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Physical and Chemical Variabilities Among Domestic Iranian Fenugreek (*Trigonella foenum-graceum*) seeds

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Abstract: The seeds of thirty-three fenugreek ecotypes collected from the main cultivation areas of Iran were analyzed to evaluate their genetic diversity in respect to seed properties and phytochemicals Trigonelline (TG) and Nicotinic acid (NA) contents. One thousand seed weight varied from 15.35 g in Ge 6 to 7.15 g in Ge 21. Phytochemical evaluation by HPLC showed that there is a big variation in alkaloid contents sum of Trigonelline (TG) and Nicotinic acid (NA) among different ecotypes. The sum of TG and NA varied from 0.248% (w/w) in Ge 14 from warm arid region up to 0.653% (w/w) in Ge 18 from dry cold region. Most of the ecotypes were rich in trigonelline and nicotinic acid and their recorded values were higher than standard pharmaceutical levels. In cluster analysis, the sum of TG and NA was the highest for group C with mean value of 12.72 g for 1000 seed weight. There were no direct correlations between TG and NA contents and 1000 seed weight. No significant relationship was observed between regions of cultivation and alkaloids (trigonelline and nicotinic acid) contents in seeds. Based on the results of this experiment, it could be concluded that the seed alkaloid contents in fenugreek ecotypes are mainly controlled by genotypic characteristics rather than ecological ones.

Key words: *Trigonella foenum-graceum*, ecotypes, phytochemical, seed properties, trigonelline

INTRODUCTION

Fenugreek *(Trigonella foenum-graecum* L.) is an annual medicinal plant belonging to the leguminous family. This crop is native to an area extending from Iran to northern India but it is now widely cultivated in China, North and east Africa, Ukraine and Greece for the Medicinal seeds and fodder (Petropoulos, 2002). One of most important ingredients of fenugreek is trigonelline witch its hypoglycemic effect was first reported by Kinsky *et al.* (1967). Fenugreek leaves and seeds are used extensively to prepare extracts and powders for medicinal uses (Basch *et al.*, 2003) such as anti-diabetic, anti-fertility, anticancer, anti-microbial, anti-parasitic and hypocholesterolaemic effects (AL-Habori and Raman, 2002). It is well known that fenugreek genotypes differ in morphology, growth habit, biomass, seed production capability and chemical constituents (Taylor *et al.*, 1997, 2000).

There are significant variations exhibited among fenugreek biotypes for growth habit, flowering time, seed color, seed size, biomass and seed yield (McCormick *et al.*, 2009a). Variation in accession traits showed the highest phenotypic diversity among fenugreek biotypes from Turkey and Iran (McCormick *et al.*, 2009a). Genotype×environment and genotype×time interactions indicate that fenugreek seed production will not be of similar quality in all environments and also all genotypes will not produce high quality seed every year (Acharya *et al.*, 2006). The reports have indicated that genotypic variance among fenugreek genotypes were greater than environmental variance in flowering date and early vigor (McCormick *et al.*, 2009b). The effect of environmental factors on plant biomass, plant height at late flowering stage, biomass yield, seed weight and harvest index have always been greater than genetic variations among fenugreek ecotypes. Furthermore, the variability of morphological traits among fenugreek accessions have been reported by Kakani *et al.* (2010)

It is a common understanding that chemical constituents of medicinal plants and so their biological activities are influenced by the genetic and environmental factors (Heywood, 2002). Therefore, to identify superior types of fenugreek, more needs to be known about the functional traits that are associated with yield and biomass production (McCormick *et al.*, 2009a).

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The study of genetic variability in different traits of available fenugreek ecotypes is a necessity for further crop improvement. To achieve this goal, the genetic variation and diversity for different traits including botanical characteristics, trigonelline content and common phytochemical characteristics among seeds of 33 ecotypes collected from different ecological regions of Iran, were evaluated in this research. So the main aim of this research was quantization of major compounds including trigonelline and nicotinic acid of the collected samples and evaluating them for their botanical characteristics.

MATERIALS AND METHODS

Seed collection procedures: A group of 33 seed batches of fenugreek ecotypes were collected from different ecological regions of Iran. The sampling sites were selected according to the information gathered from local Agricultural Extension Offices from all over the country (Fig. 1). The seed samples were cleaned manually to remove all foreign materials (dust, dirt and none fenugreek seeds) as well as broken and immature fenugreek seeds. Geographical origins of the 33 fenugreek ecotypes are listed in Table 1.

Determination of physical properties: To measure the physical properties such as length, width, thickness of seeds, a digital caliper with an accuracy of 0.01 mm was used. To measure the 1000 seed-weight, 100 seeds were randomly selected in two replications from the bulk and after weighing the samples by an electronic balance (with an accuracy of 0.001 g), the results were averaged and multiplied by 10 (Anonymous, 2002).

Determination of moisture and ash: The moisture and ash of seeds was measured as following, A 4 g sample of seeds was accurately weighed and placed in an oven at 105°C for two hours by calculating weight loss the moisture percent was obtained. To determine the weight of the ash, the dried seeds were placed in tarred crucible, and incinerated gently and then gradually increased the temperature to 675±25°C, until it was free from carbon.

Alcohol soluble materials: This measurement was done according to United States Pharmacopeia by cold extraction method: About 4 g of air-dried, coarsely powdered material, accurately weighed, was transferred to a glass-stopper conical flask. One hundred milliliter of alcohol was added; a stopper was inserted into the flask and was macerated for 24 h with shaking frequently during the first 8 h and then allowed to stand for 18 h. The

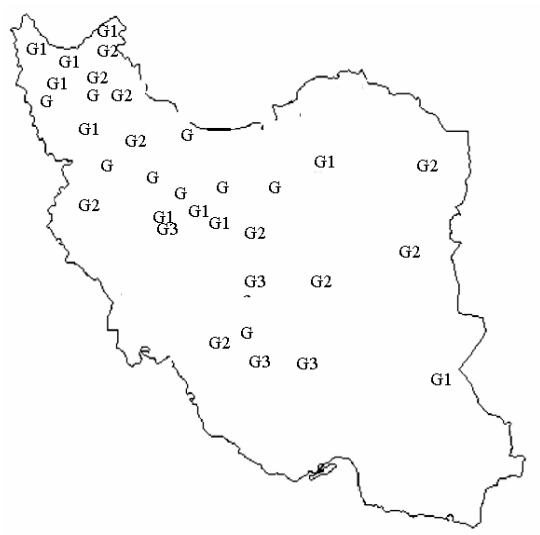

Fig. 1: The geographical origins of Iranian fenugreek ecotypes used in this study

solution was filtered rapidly taking precautions against loss of alcohol. Twenty five milliliter of the filtrate was evaporated to dryness in a tarred, flat-bottomed, shallow dish and dried at 105°C to constant weight. The content was calculated in mg per g of alcohol-extractable matter in the test specimen.

Water soluble materials: This process was performed according to United States Pharmacopoeia by cold extraction method as directed for alcohol soluble material, except to use water in place of alcohol.

Quantization of Trigonelline (TG) and Nicotinic Acid (NA): For measurement of trigonelline and nicotinic acid in the seeds samples, method (Zheng and Ashihara, 2004) method was modified. Seeds were ground with 80% Methanol and Magnesium Oxide (MgO) in a mortar and pestle. After incubation at 60°C for 30 min, the homogenates were centrifuged and the supernatant was collected. After complete evaporation of methanol, the methanol-soluble extracts were dissolved in distilled water. The samples were filtered using a disposable syringe filter unit and the aliquots were used for determination of Trigonelline (TG) and Nicotinic acid (NA) by HPLC. The analysis of the samples was carried out using a Knauer K2600A liquid chromatography (Germany), equipped with a Nucleosil C18 (150×4.6 mm I.D, 5 μm) column. A mixture of methanol: water (50:50 v/v) served as the mobile phase and pH of solution adjusted to 5.0 with 50 mM sodium acetate. The elution has been made in an isocratic mode at a flow rate of 1 mL min^{-1} and the detection made at 268 nm by UV

Table 1: Geographical origins of Fenugreek ecotypes

Ecotype no.	Region originated	Climate[a]	Latitude	Longitude	Altitude (m)
Ge1	Varamin	Temperate-semi arid	35°19'N	51°39'E	918
Ge2	Semnan	Temperate-semi arid	38°06'N	46°26'E	1310
Ge3	Gazvin	Temperate- semi arid	36°15'N	50°01'E	1800
Ge4	Rasht	Temperate- humid	37°18'N	49°36'E	10
Ge5	Saveh	Temperate-semi arid	36°10'N	50°01'E	1800
Ge6	Orumiye	Temperate-semi humid	37°34'N	44°58'E	1366
Ge7	Tabriz	Temperate-Semi humid	36°58N	46°58E	1500
Ge8	Shiraz	Temperate -semi humid	29°39'N	52°35'E	1486
Ge9	Hamedan	Tem prate-semi humid	35°15'N	34°34'E	1366
Ge10	Arak1	Temperate -semi arid	34°05'N	44°41E	1760
Ge11	Damghan	Temperate-semi humid	36°15'N	50°01'E	1800
Ge12	Zahedan	Temperate-warm arid	29°32'N	60°54'E	1385
Ge13	Kashan	Temperate- semi arid	51°27'N	59°33'E	912
Ge14	Ghom	Temperate -dry cold	34°49'N	50°56'E	790
Ge15	Khoy	Temperate -semi humid	38°56'N	44°28'E	1193
Ge16	Miandab	Temperate -semi humid	38°01'N	40°30'E	1500
Ge17	Poldasht	Temperate -semi humid	21°39'N	45°04'E	815
Ge18	Makoo	Temperate- semi arid	39°18'N	44°30'E	1294
Ge19	Dashte-Moghan	Temperate-humid	39°10'N	47°30'E	500
Ge20	Ardabil	Temperate- semi arid	36°58N	46°58E	1500
Ge21	Birjand	Temperate- semi arid	32°53'N	59°13'E	1470
Ge22	Kermanshah	Temperate- semi arid	34°23'N	47°03'E	1366
Ge23	Mashhad	Temperate- semi arid	36°19'N	59°37'E	985
Ge24	Sarab	Temperate- semi humid	37°32'N	47°14'E	1650
Ge25	Ahar	Temperate- semi humid	28°38'N	47°04'E	1360
Ge26	Yazd	Temperate- arid	32° 01'N	54°3'E	1366
Ge27	Esfahan-Ardestan	Temperate- semi arid	32°50'N	51°55'N	1200
Ge28	Zanjan	Temperate- semi arid	36°41'N	48°29'E	1700
Ge29	Kazeron	Temperate-warm	29°39'N	51°39'E	732
Ge30	Arak2	Temperate- semi arid	34° 41'N	50° 01'E	1980
Ge31	Darab	Temperate- semi arid	28°02'N	54°30'E	1150
Ge32	Kovar	Temperate- semi arid	11°29'N	42°52'E	1500
Ge33	Eghlid	Temperate- semi arid	30°53'N	52°41'E	2320

A: Annual mean temperature in warm, temperate and cool climates is 15-25, 10-15 and 0-5°C, respectively. Annual mean rainfall in semi humid, semi arid and arid climates is 600-1400, 300-600 and 100-300 mm, respectively

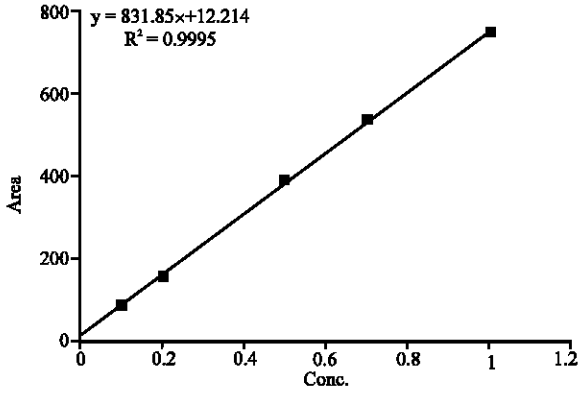

Fig. 2: Calibration curve using Trigonelline as standard

detector from the above mentioned company. One analysis requires 20 min. The retention time (min) of these alkaloids were as follows: Trigonelline 4.4 min and nicotinic acid 5.1 min. Before carrying out HPLC analysis, we made calibration curve by using different concentrations (0.1, 0.2, 0.5, 0.7 and 1.0 mg mL^{-1}) of both trigonelline and nicotinic acid in mobile phase media. Then Calibration curve made with TG and NA and the correlations were excellent for both two alkaloids (r^2 = 0.9995) (Fig. 2).

Cluster analysis: Two cluster analyses of the data were performed based on physical properties (1000 seed weight) and pharmaceutical characteristic (Sum of TG and NA). The outweighed pair-group method arithmetic average (UPGMA) was used.

RESULTS AND DISCUSSION

Seed physical and chemical properties: The physical and chemical characteristics; moisture content, water soluble materials, alcohol soluble materials and ash content in seeds of 33 fenugreek ecotypes were measured and analyzed. The results are summarized in Tables 2 and 3. The 1000 seed weight varied from 7.15 g in Ge 21 to 15.35 g in Ge 6. There was a significant positive correlation between 1000 seed weight and alcohol soluble materials ($p<0.05$ and $r = +0.3$). However, no significant correlation was observed between 1000 seed weight and sum of TG and NA contents (Table 4). The color of the seeds in most of the ecotypes was yellow to brown and only in few cases it was green or dark green (Table 2).

Trigonelline (TG) and Nicotinic Acid (NA) content: HPLC analysis was set up for differentiation and simultaneously quantization of both TG and NA. The method was good

Table 2: Seed properties of Iranian fenugreek ecotypes

Ecotype no	Seed size	1000 seed weight (g)	Seed color	Water soluble materials (%)±Sd	Alcohol soluble materials (%)±Sd
Ge1	1.42	12.38	Light green	29.23±0.58	12.11±1.02
Ge2	1.37	11.82	Yellow	29.38±0.62	10.71±1.01
Ge3	1.42	12.90	Yellow	29.20±1.47	11.36±0.73
Ge4	1.70	11.69	Brilliant yellow	28.60±0.90	11.35±0.85
Ge5	1.67	12.20	Brilliant yellow	32.45±0.86	11.90±0.91
Ge6	1.50	15.13	Beige to brown	29.79±1.25	13.43±0.64
Ge7	1.62	12.44	Yellowish brown	37.26±0.96	9.53±0.92
Ge8	1.47	12.18	Yellow to amber	35.14±0.94	10.42±1.03
Ge9	1.65	12.13	Yellowish brown	34.64±1.02	10.10±0.90
Ge10	1.45	12.37	Yellow to amber	28.51±1.62	11.17±0.86
Ge11	1.55	14.62	Beige to brown	31.73±0.89	13.20±1.05
Ge12	1.45	12.66	Yellowish brown	30.69±0.71	12.66±0.90
Ge13	1.57	14.46	Brown	32.61±0.77	12.11±0.22
Ge14	1.37	14.05	Beige to brown	35.59±0.69	11.45±0.57
Ge15	1.67	13.61	Dark green	33.30±0.69	10.62±0.39
Ge16	1.47	11.85	Yellowish brown	28.59±1.12	11.58±0.73
Ge17	1.62	13.87	Green to brown	30.69±0.25	12.59±0.61
Ge18	1.70	15.35	Dark green	29.84±0.97	13.63±0.73
Ge19	1.55	12.40	Yellowish brown	29.93±1.62	12.40±0.70
Ge20	1.60	13.66	Yellowish brown	31.49±0.74	11.76±0.80
Ge21	1.45	7.15	Dark green	31.15±1.33	11.78±0.90
Ge22	1.40	13.16	Light brownish	29.68±1.02	11.02±0.90
Ge23	1.40	11.73	Brilliant yellow	33.75±0.49	12.37±0.38
Ge24	1.75	13.84	Dark brown	35.56±0.79	11.39±0.97
Ge25	1.55	12.38	Light brownish	30.29±0.69	12.57±1.00
Ge26	1.55	11.40	Brilliant yellow	33.45±0.60	11.58±0.72
Ge27	1.42	8.41	Bright green	37.41±0.78	11.16±0.84
Ge28	1.52	11.48	Yellowish brown	34.41±1.23	11.75±0.82
Ge29	1.47	13.59	Brilliant yellow	31.37±1.06	11.11±1.00
Ge30	1.57	11.42	Yellowish brown	28.99±1.55	10.31±0.63
Ge31	1.47	11.07	Yellow brown	31.76±0.80	10.21±0.68
Ge32	1.50	13.19	Yellowish brown	34.36±0.73	11.33±0.68
Ge33	1.40	12.97	Bright green	30.61±0.87	11.36±0.73

Table 3: Simple correlation (r) between seed properties of Iranian native fenugreek ecotypes

	1000 seed weight	Water soluble (%)	Alcohol soluble (%)	Sum Tg and NA
1000 seed weight		-0.13	0.375*	-0.00
Water Soluble (%)	-0.13		-0.34	-0.360*
Alcohol Soluble (%)	0.375*	-0.34		0.077
Sum TG and NA	-0.00	-0.36*	0.077	

*Correlation is significant at the 0.05 lev 92-tailed, **Correlation is significant at the 0.01 lev 92-tailed

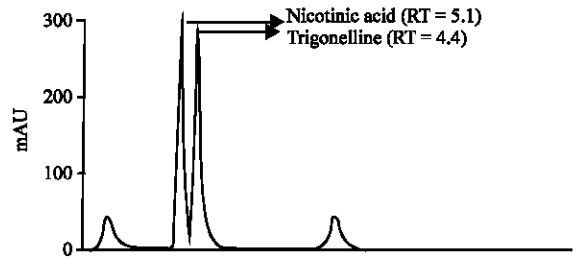

Fig. 3: HPLC chromatogram of trigonelline and nicotinic acid in test solution

and the resolution between two substances was greater than 1. A sample of HPLC chromatogram is shown in (Fig. 3). The results of analyses of TG content, NA content and sum of both in 33 ecotype seeds are presented in Table 5.

Trigonelline, N-methyl nicotinic acid, was first noted in the seeds of *Trigonella foenum-graecum* and has since been found in the seeds and tubers of many species as well as in leaves of one species of pea (Joshi and Handler, 1960). TG concentration was recorded for *Mirabilis violacea* (Nyctaginaceae) (0.3%), *Euonymus europaeus* (Celastraceae); (0.1%), *Bourgainvillia* sp. (Nyctaginaceae); (0.1%) and *Solanum wendlandii* (Solanaceae); (0.1%) (Blunden *et al*., 2005).

Surprisingly in our study the recorded TG and NA contents in local ecotype seeds were above pharmaceutical levels (Table 5). According to the statistical analysis of quantitative data, the highest TG and NA content (0.65%) belonged to Ge 3, a local selected clone from Qazvin region of Iran (Table 5). There were some variations in TG and NA content among ecotypes belonging to the same geographical regions such as Ge 5 to Ge 28 (Fig. 1) which could be explained by their genetic

Table 4: Trigonelline and Nicotinic acid content in native Iranian fenugreek ecotypes seeds (Duncan's multiple range tests with standard deviations (Sd)

Ecotype no.	Trigonelline (%)	Nicotinic acid (%)	Sum of TG and NA
Ge 1	0.197± 0.012	0.268±0.020	0.465±0.017
Ge 2	0.223± 0.013	0.285±0.015	0.508±0.020
Ge 3	0.288±0.021	0.365±0.017	0.653±0.021
Ge 4	0.193±0.016	0.221±0.015	0.414±0.016
Ge 5	0.140±0.017	0.181±0.011	0.321±0.015
Ge 6	0.089±0.016	0.171±0.013	0.260±0.011
Ge 7	0.157±0.013	0.193±0.014	0.350±0.014
Ge 8	0.178±0.018	0.212±0.016	0.390±0.013
Ge 9	0.141±0.014	0.186±0.013	0.330±0.011
Ge 10	0.185±0.013	0.214±0.018	0.399±0.016
Ge 11	0.134±0.021	0.143±0.014	0.277±0.015
Ge 12	0.155±0.020	0.181±0.013	0.336±0.014
Ge 13	0.168±0.016	0.196±0.017	0.364±0.018
Ge 14	0.107±0.013	0.141±0.014	0.248±0.016
Ge 15	0.133±0.014	0.167±0.015	0.300±0.013
Ge 16	0.148±0.021	0.154±0.017	0.302±0.018
Ge 17	0.173±0.018	0.212±0.016	0.385±0.021
Ge 18	0.256±0.020	0.328±0.015	0.584±0.014
Ge 19	0.228±0.018	0.286±0.021	0.514±0.017
Ge 20	0.167±0.017	0.212±0.019	0.379±0.021
Ge 21	0.173±0.014	0.243±0.016	0.416±0.022
Ge 22	0.165±0.014	0.214±0.022	0.379±0.018
Ge 23	0.153±0.016	0.196±0.018	0.349±0.021
Ge 24	0.154±0.011	0.211±0.015	0.365±0.016
Ge 25	0.123±0.018	0.161±0.014	0.284±0.014
Ge 26	0.178±0.011	0.203±0.015	0.381±0.018
Ge 27	0.103±0.013	0.146±0.018	0.249±0.016
Ge 28	0.201±0.018	0.275±0.021	0.476±0.013
Ge 29	0.128±0.021	0.165±0.021	0.293±0.018
Ge 30	0.141±0.013	0.181±0.016	0.322±0.013
Ge 31	0.173±0.014	0.208±0.013	0.381±0.012
Ge 32	0.176±0.014	0.213±0.018	0.380±0.014
Ge 33	0.202±0.011	0.190±0.014	0.392±0.016

Percentage of both TG and NA are presented in w/w

Table 5: Mean values of 1000 -seed weight in each cluster group

Groups	1000-seed weight (g)
A	12.03
B	14.42
C	7.78

Table 6: Mean pharmaceutical (SUM of TG and NA) values in each group of cluster analysis

Groups	Sum of TG and NA (% w/w)
A	0.370±0.028
B	0.276±0.020
C	0.533±0.065

characterizations. These observations suggest that probably the genetic factors have more influence on TG and NA content than geographical origins. So, studying the genetic variations among the ecotypes along with their potential TG and NA contents in different areas could be recommended.

In the plants cell culture, trigonelline is made from nicotinic acid via N-methylation and this reaction is reversible; trigonelline changes to nicotinic acid by demethylation (Willeke *et al*., 1979). Both trigonelline and nicotinic acid had been considered members of the pyridine nucleotide pathway, of which the function is to generate nicotinamide adenine dinucleotide (Tramontano and Jouve, 1997). Trigonelline is a hormone which regulates the promotion of cell arrest in G2 in the cell cycle of meristematic roots (Evans *et al*., 1979). In many legumes and some species of other plant families, such as coffee (*Coffea* spp.), large amounts of trigonelline (Basch *et al*., 2003) (1-60 μmol g^{-1} fresh weight) accumulate in seeds Koshiro *et al*. (2006). Several physiological functions of trigonelline have been proposed (Zheng and Ashihara, 2004).

In a study, on sucrose, chlorogenic acids, trigonelline and caffeine variations in wild genetic resources of *Coffea arabica* and *C. canephora* by HPLC instrument, trigonelline variability was seen between 38 genotypes of both *C. arabica* and *C. canephor* from all over African countries. Minimum trigonelline content were 0.88% (w/w) and 0.75% (w/w); while the maximum content were 1.77% (w/w) and 1.24% (w/w) for *C. arabica* and *C. canephora,* respectively (Ky *et al*., 2001).

Cluster analysis: The cluster analysis of the data was performed based on 1000 seed weight. The results were shown as a dendrogram indicating the estimated relations between the fenugreek ecotypes. A dendrogram created

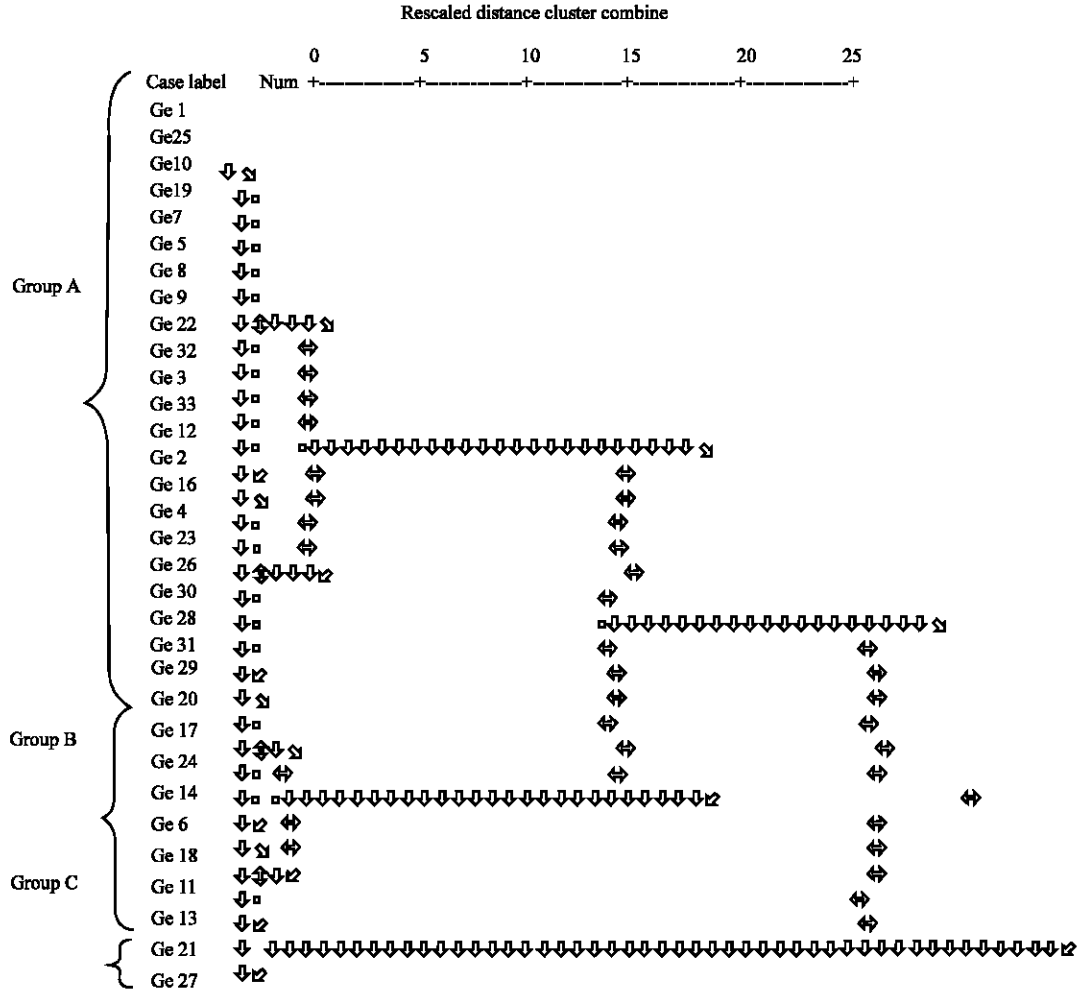

Fig. 4: Dendrogram generated by cluster analysis of 1000-seed weight. The scales portray a dissimilarity index calculated using Euclidean distance coefficient and the dendrogram was developed using UPGMA clustering proceed

by UPGMA, using 33 ecotypes, showed three main groups A, B, C (Fig. 4). Mean values of the recorded trait for each group are listed in (Table 6). The highest 1000 seed weight was observed in Group B with Mean values of 14.42 g, group A with Mean values of 12.03 g and group C with mean values of 7.78 g, respectively. The Cluster analysis of the data performed on pharmaceutical (SUM of TG and NA) traits classified the fenugreek seeds into three main groups of A, B and C (Fig. 5). The mean values of the recorded traits for each group are listed in (Table 6). Group C with a mean value of 0.533±0.065% (w/w), Group B with mean value of 0.276±0.020% (w/w) and group A with mean value of 0.370±0.028% (w/w) had the highest sum of (TG and NA), respectively. In group C (genotypes 2, 19, 1, 28, 3 and 18), the pharmaceutical grade trigonelline and nicotinic acid content was not restricted to any specific growing region. The seeds of these ecotypes (group C) were originated and collected from different areas in the center and northwest of Iran. The highest sum of TG and NA content was also observed in group C which had a mean value of 12.72 g for 1000-seed weight.

Genotype×environment interaction can play an important role in variability in quality and quantity yield among fenugreek genotypes. This is important for a crop that has a potential to be used as a neutraceutical. Also, such interaction indicates that seed produced in all environments will not be of similar quality and also all genotypes will not produce high quality seed every year. To maintain high quality in neutraceutical products, we need to produce stable cultivars showing least amount of genotype×environment interaction and continue monitoring their quality every year (Acharya *et al*., 2006). In our study most of the traits were significantly influenced by genotype×environment interactions.

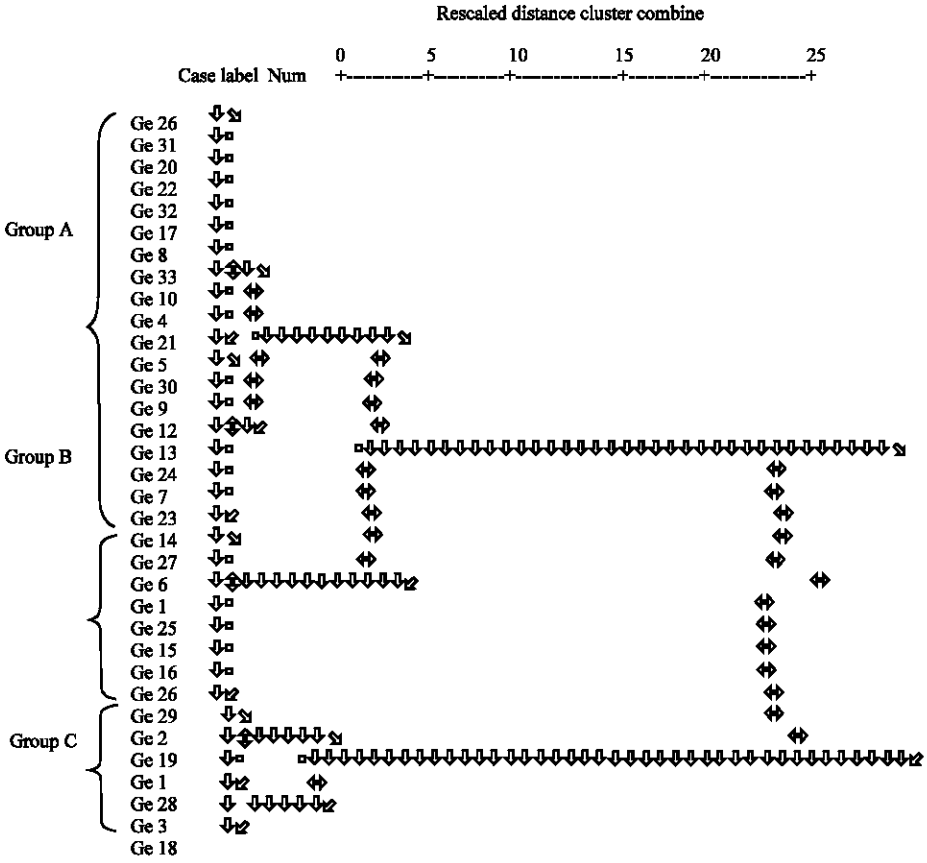

Fig. 5: Dendrogram generated by cluster analysis of pharmaceutical (SUM of TG and NA) traits. The scales portray a dissimilarity index calculated using Euclidean distance coefficient and the dendrogram was developed using UPGMA clustering proceed

The previous studies have indicated that plant growth type of fenugreek varied from semi-erect, erect to bushy types. Also, the weight per 1,000 seeds varied from 8.5 g to 21 g seed size from small to bold and seed color from yellow golden to green (Kakani *et al*., 2010).

McCormick in (2009a) reported that in a germplasm collections of 205 fenugreek accessions, significant variations were identified for all traits including growth habit, flowering time, seed color, seed size, biomass and seed yield. The accessions that fitted the description of the more diverse subsp *foenum-graecum* were mostly from Iran, Afghanistan, Turkey and several northern African countries, high yielding green-seeded types were among this group (McCormick *et al*., 2009a). Accessions from Turkey and Iran showed the most phenotypic diversity. High yielding accessions were found in germplasm from most countries and all latitude zones, although latitudes >30° provided 73% of the high yielding accessions. Our results is supported by McCormick (2009b) which reported that the genotypic variance was greater than environmental variance for flowering date and early vigor and seed size among fenugreek ecotypes.

CONCLUSION

In this study, we found variations in alkaloids (trigonelline and nicotinic acid) content among different ecotypes which gathered from all over of Iran. Sum of TG and NA varied from 0.248% (w/w) up to 0.653% (w/w) among different ecotypes. In cluster analyzing, the sum of TG and NA was the highest for group C with mean value of 12.72 g for 1000 seed weight. There was no direct correlation between TG and NA contents and 1000 seed weight. Also no correlation between original region of ecotypes and the contents of these two alkaloids were observed. So it could be concluded that the content of these alkaloids could be better explained by genotypical factors rather than ecological origins.

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