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ORIGINAL ARTICLE

Morpho-biochemical and nutritional value of some early- and late-bolting spinach (*Spinacia oleracea*) accessions

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Abstract

Spinach (*Spinacia oleracea* L.) is a well-known leafy plant with valuable nutritional properties. The nutritional and marketing values of vegetables are highly affected by bolting. So far, researchers have studied the morphological properties of spinach, but its traits and characteristics in accessions with different bolting times have not been comprehensively studied. Therefore, the study was carried out to investigate the variations in morpho-biochemical characteristics of different spinach accessions based on bolting time. This study aims to find out whether or not bolting time causes morpho-biochemical changes in different spinach accessions. Here, "Varamin 88", "Matador", "D'inverno", "Viroflay" and "Spinagh" were used as late-bolting accessions groups, while "Lorestan" and "Kashan" were applied as early-bolting accessions groups. This spring field experiment was set up in a complete randomized block of 3 replicates and 18 observations. The results revealed significant differences between the early- and late-bolting spinach accessions (comparisons between groups) in their leaf number, plant height, yield, fresh and dry shoot weight, day to bolting, flavonoid, phenol, vitamin C, carbohydrate, nitrate, calcium (Ca), and iron (Fe) content. Late-bolting spinach group had more leaf numbers (23.9% increase), yield (64.3% increase), fresh and dry shoot weight (56.4% and 74% increase), day to bolting (55.9% increase), flavonoid (40.6% increase), phenol (37% increase), vitamin C (7.2% increase), and nitrate (37% increase), while early-bolting spinach group only had more plant height (45.9% increase), carbohydrate (21.8% increase), calcium (87.5% increase), and iron content (more than 100% increase). The biplot analysis showed the superiority of the late-bolting spinach accession dry and fresh weight, flavonoid, phenol, and vitamin C in spring planting. Conclusively, it was found that early-bolting spinach accessions were taller in leaf and petiole form, which is an advantage for mechanized harvesting. Late-bolting spinach accessions, particularly "Viroflay" and "Varamin 88" among the non-Iranian and Iranian masses, respectively, were preferred for spinach production and biochemical features, according to PCA and cluster analysis. This study recommends that if the quantity of production is important, the farmers use late-bolting plants for cultivation, and if mechanization is important, they use early-bolting plants for cultivation.

Keywords Biochemical traits · Spinach bolting · Carbohydrate · Flavonoid · Vitamin C · Iron

Introduction

Spinach (*Spinacia oleracea* L.) is known as a cold-season leafy plant from the Amaranthaceae family (Caparrotta et al. 2019). This plant is a native of central Asia (Iran) and was introduced as a useful vegetable for humans due to its fiber, vitamins, antioxidants, mineral nutrients, and several health-increasing phytochemical components (Seymen 2021). Spinach is a popular leafy vegetable among people and is generally used as a raw ingredient in salads and as a cooked vegetable dish (Sabaghnia et al. 2014). In 2021, the annual world production of spinach was around 32 million tons, with most of this production reported in China and the USA (FAO 2021). According to cultivar type and environmental

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conditions, spinach yield has been reported to range from 18.6 to 44.8 tons per hectare (Kuwahara et al. 2014). However, the performance and quality of fresh consumption of spinach can be affected by various factors, such as cultivation season and bolting (Chitwood et al. 2016).

Bolting (rapid stem elongation, which eventually leads to flowering) in spinach can happen at any time of the year, although spinach planted in the spring or summer is inclined to bolt more significantly (Chitwood et al. 2016). Some leafy vegetables, such as lettuce, cabbage, celery, radish, and carrot, bolted during the growing season (Xiao et al. 2012; Nie et al. 2016). Environmental conditions with endogenous plant signals such as growth phase and age affect bolting (Nie et al. 2016). Generally, researchers have reported that the main causes of bolting depend on day length and high temperatures (Shi et al. 2016).

Spinach has different types of bolting habits, including "early bolting" (bolted earlier than 60 days after sowing), "intermediate" (bolted 60–70 days after sowing), and "late bolting" (bolted more than 70 days after sowing) (Chitwood et al. 2016). According to the report of Kuwahara et al. (2014), common spinach cultivars used in East Asia are known as early-bolting accessions, and the ones used in Europe are often late bolting. The differences between Asian and Western spinach varieties are thought to be significant in two ways. The first is the place of origin and the history of spinach, and the second is the taste of the people of these two regions (Van der Vossen 2004). Easterners prefer spinach with smooth, narrow leaves and long petioles of spinach, but Westerners prefer cultivars with wrinkled leaves (Van der Vossen 2004). Later studies reported the link between wrinkled leaves and late bolting in spinach (Sabaghnia et al. 2014; Kuwahara et al. 2014; Xu et al. 2017; Abolghasemi et al. 2021). This information can be used to improve the selection of varieties with better economic performance as well as late bolting in different environmental conditions (Abolghasemi et al. 2021). In this regard, bolting can be avoided by controlling the temperature and photoperiod in the growth chamber, using spinach cultivars with low photoperiod sensitivity, or planting 50–60 days before the start of the long summer days (Chitwood et al. 2016). Late-bolting plants have been reported as one of the most critical targets of economic production and science investigations (Chitwood et al. 2016; Abolghasemi et al. 2021).

So far, most research on spinach has been related to morphological trait evaluation (Abolghasemi et al. 2019, 2022; Arif et al. 2013; Sabaghnia et al. 2014). Abolghasemi et al. (2022) reported that the type of spinach fruit does not appear to cause differences in morphological characters. The morphological characteristics of 54 Iranian spinach showed a high variation in yield, and some of them showed a significant advantage in terms of performance, leaf number, and day to bolting (Sabaghnia et al. 2014). Comparison and classification of endemic and commercial spinach accessions collected from different parts of the world showed significant differences in agro-morphological traits under the same growth in open field conditions (Abolghasemi et al. 2019). Many scientific articles have also shown variations in biochemical features such as the levels of carotenoid, antioxidant, oxalate, and nitrate observed in spinach populations (Shi et al. 2016; Barbarin et al. 2005). Comparing the two spinach cultivars showed a significant difference in biochemical and nutraceutical traits such as phenol, flavonoid, DPPH, fiber, amino acid, carbohydrate, and Fe content (Abolghasemi et al. 2022). Shi et al. (2016) explained that leaf and petiole nitrate accumulation varied between spinach cultivars. This researcher also showed that, in terms of oxalate accumulation, Iranian accessions are among the most desirable (Shi et al. 2016). Ning et al. (2019) demonstrated that flowering spinach cultivars contain more carbohydrates, amino acids, and protein than other non-flowering cultivars. Studies by Wang et al. (2017) showed that smooth leaf spinach accessions have more vitamin C than wrinkled spinach leaf accessions. Cai et al. (2018) showed that the amount of oxalate was different in the study of two spinach accessions. Another study found that spinach grown in the winter and spring seasons had higher levels of antioxidants than spinach grown in other seasons (Mudao et al. 2019). Shi et al. (2016) reported that late-bolting spinach accessions with favorable economic characteristics and desirable quality traits such as low oxalate and nitrate levels would be very important.

Studies show that a comprehensive study in terms of comparing important biochemical traits of spinach is necessary. This study pursues this goal. Although some research has been done on spinach, there is no comprehensive comparison of the morphological and biochemical traits, with an emphasis on the bolting time. It can be accurately stated that no comparative research has been conducted so far between the important properties of spinach, focusing on the bolting time of spinach, and we intend to fill this research gap in this study.

There are different endemic accessions of spinach in Iran. It is valuable to compare these with common cultivars in the world in terms of bolting time, biochemical traits, and nutritional value. In this study, two groups of late- and early-bolting spinach (a total of seven accessions were selected to investigate their more detailed characteristics in this study) were selected from 44 accessions from those previously studied by authors from around the world (Abolghasemi et al. 2019). These seven accessions were selected based on their morphological characteristics (yield and leaf features, plant height, petiole length, fresh and dry weight, days to flowering, and percentage of the female plant), which will be further analyzed in this study focusing on bolting time comparison for biochemical and nutritional characteristics. The results of this report can be useful for further study of spinach bolting or help breeders select the good accessions regarding their high yielding, late bolting, and a lot of nutrients.

Materials and methods

Plant material and growing conditions

To evaluate the morpho-biochemical traits of early- and late-bolting spinach accessions, seven (07) accessions were selected including three (03) Iranian spinaches provided by the Seed and Plant Improvement Institute (SPII) and the Gene Bank of Iran (GBI) and four (04) foreign spinach received from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) (IPK 2018) (Table 1 and Supplementary Table 1). All accessions here were selected from 44 accessions based on these authors' previous research (Abolghasemi et al. 2019). This selection was made based on the desirable morphological features of spinach, such as leaf features, yield, and day to bolting.

Thus, two (02) early ("Lorestan" and "Kashan" with accession numberTN-69-138 and TN-69-186) and five (05) late-bolting accessions ("Varamin 88", "Matador", "D'inverno", "Viroflay" and "Spinagh" with accession number TN-69-187, SPI 131, SPI 164, SP I7 and SPI 82) were selected, and the morphology is presented in Fig. 1B, H.

On March 18, 2018, a field experiment with three replicates and 18 observations per replicate (the total number of plants grown for each accession was 54 plants) using randomized complete block design (RCBD) was conducted at Isfahan University of Technology in Isfahan, Iran (spring sowing). Spinach is sown in the climate of Isfahan from March 1, which we also planted at the same time. Rainfall in March, April and May was about 7, 15 and 40 ml, respectively. Relative humidity was 50% ± 10%. The seeds were planted in plots (plot size was 2 square meters: m^2 with a density of 20 × 20 for plants). Daylight and temperature conditions during spring spinach cultivation are shown in Fig. 2. These data were obtained from the meteorological station of the cultivated area and it is also presented in the authors' recent article (Abolghasemi et al. 2022). The soil potential of hydrogen (pH) and electrical conductivity (EC) characteristics were investigated by the soil Laboratory of Isfahan University of Technology before sowing seeds. After drying the soil in air and filtering it, some soil properties such as soil pH in saturated mud were determined by a pH meter, and the electrical conductivity of saturated extract was determined by an electric conductivity meter (Takahashi et al. 2007). The soil was sandy-clay, pH = 7 and EC = 2 dS m^{-1}. Irrigation and fertilization were conducted like commercial production in the Isfahan region. To carefully study the

Table 1 Qualitative and morphological features of early- and late-bolting spinach accessions, according to the spinach descriptor (Arif et al. 2013; Sabaghnia et al. 2014; Abolghasemi et al. 2019)

Bolting patterns	Accession name	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
Early bolting	Kashan	Smooth	Yellow-green	Erect	Low	Fragile	Erect	Triangular	Intermediate green	Concave	Flat	Angled	No
	Lorestan	Smooth	Gray-green	Semi-erect	Intermediate	Intermediate	Semi-erect	Broad oval	Intermediate green	Concave	Bend	Circular	Yes
Late bolting	Varamin 88	Smooth	Gray-green	Erect	Intermediate	Thick	Erect	Broad oval	Very dark green	Bulgy	Bend	Circular	Yes
	Matador	Smooth	Gray	Horizontal	High	Very thick	Horizontal	Oval	Intermediate green	Concave	Upward	Circular	No
	D'inverno	Smooth	Gray	Horizontal	High	Very thick	Horizontal	Broad oval	Very dark green	Concave	Upward	Circular	Yes
	Viroflay	Smooth	Gray	Semi-erect	Intermediate	Thick	Semi-erect	Broad oval	Very dark green	Concave	Upward	Circular	Yes
	Spinagh	Prickly	Gray	Erect	Low	Fragile	Erect	Triangular	Very light green	Flat	Flat	Sharp	Yes

Fig. 1 Male and female bushes of spinach (**A**), **a** in this study, each spinach plant was either male or female (dioecious). The morphology of the early-bolting accessions: Loresten (**B**) on the 49th day, Kashan (**C**) on the 43th day, and late-bolting accessions: Varamin 88 (D) after 70 days, Matador (**E**) after 70 days, D'inverno (**F**) after 70 days, Viroflay (**G**) after 70 days, Spinagh (**H**) after 70 days. 18 plant bushes (observations) in each plot were selected for morphological assay of spinach accessions

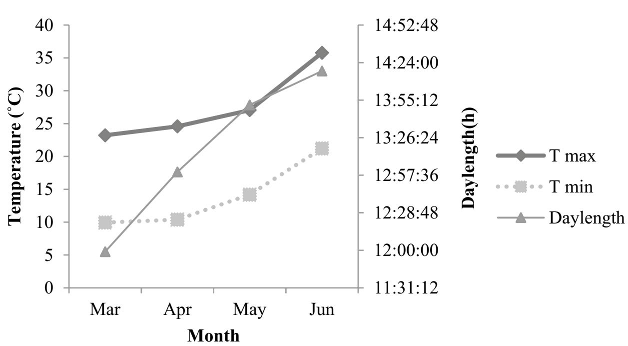


Fig. 2 Changes in temperature and day length in spinach in spring cultivation. As the summer season approaches, the length of the day and the temperature increase. These data were obtained from the meteorological station of the cultivated area (Abolghasemi et al. 2022)

biochemical characteristics of this experiment and the mismatch of the effects of fertilizers, we did not apply any fertilizer to plants and irrigation of plants was done every 6 days according to the method of farmers in the region. After seed germination and plant growth (when the plant reached the 6- to 9-leaf stage), 18 plant bushes (observations) in each plot were selected for morphological and 3 plant bushes in each plot were selected for biochemical assays of spinach accessions.

Measured parameters

Morphological characterization

The morphological characteristics of spinach accessions were investigated at commercial harvest time (35–50 days after planting). Different leaves from all directions of the plant were selected for measured traits. The qualitative characteristics of accessions were evaluated according to the descriptor of Bioversity International Plant Genetic Resources Institute (IPGRI) (Arif et al. 2013; Sabaghnia et al. 2014; Abolghasemi et al. 2019). In their earlier published articles, the authors provided a detailed explanation of how to measure the morphological features (Abolghasmi et al. 2019, 2022).

Biochemical characterization

Leaf pigments

Chlorophyll and carotenoid concentrations were extracted with acetone (80% v/v) and its absorbance was measured with a spectrophotometer (UV160A-Shimadzu Crop., Kyoto, Japan) at 663, 645 and 470 nm (Lichtenthaler and Wellburn 1985).

$$\text{Chlorophyll } a = (19.3 \times A663 - 0.86 \times \text{A645})\text{V}/100\text{W},$$

$$\text{Chlorophyll } b = (19.3 \times A645 - 3.6 \times A663)\text{V}/100\text{W},$$

$$\text{Carotenoids} = 100(A470) - 3.27(\text{mg chl. } a) - 104(\text{mg chl. } b)/227.$$

Antioxidant activity

The antioxidant activity of spinach was measured by the 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) method (Prasad et al. 2008). In 1.0 mL of MeOH, fresh tissue (0.1 g) was mixed. After 2 h of shaking, the samples were centrifuged at 12,000*g* for 30 min, and 0.5 mL of extract was added to 2.8 mL of DPPH solution (0.1 mM) and incubated at room temperature for 30 min. A spectrophotometer (UV 160A-Shimadzu Crop., Kyoto, Japan) was used to measure the absorbance at 517 nm. Finally, 2.8 mL of DPPH and 0.5 mL of MeOH comprised the control sample. The following equation was used to calculate the scavenging activity (Stojichevich et al. 2008):

$$\text{DPPH inhibition}(\%) = \{(A0 - A1)/A0\} \times 100,$$

$$A0 = A \text{ control},$$

$$A1 = A \text{ sample}.$$

Total flavonoid content

The alcoholic extract (0.30 mL) was mixed with 0.50 mL of $NaNO_2$ (5%) in a mixer. After that, 0.2 ml of $AlCl_3 \cdot 6H_2O$ (10%) and 0.4 mL NaOH (1.0 M) were added. After that, a spectrophotometer (UV 160A-Shimadzu Crop., Kyoto, Japan) was used to measure its absorption at 510 nm (Krizek et al. 1998).

Total phenol content

The total phenolic content was determined using the Folin–Ciocalteu method. The prepared samples were read at room temperature after cooling with a spectrophotometer (UV 160A-Shimadzu Crop., Kyoto, Japan) at 765 nm. Gallic acid solution was used to plot the standard curve (Raven 2003).

Vitamin E content

0.2 mL of alcoholic extract was mixed with 5.0 mL of toluene, 1.0 mL of ferric chloride·$6H_2O$, and 3.5 mL of 2, 2-bipyridine solution to determine the vitamin E (α-tocopherol compounds). A spectrophotometer (UV 160A-Shimadzu Corp., Kyoto, Japan) was used to measure the absorbance at 525 nm after it had been diluted with 95% ethanol for 2 min. With vitamin E, the standard curve was plotted: $y = 0.0081x + 0.0004$ ($r^2 = 0.996$) (Wang and Galletta 2002).

Vitamin C content

Fresh leaves (1.0 g) were mixed with phosphoric acid (6%), kept 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 of dichlorophenol indophenol solution (DCPIP) (0.025%). Using a spectrophotometer (UV 160A-Shimadzu Corp., Kyoto, Japan), the absorbance at 515 nm was determined. Plotting the standard curve was done with various vitamin C concentrations (Djioua et al. 2009).

Total soluble carbohydrate content

The anthrone method was used to determine the total amount of soluble carbohydrate content (Mc-Cready et al. 1950). The samples' alcoholic extract was supplemented with 2.0 mL of anthrone. Then, samples were boiled for 2 min in a water bath. Using a spectrophotometer (UV 160A-Shimadzu Crop., Kyoto, Japan), the absorbance was measured at 625 nm following the color phase's formation and cooling. The glucose standard was used to plot the standard curve (Mc-Cready et al. 1950).

Total amino acid content

Free amino acids were measured using the ninhydrin method. 1 mL of ninhydrin was added to the powdered samples' ethanolic extract. A spectrophotometer (UV 160A-Shimadzu Corp., Kyoto, Japan) reads absorption at a wavelength of 575 nm (Shih-wen et al. 2006).

Protein content

The protein content of extracts was determined by the method of Bradford (1976). An amount of 0.1 g of each sample was mixed with phosphate buffer (pH 7.6). Then Coomassie brilliant blue solution was added to each sample, and absorbance at 595 nm was read against the blank sample with a spectrophotometer (UV 160A-Shimadzu Corp., Kyoto, Japan). For the standard curve, bovine serum albumin was used in this method.

Determination of crude fiber

1.0 g of the dried leaves were boiled with 200 mL of H_2SO_4 for 30 min. To remove acid, the extract was filtered and washed with boiling water. After that, the residue was once more filtered after being boiled for 30 min in 200 mL of NaOH. Finally, the residue was oven-dried (550 °C) for 2 h. The following equation (Misurcova 2008) was used to determine the proportion of crude fiber:

$$\% \text{ Crude fiber content} = ((W2-W3)/W1) \times 100,$$

W2 = weight of crucible with fiber, W3 = weight of crucible with ash, W1 = weight of the sample.

Nitrate concentration

Nitrate concentration was assessed by the technique of Narayana and Sunil (2009). Briefly, the leaf water extract (0.1 g of fresh leaf in deionized water) was centrifuged at 4000 rpm for 30 min in a boiling water bath. After that, the extract was cooled to room temperature and mixed with 0.8 mL of concentrated sulfuric acid (95%) and 5% salicylic acid. After that, 19 mL of sodium hydroxide (NaOH, 2N) was added, and a spectrophotometer (UV 160A-Shimadzu Corp., Kyoto, Japan) was used to measure the absorbance at 410 nm. The standard curve was plotted for various concentrations of KNO3: $y = 0.053x + 0.035$ ($r^2 = 0.997$).

Nitrate reductase (nitrate RD) activity

100 mg leaf sample was suspended in 5.0 mL of 0.1 M phosphate buffer, KNO3 (0.02 M), and propanol (5%) for nitrate reductase activity. For 30 min, the solution was kept at 37 °C in a dark water bath. After that, the solution was treated with 1.0 mL each of *N*-1-naphthyl-ethylenediamine (0.02%) and sulfanilamide (1%). A spectrophotometer (UV 160A-Shimadzu Corp., Kyoto, Japan) was used to measure the absorbance at 540 nm, 15 min later. The KNO_2 solution was used to plot the standard curve: $y = 0.0049x + 0.0092$ ($r^2 = 0.988$) (Narayana and Sunil 2009).

Oxalic acid content

After combining the dry powder sample (0.5 g) with 30 mL of HCl (0.25N), it was placed in the water bath for 15 min. After that, the supernatant was filtered and mixed with 2.0 mL of potassium permanganate ($KMnO_4$) (0.003 M) and 5.0 mL of sulfuric acid (2N). The absorbance was measured at 528 nm after 10 min: $y = 0.9126x - 0.0705$ ($r^2 = 0.9327$) (AOAC 1970).

Determination of nutrition element uptake

Deionized water was used to wash the harvested leaves before they were oven-dried for 24 h at 65 °C for nutrition elements. After that, 0.5 g of powdered samples were digested for 12 h in an open hot plate using (7.5 mL) 36% HCl and 65% HNO_3 at room temperature, followed by 2 h at 105 °C. The content of Fe was determined by an atomic absorption spectrophotometer (Perkin Elmer, 3030, Netherland). The desired 4.0 ml solutions were pipetted and diluted in a 10 ml test tube with deionized water. Next, the contents of Ca were estimated by atomic absorption spectrometer (Perkin Elmer, 3030, Netherland) utilizing a calcium lamp at a frequency of 422.7 nm. Using flame spectrometry (Model PFP7, Jenway, England), K and Na were detected. All measurements were done in three replicates, as described by Novoa-Munozet al. (2008).

Statistical analysis

SAS "version 9.4" was used to analyze the data, and the least significant difference (LSD) test at the 0.05 level of probability was used to compare the means. Regression and correlation analysis between various morpho-biochemical characteristics was performed utilizing Statistix 8 (Tallahassee, FL, USA). Using Statistix 8, a *T* test was used to compare the two groups of accessions (early bolting and late bolting). Analysis of principal components (PCA) was also performed by Statgraphics (XVII) software. Considering the importance of this study for inter- and intra-group evaluation of different spinach accessions, various statistical methods and software were used to draw the best conclusions and all possible relationships between traits and accessions.

Results and discussion

Morphological characterization

The qualitative and quantitative characteristics of the accessions are described in detail in previously published works (Abolghasmi et al. 2019, 2022), so this study focuses on the comparison of late- and early-bolting groups. The qualitative characteristics of accessions are shown in Table 1. Early-bolting accessions had fewer wrinkles and lighter color rates than late-bolting accessions (Table 1). All of the accessions had a favorable green color except the "Spinagh" accession (Table 1). In confirmation of the results of this study, other researchers also reported differences in the qualitative traits of spinach in the study of different accessions (Shi et al. 2016; Madiha et al. 2020). Leaf color is a significant marketable feature in leafy vegetables like spinach and green, which are usually associated with chlorophyll pigments (Madiha et al. 2020). In Western countries, wrinkled leaves of spinach with a green color are marketable and suitable for storage because they get better ventilated, although they are hard to wash (Dong 2019; Kuwahara et al. 2014), while in Eastern countries, especially Iran, the majority of spinach has broad, fleshy leaves with a low leaf wrinkle (Sabaghnia et al. 2014). "Varamin 88" accession has several qualitative traits together, including erect petiole and leaf sheath, intermediate wrinkles of leaf, and desirable color of leaf, and can be recommended for mechanical harvesting (Table 1). Accordingly, this accession is very marketable and desirable in Iran. The researchers reported that these qualitative traits are important for mechanical harvesting (Madiha et al. 2020). The researchers express that spinach is a dioecious plant and male and female spinach plants appear entirely different from each other (Dong 2019), which was confirmed in this study and can be seen in Fig. 1A.

Analysis of variance showed a significant difference among accessions in petiole length, dry weight, leaf area, 1000-seed weights, germination percentage, day to bolting, percentage of male and female plants at 1% probability level, and 5% for plant height, leaf length, petiole diameter, yield, and fresh weight (Table 2). The results of the *t* test analysis showed significant differences in leaf number, plant height, yield, fresh and dry weight, and day-to-day bolting traits (Table 3). In this study, we examine comparisons between groups using the *t* test, following detailed reports of morphological features in the author's previously published articles (Abolghasmi et al. 2019, 2022). The *t* test results indicated the early-bolting group had significantly fewer leaves than the late-bolting group (Table 3). This result shows that early-bolting accessions with fewer leaves and late-bolting accessions with more leaves entered the flowering stage. Our results confirmed previous research, which revealed that spinach plants enter the reproductive phase in the range of 12–23 numbers of leaves (Liang et al. 2018). As shown in Table 3, the *t* test showed that plant height was higher in the early-bolting group compared to the late-bolting group. According to that, the accession of "Kashan" showed the highest plant height (Table 3). These results are consistent with other researchers who stated that different heights of spinach accession could be due to time to bolting (Madiha et al. 2020). The height, shape, or form of plants and petiole length of spinach increased with time to bolting (Xia et al. 2015), and these features were seen in the present study. In a study of early- and late-flowering lettuce lines, early-bolting lettuce lines showed faster growth and higher plant height than late-bolting plants (Han et al. 2016), confirming the results of the present study. Spinach accessions with upright plants, petiole standing, and leaf sheath standing are suitable for mechanical harvesting (Madiha et al. 2020). In the present study, it was also determined that early bolting with high plant height, erect petioles, and leaves, "Varamin 88", and "Viroflay" accessions (late bolting) with erect leaves can be considered suitable accessions for mechanical harvesting (Table 3, Fig. 3).

The *t* test results showed that the highest fresh weight, dry weight, and yield were observed in the late-bolting group ("Viroflay" accession with an average of 71,224 kg ha^{-1})

Table 2 Analysis of variance for morphological traits of early- and late-bolting spinach accessions

Source	DF	Leaf number	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Petiole diameter (mm)	Yield (kg ha^{-1})	Fresh weight (g)	Dry weight (g)	Leaf area (mm^2)	1000-seed weights (g)	Germination percentage (%)	Days to bolting	Male plant (%)	Female plant (%)
Blocks	2	6.66	26.84	1.11	2.11	0.42	0.20	211,308	798.7	21.75	743,889	1.46	47.82	8.6	1.92	15.96
Accessions	6	22.20^{ns}	238.31*	10.51*	3.39^{ns}	24.97**	3.61*	829,109*	3134.6*	98.41**	115,896**	8.27**	341.9**	1033.8**	356.7**	295.2**
Error	12	19.66	24.08	1.19	1.25	1.67	0.77	842,723	318.6	4.81	565,130	0.005	7.6	2.39	5.56	6.76
CV		18.43	18.24	7.21	12.47	11.28	19.85	19.37	19.37	15.48	8.81	7.8	3.17	2.16	6.34	4.07

ns: no significant. * and ** significant at 5%, 1% levels of probability, respectively

Table 3 Mean value for morphological traits of early- and late-bolting spinach accessions

Female plant (%)	Male plant (%)	Days to bolting	Germination percentage (%)	1000-seed weights (g)	Leaf area (mm^2)	Dry weight (g)	Fresh weight (g)	Yield (kg ha^{-1})	Petiole diameter (mm)	Petiole length (cm)	Leaf width (cm)	Leaf length (cm)	Plant height (cm)	Leaf number	Accessions
56.9d	47.1b	43.0f	97.56a	7.00f	7966b	9.62 cd	66.50 cd	34204 cd	4.00bc	15.7a	9.1abc	15.86ab	45.06a	14.4a	Kashan
70.7b	30.1d	49.0e	91.10b	8.65d	7613b	5.77d	51.47d	26473d	4.91ab	12.4b	9.8ab	17.53a	28.33bc	15.8a	Lorestan
63.8a	38.6a	46a	94.33a	7.82a	7789.8a	7.69b	58.98b	30,338.5b	4.45a	14.0a	9.4a	16.69a	36.69a	15.1b	Early bolting
60.4 cd	38.1c	72.0d	83.88c	11.91a	10657a	15.84b	102.2b	52590b	3.81bc	12.9b	8.8abc	16.40ab	29.40b	19.0a	Varamin 88
62.8c	36.0c	83.6b	65.99d	7.50e	8489b	16.11b	94.18bc	48436bc	5.81a	7.0d	10.6a	15.00bc	20.60 cd	19.6a	Matador
76.3a	23.6e	89.6a	96.03ab	9.95b	11483a	15.79b	124.9ab	64259ab	5.78a	9.6c	9.0abc	15.86ab	21.86bcd	22.5a	D'inverno
71.4b	29.7d	87.3a	85.86c	9.96b	7800b	23.79a	138.48a	71224a	3.81bc	9.5c	7.4c	13.47 cd	18.73d	16.6a	Viroflay
47.4e	55.1a	76.0c	91.78b	9.11c	5659c	12.21bc	67.08 cd	34503 cd	2.90c	12.9b	8.0bc	12.00d	24.33bcd	18.4a	Spinagh
63.7a	36.5a	81.7b	82.94a	9.68a	8818.2a	16.74a	105.38a	59,127.2a	4.42a	10.4a	8.7a	14.54a	22.98b	19.2a	Late bolting
6.76	5.56	2.39	7.69	0.005	56,513	4.81	318.6	842,723	1.70	1.60	4.20	6.20	15.4	18.5	Standard Error

Different letters within the same column indicate significant difference of each type at $P \leq 0.05$ by the LSD test. "Early-bolting" and "Late-bolting" rows show average values. Similar letters for "Early-bolting" and "Late-bolting" indicate statistically insignificance 0.05 probability level by *T* test. It should be noted that the mean value yield is the, fresh and dry weight of plant obtained from the harvest of all spinach plants in one harvest

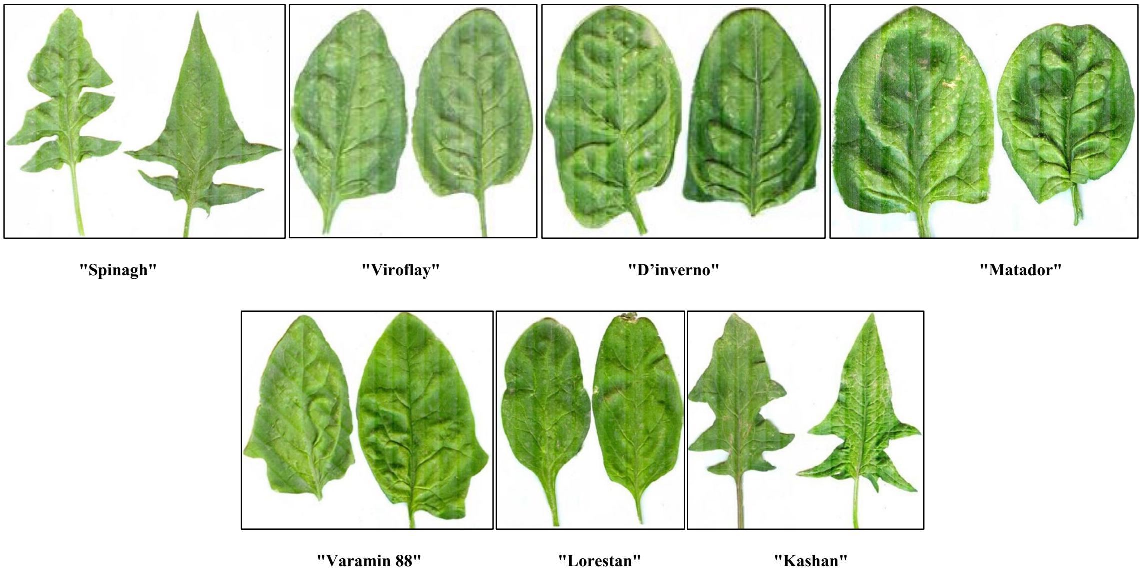


Fig. 3 Morphological features of the leaves of five late and two early-bolting accessions. Both leaves in these pictures show the appearance of the leaves of each accession

(Table 3). It has been reported that the number of spinach leaves affects the yield (Xia et al. 2015). Similarly, in the current study, the late-bolting group had more leaves and therefore higher yields. It can be concluded that the more leaves there are, the higher is the yield of spinach. For vegetative crops like spinach to be productive, the timing of bolting is crucial (Ghorbani et al. 2021). In this study, the difference in bolting time was statistically significant. The early-bolting group entered the flowering stage less than 50 days after planting, with the "Kashan" accession being the earliest one, and the late-bolting accessions of spinach entered the reproductive phase after at least 70 days, with the "D'inverno" accession being the latest one (Table 3). Since spinach is a vegetable with leaves, more seedlings are desirable. To expand the spinach creation period, utilizing the late-bolting varieties is vital (Li et al. 2020; Abolghasemi et al. 2019) and economically desirable because their flowering stems would appear late (Li et al. 2020). Both "D'inverno" and "Viroflay" accessions had the longest period of growth with a good yield and fresh and dry weight (Table 3).

Biochemical characterization

Analysis of variance showed differences between amino acid, fiber, and Fe concentration at 1% probability level and 5% probability level in chlorophyll, DPPH, flavonoid, phenol, carbohydrate, protein, nitrate, nitrate reductase, and Ca concentration. Also, there was no significant difference in carotenoid, vitamin E, vitamin C, oxalic acid, potassium, or Na concentrations between all of the accessions (Table 4). The highest chlorophyll content belonged to the "Viroflay" accession, and the lowest amount of this pigment belonged to the "Spinagh" and both these accessions belonged to the late groups (Table 5). This result was consistent with what was observed in the qualitative table of morphological characters (Table 1), where "Spinagh" had a very light green color.

Bolting is seen to be the biggest threat to vegetable crop yields, particularly for commercial and food crops like spinach, where only limited research has been done to date on the differences between early- and late-bolting accessions (Ghorbani et al. 2021). The high amount of chlorophyll helps to maintain the photosynthetic capacity (Ning et al. 2019) and plant dry weight production. The "Viroflay" accession in this study had the highest amount of chlorophyll as well as dry weight. However, this difference was not statistically significant among the group members late bolting (Tables 3, 5). The comparison of the early- and late-bolting lettuce accessions showed that the early accession had a higher chlorophyll content (Ning et al. 2019). In contrast, the *t* test result showed there were no statistically significant differences between the early- and late-bolting groups on the pigments of spinach under field conditions (Table 5). There were no differences observed between the spinach varieties on chlorophyll and carotenoid (Dong 2019) or between Iranian and foreign accessions (Sabaghnia et al. 2014). DPPH did not show significant differences between the early- and late-bolting groups, but this trait had the highest amount in "Varamin 88" (Table 5). The late-bolting group had significantly higher amounts of flavonoids and phenolic compounds, while "Lorestan" had the lowest amounts of flavonoids and phenolic compounds (Table 5). It

Table 4 Analysis of variance for biochemical traits of early- and late-bolting spinach accessions

Source	DF	Chlorophyll (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)	DPPH (%)	Flavonoid (%)	Phenol (mg g^{-1})	Vit E (mg 100 g^{-1} FW)	Vit C (mg 100 g^{-1} FW)	Carbohydrate (mg g^{-1} FW)	Amino acid (µg g^{-1} FW)	Protein (mg g^{-1} FW)	Fiber (%)	Nitrate (mg NO_3 g^{-1} FW)	Nitrate RD (µgr NO_2^-g^{-1} FW h)	Oxalic acid (mg g^{-1} FW)	Ca (mg. g^{-1}DW)	Fe (mg. g^{-1}DW)	K (mg. g^{-1}DW)	Na (mg. g^{-1}DW)
Blocks	2	0.34	0.06	8.92	0.006	666.4	0.05	0.14	0.006	0.0003	0.005	0.01	387.9	15.4	1.11	0.04	0.10	5.1	9.35
Accessions	6	3.80*	0.15^{ns}	70.02*	0.07*	1133.4*	0.07^{ns}	2.43^{ns}	0.02*	0.003**	0.06*	0.2**	1523.4*	465.8*	2.49^{ns}	3.86*	2.49**	23.5^{ns}	18.1^{ns}
Error	12	0.49	0.05	8.77	0.01	129.3	0.03	1.43	0.01	0.0001	0.02	0.006	261.2	35.2	0.88	0.2	0.13	25.8	7.75
CV		11.07	16.02	4.15	16.73	14.03	18.65	6.16	13.74	15.75	17.9	4.35	17.26	17.92	18.43	18.9	18.65	18.9	12.16

In statistics, DF means degrees of freedom, blocks means arranging of experimental units in groups, CV means coefficient of variation. ns: not significant * and ** significant at 5%, 1% levels of probability, respectively

Table 5 Mean value for biochemical traits of early- and late-bolting spinach accessions

Oxalic Acid (mg g^{-1} FW)	Nitrate RD (µgr NO_2^-g^{-1} FW h)	Nitrate (mg NO_3 g^{-1} FW)	Fiber (%)	Protein (mg g^{-1} FW)	Amino acid (µg g^{-1} FW)	Carbohydrate (mg g^{-1} FW)	Vit C (mg 100 g^{-1} FW)	Vit E (mg 100 g^{-1} FW)	Phenol (mg g^{-1})	Flavonoid (%)	DPPH (%)	Carotenoid (mg g^{-1} FW)	Chlorophyll (mg g^{-1} FW)	Accessions
2.3a	44.86a	72.49c	1.84c	0.63abc	0.083bc	0.67ab	19.0a	0.64a	62.4bc	0.58bc	69.61bc	1.46a	5.76bc	Kashan
3.16a	27.22bc	68.82c	1.58d	0.79a	0.15a	0.76a	18.5a	0.81a	59.9c	0.40c	69.11bc	1.40a	5.93bc	Lorestan
2.73a	36.04a	70.65b	1.71a	0.71a	0.11a	0.71a	18.7b	0.72a	61.1b	0.49b	69.36a	1.43a	5.84a	Early bolting
4.91a	17.04c	95.21bc	2.36a	0.50bc	0.089b	0.62abc	19.9a	0.66a	111.7a	0.86a	80.22a	1.82a	7.78a	Varamin 88
4.48a	42.81a	136.2a	1.99b	0.71ab	0.071bc	0.52bc	20.6a	0.64a	102.3a	0.81a	74.05b	1.19a	5.41c	Matador
3.58a	22.81bc	88.54bc	1.77c	0.74ab	0.063 cd	0.51c	21.1a	0.87a	81.5b	0.70ab	72.94b	1.53a	6.71ab	D'inverno
4.57a	50.76a	89.25bc	2.14b	0.36c	0.089b	0.63abc	19.5a	1.05a	74.9bc	0.73ab	66.00c	1.77a	7.87a	Viroflay
3.95a	28.23b	104.7b	1.74c	0.55abc	0.041d	0.58bc	19.7a	0.68a	74.2bc	0.60b	67.50c	1.33a	5.04c	Spinagh
4.29a	31.93a	102.7a	2.0a	0.57a	0.07a	0.57b	20.1a	0.78a	88.9a	0.74a	72.14a	1.52a	6.56a	Late bolting
0.88	35.03	261.2	0.006	0.022	0.0017	0.025	1.43	0.035	129.3	0.012	8.77	0.058	0.49	Standard error

Different letters within the same column indicate significant difference of each type at $P \leq 0.05$ by the LSD test. "Early-bolting" and "Late-bolting" rows are average values. Similar letters with "Early bolting" and "Late bolting" indicate statistically insignificant 0.05 probability level by T test has been reported that flavonoids, like other phenolic compounds, are strong antioxidants in spinach and have wide pharmacological and biochemical applications (Li et al. 2020). Every region's phenolic and antioxidant properties are influenced by a variety of factors, including climate, soil, altitude, and various plant species (Shi et al. 2016). It has been reported that in normal conditions, genotype variation causes different antioxidant levels (Dong 2019). Different varieties of spinach were found to have distinct antioxidant, flavonoid, and phenolic activity under non-stress conditions (Dong 2019). It was observed in these studies that phenolic and antioxidant compounds were more abundant in the late-bolting group than in the early-bolting group (Table 5). A study by Ma et al. (2023) on the flowering stages of *Angelica sinensis* found that early bolting resulted in reduced accumulation of biochemical compounds and antioxidants such as ferulic acid and flavonoids. Similarly, in the present study, the early-bolting group showed reduced levels of flavonoid, phenol, and vitamin C compared to the late-bolting group. A *t* test between groups showed higher amounts of vitamin C in the late-bolting group, but no statistically significant differences were observed among the seven individual accessions examined in this study (Table 5). In confirmation of these results, Barbarin et al. (2005) found no difference in vitamin C and E (α-tocopherol) levels in the study of biochemical traits of spinach varieties under non-stress conditions.

Soluble carbohydrates are nutrient chemicals as a source of plant energy that is valuable to humans, and many studies showed that spinach has very important carbohydrates (Li et al. 2020). During flowering time, high levels of carbohydrates were reported; in fact, changes have been reported to occur at the time of flowering, although this mechanism is not clear (Xia et al. 2015). Carbohydrates have been implicated in the nutritional–reproductive transition as an energy reserve for inflorescence development and signaling molecules, according to prior research (Ghorbani et al. 2021; Abolghasemi et al. 2021). In this regard, the early-bolting group had the highest average carbohydrate content (average: 0.71 mg g^{-1} FW) (Table 5). Research has shown that early-bolting spinach had a higher carbohydrate content (Li et al. 2020), which was also confirmed in the present study because it seems that soluble carbohydrate production and concentration occur faster in the early-bolting group (Table 5). It is interesting to note that carbohydrates can promote growth by up-regulating flowering promoters (Ghorbani et al. 2021). A study of early- and late-bolting lettuce lines showed that early-bolting lettuce lines had a higher carbohydrate than late-bolting plants, confirming the results of this study (Han et al. 2016). Amino acids play a role in the structure of spinach protein, and about 30% of spinach dry matter is made up of amino acids (Ning et al. 2019). The "Lorestan" accession, which belonged to the early-bolting group, contained the most amino acids and protein content (Table 5). Changes in carbohydrate and amino acid levels during flowering in this study were similar to those in a recent study by Ma et al. (2023). They found that early-bolting *Angelica sinensis* plants had higher carbohydrates, and there was not much difference in the trend of amino acids between early- and late-bolting plants.

According to our results, Xia et al. (2015) also reported that an increase in the amount of protein is necessary for bolting. The highest amount of fiber was in "Varamin 88" accessions that belong to the late-bolting group; however, there was no significant difference between the two groups (Table 5). Until now, the difference in fiber content of early- and late-bolting spinach accession has not been studied, but in this study, it was observed that "Varamin 88" accession, which was part of the late-bolting group, had the highest fiber content. In a study by Tezera et al. (2023), they found that late flowering in alfalfa increases fiber in this plant, and by blocking a gene that affects early flowering in this plant, they were able to increase the amount of fiber in alfalfa. The "Matador" accession, which is a member of the late-bolting group, had the highest nitrate content, while the "Lorestan," which is a member of the early-bolting group, had the lowest level of this characteristic. "Lorestan" and "Kashan" were not significant, and both of them showed the lowest levels of nitrate accumulation (Table 5). Due to its highly effective absorption system and ineffective nitrate recovery system, spinach is one of the largest nitrate accumulators (Shi et al. 2016). Nitrate accumulation in vegetables is affected by cultivars, ploidy levels, and environmental conditions (Hosseini-Darani et al. 2013). The accumulation of nitrate in wrinkled and smooth leaves differs, and in spring cultivation, nitrate accumulation is lower than in autumn cultivation (Hosseini-Darani et al. 2013; Bian et al. 2015). There is a direct correlation between leaf wrinkles and nitrate content, and spinach cultivars with more leaf wrinkles contain more nitrate (Bian et al. 2015). In this regard, in this study, early-bolting group accessions with the lowest leaf wrinkle had the lowest nitrate content (Tables 1, 5). The highest nitrate content was observed in the "Matador" accession, which was highly wrinkled (Table 5). In terms of nitrate accumulation, this study's findings were in line with those of other researchers (Shi et al. 2016; Bian et al. 2015).

One of the reasons for nitrate accumulation may be related to nitrate reductase activity because nitrates operate as a signal for nitrate reductase activity (Bian et al. 2015). The late-bolting group had high leaf shrinkage, low nitrate reductase activity, and, consequently, high nitrate accumulation. However, there was no significant difference between these two groups (Table 5). In this regard, the activity of nitrate reductase was higher in the early-bolting group, leading to a decrease in nitrate accumulation. It has been well established that nitrate uptake and nitrate reductase activities are related processes (Bian et al. 2015). Spinach had the highest oxalate concentration of all the plants (Anwar et al. 2015). Low oxalic acid accessions are widely used for identifying effective genes and breeding (Anwar et al. 2015). As a result, accessions of spinach with low levels of oxalate may be identified and selected with the assistance of this study. "Kashan" accession (with an average: of 2.3 mg g^{-1} FW) had the lowest amount of oxalic acid (Table 5). As reported, oxalate, like nitrate, has a defined range (596–704 mg NO_3 g^{-1} FW) and more than that causes harmful effects on the human body (Hosseini-Darani et al. 2013). Therefore, in the present study, late bolting equals the possibility of more oxalate accumulation, such as nitrate accumulation (Table 5). Iranian accessions were among the least oxalate accumulators (Shi et al. 2016).

Spinach is abundant in minerals among plants (Sabaghnia et al. 2014). The early-bolting group contained the most Ca and Fe (average: 3.58 and 2.25 mg g^{-1} DW) (Table 6) and Ca and Fe levels in this study confirm the observation of other reports (Erfani et al. 2006; Li et al. 2020).

Correlation, PCA, and cluster analysis

Correlation is a useful technique for creating selection criteria that will concurrently enhance several traits and economic output by determining the key factors impacting the dependent traits. In this study, the priority in terms of correlation was to find a general relationship between different accessions that exhibit the desired characteristics. In this regard, highly significant and positive correlations were observed between the following traits. The relationship between different traits and yield in spinach is important for selection in plant breeding projects: the number of leaves with day to bolting (r = 0.75*); plant height with petiole length (r = 0.83*); spinach yield with dry weight (r = 0.91**), and fresh weight (r = 0.95**), and day to bolting (r = 0.75*), and chlorophyll (r = 0.78*). Early studies reported a significantly positive link between the yield of spinach with leaf features (Arif et al. 2013; Tanni et al. 2023). Similarly, it was reported by the researcher that the yield components were directly correlated with time to bolting (Liang et al. 2018; Abolghasemi et al. 2019, 2021). There was a relation between fresh weight and dry weight (r = 0.91**), and day to bolting (r = 0.75*), and with chlorophyll (r = 0.78*); dry weight with day to bolting (r = 0.77*); day to bolting with oxalic acid (r = 0.75*), and with vitamin C (r = 0.75*); chlorophyll with carotenoid (r = 0.94**); DPPH with phenol (r = 0.82*); flavonoid with phenol (r = 0.87**), and with fiber (r = 0.83*); carbohydrate with amino acid (r = 0.75*). Oxalic acid content correlated with the day to bolting (r = 0.75*) (Supplementary Table 2). Some researchers reported a positive correlation between oxalic acid and nitrate, in which our results for nitrate (r = 0.58) were consistent with them (Shi et al. 2016) (Supplementary Table 2). Our results regarding the morphological characteristic link were in line with other research on Chinese cabbage, Indian spinach, and red amaranth (Tanni et al. 2023; Varalakshmi 2010; Jangde et al. 2017).

On the other, the correlation analysis between all of the accessions and all of the desired characteristics showed significant and negative correlations: the number of leaves with carbohydrates (r = − 0.84*); plant height with petiole length (r = − 0.90**) and day to bolting (r = − 0.90**); petiole length with day to bolting (r = − 0.79*); spinach yield with the male plant (r = − 0.77*); day to bolting with carbohydrate (r = − 0.75*) and amino acid (r = − 0.75*); male plant with the female plant (r = − 0.95**). Nitrate reductase showed a negative correlation with day to bolting (r = − 0.44) (Supplementary Table 2), which indicates the accumulation of nitrate in late-bolting accessions and can be due to the low activity of the nitrate reductase enzyme. Researchers recommend planting female varieties for economical use because of their higher production. Also, female

Table 6 Mean value for nutrition elements uptake of early- and late-bolting spinach accessions

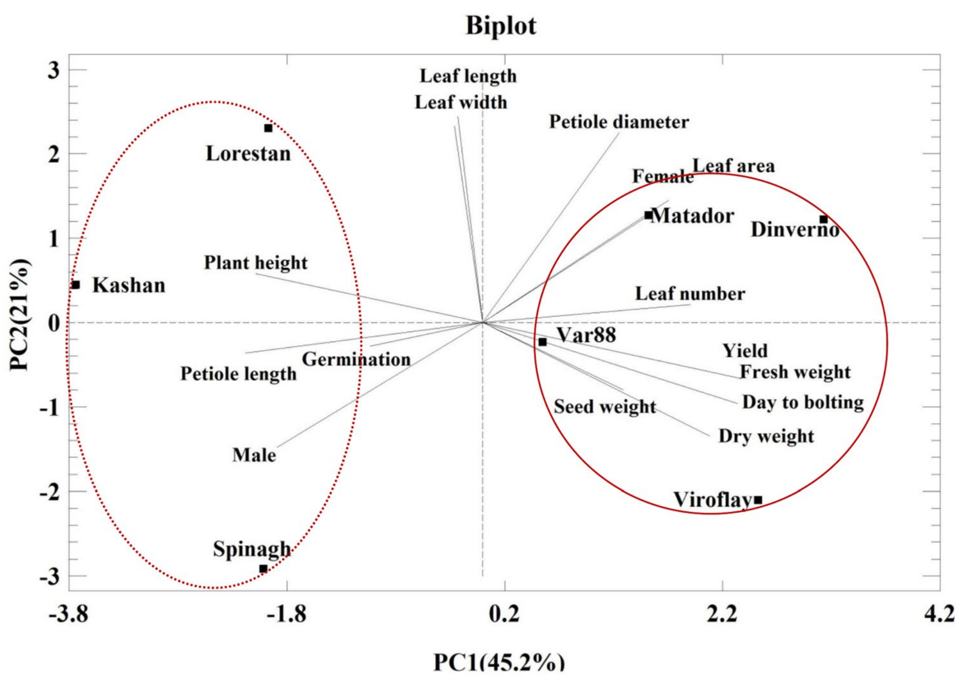
Accessions	Ca (mg g^{-1}DW)	Fe (mg g^{-1}DW)	K (mg g^{-1}DW)	Na (mg g^{-1}DW)
Kashan	4.20a	2.50a	21.03a	23.23a
Lorestan	2.97b	2.60a	21.13a	19.98a
Early bolting	3.58a	2.55a	21.08a	21.60a
Varamin 88	1.50c	1.32b	20.11a	23.05c
Matador	1.28c	0.73bc	24.08a	25.66a
D'inverno	1.67c	0.76bc	23.57a	21.92a
Viroflay	1.10a	0.59bc	23.62a	26.24a
Spinagh	1.47c	0.43bc	28.45a	20.12a
Late bolting	1.40b	0.76b	23.96a	23.39a
Standard error	0.30	0.13	25.8	7.75

Different letters within the same column indicate significant difference of each type at $P \leq 0.05$ by the LSD test. "Early-bolting" and "Late-bolting" rows are average values. Similar letters for "Early bolting" and "Late bolting" indicate statistically insignificant 0.05 probability level by T test plants look better, produce more, and are more marketable than male plants (Liang et al. 2018). "D'inverno" accession in this study had significantly more female plants than the other accessions, and the female plant was significantly correlated with yield ($r=0.76^*$) (Supplementary Table 2), so it is more commercially recommended.

Then, the second priority in terms of correlation was to find the relationship between each accession and its characteristics (this type of correlation table is not presented). In this type of correlation, a positive and significant relationship ($r=0.95^{**}$) was observed between fresh weight and yield in all seven accessions. In "Lorestan" and "Varamin 88" "Viroflay" accessions, there was a strong link between yield and chlorophyll ($r=0.95^{**}$). In "Matador" and "Viroflay" accessions, day to bolting had a significantly positive correlation with leaf length ($r=0.90^{**}$). Similar positive correlations between day to bolting and leaf length in *Amaranthus hybridus* and Chinese cabbage were discovered by researchers (Akaneme and Ani 2013; Tanni et al. 2023). The fresh and dry weight of spinach in "Varamin 88" accession showed a positive correlation ($r=0.95^{**}$). Additionally, a strong correlation was found between spinach's DPPH and vitamin C in the "Varamin 88" and "Spinagh" accessions.

Principal components analysis (PCA) has been useful in grouping germplasm in many herbs such as spinach (Sabaghnia et al. 2014). The first component (PC1) accounted for 45.2% of the changes, and the second component (PC2) accounted for 21% of the changes. Together, the first and second components accounted for 66.22% of the total data changes (Fig. 4). The researchers said that the high contribution of the two components in the changes in the total data is the reason for the stability of the results (Sabaghnia et al. 2014). In this regard, the contribution of the two components shows a relatively high part of the total data changes.

If all the accessions of this study are divided into two desirable and undesirable groups, in terms of spinach economic traits based on biplot, "D'inverno", "Matador", "Viroflay" and "Varamin 88"are in the group of desirable accession and "Lorestan", "Kashan", and "Spinagh" accessions are in the undesirable group (Fig. 4). This division was consistent with the results of the analysis of variance and correlation (Tables 3, 5, Supplementary Table 2(. The first group (desirable group) has important and favorable agro-morphological traits such as good yield, fresh and dry weight, leaf number, female plant, and day to bolting. It should also be noted that although "Spinagh" accession



		Component	Component	Component	Component
Row	Label	1	2	3	4
1	Kashan	-3.73704	0.448637	0.782602	-0.554047
2	Lorestan	-1.96889	2.30417	0.170083	-0.589062
3	Var88	0.547348	-0.231333	1.29807	1.41755
4	Matador	1.5208	1.27479	-2.89692	0.0767111
5	Dinverno	3.12872	1.22205	1.13575	0.522645
6	Viroflay	2.52591	-2.10081	0.531348	-1.65547
7	Spinagh	-2.01684	-2.9175	-1.02093	0.781668

Fig. 4 Biplot diagram and PCA tabulated data of the principal components of early- and late-bolting accessions in morphological traits and undesirable and desirable groups. As can be seen in the picture, early- and late-bolting accessions are well separated in terms of common morphological features. "Spinagh" accession was late bolting, but due to differences with other late-bolting accessions in plant height, petiole length, number of male plants, and germination percentage were separated from them and placed near early-bolting accessions. This accession also showed the highest number of male plants and therefore was placed at a distance from the early-bolting accessions. The other four late-bolting accessions were placed in the same group and close to each other due to common morphological traits

was late bolting, it was not among the favored accessions (Fig. 4). Although the second group (undesirable accession) was not considered for cultivation, they have the advantages for mechanical harvesting including plant height, leaf, and petiole length that are useful for mechanical purposes.

Based on the biplot of biochemical traits, the early- and late-bolting group was divided into separate group (Fig. 5). Useful biochemical properties such as flavonoid, phenol, and vitamin C were higher in the late-bolting accession such as "Viroflay", "Varamin 88", "Matador", and "D'inverno" accession. Similar to the biplot results of morphological traits, the "Spinagh" accession was also not favorable for biochemical traits and was in a different position than other late-bolting accessions (Fig. 5). On the other hand, amino acid, carbohydrate, and protein levels were higher in early-bolting accession (Fig. 5) (Ning et al. 2019; Xia et al. 2015). Due to the importance of biochemical traits in spinach, it seems that late-bolting accessions were more favorable than early-bolting accession in biochemical characteristics. Generally, the morphological and biochemical biplot analyses were in agreement with each other in this study, and both analyses showed the superiority of the late-bolting accession in spring planting (Figs. 4, 5).

For the objective of selecting the optimal spinach accession for future research, cluster analysis can be helpful. Because a small distance between two accessions indicates a slight difference, the cluster analysis bases its positive accession selection on a bigger gap between the accessions (Abolghasemi et al. 2019; Sabaghnia et al. 2014). The seven accessions were split into two main groups by the cluster analysis, with a distance of 55–70 (Fig. 6). The accessions "Kashan", "Lorestan" and "Spinagh" were combined into one cluster. The similarity of these three accessions' morphological characteristics (leaf shape, leaf, and petiole attitude, yield, fresh and dry weight), as well as their biochemical characteristics (chlorophyll concentration), may be the cause of their grouping together. Other accessions of this study, such as "Viroflay", "D'inverno", "Matador" and "Varamin 88", were placed in another large cluster. Figure 6 suggests that the grouping of these four late-bolting accessions may have resulted from their shared morphological and biochemical characteristics. This cluster completes earlier analyses, and the bi-plot diagram has been accepted as a result. The results were consistent with earlier research on the selection of early- and late-bolting spinach accessions (Abolghasemi et al. 2019, 2021). As can be seen, the "Virofaly" accession was at the beginning of the cluster diagram, while the "Kashan" accession was after Fig. 6.

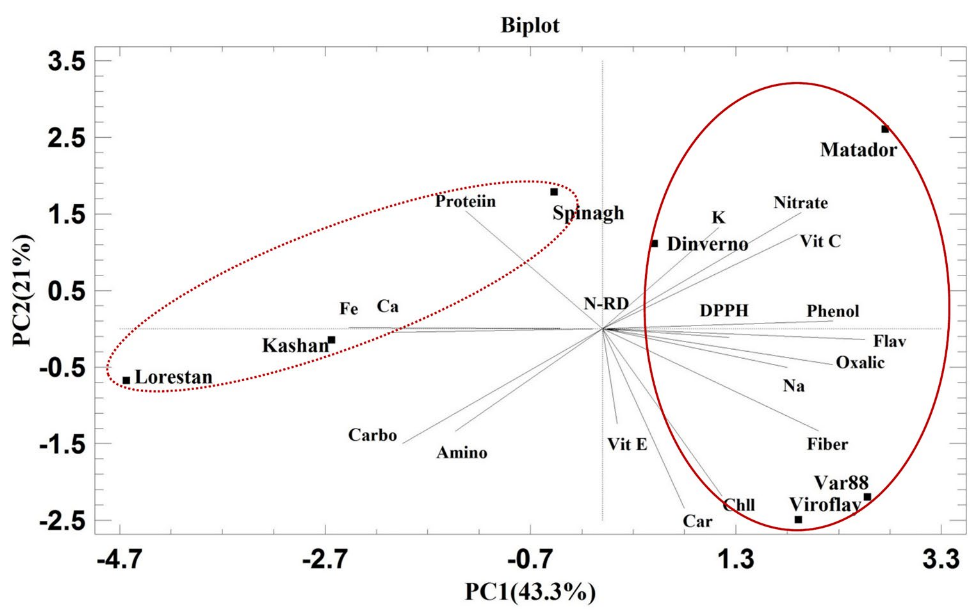


Fig. 5 Biplot diagram and PCA tabulated data of the principal components of early- and late-bolting accessions in biochemical traits. As can be seen in the figure, the four late-bolting accessions were located close to each other due to common biochemical properties. Meanwhile, "Varamin 88" and "Viroflay" accessions were close to each other in terms of chlorophyll and fiber content and showed more similarity. Early-bolting accessions were placed close to each other like morphological traits and also "Spinagh" accession was placed at a distance from other late-bolting groups

		Component	Component	Component	Component	Component
Row	Label	1	2	3	4	5
1	Kashan	-2.63842	-0.144949	0.317441	-1.70513	1.26921
2	Lorestan	-4.63399	-0.675193	-0.496258	0.401333	-0.787392
3	Var88	2.5801	-2.19399	-2.93262	-0.256479	-0.20289
4	Matador	2.75235	2.60909	0.250287	-1.15448	-0.262258
5	Dinverno	0.504227	1.11214	-0.295213	1.92947	1.48591
6	Viroflay	1.90687	-2.49185	2.96525	0.251167	-0.215271
7	Spinagh	-0.471136	1.78475	0.191109	0.534123	-1.28731

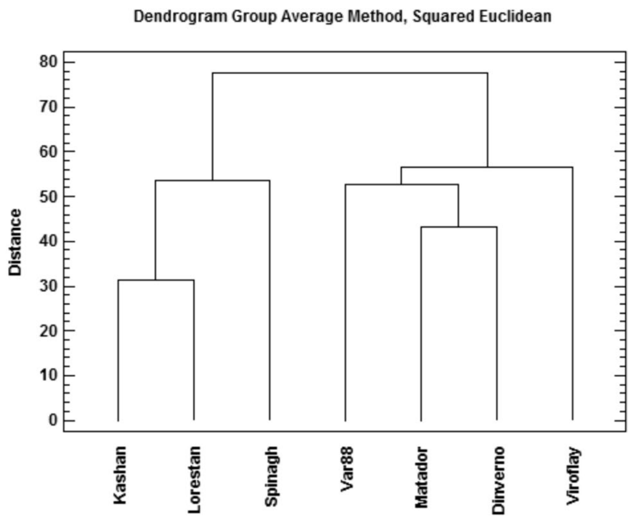


Fig. 6 Cluster analysis of the seven spinach accessions based on the average method using measured traits

Conclusion

We studied several morphological and biochemical elements of early and late spinach groups. Up to now, this study has been one of the most significant investigations of early- and late-bolting spinach accessions. A comprehensive study of the important characteristics of spinach under the influence of bolting time provides precious data for spinach breeders to select and cultivate accessions with desirable traits. Based on the number of leaves, plant height, yield, fresh and dry weight, day of bolting, and some biochemical characteristics (flavonoid, phenol, vitamin C, carbohydrate, nitrate, Ca, and Fe), there was significant variation between early and late bolting. It can be concluded that the early-bolting group was more stable in terms of appearance, especially in plant height, which is one of the advantages of mechanical harvesting. The early-bolting group was also superior in terms of some biochemical traits such as carbohydrate, iron, and calcium content. Conversely, the group that bolted later had more economic production and antioxidant properties, including yield, fresh and dry weight, flavonoid, phenol, and vitamin C. It is suggested to the farmers of the late-bolting group for production. Finally, the non-Iranian accession "Viroflay" and the Iranian accession "Varamin 88" may be used in future breeding schemes, according to this study's findings (which were based on PCA and cluster analysis).

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Author contributions The authors contributed to designing the experiment, collected samples, and analyzed and interpreted the data. The authors wrote the paper. All authors read and approved the final manuscript.

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