A study of the fatty acid and tocochromanol patterns of some Fabaceae (Leguminosae) plants from Turkey I

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1. INTRODUCTION

The Fabaceae (Leguminosae) is a family of flowering plants comprising about 269 genera and 5100 species (Mabberley, 1997) and it is one of the largest plant families in Turkey and in the world. It has 68 genera and more than 900 species in Flora of Turkey (Davis, 1970, 1988; Seçmen et al., 1989). The family is important as food plants, especially leguminate (beans, gram, peas), oil (soybean, ground nut), but also for tanbarks, timber, copal, gums, insecticides and cultivated ornamentals, as well as medicinal plants (Seçmen et al., 1989; Tsevegsuren et al., 1998).

Leguminosae is well suited with respect to chemical components. Lipids from some more common Leguminosae have been investigated to some extent, other legume lipids have not been studied in any great detail because of their low lipid content and limited or negligible use for oil purposes (Gunstone et al., 1972; Kleiman, 1988; Chowdurry, 1984, 1986, 1995; Ucciani, 1995; Grela and Gunter, 1995). Some species of the family Fabaceae (Leguminosae) are also sources of cheap protein for both humans and animals (Tewatia and Wirk, 1996).

Omega 3- fatty acids are polyunsaturated fatty acids which have been associated with many health benefits (Freeman, 2000). Linoleic acid is needed for a normal immune response and an essential fatty acid deficiency impairs B and T cell-mediated responses (Meydani et al., 1991). Tocopherols found to be highly variable among the genera investigated here. The total tocopherols was higher than the total tocotrienols. Alpha and gamma tocopherols were also the highest tocopherols present in the whole species. Beta, gamma and delta-tocotrienols were not found in most of the studied leguminous patterns. The results are discussed in view of renewable sources and chemotaxonomy.

KEY-WORDS: Chemotaxonomy - Fabaceae (Leguminosae) - Fatty acids - Tocochromanols.

SUMMARY

A study of the fatty acid and tocochromanol patterns of some Fabaceae (Leguminosae) plants from Turkey I.

In this study, the fatty acid, tocopherol, tocotrienol and plastochochromanol-8 contents of some selected Fabaceae (Leguminosae) species belonging to different genera (Colutea, Vicia, Lathyrus, Gonocytisus, Lupinus, Hedysarum, Onobrychis, Trigonella) from Turkey were determined by using GLC and HPLC techniques. Some of the studied species are endemic to Turkey. The seed oils of different Leguminous taxa contained linoleic, oleic and linolenic acids as their major components. The ratios of these fatty acids in the Leguminous genera were found to be highly variable. Palmitic and stearic acids are the major saturated fatty acids in the seed oils. Vicia and Onobrychis patterns showed high similarity in means of qualitative fatty acid concentration. The tocopherol and tocochromanol patterns of the seed oils were also found to be highly variable among the genera investigated here. The total tocopherols was higher than the total tocotrienols. Alpha and gamma tocopherols were also the highest tocopherols present in the whole species. Beta, gamma and delta-tocotrienols were not found in most of the studied leguminous patterns. The results are discussed in view of renewable sources and chemotaxonomy.
correlated with the polyunsaturated fatty acids since they counteract the potential oxidative stress caused by fats in the diet (Anttolainen et al., 1995).

Seed oils with a substantial amount of very long chain FAs have attracted attention because of their value for industrial purposes. Furthermore these compounds can be used for chemotaxonomic significance (Bagci et al., 2003). The fatty acid and tocopherol composition of plant seed oils can provide characteristic information in order to confirm taxonomical and phylogenetic relationships in the plant kingdom (Goffman et al., 1999; Bagci et al., 2004). Relatively complete data on the fatty acid composition of seed oils from Leguminosae were first used for chemotaxonomic consideration by Wolff and Kwolek, (1971).

In this study, the fatty acid, tocopherol, tocotrienol and plastochromanol content of some selected plant samples from different genera (Gonocytisus Spach., Lupinus L., Colutea L., Vicia L., Lathyrus L., Hedysarum L. Onobrychis Adans., Hedysarum L., Trigonella L.) of Fabaceae from Turkey were investigated. Among these taxa, Colutea melanocalyx, Vicia freyniana Bornm., Trigonella cretica (L.) Boiss., Hedysarum cappadocicum Boiss., Onobrychis huetiana Boiss., Gonocytisus dirimlensis Hub.-Mor. are endemic to Turkey (Davis, 1970). There are a few reports on the Turkish Fabaceae (Bagci and Vural, 2001, Akpinar et al, 2001; Azcan et al., 2001; Bagci et al., 2001, Bagci and Sahin 2004). The study on the fatty acid and tocopherols of some genera from Fabaceae was found to be of interest and fatty acid and tocochromanol contents of these genera might provide new information and some contributions on the chemotaxonomic relationships of some Fabaceae genera.

2. MATERIAL AND METHODS

2.1. Plant samples

Some seed specimens were collected in natural habitats from different regions of Turkey and some of the samples were obtained from the seed bank in Aegean Agricultural Research Institute, Izmir. Studied plant samples from different genera are listed in Table I.

2.2. Chemical Analysis

2.2.1. Oil Extraction and preparation of fatty acid methyl esters (FAME)

Impurities were removed from the seeds and the cleaned seeds were ground into powder using a ball mill. Lipids were extracted with heptane in a straight through extractor. The triglycerides were transesterified to methyl esters with potassium hydroxide in methanol according to ISO method 5509 (1989).

2.2.2. Capillary GLC

The fatty acid methyl ester composition was determined on three different gas chromatographs, Hewlett-Packard HP5890 (A), HP6890 (B) and Unicam – 610 (C) each equipped with a fused silica WCOT capillary and FID:

A) Silar 5 CP, 50 m x 0.25 mm ID, 0.24 m film thickness, nitrogen as carrier gas, 1:50 split ratio, pressure 160 kPa, oven temp.: 5 min isothermal at 163 °C, then 163 to 205 °C at 1 °C/min, Det. as 230 °C, Det. 260 °C.

B) DB-23, 60 m x 0.32 m (J&W), 0.25 m film thickness, hydrogen as carrier gas, 1:50 split ratio, pressure 69 kPa, oven temp.: 1 min isothermal at 80 °C, then 80 to 150 °C at 25 °C/min than 150 to 240 °C at 3 °C/min, 5 min isothermal, Det. 80 °C, 12 °C/s to 250 °C, 5 min isothermal, Det. 250 °C.

C) BPX-70, 15 m x 0.32 mm, carrier gas: N, ml /min- 2.5, hydrogen as carrier gas, 1:40 split ratio, oven temp.: 1 min isothermal at 80 °C, then 80 to 185 °C at 5 °C/min, 3-5 min isothermal, Det. 80 °C, 5 °C/s to 185 °C, 11 min isothermal, Det. 210 °C.

Data analysis was done with a chromatographic integrator D 2500 (Merck-Hitachi) and a Chemstation integration software, respectively. Peak identification was achieved by comparison of relative retention times with those obtained from test mixtures of known composition on three different columns.

2.2.3. Tocopherol analysis

Tocopherol were determined by high-performance liquid chromatography (HPLC) according to the procedure of Balz et al. (1992). An aliquot of a solution of 50 mg oil in 1 ml heptane was injected into an HPLC system via a Rheodyne valve with a sample loop volume of 20 µl. Tocopherols were separated on a LiChrospher 100 Diol phase, 5 m particle size (Merck, Darmstadt, Germany). HPLC column 25 cm x 4.6 mm ID with an additional guard column 4mm long and 4 mm ID, filled with LiChrospher Si 60, 5 µm particle size. The system was operated with an eluent of heptane/tert.-butyl methyl ether (96+4v/v) and detection by a fluorescent detector F-1000 (Merck, Darmstadt) at 295 nm, excitation wavelength and 330 nm emission wavelength. A D-2500 Chromato Integrator (Merck, Darmstadt) was used for data acquisition and processing. Calibration was done by external standards with α-, β-, γ- and δ-tocopherol (Calbiochem, Bad Soden, Germany). Tocotrienols are calculated with the same response factors as the corresponding tocopherols and plastochromanol-8 was calculated with the same response factor as gamma-tocopherol (Balz et al., 1992).
3. RESULTS AND DISCUSSION

In this study, the fatty acid and tocopherol compositions of the seeds of some selected Fabaceae species from Turkey were determined. The results of the fatty acid analysis and the oil yield of the taxa belonging to Gonocytisus, Lupinus, Vicia, Lathyrus, Hedysarum, Onobrychis and Trigonella genera (totally 17 taxa) are shown in Table I; the tocopherol and tocotrienol contents of the studied taxa are shown in Table II Vicia, Lathyrus, Trigonella and Onobrychis genera have the highest taxa number in Flora of Turkey in family Fabaceae (Davis, 1970; 1988).

The total lipid of the studied leguminous species were found between 18.7% and 32.3% (Vicia canescens subsp. latistipatula and Trigonella cretica, respectively) (Table I). The oil contents of the studied legumes belonging to different genera showed significant quantitative differences. Although qualitative differences were also found, the fatty acid patterns of the studied leguminous taxa belonging to different genera showed uniform fatty acid (FA) composition.

Analysis showed that low molecular acids (lauric, myristic, pentadecanoic acids) from the saturated fatty acid (SFA) were absent or present at trace levels in the Leguminosae seed oils. Palmitic acid (16:0) was the highest SFA in Trigonella cretica (12.9%), Lupinus varius (12.8%) and Colutea melanocalyx (10.7%) respectively. This is also a very constant lipid constituent in most of the Leguminous genera seed genal acid. It is possible to say that this fatty acid is not a highly variable component in the leguminous genera pattern. 16:1, 16:2, Margaric acid is not a highly variable component in the leguminous genera pattern. 16:1, 16:2, Margaric acid was not detected or in very small amounts (Table I).

Stearic acid (18:0) was generally found in a lower level. But Onobrychis hypargyrea (4.20%), Vicia cappadocica (3.91%), Lupinus sibericus (3.77%) and Trigonella (3.63 %) patterns were shown in higher concentrations. This is also reported for some Vicia sp. from Turkey like Vicia hyrcanica Fisch. et Mey., V. peregrina L. (7.3%), V. hybrida L. (9.1%) (Akpinar et al., 2001). On the other hand, this fatty acid was detected as lower in some Vicia sp. from different countries: Vicia faba (1.4%), V. sativa (1.3%). Colutea melanocalyx and Onobrychis altissima (1.38% and 1.79%) seed oils showed low stearic acid amounts. This FA was reported as 3.1% in Colutea arborescens (Ivanov & Aitzetmuller, Unpublished).

The other SFA of the legume seed oils (20:0 and 24:0, arachidic and lignoceric acid) in the studied species, were shown to be lower than 1% except V. cappadocica and Trigonella cretica. But 22:0 (Behenic acid) was different from these FA in the studied genera patterns. Trigonella, Lathyrus laxiflorus, Onobrychis major, two subspecies of V. canescens and Hedysarum species contained this FA in more than 1%. Some researchers indicated that oils with high levels of long chain SFA such as behenic acid may be difficult for the digesting enzymes in humans and animals (Hilditch et al., 1964; Balogun and Fetuga, 1985; Akpinar et al., 2001).

Among the unsaturated fatty acids (USFA), oleic and linoleic acid were the major constituents of the studied legume seed oil. The highest percentages of oleic acid was determined in Trigonella cretica (46.9%), Onobrychis hypargyrea (34.4%) and Lathyrus laxiflorus subsp. laxiflorus (30.4%) respectively (Table I). On the other hand, Vicia michauxii var. stenophylla (12.3%), Colutea melanocalyx (12.7%), Gonocytisus dirmillensis (13.19%) and Onobrychis hueltiana (13.3%) showed the lowest oleic acid composition in the seed oils.

Linoleic acid was determined as the major constituent of all the seed oils except Trigonella cretica. This fatty acid comprised more than half of the seed oil in Colutea melanocalyx (62.8%), Gonocytisus dirmillensis (67.4%), Lupinus varius (57.8%), Vicia cappadocica (50.9%) and Onobrychis major (51.7%). A high content of this component was found to be characteristic for the legume seed oil. Vicia patterns show a wide variation in this component and it was also found in small amounts in Trigonella, Vicia michauxii var. stenophylla (Table I). The seed oils of all the investigated species were richer in oleic and linoleic acid than in linolenic acid. In the Akpinar et al. (2001) study, while most of the studied Vicia samples showed this result, Vicia hybrida contrasted from the other Vicia taxa. At the same time, oleic and linoleic acids were determined to be the major unsaturated fatty acid in Psophocarpus tetragonolobus (L.) (Fabaceae) DC. (winged bean) oil which is used as a food in the nutrition of some countries (Higuchi et al., 1982).

Linolenic acid (18:3) in the legume seed oils was generally found to be lower than 10%, except in a few taxa like Hedysarum cappadocicum (21.1%), Vicia michauxii var. michauxii (39.1), two Lathyrus taxa (16.5-17.9%) and Onobrychis hueltiana (18.3%) and O. major (11.2%). In the others, it was generally lower than ten percent of the oil. This fatty acid was the most variable component among the leguminous genera when compared with the other constituents.

Total saturated fatty acid (TSFA) levels of the studied legume seed oils did not show variation between genera except in the Lupinus (17.36) and Trigonella (21.5%) patterns. These two samples had high saturated FA compositions when compared with the others. On the other hand Lathyrus laxiflorus (6.2%) and Onobrychis hueltiana (7.9%) samples were proven to have a very low concentration of TSFA in the oils. It is possible to say that the total saturated fatty acid content of the legume seed oils is between 10-20%. TUSFA composition of the seed
Table I

Fatty acid composition of some Turkish Fabaceae. Data shown are peak area - % from GC.

| plants                        | 14:0 | 16:0 | 16:1 | 17:0 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 | 22:1 | 24:0 | TSFA | TUFA | Oil Content in wt% |
|-------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|-------------------|
| Colutea melanocedys          | 0.1  | 10.7 | 0.1  | 0.2  | 0.1  | 1.4  | 12.7 | 1.5  | 62.8 | 0.3  | 0.3  | 0.1  | 0.3  | 0.0  | 0.0  | 12.8 | 86.2  | 24.6             |
| Hedysarum cappadocicum       | 0.1  | 5.6  | 0.5  | 0.0  | 0.2  | 3.2  | 21.0 | 0.9  | 40.7 | 21.1 | 0.5  | 0.5  | 0.0  | 1.2  | 0.0  | 0.0  | 10.8 | 84.8  | 31.7             |
| Lathyrus inconspicuus        | 0.5  | 9.1  | 0.0  | 0.0  | 0.0  | 3.5  | 26.2 | 1.3  | 36.9 | 17.9 | 0.0  | 1.1  | 0.0  | 0.0  | 0.0  | 13.2 | 83.2  | 27.4             |
| Lathyrus laxiflorus subsp. laxiflorus | 0.2  | 8.8  | 0.1  | 0.1  | 0.1  | 3.1  | 30.4 | 0.6  | 36.6 | 16.5 | 0.6  | 0.5  | 0.7  | 1.0  | 0.0  | 0.3  | 6.2  | 85.6  | 26.7             |
| Gonocytis dirmilensis       | 0.1  | 9.8  | 0.1  | 0.1  | 0.1  | 3.2  | 13.2 | 0.8  | 67.4 | 2.1  | 0.3  | 0.3  | 0.1  | 0.3  | 0.2  | 0.1  | 13.8 | 84.3  | 19.7             |
| Lupinus varius              | 0.00 | 12.8 | 0.0  | 0.0  | 0.0  | 3.8  | 19.1 | 0.9  | 57.8 | 2.1  | 0.0  | 0.0  | 0.0  | 0.8  | 0.0  | 0.0  | 17.4 | 79.9  | 26.1             |
| Trigonella cretica          | 0.3  | 12.9 | 0.2  | 0.4  | 0.0  | 3.6  | 46.9 | 1.9  | 24.2 | 3.5  | 0.9  | 1.0  | 0.0  | 2.7  | 0.0  | 1.3  | 21.5 | 78.0  | 32.3             |
| Vicia cracca var. sternophylla | 0.2  | 7.4  | 0.0  | 0.6  | 0.2  | 3.5  | 12.3 | 4.0  | 20.1 | 39.1 | 0.7  | 0.2  | 0.1  | 0.7  | 0.0  | 0.2  | 12.8 | 76.5  | 24.3             |
| V. cappadocica              | 0.4  | 6.6  | 0.0  | 0.0  | 0.0  | 3.9  | 25.3 | 1.1  | 50.9 | 9.2  | 1.5  | 0.7  | 0.0  | 0.0  | 0.0  | 0.0  | 13.0 | 87.2  | 20.2             |
| V. freyniana                | 0.2  | 9.8  | 0.0  | 0.2  | 0.2  | 2.8  | 18.8 | 0.8  | 44.7 | 11.0 | 0.0  | 0.9  | 0.0  | 0.4  | 0.0  | 0.0  | 13.4 | 76.4  | 23.0             |
| V. canescens subsp. gregaria | 0.2  | 8.2  | 0.0  | 0.1  | 0.0  | 3.8  | 19.4 | 0.9  | 35.7 | 9.8  | 0.4  | 0.8  | 0.0  | 0.9  | 0.0  | 0.0  | 13.5 | 66.4  | 19.1             |
| V. canescens subsp. leucophaea | 0.2  | 7.4  | 0.0  | 0.2  | 0.0  | 2.0  | 28.0 | 1.0  | 38.1 | 8.8  | 0.5  | 0.4  | 0.0  | 1.2  | 0.0  | 0.0  | 11.1 | 76.4  | 18.7             |
| V. narbonensis var. narbonensis | 0.3  | 9.3  | 0.0  | 0.0  | 0.0  | 2.7  | 21.0 | 1.0  | 41.0 | 8.0  | 0.3  | 0.3  | 0.0  | 0.3  | 0.0  | 0.0  | 11.9 | 72.5  | 21.2             |
| Onobrychis major            | 0.9  | 8.8  | 0.0  | 0.0  | 0.0  | 2.1  | 19.8 | 0.9  | 51.8 | 11.2 | 0.8  | 0.8  | 0.0  | 1.1  | 0.0  | 0.0  | 13.8 | 84.4  | 29.1             |
| O. hueiana                  | 0.3  | 4.9  | 0.0  | 0.1  | 0.1  | 2.2  | 13.3 | 0.7  | 31.5 | 18.3 | 0.1  | 0.5  | 0.0  | 0.4  | 1.6  | 0.0  | 0.0  | 79   | 65.9  | 23.7             |
| O. altissima                | 0.2  | 8.8  | 0.1  | 0.2  | 0.1  | 1.8  | 25.8 | 1.0  | 49.6 | 9.1  | 0.4  | 0.5  | 0.0  | 0.6  | 0.0  | 0.0  | 11.9 | 86.3  | 23.2             |
| O. hypercynica              | 0.2  | 8.8  | 0.0  | 0.0  | 0.2  | 4.2  | 34.4 | 1.1  | 40.5 | 7.8  | 0.8  | 0.6  | 0.0  | 0.7  | 0.0  | 0.0  | 14.8 | 84.4  | 24.7             |

TSFA: Total saturated fatty acid  TUFA: Total unsaturated Fatty acid
oils is generally between 75-90% in the oil for most of the studied specimens. But Vicia patterns and Onobrychis huetiana contained lower than 70% TUFA (Table I).

Eruic acid (22:1) has undesirable effects on the metabolisms of animals and humans (Baudet, 1976; Feil and Slamp, 1993; James, 1994). But it is not detected in most of the legume seed oils except Onobrychis dirmilensis (0.2%) and Onobrychis huetiana (1.6%). Garcia-Lopez et al. (2001) reported that some Lupinus sp. oils contained high concentrations of palmitic and linoleic acid and that erucic acid was not reported in any of the lupin oils analyzed while eicosanoic acid (22:0) was found only in Lupinus mexicanus. Some Lupinus seed oils (Lupinus albus L., L. angustifolius L., L. luteus L., and L. mutabilis Sweet. (lupin oil, Tarwi seed oil) are reported as oil plants in the world (Aitzetmuller, 1997).

The seed oils of the leguminous species contained very low levels of monoenoic fatty acids which were lower than polyenoic FA. (Table I). This is important for the quality of oils consumed as a food resource.

The results of the present study, as far as unsaturated fatty acid content is concerned, are supported by previous Leguminous studies (Sengupta and Basu, 1978; Daulatab et al., 1987; Tharib and Veitch, 1983; Hamberg and Fahlstadius, 1992; Liu et al., 1995). All these studies showed that the saturated and particularly unsaturated FA contents of Fabaceae seed oils are closely allied to each other and that the main components in the oils are linoleic–oleic type fatty acids. Tocopherols, together with tocotrienols and plastoquinones, are known as tocochromanols (Seher and Ivanov, 1973; Velasco et al., 2000) and some of them exhibit vitamin E activity. With this study some Turkish leguminous seed oils were examined as potential sources of natural tocopherols and tocotrienols. The tococromanol (tocopherol and tocotrienol) derivatives α, β, γ and δ-tocopherols and tocotrienols and δ-plastochochromanol were detected in some of the studied Fabaceae seed oils (Table II). α and γ–tocopherol were detected as the most abundant tocopherol components in all of the studied taxa except Lathyrum inconspicuos, Onobrychis huetiana, and O. hypargyre. This is also reported for some Fabaceae taxa from Bulgarian Flora (Arachis hypogaea, Gleditsia triacanthos, and Robinia pseudacacia) (Ivanov and Aitzetmuller, 1998). Colutea and Trigonella species had the highest tocopherol level (85.7 and 89.4% respectively) in all of the studied taxa. On the other hand, Hedysarum cappadocicum (69.9%), Vicia mitchulii var. stenophylla (80.2%), V. cappadocica (93.4%), and in general Onobrychis species (except O. huetiana and O. hypargyre) were characterized by the high content of gamma tocopherol in each oil (Table II). tocopherol was determined in the Lathyrus inconspicuos (51.7%), L. laxiflorus subsp. laxiflorus (46.7%) and O. huetiana (34.3%) at a high level, but it was not detected in most of the others (Table II). Onobrychis genera patterns showed the highest fatty acid and tococromanol variability in the studied Leguminosae. This requires further investigation in view of both fatty acid and vitamin resources as well as chemotaxonomic relationships.

The tococromanol analysis of the Fabaceae seed oils from Turkey showed that they contained low percentages of tocotrienols. The highest tocotrienol was found in Onobrychis hypargyre (65.6%), Lupinus varius (45.9%) and Gonocytisus dirmilensis (23.3%) and the other taxa had lower than 10%. Other tocotrienols were either not found or determined in very small amounts. Plastochochromanol-8- was found in trace amounts in most of the studied species, except two Lathyrus taxa (Table II). The analysis results showed that the total tocopherol content of the studied Fabaceae species was higher than the total tocotrienol level (Table II). On the other hand, there are some clues on the variation of the tococromanol in family patterns. Lupinus, Gonocytisus and Onobrychis genera were demonstrated to have different tocopherol and tocotrienol patterns in means of individual and total amounts compared to other genera. It can be possible to differentiate the taxa in the following groups: 1) Having high tocopherol, like Colutea and Trigonella patterns. 2) Having high γ – tocopherol, as in Hedysarum, Vicia and some Onobrychis patterns. It is determined that linoleic and oleic acid are abundant components in most of the leguminous genera and this may be a characteristic of the family or of some genera. But some results showed that the linoleic – palmitic type FA pattern is typical for some genera like Cassia nodosa, Berlinia auriculata, Bauhinia monandra, Parkia clappertoniana (Balogun and Fetuga, 1985), some Astragalus sp (Bagci and Vural, 2001), some Ebenus species reported from Turkey (Azcan et al., 2001) or linoleic - oleic - palmitic type, like in some Lathyrus species (Bagci et al., 2001; Bagci and Sahin, 2004) and some Crotalaria species (Fabaceae) (Chowdury and Banerjii, 1995). The seed oils of all leguminous members contained very low levels of linolenic acid, both in this study and in reports from the literature, cited above (Hemavathy and Prabhakar, 1989).

Such a favourable composition of USFA in most of the legume seed oils suggests that some of these species may have potential as renewable sources in oilseed crop for the food and oil industry, if growth and different regional yields can be improved. The present study shows the infrafamilial variability for fatty acid and tococromanols in Fabaceae. With this study, it was confirmed that patterns of fatty acids

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and tocochromanols may be a good chemotaxonomic criterion for this family, particularly with regard to the studied taxa. However, further studies are required to confirm the results obtained so far. The evaluation of fatty acids and tocochromanols in a wider range of species of the Fabaceae is suggested as a powerful tool that might contribute to characterize the chemotaxonomic and evolutionary relationships among the tribes and genera of Fabaceae.

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