



Integrated bioagent-organic formulations for enhancing seed quality and suppressing soil-borne pathogens in legumes under biotic stress

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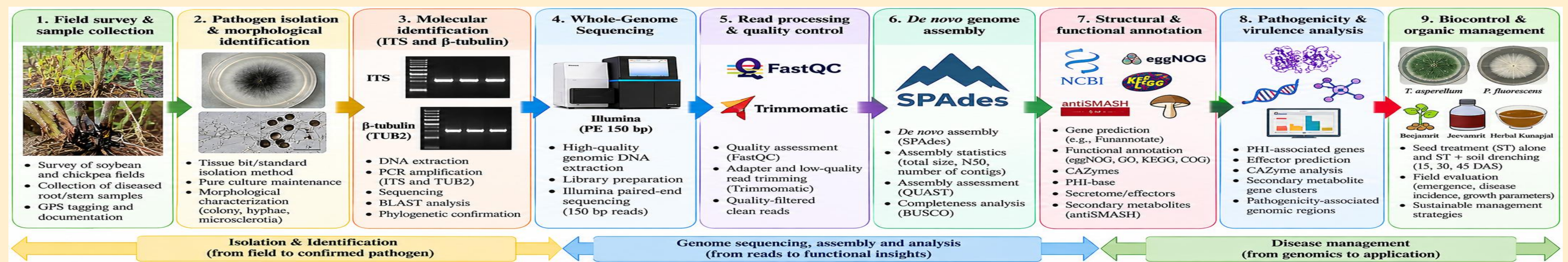
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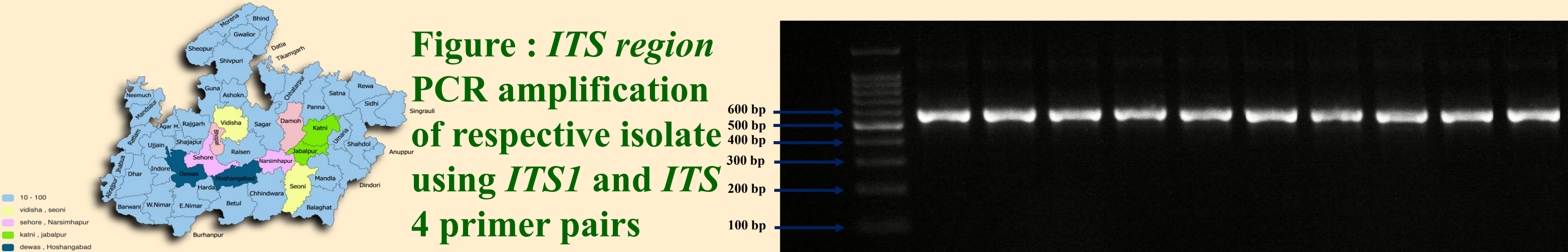
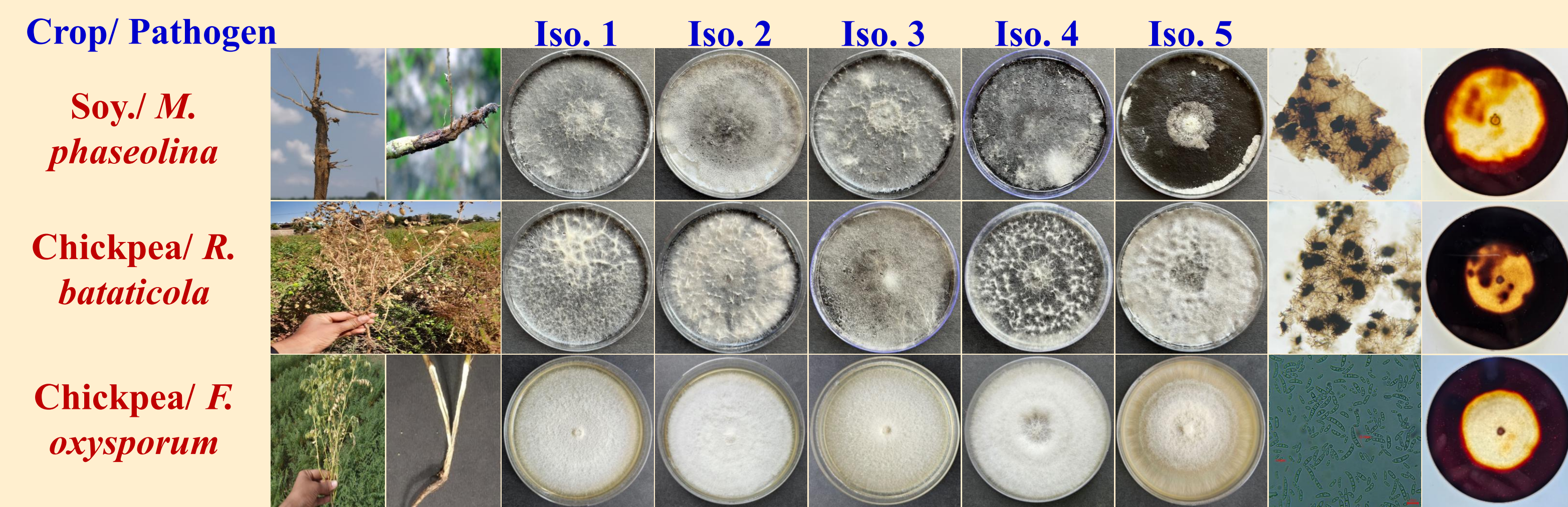
Abstract Three field experiments evaluated bioagents and organic formulations against soil-borne pathogens of soybean and chickpea. *Trichoderma asperellum* seed treatment + soil drenching increased soybean emergence from 65% to 85% and reduced *Macrophomina phaseolina* incidence by 69.28%. In chickpea, disease reduction reached 71.25% (*F. oxysporum*) and 73.96% (*R. bataticola*), demonstrating strong potential for sustainable management.

Introduction Soil-borne pathogens pose major constraints to sustainable legume production, particularly soybean. *Macrophomina phaseolina* causes charcoal rot, leading to significant yield losses under conducive conditions. This study followed a systematic approach encompassing pathogen isolation, morphological and molecular identification, whole-genome sequencing (WGS), and evaluation of bioagents and organic formulations for effective disease management.

Scheme for Whole Genome Sequencing and Management



Isolation, Pectinase activity and identification of test pathogens



Integrated bioagent-organic formulation based management



Among the bioagents and organic formulations, *T. asperellum*, Beejamrit and Herbal Kunapjal, applied as seed treatment and soil drenching, were more effective in reducing disease incidence and promoting plant growth.

Treatment details	Soybean (Charcoal rot)			Chickpea (dry root rot)			Chickpea (Fusarium wilt)		
	Emer.	PDI	PDD	Emer.	PDI	PDD	Emer.	PDI	PDD
<i>T. asperellum</i> ST	84 (66.42)	24.95 (29.97)	57.05	89 (70.63)	21.95 (27.94)	64.18	82 (64.90)	22.65 (28.42)	62.10
<i>P. fluorescens</i> ST	75 (60.00)	39.52 (38.95)	32	80 (63.43)	26.45 (30.95)	56.81	74 (59.34)	26.65 (31.08)	55.10
Beejamrit ST	81 (64.16)	25.78 (30.51)	55.63	87 (68.87)	22.75 (28.49)	62.86	82 (64.90)	23.65 (29.10)	60.55
Jeevamrit ST	79 (62.73)	39.75 (39.09)	31.58	84 (66.42)	26.25 (30.82)	57.14	80 (63.43)	27.15 (31.40)	54.20
H. Kunapjal ST	85 (67.21)	28.90 (32.52)	50.26	86 (68.03)	23.25 (28.83)	62.04	83 (65.65)	24.65 (29.77)	59.85
<i>T. asperellum</i> ST & SD	85 (67.21)	17.85 (24.99)	69.28	88 (69.73)	15.95 (23.54)	73.96	84 (66.42)	17.55 (24.77)	71.25
<i>P. fluorescens</i> ST & SD	78 (62.03)	23.45 (28.96)	59.64	79 (62.73)	21.05 (27.31)	65.63	75 (60.00)	22.05 (28.01)	61.15
Beejamrit ST & SD	83 (65.65)	19.85 (26.46)	65.84	86 (68.03)	17.75 (24.92)	71.02	84 (66.42)	18.65 (25.59)	69.25
Jeevamrit ST & SD	81 (64.16)	20.85 (27.17)	64.11	85 (67.21)	20.85 (27.17)	65.96	81 (64.16)	21.35 (27.52)	63.55
H. Kunapjal ST & SD	84 (66.42)	20.75 (27.10)	64.28	87 (68.87)	20.15 (26.67)	67.1	82 (64.90)	21.05 (27.31)	65.05
Control	65 (53.73)	58.10 (49.66)	-	57 (49.02)	61.25 (51.50)	-	47 (43.28)	61.85 (51.85)	-
S.E.m±	3.05	1.65	-	2.95	1.6	-	2.75	1.52	-
C.D. @ 5 %	9.02	4.85	-	8.65	4.75	-	8.05	4.48	-

ST = Seed treatment; SD = Soil drenching; PDI = Per cent disease incidence; PDD = Per cent disease decrease over control; Emer. = Field emergence

Features: Whole Genome Sequencing of *M. phaseolina* (Soybean)

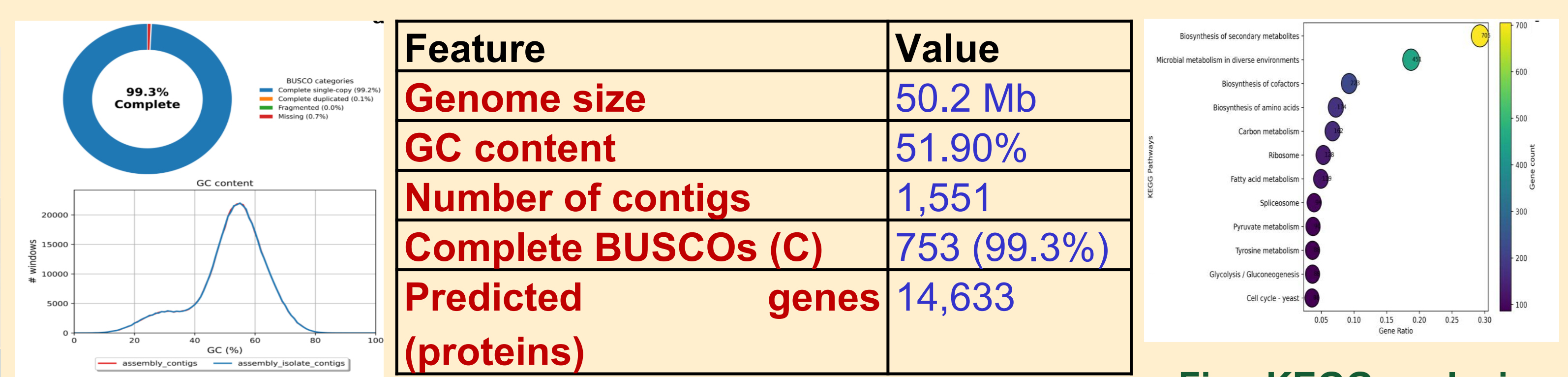


Fig.: BUSCO and GC-content

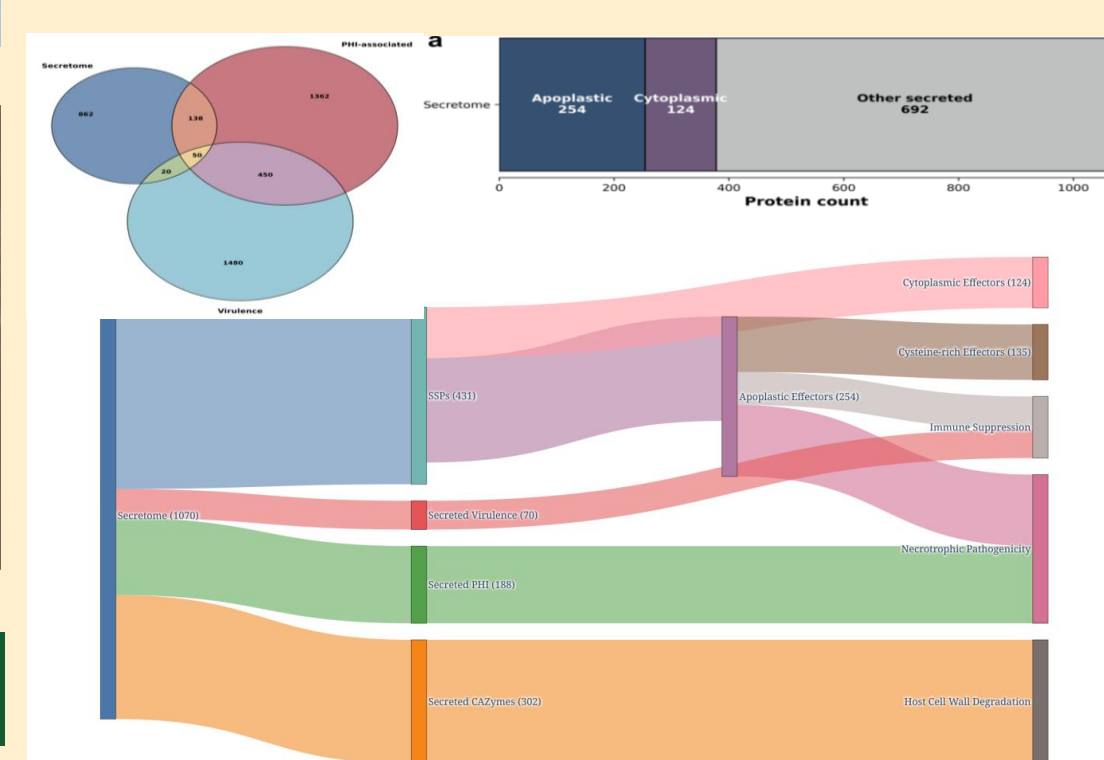


Fig.: Distribution of secreted proteins and Overlap among secretome associated, PHI-associated and virulence-related proteins; Relationship between secretome components and pathogenicity associated functional categories

Major clusters included terpenes (21), NRPS/NRPS like (21) and PKS associated clusters (20). 639 CAZyme genes were identified, including 302 secreted and 209 PHI-associated CAZymes, indicating strong degradative potential of cell-wall degradation, nutrient acquisition, host tissue maceration and aggressive necrotrophic colonization

The 50.2 Mb genome with 99.3% BUSCO completeness indicates high recovery of conserved fungal gene space, although the 1,551-contig assembly reflects fragmented genome structure.

Overall, *M. phaseolina* pathogenicity appears to be multifactorial, involving effectors + hydrolytic enzymes + virulence regulators + secondary metabolites, contributing to its broad host range and environmental adaptability.

Conclusion

M. phaseolina possesses a highly conserved yet metabolically versatile genome, with diverse CAZymes, effectors, virulence-associated proteins, signaling pathways and secondary-metabolite clusters supporting host colonization, stress adaptation and pathogenicity. The pathogen's virulence is multifactorial, involving the coordinated action of hydrolytic enzymes, secreted effectors, virulence regulators and secondary metabolites. *T. asperellum* ST + SD was the most effective eco-friendly treatment for management of charcoal rot in soybean and wilt, dry root rot in chickpea with significant growth promotion. Herbal Kunapjal and Beejamrit also demonstrated promising disease suppression and growth promotion activity and identified as potential organic formulations.

Acknowledgement

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Fig.: KEGG analysis

10,954 genes functionally annotated
Carbohydrate transport & metabolism was the most abundant COG category (648 genes).
2,000 PHI-base homologs indicated a strong repertoire of potential virulence-associated genes.
1,070 secreted proteins, including 431 small secreted proteins (SSPs)
73 secondary metabolite biosynthetic gene clusters

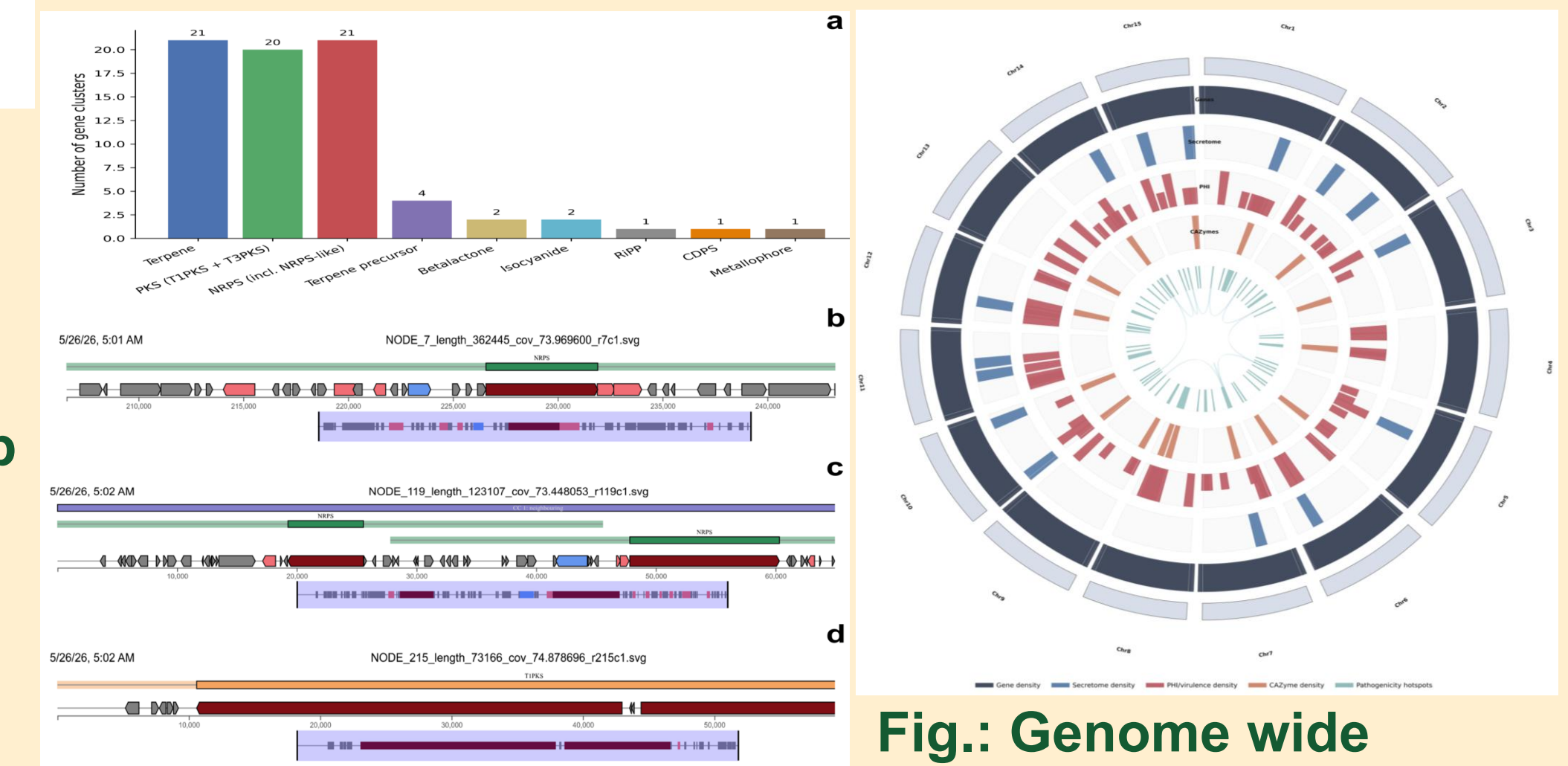


Fig.: Genome wide visualization of pathogenicity associated features highlighting regions enriched with PR factors involved in fungal virulence, host adaptation, extracellular degradation and necrotrophic infection. Outer rings : Chromosomal scaffolds & gene density
Inner tracks: Secretome proteins, PHI genes, virulence determinants, CAZymes, pathogenicity hotspots.