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Morphological and Molecular Characterization of Some Egyptian Barley Cultivars under Calcareous Soil conditions

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ABSTRACT

Agro-morphological and molecular studies were performed to evaluate 15 Egyptian barley cultivars. The cultivars were grown under two different field experiments at Sakha agricultural research station as a normal soil and EL-Nobraia agricultural research station as calcareous soil during two seasons 2014/2015 and 2015/2016 using randomized complete block design. Results showed that there were significant differences among the 15 barley cultivars for most of the traits. High phenotypic coefficient of variability (PCV) and genetic coefficient of variability (GCV) were found for most of the studied traits. Heritability estimate were ranged from moderately (0.65 %) for days to maturity to very high (0.98%) in both biological yield and plant height .Principal component analysis (PCA) was clarifying about 55.69 % of total variance. Biplot and Cluster analysis revealed four distinct groups were clustered according to their morphological traits. Ten ISSR markers were used to assess genetic variation of cultivars under calcareous soil. The highest number of ISSR fragments was presented by Giza 126 (52 band) with high polymorphism (78.7%) and high Shannon's information index (3.951). The total fragments of ten ISSR primer was 66 bands with an average (6.6%) per primer. Highest polymorphism was (87.5% for ISSR,3) with an average(59.0%).Highest Polymorphic information content (PIC) was 0.89 recorders by (ISSR3).The Dendrogram tree and genetics similarity based on the ten ISSR markers revealed two main genetic clusters, each cluster divided into two sub cluster includes the cultivars according their response and performances under calcareous soil.

Key words: Hordeum vulgar, calcareous soil, agro- morphological traits, principal components analysis, ISSR markers ,PIC, UPGMA cluster analysis

Introduction

Calcareous soil cover more than 30% of the world surface and their CaCO3 content differed from a low percent to 95% (Marschnen, 1995). It arises physically in arid and semi-arid regions as well as humid and semi-humid regions, where pH is high and $CaCo_3$ is dominated (Brady and Weil, 1999).

In Egypt the newly reclaimed soils at Nubaria region cover more than 900,000 fed. of which 290,000 fed. are calcareous soils (Moursy, 2002). Calcareous soils are poor and suffer from water shortage, poor fertility, and low organic matter content and poor physical characters (El-Banna *et al.*2011).Barley (*Hordeum vulgare* L.) consider the best choice crop for calcareous soil areas in Egypt. Barley is one of the five major crop species of the world which widely used for stock feed, human food and malting. Therefore, the important tasks for most breeders were to increase yield per unit area by developing high tolerant cultivars to be suitable for sowing on poor soils and under stress conditions (Guasmi *et al.*2009). Many studies have been conducted to reveal the substantial level of genetic diversity in barley using morphological markers (Lasa *et al*. 2001) as well as DNA markers (Tanyolac, 2003). Despite the fact that morphological markers are economical and easily implemented, but not always reflect the actual genetic relationship, due to the high genotype by location interactions and to limited number of traits studied (Slatkin and Barton 1989). DNA markers have been proved to be valuable tools used in evaluation of genetic diversity which not commonly affected by environment, selection, and are available in almost unlimited numbers (Russell *et al.*

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2002). Inter Simple Sequence Repeat Polymorphic (ISSR) is molecular marker techniques, which involve PCR amplifications of DNA using a primer composed of a microsatellite sequence by 2-4 arbitrary could be used to assess genetic diversity (Qian *et al*. 2001). ISSR has been widely used for genetic diversity in barley (Fernandez *et al*. 2002, Tanyolac, 2003, Hou *et al*. 2005 and Khatab and Mariey 2013). In this study, we aimed to assess the genetic diversity and relationships among 15 barley using ISSR marker as efficient DNA marker tools to be used in barley breeding programs under calcareous soils in Egypt.

Material and Methods

Fifteen barley cultivars (*Hordeum vulgare* L.), were used in this study in (Table1). The cultivars were grown in two locations; Sakha Agricultural Research station (as a normal soil) and at El-Nubaria Agricultural Research station (as a Calcareous soil) during two growing seasons; 2014/2015and 2015/2016, to evaluated some agro-morphological traits as well as molecular markers. Randomized complete block design with three replications was used. Plot size (experimental unit) was 1.8 m2 (6 rows x 0.2 m x 1.5 m).Ten Studied traits were measured i.e., germination rate, days of heading, days to maturity ,plant height (cm), spike length (cm), number of spikes m^{-2}, number of grain $spike^{-1}$,grain yield (t fed^{-1}), biological yield (t fed^{-1}) and harvest index%. The chemical analysis of soil samples from the two experimental sites during the two seasons, were presented (Table 2) and analyzed according to Black *et al.,* (1965).

Table 1: Name and pedigree of 15 barley cultivars used under study

No.	Name	Pedigree
1	Giza 123	Giza 117/FAO 86
2	Giza 124	Giza 117/Bahteem 52// Giza 118/FAO 86
3	Giza 125	Giza117 / Bahteem52// Giza118 /FAO86(sister line to G.124
4	Giza 126	BaladiBahteem/S D729-Por12762-BC.
5	Giza 127	W12291/B0gs//Hamal-02
6	Giza 128	W12291/4/11012-2170-22425/3/"Apam"/"B65"//"A16"
7	Giza 129	DeirAlla 106/Cel//As46/Aths*2"
8	Giza 130	Comp.cross"229//Bco.Mr./DZ02391/3/DeirAlla 106
9	Giza 131	CM67B/CENTENO//CAMB/3/ROW906.73/4/GLORIABAR/ COME-B/5/FALCON BAR/6/LINO
10	Giza 132	Rihane-05//AS 46/Aths*2Athe/ Lignee 686
11	Giza 133	ICB91-0343-0AP-0AP-0AP-281AP-0AP
12	Giza 134	ICB91-0343-0AP-0AP-0AP-289AP-0AP
13	Giza 135	ZARZA/BERMEJO/4/DS4931//GLORIABAR/COPAL/3/SEN/5/AYAROS
14	Giza 136	PLAISANT/7/CLN-B/LIGEE640/3/S.P-B//GLORIAAR/ COME B/5/FALCONBAR/6/LINOCLN-B/A/S.P/LIGNEE640/3/S.P-B//GLORIA-BAR/COME B/5/FALCONBAR/6/LINO
15	Giza 2000	Giza117/Bahteem52// Giza118/ FAO86 / 3/Baladi16/ Gem

Molecular Analysis

DNA isolation and ISSR–PCR amplification

DNA was isolated by CTAB method (Doyle and Doyle, 1990). Ten ISSR primers were used. PCR amplification was performed in a total volume 20 µl containing , 2.5 µl 10 X buffer, 1 µl 25 mM MgCl2, 2 µL 2.5 mM dNTPs, 2 µl 10 pmol primer, 1 µl 50 ng of DNA and 0.16 µl Taq DNA polymerase (5 units/µl). PCR amplifications were performed in PCR thermal cycler system, with initial denaturation at 94◦C for 5 min followed by 35 cycles: denaturation at 94◦C for 1min, annealing at 36◦C for 1min, extension at 72◦C for 2min, with final extension at 72◦C for 7min. PCR products were separated on 2% agarose gels, stained with ethidium bromide, and visualized on UV. The gel was photographed using Gel DOC

Data Analysis

Agro- morphological traits analysis,

Data were collected from the two field experiments and statistically analyzed as a randomized complete block design (RCBD) using analysis of variance (ANOVA) for each season as well as combined analysis according Steel *et al*. (1997).The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were calculated according to Burton (1952). Heritability was estimated according to Hanson *et al.* (1956). Simple correlation (r) coefficients among all studied traits calculated according to Kearsey and Pooni (1996).Multivariable analysis were calculated using computer software program Minitab v.12. Cultivars were clustered using un–weighted pair group method using arithmetic average as outlined by Kovach (1995).

Table 2: Some physical and chemical properties from the field experiments site locations during the two consecutive seasons, 2014/15 and 2015/16.

Chemical properties	2014/ 2015		2015/2016	
	Sakha	El -Nobria	Sakha	El- Nobria
pH	7.1	8.10	8.0	7.96
ECe (dsm-^{1})	3.0	8.20	3.7	8.10
$CaCO_3$ %	0.0	29.40	0.0	30.0
Soluble cations meq100 ^{1} g soil				
Ca^{++}	4.6	19.02	4.8	2.02
Mg^{++}	2.5	11.46	5.9	11.02
Na^{++}	14.8	50.42	14.9	48.5
K^{+}	0.2	1.10	0.5	1.20
Soluble anions meq100^{-1} g soil				
SO_4	18.2	20.43	7.1	21.4
Cl^{-}	11.2	6.30	10.3	7.01
HCO_3	5.5	1.30	5.3	1.32
CO_3	0.00	0.00	0.00	0.00
Texture class	Clay soil	Sandy loam	Clay soil	Sandy loam

ISSR Markers Analysis

DNA profiling was recorded as discrete variables: 1 for the presence and 0 for the absence of band. The Polymorphic information content value (PIC) was calculated according Anderson *et al.* (1993) using the equation: PIC = 1-$\Sigma^{n}{}_{Pij2j=1}$ Where, PICi is the polymorphic information content of a marker i; Pij is the frequency of the jth pattern for marker i and the summation extends over n patterns. Phylogenetic trees were constructed based on Jaccard similarity matrix using PAST program (PAleontological Statistics Version 1.94b) adapted by Hammer *et al.* (2001). Cluster analysis was performed to produce a dendrogram using UN weighted pair-group method with arithmetical average (UPGMA).

Results and Discussion

Analysis of variance of morphological traits

Data in (Table 3) revealed a significant difference among all studied cultivars for all studied traits. A significant interaction between two seasons (S) and cultivars (C) were found for all traits expect days to heading and no spikes m^{-2}.

Table 3: Analysis of variance of ten traits for 15 tested barley cultivars under the two locations during the two growing seasons 2014/2015 and 2015/2016

Morphological Traits	Cultivars	Locations	Seasons	Season X Cultivar	Location X Cultivar	Location X Cultivar X seasons
Germination /%	**	**	**	**	**	**
Days to heading (days)	**	**	**	Ns	**	Ns
Days to maturity (days)	**	**	**	**	**	*
Plant height (cm2)	**	**	**	**	*	*
Spike length (cm2)	**	**	**	**	**	Ns
No. grains spike-1	**	**	**	**	*	*
No. spikes m-2	**	**	**	Ns	**	Ns
Biological yield (ton/fed)	**	**	**	**	*	*
Grain yield (ton/fed)	**	**	**	**	**	*
Harvest index %	**	**	*	*	NS	Ns

*Which *and **; indicate significance at 0.05 and 0.01levels, respectively*

Significant interactions between locations (L) and cultivars (C) were fond for all traits expect for harvest index. Interaction between seasons (S), locations (L) and cultivars (C) were significant for germination rate %, plant height, days to maturity, No grain spike^{-1}, biological yield and grain yield, while other traits were in significant. The result about the significant traits offers a confirmation about the possibility and efficient of these traits using in selection breeding programs under El- Nubaria conditions. These results were similar to those obtained by Moursy (2002), El-Banna *et al.* (2011). They found that interaction between seasons x genotypes was in significant for days to heading and number of spikes m^{-2} under calcareous soil at El-Nobria station

Analysis of means performances of morphological traits

Data in (Table 4) indicated that the studied cultivars differed significantly in germination rate %. The Egyptian cultivars Giza 125 gave the highest germination rate during the two seasons and across their combined (85.3, 90.7and 88.1%) respectively. However Giza 124 gave the lowest germination percentage during the two seasons and across their combined recording (58.3, 66.0 and 62.2%) respectively.

The results varied significantly in days to heading (Table 4), Giza 129 was the earliest cultivars in both seasons and their combined with values of (86.0, 88.3and87.2days) respectively. While the latest cultivars was Giza 132 during two seasons and across their combined.

Table 4: Combined mean performance of the germination percentage , days to heading ,days to maturity, plant height and spike length, under normal and calcareous soil during 2014/2015 and 2015/2016 seasons

Cultivars	Germination %			Days to heading (Days)			Days to maturity (days)			Plant height (cm)^2			Spike length (cm)^2		
	Ses1	Ses2	Comb.	Ses1	Ses2	Comb.	Ses1	Ses2	Comb.	Ses1	Ses2	Comb.	Ses1	Ses2	Comb.
Giza 123	76.7	78.7	77.7	89.3	94.0	91.7	132..3	135.0	133.7	95.0	100.0	95.0	5.7	8.0	6.8
Giza 124	58.3	66.0	62.2	90.0	91.0	90.5	131.0	132.3	131.7	91.7	93.3	92.5	5.3	5.0	5.2
Giza125	85.3	90.7	88.1	90.3	94.3	92.3	131.3	127.7	129.5	100.0	110.0	105.0	6.0	7.0	6.5
Giza126	85.0	88.7	86.8	90.0	93.0	91.5	130.0	135.3	132.7	90.0	90.0	90.0	5.7	7.3	6.5
Giza127	84.3	89.3	86.8	88.3	90.3	89.3	131.0	133.7	132.3	95.0	105.0	100.0	7.0	7.0	7.0
Giza128	76.7	90.0	83.3	89.3	90.0	89.7	126.0	129.7	127.8	95.0	96.7	95.8	7.7	9.3	8.5
Giza 129	79.0	88.3	83.7	86.0	88.3	87.2	128.3	134.7	131.5	101.7	103.3	102.5	5.3	5.3	5.3
Giza 130	85.0	80.0	82.5	90.0	91.0	90.5	131.0	133.0	132.0	98.3	96.7	97.5	5.3	6.0	5.7
Giza 131	84.7	88.0	86.3	90.0	90.3	90.2	132.0	128.3	130.0	103.3	105.0	104.0	5.0	8.3	6.7
Giza 132	78.3	88.3	83.3	91.0	95.0	93.0	128.2	134.7	131.7	83.3	85.0	84.2	5.0	7.3	6.2
Giza 133	80.0	90.3	85.2	90.7	92.0	91.3	130.7	131.7	131.2	106.7	110.2	108.4	5.3	5.3	5.3
Giza 134	85.0	88.3	86.7	89.7	90.3	90.0	130.0	132.7	131.3	95.0	104.3	99.7	5.7	6.0	5.8
Giza 135	80.0	87.0	83.5	89.7	90.7	90.2	131.7	132.3	132.0	105.0	108.3	106.7	6.3	7.0	6.7
Giza 136	75.7	69.0	72.3	90.0	94.0	92.0	132.0	133.0	132.5	95.0	103.0	99.0	7.3	8.0	7.7
Giza2000	68.3	85.0	76.7	87.7	90.3	89.0	130.7	134.0	132.3	90.0	120.0	105.0	7.3	7.7	7.5
average	**77.6**	**84.7**	**81.1**	**89.0**	**91.3**	**90.2**	**129.7**	**132.1**	**13.9**	**93.2**	**102.5**	**97.8**	**5.9**	**6.9**	**6.4**
C.V%	**5.23**	**3.8**	**7.6**	**1.62**	**1.63**	**2.2**	**1.65**	**1.73**	**5.9**	**3.0**	**2.0**	**8.75**	**12.7**	**12.3**	**14.4**
L.S.D	**9.3**	**7.4**	**9.4**	**3.9**	**3.3**	**13.1**	**4.8**	**5.2**	**11.9**	**6.4**	**4.6**	**12.9**	**1.7**	**2.8**	**1.8**

Which ses1: season 1 2014/2015, ses2: season 2 2015/2016 and comb.: combined between the two seasons

Concerning days to maturity as shown in (Table 4), in the first season the earliest cultivar was Giza 128 (126 days), while latest cultivar was Giza 123 (132.3 days). However, in the second season Giza 125 was the earliest cultivar (127.7 days). Moreover, across the two seasons the means ranged between the earliest cultivar Giza 128 (127.8 days) to latest cultivar Giza 123 which gave 133.7 days. Means performance of plant height (Table 4) clearly indicated that the cultivar Giza 133 was the tallest for each individual season and their combined were (106.7, 110 and 108.3 cm,) respectively. However Giza 132 was the shortest cultivar for each individual season and their combined were (83.2, 85.0 and 84.2 cm) respectively.

Regarding spike length, results in (Table4) indicated that cultivar Giza 128 had the highest spike length in both seasons and across two seasons were (7.7, 9.3 and 8.5 cm), respectively.

However Giza 124 had the lowest spike length in first, second season and their combined were (5.3, 5.0 and 5.2 cm) respectively.

The mean performance of number of grains spike^{-1}(Table 5) in first season , second season and their combined indicted that Giza 2000 gave the highest no. of grains spike^{-1} were (64.5, 65.5 and 65.0 grains spike^{-1}and).However, the lowest no. of grains spike^{-1} was produced by Giza 132 gave (57.5, 59.5 and 58.5 grains spike^{-1}). Concerning number of spikes m^{-2}, the means of the cultivars given in (Table 5) showed that the cultivar Giza 129 gave the highest number of spikes m^{-2} in the first, second season and their combined (414, 421and 418 spikes m^{-2})respectively. On the other hand, the lowest number of spikes m^{-2}was obtained by cultivar Giza 132 with values of (261, 297.9 and 274.6 spikes m^{-2}) in both seasons and their combined respectively.

Mean values of biological yield (Table 5) indicted that Giza 126 gave the highest biological yield in first season and their combined with values (12 and 12.2 t fed^{-1}) respectively. Moreover, Giza 129 and Giza 135 gave the same highest values of biological yield in second season was (13.4 t fed^{-1}). Regarding grain yield, data in (Table 5) indicated that cultivars Giza 126 and Giza 136 gave the highest value in first season (3.7 t fed^{-1}). Moreover, in the second season both Giza 135 and Giza 123 gave the maximum value (4.2 t fed^{-1}). As well as over the two seasons Giza128 had maximum value (3.8 t fed^{-1}). However, Giza 143 had minimum values of grain yield during the first, second and their combined with values (1.4, 2.1 and 1.8 t fed^{-1}) respectively. Data in (Table 5) indicate that Giza 128 had the highest harvest index (HI) in first, second season and their combined were (39.6, 39.5and 39.6%) respectively.

Table 4: Combined mean performance of the no. of grains spikes^{-1}, no. of spikes m^{-2}, biological yield, grain yield and harvest index under normal and calcareous soil during 2014/2015 and 2015/2016 seasons

cultivars	No .of grain spike^{-1}			No. of spike m^{2}			Biological yield (t fed^{-1})			Grain yield (t fed^{-1})			Harvest index %		
	Ses1	Ses2	Comb.	Ses1	Ses2	Comb.	Ses1	Ses2	Comb.	Ses1	Ses2	Comb.	Ses1	Ses2	Comb.
Giza 123	61.8	62.2	62.0	281.1	308.1	294.6	8.2	12.3	10.3	3.0	4.2	3.6	36.8	34.2	35.5
Giza 124	60.8	62.2	61.5	276.9	330.0	303.6	8.4	12.6	10.5	2.3	2.3	2.3	28.0	32.1	30.1
Giza125	62.2	61.5	61.8	332.1	405.9	369.0	10.7	12.1	11.4	3.3	3.7	3.5	30.5	30.8	30.6
Giza126	60.8	61.8	61.3	365.1	369.0	366.9	12.0	12.5	12.2	3.7	3.7	3.7	31.2	30.1	30.6
Giza127	63.5	65.2	64.3	378.9	396.9	387.9	11.5	12.3	11.9	3.6	3.7	3.6	31.1	30.7	30.9
Giza128	61.8	61.5	61.7	339.9	344.1	342.0	106	10.6	10.6	3.5	4.0	3.8	39.6	39.5	39.6
Giza 129	58.5	60.5	59.5	414.0	422.0	418.0	10.0	13.4	11.7	3.4	3.3	3.3	34.4	32.9	33.6
Giza 130	60.5	63.5	62.0	300.0	401.1	350.4	9.6	13.1	11.4	3.3	3.8	3.5	33.9	29.3	31.6
Giza 131	62.2	62.8	62.5	368.1	378.0	372.9	10.7	11.4	11.1	3.4	3.1	3.3	32.1	27.2	29.6
Giza 132	57.5	59.5	58.5	261.0	297.9	279.6	8.0	12.9	10.4	2.9	2.9	2.9	36.5	22.6	29.5
Giza 133	61.8	63.2	62.5	386.1	375.9	381.0	9.2	12.6	10.9	3.3	3.4	3.4	36.4	26.8	31.6
Giza 134	58.5	59.5	59.0	333.0	342.9	338.1	8.6	11.8	10.2	1.4	2.1	1.8	16.6	17.9	17.3
Giza 135	62.5	64.5	63.5	348.9	420.9	384.9	9.8	13.4	11.6	3.2	4.2	3.7	32.6	31.5	32.1
Giza 136	60.5	64.5	62.5	368.1	390.0	378.9	11.4	12.0	11.7	3.7	3.4	3.5	32.4	28.6	30.5
Giza2000	64.5	65.5	65.0	374.1	336.9	355.5	10.6	12.0	11.3	3.2	3.5	3.3	29.9	29.2	29.6
C.V%	**2.7**	**2.4**	**3.1**	**2.9**	**3.5**	**8.7**	**6.11**	**11.4**	**15.6**	**7.9**	**7.1**	**14.8**	**11.1**	**13.4**	**15.9**
L.S.D	**3.7**	**3.4**	**2.9**	**7.4**	**9.7**	**15.4**	**1.4**	**3.2**	**2.6**	**0.56**	**0.57**	**0.77**	**8.1**	**8.9**	**7.6**

In the present study, high genetic variations for ten agro- morphological traits were detected among all the 15 cultivars. The differences among cultivars depend on the ability of cultivars to transfer dry matter accumulation to grains. The results were in closes agreement with obtained by Moursy (2002), Abou –Hussien *et al*. (2010), El-Banna *et al*. (2011) and Amer and Khamis(2015) they found high genetic variation for most of agro- morphological traits among barley genotypes under calcareous soil

Simple Correlation coefficient between all traits under study

The correlation coefficients among all studied traits of fifteen barley cultivars were shown in Table (6). A Significant and positive correlation was detected between plant height and number of grains spike^{-1}.

Positive correlation between number of grains spike^{-1} with each of biological yield, grain yield and harvest index.

Significant positive correlations were observed between number of spikem^{-2}and both of biological yields and grain yield.

Biological yield showed positive with grain yield and harvest index. Grain yield showed positive and significant correlation with spike length, number of grains spike $^{-1}$, number of spikes m^{-2}, biological yield and harvest index.

From the aforementioned results, positive correlation between grain yield and one or more traits of its components were found. These results were in close agreement with other researchers Najeeb and Wani (2004), Madic *et al.* (2005), Singh *et al.* (2008), Lodhi *et al.* (2015) and Singh et al. (2015) also reported significant positive association of grain yield and most of its components. The knowledge of association and relationship between grain yield and its component is important information in plant breeding programs.

Table 6: Simple correlation coefficient for all studied traits among 15 barley cultivars

traits	GR%	HD	MD	PH	SPL	GS^{-1}	SPm^{-2}	BY	GY
HD	-0.220								
MD	-0.474	0.064							
PH	0.483	-0.307	-0.023						
SPL	-0.223	-0.097	0.337	0.028					
GS^{-1}	0.223	-0.265	0.102	0.617*	0.373				
SPm^{-2}	-0.030	-0.247	0.217	0.518	-0.163	0.079			
BY	-0.403	-0.298	0.269	0.237	-0.056	0.249**	0.701**		
GY	-0.284	0.158	0.312*	-0.055	0.574*	0.112*	0.388*	0.429**	
HI	-0.134	0.288	0.177	-0.216	-0.612*	-0.247	0.063	0.017*	0.890**

Where GR%: germination rate , HD: days to heading ,MD :days to maturity, PH: plant height, SPL : spike length, GS^{-1} :umber of grains spike $^{-1}$, SPm^{-2}number of spikes m^{-2} , BY: biological yield , HI: harvest index and GY: grain yield ,

Analysis of variability, heritability and selection gain

The greatest variability at the phenotypic coefficients (PCV) and genotypic coefficients (GCV) levels (Table 7) were recorded for grain yield followed by harvest index and number of spikes m^{-2} under normal and calcareous soil in both seasons.

Moderate GCV and PCV values were observed for germination percentage, spike length, plant height, number of grains spike^{-1} and biological yield under normal and calcareous soil in both seasons. Low GCV and PCV were observed for days to heading and days to maturity under two locations during the two growing seasons.

PCV values were a little greater than GCV for all traits under normal and calcareous soil during both seasons, indicting a very little control of environment effects on all studied traits.

Genetic variations playing an important role in inheritance of grain yield, number of spike m^{-2} and harvest index, since high GCV were observed for that traits. These results were in close agreement with the findings workers, Mishra *et al*. (2007), Pal et al. (2010), Kumar and Shekhawat, (2013) ,kumar *et al*. (2013), Singh *et al.* (2014), Aynewa *et al*.(2015), Shrimali *et al*. (2017) and El-Hashash and Agwa (2018).

Heritability in broad sense % under normal soil (Table 7) was high for all studied traits during two growing seasons. However under calcareous soli heritability in broad sense % were high for all studied expect days to heading and days to maturity were moderated heritability at both seasons. Substantiate results were reported by Kishor *et al*. (2000), Singh (2011), Al-Tabbal and Al-Fraihat (2012), and Kumar and Shekhawat (2013) and Sunil *et al*. (2017).

High expected genetic advance (GA) from selection based on 10% selection intensity were observed for grain yield, biological yield, harvest index , number of grains spike^{-1} and number of spikes m^{-2} under normal and calcareous soil during two seasons (Table 7).

Low Genetic advance percentages were observed for days to heading and days to maturity under normal and calcareous soil during two seasons.

Genetic gains as a parameter for selection effectiveness were linked to genetic variability, heritability and selection intensity. Low genetic gains with moderated heritability were obtained for days to heading and days to maturity indicates that the environmental variation effects on its phenotypic. It is concluded that all traits under study except days to maturity and days to heading can be considered as suitable selection criteria for the development of high yielding barley breeding program under calcareous soil. Our result was confirmed by Kumar and Prasad (2002), and Kumar and Shekhawat, (2013) and Sunil *et al*. (2017).

Table 7: Heritability and genetic parameters of studied characteristics of 15 barely cultivars under normal and calcareous soil across two cropping seasons

Traits	Phenotypic Coefficient of Variability (PCV)				Genotypic Coefficient of Variability (GCV)				Heritability in the broad sense h2b (%)				Expected genetic advance from selection (GA %)			
	2014/2015		2015/2016		2014/2015		2015/2016		2014/2015		2015/2016		2014/2015		2015/2016	
	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S
GP	13.0	12.3	10.2	11.5	12.9	11.1	9.4	10.9	95.2	90.2	92.9	94.2	24.4	22.9	19.4	22.4
HD	5.9	2.2	5.7	2.9	5.8	1.1	5.6	2.4	98.4	49.1	99.1	50.4	11.9	2.2	11.6	4.9
MD	2.6	2.0	2.0	2.3	2.5	1.1	1.8	2.0	96.5	56.2	92.9	57.1	5.3	2.3	3.8	4.1
PH	10.7	12.6	10.4	10.1	10.5	12.6	10.3	10 .0	98.3	99.6	99.2	99.1	21.7	26.0	21.2	16.4
SL	17.0	20.9	17.6	15.5	16.2	16.5	16.8	17.8	95.1	78.6	95.4	69.8	33.4	33.9	34.7	36.6
GS^{-1}	27.7	24.0	27.8	23.9	27.6	23.1	27.7	23.2	99.6	87.2	99.5	81.3	56.9	52.4	57.0	51.5
SM^{-2}	36.3	35.7	36.3	34.8	35.9	25.6	35.9	24.6	99.1	98.4	99.1	96.3	74.0	71.5	74.0	69.5
BY	25.2	15.4	25.2	19.6	25.0	24.3	25.0	24.9	99.2	93.0	99.2	84.6	51.6	29.4	51.6	20.0
GY	58.1	53.2	58.0	55.8	57.9	51.6	57.8	50.6	99.6	94.0	99.6	93.9	89.2	44.5	87.1	40.3
H1	36.6	33.2	36.6	32.9	36.2	31.9	36.2	31.5	98.8	85.8	98.8	80.5	74.6	41.0	74.5	38.0

Which *N : normal (sakha station) , S : stress (EL-Nobria station) Gp %: germination percentage , HD: days to heading ,MD :days to maturity, PH: plant height, SPL : spike length,* GS^{-1} *:umber of grains spike* $^{-1}$ *,* SPm^{-2}*number of spikes* m^{-2} *, BY: biological yield , HI: harvest index and GY: grain yield*

Multivariable analysis

Principal component Analysis (PCA)

Principal component PCA analysis was performed using Nei's (1973) distance matrix, was justifying about 55.69 % of total variance Fig1 (a & b). The first PCA1 clarified 33.55 % of total variation prejudiced by days to heading, days to maturity ,number spikes m^{-2}, biological yield , harvest index and grain yield of .The second PCA2 clarified 22.14 % of the total variability influenced by germination rate ,plant height, number grains spike $^{-1}$ and spike length .

Cluster and Biplot analysis

Biplot analysis has ability to identify the superior genotypes for both stress and non-stress conditions. Cultivars subjected to biplot analysis, are compared for assessing relationships between all the attributes at once. Biplot and cluster analysis using (Euclidean distance matrix and average Linkage) based on their ten agro morphological traits and PCA values had clustered all 15 cultivars into four groups Fig.1 (b & c) named A,B,C,D groups. A group includes the cultivars had higher grain yield and higher values of PCA1 in both two locations under normal and stress conditions which we could consider them as a tolerant cultivars such as (Giza 16 and Giza 18). However D group includes cultivars with lower grain yield and higher PCA2 values as a sensitive cultivars such as (Giza 124, Giza 132 and Giza134) .B group included the moderated tolerant cultivars and C group included the moderated sensitive cultivars.

Data in (Table 8) showed the similarity level among the 15 Egyptian barley based on their morphological traits using Euclidean distance matrix and average Linkage. The highest similarity level was recorded between two cultivars Giza 135 and Giza 133 equaled 92.49%.While the lowest similarity levels was obtained between Giza 123 and Giza 124 recorded 33.13% similarly. It is prominent that biplot and cluster analysis has been widely used for description of genetic diversity and grouping based on morphological traits which was considered as helpful tool for breeders to design successful breeding programs for stress conditions. These results were in a good harmony with Sharafi *et al.* (2014), Solonechnyi *et al.(*2015), Ashraf *et al.* (2015), Kendal, (2016),Meng *et al.*(2016), Mariey and Khder (2017) and Arshadi (2018).They study genetic diversity in barley using multivariable analysis to grouping cultivars based on morphological traits.

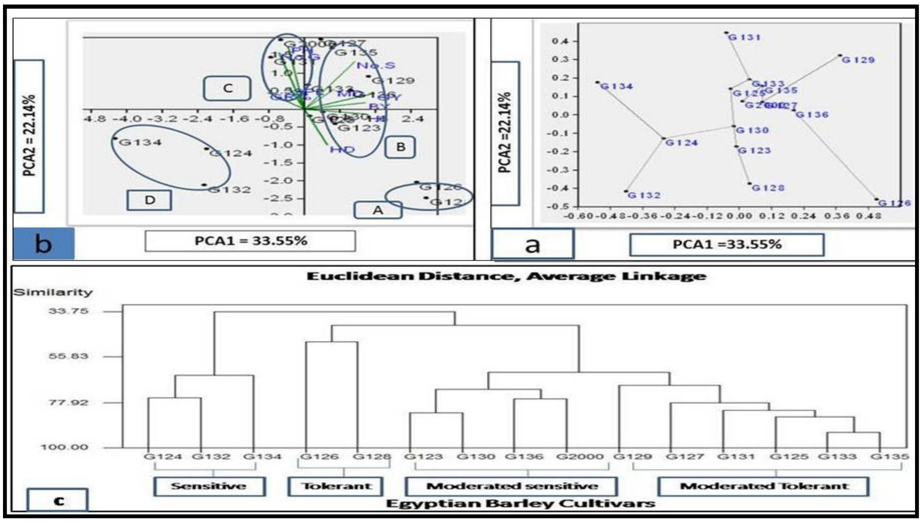

Fig. 1 (a): Principal component analysis; (b) biplot diagram based on Principal component analysis and (c) Dendrogram resulting from an UPGMA cluster analysis 15 barley cultivars based on the morphological traits

Table 8: Cluster analysis to classify 15 barley cultivars based on agro–morphological traits under different normal and calcareous during two seasons

Number clusters	Similarity level	Distance level	Joined cluster		New Clusters	No. of entries in new cluster
14	92.49	3.624	G133	G135	G133	G124
13	85.04	7.225	G125	G133	G125	G125
12	81.67	8.848	G125	G131	G125	G126
11	78.14	10.553	G125	G127	G125	G127
10	78.14	10.555	G130	G2000	G130	G124
9	76.1	11.541	G123	G124	G123	G14
8	73.26	12.908	G130	G2000	G130	G125
7	72.61	13.226	G123	G132	G123	G125
6	69.63	14.662	G125	G129	G125	G127
5	66.55	16.152	G125	G130	G125	G131
4	56.12	21.184	G123	G128	G123	G126
3	54.56	21.938	G125	G134	G125	G132
2	36.84	30.493	G123	G125	G123	G136
1	31.37	33.134	G123	G124	G123	G2000

Molecular analysis

ISSR markers analysis

The highest number of ISSR-PCR amplified fragments using ten primers among all the fifteen cultivars (Table 9 & Fig 2) was presented for Giza 126 (52 band) with highest percentage of polymorphic loci was (78.7%) and highest Shannon's information index was (3.951). However the lowest number of ISSR-PCR amplified fragments was definite by Giza 124 (40 band) with lowest percentage of polymorphic loci was (60.0 %) and lowest Shannon's index was (3.689).

The polymorphism level of studied ISSR primers was shown in (Fig.2 & Table 10). The total fragments of all ISSR primer were 66 bands, divided to39 bands polymorphic and 27 bands monomorphic with total polymorphism (0.59%). The band number for each primer was ranged from five to eight bands with an average (6.6%) per primer. The percentage of polymorphism for each primer varied from lowest percentage (33.3% for ISSR 9) to highest percentage (87.5% for ISSR, 3 (Fig.2, A) with an average (59.0%).

Polymorphic information content (PIC) values were estimated to evaluate the genetic diversity of the ten ISSR primers (Table 10).The highest PIC was 0.89 recorder by (ISSR3), while lowest PIC was 0.34 % related to primer (ISSR9).

Table 9: The genetic diversity among 15 barley cultivars using ten ISSR primers

Cultivars	Total polymorphic band	Percentage of polymorphic fragments	Shannon's information index
G2000	46	69.7	3.871
G123	45	68.1	3.807
G124	40	60.0	3.689
G125	46	69.7	3.807
G126	52	78.7	3.951
G127	49	74.2	3.892
G128	50	75.7	3.932
G129	48	72.2	3.871
G130	46	69.6	3.801
G131	48	72.2	3.871
G132	42	63.6	3.691
G133	48	72.2	3.871
G134	41	62.1	3.690
G135	48	72.2	3.871
G136	49	74.2	3.892
Average	46.54	71.49	3.83

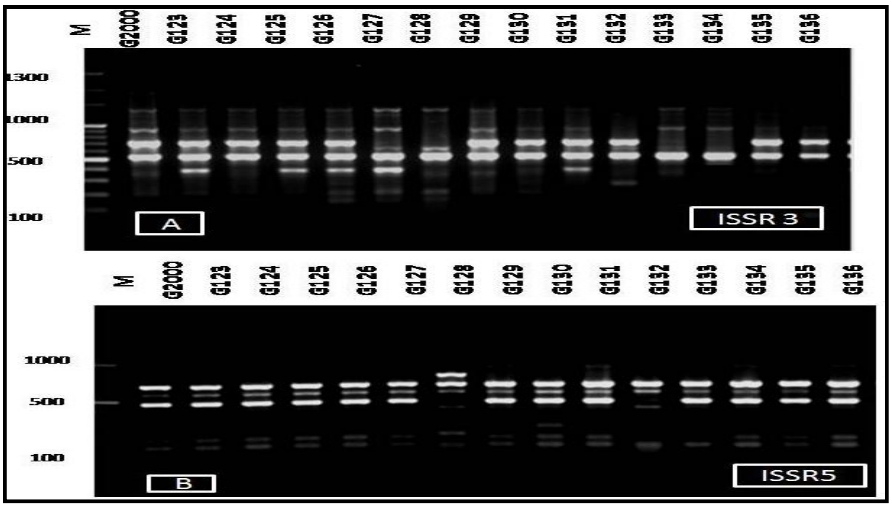

Fig 2: Amplification results of the primers (A) ISSR 3 and (B) ISSR5 in 15 Egyptian barley cultivar

Table 10: ISSR primers sequences, number of total fragment, number of polymorphic fragments, percentage of polymorphic fragments and polymorphic information content (PIC)

No.	Name	primers Sequence of primer (5⁻ - 3⁻)	Number of Total fragment	Number of polymorphic fragments	Percentage of polymorphic fragments	polymorphic information content (PIC)
1	ISSR 1	5'-ACACACACACACACACG-3'	6	3	50.0%	0.50
2	ISSR 2	5'-TCTCTCTCTCTCTCTCC-3'	5	3	60.0%	0.56
3	ISSR 3	5'-TGTGTGTGTGTGTGTGG-3'	8	7	87.5%	0.89
4	ISSR 4	5'-ACACACACACACACACGA-3'	8	5	62.5%	0.61
5	ISSR 5	5'-GAGAGAGAGAGAGAGAC-3'	7	4	57.1%	0.60
6	ISSR 6	5'-ACACACACACACACACC-3'	6	3	50.0%	0.53
7	ISSR 7	5'-GAGAGAGAGAGAGAGAGG-3'	5	3	60.0%	0.61
8	ISSR 8	5'-CACACACACACACACAAG-3'	8	5	62.5%	0.66
9	ISSR 9	5'-CTCTCTCTCTCTCTCTG-3'	6	2	33.3%	0.34
10	ISSR 10	5'-GAGAGAGAGAGAGAGAT-3'	7	4	57.1%	0.58
Total			66	39	59	0.63
Average			6.6	3.9		0.59

Phylogenetic trees and Genetic Similarity Analysis

The Dendrogram and genetics similarity based on all studied ISSR revealed two main genetic clusters (Fig.3). The first cluster divided into two sub cluster includes the tolerant cultivars under calcareous in one sub-cluster as (Giza 126 and Giza 128) with highest genetics similarity value was 0.92 % (Table11) and moderated tolerant cultivars were found in second sub cluster. The second cluster divided into two sub cluster. First sub-cluster include the sensitive cultivars (Giza 124, Giza 132 and Giza 134), and other sub cluster included the moderated sensitive cultivars, with high genetic similarity between them were (0.83% between Giza 124 and Giza 132 , 0.80% between Giza 124 and Giza 134 and 0.82% between Giza 132 and Giza 134).

The results in the present study confirmed that ISSR was useful method to detect differences and genetics diversity among 15 Egyptian barley cultivars. ISSR data separated the all the cultivars into two major clusters according to their response to calcareous conditions. Similar ISSR results were confirmed by Russell *et al*.(1997), Fernandez *et al*. (2002), Tanyolac, (2003), Brantestam et al (2004) Hou *et al*. (2005), Guasmi *et al*. (2009), Guasmi *et al*. (2012), El-Awady *et al* .(2012) and Rahimi *et al.* (2014) whom studied the genetic diversity in barley from different countries and they reported that the ISSR markers were superior to other markers in the capacity of revealing and more informative bands in a single amplification. ISSR marker could use as DNA marker tools in taxonomy in barley genotypes breeding under stress conditions.

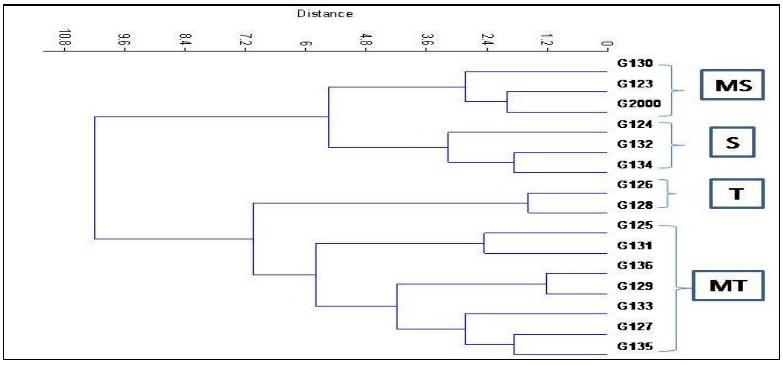

Fig. 3: Dendrogram of 15 Egyptian barley cultivars clustered using ten ISSR markers

Table 11: The genetic similarity coefficient among the 15 barley cultivars

cultivars	Giza 2000	Giza 123	Giza 124	Giza 125	Giza 126	Giza 127	Giza 128	Giza 129	Giza 130	Giza 131	Giza 132	Giza 133	Giza 134	Giza 135
Giza123	0.89													
Giza124	0.76	0.77												
Giza125	0.72	0.79	0.64											
Giza126	0.70	0.73	0.67	0.76										
Giza127	0.83	0.84	0.75	0.80	0.77									
Giza128	0.67	0.74	0.64	0.76	0.92	0.78								
Giza129	0.78	0.79	0.69	0.81	0.72	0.87	0.73							
Giza130	0.87	0.91	0.75	0.74	0.68	0.81	0.68	0.73						
Giza131	0.67	0.70	0.60	0.83	0.70	0.77	0.67	0.79	0.65					
Giza132	0.75	0.82	0.83	0.72	0.66	0.76	0.67	0.75	0.80	0.66				
Giza133	0.71	0.78	0.68	0.77	0.78	0.86	0.78	0.77	0.75	0.71	0.74			
Giza134	0.75	0.82	0.80	0.69	0.66	0.76	0.70	0.68	0.80	0.63	0.88	0.74		
Giza135	0.72	0.76	0.73	0.79	0.83	0.88	0.84	0.78	0.74	0.76	0.75	0.85	0.75	
Giza136	0.78	0.79	0.73	0.81	0.72	0.87	0.73	0.69	0.76	0.75	0.78	0.80	0.71	0.82

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