

Heritability and correlation studies of fatty acid composition within *Hyoscyamus* accessions

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Abstract

In the present study, the variability among the 14 accessions was assessed through analysis of variance, which showed significant differences among the accessions for 11 fatty acids and oil content. Variability in different traits was due to genotypic effects, since phenotypic coefficient of variation (PCV) was higher than those of genotypic coefficient of variation (GCV) with very small differences. The range of genetic similarity was obtained between 0.05 and 0.99 among various comparisons. Fatty acid composition significantly varied with locations. The dendrogram revealed that genotypes h1, h2, h3, h4, h7, h8, h9, h10, h13 and h14 are the most distantly related. Hence, these genotypes could be considered for future breeding programs to create higher amount of genetic variability in Iranian germplasm of *Hyoscyamus*

Keywords: *Hyoscyamus*; fatty acids; Genetic variability

Introduction

The genus *Hyoscyamus* L. belongs to the tribe Hyoscyameae Miers of Solanaceae family with 20 species all over the world (Yousaf et al., 2008) and 13 species in Iran (Khatamsaz, 1998). Structurally, fatty acids are the straight-chain monounsaturated and polyunsaturated chain building blocks of dietary fats and oils (Wolfum and Spener, 2000). Linoleic and oleic acids are two long-chain unsaturated fatty acids that are essential for human diets. As such, a lack of dietary essential fatty acid on efficient metabolism has been implicated in the etiology and progression of disease (Brown, 2005). There is evidence that a MUFA (Monounsaturated Fatty Acids) rich diet can lower the risk on cardio vascular disease and also has preventive effects on atherosclerosis (Kris- Etherton, 1999).

The species of *Hyoscyamus* L. were characterized by high unsaturation ratio. Linoleic acid was the dominant fatty acid of the oil, which had bioactivities of lowering blood pressure and construction of smooth muscle. *H.niger* seeds give considerable yield of oil which seems to be a good source of essential fatty acids and lipid-soluble bioactive compounds (Li et al., 2011). It is well known that quantitative variation in seed oil fatty acid composition can be related to habitat, environment and genetic differences (Levin, 1974; Linder, 2000; O'Neill et al., 2003). Since the dominant seed oil constituent in angiosperms is Linoleic acid (C18:2) (Graham and Kleiman, 1992), most published studies deal with the effect of these factors on the proportions of saturated to unsaturated fatty acid produced.

Among environmental components, temperature is considered influential. The proportion of saturated to unsaturated fatty acids produced by seeds increases at high temperatures; conversely, more unsaturated fatty acids are produced by the same species at lower temperatures (Canvin, 1965; Linder, 2000). Pritchard et al, 2000 determined the effect of environment on the quality of canola oil. They reported correlation of oil contents were correlated with cooler spring temperatures and higher spring rainfall.

Studies on various chemical and fatty acid contents of *Hyoscyamus* in the world have not been reported and also the literatures don't contain any information of environmental effect on fatty acids composition. Therefore, the objective of this study was to evaluate *Hyoscyamus* fatty acids composition within 14 Iranian *Hyoscyamus* accessions. We also attempted to find correlation between altitude, rainfall and mean annual temperature and fatty acids levels to provide information on the influence of the environment on fatty acid production.

Materials and Methods

Five *Hyoscyamus* species including *H.niger* L., *H.reticulatus* L., *H.pusillus* L., *H.arachnoideus* POJARK. and *H.kurdicus* Bornm. were collected from natural habitats at 14 regions of Iran (Table1). In each region, seeds of *Hyoscyamus* species were randomly harvested. Plants were collected during fruit formation with 3 replications from 27 to 30 June 2011, depending to the sites.

Table 1. *Hyoscyamus* accessions, collected sites and climate data.

Codes	<i>Hyoscyamus</i> accessions	Collection site	Altitude (m)	Mean annual temperature (°C)	Total Rainfall (mm)
h1	<i>H.niger</i>	Ardabil	2100	18.69	229.05
h2	<i>H.niger</i>	Ardabil:	2200	8.23	378.57
h3	<i>H.niger</i>	Salmas	1526	11.39	227.5
h4	<i>H. niger</i>	Esphahan	1550	16.2	122.8
h5	<i>H.niger</i>	Gazvin	1279.2	14.3	316
h6	<i>H.niger</i>	Gilan	5	16.2	1359
h7	<i>H.reticulataus</i>	Ardabil	2100	8.23	378.57
h8	<i>H.reticulataus</i>	Urmia	1480	10.3	358.8
h9	<i>H.reticulataus</i>	Nagade	1389	12.55	358.73
h10	<i>H.pusillus</i>	Ardabil	1900	9.1	302.4
h11	<i>H.pusillus</i>	Urmia	1500	10.3	358.8
h12	<i>H.pusillus</i>	Miandoab	1463	14.14	300
h13	<i>H.arachnoideus</i>	Ardabil	2200	9.1	302.4
h14	<i>H.kurdicus</i>	Ardabil	2300	9.1	302.4

Lipid analysis

Total lipids were extracted (López-Martínez et al., 2004). The formation of FAME (Fatty Acid Methyl Esters) was carried out according to the procedure described by Desvilettes et al. (1994). The sample was saponified with methanolic sodium hydroxide and the fatty acids were esterified with methanolic sulfuric acid. FAME were analyzed with a 6890 N GC–FID (Agilent Technologies, Wilmington, DE, USA) fitted with a J&W DB-Wax capillary column (30m, 0.25 mm id., 0.25 mm film thickness), a split– splitless injector with Agilent tapered liner (4mm id) and flame ionization detector. The initial column temperature was maintained at 100°C for 1 min and then raised at 25°C/min to 190°C and held for 10 min and then raised to 220°C and held for 15 min. Nitrogen was used as carrier and makeup gas, at flow rates of 1.0 and 45 mL/min, respectively. The injector and detector temperature were held at 250 and 260°C, respectively. Chem. Station software was used for online data collection and processing. Individual FAME was identified by comparison to known standards (Sigma, Chemical Co. St. Louis).

Statistical analysis

All analyses were done on a randomized block design. Each data was the mean of three replicates and means were considered as significant differences at $p < 0.05$ level. All statistical analyses were performed using NTSYS pc 2.02 and SAS software 9.1.3. Degree of correlation between fatty acid profiles contents and environmental data was analyzed using correlation analysis.

Results and discussion

The fatty acid composition of the seed oil was determined by Gas Chromatography (Table 2 & Figure1).

In the investigated *Hyoscyamus* accessions, the lowest oil yield was obtained from *H.niger* collected from Khalkhal region (15.03 %), and *H.niger* seeds from Salmas region gave the highest oil yield (25.4%) (Table2).

Table 2. Mean amounts of fatty acid compositions (%) and oil content (%) of 14 *Hyoscyamus* accessions. Codes represent accessions of *Hyoscyamus* listed in table 1.

Codes/ fatty acid	14:0	14:1	16:0	16:1	18:0	18:1 ω9	18:2 ω6	18:3 ω3	20:0	18:3 ω6	18:4 ω3	Oil percent (%)	ΣSFA/ ΣUSFA
h1	0.2± 0.01	0.02± 0.0005	3± 0.1	0.03± 0.003	2.6± 0.1	16.1± 0.1	70.2± 0.02	1.3± 0.01	0.05± 0.0002	0.48± 0.03	0.3± 0.02	25.4± 0.1	0.06
h2	1.49± 0.005	0.03± 0.006	4.56± 0.01	0.05± 0.005	3.06± .05	16.9± 0.1	66.9± 0.01	1±0.1 0.01	0.07± .001	0.45± .005	0.29± .01	23.1± 0.1	0.1
h3	2.11± 0.005	0.22± .01	5.83± 0.05	0.36± 0.005	3.19± .01	17.43± 0.01	64.4± 0.01	0.3± 0.02	0.37± .01	0.43± .01	0.21± .01	20.13± 0.05	0.13
h4	7.38± 0.01	0.28± .02	6.18± 0.01	0.55± 0.01	3.25± .005	18.64± 0.005	63.56± 0.14	0.28± .01	0.52± .01	0.39± .01	0.19± .005	18.06± 0.11	0.2
h5	8.63± 0.01	0.36± .8E-17	6.43± 0.005	0.6± 0.01	3.85± .05	18.88± 0.01	41.7± 0.04	0.27± .01	1.3± 0.01	0.37± .01	0.18± .005	16.26± 0.25	0.32
h6	9.32± 0.005	0.98± .01	12.08± 0.01	0.7± 0.01	4±0.1 0.01	19.01± 0.01	40.2± 0.1	0.26± .02	1.65± .04	0.35± .35	0.15± .02	15.03± 0.05	0.43
h7	0.73± 0.03	0.03± 0.01	3.78± 0.02	0.2± 0.005	1.92± .01	3.62± 0.02	66.3± 0.01	15.2± .2	0.06± .01	0.56± .03	0.39± .005	24.06± 0.11	0.07
h8	0.87± 0.01	0.04± 0.01	5.83± 0.03	2.03± 0.2	2.87± .01	9.65± 0.05	65.1± .1	14.16± 0.005	0.07± .005	0.24± .01	0.34± .005	21.5± 0.1	0.073
h9	0.9± 0.1	0.72± 0.01	6± 0.29	2± 0.05	3±0.1 0.1	11± 0.05	63.0± 0.05	12.06± 0.01	0.89± .005	0.2± .02	0.33± .05	19.1± 0.17	0.12
h10	4.01± 0.01	0.2± 0.005	3.77± 0.01	0.85± 0.005	0.93± .01	4.56± 0.05	51.1± 0.005	0.37± .02	3.75± .005	10.8± .005	3.74± .005	23.1± 0.17	0.17
h11	5.41± 0.02	0.24± 0.01	7.17± 0.03	1.18± 0.01	1.25± .07	17.89± 0.01	44.5± 0.005	0.29± .005	2.85± .01	5.06± .15	2.23± .05	19.1± 0.1	0.23
h2	8.87± 0.005	0.29± 0.01	8.22± 0.005	1.71± 0.01	3.04± .005	18.13± 0.01	21.0± 0.07	0.21± .005	1.13± .01	4.21± .01	1.43± .05	18.46± 0.05	0.45
h13	0.5± 0.01	0.17± 0.005	5.72± 0.005	0.32± 0.01	2.99± .005	1.93± 0.01	0.3± 0.01	0.07± .005	0.2± 0.01	0.44± .01	0.2± .02	19.46± 0.05	0.61
h14	1.04± 0.01	0.24± 0.005	6.18± 0.005	0.68± 0.01	2.64± 0.02	70.1± 0.1	8± 0.1	0.35± 0.01	0.1± 0.005	0.42± 0.01	0.33± 0.02	19.06± 0.05	0.12

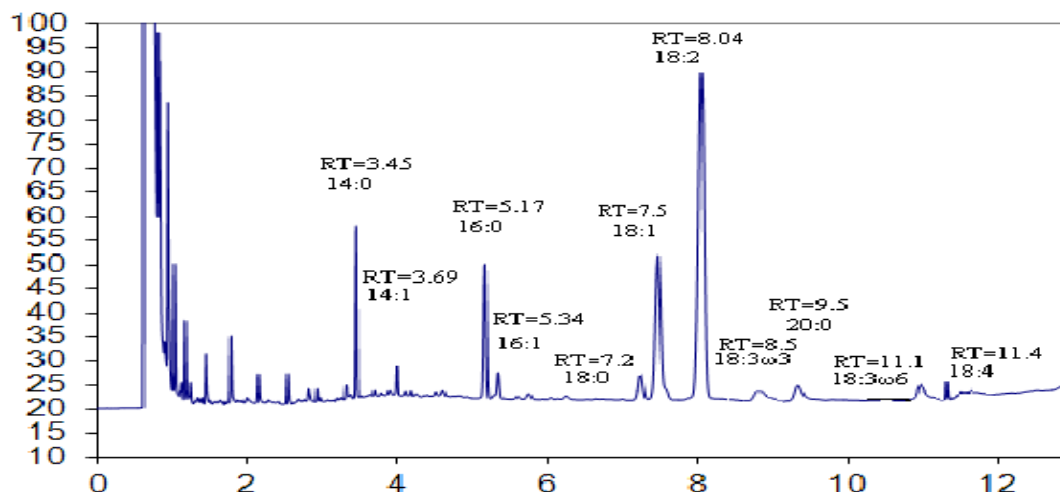


Figure 1: GC chromatograms of *Hyoscyamus pusillus*.

Accessions variability

The variability among the 14 accessions was assessed through analysis of variance, which showed significant differences among the accessions for 11 fatty acids and oil content (Table3). Of the eleven fatty acids studied, C18:0 (31%) and C18:3 (170%) showed the lowest and highest variability among the accessions, respectively (Table 3).

Hyoscyamus niger

In the seed oil of all accessions of *Hyoscyamus niger*, Linoleic acid was the main fatty acid identified by comparison to the fatty acid methyl ester standards. There were 4 saturated fatty acids including 14:0 (Myristic acid), 16:0 (Palmitic acid), 18:0 (stearic acid) & 20:0 (Arachidic acid) in the samples, of which, the total percentage of them was 5.85-27.05 %.

Of the unsaturated fatty acids, ω6 was the predominant ones (40.2-70.24 %) with total percentage of 61.66-88.38 %. The ratio of saturated fatty acids to the unsaturated (SFA/USFA) was the least in *H.niger* from Ardabil (0.06) followed by Khalkhal (0.1), Salmas (0.13), Esphahan (0.2), Gazvin (0.32) and Gilan (0.43).

H.reticulatus

Linoleic acid was the main fatty acid among all accessions of *Hyoscyamus reticulatus*. The total percentage of 6.49-10.79 % obtained for 4 saturated fatty acids including 14:0, 16:0, 18:0 & 20:0. Linoleic acid (63.03-66.37 %) was the predominant fatty acid among UFAs with the total percentage of 86.37-91.58 %. The ratio of saturated fatty acids to the unsaturated (SFA/USFA) was the least in *H.reticulatus* from Khalkhal (0.07) followed by Neichalan (0.073) and Nagadeh (0.12).

H.pusillus

The main fatty acid in the seed oil of *Hyoscyamus pusillus* accessions identified as Linoleic acid. This fatty acid was predominant (21.08-51.12 %) with total percentage of 47.05 to 71.59 %. *H.pusillus* samples contained four saturated fatty acids (14:0, 16:0, 18:0 & 20:0) with the total percentage of 12.46-21.26 %. The ratio of saturated fatty acids to the unsaturated (S/U) was the most in *H.pusillus* from Miandoab (0.45) followed by Rikan (0.23) and Khalkhal (0.17).

H.arachnoideus

Linoleic acid was the main fatty acid among all accessions of *Hyoscyamus arachnoideus*. The total percentage of 9.44 % obtained for 4 saturated fatty acids including 14:0, 16:0, 18:0 & 20:0. Linoleic acid (45.3 %) was the predominant fatty acid among UFAs with the total percentage 15.42 %. The ratio of saturated fatty acids to the unsaturated (SFA/USFA) was 0.61.

H.kurdicus

Oleic acid was the main fatty acid among all accessions of *Hyoscyamus kurdicus*. The total percentage of 9.69% was obtained from four fatty acids including 14:0, 16:0, 18:0 & 20:0 in the samples. Oleic acid (70.1 %) was the predominant fatty acid among UFAs with the total percentage of 80.02 %. The ratio of saturated fatty acids to the unsaturated (SFA/USFA) was 0.12.

Khan et al (1992) reported that the seeds of *H.niger* contain 14.85-22.13% of oil belonging to the Oleic and Linoleic groups. The content of Oleic and Linoleic acids were recorded 16.32 and 74.81% in the study of Sun (2000).

Our finding also supports high level of Oleic acids in studied *Hyoscyamus* accessions except *H.arachnoideus* & *H. reticulatus* from Khalkhal regions. Linoleic content was also high in all accessions of *Hyoscyamus* except *H.arachnoideus*.

Heritability

To achieve the existing variability among the accessions for particular traits, different genetic parameters were estimated (Table 3). In order to assess the heritable portion of total variability, phenotypic variance (σ^2_p) was partitioned into genotypic (σ^2_g) and error variance (σ^2_e), which clearly indicated that variability exists in the accessions, are mainly due to genotypic variance as the error variance values are very less (Yadav et al., 2006).

However, phenotypic coefficient of variation (PCV) was higher than those of genotypic coefficient of variation (GCV) for all traits of accessions, though the differences were very small since the variability in different traits is due to genotypic effects. Therefore, genetic improvement for these traits can easily be achieved by selection of promising plant types and also through crossing the desirable accessions among themselves followed by selection in segregating generations.

The knowledge of heritability of a character is important as, it indicates the possibility and extent to which improvement is possible through selection (Yadav et al, 2006). The heritability estimates were high for all the traits and showed high heritability, $h^2 = 0.99703$, for Palmitoleic acid (C16:1) to 0.99999 for Oleic acid (C18:1) and Linoleic acid (C18:2). Therefore, the fatty acid content (% in oil) was environmentally stable at all locations tested and environment conditions had minor effect on fatty acids content.

Table3. Estimates of variance components and heritability in *Hyoscyamus* accessions.

Traits	δ^2_g	δ^2_e	δ^2_p	heritability	GCV	PCV	CV (%)	F value
C14:0	1.099836	0.000921	1.100757	0.999163	0.285092	0.285211	93	*40268.87
C14:1	0.148537	0.0001	0.148638	0.999326	1.404759	1.405232	97	*2213.814
C16:0	41.31646	0.007186	41.32365	0.999826	1.061358	1.061451	36	*2074.822
C16:1	1.120332	0.003336	1.123668	0.997031	1.312796	1.314749	82	*413.9663
C18:0	7.829991	0.003	7.832991	0.999617	1.01437	1.014564	31	*755.285
C18:1	498.1085	0.00339	498.1118	0.999993	1.281261	1.281265	92	*236942.3
C18:2	2284.511	0.00455	2284.515	0.999998	1.003723	1.003724	47	*352104
C18:3 ω^r	38.98671	0.00396	38.99067	0.999898	1.891555	1.891651	170	*25060.29
C20:0	1.99907	0.000182	1.999252	0.999909	1.517393	1.517462	120	*21675.51
C18:3 ω^l	11.68621	0.002045	11.68826	0.999825	1.957963	1.958134	169	*13491.31
C18:4	1.571737	0.000879	1.572616	0.999441	1.696905	1.697379	138	*3745.595
Oil content	369.4214	0.014762	369.4362	0.99996	0.954653	0.954672	14.73	*1788.491

GCV: Genotypic coefficient of variability; PCV: Phenotypic coefficient of variability; CV: coefficient of variability

*significant at p<0.05

Cluster analysis

The results of cluster analysis based on UPGMA illustrated the distribution of genotypes in three main clusters (Fig 2). Cluster I was divided into two sub-clusters. The h1, h2, h3, h4, h5, h6, h7, h8, h9, h10, h11 and h12 were grouped in the first cluster at the similarity of 0.8. The second cluster consisted of genotype labeled as h14 at the similarity of 0.29. Cluster III comprised of genotype h13 at the similarity of 0.21. *H.pusillus* from Rikan region was close to *H.niger* accessions (h1, h2, h3, h4 and h5). *H.pusillus* from Khalkhal and Miandoab regions were grouped in the same cluster with *H.reticulatus* accessions (h7, h8 and h9). *H.kurdicus* (h14) was classified at the second main cluster. *H.arachnoideus* was classified at the third main cluster.

Genetic variability among the genotypes was estimated using CORR similarity coefficient. Genetic similarity ranged from 0.05 to 0.99 among various comparisons.

It is evident from the dendrogram that ten genotypes h1, h2, h3, h4, h7, h8, h9, h10, h13 and h14 are most distantly related. Hence, these genotypes could be considered for future breeding programs to create higher amount of genetic variability in Iranian germplasm of *Hyoscyamus*.

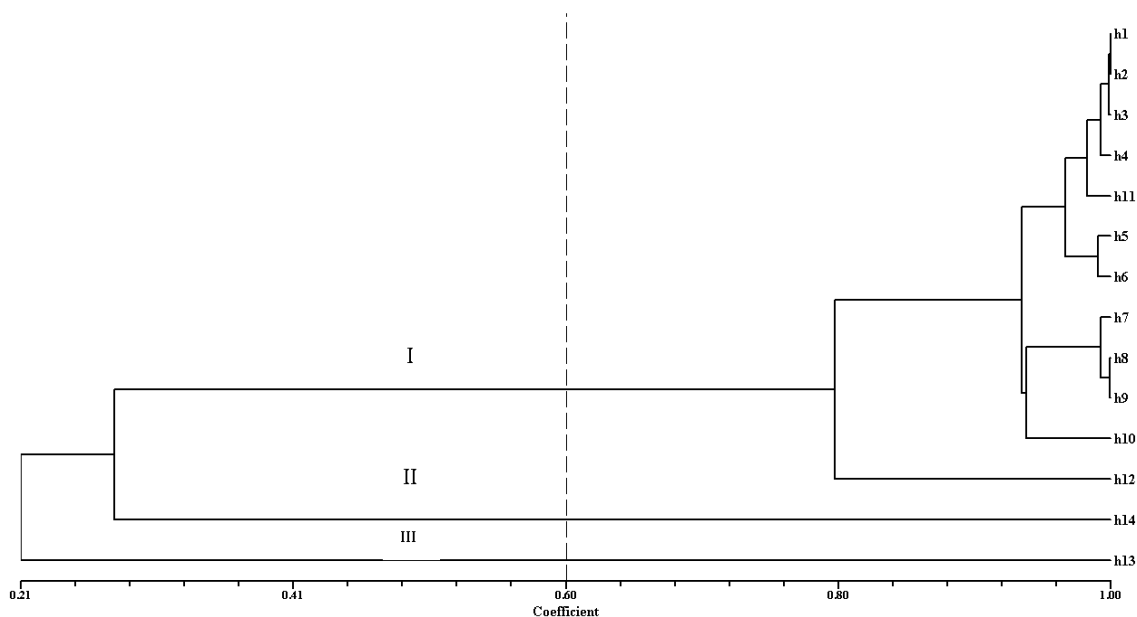


Figure 2. UPGMA dendrogram based on CORR coefficient among *Hyoscyamus* accessions studied.

Correlation coefficient analysis

Correlation analysis was performed to clarify the relations among parameters evaluated in this study (Table 4). As shown in table 4, fatty acid composition varied with locations, significantly. In general, the locations with high temperatures, little rainfall and lower altitudes such as two accessions of *H.niger* from Gazvin & Gilan regions and one accession of *H.reticulatus* from Nagadeh region and also *H.pusillus* from Miandoab region produced high 14:0, 16:0, 16:1, and 18:0, 18:1 ω 9 and 20:0 acids, however, low 18:2 ω 6, 18:3 ω 6 and 18:4 ω 3 acids.

Environmental factors such as climatic have played an important role in change of fatty acid composition and temperature is the most important factor affecting fatty acid composition (Baydar and Turgut, 1999).

Oil percent showed significant positive correlation with six fatty acids: C14:1, C16:1, C18:1 ω 9, C18:2 ω 6, C18:3 ω 3, C18:3 ω 6 and C18:4 ω 3 but negative correlation with C14:0, C16:0, C18:0 and C20:0.

The amount of altitude showed significant positive correlation with C18:2 ω 6, C18:3 ω 3, C18:3 ω 6 and C18:4 ω 3 but negative correlation with C14:0, C14:1, C16:0, C16:1, C18:0, C18:1 ω 9 and C20:0.

Mean annual temperature data showed significant positive correlation with C14:0, C14:1, C16:0, C16:1, C18:0, C18:1 ω 9 and C20:0 but negative correlation with C18:2 ω 6, C18:3 ω 3, C18:3 ω 6 and C18:4 ω 3.

Rainfall value showed significant positive correlation with C18:2 ω 6, C18:3 ω 3, C18:3 ω 6 and C18:4 ω 3 but negative correlation with C14:0, C14:1, C16:0, C16:1, C18:0, C18:1 ω 9 and C20:0.

Beringer (1971) found that low growth temperature (12°C) increases the oil content of oats and results in greater unsaturated fatty acid content compared with higher growth temperature (28°C). Also, Welch (1975) found that low temperatures cause a higher synthesis of unsaturated fatty acids in oats.

Oleoyl desaturase and linoleoyl desaturase enzymes convert Oleic acid to Linoleic and Linoleic acid to Linolenic acids, respectively. The activities of both enzymes have been decreased by high temperatures resulting in the decrease in Linoleic and Linolenic acid synthesis and the increase in Oleic acid synthesis (Pleines and Friedt, 1989). In the light of these findings, it can be concluded that an increase in temperature promotes a higher synthesis of Oleic acid but a lower synthesis of Linoleic acid.

The proportion of saturated to unsaturated fatty acids produced by seeds increases at high temperatures; conversely, more unsaturated fatty acids are produced by the same species at lower temperatures (Canvin, 1965; Linder, 2000). Ecological conditions, variety, location and technical and cultural practices can affect the quality and fatty acid composition of hazelnut (Beyhan et al., 2011). Also, Parcerisa et al (1993) reported that the composition of hazelnut oil is influenced by the geographical origin.

This has been demonstrated in commercial oil seed crops such as castor (*Ricinus communis* L.), sunflower (*Helianthus annuus* L.) and flax seed (*Linum usitatissimum* L.) (Harris and James, 1969; Sobrino et al., 2003), corn (*Zea mays* L.) (Thompson et al., 1973), peanut (*Arachis hypogaea* L.) (Young- Kyoo and Joeng, 1995) and rapeseed (*Brassica napus* L.) (Tremolieres et al., 1982). In sunflowers, this result is attributed to the reduction in activity of desaturase enzymes under high temperatures (Harris et al., 1978).

Oil contents were lowest in canola grown in hotter regions during dry years and highest in cooler and wetter regions. Similarly, Deng and Scarth (1998) reported that the contents of saturated and monounsaturated fatty acids in seed oil increased when the seeds produced under high temperatures.

In general, increase in land elevation corresponds to decreases in environmental temperature and the numbers of the basic physiological metabolic process of plants have been demonstrated. It was reported for a number of crops, such as jojoba or chia, that an increase in growing temperature is related with a decrease in oil content, and an increase in oil saturation with a concomitant decrease in oil unsaturation level (Ayerza 2001, 2009).

There was no study reported about effects of environment on seed oil of *Hyoscyamus* species in the literatures hence, we were not able to compare our results with previous researches.

Table4. Correlation coefficient between pair wise traits: Fatty acids, oil percent, mean annual temperature and rainfall in seeds of *Hyoscyamus* accessions.

Traits	Oil percent	Altitude	Annual temperature	Rainfall
F1	-0.68	-0.23	-0.35	-0.01
F2	0.86	-0.11	-0.04	0.09
F3	-0.93	-0.16	0.02	-0.02
F4	0.27	-0.16	-0.34	-0.37
F5	-0.50	-0.40	-0.12	0.18
F6	0.05	-0.49	0.24	-0.34
F7	0.18	0.63	-0.48	0.22
F8	0.13	0.53	-0.02	-0.11
F9	-0.29	-0.24	-0.12	-0.19
F10	0.11	0.30	-0.05	-0.19
F11	0.13	0.33	-0.05	-0.22

F1: 14:0, F2:14:1, F3: 16:0, F4:16:1, F5:18:1 ω9, F6:18:2 ω6, F7:18:3 ω3, F8:20:0, F9: 18:3 ω6, F10: 18:4 ω3 and F11:18:4

Acknowledgements

The authors have special thanks to Dr. Mozaffarian from the Research Institute of Forests and Rangelands for his kind assistance.

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