

Multivariate Analysis Reveals the Variability in Morphological and Chemical Characteristics of Fenugreek (*Trigonella foenum-graecum* L.) Populations

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On January 2, 2024, Ziba Bakhtiar and his research team published an article titled "Variability in proximate composition, phytochemical traits, and antioxidant properties of Iranian agro-ecotypic populations of fenugreek (*Trigonella foenum-graecum* L.)" in the journal Scientific Reports. The article investigates the variability in proximate composition, phytochemical characteristics, and antioxidant properties of different agro-ecotypic populations of fenugreek (*Trigonella foenum-graecum* L.) in Iran. By analyzing leaf and seed samples from 31 Iranian fenugreek agro-ecotypic populations, the study found that the seeds contain higher levels of ash, fat, crude fiber, protein, and carbohydrates, and their energy value is significantly higher than that of the leaves. The antioxidant activity and capacity of the leaves were also studied in detail, revealing a positive correlation with total phenolic and total flavonoid contents. The research demonstrates significant differences and correlations among these traits, which are important for further studies on food production systems.

1 Interpretation of Experimental Data

The study employed multivariate statistical methods to analyze the morphological characteristics, proximate composition (including moisture, crude fiber, protein, fat, carbohydrates, and energy value), total phenolic and flavonoid contents, and antioxidant properties of 31 agro-ecotypic populations of fenugreek. Data analysis indicated significant differences in morphological characteristics and proximate composition among the different populations. For example, in seed samples, the highest ash content was 3.94%, the highest fat content was 7.94%, the highest crude fiber content was 10.3%, the highest protein content was 35.41%, and the highest carbohydrate content was 50.5%.

Figure 2 shows the total phenol content (TPC), total flavonoid content (TFC), and their radical scavenging activities (DPPH and FRAP) of the leaves and seeds of *Trigonella foenum-graecum* from 31 agro-ecotypic populations. From (a), it can be seen that there are significant differences in TPC and TFC content between the leaves and seeds of different ecotypic populations, with the leaves generally having higher TPC and TFC than the seeds. (b) shows the DPPH and FRAP activities, indicating that the antioxidant activity of the leaves is generally higher than that of the seeds, especially in populations such as Ahvaz and Kermanshah. These data suggest that the leaves of *Trigonella foenum-graecum* have advantages over the seeds in terms of antioxidant activity and phenolic content.

Figure 3 shows the bi-plot graph and correlation plot for the principal component analysis (PCA) based on the proximate composition, total phenolic content, total flavonoid content, and antioxidant activities of the leaves and seeds of *Trigonella foenum-graecum* from 31 agro-ecotypic populations. The PCA in (a) and (b) demonstrates significant differences in composition among different ecotypic populations, especially in crude fiber, protein, and carbohydrates. The correlations in (c) and (d) reveal the relationships between total phenolics, total flavonoids, and antioxidant activities. The antioxidant activity of the leaves (DPPH and FRAP) is positively correlated with total phenolics and total flavonoids (correlation coefficients of 0.50 and 0.68, respectively), while this correlation

is relatively lower in seeds. These results indicate that the leaves have more pronounced antioxidant properties than the seeds, and there are significant associations among the components.

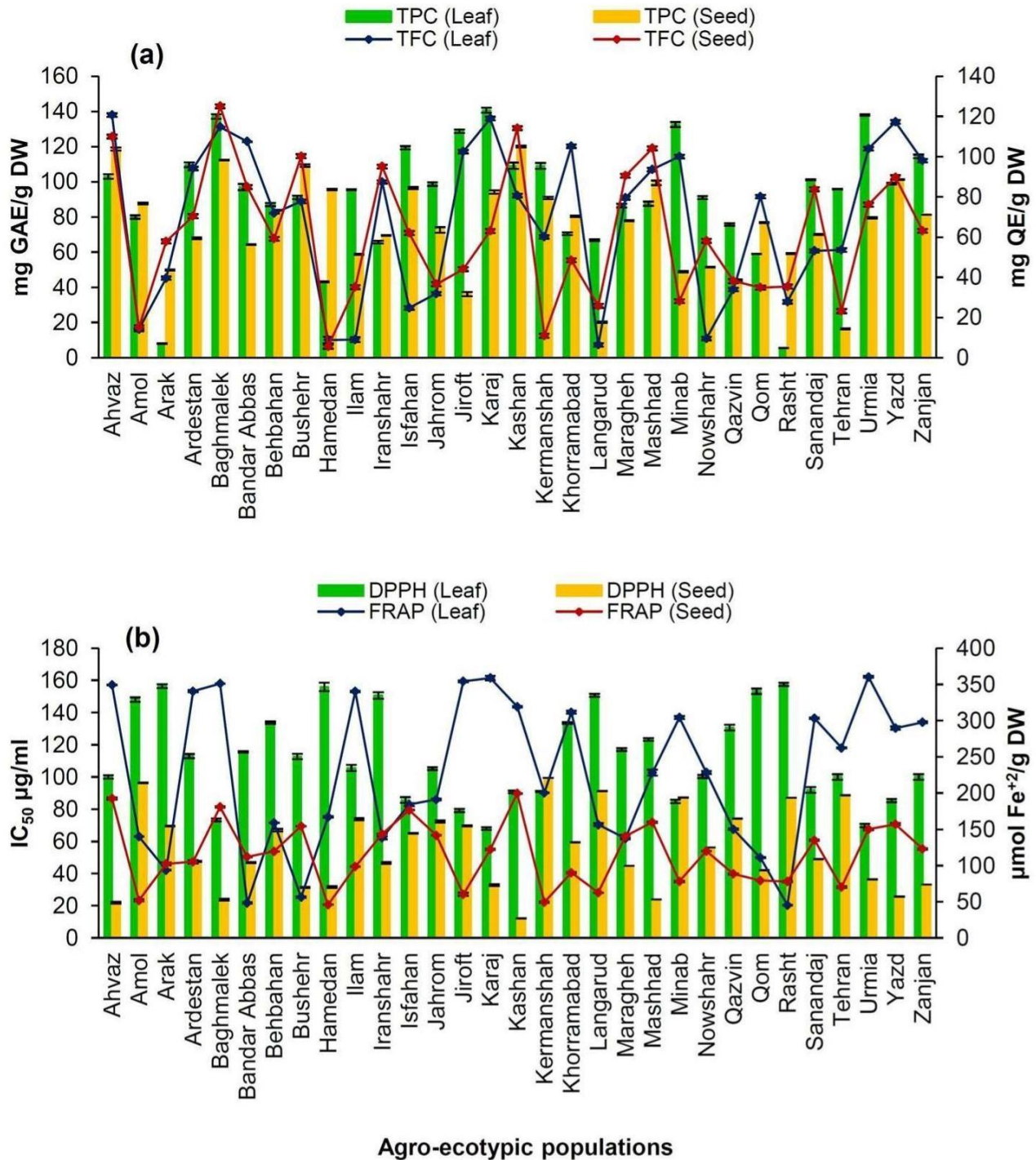


Figure 2 Histograms of total phenol content (TPC), total favonoid content (TFC) (a), and radical scavenging activities (b) of leaf and seed extracts from 31 agro-ecotypic populations of *Trigonella foenum-graecum*

2 Insights of Research Results

Through an in-depth analysis of the experimental data, they found a significant correlation between the morphological characteristics and proximate composition of different agroecotypes. Specifically, leaf protein content showed a significant positive correlation with seed protein content and seed energy value, with correlation coefficients of $r=0.78$ and $r=0.64$, respectively. This indicates that higher leaf protein content corresponds to higher seed protein content and energy value. Additionally, they observed a significant negative correlation between leaf moisture content and both leaf protein content and leaf energy value, with correlation coefficients of

$r=-0.65$ and $r=-0.57$, respectively. This means that populations with higher leaf moisture content have relatively

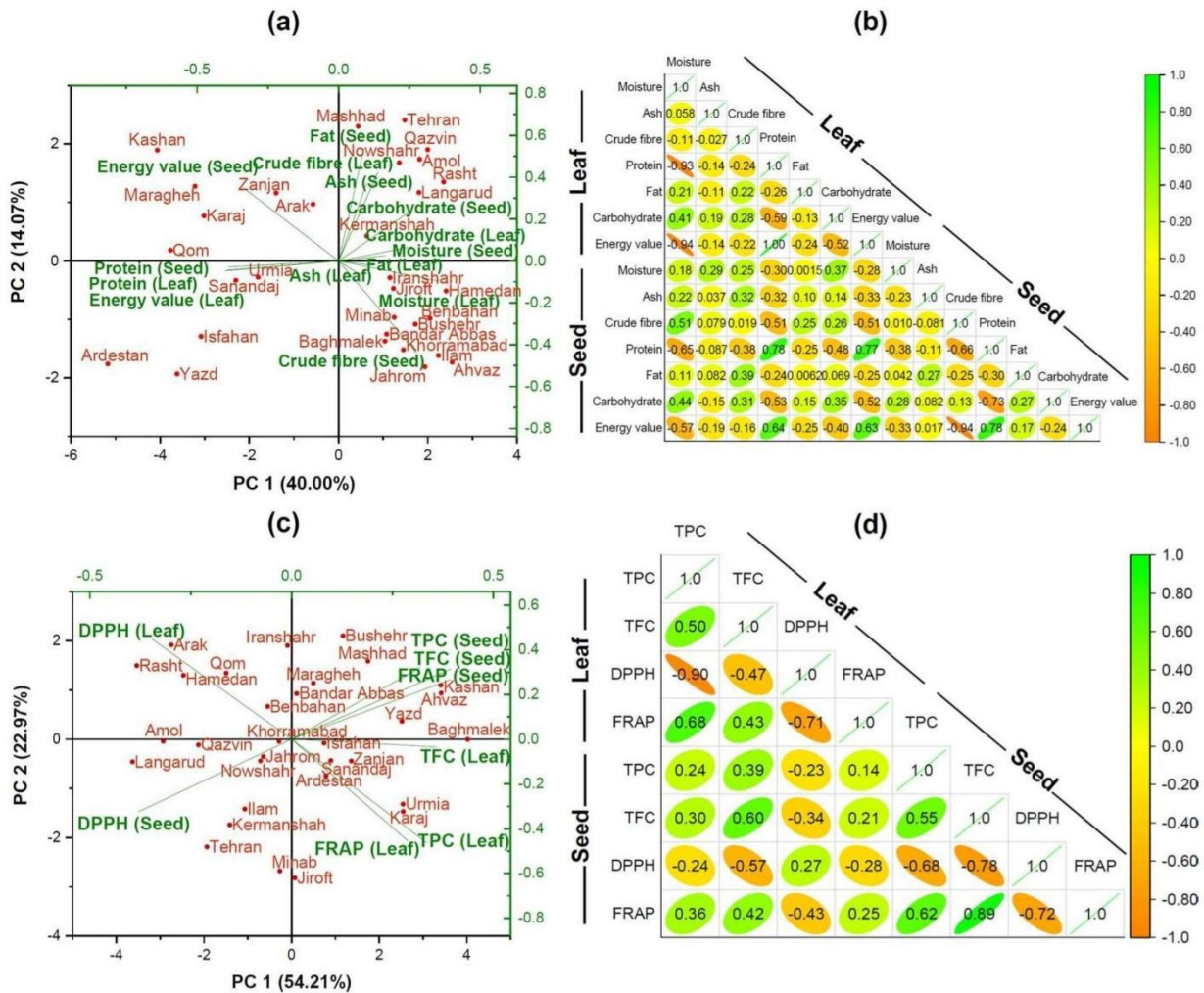


Figure 3 Bi-plot graph and correlation plot for the first and second principal components based on the proximate composition (a, b), and total phenolic, flavonoid content and antioxidant activities (c, d) for 31 agroecotypic populations of *Trigonella foenum-graecum* (Significant level: 0.05)

lower leaf protein content and energy value. Further research also revealed a significant correlation between total phenolic and flavonoid contents and antioxidant performance. Specifically, the higher the total phenolic and flavonoid contents, the stronger the antioxidant performance of fenugreek. This finding indicates that these compounds significantly contribute to the antioxidant capacity of fenugreek, further supporting its potential as a functional food ingredient. Through these correlation analyses, they can better understand the relationship between the morphological and chemical characteristics of different agroecotypes, providing important insights for further breeding and functional studies.

3 Evaluation of the Research

This study, through a comprehensive analysis of different agroecotypes of fenugreek, reveals significant variability in morphological characteristics and chemical composition among these populations. The research methodology is scientifically rigorous, the data analysis is thorough, and the results are highly reliable. Experimental data indicate significant differences between populations in the proximate composition of leaves and seeds, total phenolic and flavonoid contents, and antioxidant performance. For instance, the seeds have higher protein, fat, and carbohydrate content, as well as significantly higher energy values compared to the leaves. These findings suggest that fenugreek seeds have important application potential in the food industry. Moreover, the study shows that the antioxidant performance of fenugreek leaves and seeds is closely related to their total

phenolic and flavonoid contents, providing scientific evidence for the application of fenugreek in functional foods. Notably, the leaves of the Karaj ecotype and the seeds of the Mashhad ecotype, due to their superior nutritional and antioxidant properties, have potential for further commercial development.

4 Concluding Remarks

This study demonstrates significant differences in morphological characteristics, proximate composition, total phenolic and flavonoid contents, and antioxidant performance among different agroecotypes of fenugreek in Iran. The results indicate that fenugreek seeds are rich in nutrients, with high levels of protein, fat, and carbohydrates, as well as high energy values. The leaves and seeds of these populations exhibit significant differences in antioxidant activity, mainly related to their total phenolic and flavonoid contents. There is notable variability in the ash, fat, crude fiber, protein, and carbohydrate contents of seeds among different populations, giving certain populations an advantage in specific nutritional components. For example, the seed protein content of some ecotypes is as high as 35.41%, while the fat content can reach 7.94%. This study provides scientific evidence for the application of fenugreek in the food industry and functional foods. The high nutritional content and antioxidant performance make fenugreek seeds have broad application potential, especially in the development of foods with high nutritional value and antioxidant functions.

5 Access Original Paper

Bakhtiar Z., Hassandokht M., Naghavi M.R., and Mirjalili M.H., 2024, Variability in proximate composition, phytochemical traits and antioxidant properties of Iranian agro-ecotypic populations of fenugreek (*Trigonella foenum-graecum* L.), *Sci. Rep.*, 14(1): 87. <https://doi.org/10.1038/s41598-023-50699-9>

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