Biochemical study of some populations of *Gundelia tournefortii* L. in West Azarbaijan

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Abstract

Some populations of *Gundelia tournefortii* were compared based on chemotaxonomical characteristics. In this study, some biochemical compounds such as total protein and storage proteins of shoot were studied. The populations were compared on the basis of biochemical characteristics using statistical methods. Different populations were collected from mountains of west Azarbaijan province. Total protein content was assayed through Lowry method and storage proteins content was determined by through electrophoresis via silver nitrate staining method. The results of electrophoresis showed that populations could be classified in 3 clusters. Orumieh, Ghushchi and Shahindej populations were included in cluster 1. Also cluster 2 comprised population of Bukan and the remaining populations including Mahabad and Takab were classified in cluster 3. The results of total protein showed the maximum total protein in Shahindej population (739 mg/100 g) and minimum total protein in Takab population (465 mg/100g).

Keywords: SDS-PAGE; storage proteins of shoot; total protein; silver nitrate staining

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Introduction

Gundelia tournefortii L. (Compositea) is a medicinal plant, native to the Asian temperate zones of Western Asia, namely Cyperus, Egypt, Iran, Israel, Turkey, Azarbaijan and Turkmenistan. *G. tournefortii* locally known as 'Kangar' in Iran is found as a wild herb growing during late winter and early spring in the hills in the western and southern parts of Iran as an occasional food in different forms and also as a folk remedy. In this study, based on chemotaxonomical characteristics, 6 populations of *G. tournefortii* were collected from mountains of West Azarbaijan and compared. These included Orumieh, Ghushchi, Shahindej, Bukan, Mahabad and Takab populations. Several biochemical methods, mostly electrophoresis, have been used to estimate the genetic diversity among different plant species (Hames and Richwood, 1990). Estimating the genetic diversity has been used in several approaches, such as phylogenetic reconstruction (Fatokun et al., 1992; Kaga et al., 1996; Manghan et al., 1996), plant breeding and relationships between agricultural varieties (Sakar and Bose, 1984; Menancio et al., 1993; Kaja et al., 1999), linkage maps, and identification of markers connected with resistance genes against pests and diseases.

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Materials and Methods

Total protein assay

Folin Lowry method was used for detection of proteins. This method is based on protein hydrolysis and release of the amino acids using Folinciocalteo and then the resulting color is assayed by spectrophotometer. For each population, 0.07 g of shoot was weighed. It was then ground with mortar and pestle and 5 ml Tris-HCl buffer (50 ml Tris 0.2N, 26.08 ml Hcl, 17.2 g sucrose, 1 g ascorbic acid) was added to it. 100 ml solution was obtained by adding sterile water. The solution was then blended and put in the centrifuge 5000 g for 30 minutes. For each case, 1 ml of upper phase was taken up and 4 ml of. After 10 minutes, 1.5 ml Folin solution was added to each case and they were put in dark for 30 minutes. Then by means of a spectrophotometer (Biowave, S2100 Diode Array, UK) absorbance of each case was recorded at 660 nm. Standard curve was depicted and total protein was calculated.

SDS-PAGE

Protein extraction

For analysis of protein 0.07 g shoot of the plant was weighed for each population and ground with mortar and pestle. Protein content was then extracted with 100 ml extraction buffer (0.09M Tris, 0.08 M Boric acid, 0.93 g/l Na_2EDTA). Samples were then centrifuged at 5000 g for 30 minutes (the volume of the solution was adjusted to 100 ml). After centrifuge, supernatant solution which contained protein, was mixed with the equal volume of lammeli solution (1.25 ml Tris pH=6.8, 0.4 g SDS, 0.8 ml glycerol, 0.9 ml 2-mercaptpetanol, 0.1 ml blue bromophenol 0.5%) and was put in bain-marie for 2 minutes to denaturize its proteins. The extraction solutions were kept in refrigerator for electrophoresis.

Gel preparation and electrophoresis

Running and stacking gels were prepared with different concentrations of the same constituents, i.e., acrylamide, bisacrylamide, Sodium dodecyle sulfate, Tris-HCl, strilled water and TEMED. Electrophoresis was performed based on Lammeli metod (Creighton, T.1997)

Silver nitrate staining

Silver staining is one of the procedures that are available for detecting proteins separated by gel electrophoresis. Switzer et al. (1979) first *introduced* silver staining, a technique that today provides a very sensitive tool for protein visualization with a detection level down to the 0.3-10.

Preparing of solutions

Fixation solution

The fixation solution consisted of 50% ethanol (or methanol), 12% acetic acid and 0.05% formalin.

Washing solution

Washing solution consisted of 20% ethanol (or methanol).

Sensitizing solution

Sensitizing solution comprised 0.02% (w/v) sodium thiosulfate ($Na_2S_2O_3$).

Staining solution

Staining solution consisted of 0.2% (w/v) silver nitrate ($AgNO_3$) and 0.076% formalin make this solution.

Developing solution

Developing solution consisted of 6% (w/v) sodium carbonate (Na_2CO_3), 0.0004% (w/v) sodium thiosulfate ($Na_2S_2O_3$) and 0.05% formalin are particles of the solution.

Terminating solution

Terminating solution was made with 12% acetic acid.

Drying solution

Drying solution was made with 20% ethanol.

Staining and destaining

After electrophoresis, the gel was removed from the cassette and placed into a tray containing appropriate volume of fixing solution. After 2 hours fixative solution was discarded and the gel was washed in 20% ethanol for 20 min. Then ethanol solution was discarded and sensitizing solution was added. After 2 hours, the sensitizing solution discarded and the gel was drown in deionized water twice, each time1 minutes. The water was then discarded. The cold silver staining solution was added and shaken for 20 min. When staining was completed, the gel rinsed with a large volume of deionized water for 20-60 seconds. Then it was shortly rinsed with the developing solution. Then, the solution was discarded. New portion of the developing solution was added and the protein image was developed by incubation of the gel in 300 ml of developing solution for 2 - 5 min. The reduction reaction was stopped by adding 50 ml of terminating solution.

Results

SDS-PAGE

The results obtained from SDS-PAG electrophoresis (Fig. I) showed that the method provides a powerful tool for reliable population identification based on genetic differences in shoot protein composition among different populations of *Gundeliatournefortii.* Population identification was possible in all samples using SDS-PAGE electrophoresis of shoot proteins. The populations 5 (Mahabad), and 6 (Takab) showed the most (16) and populations 1 (Orumieh) and 2 (Ghuschi) showed the least (9) protein bands. The obtained dendrogram classified the populations into 4 classes (Fig. II): class I included the populations 1 and 2 (Orumieh, Ghushchi, respectively); class 2 included solely population 3 (Shahindej), class 3 included population 4 (Bukan) and class 4 included populations (Mahabad and Takab, respectively). Class 1 populations showed

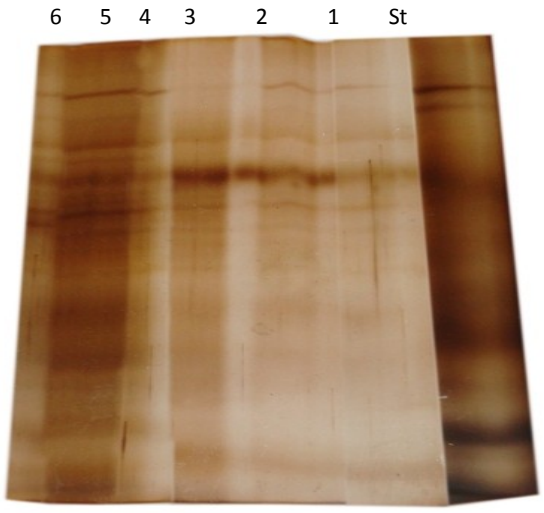

Fig I. Shoot protein banding patterns of *Gundelia tournefortii* populations. Numbers at top of the columns indicate the populations: 1- Orumieh, 2- Ghushchi, 3- Shahindej, 4- Bukan, 5- Mahabad, 6- Takab. St: Standard proteins.

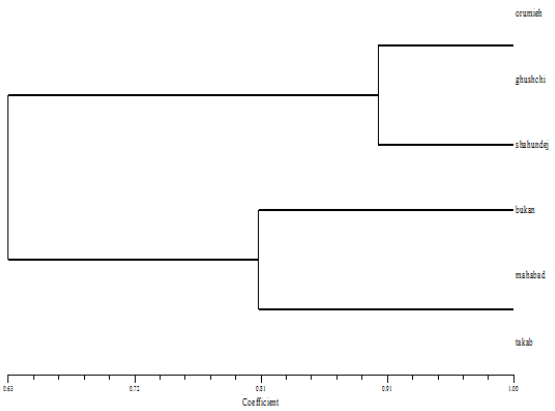

Fig. II. Dendrogram obtained from UPGMA based on scoring of SDS-PAGE gel electrophoresis shown in Fig. I.

Table 1
Total shoot protin (gr per 100 gr dry weight) of 6 populations of *Gundeliatournefortii*

Population	Mean ± Standard deviation
Orumieh	0.059 ± 0.0040
Ghushchi	0.064 ± 0.0040
Shahindej	0.073 ± 0.0040
Bukan	0.057 ± 0.00451
Mahabad	0.066 ± 0.0020
Takab	0.046 ± 0.0040

the least genetic diversity and consequently have the strongest genetic linkage. Moreover, class 2

had the most linkage with class 1 and the least linkage with the other classes.

Total protein assay

The results of total shoot proteins are shown in Table 1. As the Table suggests, Shahindej population has the strongest total protein (0.073 mg/100 g) and Takab population has the least total protein (0.046 mg/100 g).

Discussion

It was understood from biochemical studies that class 1 populations showed the least genetic diversity *among Gundeliatournefortii* populations and consequently have the strongest genetic linkage. Moreover, class 2 had the strongest linkage with class 1 and the weakest linkage with the other classes. Also, total protein assay showed that *Gundelia* can be a good source of protein. Similar research studies were done in this field. Nasr et al. (2006) used SDS-PAGE electrophoresis to show genetic differences in seed storage protein compositions among different varieties of *Brassica napus.* The varieties were classified in 5 clusters. AL-Barzinjy et al. (2003) reported protein content of *Brassica napus* in a range of 18.9-19.5. Dendrogram which was obtained from anatomical studies showed that populations 2 and 6 had the least genetic distance with each other and also populations 4 and 5. Population 3 on the other hand, had the most genetic distance with other populations.

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