Multi-Locus Sequence Analysis revealing Population and Genetic diversity of Fusarium graminearum species complex in India

Noyonika[1, 2]✉*, Prem Lal Kashyap[1], Deepti Singh[2], Sudheer Kumar[1], Gyanendra Pratap Singh[1]

[1]Department of Crop Protection, ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal 132001, Haryana, India
[2]Amity Institute of Microbial Technology (AIMT), Amity University, Jaipur 303007, Rajasthan
Corresponding and Presenting Author
e-mail:- kaulnoyonika@gmail.com Tel. (+91) 9996961632

INTRODUCTION

- Fusarium head blight (FHB) incited by Fusarium graminearum (FG) fungus is ranked as the one of the prime annihilating fungal diseases of wheat (Triticum aestivum L.) globally.
- Molecular marker-driven technologies play a significant role in species identification because of their potential usage in exploring the population structure and genetic diversity within the fungal species and their isolates

OBJECTIVE: To improve the understanding of the ecology of the plant pathogen and devise a better management strategy for the management of FHB

MATERIALS & METHODS

- One hundred and twenty three isolates of FG were used in the present study

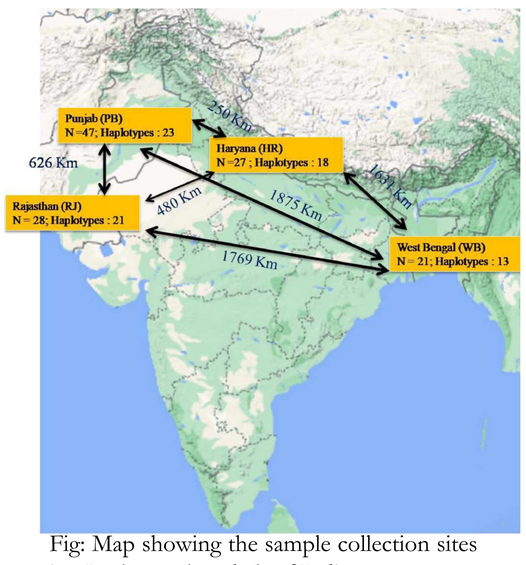

Fig: Map showing the sample collection sites in Northern wheat belt of India

Fig: Typical symptoms of wheat head scab disease

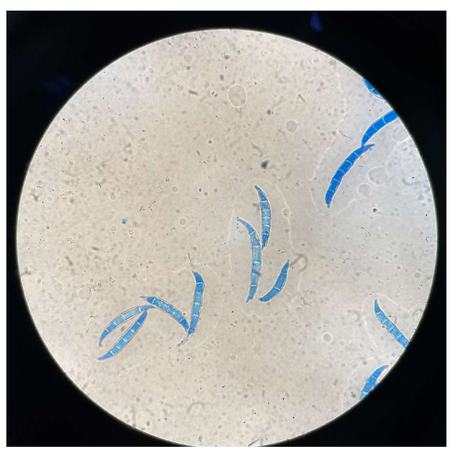

Fig: Sexual spores(ascospores) of Fusarium graminearum

- These isolates were collected during field surveys conducted from 2017 to 2022 in different wheat-growing fields in the four different states of India.
- Infected wheat samples were sliced into minute pieces of 2–3 mm and later surface-sterilizated with ethanol (70%) for 30s followed by NaOCl (1%) treatment for 1 min.
- The determination of total genomic DNA extraction and concentration was performed.

Gene Region	Sequence (5′-3′)	Product Size (bp)	Optimized PCR Conditions
Translation elongation factor 1 alpha (TEF)	TEF1: ATGGGTAAGGAGGACAAGAC	≈700	95 °C: 5 min, (95 °C: 30 s, 56 °C: 30 s, 72 °C: 1 min) × 35 cycles 72 °C: 10 min
	TEF2: GGAAGTACCAGTGATCAT GTT		
Histone (HIS)	CYLH3F: AGGTCC ACTGGTGGCAAG	≈500	95 °C: 5 min, (95 °C: 30 s, 55 °C: 50 s, 72 °C: 1 min) × 35 cycles 72 °C: 5 min
	H3-1b: GCGGGCGAGCTGGATGTCCTT		
Beta-tubulin (TUB)	BT2a:GGTAACCAAATCGGTGCTGCTTTC		94 °C: 5 min, (94 °C: 30 s, 54 °C: 50 s, 72 °C: 1 min) × 35 cycles 72 °C: 10 min
	Bt2b: ACCCTCAGTGTAGTGACCCTTGGC	≈350	

Fig: Gene regions and primer pairs used in the current study

- Per cent disease severity or per cent infected spikelets were determined by following the below mentioned formula: Disease severity (%) = [Total infected spikelets / Total spikelets per spike)] X 100.
- DnaSP version 5 tool was also employed to determine the partitioning between populations and build phylogenetic tree with the help of MEGA7 software

RESULTS

- All the isolates based on the comparison of genomics regions for all the three loci (700bp TEF, 500 bp β-tubulin, and 350 bp HIS) confirmed their identity as F. graminareum.
- The 123 FG isolates were groups in two major clusters: Cluster I (115 isolates) and Cluster II (8 isolates)
- There were total 30 haplotypes observed in all the 123 FG isolates

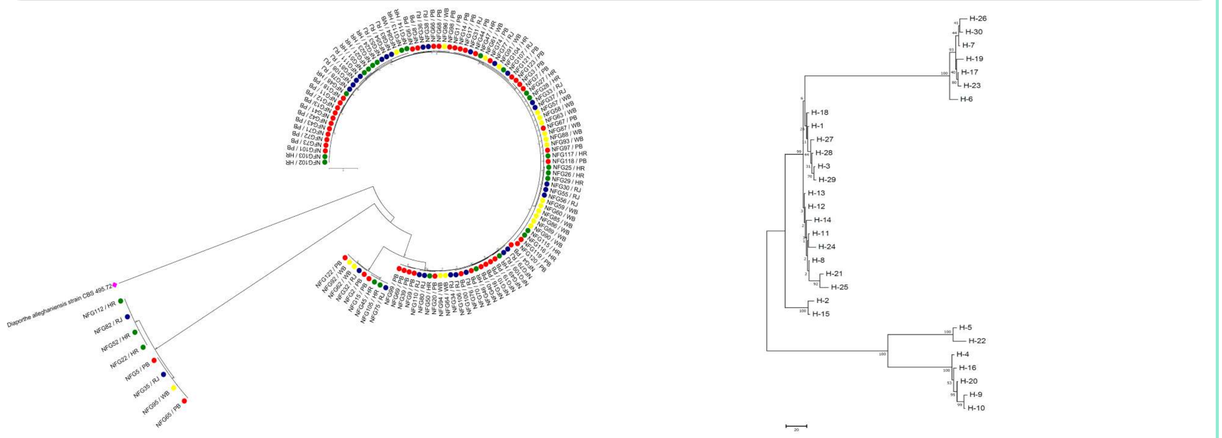

Fig: Phylogenetic relationship determined by using combined sequence of three gen loci. Unrooted maximum parsimony (MP) tree of haplotypes

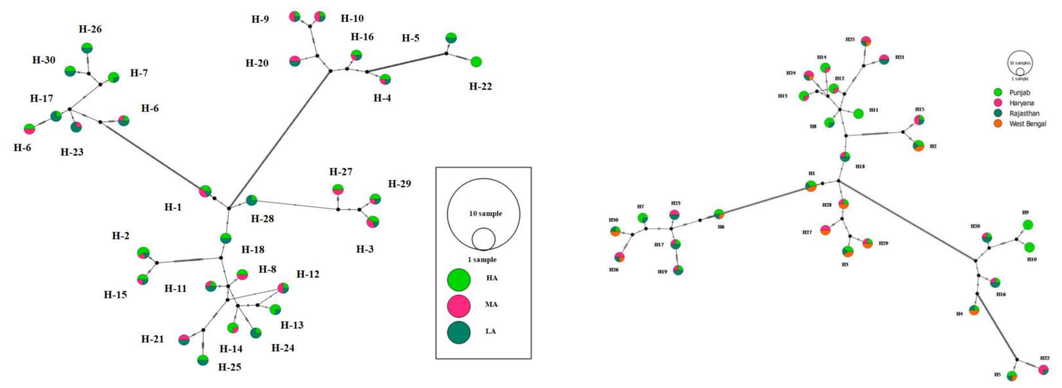

Fig: Median joining network according to different categories of virulence of FG haplotypes and Median joining network of different haplotypes of FG population.

Parameters	TUB	TEF	HIS	Combined
Number of sites	823	993	460	2045
Theta (per site) from Eta	0.012	0.120	0.003	0.083
Theta (per sequence) from Eta	1.857	38.995	1.300	90.059
Total number of mutations (Eta)	10.000	210.000	7.000	485.000
Fu and Li's F *	1.333	1.184	1.703	2.718
Fu and Li's D *	1.361	2.697	1.178	2.817
Average number of pairwise nucleotide differences, k	2.367	25.969	2.408	133.948
Total number of mutations, Eta	10	210	7	485
Minimum number of Recombination events, Rm	4	4	4	12
Tajima's D	0.6814	−1.1005	1.93605	1.62305

Fig: DNA polymorphism data for F. graminearum isolates

CONCLUSION

- TEF is highly recommended for species delineation in offering superior taxa resolution for several of the fungal genera
- Crucial to ascertain the DNA polymorphism for the identification of intraspecies kinships and inferring phylogenetic lineages
- Minimum recombination events based on combined three gene loci have been observed which clearly reflects the mechanism of intragenic recombination behind the genetic variability in pathogen population in the regions.

SIGNIFICANCE

- The greater possibility of sexual reproduction in the regions of FG isolates
- Existence of multiple founder populations,which resulted in population admixture as well as dispersion due to the assemblage of different alleles in FG populations

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