International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-8, Issue-4, 2017, pp1036-1042
http://www.bipublication.com

Research Article

The study of the Genetic Diversity of Ferula Haussknechtii in Neyshabour Province with RAPD Molecular Markers Help

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ABSTRACT:
Ferula haussknechtiiis one of the most important pasture plants that has an important role to create a dry pasture and forage production. In the present research, RAPD molecular markers were used to determine the genetic variation of 13 type of Ferula haussknechtii population in Baghee, Bar, Ariyeh and Mir-abad villages of neyshabour province. Between seven used primer, primer number 4 called S4 could vocalize all collected samples to the 67 bands Polly morph. To determine the extent of the similarity between the populations, the Jacaurd coefficient of similarity was used. Maximum amount of similarity (80) between two samples,was observed in Relive 2 and Relive3 . Using UPGMA algorithm a dendrogram based on similarity matrices was prepared. The cluster analysis, classification Ferula haussknechtii to the 9 clusters. The results of this study were to determine the efficiency of RAPD markers representative of genetic diversity of the Ferula haussknechtiipopulation.

Key words: RAPD markersGenetic diversity, Ferula haussknechtii, Neyshabour, Ferula

INTRODUCTION:

Despite the effectiveness of chemical drugs but they have frequency of adverse effects. This issue has caused researchers more attention to natural ingredients (which has less adverse effects), that nowadays in most countries use medicinal plants in the therapeutic process in a variety of common.(Zabeti,2014). The use of medicinal plants as a byproduct of pastures, has a very long history in our country and determine the genetic properties and eco-physiology, Physiology and ecology of these plants in order to maintain stable economic operation, along with the existing diversity in natural areas is crucial to Iran's rangelands of the extinction of species is unique and prevent different genetically and also create employment and increase the income range, the species for future generations and balances of nature (Talebi Kuikhy,2007). *Ferula haussknechtii* is a plant of the Apiaceous dark that investigations of this taxon from other taxon have been based on cognitive traits, reproductive characteristics and morphology. (Color flora of Iran,1975-1990). Given that the existing flora in this species was not reported in the East of Iran, therefore, with the emergence of this species by the author for the first distribution of its low altitude in the area of the North and North West of Neyshabour, pay attention to maintaining the genetic resources of this plant is very important. RAPD markers Including indicators that are widely used in genetic because they are capable of using low amounts of DNA, the differences between the plants identified in DNA and do not need to have previous information about the

Genome (Talebi Kuikhy,2007).Use this marker firstly is quick, secondly need a small amount of DNA, and do not require previous information about the genome and thirdly do not the need to radioactive material. (Mandolkanee,2002ء).

MATERIALS AND METHOD:

in this study 15 kinds of Ferula haussknechtii that collected from mountainous areas in North and North West of Neyshabour include: Mir-abad, Aryeh,Baghiee and Bar for DNA extraction. In order to extract the DNA in this research, method of Sharma et all (2012) was used with a little change in the following order.

The materials used in the Ferula haussknechtii sample extraction buffer into a 100ml balloon and then put in ben mari at the 60 centigrade temperature for 15 minutes.

Table 1- mix of extraction buffer for 100 ml

Compounds	Final concentration	gr-ml
CTAB	2	2gr
PVP	3٪	3ml
-mercaptoethanolß	2٪	2ml
Nacl	1.4M	8.1816gr

- 5 g fresh leaf of any population of *Ferula* haussknechtii powder well with liquid nitrogen and was removed to 50 ml falcon pipes.
- The amount of 15 ml extraction buffer add slowly to the second stage tubes and keep in 60 centigrade for 30 to 60 minutes and vertex every 5 minutes.
- The volume of third stage solution, chloroform ISO-Amyl alcohol ratio (24:1) added and three minutes of vertex was produce emulsion.
- Pipes Falconof part 4 centrifuges under the terms of 8000 rpm for 20 minutes and 25centigrade.
- After the completion of centrifuge three phases was formed that removed upper phase and moved to 15ml falcon.
- 1/5volume of liquid contained in the step 6 falcon, was added cool isopropanol and vertex slowly for 5 minutes then keep in freezer with -20 centigrade temperature for 3 hours (note: at this point the maintenance solution do not keep more than 3 hours in freezer) and after leaving freezer centrifuges in terms of 9000 rpm for 12 minutes and the temperature of the 40 centigrade.
- After centrifuges the upper solution was discarded and the lower phase washed by 70% ethanol, then to dry alcohol well implemented at room temperature.
- After drying the 4ml TE buffer added into the Falcon for one night at room temperature .Inthe event of the formation of the Brown deposition that there are signs of contamination in the samplewas done 10 steps to the next.
- 2ml phenol, 2 ml chloroform and 3 ml of soluble PVP 3% were added to Milky emulsion very slowly and vertex (at least 15 minutes)and was ready for the centrifuges.
- Centrifugein conditions 8000 rpm for 20 minutes and the temperature of the 25 centigrade.
- Aftercentrifuge, an upper phasewastook and the volumechloform-isoamilalcoholand 3 ml of PVP3% was added and vertex very slowly up toa milky emulsion for 15 minutes to prepare for thecentrifuges.
- Then under the terms of 9000 rpm for 12 minutes and was centrifuge at the temperature of 4centigrade.
- After centrifuges the upper phase were removed and the volume of cold ethanol and 0.1 volume sodium acetate was added and was hold in the freezer for 3 hours. (Note: at this stage, there was no problem ifa solution staya day alsoThe deposition could not be seen with

the eyes .The reason cannot be based on the absence of DNA).

- After withdrawing from the freezer, the upper phase was discard and the lower phase wash with 70% ethanol and dry at room temperature.
- One ml of TE buffer was added and was kept for 1 hour at room temperature for goodness DNA solution deposition.

In order to check the quality and quantity of DNA extracted , the spectrophotometer Jenway model 6305 device was used in the range of 260 to 280 nm absorbed by 1% agarose gel (Figure 1) that the number 13 sample (table 2) has the right to conduct DNA PCR reaction was determined.

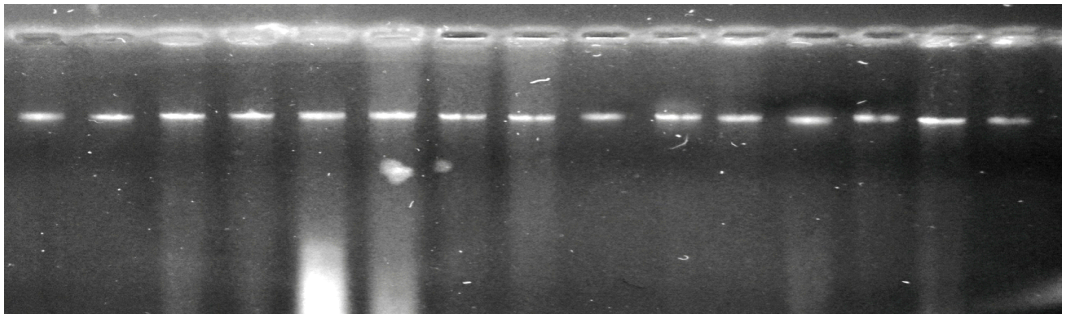

Figure 1-the pattern of the DNA extracted from the different regions sample of the *Ferula* haussknechtii

Table2 -The right DNA samples used for PCR reaction

Plot orRelive	(Altitude above sea level (m	Collecting area
)(Plot1) Or (Releve1	2150	EastBaghiee
(Plot2) Or (Releve2)	2250	EastBaghiee
(Plot3) Or (Releve3)	2250	WestBaghiee
(Plot4) Or (Releve4)	2150	WestBaghiee
(Plot5) Or (Releve5)	2240	East Bar
(Plot6) Or (Releve6)	2340	East Bar
(Plot7) Or (Releve7)	2340	West Bar
(Releve8) Or(Plot8)	2240	West Bar
(Plot9) Or (Releve9)	2315	East Ariye
(Plot12) Or (Releve12)	2315	West Ariye
(Plot13) Or (Releve13)	2213	East Mirabad
(Plot14) Or (Releve14)	2313	East Mirabad
(Plot15) Or (Releve15)	2313	West Mirabad

In order to perform a PCR reaction (Polymerase Chain Reaction) , in this research Metabion company of Germany 7 primer which holds 10 sequences random nucleoid (table3) and using Eppendorf factory Master cycler gradient thermo cycler with a thermal rotation of table4 and also for each sample of table 5 using 25 microliter reaction volume was used.

Table 3-The German Mutation company making consumer primer sequence

Percent GC	Molecular weight	Melting point ^{o}C	Concentration Mg/MI	OD	Name	Sequence
70	3013	34	118.2	4.1	S1	5/-GTCCCGACGA-3/
70	2924	34	102.1	3.1	S2	5/-CTCACCGTCC-3/
60	3090	32	116.8	3.9	S3	5/-GGGGGTCTTT-3/
60	2948	32	103.2	3.5	S4	5/-CCGCATCTAC-3/
60	3037	32	119.7	4.5	S5	5/-GAACGGACTC-3/
60	3028	32	111.8	4.0	S6	5/-TTCGAGCCAG-3/
70	3084	34	102.7	3.6	S7	5/-GTGAGGCGTC-3/

Table 4 - The thermal device rotation program thermo cycler

Phase	Temperature (centigrade)	Time (minute)
Denaturation	94	30s
Annealing	35	30s
Extension	72	45s
Final Extension	72	10Min

Table 5- The volume PCR consumables for 25 microliter

Compounds	Final concentration	In terms of volume µL
Deionized water		10.55
10X PCR Buffer	10µL	1
50mM $Mgcl_2$	3µl	4
10mM dNTP mix	2µL	0.2
Template DNA		5
Primer		3.25
Taq DNA polymerase	5 units/µL	1

In order to check the product of the PCR reaction was used from the agarose gel 1.5% in the presence of TAE electrophoresis buffer 1X. Bands of DNA after ethidium bromide staining the gel with the solution for 15 minutes, was photographed.(Figure 2)Using Excel software related variables Primer in the rows and columns in the name of the genotype, in order to seriously address violations bands, band score in each position as a number one and the lack of the band was considered to be zero, and ultimately was a form of zero and one matrix. The amount of similarity between both two samples using Jacard similarity matrix methods and calculation cluster FIND and dendrogram based on UPGMA drawing algorithm.These calculations were done using the NTSYS software. (Talebi Kuikhy,2007)

RESULTS:

The results related to the investigation of genetic diversity (*F. haussknechtii*) between the regions reviewed on agarose gel (figure2) indicates that: between the seven used primer, primer No. 4 called S4 , was able to Polly morph with all the samples collected to the number 67 bands . Polly morph in the examples column bands is 12 bands. The results indicates that Relive 8 with ten bands most runway with a variety and Rollo 6 the lowest variation indicated and also band 10 most gang presence in the samples (in 1,2,3,4,7,8,9,12,13,15 Relive) and the lowest attendance in the bands 2 and 3 samples (Relive 9).

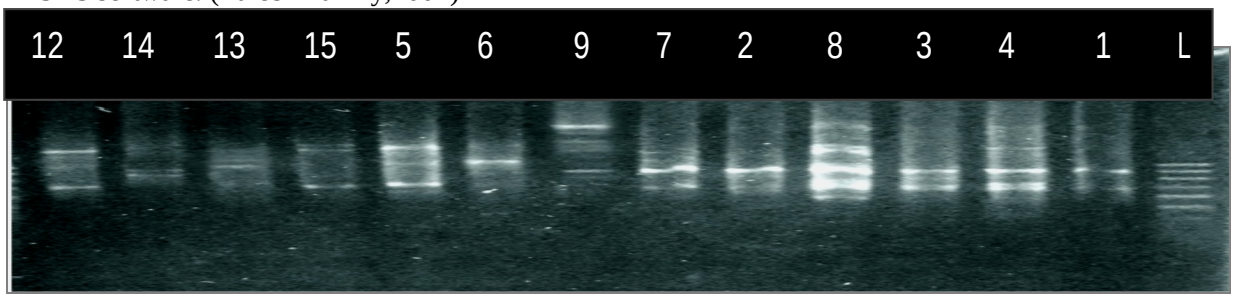

Figure 2-the pattern of gang 13 *Ferulahaussknechtii*using primer S4 indicates the size of the marker and L represent the agar control.

The results of the dendrogram revealed that all specimens are located in 9 cluster.(figure3). If the technical line draws in the range of 0.6 to 0.7 , we will have three cluster so that taken place Rollo 4,8 and 9 in one cluster and Relive 1,2,3,7 and 14 in the second cluster and Relive 5,6,12,13 and 15 in a third cluster. In the first cluster Relive 4,8 showed lowest genetic distances , in the second cluster Relive 2,3 showed lowest genetic distances , in the third cluster Relive 12,13 showed lowest genetic distances. GenerallyRelive 2 with Relive 3 the least genetic distance and Relive 12 with Relive 4 most genetic distance.

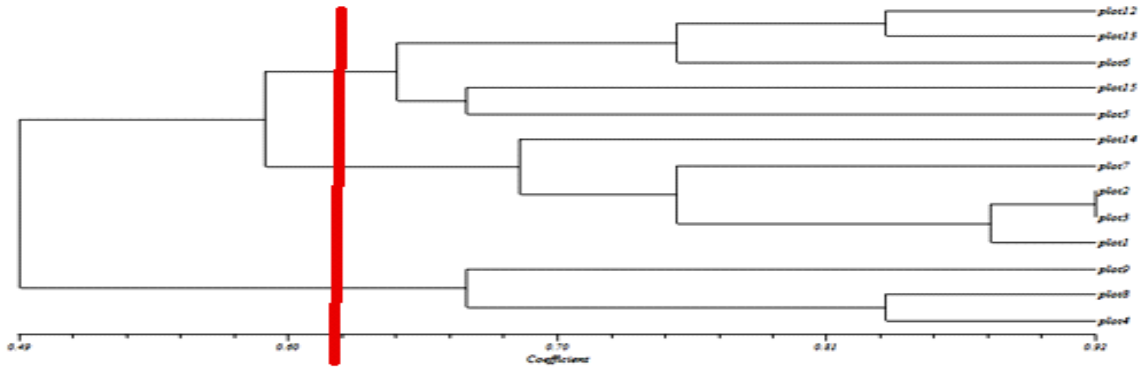

Figure 3- Genetic diversity of the calculated F haussknechtii species based on Jacard index.

A number of Parallels between the pair of samples using the Jacard coefficient in accordance with table 6 shows that the greatest amount of similarity (0.80) between two samples Relive 2 and Relive 3 and the lowest amount of similarity (0.07) between Relive 6 with all the plot.

Table 6 - the amount of Jacard similarity, between the examples (F. haussknechtii) investigated areas

Rows\Cols	plot12	plot14	plot13	plot15	plot5	plot6	plot9	plot7	plot2	plot8	plot3	plot4	plot1
plot12	1												
plot14	0.182540	1											
plot13	0.600000	0.182540	1										
plot15	0.392857	0.182540	0.392857	1									
plot5	0.392857	0.182540	0.392857	0.428571	1								
plot6	0.078571	0.078571	0.078571	0.078571	0.078571	1							
plot9	0.348799	0.182540	0.348799	0.348799	0.348799	0.078571	1						
plot7	0.348799	0.182540	0.348799	0.348799	0.348799	0.078571	0.579293	1					
plot2	0.348799	0.182540	0.348799	0.348799	0.348799	0.078571	0.442857	0.442857	1				
plot8	0.348799	0.182540	0.348799	0.348799	0.348799	0.078571	0.579293	0.683333	0.442857	1			
plot3	0.348799	0.182540	0.348799	0.348799	0.348799	0.078571	0.442857	0.442857	0.800000	0.442857	1		
plot4	0.348799	0.182540	0.348799	0.348799	0.348799	0.078571	0.579293	0.683333	0.442857	0.800000	0.442857	1	
plot1	0.348799	0.182540	0.348799	0.348799	0.348799	0.078571	0.442857	0.442857	0.675000	0.442857	0.675000	0.442857	1

DISCUSSION:

Genetic diversity indicates the differences and diversity of genes within a species. The existence of genetic variation in different populations of the species studied showed adaptation to the environmental conditions and of the most important factors affecting genetic diversity may be the climatic conditions (Mirzaee& et al,2006) topography (Hassani& et al,2007) and soil (Taleshi,2011) . The results of this study indicate the existence of genetic variation among echo type populations of *F.haussknechtii*. According to figure 3 dendogram, it seems that in general the genetic variation among populations of this species on Earth is not match-conditions of the regions and the environmental conditions also affect on it. Graphic dendrogram Figure 3 suggests that Releve 2 and 3 is shown the lowest genetic distance and Releve 12 and Releve 4 is shown the maximum genetic diversity. Given that the genetic dendrogram of the States that are located on a cluster 1,2,3,4,7,8 but Relive 5,6,9 located in another cluster that other relevant climatic conditions and the direction of the slope and elevation and is not only depend on the dirt factor.In north of the range, due to the low radiation, mean intake temperature is less that appears to be increasing the height of the southern slope of the decreased temperature and increased humidity environment. Therefore, the existence of similar climatic conditions in these regions partly

reflects a kind of adaptation of this species population phenotypic correlation. Based on regional observations, it seems that in those geographical areas where the distance is relatively less (Aryehwith Mir-abad) and (Aryehwith bar) and (Bar with Baghiee) the possibility of gene flow rate and migration between pollen and seed in *F.haussknechtii* populations in these areas and provides every year many honeybees beekeepers hive by Aryeh, Baghiee, Mir-abadand Bar and the times of the mountain between the areas that can be pasted into the possibility of exchanging pollen and PCR in the exchange between populations of this species causes genetic variation.

But as the geographic distance is relatively high and the differences inherent in the baggie region with a slight climatic regions of Aryeh and Mir-abador Bar with Mir-abad the possibility to place pollen and seeds dropped and reproductive characteristics in the area of the plant dry phases.

Overall conclusions:

Iran because of Climatic conditions and topography and different characteristics of ecologic has different ecosystems. Using ecological studies and sociology, including the recognition of the characteristics of the vegetation to environmental characteristics, genetic diversity, biodiversity, environmental factors and so on may be the fundamental factor in maintaining, renewing and reviving the natural resources managing. Investigation, identification, preservation and maintenance of plant species, particularly rare and useful species in the world has great importance and is the basis of any sustainable development and appropriate and reasonable utilization of nature and natural resources, and infrastructure preservation and protection of plant species and introduce gene. In recent years, because of the exploitation of non-renewable natural resources of appropriate lubricating and especially soil destruction, rangeland degradation and devastating floods was occurred in the country. Undoubtedly, to prevent damage and degeneration of pastures have been corrected, the best way,is identification of other local plant species, plant communities and regions with similar ecological capability and the conditions of their use in restoration of pastures. Genetic diversity is the most important factor in the survival of organisms including plants resistant to pest and environmental conditions. Awareness of the extent of genetic diversity and genetic relationships of the reserves is one of the primary needs of the modified plant species. Generally, the resulting ecosystem structure and stability in operation of the various components is because of the interaction between the sloppy. In difficult environmental conditions in arid zones, it is important to have this interaction So that the low order of anesthetic Irregularity in an ecosystem component may be fundamental changes in the other component.

In fact in these ecosystems there is balancing biological components of the ability for survivaland conditional limited environmental resources. Partial changes in the physical environment causeschanges in the biological components in plant life and it is completely under the influence of it. The man have intervene in natural balance between ecosystem components and therefore liable to decline it. One of the most important factors that human activities threaten plant life in the community is supposed to be about to agricultural activities, livestock grazing and cropping practice of analgesia of medicinal plants, edible and industrial ranges. since*F.haussknechtii* The pans was not in eastern Iran, it is essential to maintain it in a development program of interest. The medicinal value of pasture and the tagzon can be used as catalyst for the native people in the business areas of interest. Therefore, identification, preservation and development of the diversity in the crowd felt *F. haussknechtii.*

The results of a survey of genetic variation among populations of this species represent the subject, which is part of the genetic diversity of climatic conditions and topography related. The climatic conditions are similar in these regions partly reflects a kind of Phenotypic adaptation (plasticity

among)in the population of this species. There seems to be a genetic distance in some of the samples due to the spatial distance in this example is so that in areas where the lowest geographic distances are possible to accommodate the Exchange was due to pollen and *F.haussknechtii* in the populations between the genomic diversity of genetic causes while in remote areas of the plant and the possibility of exchanging pollen dropped earlier entered the phase of the reproductive characteristics and dried.

REFERENCES:

1. Ahmadi.M, Ghasemkhani.T, Atree.M, Chehregani.A (2014). Taxonomic study focussed on indicator species in the province Tanacetupolycephalum Shultz Bip-floristic and using special station method, the ECO Journal of Plant Physiology. Volume 6, Number 7.
2. Zabeti.S ,Esmailee.A , MaddahArefi.H, Nazarian .F, Mojiri. F (2014). Separation of secondary metabolites in the process of extracting DNA from Thyme and analysis by GC-Mass, (Journal of cellular and molecular (Biology Journal of Iran). published 27, Issue 1.
3. Talebi Kuikhy.A , Mohammad Aliha.M , Naghavi.M(2007). Genetic diversity of Iranian galbanum (Ferula gummosaBoiss.) Using molecular markers RAPD ", Journal of Medicinal and Aromatic Plants Research, 23 (4): 514-522.
4. Taleshi.H ,Akbarinia.M(2011). Woody and herbaceous species diversity in relation to environmental factors in the Paeen Band forests of East Branch , Journal of Biology Iran.published 24, No. 5, pp. 777 -776.
5. 5Ghahramani.A(1975-1990). Color flora of Iran, Volume 20-1, Research Institute of Forests and Rangelands, Tehran.
6. Mandolkanee.B, SeyyedTabatabaee.B, NejatBushehri.A, Ghannadha.M, Omidi.M(2002). The study yielded the wheat genetic diversity relationships ((Triticumaestivum with markers RAPD, Journal of Agriculture Science Volume 34, Issue,pp: 447 -454.
7. Mirzaee.J, Akbarinia.M, Hosseiiny.S.M, Sohrabi. H &Hosseiinzadeh. J,(2006) The (Arghavan ecological forest habitat) in the north of Ilam", Journal of Scientific Research and spruce forests Iran, 14 (4)-pp: 371-381.
8. Mirzaee.J, Akbarinia.M, Hosseiiny.S.M, Sohrabi. H &Hosseiinzadeh. J,(2006) "Herbaceous plant diversity in relation to physiographic factors in forest ecosystems in central Zagros", Iranian Journal of Biology, 20 (4)-pp: 382-375.
9. Mirzaee.J, Akbarinia.M, Hosseiiny.S, Tabaree.M, Jalali.GH (2006) "Comparison of natural breeding density of woody species in relation to physiographic and soil factors in the Zagros forests", Journal of Construction Research, 77: 23-16.
10. Hassani, J. and A. Shahmoradi. (2007). Autecology of *Prangosferulacea* in Kurdistan province". *Rangeland and desert research of Iran*., Vol. 2, No. 14, pp. 171-184.
11. Sharma,P.,Khajuria,A.(2012)." Genomic DNA Extraction From *Ferula jaeschkenana* Vatke (Apiaceae)Optimized for Random Amplified Polymorphic Dna Polymerase Chain Reaction (RAPD-PCR) analysis" .Proceedings of National Academy of Sciences, India, Section B Biological sciences. DOI 10. 1007/s40011-012-0142-x, 83, 3; 341-346.