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Wild almond (*Prunus scoparia* L.) as potential oilseed resource for the future: Studies on the variability of its oil content and composition

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ABSTRACT

Wild almond genetic resources have still not received considerable attention for oil chemical compositions and uses. The aim of this study was to assess the levels of variation in oil content and fatty acid composition in forty Iranian accessions of *Prunus scoparia* L. (Spach) to identify genotypes with desirable traits in terms of oil quantity, quality and industrial utilization. Oil parameters and indices were measured, and fatty acid methyl ester analysis was carried out by gas liquid chromatography. Oleic and linoleic fatty acids showed high variability among accessions, ranging from 232.4 to 359.6 g/kg oil and from 190.7 to 348.8 g/kg oil, respectively. Total unsaturated fatty acid fraction was higher than total saturated fatty acid. The ranges of saponification number (199.2–202.1), iodine value (104.8–125.7 kg I2/kg) and cetane number (43.8–48.8), confirmed that the oils have industrial potentialities. Results could contribute to select wild almond genotypes as genetic sources for oil production.

1. Introduction

Almonds (*Prunus amygdalus* L.) are native to the Western and Central Asia, including eastern China, Kurdistan, Turkestan, Afghanistan and Iran (Martínez-Gómez et al., 2007; Sathe, Teuber, Gradziel, & Roux, 2001; Zeinalabedini et al., 2008). Wild almond species commonly grow in areas between 28° and 38°N and 41° and 54°E and from 1100 to 2700 m.a.s.l. (Browicz, 1969), in regions characterized by a subtropical Mediterranean climate, with mild wet winters and warm, dry summers. Nearly 20 almond wild species have been reported in Iran (Sorkheh et al., 2009), indicating that this country is within the center of origin of almond. Indeed, Iran has primarily a subtropical climate in the south of the country, temperate in the north part, with mostly desert in the middle, and the resultant variability in environment and climate made possible an extensive diversity of almond germplasm (Sorkheh et al., 2009). In 2004, world production of cultivated almond was approximately 2,917,894 tonnes, of which Iran produced 87,281 tonnes (FAO STAT, 2013). Iranian almond production is mainly based on locally adapted clones, with minimum to no inputs, and traditional management (Sorkheh et al., 2009).

The almond is considered a pleasant nut throughout the world with applications in food, pharmaceutical and cosmetic industries. It is used as an ingredient in many snacks and other processed foods (Zhang et al., 2009). As is the case with other nuts, an almond-based diet reduces the risk of cardiovascular diseases (Chen, Lapsley, & Blumberg, 2006). This is attributed to the hypocholesterolemic effect of high levels of fiber, sterols, ratio of total unsaturated fatty acids (TUSFA) to total saturated fatty acids (TSFA), and also to the antioxidant capacity of vitamin E and sphingolipids present in almonds (Chen et al., 2006; Maguire, O'Sullivan, Galvin, O'Connor, & O'Brien, 2004).

At present, a few crop species such as soybean (*Glycine max*), oilseed rape (*Brassica napus*) and sunflower (*Helianthus annuus*) dominate the international edible oilseed market. However, with a continuous increase in population, the demand for high-quality seed oils is also increasing. To meet the demand, there is a need not only to increase the production of the major oilseed crops but also to diversify the sources by popularizing and increasing the production of minor crops. Although numerous studies have been reported on the characteristics of the oil and other components of almond species (Farhoosh & Tavakoli, 2008; Kiani, Rajabpoor, Sorkheh, & Ercisli, 2015), a complete investigation on the nutritional and chemical compositions of wild almond species is not currently provided in the literature. The wide adaptation of wild almond indicates its potential as sources for resistance to abiotic and biotic stresses, as well as for advantageous nut traits.

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Efforts have been made to evaluate and utilize the available germplasm, particularly in terms of fat and fatty acid profiles (Kiani et al., 2015). Despite the wide distribution of wild almond species having high nutritional values, they have not been fully utilized for industrial applications. Therefore, a thorough investigation on the chemical compositions of the oils extracted from wild almond species could significantly contribute to the current limited data available for their potential use as raw materials in food industry.

Particularly, *Prunus scoparia* L. (Spach) is a wild almond species of the taxonomic section 'Spartioids' and it is distributed as an oilseed crop in Iran. *P. scoparia* seeds are used in human food mainly as a spice in the frying of pulses, vegetables and also in pickles (Kiani et al., 2015). Its seed oil is used for various industrial products, such as soaps, paints, and biodiesel. In addition, this crop is an important source of seed protein that significantly contributes to the human dietary protein intake. Despite its great advantages in comparison with other wild almond germplasm, including low bitterness, evergreen habitus, high values of net photosynthesis, and high yields of seed and oil, *P. scoparia* suffers from a lack of improvement program through modern breeding efforts (Sorkheh et al., 2009).

On this basis, the aim of this study was to assess the levels of variation in oil content and fatty acid composition among accessions of *P. scoparia* in order to identify genotypes with desirable oil quality for consumption, industrial use or biodiesel production. This information could be used for increasing oil content and diversifying oil quality in wild almond.

2. Materials and methods

2.1. Plant materials

Forty accessions from seven populations of *Prunus scoparia* L. (Spach) were collected in seven different parts of Chaharmahal-e Bakhtiari province (Iran; 32°19′32″ N, 50°51′52″ E; average rainfall 321.5 mm) (Table 1 and Supplementary Fig. 1) for two consecutive years (2012 and 2013) and used for this study. Field expeditions were carried out according to Sorkheh et al. (2009). Sites were selected based on previous literature, indigenous information, or conspicuous presence. Collections were made from both wild and cultivated habitats from trees at the same physiological stage. The detailed procedure is available in Sorkheh et al. (2009). Mature plants were harvested by cutting plants at 15–20 cm above ground level and placing them in plastic bags before taking them to the laboratory. Most of these accessions were previously morphologically, genetically and biochemically characterized (Sorkheh et al., 2009). All the samples were stored under refrigerated conditions (4 °C) until used in the experiments.

2.2. Oil extraction and analysis

Seeds were manually separated from the rest of the plant and cleaned of impurities. The seeds were oven-dried at 60 °C, and then stored in desiccators until analysis. Seeds were ground in a laboratory rotor mill (model Pulverisette 14; Fritsch GmbH, Markt Einersheim, Germany) to particle size <200 μm. Seed moisture content was determined before oil extraction by weighing about 5 g of ground seed in pre-calibrated porcelain capsules and placing in thermoventilated oven at 105 °C until constant weight was reached. Oil extraction was performed using a Soxhlet apparatus with about 20 g of ground seeds and *n*-hexane as solvent applying the method reported by Zhang et al. (2009). The solvent was then removed under vacuum rotary evaporation at 40 °C and the percentage of recovery was meanly 80–85% for all the samples; the flask containing the extracted oil was placed in an oven at 70 °C for 4 min and weighed after cooling in a dryer. The oil content was determined using the following relation:

$$\text{Oil content} = [(P_1 - P_2)/P]100$$

where P is the weight of the dry seed, P_1 the weight of the flask containing the oil, and P_2 the weight of the empty flask.

The pure oil was transferred into a small glass vial, flushed with nitrogen and maintained at −20 °C until analyzed for, iodine value (IV), saponification number (SN) and cetane number (CN). The values of IV and SN were determined according to the methods 920.158 and 920.160, respectively, both from the official methods of analysis of AOAC International (2005). CN was determined according to Krisnangkura (1986).

Table 1
Prunus scoparia accessions with the respective codes and collection site characteristics.

Code	Number of accessions	Collection site	Latitude N	Longitude E	Elevation (m)	Annual rainfall (mm)
PSA	7	Ardal	32.01	49.50	1850.0	320.2
PSB	8	Broojen	31.57	51.18	2197.0	254.3
PSFA	6	Farsan	32.15	50.35	2250.0	275.4
PSFE	4	Felard	31.17	51.22	1970.0	456.8
PSKB	4	Kareh-e-Base	31.30	45.54	2700.0	283.2
PSKO	5	Kohreng	32.26	50.70	2285.0	1441.8
PSLO	6	Lordegan	31.30	50.59	2085.0	280.3

2.3. Fatty acid methyl ester (FAME) analysis by gas liquid chromatography

Kernels were freshly ground with a hand homogenizer (model 400010; Bioreba AG, Reinach, Switzerland) and weighed to obtain 40 mg oil when extracted with 10 mL solvent mixture consisting of chloroform:hexane:methanol (8:5:2, v/v/v). The extracts obtained were dried at 60 °C in nitrogen gas for 30 min. Methyl esters of oil samples were prepared according to the method of Sarin, Sharma, and Khan (2009). An aliquot of the hexane extract (1 mL) was injected into a highly polar HP Innowax capillary column of 30 m length (inner diameter: 0.32 m, film thickness: 0.5 mm, split: 1:80). A gas chromatograph with flame ionization detector (FID) was used (Agilent 6890 GC Gas Chromatograph Series; Varian Inc., Walnut Creek, CA, USA). The injector and detector temperatures were 260 °C and 275 °C, respectively. Oven temperature was held at 150 °C for 1 min, ramped to 210 °C at 15 °C/min and then to 250 °C at the rate of 5 °C/min, with a final hold at 250 °C for 12 min. Peaks of fatty acid methyl esters were identified by comparing their retention time with that of the known standards, were run under the same conditions. Peak integration was performed applying instrument software.

2.4. Oil indices

Stability index was defined as the ratio of oleic to linoleic acid (O/L). Correlation coefficients were calculated for the various traits studied (Yadav et al., 2010). Degree of unsaturation (DU) among various oil samples was derived taking into account the amount of monounsaturated and polyunsaturated fatty acids present in the oil (Ramos, Fernández, Casas, Rodríguez, & Pérez, 2009.).

2.5. Statistical analysis

The experiments were organized in a randomized block design. Data are the average of two crop years and, for each crop year (2012 and 2013), five kernel samples were taken from each acces-