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Genetic variation and interrelationships of sainfoin genotypes as revealed by morphologic and agronomic markers

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Abstract: Sainfoin (*Onobrychis viciifolia* Scop.) as an ancient forage crop has garnered interest due to its desirable characteristics and adaptability. This investigation was designed to study genetic variation and interrelationships of sainfoin via factor analysis and clustering methods. Thirty-two local native genotypes were collected from various geographical regions and assessed based on a randomized block scheme with four replicates. The first five factors explained 85% of the variance and were named yield performance, plant morphology, powdery mildew, forage quality and forage palatability. The first factor described 58% of the variability, encompassed forage yield and its related characteristics like stem number per plant, main stem's nodes, length of the longest stem, main stem's internodes, stem's leaves, stem fresh weight, leaf fresh weight, inflorescence fresh weight, stem dry weight, leaf dry weight, fresh weight and dry weight per unit area. The dendrogram of sainfoin genotypes is categorized genotypes into three groups, which is confirmed via the Wilks' lambda of multivariate analysis of variance. Group-1 contains 10 genotypes with the highest forage yield performance, and high amounts for most of the other remained traits. Group-2 contains 9 genotypes with the lowest performance for most traits of powdery mildew, suggesting their good potential for powdery mildew tolerance, while Group-3 contains 13 genotypes and indicated moderate performance for measured traits of sainfoin. The ten desirable genotypes of Group-1 mostly from northwest Iran, were good candidates for commercial release in Iran because they performed well and had high values of most traits, so they can be advised for cultivation in cool upland rainfed conditions of semi-arid regions.

Keywords: *Onobrychis viciifolia* Scop., genetic diversity, cluster analysis, factor analysis.

Introduction

Sainfoin (*Onobrychis viciifolia* Scop.) from the Fabaceae, as a perennial forage has high protein content and nutritional value. Believed to have originated in the Middle East or Central Asia, probably Iran, it was cultivated in past centuries (Mora-Ortiz & Smith 2018; Sayar et al. 2022). Recently, there has been renewed interest in sainfoin due to its health options for animals, attributed to condensed tannins and other phytochemicals that provide anthelmintic properties and prevent lethal bloat, unlike most forage legumes (Wijekoon et al. 2021; Yılmaz et al. 2023). Also, sainfoin had some beneficial agronomic properties such as atmospheric nitrogen fixation, reducing the need for chemical nitrogen fertilizers. Its capacity to improve soil properties and provide quality forage for animals makes sainfoin advantageous for grassland use (Kölliker et al. 2017; Bhattarai et al. 2024). Additionally, sainfoin blossoms produce large amounts of nectar, attracting pollinating insects like honeybees.

The challenges with sainfoin include susceptibility to waterlogging and freezing and weak competing potential during seedling development stages (Carbonero et al. 2011). Consequently, targeted breeding efforts are necessary to develop sainfoin varieties which had adaptability to the changes of environments. In Iran, different genotypes of sainfoin have been developed for a long time and have evolved under domestication so, they have good adaptability properties (Sedeh 2017). Farmers in northwest and west Iran continue to cultivate old ecotypes with good tolerance to abiotic stresses, representing valuable genetic resources. Collecting and evaluating these promising genotypes is essential for future breeding programs.

Determining the components of yield is crucial for advancing sainfoin breeding research. Baghainiya et al. (2012) demonstrated a significant association of forage yield with stem percentage, plant number per area, and node number per main stem and emphasized that utilizing them can be beneficial in future sainfoin breeding programs, aiding in the development of genetically improved cultivars with a broad genetic base. Zarabiyan et al. (2016) investigated the genetic diversity of 56 cultivars of sainfoin using yield components and morphological traits and reported high heritability for stem length and stem number, which indicates the major role of genes in controlling these traits. Zavoshti et al. (2023) found that the number of leaflets per leaf and length of the longest stem affected the forage yield performance in sainfoin which are followed by the number of stems, number of leaves per branches, length of inflorescence and stem/leaf ratio. Although, sainfoin breeding faces some limitations due to issues such as genetic complexity, perennial nature, and open pollination characteristics, which are typical of most fodder plants (Annicchiarico et al. 2015), the creation of new synthetic cultivars is still the most common breeding method in sainfoin and other fodder legumes. In this regard, knowledge of the degree of genetic diversity and the way traits are related to each other, important and economic characteristics like forage yield, can provide the basis for a faster and more accurate identification and selection of parents.

The use of multivariate tools provides the possibility of accurate classification of the samples under evaluation and helps the breeder to identify the genetic material which are needed for the next programs and to advance the breeding goals faster (Sabaghnia et al. 2024). The goal of this research was to evaluate the associations for morphological traits of some sainfoin genotypes to identify the most important traits for the selection process of high forage yield, and obtained information can then be used in breeding projects to develop new sainfoin cultivars.

Material and Methods

Trial

The trial involved assessing 32 local genotypes of sainfoin (Tab. 1), which were collected from various geographical locations of Iran (Fig. 1), in a field located in Maragheh (37°23'N 46°14'E), in the north-west of Iran with cool semi-arid upland characteristic and Regosols soil type. This area is in the southwest of Sahand mountain and has long winters. Most of the precipitation occurs in autumn and winter in the form of 70 % snow and 30 % rain. The field was fertilized by nitrogen and phosphorus pentoxide (30 and 50 kg ha^{-1}, respectively) on field preparation. The trial was ordered in a randomized block scheme with four replicates. Four 2-m long rows, with 30 cm intra-space rows and 20 cm inter-space rows were generated in experimental plots.

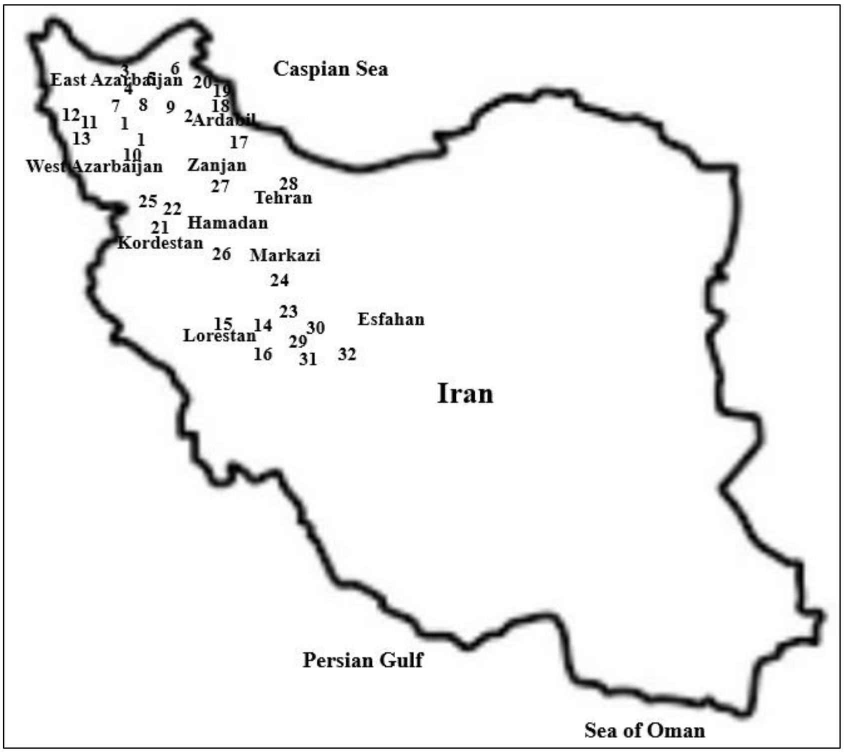

Fig. 1 Map of Iran which showing the geographical positions of collocated of sainfoin (*Onobrychis viciifolia* Scop.) genotypes.

Tab. 1 Code, name of the regions, and coordinates of where the sainfoin genotypes were collected.

Code	Origin	Coordinates	Code	Origin	Coordinates
G1	Bonab	37°20′N 46°03′E	G17	Khalkhal	37°37′N 48°31′E
G2	Sarab	37°56′N 47°32′E	G18	Garjan	38°18′N 48°12′E
G3	Marand	38°25′N 45°46′E	G19	Kahlaran	38°18′N 48°12′E
G4	Zonuz	38°35′N 45°49′E	G20	Meshginshahr	38°23′N 47°40′E
G5	Varzaqan	38°30′N 46°39′E	G21	Sanandaj	35°18′N 46°59′E
G6	Ahar	38°28′N 47°04′E	G22	Divandarreh	35°54′N 47°01′E
G7	Azarshahr	37°45′N 45°58′E	G23	Khomeyn	33°38′N 50°04′E
G8	Tabriz	38°04′N 46°18′E	G24	Arak	34°05′N 49°41′E
G9	Heris	38°14′N 47°06′E	G25	Saqqez	36°14′N 46°15′E
G10	Miandoab	36°58′N 46°06′E	G26	Asadabad	34°46′N 48°07′E
G11	Urmia	37°32′N 45°04′E	G27	Zanjan	36°40′N 48°29′E
G12	Silvaneh	37°25′N 44°51′E	G28	Damavand	35°43′N 52°03′E
G13	Oshnavieh	37°02′N 45°05′E	G29	Faridan	32°59′N 50°24′E
G14	Azna	33°27′N 49°27′E	G30	Khansar	33°13′N 50°18′E
G15	Khorramabad	33°29′N 48°21′E	G31	Fereydunshahr	32°56′N 50°07′E
G16	Aligudarz	33°24′N 49°41′E	G32	Kabutarabad	32°29′N 51°49′E

Traits

The centre rows were used for harvesting and measuring plant number per area (PNA), fresh weight (FW) and dry weight (DW), and then computing leaf/stem ratio (LSR), and dry/fresh ratio (DFR) and declared as unit area, number, kg and percent. The occurrence of powdery mildew (PM) was recorded visually as a percent of each experimental unit. About ten samples randomly chosen from the mid-rows of plots to recording stem number per plant (SNP), main stem's nodes (MSN), length of the longest stem (LLS), length of the peduncle (LP), length of inflorescence (LI), main stem's internodes (MSI), elongation of internode (EI), main stem's leaves (MSL), stem's leaves (SL), leaflets number of the leaf (LNL), stem fresh weight (SFW), leaf fresh weight (LFW), inflorescence fresh weight (IFW), stem dry weight (SDW), leaf dry weight (LDW), and inflorescence dry weight (IDW).

Statistical analysis

The recorded data was examined for normality via the procedure of Kolmogorov-Smirnov via SPSS version 22.0 (IBM-SPSS, USA). Factor analysis as a powerful way for reducing numerous correlated traits to a smaller set of independent factors, was employed to identify the most desirable traits. By utilizing the varimax rotated coefficients, factors with eigenvalues exceeding unity were identified and analysed as the most significant ones via Minitab (Minitab Inc., USA). Additionally, the magnitudes of common factors or communality were calculated to determine the extent to which each trait contributes to the variation observed in the data. Agglomerative hierarchical cluster analysis was used to group genotypes according to standardized squared Euclidean distances using the obtained dataset. The computed standardized squared Euclidean distances were merged by the minimum variance of the Wards’ method and dendrogram diagrams were generated to represent the patterns and relations among genotypes and traits with the Mult/Exploratory statement in STATITICA software version 14.0 (TIBCO Inc., USA). To verify the number of clusters showing the significant partition in the dendrogram diagrams, the multivariate analysis of variance was used via the Wilks' lambda which was applied to the original dataset for all measured traits with the Multivariate statement in SPSS, so the significant borders were selected as the final cutoff point.

Results

Some descriptive indices for the sainfoin genotypes like coefficient of variation (CV) are given in Tab. 2, which indicates the magnitudes of CV were high (>20%) for all the traits except stems number per plant (SNP), main stem's internodes (MSI), main stem's leaves (MSL), leaflets number of leaf (LNL), leaf/stem ratio (LSR), and dry/fresh ratio (DFR). Also, the amounts of CV were moderate (10%>CV>20%) for the above-mentioned traits while the values of CV were not low (<10%) for any of the sainfoin traits (Tab. 2). Thus, the genetic diversity of sainfoin genotypes was high and can be used to achieve a favourable combination of traits. The dry weight (DW) performance ranged from 0.17 to 2.2 kg m^{-2} and the fresh weight (FW) performance ranged from 0.26 to 3.04 m^{-2}. The CV of DW and FW as important economic yield performances were very high (18.5 and 53.1 %) followed by the other yield components like plants number per area (40.1 %), stem's leaves (37.2 %), stem fresh weight (48.4%), leaf fresh weight (49.2 %), inflorescence fresh weight (50.4 %), stem dry weight (47.6 %), leaf dry weight (56.4 %), and inflorescence dry weight (88.8 %). Current remarkable variability among sainfoin genotypes, demonstrated genetic changes and provides a unique opportunity for genetic improvement projects and management of genetic resources in germplasms.

The sufficiency of the dataset for factor analysis was assessed by indices of the Kaiser-Meyer-Olkin (KMO) which was bigger than 0.50 (0.71), and the significance of sphericity exam of Bartlett (χ^2= 864, P<0.01). In other words, this test was performed to ensure the efficiency of the factor analysis method, so, there was enough associations among the variables as well as ample correlation among the traits. The factor analysis indicated that the first five eigenvalues exceeded 1.0, explaining 85 % of the variance (Tab. 3). The first one with a description of 58 % of the variability, encompassed forage yield and its related characters like stem number per plant (SNP), main stem's nodes (MSN), length of the longest stem (LLS), main stem's internodes (MSI), stem's leaves (SL), fresh weight (FW), stem fresh weight (SFW), leaf fresh weight (LFW), inflorescence fresh weight (IFW), dry weight (DW), stem dry weight (SDW), and leaf dry weight (LDW), suggesting a focus on yield potential and can be named as yield factor.

Conversely, the second factor, which accounted for 10 % of the variation, showed relatively high values for traits such as length of peduncle (LP), length of inflorescence (LI), and inflorescence dry weight (IDW) following to main stem's internodes (MSI) and main stem's nodes (MSN), which shows plant morphology is

Tab. 2 Descriptive indices of 22 traits of 32 sainfoin (*Onobrychis viciifolia* Scop.) genotypes.

Traits†	Mean	Min	Max	CV‡
PNA	20.97	8.75	35.67	40.1
SNP	5.80	4.24	7.45	16.1
MSN	6.23	4.54	7.65	14.0
LLS	44.62	20.07	62.95	26.0
LP	15.87	8.4	21.34	21.8
LI	6.93	4.5	9.82	20.7
MSI	5.23	3.54	6.65	16.6
EI	9.40	4.86	24.75	34.7
MSL	6.09	4.41	9.23	18.1
SL	22.52	10.6	36.27	37.2
LNL	13.19	9.8	18.41	14.2
PM	0.68	0.13	1.00	46.9
FW	1.29	0.26	3.04	48.5
SFW	0.42	0.09	1.04	48.4
LFW	0.80	0.16	1.85	49.2
IFW	0.06	0.01	0.14	50.4
DW	0.85	0.17	2.20	53.1
SDW	0.27	0.06	0.64	47.6
LDW	0.55	0.1	1.48	56.4
IDW	0.04	0.01	0.2	88.8
LSR	1.98	1.32	2.6	15.3
DFR	0.65	0.48	0.8	11.3

†Traits are: plants number per area (PNA), stems number per plant (SNP), main stem's nodes (MSN), length of the longest stem (LLS), length of peduncle (LP), length of inflorescence (LI), main stem's internodes (MSI), elongation of internode (EI), main stem's leaves (MSL), stem's leaves (SL), leaflets number of leaf (LNL), powdery mildew (PM), fresh weight (FW), stem fresh weight (SFW), leaf fresh weight (LFW), inflorescence fresh weight (IFW), dry weight (DW), stem dry weight (SDW), leaf dry weight (LDW), inflorescence dry weight (IDW), leaf/stem ratio (LSR), and dry/fresh ratio (DFR).
‡ CV, coefficient of variation.

suitable for this factor (Tab. 3). Thus, in any plant breeding program, the morphologic characteristics must be regarded for obtaining the ideotype plant for sainfoin. The third factor, explaining 7 % of the variance, comprised powdery mildew (PM), leading to the designation of powdery mildew disease for this factor (Tab. 3). Meanwhile, the fourth factor, which accounted for 5 % of the variability, included traits like leaf/stem ratio (LSR) and inflorescence dry weight (IDW), hence it was labelled as the forage quality factor. Since factor analysis reveals more information regarding functional components and morphological structures in crops, so using this information, breeders will be able to select the most favourable traits that increase yield performance. The fifth factor, explaining 5 % of the variance, exhibited positive values for dry/fresh ratio (DFR) and elongation of internode (EI) while it indicated negative values for the length of inflorescence (LI), stem's leaves (SL), and leaflets number of the leaf (LNL), which indicates the negative relationship between forage

Tab. 3 The varimax rotated scores of first five factors and of communality values.

Traits†	Factor1	Factor2	Factor3	Factor4	Factor5	Communality
PNA	0.732	-0.169	-0.251	-0.186	0.041	0.167
SNP	**0.864**	0.159	-0.172	0.076	-0.153	0.774
MSN	**0.834**	**0.387**	-0.074	0.159	0.165	**1.471**
LLS	**0.859**	0.224	-0.195	-0.077	0.026	0.837
LP	0.716	**0.467**	-0.051	0.050	-0.081	**1.101**
LI	0.755	**0.384**	-0.203	-0.146	**-0.238**	0.552
MSI	**0.835**	**0.380**	-0.083	0.159	0.165	**1.456**
EI	0.393	0.153	-0.369	-0.533	**0.484**	0.128
MSL	0.771	0.192	-0.331	-0.192	0.006	0.446
SL	**0.821**	0.193	-0.058	0.258	**-0.257**	0.957
LNL	0.666	-0.463	0.113	-0.141	**-0.279**	-0.104
PM	-0.132	0.293	**0.589**	0.147	0.157	**1.054**
FW	**0.940**	-0.169	0.264	-0.105	-0.009	0.921
SFW	**0.918**	-0.101	0.292	-0.121	-0.043	0.945
LFW	**0.933**	-0.203	0.241	-0.127	-0.007	0.837
IFW	**0.808**	-0.144	0.304	0.222	0.063	**1.253**
DW	**0.934**	-0.269	0.191	-0.043	0.072	0.885
SDW	**0.909**	-0.223	0.248	-0.155	0.081	0.860
LDW	**0.932**	-0.295	0.156	-0.014	0.067	0.846
IDW	0.502	**0.338**	0.106	**0.397**	-0.052	**1.291**
LSR	0.526	-0.384	-0.383	**0.515**	0.085	0.359
DFR	0.257	-0.61	-0.458	0.296	**0.387**	-0.128
Eigenvalue	12.79	2.17	1.59	1.18	1.02	
Variance%	0.58	0.10	0.07	0.05	0.05	
Cumulative	0.58	0.68	0.75	0.81	0.85	

The bold cases are the highest scores in each factor.

†Traits are: plants number per area (PNA), stems number per plant (SNP), main stem's nodes (MSN), length of the longest stem (LLS), length of peduncle (LP), length of inflorescence (LI), main stem's internodes (MSI), elongation of internode (EI), main stem's leaves (MSL), stem's leaves (SL), leaflets number of leaf (LNL), powdery mildew (PM), fresh weight (FW), stem fresh weight (SFW), leaf fresh weight (LFW), inflorescence fresh weight (IFW), dry weight (DW), stem dry weight (SDW), leaf dry weight (LDW), inflorescence dry weight (IDW), leaf/stem ratio (LSR), and dry/fresh ratio (DFR).

quality and yield performance in sainfoin (Tab. 3). This factor can be named as forage palatability factor. Regarding communalities, main stem's nodes (MSN) and the main stem's internodes (MSI) following to inflorescence fresh weight (IFW), inflorescence dry weight (IDW), length of peduncle (LP) and powdery mildew (PM) demonstrated good reliability, underscoring their genetic consistency. However, it seems that the first which is named as yield factor is more important because it explains more than half of the data variation and is related to economic and target traits or yield performance. Also, in terms of communalities, all of the other remained traits except plant number per area (PNA), elongation of internode (EI) and dry/fresh ratio (DFR), indicated relatively high reliability, underscoring their genetic consistency. These high communalities suggest that the five identified factors effectively described the variability of traits, accounting for more than 85 % to nearly 100 % of the variability, and they provided a comprehensive description of trait variabilities.

The dendrogram was generated to explore the structure among traits of sainfoin (Fig. 2), the cutoff point is confirmed via the multivariate analysis of variance; the Wilks' lambda was significant and determined the position of the cutoff point in the dendrogram. The averages of the sainfoin traits in identified clusters are presented in Tab. 4 which explains the features of the identified clusters. dry/fresh Group-1 consisted of 10 genotypes which indicated the highest forage yield performance as well as the high amounts for all of the other remained traits except four traits including elongation of internode (EI), main stem's leaves (MSL), leaflets number of the leaf (LNL), plants number per area (PNA) and dry/fresh ratio (DFR). Thus, these genotypes have the same properties and show more superiority in most traits especially forage yield. Group-2 contains 9 genotypes with the lowest performance for all traits except PNA, EI, MSL, LNL and DFR, so these traits were tolerant to powdery mildew (PM) while they can be supposed as the complementary of Group-1 (Tab. 4). Also, the 13 genotypes of the Group-3 had moderate performance for measured traits of sainfoin, but they were suspectable to powdery mildew (PM). The research permits a better grasp of the sainfoin genotypes via agronomically morphologic traits and demonstrates the beneficial aspect of statistical tools like cluster analysis was useful in the identification of the most variable traits within

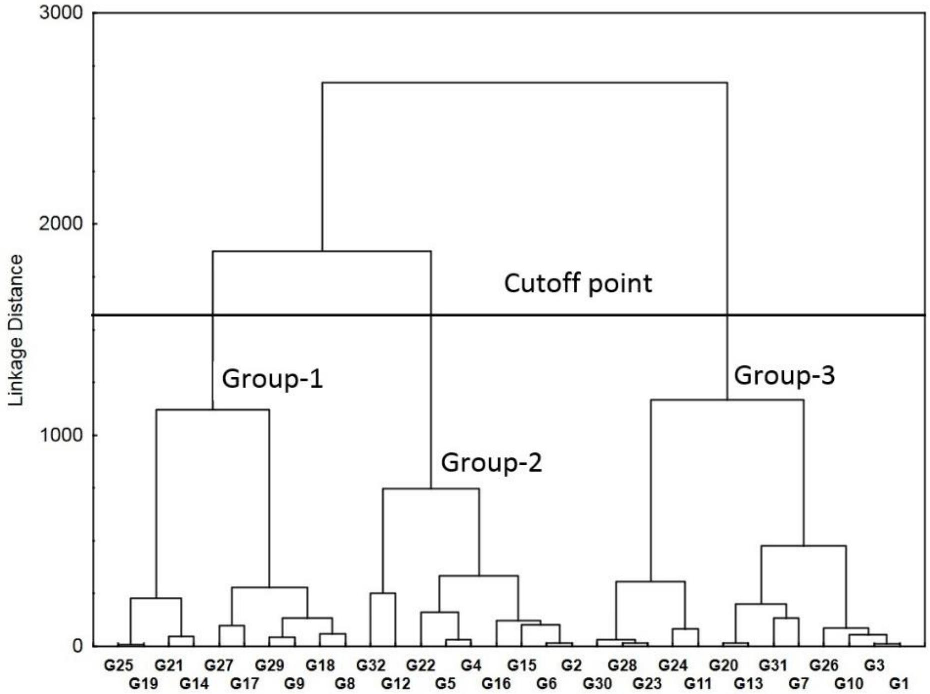

Fig. 2 Dendrogram of clustering for 32 genotypes of sainfoin (*Onobrychis viciifolia* Scop.), and the three identified cluster were determined based on Wilks' lambda statistics.

Tab. 4 Means of traits for three identified groups of sainfoin genotypes according to cluster analysis.

Traits†	Group-1	Group-2	Group-3
PNA	23.11	28.88	13.86
SNP	6.69	6.05	4.94
MSN	7.12	6.36	5.45
LLS	56.63	47.70	33.25
LP	18.64	16.63	13.21
LI	8.35	7.12	5.72
MSI	6.11	5.36	4.45
EI	9.88	11.26	7.73
MSL	6.85	6.53	5.21
SL	30.90	23.88	15.12
LNL	14.19	13.54	12.17
PM	0.71	0.54	0.75
FW	1.88	1.32	0.80
SFW	0.63	0.42	0.26
LFW	1.16	0.84	0.50
IFW	0.09	0.06	0.04
DW	1.24	0.89	0.52
SDW	0.37	0.29	0.18
LDW	0.81	0.56	0.33
IDW	0.07	0.04	0.02
LSR	2.12	2.02	1.85
DFR	0.64	0.67	0.65

†Traits are: plants number per area (PNA), stems number per plant (SNP), main stem's nodes (MSN), length of the longest stem (LLS), length of peduncle (LP), length of inflorescence (LI), main stem's internodes (MSI), elongation of internode (EI), main stem's leaves (MSL), stem's leaves (SL), leaflets number of leaf (LNL), powdery mildew (PM), fresh weight (FW), stem fresh weight (SFW), leaf fresh weight (LFW), inflorescence fresh weight (IFW), dry weight (DW), stem dry weight (SDW), leaf dry weight (LDW), inflorescence dry weight (IDW), leaf/stem ratio (LSR), and dry/fresh ratio (DFR).

Tab. 5 Mean values of the most important traits related to yield performance in the most favourable genotypes of Group-1.

Code	Yield characteristics	Mean
SNP	Stems number per plant	6.69
FW	Fresh weight	1.88
SFW	Stem fresh weight	0.63
LFW	Leaf fresh weight	1.16
DW	Dry weight	1.24
SDW	Stem dry weight	0.37
LDW	Leaf dry weight	0.81
LSR	Leaf/stem ratio	2.12
DFR	Dry/fresh ratio	0.64

sainfoin and can be useful in the future to succeed in genetic improvement projects. It was found that, each genotype has a similar magnitude of genetic variation which may be found and the distances among genotypes may be restricted. The association among traits supports the idea that only a few heritable traits are needed to explain the genetic variation within the sainfoin germplasm. These traits may engage plant breeders for effective germplasm management and evaluation. The ten desirable genotypes of Group-1; G8 from Tabriz; G9 from Heris; G14 from Azna; G17 from Khalkhal; G18 from Garjan; G19 from Kahlaran; G21 from Sanandaj; G25 from Saqqez; G27 from Zanjan; and G29 from Faridan, are good candidates for commercial release in Iran. They performed very well and showed high amounts of most target traits, so they can be advised for cultivation in cool upland rainfed conditions of semi-arid environments.

Discussion

Morphological traits have been used to explain variability in various genotypes, though research on sainfoin germplasm remains limited. Previous studies have highlighted significant variability in traits among sainfoin accessions from various geographical areas (Çaçan et al. 2023; Zavoshti et al. 2023), and this research corroborates this by showing substantial variation among the 32 common sainfoin genotypes assessed. We evaluated a total of twenty-two traits, underscoring the potential for improving this species through targeted programs. In selecting desirable traits in sainfoin, it is crucial to understand the available variation in yield components. Previous studies have reported positive associations between the number of stems with forage yield (Bhattarai et al. 2018). These bits of knowledge propose that whereas characteristics just like the number of stems are critical for scrounge abdicate, their effect may be more complex, interceded through other characteristics. Noteworthy affiliations between scrounge surrender and the number of stems, the number of branches, and the number of flowers were detailed by Hasanzadeh-Gorttapeh et al. (2014). Veisipoor et al. (2012) reported the stems number and dry yield as key traits affecting forage performance of sainfoin. Conversely, Najafipoor et al. (2017) highlighted the number of stems and seeds as critical traits for seed yield performance. These differences highlight the complexity of yield formation in common sainfoin and underscore the importance of considering multiple traits and their interactions when evaluating and selecting for improved forage yield.

The first five factors explained 85 % of the variance as yield factor, and similarly, Dadkhah et al. (2011) studied 21 local genotypes of sainfoin via factor analysis and found that the first factor explained 33 % of variability, dry forage yield, fresh forage yield, percentage of stems and number of stems per unit area have a positive and large factor loadings, which was named as forage yield. Also, Basafa & Taherian (2009) investigated 21 alfalfa ecotypes in cold regions and found that the percentage of plant components, including the percentage of stems and leaves, have the highest factor loading in a factor. The grouping of traits based on relationships is useful for identifying the main components of performance and examining diversity, but the physiological interpretation of the factors is useful. However, such interpretation depends on the studied traits, environmental conditions and their interaction (Tanner & Dowd 2019). Finally, Basafa & Taherian (2009) reported relatively similar findings in alfalfa, identifying six factors such as forage yield, forage quality, phenology, etc., which collectively explained 80 % of the observed variation.

This investigation demonstrated the utility of factor analysis as an effective multivariate statistical tool for exploring the pattern of genetic diversity in morphological characteristics of sainfoin and provided a comprehensive understanding of both the measured traits and genotypes, which is crucial for the effective utilization of sainfoin germplasm. The suitability of factor analysis for this sainfoin dataset was confirmed by the KMO measure and Bartlett's test. Five factors were identified, named as follows: (i) yield performance, (ii) plant morphology, (iii) powdery mildew, (iv) forage quality and (v) forage palatability. These factors offer important aspects into the grasp pattern of genetic diversity in sainfoin and facilitate targeted breeding efforts to enhance desired traits. Factor analysis serves as a valuable tool for condensing numerous variables into a limited set of underlying factors, categorizing traits according to their interrelations, pinpointing the primary components of performance, and assessing genetic diversity (Sabaghnia 2010). However, the outcomes are contingent upon the specific traits and genotypes being studied, as well as the environmental conditions present. Consequently, the findings derived from this research apply only to the conditions under which the study was conducted and similar environments. It was observed that forage yield performance was grouped with yield components as the primary factor. Similarly, Majidi & Arzani (2009) employed factor analysis in the evaluation of ten local ecotypes of sainfoin, identifying the characteristics of node number, plant height and sub-branches number in the stem had a large and positive factor loading in the second factor, and as a result, it can be considered as a plant morphology factor, so morphologic properties of sainfoin are very important and had large variation in various genotypes.

The dry weight ranged from 0.17 to 2.2 kg m^{-2} and fresh weight ranged from 0.26 to 3.04 kg m^{-2} while Basafa & Taherian (2009) reported 0.28 to 0.42 kg m^{-2} for the dry yield and 1.1 to 1.7 kg m^{-2} for the fresh yield. Finally, stem number per plant (SNP), main stem's nodes (MSN), length of the longest stem (LLS), main stem's internodes (MSI) and stem's leaves (SL), inflorescence were identified variables associated with forage yield performance as well as directly influencing traits like fresh weight (FW), stem fresh weight (SFW), leaf fresh weight (LFW), inflorescence fresh weight (IFW), dry weight (DW), stem dry weight (SDW), and leaf dry weight (LDW). These traits are crucial for selection in breeding programs and focusing on these traits can significantly enhance common sainfoin forage yield, particularly under semi-arid conditions. Regarding communalities, the main stem's nodes and the main stem's internodes demonstrated good reliability, underscoring their genetic consistency. However, most of the traits indicated relatively high magnitudes of communalities and reflected high reliability, showing their genetic consistency, so their contributions in total variation are high and regarding this issue, they can be used for the determination of selection indices in breeding programs. Results of factor analysis showed that a major part of community variance was contributed by most of the traits especially inflorescence fresh weight, inflorescence dry weight, length of the peduncle, powdery mildew main stem's nodes and main stem's internodes, which were the effective traits in identified factors 1-6. These characters had major effects on classifying sainfoin genotypes. According to cluster analysis, 32 sainfoin genotypes were classified into three groups based on their agronomic and morphologic similarities. The 10 genotypes in Group-1 were from northwest Iran and were suitable for forage yield and its components. The 13 genotypes in Group-2 were from the west and northwest and were realized as suitable for improving powdery mildew tolerance because their mean value for disease was lower than the means of other genotypic groups. Group-3 was distinguished from other groups with 9 genotypes from the west and centre of Iran, which indicated moderate values for measured traits. Evaluation of the amount of genetic variation is the key first step in the variability assessment of various genotypes and for attaining this breeding target, multivariate methods were required to indicate the outputs more intelligibly (Silberstein et al. 2021; Poudel et al. 2023). Cluster analysis indicated that genotypes that were collected from various regions, especially ecotypes in the centre, west and northwest have agronomic and morphological dissimilarities and similarities which may be the result of transferring from their place of origin to other cold and warm regions. Clustering results show a variation among genotypes, associated with the selection pressures in the breeding programs (Ayalew et al. 2020). Clustering recognizes genotypes by gathering them based on their similitude in terms of characteristics which makes a difference in deciding the degree of hereditary differences among the genotypes. Also, remarkable genetic variation may exist within sainfoin genotypes, so hybridization is possible for obtaining new sources for breeding (Smýkal et al. 2015; Sutcu et al. 2022). This report emphasizes the wealthy morphological differing qualities displayed in current sainfoin genotypes, proposing sufficient openings for hereditary change activities. These experiences hold significance for future breeding endeavours pointed at upgrading particular characteristics through key crossbreeding of parent plants.

Conclusions

The factor analysis indicated that the first five factors, explained 85 % of the variance as yield performance, plant morphology, powdery mildew, forage quality and forage palatability. Cluster analysis categorized 32 sainfoin genotypes into Group-1 (high forage yield and its components), Group-2 (tolerant to powdery mildew), and Group-3 (moderate values for most traits). For future breeding efforts, using stems, its nodes and internodes as well as stem length and leaves can be useful considering, fresh and dry weight of stem and leaves, and result in achieving high forage yield performance.

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