




Assessment of genetic variation in sainfoin landraces based on agronomic characteristics using a genotype-by-trait biplot model

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ABSTRACT

A mini collection comprising 32 sainfoin genotypes was cultivated using a randomized complete block design with four replicates. Various parameters were recorded, including the number of plants per area (NPA), total dry yield (TDY), thousand seed weight (TSW), number of main stems (NMS), petiole length (PL), length of inflorescence (LI), number of leaflets per leaf (NLL), leaves per main stem (LMS), number of leaves per stem (NLS), stem dry weight (SDW), leaf dry weight (LDW), inflorescence dry weight (IDW), and number of florets per inflorescence (NFI). The first and second components of the biplot accounted for 88% of the variability in the dataset, with 70% attributed to the first component and 18% to the second. A pentagon was identified, featuring two distinct sections with genotypes 16 and 25, as well as genotype 14, serving as vertex entries. Notably, genotype 14 (Azna) excelled in three traits: NLL, LMS, and NLS. Additionally, vertex genotypes 16 and 25 demonstrated superior performance in other measured traits, including economically significant traits such as total dry yield. In accordance with the ideal entry, genotypes 13, 14, and 19, along with genotypes 16 and 25, exhibited greater favorability compared to other sainfoin genotypes regarding variability in the measured traits. Based on the ideal tester, total dry yield, number of leaves per stem, and number of florets per inflorescence were identified as key factors for assessing variation among genotypes. An examination of genotypes based on total dry yield indicated that genotypes 16 and 25, followed by genotypes 14 and 16, were the most desirable.

Key words: Forage yield, Genetic diversity, Ideal entry, Ideal tester.

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INTRODUCTION

Sainfoin (*Onobrychis viciifolia*) is recognized as a significant pasture and fodder species, with its distribution extending from the Mediterranean region to Central Asia. Notably, many species are predominantly located in northwest Asia, particularly in Iran and Türkiye (Poudel *et al.*, 2023). These areas are regarded as primary centers of origin and diversity for sainfoin. Due to its high protein content, sainfoin plays a critical role in animal nutrition, soil enrichment, and the enhancement of the nutritional value of drought-resistant pastures. As a crop, sainfoin exhibits remarkable resistance to both biotic and abiotic stresses, rendering it essential for fodder production in various pasture environments (Tan and Yolcu, 2021). One strategy to mitigate fodder shortages involves the development of high-yielding cultivars that are compatible with specific regional conditions. The plant's long, thick, robust, and straight root system contributes to its resilience and adaptability, enabling it to thrive in shallow and harsh soil conditions. Sainfoin is characterized as a high-yielding, drought-resistant, and palatable species, suitable for diverse environmental contexts. Globally, sainfoin is cultivated in multiple regions and naturally occurs in the steppe and semi-steppe pastures of Central and Southwest Asia. Its nutritional value exceeds that of alfalfa, it demonstrates superior adaptability to various soil types, and it incurs lower maintenance costs (Gruffat *et al.*, 2020). Furthermore, sainfoin is particularly advantageous for pasture systems as it does not induce flatulence in livestock when grazed directly, distinguishing it from other fodder crops. Consequently, the preservation of sainfoin's genetic resources and their appropriate utilization are crucial for the revitalization of pastures and the enhancement of fodder production.

The protection and recognition of valuable crop resources for the development and production of high-yield varieties represent a primary objective that motivates many breeders to investigate genetic resources. Genetic diversity within germplasm constitutes the foundation of breeding programs. Neglecting the agronomic, biological, and biochemical characteristics of germplasm restricts its utilization, resulting in the squander of valuable plant resources (Vaccino *et al.*, 2024). Improvement programs necessitate genetic diversity, which can be evaluated through morphological characteristics or genetic markers. Germplasm with well-documented genetic diversity serves as an appropriate starting material for hybridization programs. By leveraging information on genetic diversity, breeders can select superior

genotypes to serve as parental lines in hybridization, ultimately leading to the development of new cultivars. In the context of sainfoin breeding, key objectives include enhancing fodder yield, improving resistance to environmental stresses, and ensuring yield stability (Tulu *et al.*, 2023). The scarcity of fodder results in excessive livestock pressure on pastures, which can lead to their degradation. A fundamental approach to addressing fodder demand is the production of high-yielding and high-quality cultivars. Yield performance is a complex trait governed by numerous genes, rendering selection based on a single trait often ineffective. Therefore, comprehensive breeding strategies that consider multiple traits are essential for achieving successful outcomes in genetic improvement programs.

The identification of high-yielding plant stands requires an analysis of traits that demonstrate a significant correlation with yield performance. By selecting or discarding these traits, it is possible to accumulate desirable genes in enhanced cultivars. The examination of morphological traits is a prevalent method for exploring genetic diversity and evaluating a genotype's performance under specific environmental conditions (Sutcu *et al.*, 2022). Although morphological and agronomic traits are influenced by environmental factors and exhibit low heritability, they encompass essential economic characteristics and are extensively utilized to assess genetic diversity. Additionally, techniques such as molecular markers are regarded as complementary to this methodology.

In a study conducted by Delgado *et al.* (2008) involving 36 Spanish and 44 exotic sainfoin genotypes, significant genetic diversity was identified in several traits, including flowering percentage, autumn growth, stem length at the end of winter, and growth score following the first harvest. Similarly, Behrouz *et al.* (2010) reported the highest coefficient of variation for the harvest index and the lowest for the number of days until the conclusion of the flowering period while examining the genetic diversity of 36 sainfoin ecotypes, which were categorized into three distinct groups. Additionally, a study by Dadkhah *et al.* (2011) assessed twenty-one sainfoin genotypes and found a positive correlation between fodder yield and both stem percentage and the number of stems per unit area. These traits, along with plant height, contributed significantly to the observed diversity in fodder yield and may be recommended as selection indices in sainfoin breeding programs.

The improvement of forage crops has historically

received less attention compared to other crop categories, such as cereals and oilseed crops. The evaluation of genetic diversity based on specific traits can encompass morphological, physiological, or molecular assessments. This information is vital for the organization of germplasm, the selection of appropriate parental lines for breeding programs, and the management of segregating populations. It is imperative to differentiate between genetic and environmental contributions to diversity, as only the genetic component can be effectively utilized in breeding initiatives. Zarrabian *et al.* (2016) conducted an investigation into the genetic diversity of 56 agricultural sainfoin genotypes, employing morphological traits such as forage yield and its components. Their findings indicated a substantial level of diversity in the assessed traits within the germplasm, with high heritability observed for the number of stems per plant, suggesting significant genetic control over this trait. Given the high efficiency and low cost associated with morphological markers, the genetic diversity of certain native sainfoin genotypes was also explored. Consequently, the objective of this research is to identify genetic diversity within sainfoin based on morphological markers, to highlight traits exhibiting the highest genetic diversity, and to propose populations for crossbreeding that may demonstrate the greatest potential for heterosis.

MATERIALS AND METHODS

The current investigation involved assessing 32 native landraces of sainfoin (Table 1) in an experimental field located in Maragheh, Iran. This region is characterized by a cool, semi-arid upland climate and is predominantly composed of Regosols, a soil type typically found in areas with limited organic matter and well-defined horizons. To improve soil fertility, the experimental plots were fertilized with nitrogen and phosphorus pentoxide at application rates of

30 kg ha⁻¹ and 50 kg ha⁻¹, respectively, during the preparation phase. The experimental design employed a randomized complete block arrangement with four replicates to ensure the precision of the results and to yield reliable data. Each plot comprised four rows, each measuring 2 meters in length, with a spacing of 30 cm between rows and 20 cm between plants within rows. This configuration facilitated optimal plant growth and provided ease of access for measurements and harvesting. The central rows were designated for the harvesting and measurement of total dry yield (TDY), while the thousand seed weight (TSW) was determined from three random seed samples. Additionally, approximately ten samples were randomly selected from the mid-rows of the plots to record various parameters, including the number of main stems (NMS), petiole length (PL), length of inflorescence (LI), number of leaflets per leaf (NLL), leaves per main stem (LMS), number of leaves per stem (NLS), stem dry weight (SDW), leaf dry weight (LDW), inflorescences dry weight (IDW), and the number of florets per inflorescence (NFI).

A formula that incorporates both genotype and trait information within a biplot framework was employed to illustrate the pattern of genotype-by-trait (GT) interaction through a genotype-by-trait biplot derived from principal component analysis (PCA). The overarching methodology and formula for constructing such a biplot, as outlined by Yan (2024), are as follows:

$$(1) \quad \frac{X_{ij} - \mu_j}{S_j} = \sum_{n=1}^2 \alpha_n \beta_{in} \gamma_{jn} + E_{ij}$$

In the provided relationship X_{ij} represents the genotype i for character j , μ_j is the mean of genotypes for character j , S_j is the root square of variance in character j across genotypes, α_n is the singular value, β_{in} and γ_{jn} are PCA-values of genotypes and characters, E_{ij} is the error amount of the fitted modeling. For obtaining

Table 1. Geographic origins of the 32 sainfoin (*Onobrychis viciifolia* Scop.) genotypes collected from Iran.

Code	Origin	Code	Origin	Code	Origin	Code	Origin
G1	Bonab	G9	Heris	G17	Khalkhal	G25	Saqquez
G2	Sarab	G10	Miandoab	G18	Garjan	G26	Asadabad
G3	Marand	G11	Urmia	G19	Kahlaran	G27	Zanjan
G4	Zonuz	G12	Silvaneh	G20	Meshginshahr	G28	Damavand
G5	Varzaqan	G13	Oshnavieh	G21	Sanandaj	G29	Faridan
G6	Ahar	G14	Azna	G22	Divandarreh	G30	Khansar
G7	Azarshahr	G15	Khoramabad	G23	Khomeyn	G31	Fereydunshahr
G8	Tabriz	G16	Aligudarz	G24	Arak	G32	Kabutarabad

symmetrical amounts in the recorded data for genotypes and characters, the singular value α_n requires to be corrected through involving the related vectors (β_{in} and γ_{jn}). The mentioned adjustment helps in obtaining a balanced representation of genotypes and traits in the analysis; $\beta_{in}^* = \sqrt{\lambda_n} \xi_{in}$ and $\gamma_{jn}^* = \sqrt{\alpha_n} \gamma_{jn}$. The GT biplot figures were produced by the symmetrical values which were plotted versus each other. Thus, genotypes and traits are shown by a special sign, permitting for a visual presentation of the relations among genotypes as well as traits. This comprehensive approach allowed for a detailed exploration of the relationships between genotypes and traits and data were analyzed by GGEbiplot software.

RESULTS AND DISCUSSION

The first and second PCs, derived from the fitted model, collectively accounted for 88% of the variability in the dataset (Figure 1). Specifically, PC1 contributed 70%, while PC2 contributed 18% to the overall explained variation. The significant amount of GT interaction observed suggests the presence of

both additive and crossover interactions within our dataset, indicating differential rankings of measured traits across the genotypes of sainfoin. This finding is consistent with similar observations in other crops (Ebrahimi *et al.*, 2023; Sabaghnia *et al.*, 2024), underscoring the challenge of achieving an indirect response to selection across all genotypes without accounting for the interaction between genotypes and traits. Given the importance of GT interaction, this research employed a site regression model, in accordance with the recommendations of Yan (2024), as a suitable approach for biplot presentation. This method is effective in identifying GT interactions and can provide valuable insights, as illustrated in Figure 1, which demonstrates the genotypes that excelled in specific measured traits of sainfoin. All traits, with the exception of the number of leaflets per leaf (NLL), leaves per main stem (LMS), and number of leaves per stem (NLS), were categorized within the genotypes 16 and 25 (Aligudarz and Saqqez) as the best vertex entries. Consequently, these genotypes exhibited superior performance in the aforementioned traits, which are critical economic characteristics such

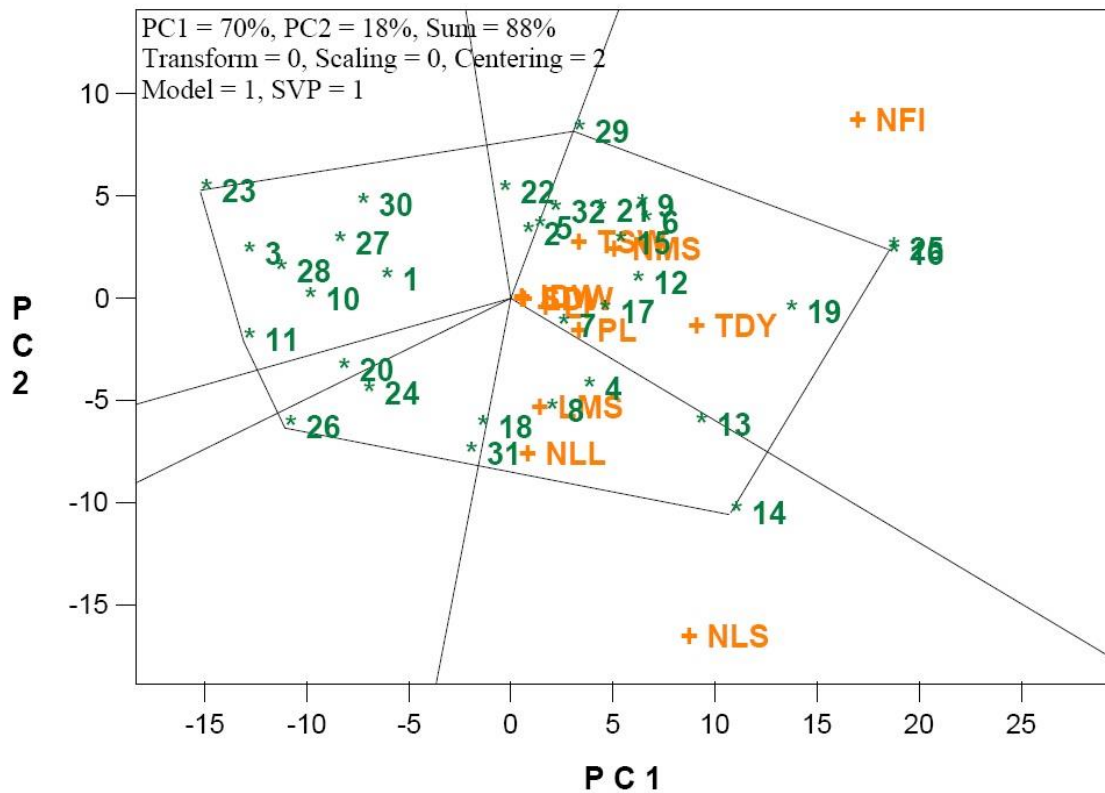


Figure 1. The polygon view of the biplot, illustrating the superior sainfoin genotype(s) for each trait. The traits under consideration include: TDY: Total dry yield, TSW: Thousand seed weight, NMS: Number of main stems, PL: Petiole length, LI: Length of inflorescence, NLL: Number of leaflets per leaf, LMS: Leaves per main stem, NLS: Number of leaves per stem, SDW: Stem dry weight, LDW: Leaf dry weight, IDW: Inflorescences dry weight, and NFI: Number of florets per inflorescence.

as forage yield. In contrast, other genotypes within this section (e.g., 7, 12, and 17) demonstrated lower performance for the traits mentioned compared to the vertex genotypes. In the section corresponding to genotype 14 (Azna), only three traits—NLL, LMS, and NLS—were identified, with this genotype exhibiting the highest values for these traits, outperforming other genotypes in this section, such as 4 and 8 (Figure 1). Finally, the remaining four sections, which included genotypes 11, 23, 26, and 29 as vertex entries, did not contain any measured traits of sainfoin, indicating that they were not optimal for any of the characteristics assessed. Therefore, to achieve optimal performance, the utilization of genotypes 16 and 25 is recommended for promoting high yield, while genotype 14 is suggested for enhancing the quality of sainfoin forage.

Figure 2 illustrates the relationships among the traits of sainfoin, with lines connecting each characteristic

to the origin of the plot. The length of the vectors serves as an estimate of the mean squares of error for the respective traits, while the cosine of the vectors reflects the strength of these associations.

As depicted in Figure 2, the traits of thousand seed weight (TSW), inflorescences dry weight (IDW), and number of main stems (NMS) demonstrate a strong positive correlation. This suggests that these traits provide similar insights regarding the variation among genotypes, a finding that is consistent with the research conducted by Abbasi-Holasou *et al.* (2019), who examined the associations among 34 populations of sainfoin to identify key traits influencing yield performance. Additionally, comparable positive associations were observed among the number of florets per inflorescence (NFI), number of plants per area (NPA), total dry yield (TDY), leaf dry weight (LDW), stem dry weight (SDW), length of inflorescence (LI),

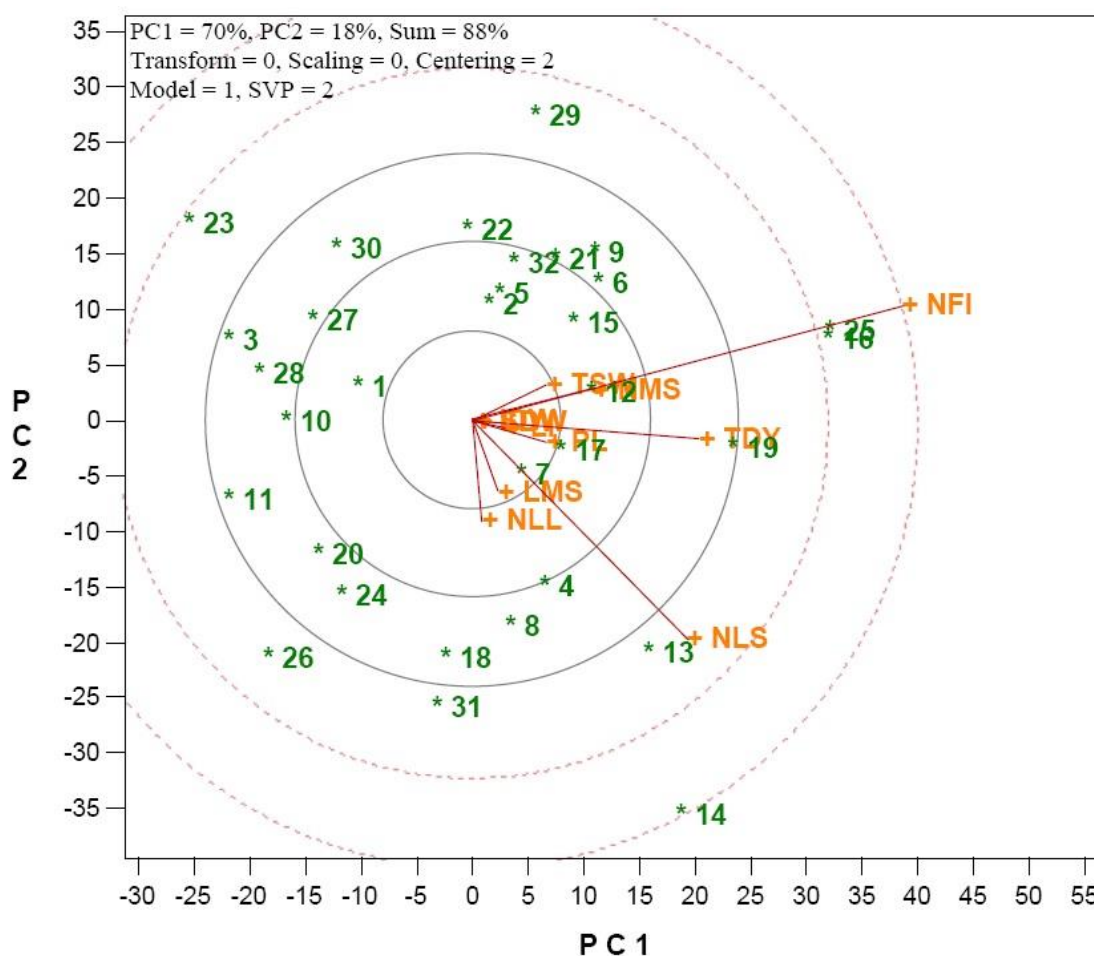


Figure 2. Vector-view indicating associations among thirteen traits of sainfoin. The traits examined include: TDY: Total dry yield, TSW: Thousand seed weight, NMS: Number of main stems, PL: Petiole length, LI: Length of inflorescence, NLL: Number of leaflets per leaf, LMS: Leaves per main stem, NLS: Number of leaves per stem, SDW: Stem dry weight, LDW: Leaf dry weight, IDW: Inflorescences dry weight, and NFI: Number of florets per inflorescence.

and petiole length (PL), as indicated by the acute angles of the vectors (Figure 2). Borreani *et al.* (2003) reported a strong relationship between forage yield and both leaf and stem weight in sainfoin, providing a morphological descriptor for this forage crop that can be applied in practical agricultural settings. Furthermore, the number of leaflets per leaf (NLL) and the number of leaves per main stem (LMS) exhibited a positive association, as evidenced by the relatively close angles of their vectors (Figure 2). An increase in one trait is likely to result in an increase in the other, thereby enhancing the quality of sainfoin. Notably, there appears to be a near-zero correlation between NLL and LMS with other measured traits, with the exception of NLS, as indicated by the relatively stable lines in Figure 2. This suggests that breeding for the number of leaflets per leaf and the number of leaves per main stem can be conducted independently. Most of the aforementioned conclusions can be substantiated by the correlation values presented in Table 2. However, it is important to note that certain discrepancies between the graphical model predictions and the numerical data were anticipated. This is due to the fact that the applied GT biplot model accounts for less than 100% of the variance detected in the dataset (74%), thus not capturing the entirety of the variability. This limitation has been acknowledged and corroborated in the application of the GT biplot model to other crops, such as safflower (Ebrahimi *et al.*, 2023), wheat (Elfanah *et al.*, 2023), and Kabuli chickpea (Sabaghnia *et al.*, 2024).

The arrow located at the center of the circles in Figure 3 indicates the position of an ideal entry, characterized

by the largest vector of genotypes exhibiting the highest performance and a minimal projection onto the other axis. Consequently, a genotype is deemed favorable if it is situated closer to the ideal entry location. In this context, genotype 14, followed by genotypes 13, 19, and 16, as well as genotype 25, demonstrated greater favorability compared to other sainfoin genotypes. Conversely, genotype 23, along with genotypes 3, 11, and 23, was identified as undesirable due to their significant distances from the ideal entry position (Figure 3). It is evident that genotypes 14 (Azna), 16 (Aligudarz), 19 (Kahlaran), and 25 (Saqqez), which exhibit the highest potential for forage yield and quality, should be considered as candidates for sainfoin production. These genotypes may also serve as reference points for the identification of various traits in future studies, as they demonstrate considerable variation among traits and possess high genetic potential concerning the measured characteristics of sainfoin. For the traits evaluated, an ideal tester should effectively differentiate genotypes based on their alignment with the ideal or perfect trait. The arrow in the center of the circles in Figure 4 represents the largest vector of traits with positive projections onto the average coordinate axis of traits. A trait is considered more favorable if it is positioned closer to the perfect trait. In this analysis, the number of florets per inflorescence (NFI), the number of leaves per stem (NLS), and total dry yield (TDY) were identified as favorable traits. In contrast, the remaining traits were classified as relatively acceptable for representativeness, as they were located above the average axis (Figure 4).

Table 2. Correlation coefficients among thirteen distinct traits of sainfoin genotypes.

	NPA†	NMS	PL	LI	LMS	NLL	NLS	SDW	LDW	IDW	TDY	NFI
NMS	0.52‡											
PL	0.35	0.66										
LI	0.41	0.68	0.81									
LMS	0.07	0.04	0.31	0.18								
NLL	-0.10	-0.19	0.09	0.07	0.70							
NLS	0.47	0.41	0.62	0.54	0.70	0.57						
SDW	0.70	0.62	0.56	0.54	0.19	-0.07	0.72					
LDW	0.53	0.63	0.62	0.66	0.04	0.00	0.60	0.78				
IDW	0.49	0.84	0.59	0.53	-0.08	-0.24	0.40	0.69	0.75			
TDY	0.57	0.84	0.74	0.71	0.11	-0.09	0.62	0.79	0.86	0.91		
NFI	0.45	0.79	0.58	0.55	0.12	-0.05	0.49	0.62	0.48	0.76	0.70	
TSW	0.52	0.85	0.46	0.48	0.00	-0.25	0.25	0.54	0.48	0.67	0.65	0.75

† Traits are: NPA: Number plants per area, TDY: Total dry yield, TSW: Thousand seed weight, NMS: Number of main stems, PL: Petiole length, LI: Length of inflorescence, NLL: Number of leaflets per leaf, LMS: Leaves per main stem, NLS: Number of leaves per stem, SDW: Stem dry weight, LDW: Leaf dry weight, IDW: Inflorescences dry weight, and NFI: Number of florets per inflorescence.

‡Critical amounts of associations $P < 0.05$ and $P < 0.01$ (degrees of freedom=30) are 0.35 and 0.45, respectively.

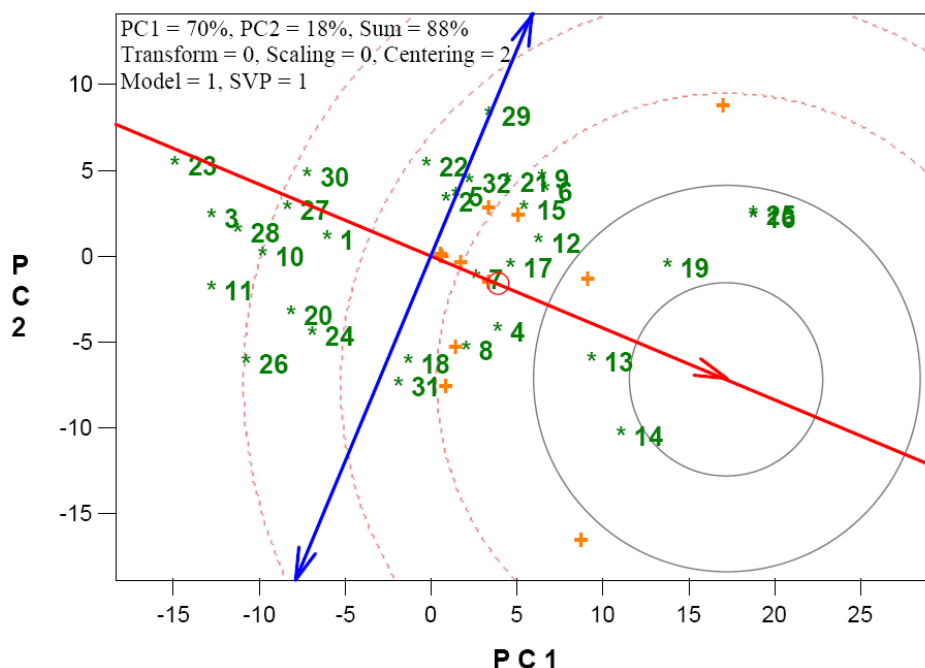


Figure 3. Ranking of entries (sainfoin genotypes) based on the performance of testers across twelve measured traits.

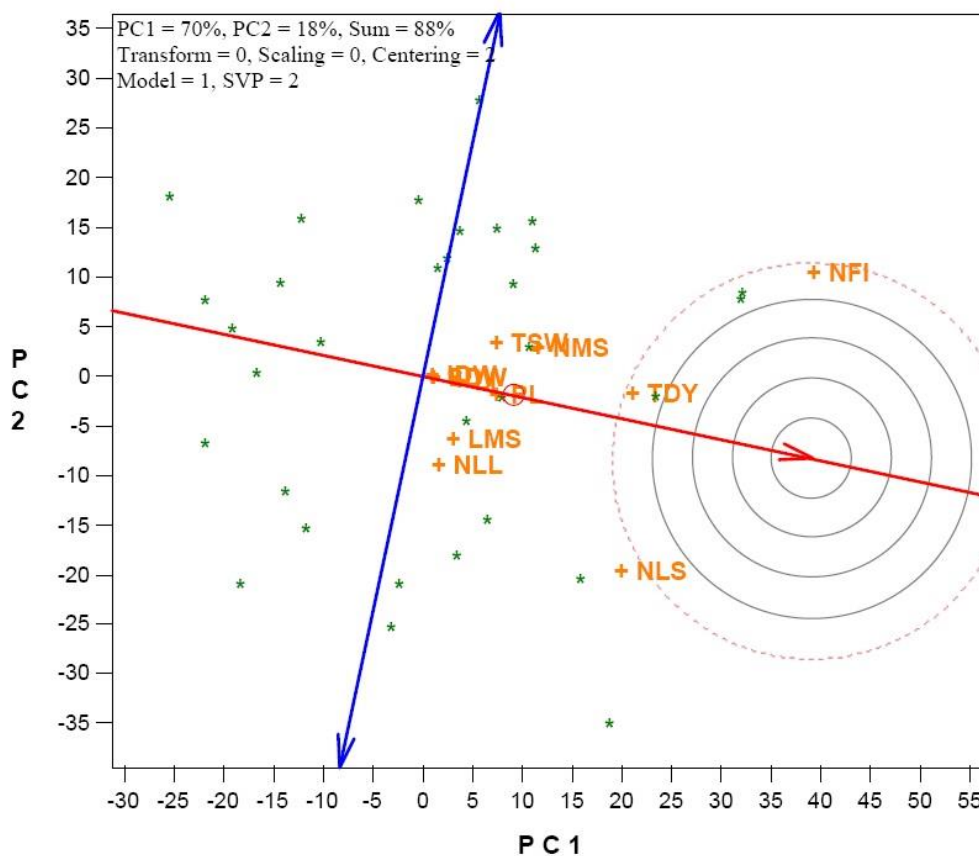


Figure 4. Ranking testers based on twelve measured traits of sainfoin, evaluated for their discriminative and representativeness properties.

The traits assessed include: TDY: Total dry yield, TSW: Thousand seed weight, NMS: Number of main stems, PL: Petiole length, LI: Length of inflorescence, NLL: Number of leaflets per leaf, LMS: Leaves per main stem, NLS: Number of leaves per stem, SDW: Stem dry weight, LDW: Leaf dry weight, IDW: Inflorescences dry weight, and NFI: Number of florets per inflorescence.

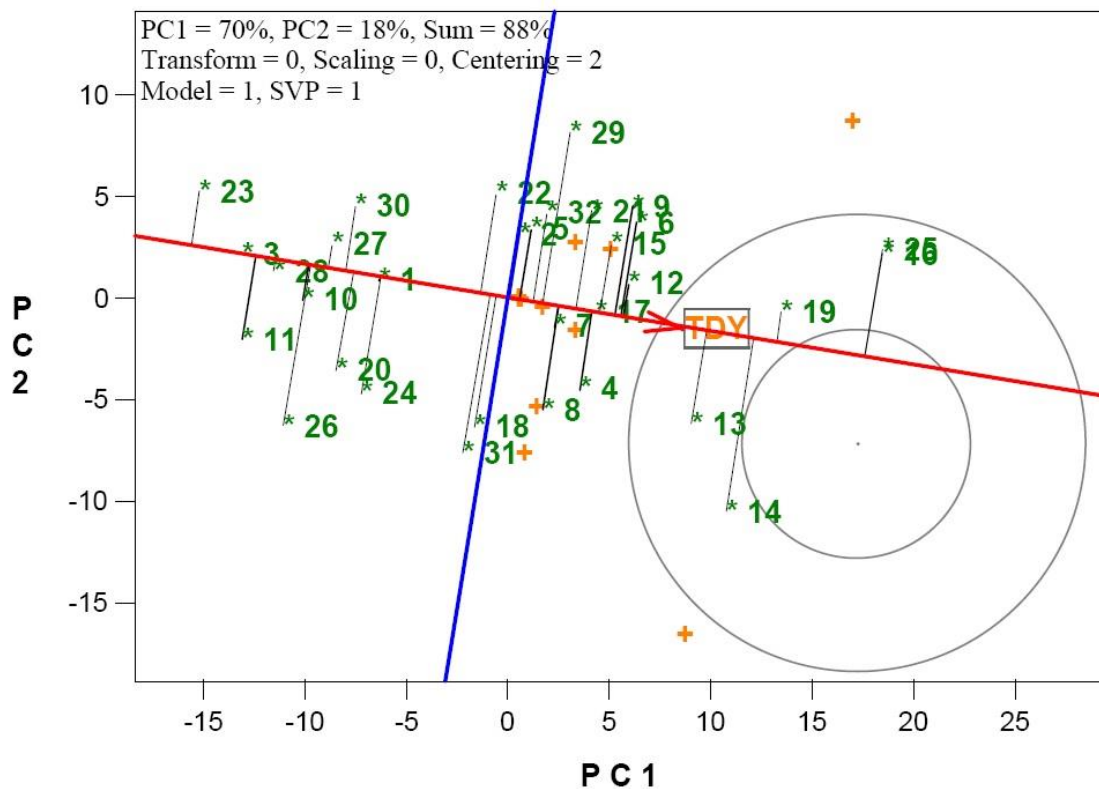


Figure 5. Assessment of total dry yield (TDY) production across 32 genotypes of sainfoin.

The performance of various genotypes concerning the yield attribute, specifically total dry yield (TDY) as a final economic trait, is illustrated in Figure 5. In this figure, a lateral line extends from the plot origin to the final trait, allowing for the ranking of genotypes. Genotypes 16 and 25, followed by genotypes 19, 14, and 13, emerged as the most desirable in terms of TDY. Conversely, genotype 23, along with genotypes 3 and 2311, were identified as the least desirable in relation to TDY (Figure 5). Furthermore, the distance of each genotype from the horizontal axis serves as a measure of variability; specifically, genotype 19 exhibits less variability in performance, whereas genotype 14 demonstrates greater variability. The lateral axis categorizes genotypes based on their performance relative to the mean TDY, with 19 genotypes performing above the mean and the remaining 13 genotypes performing below the mean (Figure 5).

Successful sainfoin genotypes must be developed using efficient selection tools to ensure optimal performance and economic viability. The graphical model employed in this study serves as a valuable method for the visual analysis of GT interactions, offering advantages over traditional models (Yan,

2021). These advantages include enhanced graphical representation, improved comprehension of genotype composition, increased interpretative capacity, simplified comparisons of genotypes and traits, categorization of genotypes based on specific traits, and the identification of clusters of similar genotypes or traits. In the context of genetic improvement projects for sainfoin aimed at increasing forage yield, it is essential to consider leaf and floret numbers. Additionally, the length of inflorescence and petiole should be incorporated into the definition of selection indices. Davazdahemami *et al.* (2019) also identified leaf number as a significant contributing trait to sainfoin yield. The pentagon-shaped GT biplot model was utilized to identify distinct heterotic sections, revealing three distinct sections. The genotypes within each section are suitable candidates for achieving high heterosis, which is critical for developing cultivars with superior forage performance and quality. Consequently, crossing genotypes 25 and 16 with genotype 14 may yield a segregating population that can be managed to produce the most favorable cultivars. Thus, the pentagon representation of the GT biplot proves to be an effective tool for identifying heterosis structures within sainfoin breeding programs.

CONCLUSION

The two identified sainfoin genotypes, specifically 16 from Aligudarz and Saqqez and 25 from Saqqez, demonstrate significant potential for broad recommendation to farmers based on their total dry yield traits. The current investigation highlights the efficacy of the genotype-by-trait biplot as an exceptional visual tool for dataset interpretation. Furthermore, the forage dry yield exhibited a positive correlation with several traits, including the number of florets per inflorescence, leaf dry weight, stem dry weight, length of inflorescence, and petiole length. Consequently, these traits should be utilized as selection indices in the breeding programs for sainfoin.

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