

## EVALUATION OF KASURI METHI *TRIGONELLA FOENUM- GRAECUM* L. VAR. TO ESTABLISH GI RIGHT OF PAKISTAN

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**ABSTRACT:-** Geographical indicator (GI) of crops create a positive impression of the product quality, the environmental virtue and human skill of the area. In the present study, comparative analysis of total seed proteins of Kasuri methi (GI of Kasur, Pakistan) was evaluated with other *Trigonella* genotypes by SDS-PAGE. Results showed that at protein level Kasuri methi acquired a unique status as a G.I of Kasur. Cluster analysis (UPGMA) of 28 genotypes including both *methi* and *methray* from various agro ecological zones of Pakistan were interlinked to some extent however Kasuri methi make their identity by standing alone among tested genotypes on the dendrogram, due to the geographical environment of Kasur in Punjab. In this connection 'Benefit sharing' mechanism can be accelerated by escalating farmer's participation for more cultivation in the interest of country.

*Key Words: Trigonella foenum-graecum; Seeds; Diversity; Geographical Indicator; Phenotype; Pakistan.*

### INTRODUCTION

Fenugreek, *Trigonella foenum-graecum* L. is an ancient and annual legume crop mainly grown for multiple uses in many parts of the world. *Trigonella*, comes from Latin meaning 'little triangle', refers to the triangular shape of its small yellowish-white flowers. The species epithet *foenum-graecum* means 'Greek hay' (Rosengarten, 1969). It is also called 'ox horn' or 'goat horn' because of the two seed pods projecting in opposite directions usually from the nodes of the stem base that resemble ox or goat horns (Petropoulos, 2002). Landraces and species of *Trigonella* have been found in parts of Europe, northern Africa, west and south Asia, north and south America and Australia (Basu, 2006; Acharya et al., 2006).

Fenugreek leaves and seeds are consumed in different countries around the world for different purposes such as medicine (anti-diabetic, lowering blood sugar and cholesterol level, anti-cancer, anti-microbial, etc.), making food (stew with rice in Iran, flavor cheese in Switzerland, syrup, mixed seed powder with flour for making flat bread in Egypt, curries, dyes, young seedlings eaten as a vegetable, etc.), roasted grain as coffee-substitute (in Africa), controlling insects in grain storages, perfume

industries, etc. Pharmaceutically medicinal species of the genus *Trigonella* are *T. foenum-graecum* L., *T. balansae*, *T. corniculata*, *T. maritima*, *T. spicata*, *T. occulta*, *T. polycerata*, *T. calliceras*, *T. cretica*, *T. caerulea*, *T. lilacina*, *T. radiata*, *T. spinosa* (Petropoulos, 2002; Basu, 2006). The biological and pharmacological actions of fenugreek are attributed to the variety of its constituent, namely, steroids, N-compounds, polyphenolic substances, volatile constituents, amino acids, etc. (Mehrafarin et al., 2010).

Fenugreek seed contains 45-60% carbohydrates, mainly mucilaginous fiber (galactomannans), 20-30% proteins high in lysine and tryptophan, 5 - 10% fixed oils (lipids), pyridine alkaloids, mainly trigonelline (0.2 - 0.38%), choline (0.5%), gentianine and carpaine, the flavonoids apigenin, luteolin, orientin, quercetin, vitexin and isovitexin, free amino acids, such as 4-hydroxyisoleucine (0.09%), arginine, histidine and lysine, calcium and iron, saponins (0.6 - 1.7%), glycosides yielding steroidal sapogenins on hydrolysis (diosgenin, yamogenin, tigogenin, neotigogenin), cholesterol and sitosterol, vitamins A, B1, C and nicotinic acid and 0.015% volatile oils 'n-alkanes' and 'sesquiterpenes' (Budavari, 1996; Newall et al.,

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1996; Mehrafarin et al., 2010).

Most renowned Pakistani fenugreek with remarkable aroma cultivated in the Kasur district of Punjab province well known as 'Kasuri methi'. Kasur (total area 3,995 km<sup>2</sup>, elevation 218 m.a.s.l). Methi (fenugreek) from Kasur is very famous for its fragrance throughout the country and became geographical indicator of Kasur as Kasuri Methi, so it can be claimed/patent for GI or AO. 'Appellation of Origin' (AO) means that a product originates in a specific geographic region and the characteristic qualities of the product are due to the geographical environment, including natural and human factors (Nagarajan, 2005). The farming community contributions have to be recognized and rewarded and benefit sharing should become mandatory. Relationship between farmers' variety (FV) and GI provides certain rights (Plant Protection Variety and Farmers Rights Act 2001 in India) to farmers, such as to save, use, sow, re-sow, exchange, share or sell his farm produce including that of the registered variety. In Pakistan, farmers who develop new plant varieties like any other plant breeder can apply their material for the conduct of Distinctness, Uniformity and Stability (DUS) testing and registration. In the last hundred years there has been a drive for improved agriculture and that has replaced farmers' variety in several crops with new varieties developed by the plant breeders. Yet farmer's variety is still dominant in pulses, vegetables, melons, etc. There are lots of controversial patent cases involving traditional Knowledge and Genetic Resources including *hoodia cactus*, *turmeric*, *neem*, *ayahuasca* and basmati rice case, facing problems for their patent rights as geographical indicators. The GI for agricultural goods like Basmati rice, coffee, tea, wine, etc. revolve around consumer preferences for the palate feeling, aroma and physical appearance that enhances the appetite. An ideal mixture of all these attributes raises the value of the product due to reasons of consumer preference (Nagarajan, 2005). Lack of documentation of indigenous farmers' plant breeding is

attributed as a major reason for failure to consider the possibility that farmers have an intellectual investment in their folk varieties (Cleveland and Stephen, 1997; Nagarajan, 2005).

The objective of the study is to evaluate the *Trigonella* species collected from various agro ecological zones of Pakistan and to distinguish Kasuri methi at protein level by SDS- PAGE.

## MATERIALS AND METHODS

The research was conducted at National Agricultural Research Centre, Islamabad, Pakistan. Seeds of 28 genotypes of *Trigonella* (*Trigonella foenum graecum*-Methi and *Trigonella corniculata*-Methray) were collected from various agroecological zones of Pakistan including Mianwali, Pakpattan, Bahawalpur, Faisalabad, Shorkot, Narowal, Karachi, Okara, Talwandi, Bahawalnagar, Daska, Sargodha, Quetta and Kasur according to the quadrat method. The current study was initiated to investigate the potential electrophoresis for intra specific characterization of *Trigonella* on the basis of their total seed protein and explore distinctiveness of kasuri methi (landrace) with other genotypes of *Trigonella*.

For judgment of Kasuri methi as a G.I., seeds samples were divided into two groups. Group I consists of 22 seed samples of *Trigonella foenum graecum*-Methi and Group II comprises seed samples of *Trigonella corniculata*-Methray, along with Kasuri methi seed sample in both the groups.

Genetic diversity of *Trigonella* species evaluated by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the standard method (Leammli, 1970; Rabbani et al., 2001; Ahmed et al., 2009 and 2010; Turi et al., 2010; Cheema et al., 2010). The subunit molecular weight of the protein bands was determined according to Weber and Osborne (1969). Each band was considered as a character for which the presence or absence was coded 1 or 0 respectively in a data matrix for numerical analysis. Density of the protein band was not considered.

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Unweighted pair group method with an arithmetic average (UPGMA) cluster analysis was used to infer genetic relationships and phylogeny among the genotypes of *Trigonella*. All computations were done by using the NTSYS-pc, Version 2.2 package (Rohlf, 2005; Rabbani et al., 2001 and 2008). For an effective breeding programme, information regarding the extent and nature of genetic diversity within a crop species is essential. It is particularly very useful for characterization of individual accessions and as a guide in the selection of parents for hybridization programme.

## RESULTS AND DISCUSSION

Seed protein analysis by SDS-PAGE has proved to be an effective way of revealing the differences and relationship between taxa. The high stability of the seed protein profile and its additive nature make seed protein electrophoresis a powerful tool in elucidating the origin and the evolution of cultivated plants. The present investigation revealed variation in the evaluated 28 accessions of *Trigonella* species collected from various ecological zones of Pakistan (Table 1, Figure 1 and 2) and to make a distinction of Kasuri methi from Kasur with other genotypes through slab type SDS-PAGE using 12.25% polyacrylamide gel.

Initially, 28 seeds samples were divided into two groups. Group I, (22 samples of methi i.e., *Trigonella foenum graecum*) and group II (six samples of methray i.e., *Trigonella corniculata*) including seed sample of Kasur in both groups. The data were scored for the presence (1) and absence (0) of the bands and entered in a binary data matrix. Based on the results of electrophoretic band spectra, similarity index was calculated for all possible pair of electrophoregrams. The similarity matrix thus generated was converted to a dissimilarity matrix and used to construct the dendrogram. Studying genetic diversity and taxonomic relationships of plants previously scored for many plants (Bult and Kiang, 1992; Zviniene and Pank, 1996). Results of Group I showed that limited interspecific diversity was found at protein level. Three

major groups were observed on the dendrogram (Figure 1), where first and third group showed 100% similarity among themselves (including 1,2,3,4,5,19,18,17,16,15,14,6,7,8 and 20,21) while 23% deviation was observed in the second group (9,13,11,10 and 12) whereas the genotype No. 22, popularly known as "Kasuri Methi" from Kasur (22) stand alone and expressed itself as a G.I even at protein level.

The variable banding pattern shown by Kasuri methi made it different from other genotypes. As proteins are the translational products of genes therefore, the differences in proteins could be related with such differences in genes responsible for synthe-

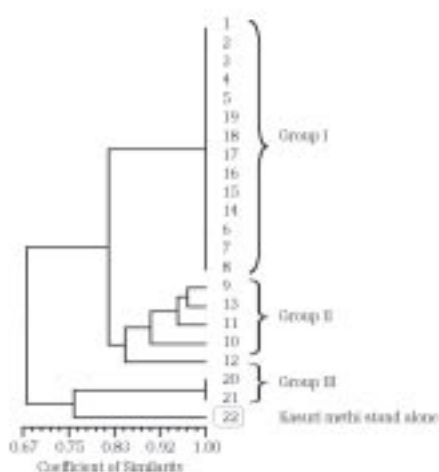
**Table 1. Passport data of *Trigonella* germplasm**

Accession	Town/city
<b>Group I</b>	
PAK 020978	Mainwali
PAK 021703	Pakpattan
PAK 022254	Bahawalpur
PAK 021131	Faisalabad
PAK 021156	Faisalabad
PAK 021330	Shorkot
PAK 021675	Narowal
PAK 021986	Karachi
PAK 021748	Karachi
PAK 021711	Narowal
PAK 021696	Okara
PAK 021117	Faisalabad
PAK 022260	Talwandi
PAK 022257	Bahawalnagar
PAK 022219	Daska
PAK 021908	Sargodha
PAK 021900	Sargodha
PAK 021889	Sargodha
PAK 021882	Faisalabad
Donated	Kasur
Donated	Kasur
Native/indigenous	Kasur
<b>Group II</b>	
21879	Faisalabad
20595	Faisalabad
21947	Karachi
21857	Quetta
20593	Faisalabad
11122	Native/indigenous of Kasur

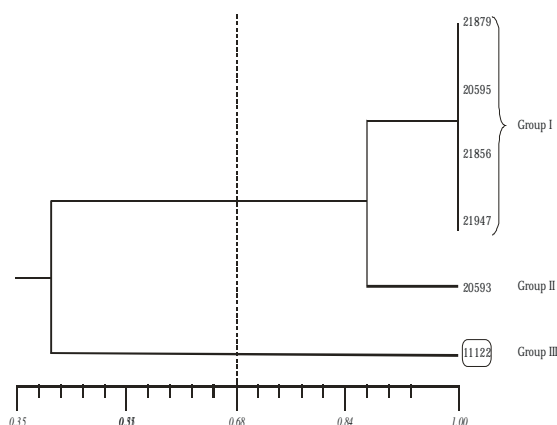
sis of these proteins. The molecular techniques including SDS-PAGE are very strong to determine among the biological organisms based on such molecular markers. Hence are very common to investigate the genetic diversity among the species, varieties and genotypes (Irfan, 2000; Ahmad and Kamal, 2002). The SDS-PAGE techniques have more advantages in the classification of genotypes (Abd-El-Zaher et al.,

2006). Inter and Intra- specific variation in SDS-PAGE electrophoregrams of total seed proteins in wheat, barley and their wild relatives was reported by Masood et al. (1994). Moreover, electrophoresis of seed proteins was previously used for cultivar identification for many other plants as *Vicia faba* (Stegmann et al., 1980) and *Linum usitatissimum* (Sammour, 1988). The technique had been utilized by Masood et al., (2005) for differentiation in different genotypes of wheat and mustard (Rabbani et al., 2001), Irfan (2000) in *Adhatoda vesica*, Ahmad and Kamal (2002) in *Hyppophae rhamnides*, *Vigna mungo* (Ghafoor et al., 2002), Mentha (Badr et al., 2003), *Raphanus sativus* (Jatoi et al., 2003), Pyrus (Ahmed et al., 2009), *Triticum aestivum* (Ahmed et al., 2010), Castor bean (Cheema et al., 2010), Brassica species (Turi et al., 2010).

Results of Group II explained by a similarity matrix based on the proportion of shared peptide fragments is used to establish the level of relatedness between Methi and Methray. Paired wise estimates of similarity ranged from 0.40 to 1. Total three groups emerged on the dendrogram (Figure 2). Group I showed 100% similarity among themselves. Group II is also the representative of accessions of Methray. Third independent group represent the accession collected from Kasur commonly known as Kasuri Methi. Comparative analysis of protein profile of Kasuri Methi with Methray (Group I and II) showed that it is 60% dissimilar (Figure 2). Accession 21879, 20595 belongs to Faisalabad, 21947 was from Karachi and 21856 from Quetta. The accession 20593 from Faisalabad depicted some distinctness as compared to other accessions from Faisalabad indicating intra species divergence. The similarity among the accessions from Faisalabad, Karachi and Quetta may be due to their common origin. Karachi and Quetta are not major agricultural production areas but due to high demand for Methi in local market the farmers may have obtained seeds from Faisalabad which has been a centre of agricultural research and production of ma-



**Figure 1. UPGMA cluster analysis showing the diversity among *Trigonella* landraces of Pakistan based on seed proteins with unique position of methi belongs to Kasur No.22**



**Figure 2. Comparative analysis of methray (*Trigonella corniculata*) with Kasuri Methi (11122)**

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for and minor crops.

While accession 11122 showing the isolated group among all belongs to Kasur. Kasuri Methi (Accession 11122) was very distinct as compared to other accessions evaluated in this study which shows its specific adaptation to Kasur environment and specificity of gene expression and gene ecology.

Comparative study of protein profile of *Trigonella* genotypes with Kasuri methi showed that diversity existed at inter and intra specific levels among 28 tested genotypes. However, the G.I of Kasur (Kasuri methi) revealed its complete distinctness as compared to other evaluated accessions from different ecological areas of Pakistan. These results clearly indicate that there is a need to patent the rights of farmer and promote benefit sharing.

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