Genetic Divergence and Path Analysis in Fenugreek

(*Trigonella foenum-graecum* L.)

eFkh ¼*VkbxksusyksQkbue&xzkde* ,y-½ e vkuof'kd foHkn ,o iFk fo'ys"k.k

ANIL SWAMI

Thesis

Master of Science in Agriculture

(Plant Breeding and Genetics)

2009

Department of Plant Breeding and Genetics
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Thesis

Submitted to the

Maharana Pratap University of Agriculture and Technology

Udaipur

In Partial Fulfilment of the Requirement for

The Degree of

Master of Science in Agriculture

(Plant Breeding and Genetics)

By

ANIL SWAMI

2009

Maharana Pratap University of Agriculture and Technology, Udaipur
Rajasthan College of Agriculture, Udaipur

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Dated: 21/11/2009

This is to certify that **Mr. Anil Swami** has successfully completed the Comprehensive Examination held on 12-03-2009 as required under the regulation for degree of **Master of Science in Agriculture.**

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This is to certify that the thesis entitled "**Genetic Divergence and Path Analysis in Fenugreek (*Trigonella foenum-graecum* L.)**" submitted for the degree of **Master of Science in Agriculture** in the subject of **Plant Breeding and Genetics**, embodies bonafied research work carried out by **Mr. Anil Swami** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The performance of the candidate in the oral examination on his thesis has been found satisfactory. The draft of this thesis was also approved by the advisory committee on 21/11/2009.

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ACKNOWLEDGEMENTS

It is a great privilege for me to express my esteemed and profound sense of gratitude to the major advisor **Dr. S.R. Maloo, Prof. & Head** Department of Plant Breeding and Genetics, Head, Department of Molecular Biology & Biotechnology and Associate Director Seeds, MPUAT, Udaipur, whose unceasing interest, constant endeavor and valuable guidance have made this work a success.

I cannot refrain to accord my humble thanks to **Dr. M.C. Vyas**, **Dr. N.K. Jain** and **Dr. T. Hussain,** members of my advisory committee for their untiring and genuine help, useful suggestions for the present investigation and kind encouragement during this study.

I am also indebted to **Dr. V.N. Joshi**, Dean, Rajasthan College of Agriculture, for providing adequate facilities for carrying out this work successfully. I wish to accord my warmest thanks to **Dr. Pushpa Seth, Dr. J.F. Munot** and **Dr. S.K. Khandelwal** members of Department of Molecular Biology & Biotechnology for their even willing generous help and valuable suggestions during the entire tenure of my study in the MBBT department.

I am also thankful to **Dr. Abhay Dashora** and other staff members of methi project, who helped me morally as well as materially during my research work.

I am also thankful to my seniors **Mr. Vishnu Goyal**, **Mr. Tara Chand Bochaliya**, **Mr. Vipul Gaur** and **Mr. Vineet Kaswan** for their moral support and kind patronage.

I have been indeed fortunate to have friends like **Vikas**, **Vikram**, **Rajesh Faujdar**, **Harjindra**, **Sanjeev**, **Ramprakas**, **Beerchand**, **Rupesh**, **Rajveer** and **Kuldeep** for extending their generous help and support during this entire course of work.

No words will be sufficient to express my gratitude to my parents **Shri Banwari Lal Swami** and **Smt Sumitra**, Sister **Babita**, Brother **Prem**, **Jeetu and Rajesh** and my father-in-law and mother-in-law for their blessings, continuous moral support and never ending sacrifices.

Still there are many more well-wishers, friends who directly or indirectly rendered me valuable help to complete this thesis academic endeavor, and I have deep sense of reverence for all of them.

Date:21/11/2009

Place: Udaipur **(Anil Swami)**

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1. INTRODUCTION

India is a land of seed spices. It is the largest producer, consumer and exporter of seed spices in the world. The global demand estimated for seed spices crop is 1, 50,000 m t, of which India contributes 83,550 m t annually accounting for 55.7 per cent of the total world trade (Malhotra *et al.* 2007).

Fenugreek (*Trigonella foenum-graecum* L**.)** is one of the important autogamous seed spices and belongs to the family *Fabaceae.* It is a diploid species with the chromosome number of 2n = 16. It is native of South Eastern Europe and South Western Asia. It is cultivated mainly in India, Pakistan, France, Argentina and North African countries (Thamburaj and Narendra, 2003).

Fenugreek ranks third in area and fourth in production among all seed spices grown in the country. In India, It is mainly cultivated in Rajasthan, Gujarat and Madhya Pradesh and to the limited extent in Andhra Pradesh, Tamil Nadu, Haryana, Maharashtra and Punjab covering an area of 53596 m ha with an annual production of 64102 m t. India exported 9050 m t of fenugreek valued Rs 1787.5 lakhs (Anonymous, 2007).

Rajasthan is considered as “fenugreek bowl” of the country and contributes about 90 per cent to the country's production. In Rajasthan, it is mainly grown in Sikar, Chittorgarh, Nagaur and Kota districts covering an area of 40495 m ha and produces 47228 m t with 1166 kg ha^{-1} productivity (Anonymous, 2007).

Fenugreek is a multipurpose crop and every part of its plant is utilized in one or the other forms. It is an annual herbaceous plant mainly cultivated for seed as well as leaves (fresh or dried). Seeds are rich in proteins (26.2 %) and used as spice for flavouring various food preparations, while young plants are used as vegetable, forage and concentrate for cattle. Its fresh tender leaves and pods are rich in iron, calcium, protein, vitamins (A and C) and essential amino acids. Besides this, it is an important ingredient in masala mixes, curry mixes and powders, pickle mixes etc. Being a legume seed spice, it has high nutritive value and used in the Middle and far East countries in preparation of meatless diets during social and religious function.

Medicinally, the seeds are carminative, aromatic, galactagogue and tonic. They are used externally in poultices for boils, abscesses and ulcers and internally as

emollient for inflammation of intestinal tracts. Thus, they are used as an adjuvant in pharmaceutical preparation and as an ingredient in several Ayurvedic medicines. This anti-diabetic herb can lower blood glucose and cholesterol. In fenugreek, the bitterness is attributed to the presence of two alkaloids, trigonelline and choline. Fenugreek seeds also contain diosgenin, a steroidal substance which is used for the synthesis of sex harmones and contraceptives. Diosgenin concentration varies from 0.68 to 2.2 per cent in seeds (Pruthi, 2001). Now days, the fenugreek seeds are regarded as a new potential source for the production of corticosteroid.

Fenugreek has got low productivity due to several reasons such as its cultivation on marginal lands with poor fertility, lack of improved varieties, susceptibility to disease like powdery mildew, wilt, root rot and poor adoption of improved agronomic package and practices. Inspite of multifarious importance of this crop, the attempts to improve its genetic potential are limited primarily because of narrow genetic variability in respect of various characters.

For developing improved varieties, exploitation of available genetic variability/diversity as well as knowledge of various associations between polygenic traits is important. Further genetic diversity among genotypes analysed for morphological/phenotypic level is subjected to the environmental fluctuation. To determine diversity and genetic relationship among genotypes more precise methodology at biochemical level viz., protein profile through SDS-PAGE has been found efficient and effective (Davis, 1964 and Sipra, 2000).

With this in view, an attempt has been made in fenugreek with following objectives to estimate:

i. Genetic variability, correlation and path coefficient for seed yield and its component characters,
ii. Genetic divergence through D^2 analysis, and its further confirmation through protein profiling through SDS- PAGE.

2. REVIEW OF LITERATURE

The genetic improvement of crop can be achieved by breeding high yielding varieties with the improved quality. A detailed knowledge of nature and magnitude of genetic diversity and its heritable portion in economic traits and information regarding inter-relationship among the component characters and direct and indirect contribution of important characters towards yield are the prime requisite of any efficient breeding programme. The findings related to the present investigation have been reviewed under following heads:

2.1 Variability parameters

2.2 Correlation and path coefficient analysis

2.3 Genetic divergence, and

2.4 Protein profiling through SDS-PAGE

2.1 Variability Parameters

A detailed study of the extent of variability for seed yield and its contributing characters is the prime requisite for an efficient breeding programme. Phenotypic variability includes both genotypic and environmental components of variation. Genotypic variation is due to genotypic differences among individuals within a population and is the main concern for plant breeders.

Shukla and Sharma (1978) studied 39 accessions of fenugreek and reported significant variability for days to 50 per cent flowering, plant height, pod length, seed weight and seed yield per plant.

Sharma and Bhati (1987) evaluated 12 cultivars of fenugreek (*Trigonella foenum-graecum* L.) for days to 50 per cent flowering, plant height, branches per plant, pods per plan, pod length, seeds per pod and seeds per plant. The highest mean seed yield was obtained from the cultivars NLM (Prabha), UM-34 and UM-35.

Kohli *et al*. (1988) evaluated 15 cultivars of fenugreek (*Trigonella foenum-graecum* L.) and reported significant variability for seed yield per plant, days to flowering, plant height, pods per plants and pod length. However, 100 seed weight showed non-significant variability.

Reddy and Reddy (1991) reported high coefficient of variation for pods per plant, test weight and seeds per pod. Seed yield had highest genotypic and phenotypic

coefficients of variation. Test weight and pod length had the highest and lowest heritability, respectively. Seed yield, plant height and pods per plant also exhibited high variability, while heritability was low for pod length and days to maturity. High genetic advance was recorded for seed yield, pods per plant, seeds per pod and test weight.

Arora and Lodhi (1993) recorded maximum range of variation for pods per plant followed by biological yield and minimum for harvest index and 100-seed weight while evaluating 20 diverse genotypes of *Trigonella foenum-graecum* L. Heritability and genetic advance were high for number of pods per plant and biological yield. They reported high heritability for days to 50% flowering, branches per plant, seeds per pod, biological yield, seed yield, harvest index and 100-seed weight.

Hariharan and Vijay Kumar (1997) evaluated 60 genotypes of fenugreek for variability of 18 component characters. Heritable variability in the breeding materials was recorded for seeds per pod, productive branches per plant, 100-seed weight, dry matter accumulation and seed yield per plant. This could be exploited for improvement through selection.

Kailash *et al.* (2000) evaluated 72 lines of fenugreek (*Trigonella foenum-graecum* L.) and recorded high variability for seed yield, pods and protein content. High broad sense heritability and genetic advance estimates were obtained for protein content and pods per plants.

Dash and Kole (2001) reported high broad sense heritability estimate for pods per plant, test weight, seeds per pod, pod length and days to flowering in 15 fenugreek genotypes. High heritability in conjunction with high to moderate genetic advance for pods per plant, seeds per pod and test weight indicated the predominant role of additive gene action for expression of these characters.

Kaushik and Dashora (2001) evaluated variability in 29 fenugreek lines and observed significant variability for days to flowering, plant height, pods per plant, seeds per pod, test weight, seed yield per plot and protein content. High genotypic and phenotypic coefficients of variation were observed for protein content, pods per plant, seed yield per plot and branches per plant. High broad sense heritability with high genetic advance was recorded for protein content and pods per plant.

Saha and Kole (2001) studied genetic variability in 15 genotypes of fenugreek and reported high phenotypic and genotypic coefficients of variation for biological and seed yield per plant and moderate for branches per plant, pods per plant and harvest index.

Kumar *et al*. (2002) studied 19 characters in 12 fenugreek cultivars and observed high heritability for pods per plant followed by 1000-seed-weight. They reported moderate to high genotypic coefficient of variation and genetic gain for pods per plant, 1000-seed weight, pod length and seeds per pod.

Rakesh and Korla (2003) reported high coefficient of variation for the economic and biological yield in fenugreek (*Trigonella foenum-graecum* L.). Heritability in the broad sense was high for plant height, seed vigour and pods per plant and moderate for 1000-seed weigh, branches per plant, total seedling length and germination percentage. Economical yield, biological and pod length exhibited low heritability.

Banerjee and Kole (2004) evaluated 22 genotypes of fenugreek (*Trigonella foenum-graecum* L.) and reported high phenotypic and genotypic coefficient of variation in fenugreek for stem weight, moderate for plant height, branches per plant, days to flowering, duration of flowering, test weight and low for pod length. High to moderate estimates of heritability coupled with moderate to high genetic advance were recorded for plant height, days to flowering, duration of flowering and test weight.

Datta *et al*. (2005) assessed 20 genotypes of fenugreek (*Trigonella foenum-graecum* L.) and observed high genotypic coefficient of variation for secondary branches, pods per plant and seed yield in fenugreek. Heritability and genetic advance were found maximum for the secondary branches per plant.

Kole and Mishra (2006) revealed high to moderate values for genotypic and phenotypic coefficients of variation for branches per plant, pods per plant, seeds per pod, 100-seed weight, pod weight, biological yield and seed yield per plant. High to moderate estimates of heritability coupled with high to moderate value of genetic advance were observed for 100-seed weight, pod weight and seeds per pod indicating predominating role of additive genetic effects. On the basis of variability the traits

such as pods per plant, seeds per pod, biological yield may be considered for the improvement of seed yield of fenugreek.

Prajapati *et al.* (2007) studied 64 genotypes of fenugreek (*Trigonella foenum-graecum* L.) and reported high estimates of phenotypic coefficients of variation, genotypic coefficients of variation, heritability and genetic advance for seed yield per plant, pods per plant and primary branches per plant in fenugreek.

Singh and Kaur (2007) evaluated 30 diverse genotypes of fenugreek for seed yield and quality attributes. Significant genetic differences were observed amongst genotypes for all the traits. Heritability estimates were high for the entire trait except for pod length.

2.2 Correlation and Path Coefficient Analysis

Correlation coefficient analysis measures the mutual relationships between various plant characters and determines the component characters on which selection can be based for improvement in yield. Further, path coefficient analysis is simply standardized partial regression coefficient which splits the correlation coefficient into the measures of direct and indirect effects of a set of independent variables on a dependent variable. Path analysis was initially suggested by Wright (1921) but was applied for first time in plant breeding by Dewey and Lu (1959).

Shukla and Sharma (1978) evaluated 39 accessions of fenugreek (*Trigonella foenum-graecum* L.) and reported moderate to high correlation of plant height, pods per plant and pod length with seed yield, whereas days to flowering showed negative association.

Pant *et al*. (1983) reported high positive association between pod length and seeds per pods, while high negative correlation was recorded for pod length with pods per plant in fenugreek.

Mehta *et al*. (1992) revealed that branches per plant and pods per plant had maximum association with seed yield. However, pods per plant recorded highest positive direct effect on yield.

Singh *et al.* (1993) determined correlation between seed yield and its components in 32 genotypes of fenugreek of exotic and Indian origin evaluated under two dates of sowing. Correlation analysis indicated that seed yield could be improved

by selecting for greater plant height, more tertiary branches, longer pods, more seeds per pod and greater seed weight.

Bajiya and Pareek (1996) reported that seed yield was positively correlated with pods per plant, seeds per pod and test weight.

Sade *et al.* (1996) observed that seed yield per plant was significantly correlated with branches per plant, pods per plant, pod length, seeds per pod and seed weight in fenugreek.

Lowanshi *et al.* (1998) carried out path coefficient analysis for different morphological and sink parameters in 16 varieties of fenugreek. The number of pods per plant had the highest direct effect on seed yield while low positive indirect effects were observed for pods per plant via-100 seed weight.

Kailash *et al.* (2000) showed through path coefficient analysis that the pods per plant, test weight and plant height were the most important traits that directly or indirectly influenced seed yield in fenugreek (*Trigonella foenum-graecum* L.).

Dash and Kole (2001) determined correlation and path analysis in 15 genotypes of fenugreek (*Trigonella foenum-graecum L*). The characters viz., pods per plant, seeds per pod, biological yield and harvest index showed significantly positive correlations with seed yield. Path coefficient analysis indicated direct positive effects of straw yield, test weight, seeds per pod and pods per plant on seed yield per plant.

Saha and Kole (2001) studied 15 genotypes of fenugreek. They observed significant positive phenotypic and genotypic correlations of days to flowering, plant height, pods per plant, pod length, seeds per pod, straw yield, biological yield and harvest index with seed yield per plant indicating the importance of these characters in the improvement of seed yield.

Kumar *et al.* (2003) observed that seed yield was positively and significantly correlated with plant height, pod length, seeds per pod in fenugreek

Mahey *et al.* (2003) evaluated 30 F_3 generation families of a fenugreek cross (UM-305 X UM-117) for seed yield and its components. Path coefficient analysis showed that plant height, numbers of pods per plant and pod length had positive direct effect at the genotypic and phenotypic level on seed yield per plant.

Ayanoglu *et al.* (2004) determined correlation and path analysis in 35 genotypes of fenugreek. Seed yield was positively correlated with pods per plant and negatively correlated with days to flowering. They revealed that the pods per plant, pod length, seeds per pod and 1000-seed weight had positive direct effect on seed yield whereas days to flowering, plant height and branches per plant had negative direct effect on seed yield.

Banerjee and Kole (2004) studied correlation in 22 genotypes of fenugreek. Seed yield was positively correlated with branches per plant, pods per plant, pod length, seeds per pod, seed weight, biological yield and harvest index at both phenotypic and genotypic levels. Days to flowering, pods per plant, pod length and seeds per pod were the important characters in determining seed yield in fenugreek.

Datta *et al.* (2005) studied 20 genotypes of fenugreek (*Trigonella foenum-graecum* L.) and showed that pods per plant had the highest direct effects on seed yield followed by the leaf yield and seeds per pod. The secondary branches per plant had high negative direct effects on seed yield.

Kole and Mishra (2006) assessed 20 genotypes of fenugreek (*Trigonella foenum-graecum* L.) and observed that branches per plant, pods per plant, seeds per pod, biological yield, and harvest index showed high positive correlation with seed yield while days to flowering, days to maturity and plant height had negative correlation with seed yield.

Prajapati *et al.* (2007) assessed 64 genotypes of fenugreek for direct and indirect effects on seed yield. Seed yield had positive and significant correlation with pods per plant, seeds per pod and 1000 seed weight. Path coefficient analysis revealed that pods per plant, days to 50 per cent flowering and test weight had the highest positive direct effects on seed yield while days to maturity and branches per plant had negative effects on seed yield.

Paramjit and Amardeep (2007) revealed that seed yield was significantly correlated with plant height, pods per plant, seeds per pod, 1000-seed weight, and harvest index. Path analysis revealed that plant height, pods per plant, seeds per pod, 1000-seed weight and harvest index had direct effects on seed yield.

Singh and Kaur (2007) reported that seed yield was found to be positively and significantly correlated with plant height, pods per plant, seeds per pod, 1000-seed weight and harvest index 30 diverse genotypes of fenugreek.

Singh *et al*. (2007) evaluated 38 genotypes of fenugreek (*Trigonella foenum-graecum* L.) and indicated that seed yield per plant had positive and significant association with pods per plant, seeds per pod and 100-seed weight in fenugreek. Similarly, path analysis for seed yield revealed that pods per plant had maximum direct positive effects followed by 100-seed weight, pod length and seeds per pod.

2.3 Genetic Divergence

The variability present among different genotypes of a species is known as genetic diversity. Genetic diversity arises due to geographical separation or due to genetic barriers to crossability. D^2 statistic found to be a powerful tool to measure genetic divergence among set of genotypes (Mahalanobis, 1936). This technique measures the forces of differentiation at two levels, namely intra-cluster and inter-cluster levels, and thus helps in selection of genetically divergent parents for exploitation in hybridization programme.

In addition to aiding in selection of divergent parents for hybridization, statistics measures the degree of diversification and determines the relative proportion of each component character to total divergence. The genotypes grouped together are less divergent than the one, which are separated by the greatest statistical distance, shows the maximum divergence.

Several statistical procedures using multiple measurements (Mahalanobis, 1936, Fisher, 1954, Rao, 1952) were developed to measure the divergence among populations. However, Mahalanobis's (1936) D^2 statistic, based on means and variances of populations, remains of considerable significance in several biological populations.

Mathur (1992) evaluated 50 genotypes of fenugreek (*Trigonella foenum-graecum* L.) for genetic diversity. There were significant differences between the genotypes for all the 10 characters studied. The genotypes were grouped in 15 clusters using multivariate analysis. The pattern of distribution of genotypes from different geographical regions into various clusters was random, suggesting that geographical distribution is not the factor responsible for genetic diversity.

Ali *et al*. (2000) evaluated 20 genotypes of coriander and revealed that cluster I contained the maximum of 13 genotypes belonging to different geographical origins. Cluster II contained 2 genotypes. The clusters III, IV, V, VI and VII contained one genotype each. Genotypes (CS-193 and Tikamgarh Local) were quite divergent and appeared promising for further improvement.

Kumar *et al.* (2000) studied 19 genotype of urdbean and revealed significantly genotypic differences which were classified in 5 clusters. Cluster I was the largest with 10 genotypes and cluster V contained only one most divergent genotype. The interaction distance was minimum between cluster I and cluster II and maximum between cluster II and cluster III.

Patel *et al*. (2000) reported 48 genotypes of coriander for genetic divergence, which were grouped into 9 clusters. Seed yield per plant had highest contribution towards genetic divergence followed by secondary branches and umbels per plant.

Kole and Mishra (2002) raised 20 genotypes of fenugreek (*Trigonella foenum-graecum* L.) and were grouped into 4 clusters. Cluster I was the largest with 15 genotypes and clusters III and IV were monogenotypic. Inter-cluster distance (D^2) was higher involving cluster IV than other combinations. Seeds per pod, 100-seed weight, grain yield, straw yield and branches per plant were the major characters contributing towards genetic divergence.

Kole *et al*. (2003) evaluated 15 genotypes of fenugreek for D^2 analysis which were grouped in to 4 different clusters. Cluster I had maximum number of 9 genotypes, while cluster IV was monogenotypic. The intra-cluster distance was maximum in cluster III and the inter-cluster distance was maximum in between clusters II and III. Pods per plant, straw yield, test weight, days to flowering, days to maturity and grains per pod contributed highly towards genetic divergence.

Banerjee and Kole (2004) raised 22 genotypes of fenugreek for genetic divergence for 8 quantitative characters, which were grouped into 6 clusters. Cluster l consists of the maximum number of 13 genotypes followed by cluster II and cluster III with 4 and 2 genotypes. 3 clusters were mono-genotypic in nature. The Inter-cluster distance was the highest between cluster III and cluster VI, while intra-cluster distance was the highest in cluster III. Plant height, pods per plant, days to flowering and test weight were the major source for divergence.

Vijayalatha and Chezhiyan (2005) raised 224 genotypes of coriander for genetic divergence, which were grouped into 3 clusters. Cluster I consisted of 130 types, cluster II had 2 types and cluster III retained 92 types. Genetic divergence was the largest between I and III and II and III respectively. Cluster III expressed the highest mean value for seed yield while cluster I exhibited the largest value for plant height, number of primary and secondary branches. Clusters II and I, which had the lowest divergence.

Jain *et al*. (2006) classified 36 genotypes of fenugreek into 6 clusters. There was no significant relationship between genetic diversity and geographical diversity. The Intra-cluster distance was the greatest in cluster I followed by cluster II. The inter-cluster distance was maximum between cluster IV and II followed by cluster III and cluster II. The results indicated that for obtaining heterotic response and better segregants, intermating between the genotypes of diverse cluster may be undertaken in breeding programme for improvement of yield and quality traits.

Paliwal and Jain (2006) classified 58 genotypes of ajowan into 7 clusters. There was lack of parallelism between genetic and geographic diversity. The inter-cluster D^2 values ranged from 16.41 to 45.23 suggesting considerable diversity among the groups of the genotypes. Among the seven characters studied for genetic divergence, fatty oil contributed the maximum, accounting for 56.29% of the total divergence followed by seed yield per plant. Based on cluster means, characters such as umbels per plant and umbellets per umbel were major factors of differentiation among genotypes, which may be taken into account while selecting parents for hybridization programmes.

Rao *et al*. (2006) evaluated 60 genotypes of mungbean (*Vigna radiata*) for 13 characters and observed considerable genetic divergence among the genotypes. They grouped these genotypes into 8 clusters. Days to maturity, 100 seed weight, pods per plant and total dry matter contributed maximum towards diversity.

Shanthi *et al.* (2006) studied genetic divergence for 60 urdbean genotypes and grouped them into 17 clusters. Among the characters studied the number of pods per plant had the greatest effect on genetic divergence followed by number of branches per plant and seed yield per plant.

2.4 Protein profiling through SDS-PAGE

Echart *et al.* (2000) analyzed the hordein polypeptide patterns of Brazilian barley varieties (*Hordeum vulgare*) and of two native species of *Hordeum* from southern Brazil (*Hordeum euclaston* and *Hordeum stenostachys*) by using SDS-PAGE 40 different hordein polypeptide bands with molecular weight ranging from 30 to 94 KD were found in the seeds of three species studied. 14 varieties examined showed intra-varietal polymorphism. The numbers of bands ranged from 10 to 17, depending on the variety, and from 3 to 13 among individual seeds, with a total of 26 bands in *Hordeum vulgare.*

Haider and Shanshoory (2000) studied relationship among 20 samples belonging to 6 sub-species of *Vicia sativa* based on the variability of seed storage protein and esterase isozyme. Electrophoresis protein profile of different accessions of same species showed identical pattern, confirming the stability of seed storage protein within these species esterase pattern revealed a sharp distinction for sub-species according to the number of loci of allelic bands.

Chand and Kole (2002) found SDS-PAGE analysis of seed albumins as protein markers to distinguish between CLS resistant and susceptible genotypes in mungbean. Total 23 polypeptide bands of wide range of RM values were obtained.

Pattnaik and Kole (2002) detected a seed protein marker to distinguish between MYMV resistant and susceptible genotype in mungbean by SDS-PAGE analysis of seed albumins. Differential expressions of polypeptide bands in resistant and susceptible genotypes were recorded.

Singh *et al.* (2002) reported variation in total protein and peroxidase activity among the 6 genotypes of *Catharanthus rosesus.* SDS-PAGE electrophoresis analysis revealed that polypeptide bands in all the parents were quantitatively identical with minor variations in the band pattern and intensity.

Goyal and Sharma (2003) distinguished 6 cluster-bean varieties by SDS-PAGE profile of seed protein. The band profile for one variety was clearly distinguishable from another. Such a biochemical approach offers a useful and rapidly performed adjunct to the more traditional method of varietal identification.

Licen *et al*. (2004) studied the endosperm protein polymorphism of 7 accessions of common buck wheat and 2 accessions of tartary buckwheat by SDS – PAGE electrophoresis. The results indicated significant higher protein polymorphism of endosperm within individuals accessions than among accession of common buck wheat.

Sultana *et al*. (2006) studied seed protein profile in 144 lentil accessions intensively collected from all over Pakistan. Heterogeneous population was isolated on the basis of SDS-PAGE and 13 polymorphic protein peptides were found representing almost all the variation reported so far in lentil. The low diversity of accessions from the Northern Area and North Western Frontier Province, the most geographically diverse areas, suggested the need for more exploration so that the maximum genetic diversity of the areas can be truly represented.

Nwabueze (2009) studied relationship among 43 cassava mosaic distance (CMD) resistant varieties. They reported distance coefficient generated between the 43 CMD resistant varieties ranged from 0.00 to 89.12. Six (6) distinct groups were identified at 0.97 coefficients. A dendrogram of the data indicated that cases with low distance are close together with a line linking them. It was observed that the line was a short distance from the left of the dendrogram indicating that they were agglomerated in a cluster at a low distance coefficient.

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3. MATERIALS AND METHODS

The present investigation entitled **"Genetic Divergence and Path Analysis in Fenugreek (*Trigonella foenum-graecum* L.)"** was carried during ***rabi,*** 2007-08 and 2008-2009 at Instructional Farm of Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur. Udaipur is situated at an elevation of 528.17 meter above mean sea level at a latitude of 24^{o} 34' North and longitude of 74^{o} 42' East.

3.1 EXPERIMENTAL MATERIAL

The experimental material consisted of 20 diverse genotypes of fenugreek which were received from different sources viz., Udaipur, Jobner (Jaipur), Jodhpur, Nagaur, Jhalawar (Rajasthan), Jagudan (Gujarat) and Coimbatore (Tamil Nadu). The details of the genotypes are given in Table 1.

3.2 EXPERIMENTAL DESIGN

These entries were planted earlier during *rabi,* 2007-08 in randomized block design with 3 replications by Fenugreek Project, Department of Plant Breeding and Genetics, Rajasthan College of Agriculture, Udaipur. These entries were again sown in *rabi,* 2008-09 in same design in 3 rows of 4 meter were planted for each entry with row to row and plant to plant spacing of 30 x 10 cm. All recommended agronomical practices and plant protection measures were adopted to raise the healthy crop in both the crop seasons.

3.3 CHARACTER STUDIED

(A) Field Studied:

The observations were recorded on following characters on 5 randomly selected competitive plants for each plot/replication except for days to 50 per cent flowering and days of 75 per cent maturity, where observations were recorded on plots basis. The detailed procedure is given below:

1. Days to 50 per cent flowering:

Number of days, from date of sowing to the date on which 50 per cent of plants completed the opening of first flowering was recorded on plot basis.

Table 1. Details of fenugreek genotypes

S. No.	Genotypes/strains	Source
1	NS 2006-1	Jobner, Rajasthan
2	NS 2006-2	Jobner, Rajasthan
3	NS 2006-3	Jobner, Rajasthan
4	NS 2006-4	Jobner, Rajasthan
5	NS 2006-5	Jobner, Rajasthan
6	NS 2006-6	Jobner, Rajasthan
7	NS 2006-7	Jobner, Rajasthan
8	UM 134	Baroda (Gujarat)
9	UM 152	Coimbatore (Tamil Nadu)
10	UM 163	Coimbatore (Tamil Nadu)
11	UM 189	Coimbatore (Tamil Nadu)
12	UM 202	Jagudan (Gujarat)
13	UM 353	Bhawani Mandi, Jhalawar
14	UM 354	Bhawani Mandi, Jhalawar
15	RMt 143	Jodhpur, Rajasthan
16	JFG 244	Jagudan (Gujarat)
17	RMt 1	Nagaur, Rajasthan
18	RMt 303	Mutant of RMt-1
19	RMt305	Mutant of RMt-1
20	RMt 351	Mutant of RMt-1

2. Days to 75 per cent maturity:

Number of days, from date of sowing to 75 per cent plants in each genotypes attained maturity was recorded

3. Plant height:

Height was measured in centimeters (cm) from the ground level to the apex of the main stem at maturity.

4. Branches per plant:

The total number of primary and secondary branches were counted in each selected plant.

5. Pods per plant:

The total number of pods were counted in each selected plant and averaged.

6. Pod length:

Length in centimeters (cm) of five randomly selected pods from each plant was measured and averaged.

7 Seeds per pod:

Total number of seeds were counted from five randomly selected pods in each selected plant and average was obtained.

8. 100-seed weight:

The weight of 100-seeds from each entry in each replication was recorded on electronic pan balance in grams.

9. Biological yield:

The randomly selected plants from each plot were harvested separately. These plants were sun dried, weighted in grams (g) and average value was workout to obtain biological yield per plant.

10. Seed yield per plant:

Total seeds obtained from five randomly selected plants of each entry from each replication were weighted separately and their average was recorded in grams.

11. Harvest index (%):

Harvest index is the ratio of seed yield to biological yield. It was calculated by using the following formula:

$$\text{Harvest index (\%)} = \frac{\text{Seed yield per plant (g)}}{\text{Biological yield per plant (g)}} \times 100$$

(B) Laboratory Studied:

12. Seed protein content:

Nitrogen content of seeds was estimated in duplicate by the standard micro-Kjeldahl method. Value of N so obtained was converted to crude protein percentage by multiplying with a factor 6.25.

13. Protein profile through SDS-PAGE:

Electrophoresis is mainly used to separate and characterize protein by applying electric current. Protein profile between the genotypes indicates a less diverse and more homogeneity or a close evolutionary relationship among them. Analysis of protein profile in their native form is carried out in polyacrylamide buffer gel. The classical disc electrophoresis using cylindrical gel has been described by Davis (1964). SDS-PAGE is undoubtedly versatile technique for the characterization of protein both quantitatively and qualitatively. Protein polymorphism by SDS-PAGE gives better information on genetic diversity and relationship among genotypes (Sipra *et al.* 2000). Electrophoresis method is relatively easy to carry out and provides rapid and authentic criterion for differentiating variation between different genotypes.

3.4 STATISTICAL METHODS

1. Analysis of Variance:

To test the variation among the genotypes analysis of variance was carried-out separately for each year and over pooled basis as per method suggested by Panse and Sukhatme (1985). Skelton ANOVA is given as under:

Source	d.f.	S.S.	MSS	Expected MSS
Replication	(r – 1)	A	al	$\sigma^2e + \sigma^2r$
Treatment	(g – 1)	B	bl	$\sigma^2e + \sigma^2t$
Error	(r – 1)(g – 1)	C	cl	σ^2e
Total	r.g. – 1			

Analysis of Variance pooled over years:

Source of Variation	d.f.	S.S.	M.S.	Expected M.S.
Environment	(s – 1)	SS_1	M_1	$\sigma^2 + r\sigma^2_{GE} + gr\sigma^2_E$
Rep./Env.	S(r – 1)	SS_2	M_2	$\sigma^2 + g\sigma^2_R$
Genotype	(g – 1)	SS_3	M_3	$\sigma^2 + r\sigma^2_{GE} + rs\sigma^2$
GXE	(g – 1) (S-1)	SS_4	M_4	$\sigma^2 + r\sigma^2_{GE}$
Pooled Error	S(g - 1) (r - 1)	SS_5	M_5	σ^2

Where, r = Number of replication, g = Number of genotypes, and

s = number of environments

Standard error for differences between treatment means was calculated as

$$SE\ (\text{diff.}) = \frac{\sqrt{2\,EMS}}{r}$$

Where,

EMS = Error mean sum of square for the experiment, and

r = Number of replication

Coefficient of variation was calculated as

$$CV = \frac{\sqrt{EMS}}{\overline{X}} \times 100$$

Where,

CV = Coefficient of variation, and $\overline{X}$ = Population mean

2. Estimation of variability parameters

(a) Genetic variability: It is the variance contributed by genetic causes or the genetic occurrence of difference among the individuals due to their genetic make up. It was calculated using following formula given by Panse and Sukhatme (1985).

$$Vg = \frac{MSV - VE}{r} = \frac{b1 - C1}{r}$$

Where,

Vg = Genotypic variance, MSV = Mean sum of square for varieties,

Ve = Error variance, and r = Number of replication

(b) Phenotypic variability: It is the sum of variances contributed by genetic causes and environmental factors and was computed as:

$$V_p = V_g + Ve$$

Where,

V_p = Phenotypic variance, V_g = Genotypic variance, and

V_e = Error variance

(c) Genotypic coefficient of variation (GCV): The magnitude of genetic variation existing in a character was estimated by the formula given by the Burton (1952).

$$GCV = \frac{\sqrt{V_g}}{\overline{X}} \times 100$$

Where,

V_g = Genotypic variance, and $\overline{X}$ = Population mean of the character

(d) Phenotypic coefficient of variation (PCV): The magnitude of phenotypic variation existing in a character was estimated by using the following formula (Singh and Choudhary, 1985).

$$PCV = \frac{\sqrt{V_p}}{\overline{X}} \times 100$$

Where,

V_p = Genotypic variance, and $\overline{X}$ = Population mean of the character

(e) Heritability: It is the proportion of total variability which is heritable in nature. It was estimated in broad sense by using following suggested by Burton and De Vane (1953) and Johnson *et al.* (1955).

$$H = \frac{V_g}{V_p} \times 100$$

Where,

H = Heritability in broad sense, Vg = Genotypic variance, and

V_p = Phenotypic variance

(f) Genetic gain: It is the percentage of expected genetic advance based on the mean of a computed using following formula suggested by Johnson *et al.* (1955).

Genetic advance as percentage of mean (Genetic gain) = $\frac{GA}{\overline{X}} \times 100$

Where,

GA = Genetic advance is the shift in a population towards superior side under some selection pressure. It was measured by the following formula suggested by Lush (1949) and Johnson *et al.* (1955) at 5 per cent selection pressure using the constant K as 2.06 given by Allard (1960).

$$GA = K \frac{V_g}{\sqrt{V_p}}$$

Where,

Vg = Genotypic variance,

Vp = Phenotypic variance, K = Selection differential at 5 per cent, and

$\overline{X}$ = Population mean of the character under study

3. CORRELATION COEFFICIENT:

Genotypic and phenotypic correlation coefficients of seed yield with its contributing characters and among themselves were calculated by using the genotypic and phenotypic variances and covariances values in the formula suggested by Fisher (1954) and Al- Jibouri *et al.* (1958). These genotypic and phenotypic covariances were worked-out between pairs of characters with the analysis techniques as used for variance calculation. Mean product expectations of covariance analysis are analogues of the mean square expectation of the analysis of variance.

(a) Genotypic correlation coefficient:

$$r_{xy}(g) = \frac{Cov.xy(g)}{\sqrt{Vx(g).Vy(g)}}$$

(b) Phenotypic correlation coefficient:

$$r_{xy}(p) = \frac{Cov.xy(p)}{\sqrt{Vx(p).Vy(p)}}$$

Where,

r_{xy} (g) = Genotypic correlation between x and y traits

r_{xy} (p) = Phenotypic correlation between x and y traits

$Cov._{xy}$ (g) = Genotypic covariance for x and y traits

$Cov._{xy}$ (p) = Phenotypic covariance for x and y traits

V_x (g) = Genotypic variance for x traits

V_y (g) = Genotypic variance for y traits

V_x (p) = Phenotypic variance for x traits

V_y (p) = Phenotypic variance for y traits

The significance of correlation was tested by formula –

$$t_{(n-2)} = r\sqrt{\frac{n-2}{1-r^2}}$$

Where,

r = Correlation coefficient

t = Test of significance, and

n = Total number of observations

The calculated value of 't' (cal.) was tested against the tabulated values of 't' with (n-2) d.f. at 0.1 and 0.5 per cent level of significance.

4. PATH COEFFICIENT ANALYSIS:

Path coefficient can be defined as the ratio of the standard deviation of the effect due to given cause to the total standard deviation of the effect i.e., if y is the effect and x is the cause, the path coefficient for the path from cause x_1 to the effect y_1 is $\sigma x_1 / \sigma y_1$.

The principles and techniques suggested by Wright (1921), Li (1955) and Dewey and Lu (1959) to assess direct and indirect effects of variable on seed yield as well as on seed protein content separately for two years and over pooled basis.

(i) For seed yield path coefficient were analyzed using nine characters viz., days to 50 per cent flowering, days to 75 per cent maturity, plant height, pods per plant, pod length, seeds per pod, 100-seed weight and biological yield per plant.

Nine simultaneous equations generated for seed yield were presented in matrix form and solved as per procedure given below:

$R_{1.}9$	$r_{1.}1\ r_{1.2}$ …….$r_{1.}9$	$P_{1.}9$
$R_{1.}9$	$r_{1.}1\ r_{2.2}$ …….$r_{2.}9$	$P_{2.}9$
.	…..	…
.	…..	…
.	…..	…
.	.....	.....
.	.....	.....
.	.....	.....
.	.....	.....
.	.....	.....
$R_{8.}9$	$r_{8.}1\ r_{8}2$ …….$r_{8.}2$	$P_{8.}9$

Or

A = B.C.

Value of C vectors were obtained as $C = B^{-1}.A$

Where B^{-1} is the inverse of mutual correlation matrix of character. The inversion matrix was carried out by Pivotal Condensation Method.

The residual effect was computed from the following algebraic relationship.

$$1 = R^2 + r_{1.}9\ P_{1.}9 + r_{2.}9\ P_{2.}9 + r_{3.}9\ P_{3.}9 + r_{4.}9\ P_{4.}9 + r_{5.}9\ P_{5.}9 + r_{6.}9\ P_{6.}9 + r_{7.}9\ P_{7.}9 + r_{8.}9\ P_{8.}9$$

$$R = \sqrt{1 - (r_{1.}9\ P_{1.}9 + r_{2.}9\ P_{2.}9 + r_{3.}9\ P_{3.}9 + r_{4.}9\ P_{4.}9 + r_{5.}9\ P_{5.}9 + r_{6.}9\ P_{6.}9 + r_{7.}9\ P_{7.}9 + r_{8.}9\ P_{8.}9)}$$

Where,

R = Residual effect

(ii) For seed protein content path coefficient was computed using eleven characters viz., days to 50 per cent flowering, days to 75 per cent maturity, plant height, pods per plant, pod length, seeds per pod, 100-seed weight and biological yield per plant, seed yield per plant and harvest index.

Eleven simultaneous equations generated for seed protein content were presented in matrix form and solved as per procedure given below:

$R_{1.}11$	$r_{1.}1\ r_{1.}2$ ……$r_{1.}11$	$P_{1.}11$
$R_{1.}11$	$r_{1.}1\ r_{2.}2$ ……$r_{2.}11$	$P_{2.}11$
.	…..	…
.	…..	…
.	…..	…
.	.....	…..
.	.....	…..
.	.....	…..
.	.....	…..
.	.....	…..
$R_{10.}11$	$R_{10.}1\ r_{10.}2$ ……$r_{10.}10$	$P_{10.}11$

Or

A = B.C.

Value of C vectors were obtained as $C = B^{-1}.A$

Where B^{-1} is the inverse of mutual correlation matrix of character. The inversion matrix was carried out by Pivotal Condensation Method.

The residual effect was computed from the following algebraic relationship.

$$1 = R^2 + r_{1.}11\ P_{1.}11 + r_{2.}11\ P_{2.}11 + r_{3.}11\ P_{3.}11 + r_{4.}11\ P_{4.}11 + r_{5.}11\ P_{5.}11 + r_{6.}11\ P_{6.}11 + r_{7.}11\ P_{7.}11 + r_{8.}11\ P_{8.}11 + r_{9.}11\ P_{9.}11 + r_{10.}11\ P_{10.}11$$

$$R = \sqrt{1 - (r_{1.}11\ P_{1.}11 + r_{2.}11\ P_{2.}11 + r_{3.}11\ P_{3.}11 + r_{4.}11\ P_{4.}11 + r_{5.}11\ P_{5.}11 + r_{6.}11\ P_{6.}11 + r_{7.}11\ P_{7.}11 + r_{8.}11\ P_{8.}11 + r_{9.}11\ P_{9.}11 + r_{10.}11\ P_{10.}11)}$$

Where,

R = Residual effect

5. GENETIC DIVERGENCE:

Genetic divergence was calculated for twelve characters by Mahalanobis D^2 statistics (1936). It is analyzed under following heads:

(i) Test of Wilk's criterion

(ii) Pivotal condensation method of transformation

(iii) Relative contribution of each trait and D^2 statistics, and

(iv) Group constellation

(i) Test of Wilk's criterion

A 12 X 12 common dispersion matrix was used for simultaneous test of significance of difference in mean values of characters from 20 varieties for each year using Wilk's criterion as described by Rao (1952) [Singh and Choudhary, 1985].

As there are 12 mutually correlated variables, it resulted into 12 sums of squares and 36 sum of product. Sum of squares were obtained from analysis of variance table and sum of product were calculated by:

$$\sum_{XY} = \frac{(\sum_X)(\sum_Y)}{n}$$

Where X and Y are two variables and n is the number of observations. Using these values dispersion table was constructed as follows:

Dispersion due to	**d.f.**	S.P. Matrix					
		X_1^2	X_2^2	X_3^2	X_1X_2	X_1X_3	X_2X_3
Replication	r – 1	A	B	C	d	e	f ...
Between treatment (Q)	Q	a'	b'	C'	d'	e'	f' ...
Within treatments (W)	Diff	A-(a+a')	B-(b+b')	C-(c+c')	D-(d+d')	E-(e+e')	F-(f+f') ...
Total	N	A	B	C	D	E	F

The Wilk's test is:

$$V = - m \log_c A$$

Where,

$$A = \frac{|W|}{|W + Q|} = \frac{\text{determinant of error S.S. and S.P. matrix}}{\text{determinant of (variety + S.S. and S.P. matrix)}}$$

and

$$m = n - \frac{q + k + 1}{2}$$

Where,

n = Total number of observations minus one

q = Number of variables minus one, and k = Number of characters under study

Using V as X^2 for 2qk degrees of freedom, probability was calculated

(ii) Pivotal condensation method of transformation

Let dispersion matrix of original variables X_1, X_2 ... X_p, be

$$\begin{bmatrix} \lambda_{11} \ldots\ldots\ldots \lambda_{lp} \\ \ldots\ldots\ldots\ldots\ldots \\ \lambda_{p^1} \ldots\ldots\ldots \lambda_{pp} \end{bmatrix}$$

and consider the extended matrix

$$\begin{bmatrix} \lambda_{11}.....\lambda_{lpx_1} \\ ..... \\ \lambda_{p}{}^{1}.....\lambda_{ppx_p} \end{bmatrix}$$

taking λ_{11} as the first pivotal element, where first row was replaced by

$$1 \ \frac{\lambda_{12}}{\lambda_{11}} \ ..... \ \frac{\lambda_{1p}}{\lambda_{11}} \quad \frac{X_1}{\lambda_{11}}$$

Sweeping out the first column and using the first pivotal row, reduced matrix was obtained as:

$$\begin{bmatrix} \lambda_{22}.....\lambda_{2p'}\lambda_{x_2'} \\ ..... \\ \lambda_{p2'}.....\lambda_{pp'}\lambda_{xp'} \end{bmatrix}$$

Where, $\lambda_{ij} = \lambda_{ij} - \frac{\lambda_{ij}}{\lambda_{11}}\lambda_{ij}X'_i = X_i - \frac{\lambda_{ij}}{\lambda_{11}}\lambda_{ij}X_1$

Now

$$V(X'_i) = V(X_i)\ \frac{2\lambda_{il}}{\lambda_{11}}\ \text{Cov}\ (X_iX_l) + \frac{(\lambda_{il})}{(\lambda_{11})}\ V\ (X_1)$$

$$= \lambda_{ii} - \frac{\lambda_{il}^2}{\lambda_{11}} = \lambda_{ii'}$$

Similarly $\text{Cov}\ (X'_i\ X'_i) = \lambda_{ij}$

also

$$\text{Cov}\ (X_1X'_i) = \text{Cov}\ (X_1X_i)\ \frac{\lambda_{11}}{\lambda_{11}}\ V\ (X_1)$$

$$= \lambda_{il} - \lambda_{il} = 0$$

So, the new variables were uncorrelated with the variable of the first pivotal row. Considering the second pivotal row,

$$\frac{\lambda_2{}^{3'}}{\lambda_2{}^{2'}}..... \ \frac{\lambda_2{}^{p'}}{\lambda_2{}^{2'}} \quad \frac{X'_2}{\lambda_2{}^{2'}}$$

Further, reduced matrix was obtained as:

$$\begin{bmatrix} \lambda_{33''}.....\lambda_{3p''}X_{3''} \\ ..... \\ \lambda_{p3''}.....\lambda_{pp''}X_{p''} \end{bmatrix}$$

Resulting into variables

$$X_1, X'_{2'}, X''_{3''} \ldots\ldots\ldots\ldots$$

$$\lambda_{11}, \lambda_{22'}, \lambda_{33''}, \ldots\ldots\ldots\ldots$$

They were all mutually uncorrelated as shown above and further X_2 depended on X_1 and X_2; X_3" on X_1, X_2 and X_3 and so on.

(iii) Relative contribution of each trait and D^2–statistics

Data of plot means were transformed into uncorrelated means by pivotal condensation method over pooled years. After analysis the difference of uncorrelated means between pairs for all the characters for all the pairs and numbers of first ranks were summed for every character. Finally, the percentage of first rank of each character was calculated.

D^2 values were analyzed by making sum of squares of the difference between uncorrelated means for each character and all the combinations of pairs (Rao, 1952; Singh and Choudhary, 1985).

$$D^2_p = \sum_{i=1}^{p} \sum_{j=1}^{p} W_{ij} \left(\overline{X}_{ij} - \overline{X}_{i2}\right)\left(\overline{X}_{j1} - \overline{X}_{i2}\right)$$

Where W^{ij} is the reciprocal matrix of W^{ij}.

$\overline{X}_{i1}$ and $\overline{X}_{i2}$ are two sample means of the i^{th} property and

(i, j = 1, 2, ... P).

Since, computation with this formula is cumbersome, hence transformation of character means of the variety into uncorrelated variables was performed. This made the D^2 values as a simple sum of square of differences in transformed values for various characters.

(iv) Group constellation:

There is no particular rule of grouping the clusters. Mostly any two varieties belonging to the same cluster show a smaller D^2 values than those belonging to two different clusters. K.D. Tocher (Rao, 1952; Singh and Chaudhary, 1985) suggested a technique which was used for grouping various varieties into different clusters. In this method the two populations having smallest distance from each other were considered first to which a third population having smallest average D^2 value from the first two populations is added. Similarly, the next population was added the process continued till the average D^2 value increased obviously. Generally, this increased level should be

approximately near to maximum D^2 value between any two populations in the first row of the table in which the D^2 values arranged in increasing order.

The spatial distances between clusters were arrived at by taking square root of the average D^2 values of intra and interclusters.

6. PROTEIN PROFILING:

(a) Extraction of water soluble protein from seeds:

The seeds were sterilized with 0.1 per cent mercuric chloride solution for 2 minutes and used for protein extraction. Seeds of fenugreek varieties were homogenized in glass pestle and mortar with phosphate buffer (0.2 M and pH 7.4) (Seth and Khandelwal, 2008) the homogenized was centrifuged at 5000 RPM for 15 minutes. The supernatant obtained was dialyzed, lyophilized and used for protein profiling.

(b) Dialysis:

Dialysis was carried out in semi-permeable dialyzing tubes having dimension of 15 cm long, 2.5 cm in diameter. The dissolved precipitate was dialyzed against distilled water. The dialysis was carried out for 24 hours with four change of water at interval of 6 hours. A small quantity of precipitate formed at this stage was removed by centrifugation.

(c) Lyophilization:

The frozen supernatants were lyophilized in separate petri plates to concentrate and dry them. Since material was very hygroscopic, it was to be handled very carefully. The dried samples were immediately packed in airtight bottles and concentration of protein was determined.

(d) Protein profile on sodium dodecyl sulphate polyacrylamide gel electrophoresis of storage seed protein:

Disc gel electrophoresis using sodium dodecyl sulphate to be obtained the ptotein banding pattern for the crude seed protein was done as method suggested by Davis (1964).

(i) Preparation of separating gel (10 %):

The 10 per cent gel was prepared by mixing 13.3 ml of stock acrylamide solution, 8 ml tris HCL (8.9) and 18.1 ml of water. To it 0.2 ml of ammonium per sulphate solution, 0.4 ml 10 per cent SDS and 20 µl TEMED were added.

(ii) Preparation of stacking gel:

The 3 per cent Stacking gel was prepared by addition of 4.0 ml of stock acrylamide solution, 2 ml tris HCL (6.7), 2ml of riboflavin solution and 8 ml of water. To it 0.1 ml of 10 per cent SDS and 20 µl TEMED were added.

(iii) Preparation of sample:

48 µl of extracted protein sample was mixed with 12 µl of sample buffer (5x) and heated in boiling water bath for 2-3 minutes to ensure complete interaction between protein and SDS. Sample was cooled to room temperature and 50 µl of sample was located in the well.

(iv) Casting of gel:

The glass plates and spacers were cleaned and dried, and then they were assembled properly.

- The separating gel solution was then poured between the glass plates leaving the space for stacking gel. Distilled water overlaid to form about a 2-5 mm layer and left undisturbed for 30 minutes at room temperature in light to polymerize.
- After polymerization of the separating gel, the water overlay was carefully removed by absorbing it with a piece of tissue paper. Stacking gel solution was poured and the comb was placed in stacking gel and the gel was allowed to set at room temperature in fluorescent light for 1 hr.
- After polymerization of stacking gel comb was removed carefully without disturbing the shape of wells. Wells were washed with distilled water using a syringe.
- The gel assembly was removed from the casting stand and fitted in to vertical electrophoresis unit.
- Prepared samples (50-60 µl) were then loaded in the wells and protein molecular marker (PMW-M molecular weight 14.3-66.0 kd purchased from Banglore genei) was also loaded in one well.
- Upper and lower tanks were then filled with electrode buffer upper tank was connected to cathode and lower tank was connected to anode. The voltage was adjusted to 50-75 V until the samples reached in

separating gel. Then continued the run at 100 V until the bromophenol blue reached the bottom of the separating gel.

- After completion of the electrophoresis, the gels were removed carefully and immersed in the staining solution (commasive brilliant blue) for 1 hr.
- After complete staining the gel was transferred in destaining solution. Dye that is not bound to the proteins is thus removed by the destaining solution and was changed many times until the background of the gel was clear.
- Photographs of the gels were taken directly on to the transilluminator.
- The photographs of SDS-PAGE gel was used to study the protein profile of all varieties. The bands were designated on the basis of their molecular weight. The presence of protein band was scored as '1' and its absence as '0'.

4. RESULTS AND DISCUSSION

In the present study, an attempt was made to estimate various genetical parameters, genetic divergence and protein profiling through SDS-PAGE in twenty genotypes of fenugreek (*Trigonella foenum-graecum* L.), which would not only help in framing an effective breeding programme but also results in making certain improvements in the yield level and quality of fenugreek. Further, this would lead to successful evaluation of genetically diverse and superior genotypes, which would be used as parents in hybridization programme.

Twenty genetically diverse genotypes of fenugreek were planted during winter crop seasons viz., *rabi*, 2007-08 (Y_1) and 2008-09 (Y_2) in randomized block design with three replications. The results obtained in the present investigation are discussed under the following heads:

4.1 Variability parameters

4.2 Correlation coefficient analysis

4.3 Path coefficient analysis

4.4 Genetic divergence, and

4.5 Protein profiling through SDS-PAGE

4.1 VARIABILITY PARAMETERS

Success of breeding programme is largely dependent on the extent of genetic variability present in the material, greater the diversity in the material, better the chance for evolving promising and desired types, since environment has a great influence on quantitative and qualitatively characters. The observed variability can be grouped under heritable and non-heritable components and this can be estimated by the parameters like genetic coefficient of variation (GCV), heritability (broad-sense) and genetic gain. This would help the breeder in developing and formulating selection programme for genetic improvement of crop.

Mean values of 20 fenugreek genotypes for various characters in two crop seasons viz., 2007-08 and 2008-09 are given in Appendix II, III, IV and V. In general based on the performance of crop for various characters, crop seasons 2007-08 appeared favorable as compared to 2008-09.

The mean performance of the best varieties/entries of each year as well as their consistent and high *per se* performance in each year and over pooled basis for 12 characters is given in Table 4. Wide range of variability was conspicuous for almost all the characters including seed yield. Varieties NS 2006-3, UM-134, UM-353 and NS 2006-1 exhibited high *per se* performance for seed yield and most of its components. Varieties RMt-1, RMt-303, NS 2006-3 and RMt-305 exhibited high *per se* performance for seed protein content. Therefore, these varieties could be gainfully utilized in breeding programmes.

Analysis of variance (Table 2) revealed significant differences among the genotypes for different characters indicating presence of sufficient variability in the material in each crop season. However, characters like days to 50 per cent flowering (Y_1), branches per plant (Y_1 & Y_2) and 100-seed weight (Y_1) were found non-significant.

Mean, standard error (S.E), range, genotypic and phenotypic coefficient of variation, heritability (broad sense) and genetic gain were analyzed separately for each year and over pooled basis are shown in Table 5.

Phenotypic coefficient of variation estimated was higher than genotypic coefficient of variation for all the characters but relatively little difference between PCV and GCV was recorded for days to 50 per cent flowering, days to 75 per cent maturity, pod length and seeds per pod in both years while for 100-seed weight (Y_1) indicated that the variability was primarily due to genotypic difference. For rest of the characters large differences between PCV and GCV existed suggesting the predominance of environmental differences hence selection based on such characters be performed carefully considering environmental factors.

High GCV and PCV were recorded for harvest index followed seed protein content for each year separately and over pooled basis as also reported by Kaushik and Dashora (2001). Moderate GCV as well as PCV were recorded for seed yield per plant followed pods per plant and plant height in each year separately and over pooled basis as also reported by Saha and Kole (2001) for pods per plant and harvest index, Banerjee and Kole (2004) for plant height, 100-seed weight and pod length and Kole and Mishra (2006) for 100-seed weight and seeds per pod in fenugreek.

On the other hand, character like days to 50 per cent flowering, days to 75 per cent maturity, pod length and seeds per pod showed low genotypic as well as phenotypic coefficient of variation indicating that these characters were highly influenced by the environmental fluctuations.

Estimates of genotypic and phenotypic coefficient of variation alone do not assess the amount of heritable variation which in turn can be estimated by heritability. High heritability in broad sense (above 75 %) was recorded for seed protein content, harvest index, seed yield per plant and biological yield per plant for at least one year and over pooled basis. Plant height, pods per plant, pod length and days to 75 per cent maturity exhibited moderate to high heritability for at least one year and over pooled basis. This indicated high transmission index for the characters. Similar findings were also reported by Dash and Kole (2001) for pods per plant and seeds per pod, Kaushik and Dashora (2001) for seed protein content and pods per plant, Arora and Lodhi (1993) for biological yield per plant, seed yield per plant and harvest index in fenugreek.

Burton (1952) suggested that GCV along with heritability estimates would give a better idea about the efficiency of selection as it simply depicts the amount of genetic variation, while heritability measures the proportion to which the variability of a character is transmitted to its progenies. However, Johnson *et al*. (1955) suggested that variability and genetic advance when calculation together would become more useful in predicting the resultant effect of selection on phenotypic expression.

While assessing over all position in each year separately and over pooled basis, the present study revealed high genetic advance as percentage of mean (genetic gain) along with high estimates of heritability and GCV for seed protein content and biological yield per plant. While, moderate GG and GCV with high heritability for pods per plant, seed yield per plant and plant height. Similar to the present findings high estimates of GCV, GG and heritability were also reported by Arora and Lodhi (1993) and Kaushik and Dashora (2000) in fenugreek. These characters might be exhibiting predominance of additive gene effects, thereby selection for these traits would be effective for genetic improvement of seed yield in fenugreek.

On the other hand, characters like days to 50 per cent and days to 75 per cent showed low GCV and GG and moderately high heritability. Further pod length and

seeds per pod exhibited low GCV, GG and heritability, hence these characters seemed to be greatly affected by environment and strong evaluation programme is to be taken for their exploitation.

4.2 CORRELATION COEFFICIENT ANALYSIS

The knowledge of genetic correlations for grain yield, its components and various quality characters become very important when the breeder is confronted with problem of combining high yield potential with desirable agronomic and quality parameters. Association studies would provide reliable information on nature, extent and direction of selection.

Genotypic and phenotypic correlation coefficients of different characters with seed yield and among themselves were estimated in the present study for each year separately using variance techniques.

Table 6 clearly indicated that, in general, a close agreement existed between genotypic and phenotypic correlation for almost all characters in two years. It was further noted that characters exhibited slightly higher genotypic correlations in comparisons to their corresponding phenotypic correlations in all the two years of experimentation. This revealed that the environmental factors affected both variables taken at a random indicating lack of correlations at environmental level.

In the present study as obvious from Table 6, seed yield per plant was positively correlated only with plant height, branch per plant biological yield per plant and harvest index at genotypic and phenotypic level in at least one year and over pooled basis as also reported by Sade *et al*. (1996), Kumar *et al*. (2003) and Banerjee and Kole (2004) for above characters.

It is interesting to note that seed protein content exhibited negative correlation with seed yield per plant at genotypic as well as phenotypic level in Y_1 and over pooled basis. Plant height showed significant positive correlation with days to 50 per cent flowering, pod length and biological yield per plant at genotypic and phenotypic level in at least one year and over pooled basis as also reported by Saha and Kole (2001) and Prajapati *et al*. (2007). Seed yield showed negative correlation with days to 75 per cent maturity, pods per plant, seed per pod, branches per plant, pod length, biological yield per plant and harvest index at genotypic and phenotypic level in both years and over pooled basis as also reported by Pant *et al.* (1983) and Berwal (1996).

Variable results were obtained with respect to mutual correlations between different characters in two years separately and over pooled basis (Table 6). Character like Plant height, days to 50 per cent flowering, pod length and biological yield per plant showed strong association with each other at genotypic and most of the characters at phenotypic level in two years and over pooled basis as also reported by Saha and Kole (2001).

Seed protein content showed significant positive genotypic correlation with days to 50 per cent flowering over pooled basis and branch per plant in Y_2 year, whereas negatives correlation with plant height, pod length, seeds per pod and harvest index index at genotypic and phenotypic level in both years and over pooled basis. 100-seed weigh showed significant positive genotypic correlation with days to 50 per cent flowering, pods per plant, biological yield per plant and harvest index in both the years separately as also reported by Kole and Mishra (2006). Biological yield showed significant positive correlation with plant height, pods per plant, pod length, 100-seed weight and harvest index at genotypic and phenotypic level in both years and over pooled basis.

On the basis of association studies, it could be concluded that seed yield per plant in fenugreek was correlation with plant height, branch per plant and biological yield per plant. On the other hand plant height was positively correlated with days to 50 per cent flowering, pod length and biological yield per plant. Most of these characters were also mutually correlated. Hence, Simultaneous selection for all these would result in the genetic improvement of seed yield in fenugreek.

4.3 PATH COEFFICIENT ANALYSIS

Path coefficient analysis is an effective method provides cause and effect relationship for understanding yield behavior of genotypes of population. Correlation estimates are known to vary from environment to environment because of differential gene expression in different environment. Hence, the direct and indirect effect is also get affected by environment with this in view, the present investigation was carried out in two years to find out the direct and indirect effects of yield attributes on seed yield and seed protein content.

Path coefficient analysis was computed for seed yield per plant and seed protein content in each year and over pooled basis using genotypic correlations.

(a) PATH ANALYSIS FOR SEED YIELD PER PLANT

The characters studied were days to 50 per cent flowering, days to 75 per cent maturity, plant height, pods per plant, pod length, seed per pod, 100-seed weight and biological yield per plant.

As observed from the correlation studies plant height and biological yield per plant were associated with seed yield per plant at genotypic level and most of the cases at phenotypic level in each year separately and over pooled basis. Table 7 revealed direct and indirect contribution of component traits on seed yield per plant. Maximum direct effects on seed yield were recorded by plant height, pods per plant and pod length in two years separately and over pooled basis as also reported by Mahey *et al*. (2003) and Ayanoglu *et al*. (2001).

Significant and positive correlation of plant height with seed yield per plant was mainly due to its direct effect and indirect effect via pods per plant, biological yield per plant, seeds per pod and pod length in at least one year as also reported by Paramjit and Amardeep (2007). Biological yield per plant exhibited strong positive correlation with seed yield per plant which was mainly due to direct effect and indirect effect via pods per plant, plant height and days to 75 per cent maturity as also reported by Banerjee and Kole (2004).

The values of residual effect (0.39 Y_1, 0.33 Y_2 & 0.51 P) revealed that variability (61% Y_1, 67% & 49% P) was due to the variability in characters under study.

Hence from path analysis studied for seed yield per plant, it could be concluded that plant height followed by biological yield per plant, pods per plant, seeds per pod and days to 75 per cent maturity contributed to seed yield per plant directly as well as indirectly. Therefore, due emphasis should be placed on these characters while breeding for high yield in fenugreek.

(b) PATH ANALYSIS FOR SEED PROTEIN CONTENT

The characters studied were days to 50 per cent flowering, days to 75 per cent maturity, plant height, pods per plant, pod length, seed per pod, 100-seed weight, biological yield per plant, seed yield per plant and harvest index.

Direct and indirect contribution of component traits on seed protein content in each year and over pooled basis are presented in Table 8. Maximum direct effects on seed protein content were recorded by days to 50 per cent flowering in Y_2 and seed yield per plant in Y_2. These characters showed positive genotypic correlations and high direct effects because of their indirect contribution through one another.

Days to 50 per cent flowering showed positive direct effects and it was positively correlated with seed protein content in Y_2 the environment and over pooled basis. Seed yield per plant showed negative indirect effects with seed protein content in at least one year and over pooled basis as also reported by Ayanogly *et al*. (2004). Negative effects of indirect contribution to majority of characters towards negative direction and its significant negative association with seed protein content.

Days to 50 per cent flowering exhibited positive correlation and high direct effect followed by seed yield per plant on seed protein content because of indirect contribution through harvest index and days to 75 per cent maturity in at least one year and over pooled basis. Seed yield per plant exhibited positive correlation and direct effect on seed protein content due to its indirect contribution through harvest index and seeds per pod in at least one year and over pooled basis.

The values of residual effect (0.66 Y_1, 0.52 Y_2 & 0.71 P) revealed that variability (34% Y_1, 48% Y_2 & 29% P) was due to the variability in characters under study. Hence from the path analysis studied for seed protein content it could be concluded that seed yield per plant, days to 50 per cent flowering, harvest index and seeds per pod contributed to seed protein content directly as well as indirectly. Therefore due emphasis showed be placed on these characters while breeding for high seed protein content in fenugreek.

Therefore on the basis of path coefficient analysis, plant height, biological yield per plant, pods per plant and seeds per pod contributed to seed yield directly as well as indirectly. Further, days to 50 per cent flowering, seed yield per plant, harvest index and seeds per pod were the main contributory characters for seed protein content.

4.4 GENETIC DIVERGENCE

In any crop breeding programme, genetic diversity is an essential pre-requiste in selecting parents for hybridization and evolving high yielding genotyping. The concept of D^2 was originally developed by P.C. Mahalanobis in 1928 but the application of this technique for the assessment of genetic diversity in plant breeding was suggested by Rao (1952). Higher the genetic diversity between the parents greater are the chances of achieving transgressive segregants, progenies derived from diverse cross are expected to show broad spectrum of genetic variability, providing greater scope for isolating high yielding segregates in advance generation. D^2 statistics is a potential tool for obtaining quantitative estimates of divergence between biological populations and has extensively been applied to assess diversity.

Mahalanobis (1936) D^2 statistics, based upon the mean and variance of the population was used to work out genetic divergence between 190 possible pairs of 20 varieties of fenugreek over pooled basis based on all the 12 characters viz. days to 50 per cent flowering, days to 75 per cent maturity, plant height, number of branch per plant, pods per plant, pod length, seeds per pod, 100-seed weight, biological yield per plant, seed yield per plant, harvest index and seed protein content. The results obtained are presented as under:

(i) Wilk's test:

Using V-statistics which in turn, utilizes wilk's criterion, a simultaneous test for divergence in 20 varieties with respect to characters studied was conducted. Values of V-statistics (826.37) which followed chi-squares distribution with (228) degree of freedom showed highly significant difference among the varieties for aggregate of all characters. This indicate that sufficient diversity was present among 20 varieties of fenugreek which ranged from 6.55 (between NS 2006-3 and UM 134 of cluster I) to 141.88 (between NS 2006-5 of cluster II and RMT-1 of cluster VI) as evident from Table 9 in which D^2 values (arranged in ascending order) are presented.

(ii) Composition of clusters:

Twenty varieties were grouped into 9 clusters on the basis of observed smaller D^2 values among varieties within a cluster as compared to varieties in other clusters. cluster I contains maximum number of genotypes i.e. 6 followed 4 in cluster II, 3 in cluster III, 2 in cluster IV and 1 in cluster V, VI,VII, VIII and IX. The clustering

pattern revealed that in general varieties from same original showed no tendency to be in same cluster.

Looking to the pattern of varietal distribution into different clusters in the present study, it appeared that geographical distance between the varieties had no relation with the genetic divergence as the varieties from same source had fallen into different clusters as well as the same cluster contained varieties from different sources.

(iii) Intra and inter-cluster Divergence:

Average inter-cluster values were maximum between cluster VI and cluster IX (105.31) and minimum between clusters III and V (25.49) at intra-cluster level maximum values was recorded for cluster III (22.21) followed by cluster I (20.79), cluster IV (18.79) and cluster II (17.46) while cluster V, VI, VII, VIII and IX values were 0.00 respectively. The spatial distance between clusters were arrived at by taking squares root of the average D^2 values if intra and inter-clusters. This has been highlighted through Table 12. Jain *et al*. (2006) also reported maximum and minimum inter and intra-cluster distance.

(IV) Contribution of characters to Divergence:

As evident from Table 13, branches per plant ranked first followed by 100-seed weight, harvest index and days to 75 per cent maturity.

The utility of multivariate analysis for measuring the degree of divergence between biological population and for assessing the relative contribution of different characters was established by Banerjee and Kole (2004). The importance of genetic divergence and its use in manifestation of heterosis is obvious. Maximum amount of heterosis and variability will be manifested in a cross involving parents belonging to most divergent cluster.

On the basis of divergence studies in fenugreek and its clustering pattern for seed yield and its component traits revealed that genetic diversity did not show strong association with geographical diversity characters like number of branches per plant, 100-seed weight, harvest index and days to 75 per cent maturity appeared promising. 20 varieties of fenugreek were grouped into 9 clusters and clusters VI and IX had maximum inter-cluster distance, therefore, the genotypes belonging to their clusters namely RMt-1 and RMt-305 having high *per se* performance could be utilized in breeding programme.

4.5 PROTEIN PROFILING

Protein profile is a powerful tool to distinguish genetic diversity and relationship among the genotypes (Sipra, 2000). It appeared to be a suitable method which covers more precise information regarding the variation due to genes as this is relatively less influence by the environment (Rao *et al*., 2001). Protein profile obtained on SDS-PAGE with 10 per cent gel was also used to find out genetic relationship among the genotypes.

4.5.1 Preparation of protein samples:

Soluble seed proteins from seeds of fenugreek genotypes were extracted by using 0.2 M phosphate buffer (pH 7.4). The extract was dialyzed against distilled water and followed by layophilization. The layophililzed sample weighed and dissolved in buffer solution.

The protein solution was subjected to 10 per cent polyacrylamide gel was used to resolved polypeptides through SDS-PAGE with a protein molecular weight marker [PMW-M (14.3-66.0 kd) from Banglore Genei].

4.5.2 Genetic relationship among the genotypes and cluster analysis:

The molecular weight of 88 resolved polypeptides ranged from 14 kd to 98 kd in 20 genotypes. The polypeptide having molecular weight 14 and 20 kd appeared in all the genotypes, while 94, 66, 43 and 30 kd were appeared only in $G_{18,}$ G_2, G_4 and G_{12} respectively. The numbers of scorable bands were found to be 15 and out of these 4 were polymorphic. Maximum and minimum bands were recorded in G_{19} (RMt 305) and G_9 (UM 152) respectively. The band which was having the highest MW of 98 kd, was found in genotypes $G_{4,}$ $G_{5,}$ $G_{6,}$ $G_{12,}$ $G_{13,}$ G_{19} and G_{20} respectively. While the lowest MW of 14 kd was present in all the genotypes.

Hierarchical agglomeration schedule coefficient based on Square Euclidean Distance Measure (SEDM) was used for cluster analysis to genetic relationship Nwabueze (2009). The dendrogram is a hierarchical tree diagram which shows the relative size of the proximity coefficient at which genotypes were combined. Genotypes with low distance are close together with a line linking them. A short distance from the left of the dendrogram indicates that they are agglomerated into a cluster at a distance coefficient indicating similarity. The coefficient generated between the 20 fenugreek genotypes ranged from 0.00 to 4.29 (Table 15). Figure 3

showed the dendrogram of the pattern of clustering among the varieties with connecting lines further to the right indicating more distance between genotypes and clusters.

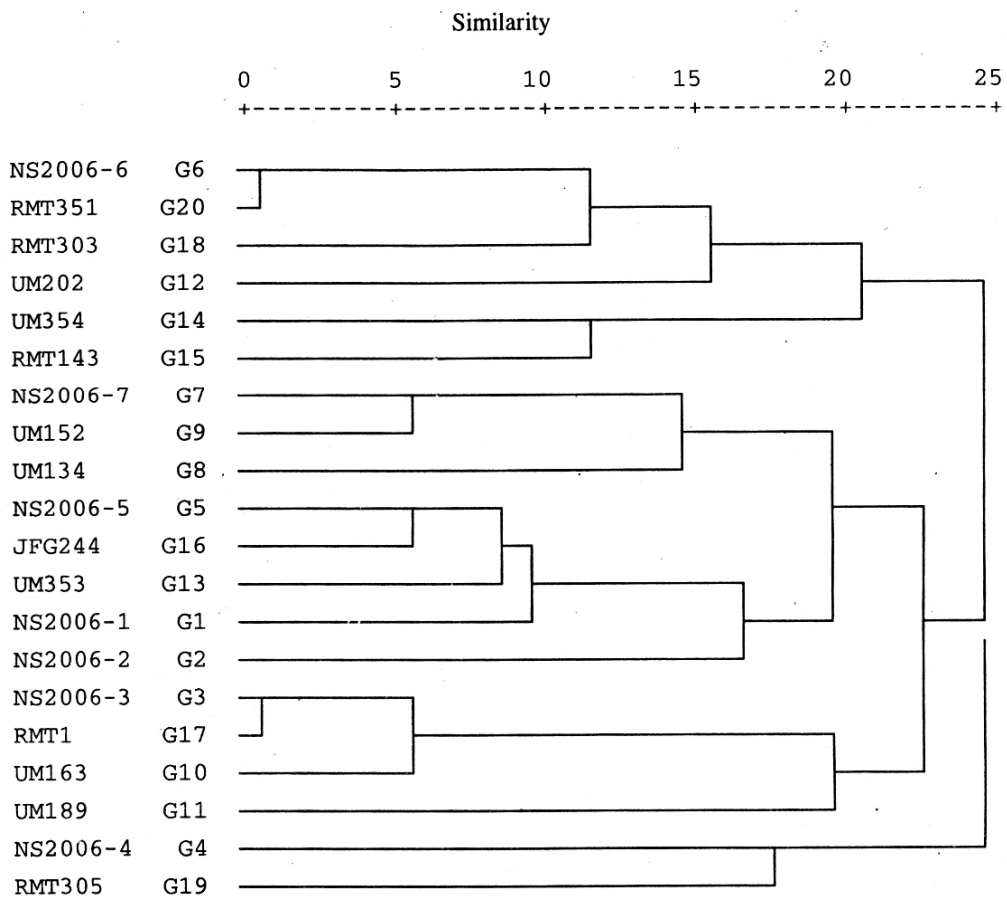
The dendrogram clearly indicated 3 main clusters. Cluster I included genotypes viz., G_6/G_{20}, G_{18}, G_{12}, G_{14}, and G_{15}. It contained 4 subclusters. G_6 and G_{18} were related to each other with 2.00 agglomeration coefficient. Cluster II included G_7, G_9, G_8, G_5, G_{16}, G_{13}, G_1, G_2, G_3, G_{17}, G_{10}, and G_{11}. G_7 related to G_9, G_5 related to G_{13} and G_3 related to G_{10} genotypes with 1.00, 1.5 and 1.00 agglomeration coefficients, respectively. In cluster III fenugreek entries included were G_4 and G_{19} with 3.00 agglomeration coefficients. Minimum distance was between G_6 (NS 2006-6) and G_{20} (RMt-351), G_3 (NS 2006-3) and G_{17} (RMt-1) and maximum distance was between G_1 (NS 2006-1) and G_4 (NS 2006-4) genotypes of these stages exhibiting high *per se* performance, which could be utilized in breeding programme.

Table 15. Hierarchical agglomeration schedule coefficient for fenugreek genotypes using Square Euclidean Distance Measure (SEDM)

	Cluster Combined		Coefficients	Stage Cluster		Next Stage
Stage	Cluster I	Cluster II		Cluster I	Cluster II	
1	6	20	0.00	0	0	8
2	3	17	0.00	0	0	4
3	5	16	1.00	0	0	6
4	3	10	1.00	2	0	15
5	7	9	1.00	0	0	10
6	5	13	1.50	3	0	7
7	1	5	1.67	0	6	12
8	6	18	2.00	1	0	11
9	14	15	2.00	0	0	16
10	7	8	2.50	5	0	14
11	6	12	2.67	8	0	16
12	1	2	2.75	7	0	14
13	4	19	3.00	0	0	19
14	1	7	3.27	12	10	17
15	3	11	3.33	4	0	17
16	6	14	3.50	11	9	18
17	1	3	3.88	14	15	18
18	1	6	4.19	17	16	19
19	1	4	4.29	18	13	0

On the basis of D^2 analysis, 20 genotypes of fenugreek were grouped into 9 clusters. Further, the clustering of genotypes based on morphological diversity i.e. D^2 analysis and biochemical analysis viz., protein profiling is not fully concurrent which might be on account of their genetic make-up and expression under specific environmental situations. While critically examining the protein profile pattern along with D^2 analysis (Fig 3, Table 15), it was observed that genotypes RMt-303 was present in cluster I in both cluster patterns. Further genotypes viz., NS 2006-5, UM-152, UM-163 and JFG-244 were also present in cluster II in both cluster patterns and similarly NS 2006-4 was also present in cluster III in both cluster patterns. Therefore, these genotypes appeared divergent as well as superior with respect to their *per se* performance, hence could be efficiently utilized in future breeding programmes.

Fig.3 Dendogram generated for fenugreek genotypes using UPGMA cluster analysis based on hierarchical agglomeration coefficient (protein profile)



5. SUMMARY

The present study entitled **"Genetic Divergence and Path Analysis in Fenugreek (*Trigonell foenum-graecum* L.)"** was undertaken with the specific objectives to estimate variability parameters, correlation, path analysis, genetic divergence. Protein profiling of genotypes was also obtained through SDS-PAGE.

The experimental material consisted of 20 diverse genotypes/strains of fenugreek which were received from different sources. The material was planted during two crop seasons viz., *rabi*, 2007-08 and 2008-09 at Instructional Farm of Rajasthan College of Agriculture, Udaipur. The experiment was conducted in randomized block design with three replications following uniform and recommended agronomic practices to raise the healthy crop during both the crop seasons.

Observations were recorded on five randomly selected competitive plants in each replication for 12 characters viz., plant height, branches per plant, pods per plant, pod length, seeds per pod, 100-seed weight, biological yield per plant, seed yield per plant, harvest index and seed protein content except days to 50 per cent flowering and days to 75 per cent maturity.

Variability parameters, correlation and path coefficient were computed for each of the two years separately and over pooled basis. While genetic divergence was analyzed using the method proposed by Mahalanobis (1936) over pooled data.

1) (i) On the basis of the performance of crop for various traits, crop seasons 2007-08 appeared relatively favorable as compared to *rabi*, 2008-09.

   (ii) Superior strains NS 2006-3, UM-134, UM-353 and NS 2006-1 were identified on the basis of their consistent *per se* performance in both years and over pooled basis for seed yield and its components. Similarly, NS 2006-3, RMt-1, RMt-303 and RMt-305 exhibited high *per se* performance for seed protein content. Fenugreek entry NS 2006-3 displayed superiority for seed yield as well as seed protein content.

2. (i) Analysis of variance showed presence of sufficient variability among the genotypes for all the characters studied. Characters like days to 50 per cent flowering (Y_1), branches per plant (Y_1 & Y_2) and 100-seed weight (Y_1) were non-significant.

(ii) Harvest index and seed protein content exhibited high estimates of genotypic and phenotypic coefficients of variation for each year separately and over pooled basis, while, moderate GCV and PCV were recorded for seed yield per plant, pods per plant and plant height in each year separately and over pooled basis.

(iii) High heritability estimates (broad sense) were recorded for seed protein content, harvest index, seed yield and biological yield per plant in each year separately and over pooled basis.

(iv) High genetic advance as per cent of mean (genetic gain) along with high estimates of heritability and GCV were recorded for seed protein content (Y_1 & Y_2) and biological yield per plant (Y_1 & Y_2), while, moderate GG and GCV with high heritability for pods per plant (Y_1 & Y_2), seed yield per plant (Y_1) and plant height (Y_2).

3. (i) Association studies revealed a close agreement between genotypic and phenotypic correlations. The genotypic correlations were slightly higher than their respective phenotypic correlations.

(ii) Seed yield per plant showed positive association with plant height, branches per plant, biological yield per plant and harvest index at genotypic and phenotypic level in at least one crop season and over pooled basis. Similarly, plant height showed significant positive genotypic correlation with days to 50 per cent flowering, pod length and biological yield per plan at genotypic and phenotypic level. Most of these characters were mutually correlated.

4. Path analysis was computed for seed yield and seed protein content separately for each crop seasons.

(i) Path analysis for seed yield revealed high direct contribution of plant height followed by indirect contribution of pods per plant, biological yield per plant, seeds per pod and pod length.

(ii) Path analysis for seed protein content depicted high direct contribution of seed yield per plant followed by indirect effects of harvest index and seeds per pod.

5. (i) D^2 values ranged from 6.55 to 141.88 and 20 fenugreek entries were grouped into 9 clusters. Cluster I had maximum number of genotypes (6

genotypes), followed by cluster II (4 genotypes), cluster III (3 genotypes), cluster IV (2 genotypes) and one in cluster V, VI, VII, VIII and IX.

(ii) Inter-cluster distance was maximum between cluster VI and IX. While intra-cluster distance was maximum in cluster III followed by cluster I, cluster IV and cluster II respectively. Characters contributing maximum to divergence were branches per plant, 100-seed weight, harvest index and days to 75 per cent maturity.

(iii) On the basis of divergence studied, it was concluded that there was no relationship between genetic and geographical diversity. Genotypes of these clusters with high *per se* performance could be utilized in breeding programmes.

6. (i) The dendrogram obtained through biochemical analysis (Protein profiling-SDS PAGE) clearly grouped genotypes in 3 main clusters. Cluster I included G_6/G_{20}, G_{18}, G_{12}, G_{14}, and G_{15}. It contained 4 subclusters. G_6 and G_{18} are related to each other with 2.00 agglomeration coefficient. Cluster II included G_7, G_9, G_8, G_5, G_{16}, G_{13}, G_1, G_2, G_3, G_{17}, G_{10}, and G_{11}. G_7 related to G_9, G_5 related to G_{13} and G_3 related to G_{10} with 1.00, 1.5 and 1.00 agglomeration coefficients respectively. In cluster cluster III fenugreek entries included G_4 and G_{19} with 3.00 coefficients.

(ii) In protein profiling the dendrogram is used for cluster analysis based on UPGMA method. The distance coefficients generated between the genotypes ranged from 0.00 to 4.29. Minimum distance was between G_6 (NS 2006-6) and G_{20} (RMt-351), G_3 (NS 2006-3) and G_{17} (RMt-1) and maximum distance was between G_1 (NS 2006-1) and G_4 (NS 2006-4).

(iii) On the basis of D^2 analysis, 20 genotypes of fenugreek were grouped into 9 clusters. Further, the clustering of genotypes based on morphological diversity i.e. D^2 analysis and biochemical analysis viz., protein profiling is not fully concurrent which might be on account of their genetic make-up and expression under specific environmental situations. While critically examining the protein profile pattern along with D^2 analysis (Fig 3, Table 15), it was observed that genotypes RMt-303 was present in cluster I in both cluster patterns. Further genotypes

viz., NS 2006-5, UM-152, UM-163 and JFG-244 were also present in cluster II in both cluster patterns and similarly NS 2006-4 was also present in cluster III in both cluster patterns. Therefore, these genotypes appeared divergent as well as superior with respect to their *per se* performance, hence could be efficiently utilized in future breeding programmes.

Therefore on the basis of present study conducted in two crop seasons (*rabi*, 2007-08 and 2008-09) revealed that fenugreek genotypes viz., NS 2006-3, UM-134, UM-353 and NS 2006-1 appeared as the most promising for increasing seed yield levels. Genotypes NS 2006-3, RMt-1, RMt-303, and RMt-305 also possessed high seed protein content as well. Genotype NS 2006-3 displayed superiority for seed yield as well as seed protein content. On the basis of variability parameters, correlation and path analysis, it was concluded that character like plant height, pods per plant, and pod length turned out to be the most important contributing traits for enhancing yield levels in fenugreek as they showed high to moderate GCV as well as heritability. Genetic divergence studies displayed that there was no relationship between genetic and geographic diversity. These 20 varieties/strains were grouped into 9 clusters. Maximum inter-cluster distance was between cluster VI and IX and genotypes of these clusters with high *per se* performance could be utilized in hybridization. Characters contributing the highest to genetic divergence were branches per plant, 100-seed weight, harvest index and days to 75 per cent maturity.

On the basis of protein profile, it was concluded that these 20 varieties/strains were grouped into 3 clusters. Maximum distance was observed between G_1 (NS 2006-1) and G_4 (NS 2006-4) genotypes. Therefore, these genotypes including NS 2006-3, UM-134, UM-353, RMt-1, RMt-303 and RMt-305 appeared divergent as well as superior with respect to their *per se* performance over two crop seasons hence could be efficiently utilized so as to ameliorate the productivity and quality of fenugreek.

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* Original not seen

Table 2. Analysis of variance for 12 characters in fenugreek in two crop years (Y_1 & Y_2)

Characters	Year	Replication	Genotype	Error
		[2]	[19]	[38]
Days to 50 % flowering	Y_1	0.02	2.26	1.42
	Y_2	1.40	11.94**	2.66
Days to 75 % maturity	Y_1	0.35	12.95**	4.86
	Y_2	7.11	5.70*	2.85
Plant height	Y_1	16.06	88.96**	18.63
	Y_2	3.98	192.74**	8.99
Branches/plant	Y_1	0.31	0.58	0.93
	Y_2	0.05	1.36	0.87
Pods/plant	Y_1	16.46	45.28**	16.31
	Y_2	2.25	49.04**	8.27
Pod length	Y_1	0.33	0.98**	0.38
	Y_2	1.63**	0.32**	0.10
Seeds/pod	Y_1	1.72	1.49*	0.73
	Y_2	3.05*	2.03**	0.70
100-seed weight	Y_1	0.01	0.02	0.02
	Y_2	0.01	0.04*	0.02
Biological yield/plant	Y_1	6.71	29.06**	3.91
	Y_2	4.61	41.08**	2.49
Seed yield/plant	Y_1	1.07*	2.33**	0.31
	Y_2	0.19	1.15**	0.39
Harvest index	Y_1	38.77	157.79**	16.17
	Y_2	39.14	95.45**	21.10
Seed protein content	Y_1	2.32	54.37**	4.16
	Y_2	1.66	71.32**	0.85

*, ** significant at 5% and 1% level, respectively

Y_1 = 2007-08; Y_2 = 2008-09

Table 3. Pooled analysis of variance for 12 characters in fenugreek in two crop years

Characters	Year	Rep/Year	Genotype	G x E	Pooled error
	[1]	[4]	[19]	[19]	[76]
Days to 50 % flowering	124.03**	0.71	8.04**	6.16**	2.04
Days to 75 % maturity	585.21**	3.73	13.39**	5.26	3.86
Plant height	65.86*	10.02	244.30**	37.40**	13.81
Branches/plant	2.13	0.18	1.04	0.90	0.90
Pods/plant	736.06**	9.36	87.26**	7.05	12.29
Pod length	19.60**	0.98**	1.01**	0.29	0.24
Seeds/pod	69.01**	2.38*	2.52**	1.01	0.72
100 seed weight	0.61**	0.01	0.04*	0.02	0.02
Biological yield/plant	50.70**	5.67	65.18**	4.96	3.20
Seed yield/plant	20.02**	0.63	2.03**	1.46**	0.35
Harvest index	56.51	38.95	219.35**	33.89*	18.63
Seed protein content	7.62	1.99	122.86**	2.83	2.50

*, ** significant at 5% and 1% level, respectively

Table 4. Varieties/strains classified according to their high *per se* performance and consistent/uniform performance for two crop seasons (Y_1 & Y_2) and over pooled basis (P) in fenugreek.

Characters	Best genotypes			Genotypes/strains showing consistent/uniform high *per se* performance
	Y_1 (2007-08)	Y_2 (2008-09)	P	
Days to 50 % flowering	NS 2006-7	UM-202, UM-353	UM-202	NS 2006-7, UM- 202, UM-353, JFG-244
Days to 75 % maturity	UM-152	NS 2006-5	NS 2006-5	NS 2006-5, UM-152, UM-163, UM-134
Plant height	NS 2006-1	NS 2006-1	NS 2006-1	NS 2006-1, NS 2006-2, NS 2006-3, NS 2006-6
Branches/ plant	RMt-1	NS 2006-3, UM- 189, UM-202, UM-353	UM-353	NS 2006-3, NS 2006-6, UM-189, RMt-1
Pods/plant	NS 2006-7	NS 2006-7	NS 2006-7	NS 2006-1, NS 2006-3, NS 2006-7, RMt-1
Pod length	NS 2006-3	NS 2006-5	NS 2006-5	NS 2006-1, NS 2006-3, NS 2006-5, UM-152
Seeds/pod	UM-134, UM- 163, RMt-303, RMt- 305	NS 2006-5, RMt-1, RMt-303	RMt-303	UM-134, UM-163, RMt-303, RMt-305
100-seed weight	UM-152	RMt-1	JFG-244	NS 2006-5, UM-152, UM-354, UM-134
Biological yield/plant	UM-152	UM-134, UM-353	UM-134, UM-303	UM-134, UM-152, NS 2006-3, RMt-303
Seed yield/ plant	RMt-143	UM-353	UM-353	NS 2006-3, UM-134, UM-353, NS 2006-1
Harvest index	UM-354	RMt-305	RMt-305	NS 2006-5, UM-354, RMt-305, NS 2006-1

Seed protein content	RMt-1	RMt-1	RMt-1	NS 2006-3, RMt-1, RMt-303, RMt-305

Table 5. Mean, standard error (SE ±) range, coefficient of variation (CV), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (H) and genetic gain (GG) for 12 characters in fenugreek for two crop seasons (Y_1 & Y_2) and over pooled basis (P)

Characters	Year	Mean± SE	Range	CV %	GCV %	PCV %	Heritability %	Genetic gain %
Days to 50% flowering	Y_1	48.48±0.69	47.00-50.00	2.46	1.09	2.69	16.50	0.91
	Y_2	46.45±0.94	43.67-50.67	3.51	3.79	5.16	53.73	5.72
	P	47.47±0.58	45.83-50.00	3.01	1.18	4.07	8.45	0.71
Days to 75% maturity	Y_1	106.10±1.27	101.67-111.00	2.08	1.55	2.59	35.70	1.91
	Y_2	110.52±0.98	108.00-114.00	1.53	0.88	1.76	24.96	0.91
	P	108.31±0.80	106.00-111.83	1.81	1.07	2.20	23.86	1.08
Plant height	Y_1	78.78±2.49	62.00-84.67	5.77	6.47	8.67	55.72	9.96
	Y_2	76.27±1.73	53.53-88.00	3.93	10.26	10.99	87.20	19.74
	P	75.52±1.52	58.27-86.33	4.92	7.78	9.92	61.41	12.55
Branches/plant	Y_1	4.98±0.56	4.00-6.00	19.36	0.00	18.10	-	-
	Y_2	5.25±0.54	3.67-6.00	17.81	7.69	19.40	15.72	6.28
	P	5.12±0.39	4.33-5.67	18.57	2.89	18.80	2.37	0.92
Pods/plant	Y_1	35.32±2.33	28.33-42.33	11.43	8.80	14.43	37.19	11.05
	Y_2	30.36±1.66	23.53-40.20	9.47	12.14	15.40	62.18	19.72
	P	32.84±1.43	26.97-41.27	10.67	11.13	14.79	55.91	17.15
Pod length	Y_1	10.24±0.36	9.00-11.23	6.06	4.34	7.45	33.92	5.21
	Y_2	9.43±0.18	8.87-10.13	3.39	2.87	4.44	41.70	3.82
	P	9.84±0.20	8.97-10.55	5.02	3.54	6.26	31.96	4.12
Seeds/pod	Y_1	15.27±0.49	14.33-16.33	5.61	3.30	6.51	25.67	3.44
	Y_2	13.75±0.48	12.00-15.00	6.08	4.85	7.78	38.83	6.22
	P	14.51±0.35	13.33-15.67	5.84	3.46	7.11	23.60	3.46
100-seed weight	Y_1	1.07±0.07	0.95-1.21	12.05	2.01	12.22	2.70	0.68
	Y_2	0.93±0.09	0.63-1.17	16.41	9.42	18.92	24.78	9.66
	P	1.00±0.06	0.82-1.09	14.12	4.92	15.49	10.08	3.22
Biological yield/plant	Y_1	20.88±1.14	15.33-25.67	9.47	13.87	16.79	68.20	23.59
	Y_2	19.58±0.91	12.33-25.00	8.06	18.31	20.01	83.76	34.53
	P	20.23±0.73	13.83-23.83	8.84	15.66	18.38	72.59	27.48
Seed yield/plant	Y_1	7.18±0.32	5.09-8.24	7.81	11.42	13.83	68.16	19.42
	Y_2	6.37±0.36	5.54-8.16	9.78	7.93	12.59	39.66	10.28
	P	6.78±0.24	5.58-8.19	8.74	4.55	13.32	11.68	3.21

Harvest index	Y_1	35.09±2.32	23.24-47.43	11.46	19.58	22.69	74.49	34.82
	Y_2	33.71±2.65	26.97-45.72	13.62	14.77	20.09	54.02	22.36
	P	34.40±1.76	26.40-44.28	12.55	16.16	21.49	56.58	25.04
Seed protein content	Y_1	24.90±1.18	18.25-31.84	8.19	16.43	18.36	80.10	30.29
	Y_2	24.40±0.53	16.89-33.10	3.78	19.87	20.22	96.50	40.20
	P	24.65±0.65	17.57-32.47	6.42	18.15	19.22	88.44	35.15

Table 6. Genotypic and phenotypic correlations between 12 characters in fenugreek for two crop seasons (Y_1 & Y_2) and over pooled basis (P)

Character	Year	Days to 50 % flowering		Days to 75 % maturity		Plant height		Branches/ plant		Pods/plant		Pod length		Seeds/pod		100-seed weight		Biological yield/ plant		Seed yield/ plant		Harvest index		Seed protein content	
		G	P	G	P	G	P	G	P	G	P	G	P	G	P	G	P	G	P	G	P	G	P	G	P
Days to 50 % flowering	Y_1	-	-	0.54*	0.00	0.18	0.16	-	-	-0.42	-0.18	0.29	0.03	0.76**	0.06	0.52*	0.04	0.05	0.14	0.11	0.11	0.05	-0.13	-0.03	0.03
	Y_2	-	-	0.41	0.36	0.45*	0.37	-0.22	-0.08	0.14	0.02	0.25	0.20	-0.19	0.03	-0.50*	-0.33	0.26	0.11	0.14	0.01	-0.31	-0.18	0.37	0.24
	P	-	-	-	0.18	0.77**	0.30	0.09	-0.08	0.25	-0.05	0.87**	0.10	-0.04	0.04	-	-0.22	0.42	0.12	-	0.04	-0.82**	-0.15	0.64**	0.17
Days to 75 % maturity	Y_1			-	-	-0.03	-0.05	-	-	-0.11	-0.11	-0.35	-0.10	0.35	0.00	-	-0.17	-0.39	-0.19	-0.07	-0.04	0.25	0.11	0.38	0.19
	Y_2			-	-	0.12	0.04	0.80**	0.01	-0.43	-0.13	-0.33	-0.20	-0.55*	-0.20	-0.37	-0.33	0.05	0.09	0.21	0.11	-0.02	-0.03	0.43	0.17
	P			-	-	-0.01	-0.01	-	0.06	-0.31	-0.12	-0.27	-0.13	-0.26	-0.09	-	-0.23	-0.23	-0.06	-0.10	0.01	0.23	0.06	0.40	0.18
Plant height	Y_1					-	-	-	-	1.02	0.30	0.62**	0.25	0.05	-0.16	-0.13	0.15	0.50*	0.35	0.08	0.09	-0.40	-0.24	-0.14	-0.09
	Y_2					-	-	0.01	0.03	0.28	0.22	0.23	0.20	-0.51*	-0.28	0.22	-0.02	0.64*	0.54*	0.49*	0.30	-0.62**	-0.46*	-0.19	-0.18
	P					-	-	-0.22	0.05	0.51*	0.25	0.63**	0.21	-0.34	-0.23	0.21	0.04	0.58**	0.46*	0.74**	0.19	-0.52*	-0.35	-0.16	-0.14
Branches/plant	Y_1							-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Y_2							-	-	0.32	0.19	-0.45*	-0.13	-0.72**	-0.01	-0.48*	-0.02	0.43	0.12	0.58**	0.08	-0.12	-0.12	0.33	0.74**
	P							-	-	0.44	0.13	-0.29	-0.01	-0.90**	-0.02	-0.65**	-0.01	0.05	0.11	0.06	-0.06	-0.14	-0.18	0.23	-
Pods/plant	Y_1									-	-	0.39	0.12	0.34	0.07	0.17	0.00	0.67**	0.33	-0.49*	-0.29	-0.79**	-0.41	0.18	0.01
	Y_2									-	-	0.38	0.20	0.38	0.22	0.60**	0.17	0.50*	0.33	-0.14	0.06	-0.74**	-0.32	0.15	0.10
	P									-	-	0.32	0.14	0.40	0.14	0.35	0.09	0.53	0.33	-0.68**	-0.14	-0.73**	-0.37	0.12	0.06
Pod length	Y_1											-	-	-0.59**	-0.23	-	0.05	0.25	0.27	0.06	0.05	-0.23	-0.13	-0.34	-0.17
	Y_2											-	-	0.38	0.20	0.14	0.21	0.56	0.33	0.37	0.19	-0.59**	-0.28	-0.07	-0.08
	P											-	-	0.14	-0.07	0.39	0.11	0.50*	0.27	-0.31	0.09	-0.59**	-0.17	-0.27	-0.13
Seeds/pod	Y_1													-	-	-0.51*	0.12	0.26	0.07	-0.55**	-0.20	-0.41	-0.19	0.13	0.03
	Y_2													-	-	0.20	-0.09	-0.15	-0.14	-0.75**	-0.13	-0.16	-0.03	-0.05	-0.01
	P													-	-	0.12	0.00	0.05	-0.04	-1.03	-0.16	0.27	-0.11	0.14	0.01
100-seed weight	Y_1															-	-	0.52*	0.05	-	0.11	0.77**	0.03	-	-0.30
	Y_2															-	-	0.29	0.21	0.40	0.04	-0.38	-0.17	0.10	0.05
	P															-	-	0.39	0.14	-0.10	0.07	-0.49*	-0.08	-0.44	-0.09
Biological yield/ plant	Y_1																	-	-	-0.15	-0.07	-0.87**	-0.78**	0.25	0.12
	Y_2																	-	-	0.74**	0.42	0.96**	-0.79**	0.17	0.15

	P	-	-	0.37	0.16	-0.95**	-0.78**	0.19	0.13
Seed yield/plant	Y_1			-	-	0.65**	0.56*	-0.41	-0.44
	Y_2			-	-	-0.48*	0.12	0.23	0.23
	P			-	-	-0.06	0.38	-0.50*	-0.50*
Harvest index	Y_1					-	-	-0.40	-0.32
	Y_2					-	-	-0.08	0.09
	P					-	-	-0.29	-0.21
Seed protein content	Y_1							-	-
	Y_2							-	-
	P							-	-

*, ** Significant at 5% and 1% level, respectively Y_1 = 2007-08 Y_2 = 2008-09 P = Pooled

Table 7. Path analysis showing direct and indirect effects of 9 characters on seed yield per plant in fenugreek for two crop seasons (Y_1 & Y_2) and over pooled basis (P)

Character	Year	Direct effect	Days to 50% flowering	Days to 75 % maturity	Plant height	Pods/ plant	Pod length	Seeds/ pod	100-seed weight	Biological yield/ plant	r
Days to 50% flowering	Y_1	-	-	-	-	-	-	-	-	-	-
	Y_2	**-1.86**	-	-0.06	0.69	0.01	0.32	0.15	0.49	-0.16	0.14
	P	**-0.02**	-	0.21	3.93	-0.97	-2.94	-0.11	-1.60	0.34	-1.38
Days to 75% maturity	Y_1	**-3.16**	-	-	0.02	-0.51	1.82	1.65	-	0.49	-0.07
	Y_2	**-0.16**	-0.76	-	0.18	-0.05	-0.42	0.43	0.36	-0.03	0.21
	P	**0.09**	-0.05	-	-0.03	1.21	0.90	-0.69	-1.20	-0.19	-0.10
Plant height	Y_1	**-0.71**	-	0.09	-	4.67	-3.23	-0.25	-	-0.63	0.08
	Y_2	**1.53**	-0.84	-0.02	-	0.03	0.29	0.40	-0.21	-0.40	0.49*
	P	**5.08**	-0.02	-0.00	-	-1.98	-2.15	-0.89	0.17	0.48	0.74**
Pods/plant	Y_1	**4.58**	-	0.35	-0.73	-	-2.06	-1.60	-	-0.85	-0.49*
	Y_2	**0.11**	-0.26	0.07	0.43	-	0.49	-0.29	-0.59	-0.31	-0.14
	P	**-3.88**	-0.01	-0.03	2.60	-	-1.10	1.06	0.28	0.43	-0.68**

Pod length	Y_1	**-5.23**	-	1.10	-0.44	1.80	-	2.80	-	-0.32	0.06
	Y_2	**1.29**	-0.46	0.05	0.35	0.04	-	-0.30	-0.14	-0.35	0.37
	P	**-3.40**	-0.02	-0.02	3.22	-1.26	-	0.36	0.31	0.41	-0.31
Seeds/pod	Y_1	**-4.71**	-	1.11	-0.04	1.55	3.11	-	-	-0.33	-0.55**
	Y_2	**-0.78**	0.36	0.09	-0.78	0.04	0.50	-	-0.19	0.09	-0.75**
	P	**2.64**	0.00	-0.02	-1.71	-1.56	-0.46	-	0.10	0.04	-1.03
100-seed weight	Y_1	-	-	-	-	-	-	-	-	-	-
	Y_2	**-0.98**	0.92	0.06	0.33	0.06	0.18	-0.15	-	-0.18	0.40
	P	**0.80**	0.05	-0.14	1.08	-1.37	-1.31	0.32	-	0.31	-0.10
Biological yield/plant	Y_1	**-1.27**	-	1.22	-0.36	3.06	-1.33	-1.23	-	-	-0.15
	Y_2	**-0.62**	-0.48	-0.01	0.99	0.05	0.72	0.11	-0.29	-	0.74**
	P	**0.82**	-0.01	-0.02	2.97	-2.05	-1.70	0.12	0.31	-	0.37

*, ** Significant at 5% and 1% level, respectively

Y_1 (2007-08) Residual effect: 0.39 Y_2 (2008-09) Residual effect: 0.33 P Residual effect: 0.51

Table 8. Path analysis showing direct and indirect effects of 11 characters on seed protein content in fenugreek for two crop seasons (Y_1 & Y_2) and over pooled basis (P)

Character	Year	Direct effect	Days to 50% flowering	Days to 75 % maturity	Plant height	Pods/ plant	Pod length	Seeds/ pod	100-seed weight	Biological yield/ plant	Seed yield/ plant	Harvest index	r
Days to 50% flowering	Y_1	-	-	-	-	-	-	-	-	-	-	-	-
	Y_2	**1.03**	-	-0.10	-0.57	-0.02	-0.19	0.01	-0.13	-0.37	0.14	0.57	0.37
	P	**0.08**	-	0.64	-0.50	0.06	-0.22	0.01	0.09	-0.28	-0.29	1.05	0.64**
Days to 75 % maturity	Y_1	**0.18**	-	-	-0.00	0.02	0.26	0.19	-	-0.00	-0.01	-0.26	0.38
	Y_2	**-0.25**	0.42	-	-0.15	0.05	0.26	0.03	-0.10	-0.07	0.22	0.03	0.43
	P	**0.28**	0.18	-	0.00	-0.07	0.07	0.04	0.07	0.15	-0.02	-0.29	0.40
Plant height	Y_1	**0.14**	-	-0.01	-	-0.20	-0.47	-0.03	-	0.00	0.01	0.41	-0.14
	Y_2	**-1.26**	0.46	-0.03	-	-0.03	-0.18	0.03	0.06	-0.92	0.52	1.15	-0.19
	P	**-0.64**	0.06	-0.00	-	0.12	-0.16	0.05	-0.01	-0.38	0.16	0.66	-0.16
Pods/plant	Y_1	**-0.20**	-	-0.02	0.14	-	-0.30	-0.18	-	0.00	-0.06	0.79	0.18
	Y_2	**-0.11**	0.14	0.11	-0.35	-	-0.30	-0.02	0.16	-0.71	-0.14	1.37	0.15
	P	**0.23**	0.02	-0.09	-0.33	-	-0.08	-0.06	-0.02	-0.35	-0.14	0.93	0.12

Pod length	Y_1	**-0.75**	-	-0.06	0.08	-0.08	-	0.32	-	0.00	0.01	0.23	-0.34
	Y_2	**-0.79**	0.25	0.08	-0.28	-0.04	-	-0.02	0.04	-0.80	0.40	1.09	-0.07
	P	**-0.25**	0.07	-0.07	-0.41	0.07	-	-0.02	-0.02	-0.33	-0.07	0.75	-0.27
Seeds/pod	Y_1	**-0.54**	-	-0.06	0.01	-0.07	0.45	-	-	0.00	-0.07	0.41	0.13
	Y_2	**-0.05**	-0.20	0.14	0.64	-0.04	-0.30	-	0.05	0.21	-0.79	0.30	-0.05
	P	**-0.14**	-0.00	-0.07	0.22	0.09	-0.03	-	-0.01	-0.03	-0.22	0.34	0.14
100-seed weight	Y_1	**-**	-	-	-	-	-	-	-	-	-	-	-
	Y_2	**0.27**	-0.51	0.09	-0.27	-0.06	-0.11	-0.01	-	-0.42	0.42	0.70	0.10
	P	**-0.05**	-0.16	-0.42	-0.14	0.08	-0.10	-0.02	-	-0.25	-0.02	0.62	-0.44
Biological yield/plant	Y_1	**0.01**	-	-0.07	0.07	-0.13	-0.19	-0.14	-	-	-0.02	0.88	0.25
	Y_2	**-1.42**	-0.27	-0.01	-0.81	-0.05	-0.44	0.01	0.08	-	0.78	1.78	0.17
	P	**-0.65**	0.03	-0.06	-0.37	0.12	-0.13	-0.01	-0.02	-	0.08	1.21	0.19
Seed yield/plant	Y_1	**0.12**	-	-0.01	0.01	0.10	-0.04	0.30	-	-0.00	-	-0.65	-0.41
	Y_2	**1.06**	0.14	-0.05	-0.62	0.01	-0.30	0.04	0.11	-1.05	-	0.89	0.23
	P	**0.21**	-0.11	-0.03	-0.48	-0.15	0.08	0.14	0.00	-0.24	-	0.07	-0.50*
Harvest index	Y_1	**-1.01**	-	0.05	-0.06	0.15	0.17	0.22	-	-0.01	0.08	-	-0.40
	Y_2	**-1.85**	-0.31	0.00	0.78	0.08	0.46	0.01	-0.10	1.36	-0.51	-	-0.08
	P	**-1.27**	-0.06	0.06	0.33	-0.16	0.15	0.04	0.02	0.62	-0.01	-	-0.29

*, ** Significant at 5% and 1% level, respectively Y_1 (2007-08) Residual effect: 0.66 Y_2 (2008-09) Residual effect: 0.52 P Residual effect: 0.71

Table 9. D^2 values based on 12 characters pooled over two crop seasons arranged in ascending order in fenugreek

Rank	G1		G2		G3		G4		G5		G6		G7		G8		G9		G10	
1	0.00	(1)	0.00	(2)	0.00	(3)	0.00	(4)	0.00	(5)	0.00	(6)	0.00	(7)	0.00	(8)	0.00	(9)	0.00.	(10)
2	15.39	(11)	17.64	(8)	6.55	(8)	14.02	(5)	14.02	(4)	11.64	(3)	11.19	(3)	6.55	(3)	9.49	(16)	8.51	(11)
3	18.79	(6)	21.25	(18)	10.80	(11)	15.44	(10)	15.84	(9)	11.69	(11)	13.22	(8)	13.22	(7)	15.84	(5)	12.71	(16)
4	23.16	(3)	23.51	(7)	11.19	(7)	17.24	(12)	21.90	(12)	13.29	(14)	13.44	(17)	15.73	(18)	18.36	(10)	15.44	(4)
5	24.05	(10)	23.57	(3)	11.64	(6)	19.79	(15)	22.98	(16)	18.79	(1)	18.69	(18)	17.64	(2)	21.66	(11)	15.98	(15)
6	25.43	(13)	23.72	(6)	16.80	(13)	22.10	(16)	25.40	(10)	19.04	(10)	19.45	(11)	18.78	(11)	26.37	(4)	16.22	(14)
7	27.76	(4)	29.26	(20)	23.16	(1)	25.33	(11)	44.54	(11)	20.27	(15)	20.86	(6)	20.41	(6)	30.48	(1)	16.26	(13)
8	30.48	(9)	29.75	(17)	23.57	(2)	26.37	(9)	46.00	(1)	20.41	(8)	23.51	(2)	21.11	(13)	32.31	(13)	18.36	(9)
9	35.79	(8)	42.50	(14)	23.99	(14)	27.38	(14)	48.26	(15)	20.86	(7)	37.17	(1)	26.76	(17)	44.40	(12)	19.04	(6)
10	37.17	(7)	43.62	(11)	24.87	(10)	27.76	(1)	56.37	(13)	23.72	(2)	37.77	(14)	28.00	(14)	44.85	(3)	24.05	(1)
11	38.05	(16)	47.88	(1)	26.85	(18)	28.31	(6)	57.18	(6)	27.31	(4)	40.76	(13)	31.57	(10)	46.04	(6)	24.87	(3)
12	38.11	(14)	49.73	(13)	28.99	(17)	37.34	(13)	58.88	(14)	28.56	(13)	43.54	(10)	35.79	(1)	50.37	(15)	25.40	(5)

13	43.33	(15)	55.92	(15)	31.74	(15)	48.04	(19)	70.88	(3)	31.93	(17)	54.77	(20)	37.41	(20)	59.84	(14)	31.44	(12)
14	46.00	(5)	63.03	(10)	39.28	(16)	49.13	(3)	80.02	(19)	32.61	(18)	56.27	(15)	39.53	(15)	60.46	(8)	31.57	(8)
15	47.88	(2)	92.55	(16)	41.97	(20)	67.46	(8)	94.14	(8)	39.56	(20)	69.23	(16)	52.69	(16)	70.21	(7)	43.54	(7)
16	69.67	(18)	93.34	(4)	44.85	(9)	77.22	(7)	108.78	(7)	41.19	(16)	70.21	(9)	60.46	(9)	95.08	(19)	53.06	(19)
17	70.48	(12)	104.97	(9)	49.13	(4)	92.83	(20)	138.60	(2)	46.04	(9)	77.22	(4)	67.46	(4)	98.09	(18)	58.67	(18)
18	75.68	(17)	116.83	(19)	70.88	(5)	93.34	(2)	139.91	(20)	56.78	(12)	108.78	(5)	94.14	(5)	104.97	(2)	63.03	(2)
19	87.00	(20)	135.47	(12)	78.56	(12)	103.76	(18)	140.29	(18)	57.18	(5)	117.62	(19)	100.40	(19)	107.19	(17)	64.58	(20)
20	103.13	(19)	138.60	(5)	93.50	(19)	106.77	(17)	141.88	(17)	59.01	(19)	117.72	(12)	101.64	(12)	123.76	(20)	64.80	(17)

Contd...

Rank	G11		G12		G13		G14		G15		G16		G17		G18		G19		G20	
1	0.00	(11)	0.00	(12)	0.00	(13)	0.00	(14)	0.00	(15)	0.00	(16)	0.00	(17)	0.00	(18)	0.00	(19)	0.00	(20)
2	8.51	(10)	17.24	(4)	11.96	(11)	7.96	(15)	7.96	(14)	9.49	(9)	9.47	(18)	9.47	(17)	29.30	(12)	29.26	(2)
3.	10.80	(3)	21.90	(5)	16.26	(10)	13.29	(6)	15.98	(10)	12.71	(10)	13.44	(7)	15.73	(8)	37.49	(15)	30.54	(14)

4	33.01	11.69	(6)	27.02	(16)	16.80	(3)	16.22	(10)	19.89	(4)	18.53	(11)	26.76	(8)	18.69	(7)	39.14	(14)	(18)	
5	37.41	11.96	(13)	29.30	(19)	18.56	(16)	20.27	(11)	20.27	(6)	18.56	(13)	28.99	(3)	21.25	(2)	48.04	(4)	(8)	
6	38.43	15.39	(1)	29.52	(15)	21.11	(8)	23.99	(3)	22.18	(13)	22.10	(4)	29.75	(2)	26.85	(30)	53.06	(10)	(15)	
7	39.56	18.53	(16)	31.44	(10)	22.18	(15)	27.38	(4)	22.54	(11)	22.98	(5)	31.93	(6)	32.61	(6)	59.01	(6)	(6)	
8	41.97	18.78	(8)	41.13	(14)	25.43	(1)	28.00	(8)	26.91	(16)	26.91	(15)	43.26	(20)	33.01	(20)	64.44	(20)	(3)	
9	43.26	19.45	(7)	44.40	(9)	28.35	(14)	28.35	(13)	29.52	(12)	27.02	(12)	44.56	(11)	41.01	(11)	65.91	(16)	(17)	
10	54.77	20.27	(14)	52.88	(11)	28.56	(6)	30.54	(20)	31.74	(3)	38.05	(1)	44.92	(14)	45.48	(14)	70.98	(11)	(7)	
11	59.37	21.66	(9)	56.78	(6)	32.31	(9)	37.77	(7)	37.49	(19)	39.28	(1)	64.80	(10)	58.67	(10)	80.02	(5)	(13)	
12	60.66	22.54	(15)	61.22	(13)	37.34	(4)	38.11	(1)	38.43	(20)	41.15	(14)	71.23	(15)	58.91	(13)	87.27	(13)	(11)	
13	64.44	25.30	(4)	70.48	(1)	40.76	(7)	39.14	(19)	39.53	(8)	41.19	(6)	73.73	(13)	66.95	(15)	93.50	(3)	(19)	
14	64.58	41.01	(18)	78.56	(3)	49.71	(2)	41.13	(12)	43.33	(1)	52.69	(8)	75.68	(1)	69.67	(1)	95.08	(9)	(10)	
15	86.75	43.62	(2)	97.34	(20)	56.37	(5)	41.15	(16)	48.26	(5)	65.91	(19)	100.12	(16)	87.60	(16)	95.73	(18)	(16)	
16	87.00	44.54	(5)	101.64	(8)	58.91	(18)	42.50	(2)	50.37	(9)	69.23	(7)	105.31	(19)	95.73	(19)	100.40	(8)	(1)	
17	92.83	44.56	(17)	117.72	(7)	59.37	(20)	44.92	(17)	55.92	(2)	86.75	(20)	106.77	(4)	98.09	(9)	103.13	(1)	(4)	
18	97.34	52.88	(12)	130.22	(18)	61.22	(12)	45.48	(18)	56.27	(7)	87.60	(18)	107.19	(9)	103.76	(4)	105.31	(17)	(12)	

19	60.66	(20)	131.80	(17)	73.73	(17)	58.88	(5)	66.95	(18)	92.95	(2)	131.80	(12)	130.22	(12)	116.83	(2)	123.76	(9)
20	70.98	(19)	135.47	(2)	87.27	(19)	59.84	(9)	71.23	(17)	100.12	(17)	141.88	(5)	140.29	(5)	117.62	(7)	139.91	(5)

Table 10. Fenugreek varieties included in each cluster

Clusters	Number of varieties	Varieties/genotypes
I	6	NS 2006-2, NS 2006-3, NS 2006-7, UM- 134, UM- 189, RMt- 303
II	4	NS 2006-5, UM 152, UM 163, JFG 244
III	3	NS 2006-4, UM 202, RMt 143
IV	2	NS 2006-1, NS 2006-6
V	1	UM 354
VI	1	RMt-1
VII	1	UM 353
VIII	1	RMt-351
IX	1	RMt-305

Table 11. Average intra and inter cluster D^2 values in 20 varieties of fenugreek

Clusters	I	II	III	IV	V	VI	VII	VIII	IX
I	20.79	66.16	72.53	29.16	33.01	25.49	33.21	42.84	99.17
II		17.46	28.68	37.75	44.02	103.49	30.87	103.75	73.51
III			22.21	41.15	25.49	103.26	40.24	76.20	38.27
IV				18.79	25.70	53.80	26.99	63.28	81.07
V					0.00	44.92	28.35	30.54	39.14
VI						0.00	73.73	43.26	105.31
VII							0.00	59.37	87.27
VIII								0.00	64.44
IX									0.00

Table 12. Average intra and inter cluster distance (D) = ($\sqrt{\ }$ D^2) values

Clusters	I	II	III	IV	V	VI	VII	VIII	IX
I	4.55	8.13	8.51	5.40	5.74	5.04	5.76	6.54	9.95
II		4.17	5.35	6.14	6.63	10.17	5.55	10.18	8.57
III			4.71	6.41	5.04	10.16	6.34	8.72	6.18
IV				4.33	5.06	7.33	5.19	7.95	9.00
V					0.00	6.70	5.32	5.52	6.25
VI						0.00	8.58	6.57	10.26
VII							0.00	7.70	9.34
VIII								0.00	8.02
IX									0.00

Table 13. Contribution of characters to divergence

Characters	Rank total	Rank
Days to 50 % flowering	1364	5
Days to 75 % maturity	1431	4
Plant height	934	10
Branches/plant	1680	1
Pods/plant	1035	9
Pod length	1336	7
Seeds/pod	1360	6
100 seed weight	1641	2
Biological yield/plant	826	11
Seed yield/plant	1126	8
Harvest index	1583	3
Seed protein content	510	12

Table 14. Protein profiling on polyacrylamide (fenugreek genotypes 10% Gel)

MW (Kd)	NS 2006-1	NS 2006-2	NS 2006-3	NS 2006-4	NS 2006-5	NS 2006-6	NS 2006-7	UM 134	UM 152	UM 163	UM 189	UM 202	UM 353	UM 354	RMt 143	JFG 244	RMt 1	RMt 303	RMt 305	RMt 351
98	0	0	0	1	1	1	0	0	0	0	0	1	1	0	0	1	0	0	1	1
97	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
81	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
68	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
66	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	1	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0
43	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	0	1
36	1	1	0	0	1	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0
30	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
29	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	1	0	1	0
24	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
No. of bands	4	4	4	5	5	4	4	5	3	5	5	4	4	5	5	4	4	4	6	4

ABSTRACT

Anil Swami* **Dr. S.R. Maloo****

P.G. Research Scholar **Major Advisor**

The present study entitled as "**Genetic Divergence and Path Analysis in fenugreek (*Trigonella foenum-graecum* L.)"** was carried out with 20 diverse genotypes/strains of fenugreek collected from different sources. The material were planted during two crop seasons viz. *rabi,* 2007-08 and 2008-09 at Instructional Farm of Rajasthan College of Agriculture, Udaipur. The experiment was conducted in Randomized Block Design with three replications following uniform and recommended agronomic practices. Observations were recorded for 11 yield contributing characters and seed protein content in each year.

Variability parameters, correlation were computed for each year while path analysis was estimated for seed yield per plant as well as seed protein content for each year. Genetic divergence was computed for seed yield per plant over pooled basis using Mahalanobis (1936) D^2 statistics and protein profile were distinguished through SDS-PAGE by Davis (1964).

Analysis of variance showed presence of sufficient variability among the genotypes. Superior varieties/strains viz. NS 2006-3, UM-134, UM-353 and NS 2006-1 were identified on the basis of their high *per se* and consistent performance for seed yield and most of their component characters. Varieties/strains NS 2006-3 displayed superiority for seed yield as well as seed protein content. Plant height, pods per plant and pod length turned out to be the most important contributing traits for enhancing yield level in fenugreek. High GG along with high estimates of heritability and GCV for seed protein content and biological yield per plant. While, moderate GG and GCV with high heritability for pods per plant, seed yield per plant and plant height suggested for these traits would be effective for genetic improvement of seed yield in fenugreek.

Association studied revealed that seed yield per plant showed positive correlated with plant height, branches per plant, biological per plant and harvest index

at genotypic and phenotypic level in at least one year and over pooled basis. Most of these characters were mutually correlated. Path analysis studied for seed yield revealed direct and indirect contribution of plant height, pods per plant, biological yield per plant, seeds per pod and pod length while for seed protein content it revealed direct as well as indirect contribution of seed yield per plant, harvest index and seeds per pod. These characters showed strong association with seed yield and seed protein content

On the basis of divergence studied, it was concluded that there was no relationship between genetic and geographic diversity. These 20 varieties/strains were grouped into 9 clusters. Maximum inter-cluster distance was between cluster VI and IX and genotypes of these clusters with high *per se* performance could be utilized in breeding programmes. Character contributing maximum to seed yield were branches per plant, 100-seed weight and harvest index. Genetically diverse and high yielding varieties/strains of fenugreek like RMt-1, RMt-305, NS 2006-3, UM-134 and UM-353 could be used for breeding programme so as to ameliorate the productivity and quality of fenugreek. In protein profiling the dendrogram is used for cluster analysis based on UPGMA method. These 20 varieties/strains were grouped into 3 clusters. The distance coefficients generated between the 20 fenugreek genotypes ranged from 0.00 to 4.29. Maximum distance was recorded between G_1 (NS 2006-1) and G_4 (NS 2006-4) genotypes of these stages with high genetic divergent as well as *per se* performance could be utilized in future breeding programme.

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vfuy Lokeh* Mk- ,l-vkj- eky**
_ukrdkÙkj 'kk/k Nk= e[; 'kk/k lykgdkj

oreku vul/kku ^^eFkh ¼Vkbxkuyk Qkbue&xbde ,y-½ e vkuof'kd foHkn ,o iFk fo'y"k.k** uked 'kh"kd eFkh dh 20 fofHkUu fdLek dk fofHkUu L=krk l ,d= fd;k x;kA bu fdLek dk nk fofHkUu o"kk jch 2007&08 vkj 2008&09 e jktLFkku Ñf"k egkfo|ky; d f'k{k.k Qke ij yxk;k x;kA bl ç;kx dk ;knfPNd [k.M vfHkdYiuk e rhu iujkofÙk;k d lkFk ,d leku Ñf"k i)fr;k e vk;kftr fd;k x;kA çR;d tyok; {k= e X;kjg mit v'knku dju oky y{k.k rFkk cht çkVhu ij ç{k.k fy; x;A

fofHkUu fLFkjkdk ,o lg&lEcU/kk dh x.kuk çR;d o"k e vyx l dh xbA iFk fo'y"k.k dk voykdu cht mit vkj çfr cht çkVhu dh ek=k gr çfr o"k vyx l fd;k x;kA vkuof'kd foHkn dh lx.kuk cht mit y{k.kk d fy, egkykukfcl ¼1936½ Mh ox ifjx.kuk }kjk fd;k x;k vkj çkVhu lLrjhdj.k dh igpku ,l- Mh- ,l- ih- ,- th- b- d ek/;e l Mfol dh dh FkhA

fopj.k fo'y"k.k }kjk fdLek@foHknk e i;kIr ifjoru'khyrk n[kh xbA cht mit vkj T;knkrj mit vo;ok dh nf"V l ,u-,l- 2006&3] ;-,e- 134] ;-,e- 353 vkj ,u-,l- 2006&1 dk p;u mudh fLFkj x.koÙkk d vk/kkj ij fd;k x;kA vul/kku çfØ;k d nkjku fofnr gvk fd ,u-,l- 2006&3 fdLe mit rFkk cht çkVhu d fy, J"B gA ik/k dh Åpkb] çfr ik/kk Qfy;k vkj ik/k dh yEckb eFkh e cht mit c<ku e lgk;d fl) gbA çfr ik/kk tfod mit vkj cht çkVhu dh ek=k d fy, mPpLrjh; vkuokf'kd ykHk d lkFk vkuokf'kd fofHkUu fLFkjkd ,o o'kkuxfr fLFkjkd çkIr g, tcfd çfr ik/kk Qfy;k] çfr ik/kk cht mit vkj ik/k dh Åpkb d fy, e/;e vkuof'kd ykHk ,o vkuof'kd fofHkUu fLFkjkd d lkFk gh vf/kd o'kkuxfr fLFkjkd çkIr g, tk fd lfpr djr g fd cht mit d vkuof'kd l/kkj d fy, bu x.kk dk puko çHkko'kkyh gkxkA

lg&lEcU/kk d ifjdyu l ;g Kkr gkrk g fd çfr ik/kk cht mit vkuof'kd ,o n';:ih nkuk Lrj ij de l de ,d o"k@tyok; ifjfLFkfr e vyx l ,o ,d lkFk x.kuk d vk/kkj ij ik/k dh Åpkb] çfr ik/k Qfy;k] çfr ik/kk tfod mit vkj dVkb lpdkd l lg&lEcfU/kr gA bue l T;knkrj y{k.k vkil e Hkh lg&lEcfU/kr gA

çfr ik/kk cht mit d fy, iFk fo'y"k.k d v/;;u l Kkr gvk fd ik/k dh Åpkb] çfr ik/kk Qfy;k] çfr ik/kk tfod mit] çfr Qyh chtk dh l[;k] Qyh dh yEckb çfr ik/kk cht mit ij çR;{k ,o vçR;{k çHkko n'kkr gA blh çdkj cht çkVhu d fy; iFk fo'y"k.k dk v/;;u dju l Kkr gvk fd çfr ik/kk cht mit] dVkb lpdkd vkj çfr Qyh chtk dh l[;k] cht çkVhu ij çR;{k ,o vçR;{k çHkko n'kkr gA ; y{k.k çfr ik/kk cht mit rFkk cht çkVhu d lkFk çcy lg&lEcU/k fn[kkr gA

vkuof'kd foHkn v/;;u e vkuof'kd vkj Hkxkfy; fofo/krk e dkb lEcU/k ugh ik;k x;kA lHkh 20 thu çk:i 9 fofHkUu legk e foHkä fd; x;A lokf/kd vUrj&legh; njh leg VI vkj lX d chp ik;h xbA tk ;g n'kkrh g fd bu legk d thu çk:i fofHkUu çtuuh; dk;Øek e ç;kx fd;

tk ldr gA vkuof'kd foHkn vkj vf/kd mRikndrk okyh eFkh dh itkfr;k vkj- ,e- Vh- 1] vkj- ,e- Vh- 305] ,u- ,l- 2006&3] ;- ,e- 134 vkj ;- ,e- 353 ox ldjhdj.k dk;de e i;kx dh tk ldrh g rkfd eFkh e mRikndrk ,o ik"kd x.kk dk mUu;u gk ldA

çkVhu e M.Mkxke : ij[kk ;- ih- th- ,e- ,- i)fr ij vk/kkfjr leg fo'y"k.k d fy, ç;kx fd;k tkrk gA bu lHkh 20 thu çk:i dk 3 fofHkUu legk e foHkä fd; x;A bu 20 thu çk:i e njh x.kkd foLrkj 0-00 l 4-29 gA lokf/kd njh G_1 ¼,u- ,l- 2006&1½ vkj G_4 ¼,u-,l- 2006&4½ d chp e g] tk ;g n'kkrh g fd bl voLFkk e thu çk:i fofHkUu çtuuh; dk;Øek e ç;kx fd; tk ldr gA

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* Lukrdkj 'kk/k Nk=] ikni çtuu ,o vkuokf'kdh foHkkx] jktLFkku Ñf"k egkfo|ky;] mn;ijA

** vkpk; ,o foHkkxk/;{k] ikni çtuu ,o vkuokf'kdh foHkkx] jktLFkku Ñf"k egkfo|ky;] mn;ijA