

Genetic variability in Iranian sainfoin (*Onobrychis viciifolia* Scop.) landraces by morphological characteristics

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Abstract

The sainfoin (*Onobrychis viciifolia* Scop.) is an ancient fodder crop traditionally cultivated in Mediterranean climates, yet its cultivation has dwindled in favor of other forage options across many regions. We investigated the diversity among 32 sainfoin landraces sourced from various parts of Iran, analyzing 18 distinct traits over two seasons (2022 and 2023) using a randomized complete block scheme. Notable variations were observed across the majority of measured characteristics, whereas the coefficient of variation ranged from 10.5–6.8% (for days to seed filling initiation) to as high as 63.2% and 70.8% (for inflorescence dry weight) in the first and second years, respectively. Principal Component Analysis (PCA) highlighted significant variability, with the first four components explaining 83% of the variance in 2022 and 78% in 2023. When plotting PCA1 against PCA2 for both years, distinct groups emerged, revealing three discernible groups of sainfoin landraces. Notably, certain landraces consistently grouped together across both years, including G1 (Bonab), G3 (Marand), and G11 (Urmia) from the northwest, G23 (Khomeyn), G24 (Arak), and G30 (Khansar) from the central region, and G26 (Asadabad) from the western, alongside G28 (Damavand) from the north. These landraces, originating from diverse geographical locations, were identified as particularly promising due to their distinct traits. Our findings underscore the rich morphological diversity present in current sainfoin landraces, suggesting ample opportunities for genetic improvement initiatives. These insights hold relevance for future breeding efforts aimed at enhancing specific traits through strategic crossbreeding of parent plants.

Introduction

Common sainfoin stands out as a perennial valuable forage plant with promising production ability option. Notably, it exhibits a remarkable resilience to environmental stresses such as low temperatures, making it suitable for cultivation even in challenging soil conditions (Radovic et al., 2019). Despite being less renowned in comparison to popular fodder plants, common sainfoin has garnered increased attention worldwide in recent years. This resurgence in interest can be attributed to its unique characteristics, including its non-bloating nature in grazing animals and its ability to produce high-quality forage (Bhattarai et al., 2018). It's worth noting that while common sainfoin offers several advantages, its regrowth rate is relatively slow. Therefore, it's essential to allow ample time for root reserves to replenish, ensuring the crop's durability. Moreover, the blossoms of common sainfoin yield copious amounts of nectar, making it highly noteworthy to pollinators like honey bees. This aspect further underscores its ecological value and contribution to supporting pollinator populations.

Sainfoin is widely distributed as landraces across the cold regions of the world. Despite alfalfa's dominance as the most prevalent legume forage, the ability of sainfoin to prevent frothy bloat, a potentially fatal metabolic disorder, has sparked new attention in the sainfoin (Craine et al. 2023). Incorporating sainfoin into cattle feeding programs helps mitigate flatulence, and decreasing losses of cattle herds. Moreover, its profits extend beyond production, making it a compelling choice for consideration. However, there are agronomic challenges hindering the widespread adoption of sainfoin in forage rotations. These challenges include issues with persistence and the higher seed cost regarding the

other forage options. Addressing these challenges through targeted breeding goals of sainfoin, particularly in cold regions is crucial for maximizing the next outcomes of farming of this crop (Sheppard et al. 2019). Currently, the cultivation of common sainfoin is not widespread, primarily due to its reliance on existence of local varieties whose long-term viability remains uncertain. To expand common sainfoin production, there is a pressing need to identify and characterize genetic resources, both to safeguard them and to explore their potential utilization (Carbonero et al., 2011).

As a cross-pollinated crop, it is indigenous to Asia's south-central regions as well as the east of Mediterranean areas and Asia's west regions, such as Iran, assumed as one of the important candidates for center of diversity in sainfoin. In the northwest and west regions of Iran, ancient common sainfoin genotypes are cultivated that exhibit robust ability to tolerate to environmental stresses, serving as valuable genetic reservoirs. Therefore, the collection and evaluation of these promising ecotypes hold significant promise for future breeding programs. By systematically gathering and assessing these ecotypes, valuable insights can be gained to inform and enhance common sainfoin breeding initiatives, ensuring its resilience and productivity in diverse agricultural landscapes (Zarrabian et al. 2013). In future common sainfoin breeding research efforts, determining dry weight yield components will yield significant benefits. While correlation coefficients play a crucial role in plant breeding programs by quantifying the genetic and non-genetic associations between traits and enabling indirect selection, relying solely on this information may not result in ideal findings due to the failure to distinguish multivariate aspect of yield performance. Instead, selection indices are utilized to prioritize traits based on their effects rather than individual correlation coefficients (Sharifi and Ebadi, 2018).

This underscores the importance of considering traits' effects in breeding programs to enhance the efficiency and effectiveness of trait selection in common sainfoin. Baghainiya et al. (2012) further highlighted the significant positively association for the dry biomass with number of stems, plants and nodes in common sainfoin. Leveraging such insights can greatly benefit future plant breeding programs by facilitating the release of new varieties with a broader genetically base. The objective of the investigation was to assess the interrelationships among morphologic characteristics of local landraces of common sainfoin, with the aim of identifying key traits for selecting forage yield performance. These findings can then inform future breeding projects, guiding the selection of traits that contribute most significantly to overall yield performance in common sainfoin.

Material and methods

The 32 local landraces of sainfoin (*Onobrychis viciifolia* Scop.) were sourced from various geographical regions of Iran and planted in the experimental fields of the University of Maragheh in 2021. The trial followed a randomized complete block scheme with four replication. Maragheh experiences a cool sub-humid climate with loamy sand soil classified as Regosols. Hence, tillage was conducted in autumn, with 30 kg ha⁻¹ nitrogen and 50 kg ha⁻¹ phosphorus pentoxide used. Field plots consisted of four rows, each two meters long, spaced 25 cm apart of rows with 20 cm distance within rows. Initial irrigation was performed at sowing, followed by subsequent irrigations at weekly intervals, with manual weed control as

necessary. Days to initiation of flowering (DIF) and days to initiation of seed filling (DSF) were recorded, with March 20th considered as the reference day. After crop establishment in 2021, measurements were taken during the 2022 and 2023 growing seasons, focusing on characteristics observed from the average of the second harvest. About fifteen random samples were selected from the center rows of each plot for measurement. These included peduncle length (PL), number of nodes per main stem (NMS), length of inflorescence (LI), number of leaves per stem (NLS), powdery mildew percentage (PMP), stem dry weight (SDW), leaf dry weight (LDW), inflorescence dry weight (IDW), dry leaf to dry stem ratio (LSR), number of florets per inflorescence (NFI), number of seeds per inflorescence (NS) and number of stems per plant (NSP). Ripened inflorescences were collected for seed yield (SY) and thousand seed weight (TSW) measurements. The total fresh yield (TFY) of the harvested two mid-rows was weighed, followed by drying in the shade for three days to record the total dry yield (TDY).

The normal shape of dataset distribution was rigorously assessed via the Ryan-Joiner method to ensure the appropriateness of subsequent multivariate analyses. Following this step, principal component analysis (PCA) was chosen as the method to delve into the common variability among the various sainfoin landraces. The PCA, a powerful statistical technique, works by extracting underlying patterns and relationships among variables. In this case, it was applied to the coefficients derived from the simple correlation table, providing insights into the intricate structure of variability present in the measured characteristics of sainfoin. By doing so, it simplifies the complexity of the dataset, making it more manageable and interpretable. To visually represent the outcomes of PCA, a plot of PCA1 versus PCA2 was meticulously crafted. This plot offers an intuitive depiction of the associations among the various characteristics measured, as well as the grouping patterns observed among the diverse sainfoin landraces. Through this visual representation, researchers can gain valuable insights into the underlying structure of variability present in the dataset. The entire analytical process, from data assessment to PCA and subsequent visualization, was meticulously conducted using industry-standard statistical software packages, including STATISTICA 14.0 (TIBCO, 2020) and MINITAB 21.3.1 (2022). These robust tools provided the necessary functionalities to perform comprehensive multivariate analyses and derive meaningful interpretations from the data.

Results

Table 2 presents descriptive indices for various characteristics of sainfoin, with the coefficient of variation (CV) showcasing notable variability. In the year 2022, CV ranged from 10.5% to 10.9% for traits such as days to seed filling (DSF) and dry leaf to dry stem ratio (LSR), while it reached higher levels of 63.2%, 50.0%, and 51.1% for traits like inflorescence dry weight (IDW), total fresh and dry yield (TFY and TDY), respectively. Similarly, in 2023, CV ranged from 7.9% to 6.8% for days to initiation of flowering (DIF) and DSF, while it peaked at 70.8%, 51.5%, and 50.2% for IDW, TFY, and TDY, respectively. Notably, environmental conditions in the second year exhibited less variability compared to the first year, resulting in relatively lower CV values in the second year across all characteristics. The most variable traits, as highlighted in Table 2, included number of nodes per main stem (NMS), powdery mildew percentage (PMP), dry weight of stems and leaves (SDW and LDW), number of florets per inflorescence (NFI),

thousand seed weight (TSW), and seed yield (SY). The wide range of variations observed in these characteristics indicates a high level of diversity among the sainfoin landraces. Moreover, the greater variability observed in the year 2023 compared to the year 2022 underscores the rich diversity present in the available sainfoin germplasm, suggesting its potential utility in future breeding programs. Considering the general heritability of sainfoin characteristics across the two years, relatively high heritability was observed for most traits, particularly in the first year (Table 2). The high levels of general heritability suggest that the influence of the environment on the estimation of this genetic parameter is minimal. This finding aligns with the findings of Majidi (2011), who reported a low effect of the environment on the estimation of genetic parameters in sainfoin under salt stress conditions.

The first four principal component analyses (PCAs) with eigenvalues greater than unity explained 83% of the variation in the year 2022, while the first four factors accounted for 78% of the variation in the year 2023 (Table 3). Additionally, the first two PCAs explained 70% of the variation in 2022 and 65% in 2023. To visually represent the relationships among the characteristics, they were shown in a plot of PCA1 versus PCA2. Since the first two PCAs captured a significant magnitude of the variability, the association between any two characteristics can be guessed by the cosine of their angles. From the plot for the first year (Fig. 1), several prominent associations were observed: a strong positive association between days to imitation of flowering (DIF) and days to imitation of seed filling (DSF); between peduncle length (PL) and number of stems per plant (NSP); between stem dry weight (SDW), LI, and NLS; and among number of nodes per main stem (NMS), inflorescence dry weight (IDW), number of florets per inflorescence (NFI), total dry yield (TDY), and total fresh yield (TFY). Conversely, there was a near-zero correlation between dry leaf to dry stem ratio (LSR) with SDW, LI, and NLS; between seed yield (SY) with DIF and DSF; and between powdery mildew percentage (PMP) with PL and NSP. Furthermore, a negative correlation was observed between LSR and PMP in the first year. It's worth noting that some disagreements between the graphic findings and numerical dataset were seen because the PCA described for less than 100% of the variability. Similarly, in the year 2023 (Fig. 2), notable associations were observed: a positively relation among DIF, DSF, and NSP; between NLS and SDW; between number of seeds (NS) and SY; and among TDY, PL, leaf dry weight (LDW), IDW, TFY, NMS, NFI, and LI. Conversely, there was a no relation between thousand seed weight (TSW) and LSR; between DIF, DSF, and NSP with TSW; and between TDY, PL, LDW, IDW, TFY, NMS, NFI, and LI with PMP. Additionally, a negative correlation was observed between LSR and DIF, DSF, and NSP in the year 2023. Furthermore, Veisipoor et al. (2012) identified stem number and dry biomass as the important characteristics influencing yield performance of sainfoin, and Najafipoor and Majidi (2017) reported stem and seed numbers as the important characteristics influencing seed yield of sainfoin.

To gain a deeper understanding of the relationships among the characteristics, they were graphically displayed as a plot of PCA1 versus PCA2. In the plot for the first year (Fig. 3), three distinct groups were identified: group A consisting of genotypes G14, G19, and G25; group B comprising fifteen genotypes; and group C comprising fourteen genotypes. In the plot for the second year (Fig. 4), similar groups were observed with some changes: group A now included genotypes G14, G16, G19, and G25; group B containing of nineteen genotypes; and group C containing of of nine genotypes. Comparison of genotype

grouping across the two years revealed that thirteen genotypes from group B, as well as eight genotypes from group C (G1, G3, G11, G23, G24, G26, G28, and G30), exhibited similar responses across both years, indicating minimal genotype by environment interaction. Notably, three genotypes from the same landraces in both years; G1 (Bonab), G3 (Marand), and G11 (Urmia); were from the northwest of Iran, characterized by semi-arid and cold climates. Similarly, three genotypes; G23 (Khomeyn), G24 (Arak), and G30 (Khansar); were from central Iran, known for its arid and warm climates. Additionally, one genotype from the same landraces in both years, G26 (Asadabad), hailed from western Iran with a Mediterranean climate featuring hot summers, while the other genotype, G28 (Damavand), originated from northern Iran with a humid continental climate characterized by warm summers. These findings underscore the acceptable genetic variability present in current sainfoin landraces, highlighting the diverse origins of the most favorable genotypes.

Table 4 displays the averages of the characteristics for each of the three groups across both years. Genotypes in group A exhibited lower values for most characteristics across both years, making them promising candidates for genetic improvement of early maturity in sainfoin, attributed to their lower amounts of days to imitation of flowering (DIF) and days to imitation of seed filling (DSF). Additionally, these genotypes demonstrated low sensitivity to powdery mildew (PMP), which is a favorable trait in target regions. In contrast, genotypes in group B displayed moderate values for most characteristics across both years. This suggests that they could be further investigated for other traits not measured in this research, potentially contributing to future improvement programs for sainfoin (Table 4). Accessions in group B show significant potential for various traits, particularly in terms of economic characteristics such as total fresh and dry yield (TFY and TDY), seed yield (SY), and thousand seed weight (TSW). Therefore, these genotypes merit further exploration and consideration in breeding programs aimed at enhancing the overall performance and productivity of sainfoin.

Discussion

Morphological and molecular markers have been extensively employed to elucidate the diversity of various crop landraces, although such investigations have been lacking for sainfoin landraces. Studies across different crops have consistently revealed a high diversity of characteristics among landraces, influenced by their origin and geographic properties (Casañas et al. 2017; Royo et al. 2022). In our examination of sainfoin landraces, utilizing eighteen characteristics, we observed significant variation across both experimental years. Notably, traits such as dry weight of stems, leaves and inflorescences (SDW, LDW and IDW), along with total fresh and dry yield (TFY and TDY), exhibited considerable diversity among the genetic materials assessed. This diversity presents opportunities for utilizing these landraces in breeding programs to identify the most favorable genotypes. Furthermore, we observed noteworthy diversity in tolerance/resistance to powdery mildew within our sainfoin collection, suggesting its potential as a candidate for breeding tolerant/resistant cultivars against this disease. Currently, sainfoin production faces challenges due to susceptibility to powdery mildew, as well as its inferior performance compared to alfalfa and its limited competitive potential during early establishment. Therefore, breeding programs should prioritize genetic improvement of yield performance and tolerance/resistance to powdery mildew.

Traditionally, sainfoin breeding efforts have focused on enhancing yield, winter survival, compatibility with other species, and disease resistance. However, recent research has highlighted its potential for human consumption as a perennial pulse crop, owing to its high crude protein content (approximately 40%) and acceptable levels of fat and starch in seeds. Moreover, studies have shown that alfalfa, sainfoin's main competitor, exhibits auto and allelopathic effects that hinder seed germination and productivity of companion crops such as sainfoin. Therefore, genetic improvement programs are targeting both dry matter yield and compatibility simultaneously.

Given that the first year after crop establishment is influenced by environmental effects to a greater extent, more emphasis should be placed on estimates from the second year. The high general heritability values observed in the second year for traits such as number of leaves per stem (NLS), powdery mildew percent (PMP), inflorescence dry weight (IDW), TFY, TDY, floret and seed numbers (NFI and NS), seed yield (SY), and thousand seed weight (TSW) underscore the significant role of genetic effects in controlling these traits. As these traits are also components of yield performance, indirect selection through them can enhance the efficiency of selection to improve overall performance. In a study by Zarabiyan et al. (2016), high heritability of dry yield performance in spruce was reported, suggesting the potential efficacy of selection methods in improving some characteristics such as stem and nodes numbers. Similarly, our findings support the importance of genetic factors in influencing sainfoin traits and suggest avenues for improving sainfoin performance through targeted breeding efforts.

We observed a high positive correlation between the node and leaf numbers (NMS and NLS) with total fresh and dry yield (TFY and TDY) in both years, consistent with findings by Hasanzadeh-Gorttapeh et al. (2014). Similarly, Zavoshti et al. (2023) reported a strong association between forage yield and traits such as leaflet number, peduncle length, and the ratio of dry to fresh weight. Such strong positive correlations suggest the presence of gene linkage with multiple effects, enabling more accurate indirect selection across all traits. Seed yield exhibited positively relation with the seed number per inflorescence and thousand seed weight, while showing negative and significant correlations with powdery mildew and no correlation with days to flowering and seed filling. This is consistent with findings by Najafipoor and Majidi (2017), who reported similar associations between seed yield and related traits. Furthermore, our correlation analysis highlighted the importance of NMS and NLS as key components of forage yield, along with the dry weight of stems, leaves, and inflorescence (SDW, LDW, and IDW). Additionally, traits such as peduncle length (PL) and length of inflorescence (LI) exhibited positive correlations with forage yield, suggesting their potential as indirect selection criteria to enhance crop performance.

Overall, our correlation analysis suggests that employing selection indices may be more advantageous for breeding efforts aimed at improving forage and seed yield as secondary preferences. Carbonero et al. (2011) similarly reported positively relations between the inflorescence per stem and traits such as leaf number, length of inflorescences, and stem number in sainfoin. This correlation between traits may be attributed to gene linkage on a single chromosome or pleiotropy, where a single gene simultaneously controls multiple traits. These findings underscore the importance of considering trait correlations in breeding programs to effectively improve overall sainfoin performance. Genetic diversity is crucial for

designing effective breeding programs, facilitating crossbreeding between individuals, and developing new sainfoin varieties. Genotypes with significant genetic distance can be particularly valuable for breeding, leading to the creation of high-performance cultivars and providing suitable populations for mapping purposes. Our principal component analysis (PCA) results consistently categorized sainfoin genotypes into three distinct groups across both years. The first group comprised genotypes exhibiting early flowering and maturity traits, the second group included those with moderate performance across most characteristics, while the third group comprised genotypes demonstrating high performance in both forage and seed yield.

Similar to findings by Zarabiyan et al. (2016), PCA proved effective in grouping sainfoin genotypes based on distinct characteristics. Our results suggest that the variation structure among sainfoin landraces is relatively consistent across seasons, indicating minimal environmental influence on genetic diversity patterns. Interestingly, sainfoin landraces did not segregate strictly based on geographic origin, implying either mixing of landraces or shared characteristics among diverse origins. This discrepancy between geographic and genetic diversity highlights the complexity of sainfoin's genetic makeup and its pollination behavior as an allogamous and self-incompatible species reliant on insect pollination. While geographic origin is informative, it may not fully capture genetic variation, as observed in our study and noted by Nosrati et al. (2012). Molecular marker analysis is warranted to delve deeper into genetic diversity assessment, facilitating better categorization of landraces and informing conservation efforts. Molecular markers can help discern whether seemingly similar landraces possess distinct genetic profiles, providing insights into their evolutionary history and aiding conservation strategies (Zhang et al. 2024).

In planning for the future breeding of sainfoin, several strategies can be employed to enhance its genetic diversity, improve desirable traits, and develop cultivars better suited to various environmental conditions and agricultural needs. Here are some key approaches for sainfoin breeding: (i) genetic diversity assessment by conducting comprehensive assessments of genetic diversity among sainfoin landraces using molecular markers, this will provide insights into the genetic structure, population dynamics, and relationships among different sainfoin genotypes (Bhattarai et al. 2018). (ii) germplasm collection and preservation, by continuing collecting and preserving diverse sainfoin germplasm from various geographic regions, especially from areas known for their unique environmental conditions or adaptive traits, and establishing and maintaining germplasm banks to conserve genetic resources for future breeding efforts (Karabulut et al. 2023). (iii) trait evaluation and selection, by identification and prioritizing key agronomic traits relevant to sainfoin productivity, quality, and resilience under different growing conditions. Traits may include yield components (e.g., leaf and stem dry weights), disease resistance (e.g., powdery mildew resistance), and environmental adaptation (e.g., drought tolerance) as advised by Irani et al. (2015). Also, rigorous phenotypic evaluations should be conducted and field trials to assess the performance of sainfoin accessions across diverse environments and management practices for selecting superior genotypes exhibiting desirable traits for further breeding programs. (iv) breeding for multiple objectives, by implementing breeding strategies that address multiple objectives, including enhancing forage yield and quality, improving disease and pest resistance, increasing environmental resilience, and promoting sustainable agricultural practices (Kölliker et al. 2017). Integrate traits related to

both forage and seed production to develop versatile cultivars suitable for diverse end-uses. By employing these breeding strategies, researchers and breeders can work towards developing improved sainfoin varieties with enhanced agronomic performance, resilience, and adaptability, ultimately contributing to the sustainable intensification of forage and seed production systems.

Conclusions

In summary, our study underscores the significant diversity among sainfoin genotypes in terms of seed and fodder characteristics, presenting opportunities for varietal improvement. Furthermore, the moderate correlation between seed and fodder performance suggests the possibility of simultaneous enhancement of these traits. Graphical plots generated through multivariate methods effectively distinguished sainfoin genotypes, aligning well with their observed characteristics. These findings provide valuable insights for future breeding endeavors aimed at enhancing sainfoin cultivars.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

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Author Contribution

All authors contributed to the study conception and design. Material preparation, data recording and analysis were performed by Naser Sabaghnia, Amin Ahadnezhad, Farid Normand Moayed, and Mohsen Janmohammdi. The first draft of the manuscript was written by Naser Sabaghnia and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

1. Baghainiya M, Majidi MM, Mirlohi A (2012) Evaluation of agro-morphological traits of sainfoin genotypes (*Onobrychis viciifolia*) with natural and induced genetic variability. Iran J Field Crop Sci 42(4):695–704.
2. Bhattarai S, Coulman B, Beattie AD, Biligetu B (2018) Assessment of sainfoin (*Onobrychis viciifolia* Scop.) germplasm for agro-morphological traits and nutritive value. Grass Forage Sci 73(4):958–966.
3. Carbonero CH, Mueller-Harvey I, Brown TA, Smith L (2011) Sainfoin (*Onobrychis viciifolia*): a beneficial forage legume. Plant Genet Resour 9(1):70–85.

4. Casañas F, Simó J, Casals J, Prohens J (2017) Toward an evolved concept of landrace. *Front Plant Sci* 8:145.
5. Craine EB, Şakiroğlu M, Peters, TE, Barriball S, Schlautman B (2023) Nutritional quality of *onobrychis viciifolia* (Scop.) seeds: a potentially novel perennial pulse crop for human use. *Legum Sci* e189.
6. Hasanzadeh-Gorttapeh A, Abbasi-Holasou H, Abbasi MR (2014) A study of agronomy, morphological and phenological traits of sainfoin (*Onobrychis viciifolia* Scop) using multivariate analysis. *Appl Crop Breed* 2(2):153–169.
7. Irani S, Majidi MM, Mirlohi A, Karami M, Zargar M (2015) Response to drought stress in sainfoin: Within and among ecotype variation. *Crop Sci* 55(5):1868–1880.
8. Karabulut E, Erkoç K, Acı M, Aydın M, Barriball S, Braley J, ... Şakiroğlu M (2023) Sainfoin (*Onobrychis* spp.) crop ontology: supporting germplasm characterization and international research collaborations. *Front Plant Sci* 14:1177406.
9. Kölliker R, Kempf K, Malisch CS, Lüscher A (2017) Promising options for improving performance and proanthocyanidins of the forage legume sainfoin (*Onobrychis viciifolia* Scop.). *Euphytica* 213:1–15.
10. Majidi MM (2011) Evaluation of genetic diversity in breeding genotypes of sainfoin under salt conditions. *Iran J Field Crop Sci* 41(4):645–653.
11. Minitab (2022) Minitab user's guide, version 21.3.1. Minitab Institute, Harrisburg, Pennsylvania, USA.
12. Najafipoor A, Majidi MM (2017) Genetic analysis of seed yield components and its association with forage production in wild and cultivated species of sainfoin. *J Crop Prod Process* 6(22):1–17
13. Nosrati H, Feizi MAH, Tarrah SS, Haghighi AR (2012) Population genetic variation in sainfoin (Fabaceae) revealed by RAPD markers. *Analele Univ din Oradea Fasc Biol* 1:11–16
14. Radovic J, Lugic Z, Sokolovic D, Babic S, Jevtic G, Andjelkovic S (2019) Phenotyping diversity within a collection of sainfoin (*Onobrychis sativa* L.) populations. Research Collection, 400. Improving sown grasslands through breeding and management Proceedings of the Joint 20th Symposium of the European Grassland Federation and the 33rd Meeting of the EUCARPIA Section Fodder Crops and Amenity Grasses, Zürich, Switzerland, 24–27 June.
15. Royo C, Soriano JM, Rufo R, Guzmán C (2022) Are the agronomic performance and grain quality characteristics of bread wheat Mediterranean landraces related to the climate prevalent in their area of origin?. *J Cereal Sci* 105:103478.
16. Sharifi P, Ebadi AA (2018) Relationships of rice yield and quality based on genotype by trait (GT) biplot. *An Acad Bras Cienc* 90:343–356.
17. Sheppard SC, Cattani DJ, Ominski KH, Biligetü B, Bittman S, McGeough EJ (2019) Sainfoin production in western Canada: A review of agronomic potential and environmental benefits. *Grass Forage Sci* 74(1):6–18.
18. TIBCO (2020) STATISTICA for Windows (data analysis software system) from StatSoft, version 14.0., Tulsa, OK, USA
19. Veisipoor A, Majidi MM, Mirlohi A (2012) Traits relationship in Sainfoin (*Onobrychis viciifolia*) under normal and water stress conditions. *Iran J Field Crop Sci* 42(4):745–756

20. Zarabiyani M, Majidi MM, Amini H (2016) Genetic diversity of Iranian and exotic sainfoin accessions (*Onobrychis viciifolia* Scop) based on morphological traits. J Crop Prod Process 6(21):1–14

21. Zarrabian M, Majidi MM, Ehtemam MH (2013) Genetic diversity in a worldwide collection of sainfoin using morphological, anatomical, and molecular markers. Crop Sci 53(6):2483-2496

22. Zavoshti SA, Sabaghnia N, Moayed FN (2023) Correlation and path coefficient analyses of dry weight yield components in the common sainfoin (*Onobrychis viciifolia* Scop.). J Agric Sci (Belgrade) 68(2):155–169

23. Zhang T, Lu L, Song Y, Yang M, Li J, Yuan J, ... Gu L (2024) Non-destructive identification of *Pseudostellaria heterophylla* from different geographical origins by Vis/NIR and SWIR hyperspectral imaging techniques. Front Plant Sci 14:134297

Tables

Table 1 The name and geographical properties of 32 Iranian sainfoin (*Onobrychis viciifolia* Scop.) genotypes

Code	Name	Altitude	Coordinates	Code	Name	Altitude	Coordinates
G1	Bonab	1290	37°20'N 46°03'E	G17	Khalkhal	2243	37°37'N 48°31'E
G2	Sarab	1650	37°56'N 47°32'E	G18	Garjan	1500	38°18'N 48°12'E
G3	Marand	1344	38°25'N 45°46'E	G19	Kahlaran	1500	38°18'N 48°12'E
G4	Zonuz	1700	38°35'N 45°49'E	G20	Meshginshahr	1400	38°23'N 47°40'E
G5	Varzaqan	1670	38°30'N 46°39'E	G21	Sanandaj	1450	35°18'N 46°59'E
G6	Ahar	1341	38°28'N 47°04'E	G22	Divandarreh	1850	35°54'N 47°01'E
G7	Azarshahr	1384	37°45'N 45°58'E	G23	Khomeyn	1830	33°38'N 50°04'E
G8	Tabriz	1348	38°04'N 46°18'E	G24	Arak	1743	34°05'N 49°41'E
G9	Heris	1900	38°14'N 47°06'E	G25	Saqquez	1476	36°14'N 46°15'E
G10	Miandoab	1314	36°58'N 46°06'E	G26	Asadabad	1607	34°46'N 48°07'E
G11	Urmia	1332	37°32'N 45°04'E	G27	Zanjan	1663	36°40'N 48°29'E
G12	Silvaneh	1587	37°25'N 44°51'E	G28	Damavand	2051	35°43'N 52°03'E
G13	Oshnavieh	1411	37°02'N 45°05'E	G29	Faridan	2390	32°59'N 50°24'E
G14	Azna	1871	33°27'N 49°27'E	G30	Khansar	2215	33°13'N 50°18'E
G15	Khorramabad	1147	33°29'N 48°21'E	G31	Fereydunshahr	2530	32°56'N 50°07'E
G16	Aligudarz	2022	33°24'N 49°41'E	G32	Kabutarabad	1545	32°29'N 51°49'E

Table 2 Some descriptive indices and general heritability ($h^2_{B,S}$) of the 18 characteristics of 32 Iranian sainfoin (*Onobrychis viciifolia* Scop.) genotypes

	Year-2022					Year-2023				
	Mean	Min	Max	CV	$h^2_{B,S}$	Mean	Min	Max	CV	$h^2_{B,S}$
NSP	5.59	4.01	7.16	12.2	94.5	6.35	4.18	9.71	17.2	62.6
NMS	6.23	3.70	9.51	22.7	98.1	7.30	3.49	15.15	33.4	86.3
PL	14.99	10.18	18.99	15.1	98.8	16.90	13.21	21.26	10.3	85.8
LI	6.78	4.72	8.73	14.0	97.0	7.82	5.66	9.69	10.4	80.4
NLS	19.03	10.50	32.90	26.4	98.4	22.58	12.10	35.90	22.7	89.8
PMP	0.618	0.100	0.910	43.1	98.8	0.670	0.100	1.000	42.1	97.6
SDW	0.250	0.106	0.402	28.5	95.6	0.300	0.163	0.466	21.9	72.1
LDW	0.501	0.222	0.895	30.5	97.2	0.597	0.359	0.954	18.4	81.2
IDW	0.037	0.005	0.101	63.2	97.5	0.047	0.005	0.145	70.8	91.7
LSR	1.92	1.45	2.63	10.9	83.6	2.22	1.27	3.22	14.7	56.5
TFY	11.21	2.76	28.19	50.0	98.5	12.98	3.40	32.86	51.5	98.3
TDY	7.33	1.83	17.90	51.1	98.6	8.49	2.29	20.21	50.2	98.0
NFI	39.78	20.96	64.31	27.4	88.2	45.35	30.73	66.99	16.6	90.0
NS	24.21	15.59	31.89	15.9	97.5	27.10	19.97	32.88	10.6	98.1
TSW	12.55	7.97	17.56	20.5	99.0	13.51	10.61	16.89	12.0	99.5
SY	0.181	0.091	0.288	28.4	99.2	0.215	0.131	0.330	23.6	98.7
DIF	41.44	29.00	51.00	13.1	99.3	49.08	37.00	57.00	7.9	88.2
DSF	48.23	36.00	57.00	10.5	99.0	54.23	44.00	64.00	6.8	74.0

Table 3 The magnitudes of eigenvalue and explained variance of for first five principal components (PCAs)

	PCA1	PCA2	PCA3	PCA4	PCA5
Year 2022					
Eigenvalue	10.30	2.27	1.34	1.12	0.89
Proportion	0.57	0.13	0.07	0.05	0.05
Cumulative	0.57	0.70	0.77	0.83	0.88
Year 2023					
Eigenvalue	8.73	2.78	1.34	1.19	0.82
Proportion	0.49	0.16	0.07	0.06	0.05
Cumulative	0.49	0.65	0.71	0.78	0.84

Table 4 The averages of the characteristics of sainfoin (*Onobrychis viciifolia* Scop.) for each of three detected sections of PCA

	Year-2022			Year-2023		
	A	B	C	A	B	C
NSP	5.15	5.82	6.54	5.57	6.68	6.59
NMS	5.00	6.96	8.32	5.03	7.64	10.80
PL	13.20	16.11	17.79	15.15	17.24	19.25
LI	6.03	7.23	8.11	7.08	7.99	8.68
NLS	15.07	20.69	29.28	18.56	23.05	29.38
PMP	0.697	0.535	0.680	0.660	0.649	0.795
SDW	0.192	0.277	0.373	0.244	0.306	0.390
LDW	0.386	0.543	0.830	0.510	0.603	0.760
IDW	0.021	0.041	0.083	0.019	0.047	0.110
LSR	1.93	1.88	2.10	2.33	2.19	2.12
TFY	6.73	12.66	24.86	7.07	13.10	25.70
TDY	4.40	8.24	16.50	4.36	8.64	17.10
NFI	31.36	45.13	52.36	38.59	46.45	55.34
NS	21.89	25.79	27.23	26.89	26.84	28.84
TSW	10.39	13.94	15.70	12.52	13.55	15.57
SY	0.142	0.211	0.213	0.200	0.219	0.230
DIF	38.11	44.05	43.92	44.86	50.72	50.75
DSF	44.93	50.80	50.75	50.58	55.78	55.13

Figures

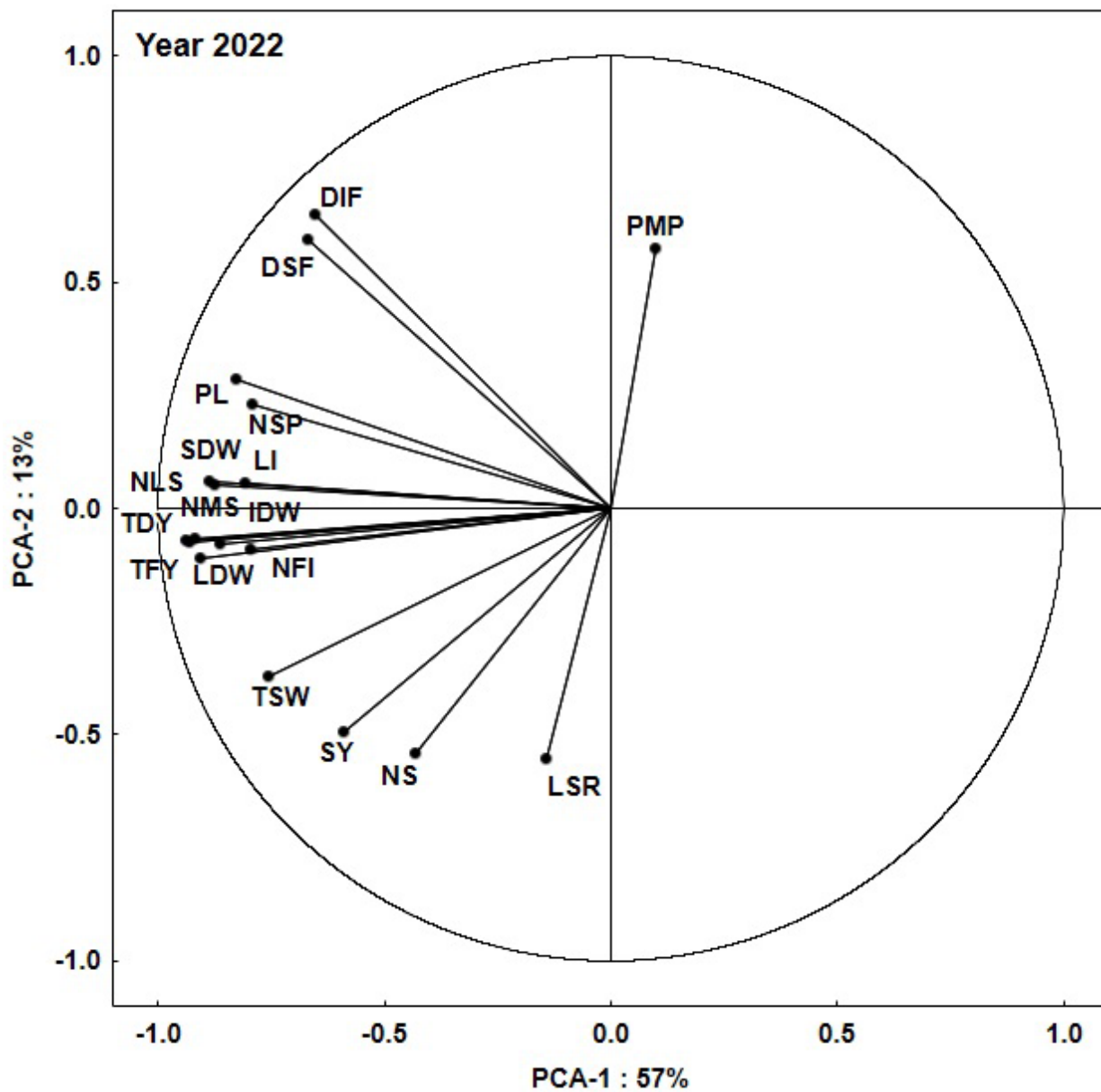


Figure 1

Plotting of PCA-1 versus PCA-2 for the 18 characteristics of sainfoin at the first year (2022). Refer to the text for abbreviation of characteristics

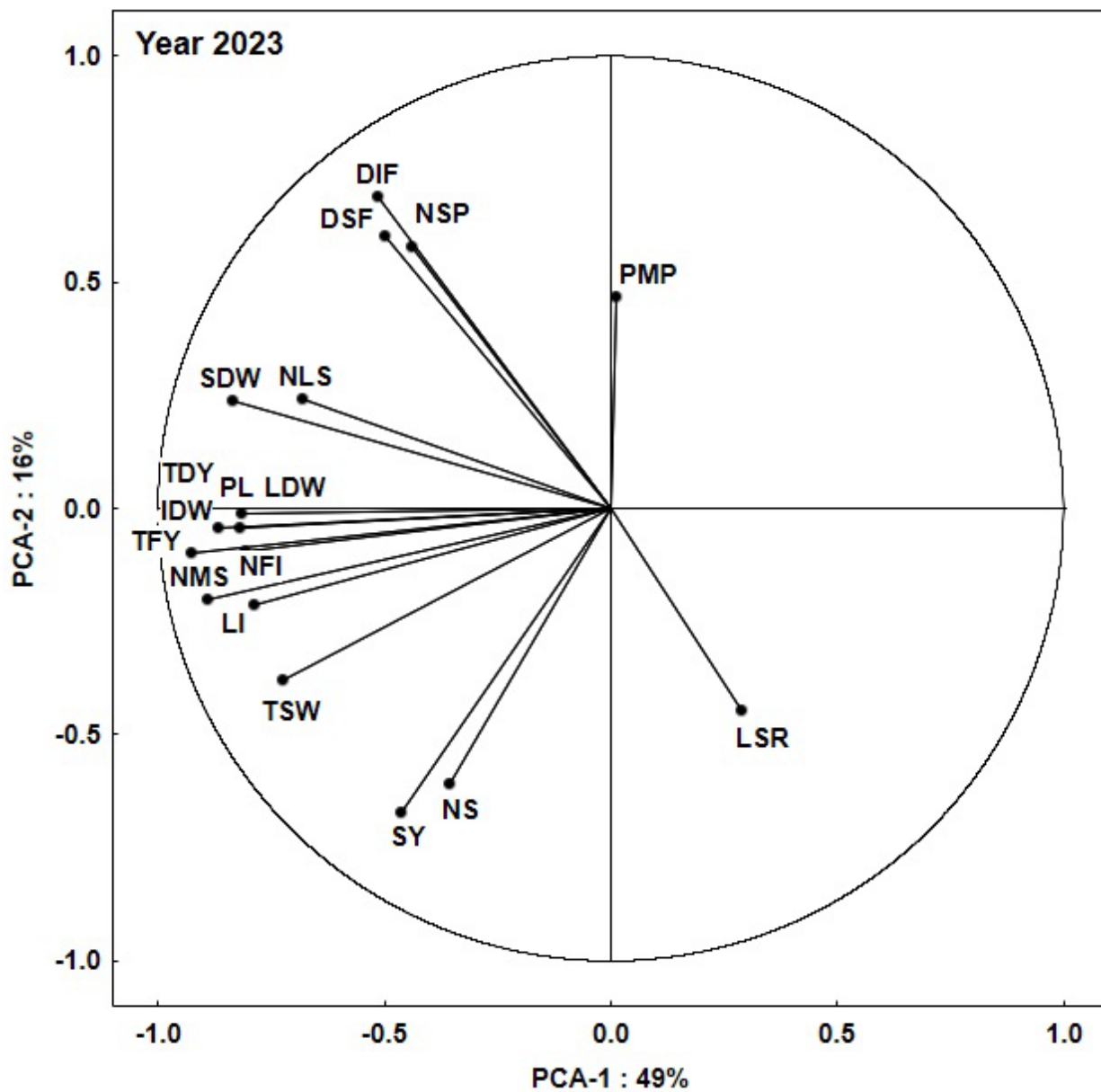


Figure 2

Plotting of PCA-1 versus PCA-2 for the 18 characteristics of sainfoin at the second year (2023). Refer to the text for abbreviation of characteristics

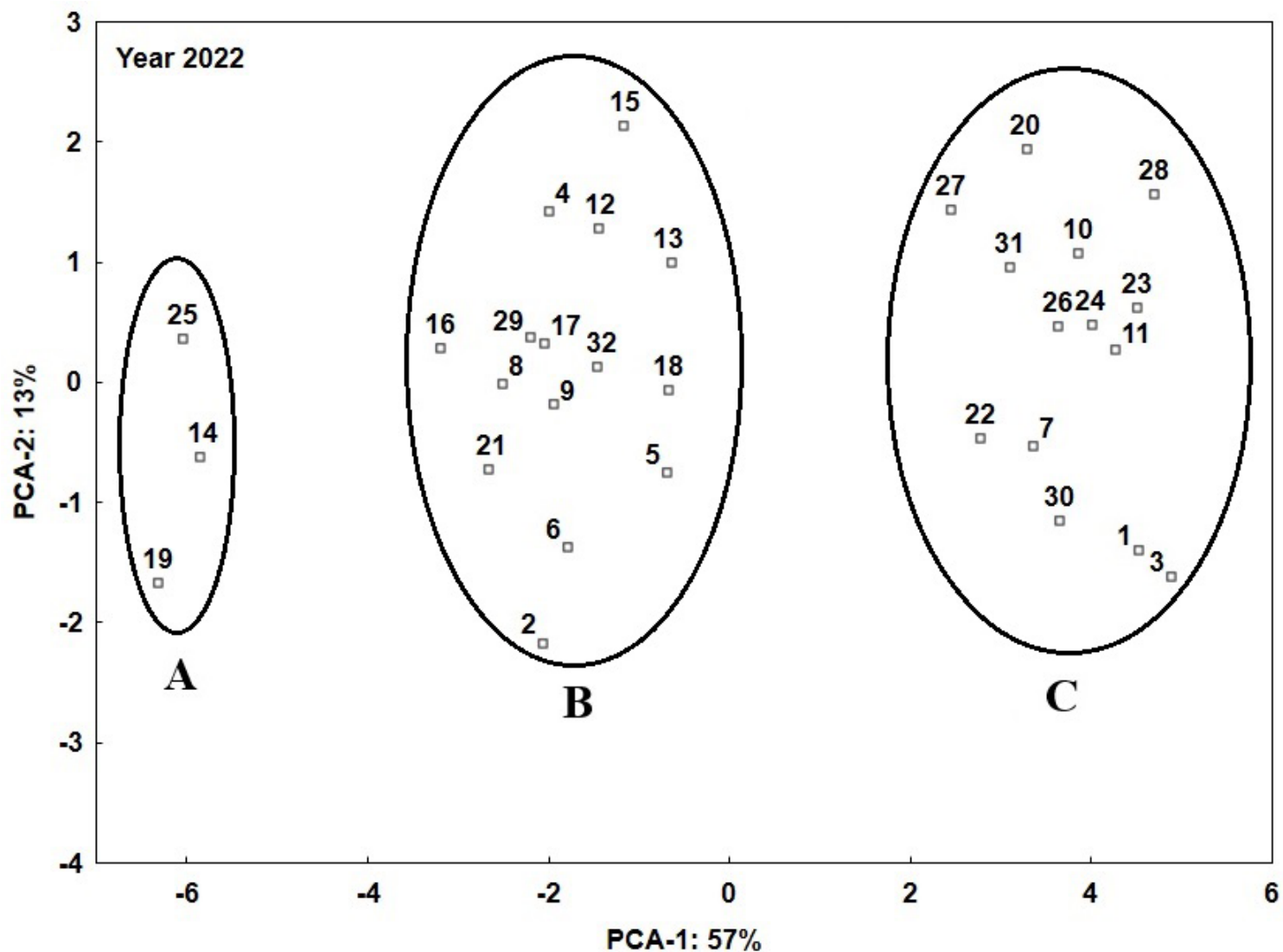


Figure 3

Plotting of PCA-1 versus PCA-2 for the grouping of 32 Iranian sainfoin (*Onobrychis viciifolia* Scop.) genotypes at the first year (2022)

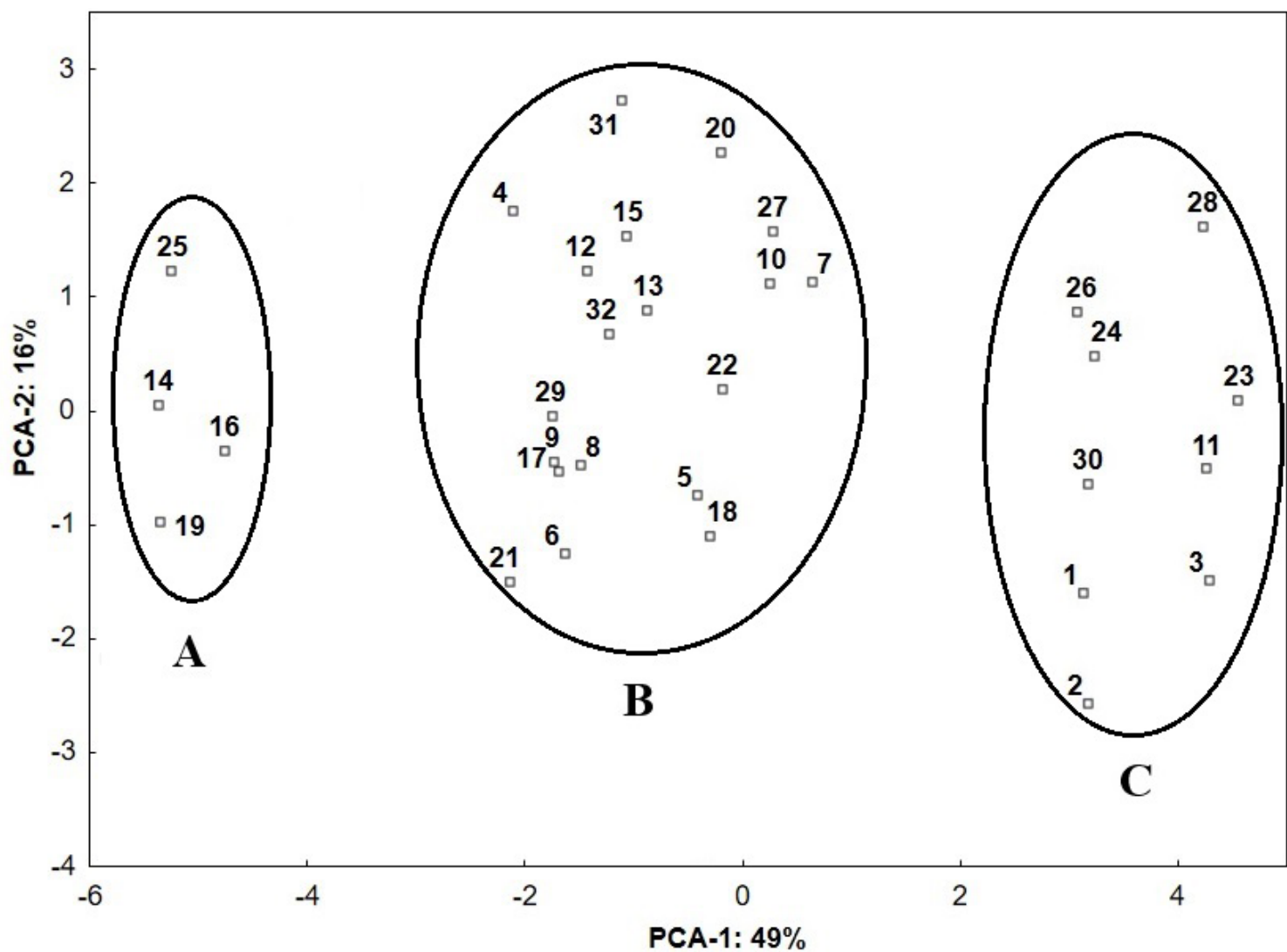


Figure 4

Plotting of PCA-1 versus PCA-2 for the grouping of 32 Iranian sainfoin (*Onobrychis viciifolia* Scop.) genotypes at the second year (2023)