Plant Archives Vol. 19, Supplement 1, 2019 pp. 1427-1432 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

GENETIC RELATIONSHIPS AMONG *PLANTAGO SPP*. GROWING IN IRAQ USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS

Mohammed F. Hamdi[1], Dhia S. Hassawi[1] and Athiya N. Salman[2]

[1] Biology Department, College of Sciences, Anbar University, Iraq

[2] Biology Department, College of Education for Pure Sciences,University of Baghdad, Iraq

mohammedkarbooly093@gmail.com

Abstract

The study dealt with the genetic relationships among 13 species of *Plantago* L. genus. Field surveys were conducted for Iraqi districts to species collection. Leaves collected and DNA extracted. RAPD marker used to detect different relations among species. Through similarity coefficient cluster; the species divided into five groups depending on the relationships between species, the highest related were observed among *P.lanceolata* L. and *P. major* L., at 91% similarity coefficient, while *P. cretica* L. and *P. crypsoides* Bois., at 73% similarity coefficient. More distantly between some species in genetic diversity considered very useful in programs of genetic improvement and the linking of useful germplasm, especially for species of economic importance.

***Keywords*:** *Plantago*, RAPD technique, molecular markers, genetic relationships.

Introduction

Plantago genus descends from Plantaginaceae family, includes herbs and shrubs plants that widely spreading in the temperate and tropics areas; it included about 15 species in Iraq and about 400 species in the world. This genus owned several species of medical importance. In general, the genus has many types of compounds against certain diseases such as urinary tract infection, reduce the proportion of cholesterol, reduce sugar in the blood, and wounds healing. Due to the presence of some compounds of medical importance in their leaves, its compound such as flavonoid structure in *P.lanceolata, P. afra* and *P. coronopus* considered as inhibitory for renewal cancer disease (Galvez *et al*., 2003). *P. major* have the essential fatty acid and carotenes (Guil-Guerrero and Rodríguez-García, 1999).The study of Chee and Noor, 2000 showed a significant effect in lowering sugar levels in infected rats by using *P.major* extracted, this species used for treatment of more cases from viral infections, such as coldness and hepatitis as a part from traditional uses in china (Chiang *et al.*, 2002). Ferulic acid concentration is high in *Plantago* species (especially in seeds); several studies showed that this acid works against breast and liver cancer (Valentão *et al*., 2001; Pierre *et al*., 2006). Flavonoids found in most species of *Plantago genus*; several studies demonstrated the effectiveness of flavonoids as inhibitor factor of cancer evolution (Hertog *et al*., 1993; Hertog, 1996). Other studies showed the importance of *P. ovata* seeds in colon cancer prevention, particularly control of cholesterol, maintains blood sugar balance, and a good laxative (Kumar *et al.*, 2017).

DNA technology markers is important evidence for studying genetic diversity because of the abundance of polymorphisms cases (Gepts, 1993). PCR- based DNA techniques such as Random Amplified Polymorphism DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) are useful in determining the genetic relationships of a wide range of different organisms. These markers are simple and do not need a background about the genome for organisms (Williams *et al*., 1990 and Vos *et al*., 1995). The molecular markers considered as a good evidence of genetic diversity because they are not affected to environments conditions and they are used for whole genomic DNA (Khurana-Kaul *et al*., 2012). RAPD is preferred for several reasons, the most important is that it is inexpensive and it does not need a large amount of plant material, but this technique is not a favorite for plants with complex genomes (Devos and Gale, 1992).

Maintaining genetic diversity within species is very important because loss of diversity leads to a reduction in the ability of these organisms to adapt to environmental conditions under the rule of survival (Yamagishi *et al*., 2010). The variations of environmental factors lead the species to evolve in response to these conditions (Storfer *et al*., 2007; Manel *et al*., 2012). Molecular markers have been widely used in the class identification, population studies, and phylogenetic studies and in drawing genetic maps (Williams *et al*. 1990). Many techniques have been used to detect the genetic variations of species in their natural

environments,as well as the possibility of the incorporation of the RAPDs in detecting these variations (Lashermes *et al.*, 1993; Wilkie *et al*., 1993). RAPDs considered a good marker in identified relationships among medicinal plant species; as reported by Devaiah *et al*., 2008; Sarwat *et al*., 2008; and Shi *et al*., 2008. This study wasconducted for finding the genetic relationships among 13 species of *Plantago* genus using RAPD technique.

Materials and Methods

Plant Materials and DNA Isolation

Thirteen species of *Plantago genus* were studied; they were collected through field trips of the Iraqi distract. Genomic DNA was extracted from leaves samples using GENEAID plant kit (bioneer. Korea). Four gm for each sample was grinded with liquid nitrogen by a pestle and mortar to become a powder. 100 µl from GBX1 solution was added to the leaves tissue powder with a simple grinding by micro pestle inside Eppendorf tubes. 30 µl of proteinase solution plus 200 µl from each GBXI and GBI solutions were added and the mixture was vortex. The mixture was transferred to the water bath and kept for 1 hour at 60 ºC, followed by adding 100 µl from GB2, and then vortex. The collection tubes were prepared and put inside it the GD column; followed by pouring the mixture inside GD column, and then centrifuged at 1000 rpm, for 1 minute. 700 µl from GB3 was added to the residual mixture and vortex. The mixture was transferred to silica column to retain the genomic DNA and get rid of other materials. The mixture was centrifuged at 1350 rpm, for 1 minute. 400 µl from washing solution was added to the suspended DNA and centrifuged at 1350 rpm for 30 seconds. 600 µl from washing buffer was added to the suspended DNA and centrifuged for 30 seconds at 1350 rpm. The genomic DNA was dried by centrifugation for 3minute at 1350 rpm. 50 µl from elution buffer was added to the DNA suspension (pure DNA) and centrifuged for 5 minute at 1350 rpm. The samples were kept at 4^0C till using.

PCR Reactions

PCR reactions of the DNA samples were done by transferring 8 µl of extracted DNA to Eppendorf tubes. Two µl from each primer solution plus 5 µl from GENEAID premix solution (Bioneer, Korea) were added and the volume was completed to 25 µl by adding 10 µl of deionized water. The mixture was transferred to Exispin centrifuge for mixing. The PCR program for DNA amplification was applied as follow: 35 cycles at 95 ºC for 5 minutes for initial denaturation, then (95 ºC for 1 minute for denaturation, 36 $C^o$for 1 minutefor annealing, 72 ºC for 5 minutes for extension). Five µl from the PCR products was loaded into 1.5 gm agarose gel electrophoresis (Bio basic, Canada) that dissolved in 10 ml of Tris Borate EDTA (TBE) buffer (Bio basic, Canada)and plus 90ml of distilled water. The agarose gels stained with ethidiumbromide then examined under UV light. The products sizes of PCR were compared with 1Kb DNA ladder (Gen direct, Korea). All PCR reactions were repeated for two times.

Scoring and Data Analysis

The scoring data of each primer was recorded as 1 or zero; where 1 represented presence of band in a certain size and zero represented absence of band. All the banding patterns were used for determination the similarity coefficient, and then the dendrogram derived from the similarity coefficient data.

Result and Discussion

Ten primers were used for recognition of 13 genotypes belong to *Plantago genus*. The bands size ranged from 150to1500 P; the maximum size was recorded with OPF 03, while the minimum size was recorded with OPC 8.The monomorphic bands considered as bands with the same mobility in gel electrophoresis, while the polymorphic bands means that the difference bands for different samples appear in difference locations on the electrophoretic gel. The maximum products of polymorphic DNA bands observed with primer OPF-03 and the minimum in primer OPC- 8(Table1). The maximum total number of bands was 46 with all primers in *P. crypsoides*, while the minimum reached 7 with all primers in *P. loeflingii* (Table 2).

Differences among species in genetic relationships came from highest polymorphic and unique bands.Most of species in this study varied in the number of specific amplified fragments for some primers, such as OPD11, OPF02, and OPF03; while other primers were not detected in some species (Figures 1 and 2). According to the similarity coefficient and dendrogram data, the genetic relationships among 13 species of *Plantago genus* divided the species into five groups(Table 3 and Figure 5). The first group included three species: *P. lanceolata*, *P. major* and *P. lagopus* that were closely related to each other. *P. lanceolata* and *P. lagopus* showed 88% similarity coefficient, this reflects the similarities in morphological characters between these two species. The similarity coefficient between *P. lanceoata* and *P. major* was 91%, while high distant between these two species was observed in study of Woolf and Schaall (1992) who studied chloroplast DNA (cpDNA) for these species. Many studies have shown that there is no affinity between the morphological results and the genetic similarities of certain plants (Steiner and Santos, 2001; Greene *et al*., 2004).

The second group included four species: *P. amplexicaulis*, *P. psammophila*, *P. loeflingii* and *P. afra*. The first two species related to each other with 82 % similarity coefficient; for morphological characters, these two species are closely to each other in some characters, such as leaves shape, color of fruits and seeds, and are of desert species. *P.loeflingii* and *P.afra* are mountain species, especially in northern districts; these two species related to each other at 87% similarity coefficient. For morphological features, these two species are small in size, leaves are sessile, and have tight lanceolate.

The third group included two species: *P. boissieri* and *P. ciliata*, with 83% similarity coefficient; these two species grow in desert and have differences, especially in leaves shape.

The fourth group included two species: *P. ovata* and *P. coronopus*, with 81% similarity coefficient. In study of Samantaray *et al*. (2010), *P. ovata* and *P. lanceolata* included in one group at 8% similarity coefficient, while in this study, *P. lanceolata*and *P. ovata* included in separated groups, with 82% similarity coefficient.

The fifth group included two species: *P. cretica* and *P. crypsoides*; this group included species that are distant from each other, with 73% similarity coefficient. This may confirmed the unique morphological characters in these species compared to the other species under study.

This study showed that the similarity coefficient between *P. coronopus* and *P. crypsoides* was 77%. This result supports the separation of these two species from each other and this in agreement with Boissier (1879). The molecular separation is confirmed by the morphological results **(Figure 3 and 4)** that showed differences in plant parts and pollen characters. Also, these two species showed 16% similarity in the study of Shalabi and Abou-El-Enain (2013).

Table 1: Data of RAPD test of 13 genotypes of *Plantago* species using 10 primers

Primer No.	Primer name	Amplicon size range (bp)	Total No. of bands	No. of bands/ genotype*	No. of different size markers	No. of monomorphic markers	% of monomorphic markers	% of polymorphic markers
1	OPD 11	200 - 1500	59	4.5	59	0	0%	100%
2	OPF 02	150-1200	64	4.9	64	0	0%	100%
3	OPF 03	150-1500	79	6	79	0	0%	100%
4	OPF 20	200-1500	56	4.3	56	0	0%	100%
5	OPP 16	200-1250	25	1.9	25	0	0%	100%
6	OPC 8	300-900	14	1	14	0	0%	100%
7	OPA 18	150-1000	31	2.4	31	0	0%	100%
8	OPC 18	200-1500	31	2.4	31	0	0%	100%
9	UBC 54	250-1000	18	1.4	18	0	0%	100%
10	OPN 08	200-1100	27	2	27	0	0%	100%
Total	**-**		**404**	-	**404**	-	-	-

Table 2: Total and unique band numbers of *Plantago* species with 10 primers

No.	Species	Total bands	Unique bands
1	*Plantago lanceolate* L.	30	1
2	*P. lagopus* L.	40	4
3	*P. major* L.	31	4
4	*P. coronopus* L.	33	6
5	*P. crypsoides* Boiss.	46	10
6	*P. ovata* Forssk.	32	5
7	*P. amplexicaulis* Cav.	41	4
8	*P. boissieri* Haussk & Bornm	38	4
9	*P. ciliata* Desf	27	6
10	*P. psammophila* Agnew & Chalabi	18	5
11	*P. afra* L	7	3
12	*P. cretica* L.	25	6
13	*P. loeflingii* L.	36	2

Table 3 : Similarity coefficient matrix of pair wise comparisons for 13 *Plantago* species

Genotypes	*P. Lanceolata*	*P. major*	*P. lagopus*	*P. boissieri*	*P. crypsoides*	*P. coronopus*	*P. ovata*	*P. ciliata*	*P. amplexicaulis*	*P psammophila*	*P. loeflingii*	*P. afra*	*P. cretica*
P. Lanceolata	1.00												
P. major	0.91	1.00											
P. lagopus	0.88	0.83	1.00										
P. boissieri	0.84	0.79	0.82	1.00									
P. crypsoides	0.82	0.77	0.79	0.78	1.00								
P. coronopus	0.83	0.77	0.79	0.79	0.77	1.00							
P. ovata	0.82	0.80	0.78	0.82	0.77	0.81	1.00						
P. ciliata	0.83	0.79	0.79	0.83	0.76	0.79	0.77	1.00					
P. amplexicaulis	0.81	0.78	0.83	0.82	0.75	0.83	0.78	0.81	1.00				
P. psammophila	0.82	0.80	0.82	0.84	0.75	0.81	0.80	0.79	0.82	1.00			
P. loeflingii	0.85	0.82	0.84	0.83	0.80	0.85	0.80	0.81	0.87	0.90	1.00		
P. afra	0.85	0.82	0.84	0.83	0.80	0.80	0.78	0.83	0.84	0.85	0.87	1.00	
P.cretica	0.79	0.75	0.80	0.78	0.73	0.77	0.77	0.80	0.80	0.80	0.84	0.80	1.00

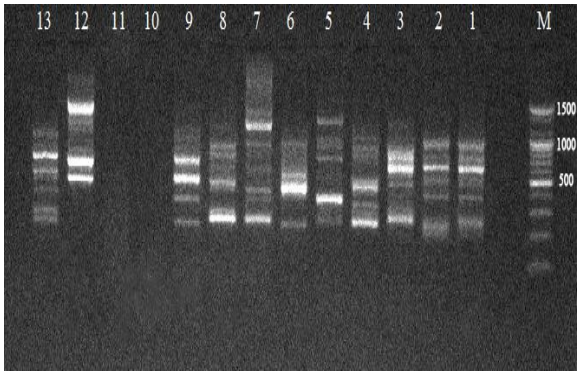

Fig. 1 : Agarose gel electrophoresis showed RAPD pattern of different *Plantago spp*. with primer OPD 11: lane M, 1Kb DNA marker; lane 1, *P.lanceolata;* lane 2, *P.major;* lane 3, *P.lagopus;* lane 4, *P.boissieri;* lane 5, *P.crypsoides;* lane 6, *P.coronopus;* lane 7, *P.ovata;* lane 8, *P.ciliata;* lane 9, *P.amplexicaulis;* lane 10, *P.psammophila;* lane 11, *P.loeflingii;* lane 12, *P.afra;* lane 13, *P.cretica.*

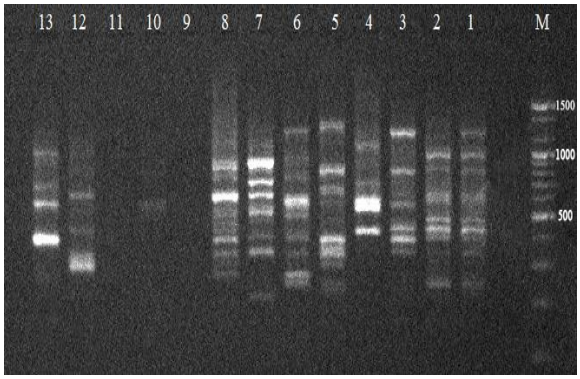

Fig. 2 : Agarose gel electrophoresis showed RAPD pattern of different *Plantagospp*. with primer OPF 02: lane M, 1Kb DNA marker; lane 1, *P.lanceolata;* lane 2, *P.major;* lane 3, *P.lagopus;* lane 4, *P.boissieri;* lane 5, *P.crypsoides;* lane 6, *P.coronopus;* lane 7, *P.ovata;* lane 8, *P.ciliata;* lane 9, *P.amplexicaulis;* lane 10, *P.psammophila;* lane 11, *P.loeflingii;* lane 12, *P.afra;* lane 13, *P.cretica.*

Fig. 3 : The general morphological shape of *P. coronopus.*

Fig. 4: The general morphological shape of *P. crypsoides*.

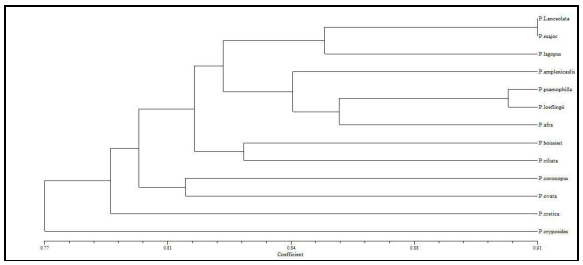

Fig. 5: Dendrogram generated from RAPD markers data showing the genetic relationship among 13 *Plantago*s pecies

Acknowledgements

The authors extend his thanks to the participants in the research, as well as thanks to the sub-official of Pioneer Company in Iraq to assist in the completion of research.

References

Boissier, E. (1879). Flora Orientals. Vol. 4, Geneva etBasileae, H George. Bibliopolam Lugdunt, 876-898.

Cheebeng-jin and Noor Hamdan (2000). Medicinal Properties of *Plantago major*: Hypoglycemic and Male Fertility Studies. PertanikaJ. Trap. Agric. Sci. 23(1): 29 – 35.

Chiang, L.C.; Chiang, W.; Chang, M.Y.; Ng, L.T. and Lin, C.C. (2002). Antiviral activity of *Plantago major* extracts and related compounds in vitro. *Antiviral Research*, *55*(1): 53-62.

Devaiah, K.M. and Venkatasubramanian, P. (2008). Genetic characterization and authentication of *Embelia ribes* using RAPD-PCR and SCAR marker. *Planta Medica,* 74: 194–196.

Devos, K.M. and Gale, M. (1992). The use of random amplified polymorphic DNA markers in wheat. *Theoretical and Applied Genetics*, *84*(5-6): 567-572.

Galvez, M.; Martin-Cordero, C.; Lopez-Lazaro, M.; Cortes, F. and Ayuso, M.J. (2003). Cytotoxic effect of *Plantago spp.* on cancer cell lines. J Ethnopharma 88: 125–130.

Gepts, P (1993). The use of molecular and biochemical markers in crop-evolution studies. In: Hecht MK (ed) Evolutionary biology. Plenum Press, New York, 51–94.

Greene, S.L.; Gritsenko, M. and Vandemark, G. (2004). Relating Morphologic and RAPD marker variation to collection site environment in wild populations of Red Clover (*Trifolium pratense* L.). *Genetic resources and crop evolution*, 51(6): 643-653.

Guil-Guerrero, I. and Rodríguez-García, J. (1999). Lipids classes, fatty acids and carotenes of the leaves of six edible wild plants. Eur Food Res Technol, 209: 313–316.

Hertog, M.G.L. (1996). Epidemiological evidence on potential health properties of flavonoids. Proceedings of the Nutrition Society of Australia 55: 385–397.

Hertog, M.G.L.; Hollman, P.C.H.; Katan, M.B. and Kromhout, D. (1993). Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. Nutrition Cancer 20: 21– 29.

Khurana-Kaul, V.; Kachhwaha, S. and Kothari, S.L. (2012). Characterization of genetic diversity in *Jatropha curcas L.* germplasm using RAPD and ISSR markers. Indian J. Biotechnol. 11: 54-61.

Kumar, D.; Pandey, J.; Kumar, P. andRaj, V. (2017). Psyllium Mucilage and Its Use in Pharmaceutical Field: An Overview. *Curr Synthetic Sys Biol*, *5*(134): 2332-0737.

Lashermes, P.; Cros, J.; Marmey, P. and Charrier, A. (1993). Use of random amplified DNA markers to analyse genetic variability and relationships of *Coffee* species. *Genetic resources and crop evolution*, 40(2): 91-99.

Manel, S.; Gugerli, F.; Thuiller, W.; Alvarez, N.; Legendre, P.; Holderegger, R.; Gielly, L. and Taberlet, P. (2012). Broad-scale adaptive genetic variation in Alpine plants is mainly driven by temperature and precipitation. Mol. Ecol 19: 3824–3835.

Pierre, G.; McKinnon and Carole, M. (2006). Effect of wheat variety, farming site, and bread-baking on total phenolics. International Journal of Food Science and Technology 41 (3): 329.

Samantaray, S.; Dhagat, U.M. and Maiti, S. (2010). Evaluation of genetic relationships in *Plantago* species using Random Amplified Polymorphic DNA (RAPD) markers. *Plant Biotechnology* 27: 297–303.

Sarwat, M.; Das, S. and Srivastava, P.S. (2008). Analysis of genetic diversity through AFLP, SAMPL, ISSR and RAPD markers in *Tribulus terrestris*, a medicinal herb. *Plant Cell Rep* 27: 519–528.

Shalabi, L.F. and Abou-El-Enain, M.M. (2013). Morphological Variations and Morphometry in the Genus *Plantago.* 1: 468-472.

Shi, W.; Yang, C.F.; Chen, J.M. and Guo, Y.H. (2008). Genetic variation among wild and cultivated populations of the Chinese medicinal plant, *Coptis chinensis* (Rananculaceae). *Plant Biol.* 10: 485–491.

Steiner, J.J. and delos Santos, G.G. (2001). Adaptive ecology of *Lotus corniculatus* L. genotypes. *Crop Science*, 41(2): 552-563.

Storfer, A.; Murphy, M.A.; Evans, J.S.; Goldberg, C.S.; Robinson, S.; Spear, S.F. and Waits, L.P. (2007). Putting the landscape in landscape genetics. *Heredity*, 98(3): 128.

Valentão, P.; Fernandes, E.; Carvalho, F.; Andrade, P.B.; Seabra, R.M. and Bastos, M.L. (2001). Antioxidant Activity of *Centaurium erythraea* infusion Evidenced by Its Superoxide Radical Scavenging and Xanthine Oxidase Inhibitory Activity". Journal of Agricultural and Food Chemistry, 49(7): 3476–9.

Vos, P.; Hogers, R.; Bleeker, M.; Reijans, M.; Lee, T.V.D.; Hornes, M. and Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic acids research*, 23(21): 4407-4414.

Wilkies, S.E.; Isaac, P.G. and Slater, R.J. (1993). Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium*. *Theoretical and Applied Genetics*, 86(4): 497-504.

Williams, J.G.; Kubelik, A.R.; Livak, K.J.; Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids research*, *18*(22): 6531-6535.

Wolff, K. and Schaal, B. (1992). Chloroplast DNA variation within and among five *Plantago* species. *J Evol Biol* 5: 325–344.

Wolff, K. and Schaal, B. (1992). Chloroplast DNA variation within and among five *Plantago* species. Journal of evolutionary biology, 5(2): 325-344.

Yamagishi, M.; Nishioka, M. and Kondo, T. (2010). Phenetic diversity in the *Fritillaria camschatcensis* population grown on the Sapporo campus of Hokkaido university. Landscape *and ecological engineering*, 6(1): 75.