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Study genetic diversity in maize inbreds and improved hybrid vigor in hybrids produce to determine commercial hybrids in Iraq

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Abstract

A field experiments was conducted in greenhouse to determinate the genetic diversity among 7 genotypes from maize(4 inbreds and 3 hybrids) by using molecular markers with Random Amplified polymorphic DNA(RAPD),that shown high level of polymorphism among genotypes of maize ,where the percentage of polymorphism ranged from(66%) and (83.33%) the highest number of polymorphism band (16) and size fragment ranged between (3800 bp) with the primer (Bnlg 1185) and the lowest 180 with the primer(Bnlg 1464).The genetic distance value ranged between (0.3451) and (0.6534) ,where the lowest genic distance between (k1 and k2),while the highest genetic distance between(k4) and (k3xk4).In this study RAPD markers were shown to be powerful to detect genetic diversity and provided us high polymorphism values within genotypes of maize . conclusion of this study for using those primers and determine genetic diversity a among maize inbreds and then use these inbreds in plant breeding programs to develop synthetic cultivars or improved cultivars from maize to adapt these cultivars for biotic and abiotic stress ,so as to determinate which cultivar boss high yield and stability under stress factors .

Key words: Maize; genetic diversity; polymorphisms; RAPD- PCR

Academics discipline and sub-discipline

Plant Breeding

Subjected classification

Genetic Diversity in Maize

Type method

RAPD –PCR (random amplification polymorphism diversity)

Introduction

Genetic diversity is the most important element for maize breeding programs , for developing new cultivars and pure lines depend on gene combinations for producing commercial hybrids , synthetics varieties and cultivars with desirable traits such as stay –green , high yield and biotic and abiotic stress (Enoki et al,2002; Hoxha et al,2004).Several methodologies include to estimate the genetic diversity in maize based on morphological traits are affected by environmental factors and expensive time (Agruirri et al,2000).Molecular genetic techniques can be applied for assessment the genetic diversity to complementary the strategies of conventional approaches in conservation and utilization of plant genetic resources in plant breeding programs(Wu and Tanksify,1993; Gauthier et al,2002) .DNA Markers provide more information on genetic diversity and relationships between pure line of maize with origin population ,so this technique is useful to remove any duplicates that have might introduce in genotypes collection and exchange it, Furthermore the useful of these information in plant breeding programs to assess the genetic diversity values of germplasm subjected to selection ,mutations and hybridization (Senior and Heun,1993; Hosprral et al,2002).DNA Markers may be used for development pure lines of local population that are known to be tolerant of biotic and abiotic stress(Bardosa-Neto et al ,1997; Reif et al,2003).The aim of this study to estimate the genetic diversity among 10 pure line of maize ,and knowledge the genetic diversity and polymorphism can be used for developing inbreds , As well as the advantage in the production of genetically improved varieties.

Material and Method

1- Plant Materials

Seven genotypes of corn were evaluated in this study which includes 4 inbreds (k1,k2,k3,k4) and 3 hybrids(k1xk2),(k2xk3)and(k3xk4).These genotypes were growing in greenhouse in order to assess their efficiency form yield and component yield and one of physiological trait (leaf area m2) (Table 1).

Table 1. some components yields and leaf area (m2)

Genotypes	Leaf area (m2)	Weight grain(mg)	Yield ton.ha-1
K1	0.32	273.00	3.78
K2	0.30	245.66	4.11
K3	0.33	237.66	3.26
K4	0.30	248.00	3.43
K1xk2	0.36	277.34	4.65
K2xk3	0.40	285.07	4.77
K3xk4	0.44	293.00	5.75

2- DNA extraction

Maize seeds were planted in trays and grown in the greenhouse at 25 C for (15 days).Leaves (reach 8 cm in length) were samples into 2ml eppendorf tubes and then put in liquid nitrogen ,and then freeze dried ,also maintained on silica gel at-80 C after that the samples were milled by using a Restch MM400 shaker (Hann ,Germany).DNA extraction from leaves of 7 genotypes(inbreds and hybrids) ,with using acetyl trimethyl ammonium bromide (CTAB) protocol ,that reported from Bekele et al(2007).the purity and quality of DNA was analyzed by using agarose gel electrophoresis at wave 260 - 280nm(Sambrook et al., 1989 ; Maniatis et al.,1982 ;Smith and Smith ,1991).

3- RAPD analysis

Nine primers of oligonucleotide have been provided by company(Alpha DNA) ,these primers and their sequences were represented in table(2).The PCR was performed in volume 25µL reaction, containing 12.5µL of Taq DNA polymerase(promega USA),with concentration (1x) containing 10mM Tris HCL (PH=8.3),50mM kcl ,2 mM Mgcl2 , and 200mM of each dNTPs ,and 1 unit Taq of DNA polymerase , and added 10 pmol of each primer with added 10ng of template DNA .DNA Amplification was conducted by using master cycler and thermo cycler with following :

1 cycle of 3minutes at 95 C0 for separating initial of DNA strands
4 cycles of 30 s for denaturation and 30 s for annealing and 72 C0 for primer extension
1 cycle of 20 minute at72 C0 for final extension .

12 microliter of PCR product and analyzed by using electrophoresis on 1.5 % agrarose gel at and voting 2 hours with added 0.5 TBE(10m for 25 minutes .After electrophoresis ,look images of gels were taken by using gel documentation (E-graph,Korea)

Data analysis

1-Molecular weight

Molecular weight was calculated by computer software(M.W) program depend on comparison PCR product with known DNA fragment of DNA ladder size.

2-Gentic distance and polymorphisms

The data were analyzed to detect polymorphic fragments ,which amplification of all genotypes in this study for each primer compared with others ,where the presence of bands gave number(1),while the absence of the same band of the same size in other genotypes gave number (0).Genetic distance was estimated between all pairs of genotypes according of data matrix(Nei and Li ,1979 ; Schut et al,1997)

$$G.D = 1- [2 Nab / (Na + Nb)]$$

Where

Na = the numbers of fragments were detected in individual "a".
Nb = the numbers of fragments were shown by individual "b".
Nab = the numbers of fragment participated between individual "a" and "b".

Result and discussion

DNA amplification and genotypes identification

The genetic distance among 7 genotypes of maize were estimated by RAPD-PCR technique with using 9 random primers (the name all primers and their sequence are shown in table 2). The total number of main bands that were produced by all primers was 114 bands, involving 87 polymorphic bands with percentage 76.63%, while the other bands (27 bands or 23.68%) were monomorphic, the size of DNA fragments ranged from 2750 bp for primer Bnlg1194 to 200 bp for primer Bnlg1108 . The number of polymorphic bands ranged between 7-13 bands, the primer Bnlg1108 produced highest number of polymorphic bands amount to 16 bands, while the primers Phi32 gave the lowest number of bands amount to 10 bands of polymorphism (Table 2, Figure A). The average of polymorphic bands equal to 9.33 per primers among seven maize inbred lines. The highest percentage of polymorphism was 83.33% obtained by the primer Bnlg1185, while the lowest percentage was 66% produced by Pimer 6.

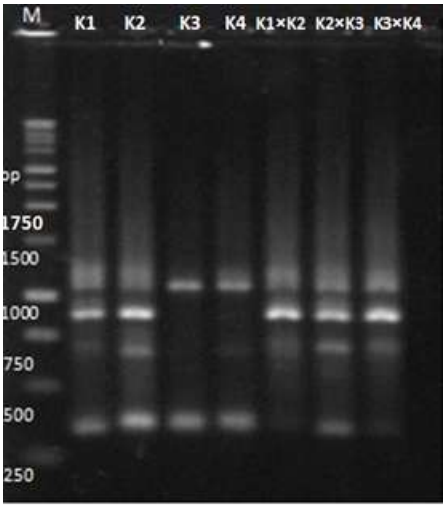


Figure 1. Agarose gel electrophoresis of a RAPD-PCR reaction for DNA Primers samples of genotypes of maize . (Bands were separating by electrophoresis on 1.5% agarose gel (2hr, 5V/cm, and visualized by ethidium bromide staining. M: 1kb ladder; Samples(k1,k2,k3 and k4) and hybrids (k1xk2),(k2xk3) and (k3xk4)

Table 2. Primers and their sequences, number of main and polymorphic bands and their percentage of polymorphism across the genotypes of maize.

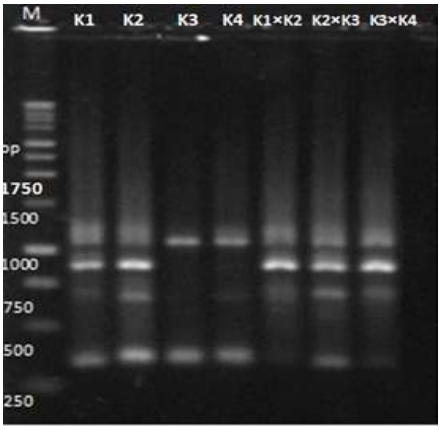
No.	Primer	Sequence (5'~3')	Number of main bands	Number of polymorphic bands	Polymorphism %
1	Bnlg1429	CTCCTCGAAGGATTAC	11	8	72.72
2	Phi402893	GCCAAGCTCAGGAGGG	14	10	71.42
3	Phi251315	GGATTCCTTATGACGGGT	16	12	75
4	Bnlg1108	GGATTCCTTATGACGGGT	16	13	81.25
5	Bnlg1867	CCACCACCATCGTAGGAT	10	8	80
6	P-umei6	CAGGTAACGACGCAGCA	12	8	66
7	Bnlg1194	GCGTTATTGGACGCAGCA	13	9	69
8	Phi32	AAGCTAAGGCCGGTATCC	10	7	70
9	Bnlg1185	CGGTCCAGGCAGGTTAA	12	10	83.33
Total			114	87	___

2-Genetic distance

The genetic distance in genotypes of maize or a group of population depend on large number of alleles ,that caused by allelic frequency among of maize population (Hoxha et al ,2004).The RAPD genetic distance value ranged from(0.3451) to (0.6523) shown in (Table 3).the highest value of genetic distance between (k1xk2) and (k2xk3),while the lowest value of genetic distance between (k1xk2) and (k2xk3),the results shown that inbred maize (k3) gave big distance value (0.5401) with inbred(k2) and (k1).Therefore we can used (k3) in hybridization and selection programs for exploiting heterosis so that to produce commercial hybrids of maize (Doubley and Stec ,1991),moreover ,the genetic distance values between inbeds(k1)and(k2) was (0.3451) .there is no heterosis to predicate from crossing between them ,these results was similarity of a lots of experiences in genetic diversity of population of maize(Smith and Smith,1991).

Table 3.values of genetic distances among some genotypes of maize estimated according to(Nei and Li ,1979)

	K1	K2	K3	K4	K1×k2	K2×k3	K3×k4
K1	0.00						
K2	0.3451	0.00					
K3	0.5242	0.5401	0.00				
K4	0.4321	0.5398	0.54234	0.00			
K1×k2	0.4672	O.4327	0.6321	0.3456	0.00		
K2×k3	0.5623	0.4451	0.5043	0.5432	0.4325	0.00	
K3×k4	0.5843	0.5709	0.40577	0.6523	0.6534	0.5392	0.00



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Conclusion

The conclusion of this study that use the primers to identify the genetic diversity among maize inbreds and determinate the high polymorphism ratio that can useful in maize programs and improved cultivars that will boss between high yield under stress factors.

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References

1. Enoki H., Sato H. and Koinuma K.2002.SSR analysis of genetic diversity a mong maize inbred lines adapted to cold regions of Japan. THeor .Appl.Genet.104:1270-1277
2. Hopstal,F.,Moreau,I., Lacoudrf ,F., Charcossef ,B A., Gallais,A. 1997 . More on the efficiency of marker –assisted selection . Theor Appl. Genet 95:229-236
3. Aguirre J.A., Bellon M.R., Smalf, M.2000 .A regional analysis of maize biological diversity in southeastern Guanajuato Mexico Economic Botany 54:60-70
4. Wu. K .S. and Tansksify S.D.1993. Abundance ,polymorphism and genetic mapping of microsatellites in rice Mol .Gen .Genet.241:225-235
5. Gauthier , P., Gouesnard B., Dallard H., Redafll, I., Reburg., C.,Charcoss E.,T., Boyat, A .2002. RFLP diversity and relationships among traditional Eroupean maize populations .Theor .Appl.Genet 105:91-99.
6. Senior M.L. and Heun.M1993.Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CTP primer .Genome 36:884-889.
7. Hopstal,F.,Moreau,I., Lacoudrf ,F., Charcossef ,B A., Gallais,A. 1997 . More on the efficiency of marker –assisted selection . Theor Appl. Genet 95:229-236.
8. Bardosa-Neto J.F. Heranandez L.S. and Odonoughuf M.E.1997.Precision of genetic relationships estimates based on molecular markers .Euphytica 98:59-67
9. Reif J.C.,Melchinger,X.C.,Xia M,I.,Warburton,D.A.,Hoisington,S.K.,Vasal,G,Srinivasan,M
10. Bohn,M., Frisch, M (2003).Genetic distance based on simples sequences repeats and
11. Heterosis in tropical maze population .Crop Sci.43:1275-1282
12. Bekele E, Geleta M, Dagne, K(2007).Molecular phylogeny of genus Guizotia (Asterataceae) using DNA sequences derived from IST.Gent Resources Crop Evol. 54:1419-1427
13. Sambrook, J., Fritsh, E.F. and Maniatis, T. 1989. Molecular Cloning; A Laboratory Harbor Laboratory press, cold spring Harbor, N.Y.P.253
14. Maniatis, T., Fritsch, E. F. and Sambrook, J.1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, N.Y. p.545.
15. Smith O.S., Smith J.S.C(1992).Measurement of genetic diversity among maize hybrids ; A comparison of isozymic RFLP,pedigree and heterosis data ,Maydica 37:53-60
16. Nei, M. and Li, W1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA, 76: 5256-5273.
17. Schut J.W., Qi W. and Stam P.1997.Associatian between relationship measures based on REFLP markers ,pedigree data and morphological traits in barley .Theor .Appl.Genet. 95 :1161-1168.
18. Doubly J.F,Stec A.1991.Genetic analysis of the morphological differences between maize and teosinte .Genetics 129:285-295.
19. Smith O.S. and Smith J.S.C.1991.Restraction fragment length polymorphism can differentiate among U.S maize hybrids .Crop Sci 31:893-899

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