Assiut J. Agric. Sci., 52 (4) 2021 (53-65) *ISSN: 1110-0486*
Website:www.aun.edu.eg/faculty_agriculture/journals_issues_form.php *E-mail: ajas@aun.edu.eg*

Assessment of Genetic Diversity Using SCoT Markers and Some Morphological Traits in Ten Lines of Barley (*Hordeum vulgare* L.)

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Accepted for publication on: 31/12/2021

Abstract

To assessment molecular and phenotypic diversity for ten barley lines belong to *Hordeum vulgare* L., ten SCoT primers were used and 12 morphological traits were estimated in two seasons (2018/2019 and 2019/2020). The SCoT primers succeeded in generating reproducible and reliable amplicons. SCoT technique showed that 66.67 % to 100% of polymorphism. The resolving power (Rp) value varied from 4 to 11.40. In addition, the 10 lines were characterized by 41 unique markers (22 positive and 19 negative). B_6 had the highest numbers of positive markers (six). According to phenotypic evaluation, the mean squares for genotypes were highly significant for all studied traits from combined data over two seasons. The heritability values in broad sense (h^2_b %) ranged from 40.63 (100-grain weight) to 99.22 (Days to heading). The P_7 gave desired value in four traits (NT/P, NS/P, NG/S and GY/P g) and the other lines showed desired value in one or two trait, thus all traits which detected in the ten lines might be associated with all unique markers distinguished in this study. The inbred line P_6 showed the highest number of unique markers (6 positive), one or some of which may be linked with grain filling period (GFP day) trait that showed in line desirable value. Consequently, these markers may be used as selectable markers for genetic improvement of these traits in barley.

Keywords: *SCoT technique, barley, Genetic diversity, Molecular distance, Cluster analysis.*

Introduction

Barley (*Hordeum vulgare*) was one of the primary cultivated grains, as early as 10,000 years ago. It is a major cereal grain grown in temperate climates globally, self-pollinated and diploid (2n = 14). In the ancient, barley flour was the main used for breads. Malt is used to produce purified alcohol, malt syrup and malted milk. Co-products from malting and brewing are also used in fodder manufacture.

Assessment of genetic diversity and phenotyping of germplasm provide information about traits variability in any crop species and offers a basis for planning future strategies for crop development. Thus, used of molecular markers via marker-assisted selection may be improve the main traits in barley such as yield components traits. The relationship between molecular markers and morphological trait evaluation is one of the most important factors in the plant breeding and molecular genetics. It delivers important landmarks for exposition of genetic variability and discovery of genomic regions that are responsible for the morphological trait, which plays an important role in the development of barely using marker-assisted selection Dora *et al*. (2017)

and Adawy *et al*. (2008). In barley, The genetic diversity has been investigated using diverse molecular techniques as SCoT, ISSR and RAPD markers such as Aboulila and Mansour (2017), Amer *et al.* (2017), Dora *et al*. (2017) and Fernandez *et al*., (2002). SCoT is one of molecular marker system described by Collard and Mackill (2009). It built on the small preserved regions of genes are flanked by the ATG start codon of translation. SCoT uses single primer designed to anneal the around regions of the ATG on the two strands of DNA. SCoT technique is dominant marker like RAPDs (Xiong *et al*., 2011) and might be used for genetic analysis, QTL mapping and bulk segregation analysis.

The present study aimed to study the genetic diversity in ten barley lines at molecular and phenotypic levels using SCOT molecular markers. Thus, it would be determine the molecular markers that can be linked with distinguished traits in each studied line.

Materials and Methods

This study was carried out at the molecular genetic laboratory Genetics Department and Experimental Farm at Agronomy Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt. The plant material used in this study comprised of ten Libyan local lines of barley belong to species *Hordeum vulgare* were supplied from the Faculty of Agriculture, Benghazi Univ.

Molecular evaluation:

DNA was isolated from fresh leaves by DNeasy plant mini kit (QIAGEN). Genomic DNA was used as a template for Polymerase Chain Reaction using ten SCoT primers in molecular evaluation for 10 lines of barley. Amplification reactions for SCoT technique were carried out in Techni TC-512 Thermal Cycler according to Abd El-Aziz *et al*. (2019). The PCR cycles were carried out according to Rehab *et al.* (2020). DNA profiles were photographed using Bio-1D Gel Documentation system and analyzed by Gel Analyzer 3 software. DNA-profiles were done for SCoT technique according to Adhikari *et al.* (2015). Polymorphic Information Content (PIC) and Diversity Index (DI) were calculated according to, $PIC = 1-p^2-q^2$ (Gorji *et al.,* 2011). In addition, the capability of each primer to distinguish between lines was assessed according to resolving power value (Rp) calculated as described in Prevost and Wilkinson (1999). Molecular distances (MD) were calculated by Dice coefficient (Nei and Li, 1979) and cluster analysis was done using XLSTAT.7 software.

Phenotypic evaluation:

A two-year field trial was conducted at Experimental Farm, Agronomy Department. During two successive growing seasons, 2018/2019 and 2019/2020, ten barley lines were evaluated in a randomized complete block design (RCBD) with three replications. Lines were evaluated using 12 agro-morphological traits. Analysis of variance was employed in order to test the significance of the difference between the lines for the various traits across the two years. In addition, a combined analysis of variance for genotypes over the two years was made for the studied traits according to Steel and Torrie (1960). The least significant difference (LSD) test for mean comparisons was down using SAS (Ver. 9.1). The data were recorded on ten guarded randomly chosen plants per plot for all genotypes. The same procedure was followed in

the two seasons. The studied traits were: Plant height (PH cm), Number of tillers (NT/P), Number of spikes/plant (S/P),Spike length (SL cm), Number of grains/spike (NG/S), 100-grain weight (100/GWT g), Flag leaf area (LA cm2), Days to heading (DH day), Days to maturity (DM day), Grain filling period (GFP day), Grain filling rate (GFR g/day) and Grain yield per plant (GY/P g). Finally, the heritability was estimated on the basis of entry mean following $\sigma^2g/(\sigma^2g + \sigma^2gy/ry + \sigma^2e/r)$, where σ^2g, σ^2gy are the variance component due to genotypes, genotypes by years interaction and σ^2e is variance components due to unexplainable error. r, y are the number of replicates and years.

Resultes and Discussion

Ten SCoT primers were used to study the genetic differences and relationships among the 10 barley genotypes as shown in Fig. 1 and Table 1. The molecular sizes of the amplified bands varied with the different SCoT primers and ranged from 182 bp to 2203 bp. All these SCoT primers generated good banding patterns that indicated the influence of SCoT marker in fingerprinting and genetic diversity.

A total of 180 major SCoT amplified fragments were produced, out of them 143 (79.44%) were polymorphic and the polymorphism percentage ranged from 66.67 % (SCoT-1) to 100% (SCoT-9). Similarly, high level of polymorphism in barley was founded by Amer *et al*. (2017) and Dora *et al*. (2017) who found that level of polymorphism ranged from 55.56% to 91.67%. In tomato genotypes, polymorphism was 100% founded by Henareh *et al*. (2016) and 80 to 100% founded by Rehab *et al*. (2020).While, low level of polymorphism was found by Shahlaei *et al*. (2014) (23.25%). The total number of polymorphic fragments of DNA ranged from low scored by the two primers SCoT-1 and SCoT-3 (10), to high scored by the primer SCoT-12 (22). All these primers generated good banding patterns that illustrated the rule of SCoT marker in fingerprinting and diversity analyses. These differences in the number of amplified bands by altered SCoT primers are influenced by variable reasons such as number of annealing sites in the genome and primer structure (Kernodle *et al*., 1993).

The polymorphism index content (PIC) analysis was carried out to conclude the efficiency of each primer SCoT to express polymorphic loci in barley. The calculated PIC values for primers SCoT ranged from 0.222 to 0.348. The primer SCoT-3, which produced the lowest mean PIC value of 0.222, was the least polymorphic. Dora *et al*. (2017) found that the PIC ranged from 0.18 to 0.33. Resolving power (RP) is used to describe the capacity of the primer/marker combination to detect the differences among various genotypes (Prevost and Wilkinson, 1999). RP values of the ten SCoT primers ranged from 4 to 11.40 characteristic the different genotypes whereas the average was 7.76 per SCoT primer. The highest RP values were detected with the primer SCoT-12 (11.40) and the lowest with the primer SCoT-3 (4). In this respect, Dora *et al*. (2017) found that the RP values for SCoT markers was ranged from 9.6 to 12.7 in barley.

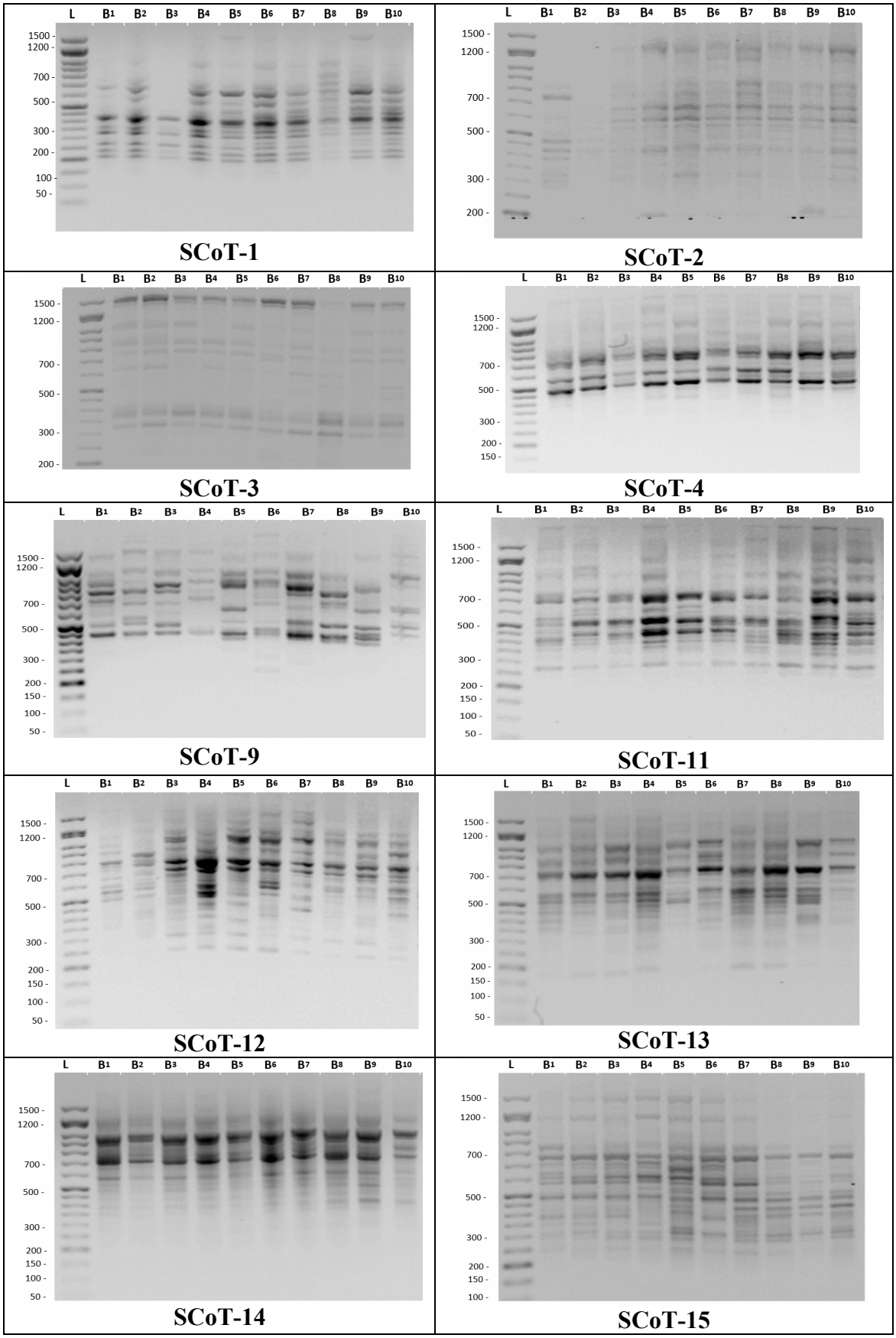

Fig. 1: Banding patterns of SCoT-PCR products for ten barley lines, M, 1.5 K bp ladder and lanes 2 to 11 represent the ten genotypes.

Table 1: Molecular data estimated from banding patterns of SCoT technique.

Primer		Amplicons								
Name	Sequence (5´→ 3`)	Molecular size range	Monomorphic	Polymorphic			Total	Polymorphism %	Polymorphic index content (PIC)	Resolving power Rp
				Without unique	Unique +	Unique -				
SCoT-1	CAACAATGGCTACCACCA	190:1623	5	7	-	3	15	66.67	0.231	5.00
SCoT-2	CAACAATGGCTACCACCC	196:1540	1	10	2	3	16	93.75	0.316	7.80
SCoT-3	CAACAATGGCTACCACCG	223:1523	3	4	4	2	13	76.92	0.222	4.00
SCoT-4	CAACAATGGCTACCACCT	454:2122	5	10	2	-	17	70.59	0.278	7.60
SCoT-9	CAACAATGGCTACCAGCA	239:2203	-	12	6	1	19	100.00	0.348	10.20
SCoT-11	AAGCAATGGCTACCACCA	222:2065	5	12	1	1	19	73.68	0.306	9.40
SCoT-12	ACGACATGGCTACCAACG	251:1674	2	17	2	3	24	91.67	0.324	11.40
SCoT-13	ACGACATGGCGACCATCG	182:1866	6	11	1	2	20	70.00	0.278	8.80
SCoT-14	ACGACATGGCGACCACGC	286:1539	5	8	1	2	16	73.68	0.234	5.40
SCoT-15	ACGACATGGCGACCGCGA	244:1519	5	11	3	2	21	76.19	0.257	8.00
Overall		**182:2203**	**32**	**102**	**22**	**19**	**180**	**79.32**	**0.28**	**7.76**

The genetic fingerprints for all ten lines of barley were performed as DNA-profile diagram (Figure 2) based on 180 amplicons obtained using 10 SCoT primers. This profile showed that the amplicons per lines were variously ranged from 94 (for B_1) to 120 (for B_7). In addition, the 10 lines were categorized by 41 unique bands (markers) (22 positive and 19 negative). B_6 had the highest numbers of positive bands (six). These markers were spread over these lines variously differentiate each lines from the other. These unique bands may be suitable as unique markers as clarified by Abd El-Aziz *et al*. (2016) and Rehab *et al*. (2020) in tomato, Abd El-Aziz and Rehab (2016) in canola and Abd El-Hadi *et al*. (2017) in squash. These results indicated that DNA-profiling diagram also is a good tool for molecular identification for ten barley genotypes. Thus, it was assumed that SCoT primers used in this study were with high degree of confidence for the molecular identification. These results agree with Aboulila and Mansour (2017) who studied the genetic diversity between ten barley genotypes using SCoT marker, and they described that SCoT marker is an effective tool for finding new fingerprint of barley.

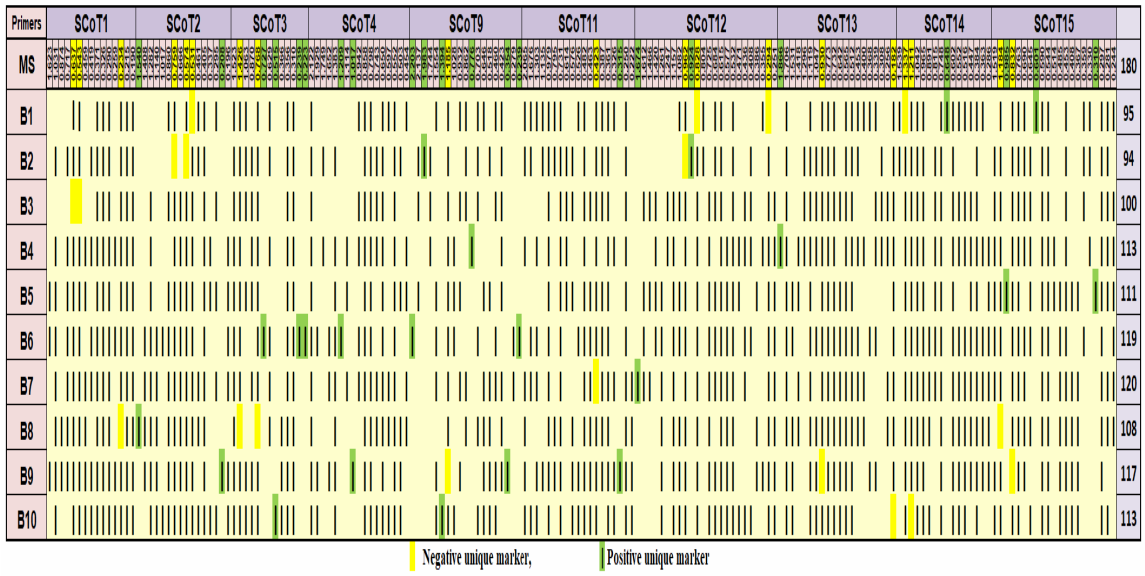

Fig. 2: DNA-profile representations of SCoT fingerprint of ten barley lines based on 180 amplicons 41 of them were marker loci.

According to Table 3, the molecular distance (MD) among all studied lines based on SCoT data ranged from 0.447 to 0.839. The highest molecular distance (MD) was among B_9 and B_2 (0.839) followed by B_5 and B_1 (0.830). While the lowest MD was between B_8 and B_7 (0.447) followed by B_{10} and B_9 (0.529). This means that B_9 and B_2 were the best genotypes that can be used in breeding programs to obtain the genetic variability and/or hybrid vigor from hybridization between them.

Table 3. The molecular distance (MD) between all ten barley lines based on SCoT data.

	B1	B2	B3	B4	B5	B6	B7	B8	B9
B2	0.590								
B3	0.614	0.535							
B4	0.738	0.609	0.532						
B5	0.830	0.725	0.532	0.638					
B6	0.737	0.815	0.601	0.677	0.695				
B7	0.630	0.780	0.613	0.594	0.539	0.585			
B8	0.705	0.714	0.680	0.690	0.571	0.698	0.447		
B9	0.738	0.839	0.766	0.818	0.596	0.615	0.605	0.553	
B10	0.784	0.747	0.671	0.737	0.567	0.604	0.569	0.573	0.529

According to the UPGMA clustering algorithm from SCoT markers, the 10 lines of barley were divided into three major clusters (Fig. 3). The first major cluster was divided into two sub-clusters. The first sub-cluster consisted of lines B_4 and B_3 and the second sub-cluster consisted of lines B_2. The second main cluster consisted of one lines B_1. In addition, the third main cluster was divided into two sub-clusters. The first sub-cluster contained of lines B_8, B_7, B_5, B_{10} and B_9 and the second sub-cluster contained of one line B_6 which was substantiated before by the presence of

highest number of specific unique bands over other tested lines.

In conclusion, this study confirms the capability of SCoT as a good marker to examine the genetic relationships between different lines of barley and obtaining new specific markers. Documentation of new specific markers is very significant for breeders to evaluate barley genotypes for breeding programs.

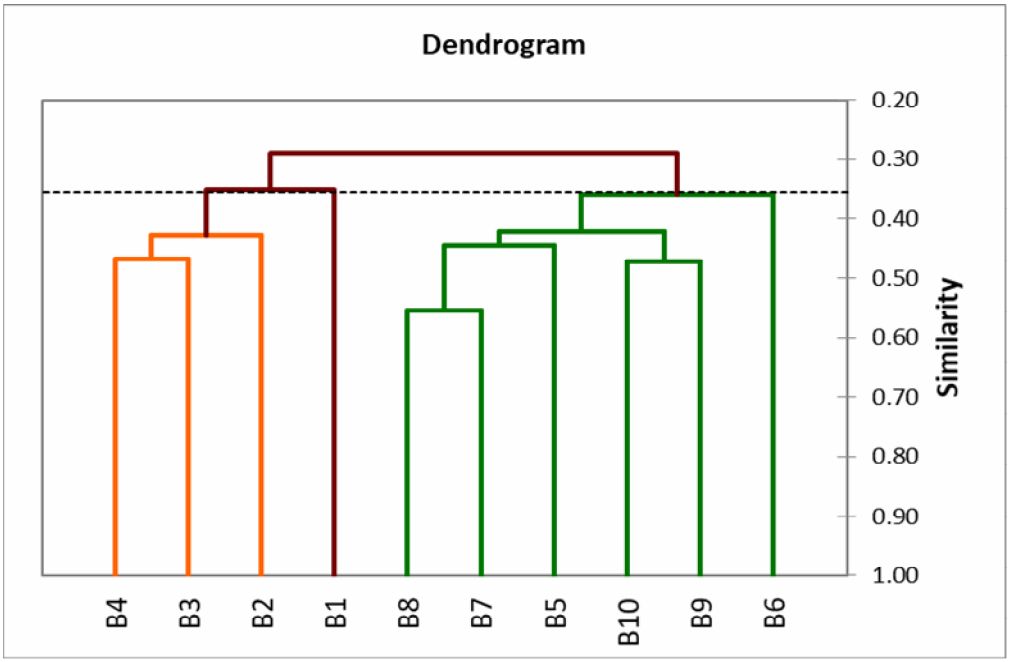

Fig.3: Dendrogram derived by UPGMA method using Dice-similarity coefficient for binary data of SCoT technique for ten barley lines.
Legend: TL represents truncated line at a coefficient of Similarity=0.35

Phenotypic evaluation:

The combined analyses of variance for the agronomic traits are shown in Table 4. The obtained results revealed that the magnitudes of the mean squares for genotypes were highly significant for all studied traits, indicating the presence of genetic differences among barley genotypes for the studied traits. These result in agreement with Pesaraklu *et al*. (2016). As well as, the mean squares due to genotypes by year interaction were highly significant for plant height (PH cm), number of tillers (NT/P), and number of spikes/plant (NS/P). The mean squares due to years were highly significant for number of tillers (NT/P) and number of spikes/plant (NS/P). While, it was significant for plant height (PH cm) and Spike length (SL cm). These results suggest that these lines of barley showed different performances at different environments conditions and may have different combining ability pattern and in crosses depending on type of other parent. In addition, the heritability values in broad sense (h^2_b %) ranged from 40.63 (100-grain weight) to 99.22 (Days to heading). Pesaraklu *et al.* (2016) found that the heritability values in broad sense (h^2_b %) ranged from 47.7 (Plant height) to 87.7 % (Grains per spike)

Table 4. Estimated mean squares and broad-sense heritability (h^2b %) of different agronomic traits in ten barley lines from the combined data over two growing seasons

S.O.V	d.f	PH (cm)	NT/P	NS/P	SL (cm)	NG/S	100/GWT
Rep/Years	4	60.01**	6.07	3.27	0.77	111.03	0.12
Years (Y)	1	38.40*	70.42**	62.02**	1.66*	0.00	0.01
Genotypes(G)	9	418.67**	52.83**	59.68**	7.11**	687.42**	0.97**
G/Y	9	90.94**	22.42**	21.31**	0.44	0.000	0.45
Error	36	12.39	4.03	3.58	0.38	87.89	0.31
h^2_b %		76.03	53.48	60.65	89.05	79.64	40.63
S.O.V	**d.f**	**LA**	**DH day**	**DM day**	**GFP**	**GFR**	**GY/P**
Rep/Years	4	2.12	0.93	1.38	4.27	0.01	7.19
Years (Y)	1	0.51	0.00	20.42	24.07	0.008	0.001
Genotypes(G)	9	484.71**	752.96**	464.91**	252.51**	0.47**	1146.46**
G/Y	9	0.17	3.11	1.75	4.92	0.003	5.52
Error	36	15.16	2.80	2.88	6.03	0.01	9.13
h^2_b %		94.11	99.22	98.77	95.36	95.89	98.42

Plant height (PH cm), Number of tillers (NT/P), Number of spikes/plant(S/P),Spike length (SL cm), Number of grains/spike (NG/S), 100-grain weight (100/GWT g), Flag leaf area (LA cm^2), Days to heading (DH day), Days to maturity (DM day), Grain filling period (GFP day), Grain filling rate (GFR g/day) and Grain yield per plant (GY/P g).

The results from Table 5 showed the lowest and highest mean performance values for studied phenotypic traits of ten barley lines. In all traits, the favorable genotypes were the ones with higher values of the traits except for plant height, days to heading, days to maturity, grain filling period and grain filling rate.

Table 5. Mean performance range and desirable values for all studied traits in ten lines

desirable value	High		Low		Traits
	Lines	Value	Lines	Value	
Low	B_6	124.41	B_3	99.93	PH cm
High	B_7	30.00	B_8	20.00	NT/P
High	B_7	28.67	B_8	18.67	NS/P
High	B_1	11.67	B_{10}	8.17	SL cm
High	B_7	88.00	B_6	52.66	NG/S
High	B_5	7.44	B_9	6.10	100/GWT g
High	B_{10}	51.55	B_2	22.13	LA cm^2
Low	B_7	95.00	B_3	62.33	DH day
Low	B_{10}	156.50	B_2	131.33	DM day
Low	B_3	73.50	B_6	53.00	GFP day
Low	B_7	1.66	B_8	0.04	GFR g/day
High	B_7	91.90	B_8	39.45	GY/P g

Plant height (PH cm), Number of tillers (NT/P), Number of spikes/plant(NS/P),Spike length (SL cm), Number of grains/spike (NG/S), 100-grain weight (100/GWT g), Flag leaf area (LA cm^2), Days to heading (DH day), Days to maturity (DM day), Grain filling period (GFP day), Grain filling rate (GFR g/day) and Grain yield per plant (GY/P g).

In the present study, the tested SCoT primers showed high efficiency in generating specific unique markers for all tested lines of barley. Based on desirable performance of ten lines for studied traits, data presented in Table 6 clearly reflected that plant height (PH cm), number of tillers (NT/P), number of spikes/plant (NS/P), spike length (SL cm), number of grains/spike (NG/S), 100-grain weight (100/GWT g), flag leaf area (LA cm^2), days to heading (DH day), days to maturity (DM day), grain filling period (GFP day), grain filling rate (GFR g/day) and grain yield per plant (GY/P g) traits which showed desirable values could be associated with some unique markers detected in this study. These unique markers were 19 negative and 22 positive SCoT DNA fragments. The line P_6 showed the highest number (six) of positive unique markers where one or more of them may be linked with grain filling period (GFP day) trait that indicated in this line a desirable value. Similarly, the line B_1 which exposed two positive unique markers at least one of them may be linked with spike length (SL cm) trait. Also, B_5 showed two positive unique markers one or more of them may be associated with 100-grain weight (100/GWT g) trait. These results illustrated that some of these markers may be used as markers assisting selection in the breeding program to improve barley lines. Similar conclusion was obtained by Giancarla *et al*. (2012) in barely, Abd El-Aziz *et al*. (2017) in okra and Abd El-Hadi *et al.* (2017) in squash.

Table 6: The relationship between molecular markers and distinguished traits

Distinguished traits		Unique markers				Inbred lines
Mean per-formance	Trait	Total	Type	Molecular size	Primer	
11.67	SL cm	6	- - + - +	531 294,828 648 1337 310	SCoT-2 SCoT-12 SCoT-14 SCoT-14 SCoT-15	B_1
131.33	DM day	5	- - + -	574,759 1983 924 962	SCoT-2 SCoT-9 SCoT-12 SCoT-12	B_2
99.93 62.33	PH cm DH day	2	-	543,637	SCoT-1	B_3
---	--	2	+ +	776 1866	SCoT-9 SCoT-13	B_4
7.44	100/GWT g	2	+	310, 985	SCoT-15	B_5
53.00	GFP day	6	+ + +	223,239,727 1209 239, 2203	SCoT-3 SCoT-4 SCoT-9	B_6
30.00 28.67 88.00 91.90	NT/P NS/P NG/S GY/P g	2	- +	423 1674	SCoT-11 SCoT-12	B_7
0.04	GFR g/day	5	- + - -	234 1540 768,1426 1184	SCoT-1 SCoT-2 SCoT-3 SCoT-15	B_8
---	---	7	+ - + - + - -	208 1017 354 1185 315 930 832	SCoT-2 SCoT-4 SCoT-9 SCoT-9 SCoT-11 SCoT-13 SCoT-15	B_9
51.55	LA cm^2	4	+ + - -	515 1394 182 1211	SCoT-3 SCoT-9 SCoT-13 SCoT-14	B_{10}

Plant height (PH cm), Number of tillers (NT/P), Number of spikes/plant(NS/P),Spike length (SL cm), Number of grains/spike (NG/S), 100-grain weight (100/GWT g), Flag leaf area (LA cm^2), Days to heading (DH day), Days to maturity (DM day), Grain filling period (GFP day), Grain filling rate (GFR g/day) and Grain yield per plant (GY/P g).

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Assiut J. Agric. Sci., 52 (4) 2021 (53-65) *ISSN: 1110-0486*
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تقدير التنوع الوراثى باستخدام واسمات SCOT وبعض الصفات المورفولوجية فى عشرة سلالات من الشعير

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الملخص

لتقدير التنوع الجزيئى والمظهرى لعشرة سلالات من الشعير (*Hordeum vulgare* L.)، تم استخدام عشرة بادئات من SCOT بالاضافة لتقدير ١٢ صفة مورفولوجية فى موسمين (٢٠١٨/٢٠١٩ و ٢٠١٩/٢٠٢٠). هذه البادئات نجحت فى انتاج العديد من تتابعات DNA المتباينة. أظهر تكنيك SCoT تنوع جزيئى يتراوح من ٦٦,٦٧ الى ١٠٠%، وكانت قيمة متوسط قوة التحليل Rp تتراوح من ٤ الى ١١,٤٠. بالاضافة الى ان السلالات العشر اظهرت ٤١ واسمة جزيئية متنوعة ومنفردة (٢٢ موجبة و ١٩ سالبة)، اعطت السلالة B_6 اعلى عدد من الواسمات الجزيئية الموجبة.

تبعا للتقييم المظهرى، كان التباين الراجع للتراكيب الوراثية عالى المعنوية لكل الصفات المدروسة وكانت قيمة معامل التوريث فى المدى الواسع تتراوح من ٤٠,٦٣ الى ٩٩,٢٢%. أعطت السلالة B_7 قيما مرغوبة ومختلفة عن باقى السلالات فى أربع صفات (NT/P, NS/P, NG/S and GY/P g)، بينما اعطت كل سلالة من السلالات الأخرى قيم مرغوبة فى صفة او صفتين فقط ، ولهذا فان هذه الصفات التى تميزت بها العشر سلالات من الشعير ربما يمكن ربطها مع الواسمات الجزيئية المنفردة التى تم الحصول عليها. السلالة B_6 أظهرت اعلى عدد من الواسمات الجزيئية الموجبة والمتفردة (ستة واسمات ايجابية) فإن واحدة او اكثر من هذه الواسمات قد تكون مرتبطة مع الصفة التى أعطت فيها هذه السلالة قيم مرغوبة (GFP day) عن باقى السلالات. وهذا يدل على أن هذه الواسمات الجزيئية المنفردة ربما يمكن استخدامها كعلامات انتخابية فى برامج التربية للتحسين الوراثى لهذه الصفات فى الشعير.