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STUDY OF MOLECULAR VARIATION AMONG DIFFERENT ISOLATES OF *FUSARIUM OXYSPORUM* F. SP. *LENTIS*

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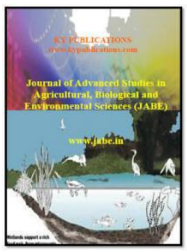

ABSTRACT

A species of *Fusarium* denoted by ten isolates were collected from diverse crops and variation among them was examined by protein profiling techniques. The SDS PAGE revealed that, each of the FOl isolates was unique in band patterns. In conclusion, cluster analysis of the protein banding patterns by SDS-PAGE, and SPSS analysis of banding patterns were found to be efficient and effective tools for finding the protein variability among the isolates isolated in the same geographical area and environmental conditions.

Keyword-isolation, variation, protein profiling ,lentil.

INTRODUCTION

Lentil (*Lens culinaris* Medik.), is a member of Leguminaceae family and it is commonly known as masoor or poor man's meat. It has a high nutritive value and major source of dietary proteins (25%) after soybeans in human and animal diet (Rahman *et al*., 2010).

In Uttar Pradesh, it is grown in 620.000 lakh/ha area with 452.000 lakh tones production and 732.0 kg/ha productivity (Ahmad, 2017). It suffers from a number of diseases. Wilt of lentil caused by *Fusarium oxysporum* f. sp*. lentis* is one of the most wide spread and destructive disease where ever crop is grown. The yield losses due to this disease as much as 50 percent have been reported in India (Anonymous, 1999) Electrophoretic analysis of proteins and isozymes can be used as an adjunct to morphological, cultural and pathogenic variability of different isolates of the pathogen (Hall, 1967). Protein and isozyme analysis on PAGE provides a well established and efficient tool for revealing genetic variability in fungal population (El-Kazaz, *et al.,* 2008). The prime temperature range for their existence and growth is 25 to 30 °C. *Fusarium oxy.* f. sp. *lentis* usually have ability to sporulate in most of the cultures. Potato dextrose agar media are found to be the most suitable media. Incubation under alternate 12 hr. light/dark circle is found to be most suitable for sporulation. Identification of variability amongst *Fusarium oxy.* f. sp. *lentis* on the basis of molecular studies are important.

MATERIALS AND METHODS

Fungal isolates and culture conditions

Ten different isolates of fungal isolates from root were included in the analysis. The isolate were collected from various sites of U.P. Strains were cultivated in the potato dextrose agar medium for 7 day at 25^{0}c. Petri disc (9mm size, 2mm thickness) containing mycelia of Fol were cut with a sterilized cork borer and one disc was inoculated in to 100ml potato dextrose broth in flasks. Each isolate was grown in triplicate (3 flasks) at room temperature in darkness (Lakshman andTavantzis, 1994). The mycelium was harvested after 7 days, washed four time with autoclaved and de-ionized water, blotted to remove excess water, lyophilized and stored in deep freeze in refrigerator until further processing.

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Protein estimation

Total Protein was estimated through the Hartree-Lowry method.

Protein profiling of fungal mycelium by SDS- PAGE: One gram mycelium of *F. o.* f. sp. *lentis* samples were grounded in mortar and pestle at 4°C in RIPA buffer (150mMNaCl, 1.0% NP-40 or 0.1% Triton X-100, 0.5% Sodium Deoxycholate, 50mM tris-HCl,pH8.0, Protease inhibitor) with 2mM PMSF and centrifuge at 12000 rpm for 15 minutes. Crude lysate was centrifuges at 10,000 rpm and supernatant was collected for further analysis. Then put into 1.5 ml of eppendorf tube and it was centrifuge at 5000 rpm for 20 minutes 4^{o}c temperature. The supernatant was transfer into the fresh eppendorf tube and store it at 4^{0}C for future use.

70µl of sample was taken in eppendorf tube in which 30µl of dye was added and denature for 5 minutes in boiled water. After the process of denaturation the sample was loaded in the well. 10% gel was prepared. After completion the process of gel electrophoresis the gel was put in staining solution and after 8 hour this gel was put into destaining solution.

RESULT AND DISCUSSION

Table-1. Molecular different variation concentration volume loaded in isolates of *Fusarium oxysporum* f.sp. *lentis*

Sl. No.	Sample	Concentration (mg/ml)	Volume loaded (µl)
1	Faizabad (FOL-1)	2.46	100
2	Amethi (FOL-2)	3.56	100
3	Raibrelly (FOL-3)	5.14	100
4	Lucknow (FOL-4)	2.29	100
5	Sonbhadra (FOL-5)	4.24	100
6	Mirzapur (FOL-6)	2.6	100
7	Bhadohi (FOL-7)	3.12	100
8	Varanasi (FOL-8)	3.08	100
9	Rath (FOL-9)	4.94	100
10	Sultanpur (FOL-10)	3.45	100

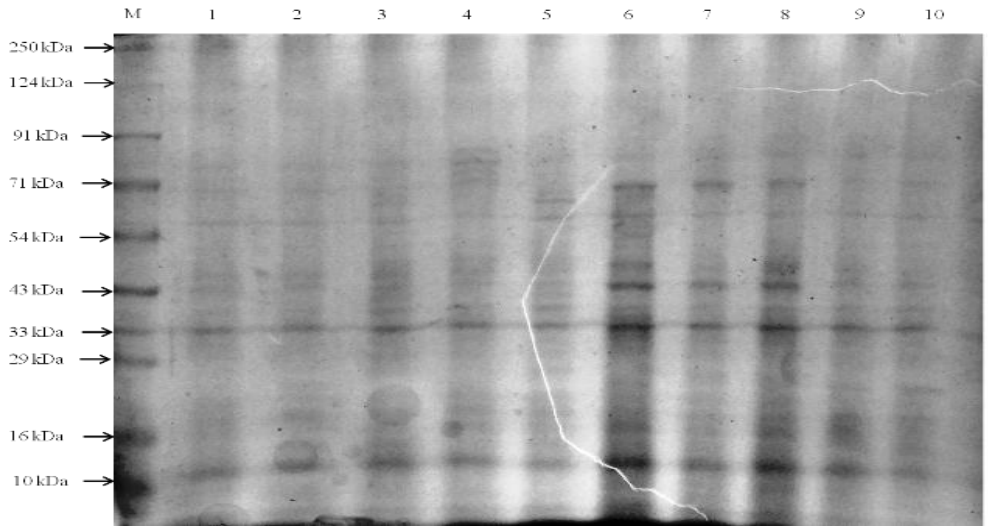

Fig.2 . The SDS-PAGE protein profile showing variability in ten isolates of *F. oxysporum* f. sp. *lentis*

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Results showed that each of *F. o.* f. sp. *lentis* isolates had their own unique protein profiles (Fig.2). The protein profile of different isolates showed common banding protein band 0f 250kDa and 10 kDa in all 10 isolates of *F. o.* f. sp. *lentis.* FOL-F1 and FOL-S10 isolates were distinct in having bands of 250kDa. FOL-F1, FOL-R3 and FOL-V8 showed similar protein banding pattern at 124kDa. Isolate FOL-L4 and FOL-S5 was unique having protein band of 71 kDa and 91kDa. Protein band pattern of 54 kDa , 33kDa, and 10kDa was present similar pattern of protien bands. Isolates FOL-B7 and FOL-V8, FOL-R9 exhibited distinct banding patterns of 43 kDa, 29kDa and 16kDa.

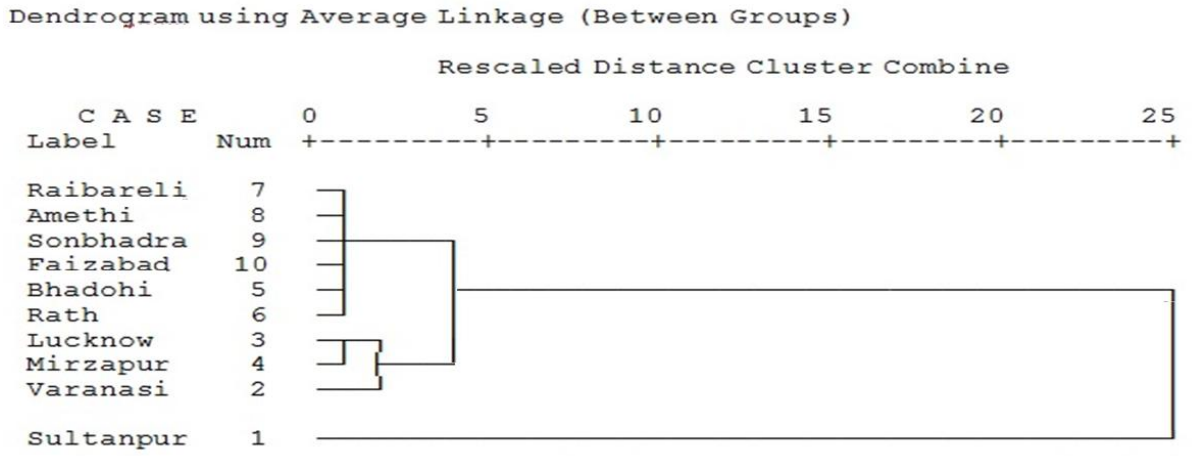

Fig.3 . The SDS-PAGE dendogram showing variability in ten isolates of *F. oxysporum* f. sp*. lentis*

The cluster dendogram analysis under pot condition at reproductive stage showed 3 clusters that were demarcated at a cutoff similarity coefficient level of 1.0, below which the similarity values narrowed conspicuously. Clusters I was the largest and included 6 district sample while clusters II included 3 district sample, Cluster III and included one district sample . A dendogram was generated by SPSS version 0.16 to depict the protein level relationships among the 10 sample of lentil under this study. The cluster analysis showed the significant protein level variation among thewilt of lentil. Clusters I having six variation sample namely FOL-Sonbhadra (9), FOL-Faizabad (10), FOL-Raibreilly (7), and FOL- Rath (6) , FOL-Amethi (8) FOL-Bhadohi (5) . Cluster II contained maximum 3 variation sample namely FOL- Lucknow (3) and FOL-Mirzapur (4), Varanasi (2). Cluster III contained 1sample FOL-Sultanpur (1) .

CONCLUSION

The protein profile of different isolates showed common banding protein band of 250kDa and 10 kDa in all 10 isolates of *F. o.* f. sp. *lentis. Three i*solates exhibited distinct banding patterns of 43 kDa, 29kDa and 16kDa. The cluster dendogram analysis showed 3 clusters that were demarcated at a cutoff similarity coefficient level of 1.0, below which the similarity values narrowed conspicuously. Clusters I was the largest and included 6 district sample while clusters II included 3 district sample, Cluster III and included one district sample.

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Reference

[1]. Abraham, R. 2015. Lentil (*Lens Culinaris Medikus*) Current Status and Future Prospect of Production in Ethiopia. Adv. *Plants Agric Res* 2 (2): 00040.

[2]. Ahmad, D. K. Sinha and K. M. Singh, 2017. Economic Analysis of Production and Instability of Lentil in Major Lentil Growing States of India. *Int. J. Pure App. Bio.sci.* 6 (1): 593-598 .

[3]. Anonymous, 1999. Technology for increasing pulses production In India. *Indian Institute of Pulses Research,* Kanpur. pp. 1-108.

[4]. Hall, R. (1967). Proteins and Catalase isozymes from *Fusarium solani* and their taxonomic significance. Australian Journal of Biological Science 20:419-428.

[5]. Rahman, T., Ahmed, A.U., Islam, M.R., and Hosen, M.I. 2010. Physiological study and both *in vitro* and *in vivo* antifungal activities against *Stemphylium botryosum* causing *Stemphylium* blight disease in lentil (*Lens culinaris*). *Plant Pathology Journal* 9 (4): 179-187.

[6]. Sumana, K.,Punith B.D.andDevaki, N.S. (2014).First report on molecular and biochemical variations among the populations of *Fusarium oxysporum* infecting tobacco in Karnataka India. *Journal of Agricultural Technology* 10 (4): 931-950.