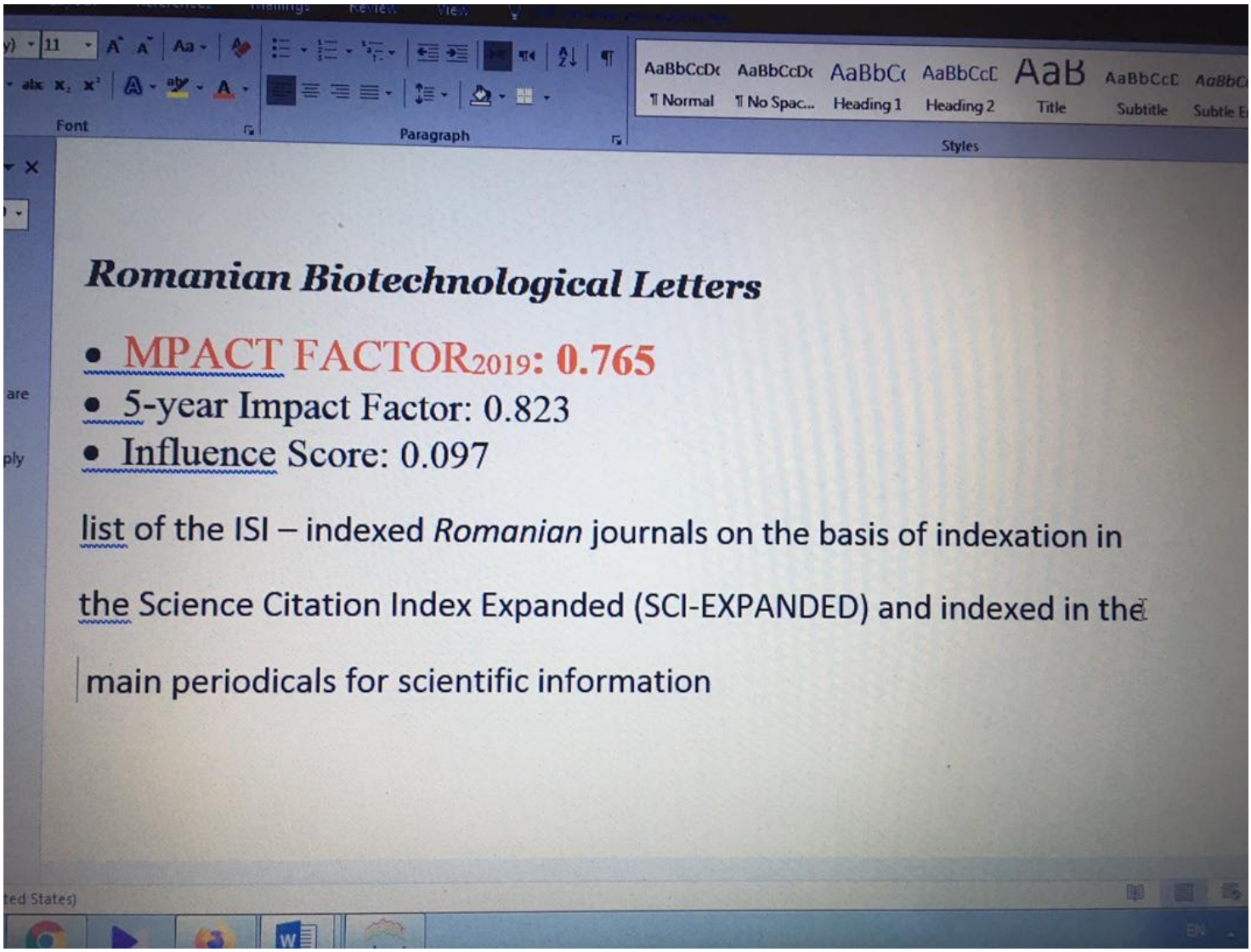
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Effects Of Eco-Geographical Factors on Genetic Diversity In *Elymus tauri* (Boiss and Balansa) Populations Using RAPD Markers

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AKBAR ABDIGAZIJAHANI[1], NAIB KHALIQ AMINOV[2], AHMAD RAZBAN HAGHIGHI[3]*, MAHMOUD MAJIDAZAR[4]
[1] *Ph.D. student at Azarbaijan National Academy of Sciences,Genetic Resources Institute, Baku,Republic of Azarbaijan*
[2] *National Academy of Sciences,Genetic Resources Institute,Baku,Republic of Azarbai*
[3] *Research Center of Agriculture and Natural Resources,Tabriz,East Azarbaijan,Iran*
[4] *Faculty Of Applied Scientific Institute of Ministry of Agriculture Jihad*
**** Corresponding author***:**Ahmad Razban Haghighi**, *Research Center of Agriculture and Natural Resources, Tabriz, East Azarbaijan, Iran, E-mail: a_razban_h@yahoo.com, Tel: +989141166390*

Abstract

In this research, eco-geographical effects on genetic diversity of Elymus tauri populations from northwestern of Iran were investigated by utilization of RAPD markers. The genetic diversity within and among populations were estimated by using Analysis of Molecular Variance ***(AMOVA)****. The* **AMOVA** *analysis based on eco-geographical regions revealed that the inter-regions genetic diversity was 13%, whereas, the intra-region genetic diversity was 87%. The lower genetic diversity and also lower level of polymorphisms in Mishow region compared to Sabalan region, are due to lower altitude, and also early grazing and overgrazing.*

Keywords: Eco-Geographical factors, *Elymus tauri*, Genetic Diversity, Polymorphism, RAPD markers

1. Introduction

Different species of *Elymus* genus grows in most of pastures in Iran. Eleven species of genus *Elymus* were reported from Iran, which grow in cold and temperate steep zones [24]. They are almost distributed in over north, northwest, and central regions of Iran. The most species of this genus are heterozygous, polyploid, and out-fertile plants, but they are not completely cross fertile [19, 25]. At the same time some of them; such as slender wheatgrass, are self pollinated species (4). *Elymus* species are resistant against abiotic stresses, rust and other pathogens [4]. *Elymus tauri* (Boiss and Balansa) is a perennial plant which has a long and strong root and resistant to overgrazing. Additionally, they have tolerance to cold and drought conditions..This species is very valuable among pasture plants due to its palatability and also special traits. So, understanding its genetic structure and selection of its superior populations could be an effective approach towards increasing qualitative and quantitative forage in pastures. This species usually grows on poor soils of steep slope foothills, rock filled mountains, and flat areas on top of hills. The eco-geographical factors are among effective factors in structure and genetic diversity of populations [3, 18]. Accidental fluctuations of ambient conditions such as fluctuations in temperature and precipitation have considerable effects on reproduction and survival rate of populations [3, 18]. The positive overlap and feedback of demographic and genetic factors may lead to lower genetic diversity, population size and eventually extinction [11]. Researchers have noticed a positive correlate-ion between

traits related to fitness and heterozygosis in some species [15, 21]. Plant species diversity represents a positive correlation with geographical dispersion (13). Plants resistance against stresses and their compatibility to habitats changes have an effective role in preservation and survival of plant populations [17, 23]. Even though the reproductive system, type and mode of pollination (Self fertility and Cross fertility, insects and animals) and human activities are the contributing factors affecting genetic diversity. The role of eco-geographical factors (elevation, slope, topography and latitude) and climate conditions (wind, temperature, humidity and precipitation) should not be overlooked, because eco-geographical and biological factors are linked together [22]. In fact, factors leading to lower genetic diversity have synergic effect on each other. Sudden changes in climatic conditions such as sudden warming up or cooling down, frost or freezing and acid rain are bio-environmental factors causing lower genetic diversity. Decreased genetic diversity leads to lower survivability, lower compatibility to environmental changes, which will eventually end up in extinction of species [6, 12]. Climatic changes may cause changes in phenology, reproduction system, pollination, pollen and seed dispersal [2]. The reproductive system, in short term, may also affect diversity patterns directly [5, 7]. The pollination system itself, directly and also indirectly, causes lower genetic diversity. Self fertilization increases the frequency of homozygous in short term, but in longer term may cause lower effective population. Study of genetic diversity pattern, effect of eco-geographical and regional factors in populations are necessary in comprehending evolution and management approach of conservation programs [8]. The purpose of this study was to investigate the effects of eco-geographical factors on genetic diversity within and among populations of *Elymus tauri* in Mishow and Sabalan regions using RAPD molecular markers.

2. Materials and Methods

The seeds of ten native populations were collected from two different eco-geographical zones (six from Mishow region and four from Sabalan region) (Fig1). We have used sees as a material for all genetic analysis. DNA extraction was carried out using seedlings, Madden method (2002) with minor modifications [16]. Twenty RAPD primers were tested (CinnaGen Company, Iran) but only five primers showed the highest and sharp polymorphic bands, which were chosen for further analysis (Table1). The PCR reaction was activated based on the William standard protocol (1990) with minor modifications [26]. The PCR reaction was included RAPD primer 1 µl, template DNA (30 ng) 1 µl, double distilled water 10 µl, and Master Mix (CinaGen PCR Masterkit.Cat.No.PR8250C) 13 µl. All PCR reactions were done on Quanta Biotech thermal cycler machine. The amplification condition was composed of a Quanta Biotech thermal cycler based on the following conditions.

1- The initial denaturation at 95°C for 4 min.

2- The denaturation at 94°C for 1 min. Annealing at 37°C for 2 min and the extension at 72°C for 1 min (followed by 40 cycles).

3- The final extension at 72°C for 10 min.

Amplified bands were segregated in 1.5% agarose gel. The segregated bands were visualized using 0.5% µgr/ml Ethidium Bromide solution under ultraviolet light. The electrophoresis set was adjusted on 120 volts. The electrophoresis was carried out for 50 minutes, and finally visualized using ultraviolet trans-laminator. The resultant bands were constructed as binary matrix by giving 0 and 1 for absence and presence bands (Fig2). Genetic diversity within and between population was carried out using AMOVA analysis as implemented in GenAlex 6.41

package [10]. The Nei genetic diversity and Shannon index were calculated using GenAlex 6.41[10].

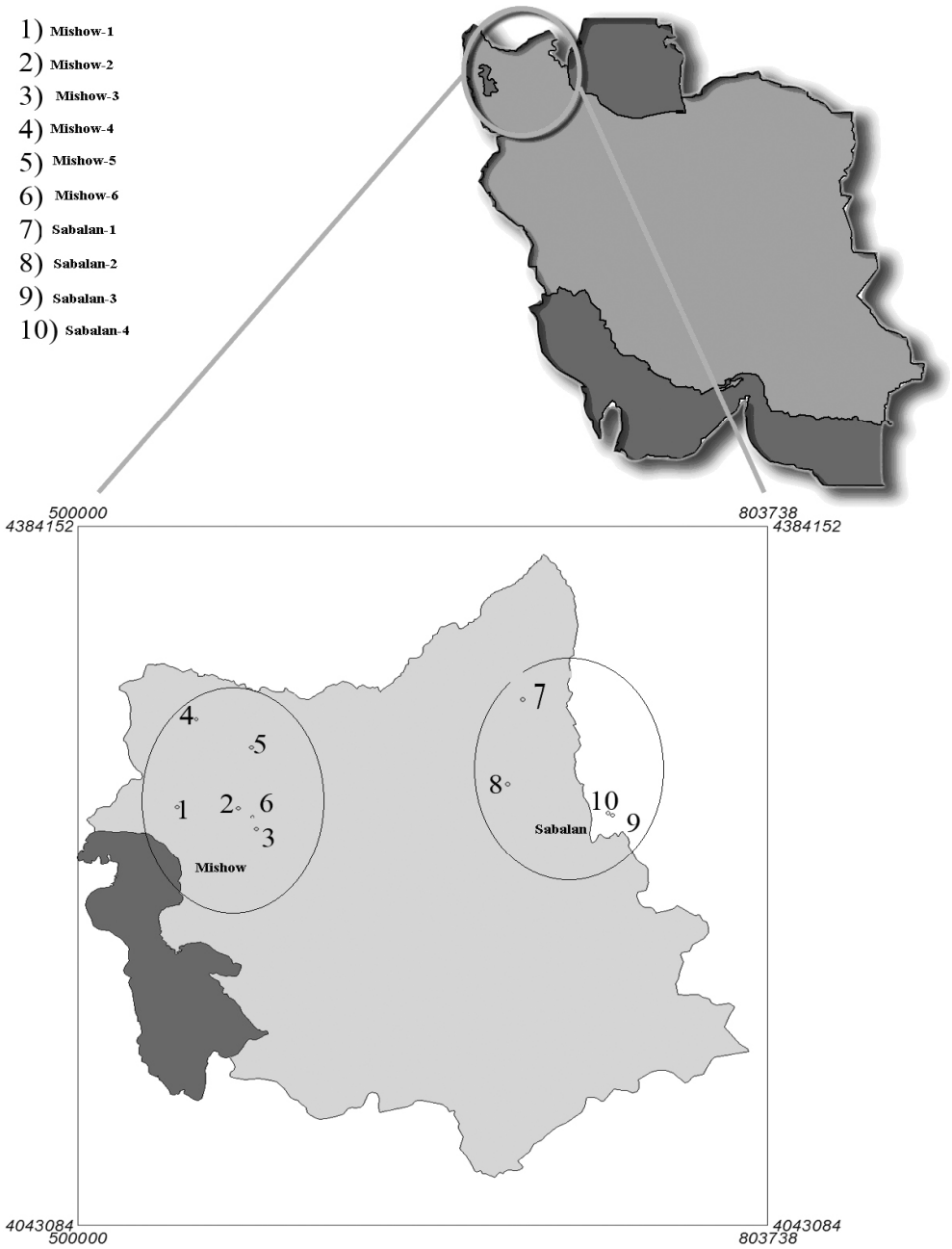

Figure 1. Sampling locations of *Eylmus tauri* populations in northwest of Iran

Table 1. Specifications of the codes and sequence of primers

Primer cod	Primer sequence($5'$-$3'$)
E-17	CCTGGGTCCA
E-18	GGTGGCGGGA
E-22	GGGCCGTTTA
E-23	ACAGGGCTCA
E-26	CCGGCCTTAG

Figure 2. Electrophoresis of the resultant bands from primer E-22 for 3 populations for Mishow region and for 3 populations for Sabalan region

3. Results and discussions

The five randomly used RAPD primers produced 55 sharp and polymorphic bands. The average number of bands in Sabalan region was 43.25, while this was 40 in Mishow region. The average polymorphism in Sabalan region was 93.73%, while for Mishow 89.90%. The average altitude in Sabalan region was 1650m, whereas, this was 1550m in Mishow region. Due to this reason, the average genetic diversity index in Sabalan region was higher than Mishow region (h=0.17, I=0.23 vs. h=0.13, I=0.2) (Table 2). The reason for lower genetic diversity and lower polymorphism in Mishow region are due to the lower altitude, early grazing and overgrazing (9). In addition to, *Elymus tauri* is a palatable pasture fodder for livestock, which are usually over-utilized. In order to evaluate the effect of the eco-geographical regions on genetic diversity, the AMOVA analysis was performed. The genetic diversity for inter-regions was 13%, while the intra-region diversity was 87%.The obtained diversities were significant with 1% probability (Table3). This research showed that the eco-geographical conditions of Mishow and Sabalan regions were different and affected the level of population diversity of *Elymus tauri* (Boiss & Balansa). These differences can also be due to the evolution potentials (23). The AMOVA analysis based on eco-geographical regions have clearly showed the existence of the same genetic differences (between region genetic diversity of 13% and within genetic diversity of 87%). The existence of large geographical distance among populations of the two regions of Mishow and Sabalan (about 150 kilometers) confirmed the obtained results. Without considering this population, the average altitude above sea level of the Sabalan populations is 1750 meters, which is in fact 180 meters higher than Mishow populations. So, the populations located in Mishow region have been prone to changes due to ease of access, early grazing, overgrazing of livestock and increased human activities, leading to lower genetic diversity. Over-utilization of Mishow region has also led to

lower seed production, reproduction and elimination of recombination, which in turn limited the population expansion [9]. This must also be stated that the above mentioned factors have taken place in recent years and subsequently led, changes in populations. The results of this study emphasizes the existing genetic differences of *Elymus tauri* populations inhabiting in Mishow region which harbours have lower genetic diversity. Therefore, it is suggested to perform management policies in order to preserve gene pool in this region.

Table 2. Polymorphic RAPDs percentage, Alitude, Shannon and Nei's genetic diversity in Mishow and Sabalan populations of *Elymus tauri* in two different regions in East Azerbaijan, Iran.

Region	Altitude	Nei index(h)	Shanon index(I)	Number of band	Polymorphism
Mishow	1550	0.131	0.198	40	89.09%
Sabalan	1650	0.169	0.226	43.25	92.73%

Table 3. Analysis of molecular variance (AMOVA) in *Elymus tauri* (Boiss & Balansa) based on eco-geographical regions of Mishow and Sabalan

S.O.V	d.f	SS	MS	Variance estimate	Percentage
Between region	1	75.71	75.71	1.38	13%**
Within region	98	903.09	9.21	9.21	87%**
Total	99	978.8	-	10.6	100

4. Conclusions

The present study showed that the eco-geographical factors have significant effect on genetic diversity of *Elymus tauri* populations in Mishow and Sabalan regions in northwestern of Iran. The lower genetic differences among populations in Misho region compared to Sabalan region represents a potential changes in populations of Mishow region over the recent years. The populations in Mishow region have undergone changes due to eco-geographical conditions, lower altitude, increased un-economical utilization, and expansion of human activities, which in turn would lead to decrease in the amount of genetic diversity.

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