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Fatty acid composition of the seed oils of selected *Vicia* L. taxa from Tunisia

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Abstract

Whole mature seeds of eight selected varieties, subspecies and accessions of three *Vicia* L. species grown in Tunisia were investigated for their fatty acid (FA) profile. The FA composition ranged from lauric (C12:0) to lignoceric (C24:0) acids. The total FA content was 1235.14 to 1580.34 mg 100 g^{-1} dry matter (DM). Linoleic acid (C18:2 *c*9*c*12; 647.87 to 801.93 mg 100 g^{-1} DM, representing >50% of total FA), oleic acid (C18:1 *c*9; 181.32 to 346.79 mg 100 g^{-1} DM - 13.2 to 24.6% of total FA) and α-linolenic acid (C18:3 *c*9*c*12*c*15; 42.01 to 97.72 mg 100 g^{-1} DM - 3.4 to 7.1% of total FA) were the most abundant unsaturated FA. Palmitic acid (C16:0; 189.86 to 281.07 mg 100 g^{-1} DM - 15.4 to 17.8% of total FA) and stearic acid (C18:0; 24.35 to 52.75 mg 100 g^{-1} DM - 2.0 to 4.0% of total FA) were the major saturated ones. The sum of all other FA did not exceed 3.0% of TFA. The favorable FA profile of the studied vetch seeds makes them interesting cheap diet components to be used in the nutrition of ruminants and non-ruminants reared in the dryland agricultural regions of Mediterranean countries.

Key words: Fatty acids, Mediterranean region, Oilseeds, *Vicia narbonensis*, *Vicia sativa*, *Vicia villosa*

Introduction

The genus *Vicia* L. belongs to the Leguminosae (Fabaceae) family and comprises about 190 species, mainly distributed in temperate areas of both hemispheres. Mediterranean and Irano-Turanian regions represent primary vocation areas for the growth of these plants (van de Wouw *et al*., 2001). Positive agronomic attributes of vetches may be ascribed to their high fodder quality (Larbi *et al*., 2010a) and their ability to preserve/improve soil fertility (Rejili *et al*., 2012).

In Tunisia, three annual species, namely *Vicia sativa* L., *Vicia villosa* Roth. and *Vicia narbonensis* L., are largely cultivated in different bioclimatic areas (semi-arid for *V. sativa*, sub-humid for *V. villosa*, and from subhumid to arid for *V. narbonensis*), particularly in the North of the country and mainly in association with oats (*Avena sativa* L.), for food and fodder production (Hassen and Zoghlami, 2004; Haffani *et al*., 2013).

Vetch seeds are considered valuable sources of protein to be used in animal nutrition (Larbi *et al*., 2010b). Some studies conducted on the potential nutritional value of vetch seeds from several species and cultivars grown in Tunisia showed that their high protein content makes them a cheap natural valid alternative to the more expensive soybean and its derivatives (Selmi *et al*., 2010). The widespread use of vetch seeds makes them also noteworthy sources of lipids for the rations of ruminants and non-ruminants (Kökten *et al*., 2010). The importance of fatty acid (FA) analysis in plant seeds relies in the possibility to select taxa characterized by a favorable FA profile (e.g., high levels of beneficial unsaturated FA) (Ryan *et al*., 2007; Kuhnt *et al*., 2012) which may lead to animal derived food products of enhanced fat quality, and to provide characteristic phenotypic information for the chemotaxonomic characterization and the phylogenetic relationships existing at different taxonomic levels (Bağci and Şahin, 2004; Koçak *et al*., 2011).

The aim of this study was to determine the FA composition of the seeds of selected varieties, subspecies and accessions of three *Vicia* L. species grown in the region of Mateur (North Tunisia) as, despite their extensive use and value as animal feed in the dryland agricultural regions of Mediterranean countries, no such data are currently available.

Materials and methods

Vicia seeds

The biological material consisted of fully matured non heat-treated whole seed samples from three *Vicia* L. species: *V. sativa* L. (common vetch, section *Vicia*), represented by three Tunisian varieties (commune, Languedoc, Mghila) and one subspecies (*amphicarpa* (Dorthes) Asch.); *V. villosa* Roth. (hairy vetch, section *Cracca*), represented by a Tunisian variety (Sejenane) and two accessions (2565 and 3615) introduced from and provided by ICARDA (International Center for Agricultural Research in the Dry Areas) in the frame of a germoplasm exchange; and *V. narbonensis* L. (narbon vetch, section *Narbonensis*). All the seeds were collected in June 2012 from certified material grown in the region of Mateur (North Tunisia) and stocked in cleaned form in the gene bank of INRAT (Institut National de la Recherche Agronomique de Tunisie, Tunis, Tunisia).

Chemical analysis

The samples were ground with a cutting mill (MLI 204 – Bühler AG, Uzwil, Switzerland) and analyzed for their dry matter content (DM) according to the AOAC Official Method 930.15 (AOAC, 2000).

The seed FA composition was assessed using a combined direct transesterification and solid-phase extraction method as described by Alves *et al*. (2008). Fatty acid methyl esters (FAME) were separated and quantified by a high resolution gas chromatograph (Shimadzu GC 2010 Plus, Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan) equipped with a flame-ionization detector, and a CP-Sil 88 capillary column (100 m × 0.25 mm ID, 0.20 µm film thickness; Varian Inc., Lake Forest, CA, USA). Injections were made in on-column mode and the injection volume was 0.5 µL. The temperatures of the injector and the flame-ionization detector were maintained at 250°C and 280°C, respectively. The column temperature was held at 45°C for 5 min, then raised 20°C min^{-1} up to 195°C and maintained for 65 min. Peaks were identified by comparing retention times to pure standards (Restek Corporation, Bellefonte, PA, USA) and by comparison with published chromatograms (Alves *et al.*, 2008). Quantification was assessed by using heptadecanoic acid (C17:0) as internal standard. The results are expressed in absolute values as mg 100 g^{-1} DM and as percentages of total detected FA. All analytical determinations were performed in triplicate.

Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics v.20 for Windows (SPSS Inc., Chicago, IL, USA). Data were subjected to one-way analysis of variance according to the following model:

$X_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where: X_{ij} = observation; μ = overall mean; α_i = effect of variety/subspecies/accession; ε_{ij} = residual error. The Kolmogorov–Smirnov test was used to check dependent variables for normality. Pairwise multiple comparisons (Tukey's test) were performed to test the difference between each pair of means. Significance was declared at $P \leq 0.05$.

Results and discussion

Differences among the analyzed vetch seeds

The DM content and the FA composition of the seeds are reported in Table 1. The considered taxa showed a very similar DM content ($P>0.05$). The FA composition of the seeds ranged from lauric (C12:0) to lignoceric (C24:0) acids. Linoleic (C18:2 *c*9*c*12), oleic (C18:1 *c*9) and palmitic (C16:0) acids were the most abundant ones. Unsaturated fatty acids (UFA) largely predominated over saturated fatty acids (SFA).

The UFA/SFA ratio varied from 3.34 to 4.44 and was significantly different among the considered seeds ($P \leq 0.001$). The concentration of total polyunsaturated fatty acids (PUFA) was from 2.2- to 4.6-fold higher than that of total monounsaturated fatty acids (MUFA).

Polyunsaturated fatty acids

Linoleic acid was predominant, comprising more than 50% of total fatty acids (TFA) in all the samples. *V. sativa* subsp. *amphicarpa* and *V. villosa* accessions 2565 and 3615 showed higher concentrations of linoleic acid compared to *V. narbonensis* ($P \leq 0.01$), while intermediate values were detected for the other seeds.

Alpha-linolenic acid (C18:3 *c*9*c*12*c*15) was also well represented, being the third most abundant UFA, after linoleic and oleic acids, in all the samples here analyzed. The concentration of α-linolenic acid was comparable among the studied seeds, with the exception of *V. narbonensis* which showed approximately half values as much as all the other vetches.

Besides linoleic and α-linolenic acids, other detected PUFA were γ-linolenic (C18:3 *c*6*c*9*c*12), eicosadienoic (C20:2 *c*11*c*14) and arachidonic (C20:4 *c*5*c*8*c*11*c*14) acids. All of them were detected only in traces. Stearidonic acid (C18:4 *c*6*c*9*c*12*c*15), a promising precursor of the endogenous synthesis of long-chain n3 FA in both animals and humans (Kuhnt *et al*., 2012), was not detected. In the current study, γ-linolenic acid was detected in all the seeds with the exception of *V. narbonensis*. The concentration of γ-linolenic acid in *V. sativa* subsp. *amphicarpa* was significantly higher if compared to *V. sativa* var. commune and var. Mghila ($P \leq 0.01$); the other seeds showed intermediate values. Eicosadienoic acid was detected in all the analyzed seeds and its concentration significantly varied among the considered taxa ($P \leq 0.001$). The highest amount was found in *V. sativa* var. Mghila, being different from the concentrations recorded in *V. villosa* var. Sejenane, acc. 2565 and acc. 3615, and *V. sativa* var. commune; the latter showed the lowest absolute concentration. Arachidonic acid was detected only in *V. sativa* subsp. *amphicarpa* and *V. villosa* var. Sejenane with relatively low and comparable concentrations.

No significant differences among the considered seeds were found in the n6/n3 PUFA ratio, with the exception of *V. narbonensis* which showed almost doubled values than the other seeds ($P \leq 0.001$). The n6/n3 FA ratio is commonly used to assess the nutritional value of lipids for human consumption. A strong imbalance towards high dietary intakes of n6 FA at the expense of n3 FA is positively correlated with a number of widespread human diseases. An optimal n6/n3 FA ratio should vary between 1:1 and 4:1, but Western diets may reach ranges of 10:1 to 20:1 (Simopoulos, 2011). None of the studied vetch seeds fell within the above-mentioned optimum recommended values.

Monounsaturated fatty acids

Compared to all other detected FA, oleic acid showed the greatest differences among the studied seeds. It ranked second after linoleic acid in the seeds of *V. villosa* accessions 2565 and 3615 and *V. narbonensis*, the latter showing the highest absolute concentration. The seeds of *V. sativa* var. commune, var. Languedoc, subsp. *amphicarpa* and *V. villosa* var. Sejenane showed significantly lower concentrations of oleic acid if compared to *V. narbonensis* and *V. villosa* accessions 2565 and 3615 ($P \leq 0.001$). Moreover, *V. villosa* acc. 2565 and *V. sativa* var. Mghila showed significantly lower values than *V. villosa* acc. 3615. No significant differences were instead observed in the concentration of oleic acid between *V. narbonensis* and *V. villosa* acc. 2565, or between the latter and *V. sativa* var. Mghila. The oleic/linoleic FA ratio was always less than one, ranging from 0.23 to 0.47.

Except for oleic acid, all other detected MUFA [*trans*-3-hexadecenoic acid (C16:1 *t*3), palmitoleic acid (C16:1 *c*9), *cis*-vaccenic acid (C18:1 *c*11) and eicosenoic acid (C20:1 *c*11)] were present only in traces in the seeds. Their sum accounted for approximately 1% of TFA. Even if at low levels, they were detected in all the analyzed samples. *V. villosa* acc. 3615 was significantly richer in *trans*-3-hexadecenoic acid than the other taxa (P≤0.001). *V. villosa* acc. 3615 showed significantly higher levels of *cis*-vaccenic acid with respect to the other seeds (P≤0.001). The lowest absolute concentration of *cis*-vaccenic acid was observed in the seeds of *V. sativa* subsp. *amphicarpa*, being significantly different from those recorded for *V. villosa* accessions 2565 and 3615 and *V. sativa* var. Mghila. Palmitoleic acid did not show any significant differences among the considered seeds. Regarding eicosenoic acid (a n9 very long chain MUFA), the seeds of *V. sativa* subsp. *amphicarpa*, *V. villosa* var. Sejenane and *V. narbonensis* showed very similar concentrations, which were significantly higher if compared to those of *V. sativa* var. commune. The other seeds showed intermediate amounts.

Erucic (C22:1 *c*13) and nervonic (C24:1 *c*15) acids were not detected in the seeds analyzed in this study.

Saturated fatty acids

Considering all detected FA, palmitic acid ranked second after linoleic acid (in *V. sativa* var. commune, var. Languedoc, var. Mghila, subsp. *amphicarpa* and *V. villosa* var. Sejenane) or third after linoleic and oleic acids (in *V. narbonensis* and *V. villosa* accessions 2565 and 3615). The concentration of palmitic acid in *V. villosa* acc. 3615 significantly differed from that of all other seeds ($P \leq 0.001$), with the exception of *V. villosa* acc. 2565. The latter showed a concentration of palmitic acid which significantly differed only from that recorded in *V. narbonensis*.

The second most abundant SFA was stearic acid (C18:0) in all the seeds. *V. sativa* var. Mghila and *V. villosa* var. Sejenane showed significantly higher values of stearic acid if compared to *V. narbonensis* and *V. villosa* accessions 2565 and 3615 ($P \leq 0.001$). *V. narbonensis* showed the lowest absolute concentration, being significantly different from all the other seeds except for *V. villosa* acc. 3615.

The sum of all other detected SFA [lauric (C12:0), myristic (C14:0), arachidic (C20:0), behenic (C22:0) and lignoceric (C24:0) acids] did not exceed 23.29 mg 100 g^{-1} DM, that is 1.74% of TFA. Low molecular weight SFA, such as lauric and myristic acids, were found in all the samples. Odd-chain SFA [pentadecanoic (C15:0), heptadecanoic (C17:0) and nonadecanoic (C19:0) acids] were not detected in the current study. Among the considered seeds no significant differences were observed in the concentration of lauric acid. On the contrary, myristic acid varied significantly: *V. sativa* var. Mghila showed the absolute highest concentration being significantly different ($P \leq 0.05$) from the concentrations recorded in *V. villosa* acc. 2565 and *V. narbonensis*. The other vetch seeds showed intermediate amounts.

Long-chain SFA levels significantly differed among the considered vetches. The concentration of arachidic acid was significantly higher ($P \leq 0.001$) in the seeds of *V. sativa* var. commune, var. Languedoc, var. Mghila, subsp. *amphicarpa* and *V. villosa* var. Sejenane if compared to *V. narbonensis* and *V. villosa* accessions 2565 and 3615. The highest and lowest absolute concentrations of behenic acid were observed in *V.*

sativa subsp. *amphicarpa* and *V. narbonensis*. Lignoceric acid was not detected in the seeds of *V. narbonensis* and *V. villosa* accessions 2565 and 3615. The other vetch seeds showed significant differences (P≤0.01) in the concentration of lignoceric acid. The highest value was detected in *V. villosa* var. Sejenane, being twice as much as that recorded in *V. sativa* var. commune. The latter showed the absolute lowest concentration.

Compared to the other taxa, the seeds of *V. narbonensis* showed a significantly lower total SFA concentration (P≤0.01).

It is known that several factors, such as genetics, geographical location, climatic settings, growing conditions and post-harvest treatments, may affect the content of FA in the seed oils of many plants (Johansson *et al*., 2000; Khan *et al*., 2012). Environmental-based factors are likely not to be significant contributors of the observed variations in seed FA among the analyzed *Vicia* taxa, as all the seeds were collected in a short period of time from the same geographical region and grew under similar climatic conditions and soil features. Therefore we conclude that genetic predisposition had a major impact on the observed variations. *V. narbonensis* provided the most considerable differences among the studied taxa, despite the lower taxonomic distance (based on morphological, cytological, biochemical, and molecular approaches) existing between *V. sativa* and *V. narbonensis* (both belonging to subgenus *Vicia*) if compared to those existing between *V. sativa* or *V. narbonensis* and *V. villosa* (the latter belonging to subgenus *Cracca*) (Mirali *et al*., 2007; Leht, 2009; Schaefer *et al*., 2012). Such hypothesis seems to be also confirmed by the results obtained in other studies where vetch seeds were collected in restricted geographical areas (Kokten *et al.*, 2010; Emre *et al.*, 2011).

Comparison with the literature data

The DM content of the analyzed seeds was comparable to previously reported literature data (Yu *et al.*, 2001; Seifdavati *et al.*, 2013).

A comparison among the mean FA percentages obtained in this study for *V. sativa* and *V. narbonensis* to data found by other authors is presented in Figure 1. To the best of our knowledge, for *V. villosa* no literature data of the seed FA profile is currently available.

Higher UFA than SFA, as well as higher PUFA than MUFA levels, were reported in the seeds of various wild and cultivated legumes in different ecological and geographical areas (Grela and Günter, 1995; Maestri *et al.*, 2002; Bağci *et al.*, 2004; Bağci and Şahin, 2004; Bağci, 2006; Yoshida *et al.*, 2007; Pastor-Cavada *et al.*, 2009a, 2009b; Kökten *et al.*, 2010; Koçak *et al.*, 2011).

The percentages of total UFA were comparable to those previously reported for other species of the genus *Vicia* (71.0 to 92.2%) (Bağci *et al.*, 2004; Pastor-Cavada *et al.*, 2009a; Kökten *et al.*, 2010; Emre *et al.*, 2011), including the species here studied. The obtained percentages were also comparable to those of the seeds of other related genera of the tribe Fabeae, such as *Lathyrus* L. (56.1 to 86.7%) (Bağci *et al.*, 2004; Bağci and Şahin, 2004; Pastor-Cavada *et al.*, 2009b; Emre *et al.*, 2010), *Lens* Mill. (73.7 to 82.5%) (Ryan *et al.*, 2007; Pastor-Cavada *et al.*, 2009b) and *Pisum* L. (75.9 to 85.3%) (Ryan *et al.*, 2007; Yoshida *et al.*, 2007; Pastor-Cavada *et al.*, 2009b; Renna *et al.*, 2012), which are used as a protein source in animal and human nutrition. The seeds of some vetches grown in the Sivas region of Turkey (namely *V. cracca*, *V. hyrcanica*, *V. galilaea* and *V. faba*) were however reported to contain <60% of total UFA (Akpinar *et al*., 2001).

The obtained percentages of total SFA were similar to those reported by Kökten *et al*. (2010) for six vetch species (18.0 to 22.4%), but slightly higher if compared to the 10-20% total SFA levels generally found by Bağci *et al*. (2004) for legume seeds.

Linoleic-oleic, linoleic-palmitic and linoleic-oleic-palmitic types FA patterns are known to be typical of many leguminous genera (Bağci *et al*., 2004). This was also confirmed by the preponderance of these three fatty acids in the analyzed Tunisian vetch seeds.

Polyunsaturated fatty acids

In the current study, the observed variations in the linoleic acid percentages among vetch seeds were less pronounced (50.75 to 57.53% of TFA) if compared to those reported in other published works. Pastor-Cavada *et al*. (2009a) and Bağci *et al*. (2004) reported more than double levels of linoleic acid (28.7 to 66.3% of TFA and 20 to 50% of TFA, respectively) among the vetch species considered in their respective studies. On a whole, linoleic acid was usually found to be the most abundant FA in vetches (Bağci *et al*., 2004; Yoshida *et al*., 2008; Pastor-Cavada *et al*., 2009a; Kökten *et al*., 2010), with few exceptions reported (Akpinar *et al*., 2001; Bağci *et al*., 2004; Pastor-Cavada *et al*., 2009a). High levels of linoleic acid are also known to be typical of the seeds of many other legumes (Maestri *et al*., 2002; Bağci *et al*., 2004; Yoshida *et al*., 2007; Pastor-Cavada *et al*., 2009b; Emre *et al*., 2010; Koçak *et al*., 2011).

Alpha-linolenic acid was found to be one of the most variable FA components in legume seeds (Bağci *et al.*, 2004). It was reported as the major FA in *V. michauxii* var. stenophylla, but more usually as the third most abundant UFA (after linoleic and oleic acids) in other vetch species (Bağci *et al.*, 2004), as also occurred in the current study. Many vetches were reported to contain less than 15% α-linolenic acid in their seeds (Akpinar *et al.*, 2001; Bağci *et al.*, 2004; Pastor-Cavada *et al.*, 2009a). Exceptions regarded few species or varieties such as *V. articulata* (16.6% of TFA) and *V. pubescens* (16.6%) (Pastor-Cavada *et al.*, 2009a), *V. ervilia* (19.7%) and *V. hybrida* (22.0%) (Kökten *et al.*, 2010) and particularly *V. michauxii* var. stenophylla (39.1%) (Bağci *et al.*, 2004). As found in the analyzed Tunisian *V. narbonensis* seeds, quite low α-linolenic acid levels (3-4% of TFA) in such species were also previously obtained by other authors (Pastor-Cavada *et al.*, 2009a; Kökten *et al.*, 2010).

The absence of stearidonic acid in the analyzed vetch seeds confirms previously published data for *V. sativa* and *V. narbonensis* oilseeds. On the contrary γ-linolenic acid, which is known to possess a therapeutic value (being able to modulate inflammatory responses) (Kapoor and Huang, 2006), was not reported in vetch seeds in previously published papers, but it was found in traces in *V. sativa* and *V. villosa* oilseeds in the current study.

Considering the vetch seeds studied by Bağci *et al.* (2004), eicosadienoic acid was detected only in one out of six species analyzed, with a percentage (0.1% of TFA) comparable to those obtained in our trial. Conversely, Akpinar *et al.* (2001) did not detect eicosadienoic acid in the seeds of *V. hybrida*, but they found a large variation in the levels of this FA (0.38 to 10.9% of TFA) among the remaining seven studied vetch species. These authors reported 9.25% eicosadienoic acid in the seeds of *V. sativa*, a value notably higher if compared to the range values (0.06 to 0.13% of TFA) found in our study. The same authors also reported notable amounts of arachidonic acid (1.23 to 6.83% of TFA) in the seeds of all examined species, which contrasts with the relatively low levels of this FA found in just two Tunisian vetch seeds in the current trial.

Monounsaturated fatty acids

Oleic acid was found to be the most abundant FA in the seeds of *V. cassubica*, *V. cracca*, *V. hyrcanica*, *V. peregrina*, *V. hybrida*, *V. sativa*, *V. galilaea* and *V. faba* by Akpinar *et al*. (2001) and in the seeds of *V. articulata* by Pastor-Cavada *et al*. (2009a). However, the oleic/linoleic FA ratio was usually reported to be less than one in the seeds of many species of the genus *Vicia* (Pastor-Cavada *et al*., 2009a) or other genera of the Leguminosae family (Maestri *et al*., 2002). As obtained in the current study, high levels of oleic acid in *V. narbonensis* seeds were already detected in different Mediterranean regions (Bağci *et al*., 2004; Pastor-Cavada *et al*., 2009a; Kökten *et al*., 2010; Emre *et al*., 2011).

The other monoenoic FA detected in the current study were either not reported (C16:1 *t*3), found in traces (C16:1 *c*9 and C20:1 *c*11) or only in small amounts (C18:1 *c*11) in the seeds of legume species, including those belonging to the genus *Vicia* (Maestri *et al*., 2002; Bağci *et al*., 2004; Bağci, 2006; Pastor-Cavada *et al*., 2009a, 2009b; Kökten *et al*., 2010; Koçak *et al*., 2011). The presence of *trans*-3-hexadecenoic acid was previously found to occur in the seeds of some Asteraceae (Hopkins and Chisholm, 1964; Morris *et al*., 1968) and, in general, in photosynthetic systems (Harwood and James, 1975). As occurred in our study, *cis*-vaccenic acid was usually found at higher levels if compared to palmitoleic and eicosenoic acids in different legume seeds (Bağci *et al*., 2004; Bağci, 2006).

The occurrence of erucic acid in vetch seeds was previously reported by Akpinar *et al*. (2001), who found percentages varying from 0.23 (in *V. hyrcanica*) to 3.01% of TFA (in *V. hybrida*), with *V. sativa* presenting levels slightly less than 1% of TFA. Bağci *et al*. (2004) revealed the occurrence of low erucic acid levels in some legumes, but not in vetch seeds. In accordance with the latter authors, erucic acid was not detected in the Tunisian vetch seeds here analyzed. Such a result seems to be of quite importance as erucic acid was reported to exert negative effects on animal and human metabolism, so that the government regulation of the European Union limits its levels for human consumption to a maximum of 5% (Kuhnt *et al*., 2012). Nervonic acid, another n9 very long chain MUFA known to derive from erucic acid, was never reported as lipid constituent in vetch seeds in previously published works, a result which is also confirmed in our study.

Saturated fatty acids

Palmitic acid is a steady lipid constituent in the seeds of various genera of the Leguminosae family (Bağci *et al*., 2004; Koçak *et al*., 2011). Confirming this, the range of palmitic acid variation among the seeds analyzed in the current study was also relatively low (15.37 to 17.79% of TFA).

As occurred in the considered Tunisian vetch seeds, various other vetches were previously found to contain stearic acid as second most abundant SFA in their seeds (Akpinar *et al*., 2001; Bağci *et al*., 2004; Pastor-Cavada *et al*., 2009a; Emre *et al*., 2011). The majority of the species of the genus *Vicia* were reported to contain less than

6.0% stearic acid, with some exceptions such as *V. pubescens* (7.5% of TFA), *V. cracca* (13.2%), *V. hyrcanica* (19.4%), *V. peregrina* (7.26%), *V. hybrida* (9.13%), *V. sativa* (7.31%), *V. galilaea* (15.94%) and *V. faba* (9.03%) (Akpinar *et al*., 2001; Pastor-Cavada *et al*., 2009b). The percentages of stearic acid obtained in our study were also similar to those previously reported for the seeds of other leguminous genera which can be used in animal and human nutrition, such as *Hedysarum*, *Lathyrus*, *Gonocytisus*, *Lupinus*, *Trigonella*, *Onobrychis*, *Lens*, *Pisum* and *Astragalus* (Bağci *et al*., 2004; Bağci, 2006; Pastor-Cavada *et al*., 2009b; Renna *et al*., 2012).

Low molecular weight SFA, such as lauric and myristic acids, were found in all the samples analyzed, as previously reported by Akpinar *et al*. (2001). The presence of odd-chain FA was noticed in some vetch seeds in other trials (Akpinar *et al*., 2001; Pastor-Cavada *et al*., 2009a). Lauric, myristic and pentadecanoic acids were not usually found or found only in traces in the seeds of other leguminous genera (Bağci *et al*., 2004).

Long-chain SFA (arachidic, behenic and lignoceric acids) were not usually found or found at low levels (<1.5% of TFA) in vetch seeds (Bağci *et al*., 2004; Pastor-Cavada *et al*., 2009a; Kökten *et al*., 2010), with only few species (mainly *V. cracca*, *V. peregrina*, *V. hybrida* and *V. galilaea)* showing more than double amounts (Akpinar *et al*., 2001). Such findings are interesting from a nutritional point of view as oils with high levels of long-chain SFA were reported to be difficult to digest in both humans and animals (Akpinar *et al*., 2001).

On a whole, Figure 1 shows that great differences exist among the studies regarding both the number of detected FA and the relative percentage of each FA relative to the TFA content. Such discrepancies may be partly explained by the different levels of accuracy in FA analysis applied in the studies. Variations in the ecological and geographical zones where the seeds were collected may also have exerted a key role as it is known, as above mentioned, that the environment can significantly affect the synthesis of FA in plants (Akpinar *et al*., 2001; Mao *et al*., 2012).

Conclusions

In the studied vetch seeds the major FA ranked in the following order: C18:2 *c*9*c*12 > C16:0 > C18:1 *c*9 [or C18:1 *c*9 > C16:0, depending on the considered subspecies/variety/accession] > C18:3 *c*9*c*12*c*15 > C18:0, which is consistent with data reported in the available literature for leguminous seeds. From a qualitative perspective, oleic, stearic, linoleic and α-linolenic acids (among individual FA) and total MUFA (among FA groups), were the most useful parameters for highlighting interspecies variability among the seeds. Arachidic acid, expressed as percentage of total detected FA, seems to be helpful to show up intraspecies variability for the three varieties/accessions of *V. villosa*. Characteristic phenotypic information was provided by i) arachidonic acid, which was only detected in the seeds of *V. sativa* subsp. *amphicarpa* and *V. villosa* var. Sejenane, and ii) lignoceric acid, which was not detected in the seeds of *V. villosa* acc. 2565 and acc. 3615. The seeds of *V. narbonensis* drew away from those of the other studied vetches, essentially due to i) their high levels of oleic acid, total MUFA, UFA/SFA and n6/n3 PUFA ratios, ii) their low levels of palmitic acid and total SFA, and iii) the absence of γ-linolenic acid.

The analyzed vetch seeds are a valuable source of UFA (both mono- and polyunsaturated ones), whose levels are comparable to those of other edible seeds. Such a favorable FA profile and the high protein levels make these seeds interesting cheap diet components for animal nutrition. Due to the higher concentration of the sum of linoleic, α-linolenic and γ-linolenic acids (about 890 mg $100g^{-1}$ DM), the seeds of *V. sativa* subsp. *amphicarpa* and *V. villosa* accession 3615 may be the most effective, among the studied ones, in improving the quality of the lipid fraction of ruminant-derived food products (raise in the content of beneficial FA such as vaccenic and conjugated linoleic acids).

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Table 1. Fatty acid composition (mg 100 g^{-1} DM and % of TFA) of the seeds of selected *Vicia* L. taxa grown in Tunisia.[a]

	V. sativa				*V. villosa*			*V. narbonensis*		
	var. commune	var. Languedoc	var. Mghila	subsp. *amphicarpa*	var. Sejenane	acc. 2565	acc. 3615	-	SEM	Sig.[b]
DM g kg^{-1}	894.7	895.0	893.3	893.8	892.4	894.8	896.6	894.1	0.43	ns
C12:0										
mg 100 g^{-1} DM	0.84	0.85	1.14	0.97	0.84	1.00	1.00	0.86	0.032	ns
% TFA	0.07	0.07	0.09	0.07	0.07	0.07	0.06	0.07	0.002	ns
C14:0										
mg 100 g^{-1} DM	2.97 ab	3.04 ab	3.46 a	2.88 ab	2.96 ab	2.54 b	3.08 ab	2.36 b	0.090	*
% TFA	0.23 ab	0.24 ab	0.26 a	0.21 bc	0.23 ab	0.18 c	0.19 bc	0.19 bc	0.007	**
C16:0										
mg 100 g^{-1} DM	219.92 bc	221.47 bc	225.73 bc	222.11 bc	224.44 bc	250.56 ab	281.07 a	189.86 c	6.629	***
% TFA	17.07 ab	17.20 ab	16.89 ab	16.15 bc	17.50 a	17.52 a	17.79 a	15.37 c	0.201	***
C16:1 *t*3										
mg 100 g^{-1} DM	0.58 b	0.59 b	0.66 b	0.32 b	0.52 b	0.79 b	1.87 a	0.70 b	0.116	***
% TFA	0.05 b	0.05 b	0.05 b	0.02 b	0.04 b	0.06 b	0.12 a	0.06 b	0.007	***
C16:1 *c*9										
mg 100 g^{-1} DM	0.68	0.51	0.60	0.45	0.48	0.52	0.44	0.50	0.034	ns

% TFA	0.05	0.04	0.05	0.03	0.04	0.04	0.03	0.04	0.003	ns
C18:0										
mg 100 g^{-1} DM	47.78 ab	46.98 ab	52.75 a	45.38 ab	49.25 a	39.70 bc	32.01 cd	24.35 d	2.374	***
% TFA	3.71 a	3.65 ab	3.95 a	3.30 b	3.84 a	2.77 c	2.02 d	1.97 d	0.194	***
C18:1 *c*9										
mg 100 g^{-1} DM	203.63 d	181.68 d	220.24 cd	181.32 d	192.19 d	262.17 bc	346.79 a	303.29 ab	15.079	***
% TFA	15.80 d	14.11 ef	16.48 d	13.19 f	14.99 de	18.35 c	21.92 b	24.55 a	0.962	***
C18:1 *c*11										
mg 100 g^{-1} DM	7.98 cd	8.27 cd	8.73 bc	7.05 d	7.58 cd	10.23 b	12.45 a	8.25 cd	0.427	***
% TFA	0.62 bc	0.64 abc	0.65 abc	0.51 c	0.59 bc	0.72 ab	0.79 a	0.67 ab	0.021	**
C18:2 *c*9*c*12 (n6)										
mg 100 g^{-1} DM	699.10 ab	717.17 ab	713.18 ab	791.18 a	695.18 ab	761.06 a	801.93 a	647.87 b	13.589	**
% TFA	54.27 bc	55.68 ab	53.37 c	57.53 a	54.21 bc	53.21 c	50.75 d	52.46 cd	0.502	***
C18:3 *c*6*c*9*c*12 (n6)										
mg 100 g^{-1} DM	0.74 bc	0.86 abc	0.71 c	1.00 a	0.93 ab	0.84 abc	0.86 abc	nd	0.028	**
% TFA	0.06	0.07	0.05	0.07	0.07	0.06	0.05	-	0.002	ns
C18:3 *c*9*c*12*c*15 (n3)										
mg 100 g^{-1} DM	84.53 a	84.74 a	84.97 a	97.72 a	83.43 a	84.06 a	85.28 a	42.01 b	4.034	***
% TFA	6.57 ab	6.58 ab	6.36 ab	7.10 a	6.51 ab	5.87 bc	5.40 c	3.40 d	0.283	***
C20:0										
mg 100 g^{-1} DM	12.77 a	12.90 a	14.62 a	13.13 a	13.48 a	9.71 b	7.26 b	8.58 b	0.655	***
% TFA	0.99 a	1.00 a	1.09 a	0.95 a	1.05 a	0.68 b	0.46 c	0.69 b	0.055	***

C20:1 *c*11										
mg 100 g^{-1} DM	2.79 b	3.28ab	3.75 ab	3.92 a	3.89 a	3.61 ab	3.74 ab	3.93 a	0.106	*
% TFA	0.22 b	0.25 ab	0.28 ab	0.28 ab	0.30 a	0.25 ab	0.24 ab	0.32 a	0.009	*
C20:2 *c*11*c*14 (n6)										
mg 100 g^{-1} DM	0.82 d	1.33 abc	1.69 a	1.37 ab	1.06 bcd	1.02 bcd	0.83 cd	1.25 abcd	0.075	***
% TFA	0.06 bc	0.10 ab	0.13 a	0.10 ab	0.08 abc	0.07 bc	0.05 c	0.10 ab	0.006	**
C20:4 *c*5*c*8*c*11*c*14 (n6)										
mg 100 g^{-1} DM	nd	nd	nd	1.07	1.22	nd	nd	nd	0.083	ns
% TFA	-	-	-	0.08	0.10	-	-	-	0.008	ns
C22:0										
mg 100 g^{-1} DM	1.98 bc	2.59 ab	2.16 bc	2.91 a	2.54 ab	2.10 bc	1.76 cd	1.34 d	0.124	***
% TFA	0.15 bcd	0.20 ab	0.16 bc	0.21 a	0.20 ab	0.15 cd	0.11 d	0.11 d	0.010	***
C24:0										
mg 100 g^{-1} DM	1.13 c	1.66 bc	1.91 ab	2.39 ab	2.47 a	nd	nd	nd	0.171	**
% TFA	0.09 b	0.13 ab	0.14 ab	0.17 a	0.19 a	-	-	-	0.013	**
ΣSFA										
mg 100 g^{-1} DM	287.38 a	289.49 a	301.76 a	289.77 a	295.96 a	305.60 a	326.17 a	227.35 b	7.217	**
% TFA	22.31 abc	22.48 ab	22.58 ab	21.07 cd	23.07 a	21.37 bcd	20.64 d	18.40 e	0.368	***
ΣMUFA										
mg 100 g^{-1} DM	215.65 d	194.33 d	233.97 cd	193.06 d	204.66 d	277.32 bc	365.28 a	316.67 ab	15.540	***
% TFA	16.74 d	15.09 ef	17.51 d	14.04 f	15.96 de	19.42 c	23.10 b	25.64 a	0.981	***
ΣPUFA										

mg 100 g^{-1} DM	785.18 ab	804.09 ab	800.55 ab	892.35 a	781.81 ab	846.98 a	888.90 a	691.13 b	16.765	**
% TFA	60.96 bc	62.43 b	59.91 c	64.89 a	60.97 bc	59.22 c	56.26 d	55.97 d	0.728	***
ΣUFA										
mg 100 g^{-1} DM	1000.83 b	998.42 b	1034.51 b	1085.40 b	986.46 b	1124.30 ab	1254.17 a	1007.79 b	23.294	**
% TFA	77.69 cde	77.52 de	77.42 de	78.93 bc	76.93 e	78.63 bcd	79.36 b	81.60 a	0.368	***
TFA										
mg 100 g^{-1} DM	1288.21 b	1287.91 b	1336.28 b	1375.16 b	1282.42 b	1429.90 ab	1580.34 a	1235.14 b	28.339	**
% TFA	100	100	100	100	100	100	100	100	-	-
ΣUFA/ΣSFA	3.48 cd	3.45 cd	3.43 cd	3.75 bc	3.34 d	3.68 bcd	3.84 b	4.44 a	0.087	***
ΣPUFA/ΣMUFA	3.65 cd	4.14 b	3.42 d	4.62 a	3.82 bc	3.06 e	2.44 f	2.18 f	0.200	***
Σn6 PUFA/Σn3 PUFA	8.29 b	8.29 b	8.43 b	8.14 b	8.37 b	9.12 b	9.43 b	15.46 a	0.598	***

[a] Abbreviations: DM, dry matter; *t*, *trans*; *c*, *cis*; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; TFA, total fatty acids; SEM, standard error of the mean; nd, not detected.

[b] Probability: *: P≤0.05; **: P≤0.01; ***: P≤0.001; ns, not significant (P>0.05). Means within a row with different letters differ significantly.

Figure 1. Comparative bar charts of the fatty acid composition of *Vicia sativa*, *Vicia villosa* and *Vicia narbonensis* oilseeds (% of TFA).[a]

Country (region):
1 □ Tunisia (Mateur), mean values found in the current study;
2 ■ Turkey (Sivas), adapted from Akpinar *et al*. (2001)[b];
3 ▩ Spain (Andalusia), adapted from Pastor-Cavada *et al*. (2009a)[c];
4 ▒ Turkey (Elaziğ), adapted from Emre *et al*. (2011)[d];
5 ▤ Turkey (various sites) adapted from Bağci *et al*. (2004)[e];
6 ▥ Turkey (Adana) adapted from Kokten *et al*. (2010).

[a] Abbreviations: *t*, *trans*; *c*, *cis*; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; TFA, total fatty acids; nd, not detected; nr, not reported.

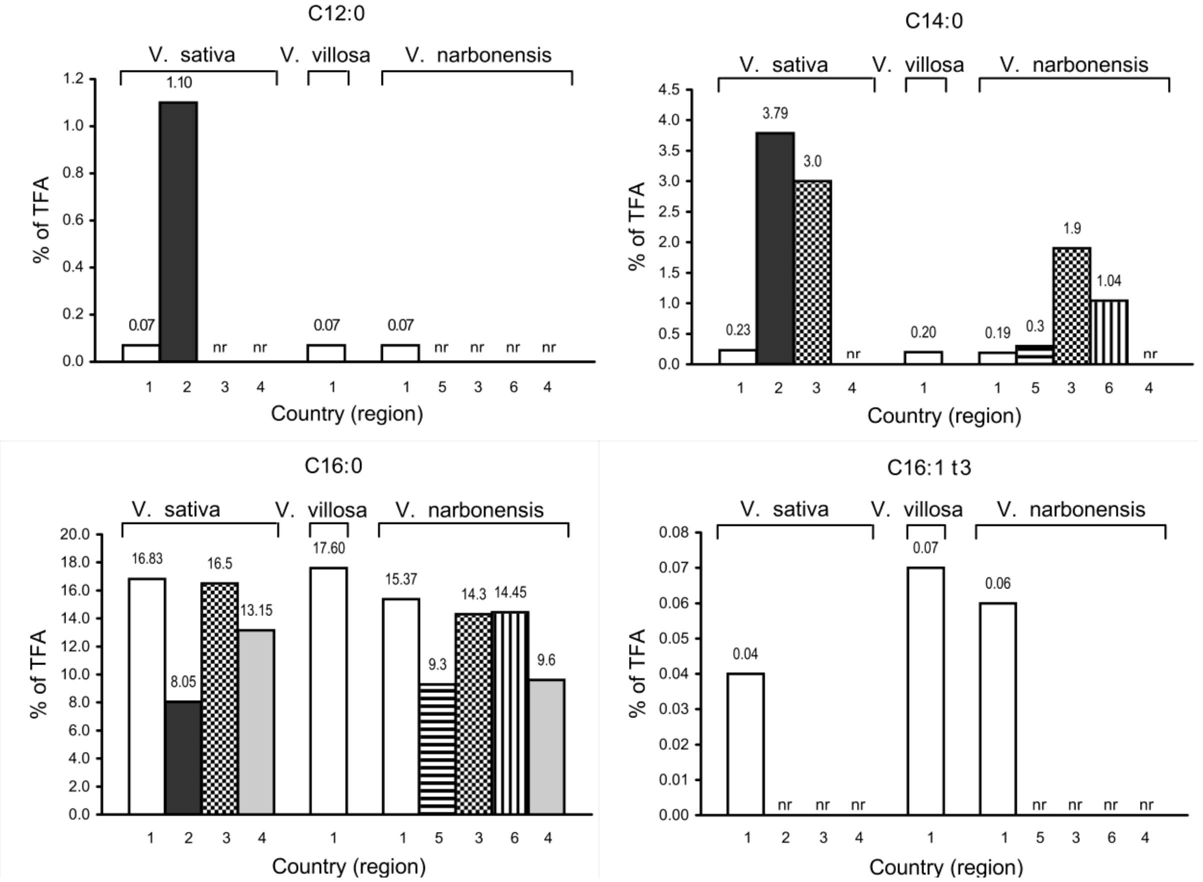
[b] *V. sativa* subsp. *nigra*.

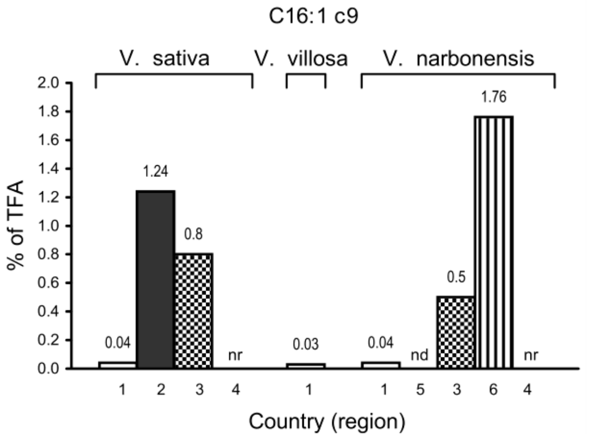
[c] for *V. sativa*: *V. sativa* subsp. *sativa*.

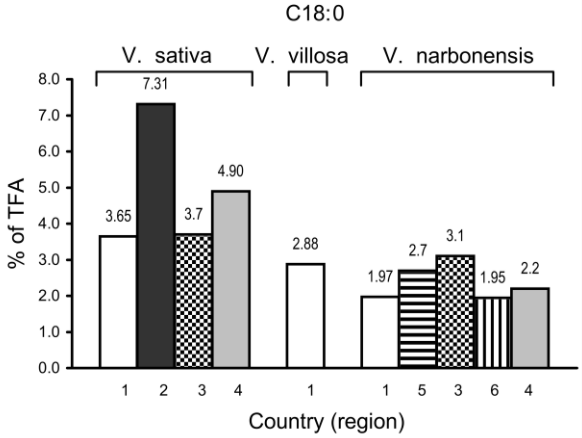
[d] for *V. sativa*: mean values of *V. sativa* subsp. *nigra* and *V. sativa* subsp. *sativa*; for *V. narbonensis*: *V. narbonensis* var. narbonensis

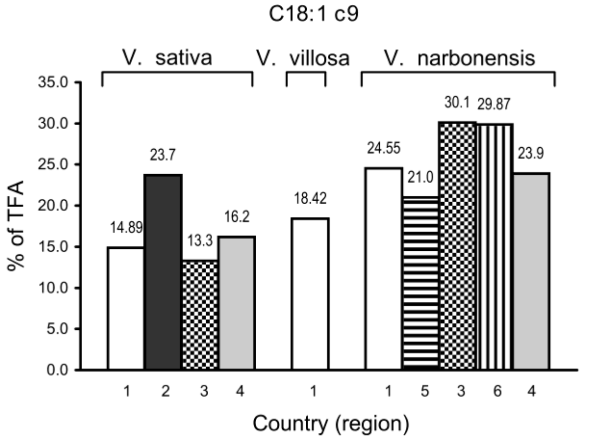
[e] *V. narbonensis* var. narbonensis.

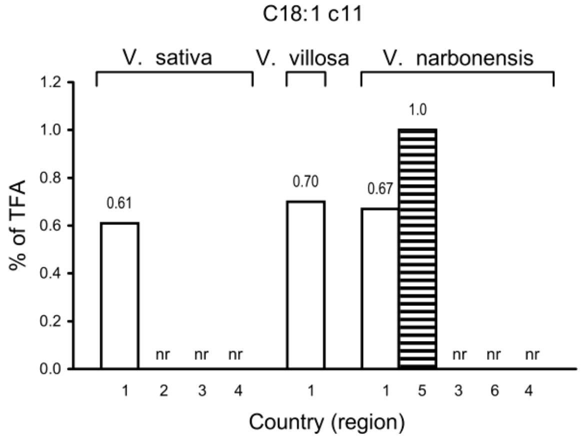
[f] Akpinar *et al*. (2001)[b]: C14:1ω5, C15:0, C16:2, C17:0, C19:0, C20:3, C22:1*c*13, C22:2, C22:4; Pastor-Cavada *et al*. (2009a)[c]: C15:0; Emre *et al*. (2011)[d]: C16:1*c*7 for *V. sativa* subsp. *sativa*.

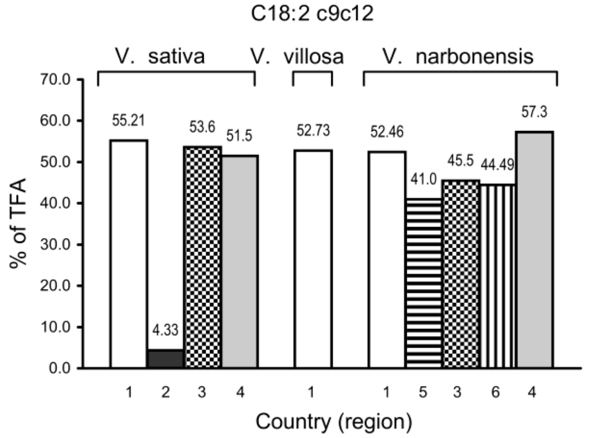


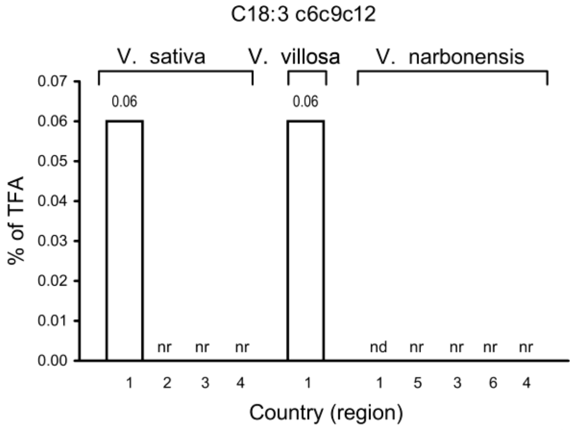

C16:1 c9
V. sativa
V. villosa
V. narbonensis
% of TFA
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.04
1.24
0.8
nr
0.03
0.04
nd
0.5
1.76
nr
1
2
3
4
1
1
5
3
6
4
Country (region)

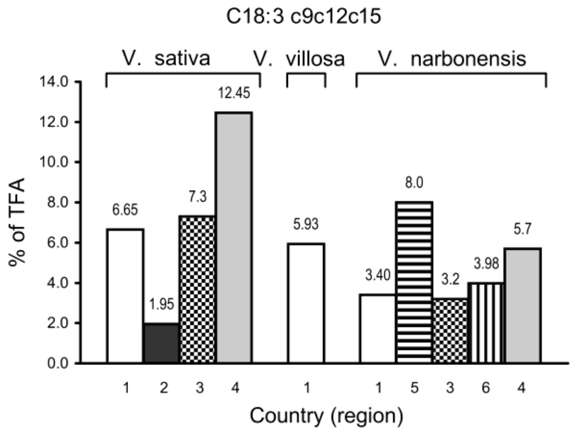

C18:0
V. sativa
V. villosa
V. narbonensis
% of TFA
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
3.65
7.31
3.7
4.90
2.88
1.97
2.7
3.1
1.95
2.2
1
2
3
4
1
1
5
3
6
4
Country (region)

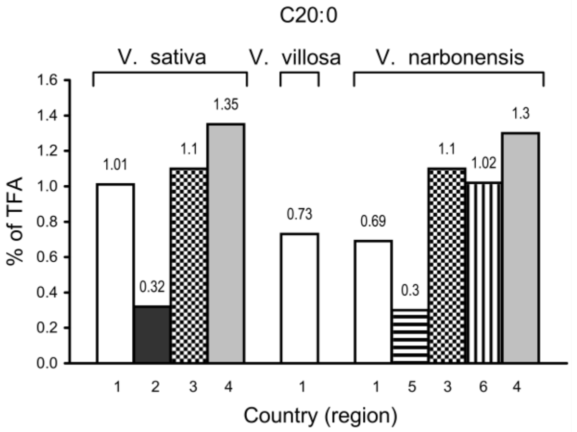

C18:1 c9
V. sativa
V. villosa
V. narbonensis
% of TFA
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
14.89
23.7
13.3
16.2
18.42
24.55
21.0
30.1
29.87
23.9
1
2
3
4
1
1
5
3
6
4
Country (region)

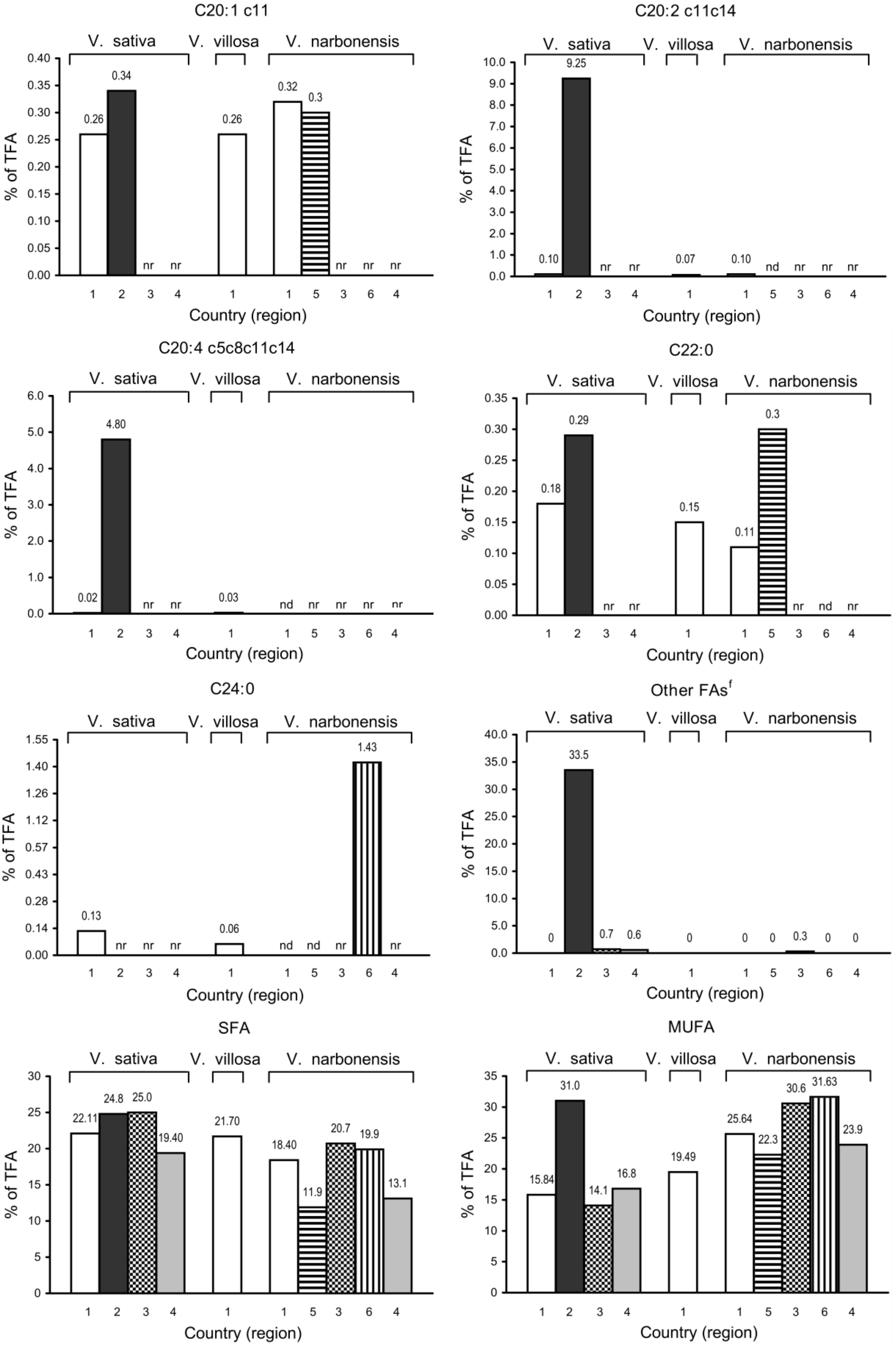

C18:1 c11
V. sativa
V. villosa
V. narbonensis
% of TFA
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.61
nr
nr
nr
0.70
0.67
1.0
nr
nr
nr
1
2
3
4
1
1
5
3
6
4
Country (region)

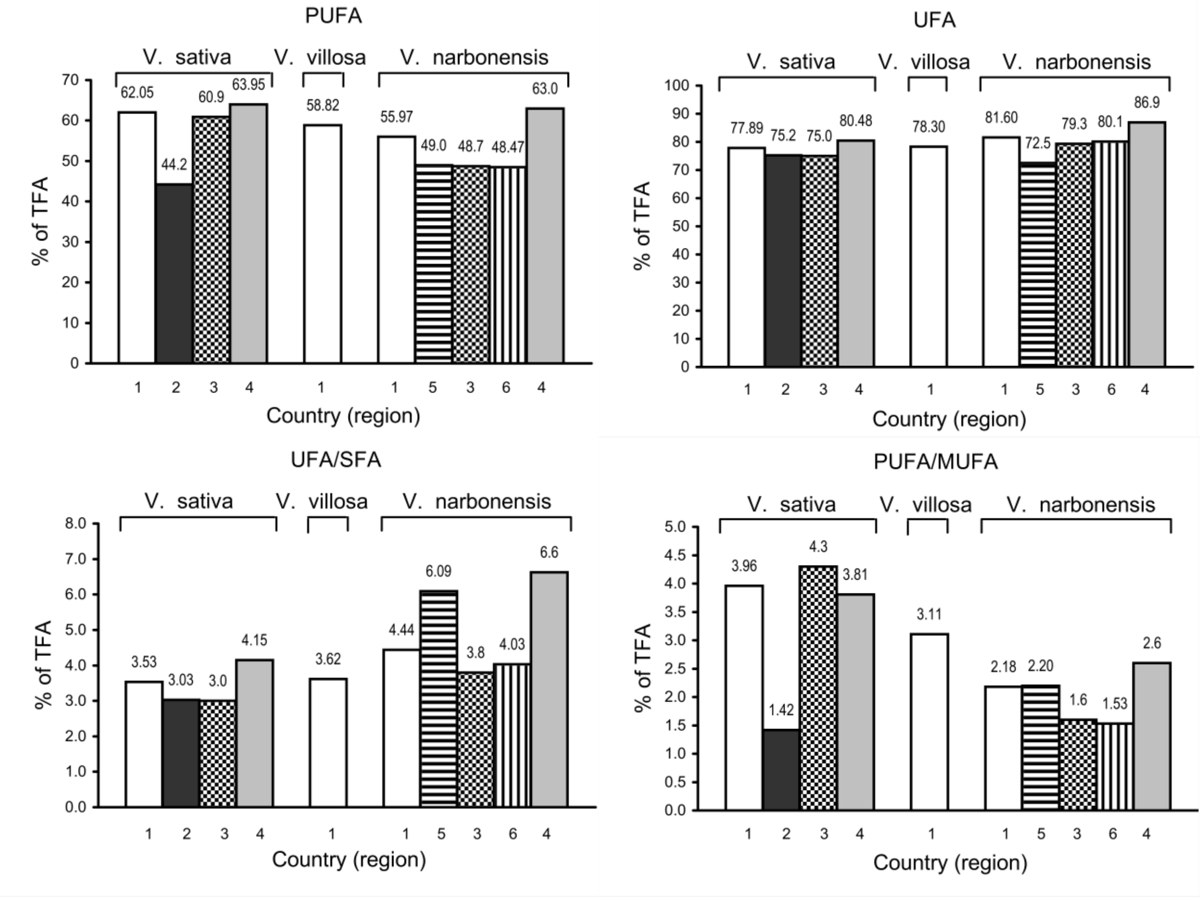

C18:2 c9c12
V. sativa
V. villosa
V. narbonensis
% of TFA
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
55.21
4.33
53.6
51.5
52.73
52.46
41.0
45.5
44.49
57.3
1
2
3
4
1
1
5
3
6
4
Country (region)


C18:3 c6c9c12
V. sativa
V. villosa
V. narbonensis
% of TFA
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0.06
nr
nr
nr
0.06
nd
nr
nr
nr
nr
1
2
3
4
1
1
5
3
6
4
Country (region)


C18:3 c9c12c15
V. sativa
V. villosa
V. narbonensis
% of TFA
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
6.65
1.95
7.3
12.45
5.93
3.40
8.0
3.2
3.98
5.7
1
2
3
4
1
1
5
3
6
4
Country (region)


C20:0
V. sativa
V. villosa
V. narbonensis
% of TFA
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.01
0.32
1.1
1.35
0.73
0.69
0.3
1.1
1.02
1.3
1
2
3
4
1
1
5
3
6
4
Country (region)


C20:1 c11
V. sativa
V. villosa
V. narbonensis
% of TFA
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.26
0.34
nr
nr
0.26
0.32
0.3
nr
nr
nr
1 2 3 4
1
1 5 3 6 4
Country (region)
C20:2 c11c14
V. sativa
V. villosa
V. narbonensis
% of TFA
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.10
9.25
nr
nr
0.07
0.10
nd
nr
nr
nr
1 2 3 4
1
1 5 3 6 4
Country (region)
C20:4 c5c8c11c14
V. sativa
V. villosa
V. narbonensis
% of TFA
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.02
4.80
nr
nr
0.03
nd
nr
nr
nr
nr
1 2 3 4
1
1 5 3 6 4
Country (region)
C22:0
V. sativa
V. villosa
V. narbonensis
% of TFA
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.18
0.29
nr
nr
0.15
0.11
0.3
nr
nd
nr
1 2 3 4
1
1 5 3 6 4
Country (region)
C24:0
V. sativa
V. villosa
V. narbonensis
% of TFA
1.55
1.40
1.26
1.12
0.57
0.43
0.28
0.14
0.00
0.13
nr
nr
nr
0.06
nd
nd
nr
1.43
nr
1 2 3 4
1
1 5 3 6 4
Country (region)
Other FAs^f
V. sativa
V. villosa
V. narbonensis
% of TFA
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0
33.5
0.7
0.6
0
0
0
0.3
0
0
1 2 3 4
1
1 5 3 6 4
Country (region)
SFA
V. sativa
V. villosa
V. narbonensis
% of TFA
30
25
20
15
10
5
0
22.11
24.8
25.0
19.40
21.70
18.40
11.9
20.7
19.9
13.1
1 2 3 4
1
1 5 3 6 4
Country (region)
MUFA
V. sativa
V. villosa
V. narbonensis
% of TFA
35
30
25
20
15
10
5
0
15.84
31.0
14.1
16.8
19.49
25.64
22.3
30.6
31.63
23.9
1 2 3 4
1
1 5 3 6 4
Country (region)


PUFA
V. sativa
V. villosa
V. narbonensis
% of TFA
0
10
20
30
40
50
60
70
62.05
44.2
60.9
63.95
58.82
55.97
49.0
48.7
48.47
63.0
1
2
3
4
1
1
5
3
6
4
Country (region)
UFA
V. sativa
V. villosa
V. narbonensis
% of TFA
0
10
20
30
40
50
60
70
80
90
100
77.89
75.2
75.0
80.48
78.30
81.60
72.5
79.3
80.1
86.9
1
2
3
4
1
1
5
3
6
4
Country (region)
UFA/SFA
V. sativa
V. villosa
V. narbonensis
% of TFA
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
3.53
3.03
3.0
4.15
3.62
4.44
6.09
3.8
4.03
6.6
1
2
3
4
1
1
5
3
6
4
Country (region)
PUFA/MUFA
V. sativa
V. villosa
V. narbonensis
% of TFA
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
3.96
1.42
4.3
3.81
3.11
2.18
2.20
1.6
1.53
2.6
1
2
3
4
1
1
5
3
6
4
Country (region)