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Characterizing Morphological Properties of Select Populations of Iranian Fiber Cannabis (*Cannabis sativa* L.)

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ABSTRACT

In this study, we investigated important morphological traits of select populations of Iranian fiber hemp. The analysis of variance showed significant diversity among the populations. Four of the eight genotypes showed a large biomass suitable for the fiber industry. Kerman, Sirjan, Dezful, and Arak genotypes had the highest plant height and the largest stem diameter. These populations had late-flowering habits with a short inflorescence length. Our classification revealed that of the four high-fibrous candidates, three were classified in one group, comprising Kerman, Sirjan, and Dezful genotypes. The fourth one, Arak, was placed in the second group. Significantly positive correlations were observed between final plant height, footstalk diameter, and number of days to first-flower emergence. A strong correlation occurred between morphological traits and geographical data pertaining to the genotypes. Our findings indicated that a high elevation and a low average annual temperature are suitable for the growth of medicinal cannabis. Warmer weather made the plants taller with thicker stems and late blooming habits, which are more relevant for use in the fiber industry.

Introduction

Cannabis (*Cannabis sativa*. L) is an annual plant of the family Cannabacea. The original habitat of Cannabis is Central Asia (Soest and Meijer, 1992; Soorni et al., 2017). Other regions, such as Iran and the Middle East, host this species in broad ranges. Despite its long cultivation history, cannabis has not earned the reputation it deserves in Iran due to legal barriers. Thus, it has never been considered an essential food source, medicinal asset, or industrial commodity. Small amounts of research have been done on its agricultural, physiological, and biochemical characteristics in Iran (Bagherpour et al., 2012). However, different provinces in Iran display rich diversity regarding this species, as it can be cultivated for various uses (Saadati et al., 2013). Some genotypes have seeds with rich unsaturated fatty acid contents. The paper and textile industries can benefit from its fiber (Wills, 2003; Russo, 2004; Jiang et al., 2006).

With the increase in global population and the growth of economic uses, the level of human intervention in natural resources and forests is increasing, and the total area of forests in the last ten years has been widely and rapidly decreasing (Williams, 2003; FAO Forestry report, 2010). Thus, a suitable alternative is required to produce the fiber humans need. Hemp has been known for centuries as a fast-replacing source of fiber, mainly because of its cheap processing and the quality of fiber it generates. Hemp fiber is natural and physically resistant, which makes it valuable for specific uses (Ranalli et al., 1999). This fiber is also more resistant, warmer, and softer than cotton and similar textiles (Shahzad, 2012). It is also recyclable and environmentally friendly and is used to produce paper, tissue, clothes, hats, and

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even shoes in many countries with this technology. According to the urgent need to reduce the exploitation of forests and the need for resources of renewable fiber in the wood and paper industry, hemp is an ideal option (Saadati et al., 2013; Mehrani et al., 2019; Mostafaei et al., 2022).
According to a relevant survey, despite the notable habitat diversity of native Cannabis populations, little evaluation of Cannabis populations in different regions of Iran has been done. An assessment of the genetic and morphological diversity of nineteen Iranian hemp plants under greenhouse conditions showed remarkable genetic and morphological diversity (Afsharian, 2013). Generally, most of the conducted research included evaluations of medicinal effects and phytochemical compounds or agronomic studies (Vosulipur et al., 2004; Asgharipur et al., 2006 and 2007; Shahverdi et al., 2011). One of the standard methods in investigating plant masses is the method of decomposition into factors. The advantage of using such a method is that it reduces the number of attributes without eliminating helpful information. Since the simple correlation of traits without considering their relationships cannot be a background for judgment, multivariate statistical methods such as factor analysis can provide accurate results (Nomani and Rashidi, 2001).
This research aimed to evaluate the relationship of traits and to determine factors explaining diverse characteristics in Iranian hemp under field conditions. For this purpose, in addition to examining trait-related linear relationships, decomposition into several factors was used for interpreting the relationships and describing the populations.

Material and Methods
This research was conducted in the spring and summer of 2019 at the research station of the Horticulture Department of Karaj Agriculture and Natural Resources Campus. Eight cannabis populations of both male and female plants were selected from different geographical regions with distinct climatic patterns. These included cold and mountainous regions of the west and northwest, hot and humid regions of the southwest, and hot and dry regions of the east and southeast (Table 1, Fig. 1). At least 15 female plants in each population were used for morphological analysis. In addition, seeds were collected from labeled local populations to be cultivated after collecting the seeds from their natural habitats. The geographical information of the sampled areas was recorded and used for determining climatic parameters.

Table 1. Geographical data of native cannabis genotypes collected from different regions of Iran

Province	Population code	Sample size	Elevation (m)	Longitude (E)	Latitude (N)	Annual Average temp (°C)	Annual Rainfall (mm)	Days to harvest
Kordestan	San-01	10	1464	46°99'	35°31	14	375.10	209
Kerman	Sir-01	10	1754	55°68'	29°43	17/81	138.52	207
Sistan and Balochestan	Zah-01	10	1352	60°86'	29°49	19/30	73.58	208
Khozestan	Rmhz-01	10	179	49°59'	31°27	27/67	280.49	210
Markazi	Ark-01	10	1722	49°42'	34°04'	14/04	297.75	200
Kerman	Krmn-01	10	1761	56°58'	30°15'	17/01	123.43	185
South Khorasan	Bsh-01	10	881	57°43'	34°03'	21/07	79.98	174
Khozestan	Dez-01	10	144	48°42”	32°38'	24/56	389/40	206

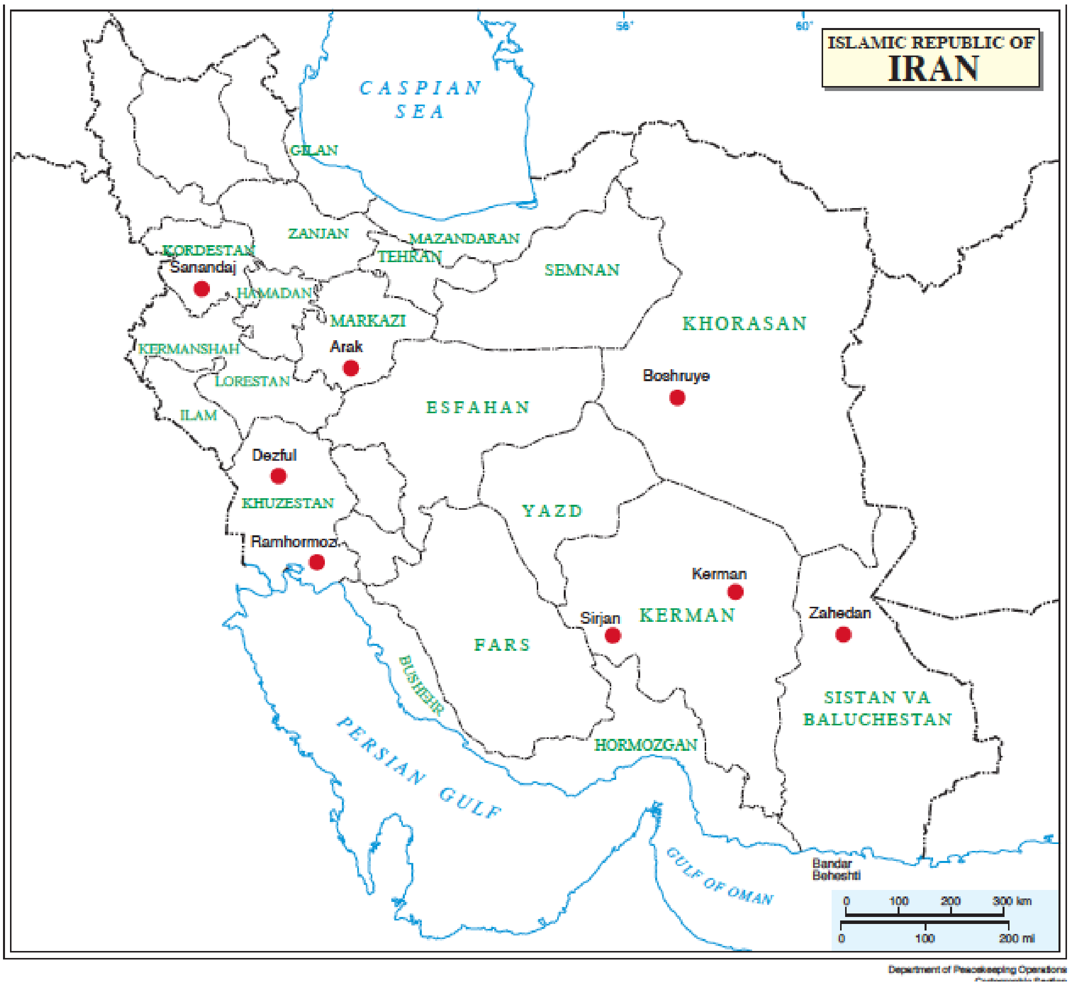

Fig. 1. Geographical distribution of 8 distinct Cannabis populations across selected regions of Iran.

This experiment was done in three replicates in a completely randomized block design. The seeds were planted in a depth of 4 cm. The distance between the rows was 52 cm and within a row the distance between the holes was 22 cm. The plants were watered regularly and twice a week using a drip irrigation system. To prevent interference and competition, we removed weeds by rooting using a shovel.
To minimize sampling bias, this study was performed on female flower buds as the main source of glandular trichomes. These comprise the main tissue of accumulation of phytocannabinoids (Flores-Sanchez and Verporte, 2008; Brusi et al., 2012). The number of days from seeding to harvest varied in the studied populations as presented in Table 1.
Vegetative and morphological traits included stem diameter at a height of 5 cm above the soil surface, number of nodes, average internode distance (cm), and final plant height (m).
Morphological characteristics of the reproductive stage were described by sex, the number of days from the time of planting to the appearance of the first blossom in males and females, the number of days until 50% opening of the flowers in females, length of inflorescence in females (cm), number of inflorescence in females, number of leaves in males and females, and crown length (cm) in females. In each block, we measured the morphological properties of 10 plants from each population with a balanced representation of each sex.
After recording and finishing the data collection, data analysis was done using Python (version 3.10). We used Python packages to perform all analysis, including the analysis of variance (Statsmodels 0.13.5; https://pypi.org/project/statsmodels/), comparison of mean values (Researchpy 0.3.5; https://pypi.org/project/researchpy/), correlation analysis of traits (Pandas 1.5.2; https://pypi.org/project/pandas/), and decomposition into factors (Sklearn 0.0.post1;

https://pypi.org/project/sklearn/).

Results

Studying morphological patterns and profiling hemp grown in highly diverse climates and geographical regions was valuable and informative as a starting point for future hemp breeding programs. Here, we studied populations diverse in morphophysiological features for the fiber industry. The structures of plants, female inflorescences, and buds in the studied populations were diversely different (Fig. 2). Descriptive statistics of the morphological traits in the examined hemp populations (Table 3) confirmed the variance analysis (Table 2). About half (121 out of 240) of the plants were female. Ten traits were measurable in female and male plants, respectively. A significant difference occurred in the number of inflorescences (p-value = 2.2e-3), the number of nodes (1.4e-4), height (9.0e-4), inter-node distance (1.0e-3), footstalk diameter (4.8e-13), days to first blooming (9.5e-5), and days to 50% bloom (6.6e-4) at the level of 5% (with Bonferroni adjustment for multiple comparisons) among the evaluated cannabis populations. Our results indicated the potential genetic population diversity agreed with previous works showing significant differences in the morphological traits of 19 Iranian hemp genotypes planted in greenhouse conditions (Afsharian, 2013). Significant variations reportedly occurred in the amount and composition of hemp oil in different regions of Iran (Vosulipur et al., 2004). The highest coefficient of variability occurred in plant height and footstalk diameter.

Table 2. Analysis of variance of the measured morphological properties across the population

	Df	Len. of inflorescence (cm)	Num. of Inflorescence	Num. of nodes	Height (m)	Num. of leaves	Inter-node distance (cm)	Footstalk diameter (cm)	Days to first blooming	Days to 50% blooming	Crown len. (cm)
						Sum of Squares (SS)					
Variable											
Block	2	31.92	27.22	3.60	0.56	9.88	1.80	23.15	135.05	325.35	193.59
Population	7	1338.07	676.06	320.20	17.47	40.27	12845.05	117.31	17970.93	2232.02	4720.32
Residual	14	3159.40	2500.86	491.03	64.53	118.33	17894.48	182.65	51690.61	21851.20	9998.25
PR(>F)		0.547	2.2e-03	1.4e-04	9.0e-04	0.0059	1.0e-03	4.8e-13	9.8e-5	6.6e-4	3.6e-2

Table 3. Comparison of mean values of morphological traits in the cannabis populations using Duncan's multiple range test (P≤0.05).

Province	Len. of inflorescence (cm)	No. of Inflorescence	No. of nodes	Height (m)	No. of leaves	inter-node distance (cm)	Footstalk diameter (cm)	Days to first blooming	Days to 50% blooming	Crown len. (cm)
Arak-01	27.50	17.17	8.07	2.24	8.62	51.03	3.18	111.40	124.62	57.00
Bsh-01	26.29	14.63	6.97	1.68	7.57	33.40	1.77	105.30	124.86	40.50
Dez-01	33.12	16.60	9.43	2.27	8.18	60.73	3.35	122.67	131.06	40.29
Krmn-01	30.00	13.73	7.60	2.31	8.43	48.40	4.19	126.90	133.43	48.93
Rmhz-01	29.06	13.80	7.10	1.83	6.88	50.33	2.48	125.30	132.88	49.12
San-02	27.06	15.73	7.63	1.78	7.38	45.97	2.83	126.27	134.75	48.31
Sir-01	36.33	18.30	10.33	2.28	8.33	54.37	3.42	124.77	136.67	60.08
Zah-01	25.60	17.90	7.00	1.69	8.07	47.30	2.40	106.63	125.20	47.40

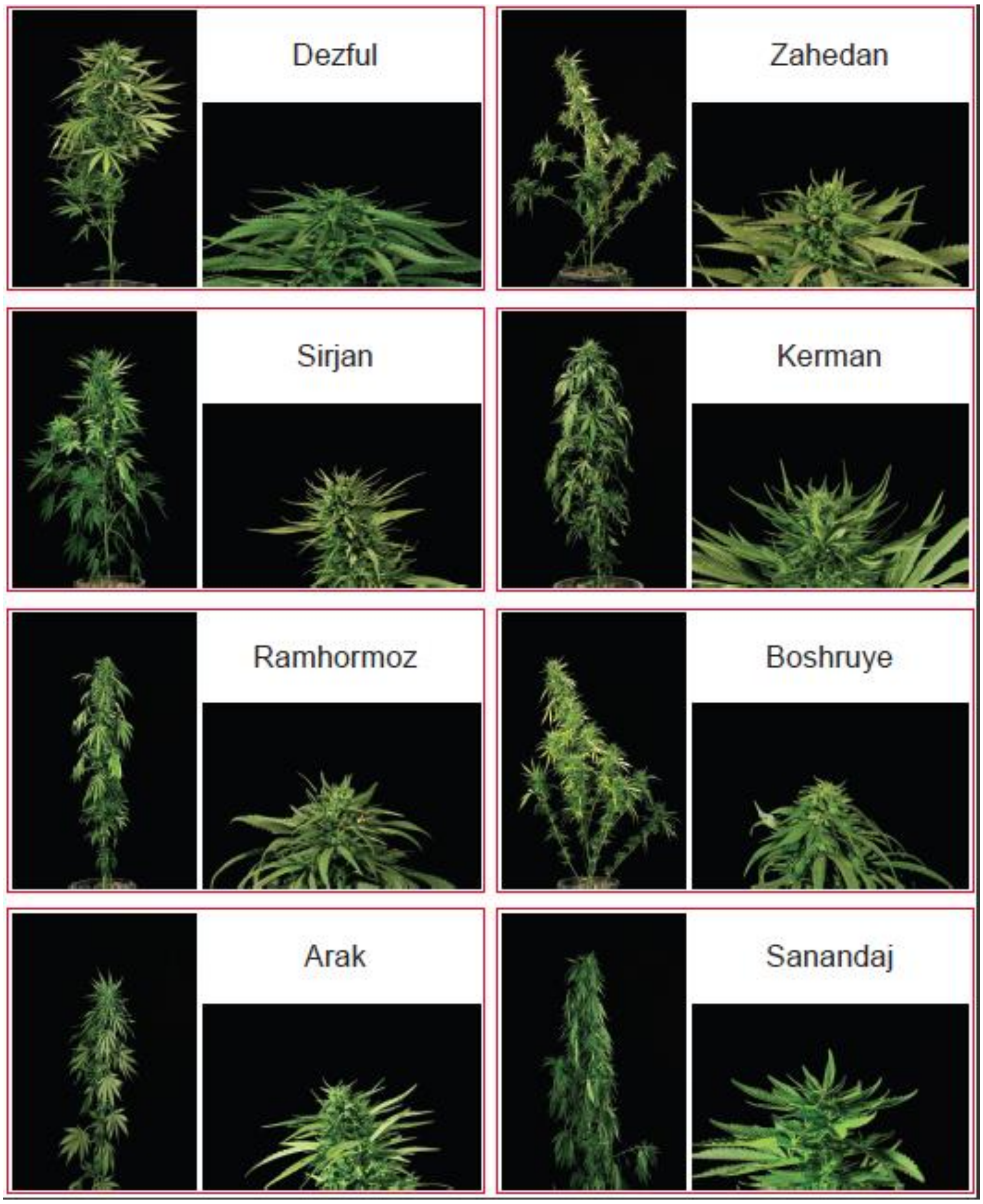

Fig. 2. Diversity in plant structure and female inflorescence of 8 natural cannabis populations distributed across Iran

The comparison of the average values for the measured traits (Table 3) showed that the level of medicinal substances in cannabis plants was highest just before seed formation. It is valuable when knowing how long blooming should take (Asadi et al., 2019). The difference in the number of days until the opening of the first and 50% of the flowers was significant in the populations. The earliest time took in Boshruye (first blooming, 105.30; 50% blooming 124.86 days) and Zahedan (first blooming, 106.63 and 50% blooming 125.20 days). For use in the fiber industry, having the tallest and the thickest plants is more beneficial. Table 3 indicates that the Kerman population was significantly taller (mean: 2.31 m) than others and had the thickest footstalk (mean: 4.19 cm). Sirjan (height: 2.28 m, footstalk diameter: 3.42 cm), Dezful (2.27 m, 3.35 cm), and Arak (2.24 m, 3.18 cm) are the next ones in the ranking. Our results confirm previous works where significant differences were observed across different Cannabis populations (Riahi et al., 2016).

Also, the amount of cannabinoids has a significant relationship with some morphological traits (Meijer et al., 1992). The lowest average internode distance occurred in Boshruye (33.40 cm) population, and the highest average internode distance was related to Dezful (60.73 cm) and Sirjan (54.37 cm). The high levels of

Delta-9-Tetrahydrocannabinol (THC), low cannabidiol (CBD) levels, small leaf area, and early flowering were the common characteristics of medicinal samples (Meijer and Keizer, 1994 and 1996; Asadi et al., 2019). In contrast, thicker stems, higher height, late flowering, and low cannabinoid content were reportedly known as fiber characteristics (Meijer and Keizer, 1994; Riahi et al., 2016). Therefore, our findings indicate that Kerman, Sirjan, Dezful, and Arak populations are the best candidates to use in the fiber industry.

Traits such as footstalk diameter and height are the components of plant performance, and in the field of fiber extraction, their high values in each sample are considered a positive factor. Finding traits having high correlations with each other in any study is a goal in breeding programs. An assessment of the correlation coefficients of the traits shown in Figure 3 revealed a positive and significant correlation between how early plants bloom and how much it takes for 50% of the flowers to appear (corr = 0.994, p-value = 2.7e-11). Moreover, plant height showed a positive and significant correlation with footstalk diameter (corr = 0.704, p-value = 8.3-6) and the number of nodes (corr = 0.296, p-value = 0.034). The inflorescence length correlated significantly with the number of nodes (corr = 0.477, p-value = 2.6e-3) and internode distance (corr = 0.537, p-value = 3.8e-3). Previous works reported similar findings, where early flowering populations showed a lower average height (Meijer and Keizer, 1996). A positive and significant correlation occurred between the flowering time and the final plant height. It was also significantly correlative between the flowering time and the first flower node height (Afsharian, 2013).

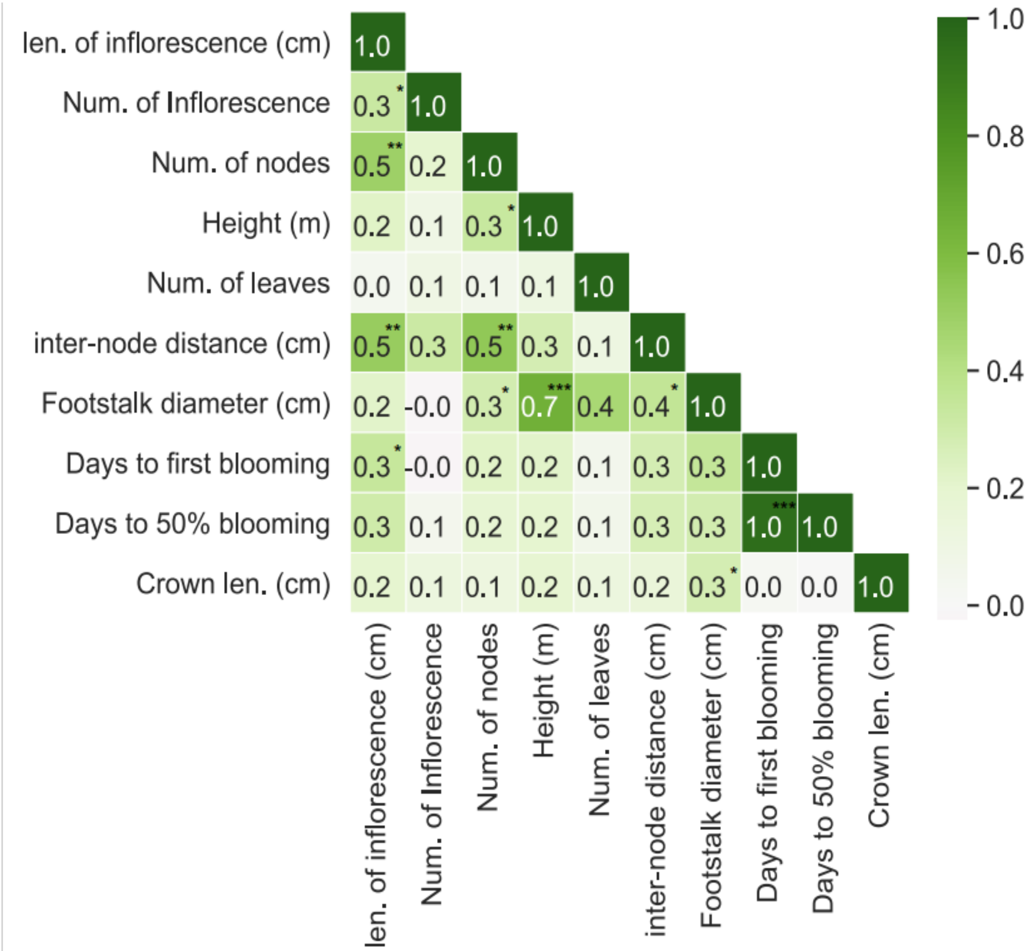

Fig. 3. Correlation coefficients between the morphological traits measured in the cannabis populations (*** p<0.001, ** p<0.01, * p<0.05).

To group the populations, a cluster analysis was used based on the standardized mean of morphological traits using the Ward method (Fig. 4; Sharma and Batra, 2019). Eight populations landed on two distinct groups based on morphological attributes. The first group consists of samples with late-flowering habits, tall and thick stems, and long inter-node distance. In contrast, the second group represents samples with shorter, thinner stems and a more significant inflorescence. Three of the four high-fibrous candidates ended up in the first group. These included the Kerman, Sirjan, and Dezful genotypes. The fourth one, Arak, was classified in the second group. The classification seems reasonable because the Arak population was the least fibrous among the four. In terms of the number of leaves, crown length, and the number of nodes of the first inflorescence, these populations did not differ significantly.

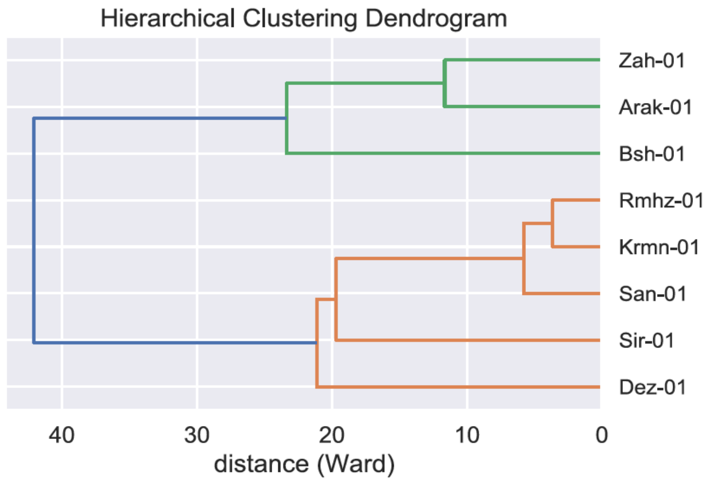

Fig. 4. Cluster analysis of investigated Cannabis stands based on morphological traits using Ward's method

The factor analysis based on the investigated quantitative traits showed that the first two factors with eigenvalues greater than one explained more than 56.48% of the variability of the traits. According to the coefficient factors for each trait, the importance and influence of each trait were in the mentioned factors (Table 4).

Table 4. Factor coefficients obtained from decomposition into factors for the investigated morphological traits in different hemp populations

Variable	First factor	Second factor
Len. of inflorescence	-0.007	1.337
Num. of inflorescence	-0.523	1.210
Num. of nodes	-0.157	0.296
Height	-0.003	0.314
Num. of leaves	-3.863*	7.061***
Inter-node distance	-0.493	0.559
Footstalk diameter	-17.467***	-1.307
Days to first blooming	-8.349**	0.563
Days to 50% blooming	-0.690	3.444**
Crown Len.	0.015	0.009

The factor analysis also revealed that the traits of footstalk diameter, number of leaves, and days to first blooming had significant and positive factor coefficients in terms of the first factor (Fig. 5). Meanwhile, in the second factor, the footstalk diameter, number of leaves, days to 50% blooming, and length and number of lateral inflorescences had significant and positive factor coefficients. The two-dimensional graph of the traits based on the first two factors, with a share of 73% of the variables, confirmed the importance and influence of the mentioned traits in the relevant factors. Plotting the studied cannabis populations based on the first two factors showed that the Boshruye and Zahedan populations had the highest values in terms of the first factor. In contrast, Sirjan and Boshruye had the highest positive and negative values, respectively, in terms of the second factor (Fig. 6). Among the four fibrous genotypes, Sirjan and Dezful populations had great second-factor values, while Zahedan had a high and positive value in terms of the first factor. The Arak population seemed to have a relatively large value for both factors but was not identified as fibrous as the other three populations.

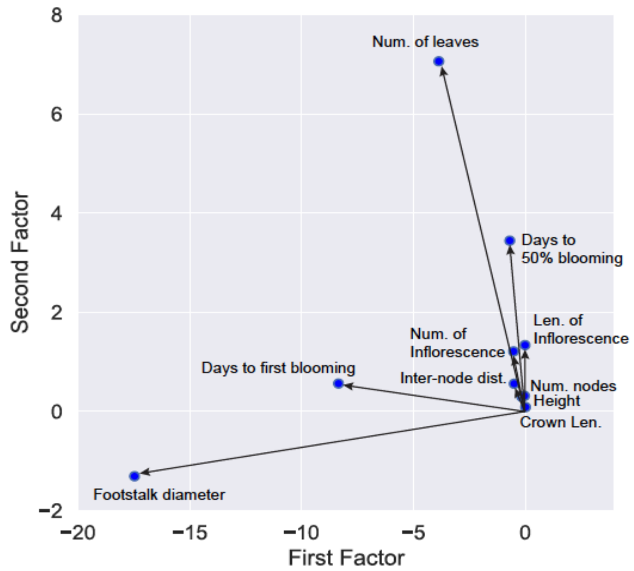

Fig. 5. Two-dimensional diagram of the attributes used in factor analysis based on the first two factors

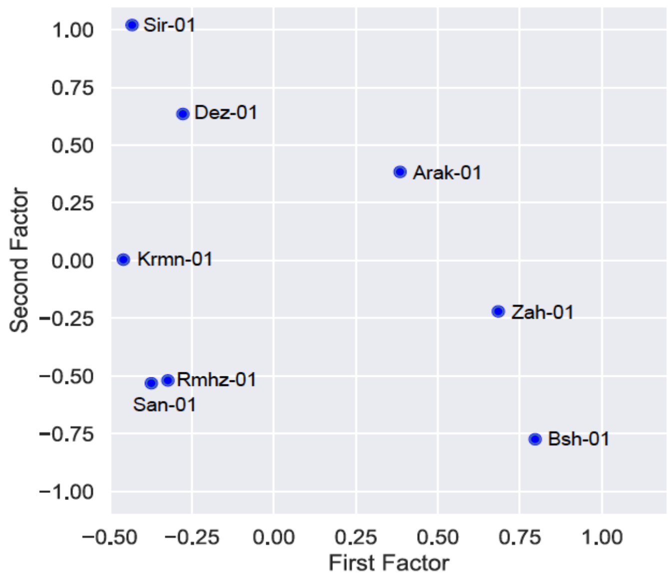

Fig. 6. The two-dimensional diagram of the investigated hemp populations based on the first two factors obtained from factorization

The late flowering period, high final height, large footstalk diameter, and multitude of nodes are associated with high-fibrous genotypes. In contrast, early flowering and a large number of long inflorescence are characteristics of medicinal genotypes. Correlation analysis of geographical data of the cannabis population with morphological parameters revealed interesting results (Fig. 7). The annual average temperature and latitude correlated negatively with the length and number of inflorescence and positively correlated with the number of days to the first and 50% blooming. It indicated that medicinal genotypes are more likely to thrive in the northern parts of Iran, with lower average annual temperatures. At the same time, more fibrous plants are more likely to grow in the southern part of the country with a higher average annual temperature.

Similarly, elevation correlated significantly and more positively with the characteristics of the medicinal genotypes, meaning that at higher elevations, the medicinal genotypes were more likely to grow. Finally, longitude and average annual rainfall are not mainly associated with any genotype. This finding is reasonable since plants were watered regularly twice a week and each time for 6-9 hours by drip irrigation. Thus, the average annual rainfall was not a determinant factor in the growth of plants.

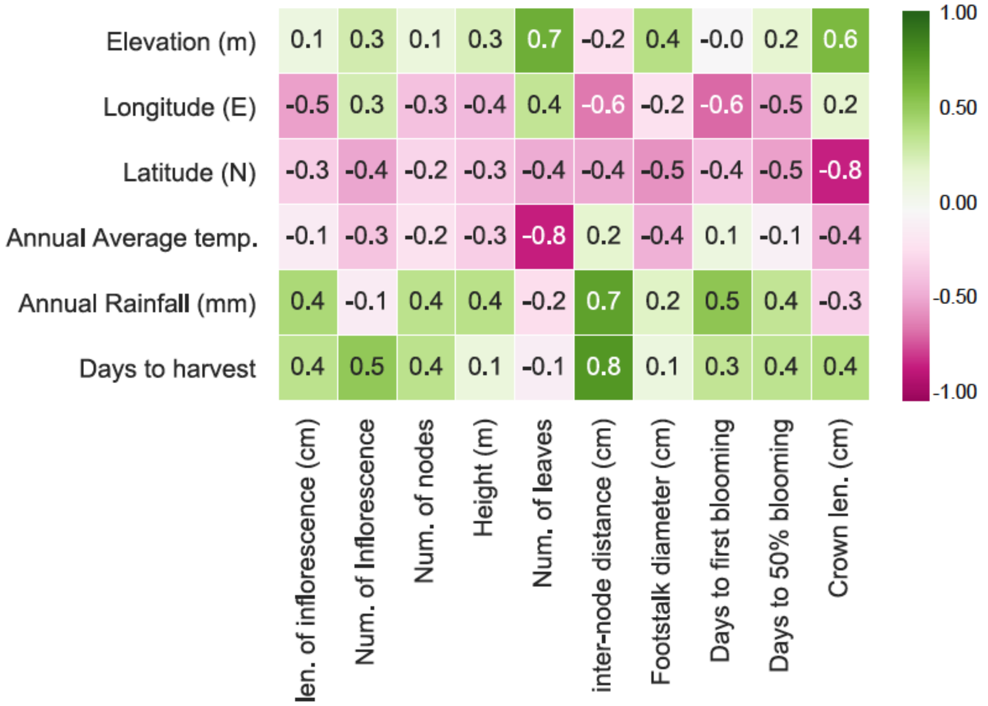

Fig. 7. Correlation coefficients between geographical data and the morphological traits measured in the cannabis population

Discussion

In this study, we presented evidence of a significant diversity among the Iranian cannabis populations. Our results showed strong correlations among some of the morphological traits and plant genotypes. Having more biomass is very important in fibrous plants. The investigated genotypes showed a positive and significant relationship between stem diameters, the number of nodes, the height of the first alternate node, and the ratio of length to width in the central leaf. Decomposition into factors was able to separate hemp masses based on morphological characteristics. Therefore, using multivariate analysis like factor analysis for data purification in the comprehensive description and grouping of different Cannabis populations can be efficient and effective. Moreover, our results indicated that high elevation and low average annual temperature are suitable for the growth of medicinal cannabis plants. In contrast, warmer weather leads to taller plants with thicker stems during the late blooming period.

Knowledge of the relationships between traits is essential in describing cannabis plants, particularly when differentiating medicinal and fibrous types from each other. For example, a significant relationship between plant height and the number of days until 50% of flowers open can be necessary because low height and early flowering are characteristics of medicinal varieties. In general, the longer the vegetative phase is, the more valuable it becomes for fiber production because the maximum growth of the stem is in this phase and before flowering. After flowering, stem growth did not continue. Longer vegetative periods meant the stem yield increased and enhanced the fiber yield (Irannezhad et al., 2007). Thus, the population of Kerman and Sanandaj, which have long growth periods, may be included in one group of the hemp population. As the stem yield of these ecotypes was taller, this issue became more prominent because stem yield correlates with high fiber yield (Asghari-Pour and Rashed-Mohassel, 2007). Phytochemical studies are needed to prove that cannabis is a fibrous or medicinal plant.

Some cannabis populations (Saghez and Sarv) are considered medicinal due to their low leaf surface, short final height, and very early flowering period (Riahi et al., 2016). The assurance of the medicinal properties of these masses depends on phytochemical analysis. Previous works showed that the highest calculated yield was found in Ramhormoz, Shiraz, Boshruye, Zahedan, and Bam genotypes, while the lowest was found in Saghez and Sarv populations (Asgharipur et al., 2007; Poisa and Adamovics, 2010; Riahi et al., 2016). Our findings are in line with previous works, where the number of cannabinoids has shown to have a significant relationship with some morphological traits (Meijer et al., 1992) or the high THC level, low leaf area, and early flowering are associated with medicinal genotypes (Meijer and Keizer, 1994 and 1996; Riahi et al., 2016; Asadi et al., 2019).

While our work did not show any significant negative correlation between traits, previous studies have shown some significant negative correlations. For example, between leaf surface area and the ratio of length to width in the central leaf, meaning that in the plants with more leaf surface, the leaflets were broader, and in the plants with less leaf surface, they were elongated and narrow (Riahi et al., 2016). These traits were absent in our study. Also, a significant negative relationship between the number of lateral flowers and the average length of the internode shows that the flowers were denser in the plants with more lateral flowers.

Overall, the results of this study show the incredible morphological diversity in the investigated Cannabis heritage stocks. However, a more detailed investigation of this variety requires phytochemical, physiological, and molecular assessment. However, the preliminary description and evaluation of Iranian hemp populations in this research can be beneficial in introducing suitable types for selecting parents and desirable genotypes for breeding programs.

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Conflict of Interest

The authors indicate no conflict of interest for this work.

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