ABSTRACT
In this study, the genetic relatedness of 12 cultivars of fig from different populations in Kurdistan region- Iraq were analyzed using eleven AFLP primers pairs combinations by using the technology of molecular analysis the DNA. Genetic similarity matrices were produced for the AFLP data to calculate genetic distances among their cultivars. Genetic similarity coefficient ranged from 0.1261 to 0.3905. The lowest genetic similarity was observed between Kola and Gala Zard (0.1261). The Hejera Rash and Shela cultivars were most similar ones with a coefficient of 0.3905. Clustering based on AFLP data for the 12 fig cultivars was identified at the 0.32 similarity level. In the developed dendogram two main groups were found, the first one combined Ketek and Shela together, while the second group contained two sub group Shingaly and Benatty combined together, while in the other sub group cluster three other sub-group were identified. The results of this study may help in the formulation of appropriate strategies for conservation and cultivar improvement in figs, for which limited knowledge of the genetic diversity is available.

Key words: Molecular Biology, DNA markers, genetic diversity, Fig.

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INTRODUCTION

The fig *Ficus carica* L. (2n= 2x =26 chromosome) (7). Fig belongs to Family: Moraceae (32), in literature it has several common names such as common fig, edible fig (22). The genus *Ficus* is made up of about 1,000 species from pan-tropical to subtropical origins (32). Fig plants are all woody in the family, from trees and shrubs to climbers (22). The name carica is named after the Caria place in Asia Minor, home of the fig. (11, 22) *F. carica* is presumed to originate from Western Asia and spread to the Mediterranean by humans (9). Today, it is considered as one of an important world crop, because of their nutritional, medicinal, food industry and ornamental values (13, 15). According to FAO reports, the planet generates more than one million tons of figs per annum (12). Large edible fig producers include Turkey, Egypt, Morocco, Spain, Greece, California, Italy, Brazil and other usually mild winters and hot dry summers (29). The available methods for fig plants diversity analysis include the classical research methods which mainly include morphological and agronomical traits, biochemical markers and cytological such as cell karyotype analysis and isoenzymes (4, 14, 19). These methods are considered as sensitive to environmental factors and the number of markers is limited, thus the research of fig diversity has been limited. Molecular marker techniques such as RFLP, ISSR, RAPD, and AFLP have vastly improved knowledge on genome structure, organization, and evolution of many cultivates plants (1, 2, 5, 10, 18, 21, 24). AFLP analysis has been used to detect DNA polymorphisms and the genetic relationships of many economically important plants including fig genotypes (8, 25). However, few applications of AFLP technology to the genetic analysis and fingerprinting of fig cultivars have been reported. AFLP technique was introduced as a reliable and reproducible marker system (31). It was favored over other DNA-based markers mainly because of its high multiplex ratio and prior sequence information is not needed (34). Distribution of fig plants throughout Iraq's Kurdistan region in rocky mountain slopes, valleys, hill sides and road sides, depleted forests of Oak and Pine. The figs were also grown in irrigated orchards in dry vineyards and as house plants (27). Most fig populations in Kurdistan have received very little attention from scientists, so they are not aware whether they are native trees or new varieties that the local people have introduced to the region for many years. The objectives of the present study were the application of AFLP markers to reveal DNA polymorphism among populations and between individuals and to determination of genetic relationships between the populations or cultivars of fig in Kurdistan region of Iraq.

MATERIALS AND METHODS

Samples collection

Samples (fresh leaves) from (12) of fig cultivars were collected from different districts in the Kurdistan Region – Iraq. These samples were obtained from the Ministry of Agriculture fields at Duhok, Erbil and Sulimania cities. The cultivars of Fig selected for this study were (Shingaly, Benatty, Ketek, Rebwary Rash, Henjeer Rash, Rash khomali, Rehan Rash, Rehan Zard, Zarda Roon, Shela, Kola and Gala Zard).

DNA Extraction

Genomic DNA was extracted from fresh healthy tissue as mentioned by Weigand, et al (33). Fresh tissue (3g) was homogenized to powder with 40 ml in liquid nitrogen. The fine powder was dissolved in a pre-heated (60°C) 2x CTAB extraction buffer (2x CTAB, 5M NaCl, 1M Tris-HCl, 0.5 M EDTA), and incubated at 60°C in a water bath with shaking for 30 min. The mixture was extracted with an equal volume of chloroform / isoamyl alcohol (24:1, v/v) (20). The mixture was then centrifuged at 4000 rpm for 30 min. The aqueous phase was transferred into fresh tube and precipitated with 0.66 volume of isopropanol. Precipitated nucleic acids were then dissolved in 500µl Tris EDTA TE-buffer (1 ml of 1M tris-HCl (PH8.0) 0.2µl of 0.5M EDTA.

PCR Amplification of AFLP- primers

The AFLP procedure was performed as described by Vos, et al (31) as follows; 500ng of DNA from each sample was double digested with 5U each of the two restriction enzymes, *Msel* (recognition site 5’T↓TAA3’) and *PstI* (recognition site 5’CTGCA↓G3’). The digestion reaction was prepared in 30µl
final volume containing, 1x one-phor all buffer (Pharmacia Biotech, Uppsala, Sweden), and incubated for three hours at 37°C. DNA fragments, were then ligated to Pst I and MseI adapters by adding 50 pmol of MseI-adapter, 5 pmol PstI-adapter in a reaction containing 1U of T4-DNA ligase, 1mM rATP and 1x of one-phor-buffer and incubated for 3hr. at 37°C. After ligation, the reaction mixture was diluted to 1:5 using sterile distilled water. Pre selective PCR amplification was performed in a reaction volume of 20 µl containing 50ng of each of the primers (P00, M00) corresponding to the MseI and Pst I adapters, 2µl of template-DNA, 1U Taq DNA polymerase, 1x PCR buffer and 5mM dNTPs. PCR amplification was performed in WMG thermal cycler using the following program: 30 cycles of 30s at 94 ºC, 1min at 60ºC, 1min at 72 ºC. Pre-amplification products were then diluted to 1:5 and 2µl were used as template for selective amplification. Selective amplification was conducted using MseI and PstI selective primer combinations, (Table 1). Amplification was performed using a selective program of 36 cycles with the following profile: a 30sec. DNA denaturation step at 94°C, 30sec. annealing step, and a 1 min extension step at 72°C. The annealing temperature in this program varied in the first cycle where it was 65ºC and in each subsequent cycle for the next 12 cycles it was reduced by 0.7ºC (touchdown PCR). Then for the remaining 23 cycles, it was 56ºC. Selective amplification products were loaded onto 6% polyacrylamid gels, and DNA fragments were visualized by silver staining kit (Promega, Madison, Wis) as described by the supplier. Silver – stained gels were scanned to capture digital images of the gels after air drying.

**Table 1. Represents the sequences of Pre-selective and Selective primers combinations used in this study**

| Pre-selective primer combinations Poo + Moo | Sequences ‘5--------3’ |
|--------------------------------------------|------------------------|
|                                            | GACTGCGTACATGCAGGGA    |
|                                            | GATGAGTCTCTGAGTAAAG    |
| 1- PGGGA /M GATA                          | GACTGCGTACATGCAGGTA    |
| 2- PGATA /M TAAG                          | GACTGCGTACATGCAGGATA   |
| 3- PTACC /M GATA                          | GACTGCGTACATGCAGTACC   |
| 4- PGATA /M GATA                          | GACTGCGTACATGCAGGATA   |
| 5- PGATA /M TACA                          | GACTGCGTACATGCAGGATA   |
| 6- PTACC /M TAAG                          | GACTGCGTACATGCAGTACC   |
| 7- PGATA /M TCA                           | GACTGCGTACATGCAGGATA   |
| 8- PTACC /M ACC                           | GACTGCGTACATGCAGTACC   |
| 9- PGGGA /M TCA                           | GACTGCGTACATGCAGGGA    |
| 10- PAATA /M GATA                         | GACTGCGTACATGCAGGTA    |
| 11- PGATA /M ACC                          | GACTGCGTACATGCAGGATA   |

**Data analysis**

The digital photographs of gels were used to score the data for AFLP analysis starting from the higher molecular weight product to lowest molecular weight product. Presence of a product was identified as (1) and absence was identified as (0). Data were scored for all genotypes, their amplification product and primers. The data then entered into NTSYS-PC (Numerical Taxonomy and multivariate
RESULTS AND DISCUSSION

The results of selective primer amplifications are shown in (Figure 1 A,B) and the presence of AFLP bands across all 12 Fig cultivars clearly indicate the successful application of AFLP marker technology. AFLP analysis (Table 2) was used eleven selective primer combinations, the experiments generated a total of 301 fragments (bands of DNA), in the rate of 27.36 band for each combination. The number of polymorphic bands was 226 representing a level of polymorphism of 75.07% and in the rate of 20.54 for each combination. Also in this study the greatest Discrimination Power appeared for the combination (P<sub>GGGA</sub>/M<sub>GATA</sub>) where it reached 15.04% and the smallest where 3.98% appeared for the combination (P<sub>GATA</sub>/M<sub>AACC</sub>) which shows the lowest number of polymorphic bands. Also the results of this study have produced the greatest number and size of bands by the two combinations (P<sub>GGGA</sub>/M<sub>GATA</sub>) and (P<sub>GATA</sub>/M<sub>TAAAG</sub>). These combinations were produce 41 bands, whereas the lowest bands 19 and 17 were produce by two combinations (P<sub>AATA</sub>/M<sub>GATA</sub>) and (P<sub>GATA</sub>/M<sub>AACC</sub>). What supports any study is the appearance of polymorphic bands or bands with different sizes that provide the database with the ability to make it eligible to carry out the necessary genetic analyzes that are consistent with the objective of the study (30, 3). So the importance of primer combinations is measured by the number of polymorphic bands, it stands out for the discrimination Power for each combination, it is compared to the total product polymorphic bands that showed by all combinations that used in any study. Another important variation by using AFLP marker as in all molecular markers is the differences in molecular weight (bp) for bands, those present on gel. In currently study the size of the AFLP amplified fragments ranged from 50bp. to 1500bp. Other study was reported by Laddomada, et al (17) to assess polymorphism and relationships among 24 fig accessions using AFLP markers; 553 amplification products of which 535 were polymorphic among the analyzed genotypes. A high degree of polymorphism was revealed by these primer combinations. The results showed (6) that using AFLP marker with Tunisian fig germplasm is characterized by having a large genetic diversity at the deoxyribonucleic acid level, as most of AFLP bands were detected. In fact, 351 (342 polymorphic) were detected using AFLP primers. AFLP markers showed the highest effective multiplex ratio (56.9). It was not accurate to identification of varieties depending on morphological traits only. May be a variety have many names in different plantation and genetically different varieties may have the same name (28). There were several different DNA marker analysis techniques that have been used to identify and characterize fruits to determine genetic diversity (16).

A- P<sub>GATA</sub>/M<sub>TAAAG</sub>
B- P<sub>GATA</sub>/M<sub>TCAG</sub>
Figure 1 A, B. Results of twelve fig cultivars using AFLP primer combinations (A- $P_{GATA}/M_{TAAG}$) & (B- $P_{GATA}/M_{TCAG}$). Lanes from 1 to 12 represent *Ficus* cultivars: 1= Shingaly, 2= Benatty, 3= Ketek, 4= Rebwar Rash, 5= Hejjeer Rash, 6= Rash khomali, 7= Rehan Rash, 8= Rehan Zard, 9= Zarda Roon, 10= Shela, 11= Kola, 12= Gala Zard. Lane M refers to molecular marker 100-2686 bp.
Table 2. Estimates of genetic diversity between *Ficus* samples studied

| Primer combinations | Total number of Bands | Number of polymorphic bands | Polymorphic percentage % | Primer Efficiency % | Discrimination Power % |
|---------------------|-----------------------|-----------------------------|--------------------------|---------------------|------------------------|
| 1-P_GGGC_MGATA      | 41                    | 34                          | 82.92                    | 13.62               | 15.04                  |
| 2-P_GATA_MGAAG      | 41                    | 25                          | 60.97                    | 13.62               | 11.06                  |
| 3- P_TACC_MGATA     | 30                    | 28                          | 93.33                    | 9.96                | 12.38                  |
| 4- P_GATA_MGATA     | 28                    | 24                          | 85.71                    | 9.30                | 10.61                  |
| 5-P_GATA_MTACA      | 27                    | 20                          | 74.07                    | 8.97                | 8.84                   |
| 6- P_TACC_MTAAAG    | 25                    | 20                          | 80.00                    | 8.30                | 8.84                   |
| 7- P_GATA_MTACG     | 25                    | 18                          | 72.00                    | 8.30                | 7.96                   |
| 8- P_TACC_MAAC      | 24                    | 20                          | 83.33                    | 7.97                | 8.84                   |
| 9- P_GGGC_MAAC      | 24                    | 16                          | 66.66                    | 7.97                | 7.07                   |
| 10-P_AATA_MGATA     | 19                    | 12                          | 63.15                    | 6.31                | 5.30                   |
| 11-P_GATA_MAAAC     | 17                    | 9                           | 52.94                    | 5.64                | 3.98                   |
| **Average**         | **27.36**             | **20.54**                   | **75.07**                |                     |                        |
| **Total**           | **301**               | **226**                     |                          |                     |                        |

**Genetic Similarity**

Genetic similarity matrices were produced for the AFLP data to calculate genetic distance. As shown in (Table 3) genetic similarity coefficient ranged from 0.1261 to 0.3905. The lowest genetic similarity was observed between Kola and Gala Zard (0.1261). The Hejeera Rash and Shela populations were most similar ones with coefficient of 0.3905. These data were used to generate a dendogram.

**Cluster analysis**

Dendogram was established with UPGMA cluster analysis based on the AFLP data using 11 combination primers. Clustering based on AFLP data for the twelve figs was identified at the 0.32 similarity level (Figure 2). In this dendogram there was two main groups, the first one combined C3 Ketek and C10 Shela together, while the second group contain two sub group C1 Shingaly and C2 Benatty combine together, while the other sub group cluster there are three other sub-group, the first one C8 Rehan Zard and C9 Zarda Roon clustered together, the other sub-group dived to more sub group which first include C5 Hejeer Rash and C6 Rash khomali together in one cluster, second C7 Rehan Rash cluster alone. The third sub group also contains C4 Rebwary Rash clustered alone. The analyzed data illustrates a good variability in the genetic pool of the common local fig making it a valuable source for incorporation into potential breeding programs for the region. The most important advantage of these markers for use in genetic diversity such as fig is that they can be used without any prior knowledge of the target template DNA sequence. These results of this study conclude the usefulness of AFLP marker characterization fig populations compared with other PCR- based techniques.

Table 3. The genetic similarity between *Ficus* samples studied

|        | Shingaly | Benatty | Ketek   | Rebwary Rash | Hejeera Rash | Rash khomali | Rehan Rash | Rehan Zard | Zarda Roon | Shela | Kola  | Gala Zard |
|--------|----------|---------|---------|--------------|--------------|--------------|------------|------------|------------|-------|-------|----------|
| Shingaly| 0.0000   |         |         |              |              |              |            |            |            |       |       |          |
| Benatty| 0.1419   | 0.0000  |         |              |              |              |            |            |            |       |       |          |
| Ketek  | 0.3328   | 0.2453  | 0.0000  |              |              |              |            |            |            |       |       |          |
| Rebwary Rash | 0.2102  | 0.2116  | 0.3291  | 0.0000       |              |              |            |            |            |       |       |          |
| Hejeera Rash| 0.2507  | 0.2817  | 0.3661  | 0.1817       | 0.0000       |              |            |            |            |       |       |          |
| Rash khomali| 0.2355  | 0.2508  | 0.2855  | 0.2240       | 0.1358       | 0.0000       |            |            |            |       |       |          |
| Rehan Rash | 0.2412  | 0.2711  | 0.3248  | 0.1948       | 0.1386       | 0.1510       | 0.0000     |            |            |       |       |          |
| Rehan Zard | 0.2991  | 0.2931  | 0.2509  | 0.2589       | 0.2211       | 0.1438       | 0.1449     | 0.0000     |            |       |       |          |
| Zarda Roon| 0.3141  | 0.2998  | 0.3135  | 0.2649       | 0.2694       | 0.2331       | 0.1972     | 0.1678     | 0.0000     |       |       |          |
| Shela   | 0.3645   | 0.3417  | 0.3177  | 0.3690       | 0.3905       | 0.2727       | 0.3076     | 0.2322     | 0.2632     | 0.0000 |       |          |
| Kola    | 0.2398   | 0.3123  | 0.3790  | 0.2563       | 0.2254       | 0.2348       | 0.2188     | 0.2613     | 0.2373     | 0.3124 | 0.0000 |          |
| Gala Zard | 0.2313  | 0.2673  | 0.3611  | 0.2614       | 0.2237       | 0.2465       | 0.2239     | 0.2664     | 0.2842     | 0.3248 | 0.1261 | 0.0000  |
Figure 2. UPGMA dendrogram of twelve fig varieties showing genetic relationships of estimated from AFLP data. The 12 Ficus cultivars are: 1= Shingaly, 2= Benatty, 3= Ketek, 4= Rebwary Rash, 5= Hejeer Rash, 6= Rash khomali, 7= Rehan Rash, 8= Rehan Zard, 9= Zarda Roon, 10= Shela, 11= Kola, 12= Gala Zard

REFERENCES
1. Abood, I. D. and H. SH. Yassien. 2016. Detection resistance genes of root knot nematode mi in some of pure lines of tomato indeterminate growth. Iraqi Journal of Agricultural Sciences. 47 (5): 1321-1327
2. Al-juboori, A. W. A.; E. N. Ismail and K. A. Alwan. 2018. Molecular and morphological indicators (Qutha ) Cucumis melo planted in Iraq. Iraqi Journal of Agricultural Sciences. 50 (3): 835- 841
3. Al-Asie, A. H. A. 2002. The use of DNA markers for genetic diversity analysis of barley (Hordeum vulgare L.). Ph. D. Thesis College of Education/Ibn AL-Haitham, University of Baghdad. (In Arabic)
4. Al-Maamory, S. M. and I. M. H. Al-bayati. 2019. Effect of foliar nutrition on fig sapling growth of cv. WAZIRY. Iraqi Journal of Agricultural Sciences. 50 (2): 356-363
5. Altameme, H. J. M. and I. A. Ibraheam. 2019. RAPD and ISSR analysis of the genetic relationship among some species in rutaceae in and apiceae in Iraq. Iraqi Journal of Agricultural Sciences. 50 (2): 608-616
6. Baraket, G.; A. Abdelkrim; M. Mars and A. S. Hannachi. 2011. Comparative Assessment of SSR and AFLP Markers for Evaluation of Genetic Diversity and Conservation of Fig, Ficus carica L. Plant Mol Biol Rep. 29: 171-184
7. Berg, C. C. 1989. Classification and distribution of Ficus. Experientia. 45: 605-611
8. Cabrita, L. F.; U. Aksoy; S. Hepaksoy and J. M. Leitão. 2001. Suitability of isozyme, RAPD and AFLP markers to assess genetic differences and relatedness among fig (Ficus carica L.) clones. Scientia Horticulturae. 87: 261-273
9. California Rare Fruit Growers, Inc. 1996. Fig Fruit Facts. The Fullerton Arboretum, CSUF, Fullerton, CA. Available: http://www.crfg.org/pubs/ff/fig.html
10. Cervera, M. T.; D. Remington; J. M. Frigerio; V. Storme; B. Ivens; W. Boerjan and C. Plomion. 2000. Improved AFLP analysis of tree species. Canadian Journal of Forest Research. 30 (10): 1608-1616
11. Dehgan, B. 1998. Landscape Plants for Subtropical Climates. University Press of Florida, Gainesville, FL
12. FAO. 2009. The FAO Statistical Database-Agriculture. 2009. Available from: www.fao.org/economic/the-statistics-division-ess/publications/studies/statistical-yearbook/fao-statistical-yearbook-2009/en/.
13. Flashman, M.; V. Rodov and E. Stover. 2008. The Fig: Botany, Horticulture, and Breeding. Horticultural Reviews. 34: 113-197
14. Fornari, B.; M. E. Malvolti; D. Taurchini; S. Fineschi; I. Beritognolo; E. McCaglia and F. Cannata. 2001. Isozyme and organellar DNA analysis of genetic diversity in natural/naturalized European and Asiatic walnut (Juglans regia) populations. Acta Hort. 544:167-178
15. Guasmi, F.; A. Ferchichi; K. Farès and L. Touil. 2006. Identification and differentiation of Ficus carica L. cultivars using inter simple sequence repeat markers. Afr. J. Biotechnol 5:1370-1374
16. Jubrael, J. M. S. 2005. Assessment of AFLP based Genetic Relationships among Date Palm (Phoenix dactylifera L.) Varieties of Iraq. J. AMER. Soc. HoRT. Sci. 130 (3): 442-447
17. Laddomada, B.; C. Gerardi; G. Mita; D. Lumare; F. Minonne; S. Marchiori and F. Fiocchetti. 2008. Molecular Characterization of Apulian Fig (Ficus carica L.) Germplasm Collection Using Fluorescence-Based AFLP Markers. J. Leitaõ and M.A. Neves Acta Hort. 798, ISHS. 205-212
18. Li, G. T.; C. X. Ai; L. S. Zhang; H. R. Wei and Q. Z. Liu. 2011. ISSR analysis of genetic diversity among seedling walnut (Juglans spp.) populations. Journal of Plant Genetic Resources. 12 (4): 640-645
19. Malvolti, M. E.; P. Pollegioni; S. Mapelli and F. Cannata. 2010. Research of Juglans regia provenances by molecular, morphological and biochemical markers: a case study in Italy. Bioremediation, Biodiversity and Bioavailability. 4: 84-92
20. Maniatis, T.; E. F. Fritsch and J. Sambrook. 2001. In vitro application of DNA by the Polymerase Chain Reaction, in Molecular Cold Spring Harbor Laboratory Press, New York, U.S.A., p. 691
21. Naroui Rad, M. R.; J. Abbaskohpayegani and G. Keykha. 2018. Assessment of genetic diversity among melon accessions using graphical principal component and cluster analysis. Iraqi Journal of Agricultural Sciences. (5): 817- 825
22. Neal, M. C. 1965. In Gardens of Hawai‘i. Bernice P. Bishop Museum, Special Publication 40, Honolulu, HI
23. Nei, M. and W. H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci.USA. 76: 5269-5273
24. Niu, J. X.; L. Wang; Y. X. Dai; R. Li; X. K. Yang and J. Q. Lv. 2007. Identification of AFLP Makers Linked to Early-Bearing Gene in Walnut. Molecular Plant Breeding. 5 (2): 266-268
25. Resta, P.; E. Ferrara; M. Roselli; R. Chaabane; F. Lamaj and G. Fanizza. 2003. AFLP Analysis of relationships among fig genotypes (Ficus carica L.) in the Apulia region. In: Proceedings of XLVII SIGA Annual Congress, Verona, 2003. Abstract no. 2.29
26. Rohlf, F. J. 1993. NT SYS-PC. Numerical Taxonomy and Multivariate Analysis System. Version 1.8 Exter Software, Setauket, New York, U.S.A
27. Shahbaz, S. E. 2010. Trees and shrubs. A field guide to the trees and shrubs of Kurdistan region of Iraq. Duhok University Press. Iraq pp601
28. Torres, A. M. and B. Tisserat. 1980. Leaf isozymes as genetic markers in date palms. Amer. J. Bot. 67:162-167
29. Tous, J. and L. Fergueson. 1996. Mediterranean fruits. In progress in New crops. Janick J. (ed.). Atlas press. Arlington, pp.46-430
30. Van Toai, T.; T. Deng; S. K. S. Martin and J. Peng. 1997. Using AFLP markers to determine the genomic contribution of parents populations. Crop. Sci. 37 (4): 1370-1373
31. Vos, P.; R. Hogers; M. Bleeker; M. Rijans; Van De Leet; M. Hornes; A. Fijters; J. Pot; J. Peleman; Kuiperem and Zabeaum. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414
32. Wagner, W. L.; D. R. Herbst and S. H. Sohmer. 1999. Manual of the Flowering Plants of Hawai‘i. 2 vols. Bishop Museum Special Publication 83, University of Hawai‘i and Bishop Museum Press, Honolulu, HI
33. Weigand, F.; M. Baum and S. Udupa. 1993. DNA molecular marker technics, technical manual, No. 20 International Center for Agricultural Research in the Dry Areas (ICARDA). Aleppo, Syria

34. Yuan, L.; M. Warburton; S. Zhang; M. Khairallah; X. Liu; Z. Peng and L. Li. 2000. Comparison of genetic diversity among maize inbred lines based on RFLP, SSRs, AFLPs, and RAPDs. Yi Chuan Bao. 2: 723-733.