PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(1): 1-8
https://doi.org/10.14719/pst.4331

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RESEARCH ARTICLE

Host infectivity of *Pyricularia* pathogen of finger millet (*Eleusine coracana* (L.) Gaertn.) on Poaceae weeds

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OPEN ACCESS

ARTICLE HISTORY
Received: 12 July 2024
Accepted: 22 October 2024
Available online
Version 1.0 : 17 January 2025

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
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Thangavelu S P, Narasimha V J, Mallian V, Kumaresan S, Iruthayasamy J, Manickam R. Host infectivity of *Pyricularia* pathogen of finger millet (*Eleusine coracana* (L.) Gaertn.) on Poaceae weeds. Plant Science Today. 2025; 12(1): 1-8. https://doi.org/10.14719/pst.4331

Abstract

Blast disease of finger millet is a serious disease all over the world and causes significant yield loss. *Pyricularia* populations consist of various pathotypes with different host ranges within the Poaceae family and express host specificities mediated by Avr and R genes. So, identification of the host range of *Pyricularia* on weeds within the poaceae family will give an idea for further research on understanding of genes responsible for host specificity reaction of *Pyricularia* among various host ranges. *Pyricularia* isolated from finger millet was tested on different weed host plants to study the infectivity of *Pyricularia* on weed species. The *Pyricularia* pathogen isolated from the infected leaf of finger millet plants having spindle-shaped lesions. The Poaceae weeds viz., *Chloris barbeta*, *Cynodon dactylon*, *Cyperus rotundus*, *Dactylacterium aegyptium,* and *Echinochloa colonum* are observed regularly in the field of finger millet. Among the weed species, spindle shaped lesion was observed in *C. barbeta*, *C. dactylon, C. rotundus* and *D. aegyptium* in leaf detachment assay with *Pyricularia* at Centre of Excellence in Millets, Athiyandal, Tiruvannamalai, India. Whereas *E. colonum* did not express any lesion. The incidence of *Pyricularia* in finger millet was studied under various weed infestation levels under field conditions during 2022-23 and 2023-24 growing seasons. It was found that the experimental plot of finger millet with all Poaceae weeds recorded the highest incidence of leaf blast and the largest number of *Pyricularia* colonies in both years. This was followed by the plot with finger millet + *D. aegyptium* alone and finger millet + *C. rotundus* alone. The leaf blast symptoms were noticed in finger millet after one week of sowing in the case of the crop with all weed species during 2022-23 and 2023-24. A significant correlation was obtained between the incidence of the leaf blast and colonies of *Pyricularia* from 28 days after sowing till the maturity of the crop in both years. Hence, the incidence, as well as colonies of *Pyricularia,* were higher in plots with finger millet + all weeds, followed by plots with finger millet + *D. aegyptium* alone and plots with finger millet + *Cyp. rotundus* alone.

Keywords

Finger millet; infectivity; Pyricularia; Poaceae weeds; correlation determination

Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn. subsp. *coracana*) is one of the popular millet crops, and grown in East Africa and India. Finger millet is a

nutrient-dense millet that is typically farmed in underdeveloped nations by small and medium-sized farmers (1, 2). The crop can adapt to a wide range of habitats and can survive unfavorable environmental conditions such as moisture stress and water stagnation (3, 4). A multitude of biotic and abiotic variables often pose challenges to crop yield under field conditions. *Magnaporthe grisea* (teleomorph: *Pyricularia grisea*) is one of the biotic factors that cause blast disease, which is a serious disease that has been observed in all finger millet growing areas (5). It affects all stages of plant growth and causes severe reductions in grain output and biomass, sometimes reaching 80–100% (6- 9). The disease can cause lesions and discoloration in a variety of plant components, including the leaves, necks, and grains (10). Eventually, the affected portions may completely dry up, which can result in a drop in test weight and seed size (11). Interestingly, the most damaging disease in rice is caused by the same pathogen that parasitizes rice (*Oryza sativa* L.) (12). In the western arid region of Rajasthan, *Pyricularia pennisetigena* is commonly found on pearl millet (*Pennisetum glaucum*) (13, 14) and *P. pennisetigena* was a pathogen from several distinct cereal hosts, such as *Echinochloa colona* (Brazil), several *Pennisetum* species (Mali), *Cenchrus echinatus* (Philippines), and *Cenchrus ciliaris* (Japan). The blast pathogen is currently present in roughly 137 grass species, indicating the infection's adaptability and dissemination (15). The pathogen *Pyricularia* has a broad host range (16-18), and many types of weeds that are present in the field could potentially serve as sources of the pathogen's inoculum. Poaceae weeds can also harbor the inoculum, which is important for the occurrence of blast disease in finger millet. Furthermore, *Pyricularia* is made up of a variety of pathotypes that have varying host ranges and are controlled by host resistance and avirulence genes. The effector proteins released by the pathogen during *Pyricularia* pathogenesis change the physiology of host plants and promote pathogen colonization, frequently deciding the success or failure of infection (19). Earlier, the host specificity of *M. oryzae* was studied by the genetic analysis of *P. oryzae* in paddy (20, 21). More recently, employing sequencing of the *Pyricularia* isolates, the cause of host specificity was investigated on several field isolates from rice as well as isolates from various grass and grain hosts (22-25). The *Pyricularia* from various crops could influence their genetic variation within these host-adapted *M. oryzae* populations (20). However, genomes of most of the species of the *Pyricularia* complex remain unexplored. The purpose and understanding of the host range of *Pyricularia* of finger millet among Poaceae weeds will give an idea for genetic analysis of finger millet and weeds of Poaceae on host-specific reaction of *P. grisea*.

Materials and Methods

Isolation of Pathogen (*Pyricularia grisea*)

To isolate the pathogens, the infected leaves of finger millet were cut into 2mm size pieces with sterilized scissors. The infected leaf samples showing typical blast lesions were collected from A block Farm, Centre of Excellence in Athiyandal, India. Those pieces were then surface sterilized using 1% sodium hypochloride (NaOCl) for 1 minute, followed by two successive cleaning with sterilized distilled water. Then, the sterilized tissues were kept in a clean, sterile Petri dish containing three layers of moistened blotting paper. The samples were then incubated at 25 to 26°C for one day. After incubation, brownish discolouration with a greyish spongy centre appeared on the inoculated area in the leaf. The conidia that emerged from the lesion area were identified using a compound microscope at 40x magnification power. From these sporulating lesions on the leaf sample, a single conidium was transferred to separate sterilized culture tubes of agar slants using an inoculation needle under aseptic conditions as per the single isolation method (26). Spreading conidia from the discrete lesions on 4% water agar with the help of an aseptic inoculating needle under a stereomicroscope to get single spore isolates. Transfer the germinating conidia aseptically to the agar plate. The plate was incubated at 25± 2°C for 72-96 hours under the incubator.

Infectivity of *Pyricularia* on Poaceae weed species by detached leaf assay

To find out the infectivity of *Pyricularia* on weed host of the finger millet ecosystem, the weed hosts of finger millet viz., *C.barbeta*, *C. dactylon*, *C. rotundus*, *D. aegyptium,* and *E. colonum* were selected. For which detached leaf assays were performed with slight modifications (27). Initially, the mycelia growth of *Pyricularia* was scrapped using 100 ml sterile water from 10 days old Petri plate culture of *Pyricularia,* and the final concentration was x 10^5 mL^{-1}.Then, the second leaf of the above-mentioned weed hosts was taken on a sterile Petri plate and drop-inoculated with 10 µL of the conidial suspension (2-3 × 10^5 mL^{-1}) of *Pyricularia* separately using a pipette. The inoculated detached leaves were incubated under 25°C with a 16 h/8 h of light/dark photoperiod. After seven days of inoculation, the disease symptoms were recorded. The experiments were repeated three times. The leaves were sprayed with sterile distilled water and kept in control. The pathogen was reisolated from the inoculated symptomatic leaves under aseptic condition and confirmed by the cultural and morphological characteristics of the isolate to satisfy Koch's postulates.

Effects of Poaceae weed species on the incidence of blast disease

Field experiments were conducted during 2022-23 and 2023-24 seasons at Centre of Excellence in Millets, Athiyandal, Thiruvannamalai, India (12° 23′N, 70°02′E, 280 m above mean sea level) to study the influence of individual Poaceae weed species against *P. grisea*. The finger millet variety CO (Ec) 14 was sown in a line spacing of 22.5 cm with a standard plot size of 4 × 3 m, implementing the recommended spacing and dosage of fertilizers. The treatments viz., T_1: Finger millet + *C. barbeta,* T_2: Finger millet + *C. dactylon,* T_3: Finger millet + *C. rotundus,* T_4: Finger millet + *D. aegyptium,* T_5: Finger millet + *E. colonum,* T_6: Finger millet + All weeds and T_7: Finger millet alone was maintained with three replications.

Maintenance of weed species in the treatment

In the above experiment, the above weed species were allowed to grow in between the line of crops in the respective treatment plot from day one of the field trial. To avoid the growth of other weed species, hand weeding was done once in five days in the field up to the harvesting stage. There was no weeding during the study period in the case of plot with finger millet + all weeds. Twenty respective weed species were maintained in one square meter area of the individual treatment plot. Two-foot space gap was maintained on all sides between treatment plots in which four rows of Sesamum (*Sesamum indicum*) were grown to prevent the movement of spores of *Pyricularia* from nearby treatment plots.

Assessment of colonies of *Pyricularia*

For monitoring the spore load of *Pyricularia* in the treatment plot, the Agroscope (AGS) spore trap model (28) was fixed in the field. The agroscope trap is the wooden board that holds the spore trap, measuring about 30 cm in length and 10 cm in width, fixed by an iron rod. A 15 cm by 11 cm by 4.5 cm metal plate was affixed vertically to the board. A second identical dish was affixed horizontally to the upper end of the metal dish. A 9 cm Petri dish filled with 15 ml of Host Extract Potato Dextrose Agar medium and 48 mL of penta chloronitrobenzene (PCNB) from Sigma-Aldrich®, India, was positioned beneath the horizontal aluminum plate and near the bottom of the vertical one. To secure the Petri dish to the board, two tiny nails were positioned nine centimeters from the bottom of the vertical aluminum plate. The aluminum dishes protected the agar plates not only from wind and rain but also from high temperatures at noon and thereby prevented the dehydration of the agar. Agroscope (AGS) spore traps were placed in between line areas where weeds were grown in the field. Three spore traps were fixed in each plot size of 4 × 3 m. The *Pyricularia* appeared as a greyish colony with pyriform conidia on the Petri plates. Every three days, plates were replaced with new ones.

Disease scoring and percent disease calculation

The assessment of leaf blast incidence and the percent disease index (PDI) were calculated at weekly intervals. The leaf blast incidence was recorded using a standard evaluation system containing 1 to scale (29).

The percent disease index was calculated using the formula mentioned below.

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves graded}} \times \frac{100}{\text{Maximum grade}}$$

Statistical analysis

The experimental data statistical analysis was carried out by adopting the standard method (30). The experiment was conducted in a completely randomized design with three replications. The data on field experiments was analysed by analysis of variance (ANOVA) of randomized block design (RBD). Data for correlation studies from each experiment were analysed by one-way analysis of variance using IBM SPSS (v. 28.0).

Results and Discussion

Infectivity of *Pyricularia* of finger millet on weed species

One of the most risks and most damaging diseases that affect major millet-growing regions worldwide is blast, which is caused by *Pyricularia* spp. (31). The disease is widespread throughout the main millet-growing regions and is also expanding to new places. Emerging pathotypes exhibit varied intensities based on the cultivar, favorable conditions, and production techniques. The pathogen parasitizes over 50 grasses and sedges in addition to causing illness in a variety of host plants, including finger millet, rice, pearl millet and foxtail millet (32). A detailed study was conducted to find out the host preference of *Pyricularia* of finger millet on various weed species. The populations of Poaceae weed species viz*., C. barbera*, *C. dactylon*, *C. rotundus*, *D. aegyptium,* and *E. colonum* were more, and frequently observed in finger millet crop ecosystem, and the weed species were taken for our study with finger millet against leaf blast pathogen. *Pyricularia* pathogen isolated from infected leaf sample of finger millet and the pure culture of the pathogen used in the study. The fungal growth showed a typical pyriform shape with a rounded base, narrow apex, two septa, three-celled, and the broader middle cells than adjacent cells of conidia. The spore suspensions of *Pyricularia* isolate were inoculated on five weed grass species to evaluate host specificity reaction as a leaf detachment method. The experimental results presented in Table 1 revealed that *Pyricularia* isolated from finger millet produced symptoms on weed species viz., *C. rotundus, C. dactylon*, *C. barbeta,* and *D. aegyptium* when inoculated as a leaf detachment method and whereas *E. colonum* did not express the symptoms. Earlier, *P. grisea* isolated from finger millet could infect rice crops, but not the other way around (33). Similarly, *P. setariae* isolated from foxtail millet can infect *D. aegyptium*, finger millet, pearl millet, and wheat (34). Despite the limited host range, there has been conjecture that *Pyricularia* populations on weed hosts may serve as a source of inoculum due to the sporadic cross-infection of weeds by isolates of finger millet (35).

Leaf blast incidence in finger millet under different weed infestation levels

Two field trials were conducted during 2022-23 and 2023-24 to find out the incidence of *Pyricularia* on finger millet at different weed species levels. The results are presented in Tables 2 & 3 and it revealed that the incidence of leaf blast is more when the finger millet is grown with all the weeds, which is followed by finger millet crop grown with *C. rotundus* alone and *D. aegyptium* alone, in both years. The leaf blast symptoms were noticed in finger millet after two weeks of sowing during 2022-23. Whereas in 2023-24, the leaf blast symptoms were noticed after one week of sowing in finger millet. The finger millet with other weeds also recorded leaf blast symptoms in the crop. An increasing trend of leaf blast PDI was observed up to 63 DAS during 2022-23 as well as 2023-24, and later PDI shows decreasing trend till maturity of the finger millet. Our results

Table 1. Infectivity of *Pyricularia* of finger millet on weed species.

S.No	Weed Species	Expression of symptoms			
		***Pyricularia* inoculation**		**Water spray**	
		Reaction	**Lesion length (cm)***	**Reaction**	**Lesion length (cm)***
1	*Chloris barbeta*	+	0.12	-	0.0
2	*Cyanodon dactylon*	+	0.32	-	0.0
3	*Cyperus rotundus*	+	0.82	-	0.0
4	*Dactylacterium aegyptium*	+	0.94	-	0.0
5	*Echinochloa colonum*	-	0.0	-	0.0

'**+**' : Symptoms observed; '**-**': No symptoms observed, *****Mean of three replications.

Table 2. Leaf blast incidence in finger millet under (cv. Co (Rg) 14) different weed infestation level during 2022-23.

Sl. No	Treatment	Incidence of leaf blast (PDI)*												
		7 DAS	**14 DAS**	**21 DAS**	**28 DAS**	**35 DAS**	**42 DAS**	**49 DAS**	**56 DAS**	**63 DAS**	**70 DAS**	**77 DAS**	**84 DAS**	**91 DAS**
1	Finger millet + *C. barbeta*	0	0	1.64	7.61	14.67	21.34	26.34	32.08	35.21	30.15	26.42	21.63	16.54
2	Finger millet + *Cya. dactylon*	0	0	1.51	5.06	10.20	15.62	18.62	20.16	23.17	21.06	18.63	12.30	11.34
3	Finger millet + *Cyp. rotundus*	0	0	3.51	11.25	22.18	28.34	34.12	41.02	42.65	38.5	36.41	31.49	26.32
4	Finger millet + *D. aegyptium*	0	0	1.34	8.02	19.31	26.49	30.18	34.62	37.43	35.29	31.02	22.48	15.32
5	Finger millet + *E. colonum*	0	0	1.85	7.49	15.62	24.91	29.05	33.41	39.01	33.46	30.49	20.61	12.30
6	Finger millet + All weeds	0	1.27	4.05	13.42	25.64	36.51	46.15	51.24	55.67	53.24	52.34	41.05	36.02
7	Finger millet alone	0	0	1.15	5.12	9.63	16.84	18.05	21.05	24.27	20.15	16.21	10.48	6.42
	SEd	--	--	7.51	6.95	8.67	16.15	18.64	21.62	19.42	16.63	14.75	16.49	10.69
	CD (0.05 % Level)	--	NS	0.71	1.32	1.48	3.45	3.84	6.25	7.30	5.94	4.68	7.40	5.34

* Mean of four replications; **PDI**: Per cent disease index; **DAS**: Days after sowing, **NS**: Non Significant.

Table 3. Leaf blast incidence in finger millet (cv. Co (Rg) 14) under different weed infestation level during 2023-24.

S.No	Treatment	Incidence of leaf blast (PDI)*												
		7 DAS	**14 DAS**	**21 DAS**	**28 DAS**	**35 DAS**	**42 DAS**	**49 DAS**	**56 DAS**	**63 DAS**	**70 DAS**	**77 DAS**	**84 DAS**	**91 DAS**
1	Finger millet + *C. barbeta*	0	0.49	2.45	5.63	8.49	13.46	15.32	18.32	18.60	17.05	15.49	12.43	10.43
2	Finger millet + *Cya. dactylon*	0	0.68	3.02	6.32	8.32	12.73	16.43	18.43	17.02	16.30	16.42	11.64	8.42
3	Finger millet + *Cyp. rotundus*	0	0.84	6.32	8.62	16.32	20.46	24.61	27.30	26.32	25.03	22.64	20.41	18.32
4	Finger millet + *D.aegyptium*	0	0.64	1.86	6.2	14.63	18.43	22.30	26.72	27.09	26.45	25.43	21.60	16.41
5	Finger millet + *E.colonum*	0	0.23	2.15	5.26	9.62	13.47	17.32	20.46	18.47	16.07	15.41	10.24	8.32
6	Finger millet + All weeds	0	0.89	5.12	9.26	18.32	24.61	30.14	33.42	33.71	30.67	28.63	23.43	21.30
7	Finger millet alone	0	0.43	2.61	4.02	6.23	11.26	12.48	15.02	16.07	13.06	12.08	8.05	7.32
	SEd	0	2.48	6.32	7.03	9.32	10.34	11.84	16.24	14.28	15.30	16.49	12.34	10.42
	CD (0.05 % Level)	0	0.15	0.85	0.94	1.62	1.24	2.08	2.61	1.87	1.28	1.41	1.32	1.68

* Mean of four replications; **PDI**: Per cent disease index; **DAS**: Days after sowing.

indicate that leaf blast incidence was higher during the vegetative stage when compared to the flowering stage.

A variety of weed hosts that are growing next to cultivated plants may act as potential sources of inoculum for the disease, giving the fungus a different way to survive (35). Although blast infects a broad range of sympatric flora, *M. grisea* populations are firmly limited by host range (36, 37). Furthermore, blast also infects weeds that typically occur in finger millet farms, including *Eleusine indica, Digitaria* spp., *Dactyloctenium* sp., and *Cyperus* sp. (38). The fungus has alternate hosts in these and other weeds. Prompt weeding stops the spread of blast propagules. Among these, the genus *Pennisetum* has approximately 100 species and is varied (39). It is currently unknown whether any *Pennisetum* species is susceptible to an infection with *Magnaporthe grisea*. According to the material at hand, *Pennisetum glaucum, P. macroforum, P. squamulatum, P. pedicellatum* and *P. ciliare* (40), and *P. purpureum* (41) are the main host organisms of the pathogen. The pathogen's collateral hosts include other graminaceous hosts such as *Panicum miliaceum, Agrostis palustris, Brachiaria mutica, Eleusine indica, Cyperus rotundus,* and *Eragrostis* sp. (42). The *M. grisea* species complex has a very wide host range, has genetic diversity, and has new strains emerging (43).

Effect of weed on colonies of *Pyricularia* in finger millet

During the field trials, colonies of *Pyricularia* were observed using Agroscope spore trap and the results are pre-

sented in Fig. 1 & 2. The results indicated that colonies were observed from 7 days after sowing in the field during 2022-23 and 2023-24. No. of colonies per plate increased from 21 DAS to 49 DAS in 2022-23 and to 63 DAS in 2023-24. The number of colonies was found to be more during 2022 -23 when compared to 2023-24. The number of fungal colonies was higher in finger millet with all the weeds in both years.

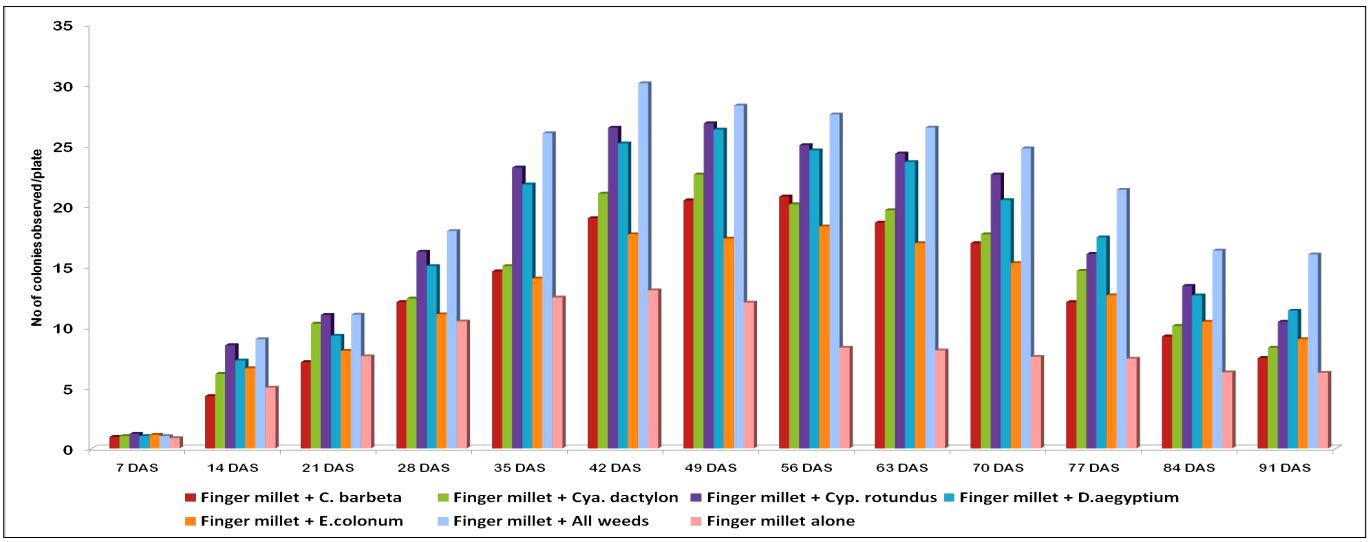

Fig. 1. Impact of weed species on colonies of *Pyricularia* in finger millet during 2022-23

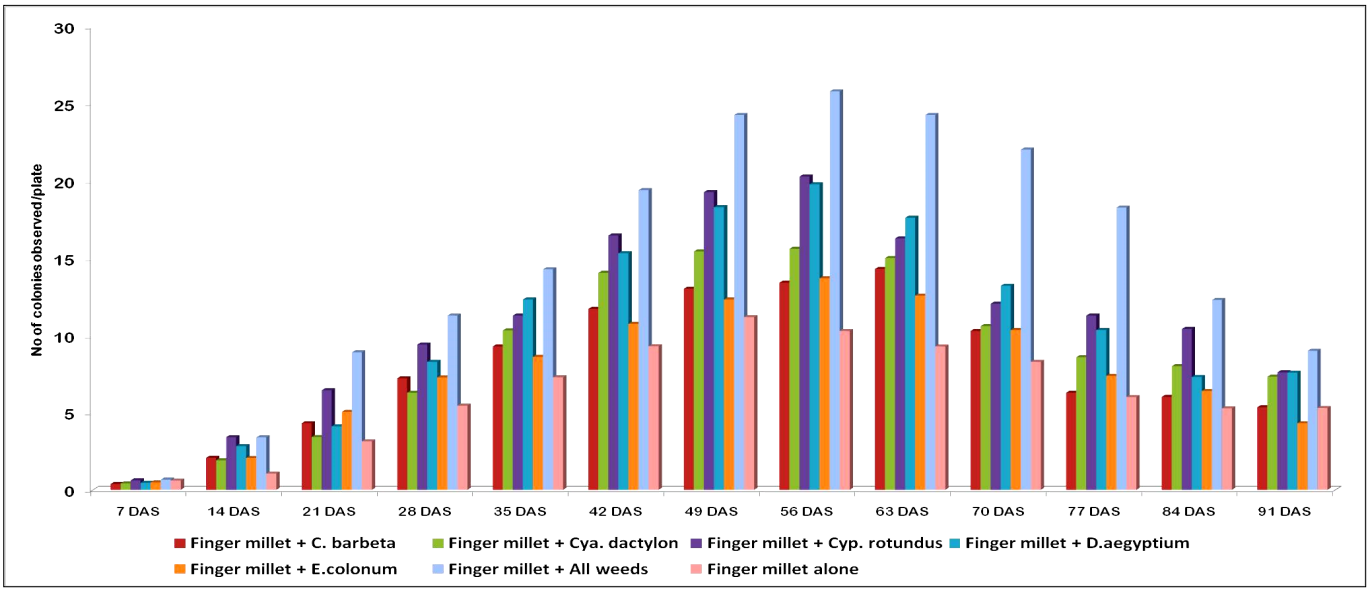

Fig. 2. Impact of weed species on colonies of *Pyricularia* in figer millet during 2023-24

Correlation interaction between percent disease index and colonies of *Pyricularia*

A significant level of correlation was observed between the PDI and colonies of *Pyricularia* during the two years from 28 DAS to 91 DAS (Table 4). There was no significant correlation observed before 28 DAS. The reason for non-significant observation before 28 DAS is that the spore load of *Pyricularia* is not sufficient to influence the disease index among the weed host plants. During the pathogenesis of *Pyricularia*, the infectious hyphae swiftly spread after they were successfully inserted, causing sores all over the leaves. The lesions are shown with many conidia within 7 days, which can start a new ease cycle (44). A wide variety of host species are susceptible to infection by various host -limited forms of *P. oryzae*, including weeds and crops like foxtail millet (*Setaria italica*), rice (*Oryza sativa*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), oat (*Avena sativa*), wild millet (*Eriochloa villosa*), green bristlegrass (*S. viridis*), crabgrass (*Digitaria sangui-nalis*), and goose grass (*Eleusine indica*) (45-48). Many *P. oryzae* isolates from various hosts had their pathogenicity investigated, and the *Pyricularia* isolates of barley, foxtail millet, crabgrass, and goose grass were virulent against rice (49, 50). Whereas *Pyricularia* of crabgrass, goose grass, green bristle grass, and foxtail millet were unable to infect rice in other investigations since they are host-specific, while barley is extremely vulnerable to several isolates from various hosts (14). The *Pyricularia* has host specificity in the ecosystem and survives in the weed hosts.

The interaction between the host plant and *Pyricularia* is well studied. Host specificities are determined by the combinations of avirulence (Avr) genes of the pathogen and disease resistance (R) genes of the host plant. In rice (*Oryza sativa*), numerous Avr genes of *P. oryzae* and R genes of the plant have been identified and these combinations explain the race–cultivar specificity (51-53). Most

Table 4. Correlation coefficient between Percent disease index and colonies of *Pyricularia* in finger millet under various weed species level.

S.No	Days after sowing	2022-23		2023-24	
		Coefficient determination	R^2 value	Coefficient determination	R^2 value
1	7	NS	NS	NS	NS
2	14	NS	NS	NS	NS
3	21	NS	NS	NS	NS
4	28	0.894**	0.799	0.917**	0.841
5	35	0.934**	0.873	0.908**	0.824
6	42	0.816**	0.667	0.931**	0.871
7	49	0.723*	0.522	0.969**	0.940
8	56	0.771*	0.603	0.978**	0.958
9	63	0.730*	0.533	0.915**	0.838
10	70	0.807**	0.652	0.867**	0.751
11	77	0.842**	0.709	0.902**	0.814
12	84	0.912**	0.831	0.807**	0.652
13	91	0.881**	0.770	0.825**	0.681

NS: Non-significant, Significant level *=p<0.001, **= p<0.

Avr genes encode signal peptides and are thought to function as effectors; therefore, these race–cultivar specificities are called effector-triggered immunity. The *P. oryzae* population comprises various pathotypes with different host ranges, such as the *Oryza, Triticum, Setaria*, *Lolium*, and *Eleusine* pathotypes (54). The majority of Avr genes contain signal peptides and are believed to serve as effectors, so effector-triggered immunity is the term used to describe these race-cultivar specificities. Strong pathotype-genus specificities are believed to result from infections and host plants coevolving together (16). The combination of the Avr and R genes also explains these specificities (48). Hence, the identification of host ranges of *Pyricularia* among weed species is also useful for studying the Avr and R genes available in the host plants.

Conclusion

Our studies clearly demonstrated that *Pyricularia* isolated from finger millet expresses host-specificity reactions. The *Pyricularia* produces symptoms on *C. rotundus* and *D. aegyptium* under artificial inoculation study and is unable to express symptoms on weeds viz., *C. barbeta*, *C. dactylon*, and *E. colonum*. Further, the analysis of Avr genes and R genes available in finger millet and in the above weed host species will give an idea for the host-specific reaction of *Pyricularia* at the molecular level.

Acknowledgements

All authors are thankful to Tamil Nadu Agricultural University, Coimbatore 641 003, India for providing the necessary support for conducting laboratory as well as field experiments and facilities to write this research paper.

Authors' contributions

The authors SPT, VJN, RM, SK, and VM were involved in the isolation of the *Pyricularia* pathogen, infectivity assay on Poaceae weeds and field experiments, and JI was involved in the design of the study and performed the statistical analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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