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HERITABILITY AND RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) OF SOME EXOTIC BARLEY (*Hordeum vulgare* L.) GENOTYPES UNDER RAS SUDR CONDITIONS SOUTH SINAI

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ABSTRACT

Four experiments were carried out at Ras Sudr Agricultural Research Station of Desert Research Center during the two successive seasons of1998/1999 and 1999/2000 to estimate the heritability values for yield and yield attributes for the four exotic barley (*Hordeum vulgare* L.) genotypes i.e. 1) ICD-84-0838-IAP-OTR, 2) CD 29706-E-Sy-3M-IY-IB-OAP, 3) L93-6AP-IAP-IAP-OAP and 4) APM-Hc-ISIS-41/Rabour/3 Arar. In addition, random amplified polymorphic DNA (RAPD) analysis was used for the assessment the genetic diversity and genetic similarity between the studied barley genotypes. Heritability estimations under the two saline water irrigation and salinity levels were generally high for all studied traits [No. of spikes / plant, No. of grains/ spike, 1000-grain weight, grain yield / plant and harvest index (%)] which ranged from 40% for No. of grains / spike to 84% for No. of spikes / plant at low irrigation water salinity level. Six random 10-mer primers were used to amplify DNA via polymerase chain reaction (PCR) and 75 RAPDs were generated the six detected polymorphism in all four barley genotypes. Out of the total amplified bands, 72% of which were polymorphic. Cluster analysis by the unweighted pair group method of arithmetic means (UPGMA) showed that the genotypes; 4 and 3 were grouped together with similar coefficient 84%. Genotype 2 was the least similar barley genotype that its similarity ranged from 49% -56% with the rest of all genotypes studied, also, it attained the lowest heritability value. The results obtained could be used for selection the promising parents to generate an effective breeding barley program under saline condition.

Keywords: *Hordeum vulgare* L. – Exotic barley lines – RAPD – Genetic similarity – Genetic diversity -Stress conditions

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important crops, ranking fourth in the world acreage (Brown 1992). Barley has great adaptive potential because it has advantage in aspects such as salt and drought tolerance (Esparza martinez and Foster 1998). Accordingly in arid and semi arid regions as Wadi Sudr (South Sinai) where there are brief rainy seasons and salinity hazard in both soil (EC 4865 ppm) as well as in irrigation water (EC = 6000-10000 ppm) growers could obtain yield under such conditions by using the most specific tolerant varieties.

Much of genetic diversity of these local and traditional cultivated barley varieties (genotypes) is being lost. In addition, the new varieties are forever more genetically homogeneous and are therefore more exposed to pathogens and adverse environmental conditions. This is confirmed with the conclusions of Asins *et al.* (1989) and De Bustos *et al.* (1998). Iqbal *et al.* (1997) which reported that any crop with a narrow genetic base is more

subjected to natural disasters such as outbreak of a diseases or in general of a biotic stresses.

Under saline conditions this has promoted the search for new source of variation and introduce exotic genotypes that might be used in elite breeding programs. However, before breeding programs for providing hybrid tolerate at least to medium as well as highly salinity levels, an accurate and reliable estimate of genetic relationships and genetic diversity of such new genotypes can be detected to supplement and refine the morphological-based data. Morphological features are the principle indicators of the genotypes but are represented by only a few loci because there is not a large enough number of characters available. Moreover, they can also be affected by environmental factors and growth practices (Iqbal *et al.* 1997 and Russell *et al.* 1997).

The realization that DNA sequence polymorphisms between genotypes could be used for the analysis of variability has been of major importance. Recently, technology for the amplification of discrete sequences using the polymerase chain reaction (PCR), has offered promising new marker systems to be used in the diagnosis of genetic diversity in breeding programs that use many genotypes (Saiki *et al.* 1988). RAPD (random amplification of polymorphic DNA), Williams *et al.* 1990, is one of these technologies.The main advantages of Thes method rises from its simplicity of use, reduced running time and lower cost. Further, since RAPD markers are dominant, attempts to diagnose genetic diversity have to be designed to take into account the fact that profiles are scored for the presence or absence of single allele.

Heritability known as the knowledge of the relative contribution of genotypic to phenotypic variances, which consider one of the important, factors in the formulation of effective breeding plant program. An effective breeding program for improving barley under saline conditions such as presented in South Sinai depends not only on the amount of variability among the diverse genotypes, but also on heritability for the traits under consideration.

The objective of the present work were; to evaluate the estimates of heritability of yield and yield attributes for the four exotic barley (*Hordeum vulgare* L.) and investigate genetic diversity and genetic similarity of the four studied genotypes using RAPD markers. Such evaluation could aid the long term objective of identifying elite diverse parental genotypes to formulate an effective breeding barley program under saline conditions without attained the main results of hybrids either in F_1 or F_2 breeding program.

MATERIALS AND METHODS

1-Materials

Grains of four exotic barley (*Hordeum Vulgare* L.) genotypes, kindly obtained from ICARDA and ACSAD were used in this study. (Table 1) represented code number, origin and pedigree of barley varieties or lines under evaluation.

Table (1): Code number, origin and pedigree of barley genotypes evaluated under Ras Sudr conditions.

Code number	Origin	Pedigree
1	Mexico/Syria	ICD-84-0838-IAP-OTR
2	Mexico/Syria	CD 29706-E-SY-3M-IY-IB-OAP
3	Mexico/Syria	L93-6AP-IAP-IAP-OAP
4	Syria	Apm-Hc. Isis-41/Rabour/3/Arar

2- Methods

2.1. Field evaluations:

The four exotic genotypes of barley were cultivated under two salinity levels of irrigation water i.e 6000 and 10000 ppm during two successive seasons 1998/1999 and 1999/2000. Each of the four genotypes was grown in a separate experiment under two locations of soil and two irrigation water salinity levels at Ras Sudr experimental station. Each experiment was designed in randomized complete block design with three replications. The plot size was 3 x2.5 m. Each row was 3m long 15cm between rows. Planting rate was about 80 Kg/fed. All agricultural practices were done as recommended for such newly reclaimed lands such as fertilization, irrigation intervals .... etc.

2.2. Yield and yield attributes measurements

Measurements were recorded on ten individual guarded plants at harvest time for yield and yield attributes. These attributes were number of spikes / plant, number of grains / spike, 1000-grain weight, grain yield / plant and harvest index was calculated for each traits and the means.

2.3. Polymerase chain reaction (PCR)technique and RAPD analysis:

2.3.1. DNA extraction

Genomic DNA was extracted from fresh young leaves of ten plants chosen randomly for each genotype, according to the method of Dellaporta *et al.* (1983).

All leaves of the ten plants for a signal experiment were bulked prior to extraction. DNA was quantitated by spectrophotometer and gel electrophoresis.

2.3.2. DNA amplification:

Oligonucleotide primers (10-mer) were purchased from Operon

Primer code	Primer sequence $5^{\prime}$.....$3^{\prime}$
B_8	GTCCACACGG
D_{20}	ACCCGGTCAC
E_9	CTTCACCCGA
Z_7	CCAGGAGGAC
Z_{10}	CCGACAAACC
Z_{13}	GACTAAGCCC

PCR amplification reactions contained 10mM Tris-HCl PH 8.8 at 25°C,50mM KCl, 2.0mM $MgCl_2$, 200μM of each of NTP, 0.2μM 10-mer primer, 10ng template DNA and 0.5 units of Taq DNA polymerase (promega). Amplifications were performed for 40 cycles of 40 s at 94° C, 1 min at 36° C, 1 min at 72° C, and ending with 6 min at 70° C. Amplified DNA products were separated on 14 g l^{-1} agarose in 1 X TAE buffer (40 mM Tris base, pH 8.0, 20 mM glacial acetic acid, 2 mM Na EDTA). The run was performed for one hour at 100V in Pharmacia submarine, then stained in an ethidium bromide soak. Bands were detected on UV-transilluminator and photographed by a Polaroid camera.

The fragment sizes of the amplification products were estimated from gel by comparison with standard molecular markers. The markers consist of 14 blunt-ended fragments with bp lengths of 10.000, 8.000, 6.000, 5.000, 4.000, 3.000, 2.500, 2.000, 1.500, 1.000, 750, 500 and 250.

2.4. Statistical analysis:

Heritability in broad sense (H_b) was calculated for all studied characters using Allard equation (1960) as follows:

Heritability in broad sense (H_b) = Genotypic variation / Phenotypic variation

DNA amplification profiles of 4 barley genotypes were compared with each other and bands of DNA fragments were scored as present 1 or absent 0. The data for all 6 primers were used to estimate genetic distances on the basis of the number of shared amplification products (Nei and Li, 1979). A dendrogram based on dissimilarity coefficients was generated by using the unweighted pair group method of arithmetic means (UPGMA).

RESULTS AND DISCUSSION

Under both water irrigation salinity levels, estimates of heritability were generally enhanced for all the studied traits of the four tested genotypes that ranged from 40% for number of grains / spike to 84% for number of spikes / plant at low salinity level. Results revealed that heritability in broad sense showed low value for the number of grains / spike that indicated the dominance of gene action compared with the other characters which surpassed and dominance of additive gene action in yield attributes i.e number of spikes / plant, 1000-grain weight and grain yield / plant as well as harvest index. Moreover, the results indicated that number of grains / spike was highly responded trait for the environmental changes for the four barley genotypes, which had the lowest h^2 estimates 41% and 42.2% under low salinity level. However, h^2 values estimated 33.6 and 38.1% under high salinity level and these was true for all studied genotypes. The data reported by Afiah *et al.* (2001) were in agreement with these obtained results and in partial agreement with those obtained by Nigem and Eissa (1988) and Abdel-Moneim (1993) durum wheat plants.

Under high salinity level of irrigation water, the studied genotypes under investigation take the same trend that obtained under low salinity level. However the heritability values were decreased by increasing salinity level and this was true for yield and its attributes in all the studied genotypes.

Out of the oligonucleotide primers screened in this study six primers revealed a scorable polymorphism between the four barley species, as well as total 75 fragments DNA bands were scored. The number of amplification products generated by each primer varied from 10 to 18 and out of the 75 bands, 54 (72%) were found to be polymorphic. However, the range of polymorphism for such six primers varied from 54% to 93%. The number of amplification products generated by each primer within barley genotypes varied from 10 to 18. The size of DNA bands ranged from 8000 to 400Kbp. marked differences were observed in the intensity of specific bands (Photo.1).

Although RAPD primers detected regions that did not otherwise scorable by RFLP probes, the information content of an individual RAPD marker is very low and therefore, it is essential to use many of these markers in the assessments of the genetic polymorphism of plant species (Welsh and Mclelland 1990; Williams *et al.* 1990). Furthermore, the data revealed that RAPD primers can discriminate between the four barley genotypes investigated (Photo. 1) and serve as useful probes for genetic typing. From these findings, Whitty *et al.* 1994; Graham and Mc Nicol 1995; Morel *et al.* 1995; Russel *et al.* 1997; Gallios *et al.* 1998 demonstrated that RAPD allow discrimination between varieties within a species and between different species. The present investigation was mainly carried out to estimate the genetic similarity of the four studied barley genotypes. The similarity matrix obtained after multi-variant analysis using Nei and Li's (1979) coefficient was shown in Table (3). These similarity coefficients were used to generate a dendrogram (Fig.2) by UPGMA analysis in order to determine the grouping of different genotypes. From similarity matrix, the least similar genotype was genotype 2, which its similarity ranged from 49.00 with genotype 1 to 63.00% with genotype 4. On the contrary, genotype 3 attained the highest similarity index, ranged from 84.00% with genotype 4 and 56.00% with genotype 2. The data in the dendrogram showed that the four genotypes clustered together. Also, the pure Syrian genotype (4) and the Mex./Syr. genotype (3) were origenated from the some source, while the Mex./Syr. genotype (2) was distantly separated from the other two Mex./ Syr. background genotypes (1 and 3) and from the Syrian genotype (4). This may be due to the relationships between the ultimate parents of these four genotypes that obtained from breeding programs don't know. Estimates of genetic similarity based on RAPDs have been developed for *Oryza* L. (Mackill 1995), *Triticum* (Chandrashekhar and Nguyen 1993) and *Hordeum vulgare* L. (Gonzalez and Ferrer 1993; Tinker *et al.* 1993).

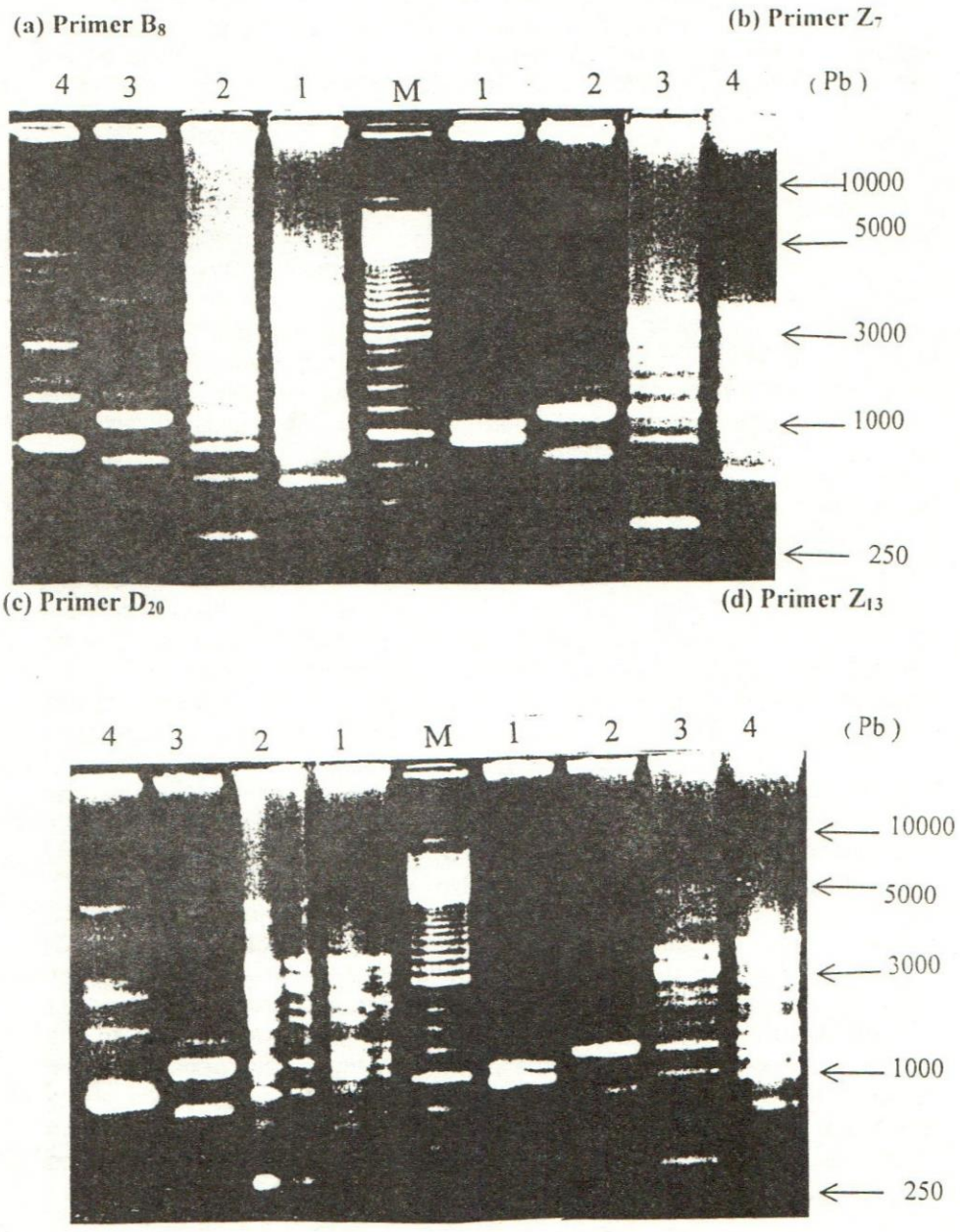


Photo (1): DNA polymorphism based on RAPD analysis for the four barley (*Hordeum vulgare* L.) genotypes 1, 2, 3 and 4, using primers B_8 (a), Z_7 (b), D_{20} (c), Z_{13} (d), Z_{10} (e) and E_9 (f). M. refer to DNA standards.

Photo (1): Continued.

(e) Primer Z_{10} (f) Primer E_g

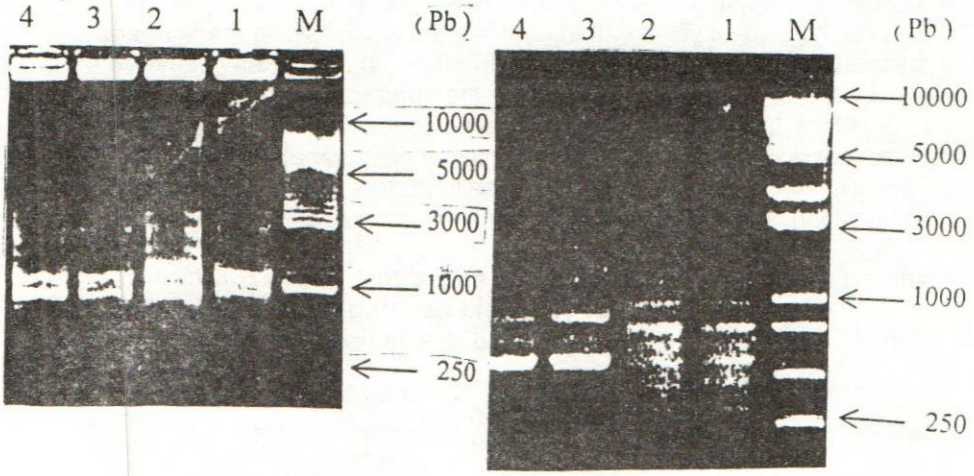
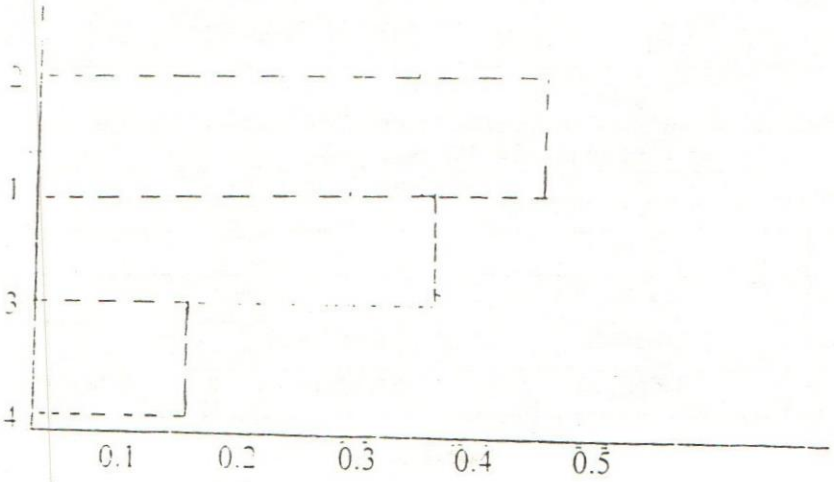


Fig. (1): Dendrogram demonstrating the genetic distance among the four barley (*Hordeum vulgare* L.) genotypes based on RAPD analysis.



Considerable interest exists in using molecular markers RAPDs to assign cultivars to heterotic groups (Boppernmaier *et al.* 1992; Dudley *et al.* 1991; Melchinger *et al.* 1991, 1992; Stuber *et al.* 1992 and Yuan 1994). The result presented here indicated that the highly similar barley genotypes 3 and 4 (Table 3 and Fig.1) had high heritability percent (81.4% - 84.0%) for the No. of spikes / plant (Table 2). Meanwhile the lowest similar barley genotype 2 attained a lower heritability value for trait No. of spikes / plant (74.4%), however, the same results were true for the other traits under investigation.

From the previous data it can be concluded that the knowledge of genetic variation and genetic relationship between genotypes and heritability values of yield and yield attributes is an important consideration for efficient rationalization and utilization of germplasm resources. Furthermore, it's

Table (2): Heritability in broad sense (H_b) of four barley (*Hordeum vulgar* L.) genotypes for yield and its attributes under two levels of salinity i.e 6000 ppm and 10000 ppm in irrigation water at Ras Sudr conditions.

Salinity level	Genotype number	No. of spikes/ plant	No. of grains/ spike	1000 grain weight (gm)	Grain yield/ plant (gm)	Harvest index (%)
6000 (ppm)	1	76.80	41.00	58.1	60.60	62.00
	2	74.70	40.00	57.4	58.80	60.10
	3	81.40	41.60	60.4	61.00	62.10
	4	84.00	42.20	64.6	61.60	63.50
10000 (ppm)	1	71.40	34.70	51.9	53.40	56.30
	2	70.40	33.60	50.1	51.70	50.30
	3	76.60	35.60	54.1	54.70	57.40
	4	80.30	38.10	60.4	55.10	60.00

Table (3): Similarity coefficients across RAPD analysis of the four barley (*Hordeum vulgare* L.) genotypes.

Case	Matrix file input		
	1	2	3
1	–		
2	0.49±0.12	–	
3	0.65±0.02	0.56±0.03	–
4	0.60±0.08	0.55±0.01	0.84±0.01

important for the optimal design of plant breeding programs influencing, the choice of the promising genotypes to cross for the development of new populations (Russel *et al.* 1997). Breeders of barely had made crosses between selected genotypes characteristics with the number of genotypes within the breeding genepool is very small. In the same manner, Graner *et al.* (1994) demonstrated that better knowledge and measurements of genetic similarity of genotypes could help to maintain genetic diversity. From such investigation molecular markers (RAPDs) were used to determine direct measurements of genetic variation and similarity between genotypes. Accordingly, the two genotypes 3 and 4 were the more similar and less diverse genotypes, whereas genotype 2 was the more diverse and dissimilar to the other studied genotypes. Also, heritability estimates of number of spikes / plant, number of grains / spike, grain yield / plant and harvest index were higher for the two similar genotypes 3 and 4 than the other two genotypes 1 and 2.

Finally, it can be concluded that the promising breeding programs for given the best variability from the different genotypes was within four and two.

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درجة التوريث والأشكال المتعددة للحمض النووى (DNA) المكبرة عشوائيا لبعض سلالات الشعير المستوردة تحت ظروف رأس سدر – جنوب سيناء

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أقيمت أربع تجارب بمحطة بحوث راس سدر الزراعية خلال الموسمين المتتاليين ١٩٩٨-١٩٩٩ و ١٩٩٩-٢٠٠٠ لدراسة قيم درجة التوريث لصفات المحصول و مساهماته لأربع تراكيب وراثية مستورده من الشعير و هي:

1) ICD-84-0838-IAP-OTR. 2) CD 29706-E-Sy-3M-IY-IB-OAP.
3) L93-6AP-IAP-IAP-OAP. 4) APM-Hc-ISIS-41/Rabour/3 Arar.

كذلك استخدم تحليل الأشكال المتعددة للحمض النووي الـــ DNA (RAPD) لتقدير التنوع البيولوجي والتشابه الوراثي بين التراكيب الوراثية للشعير تحت الدراسة.

كانت قيم درجة التوريث تحت كلا من مستويي الملوحة بماء الري (٦٠٠٠، ١٠٠٠٠ جزء في المليون) لكل من الصفات المدروسة (عدد السنابل/نبات ، عدد الحبوب/سنبلة، وزن ١٠٠٠ حبة بالجرام، إنتاج الحبوب/نبات بالجرام وكذلك دليل الحصاد% والتي تتراوح بين ٤٠% لعدد الحبوب/نبات إلى ٨٤% لعدد السنابل/نبات تحت مستوى الملوحة المنخفض).

تم استخدام ٦ بادئات عشوائية لتكبير الحمض النووي الديوكسى ريبوزى الـــ DNA بواسطة تفاعل البلمرة التسلسلي وإنتاج ٧٥د RAPD. وقد اكتشف الـ ٦ بادئات تعدد أشكال الـ DNA في كل الـ ٤ تراكيب الوراثية المستخدمة ووجد أن ٧٢% من أشرطة الـ DNA المكبرة عديدة الأشكال. كما أوضح التحليل العنقودي بطريقة المجموعات الثنائية للمتوسط الحسابي (UPGMA) تماثل التركيبين الوراثيين ٣،٤ مع بعضهم بمعدل ٨٤% وأعلى قيم لدرجات التوريث وان التركيب الوراثي ٢ كان اقل التراكيب الوراثية تماثلا وتراوحت نسبة تماثله بين ٤٩%-٥٦% مع بقية التراكيب الوراثية. كما حصل على اقل القيم لدرجات التوريث.

إن النتائج المتحصل عليها ممكن استخدامها فى اختيار الآباء المتميزة لتنفيذ برنامج تربية ذو فاعلية كبيرة وسريعة لنبات الشعير تحت الظروف الملحية.