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Application of Floristic Marker in Eco-phytosociology Method for Diagnosing Existing Intra-specific Diversity in Plants: A Case Study of *Astragalus glaucops*

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ABSTRACT

Study was carried out on 11 disperse populations of *Astragalus glaucops* in Alvand mountain. Floristic-ecologic data were collected using the unit of study as mean endogenous milieou by eco-phytosociologic method. Then data obtained from five different groups of releves for this species was analyzed by Anaphyto software with FCA method. Also the study on flavonoids of populations was carried out by thin layer choromatography. Obtained data were analyzed by MVSP software with Ward methods. Five groups resulted from floristic-ecologic study confirmed by flavonoid patterns. Results of floristic analyses showed five distinctive different groups of *A. glaucops* in the study region. Photochemical studies revealed five chemotypes, which confirmed the results of floristic studies. Between studied ecological factors, elevation was the most important ecological factor in creation intraspecific diversity. © 2010 Friends Science Publishers

Key Words: *Astragalus glaucops*; Chemotype; Eco-phytosociology; Flavonoids

INTRODUCTION

The knowledge of the floristic composition of an area is a perquisite for any ecological and phytogeographical studies and conservation management activities. For studying a particular vegetation from an ecological point view, our first step must be to determine the facts as they exist on the ground e.g., vegetation, habitat etc., (Nicholes, 1930). The floristic composition of the vegetation is more susceptible to direct study and exact characterization. In phytosociology determination of individuals association and sampling carry out by using releves and releves with similar floristic composition constitute a plant association. In new method namely eco-phytosociology (Atri, 1996 & 1999) for the determination of plant association endogenous milieus is important. Endogenous milieu determine by physiogenomic-floristic-ecologic criteria.

Establishment of releves (stands) is carried out randomly in each endogenous milieu (special station) for the study of floristic ecotypes (Atri *et al.*, 2007). In each endogenous milieu, there could be one or several releve. Finally data analyses leads to plant associations of vegetation study. Since floristic composition in each environment reflects ecological conditions that influence in plant variation, endogenous milieus with similar floristic composition are in similar environments. For studying inter- and intraspecific diversity by eco-phytosociological method, an endogenous milieu determines the base on the presence of species in its stations (Atri *et al.*, 2007).

Floristic composition as is good floristic marker, because any kind of changing floristic compositions in different endogenous milieu show the existence of different ecological factors; thereby leading to inter-and intraspecific diversity. This has been established in various studies (Fakhre-Tababaei *et al*., 2000; Safidkon *et al*., 2003 & 2005; Kalvandi *et al*., 2004). The application of endogenous milieu for collection and analysis of data permit determine existence of inter and intra-specific diversity. The aim of this project was to study the existence of intra-specific biodiversity in *Astragalus glaucops* with narrow distribution in Alvand Mountain. The determinations were made for the floristic and ecologic diversity and flavonoids accumulation in their populations. The recorded data for each species was used to distinguished intra-specific diversity of the species separately.

MATERIALS AND METHODS

Different stations of *A. glaucops* were determined by using the accessible reference, herbarium and available information. Studies show that *A. glaucops* has narrow distribution in Iran and is endemic. This species is present in many habitats with different ecological conditions in Alvand Mountain; one of the important mountain chains in Iran.

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Alvand region is located in the west of Iran, and Hamedan, Asad abad and Touyserkan cities surround it. This region is located between western longitudes 48° 10′ to 48° 40′and northern latitudes 34° 30′ to 34°50′. The precipitation ranges between 206.1 and 420.7 mm, respectively. Its maximal altitude is 3428 m. Different endogenous milieu of *A. glaucops* were determined in the study area. In each endogenous milieu, location of establishment for each releve established on base of presence of individual studied species, minimal area determined by using the area-species method with area-species curve.

All Floristic–ecologic data (the study species & companion species as floristic marker) were collected from each endogenous milieu (each releve representing endogenous milieu). Study of ecological factors included elevation, EC, texture of soil and pH in each releve. Total of 11 endogenous milieu were selected for investigation in study area. The plant leaflets of different individual of *A. glaucops* were collected in each releve, separated and ground in a grinder. Flavonoids aglycone extracted (Joseph *et al*., 2003) and flavonoid aglycone analysis was taken on all individuals *A. glaucops* (Table I) using TLC method. The analysis was performed on Silica gel plates 25 Fuelled aluminum CCM (20×20), Gel de silica 60 F254 (Merck). Then measured Rf of each band on chromograms (Medica-Saric *et al*., 2004).

Data obtain from floristic were analyzed by using Anaphyto software (Briane, 1995) by FCA (Factorielle Correspondence Analysis). Ecologic and flavonoids data were analyzed by MVSP software by means of Ward and CCA methods.

RESULTS

Results based on floristic composition analyses of 11 endogenous milieu showed five groups by using FCA method (Fig. 1), based on similarity and dissimilarity of floristic composition (as floristic marker). These groups indicated the existence of intraspecific diversity in *A. glaucops* in the study area. As for flavonoids diversity, TLC showed different bands and also different quantity of bonds in different individuals of *A. glaucops* in study area. Analysis of flavonoid data separated some groups (Fig. 2). The obtained groups of flavonoids had a good correlation with floristical composition groups that confirmed intra-specific diversity at biochemical level. Among ecological factors, elevation had the most important role in the distribution and diversity of species (Fig. 3).

DISCUSSION

The environmental factors and its influence in plant variation (plant diversity) have been extensively studied (Semmar *et al.*, 2005; Telascrea *et al*., 2006). For determination and discrimination of inter- and intra-specific diversity, use of suitable methods is important to obtain

Fig. 1: Results of floristical composition data analysis by FCA method on1, axes

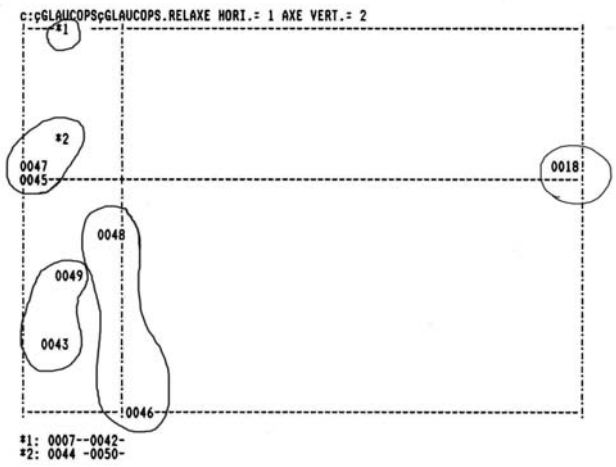


Fig. 2: Resulted cluster of aglycoside flavonoids studies of *Astragalus glaucops* individuals by Ward method

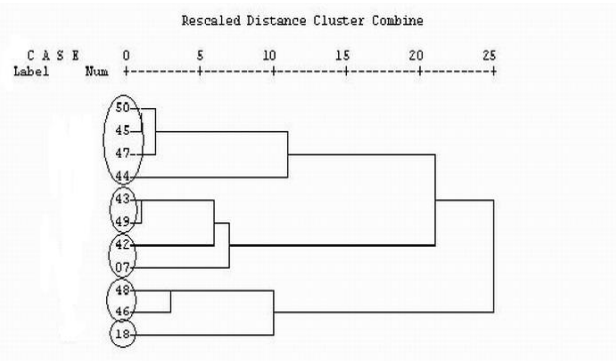


Fig. 3: Results of ecological factors studies by CCA method that show many populations has influenced by elevation factor

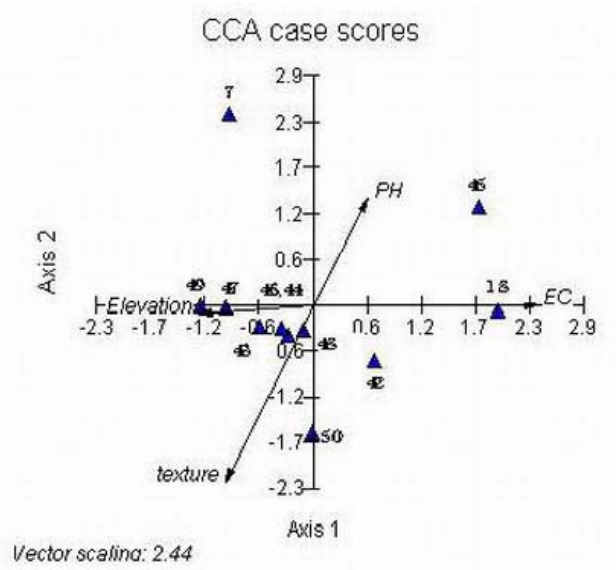
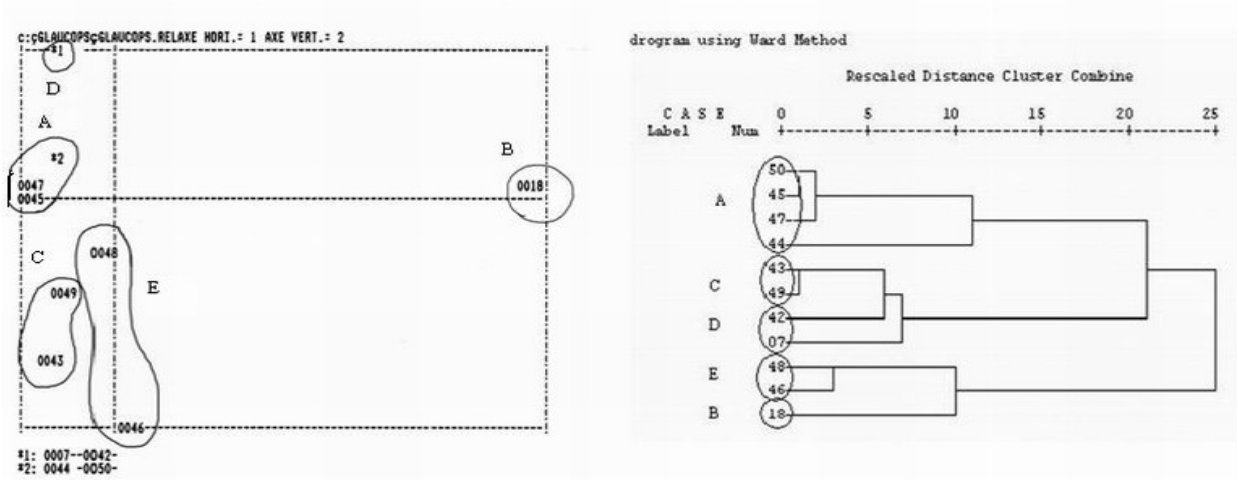


Fig. 4: Conformity of aglycoside flavonoids and floristic studies that has good correlation with other



correct and precise results (Atri *et al*., 2006). Use of floristic marker in eco-phytosociological method (endogenous milieu) in this study led to correct and precise results. Other similar studies in this field are also available (Fakhre-Tabatabaei *et al.*, 2000; Sefidkon *et al.,* 2003 & 2005; Atri *et al.,* 2007). From these findings, it can be certainly declared that floristic markers alone can determine the existence of intra-specific diversity.

For determining the kind and level of intra-specific diversity (ecophene, chemotype, cytotype, ecotype etc.) in the floristic groups, we can use other studies such as morphology, anatomy, phytochemistry, cytology and etc. Some studies that done base on this method until to now, show the high efficiency of it in determination and discrimination of inter and intra-specific diversity existence (add references). By using this method after determining floristic groups we may characterize kind and level intra-specific diversity only between floristic-ecologic groups and this in require us of testing all of the individuals of studied species. According to our results of floristic analyses there are five distinctive different groups of *A. glaucops* in the study region. At second phase, data showed different flavonoid bands and also different quantity of bands in different individuals of A. *glaucops* in study area.

Analyzing of flavonoid data revealed that the obtained groups of flavonoid results had a good correlation with floristical composition groups that confirm them and showed intraspecific diversity in chemotype level. The determination of flavonoids created five chemotypes. These five chemotypes were different regarding quality, quantity and R_f of flavonoid bands, which conform and affirm the obtained results of floristic studies (Fig. 4). The present study shows that in studying the vegetation and determining ecological factors, employing ecological and phytosociologic criteria as eco-phytosociology (Atri, 1996) are not only suitable and exact in the data collection stage to determine the placement of releves, but also it is able to provide results, which conform and agree to the rules that govern the nature in the analysis and result interpretation stage. The phenomena such as interactions, substitution, stenoece and euryece nature of species and existence of intra-specific and inter-specific relations, consideration of the ecological factors as the base and pillar by focusing on one or a number of predetermined ecological factors to study vegetation, could not express the existing reality in all times.

Table I: The different studies special stations for *Astragalus glaucops* populations in Alvand Mountain

Releve No.	Voucher No.	Altitude
7	7307	2563
18	7308	2300
42	7312	2540
43	7310	2536
44	7313	2547
45	7309	2600
46	7311	2650
47	7314	2660
48	7315	2723
49	7316	2570
50	7317	2598

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