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Variation of some seed oil components at altitidunal range in a widely distributed species, *Echium italicum* L. (Boraginaceae) from Turkey

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Abstract

Different seed oil contents were analysed for *E. italicum* L. accessions distributed in different gride squares and altitudes for observation of the intraspecific spatial variations. High levels in total unsaturated fatty acid (85,90 and 86,67%) and low percents in saturated ones (10,31-12,86%) were examined. Maximum level of α-linolenic acid (43,91%) was observed in the accession collected from 937 m (B3). As unusual fatty acids stearidonic and gamma linolenic acids were quantified at maximum levels with 13,72% at 377 m (A5) and 10,28% at 1200 m (B8) respectively. The higher values in linoleic and gamma linolenic acid as omega-6 series were detected in the accessions growing at higher altitudes, but the lower levels examined in α-linolenic and stearidonic acid as omega-3 series. Major components are β-sitosterol (53,31-59,86%), campesterol (24,80-34,72%) and Δ5-avenasterol (3,81-9,07%). Maximum total sterol amount (9960 mg/kg) was detected in A1 accession growing near sea level (21m). Minimum level (5003 mg/kg) was examined in C2(b) accession at 655m. α-tocopherol ranged from 0,40 in A5 (377 m) to 0,94 (mg/100g) in B8 (1200 m). High concentration similar with B8 accession was also detected in A1 accession (0,93 mg/100g) from near sea level (21 m). Obtained results contribute for determining the range of intraspecific variations of bioactive seed oil components of *E. italicum* L. on the altitudinal and environmental gradient, and utility its biomedical product potential as a new crop by means of selection of high yield genotypes for agricultural and biotechnological practices.

1. Introduction

Boraginaceae family includes a variety of shrubs, trees, and herbs, with 148 genera and more than 2,700 species occurring mainly in Europe, Asia and especially well-represented in the Mediterranean region. Especially, FA compositions of seed oils as characteristic parameters have utility in biochemical systematics for delineations of taxa from different hierarchical levels of Boraginaceae [1, 2]. As the valuable sources of some unusual FAs, Boraginaceae is needed to wide-range scanning in terms of taxonomical potential of medicinal and industrial utility, phylogeographical relations, ecological and spatial variations at intraspecific level. Many species are poisonous (e.g. hepatotoxic pyrrolizidine alkaloids in *Echium*), but some species have been used various purposes (*Borago officinalis*, *Lithospermum* etc) and as some important honey plants. *Echium* species with large ecological tolerance has widespread distributional pattern on various soil and habitat conditions of Turkey including sand dunes, limestone slopes, rocky places, steppe, fallow fields, disturbed ground, vast place etc., between sea level and 1,950 m. This species are contained different secondary metabolites (with no iridoid alkaloids) and unusual fatty acids (FAs) such as γ-linolenic acid (18:3n6) (GLA) and stearidonic acid (18:4n:3) (SDA) in some groups. There are very limited number of studies were published for FA

compositions of the seed oils of Turkish Boraginaceae [3,4,2,5,6,7]. Lipid components in seeds are thought to be under evolutionary selection for maximizing germination success, homeostatic control and survival in various abiotic stress conditions such as drought, colder temperature, edaphic factors etc. [8,9]. In our previous observations carried out on *E. italicum* L. populations from Turkey, significantly differences were found based on micromorphological features of the nutlets, RAPD patterns and fatty acid compositions of seed oils along with the latitudinal distributions of the populations. Large distribution of this species in various environmental conditions require high intraspecific variation in seed oil compositions that allow to select valuable allels. On the other hand, as a lipidomic marker, phytosterol composition and quantities are of great importance in taxonomy [10,11] and evaluation of food oil nutritional value as well as in oil quality control. Phytosterols with a wide range of biological activities have received particular attention for their biomedical utility and in human nutrition. Plant sterols have lipid-lowering effect among adults with hypercholesterolemia and effective and safe in treatment of infantile dyslipidemia [12]. Limited number of studies were published on phytosterol compositions of Boraginaceae taxa [13,14]. For the assesment of seed oil quality and authentication, compositions and quantities of tocopherols and tocotrienols known as vitamin E are also important biochemical parameters in addition to confirming taxonomic and phylogenetic relationships in many plant families [15]. High amounts of naturally occurring tocopherols are also valuable for the stabilization of fats and oils against oxidative deterioration in dietary, pharmaceutical and biomedical products [11]. In this study, we aimed to (i) observe intraspesific variations of some seed oil components in *E. italicum* L. populations distributing in the grid squares of three phytogeographical regions of Turkey. Obtained results were also evaluated based on altitudinal gradient in order to (ii) establish the correlation between altitude and some fatty acid ratios of the seed oils. With our observations, it is possible to (iii) select high yield genotypes of *E. italicum* L. for the production of some bioactive components such as SDA, GLA and phytosterols. Up to now, no studies have been conducted for the variations of FA compositions including unusual FAs in addition to fitosterol composition and α-tochopherol concentrations in the seed oils of *E. italicum* L. based on altitudinal gradient.

2. Material and Methods

2.1. Plant material

Nutlet specimens from 7-8 individual of *E. italicum* L. were collected at maturity from each native populations based on altitudinal gradient distributions in A1, A3, A5, A6, B3, B5, B8, B9(a), B9(b), C2(a) and C2(b) according to grid system (Table 1). Total oil, α-tocopherol quantities and fatty acid compositions in five accessions, sterol compositions in eleven accessions were determined. *E. italicum* L. was identified by the classification criteria in the Flora of Turkey (vol. 6) and by comparison with identified specimens from the ISTF (Herbarium of Faculty of Science of Istanbul University). Voucher specimens were deposited with the Division of Botany.

Table 1. *Echium italicum* L. accessions.

Accession	Coordinates	Altitude (M)	Location
A1	40º27’46” N 26º40’43” E	21	Gelibolu-Çanakkale
A3	40º51’23” N 32º29’14” E	1256	Gerede-Eskipazar road
A5	41º37’46” N 34º36’03” E	377	Boyabat-Hanönü road
A6	41º02’10” N 35º54’29” E	722	Samsun-Bolu road
B3	38º01’10” N 30º57’35” E	937	Eğirdir-Gelendost road
B5	39º11’12” N 35º14’22” E	1063	Kayseri-Sorgun road
B8	38º52’59” N 40º52’34” E	1200	Bingöl University campus
B9(a)	38º52’59” N 40º52’34” E	1511	Bitlis-Baykan road
B9(b)	38º28’57” N 42º10’15” E	1800	Bitlis-Tatvan road
C2(a)	37º07’51” N 28º22’29” E	655	Muğla-Ula road
C2(b)	36º48’48” N 29º18’48” E	1220	Fethiye-Korkuteli road

Gas Chromatography Condition

Total oil content was extracted using a Tecator Soxtec System HT (Foss Tecator AB, Horanas Sweden, Sweden). The oil was then transferred to sealed amber glass bottles, which were capped and stored at −18 °C until analysis. The IUPAC standard method was used for the preparation of fatty acid methyl esters (FAME) [16]. The FAME content was quantified by standard methods using a Perkin Elmer Auto System XL Gas chromatograph using FID (Auto system GLX, Shelton, U.S.A.) equipped with an SP™ −2380 (100 m length × 0.25 mm with a 0.25 μm film thickness; Supelco, Bellefonte, U.S.A.). Identification and quantification of FAME by FID response integration were accomplished by comparing the relative retention times of sample peaks with those of authentic standards (Sigma Code No. 189-19, Sigma-Aldrich Co.).

Phytosterol Composition

Sample preparation was performed according to Piironen et al [17] Samples of 0.5 g were saponified with 0.5 ml of saturated aqueous KOH in 8 ml of ethanol acetic acid with shaking (100 cyc/min) at 85 °C for 30 min in the presence of 200 mg of dihydrocholesterol as an internal standard. After addition of 12 ml of water, sterols were extracted into 20 ml of n-heptane/diethyl ether (1/1, v/v) and a 0.5-ml aliquot silylated using 200 ml of 1% TMCS in BSTFA and 200 ml of pyridine (Sigma-Aldrich, Inc.) at 60°C for 30 min. The resulting trimethylsilyl ether derivatives were partitioned into 200 ml of n-heptane after evaporation of the silylation reagent. Qualitative and quantitative analyses of sterol trimethylsilyl ethers were performed on a Perkin Elmer Auto System XL GC (Perkin Elmer Life and Analytical Sciences) equipped with an FID and a 30-m CP-Sil 24 CB capillary column (0.25 mm i.d. and 1.00 mm film thickness, Agilent Technologies). Identification and concentrations, based on proportional peak areas, of sterols were carried out using relative peak retention times compared with those of a standard sterol mixture (Supelco, Inc.).

α-Tocopherol

Determination of α-tocopherol was performed according to the procedure of Manz and Phillipp [18] and the AOAC [19] Official Method (992.03 and 985.30). A homogenized seed sample in a glass flask was boiled in 50 ml of methanol – ascorbic acid solution. KOH solution (5 ml) was added to nitrogen and the mixture mixed, shaken and boiled for 20 min. The resulting saponified sample was shaken at room temperature with 20 – 50 ml of H2O and then 70 ml of diethyl ether. The resulting two phases were separated after vigorous shaking for 1 min. The combined organic phases from three repeat extractions were made up to 250 ml with diethyl ether. H_2O (30–50 ml) was added for removal of remaining KOH and then dried by addition of anhydrous Na_2SO_4. An aliquot of this sample was placed into a volumetric flask, the diethyl ether evaporated at 50°C, and the residue dissolved in 10 –50 ml of n-hexane. High performance liquid chromatography (HPLC) was operated in a programmed manner with 97% n-hexane (No. 104391, Merck KGaA, Darmstadt, Germany) and 31% 4- dioxane (No. 103115, Merck KGaA) as a mobile phase. Detection was performed at excitation and emission wavelengths at 293 and 326 nm, respectively.

Data analysis

Multivariate and correlation (Pearson) analysis of experimental results were performed at the p= 0.05 significance level (SSPS 21).

3. Results and Discussion

All measurements for examined seed oil components were documented in Table 2. Significant differences in the tests of between subjects effects were calculated between the fatty acid values in the accessions collected from different grid squares and altitudes ranged from 21-1800 m ($p<0,05$), except for accession B3 from 937 m ($p=0,201$).

Table 2. The concentrations, total percents and some proportions of fatty acids, sterol and alpha-tocopherol in the seed oils of *E. italicum* accessions collected from different altitudes and grid squares

Component name	A1	A3	A5	A6	B3	B5	B8	B9(a)	B9(b)	C2(a)	C2(b)
Altitude (m)	**21**	**1256**	**377**	**722**	**937**	**1063**	**1200**	**1511**	**1800**	**655**	**1220**
Fatty Acids											
C6:0 Caproic acid	0,02	-	ND	-	ND	-	ND	-	ND	-	-
C8:0 Caprylic acid	0,01	-	ND	-	ND	-	ND	-	ND	-	-
C10:0 Capric acid	0,03	-	ND	-	ND	-	ND	-	ND	-	-
C12:0 Lauric acid	0,04	-	0,01	-	ND	-	ND	-	0,02	-	-
C14:0 Myristic acid	0,21	-	0,06	-	0,04	-	0,07	-	0,07	-	-
C15:0 Pentadecanoic	0,03	-	0,01	-	ND	-	ND	-	0,01	-	-
C16:0 Palmitic acid	8,90	-	8,02	-	6,90	-	7,82	-	8,65	-	-
C16:1 Palmitoleic acid	0,08	-	0,06	-	0,06	-	0,07	-	0,14	-	-
C17:0 Heptadecanoic	0,12	-	0,09	-	0,10	-	0,11	-	0,09	-	-
C18:0 Stearic acid	3,30	-	2,48	-	3.14	-	2,37	-	3,03	-	-
C18:1n9c Oleic acid	18,25	-	14,30	-	13,37	-	13,54	-	19,47	-	-
C18:2n6 Linoleic acid	10,65	-	11,40	-	11,14	-	16,03	-	16,63	-	-
C20:0 Arachidic acid	0,13	-	0,08	-	0,08	-	0,08	-	0,10	-	-
C18:3n6 γ- linolenic acid	6,64	-	7,45	-	6,70	-	10,28	-	8,58	-	-
C20:1 cis-Eicosenoic acid	0,73	-	0,63	-	0,56	-	0,59	-	0,57	-	-
C18:3n3 a-Linolenic a	37,46	-	40,11	-	43,91	-	35,37	-	31,71	-	-
C18:4n3 Stearidonic a	11,77	-	13,72	-	12,70	-	12,00	-	9,34	-	-
C22:0 Behenic acid	0,07	-	0,04	-	0,05	-	0,05	-	0,07	-	-
C22:1n9 Erucic acid	0,16	-	0,14	-	0,10	-	0,14	-	0,12	-	-
C20:3n3 cis-11,14,17	0,04	-	0,03	-	0.03	-	0,02	-	ND	-	-
C24:1 Nervonic acid	0,12	-	0,14	-	0,08	-	0,10	-	0,11	-	-
Saturated FA	12,86	-	10,79	-	10,31	-	10,50	-	12,04	-	
Unsaturated FA	85,90	-	87,92	-	88,65	-	88,14	-	86,67	-	
Mono-unsaturated	19,34	-	15,27	-	14,17	-	14,44	-	20,41	-	
Poly-unsaturated	66,56	-	72,65	-	74,48	-	73,70	-	66,26	-	
Unsaturated/Saturated	6,68	-	8,14	-	8,59	-	8,39	-	7,19	-	
Poly-/Mono-unsaturated	3,44	-	4,75	-	5,25	-	5,10	-	3,24	-	
OL/LA	1,71		1,25		1,20		0,84		1,17		
OA/α-LA	0,48		0,35		0,30		0,38		0,61		
α-LA/ LA	3,52	-	3,51	-	3,94	-	2,20	-	1,90	-	
Omega-3 /Omega-6	2,84	-	2,85	-	3,17	-	1,80	-	1,62	-	
SDA/ GLA	1,77	-	1,84	-	1,89	-	1,16	-	1,08	-	
Total oil (%)	11,24	-	14,96	-	12,75	-	15,70	-	11,72	-	-
Phytosterols											
24-Methylenecholestrol	0,59	1,09	0,63	ND	0,36	0,87	0,72	0,99	0,65	0,85	0,90
Campesterol	24,80	30,83	28,00	27,63	27,71	30,94	34,72	28,14	27,49	31,51	29,59
Campestanol	0,90	0,45	0,85	0,75	0,39	0,92	0,82	0,65	1,08	1,40	0,57
Stigmasterol	5,23	1,50	2,61	1,70	2,46	2,57	1,53	3,50	1,76	1,81	3,44
Δ5,23- stigmastadienone	2,38	3,16	2,32	2,31	1,86	2,56	1,60	3,94	2,77	2,69	2,70
Clerosterol	2,47	3,22	3,08	1,13	2,55	2,93	1,78	3,71	3,58	2,24	2,85
β-sitosterol	56,36	53,37	56,46	56,70	59,86	53,60	53,34	53,63	56,60	54,10	53,31
Sitostanol	1,82	0,03	0,07	0,74	0,40	1,28	1,28	0,98	2,08	1,46	0,84
Δ5-avenasterol	5,49	6,35	6,00	9,07	4,43	4,33	4,66	4,43	4,02	3,81	5,83
Total sterol (mg/kg)	9960	6886	6386	5676	5298	6292	5681	6772	6777	5003	6510
α-Tocopherol (mg/100g)											
α-Tocopherol	0,93	-	0,40	-	0,79	-	0,94	-	0,60	-	-

Each value is the average of triplicate determinations

The higher values in linoleic acid (LA) and GLA as omega-6 series were detected in the accessions growing at higher altitudes, but the lower levels examined in α-linolenic acid (α-LA) and SDA as omega-3 series. While maximum level of α-LA (43,91%) was observed in the accession collected from 937 m (B3), maximum stearidonic acid concentration (13,72%) detected in 377 m (A5) (Figure 1).

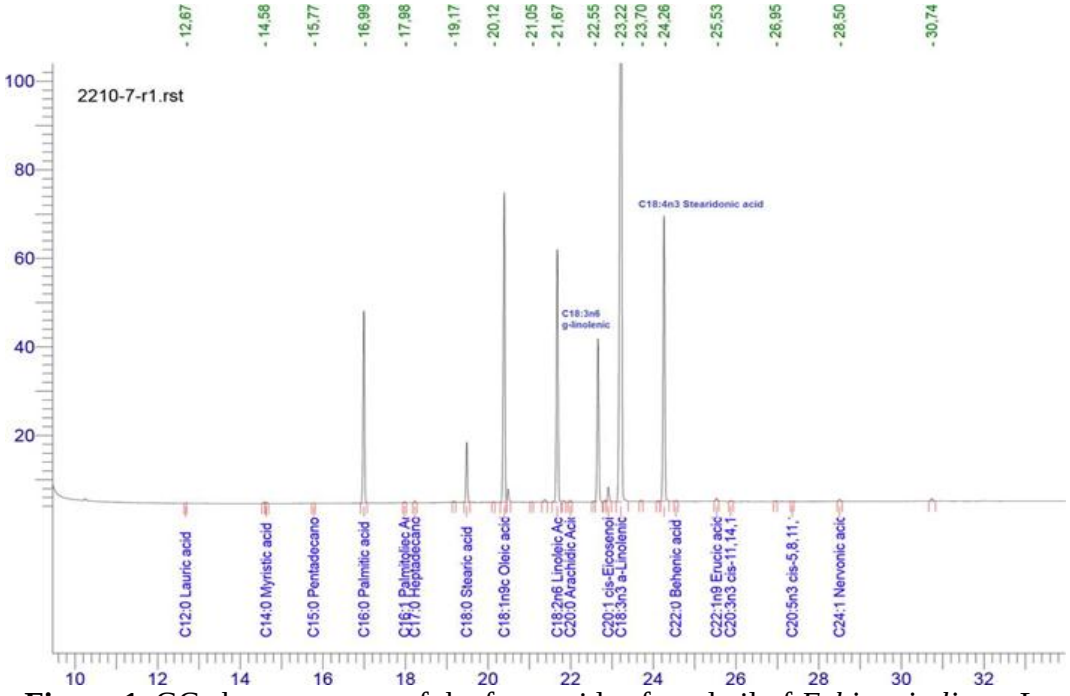

Figure 1. GC chromatogram of the fatty acids of seed oil of *Echium italicum* L.

The other accessions between 377 and 1200 m showed moderate levels of regarding fatty acids. Percentages of mono-unsaturated fatty acid showed parallely higher values (19,34 and 20,41%) in the accessions growing at the lowest (21m) and the highest altitudes (1800m). Contrary, lower percentages of poly-unsaturated FAs were examined in both margin accessions (66,26 and 66,56%), but, relatively higher values at moderate altitudes (72,64-74,48%). While total saturated fatty acid concentrations were observed at relatively higher levels in both margin accessions of the range (12,04 and 12,86%), unsaturated FAs percents are tend to be lower (85,90 and 86,67%) compare to in between accessions (87,92-88,65%) (Figure 3).

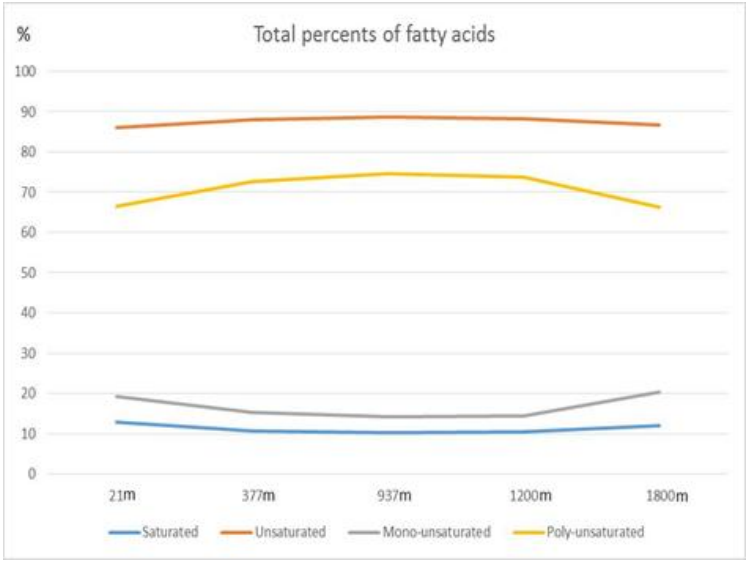

Figure 3. Total percent of fatty acids on the altitudinal gradient.

Some ratios of both margin accessions including unsaturated/saturated (6,68 and 7,19) and poly-/mono-unsaturated FAs (3,24 and 3,44) result in similarly parallel and lower values than the other accessions (Figure 4).

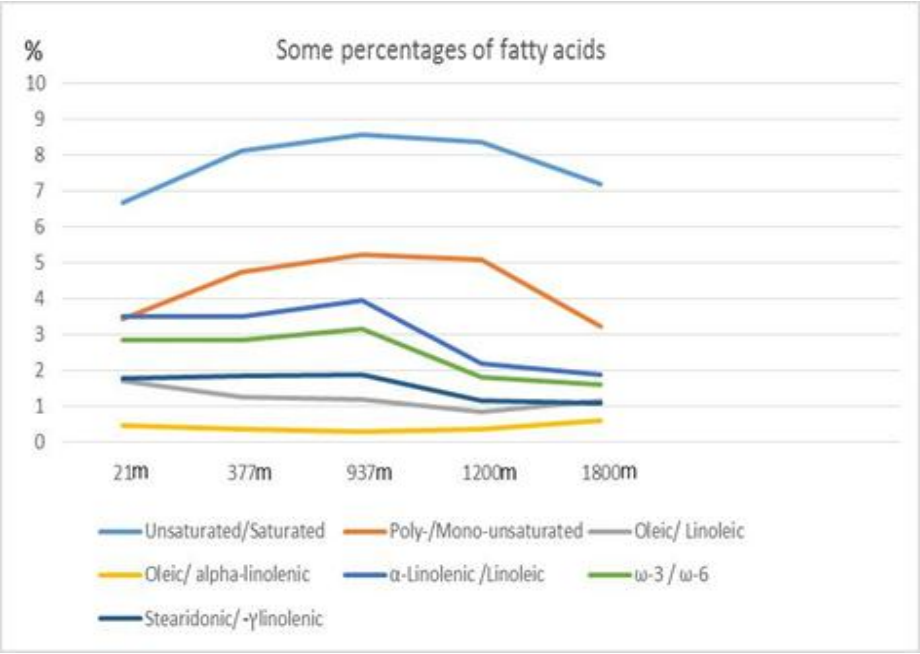

Figure 4. Some percentages of fatty acids on the altitudinal gradient (m).

Apart from fatty acid data, no significant differences between accessions were calculated based on sterol components. Major components are β-sitosterol (53,31-59,86%), campesterol (24,80-34,72%) and Δ5-avenasterol (3,81-9,07%). Stigmasterol (1,50-5,23%), Δ5,23- stigmastadienone (1,86-3,94%) and clerosterol (1,13-3,71%) exhibited lower concentrations (Figure 2).

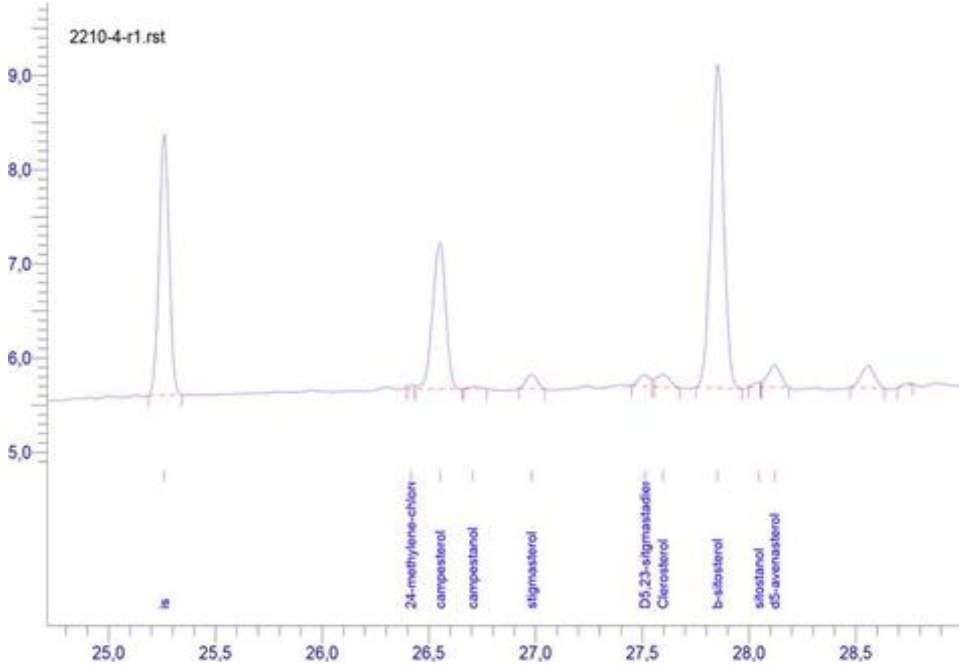

Figure 2. GC chromatogram of sterol components of the seed oil of *Echium italicum* L.

Δ5,23- stigmastadienone and clerosterol are tend to increase generally based on altitudinal gradation. Maximum concentrations of these components were observed in B9(a) accession from 1511 m. No significant correlation was found between fatty acid and sterol components of each accessions on the altitudinal gradient ($p>0,05$). In general, the concentrations of sterol components exhibited horizontal patterns along with altitudinal samplings on the graphic (Figure 5).

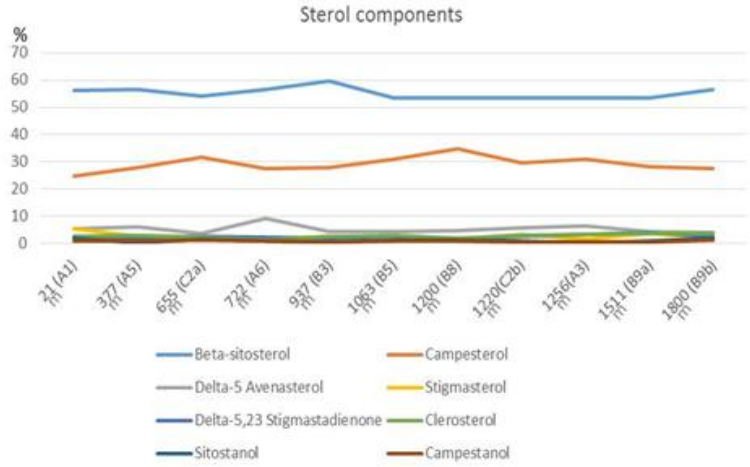

Figure 5. Sterol components based on altitudinal gradient (m)

On the other hand, α-tocopherol ranged from 0,40 in A5 (377 m) to 0,94 (mg/100g) in B8 (1200 m). Similarly high concentration with B8 accession (0,93 mg/100g) was also detected in A1 accession from near sea level (21 m).

The association of seed oil components including FAs, sterols, vitamins etc. with environmental gradient is a viewpoint for defining of some intelligible patterns of seed oil compositions. Little is known about the large-scale spatial (latitudinal, longitudinal and altitudinal) variability of seed oil FAs along with their ecological significance. Very limited number of studies were focused on a few species or cultivars [20, 21, 22]. Obtained data may be also evaluated for establishing taxonomical and phylogenetic relations, and selection of some high yield genotypes by allele mining strategy for the agricultural production in different agro-ecosystems. In the present study, spatial variation of FA, phytosterol and α-tocopherol concentrations were investigated in the seed oils of *E. italicum* L. populations growing in the broad range of ecological habitats of Turkey. Considerable differences and some correlations were observed in the examined traits. Some minor morphological differences in habitus of the collected plants as reported in the Flora of Turkey Edmondson [23], and the size and color of the nutlets are related with the biochemical variations in different habitat conditions [6]. In the five accession, Palmitic Acid (PA) and Oleic Acid (OA) values exhibited similar curve shapes based on altitudinal gradient between 21 and 1800 m. Both FAs have the highest and similar concentrations individually at the each margins of the gradient, while showing lower levels in between accessions. This observation may reflect the homeostatic balances of regarding fatty acids in the seed oils growing in the contrast habitats having completely different climatic conditions. The lower concentrations of these FAs were examined in the accession collected from 937 m (B3), parallel with PA and OA values. In this accession, omega-6 pathway may be downregulated for the benefit of omega-3 production. Because, the highest concentration of ALA and considerably higher concentration of SDA were detected in regarding accession (B3). It may be speculated that Δ-12 desaturase tend to prefer for producing ALA from OA much more efficiently instead of LA in this accession. Biological organisms are known to adjust FA composition according to temperature to maintain ideal membrane fluidity [24]. Increasing of unsaturated fatty acids relative to saturated ones was shown in acclimation to colder temperature for the homeostatic control of membrane fludity [25, 26, 27, 28]. In our observation, increasing ratio of unsaturated FAs to saturated ones was also examined along with altitudinal scala which is related to climatic conditions. It may be hypothesised that genotypic divergency including fatty acid synthesis patway become evident with increasing the effects of environmental stress factors up to alpine zone. Lipids stored in seeds, which are presumably under evolutionary selection to maximize survival and germination success of seeds, are reported to be under natural selection by climatic conditions [27, 8]. Such remarkable patterns may be explained as the respond of similar allelic profiles of the omega-3 pathway to changing climatic conditions. Another way to say, environmental factors are much more efficient in the decreasing of omega-3 fatty acids and their ratios than genetical factors. In a genetic

variability studies in seed biochemical traits of *Pongamia pinnata* (L.) Pierre accessions, significant correlations were reported between concentrations of some fatty acids, and latitude, longitude and altitude gradient distributions, and suggested that phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all the biochemical traits [29]. In our preliminary observations on *E. italicum* L populations in Turkey, high level of polymorphism within species on latitudinal gradient was found based on FA composition, RAPD profiles and micromorphological features of the nutlets, and suggested a segregation of the populations on three phytogeographical regions, in addition to differentiation of the ecological populations. For example, Mediterranean and Irano-Turanian populations exhibited relatively lower levels of SDA (8.93–9.97 %) and GLA (5.36–6.24 %) [6]. In this study, the highest level of SDA (13.72%) was examined in A5 population (at 377 m), parallel with our previous observations in A2(E) from Euro-Siberian phytogeographical region, up to 15.48%. Drought was reported to cause a reduction in seed oil saturated FAs in some species [30], although the response of seed oil fatty acid composition to drought is negligible in some crop cultivars [31]. Comparing with the other *Echium* species, *E. italicum* L. has considerably higher levels of stearidonic acid as an alternative source of terrestrial omega-3 fatty acids [2]. On the other hand, phytosterols including sterols and stanols (saturated form of plant sterols) found in plant cell membranes are biologically active components involved in human metabolism and have great importance in nutrition [32]. A number of products such as yogurts, milk, spreads and margarines are enriched in plant sterols/stanols as functional foods [33,12]. Phytosterol compositions play a critical role in cell membrane fluidity of plant species effecting water and ion exchange for optimal membran function in various habitat conditions [34]. β-Sitosterol is the individual phytosterol at the highest proportion in nearly all plants. The sterol profile for Echium oil was reported to be similar to that of traditional counterparts such as borage, blackcurrant, evening primrose and safflower [35]. In the present study, the highest level of phytosterol component was detected in β-sitosterol (53,31-59,86%) with limited range of variation on the altitudinal ordinate. As the other major component was campesterol between 24,80% (at 21 m) and 34,72% (at 1200 m), implying relation with altitude effect. Critically higher values in stigmasterol (5,23%) at nearly sea level (21 m), while the lowest value (1,50%) at 1256 m (A3) in two accessions of Euro-Siberian populations are considerable. Except for some individual critical values such as highest levels of Δ 5-avenasterol up to 9,07% in A6 accession at 722 m and some lower levels in delta 5,23-stigmastadienone (1,60% at 1200 m) and clerosterol at 722 m (1,13%) comparing to general quantities of each individual components are remarkable findings. Besides, stanol concentrations including sitostanol and campestanol were detected at very low levels with small variations generally. But, a little difference was observed at sitostanol between two accession collected in 21 (1,82%) and 1256 m (0,03%) from Euro-Siberian populations. Comparing with *E. plantagineum*, β-sitosterol has higher concentrations in *E. italicum* L., while lower levels relatively quantified in Δ-5 avenasterol and 24-methylenecholesterol, in addition to similar values in campesterol. In our observation carried out on *Bupleurum* (Umbelliferae) accessions, utility of phytosterol compositions as lipid biomarkers was suggested at specific and sectional levels of this genus [11]. On the other hand, phytosterols commercially are isolated from vegetable oils. They are not synthesized inside the human body. In the accessions of *E. italicum* L., concentratios of some phytosterols such as β-sitosterol and campesterol having activity in balancing the cholesterol in the body are comparable with soybean oil, rapeseed (canola) oil, sunflower oil or corn oil [36]. As a bioactive and therapeutical ingredient, β-sitosterol has large scale utility in various medical cases. Vitamin E is a family of related molecules called tocopherols and tocotrienols. They are antioxidants by virtue of the phenolic hydrogen, and react with the most reactive form of oxygen with protecting unsaturated FAs from oxidation. Seed oils are the most important reserve material for accumulation of vitamin E components compared with other vegetative organs [37]. Wheat germ and sunflower oils (192 mg and 59 mg/100 g respectively) are the best sources of alpha-tocopherol [38]. In different seed oils, concentrations of α-tocopherol exhibited certain differences [39,40]. Beside to genotypic characteristics, ecological factors and maturation periods of the seeds are also important factors in tocopherol accumulation. In the present study, very low levels of alpha-tocopherol were examined between 0,40 and 0.94 mg/100g in *E. italicum* L. accessions. Small variations at lower levels reveal consistent

characteristics of α-tocopherol which is slighltly effected with genotypic and environmental conditions. Considering climatic conditions at higher elevations, lower levels of alpha-tocopherol may provide sufficient protection from oxidation of the seed lipids, or the other components of tocopherols (i.e. β-, γ-tocopherol) and tocotrienols may be much more effective in the process of lipid oxidation. Large scanning is needed to establish correlation between vitamin E components and altitudinal gradient.

Conclusion

Observation on the range of intraspecific variations of seed oil components of *E. italicum* L. may provide information for defining some intelligible patterns correlated with spatial distribution of the populations and ecological variables. Lipidomic analysis at the gene pool level of any species may be useful in understanding the adaptation strategy in extreme habitat conditions, phylogeographic associations of the genotypes, chemotypic segregations at infraspecific levels and selection of suitable genotypes in agro-ecological farming and crop improvement with traditional and biotechnological approachs. SDA and GLA as unusual fatty acids have great importance from nutriceutical and biomedical point of views. Especially, SDA from omega-3 series is a terrestrial alternative for marine sources. Establishing a database with large scanning of *E. italicum* L. accessions distributing various habitat conditions in Anatolia will serve as a reference gene bank for development of omega-3 rich crops and nutraceutical products for human nutrition and health.

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