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Morphological Variability of the *Curvularia lunata* Associated with Grain Discoloration of Rice

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Rice is an important staple crop and India is the largest grower in the world. Rice suffers from many diseases caused by fungi of which grain discoloration disease is a complex disease due to infection by certain microorganisms on the glumes, kernels, or both. *Curvularia lunata* is a one of major fungal plant pathogen that infects rice and causes grain discolouration. On the basis of cultural and morphological identification, 60 isolates of *Curvularia lunata* were collected from different locations of Karnataka. These isolates were grown on PDA media and were characterized for colony morphology. The *Curvularia lunata* isolates, maximum size of conidia was observed in Cl-22 (20.30 μm long ×10.90 μm wide) and maximum dry mycelium weight was observed in Cl-15 (482.22 mg), while poor sporulations (<1-10 conidia per microscope field) was noticed in Cl-17, 19, 28, 30, 45 and Cl-50.

Keywords: Curvularia lunata; variability; cultural; morphological; grain discoloration.

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1. INTRODUCTION

Rice is one of the most important cereal crops of the country. Rice intension deeply applied has increased chemical fertilizer application and developed more rice seasons yearly. This is one of the causes to increase damage by pests and diseases. Rice yield loss due to pests and diseases has been noticed more and more seriously. Grain discoloration is considered as one of popular problems in Karnataka.

Grain discoloration of paddy is a complex disease due to infection by certain microorganisms on the glumes, kernels, or both. The fungi that were reported to be associated with discoloration of grains are *Curvularia lunata* [highest (35.30%) in Tungabhadra Project (TBP) and Upper Krishna Project (UKP) areas of North Eastern Karnataka], *Alternaria alternata, Fusarium moniliforme, Bipolaris oryzae*, *Alternaria padwickii, Pyricularia oryzae, Fusarium graminareum, Nigrospora oryzae, Epicoccum nigrum, Phoma sorghina, Dichotomophthoropsis nymphacearum* and *Heterosporium echinunulatum etc.* [1].

Grain discoloration results in seedling mortality and reduction in germination and seedling vigour [2], causing significant yield loss. Thus, the pathogens causing grain discoloration have direct influence on both quantity and quality of seeds.

In nature, plant pathogens exist as different strains that exhibit variation in their morphological and cultural characters, pathogenicity and virulence. To understand the present plant disease situations and to predict the possible future development it is essential to learn as much as possible about the variability in fungi that are pathogenic to plants. Morphological and pathogenic variations are known in many fungal pathogens. Keeping in view, the importance of the crop and severity of the disease, present investigation was taken up to study the variability in cultural and morphological characters of *Curvularia lunata* isolates.

2. MATERIALS METHODS

The grain discoloration infected panicle/seed samples were collected from 16 districts of Karnataka during survey and pure hyphal tip cultures of 60 isolates were maintained on PDA and later morphological and cultural studies were carried out in laboratory.

The growth characters of predominant pathogens were studied on potato dextrose agar. The media were sterilized at 1.1 kg/cm^2 pressure for 15 min. To carryout the study, 20 ml of each of the medium was poured in 90 mm petriplates. Such petriplates were inoculated with 5mm disc cut from periphery of actively growing culture and incubated at 27±1°C. Each treatment was replicated thrice. Observations were taken when the fungus covered complete petriplate in any one of the media. The colony diameter was recorded. The fungus colony colour, margin and sporulation were also recorded. The data on radial growth was analyzed statistically. The colonies were characterised for phenotype and growth pattern and different morphotypes, shape (irregular and regular); growing pattern (circular and feathery); texture (velvety and cottony) were observed *in vitro*. Similarly, colour was differentiated into black and greyish black.

The composition and preparations of liquid media used, were the same as that of solid media except that the agar-agar was not added. The mycelial mat was harvested, dried and weighed and results were analyzed statistically.

3. RESULTS AND DISCUSSION

3.1 Morphological Variability

Morphological characters such as colony character, colony diameter, sporulation, spore germination, size of conidia, mycelium width and dry mycelium weight of sixty monoconidial hyphal tip isolates of *C. lunata* were studied. All the sixty isolates showed considerable variability with respect to colony characters, colony diameter, sporulation, size of conidia, mycelium width and dry mycelium weight (Fig. 1a &1b).

3.2 Size of Conidia and Dry Mycelium Weight

Maximum size of conidia was observed in Cl-22 (20.30 μm long ×10.90 μm wide) followed by Cl-25 (19.80 μm long × 9.10 μm wide). Minimum size of conidia was found in Cl-54 (12.60 μm long × 7.20 μm wide). Although some of the isolates produced conidia of larger size but their width was very small and some conidia were length wise smaller but were wider comparatively thus indicating variability among the isolates with respect to conidial size. Maximum dry mycelium weight was observed in Cl-15 (482.22 mg) followed by Cl-52 (488.42 mg) and Cl-59 (466.23

mg). Minimum dry mycelium weight was observed in Cl-35 (180.96 mg).

3.3 Colony Colour

Colony colour varied from isolate to isolate i.e., light grey to black. Cl-24, 26, 46 were showed black colour colonies, while Cl-2, 4, 20, 36, 38, 40, 51 and 53 showed dark. Colony margin colour varied from isolate to isolate i.e., light grey to black. Cl-9, 10, 12 were showing light grey colour colony margins and Cl- 3, 4, 5, 49, 50, 52, 56 were showed grey colour colony margins while remaining were showed light brown, brown and black colour margins (Table 1).

3.4 Topography and Type of Margin

Topography varied from isolate to isolate i.e., fluffy, smooth to flat. Majority were showed smooth growth *viz*., Cl-10, 11, 16, 17, 19, 21, 23, 27, 28, 29, 31, 34, 35, 37, 38, 42, 46, 48, 50, 54, 55 and 56, while remaining were showed fluffy raised, raised and flat growth with regular and irregular margins (Table 1).

3.5 Colony Character

Growth of isolates *viz.,* Cl- 1, 2, 3, 4, 5, 6, 10, 12, 17, 20, 21, 22, 23, 24, 25, 26, 27, 32, 34, 35, 36, 38, 39, 40, 41, 42, 45, 46, 48, 49, 51, 52, 53, 54, 56, 57, 58, 59, 60 were excellent, fast growing and isolates Cl-19,47,50 produced good growth. Excellent sporulation was (> 50 conidia per microscope field) observed by isolates Cl-1, 2, 6, 15, 16, 20, 21, 25, 34, 35, 53, 54, 56, 57 and Cl-58, while poor sporulation (<1-10 conidia per microscope field) was noticed in Cl-17, 19, 28, 30, 45 and Cl-50 (Table 1).

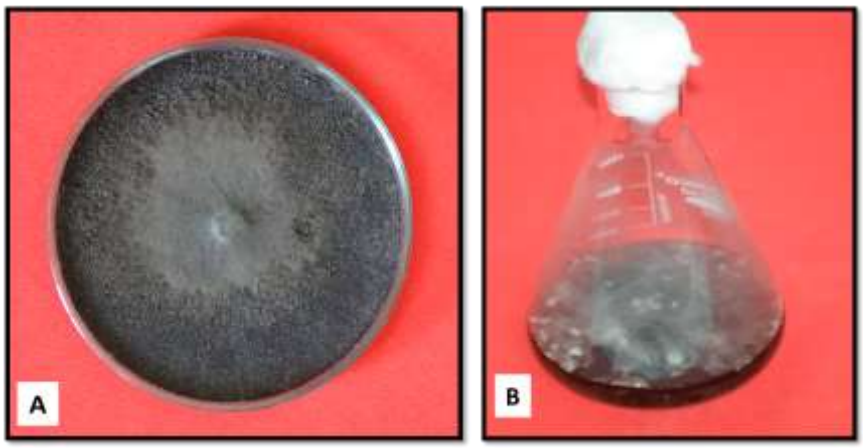

Fig 1a. Pure culture of predominant pathogen *Curvularia lunata* on *A) PDA B) PDB*

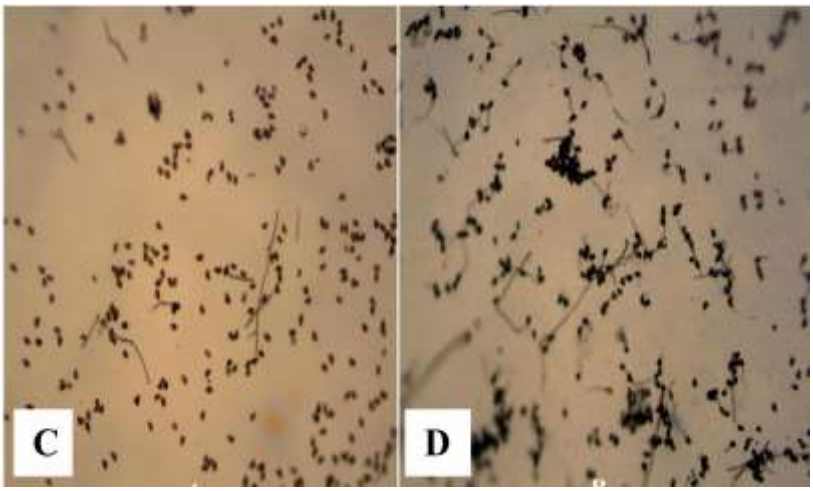

Fig 1b. Microphotograph of *Curvularia lunata* (10X magnification) *C) condia D) condia germinated*

Table 1. Morphological and cultural characteristics of *Curvularia lunata* isolates of paddy collected from different regions of Karnataka

Sl. No.	Location	Isolates	Colony Colour	Colony Growth	Type of margin	Margin colour	Growth in mm	Sporulation	Size of conidia (µm) (Length X Breadth)	Dry mycelial weight (mg)
1	Yadgir	**CI-1**	Dark gray to black	Flat	Irregular	Black	90	++++	14.40X9.50	394.44
2	Shahapur	**CI-2**	Dark gray	Fluffy raised	Regular	Black	90	++++	17.00X9.80	382.08
3	Shorapur	**CI-3**	Medium brown	Raised	Regular	Gray	80	+++	17.40x9.40	340.40
4	Shorapur	**CI-4**	Dark gray	Fluffy raised	Regular	Gray	90	+++	15.60X11.50	348.55
5	Raichur	**CI-5**	Medium gray	Fluffy raised	Regular	Gray	80	+++	18.40X10.20	320.74
6	Raichur	**CI-6**	Dark gray to black	Flat	Regular	Black	90	++++	13.00X7.40	331.05
7	Manvi	**CI-7**	Medium brown	Flat	Irregular	Black	70	++	18.00X10.20	340.56
8	Sindhnur	**CI-8**	Light Brown	Flat	Regular	Light brown	70	++	15.30X10.10	299.30
9	Devadurga	**CI-9**	Light gray	Fluffy	Regular	Light gray	60	++	14.70X8.80	240.57
10	Bellary	**CI-10**	Light gray	Smooth	Regular	Light gray	80	++	16.00X9.90	366.20
11	Hosapet	**CI-11**	Dark Brown	Smooth	Regular	Light brown	70	++	16.50X7.40	289.40
12	Siraguppa	**CI-12**	Light gray	Fluffy	Irregular	Light gray	80	+++	15.40X10.80	421.44
13.	Siraguppa	**CI-13**	Medium grey	Raised	Regular	Light brown	70	+++	17.00X10.40	432.66
14	Koppal	**CI-14**	Dark gray to black	Raised	Irregular	Black	60	+++	15.80X11.70	450.15
15	Gangavathi	**CI-15**	Dark gray to black	Flat	Irregular	Black	70	++++	14.50X7.80	466.22
16	Gangavathi	**CI-16**	Medium brown	Smooth	Irregular	Light brown	70	++++	15.10X9.80	451.39
17	Davangere	**CI-17**	Dark brown to black	Smooth	Regular	Black	90	+	18.30X10.20	327.46
18.	Harihar	**CI-18**	Dark gray to black	Raised	Irregular	Black	60	++	16.30X9.00	260.45
19	Harihar	**CI-19**	Dark Brown	Smooth	Irregular	Black	50	+	17.10X7.20	250.66

Sl. No.	Location	Isolates	Colony Colour	Colony Growth	Type of margin	Margin colour	Growth in mm	Sporulation	Size of conidia (µm) (Length X Breadth)	Dry mycelial weight (mg)
20	Shimoga	**CI-20**	Dark Gray	Fluffy raised	Regular	Black	90	++++	14.90X9.10	388.43
21	Shimoga	**CI-21**	Dark gray to black	Smooth	Regular	Brown	90	++++	16.50X8.90	376.40
22	Tirthahalli	**CI-22**	Dark Brown	Fluffy raised	Irregular	Brown	90	+++	20.30X10.90	210.33
23	Bhadravati	**CI-23**	Medium gray	Smooth	Regular	Black	90	+++	14.40X8.50	320.60
24	Hosanagar	**CI-24**	Black	Fluffy	Regular	Black	80	+++	15.20X7.10	352.44
25	Sirsi	**CI-25**	Dark brown	Fluffy	Regular	Black	90	++++	19.80X9.10	349.21
26	Sirsi	**CI-26**	Black	Fluffy	Regular	Black	90	+++	16.40X9.60	288.45
27	Yellapur	**CI-27**	Dark brown	Smooth	Regular	Brown	80	++	16.40X9.40	212.87
28	Mundgod	**CI-28**	Medium brown	Smooth	Irregular	Light brown	60	+	14.70X9.00	210.44
29	Hassan	**CI-29**	Medium brown	Smooth	Irregular	Light brown	70	++	15.10X6.30	278.50
30	Alur	**CI-30**	Medium brown	Raised	Irregular	Light brown	60	+	17.10X11.10	310.48
31	Alur	**CI-31**	Medium brown	Smooth	Irregular	Light brown	60	++	15.70X8.00	368.44
32	Sakleshpur	**CI-32**	Light brown	Fluffy raised	Regular	Brown	90	+++	15.10X10.20	466.50
33	Chikamagalur	**CI-33**	Dark gray to black	Fluffy	Irregular	Black	70	++	17.50X7.00	356.28
34	Koppa	**CI-34**	Dark gray to black	Smooth	Regular	Black	90	++++	15.70X8.00	278.36
35	Mudigere	**CI-35**	Dark gray to black	Smooth	Regular	Black	80	++++	15.40X7.70	180.96
36	Mudigere	**CI-36**	Dark gray	Fluffy raised	Regular	Brown	80	+++	18.20X8.50	248.69
37	Madikeri	**CI-37**	Dark brown	Smooth	Irregular	Brown	70	++	16.40X9.60	310.56
38	Virajpet	**CI-38**	Dark gray	Smooth	Regular	Black	90	+++	18.50X8.40	330.78
39	Virajpet	**CI-39**	Dark gray to black	Fluffy	Regular	Black	90	+++	14.00X6.40	410.95
40	Mangalore	**CI-40**	Dark gray	Fluffy raised	Regular	Brown	90	+++	17.00X6.70	421.55
41	Mangalore	**CI-41**	Dark gray to black	Flat	Regular	Black	90	+++	18.10X10.70	374.45

Sl. No.	Location	Isolates	Colony Colour	Colony Growth	Type of margin	Margin colour	Growth in mm	Sporulation	Size of conidia (µm) (Length X Breadth)	Dry mycelial weight (mg)
42	Bentval	**CI-42**	Dark gray to black	Smooth	Regular	Black	80	+++	17.70X6.70	324.88
43	Udupi	**CI-43**	Dark brown	Fluffy raised	Irregular	Brown	70	++	17.60X10.50	256.44
44	Karkal	**CI-44**	Medium gray	Raised	Irregular	Black	60	++	16.10x8.60	230.47
45	Mysore	**CI-45**	Light gray	Flat	Regular	Brown	80	+	15.30x11.50	361.85
46	Nanjangud	**CI-46**	Black	Smooth	Regular	Black	80	+++	17.30X6.00	223.54
47	Nanjangud	**CI-47**	Medium brown	Flat	Irregular	Brown	50	++	16.6x10.00	189.45
48	T.Narshipura	**CI-48**	Dark gray to black	Smooth	Regular	Black	80	+++	15.90x7.70	451.26
49	Chamarajanagar	**CI-49**	Medium gray	Fluffy	Regular	Gray	80	++	15.50X7.70	460.32
50	Yelandur	**CI-50**	Light gray	Smooth	Regular	Gray	50	+	18.40X12.00	350.91
51	Kollegal	**CI-51**	Dark gray	Fluffy raised	Regular	Brown	80	+++	16.30X9.00	366.80
52	Kollegal	**CI-52**	Dark gray	Fluffy raised	Irregular	Gray	90	+++	17.40X8.20	480.42
53	Mandya	**CI-53**	Dark gray	Fluffy raised	Irregular	Black	90	++++	18.40X9.60	446.81
54	Pandavpur	**CI-54**	Medium gray	Smooth	Regular	Brown	80	++++	12.60X7.20	382.64
55	Pandavpur	**CI-55**	Medium gray	Smooth	Irregular	Brown	60	+++	17.00X10.60	356.20
56	Malavalli	**CI-56**	Light gray	Smooth	Irregular	Gray	80	++++	16.40X11.90	288.54
57	Malavalli	**CI-57**	Dark gray to black	Flat	Regular	Black	90	++++	14.70X9.30	367.26
58	Ramanagar	**CI-58**	Dark gray to black	Fluffy raised	Regular	Black	90	++++	18.40X8.90	451.86
59	Kanakpur	**CI-59**	Dark gray to brown	Flat	Regular	Brown	90	+++	14.10X8.80	466.23
60	Kanakpur	**CI-60**	Dark brown to black	Flat	Regular	Brown	90	++	16.60X9.80	391.56

-: no spprulation, + : poor [1-10 conidia/microscopic field (10X)], ++ : fair[11-30 conidia/microscopic field (10X)], +++ : good[31-50 conidia/microscopic field (10X)], ++++ : excellent[>50 conidia/microscopic field (10X)]

3.6 Discussion

3.6.1 Morphological variability

The pathogen *C. lunata* put forth moderately rapid growth, covering the agar plate within 6-10 days. All the sixty isolates showed considerable variability with respect to colony characters, colony diameter, sporulation, size of conidia, mycelium width and dry mycelium weight [3]. The colony was sparse to dense, light grey to dark black, fair to fast growing, Excellent sporulation was (> 50 conidia per microscope field) exhibited by isolates Cl-1, 2, 6, 15, 16, 20, 21, 25, 34, 35, 53, 54, 56, 57 and Cl-58, while poor sporulation (<1-10 conidia per microscope field) was noticed in Cl-17, 19, 28, 30, 45 and Cl-50, maximum size of conidia was observed in Cl-22 (20.30 μm long ×10.90 μm wide) followed by Cl-25 (19.80 μm long × 9.10 μm wide) [4]. Minimum size of conidia was found in Cl-54 (12.60 μm long × 7.20 μm wide), maximum dry mycelium weight was observed in Cl-15 (482.22 mg) followed by Cl-52 (488.42 mg) and Cl-59 (466.23 mg). Similar discrption was given by Mathur and Sarbhoy [5]; CHANG Jia-ying et al. [6].

Similarly, Haq et al. [7] found greater mycelial growth of *A. alternata, C. lunata, M. phaseolina* and *R. solani* on glucose peptone agar and Brown's agar than on potato dextrose agar and oat meal agar. Aurangzeb et al. [8] found potato dextrose agar to be best medium for the mycelial growth and sporulation of *F. moniliforme* followed by Wakasman"s agar, basal medium, Czapek"s Dox agar and Richard"s medium. Patel [9] found potato dextrose agar superior for the growth and sporulation of *A. alternata* followed by potato carrot sucrose agar.

Morphological characterization of Curvularia species and related species is the classic approach of identification which could be aided with molecular characteristics.

4. CONCLUSION

Based on morphological and cultural, the fungus identified with high incidence associated with seeds collected in different agricultural producing regions was *Curvularia lunata*. This fungus is transmitted to plants via seeds, is pathogenic. Its gives information regarding virulence characteristic nature of pathogen to particular region.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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