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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (12): 1947-1955, 2007
ISSN 1028-8880

Determination and Discrimination of Intraspecific Diversity of *Astragalus gossypinus* by Eco-Phytosociological Method from West of Iran

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Abstract: *Astragalus gossypinus* Fischer with wide distribution in Iran belongs to the genus *Astragalus* (Fabaceae). According to existing references and information, individuals of this species are present in many stations with different ecological conditions. This study carried out for determination and discrimination of intraspecific diversity of *Astragalus gossypinus* by Eco-phytosociological method from west of Iran. In this method, the principle of data collecting and analyzing based on floristical composition (as floristical markers) of each Endogenous milieu (as the unit of study in Eco-phytosociological method). In this order, application of Endogenous milieu (special station) for data collecting and then their analyzing permit us only determine existence of inter and intraspecific diversity. Then for determinating kind and level of intraspecific diversity (Ecophene, Chemotype, Cytotype, Ecotype ...), can use other studies such as: morphological, anatomical, phytochemical, cytological and etc. In this survey, 29 special stations were studied and 195 species distinguished as companions for *Astragalus gossypinus*. Then floristic-ecologic data collected from each 29 special stations and analyzed by Anaphyto software (F.C.A, A.H.C, B.O, Marquag methods). Comparison of obtained results on multiple coordinate axes from F.C.A method with results from B.O, Marquag and A.H.C methods led to determination of 7 main groups of Endogenous milieus (special station). Flavonoid analyses were used for determinating kind and level of intraspecific diversity in 7 discriminated groups. Leaves flavonoid components of all collected individuals of *Astragalus gossypinus* were investigated by TLC method. Obtained data from flavonoid survey analyzed by SPSS and MVSP package with WARD and UPGMA methods. Finally, the results of flavonoid studies confirmed the same 7 groups that identified by floristical composition study and showed intraspecific diversity in chemotype level. So according to these results, we can introduce 7 chemotypes for *Astragalus gossypinus* from west of Iran. These chemotypes exist in different stations with various ecological conditions.

Key words: Astragalus, chemotype, endogenous milieu, eco-phytosociological method, flavonoid, floristical composition, Intraspecific diversity

INTRODUCTION

Astragalus L. (Fabaceae) is generally considered the largest genus of vascular plants with an estimated 2500-3000 species (Zarre and Podlech, 1997) and 250 sections (Mehrnia *et al*., 2005). *Astragalus* is widely distributed in temperate regions of the Northern Hemisphere. The greatest number of species are found in the arid, continental regions of Western North American (400 species) and central Asia (2000-2500 species). An additional 150 species are known from temperate South America and one species extends along the East African Mountains to Transvaal, South Africa (Liston and Wheeler, 1994).

The environmental factors and its influence in plant variation (plant diversity) have been extensively studied. Some of these studies include: (Turesson, 1922; Mooney and Billings, 1959; Rejmanek, 1996; Perez-Alonso, 2003; Koch and Bernhardt, 2004; Semmar *et al*., 2005; Telascrea *et al*., 2006).

In mentioned studies did not use a special method in plant specimens collecting process, while for collecting correct and precise floristic-ecologic data, we must apply an appropriate method that be according to factors governing nature and can be used for determination and discrimination existence of inter and intraspecific diversity. In this order, we used the unit of study (Endogenous milieu) in Eco-phytosociological

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method (Atri, 1996, 1999). Endogenous milieu in Eco-phytosociological method is an area of vegetation that is homogenous view point of Floristic-Ecologic. In vegetations study, endogenous milieu determine by physiognomic-floristic-ecologic criteria. Establishment of relieves (stands) carry out randomly in each Endogenous milieus for floristic-ecologic data collecting. Finally, data analyses lead to know plant associations of vegetation study. while for studying inter and intraspecific diversity, an Endogenous milieu (special station) determine base on the presence of individual of studied species in its stations (Atri and Asgari Nematian, 2006). Data collecting in inter and intraspecific diversity study is base on floristical composition in each special station. floristical composition as floristical marker is good marker, because any kind of changing floristical composition in different special stations show existence of different ecological factors in them, that lead to inter and intraspecific diversity. We have done some research by this method (Atri, 1996, 1999; Atri and Asgari Nematian, 2006). Some of these studies include: Distribution of *Triticum boeoticum* ssp., *Thaoudar* and its associates (*Aegilops* ssp.) in Iran (Fakhre-Tabatabaei *et al*., 2000). Contribution for the characterization of *Thymus eriocalyx* chemotypes (Sefidkon *et al*., 2003). Analysis of the essential oil of *Thymus Eriocalyx* from Iran (Kalvandi *et al*., 2004). Essential oil variability of *Thymus eriocalyx* (Ronninger) Jalas (Sefidkon *et al*., 2005). Protein fingerprinting of common wild wheat populations in Iran (Ebrahimzadeh *et al*., 2006). Introduction of the new method for determination and discrimination of inter and intraspecific diversity between different populations of plants (Atri and Asgari Nematian, 2006).

With regard to the wide distribution of *Astragalus gossypinus* in the west of Iran, we wanted to know if there is intraspecific diversity between different individuals of this species in different stations from west of Iran or not? For reach this purpose used the unit of study (special station) in Eco-phytosociological method. The aim of this research is to prove the ability of Eco-phytosociological method for determination and discrimination the existence of inter and intraspecific diversity.

MATERIALS AND METHODS

Plant materials: At the first phase, different stations of *Astragalus gossypinus* were determined in the west of Iran by using the accessible references, Herbaria and existence information. Then we referred to the different stations in study area, along 2004-2006 years, in growth season for collecting floristic-ecologic data. Totally between studied stations, 29 stations selected for investigation in Hamadan, Kermanshah, Kordestan and Markazi provinces from west of Iran (Table 1 and Fig. 1). Data collecting from 29 selected station carried out by using the unit of study in Eco-phytosociological method (Atri, 1996, 1999; Atri and Asgari Nematian, 2006) that is

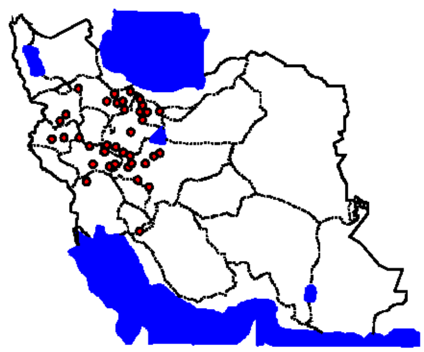

Fig. 1: The distribution map of *Astragalus gossypinus* in Iran

Table 1: The different studied stations for *Astragalus gossypinus* from west of Iran

Populations	Voucher No.	Place of collecting plant species	Altitude
A. gossypinus 9	7318	Hamadan	1764
A. gossypinus 10	7319	Hamadan	1754
A. gossypinus 11	7320	Hamadan	1713
A. gossypinus 12	7321	Hamadan	1692
A.gossypinus 13	7322	Hamadan	1615
A. gossypinus 14	7323	Hamadan	2027
A. gossypinus 15	7324	Hamadan	2015
A. gossypinus 16	7325	Hamadan	1739
A. gossypinus 18	7326	Hamadan	2035
A. gossypinus 19	7327	Hamadan	2103
A. gossypinus 20	7328	Hamadan	1898
A. gossypinus 21	7329	Hamadan	1864
A. gossypinus 22	7330	Hamadan	2033
A. gossypinus 23	7331	Hamadan	2213
A. gossypinus 24	7332	Hamadan	2220
A. gossypinus 25	7333	Kermanshah	1770
A. gossypinus 26	7334	Kermanshah	1800
A. gossypinus 27	7335	Kermanshah	1700
A. gossypinus 28	7336	Kermanshah	1900
A. gossypinus 29	7337	Kermanshah	2000
A. gossypinus 30	7338	Kermanshah	1840
A. gossypinus 32	7339	Kermanshah	1800
A. gossypinus 33	7340	Kermanshah	1700
A. gossypinus 34	7341	Kordestan	1600
A. gossypinus 35	7342	Kordestan	1680
A. gossypinus 36	7343	Kordestan	1620
A. gossypinus 39	7344	Kordestan	1940
A. gossypinus 40	7345	Markazi	2030
A. gossypinus 41	7346	Markazi	2000

named Endogenous milieu (special station). In each station, location of establishment for each relive (stand) determined on base of presence of individual study species. Then for determination of special station of individual study species, minimal area determined by using the area-species method with area-species curve and Cain method (Cain *et al.*, 1959). All ecologic-floristic data (the studied species and its companion species as floristical markers) were collected of each special station. Plant specimens deposited in the Herbarium, of Bu-Ali Sina University in Hamadan, Iran. Studied ecological factors included (elevation, pH, EC, texture of soil, slop direction and slop percent) in each special station.

Flavonoid aglycone study: The plant leaflet of different individuals of *Astragalus gossypinus*, that collected from different special stations in west of Iran, were separated and then ground in a grinder. Flavonoid aglycone analysis was taken on all individuals of *Astragalus gossypinus* listed in (Table 1). Briefly, 2 g dried powder of leaves boiled in 50 mL 2 M HCL for 45 min. Hydrolyzed leaf extracts were allowed to cool to room temperature. The extracts were then washed 3 times with equal volumes of ethyl acetate. The pooled ethyl acetate fractions were evaporated to dryness in a fume hood. The residue of each plant sample was taken up in an equal volume of 95% ethanol (Joseph *et al.*, 2003). The analysis was performed on Silica gel plates 25 Fuelled aluminum CCM (20×20), Gel de silica 60 F_{254} (Merck). Replicate plates were developed in BAW (n-butanol: acetic acid: water, 4:1:5, top layer used). Quercetin, flavone and rutin used as standards. The plates were developed at room temperature in a vertical separating chamber to the height of approximately 14 cm from the start. After drying, visualization was performed by:

- Spraying with 1% methanolic diphenylboryloxyethyl-amine
- and 5% ethanolic polyethyleneglycole 4000

Chromatograms were interpreted in long wave UV light (366 nm), then were measured R_f of each bands (Medica-Saric *et al.*, 2004).

Data analysis: For determination and discrimination of intraspecific diversity of *Astragalus gossypinus* were applied correspondence, cluster, classification and discriminate analysis. Floristical composition data (as floristical marker) analyzed by using Anaphyto software version 95 (Briane, 1995) by means FCA (Factorielle Correspondence Analysis), AHC (Ascendant Hierarchical Classification), BO (Boules Optimisees) and Marquage methods. In studying phytochemical data, once presence and absence of different bands were determined on chromatogram for different individuals of *Astragalus gossypinus*. Then phytochemical data analysis was taken by SPSS (version 10) and MVSP softwares by means PCA, UPGMA and CCA methods. Ecological data analyzed by MVSP software with CCA method.

RESULTS

Floristical results: Obtained results base on floristical composition analyses of 29 special station showed seven main groups by using FCA method (Fig. 2). Group (1) include special station number 0009, group (2) number 0010, group (3) numbers 0039, 0033, 0026, 0025, 0024, 0023, 0022, 0021, 0020, 0019, 0018, 0016, 0015, 0014, 0013, 0012, 0011, group (4) number 0032, group (5) numbers 0041, 0040, 0036, 0034, 0030, group (6) number 0035, group (7) numbers 0029, 0028 0027. It must indicate that the mentioned 7 groups obtained base on similarity and dissimilarity of their floristic composition (as floristical marker). The obtained results from FCA method completed by AHC, BO and Marquage methods (Fig. 3-5). These 7 main groups evidence existence of intraspecific diversity for *Astragalus gossypinus* in study area. As seen in (Fig. 2-5), the obtained groups by FCA method are according to defined groups of AHC, BO and Marquage methods and confirm them.

Flavonoid results: Determination of level and kind of intraspecific diversity were used by flavonoid studies. Prepared chromatograms by TLC method showed different flavonoid bands and also different quantity of bands in different individuals of *Astragalus gossypinus* in study area. Different bands and their R_f measured that their results illustrated in Table 2. Analyzing of flavonoid data separate some groups (Fig. 7). The obtained groups of flavonoid results (Fig. 6 and 7) had a good correlation with floristical composition groups (Fig. 2-5) that confirm them and showed intraspecific diversity in chemotype level. According to these results can introduce seven different kinds of chemotypes for *Astragalus gossypinus* from west of Iran. These 7 chemotypes are different regarding quality, quantity and R_f of flavonoid bands.

Ecological results: Ecological factors data that were collected by applied method analyzed by MVSP software with CCA method. The obtained results showed between

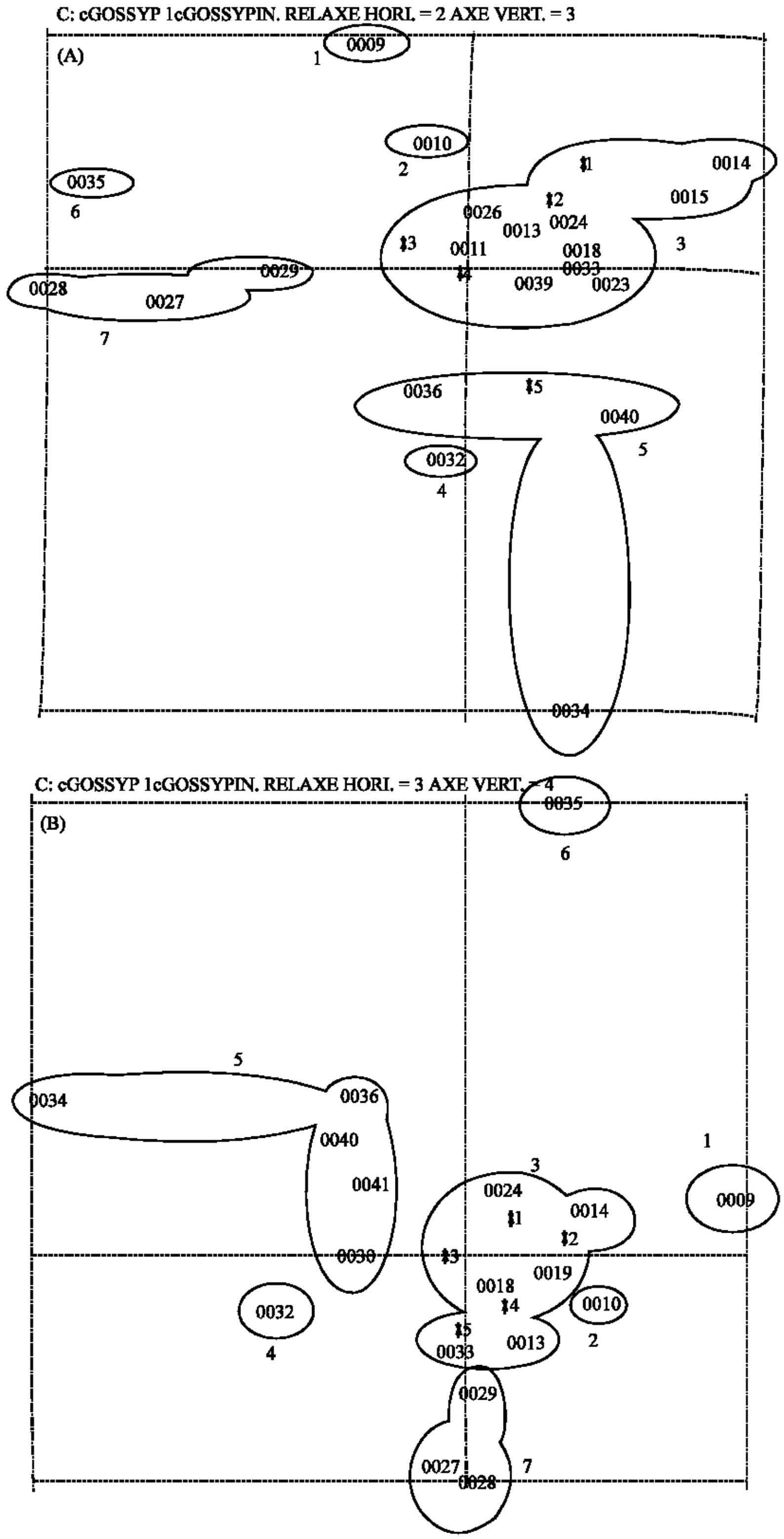

Fig. 2: A and B) Results of floristical composition data analysis by FCA method on 2,3 and 3,4 axes. These 7 groups are the same to grouping resulted by AHC, BO and Marquage methods (Fig. 3-5)

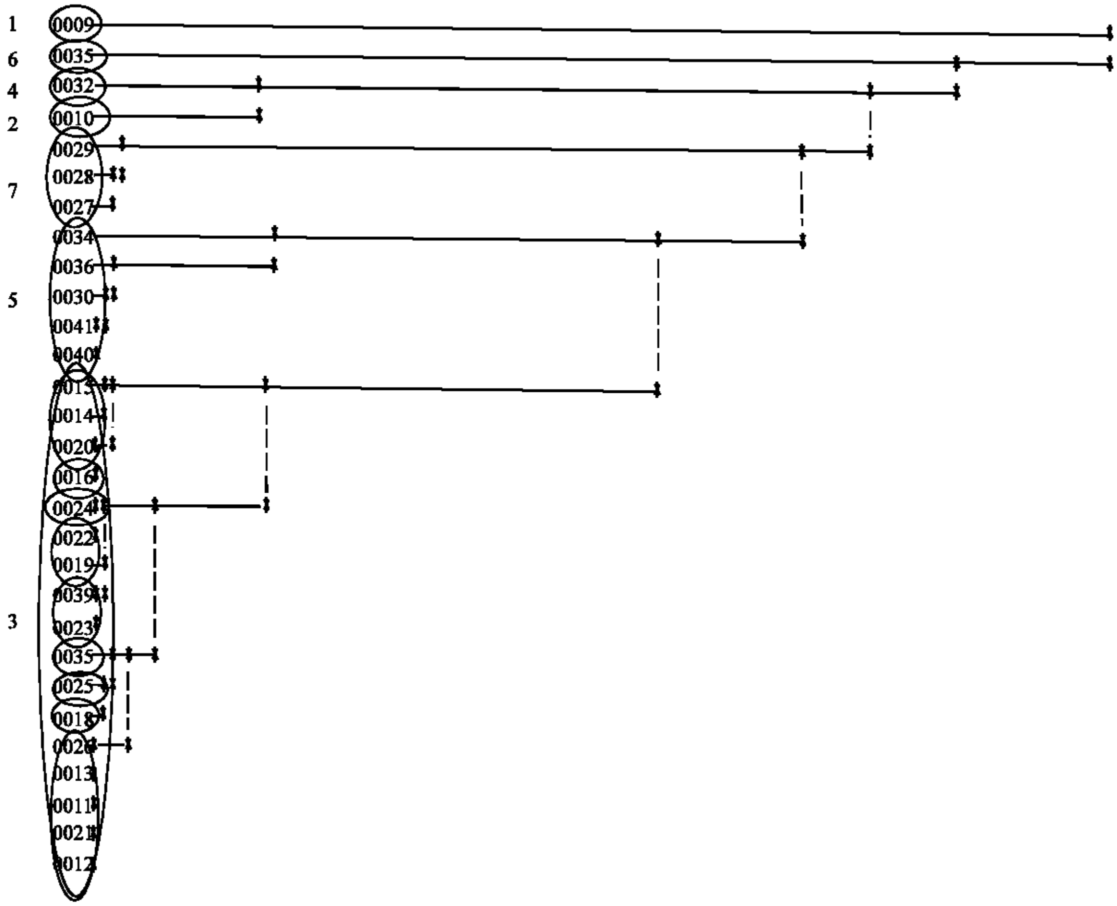

Fig. 3: Results of floristical composition data analysis by AHC method. As seen these groups are the same as groups from FCA

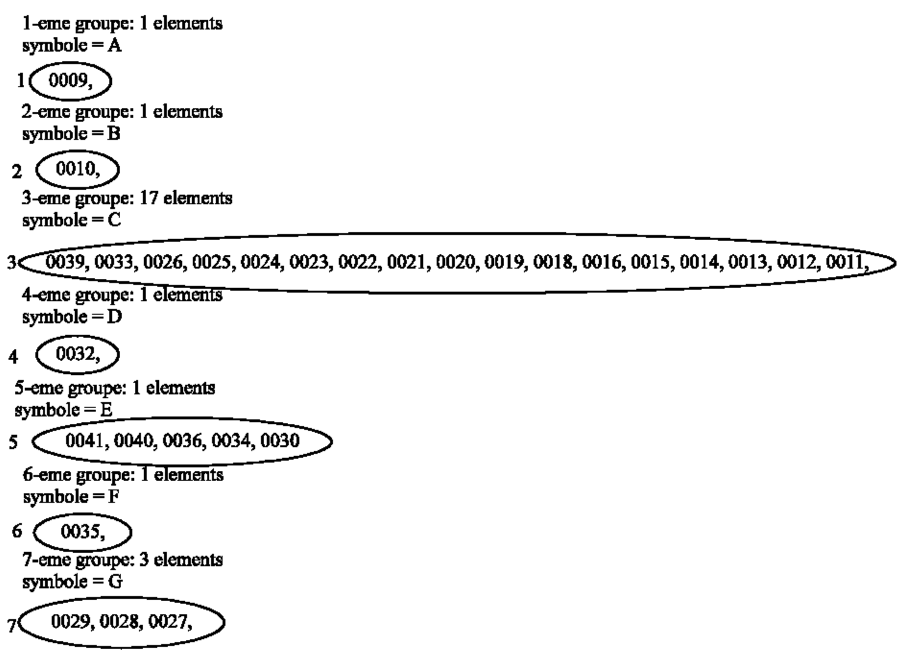

Fig. 4: Results of floristical composition data analysis by BO method, which these seven groups have good correlation with resulted groups of FCA and AHC methods

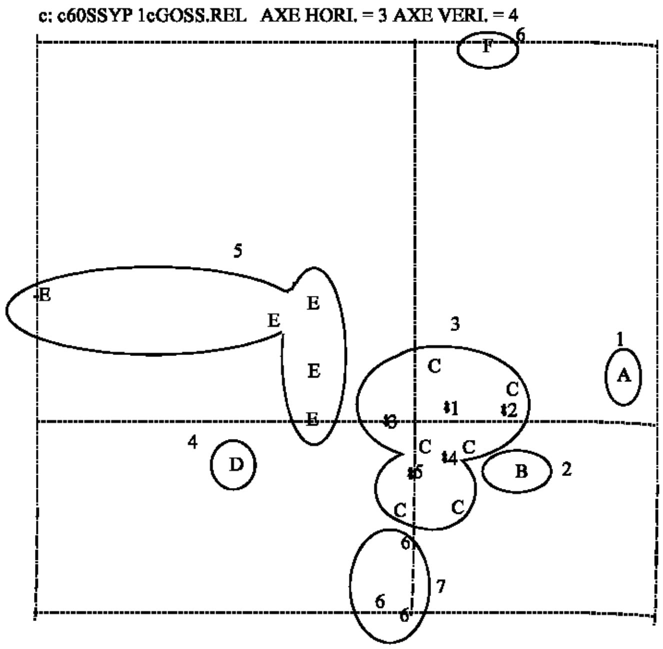

Fig. 5: Results of floristical composition data analysis by Marquage method. Grouping results were confirmed grouping resulted by methods of AHC and FCA and BO

Table 2: The table of Presence and absence flavonoid bands in one dimensional TLC related to different individuals of *A. gossypinus* in the study area

R_f	0009	0010	0011	0012	0013	0014	0015	0016	0018	0019	0020	0021	0022	0023	0024	0025	0026	0027	0028	0029	0030	0032	0033	0034	0035	0036	0039	0040	0041
07.1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
10.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12.8	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
16.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
17.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
21.4	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
22.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0
25.0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0
26.4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31.0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
32.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
35.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0
37.8	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
38.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
42.7	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
42.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
44.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
47.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1
49.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
50.0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
50.7	0	0	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
53.5	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
54.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57.1	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
59.2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
60.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
64.2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
70.0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72.8	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
75.0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
76.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
77.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
77.8	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82.8	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
92.8	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
96.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

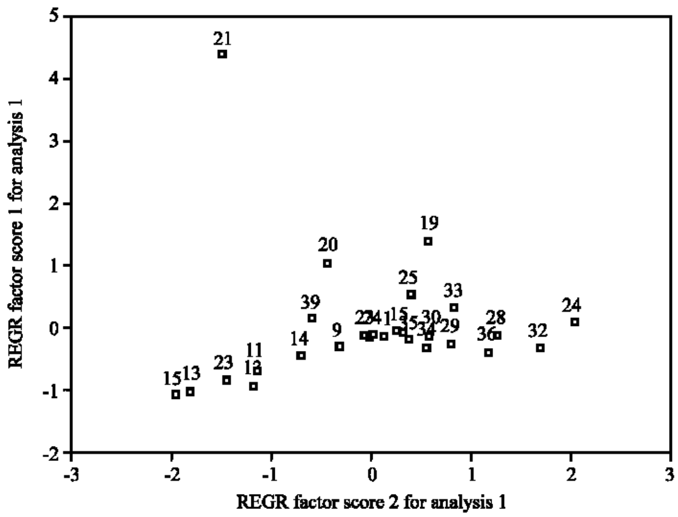

Fig. 6: Flavonoid data analysis of *Astragalus gossypinus* individuals by PCA method

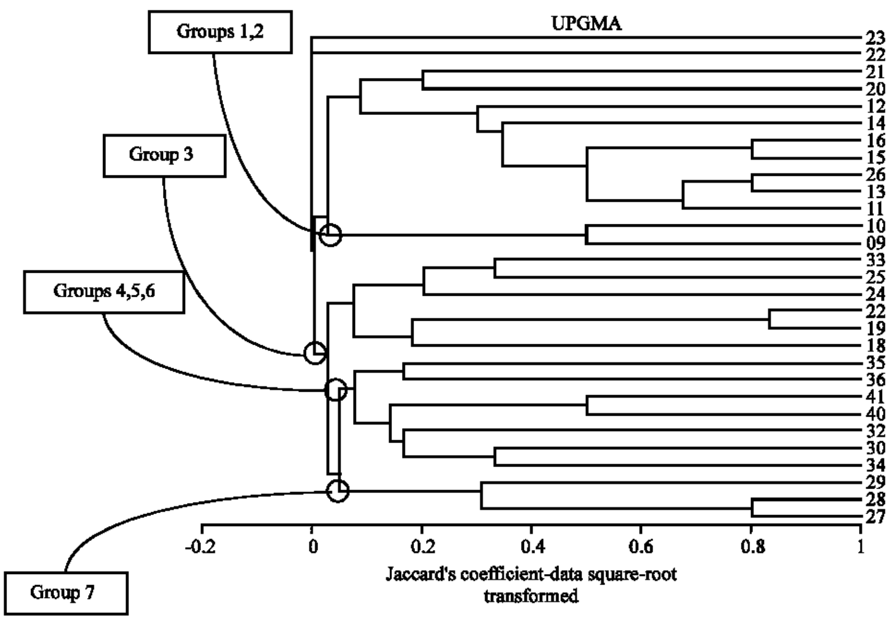

Fig. 7: Resulted cluster of aglycoside flavonoids studies by UPGMA method that has good correlation with floristical composition groupment

studied ecological factors (elevation, pH, EC, texture of soil, slop direction and slop percent) elevation factor has the most important role in separating different determined groups (Fig. 8).

DISCUSSION

Biodiversity-that is result of different ecological factors existence in various stations-shows the biological capacity and ability of each area. One of the most important reserve that led to biodiversity is inter and intraspecific diversity. Creation of inter and intraspecific diversity are the main origin and storage of speciation. In this order, creation inter and intraspecific diversity in different levels cause to richness of taxa in an area. So identification of inter and intraspecific diversity in order to knowing biodiversity is very important. For determination and discrimination of inter and intraspecific

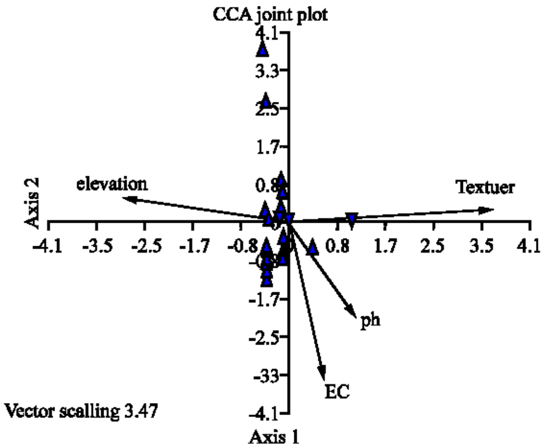

Fig. 8: Results of ecological factors studies by CCA method that show many populations has influenced by elevation factor

diversity, should applicate an suitable method that the obtained results of applied method be correct, precision and spends lower expenses (Atri and Asgari nematian, 2006).

Many studies carried out for determination intraspecific diversity (Turesson, 1922; Mooney and Billings, 1959; Perez-Alonso *et al*., 2003; Koch and Bernhardt, 2004; Semmar *et al*., 2005; Telascrea *et al*., 2006), but most of them do not apply a special method in plant data collecting process and carry out by time consumer and expensive experiments. While, application the floristical marker and the unite of study in Eco-phytosociological method (special station) in this kind of studies led to correct and precision results because that is according to factors governing nature. In the other hand, it prevents of more expenses and time consumer experiments (Fakhre-Tabatabaei *et al*., 2000; Sefidkon *et al*., 2003; Kalvandi *et al*., 2004; Sefidkon *et al*., 2005; Ebrahimzadeh *et al*., 2006; Atri and Asgari Nematian, 2006).

In regard to applied principles in this method for data collecting, we can certainly declare that floristic markers without application other markers can determine intraspecific diversity existence. Then for determinating kind and level of intraspecific diversity (Ecophene, Chemotype, Cytotype, Ecotype ...), between obtained floristical groups, we can use other studies such as: morphology, anatomy, phytochemistry, cytology and etc.

Present results show that *Astragalus gossypinus* has high diversity in the west of Iran. According to our results of floristical analyses, there are 7 distinctive different groups of *Astragalus gossypinus* individuals in study region. At second phase, phytochemical studies create seven kinds of chemotypes which conform and affirm the obtained results of floristical studies. Between studied ecological factors, elevation is the most important ecological factor in creation intraspecific diversity.

So this study and other studies that done base on this method until to now, show the high efficiency of it in determination and discrimination of inter and intraspecific diversity existence. By using this method after determinating floristic groups, we should characterize kind and level of intraspecific diversity only between obtained floristic-ecologic groups and this in require us of testing all of the individuals of studied species and expending long time and much money in this way.

REFERENCES

Atri, M., 1996. A presentation of some aspects of the application of neosigmatiste method in pedology, systematics and chorology. Iran. J. Biol., 2:

Atri, M., 1999. A new concept of ecological factors and their division in vegetation studies. Iran. J. Biol., 8: 61-73.

Atri, M. and M. Asgari Nematian, 2006. Introduction of the new method for determination and discrimination of inter and intraspecific diversity between different populations of plants. Conference on Bioprospecting of Extreme Environment and Extremophile Organisme. Organized by: UNESCO, ISESCO. November, 19-23, 2006.

Briane, J.P., 1995. Cours et TP du traitment des donnees phytosociologique sur microordinateures compatibles IBM-PC. Lab. Sys. Ecol. Veg. Ursay Univ., Paris.

Cain, S.A. O. De and G.M. Castro, 1959. Manual of Vegetation Analysis. Harper and Bros. Publishers, New York, pp: 325.

Ebrahimzadeh, H., M. Atri, A.A. Shahnejat Bushehri, S.M. Fakhre-Tabatabaei and M.R. Naghavi, 2006. Protein fingerprinting of common wild wheat populations in Iran. Cereal Res. Commun. J. (In Press).

Fakhre-Tabatabaei, S.M., M. Atri and Ramakmaasoumi, 2000. Distribution of *Triticum boeoticum* ssp. Thaoudar and its associates (*Aegilops* ssp.) in Iran. Pak. J. Bot., 32: 317-322.

Joseph, O., B. Adil, H. Rebecca, G. Margaret, S. Juliana and W. Neil, 2003. Leaf flavonoids of the cruciferous species, *Camelina sativa*, *Crambe* ssp., *Thiaspi arvense* and several other genera of the family Brassicaceac. Biochem. Syst. Ecol., 31: 1309-1322.

Kalvandi, R., F. Sefidkon, M. Atri and M. Mirza, 2004. Analysis of the essential oil of *Thymus eriocalyx* from Iran: Flavour Fragr. J., 19: 341-343.

Koch, M. and K.G. Bernhardt, 2004. Comparative Biogeography of the cytotypes of annual *Microthlaspi perfoliatum* (Brassicaceae) in Europe using isozymes and cpDNA data: Refugia, diversitr centers and postglacial colonization. Am. J. Bot., 91: 115-124.

Liston, A. and J.A. Wheeler. 1994. The Phylogenetic position of the genous *Astragalus* (Fabaceae) evidence from the chloroplast gene rpoC1 and rpoC2. Syst. Ecol., 22: 377-388.

Medica-Saric, M., I. Jasprica, A. Smolcic-Bubalo and A. Mornar, 2004. Optimization of chromatography of flavonoids and phenolic acids. Croatica Chem. Acta, CCACAA., 77: 361-366.

Mehrnia, M., S.H. Zarre and A. Sokhan Sanj, 2005. Intra and inter specific relation within the *Astragalus microcephalus* complex (Fabaceae), using RAPD. Biochem. Syst. Ecol., 33: 149-4-158.

Mooney, H.A. and W.D. Billings, 1959. Comparative physiological ecology of arctic and alpine populations of *Oxyria digyna*. Ecol. Monogra, 31: 1-29.

Perez-Alonso, M.J., A. Velasco-Negueruela, J. Pala-Paul and J. Sanz, 2003. Variations in the essential oil composition of *Artemisia pedemontana* gathered in Spain: Chemotype camphor-1,8-cineole and chemotype davanone. Biochem. Syst. Ecol., 31: 77-84.

Rejmanek, M., 1996. A theory of seed plant invasiveness: the first sketch. Biol. Conserv., 78: 171-181.

Sefidkon, F., R. Kalvandi, M. Atri and M.M. Barazandeh, 2003. Contribution for the characterization of *Thymus eriocalyx* chemotypes. The International Magazine for Cosmetics and Fragrances.

Sefidkon, F., R. Kalvandi, M. Atri and M.M. Barazandeh, 2005. Essential oil variability of *Thymus eriocalyx* (Ronninger) Jalas. Flavour Fragr. J., 20: 521-524.

Semmar, N., M. Jay, M. Farman and R. Chemli, 2005. Chemotaxonomic analysis of *Astragalus caprinus* (Fabaceae) based on the flavonic patterns. Biochem. Syst. Ecol., 33: 187-200.

Telascrea, M., C.C. Araujo, M.O.M. Marques, R. Facanali, P.L.R. Moraes and A.J. Cavalheiro, 2006. Essential oil from leaves of *Cryptocarya mandioccana* Meisner (Lauraceae): Composition and intraspecific chemical variability. Biochem. Syst. Ecol., 20: 1-11.

Turesson, G., 1922. The species and variety as ecological units, Hereditas, 3: 100-113.

Zarre, S.H. and Podlech, 1997. Problems in the taxonomy of tragacanthic *Astragalus*. Sendtnera, 4: 243-250.