

FATTY ACID PATTERNS OF THE SEED OILS OF SOME *LATHYRUS* SPECIES L. (PAPILIONIDEAE) FROM TURKEY, A CHEMOTAXONOMIC APPROACH

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Abstract

Seed oils of 16 *Lathyrus* (Fabaceae) species belonging to different sections viz., *Cicerula*, *Orobastrum*, *Orobon*, *Platystylis*, *Lathyrus* and *Pratensis* were investigated for their fatty acid composition with gas liquid chromatography. Unsaturated fatty acids, particularly linoleic and oleic acid comprised more than 50 % of seed oil. The fatty acid composition of the studied *Lathyrus* taxa were found as identical qualitatively, but some quantitative differences were observed in infrasectional and interspecific level. Linoleic acid was found major component as unsaturated fatty acid (average 48 %). Oleic and linolenic acid were the second highest major unsaturated fatty acid components. On average, palmitic acid was the major saturated acid present (average 14 %). *Lathyrus* species showed linoleic - palmitic acid type FA patterns. Similar conclusions were detected in the sectional classifications of *Lathyrus* sp., as reported earlier. The results obtained from this study were discussed with the genera and family patterns. Some differences were determined in the main type fatty acid in family patterns in particular subfamily Mimosoideae and Caesalpinioideae. Chemotaxonomic and renewable resources implications of the components of fatty acids and plant taxa are discussed.

Introduction

In response to an ever increasing global demand for food and feed resources and the need to diversify modern cropping systems, the legume genus *Lathyrus* is receiving increased attention by scientists. It includes a range of grain, forage, pasture and ornamental crops (Enneking, 1998). The Fabaceae (Leguminosae) is one of the largest plant families of flowering plants comprising of about 269 genera and 5100 species (Mabberley, 1997) in the world and also in Turkey (Davis, 1970,1988; Seçmen *et al.*, 1989). The Papilionoideae (Lotoideae) (Leguminosae, subfamily) is much larger than either of the other two subfamilies (Mimosoideae and Caesalpinioideae). About 30 genera contain 100 or more species (Heywood, 1978). *Lathyrus* L., is placed in the tribes of *Vicieae*, Fabaceae (Papilionideae subfamily). Kupicha (1983) lists 152 species in the *Lathyrus* genus (Fabaceae) which is distributed from the sub-arctic to the sub-tropics. It shows a broad diffusion throughout the world (Alkin *et al.*, 1986). Some species of this genus are very important in terms of economy and agriculture throughout the world and also in Turkey. It is distributed in the whole region of Turkey particularly in the east and southeast Anatolia and is represented by 67 –70 taxa with 8 section in Flora of Turkey (Davis, 1970, 1988).

Some species of family Fabaceae (Leguminosae) are a source of cheap protein for both humans and animals (Tewatia & Virk, 1996). The pulses are also important as potential sources of natural tocopherols, tocotrienols and fatty acid composition all over

the world (Krishna *et al.*, 1997; Bağcı *et al.*, 2003). It has been demonstrated in recent studies that *Lathyrus sativus* and *L. cicera* have considerable potential as grain legume crops on fine textured, soil types in southern Australian Mediterranean-type environments (Hanbury *et al.*, 2000). There are also some taxonomic problems on this genus. *L. cicera* resembles *L. stenophyllus* in their narrowly linear leaflets, it can be referred to *L. cicera* due to some of their morphologic properties (Davis, 1970).

The content and composition of fatty acids of seed lipids can serve as taxonomic markers in higher plants (Shorland, 1963; Harborne & Turner, 1984; Hegnauer, 1989; Aitzetmuller, 1993; Aitzetmuller & Tesevegsuren, 1994; Bağcı *et al.*, 2003). Unsaturated acids based on C16 and C18 are widespread in both leaf and seed oils and a number of rarer fatty acids are found as lipid components, many occurring characteristically in seed oils of just a few related plants (Harborne & Turner, 1984). On the other hand, seed oils with a substantial amount of very long chain of FA have attracted attention because of their value for industrial purposes (Bauman *et al.*, 1988). Furthermore these compounds can be of chemotaxonomic significance (Spitzer *et al.*, 1990).

Seed oils of plants of Leguminosae were investigated and it has attracted interest of researchers (Kleiman, 1988; Chowdury *et al.*, 1984, Chowdury & Banerjii, 1995; Ferlay *et al.*, 1993; Ucciani *et al.*, 1994; Muraliharudu & Nagaraj, 1995; Bağcı & Vural, 2001; Bağcı *et al.*, 2003). But, very scant information is available on the lipid composition of this genus particularly in Turkey (Senatore & Basso, 1994; Grela *et al.*, 1999, 2001).

In order to extend our knowledge of the FA composition of the *Lathyrus* (Fabaceae) seed oils, it was considered desirable to investigate more members of the genus and family with modern analytical methods. In this study, some selected samples from different sections viz., *Cicerula* (Medic.) Gren. & Godr., *Orobastrum* (Taub.) Boiss., *Pratensis* Bassler, *Platystylis* (Sweet) Bassler, *Lathyrus* and *Pratensis* were analysed for seed oil fatty acid. We aimed to indicate the fatty acid composition and chemical relationships among this taxa in relation to chemotaxonomy. It is reported that, especially in the interspecific hybridization studies in the genus *Lathyrus* are essential for clarifying the genetic relationships among species of this important genus (Yamamoto, 1984). The results are also important in point of renewable resources and systematic relationships in interspecific and infrasectional classification of *Lathyrus* sp.

Materials and Methods

Plant materials: Seed specimens were obtained from natural habitats of different regions of Turkey (Table 1).

Oil extraction and preparation of fatty acid methyl esters (FAME): Impurities were removed from the seeds and the cleaned seeds (5 g) were ground using a ball mill into powder. Lipids were extracted with heptane (Merck) in a straight through extractor 0.1 µl of sodium methanolate solution was added. After seeing the phase separation, aqueous layer was removed and added HCl (100 µl) containing methyl orange and centrifuged (Anon., 1989).

Capillary GLC: Fatty acid methyl ester composition was determined on gas chromatographs, UNICAM – 610 GC. BPX- 70, (15 m x 0.32 mm.,) carrier gas; N, ml/min- 2.5, 1: 40 split ratio, oven temp.: 3 min isothermal at 80 °C, then 80 to 185 °C at

Table 1. Accessions provenance and field knowledge details of *Lathyrus* sp.

Species	Region	Locality	Altitude
<i>Lathyrus saxatilis</i> (Vent).	Vis. Mersin	Silifke, Narlıkuyu beach edge.	10 m.
<i>L. vinealis</i> Boiss. & Noe.	Elazığ	Hazar lake, Forest seedling field.	1250m.
<i>L. sphaericus</i> Retz.	Burdur	Salda Lake, Forest camp	1150m
<i>L. inconspicuus</i> L.	Elazığ	F.Ü. Campus	1020 m.
<i>L. setifolius</i> L.	Isparta	Senirkantkapı Mountain edge	940 m.
<i>L. tuberosus</i> L.	Konya	Beyşehir Gölyaka,	1200 m.
<i>L. pratensis</i> L.	Isparta	Aksu Yenişarbademli	1800 m.
<i>L. digitatus</i> (Bieb). Fiori	Burdur	Salda	1200 m.
<i>L. roseus</i> Stev.	Konya	Hadım	1300 m.
<i>L. cicera</i> L.	Elazığ	Sivrice village entrance	1225 m.
<i>L. annuus</i> L.	Elazığ	İçme village	1040 m
<i>L. gorgoni</i> Parl. var. <i>gorgoni</i> Fenzl.	Antalya	Prison around.	40 m.
<i>L. sativus</i> L.	Antalya	Korkuteli, Avdan Village	1000 m.
<i>L. stenophyllus</i> Boiss. & Hedr.	Isparta	Aksu, Yenişarbademli Road	1600 m.
<i>Lathyrus hirsutus</i> L.	Isparta	Eğirdir, Balkırı Village	910 m.
<i>L. chloranthus</i> Boiss.	Isparta	Şarkikaraağaç,	1230 m.

5 °C/min, then 185°C to 220°C at 3°C/min. Peak identification was achieved by comparison of relative retention times with those obtained from test mixtures of known composition on columns and representative studies in other GC conditions. All determinations were performed in duplicate and the mean values were reported.

Results and Discussion

Fatty acids of seed oils of some *Lathyrus* taxa belonging to different sections (*Pratensis*, *Platystylis*, *Lathyrus*, *Orobon*, *Orobastrum*, *Cicercula*) from Turkey were determined. Sixteen species of *Lathyrus* genus comprising a representative sampling of these group were examined for fatty acid composition (Table 2). Studied *Lathyrus* taxa belonging to different sections showed similar fatty acid composition, with few exceptions. Wolff & Kwolek (1971) compared available data on fatty acid composition in Leguminosae. They reported that the most satisfying results, chemotaxonomically, would have been to find chemical compositions unique to particular morphologically determined taxa below and above the generic level. For this reason to obtain chemical and for some phylogenetical relationships, *Lathyrus* a large genus of Fabaceae in Turkey was selected.

From saturated acid components of the seed oils; palmitic acid (16:0) was found abundant. These results were supported by other studies (Bağcı & Vural, 2001; Bağcı *et al.*, 2003). It ranged between 11.39 and 17.64% (*L. inconspicuus* and *L. pratensis*) except for *L. roseus*. It was the highest in *L. pratensis* (17.64 %) and *L. sphaericus* (17.55%). This fatty acid is very constant lipid constituent in the studied *Lathyrus* sp.,

and also in most of the Leguminous genera seed oil (Bağcı *et al.*, 2003). 16:1 and 17:0 (margaric acid) fatty acids were not detected or were in very small amounts (Table 2). Stearic acid as second major saturated FA was found between 1.92 and 9.25 % in *L. chloranthus* and *L. cicera*, respectively. *Cicerula* section showed close relationships in this fatty acid and also *Cicerula* and *Orobastrum* sections members showed similar stearic acid composition.

In the studied *Lathyrus* species, linoleic, oleic and linolenic acids were the main USFA components. Seed oil analysis showed that unsaturated fatty acids comprised most of the oil. Oleic acid (18:1) was found very variable in the *Lathyrus* taxa particularly in infrasectional level. *L. inconspicuus* (19.7%), *L. annuus* (19.4%) and *L. gorgoni* var. *gorgoni* (19.0%) have the highest oleic acid (18:1) composition. *L. sphaericus* showed the lowest oleic acid composition (Table 2). Linoleic acid was the predominant component of seed oils of this genus. This unsaturated fatty acid (USFA) was highest in *L. roseus* (72.29%), *L. pratensis* (60.91%) and in *L. setifolius* (60.15%). The range of this FA in studied *Lathyrus* patterns was between 40.4 and 72.29 %. Linoleic acid comprised more of the half of the oils. But some members of the *Cicerula* section were below this concentration like *L. cicera*, *L. chloranthus* and some others (Table 2). This is also reported for *L. odoratus* (45.12%) (Chowdurry *et al.*, 1984), *L. laxiflorus* subsp. *laxiflorus* (36.6%) and in *L. inconspicuus* (36.9%) Bağcı *et al.*, (in press). In the *Orobastrum* and other section members of this genus showed high level of this component at above 50% of oil. This is also reported for *L. davidii* Hancl. (Gorjaev & Evdakova, 1977). The high content of this fatty acid is an important characteristic of the Fabaceae and its subfamilies, Mimosoideae, Caesalpinioideae and Lotoideae.

A number of studies suggest that the unsaturated fatty acid component of Leguminosae seed oils resemble each other and oleic and linoleic acid were the main components in seed oil (Kwiecinska & Matyka, 1986; Daulatabad *et al.*, 1987). Some groups of Fabaceae do not show these series, sometimes this grouping might be different in some genera (Table 2). Oleic and linoleic acid are the principal component acids with (about 65% of the total fatty acids). Percentages of these two acids are inversely correlated –some of the legume oils are rich in linoleic whereas in others oleic acid is in larger amount (Wolff & Kwolek, 1971).

Linolenic acid was also detected in the seed oil of *Lathyrus* sp., but it was in very low level in all of the patterns when compared with the linoleic and oleic acid. Its amount was found between 4.06% and 16.3 % in *L. roseus* and *L. chloranthus*. *L. roseus* (4.06%), *L. tuberosus* (4.71%) and *L. sativus* (4.76%) showed the lowest level of this FA. For edible purposes an oil should have a minimal amount of linolenic acid since it is commonly thought to be the prime constituent responsible for reversion to undesirable flavours in stored oils and in food products containing vegetable oils (Wolff & Kwolek, 1971).

Poly- saturated and unsaturated (PUFA) fatty acid components were found in very low amount in the *Lathyrus* seed oil. This is also characteristics of the most Fabaceae genera (Hemavathy & Prabhakar, 1989; Bağcı *et al.*, in press). However *L. hirsutus* and *L. digitatus* (1.01%) showed more than 1% amount of the 20:0 (Arachidic acid) in the studied seed oil. Behenic acid (22:0) was also detected in *L. gorgoni* var. *gorgoni*, *L. digitatus* and *L. roseus* at more than 1%. Other polyunsaturated FA components were found not more than 1 %. In some country like Japan, PUFA intake is not only from fish and shellfish but also from edible vegetable oils, exclusively rapeseed and soybean oils (Sugano & Hirahara, 2000). The polyunsaturated fatty acids 18:2 and 18:3, are

commonly found in seed oils, and they play a role in the initial oxidative processes whereby fatty acids are converted to carbohydrates, because of their ease of peroxidation (Holman, 1948). In Fabaceae 18:2 linoleic acid was predominant FA in *Lathyrus* sp. On the other hand linolenic acid was found in general lower than 10 % in most of the *Lathyrus* sp., except *L. annuus*, *L. chloranthus*, *L. vinealis* and *L. digitatus*. This is also in most of the legume seed oil. According to Wolff & Kwolek, (1971), lower than 10 % linolenic acid was determined in only Mimosoideae and Caesalpinoideae subfamily genera patterns. This high content of linolenic acid more than 10 % was found in some genera from Lotoideae like *Lupinus*, *Phaseolus*, *Vigna* and *Medicago*.

Total saturated fatty acids (TSFA) of studied *Lathyrus* sp., were found between 12.6 and 32.2%. TSFA content were not more variable among *Lathyrus* sections and species. But there are some extreme *Lathyrus* patterns like *L. roseus* and *L. cicera* (Table 2). Its amount was between 57.9 – 86.74 %. Total unsaturated FA composition (TUFA) of *Lathyrus* species also did not show more sectional differences. Similarly *L. cicera*, *L. saxatilis* and *L. roseus* showed some differences from the others. Other *Lathyrus* species were found very closely related with each other. Thus, as far as unsaturated fatty acid content is concerned, the present study is supported by previous reports (Sengupta & Basu, 1978; Tharib & Veitch, 1983; Hamberg & Fahlstadius, 1992; Bağcı *et al.*, in press), which suggested that the unsaturated FA contents of *Lathyrus* and other legume seed oils closely resemble each other and the abundant components are the linoleic – palmitic and / or oleic acids type FA. The results obtained from GC analysis showed that these triplet fatty acid components were abundant in *Lathyrus* sp., examined and their sections. But inter and intrasectional studies on this genus patterns needs detailed studies.

L. roseus (Section *Orobon*) was found different from the others. This is also reported in the sectional classification of *Lathyrus*. Section *Orobon* is monotypic (Davis, 1970). These results were confirmed with FA analysis. On the other hand, *L. sativus* and *L. cicera* were found very different in the studied *Lathyrus* species, in particular myristic (14:0) and oleic acid (18:1) in *Cicercula* section. These two *Lathyrus* sp., are reported as more complex taxa in Flora of Turkey. Jackson & Yunus (1984) studied the morphologic variation of *Lathyrus sativus* and found that *L. sativus* had high similarity between *L. cicera* and *L. gorgoni*. Chemical results obtained from this study were supported by these findings partly. It would suggest that the systematic position of *Lathyrus saxatilis* Vent. is doubtful. It was found different from the other members of *Orobastrum* section in terms of fatty acids. This species was also distinguished from *Orobastrum* section and included in a monotypic section “*Viciopsis*” by Kupicha (1983). In the karyotype analysis of *L. saxatilis*, *L. vinealis*, *L. inconspicuus* and *L. setifolius* (Şahin & Altan, 1990), chromosome number of these species were found as same $2n=14$, but some karyologic differences were found. *L. saxatilis* was different in terms of three subterminal chromosomes from other *Lathyrus* species. In the description of *Cicercula* section, it is reported that it was a rather diverse group. Doğan *et al.*, (1992) divided *Lathyrus* genus into *Orobus* and *Lathyrus* subgenus. Then they reduced the section number of *Lathyrus* from 10 to 9. The fatty acid analysis of *Lathyrus* sp., studied have given important clues in sectional classification of *Lathyrus* genus (Table 2).

Fatty acid results showed that *Lathyrus* species contains linoleic – palmitic and oleic type FA patterns. Since the FA patterns of *Lathyrus* (Papilionideae) seed oils showed a remarkable uniformity in terms of their high content of the linoleic and palmitic acids. It is proposed that this can be of chemotaxonomical interest and their quantity may indicate closer or more distant relationships between the considered members of the genus. Our

results on *Lathyrus* sp., seed fatty acids are in good agreement with some reports of Bağcı *et al.*, (2001, in press). The major unsaturated acids in ten members (*Adenanthera paronina*, *Tetrapleura tetraptera*, *Cassia nodosa* etc.) of the Leguminosae, seed oils were oleic and linoleic acids, both comprising an average of 68.4% of the component acids (Balogun & Fetuga, 1985). Some genera from subfamilies Mimosoideae, Lotoideae and Caesalpinoideae showed same type of main fatty acid (Balogun & Fetuga, 1985). But some differences particularly relative contents of linoleic, oleic – palmitic and linolenic acid were observed (Akpınar *et al.*, 2001; Bağcı *et al.*, 2001; Azcan *et al.*, 2001). Tsevegsuren *et al.*, (1998) reported that Leguminosae seeds contained two groups of seed oil fatty acid profiles which are rich in linoleic acid (*Ammopiptanthus*, *Trifolium*, *Thermopsis*) or rich in linolenic acids (*Oxytropis*). The seeds from 3 plant species belonging to *Crotalaria* was analysed by Chowdury & Banerjee (1995), for fat and fatty acids where all the seed fats resembled the simple linoleic-oleic-palmitic type.

There is some evidence that the rarer fatty acids, like non protein amino acids, may be harmful to animal eating the seeds. Erucic acid (22:1), a characteristic acid of the Cruciferae, has been described as having toxic effects in mammals if ingested in sufficient amounts (Harborne & Turner, 1984). It was not determined in this and previously studied *Lathyrus* seed oils (Bağcı *et al.*, 2001, in press).

Studies on the distribution of fatty acids of seed oils have been driven by economic and taxonomic interests. Some relatively recent examples are the interest raised by the finding of the distribution of FA in seed oils of *Cuphea* (Lythaceae) and some other genera from different families (Saturejoidae, Ajugoidae, Boraginaceae, Cannabaceae and Scutellarioideae (Marin *et al.*, 1991; Velasco & Goffman, 1999; Bağcı *et al.*, 2003). These have been shown to be of valuable taxonomic character at the infrageneric level (Graham *et al.*, 1981 a,b.; Wolf *et al.*, 1983; Graham & Kleiman, 1987; Knapp *et al.*, 1991; Bağcı & Vural, 2001). Their existence has renewed on interest in the further development of this species as a pulse crop. It is possible to say that fatty acid composition of *Lathyrus* species had given some clues on the chemotaxonomic relationships of *Lathyrus*, but it is necessary to enlarge the study with all of the genera and family patterns. The number of species sampled here was very small to draw definite taxonomic conclusions. Our study on the family is currently being carried out. However recent studies (Bağcı *et al.*, 2001; Bağcı *et al.*, in press) revealed that some underexploited leguminous seed crops contain appreciable amounts of oil which could warrant their screening for increased edible oil production in Turkey. In particular this genera patterns from Leguminous plants are very important as the food for animals. Oils rich in oleic and linoleic acids, according to Bailey (1951), are the most adaptable of all oils and are excellent edible oils. These species might have potential as a new oilseed crop for the food industry if growth and yield behavior can be improved.

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