

GENETIC DIVERSITY AND MORPHOLOGICAL TRAIT ANALYSIS OF SUMMER SAVORY (*Satureja hortensis* L.) GENOTYPES USING GT BILOT MODELING

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ABSTRACT. Current investigation aimed to assess the genetic diversity among twenty-nine summer savory (*Satureja hortensis* L.) genotypes by analyzing fourteen morphological traits using genotype by trait biplot model. The trial was performed in a randomized block scheme with three replicates. The genotype by trait interaction biplot model accounted for 76% of the observed variability, revealing six distinct genotypic groups. Genotype 5 (Mashhad) exhibited the highest values for the plant fresh and dry weight, stem fresh and dry weight, root fresh and dry weight, and root length; genotype 16 (Tabriz-A) had high amounts of plant height, stem length, distance of internodes, leaf area of main stem's leaves, and leaf area of lateral branches' leaves. Additionally, genotype 3 (Ardabil) exhibited the highest values for the leaflets per main stem and essential oil while the other three vertex genotypes (2, 18 and 19) did not display superior performance in any of the measured traits. Positive correlation coefficients were observed among the plant height, stem length, and distance of internodes, similarly such positive relation was seen between plant fresh and dry weight with stem fresh and dry weight. Based on discriminative ability, dry weight of plant and stem, following to fresh weights, are identified as the most discriminative traits, offering the greatest potential for distinguishing among the genotypes. According to the position of perfect genotype, genotype 5 (Mashhad) followed by genotype 7 (Tonkabon), indicating their superior overall performance. Comparison of the summer savory performance in

terms of essential oil indicated that genotype 3 (Ardabil) following to genotypes 1 (Shush) and 18 (Qazvin), demonstrated the most desirable essential oil performance. Genotype 5 (Mashhad) following to genotype 7 (Tonkabon-B), produced the high of plant dry weight. The remarkable diversity identified could be instrumental in enhancing the breeding and conservation of summer savory germplasm.

KEYWORDS: genotype by trait biplot, perfect genotype, perfect trait, polygon.

INTRODUCTION

Iran boasts an extraordinary diversity of medicinal, many of which belong to the Lamiaceae family, renowned for their therapeutic and culinary applications (Dini et al. 2024). *Satureja* species are predominantly indigenous to the Mediterranean region; however, they can also be located in diverse environments worldwide, especially in arid, sun-drenched, stony, and rocky terrains. One of the most recognized species in this genus is *Satureja hortensis*, commonly known as summer savory. This annual, herbaceous, aromatic plant has been traditionally used as a spice in cuisine and as a medicinal plant (Bimbiraité-Surviliené et al. 2021). Research has demonstrated that summer savory exhibits a variety of bioactive properties, such as antitumor, antifungal, antioxidant, and antibacterial effects (Abou-Baker et al. 2020). However, the growing threat of environmental degradation and overharvesting poses significant risks to the survival of medicinal plant species, including summer savory. The losing of biodiversity in cultivated plants disturb ecosystems and lead to the disappear of germplasm, which has consequences for both traditional medicine and modern drug discovery (Parveen et al. 2020). Consequently, developing strategies for the conservation, sustainable use, and management of these species is critical. One essential step toward this goal is accurately identifying and characterizing plant accessions, ensuring that valuable genetic resources are preserved. In addition to conservation efforts, understanding the genetic diversity and relationships among different accessions is crucial for developing breeding programs (Wambugu & Henry, 2022). Genetic variation provides the raw material for selection in breeding, allowing the enhancement of traits such as yield performance,

resistance to diseases, and adaptability to environmental stressors. Knowledge of genetic distances between populations enables breeders to organize germplasm more effectively and facilitates the development of new cultivars with desirable traits (Sanchez et al. 2023).

To achieve above target, multivariate analysis serves as a powerful tool for exploring genetic variation by analyzing multiple morphological traits simultaneously. Techniques such as principal component analysis are commonly employed to reduce data complexity, grouping correlated traits together while distinguishing them from unrelated traits (Sabaghnia et al. 2024). These analyses provide insight into patterns of variation that can be used to understand the genetic structure of accessions. A perfect genotype has to have favorable amounts for the traits while they are not usually correlated so breeding in one trait result in decrease in one or several traits. A genotype by trait (GT) biplot model is generated to solve this challenge which is another application of G+GE biplot model, genotype plus interaction between environment and genotype (Yan & Frégeau-Reid 2018). In this model, the genotype by trait two-way pattern is displayed in a biplot of first two principal components. The mean of traits' coordinates is used for ranking of studied genotypes according to their general desirability across the measured traits, which provide a good index for evaluating genotypes. Despite these advanced analytical tools, it is important to recognize that conserving genetic diversity also hinges on effective management practices.

Geographic distribution, ecological adaptability, and the inherent genetic makeup of each population all play a role in the success of conservation and breeding strategies (Stronen et al. 2022). In the case of summer savory, the rich genetic diversity observed across Iranian accessions offers a promising avenue for breeding programs aimed at enhancing desirable traits such as increased biomass, improved essential oil composition, and enhanced resistance to pests or environmental stresses. Hadian et al. (2010) explored the morphological and chemical variability of some summer savory accessions from different regions of Iran and indicated that association between morphological traits with the essential oil composition, so variations in plant structure could influence the chemical properties of this plant. Ramezani et al. (2016) focused on the morphological variation of summer savory in relation to its medicinal properties and highlighted how phenotypic differences, particularly in leaf morphology, correlated with the plant's antioxidant and antimicrobial activities. Thus, the main objective of

this study is to investigate the genetic variation among some collected Iranian summer savory genotypes and classify them based on morphological traits through the use of the genotype by trait biplot model.

This research aims to contribute to the sustainable management and utilization of summer savory germplasm by identifying key traits and genetic relationships. The findings could be instrumental in breeding programs, where the selection of genetically diverse accessions can enhance the success of future cultivar development. Furthermore, this study adds to the growing body of knowledge on medicinal plants' conservation and genetic diversity, emphasizing the importance of safeguarding biodiversity for future generations.

MATERIALS AND METHODS

Trial

In this study, seeds from 29 different accessions of Iranian summer savory (*Satureja hortensis*) were meticulously collected from various regions of Iran, each characterized by unique geographical and environmental conditions (Table 1). This selection aimed to capture a wide range of genetic diversity within the species. The experiment was scheduled in a randomized block scheme, a widely routine methodology that helps control for variability by randomizing the placement of treatments within blocks. Each block consisted of three replications to ensure statistical reliability. The planting setup involved sowing seeds in four rows, with a spacing of 20 cm between each row and 20 cm between individual plants within each row. This spacing was chosen to facilitate proper air circulation and light exposure, which are critical for optimal growth and development. At the flowering stage, five plants from the central row of each plot were harvested individually to ensure that measurements were taken from plants that had uniform exposure to environmental conditions, reducing variability related to edge effects.

Traits

The morphological evaluation focused on fourteen specific traits, which encompassed various aspects of plant growth and structure. The evaluation process was systematic and involved several key traits from ten randomly selected samples, including: plant fresh weight (PFW), stem fresh

weight (SFW), root fresh weight (RFW), plant height (PH), stem length (SL), root length (RL), distance of internodes (DI), leaflets per main stem (LMS), plant dry weight (PDW), stem dry weight (SDW), root dry weight (RDW), essential oil (EO), leaf area of main stem's leaves (LAMS), and leaf area of lateral branches' leaves (LALB).

Table 1. Name and geographic properties of the collected summer savory genotypes

Code	Name	Province	Coordinates	Altitude
1	Shush	Khuzestan	32° 11'N 48° 15'E	84
2	Tabriz-A	East-Azarbaijan	38° 06'N 46° 16'E	1361
3	Ardabil	Ardabil	38° 15'N 48° 17'E	1339
4	Razan-A	Isfahan	35° 23'N 49° 02'E	1854
5	Mashhad	Razavi Khorasan	36° 15'N 59° 36'E	1022
6	Sistan	Sistan and Baluchistan	27° 35'N 60° 35'E	1530
7	Tonkabon-B	Mazandaran	36° 48'N 50° 52'E	-18
8	Shiraz	Fars	39° 35'N 52° 34'E	1519
9	Qom	Qom	34° 38'N 50° 52'E	965
10	Kermanshah	Kermanshah	34° 19'N 47° 04'E	1330
11	Sanandaj	Kurdistan	35° 19'N 46° 58'E	1577
12	Parsabad	Ardabil	39° 37'N 47° 54'E	61
13	Sardasht	West-Azarbaijan	36° 09'N 45° 28'E	1528
14	Tuserkan-B	Hamadan	34° 33'N 48° 27'E	1864
15	Hamedan	Hamadan	34° 48'N 48° 30'E	1841
16	Tabriz-B	East-Azarbaijan	38° 06'N 46° 16'E	1361
17	Isfahan	Isfahan	32° 39'N 51° 39'E	1601
18	Qazvin	Qazvin	36° 16'N 49° 59'E	1307
19	Tuserkan-A	Hamadan	34° 33'N 48° 27'E	1864
20	Ilam	Ilam	33° 38'N 46° 22'E	1364
21	Borujerd	Lorestan	34° 01'N 48° 44'E	1963
22	Kelachay	Gilan	37° 05'N 50° 21'E	-13
23	Arak	Markazi	34° 05'N 49° 42'E	1737
24	Dezful	Khuzestan	32° 23'N 48° 25'E	150
25	Razan-B	Isfahan	35° 23'N 49° 02'E	1854
26	Tehran	Tehran	35° 41'N 51° 23'E	1187
27	Tonkabon-A	Mazandaran	36° 48'N 50° 52'E	-18
28	Sarab	East-Azarbaijan	37° 56'N 47° 32'E	1682
29	Qeshm	Hormozgan	26° 48'N 55° 53'E	79

The weight-related traits were measured using an electronic balance with a precision of 0.1 g, providing accurate data on the biomass of the plants. The plant's overall height was measured from the base to the apex using a ruler, providing insight into the growth potential of each genotype.

The length of stem and root as well as distance of internodes were measured by a ruler and the number of leaflets per main stem were counted. The leaf area of leaves in the main stem and lateral branches were measured from samples of the first leaf, the middle and the terminal leaf positions.

Statistical analysis

Data normality was assessed by the Anderson-Darling normality test via MINITAB 14.0 (Minitab Inc., USA) software. Pearson's simple correlation coefficients were computed for pairwise measured traits. The principal component analysis model was used for the genotype by trait biplot analysis (Yan & Frégeau-Reid 2018). For the computation of symmetrical scales, where traits are considered testers and genotypes as entries, the eigenvalue is adjusted using the process of vector absorption. The following settings were applied to achieve the scaled and centered data required for analysis: Scaling = 1 was applied to scale the data, making the variables dimensionless and comparable. Centering = 2 was used to center the data by subtracting the mean, which ensures that each trait is analyzed relative to its average performance. Singular Value Decomposition (SVP) = 2 was employed to calculate the singular scores, which is essential for decomposing the data into principal components. Additionally, SVP = 1 was applied specifically to obtain singular scores suitable for visually interpreting correlations among genotypes using what is referred to as the perfect entry view. The graphs of biplots were generated through GGEbiplot, which provides a comprehensive graphical interpretation of the GT interactions.

RESULTS AND DISCUSSION

The GT interaction biplot model accounted for 76% of the variation observed in the dataset (Fig. 1). This relatively sufficient variation highlights high associations among the traits and prepares a robust visualization of their interrelationships. In this biplot, vectors extending from the origin to each trait indicate the amount and direction of trait interactions, allowing for a detailed interpretation of how traits correlate. The fitted model demonstrated both additive and non-additive interactions, indicating that genotype rankings for different traits can alter regarding which traits are being investigated.

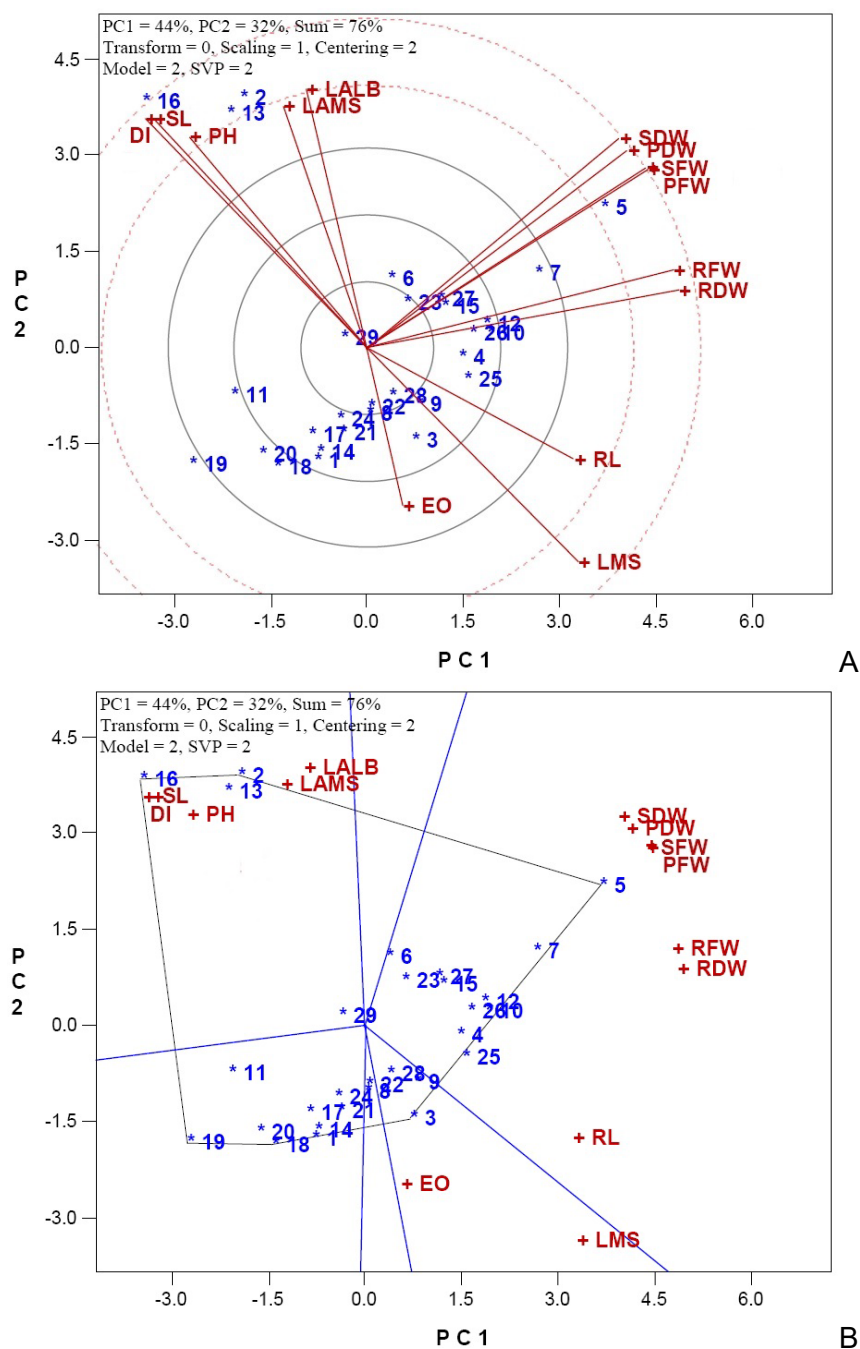


Figure 1. (A) vector-view of GT biplot for interrelationships among traits; (B) polygon-view of GT biplot for which genotype(s) won in which trait(s).

These results align with reports of Sabaghnia et al. (2015) on *Carthamus*

tinctorius and Ebrahimi et al. (2023) on *Spinacia oleracea* and emphasize the importance of entry-by-tester interaction explored during the selection of the most desirable individuals regarding various reactions to different traits. In the current investigation, the biplot model, as recommended by Yan & Frégeau-Reid (2018), effectively extracted the genotype-trait relationships, making it a valuable analytical instrument for the visualization and interpretation of complicated types of interactions in summer savory genotypes. The biplot model displayed extensive variability, as shown by the length of the trait vectors (Fig. 1A), indicating a wide range of influence for some traits. Several key associations were observed: positive correlations were found between the plant height (PH), stem length (SL), and distance of internodes (DI), suggesting that these traits are interdependent and tend to increase together. Jamshidi et al. (2009) researched the morphological variation and genetic diversity of summer savory using different morphological traits and multivariate analysis and found significant differences among the accessions regarding traits like plant height, biomass, and leaf size. Similarly, plant fresh weight (PFW), stem fresh weight (SFW), plant dry weight (PDW), and stem dry weight (SDW) were positively correlated, reflecting the close relationship between stem and plant growth. Root fresh weight (RFW) and root dry weight (RDW) were also positively linked, indicating that higher fresh weight often translates into higher dry weight in roots. Understanding the correlation of traits is essential for establishing logical relationships, which can simplify the identification of traits that may be challenging to measure directly. When certain traits exhibit significant correlations, it allows researchers to select proxy traits that are more convenient to assess, so this is particularly beneficial in scenarios where measuring a trait is resource-intensive, complex, or time-consuming (Werme et al. 2022). Consequently, by leveraging high correlations, one can infer the status of other related traits through more accessible measurements. There was a positive association between the leaf area of the main stem's leaves (LAMS) and the leaf area of lateral branches' leaves (LALB), suggesting that plants with more branches tend to produce more leaves (Fig. 1A).

Finally, relatively moderate positive correlations were found between the root length (RL), leaflets per main stem (LMS), and essential oil (EO). Near-zero correlations were seen between PFW, SFW, PDW, and SDW with traits like PH, SL, and DI, as well as LAMS and LALB, which was indicated by vectors that were nearly perpendicular to one another (Fig. 1A), showing

that these traits have little to no interaction. Such similar relations were observed for RL, LMS, and EO with traits RFW and RDW as well as PFW, SFW, PDW, and SDW. Additionally, the model revealed strong negative correlations between traits like RL, LMS, and EO with PH, SL, and DI, as demonstrated by the obtuse angles between their vectors (Fig. 1A), which shows that an increase in one set of traits is often associated with a decrease in the other. Overall, the biplot model provided a clear and informative visual representation of the relationships between traits in purslane. These findings are consistent with previous research by Nikrouz-Gharamaleki et al. (2019), who also reported positive correlations between the length of plant, stem, and internodes in summer savory, as well as positive correlations among fresh and dry weight of stems and whole plant. They also noted the lack of significant correlation between the length of plant, stem, and internodes with fresh and dry weight of stems and whole plant, aligning with the findings of this study. It is worth noting that while the biplot method offers an overall graphical depiction of trait associations, it does not always perfectly align with correlation coefficients (Table 2), which measure the direct interrelationship between individual traits. The biplot captures the broader structure of the dataset (Sabaghnia et al. 2024). Though both methods provide valuable insights, they may highlight different aspects of trait interactions depending on the context and objectives of the analysis. Fig. 1B facilitates the comparison of genotypes and helps to identify superior genotypes that may be ideal for breeding or cultivation. Thus, a polygon was constructed, with perpendicular lines drawn to its sides, which allowed for identifying vertex genotypes as the best performers for specific traits. Six key vertex genotypes were identified from the analysis: genotype 5 (Mashhad) excelled across a wide range of traits showing the highest values for the plant fresh weight (PFW), stem fresh weight (SFW), root fresh weight (RFW), root length (RL), plant dry weight (PDW), stem dry weight (SDW), and root dry weight (RDW), making it a top performer in yield performance traits (Fig. 1B). Also, genotype 16 (Tabriz-A) stood out in vegetative growth traits, particularly plant height (PH), stem length (SL), distance of internodes (DI), leaf area of main stem's leaves (LAMS), and leaf area of lateral branches' leaves (LALB), indicating its potential for size and morphology properties. Additionally, genotype 3 (Ardabil) exhibited the highest values for the leaflets per main stem (LMS) and essential oil (EO), which makes this genotype a particularly strong genotype for essential oil biomass production (Fig. 1B). Finally, three key

vertex genotypes (2, 18 and 19) did not display superior performance in any of the measured traits, suggesting it may not be ideal for selection in breeding programs of summer savory.

Table 2. Pearson's correlation coefficients for measured traits of summer savory genotypes

	PFW†	SFW	RFW	PH	SL	RL	DI	LMS	PDW	SDW	RDW	EO	LAMS
SFW	0.99‡												
RFW	0.82	0.82											
PH	-0.09	-0.10	-0.37										
SL	-0.13	-0.14	-0.46	0.95									
RL	0.27	0.23	0.51	-0.37	-0.60								
DI	-0.21	-0.20	-0.37	0.82	0.90	-0.62							
LMS	0.22	0.22	0.36	-0.61	-0.71	0.45	-0.81						
PDW	0.95	0.94	0.79	0.05	-0.02	0.32	-0.08	0.12					
SDW	0.97	0.97	0.77	0.08	0.03	0.19	-0.06	0.12	0.96				
RDW	0.80	0.81	0.90	-0.39	-0.48	0.49	-0.44	0.46	0.78	0.75			
EO	-0.15	-0.18	-0.05	-0.10	-0.20	0.25	-0.30	0.51	-0.07	-0.12	0.02		
LAMS	0.12	0.13	-0.04	0.40	0.48	-0.23	0.57	-0.69	0.18	0.17	-0.07	-0.32	
LALB	0.22	0.23	0.04	0.39	0.50	-0.36	0.53	-0.60	0.24	0.25	-0.02	-0.37	0.70

† Traits are: plant fresh weight (PFW), stem fresh weight (SFW), root fresh weight (RFW), plant height (PH), stem length (SL), root length (RL), distance of internodes (DI), leaflets per main stem (LMS), plant dry weight (PDW), stem dry weight (SDW), root dry weight (RDW), essential oil (EO), leaf area of main stem's leaves (LAMS), and leaf area of lateral branches' leaves (LALB). ‡Critical values of correlations are 0.7 and 0.47 at 0.05 and 0.01 statistical level and 27 degrees of freedom.

The discriminative ability of traits in identifying differences among genotypes is determined by the magnitude of the standard deviation. Traits with higher standard deviations possess a greater capacity to distinguish between genotypes, as illustrated in the perfect position model (Fig. 2A). In this model, traits closer to the hypothetical perfect position are more discriminative. Those further away have a reduced ability to differentiate genotypes. Based on this biplot, stem dry weight (SDW) and plant dry weight (PDW), following plant fresh weight (PFW) and stem fresh weight (SFW), are identified as the most discriminative traits, offering the greatest potential for distinguishing among the genotypes. All other remaining traits except the leaflets per main stem (LMS) and essential oil (EO), though not the most discriminative, still have above-average potential for differentiating genotypes, making them valuable for further investigation (Fig. 2A).

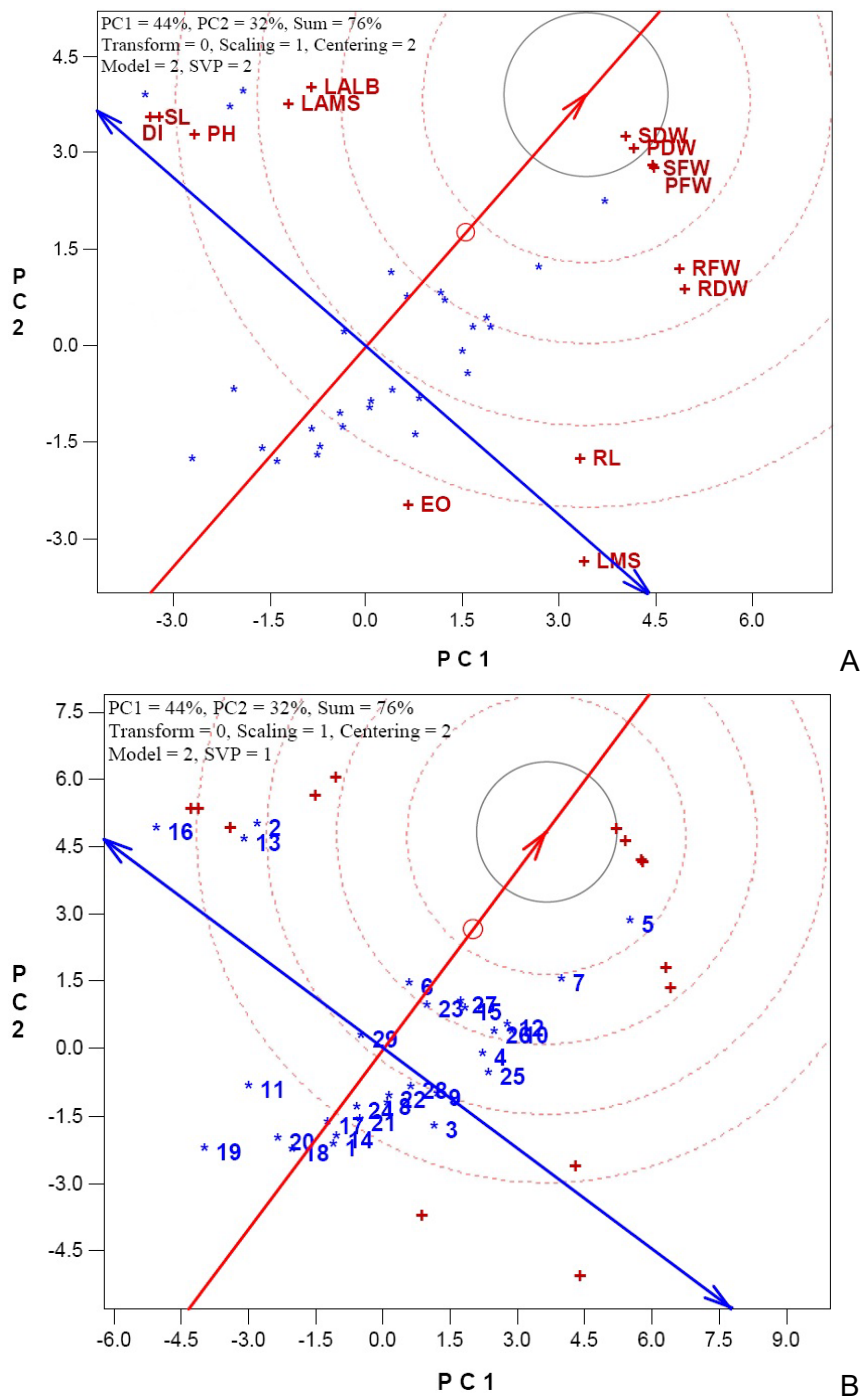


Figure 2. (A) perfect trait of GT biplot for testing discriminative ability of traits; (B) perfect entry of GT biplot for detecting the most favorable genotype(s).

However, LMS and EO have low discriminative potential and may not be as useful for future studies or breeding programs. The typical potential of each trait, representing its symbolic importance, is measured by the angle between the trait's vector and the axis of the average trait. Traits with smaller angles exhibit higher typical potential while in this study, all measured traits except SDW, PDW, PFW, and SFW, which have the smallest angles with the axis, making them the most typical traits with the highest potential for genetic improvement, the other traits with a larger angle, has lower typical potential compared to others, making it less significant for breeding considerations (Fig. 2A). Thus, SDW, PDW, PFW, and SFW with high discriminative can distinguish differences among genotypes better than the other traits, so can be advised in future evaluation studies of summer savory. Fig. 2B further visualizes the perfect genotype position, clearly indicating which genotypes come closest to this location. Genotypes nearest to this perfect position are considered to have the most favorable combination of traits. In this case, genotypes 5 (Mashhad) and 7 (Tonkabon) were closest to the perfect entry, indicating their superior overall performance. On the opposite end, genotypes such as genotype 19 (SI) flowing to genotypes 11 (Sanandaj), 18 (Qazvin), and 20 (Ilam) were positioned farthest from the perfect, marking them as less desirable for breeding based on the traits analyzed (Fig. 2B). The remaining genotypes were ranked based on their proximity to the perfect genotype falling between the top performers and the least desirable. Identifying perfect genotypes is crucial for summer savory genetic improvement programs, particularly because yield performance and quality traits like essential oil often exhibit low or even negative correlations in many plants. This means that selecting one trait might compromise another, making it difficult to achieve balanced improvements without advanced selection tools.

Multivariate statistical models, such as the GT biplot model and graphical representations, are valuable in identifying high-performing genotypes (Yari et al. 2018). These tools help breeders visualize the complex relationships among traits, providing clearer guidance for making informed decisions in the selection and breeding of summer savory, focusing on enhancing yield performance and quality. Fig. 3A focuses on the performance of summer savory genotypes in terms of essential oil (EO). The horizontal axis represents EO, and an arrow indicates the direction of increasing EO performance.

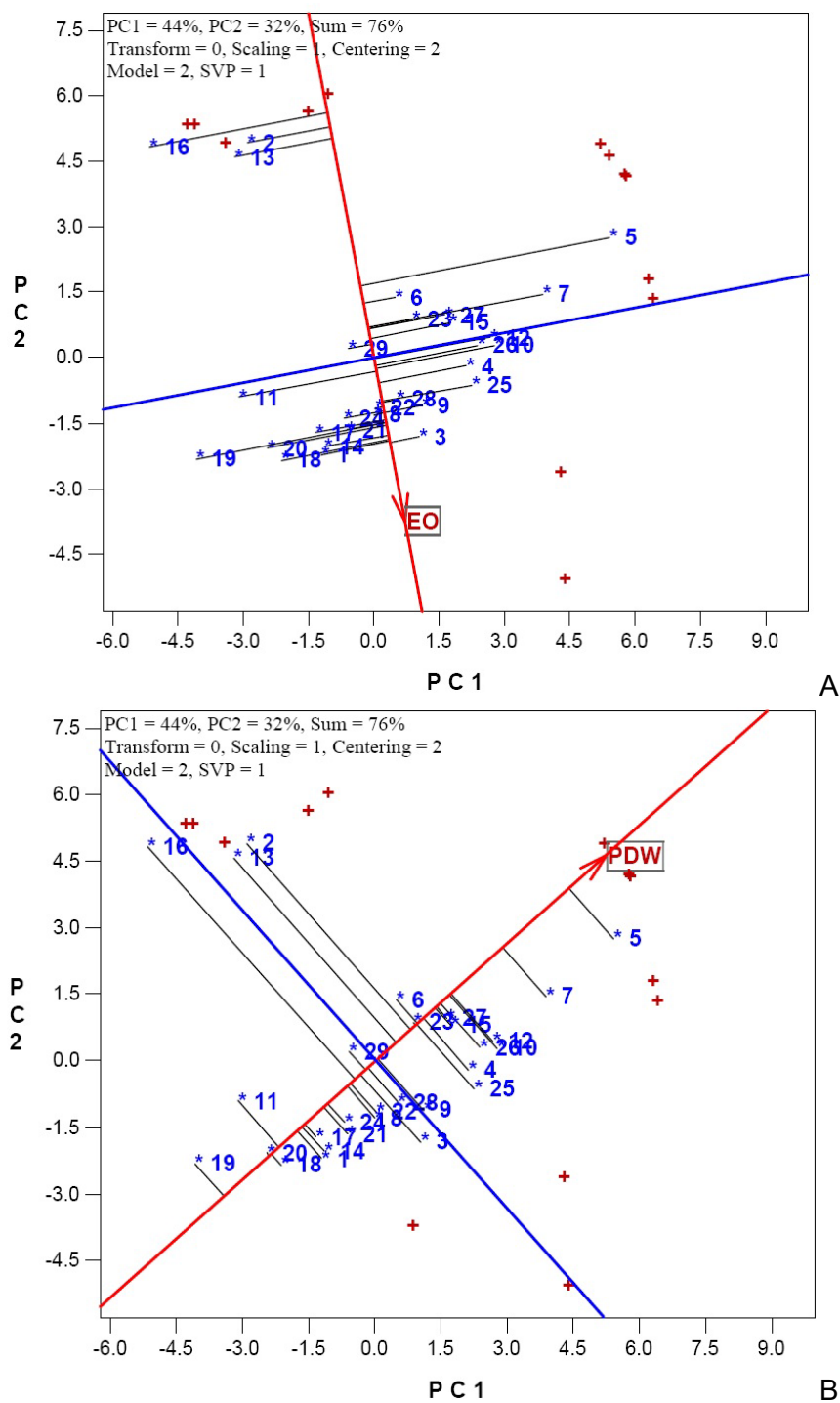


Figure 3. (A) testing of genotypes' performance for essential oil (EO); (B) examining of genotypes' performance for plant dry weight (PDW).

Genotype 3 (Ardabil) following to genotypes 1 (Shush) and 18 (Qazvin), indicated the most desirable essential oil performance, positioning them as top candidates for selection for improving essential oil. Conversely, genotypes 2 (Tabriz-A), 13 (Sardasht), and 16 (Tabriz-B) were the least favorable for essential oil. The distance of genotypes from the horizontal axis indicates their standard deviation in EO, so genotypes such as 3, 8, 21, 22, and 24 are recommended due to their low variability, making them reliable options for selection. Also, genotype 3 (Ardabil), with low EO and a greater distance from the axis, is considered one of the least desirable genotypes for EO improvement. Similar to essential oil, plant dry weight (PDW) performance, as an important target trait, is survived in Fig. 3B. In this graph, the horizontal axis shows PDW, and its arrow is the direction. The following genotypes identified: genotype 5 (Mashhad), following genotype 7 (Tonkabon-B) produced the high PDW, positioning them as top candidates for selection in genetic improvement projects aimed at improving biomass production. Conversely, genotypes 19 and 11 were the least favorable for PDW, with lower performance than other genotypes. Additionally, 15 genotypes such as 5, 7, 13, 25, and 27, showed above-average PDW performance, as they were above the average axis in Fig. 3B. The distance of genotypes from the horizontal axis shows their variability in PDW and smaller amounts signify lower variability, which is generally preferred in selection. Accordingly, genotypes such as 5 and 7 are recommended due to their low variability. Thus, these genotypes (Mashhad and Tonkabon-B), which showed both high PDW and a lower distance from the axis, are regarded as the most favorable genotypes for improving plant dry weight.

Evaluating summer savory genotypes' performance is critical for conserving genetic diversity and preventing the erosion of valuable plant materials. The observed substantial variation among summer savory genotypes in this study aligns with the findings of Fathi et al. (2021) and Dodoš et al. (2024), who also reported significant diversity within summer savory populations. The GT interaction biplot model in this study revealed a relatively high variation level, confirming simple and complex interactions among genotypes and traits. These interaction patterns have been similarly documented in other vegetable crops, such as *Capsicum annuum* (Abu et al. 2011) and *Carthamus tinctorius* (Sabaghnia et al. 2015). The complex nature of these interactions, which result in varying genotype rankings across traits, underscores the challenge of selecting the best genotypes.

Ignoring the GT interaction complicates the breeding process, significantly influencing the genetic factors underpinning trait expression. This study provides valuable insights into the discriminative and typical potential of various traits in summer savory, offering a foundation for more informed and effective breeding strategies. The identification of top-performing genotypes, such as Mashhad and Tonkabon-B for biomass yield performance and Ardabil, Shush, and Qazvin for essential oil production, holds significant promise for genetic improvement programs aimed at enhancing both biomass and essential oil traits in summer savory.

CONCLUSIONS

Significant relationships among traits were found for fourteen traits of 29 summer savory genotypes, proving to be crucial for selecting the most desirable individuals for breeding targets. Genotype 5 (Mashhad) was the best for the fresh and dry weight of stems and whole plant, genotype 16 (Tabriz-A) was good for size and morphology properties, and genotype 3 (Ardabil) exhibited the highest values for the essential oil of summer savory. Thus, they are particularly favorable candidates for cultivar release due to their outstanding trait performance. They should be prioritized for further breeding programs to optimize yield and improve overall quality in summer savory cultivation. These insights provide a strong foundation for future research and practical applications in breeding programs aimed at improving the characteristics of Iranian summer savory.

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