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Evaluation of Fenugreek (*trigonella foenum graecum*) Genotypes Against Powdery Mildew (*Erysiphe polygoni*) in Ethiopia

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Abstract:
Background and Objective: Ethiopia have suitable environmental condition for fenugreek production, it grows under agro ecologies ranging from 1800 to 2300 m.a.s.l. Fenugreek is mainly used as an herb (leaves) and as a spice (seed), medicinal value and also as nitrogen fixation and soil enrichment. Perhaps Powdery mildew caused by Erysiphe polygoni is an economically important disease especially during the flowering and pod formation stage on the fenugreek and causes significant loss in grain quality as well as quantity. Hence the present study done with the objectives to identify source of resistance among 100 Ethiopian fenugreek materials for further resistance breeding program and for identifying high yielding genotypes to ensure sustainable production and productivity. Materials and Methods: The Current study was done using lattice design during 2021/2022 cropping season under natural epidemic conditions to evaluate the fenugreek genotypes obtained from Ethiopian Bio diversity Institute at kulumsa, South Eastern Ethiopia. Result: The current evaluation of fenugreek genotypes against powdery mildew results identified genotypes namely 28601 and 29561 showing moderately resistant reaction and forty-three genotypes as moderately resistant type. The remaining 55 genotypes showed susceptible reaction. The Present result also identified the genotypes,31100, 29561 and 212775 were with lower rAUDPC which could cross with 238247 and 9239 which are relatively high yielder. Conclusion: The study results recommended having a crossing program between genotypes 31088,237983 and 20428 and 35190 and 31087 for improved resistance and high yielder. Contemporary results suggested genotypes with moderately susceptible and moderately resistant material for further resistant breeding considering other physio chemical evaluation quality mechanisms including evaluating under greenhouse condition.

Keywords: Fenugreek, Powdery mildew, evaluation, resistance, genotypes, field.

INTRODUCTION

The cognitive content of yield losses is the primary program in an effort to combat hunger, raise income and improve food security mainly in the poorest countries of the world. Crop yield losses have an impact on food security, economic development and the environment. The exact causes of yield losses vary throughout the world and are very much dependent on the specific conditions and local situation in a given country. Ethiopia has diverse agro-ecologies which enable it to grow various crop species. Although agriculture is the backbone of the Ethiopian economy, annual agricultural production and productivity growth have not been commensurate with the annual population growth rate. The production and productivity of different crops in all agro-ecologies are constrained by several biotic and abiotic stresses, which can vary across agro ecologies and from place to place within an agro ecology. Several factors including climate change which aggravates pest problems and diverse agro-ecologies that need diverse management options and low level of access to improved crop production and protection contribute to the poor performance of Ethiopian agriculture. These factors slow the growth of agricultural production in general and food grain production in particular; eventually contributing significantly to food insecurity. Therefore, the incorporation of yield-increasing technologies like disease resistant varieties and other control measures, which are environmentally safe are all crucial points to be taken into consideration.

In Ethiopia, the performance of the agricultural sector has been constrained by biotic and abiotic factors, which in turn affected its contribution to yield reduction. Powdery mildew is one of the major biotic constraints causing losses in quantities and qualities in fenugreek production. Fenugreek or *Trigonella foenum-graecum L.*, is an annual plant and is an extremely important crop for Ethiopia's economy and the country has ideal natural conditions for its cultivation. It is important in an incapacitating economy as a spice cash crop, medicinal value and also as nitrogen fixation and soil enrichment. Fenugreek now covers around 34,603.81 hectares in Ethiopia, with an average national productivity of 1.3 t/ha, which is lower than what can be obtained (1.7 t/ha) with proper management techniques[1].Due to inadequate agronomic practices, diseases (such as powdery mildew and wilt) and insect pests (borer) in the research area as well as other agro-ecological zones of Ethiopia, the large yield difference linked to the lack of stable, high yielding and disease resistant genotypes[2]. According to the study done on the crop by Fikreselassie[3]**,** powdery mildew had a significant effect on the number of seeds per pod and thousand seed weight yield parameters. Powdery mildew was prevalent on fenugreek in the central highlands of Ethiopia with an incidence of about 95% and severity ranges from 20 to 80%[4]. The disease downy mildew of fenugreek was reported from Algeria, India, Pakistan and the United Kingdom but the first report of its occurrence was from California the United States and India. It is observed that the disease commonly occurs during February and March. The pathogen causing downy mildew of fenugreek was identified as *Peronospora Trigonella Guam*[5].

The symptoms of downy mildew are quite distinct from the other diseases. The axial surfaces of the leaves showed yellow patches or small chlorotic spots which appeared at the margins often. The axial surface of the leaf showed white cottony mycelium growth which often appears as grayish violet. The disease also causes stunted growth of the plant[6].Though powdery mildew is the number one yield constraining disease of the crop; less focus is given to the management of the disease in fenugreek. In resistant breeding for powdery mildew management in fenugreek, Bélanger[7] reported that a mildew resistant fenugreek variety Chala (FG-47-01) has been developed and released for mildew risk areas. As host plant resistance is the best and most economical for plant disease management, investigations of moderately resistant genotypes are paramount in breeding for disease resistance. Therefore, a wide gap in yield is attributed to a lack of stable, high yielding and disease resistant genotypes for different agro ecological zones of Ethiopia including the study area, hence the study was designed with objective of evaluation and recommendation of potential sources of resistance fenugreek genotypes against powdery mildew under field condition.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted at Kulumsa Agricultural Research Center, South Eastern Ethiopia from July 2021 to February 2022.The representative agro ecology of Kulumsa is characterized as waterlogged Vertisols[8].

Planting Materials
One hundred fenugreek accessions along with one local check were used for this study. The majority of the accessions represent the national collection from major growing regions of Ethiopia, mainly obtained from Ethiopian Biodiversity Institute (EBI),while one local variety was obtained from a local farmer. The total lists of planted one hundred genotypes are retained along with the Author.

Experimental Design
The experiment was laid out in alpha lattice design in three replications. The plot size was a single row of 1.5 m long and spacing of 0.30 m between rows and 0.10 m intra-row spacing. A replication holds 4 blocks and contains 100 entries (genotypes) including local checks. Spacing between sub blocks was 1 m and between the two replications was 1.5 m. Therefore, the total experimental area was 11m X 30.3m = 333.3 m2. The seed and fertilizer rates were as per the national recommendation for the crop. i.e., 25 kg /ha of seed, 100 kg for NPS(Nitrogen(N), Phosphorus(P205) and Sulfur (SO3)) and 50 kg UREA /H2NCONH2 per hectare. Half the rate of UREA and full dose DAP were applied at planting time and the second half of UREA was applied on the second round.

Data Collected and Measurements
Progress of disease development in the plants was observed five times during the epidemiological period. Disease assessments were made by observing the plants that were planted on the plot or row and recorded as diseased and healthy. Disease incidence and severity were taken as Danielsen & Munk, standard procedure[9].Disease Incidence (DI): is the number of infected plants over the total number of plants per plot and Disease severity (DS) is defined as the affected leaf area, including the lesion and associated chlorosis (non-green area) as a percentage of total leaf area.

The severity score from five selected plants was converted into percent severity index (PSI) for analysis using the formula suggested by Jenkins & Wehner[10].The disease severity indexes obtained from different assessment periods were used to calculate the Area under the Disease Progress Curve (AUDPC-% day) of the recording period. Area under the disease progress curve (AUDPC) was calculated for each genotype[11].

Disease Assessment
Starting from when tillers first appeared and continuing at intervals of five days throughout the entire season, the severity of powdery mildew was measured on five randomly selected tagged plants in a plot. According to a 0–9 rating scale, the disease was scored based on the percentage of leaf area that was infected (Jenkins and Wehner,1983).0 = No illness 1= a few tiny leaf lesions (0–3), 2= a few leaf lesions (3-6) but no stem lesions,3-6 superficial stem lesions or few lesions on few leaves, 5= 25-50 Few well-formed leaf lesions or growing stem lesions,4= 12–25 few well-formed leaf lesions or superficial stem lesions 6= 50-75 a plant with more than 50% of its leaves missing, several huge leaf lesions, deep stem lesions with profuse sporulation.

Phonological Parameter
Days to 50% flowering, number of days between planting and 50% flowering of a row was recorded. Days to 95 % physiological maturity: was calculated as the number of days between planting and 95 % physiological maturity in an experimental unit.

Yield and Yield Components
Average number of pods counted from the five plants that were randomly chosen. The average number of primary branches (branch lets that extend from the main stem) was calculated for five randomly selected plants. Plant height (PH) in cm. The height of plants was measured from the ground up to the tip of the main stem at 90% physiological maturity. The difference between the days to maturity and the days to flowering (DM-DH) was used to compute the grain filling period (GFP). The average number of nodes from the five studied plants was used to calculate the number of nodes per plant (NNPPl). The average of the total number of pods on five randomly chosen plants is used to calculate the number of pods per plant (NPPPl).

Statistical Analysis
Analysis of variance (ANOVA) made using SAS version 9.2[12]. When ANOVA indicate significant difference among treatment means. Mean comparisons were carried out using the least significant difference (LSD) at a 5% level of significance. Correlation analysis was made to analyze the relation of the disease parameters to yield parameters and principal component analysis was made to group the genotypes.

RESULTS AND DISCUSSION

Disease Development and Genotype Reaction
The study revealed that there was disease reaction variation among Ethiopian fenugreek landraces. Different scholars also found that there is a variation among fenugreek genotypes in reaction to powdery mildew pathogen. The evaluated genotypes fall into four disease reaction classes namely resistant, moderately susceptible, susceptible and highly susceptible against the powdery mildew pathogen according to Jenkins and Wehner rating scale[10].Among the hundred genotypes evaluated two genotypes namely 28601 and 29561 exhibited resistance reactions against the powdery mildew pathogen with the mean disease severity of 9.49 and 7.19 respectively. Similarly, forty-three of the genotypes showed moderate resistance, fifty-four were susceptible and one genotype showed a highly susceptible disease reaction with mean disease severity of 69.92.

The current results indicated that there were high variations in disease development between the moderately resistant and susceptible genotypes which reveals resistant genotypes potentially reduce the disease severity while disease severity on susceptible genotypes. This finding was in line agreement with the finding of Raje[13], who reported that there was a heavy incidence of powdery mildew in susceptible check while less incidence in a moderately resistant material. Apart from powdery mildew other fenugreek disease like; Cercospora leaf spot and rust were recorded and considered during the experiment.

Analysis of Variance/ANOVA
ANOVA result for agronomic and disease parameters also revealed that different fenugreek genotypes responded differently to the infection of powdery mildew (Table 1). The results showed that AUDPC and all agronomic parameters except plant height and number of primary branches per plant (NPBPPl) were significantly different among tested genotypes. The analysis of variance showed that there is no significant difference among genotypes concerning plant height and number of primary branches per plant. This might be related to the late coming of the pathogen at which the plant grows to its optimum height and primary branch and the two traits were similar in fenugreek genotypes. Typical powdery mildew symptoms of infection were observed beginning from sixty-two days post-germination. The symptoms began from the lowest

leaf among the plants. The powdery mildew appeared as small white powdery spots on the lower and upper surfaces of leaves. The powdery mildew progresses to other leaves as the plants grow. These were observed virtually on all the leaves of the plants. Benagi[15] screened 110 lines of fenugreek for resistance to Erysiphe polygoni, Rhizoctonia solani and Fusarium oxysporum in Harry. None of the genotypes was completely resistant to all three pathogens However, GP75, GP82, GP94, GP and PEB were the moderately resistant lines and lines are significantly different in the yield and yield component parameters.

Table 1: Growth and phonological parameters of evaluated fenugreek germplasm reactions for powdery mildew (*Erysiphe polygoni*) under field conditions.

	DE	DF	DM	GFP	PL	NPBPPl	NSBPPl	NNPPl	NPPPl
min	5.33	44.33	130	82	6.47	3.1	0.47	13.07	3.13
max	8	49.33	135.67	90	9.63	5.4	3	41	15.73
mean	6.67	46.86	132.24	85.38	8.27	4.03	1.94	28.05	10.2
Cv	13.33	3.06	0.95	1.9	6.5	11.63	5.45	18.89	26.2
LSD	1.43	2.31	2.03	2.61	0.87	4.46	0.35	4.31	0.59
P value	0.03	0	<0.0001	0.02	<0.0001	0.55	<0.0001	<0.0001	<0.0001

Key: PL= Pod length, NPBPP= number of primary branches, NSBPP= Number of secondary branches, NNPPl=Number of nodes per plant, NPPPl =Number of pods per plant, DE= Days of emergence, DF= Days to 50% flowering, DM=Days to maturity, GFP=Grain filling period, LSD=Least significant difference.

Correlation Analysis

Pearson's correlation coefficients between possible pairs of agronomic traits and disease parameters were tested using SAS software 12. The results showed that the correlation between most of the yield and yield components in fenugreek is positive and significant(Table2).Seed yield per hector had positive and significant correlations with all paired yield component traits except pod length. The result revealed that genotypes with better (longer) grain filling periods are better in their seed yield and the plants bearing more nodes per plant, a greater number of pods per plant and a greater number of seeds per pod produce more seed yield. Indeed, genotypes with better hundred seed weights had higher above-ground biomass and seed yield per hectare. Thus, selection for better yield component traits will bring about a definite improvement in above-ground biomass and seed yield. The trait Seed Yield per hectare is non-significant with disease parameters, an area under the disease progress curve (AUDPC) and disease progress rate. The disease parameters AUDPC and disease progress rate were non-significant with most yield component parameters except grain filling period and number of seeds per pod. This result revealed that the tested genotypes were diverse in their yield traits and yield traits are genetically different because of the disease effect. Generally, this study revealed that resistant genotypes identified in this study significantly reduced the disease parameters but low yielder. This phenomenon happens in non-elite resistant material because they mobilize most of their genetic resource for disease or stress response rather than yield response. As powdery mildew is a series disease in fenugreek the resistant genetic materials identified in this study are important for cross-breeding with elite high-yielding genotypes.

Table 2: Correlation between different agronomic traits and final disease reaction to powdery mildew disease on fenugreek genotypes

	GFP	PL	NNPPl	NSPP	SYPPl	HSW	AGBM	HI	rAUDPC	DPR
PL	0.106ns									
NNPPl	0.211*	0.353**								
NPPPl	0.237*	0.348**	0.820**							
NSPP	0.356**	0.253*	0.205*	0.188ns						
SYPPl	0.168ns	0.137ns	0.342**	0.213*	0.511**					
HSW	0.193ns	0.135ns	0.107ns	0.033ns	0.515**	0.480**				
AGBM	0.274**	0.249*	0.237*	0.217*	0.799**	0.695**	0.665**			
HI	0.000ns	-0.107ns	0.143ns	0.156	-0.183	0.331**	0.037ns	-0.181ns		

Key: NSPP = number of seeds per plant, SYPPl= Seed yield per plant, HSW=Hundred seeds weight in gram, SYPH = Seed yield per hector, AGBM = above-ground biomass, HI= Harvest index, and rAUDPC= residual Area under disease progress curve, *=significant @ p<0.05; **=significant @ P, 0.01; ns=non-significant.

Cluster Analysis

Hierarchical clustering of the average linkage method with squared Euclidean distance was performed using SAS or/ and MINITAB14 software (MINITAB,2003). The distances between clusters were calculated using the average linkage method of squared Euclidean distance. The average linkage Euclidean distance technique of clustering produced a more understandable portrayal of the 100 accessions by grouping them into six clusters, whereby different members within a cluster are assumed to be more closely related in terms of the trait under consideration with each other than those members in different clusters. Similarly, members in clusters with non-significant distances are assumed to have more close to each other than they are with those in significantly distant clusters. In this study, the hundred genotypes were grouped into five clusters (Table3). The maximum distance i.e., ED=9.642 was found between cluster 3 and cluster6. On the other hand, minimum inter-cluster distance (3.700) was recorded between clusters 2 and 4 indicating their genetic related. While the highest intra clusters some of square (947.379) were recorded in cluster2 which consists of 74 genotypes.

Table 3: Clusters of 100 fenugreek genotypes into different diversity classes.

Cluster-1	Cluster-2					Cluster-3	Cluster-4	Cluster-5	Cluster-6
G1	G2	G7	G74	G75	G35	G3	G12	G23	G88
G15	G55	G8	G56	G33	G84		G14	G97	
G39	G73	G40	G62	G50	G78		G95	G100	
G91	G58	G86	G11	G71	G80		G48	G98	
G64	G69	G72	G45	G6	G77		G52		
G66	G59	G85	G22	G60	G81		G49		
G34	G93	G24	G83	G70	G20		G18		
G87	G65	G27	G45	G82	G37		G68		
G94	G13	G42	G25	G26	G28		G31		
	G46	G43	G30	G67	G92		G51		
	G93	G47	G32	G89	G99		G63		
	G5	G10	G96	G61	G54				
	G38	G17	G41	G21	G4				
	G19	G16	G44	G51	G9				
	G90	G36	G76	G79					

G= Genotype

Cluster 1: It consisted of 9 genotypes which were collected from Oromia and Amhara regions. There is a genetic distance between cluster one and cluster two. Members in this cluster laid on intermediate value in all the traits under consideration.

Cluster 2: It consisted of 74 genotypes, which were early in days to flowering, intermediate in biomass yield, number of pods and seeds per plant and number of seeds per pod. Among these clusters, the genotype/ accession, 35190 is a high yielder. Accessions in this cluster also exhibited lower with hundred seed weight, seed yield per plant harvest index and 1 accession exhibited resistance and 17 the remaining exhibited moderately susceptible as well as susceptible to powdery mildew disease.

Cluster 3: It consisted of 1 genotype characterized by late days to flowering; low seed and biomass yield and a number of seeds and pods per plant high in hundred seed weight. Accessions in this cluster also exhibited an intermediate, number of seeds per pod harvest index and resistance to powdery mildew disease.

Cluster 4: It had 11 genotypes which exhibited early growth periods, short days to flowering; low in hundred seed weight and intermediate in both biomass yield and number of pods per plant. Among these clusters, the accession,237935 showed a relatively high mean. These accessions also exhibited intermediate, seed yield per plant, number of seeds per pod and number of seeds per plant, harvest index and resistance to powdery mildew disease.

Cluster 5: It consisted of four genotypes, collected one from Bale Ginir, one from East Gojam, and one from south Gonder. The accessions under this category were relatively inferior in most of the traits investigated. It was characterized by intermediate days to flowering; exhibited lowest in all traits under study except hundred seed weight, harvest index and moderately susceptible to powdery mildew.

Cluster 6: It consisted of one genotype from Tigray. It was found to be the most superior accession regarding the traits studied. This accession was characterized by a low hundred seed weight and harvest index. However, this particular accession also required a longer period to maturity, characterized by intermediate seed and biomass yield per plant, number of seeds and pods per plant and seeds per pod and moderately susceptible response to powdery mildew. In general, the differences between the clusters were mainly attributed to the variation in all traits. Other traits such as days to flowering, biomass yield and number of seeds per plant have contributed equally well for cluster castellations.

CONCLUSIONS

The resistant and moderately susceptible genetic materials identified in this study can be used for further exploitation of the genes for disease resistance breeding in the future. I highly recommend that those materials viz 28601 and 29561 found resistant to powdery mildew, disease be used as germplasm to broaden the genetic base of fenugreek for sustainable production in the country in general. However, further evaluation of the materials under optimum disease pressure including evaluation of the materials under greenhouse conditions is recommended.

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