10th Pathology Workshop

'Understanding Sugar Cane Diseases for their Efficient Management'



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Morphological and molecular diversity among *C. falcatum* isolates causing red rot disease of sugarcane in Uttar Pradesh, India

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Sugarcane

- Important agricultural crop covering an area of about 4.2 m ha in India
- In India in tropical regions Maharashtra, Tamil Nadu, Gujrat, Karnataka and Andhra Pradesh are important cane growing states
- In subtropical regions Uttar Pradesh has the largest area under sugarcane followed by Bihar, Haryana and Punjab.
- In Uttar Pradesh average cane yield is 60 tonnes/h

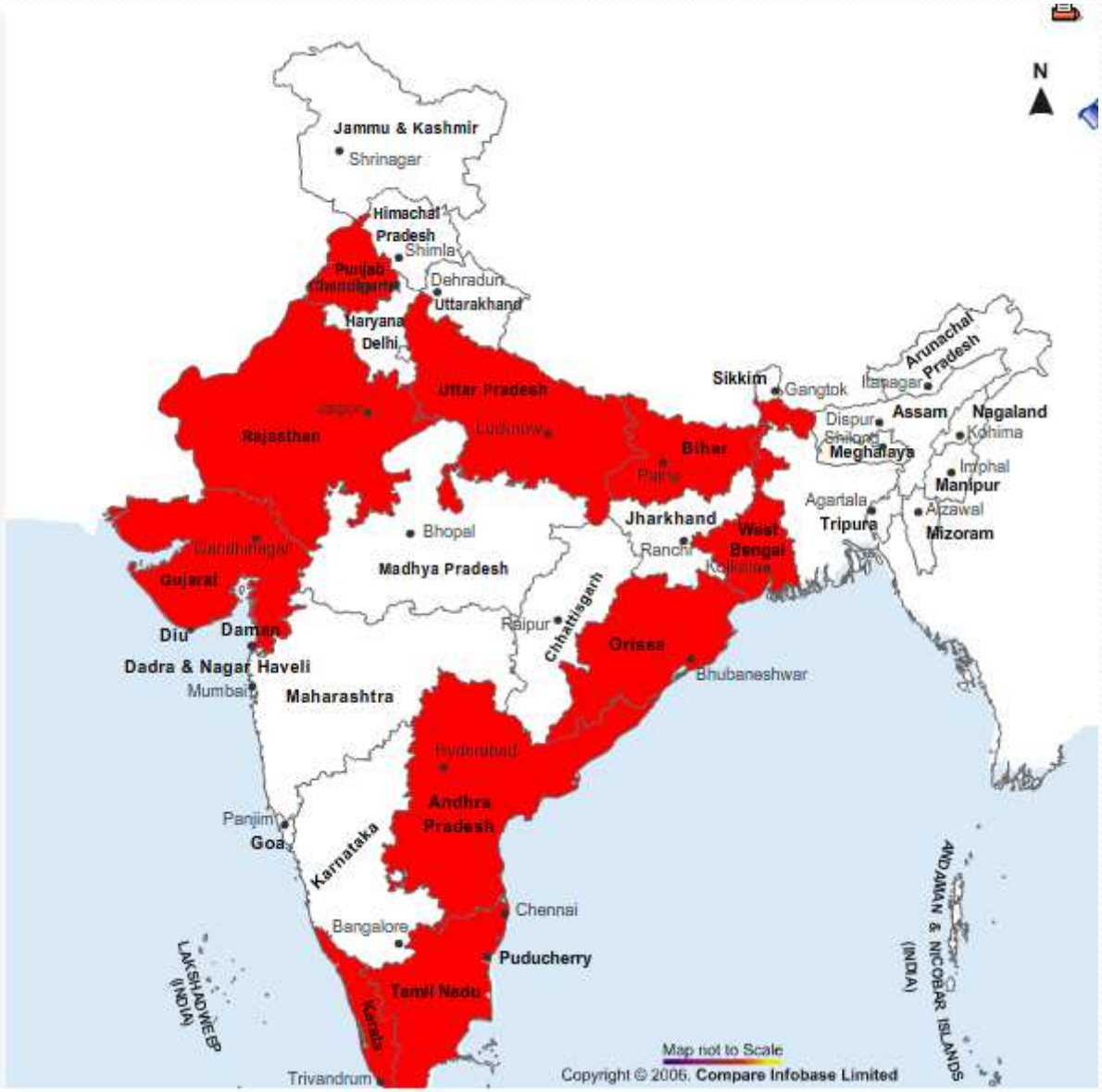
Red rot

- Caused by *Colletotrichum falcatum* Went (perfect stage as *Glomerella tucumenansis*)
- The disease was first reported from Jawa, Indonesia in 1893, within a decade it was reported from West Indies, USA, Australia, Hawaii.
- In India it was first observed in 1901 by Barber from Andhra Pradesh
- The name was given by Butler 1906
- This pathogens keeps changing in nature due to factors such as mutation, hybridization and adaptation.

Importance....

- Causes <3 dozen epidemics in Indian History
- 100% economic loss
- Widely spread in India
- emergence of newer virulent isolates regularly
- No chemical and heat therapy spread in India

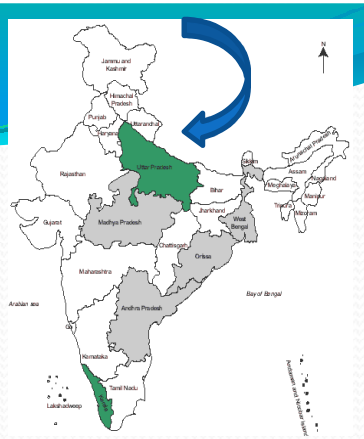
Distribution of Red rot in India



Objectives of the present investigation

- Survey of sugarcane growing areas of Uttar Pradesh to find out the red rot incidence in commercial cultivated varieties.
- Morphological characterization of red rot isolates
- Virulence of the isolates
- Molecular characterization of the isolates

Survey



- An extensive survey of sugarcane growing areas of Eastern, Central and Western regions of Uttar Pradesh viz: Basti, Maharajganj, Kushinagar, Sardarnagar and Santkabirnagar, Deoria, Gorakhpur, Khalilabad, Lucknow, Bareilly and Shahjahanpur were conducted during July and August of 2007-2008 to 2008-2009, to study the incidence of red rot disease on different growing commercial cultivars under natural field conditions.

Incidence

- During survey of sugarcane crops in different parts of UP, 100% incidence of red rot was recorded in variety CoS 8436 at Shahjahanpur and Bareilly and variety CoSe 95422 at Kushinagar, respectively.
- Besides severe incidence of red rot ranging from 7- 42 per cent was also recorded in west, central and eastern part of UP on CoSe 8436, CoS 91269, CoJ 64, UP 95422, CoS 98231 and CoLK 8102 sugarcane varieties .
- The devastating spread and serious losses to the crop prompted us to characterize the associated isolates of red rot, to know the real cause of their spread so as to formulate the significant control measures.

Collection of red rot isolates

- Ten different red rot isolates from infected symptomatic cane samples were collected from six commercial sugarcane varieties viz. CoS 8436, CoS 91269, CoS 95422, CoSe 98231, CoJ 64 and CoLK 8102 from different places.

Symptoms

The most characteristic symptoms of red rot disease in the fields were

- Discoloration of stalk
- Reddening of internodal tissues with interruption of white bands
- Yellowing of the crown leaves
- Total drying of affected stalks

Sugarcane field affected with red rot





Symptoms of red rot disease in splitted open canes



White spots in splitted open affected canes



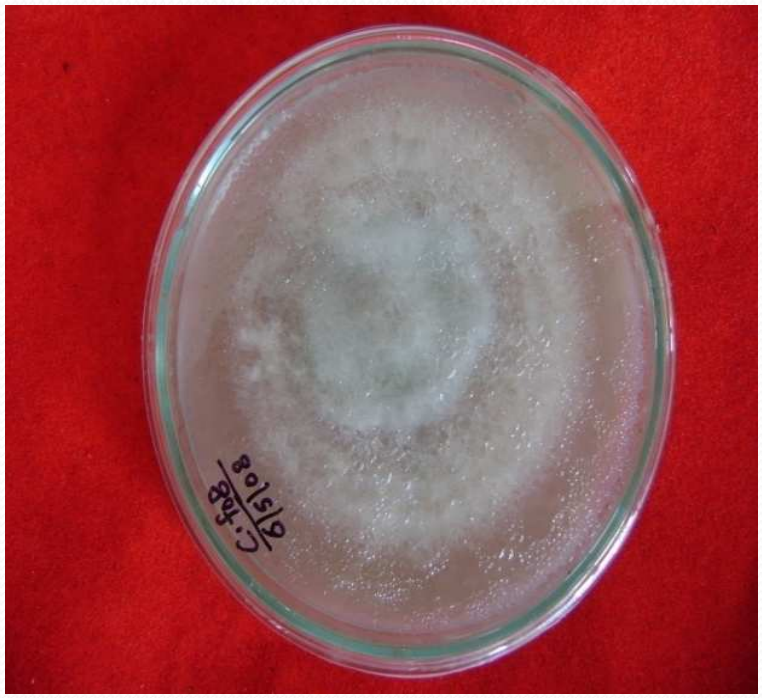
Incidence of isolates on different varieties at various location of U.P in Present investigation

S. N.	Red rot isolates	Location	Varieties	Incidence
1.	R04-01	Shahjahanpur	CoS 8436	100%
2.	Cf-1B	Bareilly	CoS 8436	5-7%
3.	Cf-2B	Bareilly	CoS 8436	100%
4.	Cf-17	Karmaha	CoSe 95422	42%
5.	Cf-Kushinagar	Kushinagar	CoSe 95422	100%
6.	Cf-18	Bilari khash	CoS 91269	11%
7.	Cf-19	Devkaiya futahva	CoJ 64	7%
8.	Cf-20	Basant Tola Sardar Nagar	CoSe 92423	22%
9.	Cf-21	Khalilabad	CoSe 92423	12%
10.	Cf-22	Basti	CoLk 8102	100%

Morphological characteristics of *C. falcatum* isolates

Red rot isolates	Mycelial growth 7^{th}day/15^{th} day	Texture and colour	Conidia size and shape
R04-01	5.5 mm/full plate	Dull white mild cottony growth , slow growing with very thin mycelial matt with even margin	absent
Cf-1B	7.0 mm/full plate	Uneven cottony fluffy white growth with uneven margins Thick mycelial matt equally distributed with even outer margin.	Length 30.0 µm Width 7.8 µm
Cf-20	7.5 mm/full plate	Fast growing cotton arranged in concentric rings at outer circle equal distribution of mycelial growth.	Length 28.5 µm Width 7.5 µm
Cf-19	6.8 mm/full plate	Slow growing cottony growth , highly over growth at centre	Length 28.8 µm Width 7.8 µm
Cf-Kushinagar	6.6 mm/full plate	Cottony growth, vertically concentrated in centre followed by surface unequal growth	Length 27.0 µm Width 6.6 µm
Cf-18	6.6 mm/full plate	Fast growing massive cottony growth mycelial thickness observed erratically	Length 28.8 µm Width 7.8 µm
Cf -17	5.5 mm/full plate	Moderately growing thick mycelial matt even distribution dense at centre Massive cottony growth unequal distribution	Length 28.2 µm Width 7.2 µm
Cf-2B	7 mm/full Plate	Thick mycelial matt equally distributed with even outer margin	Length 29.9 µm Width 7.8 µm
Cf-Basti	5.9 mm/full Plate	Massive cottony growth unequal distribution	absent
Cf-Khalilabad	6.5 mm/full	Slow growing cottony growth highly over growth at centre	absent

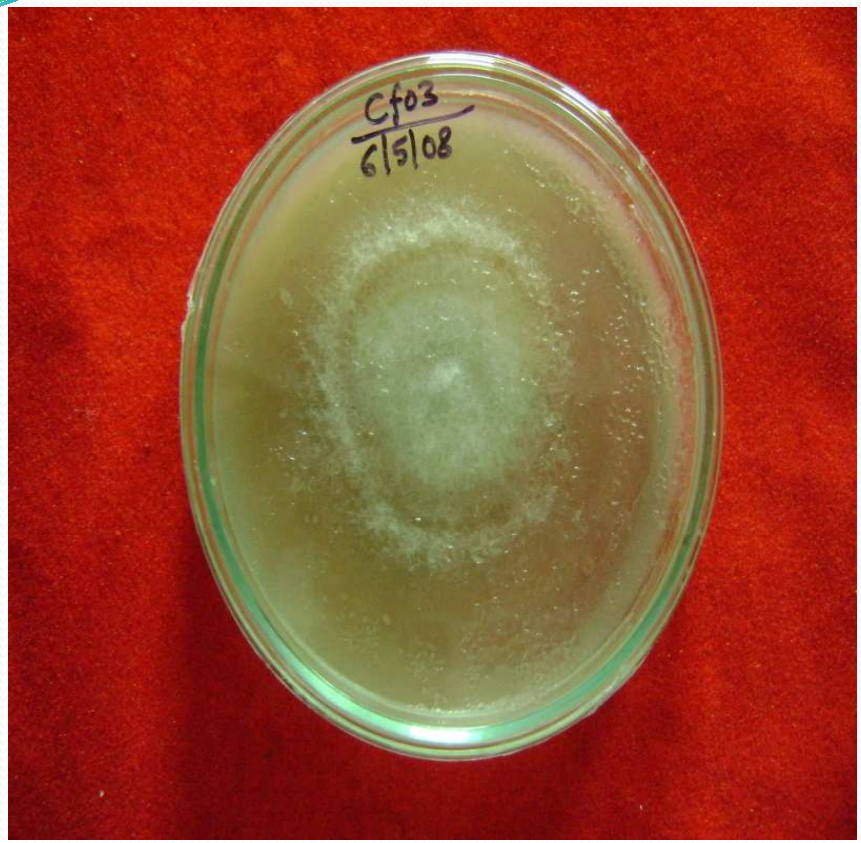
Growth Pattern of different isolates under study



Concentric with equal outer margin ring growth at early stage followed by fluffy white cottony growth with dull black swallow centre with even margin



Cottony and basal in layer at later stage the outer growth ring was white fluffy with deep centre



Dull white mild cottony growth slow growing with very thin mycelial matt with even margin



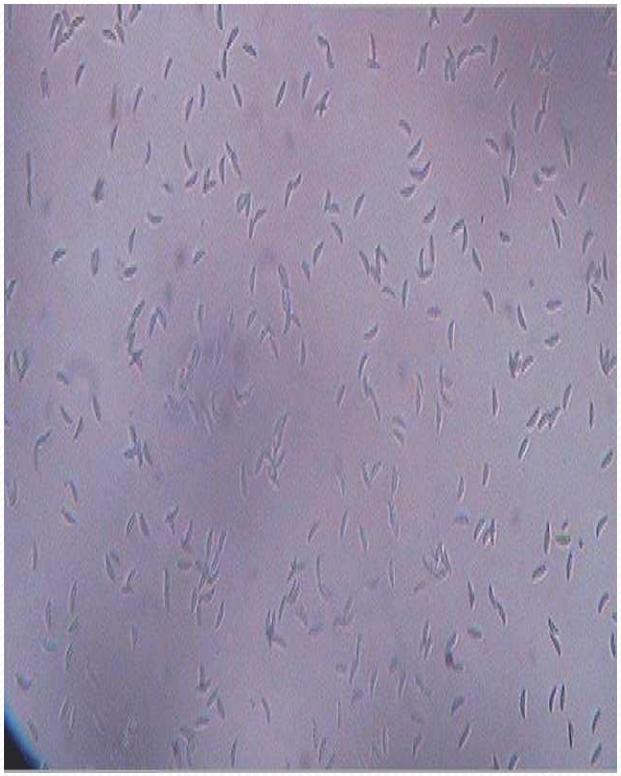
Moderately growing mycelial cottony growth with uneven spreading

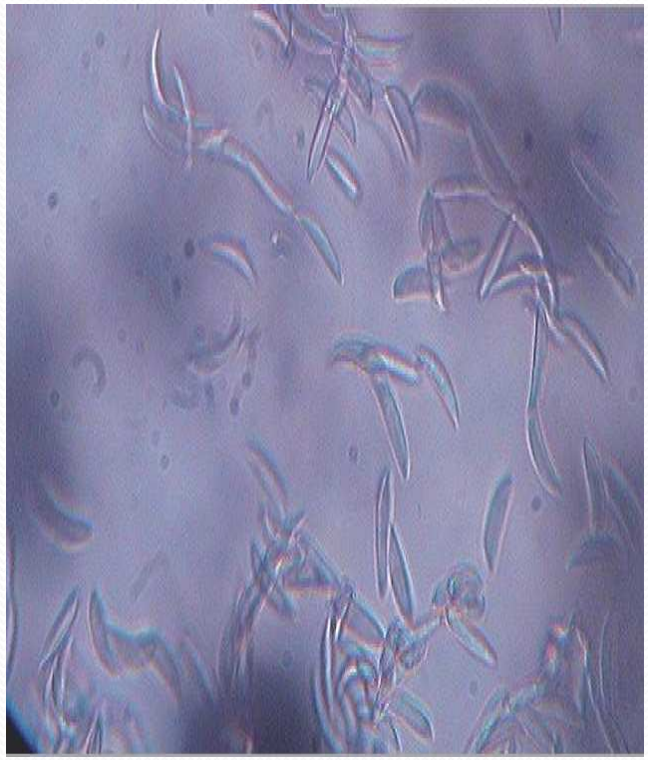
Sporulating isolates of red rot under study





Conidia observed in cultures





Size 27µm to 30µm

Morphological Characteristics of Red Rot isolates

- All the ten isolates showed tremendous variation in their radial growth on oat meal agar medium and sporulation. Nearly all the isolates were able to produce conidia in culture with a diameter ranged from 27µm to 30µm

Conclusion

- **The morphological culture characteristics results revealed a clear cut variation in mycelial growth among the tested isolates.**
- **The mycelia growth showed different patterns and colour as concentric rings, fluffy growth, moderately slant and vertical growth, uneven marsh growth with dark colour mycelium at centre with white, muddy and dull white colour.**
- **Isolates Cf-kushinagar (variety CoSe 95422) recorded highest mycelial dry weight and Cf-17 (variety CoSe 95422) recorded the least.**

Conclusion …

- **Regarding other characteristics, Cf-1B (variety Cos 8436), Cf-Kushinagar (variety CoSe 95422) and Cf- 20 (CoSe 92423) were producing thicker matty mycelium and were darker as compared to other isolates.**
- **Among the tested red rot isolates viz. Cf-20 , Cf-19, Cf-18 and Cf-1B were fast in initiating conidia production with higher conidial germination.**

Virulence behavior of red rot isolates

- In the virulency test, screening of six red rot isolates on a set of a susceptible varieties (CoS 453 , Co 312, Bo17, B0 70, CoS770, CoJ 64, CoS1158, CoS443, CoS510, UP01) indicated that the isolate Cf-Basti proved more virulent followed by Cf-Kushinagar and Cf-2B (Bareilly).
- The virulency behavior observed in tested isolates was found in the order Cf-Basti> Cf-Kushinagar > Cf-2B> Cf-20> Cf-18 > Cf-17 (Table 2).
- Our results suggested that the isolates Cf- Basti was the more virulent one and responsible for knocking down the commercial varieties of sugarcane in the affected area.

Table 2

Virulency of red rot isolates in Uttar Pradesh (0-9 scale)

Red rot isolates						
Sugarcane Variety	**Cf-20**	**Cf-2B**	**Cf-17**	**Cf-18**	**Cf-Kushinagar**	**Cf-Basti**
Co 453	**3.0**	**4.0**	**5.0**	**3.0**	**6.0**	**4.0**
Co 312	**6.0**	**5.0**	**5.0**	**3.0**	**4.0**	**6.0**
BO 17	**5.0**	**6.0**	**4.0**	**2.0**	**6.0**	**9.0**
BO 70	**5.0**	**6.0**	**5.0**	**2.0**	**6.0**	**4.0**
CoH 770	**6.0**	**5.0**	**3.0**	**3.0**	**5.0**	**6.0**
CoJ 64	**7.0**	**5.0**	**5.0**	**6.0**	**5.0**	**9.0**
Co 1158	**5.0**	**9.0**	**5.0**	**4.0**	**5.0**	**8.0**
Co 443	**7.0**	**5.0**	**5.0**	**4.0**	**4.0**	**9.0**
Co 510	**5.0**	**5.0**	**3.0**	**3.0**	**9.0**	**9.0**
UP 001	**1.0**	**2.0**	**2.0**	**2.0**	**2.0**	**5.0**

Molecular Characterization

- In the present study the most virulent red rot isolates Cf- Basti and Cf-kushinagar which are causing epidemic in the areas would alarm us to investigate on molecular approaches of characterization to know whether they are entirely new isolate/pathotypes/ strains of red rot. So that significant initiatives should be taken well in advance towards developing resistant /tolerant genotypes through effective resistant breeding crossing programmes.

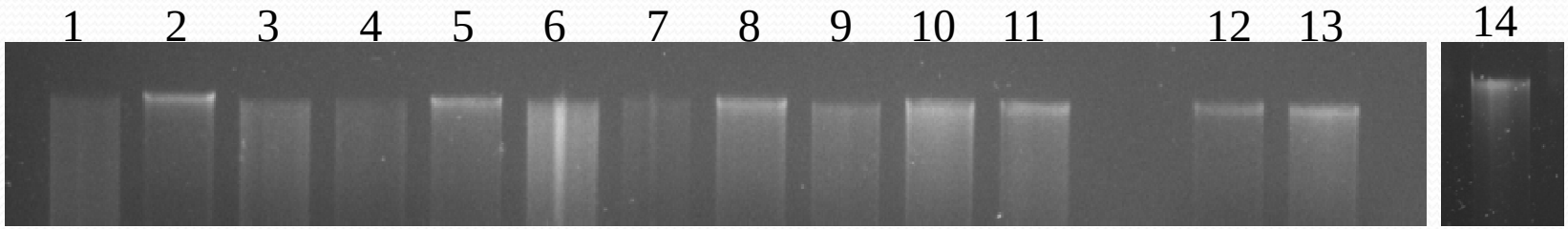
DNA Isolation and PCR amplification

- **Total DNA was isolated from collected red rot isolates of different varieties through CTAB method and Polymerase chain reaction were performed with the specific primers (ITS-1 and ITS-4).**

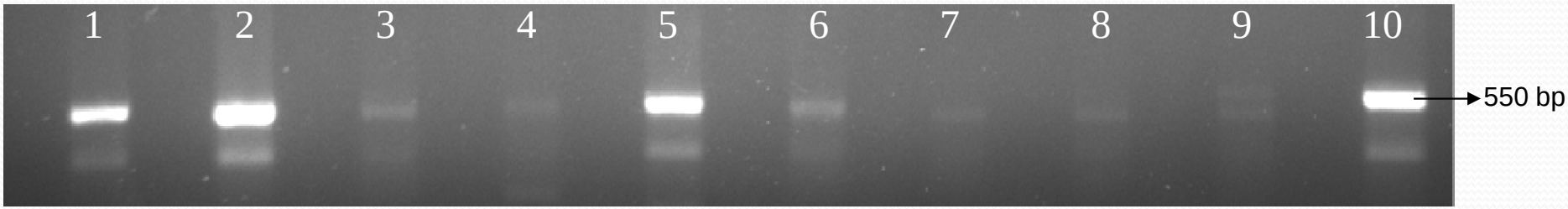
- The cycling protocol used for the PCR was as follows:

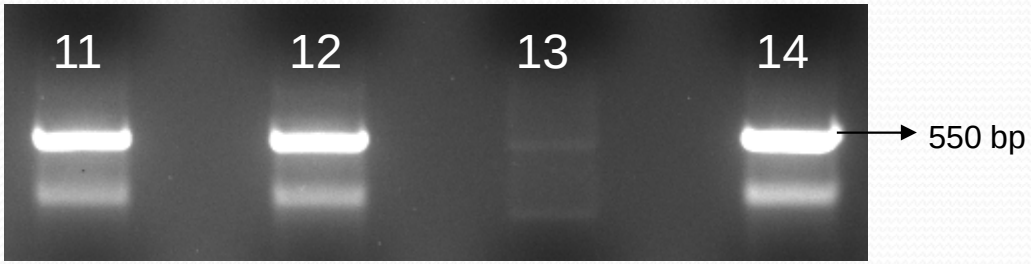
94°C	5min		
94°C	10sec	}	
60°C	10sec	}	30 cycles
72°C	20sec	}	
72°C	10min		

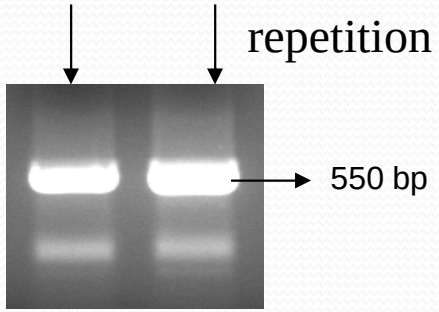
DNA:



PCR: using ITS 1 and ITS-4







1. Cf-06,
2. Cf-08,
3. Cf-20,
4. Cf-01,
5. Cf-04,
6. Cf-05,
7. Cf-09,
8. Cf-10,
9. Cf-17,
10. Cf-18,
11. Cf-19,
12. Cf-1B,
13. Cf-2B,
14. Cf-kushinagar.

PCR products of the expected size, ~550bp, respectively, were obtained from collected isolates.

Identity of present study isolates with other reported isolates from across the world

Accession number	Location	Isolate	% identity
HQ833660	Shajahanapur	Cf-04	99-98% (HQ608149, EU489905, JN207324, FR177533)
HQ833663	Kushinagar	Cf-Kushinagar	99-94% (AB455515, AB457007, AY256855, HQ833659, EU002901)
HQ833659	Shahjahanapur	Cf-08	99-94% (AB455515, AB457007, HQ608067, HQ833663)
HQ833661	Basti	Cf-10	99-98% (HQ833662, HQ833658,HM627380, HM592294, AY9447748, AY944753)
HQ833662	Karmha	Cf-17	99-98% (HQ833658, HM627380, HQ833661, HM592294, AY944747)
HQ833658	Shahjahnapur	Cf-06	99-98% (HQ833662, HM627380, HQ833661, HM592294, AY944748)

Phylogenetic analysis of the red rot isolates



Conclusion

- **On the basis of phylogenetic tree it is concluded that Cf-08 isolate collected from Shahjahanpur is very closely related to Cf-kushinagar isolate. Cf-17 isolate collected from Deoria formed another cluster showing maximum similarity with Cf-06 & Cf-10 from Coimbatore.**

- By ISSR, RAPD markers Kumar et al (2010) from Hissar characterize 25 isolates collected from UP, Haryana & Punjab and found the high genetic diversity and also recorded the presence of one new isolate.

- Suman et al (2005) detect and showed the diversity of 6 red rot isolates collected from different regions of UP by RAPD markers.

- By RAPD analysis Madan & Bikas (2000) proved the diversity of red rot isolates in North eastern India.

- **No information is available at sequence level from Uttar Pradesh regions**. The present investigation proved the high genetic diversity of the isolates collected for the different regions of the UP and also suggesting for the further characterization of the isolates from the rest part of the country.

Acknowledgements

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Thanks for your kind attention.....