for heading date, and Lines 3, 4, 8, 9, 14, 15 and 17 for maturity date. While, Lines 5, 6 and 7 for heading date and Lines 5, 6, 10, 11 and check variety Giza 2000 for maturity date which had highest values as compared to other checks varieties and lines (Fig 4, 5 and 6).

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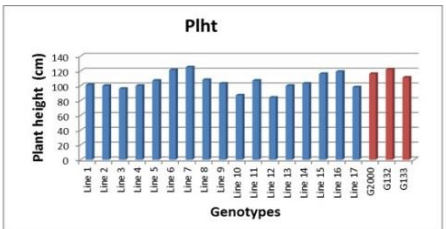

Fig 2. Average mean values of plant height (Plht) (cm) for studied genotypes of barley for two experimental seasons.

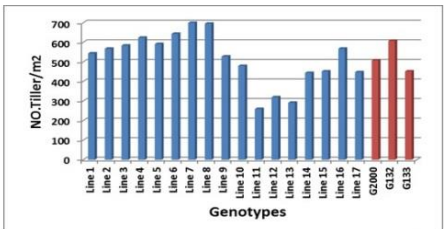

Fig 3. Average mean values of No. of tillers/m^2 for studied genotypes of barley for two experimental seasons.

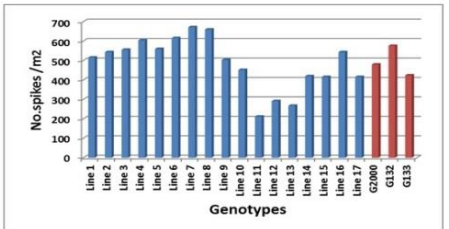

Fig 4. Average mean values of No. of spikes/m^2 for studied genotypes of barley for two experimental seasons.

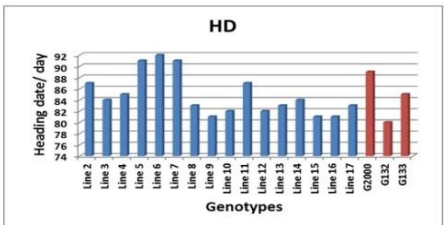

Fig 5. Average mean values of heading date/ day (HD) for studied genotypes of barley for two experimental seasons.

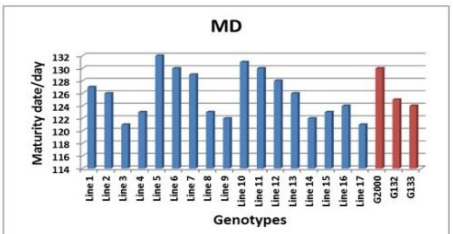

Fig 6. Average mean values of maturity date/ day (MD) for studied genotypes of barley for two experimental seasons.

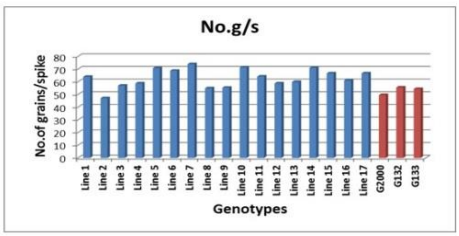

Fig 7. Average mean values of No. of grains/spike (No.g/s) for studied genotypes of barley for two experimental seasons.

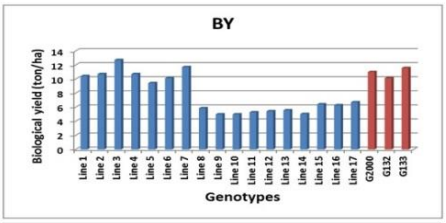

Fig 8. Average mean values of biological yield (BY) (ton/ha) for studied genotypes of barley for two experimental seasons.

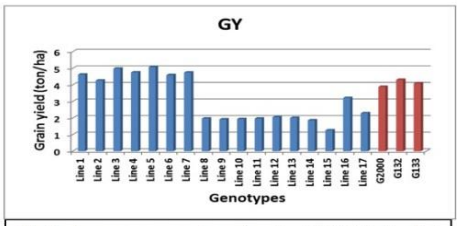

Fig 9. Average mean values of grain yield (GY) (ton/ha) for studied genotypes of barley for two experimental seasons.

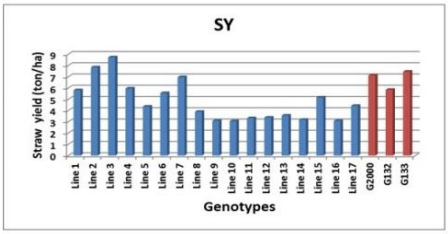

Fig 10. Average mean values of straw yield (SY) (ton/ha) for studied genotypes of barley for two experimental seasons.

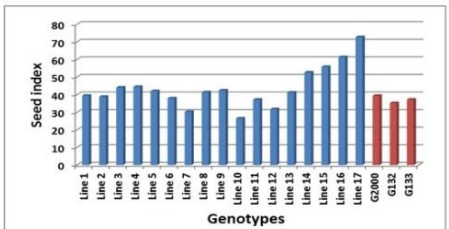

Fig 11. Average mean values of seed index for studied genotypes of barley for two experimental seasons.

Fig. 2 - 11 Barley genotypes yield components characters

Plht: Plant height (cm), HD: Heading date, MD: Maturity date, No. g/s: No. of grains/ spike, BY: biological yield (ton/ha), GY: grain yield (ton/ha) and SY: straw yield (ton/ha).

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In Fig 7, the No. of grain/ main spike trait overpassed among most of the lines, except Lines 5, 7, 10 and 14 which had highest values among all barley genotypes under study. The seed yield components (the biological yield, grain yield and straw yield (ton/ha). Lines 1, 2, 3, 4, 5, 6, and 7 had the best mean values comparing to the other lines and check varieties (Figs 8, 9 and 10). Fig 11 demonstrated the seed index (1000 seed/g) values which was the lowest values for two lines (Line 7 and 10), whereas the superior lines were recorded in Lines 14, 15, 16, 17.

c) ISSR-PCR Molecular Characterization

The fifteen ISSR markers had successful amplification patterns with the twenty barley genotypes, generated a relative high number of fragments.

Marker (842) amplified the highest number of fragments (24 fragments), which also generated the highest number of polymorphic fragments (21 fragments). While marker (AW-3) amplified the lowest number of fragments (13 fragments). The total percentage of polymorphism reached 83.62%, as it recorded for 143 polymorphic bands out of 171 total bands (Table 4).

Barley ISSR-Unique Markers

Out of the twenty genotypes, 17 genotypes were characterized by unique markers, and Lines 3, 6, and 10 had not any unique markers. The 17 characterized genotypes generated 35 unique markers (27 unique positive and 8 unique negative markers). Eight genotypes (Line 2, 4, 7, 9, 14, 15, Giza 133, and Giza 132) were characterized by only one unique positive marker. Meanwhile, Line 1 identified by 6 unique markers (4 unique positive, and 2 unique negative markers), results demonstrated in Table 5.

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Table 4 Primer name, total number of amplicons, amplicons size range (bp), number of monomorphic amplicons, number of polymorphic amplicons, and the percentage of polymorphism used to study barley genotypes

Primer name	Total number of amplicons	Amplicons size range (bp)	Number of monomorphic amplicons	Number of polymorphic amplicons	% Polymorphism
17899-B	17	394-1709	2	15	88.23
AW-3	13	424-1564	3	10	76.92
ISSR-34	19	410-1974	4	15	78.94
ISSR-35	15	278-1318	3	12	80
834	17	301-1811	2	15	88.23
841	16	381-1758	2	14	87.5
842	24	386-2081	3	21	87.5
809	15	292-1240	4	11	73.22
ISSR-4	19	403-1777	2	17	89.47
W-7	16	337-1781	3	13	81.25
8	21	398-1490	2	19	90.47
16	29	403-1426	4	25	89.28
17	19	406-1374	4	15	78.94
W844	25	442-1720	2	23	92
15	19	399-1369	4	15	78.94
Total	284		44	240	84.5
Average	18.86		2.93	16	

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Table 5 Barley genotypes characterization by unique positive and negative ISSR markers

genotype	Total	Unique positive markers (bp)	Unique negative markers (bp)
Line 1	6	ISSR-35 (822 bp), 841 (603 bp), 842 (2081 bp, 1969 bp)	ISSR-34 (1226 bp), 841 (652 bp)
Line 2	1	17 (828 bp)	---
Line 4	1	16 (489 bp)	---
Line 5	2	842 (1773 bp), W-7 (1336 bp)	---
Line 7	1	W844 (1560 bp)	---
Line 8	3	17899-B (1208 bp), 17 (1242 bp), W844 (933 bp)	---
Line 9	1	809 (542 bp)	---
Line 11	2	---	17899-B (546 bp, 473 bp)
Line 12	3	842 (512 bp), 8 (650 bp)	17 (957 bp)
Line 13	3	AW-3 (1180 bp), W844 (1600 bp)	ISSR-4 (652 bp)
Line 14	1	W844 (1491 bp)	---
Line 15	1	---	15 (677 bp)
Line 16	3	AW-3 (895 bp), 842 (386 bp), ISSR-4 (903 bp)	---
Line 17	3	8 (1100 bp), 16 (854 bp)	ISSR-35 (770 bp)
Giza 2000	2	AW-3 (977 bp), W844 (828 bp)	---
Giza 133	1	ISSR-34 (680 bp)	---
Giza 132	1	W844 (629 bp)	---
Total	35	27	8

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Barley Genetic Similarity Matrix

The genetic similarity matrix (data not supplemented) among the barley genotypes were computed using UPGMA methods based on the similarity matrix generated by the fifteen ISSR markers. It was observed that the highest similarity index was 0.84 between (Line 9 and Line 10), while, the lowest similarity index was 0.61 between (Line 6 and Line 16), (Line 6 and check variety Giza 132), (Line 7 and Line 16) and (Line 10 and check variety Giza 132).

The Phylogenetic Relationships among the Twenty Barley Genotypes

The UPGMA phylogenetic dendrogram (Fig 12) was divided into two main clusters at 0.64 similarity. The first one contained only one genotype (Line 16), while the second cluster contained the other 19 genotypes. The second cluster was subdivided (at similarity 0.69) in to two subclusters. The first subcluster contained 5 genotypes. It was observed that the three check varieties (Giza 2000, Giza 133, and Giza 132) were clustered together with 0.75 similarity, where Giza 133 and Giza 132 were closely related than Giza 2000. The second subcluster employed the 14 genotypes, where lines (Line 10 and Line 9) were the most closely related genotypes having 84% similarity index, results demonstrated in Fig 12.

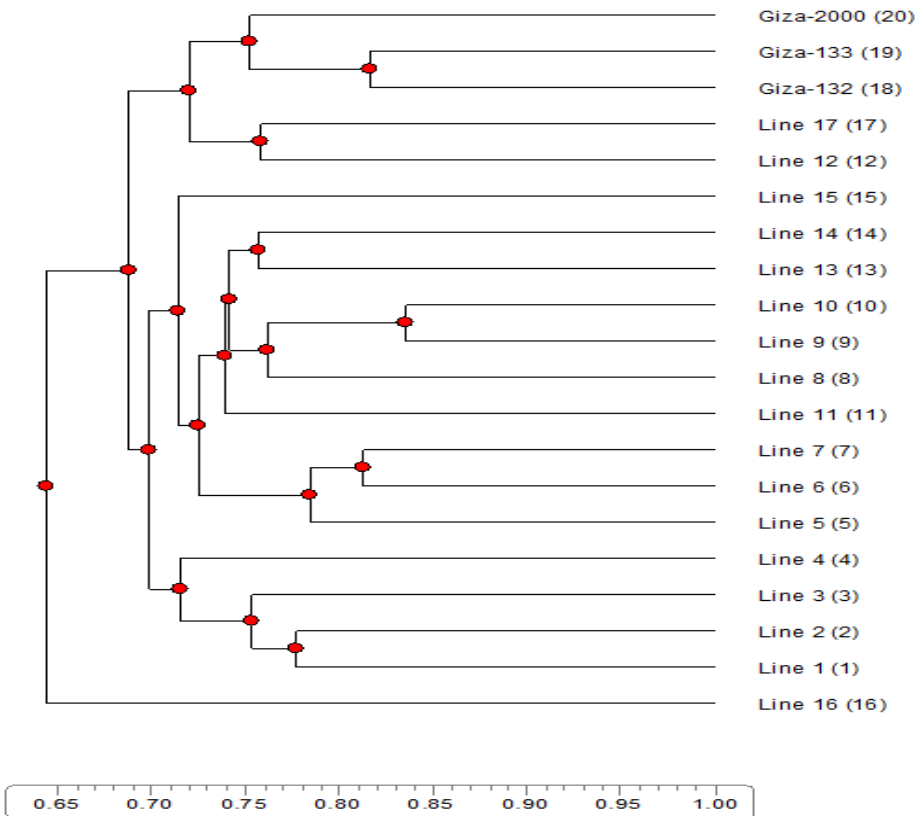

Fig. 12 The UPGMA ISSR-based phylogenetic dendrogram for the barley genotype

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d) PCA Analysis

The loadings of the first two principal components (PCs) PC1 and PC2 were presented in the horizontal axe against vertical axe as biplot graph. The sign of PC1 indicated the direction of relationship among the measured traits.

Based on the barley yield traits, out of ten revealed Eigen values, only the first three PCs were higher, with 69.67% variation among the genotypes for all studied traits (Table 6). Biplot graph illustrated MSPL, No. g/s, seed index and W. g/s traits, that placed in the left side of the graph, which were more correlated, and recorded negative correlations with other traits. However, the correlated traits BY/ton ha, GY/ton ha, No. Tiller, No. spikes, Plht and SY/ton ha, that located in the right side of the graph, had positive correlations with each other and more correlated to PC1. Based on these findings, selection of these traits could be more effective in barley development of breeding programs.

Table 6 The first important principal components (PCs) for the 10 quantitative yield traits for the studied barley genotypes

	PC1	PC2	PC3	PC4
Eigen value	4.01	1.61	1.35	0.90
Proportion%	40.05	16.09	13.53	8.99
Cumulative	40.05	56.14	69.67	78.66
Biological yield (BY) (ton/ha)	0.46	-0.16	0.08	0.29
Grain yield (GY) (ton/ha)	0.44	-0.02	0.09	0.30
Main spike length (MSPL)	-0.04	0.48	0.47	0.30
No. of Tiller/m^2	0.41	0.28	-0.16	-0.33
No. of grains/ spike (No. g/s)	-0.11	0.59	0.05	0.36
No. of spikes/m^2	0.41	0.27	-0.17	-0.33
Plant height (Plht)	0.25	0.35	-0.15	-0.08
Straw yield (SY) (ton/ha)	0.41	-0.29	0.06	0.26
Seed index	-0.11	0.20	-0.54	0.00
Weight of grains/ spike (W.g/s)	-0.05	-0.04	-0.63	0.56

Results demonstrated in Fig 13 demonstrated that Line 7, 6, 5, 8, 1 and check variety Giza 132 revealed the highest values for No. spikes, Tiller and Plht that produced increases in GY/ton ha in these genotypes. However, Line 3, 4 and checks Giza 2000 and Giza 132 were the best genotypes in BY/ton ha, No. SY/ton ha producing high GY/ton ha. Meanwhile, Line 16, 14,

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17, 9, 19 and 10 were grouped under negative correlated traits in terms of MSPL, No. g/s, seed index and W. g/s which characterized as low seed index, number and grain weight/spike indicating decreases in GY/ton ha characters. Similar results were reported by Mariey *et al.* (2021) and Verma *et al*. (2020).

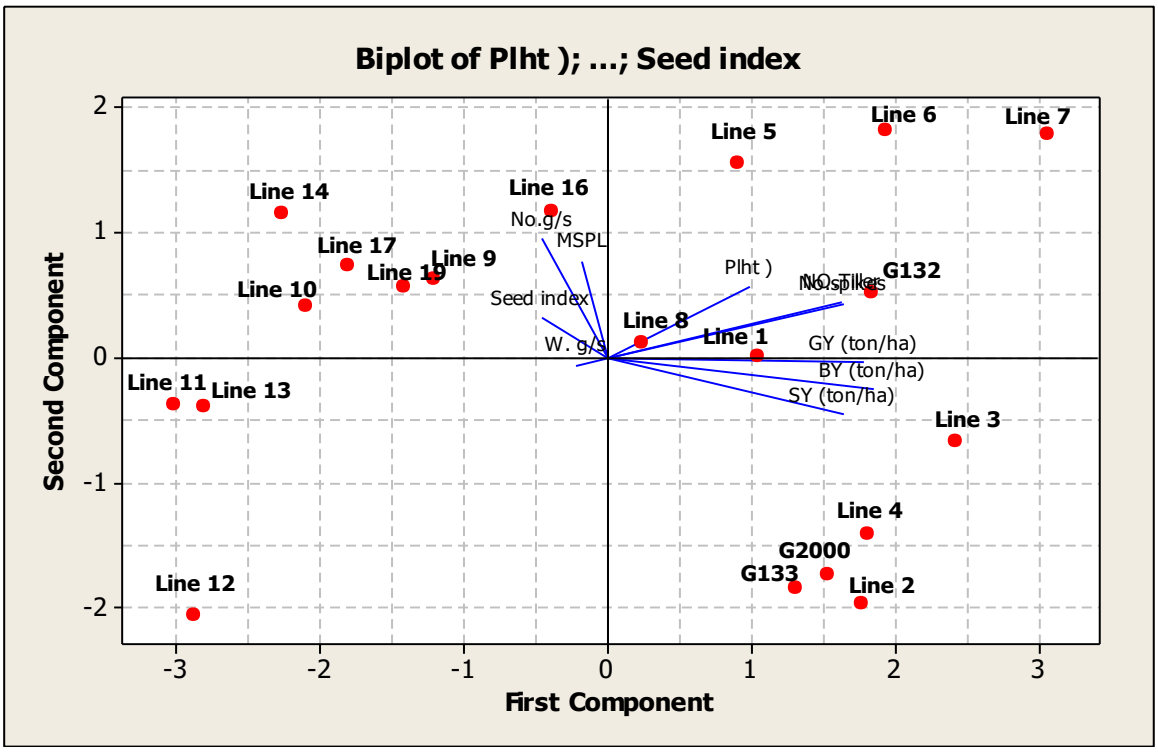

Fig. 13 Principal Components (PC) biplot, showing the correlation relationships among the yield traits and distribution of evaluated barley genotypes

Plht: Plant height (cm), MSPL: main spike length (cm), No. g/s: No. of grains/ spike, W.g/s: weight of grains/ spike, BY: biological yield (ton/ha), GY: grain yield (ton/ha) and SY: straw yield (ton/ha).

Based on the morphological measured traits of barley, which had high variation, principal component loading plot of different morphological traits for the studied barley genotypes were performed. The first seven PCs that had Eigen value more than unit accounted for 82.94 % of the total variability among the genotypes using different traits (Table 7). The first two PCs (PC1 and PC2) contributed only 37.39 % (22.29 % and 15.10 %) of the total variability (Fig 14). It was observed that %1st segment, ear traits, grain lemma, attitude, ear 1st segment, intensity tips, shape and lodicules traits were positively correlated with the other traits, and were more associated with the first principal component. Whenever, the variation in PC2 was due to flag leaves and

tips. Meanwhile, grain hair, median spikelet, plant traits, sterile spikelet, awn traits, density, emergence and glaucosity in the left side of graph had negative association with other characters located in the right side.

Table 7 The first important principal components for morphological (19 quantitative) traits in the studied barley genotypes

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Eigen value	4.23	2.87	2.21	2.02	1.67	1.48	1.27	0.81
Proportion%	22.29	15.10	11.65	10.64	8.81	7.79	6.66	4.27
Cumulative	22.29	37.39	49.04	59.68	68.49	76.28	82.94	87.21
Rachis length of first segment	0.22	0.12	-0.39	0.13	0.35	0.08	0.21	-0.24
Ear traits	0.37	-0.06	0.06	0.25	0.07	-0.02	0.14	0.34
Grain rachial hair type	-0.21	0.35	0.16	0.02	-0.24	-0.25	0.13	-0.15
Grain anthocyanin coloration of nerves of lemma	0.25	0.11	-0.19	-0.41	0.03	0.06	0.30	-0.21
Median spikelet length of glume and its awn relative to grain	-0.13	0.08	0.16	-0.03	0.15	-0.67	-0.07	0.07
Plant traits	-0.24	-0.15	0.31	0.00	0.09	-0.13	0.49	0.20
Sterile spikelet attitude (in mid-third of ear)	-0.30	0.16	-0.26	-0.28	0.11	-0.04	-0.09	0.20
Ear attitude	0.33	-0.16	0.08	-0.02	0.29	-0.10	-0.26	0.04
Awn length (compared to ear)	-0.24	-0.13	0.15	-0.25	0.40	0.21	0.22	0.12
Ear density	-0.31	-0.14	-0.10	0.26	0.07	0.16	-0.41	0.11
Rachis curvature of first segment	0.22	0.00	0.02	-0.20	0.47	-0.27	-0.15	0.20
Time of ear emergence (first spikelet visible on 50% of ears)	-0.21	0.15	-0.42	0.16	0.12	-0.12	0.38	0.23
Plant frequency of plants with recurved flag leaves	0.05	0.39	-0.12	0.47	0.13	0.02	0.07	-0.05
Ear glaucosity	-0.11	0.40	-0.03	0.10	0.14	0.11	-0.24	0.37
Plant growth habit	0.04	0.01	0.41	0.43	0.24	-0.05	0.13	-0.28
Awns intensity of anthocyanin coloration of tips	0.25	0.37	0.25	-0.15	0.01	0.17	-0.02	0.17
Grain disposition of lodicules	0.19	-0.27	-0.06	0.16	-0.29	0.07	0.22	0.53
Ear shape	0.20	-0.03	-0.26	0.01	-0.28	-0.48	-0.06	0.02
Awns anthocyanin coloration of tips	0.15	0.43	0.26	-0.14	-0.19	0.10	0.02	0.17

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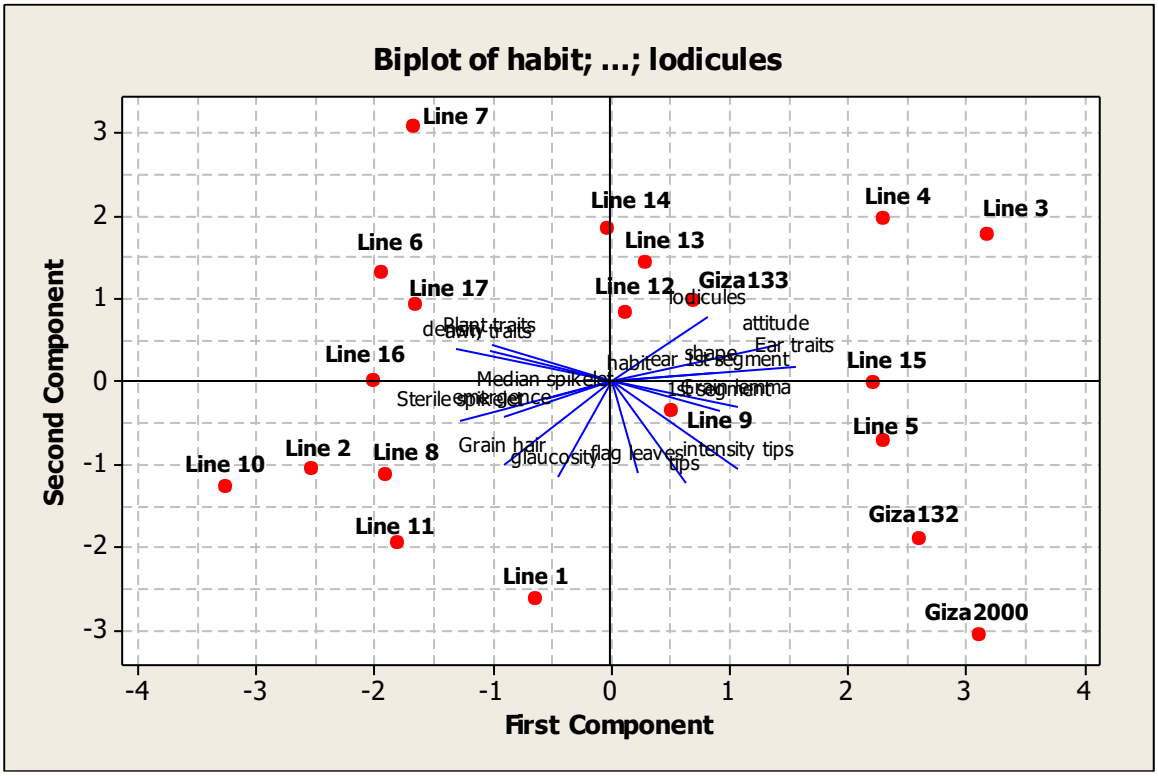

Fig. 14 Principal Component (PC) biplot, showing the correlation relationships among the morphological traits distribution of evaluated barley genotypes

Flag leaves: plant frequency of plants with recurved flag leaves, Emergence: time of ear emergence (first spikelet visible on 50% of ears), Median spikelet: Median spikelet length of glume and its awn relative to grain, Sterile spikelet: sterile spikelet attitude (in mid-third of ear), 1st segment: rachis length of first segment, Rachis curvature of first segment, Grain hair: grain rachial hair type, Grain lemma: grain anthocyanin coloration of nerves of lemma, Lodicules: grain disposition of lodicules, Attitude: ear attitude, Glaucosity: ear glaucosity, Density: ear density, Shape: ear shape, Intensity tips: awns intensity of anthocyanin coloration of tips, Awn length (compared to ear) and Awn: awns anthocyanin coloration of tips

It was noted that the previous measured traits were used to characterize and discriminate the evaluated 20 barley genotypes. Principal component analysis could be also be used in barley studying traits to determine the diversity and grouping the evaluated barley genotypes. In the upper right quarter (Fig 14), genotypes group of Line 3, 4, 15, 13, 12 and check variety Giza 133 exhibited the same advantages for traits of lodicules, attitude, ear traits, ear 1st segment and shape. Whenever, genotypes group of Line 9 and 5 with checks varieties Giza 132 and Giza 2000 could be characterized by such traits of grain lemma, 1stsegment, intensity tips, tips and flag leaves. On the left side, traits of median spikelet, emergence, sterile spikelet, grain hair and

glaucosity, may be used to distinguish some genotypes of Line 1, 11, 8, 2 and 10. Meanwhile, Line 7, 6, 17, 16 and 14 might be proposed the same characteristics of plant traits, awn traits and density traits.

Based on Table 6 and 7, No. of spikes and No. of tiller traits were the more correlated to GY/ton ha followed by plht, BY/ton ha and SY/ton ha indicated their importance as selecting criteria for yield development in barley grain yield. Generally, principal components showed clear diversity and distinguishable grouping for the studied barley genotypes to determine relationships among all traits, in order to detect the promising barley genotypes, which will be useful in breeding development programs.

Discussion

The upper mentioned results indicated that barley genotypes were highly varied in performance for morphological and agronomic traits during the two growing seasons. The crop variability is important for genetic studies and consequently improvement in selection programs, as it is a clear magnitude for parenting in barley improvement breeding programs. These results were in agreement with those reported by Ahmed *et al.* (20.23, 2021, 2018 and 2014), Ashrei *et al.* (2018) and Abo-Hegazy *et al.* (2020). There were variations within the studied genotypes in most morphological characters, such as plant frequency of plants with recurved flag leaves, awns intensity of anthocyanin coloration of tips, ear attitude, ear shape, rachis length of first segment and grain disposition of lodicules. These results were in agreement with Manjunatha *et al.* (2007) as stated much differences in lemma and grain color, awn type of investigated barley. Morphological characterization is the fundamental of genetic diversity research studies in taxonomy (Chandran *et al.*, 2000). It is still an important tool for the management of crop germplasm (Ariyo, 1993 and Annicchiarico, 1994). Also, it had been used to: 1) Identify the duplicates to discriminate material from different geographic areas, in order to establish a core collection. 2) To investigate the relationships within and between landraces and their wild. 3) To prioritize materials to be used in breeding programs. Significant amount of genetic variation was observed for most of the plant characteristics as plant height, number of tillers, No. of spikes, grain weight, heading and maturity date to all genotypes. Development of high-yielding cultivars requires an intensive knowledge of the existing genetic variation for yield and its components. These results were similar to Al-Tabba (2012) and Puri *et al*. (1982). Due to its global distribution, the evaluation of the genetic diversity in barley germplasm from different countries had been performed by many researchers (Feng *et al.*, 2003; Tanyolac, 2003; Fernández *et al.*, 2002; Liu *et al*., 2002; Matus *et al.*, 2002; Chen *et al.*, 2000b Dávila *et al.*, 1999 and 1999b; and Bjornstad *et al.*, 1997). In the present study, the total percentage of polymorphism was 84.5%, as it recorded for 240 polymorphic bands out of 284 total bands. The fifteen ISSR used primers

were able to characterize 14 lines and the 3 check varieties with total 35 unique markers (27 positive and 8 negative markers). The resulted phylogenetic dendrogram clustered the twenty genotypes into two main clusters, whereas the three check varieties grouped together with 0.75 similarity. The most noted observation was that the high level of genetic homogeneity and the high similarity matrix (ranged from 0.64 to 0.84 similarity) were observed in within and between the genotypes, which means a successful rate of crosses will occurs using these promising lines as a rich source for improving barley programs. The results which were observed by Hou *et al.* (2005) indicated that the percentage of ISSR polymorphic bands was higher (98.13%) and the mean number of amplification was 5.94, and concluded that the ISSR markers could be successfully used to investigate the genetic diversity of the barley landraces and its wild relatives. However, the results in this study suggested that the ISSR markers were superior in the capacity of revealing more informative bands in a single amplification. The similar results were observed by Fernández *et al.* (2002) and Tanyolac (2003). Bernard *et al*. (1997) analyzed the genetic diversity in 88 genotypes from 20 populations of wild barley from Israel, Turkey and Iran by RAPD markers, as the total genetic diversity was estimated, 75% of the variation detected was partitioned within the 88 genotypes and 25% among the populations. Also, the resulted variation between countries was assessed, high number of substantial differences also was found. In this study, principal components analysis cleared that the diversity and distinguishable grouping for the investigated barley genotypes to determine relationships among all traits. However, detect the promising barley genotypes, which will be useful in plant breeding programs. The observed results were in agreement with those obtained by Mariey *et al.* (2021) and Verma *et al*., (2020).

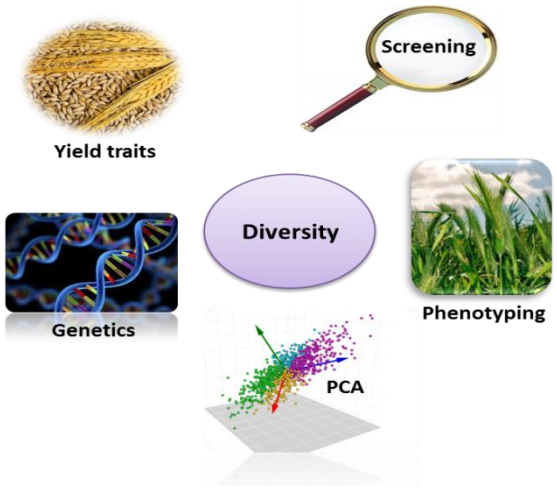

Fig. 15 Model showing the objectives of the current investigation

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Conclusion

The current investigation was conducted to evaluate 17 promising barely lines comparing to 3 barely commercial Giza check varieties, based on the morphological, agronomical, molecular levels and the principal component analysis test. By evaluating the novel barley lines, they showed a wide variance concerning the different studied traits. Also, the check Giza varieties had fluctuated performance among the studied parameters, while they had 75% similarity percentage. Based on the resulted yield component for the studied barley genotypes, it was obvious that the 17 promising lines could be considered as a rich source for the introduced breeding material by direct and indirect selection for improving grain yield quality and quantity in the future breeding programs. Therefore, it can be identified as a promising genotype in the barely breeding programs. And also, may be considered as a fertile environment for the direct and indirect selecting criteria to improving the barley grain quality and quantity.

Disclaimer

Conflict of interest: The authors declare that they have no conflict of interests, and contributed equally.

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