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Genetic Diversity among some Egyptian Barley (*Hordeum vulgare* L.) Cultivars Based on Protein and RAPD Markers

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ABSTRACT

Genetic variability of seven Egyptian barley cultivars was analyzed utilizing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and random amplified polymorphic DNA (RAPD) techniques. The results of SDS-PAGE produced 12 bands ranging from 135 to 11 KDa. Two polymorphic bands at molecular weight (MW) 13 and 12 KDs were detected with 16.6% polymorphism among barely cultivars. In the other hand, five RAPD primers were employed to assess the genetic diversity and relationships between the seven Egyptian barley cultivars. RAPD analysis exhibited 93 amplicons (86% polymorphism) with an average number of 18.6 amplicons per primer. Genetic similarity of RAPD and protein input ranged from 0.31 to 0.83. The dendrogram of combined data had clustered all the cultivars into two main clusters; the first one containing all barley cultivars except Giza 123 cultivar which formed a separate cluster indicating that the genetic background of this cultivar was distinct from all cultivars. The results obtained from RAPD and protein analysis exhibit different level of polymorphism. RAPD profile is more suitable technique than SDS-PAGE in assessing genetic variation among the seven barely cultivars. Moreover, the present results suggest that, the increasing number of primers and using more different markers could be more accurate to discriminate the genetic variance between barley cultivars. Subsequently, this could be useful to differentiate between barley cultivars in the breeding program.

Keywords: Barley, genetic polymorphism, SDS-PAGE- protein and RAPD.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the main cereal crops, the oldest and fourth largest cereal plant in the worldwide. Barley belongs to the genus *hordeum* and barley genome is a diploid (2n) which contains fourteen chromosomes. This crop is grown in arid and semi-arid regions in the Middle East and North Africa because this crop needs low cultivation inputs such as fertilizer, irrigation and insecticides. Also, tolerance to abiotic stress and containing glutenin protein is important in the industry (Hayes *et al*., 2000; Kumar *et al*., 2014 and Mariey *et al*., 2018). In addition to, diploid barley is appropriate example crop to study genome diversity and phenotypic differences that happen during transition from growing landraces to cultivation of new cultivars (Brantestam *et al*. 2007). Awareness of the nature of genetic diversity among varieties and relationships is serious for detecting the genetic variability attainable and employment in breeding programs for barley. Classical techniques of plant breeding that depend on phenotypic traits have made considerable contributions to crop improvement and development but it is very slow in aiming difficult traits such as seeds quality, abiotic stress tolerance and yield, these techniques are unsuitable to detect variance in barley plant (Buck-Sorlin 2002 and Mzid *et al*., 2016). Various methods exist to estimate genetic diversity, biochemical markers such as SDS–PAGE is a sturdy tool for characterizing patterns of genetic diversity and differentiation among plant species. Protein banding patterns reveal information about similarities and dissimilarities between cultivars, thus reflecting genome relationships in the breeding material. Also provide a basis for identifying the different cultivars of a given species (Mishra *et al*., 2010; Eid, 2018 and Ali *et al*., 2019).

In the last years, new markers were used as a powerful methods for characterization and determination of genetic variance among cultivars which are independent of environmental effects (Liu *et al*., 2006 and Velicevici *et al*., 2012). The discovery of polymerase chain reaction (PCR) make revolution in genetic field and create numerous molecular techniques like RAPD-PCR, simple sequence repeats and inter simple sequence repeat (Matus *et al*., 2002; Rashal *et al*., 2004; An *et al*., 2009 and Hasan *et al*., 2018). Nevertheless, molecular markers have been applied in linkage map construction, variety identification, detecting alien chromosome substitutions and genetic diversity in barley (Tahir 2014 and Elakhdar *et al*., 2018). RAPD profile is easy, unlimited marker numbers, fast and efficient technique. Thus, it is widely used for determining genetic variation among barley varieties (Tanyolac *et al*., 2003; Raoudha *et al*., 2010 and Saroei *et al*.,2017).The use of biochemical and modern molecular markers to identify genotypic variation between barley genotypes is an important step toward effective and specific strategies for breeding program. Thus, the target from this study was to determine the genetic diversity among some Egyptian barley cultivars based on the genetic distances obtained from SDS-PAGE and RAPD-PCR markers.

MATERIALS AND METHODS

Plant materials

Seven Egyptian barley cultivars namely; Giza 123, Giza 126, Giza 2000, Giza 129, Giza 131, Giza 132 and Giza 136 were obtained from the agricultural research center (ARC), Giza, Egypt .

Methods

SDS-protein electrophoresis

Barley leaves were grind to powder with liquid nitrogen, after that the extraction buffer (20 mM phosphate buffer, pH 7) was added to the samples at a ratio of 1:10 (w/v),vortex at 5000 rpm for 4 min at 25°C ,then put in centrifuge at 10000 rpm for 6 min; the total proteins isolated from barley were migrated using one dimensional SDS polyacrylamide gel (SDS-PAGE) as described in the methods of Laemmli (1970) and as modified by Studier (1973).To estimate the molecular weights (MW) of the migrated total proteins, the proteins marker method (Thermo Scientific PageRuler Prestained Protein Ladder) was used.

RAPD analysis

Total DNA was isolated from fresh leaves of barley cultivars using the CTAB protocol (Murray and Thompson, 1980). Five primers of RAPD obtained from Bio Basic Canada Inc. were used (Table 2). PCR multiplication was carried out in 20 µL reaction volume containing 1 µL genomic DNA (20 ng/µL), 10 µL master mix (Biotecke Corporation), 1 µL primer (100 ng/µL) and

8 µL of nuclease free water. The conditions were started with an initial step of 5 min at 94°C. After that, the multiplication reaction was carried out using 39 cycles of 1 min at 94°C for denaturation, an annealing step of 1 min at 34°C, an elongation step of 1 min at 72°C and finally a terminal extension cycle at 72°C for 5 min. The PCR products were migrated in 1.5% agarose gel in 1x TAE solution, DNA bands visualized and photographed under UV light using the gel documentation system (Bio-Rad® Gel Doc-2000). One hundred bp DNA marker was used.

Data analysis

The amplicons in the gel profiles of protein and RAPD were recorded as (1 and 0). The genetic similarity coefficients were assessed according to the Dice coefficient (Sneath and Sokal, 1973). The dendrogram based on unweighted pair group protocol with arithmetic mean algorithm (UPGMA) was produced by using the computer program systat ver. 7 (SPSS Inc. 1997 SPSS Inc.3/9`7 standard version) (Yang and Quiros, 1993).

RESULTS AND DISCUSSION

Protein banding patterns

Protein banding patterns in leaves of the seven Egyptian barely cultivars were analyzed by SDS polyacrylamide gel electrophoresis under reducing conditions. The results showed that total number of bands in the profiles of protein was 12 with approximate molecular weights ranging from 135 to 11 KDa (Fig. 1)With only two polymorphic bands at MW 13 and 12 KDs and all remaining bands were monomorphic which found in the profiles of all cultivars. These bands may represent the species specific basic proteins of Egyptian barley, and are represent conserved by gene coding (Javaid *et al*., 2004). These results are in agreement with those of Manal Eid (2018) who measured the genetic similarity among some barley varieties and noted high similarity among them. She suggested that the similarity could reflect the common origin of many of these cultivars. Electrophoretic patterns indicated a considerable similarity among barely cultivars which was expressed as a level of polymorphism of 16.6%. Ali *et al.* (2019) used SDS-PAGE to classify glutenin in barley lines. The glutenin protein was classified into five different groups on the basis of variation present in the barley lines, and different intensity of bands was observed. Moreover, Mzid *et al.* (2016) compared the genetic variance of a set of fifty Lebanese barley landraces with the wild *Hordeum spontaneum* and 2 commercial cultivars using SDS-PAGE patterns.

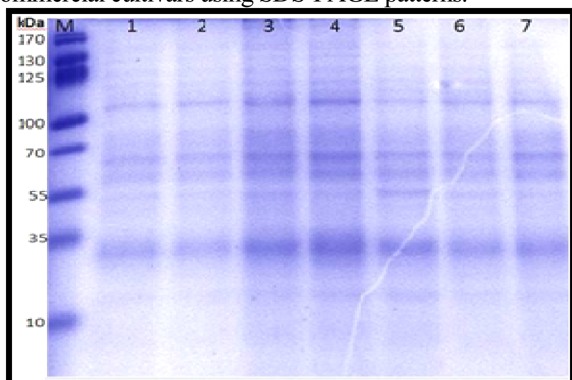

Fig. 1. SDS-PAGE patterns of total proteins in leaves of barley cultivars. Lanes 1 -7 represent (Giza 123, Giza 126, Giza 2000, Giza 129, Giza 131, Giza 132, and Giza 136, respectively). M: protein marker.

To define the relationships among barley cultivars, the collected data (1and 0) produced from the protein profiles was used to compute the similarity matrices according to coefficient of Dice (Sneath and Sokal, 1973).The results of SDS-PAGE exhibited high genetic similarity (Table 1) between barley cultivars ranged from 1 to 0.90. The highest genetic similarity (1.00) was between (Giza 123 and Giza 126), (Giza 2000 and Giza 129), (Giza 131 and Giza 132), (Giza 131 and Giza 136) and (Giza 132 and Giza 136). While, the lowest genetic similarity (0.90) was observed between (Giza-123 and Giza 2000), (Giza-123 and Giza 129), (Giza-126 and Giza 2000) and (Giza-126 and Giza 129).

Table 1. Genetic similarity matrix of barley cultivars based on protein data.

	Giza 123	Giza 126	Giza 2000	Giza 129	Giza 131	Giza 132	Giza 136
Giza 123	1.00						
Giza 126	1.00	1.00					
Giza 2000	0.90	0.90	1.00				
Giza 129	0.90	0.90	1.00	1.00			
Giza 131	0.95	0.95	0.96	0.96	1.00		
Giza 132	0.95	0.95	0.96	0.96	1.00	1.00	
Giza 136	0.95	0.95	0.96	0.96	1.00	1.00	1.00

The dendogram developed based on Dice coefficient revealed the genetic relationship among the seven barely cultivars with different linkage distance, as shown in Fig. 2. The dendogram grouped the seven cultivars in two clusters, the first one separated into 2 sub-clusters. The first sub-cluster included Giza 2000 and Giza 129.Wherease, the second sub-cluster included Giza 131, Giza 132 and Giza 136. However, the second cluster contained cultivars Giza 123 and Giza 126 only. SDS-PAGE marker was not efficient to identify and differentiate between the barley cultivars. Therefore, enhancing the available knowledge of plant genetic resources may contribute to their conservation and utilization in breeding programs (Tahir 2014; Mishra *et al*., 2010 and Ali *et al*., 2019).

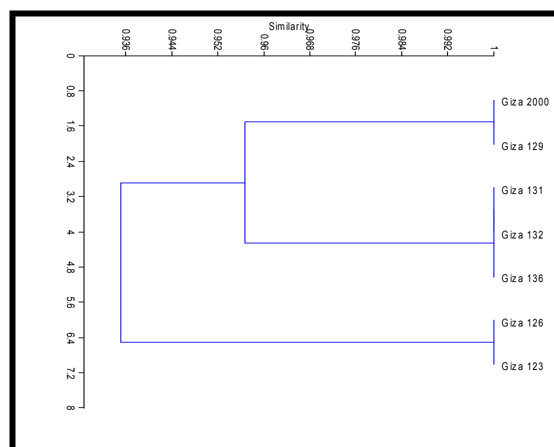

Fig. 2. Dendrogram for the barley cultivars constructed based on protein data using (UPGMA) computed according to coefficient of Dice.

RAPD-PCR profile

Five random primers were employed in the present study to identify the seven barley cultivars (Table 2 and Fig. 3). The five primers produced reproducible and scorable RAPD profiles with number of fragments ranging from 13 to 28 amplicons per primer. In this investigation, the total

number of fragments generated by the five primers was 93 with an average number of amplicons 18.6 per primer. Eighty fragments out of ninety three were polymorphic (86 %).The number of amplified DNA fragments was scored for each primer, primer H5 amplified the highest number of amplicons (28).While, the lowest number was (13) with the B12 primer. Molecular genetic techniques such as RAPD has been applied in many crop plants for genomic mapping, identification of specific cultivars related with genes of interest, and genetic variation studies, because of simplicity, versatility and ability to generate high rates of polymorphism (Chen *et al*., 2000; Aida *et al*., 2007 and Raoudha *et al*., 2010). Nevertheless, Ciulca *et al*. (2010) used RAPD marker for the recognition of relationship between barley cultivars with a view to estimate the genetic distance and genetic variation.

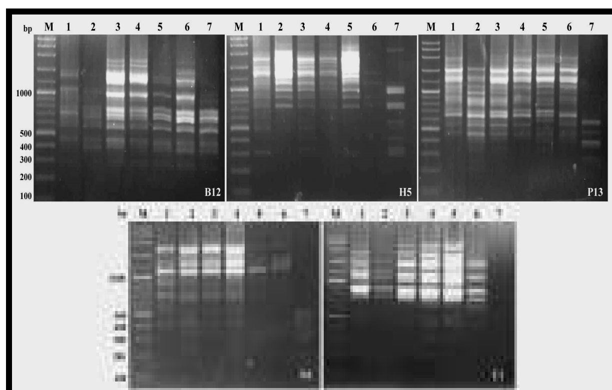

Fig. 3. RAPD profile of the barley cultivars. M, 100 bp DNA ladder marker, Lanes 1 -7 represent (Giza 123, Giza 126, Giza 2000, Giza 129, Giza 131, Giza 132, and Giza 136, respectively).

Table 2. The amplicons as revealed by RAPD markers among the seven barley cultivars.

Primer name	Sequence (5`-3`)	Total No. of amplicons	Monomorphic amplicons	Polymorphic amplicons	% of polymorphism
C1	5`TTCGAGCCAG-3`	18	5	13	72 %
P13	5`GGAGTGCCTC-3`	19	0	19	100%
N8	5`ACCTCAGCTC-3`	15	1	14	93%
B12	5`CCTTGACGCA-3`	13	5	8	61.5%
H5	5`AGTCGTCCCC-3`	28	2	26	92.8%
Total	-----	93	13	80	
Average	-----	18.6	0	16	86

The level of genetic similarity (GS) between the seven barley cultivars (Table 3) ranged from 0.28 to 0.87.The highest similarity (0.87) was observed between Giza 129 and Giza 131, while the lowest genetic similarity (0.28) was observed between Giza 123 and Giza 136. On the other hand, Giza-2000 was slightly related to Giza 129 (0.86). These findings are similar to Tahir (2014) who used the techniques of RAPD-PCR and protein banding patterns to assess the genetic variation between barley cultivars. His results showed that barley cultivars which analyzed with 18 RAPD markers, revealed 116 amplified bands that contained 63 polymorphic fragments, with an average of 3.937. Moreover, genetic dissimilarity revealed from RAPD data ranged from 0.357 to 0.767. Whereas, Karim *et al*. (2010) found that the genetic distance in Tunisian barley based on RAPD was ranging from 0.114 to 0.933.

Table 3. Genetic similarity matrix of barley cultivars based on RAPD data.

	Giza 123	Giza 126	Giza 2000	Giza 129	Giza 131	Giza 132	Giza 136
Giza 123	1.00						
Giza 126	0.29	1.00					
Giza 2000	0.36	0.76	1.00				
Giza 129	0.39	0.73	0.86	1.00			
Giza 131	0.32	0.74	0.81	0.87	1.00		
Giza 132	0.52	0.58	0.65	0.72	0.71	1.00	
Giza 136	0.28	0.67	0.70	0.76	0.80	0.68	1.00

The UPGMA dendrogram of RAPD generated from the similarity values is shown in Fig. (4). This dendrogram separated the seven cultivars into 2 clusters, the first cluster included two main sub-clusters, and the first sub-cluster included Giza126, Giza 2000, Giza129, Giza131 and Giza136.While, the second sub-cluster contained Giza 132 cultivar. On the other hand, the second cluster included Giza123 only.

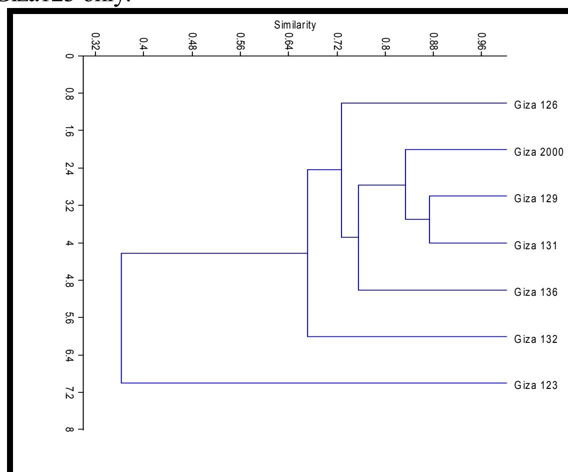

Fig. 4. Dendrogram for the barley cultivars constructed based on RAPD data using UPGMA computed according to coefficient of Dice.

Genetic relationships as revealed by protein and RAPD markers.

In this study, the genetic markers as biochemical and molecular marker, exhibited differences at the level of genetic similarity among the seven barley cultivars. This could be a result of the different mechanisms of polymorphism detection by the proteins and RAPD markers. Protein is product of gene expression, while, RAPD polymorphism results from DNA nucleotide sequence divergence at primer attached to random sites and from DNA length differences between different primer binding sites (Ovesna *et al*., 2002).

The genetic similarity estimates (Table 4) ranged from 0.45 to 0.89. The highest GS 0.83 was between Giza 129 and Giza 131, followed by (0.88) between Giza 2000 and Giza129. Whereas, the lowest genetic similarity (0.45) was detected between (Giza123 and Giza126) and (Giza123 and Giza136).

Cluster analysis based on combined proteins and RAPDs data

The dendrogram based on Dice coefficient using proteins and RAPD data showed the linkage distance between the 7 barley cultivars is illustrated in Fig. (5).The dendrogram clustered the barley cultivars into 2 clusters, the

first one divided into 2 sub-clusters. The first one included Giza 126, Giza 2000, Giza 129, Giza 131 and Giza 136 which revealed the highest genetic relationship due to the fact that they might share common ancestors. While, the second sub-cluster contained Giza 132. On the other hand, Giza 123 cultivar formed a separate cluster indicating the genetic background of the Giza 123 cultivar was distinct from all cultivars. The results almost agree with Manal Eid (2018) who studied the genetic polymorphism using SDS-proteins patterns. Her results produced 30 bands with MW ranging from 12 to 148 KD, of which only 5 bands varied among the accessions with polymorphism (83.3%).Her results also separated Giza123 cultivar from the other cultivars.

Table 4. Genetic similarity matrix computed according to coefficient of Dice based on protein and RAPD combined data.

	Giza 123	Giza 126	Giza 2000	Giza 129	Giza 131	Giza 132	Giza 136
Giza 123	1.00						
Giza 126	0.45	1.00					
Giza 2000	0.49	0.80	1.00				
Giza 129	0.51	0.78	0.88	1.00			
Giza 131	0.46	0.79	0.84	0.89	1.00		
Giza 132	0.62	0.66	0.71	0.77	0.76	1.00	
Giza 136	0.45	0.74	0.75	0.80	0.84	0.74	1.00

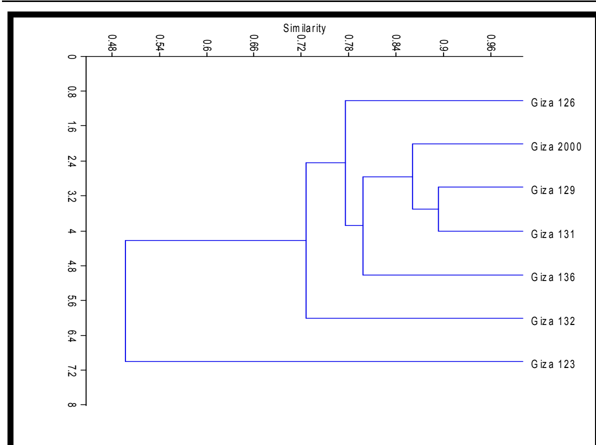

Fig. 5. Dendrogram for the barley cultivars constructed based on protein and RAPD data using UPGMA computed according to coefficient of Dice.

In the present study, the genetic diversity among seven barley cultivars was examined by using SDS-PAGE analysis and RAPD-PCR markers. The SDS-PAGE revealed low genetic polymorphism (16.6%) with high genetic similarity (1.00) and low genetic similarity (0.90).While, RAPD-PCR markers showed higher genetic polymorphism (86%) than protein analysis among barley cultivars. In this respect, Weiss *et al*. (1991) concluded that, SDS-PAGE method was not adequate to discriminate all 55 European winter and spring barley cultivars, although results showed they were genetically related. Furthermore, the cluster analysis revealed by RAPD data was not in harmony with that revealed by SDS-PAGE. However, the dendrogram of combined data was a closey related to dendrogram revealed by RAPD marker which showed a close relationship between the cultivars (Giza 129 and Giza 2000) and between (Giza 129 and Giza 131). Cluster analysis also separate Giza123 cultivar from the other cultivars which reflect the genetic distance of this cultivar. The cause to find the levels of diversity in some cultivars could be that their proteins are controlled by quantitative gene families that have arisen by divergence from an ancestral gene through chromosomes aberration. These finding agree with pervious research (Tahir 2014; Mzid *et al*., 2016 and Eid 2018).

CONCLUSION

The present results of RAPD and protein analysis exhibit difference in the degree of genetic diversity between all cultivars. Because, protein profile revealed no major differences in bands between *hordeum* cultivars. Whereas, the results obtained from RAPD markers exhibit more accurate polymorphism than SDS-PAGE analysis among barely cultivars. Furthermore, the results of dendrogram based on combined data showed that it is close to dendrogram based on RAPD analysis. RAPD profile was more efficiency than SDS-PAGE markers for determining genetic variation. The present results suggests that, the increasing number of primers and using more different markers could be more accurate to discriminate the genetic variance between barley cultivars. This finding could be useful to differentiate between barley cultivars in the breeding program.

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التنوع الوراثى بين بعض أصناف الشعير المصرى (*Hordeum vulgare* L.) المبنية على واسمات البروتين و RAPD

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تم تحليل التباين الوراثي لسبعة أصناف من الشعير المصري بإستخدام التفريد الكهربى للبروتينات (SDS-PAGE) والتضاعف العشوائى للحمض النووى (RAPD). وقد أظهرت نتائج SDS-PAGE عن وجود 12 حزمة لكل الأنواع الوراثية ،عند وزن جزيئى يتراوح ما بين 135 إلى 11 كيلو دالتون منهم حزمتين عند وزن جزيئى 13 و 12 كيلو دالتون مختلفتين فى جميع الأصناف. بالإضافة إلى ذلك ، كان مستوى التباين بين الأصناف قيد الدراسة 16.6 %. و عند إستخدام خمسة من بادئات الـ RAPD لتقييم التنوع الوراثي والعلاقات بين الأصناف السبعة للشعير المصري أظهر تحليل الـ RAPD عن وجود 93 حزمة بمتوسط عدد الحزم 18.6 للبادئ الواحد. و بذلك كانت تمثل نسبة تعدد مظاهر (86 ٪). و قد تراوحت نسبة التشابه الوراثى المحسوب من بيانات الـ RAPD وبيانات البروتين مابين 0.31 إلى 0.83. وقسمت شجرة القرابة الوراثية dendrogram الناتجة من البيانات المدمجة جميع الأنماط الوراثية إلى مجموعتين ؛ المجموعة الاولى تحتوي على جميع أصناف الشعير باستثناء صنف جيزة 123 الذى وضع فى مجموعة منفصلة مما يشير إلى أن الخلفية الوراثية لهذا الصنف كانت مختلفة عن جميع الأصناف الاخرى. و تظهرالنتائج التي تم الحصول عليها من RAPD وتحليل البروتين مستوى مختلفا من تعدد المظاهر. ومع ذلك ، فإن تكنيك الـ RAPD كان أكثر ملاءمة من SDS-PAGE في تقييم التباين الوراثي بين الأصناف السبعة. علاوة على ذلك ، فإن النتائج الحالية تقترح أنة عند زيادة عدد البادئات وإستخدام واسمات أكثر اختلافاً حيث أن ذلك يمكن أن يكون ذلك أكثر دقة لتمييز التباين الوراثي بين الآصناف المختلفة من الشعير . و الذى يعتبر مفيداً للتمييز بين أصناف الشعير فى برامج التربية.