Indonesian Journal of Chemical Research

http://ojs3.unpatti.ac.id/index.php/ijcr Indo. J. Chem. Res., 12 (1), 26-30, 2024

Comparative study of *Trigonella foenum-graecum* L. varieties in The Middle East by Using Protein Pattern and Seed Morphology

A. El-Shabasy

Department of Biology, College of Science, Jazan University, P.O. Box. 114, Jazan 45142, Kingdom of Saudi Arabia
*Corresponding author: ael-shabasy@jazanu.edu.sa

Received: December 2023
Received in revised: April 2024
Accepted: May 2024
Available online: May 2024

Abstract

This study illustrates the situation of the relation between different varieties of *Trigonella foenum-graecum* L. not in the same one country but in different countries in the Middle East area; Algeria, Egypt, Ethiopia, India, Iran, Sudan, Tunisia, Turkey and Yemen. This comparative study explained the origin of the studied plant species and the evolutionary trends by using protein pattern and seed morphology showing the ancestral origin arisen upper Middle East region. This study explored the global changes and impacts on variety distribution.

Keywords: Evolution, Ancestor, Climate changes, Global impacts, Electrophoresis.

INTRODUCTION

Study of taxonomy comprises different levels of discrimination among genus and species. Intraspecies differentiation is the hard study that needs using specific chemical studies to obtain the clear variation among studied races, varieties or tribes (Carstens et. al., 2013). Molecular biology facilitates the similarity and dissimilarity among species and exhibits the entire polymorphism and real interrelationships. Organic chemistry and biochemistry serve biological studies to achieve the taxonomic goals among all living organisms. By using DNA, RNA or even protein analysis, one can reach to the optimum evaluation in taxonomy and know the origin of evolution and causes of changes and new adaptations (Fontaneto et. al., 2015). Comparative taxonomy indicates the global alterations and implications which pave scientists to solve the main world problems like climatic changes, desertification and spread of new epidemic diseases (Eberle et al., 2020). *Trigonella foenum-graceum* is the most medicinal plant that can be used all over the world belonging to Fabaceae (Jethra et. al., 2015). The plant height reaches amost 60 cm and its stem is single, branched and erect. The leaves are egg-shaped, three-lobed, complex odd feathery, 2 cm long. The plant blooms in summer from May to June then the seeds ripen in June to July (Kaysarov & Mardonov, 2023). It heals high cholesterol, high sugar-level, skin inflammation either rashes, boils or wounds (Sumayya et. al., 2012). It has excellent adaptation abilities that let it to be in different countries of temperate climate because it can withstand the harsh conditions especially in winter (KÜLÖNBÖZŐ et. al., 2004). From this plant, one can suggest the opportunity of germplasms distribution and the ways the plant used in invasion to better conditions.

The aim of this study is to stand on how this plant distributed in a large scale covering many different countries and what the relations appear among different varieties by using two integrated methods; traditional and new ones; seed morphology and protein pattern respectively.

METHODOLOGY

Plant collection

Seeds of *Trigonella* varieties were collected from different countries belonging to the Middle East; Algeria (1), Egypt (2), Ethiopia (3), India (4), Iran (5), Sudan (6), Tunisia (7), Turkey (8) and Yemen (9) (Figure 1). All seeds were derived from the Gene Banks at the Capital of each country to bring the main ancestor like Gene Bank of Agricultural Research Center (GBARC), Institute of Plant Genetics and Crop Plant Research (IPGCPR), National Plant Germplasm System (NPGS) etc.

Seed morphology

The seed morphology was studied in two parameters; macro and micro-parameters. Macro-parameter means the morphological characterization of the seed like seed color and seed shape while micro-parameter deals with the seed and kernel dimensions.

Figure 1. The studied map of Middle East area denoting each country with a number arranged alphabetically
1; Algeria, 2; Egypt, 3; Ethiopia, 4; India 5; Iran, 6; Sudan 7; Tunisia, 8; Turkey, 9; Yemen

Protein electrophoresis

Seeds of studied plant materials were cleaned with distilled water then air drying. The seeds of each variety were grinded and homogenized separately in presence of 0.1 M Tris-HCl buffer (pH 7.5). The supernatant was obtained for each plant variety after centrifugation at 17600 rpm at 4 °C for 20 min then diluted with sample buffer containing 0.0625 M Tris-HCl pH 6.8, 2.5 % sodium dodecyl sulfate (SDS), 5 % 2-mercaptoethanol and 10 % glycerol. After that, the extract is heated in boiling water for 5 min prior to being loaded on gel. Content of total protein in the samples were estimated following the method of (Bhargava et. al., 2005). Electrophoresis was done in the modified discontinuous SDS-PAGE system of (Chadijah et. al., 2023) using 4 % stack gel (0.125 M Tris-HCl, pH 6.8) and 10 % acrylamide resolving gel (0.375 M Tris-HCl, pH 8.9). The running buffer was prepared as Tris-glycine (0.3 % Tris base, 1.44 % glycine and 0.1 % SDS, pH 8.3). Staining of the gels was carried out in 0.02 % Coomassie Brilliant Blue R-250 containing 7 % acetic acid and 55 % methanol while destaining one was prepared in the same solution but without the dye.

Cluster analysis

The molecular weights of the protein marker bands were inserted at the left side of protein pattern. The corresponding plant bands were determined and scored as presence (+) or absence (-) in a binary matrix accompanied with seed characters whether macro or micro-parameters. Cluster analysis and similarity matrix were constructed via (Atta et. al., 2022) where the distances among similarities and dissimilarities were established by using a modification of the Gower coefficient. Hierarchic nest and sequential agglomerative clustering were done by using UPGMA (El-Gazzar & Rabei, 2008).

RESULTS AND DISCUSSION

Seed morphology

The seed morphology results were classified into macro-parameters including seed color, seed shape and seed poles while micro-parameters illustrated seed length, seed width and kernel length. The seed color was either brown, pale brown or dark brown. On the other hand, other macro-parameters; seed shape and seed poles were concerned only into two variables; Rhomboid and rectangular besides rounded and truncate respectively. Furthermore, *Trigonella* varieties were able to score different values of micro-parameters. The highest seed length value was in Tunisia variety while the lowest one was India one. Ethiopia and Tunisia varieties share with the highest seed width values while Sudan one had the lowest one. Moreover, Tunisia variety had also the highest kernel length value while Algeria one had the lowest one (Table 1).

Protein electrophoresis

Protein pattern recorded 18 bands with 5 similar bands among all varieties. Hence, the polymorphism scored 72.22%. Negative Unique Parameter (NUP) is the parameter which is absent in such a taxa but present in others (EL-Atroush et. al., 2015). So, there were 5 NUPs that applied to India, Iran and Sudan

Table 1. Seed morphological traits among *Trigonella* varieties in the Middle East

Seed Morphology	1	2	3	4	5	6	7	8	9
Seed color	brown	pale brown	dark brown	brown	pale brown	dark brown	brown	pale brown	brown
Seed length (mm)	2.2±3.47	2.0±0.05	2.4±2.58	1.36±2.24	2.6± 3.47	1.5±2.98	2.8± 1.29	2.0± 2.58	1.4± 1.89
Seed width (mm)	1.2±1.58	1.4±2.36	1.6± 5.02	1.2± 2.78	1.52± 1.49	1.0± 5.12	1.6± 3.88	1.52± 3.78	1.2± 1.44
Kernel length (mm)	1.0±4.02	1.2±2.78	1.2± 1.74	1.04± 3.47	1.2± 2.47	1.3± 1.89	2.2± 4.12	1.2± 1.45	1.3± 2.88
Seed shape	Rhomboid	Rectangular	Rectangular	Rhomboid	Rectangular	Rhomboid	Rhomboid	Rectangular	Rhomboid
Seed poles	rounded	truncate	rounded	truncate	rounded	truncate	truncate	rounded	truncate

1; Algeria, 2; Egypt, 3; Ethiopia, 4; India 5; Iran, 6; Sudan 7; Tunisia, 8; Turkey, 9; Yemen varieties but Ethiopia had two NUPs (Table 2; Figure 2).

Table 2. Polymorphic protein bands scored (1; present), (0; absent) among *Trigonella* varieties in the Middle East

Protein bands	1	2	3	4	5	6	7	8	9
Band 1	1	1	1	0	1	1	1	1	1
Band 2	0	0	0	1	1	1	0	0	0
Band 3	0	0	0	0	1	1	0	0	0
Band 4	1	1	0	1	1	1	1	0	1
Band 5	0	1	1	1	1	0	1	1	1
Band 6	1	1	1	1	1	0	1	1	1
Band 7	1	1	1	1	1	1	1	1	1
Band 8	1	1	1	1	1	1	1	1	1
Band 9	1	1	1	1	1	1	1	1	1
Band 10	1	1	1	1	1	1	1	1	1
Band 11	1	1	0	1	0	0	1	1	0
Band 12	1	1	1	1	0	1	1	1	1
Band 13	1	1	0	1	1	0	0	0	0
Band 14	1	1	0	1	1	1	1	1	1
Band 15	1	1	1	1	1	1	1	1	1
Band 16	1	1	0	0	1	1	0	1	1
Band 17	0	0	0	0	0	0	0	1	1
Band 18	1	1	0	1	1	1	1	1	1

1; Algeria, 2; Egypt, 3; Ethiopia, 4; India 5; Iran, 6; Sudan 7; Tunisia, 8; Turkey, 9; Yemen

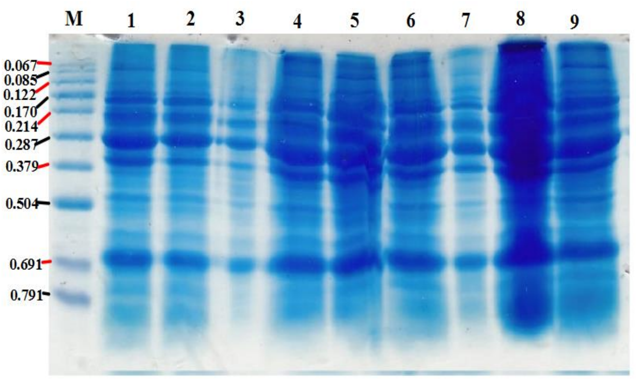

Figure 2. Protein pattern electrophoresis of *Trigonella* varieties in the Middle East
(M; Molecular marker, 1; Algeria, 2; Egypt, 3; Ethiopia, 4; India 5; Iran, 6; Sudan 7; Tunisia, 8; Turkey, 9; Yemen)

Cluster analysis

The resulting phenogram revealed that the *Trigonella* varieties in the Middle East had an average taxonomic distance of 0.82. At this level, there were two clusters, the first cluster had unigeneric variety Sudan variety (No. 1) at 0.94 while the rest of the first cluster was differentiated into one clade having Iran variety (No. 5) at 1.048 and the second clad having Egypt variety (No. 2) as a sub-clad besides Ethiopia and Turkey varieties as another sub-clad (No. 3 &8) at 1.204. Similarly, the second cluster where India variety (No. 4) was split into a separated clad at 0.88 while the rest of *Trigonella* varieties Tunisia and Yemen varieties (No. 7 & 9) were differentiated into a group at 1.12 (Figure 3). The similarity matrix indicated that Egypt and Turkey varieties had the high similarity with 0.83 with highest number of common bands while Ethiopia and Sudan had the low one with 0.46 value with lowest number of common bands (Table 3), that referring that the ancestor origin arose from upper Middle east region spreading in both east and west direction till the south one with distant relation.

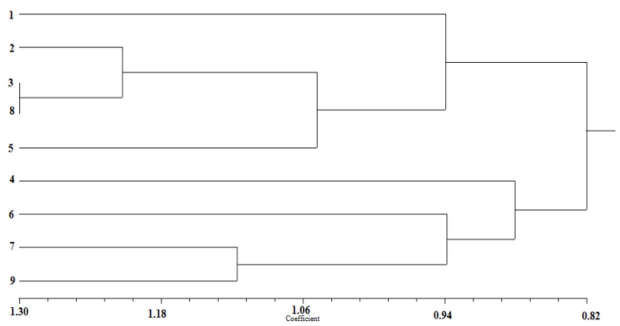

Figure 3. Phenogram of *Trigonella* varieties in the Middle East
(1; Algeria, 2; Egypt, 3; Ethiopia, 4; India 5; Iran, 6; Sudan 7; Tunisia, 8; Turkey, 9; Yemen)

Table 3. Similarity matrix of *Trigonella* varieties in the Middle East.

	1	2	3	4	5	6	7	8	9
1	1.00								
2	0.79	1.00							
3	0.58	0.71	1.00						
4	0.75	0.71	0.50	1.00					
5	0.67	0.79	0.67	0.58	1.00				
6	0.62	0.58	0.46	0.62	0.62	1.00			
7	0.75	0.79	0.67	0.75	0.58	0.62	1.00		
8	0.71	0.83	0.79	0.54	0.71	0.50	0.71	1.00	
9	0.71	0.67	0.54	0.71	0.54	0.75	0.79	0.67	1.00

1; Algeria, 2; Egypt, 3; Ethiopia, 4; India 5; Iran, 6; Sudan 7; Tunisia, 8; Turkey, 9; Yemen

The study of origin and evolution of such plant species should be covered within large areas extended whenever possible to adjacent countries. The evolutionary trends among plant species can be accomplished by knowing the ancestral and transitional species. Traditional taxonomic methods can't give the entire picture of the taxonomic origin of such a plant species. Modern technologies like protein electrophoresis can help to trace the evolutional ways in different regions and countries. From previous phenogram illustrated that Egypt, Ethiopia and Turkey varieties were related to each other. The origin of this plant species is focused in the North Africa and

South Europe. On the contrary, Tunisia and Yemen varieties were related to each other while other varieties were regarded as transition varieties between Egypt, Ethiopia and Turkey from one hand and Tunisia and Yemen in another hand. Sudan variety was the most distantly related which may refer to extend the evolutionary trend from Africa to Asia. Asian varieties were not related to each other like North African and South Europe which reflecting that the adaptational variations were originated from Asia (Al Faifi et. al., 2023). The improvement of *Trigonella* yield is done through studying the adaptation conditions and evolutionary trends (KÜLÖNBÖZŐ et al., 2004). Assessment of genetic divergence assists the studied plant species from threating of genetic erosion which spreads worldwide due to pollution impacts and desertification (Al-Maamari, Khan, Al-Sadi et. al., 2020).

The change of environmental factors can influence on the germplam of individual. The deviation of evolutionary trends from ancestor origin was undergone through impact of global changes (Kainama et. al., 2021). Alteration of physical conditions like high thermal earth degree and increasing of sea level besides chemical conditions like chemical nature composition of air and water should be understood annually to interpreting biological modification for all living organisms whether prokaryotes or eukaryotes to face the possible future challenges facing all over the countries like pandemic COVID (Istyami et al., 2024)\.

CONCLUSION

This study stands on the evolutionary situation of *Trigonella* varieties in the Middle East. It may refer to African origin where the ancestral traits can be conserved till protruding to another continent like Asia. Evolutionary trends extended from upper Middle East region towards the east and west ending in the south direction with the most distant varieties.The knowing the evolutionary trends improve the adaptitional habitats and extend the deep thinking on climate changes and their impacts.

REFERENCES

Al Faifi, T., Masrahi, A. S., & El-Shabasy, A. (2023). Mechanical structures of sidewalk plants: Anatomical evaluation. *Saudi Journal of Biological Sciences*, *30*(6), 1–8.

Al-Maamari, I. T., Khan, M. M., Al-Sadi, A. M., Iqbal, Q., & Al-Saady, N. (2020). Morphological characterization and genetic diversity of Fenugreek (Trigonella foenum-graecum L.) accessions in Oman. *Bulgarian Journal of Agricultural Science*, *26*(2), 375–383.

Atta, E., Al Faifi, T., & El-Shabasy, A. (2022). Chemotaxonomic and morphological classification of six Indigofera species in Jazan region, KSA. *Journal of Saudi Chemical Society*, *26*(3), 1–15.

Bhargava, A., Rana, T. S., Shukla, S., & Ohri, D. (2005). Seed protein electrophoresis of some cultivated and wild species of Chenopodium. *Biologia Plantarum*, *49*(4), 505–511.

Carstens, B. C., Pelletier, T. A., Reid, N. M., & Satler, J. D. (2013). How to fail at species delimitation. *Molecular Ecology*, *22*(17), 4369–4383.

Chadijah, S., Firnanelty, F., Baharuddin, M. B., & Sappewali, S. (2023). Bioelectrochemical Systems (BESs) Technology for The Production of Electrical Energy from Kepok Banana Stem. *Indo. J. Chem. Res.*, *11*(2), 112–117.

El-Gazzar A., Rabei S. (2008). Taxonomic assessment of five numerical methods and its implications on the classification of *Hyptis* sp. (Labiatae): Int. J. Botany, 4 (1): 85–92.

EL-Atroush, H., EL-Shabasy, A. E., Tantawy, M. A., & Barakat, H. M. S. (2015). Pollen Morphology and Protein Pattern of Nitraria retusa and Some Selected Taxa of Zygophyllaceae in Egypt. *Egyptian Journal of Botany*, *55*(2), 207–230.

Eberle J., Ahrens D., Mayer C., Niehuis O., Misof B. (2020). A plea for implementing a standardized set of nuclear markers in DNA taxonomy: Trends Eco.l Evol., 35 (4):336–345.

Fontaneto, D., Flot, J.-F., & Tang, C. Q. (2015). Guidelines for DNA taxonomy, with a focus on the meiofauna. *Mar Biodiv*, *45*, 433–451.

Istyami, A. N., Arif, M., Azzindi, M. I., Pratiwi, M., Adisasmito, S., Damayanti, N. Y., … Rizkiana, J. (2024). Utilization of Tamarind Seeds Extract as a Natural and Sustainable Fabric Dye. *Indonesian Journal of Chemical Research*, *11*(3), 190–198.

Jethra, G., Sharma, R., & Singhand, P. (2015). IN-SILICO ANALYSIS OF FENUGREEK (TRIGONELLA FOENUM- GRAECUM) PROTEIN. *International Journal of Advances in Science Engineering and Technology*, *3*(3), 66–67.

Kainama, H., Sohilait, H. J., & Souisa, C. J. (2021). Qualitative Protein Hydrolyzed from Nerita undata in supralittoral rocks and mezolittoral zone of Hasa Cape using TLC. *Indo. J. Chem. Res*, *9*(2), 118–123.

Kaysarov, V., & Mardonov, F. (2023). Morphoanatomic description of *Trigonella foenum-graecum* L. seeds. *E3S Web of Conferences*, *389*(03054), 1–5.

KÜLÖNBÖZŐ, G., FAJTÁK, Ö., & VIZSGÁLATA, V. (2004). Comparative Test Of Fenugreek / Trigonella Foenum-Graecum L. / Varieties Különböző Görögszéna / Trigonella Foenum-Graecum L. / Fajták Összehasonlító Vizsgálata. *Journal Central European Agriculture*, *5*(4).

Sumayya, A., Sivagami, S., & Nabeelah, A. (2012). Screening and Biochemical Quantification of Phytochemicals in Fenugreek (Trigonella foenum-graecum). *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *3*(1), 165–169.