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PHENOTYPIC AND MOLECULAR EVALUATION OF GENETIC DIVERSITY IN NS SAFFLOWER (*Carthamus tinctorius* L.) COLLECTION

ABSTRACT: Safflower (*Carthamus tinctorius* L.) belongs to the Asteraceae (Compositae) family. It is primarily grown for seeds used for bird feed or as edible oil. Stamens are used in traditional medicine and nutrition. Breeding for high resistance to dry growing conditions has initiated intensive studies of this plant species in recent years. Six safflower genotypes of different geographical origins (Ukraine, Italy, Turkey) were collected and added to the collection of less cultivated oil plant species of the Institute of Field and Vegetable Crops in Novi Sad. Phenotypic observations during two growing seasons revealed that analysed genotypes differed in flower colour (yellow, orange, red), in the presence of spines, and in seed oil and protein content. Oil and protein content differed between years and genotypes, indicating large influence of genotype and environmental conditions on variations of these quantitative traits that are negatively correlated. Genetic variability of the analysed genotypes was tested by use of molecular markers. Given that sunflower and safflower belong to the same family, the possibility of applying SSR markers developed for sunflower for molecular analysis of safflower was analysed. The obtained results proved that sunflower markers can be successfully transferred to safflower. Future studies should include larger number of markers in order to identify polymorphic and informative ones. Significant variations within a relatively small number of the analysed safflower genotypes justify further work on the evaluation of the collection, taking into account both genetic and environmental factors.

KEYWORDS: oil content, protein content, molecular marker, safflower

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INTRODUCTION

Oil crops are grown all over the world and represent a vital part of the agricultural sector in many economies. Under European agro-ecological conditions traditional oil crops are mostly annual and biannual crops such as sunflower, rapeseed, soybean, castor, poppy or pumpkin seed, but novel or specialty oil crops such as linseed, safflower, false flax, groundnut, sesame and others could be of particular interest regionally. Given their value for diverse food and non-food applications, oils are a highly desired commodity with worldwide consumption increasing by >50% during the past decades (Vollmann and Laimer 2013). Safflower (*Carthamus tinctorius* L.), also known as Dyer's Saffron, American Saffron, Fake/False Saffron, Bastard Saffron, Zaffer, Azafran (Spanish), Hong Hua (Chinese), Kesumba, Qurtum, etc., is of particular interest. Safflower possesses numerous valuable agronomic attributes that make it attractive as an alternative spring-sown crop for tight crop rotations.

Around the world, safflower is mainly grown for its edible oil for cooking, salad oil and margarine. In affluent countries, the demand for the oil increased after researches linking health and diet, because this oil has the highest poly-unsaturated/saturated ratio of all available oils. Safflower oil is stable and its consistency does not change at low temperatures, making it particularly suitable for use in chilled foods. It is nutritionally similar to olive oil, with high levels of linoleic or oleic acid, but much less costly. Safflower oil is sprayed on various edible products to prevent them from absorbing or losing water, and thus extends their shelf life. In China, safflower is grown almost exclusively for its flowers, which are used in treatment of many illnesses as well as in tonic tea. Addition of safflower florets to foods is a widespread and ancient tradition. True saffron is perhaps the world's costliest spice, and safflower is a common adulterant or substitute. Rice, soup, sauces, bread and pickles take on a yellow to bright orange colour from the florets. Health concerns regarding synthetic food colourings may increase demand for safflower-derived food colouring (Mündel and Bergman 2009).

Adapted to arid, semi-arid and saline soils, safflower is commonly grown in such unfavourable conditions where drought and salinity limit seed germination and plant growth. High resistance to dry growing conditions has initiated intensive studies of this plant species in recent years (Özel 2004; Omidi 2012). Phenotyping and evaluation of morphological traits were performed by use of different methods (Atlagić *et al*., 2009; Ada 2013; Hamza 2015), while molecular analysis included the use of various types of molecular analysis (Johnson *et al*., 2007; Yang *et al*., 2007; Amini *et al*., 2008; García-Moreno *et al*., 2010; Panahi *et al*., 2013; Kumar *et al*., 2015; Ambreen *et al*., 2015). Considering that safflower and sunflower belong to the Asteraceae family, several SSR markers developed for sunflower were chosen in order to determine whether these markers could be transferred and applied for molecular analysis of safflower.

MATERIAL AND METHODS

Six safflower genotypes of different geographical origins (Ukraine, Italy, Turkey) were collected and added to the collection of less cultivated oil plant species of the Institute of Field and Vegetable Crops in Novi Sad: Sunčana (Ukraine), Ptica and Liman (Serbia), Remzibey, Dinçer and Yenice (Turkey). Phenotypic observations of flowers and seeds during two growing periods were performed (Figure 1).

Figure 1. Safflower

Each plot in the experiment consisted of 4 rows, 0.25 m apart and 3.6 m long making the harvest area of individual plots equal to 3.6 m^2. Harvest was performed manually and all plants in each plot were threshed together.

Phenotypic observations (flower colour and presence or absence of spines) were conducted according to Dajue and Mündel (1996). At maturity, seed oil and protein content were measured in whole, unpeeled seed. The oil content was determined using the NMR (Nuclear Magnetic Resonance) method, and expressed as a percentage of seed. The protein content was determined using the classical Micro Kjeldahl method and measured only in 2015.

DNA for molecular analysis was extracted from safflower leaves using modified CTAB protocol (Permingeat *et al.*, 1998). Three SSR markers developed for sunflower were applied for molecular analysis: ORS 595, ORS 610, and ORS 1013 (Tang *et al.*, 2002). PCR was performed as described by Dimitrijević *et al.* (2010). Products of PCR amplification were run on 2% agarose gels and visualized in the BIO-Print system (Vilber Lourmat, Marne-La-Vallée, France).

RESULTS AND DISCUSSION

Phenotypic observations showed that analysed genotypes differ in flower colour. Remzibey had yellow, Liman, Ptica and Dinçer orange and Yenice and Sunčana red flowers. Genotype Liman differed from other analysed genotypes of the collection by the absence of spines, which were in all other genotypes present on branches, leaf edges and blossom.

Seed oil and protein content of the analysed safflower genotypes differed between years and genotypes (Figure 2), which indicated the impact of genotype and environmental conditions in variations of these negatively correlated quantitative traits. In the first year, the oil content ranged between 15.75% and 21.20%. The highest oil content was obtained from genotype Yenice.

In the second year, the oil contents were 12.59% to 16.81% and the highest oil content was obtained from genotype Remzibey (Figure 2). In general, the level of oil in the first year was higher than that in the second year. This may be attributed to the lower precipitation in the second year. Oil content values obtained were lower than the values reported by Çamaş *et al.* (2007) and Golkar *et al.* (2012), and higher than the values reported by Marjanović-Jeromela *et al.* (2007).

Seed protein content ranged from 14.29% (Remzibey) to 17.90% (Liman). Similar values were reported by Marjanović-Jeromela *et al.* (2007), while Golkar *et al.* (2012) reported higher protein content.

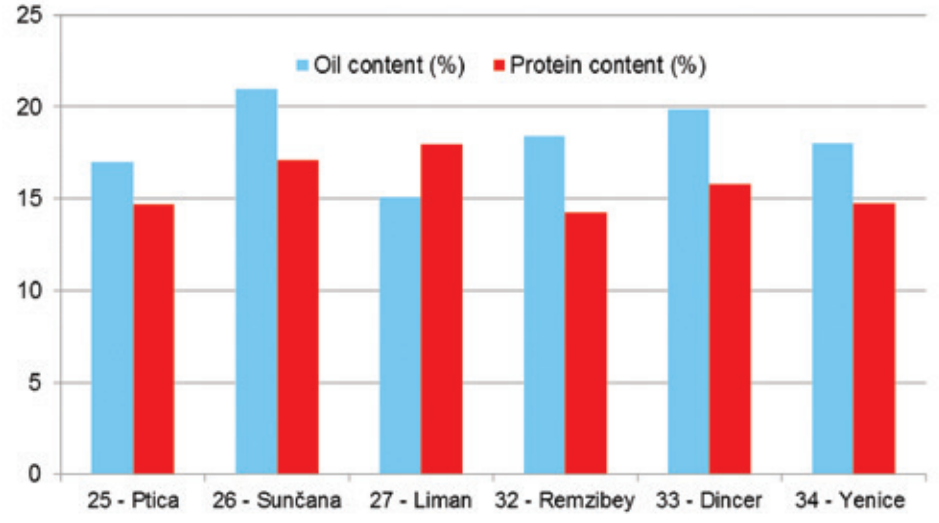

Figure 2. Seed oil and protein content obtained from six safflower genotypes of different geographical origins

Genetic variability of genotypes was analysed by use of SSR molecular markers. Given that sunflower and safflower belong to the same family, the

possibility of using sunflower SSR markers for molecular analysis of safflower was analysed.

All three tested markers amplified bands in the tested safflower genotypes. ORS 595 amplified two bands of different length, 111 bp and 150 bp, while ORS 610 and ORS 1013 were monomorphic, amplifying one band of the same length in all tested genotypes: 73 and 187 bp, respectively (Figure 3). Some unspecific bands were also observed.

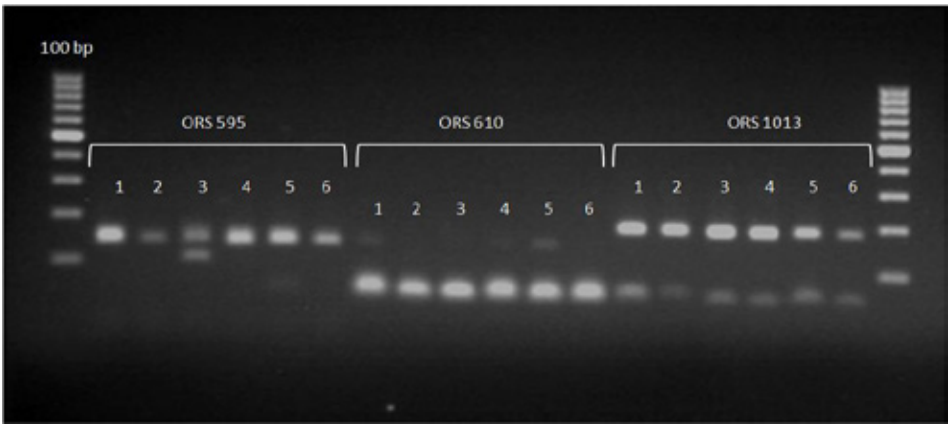

Figure 3. Amplification profiles obtained by use of sunflower SSR markers (ORS 595, ORS 610, ORS 1013). Safflower genotypes: 1 – Sunčana (Ukraine), 2 – Ptica (Serbia), 3 – Liman (Serbia), 4 – Remzibey (Turkey), 5 – Dinçer (Turkey), 6 – Yenice (Turkey). DNA ladder 100 bp (Thermo Scientific).

All tested markers successfully amplified bands in safflower. Only one marker proved to be polymorphic. Opposite to our research, ORS 595 did not amplify bands of sufficient quality for scoring in research reported by García--Moreno *et al.* (2010). In addition, ORS 610 and ORS 1013 were monomorphic, while in the work reported by García-Moreno et al. (2010) these markers were polymorphic. ORS 595 and ORS 610 amplified bands of the similar length (less than 100 bp difference) comparing to bands amplified in sunflower (Dimitrijević *et al.*, 2013). However, when comparing obtained results with those reported by Tang *et al.* (2002), this was only the case with ORS 595.

Seed oil content in safflower usually ranges between 20% and 45% (Vosoughkia *et al.*, 2011), and with decrease in seed coat it can vary between 42% and 50% (Knowles 1982). The results obtained in this trial proved that the evaluated accessions were useful for combined production of oil and proteins, while the collection could be enlarged by introducing breeding material with higher oil content.

Consequently, sunflower markers can be successfully transferred to safflower and future studies should include larger number of markers in order to identify polymorphic and informative ones.

The obtained results indicate significant variation within a relatively small number of the analysed safflower genotypes. This justifies further work on the evaluation of the collection, taking into account both genetic and environmental factors.

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ФЕНОТИПСКА И МОЛЕКУЛАРНА ЕВАЛУАЦИЈА ГЕНЕТИЧКЕ РАЗНОЛИКОСТИ НС КОЛЕКЦИЈЕ ШАФРАЊИКЕ (*Carthamus tinctorius* L.)

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РЕЗИМЕ. Шафрањика (*Carthamus tinctorius* L.) припада породици Asteraceae (Compositа). Гаји се првенствено за добијање семена које се користи за исхрану птица или производњу јестивог уља. Прашници се користе у традиционалној медицини и исхрани. Висока отпорност на сушне услове гајења иницирала је последњих година интензивнија истраживања ове биљне врсте. За колекцију мање гајених уљаних биљних врста Института за ратарство и повртарство, прикупљено је шест генотипова шафрањике различитог географског порекла (Украјина, Италија, Турска). Фенотипским опажањем у току две вегетационе сезоне утврђено је да се генотипови међу собом разликују у боји цвета (жута, наранџаста, црвена), у присуству бодљи, као и садржају уља и протеина у семену. Садржај уља и протеина се разликовао између година и генотипова, што указује на велики утицај и генотипа и спољашње средине у варирању ових квантитативних својстава која се налазе у негативној корелацији. Генетичка варијабилност генотипова је испитана молекуларним маркерима. С обзиром на то да сунцокрет и шафрањика припадају истој породици, проучавана је могућност употребе SSR маркера сунцокрета за молекуларну анализу шафрањике. Добијени резултати показали су да се маркери сунцокрета могу успешно користити за шафрањику и да будућа истраживања треба да садрже већи број маркера у циљу идентификовања полиморфних и информативних маркера. Значајне разлике у релативно малом броју анализираних генотипова шафрањике оправдавају даљи рад на евалуацији колекције, узимајући у обзир и услове средине и генетичку варијабилност.

КЉУЧНЕ РЕЧИ: садржај уља, садржај протеина, молекуларни маркери, шафрањика