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Morphological, biochemical, and nutritional value of prickly and smooth fruit spinach

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Abstract: This study aimed to investigate the morphological (qualitative and quantitative traits) and biochemical characteristics (such as leaf pigments and total antioxidant capacity, vitamin E and C content, total soluble carbohydrate, total amino acid content, nitrate concentration, nitrate reductase assay, oxalic acid content, Ca and Fe content) in spinach. The selected accessions in this study were prickly ('Varamin Prickly') and smooth ('Monatol') fruits of spinach selected among 44 accessions. This experiment was carried out in spring, arranged as a complete randomized block with three replicates and 18 observations. Results showed no significant differences between the two accessions for most qualitative and quantitative morphological traits. In contrast, biochemical characteristics showed significant differences between the two accessions. Both accessions had high yields, but the dry biomass of 'Varamin Prickly' accession was more than 'Monatol' (smooth fruit). The results indicated that the fruit type does not appear to cause variations in morphological traits, and differences in accessions could be due to genetic sources and environmental distribution. The prickly fruit accession showed a significant superiority for most qualitative nutraceutical traits, including DPPH, flavonoid, phenol, carbohydrate, amino acid, fiber, and Fe content compared to smooth fruit accession. Finally, it was found that prickly fruit accession is very suitable for mechanized harvesting and human diet due to its appropriate plant, leaf, petiole, and qualitative nutraceutical traits and can be used for breeding purposes and cultivation fields.

Key words: Iranian spinach accession; prickly fruit; smooth fruit; nutraceutical traits; *Spinacia oleracea*

Morfološka, biokemična in hranilna vrednost špinače z gladkimi in bodečimi plodovi

Izvleček: Namen raziskave je bil preučiti morfološke (kakovostne in količinske) in biokemijske lastnosti (listna barvila, celokupno antioksidacijsko sposobnost, vsebnost vitaminov E in C, celokupnih topnih ogljikovih hidratov, celokupnih amino kislin, nitrata, oksalne kisline, Ca in Fe, preiskus nitrate reduktaze) v špinači. Iz 44 akcesij so bile izbrane sorte z bodečimi ('Varamin Prickly') im gladkimi ('Monatol') plodovi. Poskus je bil izveden spomladi kot popolni naključni bločni poskus s tremi ponovitvami in 18 opazovanji. Rezultati so pokazali, da v večini kakovostnih in količinskih morfoloških lastnostih obeh tipov ni bilo značilnih razlik. Nasprotno so se vbiokemičnih lastnostih obeh tipov pokazale značilne razlike. Oba tipa akcesij sta imela velike pridelke, a je bila biomasa 'Varamin Prickly' večja kot pri akcesijah sorte Monatol. Rezultati so pokazali, da tip plodov ne povzroča raznolikosti v morfoloških lastnostih in, da bi razlike med akcesijami lahko bile genetskega ali okoljskega izvora. Akcesije z bodečimi plodovi so bile značilno superiorne v večini kakovostnih hranilnih lastnosti kot so DPPH, vsebnost flavonoidov, fenolov, ogljikovih hidratov, amino kislin, vlaknin in Fe v primerjavi z akcesijami z gladkimi plodovi. Povzamemo lahko, da bi bile akcesije z bodečimi plodovi zelo primerne v bodočih programih žlahtnenja in pridelave na polju zaradi primernosti za strojno spravilo in prehrano ljudi zaradi primernih lastnosti habitusa, listov, listnih pecljev rastlin in ugodnih hranilnih lastnosti.

Ključne besede: akcesije iranske špinače; bodeči plodovi; gladki plodovi; hranilne lastnosti; *Spinacia oleracea*

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1 INTRODUCTION

Spinach (*Spinacia oleracea* L.) is one of the critical and commercial vegetables planted worldwide for fresh and processed consumption (Morelock & Correll, 2008). As one of the origins of spinach, Iran has ranked the seventh producer globally (Shi et al., 2016). This vegetable is a fast-growing, cold season, and nutritious that is highly recommended in the human diet. Spinach has high levels of iron, dietary fiber, vitamins, antioxidants, and several phytochemical components. On the other hand, some compounds, such as nitrate and oxalate, can be high accumulated in leaves that have potential health hazards to humans (Koh et al., 2012). Therefore, selecting and introducing low-capacity nitrate and oxalate varieties is an essential aim in spinach breeding programs (Koh et al., 2012).

Pprevious studies were devoted to the genetic structure associated with the morphological traits of spinach (Arif et al., 2013; Sabaghnia et al., 2014). High variation yield was reported among 54 Iranian spinach accessions (Sabaghnia et al., 2014). Nevertheless, variation in the phenotypic characteristics of plants such as leaf type, fruit type (seed type), and even leaf color can affect plant growth and biochemical traits. Like, leaf color affects morphological and biochemical characteristics of *Sassafras tsumu* (Hemsl.) Hemsl. Jiang et al., 2016). Phenotypic differences were also reported in *Vachellia nilotica* subsp. *indica* (Benth.) Kyal. & Boatwr. due to having two types of smooth and prickly seeds. (Gorain et al., 2014). Sunflowers from different seeds differ in biochemical properties such as oil and protein content (Balalic et al., 2012).

Fruit types, leaf morphology, leaf color (green vs. purple), and day to flowering are spinach's most critical phenotypic characteristics for breeding classification and commercial purposes (Shi et al., 2016). Wrinkled leaves are marketable in the USA, whereas flat leaves are favorable in Iran (Avsar, 2011). Moreover, fruit forms used in mechanization and handy cultivation are different (Wu et al., 2015). There are two types of taxonomic varieties of spinach (*S. oleracea* var. *spinosa* Moench.) with prickly and (*S. oleracea* var. *inermis* Peterm.) with smooth fruits (Mei et al., 2010). Spinach cultivars, with prickly fruits are not suitable for mechanized cultivation. So, it has been suggested that round and smooth fruits, is suited for mechanized spinach cultivation (Morelock & Correll, 2008). Some countries have solved the prickly problem of spinach fruit by using fruit coating (Shi et al., 2016). It is reported that most European varieties of spinach fruits are smooth (Meng et al., 2017). Conversely, most prickly spinach fruits belong to the Asian region consisting of Korea, Japan, Iraq, and Iran. There is a remarkable diversity among the fruits of Asian spinach cultivars due to the vast territory and different climates (Meng et al., 2017; Shi et al., 2016).

The researchers reported that prickly fruit spinach varieties had narrow and small leaves with long petioles that are generally resistant to low temperatures but sensitive to high temperatures (Meng et al., 2017; Mei et al., 2010). On the other hand, thick and wrinkled leaves with short petioles are generally observed in smooth fruit varieties (Mei et al., 2010). It is reported that smooth fruit varieties of spinach are tolerant to high temperatures and are sensitive to low temperatures (Mei et al., 2010). So, the appearance of spinach fruit is also an essential feature for spinach classification (Wu et al., 2015; Liu et al., 2004).

Besides, the type of fruit may be affected by spinach quality. Some relationships were observed between the morphological and biochemical characteristics of spinach (Mei et al., 2010). Wu et al. (2015) investigated a wide variety of morphological traits of two smooth and prickly fruits spinach varieties. He reported significant differences in the growth habits of two fruits types, and morphological and biochemical classification of spinach fruits are critical to distinguish among them (Wu et al., 2015). Therefore, the qualitative assessment of prickly and smooth fruits of spinach, such as antioxidants, fiber, iron content, and accumulation of nitrate and oxalate, provided helpful information on cultivar selection (Wu et al., 2015).

Recently, due to the extensive cultivation of spinach, there has been a vast demand for high-quality seed material for farmers (Jafari & Jalali, 2015). There is a lack of information on the differences between smooth and prickly fruit spinach for the future breeding program. Therefore, this study seems necessary as primitive information for broadcasting future research. The present experiment was conducted to investigate and compare the morphological and biochemical characteristics of the prickly and smooth fruit accessions and the relation of fruit morphology with spinach's biochemical and nutraceutical characteristics.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL AND FIELD EXPERIMENT DESIGN

Iranian spinach accession 'Varamin Prickly' (prickly fruit) and 'Monatol' accession (smooth fruit), in text later indicated as seeds, were selected from 44 spinach accessions provided by the Seed and Plant Improvement Institute and the Gene Bank of Iran (SPII) and the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) gene bank (IPK, 2018) based on the previous author's study (Abolghasemi et al., 2019). The selection was based on spinach's yield and mechanized planting characteristics, such as leaf features, plant height, petiole length, fresh mass and yield, dry mass, days to flowering, and percentage of the female plant.

In addition, these two accessions had different seed type that was selected for this study. The experiment was designed based on a randomized complete block design (RCBD) with 3 replications and 18 observations. The seeds were sown in a field located at the Isfahan University of Technology, Isfahan, Iran, in March 2018 (spring sowing). The conditions of daylight and temperature during spring are presented in Fig 1. The soil was sandy-clay, and manure fertilizer mixed with soil 40 t ha^{-1} pre-planting. The plot size was 2 m^2. After seed germination and plant growth, 18 observations (plant bush) in each plot were selected. It should be noted that this study was carried out in the growth chamber for a more precise evaluation and better comparison of morphological and biochemical characteristics of these two accessions (smooth and prickly seed). Finally, the results of our research in controlled conditions confirmed our field cultivation results (data was not presented). Therefore, due to the similarity of the two studies, the field study results are presented in this manuscript.

2.2 MORPHOLOGICAL CHARACTERIZATION

To investigate morphological features, 35-50 days after planting (due to non-homogeneous growth of spinach), 18 plants were selected from each plot. Then four leaves were selected from four directions of plants to measure the desired parameters. In the first step, the qualitative and visual characteristics of spinach accessions were evaluated based on the descriptor of the International Plant Genetic Resources Institute (IPGRI) presented in Table 1. (Arif et al., 2013; Jafari & Jalali 2017).

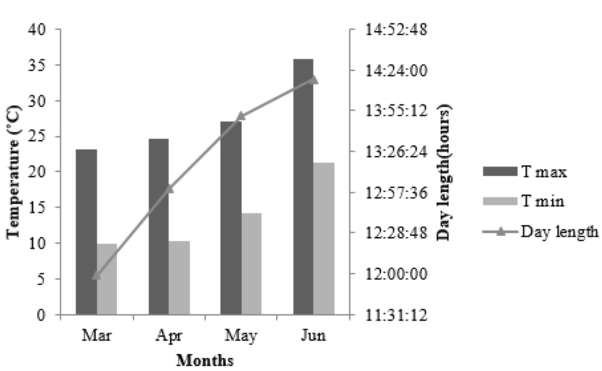

Fig 1: Changes in temperature and day length in spinach spring cultivation

Quantitative and morphological characteristics studied in this study included: 1000-grain mass, germination percentage was calculated using the formula when 50 percent of the seeds were germinated (Ikic et al., 2012):

Germination percentage = (number of germinated seeds / total number of seeds) × 100 when 50 percent of the seeds were germinated, leaf length (cm), leaf width (cm), petiole length (cm), petiole diameter (mm), leaf area (mm^2), plant height (cm), fresh and dry mass (g), yield (kg ha^{-1}), leaf numbers, male and female plants percent, days to flowering. The dry mass of the shoot was measured after putting the bush in an oven at 70 °C for 48 hours (Arif et al., 2013; Jafari & Jalali, 2017).

2.3 BIOCHEMICAL ANALYSIS

Leaf pigments**:** Some fresh leaf tissue (5 g) was mixed with 80 % acetone, then filtered and balanced to 10 ml, and its absorption was measured in 663, 647, and 470 nm with a spectrophotometer (U-2100, JASCO, Japan) (Lichtenthaler, 1987).

Chlorophyll a = (19.3 × A663 - 0.86 × A647) Volume / 100 Mass

Chlorophyll b = (19.3 × A647 - 3.6 × A663) Volume /100 Mass

Total chlorophyll = Chlorophyll a + Chlorophyll b

Carotenoids = 100(A470) - 3.27(mg g^{-1} Chl. a) - 104 (mg g^{-1} Chl. b)/227

Total antioxidant capacity**:** The total antioxidant capacity of spinach was measured by the 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) method (Prasad et al., 2008). Fresh tissue (0.1 g) was mixed in 1.0 ml MeOH and shacked for 2 hours, then centrifuged at 12000 g for 30 min. The supernatant (0.5 ml) was added to the 2.8 ml DPPH solution (0.1 mM) and incubated for 30 mat room temperature. Absorbance was read at 517 nm. The control sample was 2.8 ml DPPH, with the addition of 0.5 ml MeOH. The scavenging activity was determined by the following equation (Stojichevich et al., 2008):

Inhibition % = (A control – A sample / A control) × 100

A: Absorption at 517 nm

Total flavonoid content**:** 0.30 ml of the extract was mixed with 0.50 ml of $NaNO_2$ (5 %). After that,

$AlCl_3.6H_2O$ (10 %), 0.4 ml NaOH, and 0.20 ml were added. Then, its absorption was performed at 510 nm with a spectrophotometer (U-2100, JASCO, Japan) (Krizek et al., 1998).

Total phenolic content**:** The Folin–Ciocalteu method measured total phenolic content. We were using a spectrophotometer (U-2100, JASCO, Japan) at 765 nm. The standard curve was plotted with the Gallic acid solution (Raven, 2003).

Vitamin E content**:** 0.2 ml of alcoholic extract mixed with 5.0 ml toluene, 1.0 ml of ferric chloride, and 3.5 ml of 2, 2-Bipyridine. The mixture was diluted with 10 ml ethanol (95 %), and after 2 min, absorbance was recorded at 525 nm. The standard curve was plotted with vitamin E (Wang & Galletta, 2002).

Vitamin C content**:** Fresh leaf (1.0 g) mixed with 50 ml meta-phosphoric acid (6 %), keeps in the dark for 45 min then centrifuged at 6000 g for 15 min, then 1.0 ml of extract was added to 9.0 ml dichlorophenol indophenol solution (DCPIP) (0.025 %). The next absorbance was read at 515 nm. The standard curve was plotted with different vitamin C concentrations (Djioua et al., 2009).

Total soluble carbohydrate content**:** Total soluble carbohydrate content is measured by the anthrone method (Mc-Cready et al., 1950). For this purpose, 2.0 ml of anthrone was added to the alcoholic extract of the samples. Then, samples were boiled for 2 minutes in the water bath. After cooling and creating the color phase, the absorbance was read at 625 nm. The standard curve was plotted with the glucose standard (Mc-Cready et al., 1950).

Total amino acid content**:** The ninhydrin method was used to measure free amino acids. The ethanol extract of powdered samples was mixed with 1.0 ml of ninhydrin; after boiling and cooling, absorption was read in 575 nm wavelength by spectrophotometer (U-2100, JASCO, Japan). Calibrated curves were plotted with alanine: $y = 0.0198x - 0.0025(r^2 = 0.997)$ (Shihwen et al., 2006).

Crude fiber content**:** Dried leaves (1.0 g) boiled with 200 ml of H_2SO_4 for 30 min. The extract was filtered, washed with boiling water, the residue boiled with 200 ml NaOH for 30 min then filtered, the solids solid part removed from the filter and placed in a tarred crucible for drying and ashing. It has placed tarred crucible with residue in an oven set at 100 ˚C for 8 hours. Then weighted the crucible with fiber residue and calculated the residue mass by subtracting the empty crucible mass from the crucible and sample mass. Then, the crucible was placed with the dried residue in the oven (550 ˚C) for 4 hours for ashing. Finally, we weighted the crucible with the ash residue in the analytical balance and calculated the ash mass by subtracting the empty crucible mass from the crucible and ash mass. The percentage of crude fiber was calculated using the following equation:

% Crude fiber content = ((dried residue mass – ash mass) / sample mass) × 100

Nitrate concentration**:** Nitrate concentration was evaluated by Narayana & Sunil (2009). Briefly, water extraction (0.1 g fresh leaf mixed in deionized water) was placed in a boiling water bath for 30 min and centrifuged at 4000 g for 30 min. Then, extraction was mixed with 0.8 ml salicylic acid (5 %) in concentrated sulfuric acid (95 %) and cooled at room temperature. Then, 19 ml of sodium hydroxide (NaOH, 2N) was added, and the absorbance at 410 nm was determined with a spectrophotometer (U-2100, JASCO, Japan). The standard curve was plotted with different KNO_3 concentration using following standard formula get from standard curve; $y = 0.053x + 0.035$ ($r^2 = 0.997$).

Nitrate reductase assay**:** The leaf sample (100 mg) was suspended in 5.0 ml of phosphate buffer (0.1 M), KNO_3 (0.02M), and propanol (5 %). The solution was kept in the dark water bath (37 °C) for 30 min. The solution was treated with 1.0 ml of sulfanilamide (1 %) and N-1-naphthyl-ethylenediamine (0.02 %). After 15 min, the absorbance was measured at 540 nm with a spectrophotometer (U-2100, JASCO, Japan). The standard curve was plotted with the KNO_2 solution: $y = 0.0049x + 0.0092$ ($r^2 = 0.988$) (Narayana & Sunil, 2009).

Oxalic acid content**:** The dry sample (0.5 g) was mixed with 30 ml HCl (0.25N) and put in a water bath for 15 min. Then, the supernatant was filtered and mixed with 5.0 ml sulfuric acid (2 N) and 2.0 ml potassium permanganate (0.003 M). After 10 minutes, the absorbance was detected at 528 nm. $y = 0.9126x - 0.0705$ ($r^2 = 0.9327$) and standard oxalic acid solution (1 mg ml^{-1}) was prepared with distilled water. (AOAC, 1970).

Ca and Fe content**:** The concentration of Ca and Fe shoot was measured by atomic absorption (Perkin Elmer, 3030, Netherland) after digesting for 12 h with 2.5 ml HCl (36 %) (Nolte, 2003).

2.4 ANALYSIS OF DATA

The experiment was arranged in RCBD with three replicates. The morphological and biochemical obtained data were analyzed using analysis of variance (ANOVA) by SAS 9.4 comparison of means was performed using the least significant difference (LSD) test at a 0.05 level of probability. The correlation between morphological and biochemical traits was also tested using the least significant difference (LSD) test at $p \leq 0.05$.

3 RESULTS AND DISCUSSION

3.1 QUALITATIVE TRAITS

The qualitative characteristics of two accessions based on the spinach descriptor are shown in Table 1. Usually, smooth seeds of spinach are generally more favorable in the world. The 'Monatol' accession has a smooth seed (presented in Fig 2, C). On the other hand, prickly seed accession (Fig 2, A) is less desirable; they are challenging to plant (Asadi & Hasandokht, 2007). It has been reported that the best seed type for mechanized planting of spinach was a smooth and round seed (Morelock & Correll, 2008). In this study, both accessions had a gray background in seed color (Table 1).

It has been reported that accessions with petiole standing, leaf sheath standing, wrinkled, or slightly wrinkled, are suitable for mechanical harvesting (Mei et al., 2010). Accordingly, 'Varamin Prickly' (prickly seed) accession is more suitable for mechanical harvesting than 'Monatol' (smooth seed) accession because of petiole and leaf form (Table 1). Prickly seed accession has a feature of leaf standing, petiole standing, and low wrinkle, green leaf color appropriate, which can be used in breeding programs, mechanical harvesting, and genetic modification (Table 1). In the USA and other western countries, wrinkled leaves of spinach are more marketable than flat leaves (Kuwahara et al., 2014). In confirmation of this report, smooth seed accession had wrinkle leaves (Fig 2, C, and D).

The smooth seed accession has wrinkled dark green leaves and is more suitable for storage because the ventilation is better but hardly washable (Fig 2, D). Usually, spinach cultivars with lower leaf wrinkles have less nitrate content, and there is a direct correlation between leaf wrinkle and nitrate content (Arshi, 2000). So, no-wrinkle leaves as a desirable attribute are interested in researchers. While in Iran, the most favorable spinach has a large, fleshy, thick, low leaf wrinkle and juicy leaf (Fig 2, B), and according to Table 1, the endemic accession with prickly seed has these features. In Iran, the optimal form of spinach leaf is round, and overseas desirable triangular shapes have been reported (Kunicki et al., 2010). The seed type did not affect leaf shape (Table 1). Leaf color is critical in leafy vegetables since green pigments are desirable and marketable for fresh and frozen spinach (Eftekhari et al., 2010). In this study, both accessions had a complimentary green color, although the foreign accession with smooth seed

Table 1: Qualitative morphological features of prickly and smooth spinach accessions (Varamin Prickly and Monatol) according to spinach descriptor (Arif et al., 2013; Jafari and Jalali 2017)

Seed type	Seed color	Petiole attitude	Wrinkles of leaf	Leaf thickness	Leaf Sheath attitude	Leaf shape	Leaf color	Leaf Sheath	Lobation of leaf tip	The shape of leaf tip	Wave margin of leaf
Prickly seed	Gray-yellow	Erect	Very Low	Thick	Erect	Broad oval	Dark green	Concave	Bend	Circular	Yes
Smooth seed	Gray-yellow	Horizontal	High	Very thick	Horizontal	Broad oval	Very dark green	Concave	Upward	Circular	No

was darker in color (Table 1, Fig 2). The leaf shape, leaf sheath, and leaf tip shape were similar in both accessions. In general, although the type of seed did not affect most of the quality characteristics of spinach in this study, the prickly seed type of spinach was much more desirable for mechanical harvesting than the smooth seed due to the attitude of the petiole and leaf sheath (Table 1). Researchers have reported that prickly seed spinach has better growth in appearance characteristics (Wu et al., 2015; Amoli, 2012) that can be observed in mechanical harvesting for the prickly seed (Table 1).

3.2 QUANTITATIVE TRAITS

Analysis of variance showed a significant difference between the two accessions in dry mass, 1000-grain mass, germination percentage, male and female plants at 1 % probability level, and 5 % for petiole length (data was not presented). Mean comparison in Table 2 showed no significant difference in leaf number, plant height, leaf length and width, petiole diameter, yield, fresh mass, leaf area, and day to flowering between prickly and smooth seed accessions. There is not much similarity between the investigations that can be reported in each region according to different spinach weather and growth conditions. In this study, petiole length was more than 100 % higher in the prickly seed accession (Iranian accession) than in the smooth accession, indicating that the plant's form was more stable in the prickly seed than the smooth seed accession and more suitable for mechanical harvesting (Table 2). Confirming this, it has been reported that plant shape and petiole length are crucial for mechanical harvesting (Shi et al., 2016). Dry mass was the highest in prickly seed accession (Table 2). So, the highest amount of dry mass (14.77 g) was observed in 'Varamin Prickly' accession (Table 2). yield, fresh and dry mass of 'Varamin Prickly' was more suitable (Asadi & Hasandokht, 2007; Eftekhari et al., 2010; Jafari & Jalali, 2015). One of the desirable traits for spinach processing and packaging is dry mass, which directly relates to the smoothness of the leaf spinach (Arshi, 2000; Eftekhari et al., 2010). It can also be stated that the different water content in the tissues of these two accessions leads to the difference in dry mass. In Asadi & Hasandokht's (2007) study, the highest dry mass in 'Qom' accession was reported with prickly seed. The mass of 1000-grains of prickly seed accession was 16.81 g, which had bigger seeds than the smooth seed accession 48.3 % more than the 1000-grain mass of smooth seed accession (Table 2). According to our results, Eftekhari et al. (2010) reported that the 1000-grain mass of prickly seed accessions was higher than that of smooth seed accession. Prickly seeds appear larger in appearance than smooth ones. The percentage of seed germination of smooth seed accession was higher than the prickly seed accession (showed a 52.3 % increase in germination). There was a significant difference between the two accessions (Table 2), which may be due to more water absorption of smaller seeds during germination. Although prickly seeds are high in mass, it is hard to say that prickly seeds have a much larger surface area than smooth seeds. It is believed that the small seed varieties have higher water absorption capacity and better establishment due to the higher surface area to volume ratio (Zaferaniye, 2015). Observations on the germination process of spinach seeds have shown that genotypes with better germination had better vegetative growth and yield. (Jaliliyan, 2009; Zaferaniye, 2015). However, this study's performance was not statistically significant (Table 2). Spinach is a leafy vegetable, so longer vegetative growth is desirable. It is reported that the late-flowering varieties are a priority to increase the spinach production period (Jaliliyan, 2009). In this regard, this study classified prickly and smooth seed accessions as spring late flowering (near to 70 days) (Table 2). The researchers reported that the late-flowering accessions were mostly economically desirable (Asadi & Hasandokht, 2007). Therefore, spinach with better vegetative growth, yield, and appearance is more favorable to farmers. It should

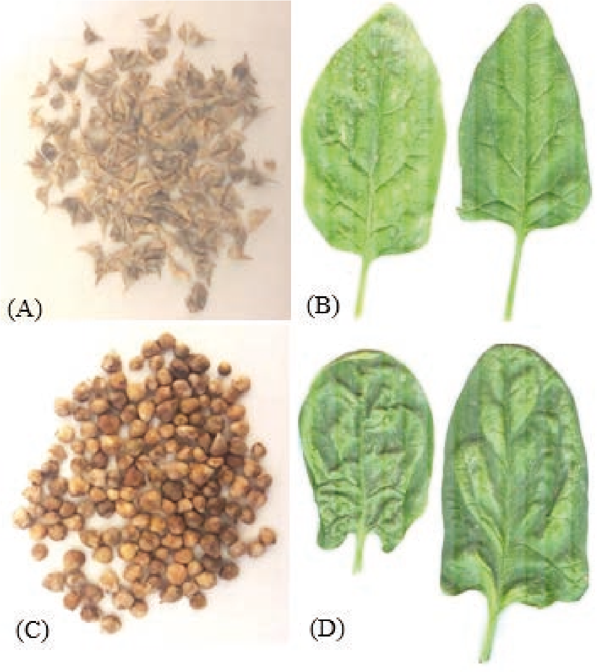

Fig 2: Comparison of 'Varamin Prickly' spinach seed (A); leaf (B) and 'Monatol' spinach seed (C); leaf (D)

Table 2: Morphological traits of prickly and smooth seed spinach accessions

Seed type	Leaf number	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Petiole diameter -(mm)	Yield (kg h^{-1})	Fresh mass (g)	Dry mass (g)	Leaf area (mm^2)	1000-grain mass (g)	Germination percentage (%)	Days to flowering	Male plant (%)	Female plant (%)
Prickly seed	15.66^a	25.00^a	13.73^a	7.33^a	12.66^a	4.35^a	30867^a	60.02^a	14.77^a	48015^a	16.81^a	64.71^b	69.66^a	51.86^a	49.59^b
Smooth seed	20.20^a	19.66^a	12.00^a	9.06^a	6.20^b	4.75^a	42056^a	81.77^a	8.63^b	60085^a	11.33^b	98.58^a	69.00^a	3.82^b	95.96^a
Standard Error	28.40	8.48	5.24	1.28	2.84	0.26	68595	259.2	1.14	57668	0.04	13.2	3.16	0.44	11.2

Different letters within the same column indicate significant differences of each type at $p \leq 0.05$ by the LSD test

be noted that the result of two traits of yield and day to the flowering of spinach in two different seasons (spring and autumn) can be different because these two traits are affected by the type of growing season (Asadi & Hasandokht, 2007). In this study, both Iranian and foreign accessions (the prickly and smooth seed) were similar in day to flowering and yield (Table 2)). In confirmation of various reports of spinach being dioecious , both of the accessions were male or female in this study as well (Morelock & Corell, 2008). As can be seen in table 2, the smooth seed accession had significantly more female plants than the Iranian prickly seed accession. So smooth seed accession showed 93.5 % more female plants (Table 2). According to studies, spring spinach cultivation is valuable if the plant can flower later with female flowers (Zaferaniye, 2015). Jaliliyan (2009) recommends planting female varieties for economic yield. As a result, the smooth seed accession in this study confirms this statement. It has been reported that prickly seeds in winter and spring conditions usually have better growth than smooth seeds (Wu et al., 2015). It can be concluded that prickly seed accession was similar to the smooth seed accession in many traits in spring cultivation.

According to the spinach descriptor coding (Table 5), prickly seed accession showed higher valuable traits (a score of 33) in yield. Smooth seed accession has more scores in the mechanical harvesting traits (rated 31), indicating the superiority of mechanical harvesting. According to qualitative characteristics (Table 1), these results are related to more standing leaves and petiole in the Iranian accession, than in the foreign accession. Our results confirm previous reports on favorable vegetative and mechanical harvesting characteristics for prickly seed accessions (Asadi & Hasandokht, 2007; Eftekhari et al., 2010; Jafari & Jalali, 2015). In the category of breeding traits, smooth seed accession (with a score of 13) was better than the Iranian accession (with a score of 12), which indicates that the foreign accession is better for breeding traits (Table 5). However, it seems that prickly seed accession should also be considered in terms of breeding characteristics due to near score according to the descriptor scale to smooth seed accession (Table 5).

3.3 BIOCHEMICAL TRAITS

Currently, many domestic and wild spinach accessions in the country may have more favorable biochemical characteristics than foreign accessions. Identifying these genotypes and crossing them improved cultivars with good biochemical characteristics (Sabaghnia et al., 2014). Results of the analysis of variance showed that there were differences between DPPH, flavonoids, amino acid, and iron content in a 1 % probability level and 5 % probability level in phenol, carbohydrate, and fiber content. No significant differences were observed in some biochemical traits such as photosynthetic pigments, vitamins E, C, nitrate and nitrate reductase activity, and the amount of oxalic acid. (data was not presented).

There were significant differences between DPPH, flavonoids, and phenolic compounds they are increased in prickly Iranian accession (69.38 %, 0.76 %, and 79.55 mg 100 g^{-1} fresh mass, respectively, Table 3). Phenol and antioxidant properties of every region depend on many parameters such as climate, soil, altitude, and different species of plants (Mirzaei et al., 2010). Phenolic and antioxidant compounds of Iranian accessions 'Saleh-abad' and 'Langrood' had the highest amount of antioxidant compounds, and both of them had prickly seeds (Yosefi et al., 2010). Accordingly, the antioxidant capacity of prickly seed accession was 6.5 % higher than smooth seed accession (Table 3). Plants are one of the important sources of antioxidants compounds that can protect cells from oxidative damage. Secondary plant metabolites such as total phenols and flavonoids derived from plants have strong potential for free radical sweeping in different parts of the plant, such as spinach leaves. Iranian spinach is rich in phenolic compounds with antioxidant properties (Chan et al., 2009).

Spinach leaves contain active components of flavonoids, high antioxidants, and wide pharmacological and biochemical applications, including anti-allergic, anti-viral, and anti-cancer (Lamnitski et al., 2003; Bergman et al., 2001). In the present study, the content of flavonoids in prickly seed accession was 67.1 % higher than in smooth seed varieties (Table 3). Flavonoids are one of the most widespread and diverse natural compounds that can absorb free radicals like other phenolic compounds. In oxidative stresses, phenolic compounds, especially flavonoids, can interact with membrane phospholipids through hydrogen bonding to the polar heads of phospholipids, thereby contributing to membrane integrity (Mirzaei et al., 2010). Under non-stress conditions, there were differences in antioxidant, flavonoid, and total phenolic activity in different spinach varieties (Barbarin et al., 2005).

Soluble carbohydrates are nutrient chemicals valuable to humans and a source of plant energy. Carbohydrates of spinach are very important (Bavec et al., 2010). The highest amount of carbohydrate was observed in prickly seed accession with 0.64 mg g^{-1} fresh mass of spinach (Table 3). Hagen et al. (2009) reported that the carbohydrate content of leafy vegetables varied among the different accessions under the same growth conditions. The content of soluble sugars has been affected by pre-harvest growth temperature (Steindal et al., 2015).

Amino acids are involved in the structure of spinach protein, and amino acids make up about 30 % of all spinach dry matter (Lisiewska et al., 2011). Few articles have been published investigating factors affecting spinach amino acid content (Trejo-Tellez et al., 2005). The level of amino acids in spinach has been correlated with the amount of nitrogen content (Trejo-Tellez et al., 2005). Although nitrogen was not used as fertilizer in the present study, amino acid content was higher in Iranian prickly seed accession (Table 3) (66.6 %) than in other cultivars (Table 3).

The studied accessions had significant differences in total fiber content. Prickly Iranian accession showed the highest fiber content (2.03 %) (Table 3). One of the causes of vegetable consumption is fiber. In this opinion, the studied Iranian spinach has superiority over the foreign accession in fiber content was more suggested for consumption as a diet food (Erfani et al., 2006). In confirming Iranian spinach accession showed 27.6 % more crude fiber content (Table 3).

Spinach is one of the most significant nitrate accumulators because it has a very efficient absorption system and an inefficient nitrate recovery system (Cantliffe, 2005). According to the results of the analysis of variance, nitrate accumulation and nitrate reductase activity in prickly and smooth seed accessions were not significant (data was not presented). It has been reported that there is a difference in nitrate accumulation only in smooth and wrinkled leaves (Arshi, 2000). Although the difference in leaf wrinkling between the two accessions was obvious, their leaves were not sufficiently different in this trait. Probably, nitrate accumulation was not statistically significant (Table 3). Nitrate accumulation in vegetables is also reported to be affected by cultivars and even specific genotypic differences such as ploidy levels (Alamian et al., 2014).

The studied accessions had significant differences in Fe content (Fig 3). Zaferaniye's (2015) study reported that the Iranian accessions have higher iron content than the foreign accessions. In the present study, the Iranian spinach accession had higher Fe content, which was 62.1 % more than the foreign accession (Fig 3). Also, the concentration of iron in the Iranian and foreign accessions of spinach varied from 30 to 50 mg g^{-1}, which was consistent with the amount of iron in this study (Fig 3). Prickly accession was superior in morphological traits in most studies (Asadi & Hasandokht, 2007), and the present study also showed the superiority of nutraceutical traits in prickly seed accession. In the nutraceutical category, the superiority of the

Table 3: Biochemical traits of prickly and smooth seed spinach accessions

Seed type	Chlorophyll (mg g F M-1)	Carotenoid (mg g F M-1)	DPPH (%)	Flavonoid (%)	Phenol (mg/100 g F M-1)	Vit E (mg 100 g F M-1)	Vit C (mg 100 g F M-1)	Carbohydrate (mg g F M-1)	Amino acid (µg g F M-1)	Fiber (%)	Nitrate (mg NO_3 g-1 F M)	Nitrate Reductase (µgr NO_2^- g F M-1 h)	Oxalic Acid (mg g-1FM)
Prickly seed	7.27^{a}	1.65^{a}	69.38^{a}	0.76^{a}	79.55^{a}	1.04^{a}	20.54^{a}	0.64^{a}	0.05^{a}	2.03^{a}	106.2^{a}	29.11^{a}	2.09^{a}
Smooth seed	6.1^{a}	1.43^{a}	65.11^{b}	0.25^{b}	78.75^{b}	0.76^{a}	19.49^{a}	0.36^{b}	0.03^{b}	1.59^{b}	107.2^{a}	38.03^{a}	3.02^{a}
Standard Error	0.63	0.025	2.25	0.003	29.8	0.025	1.52	0.007	0.005	0.041	465.3	240.8	0.31

Different letters within the same column indicate significant differences of each type at $p \leq 0.05$ by the LSD test

prickly seed accession over the smooth seed accession was quite evident (Table 5). Therefore, it is concluded that prickly seed accession had significant antioxidants, flavonoids, total phenols, carbohydrates, amino acids, fibers, and iron content, which was higher than the foreign accession of smooth seed. Following the results of other researchers, Iranian accessions, including 'Varamin Prickly' have a significant superiority in terms of nutritional and functional traits compared to imported foreign accessions, which can be emphasized in the selection and improvement of Iranian spinach accessions (Erfani et al., 2006; Zaferaniye, 2015).

3.4 CORRELATION AMONG THE MORPHOLOGICAL AND BIOCHEMICAL TRAITS

Morphological characteristics of leaves in spring conditions were impressed, as a positive correlation was observed between leaf length and leaf width (r = 0.79^{*}) (Table 4). Similar to our results, Eftekhari et al. (2010) reported a significant correlation between leaf length and width in spinach accessions. Plant height was positively but not significantly correlated with petiole length (r = 0.74), indicating that increasing petiole length was desirable for mechanized harvesting (Table 4). Leaf number is one of the most important yield components in spinach (Jafari & Jalali, 2015), and as shown in table 4, this trait had a significant correlation with yield (r = 0.85^{*}). Also, a significant positive relationship was observed between fresh and dry mass (r = 0.86^{*}). There was also a positive relationship between iron content, leaf area (r = 0.79^{*}) and dry mass (r = 0.89^{**}). A good correlation was observed between female plants and the amount of antioxidants' activity (r = 0.81^{*}), flavonoids (r = 0.96^{**}) and total phenols content (r = 0.61) (Table 4). Also, there was a significant correlation between the percentage of the female plant

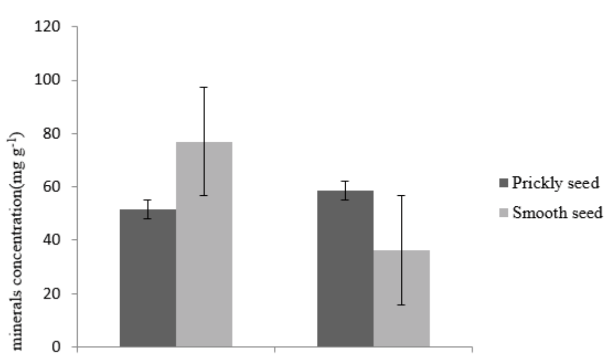

Fig 3: The mineral concentration of prickly and smooth seed spinach accessions

Table 4: Correlation coefficients 30 morphological and biochemical traits studied on prickly and smooth seed spinach accessions

Traits	L.N	P.H	L.L	L.W	P.L	P.D	Y	F.W	D.W	L.A	S.W	G.P	D.F	M	F	Ch	Car	DPPH	Flav	Ph	Vit E	Vit C	Carb	A.A	Fib	Nit	NitRD	OA	Ca
L.N	1																												
P.H	-0.52	1																											
L.L	-0.83*	0.70	1																										
L.W	0.72	-0.24	0.79*	1																									
P.L	-0.75	0.74	0.70	-0.55	1																								
P.D	0.29	0.07	0.22	0.17	-0.34	1																							
Y	0.85*	0.38	0.14	0.52	-0.44	0.44	1																						
F.W	0.79	0.40	0.26	0.42	-0.39	0.55	0.98**	1																					
D.W	0.64	-0.09	-0.10	0.68	-0.74*	0.72	0.85*	0.86*	1																				
L.A	0.46	-0.57	-0.28	0.48	-0.79*	0.64	0.38	0.43	0.76	1																			
S.W	0.58	-0.49	-0.48	0.77	-0.94**	0.48	0.58	0.56	0.85*	0.91*	1																		
G.P	0.64	-0.57	-0.60	0.82*	-0.98**	0.38	0.46	0.43	0.77	0.87*	0.98**	1																	
D.F	0.53	-0.15	-0.41	-0.06	0.13	-0.08	0.68	0.50	0.54	0.39	0.69	-0.25	1																
M	-0.58	0.61	0.55	-0.74	0.94**	-0.39	-0.45	-0.43	-0.76	-0.91**	-0.88*	-0.98**	0.28	1															
F	0.63	-0.57	-0.57	0.78*	-0.96**	0.41	0.50	0.48	0.79*	0.89*	0.69	0.98**	0.79*	-0.99**	1														
Ch	-0.76	-0.02	0.39	-0.76	0.69	-0.64	-0.57	-0.54	-0.78	-0.47	-0.63	-0.60	-0.23	0.53	0.61	1													
Car	-0.74	0.01	0.37	-0.71	0.64	-0.64	-0.34	-0.34	-0.64	-0.43	-0.52	-0.25	-0.17	0.46	0.51	0.9*	1												
DPPH	-0.45	0.16	0.12	0.55	0.72	-0.79	-0.74	-0.77	0.95*	0.65	0.66	-0.76	0.22	0.79	0.81*	0.74	0.57	1											
Flav	-0.68	0.54	0.59	0.54	0.48	-0.44	0.43	0.41	0.78*	0.56	0.66	-0.69	0.23	0.56	0.96**	0.65	0.65	0.76	1										
Ph	-0.25	-0.59	-0.21	-0.29	0.11	-0.67	0.46	0.49	0.5	0.03	0.08	-0.03	0.11	0.04	0.61	0.73	0.77	0.40	0.13	1									
Vit E	-0.73	0.24	0.32	-0.60	0.73	-0.83*	-0.47	-0.50	-0.83*	-0.77	0.70	-0.72	-0.12	0.30	0.74	0.89*	0.82	0.88*	0.76	0.56	1								
Vit C	0.28	-0.04	-0.29	-0.11	0.26	-0.27	-0.74	-0.77	-0.61	-0.51	0.53	-0.39	0.66	0.48	0.47	0.04	-0.20	0.62	0.31	-0.12	0.19	1							
Carb	-0.36	0.09	0.20	-0.76	0.39	-0.55	-0.64	-0.66	-0.85*	-0.71	0.82	-0.81*	0.54	0.57	0.77	0.63	0.69	0.73	0.85*	0.42	0.67	0.39	1						
A.A	-0.62	0.2	0.43	-0.87*	0.55	-0.54	-0.53	-0.52	-0.82*	-0.69	0.43	-0.86*	0.25	0.49	0.81*	0.78	0.84*	0.72	0.91*	0.47	0.78	0.18	0.94**	1					
Fi	-0.42	0.65	0.85	0.37	0.66	-0.11	-0.30	-0.21	-0.57	-0.66	0.56	0.51	-0.04	-0.78*	0.90*	0.59	0.49	0.57	0.89*	-0.08	0.60	0.17	0.59	0.72	1				
Nit	0.35	-0.41	-0.05	-0.26	0.63	0.51	-0.51	-0.41	-0.08	0.31	-0.007	0.008	0.44	0.33	0.21	-0.12	-0.28	-0.11	-0.07	-0.13	-0.41	-0.41	0.11	-0.01	0.05	1			
NitRD	0.002	-0.43	-0.46	0.42	0.41	-0.55	-0.04	-0.12	-0.04	0.14	0.31	0.43	-0.48	0.40	0.35	0.26	0.16	0.16	-0.38	0.57	0.29	-0.11	-0.31	-0.26	-0.46	-0.48	1		
OA	0.02	-0.18	-0.18	0.47	-0.54	-0.01	0.70	0.64	0.58	0.49	0.68	0.60	-0.50	0.65	0.66	-0.16	0.11	-0.55	-0.51	0.27	-0.18	0.85*	-0.44	-0.32	-0.55	-0.50	0.44	1	
Ca	0.16	-0.31	-0.49	0.50	-0.48	-0.41	0.41	0.29	0.26	0.21	0.49	0.49	-0.20	-0.50	0.53	-0.05	0.21	-0.22	-0.38	0.47	0.03	-0.52	-0.18	-0.16	-0.62	-0.67	0.60	0.87*	1
Fe	0.75	-0.26	-0.40	0.63	-0.33	0.45	0.59	0.57	0.89**	0.79*	0.65	0.51	-0.04	-0.79*	0.83*	0.50	-0.31	-0.44	-0.20	-0.50	0.38	0.28	-0.74	0.54	0.62	-0.64	-0.14	0.34	0.14

**indicates significant correlations at the level of $p < 0.01$; * indicates significant correlation at the level of $p < 0.05$. L.N: Leaf number; P.H: Plant height;L.L: Leaf length; L.W: Leaf width: P.L: Petiole length; P.D: Petiole diameter, Y: Yield; F.M: Fresh mass; D.M: Dry mass; L.A: Leaf area; S.W: 1000-grain mass; G.P: Germination percentage; D.F: Day to flowering; M: Male; F: Female; Ch: Chlorophyll; Car: Carotenoids; Flav: Flavonoids; Ph: Phenols; Carb: Carbohydrates; A.A: Amino acids; Fi: Fiber; Nit: Nitrate; NitRD: Nitrate reductase; OA: Oxalic acid

Table 5: Descriptive statistics of the measured traits in smooth and prickly seed of spinach accessions (Sabaghnia et al., 2014; Jafari and Jalali, 2015)

Seed type	Nutraceutical attributes															Total grade
	Chlorophylls	Carotenoids	DPPH	Flavonoids	Phenols	Vit E	Vit C	Carbohydrates	Amino acids	Fiber	Nitrate	Nitrate reductase	Oxalic acid	Ca	Fe	
Prickly seed	3	2	4	4	3	3	4	4	4	5	2	1	3	3	4	48
Smooth seed	2	1	3	2	3	2	3	3	2	2	1	2	2	4	2	34
	Yield and mechanical harvesting attributes															
	Leaf number	Plant height	Leaf length	Leaf width	Petiole length	Petiole diameter			Yield	Fresh mass	Dry mass	Leaf area	Germination			
Prickly seed	3	4	4	1	3	2			3	3	4	3	3			33
Smooth seed	4	2	2	2	1	2			4	4	1	4	5			31
	Breeding features															
	Seed type	Seed weight	Day to flowering	Male	Female											
Prickly seed	2	4	2	2	2											12
Smooth seed	1	2	2	4	4											13

† (1 = Low point; 2 = Medium point; 3 = High point; 4 = very high point)

with antioxidant properties and leaf iron content (Table 4). In confirmation of our results, Asadi & Hasandokht (2007) reported that female plants of spinach had more leafy, yield, and antioxidant properties.

A significant correlation was observed between chlorophyll and carotenoid (r = 0.90*). Carotenoid has been reported to have a protective effect on chlorophyll (Macfarland & Burchett, 2001).

A positive correlation was observed between nitrate and petiole length (r = 0.63). However, this relationship was not significant but consistent with other researchers' reports about the direct relationship between nitrate accumulation and petiole length (Asadi & Hasandokht, 2007; Jafari & Jalali, 2015).

The correlation in Table 4 shows that vitamin C has a negative relationship with nitrate (r = -0.41) and a positive relationship with oxalic acid (r = 0.85*). Increasing nitrate reduces vitamin C in spinach leaves. No significant negative relationship was observed between oxalic acid and nitrate (r = -0.50). According to studies, different results have been reported on nitrate and oxalate relation; (Kaminishi & Kita, 2006) reported a negative relationship between nitrate and oxalate in spinach. Koh et al. (2012) and Zhang et al. (2005) reported a positive correlation between these traits. These reports may indicate a complex pathway involved in nitrate and oxalate metabolism.

Fiber content was positively correlated with female plant percentage (r = 0.90*) and negatively with male plant percentage (r = -0.78). It seems that in addition to photosynthetic pigments (Asadi & Hasandokht, 2007), the fiber content will also increase in the female bushes.

4 CONCLUSION

It can be concluded that although the foreign smooth seed accession was better in breeding characteristics such as 1000-grain mass, germination percentage, leaf thickness, and female plant %, 'Varamin Prickly' (prickly seed) accession was significantly superior to nutraceutical traits such as antioxidant activity, total phenols, fibers, and iron content. Therefore, to enhance the quality traits in new spinach varieties, 'Varamin Prickly' (prickly seed accession) is suitable for transferring these traits and breeding purposes. These features may help obtain more information on the qualitative characteristics of Iranian spinach accessions or clarify the factors that influence the nutraceutical properties of Iranian accessions. Therefore, desirable Iranian accessions for nutraceutical and morphological traits can be used in breeding programs for spinach cultivar production.

5 AUTHOR CONTRIBUTION STATEMENTS

Each named author has contributed to conducting the underlying research and drafting this manuscropt.

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7 COMPLIANCE WITH ETHICAL STANDARDS (E.G., CONFLICT OF INTEREST)

To the best of our knowledge, the named authors have no conflict of interest, financial or otherwise.

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